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Manual

ZEN 2011 (blue edition)

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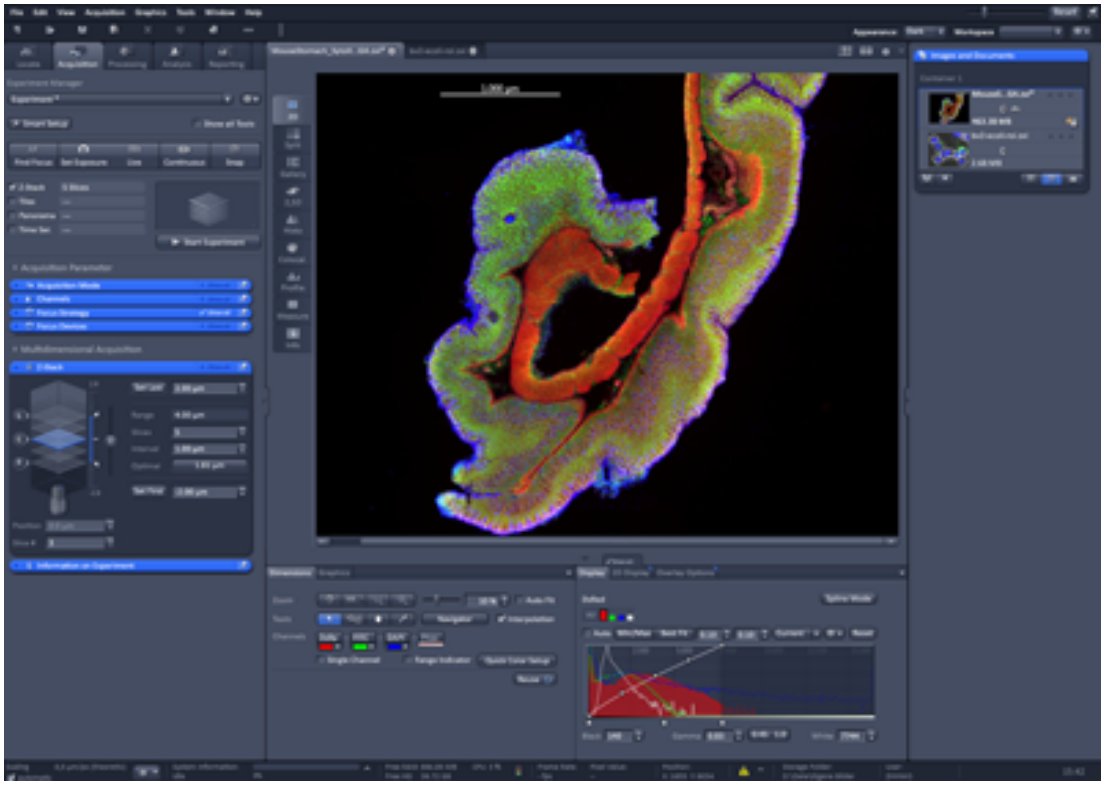
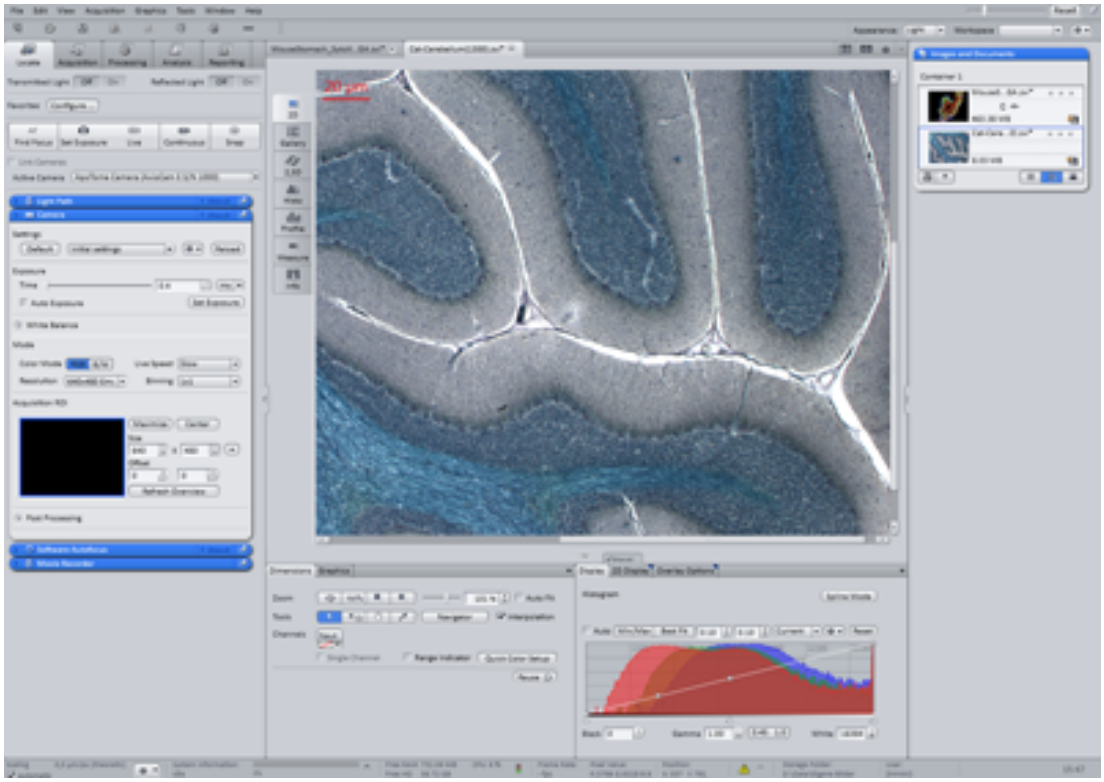
2 Introduction

2.1 Whats new?

2.1.1 Design dark/light

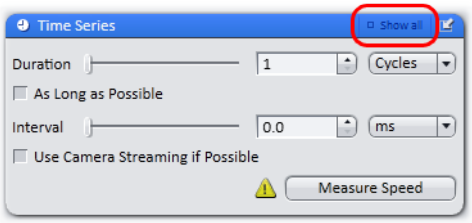


Design dark

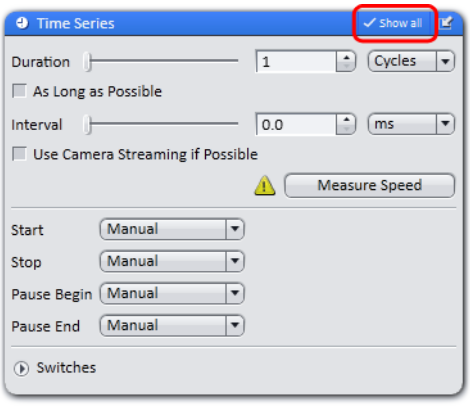


Design light

2.1.2 Show all mode

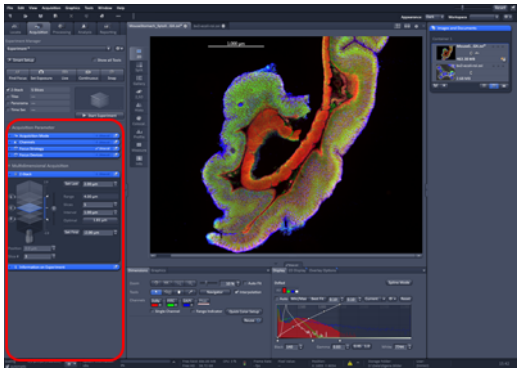


Show all mode deactivated

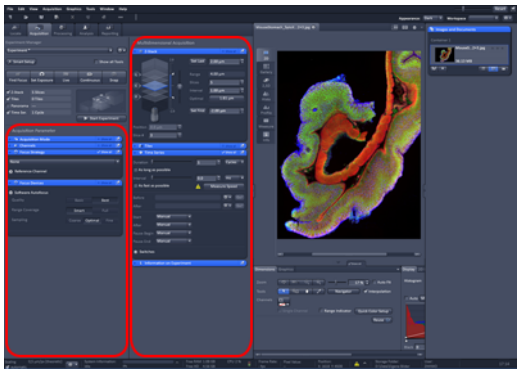


Show all mode activated

2.1.3 Multi columns layout

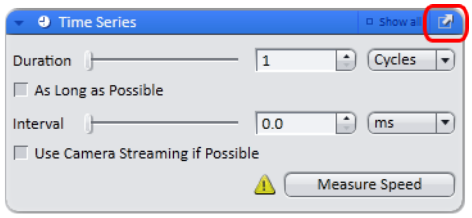


Single column

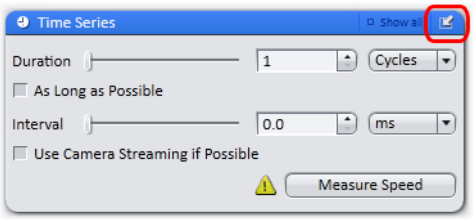


Second column

2.1.4 Undock/dock function

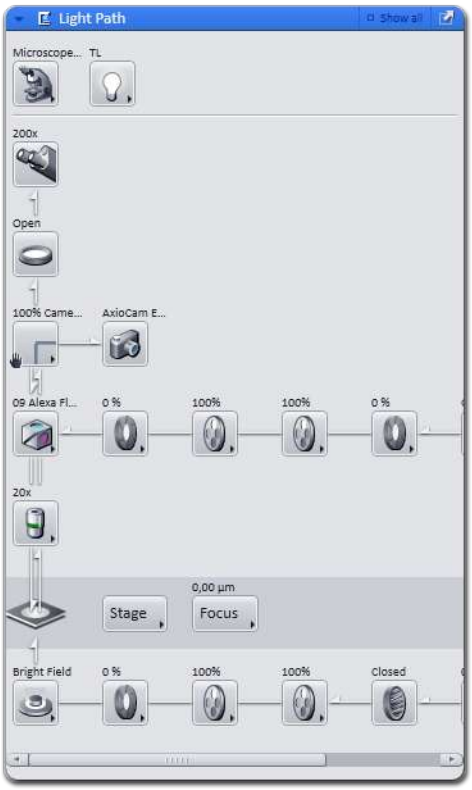


Undock function



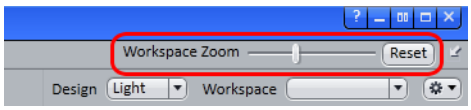
Dock function

2.1.5 Interactive lightpath configuration



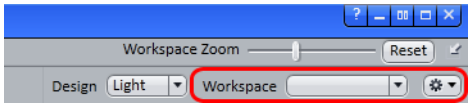
Lightpath tool

2.1.6 Workspace zoom



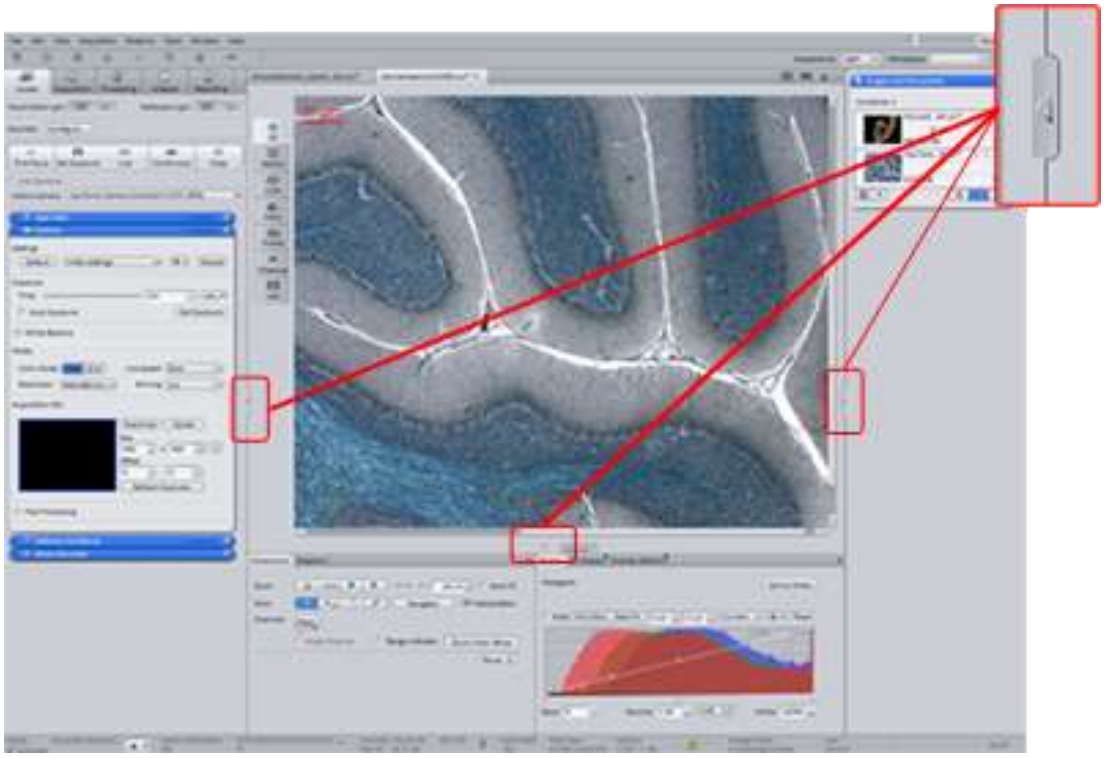
Workspace Zoom

2.1.7 Workspace configuration



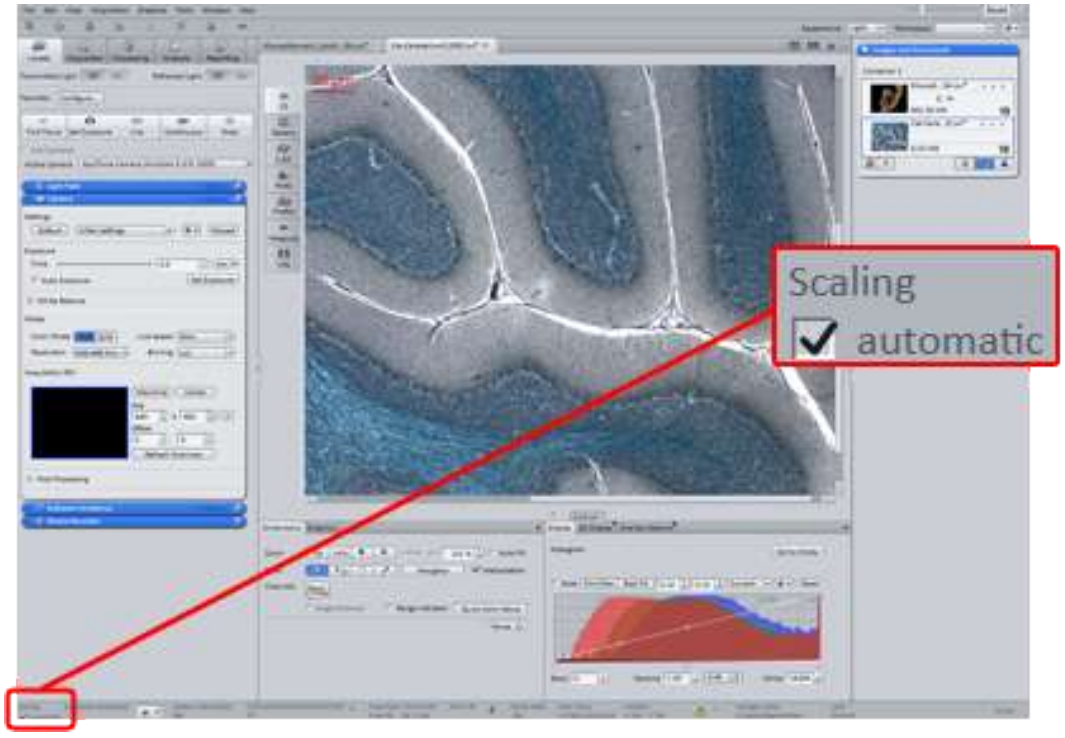
Workspace configuration

2.1.8 Show/hide tool areas



Show/Hide tool areas

2.1.9 Automatic scaling



Automatic scaling

2.2 Concept

ZEN is a modular image-processing and analysis software for modern microscopy from **Carl Zeiss**. In addition to basic functionality for image acquisition and microscope definitions, elementary image processing and annotations, image analysis and documentation optional modules for specific tasks are available.

2.2.1 Image Acquisition

A range of different camera types can be used with **ZEN**, from simple TV cameras through to high-resolution and high-sensitivity cameras. The cameras of the Carl Zeiss **AxioCam** family guarantee optimum integration.

2.2.2 Digital Movie Recorder

This allows the recording of image sequences with high speed and outstanding image quality. The raw data will be saved immediately on the hard drive. Finally the movie may be saved in the CZI-image format or exported as an AVI-movie.

2.2.3 Microscope Parameters

With **ZEN** it is possible to control any **Carl Zeiss** microscope interactively, manual standard microscopes as well.

2.2.4 Image Processing

The acquired image is immediately displayed on the monitor. It can then be optimized using a wide range of techniques:

- Contrast, brightness and color adjustment
- Noise suppression, smoothing and contour enhancement
- Sharpness enhancement/emphasizing of details
- Correction of illumination influences and white balance

ZEN can also be used to add any annotations that you may require to the images. All elements, from scale bars and colored markings through to text and graphics, have been integrated into the program.

2.2.5 Image Analysis

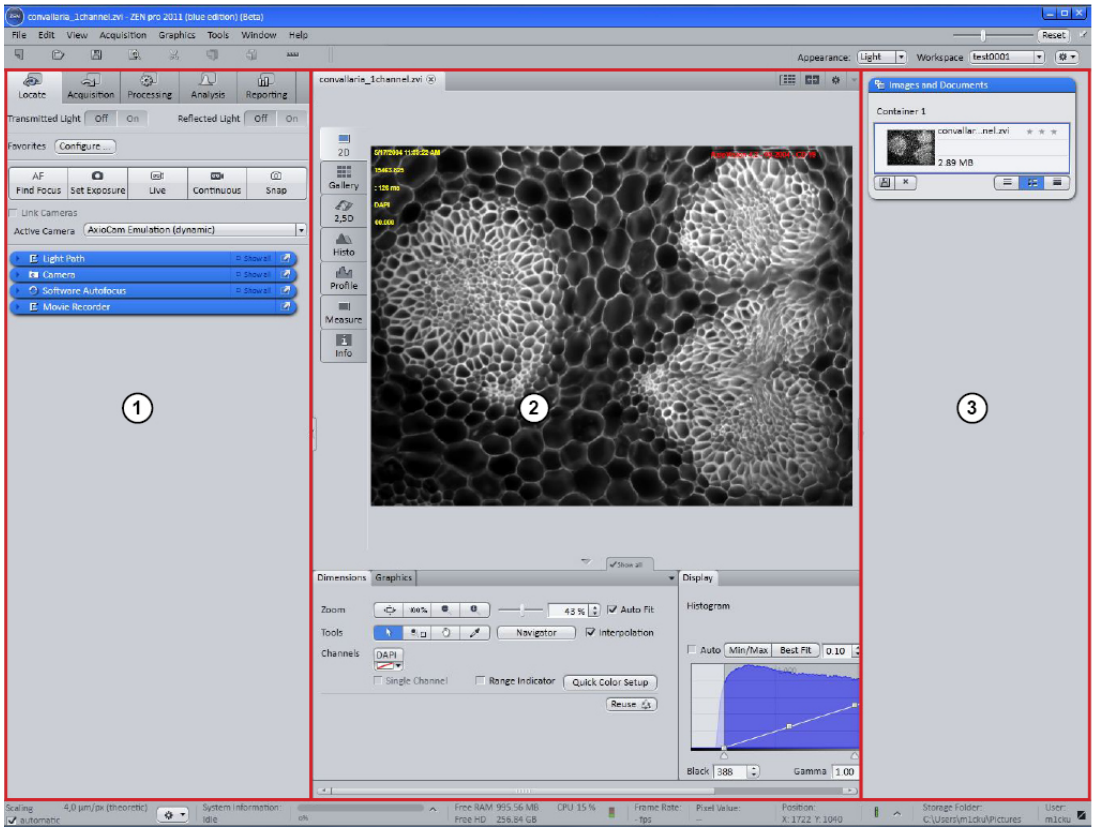
You are able to perform simple interactive measurements in the basic program. The measured values (e.g. lengths, areas and perimeters) are made available in a data table, and can be processed further using spreadsheet programs. The interactive measurement can be executed via the Graphics menu from the menu bar, or via the **Graphics** tab in the view controls of the **Measurement** View.

2.2.6 Documentation

Besides the image itself, the **ZEN** image format CZI also saves additional data, such as the image number, date of acquisition, microscope settings, exposure values, size and scale details, contrast procedures used etc. Annotations and measured values are also saved with the image.

2.3 Program interface

The ZEN (blue edition) program interface is divided into three main areas. Via the tabs in the **left tool area** you can access all the main tools for microscope control (Locate tab), acquisition (Acquisition tab), image processing (Processing tab), image analysis (Analysis tab) and report generation (Reporting tab). The **center screen area** is used to display your images, while the **right tool area** provides you with an overview of all open documents and is used for advanced file management.



Program interface

1	Left tool area
2	Center screen area
3	Right tool area

2.3.1 Title bar

Title bar

Question mark

Activates the "drag & drop“ help function. A question mark appears beside the mouse pointer. Move the mouse pointer to a place in the software where you need help. Left-click on the desired location. The online help opens.

Minimize

Minimizes the program window.

Maximize Across 2 Screens

Maximizes the program window across 2 screens. This option is only possible if you are working with 2 screens.

Maximize

Maximizes the program window to the main screen.

Close

Closes the program window.

2.3.2 Menu bar

The menu bar contains all the menus you need to manage, edit and view your projects.

File menu

New menu item (Ctrl+N)

Opens the **New Document** dialog window.

Open menu item (Ctrl+O)

Opens the **Open Document** dialog window. Here you can select the file you want to open.

Save menu item (Ctrl+S)

Saves the selected file.

Save As menu item

Saves the selected file under a new name.

Rename menu item

Opens the **Rename** dialog window. Enter a new name for the file. Confirm the entry with **Yes**.

Delete menu item

Deletes the selected file.

Export/Import menu item

Opens the relevant parameters on the **Processing** tab | **Method Selection** | **Method** | **Extras** | Export/Import [→ 151].

Close menu item (Ctrl+F4)

Closes the selected file.

Save All menu item

Saves all open files.

New File Browser menu item

Opens the **Browser window** in the **center screen area**.

Open Parent Folder menu item

Opens the folder in which the selected file is located.

Last Files Used menu item (Ctrl+R)

Opens the Last Files Used [→ 19] dialog window.

Log In menu item

Opens the Log In [→ 20] dialog window.

Exit menu item (Alt+F4)

Exits the program.

File menu dialog windows

New Document dialog window

In the **New Document** dialog window you can create new, empty documents.

Image button

Creates a new, empty image.

Table button

Creates a new table.

The following elements are only visible if you have clicked on the **Table** button:

Name input field

Here you can enter the name of the new table.

Columns input field

Enter the number of columns that you want the new table to have in the spin box/input field.

Rows input field

Enter the number of rows that you want the new table to have in the spin box/input field.

Column Definition list

In this section you can define the name, data type and a default value for each column.

Column Name input field

Here you can enter the name of the column.

Column Type dropdown list

Select the desired data type from the dropdown list. The following types are available for selection:

- Text
- Integer
- Floating point number

Default Value input field

Here you can enter the value that you want the cell to contain.

Last Files Used dialog window

The **Last Files Used** dialog window displays the last files you have used, separated according to file type.

Select File Type section

In the **Select File Type** section you can select which of the last files you have used are listed.

Images button

Click on the **Images** button to display the list of the last images opened.

Tables button

Click on the **Tables** button to display the list of the last tables opened.

Reports button

Click on the **Reports** button to display the list of the last reports opened.

Files list

Here you can select the files you want to open. To do this, click on the relevant file name. Hold down the CTRL or SHIFT key to select several files.

Open button

Click on the **Open** button to open the selected files.

Cancel button

Click on the **Cancel** button to exit the dialog window without opening a file.

Log In dialog window

Here you can decide how you want ZEN (blue edition) to start.

Start System button

Starts the system with its full functionality. You can control your microscope, cameras and all other connected devices.

Image Processing button

Starts the software with the image processing functionality only.

Don't Show This Dialog Window checkbox

Activated: The next time you start ZEN (blue edition) the **Log In** dialog window is no longer displayed.

Edit menu

Undo (Ctrl+Z)

Undoes the last action.

Redo (Ctrl+Y)

Redoes the last action.

Cut

Cuts the selected graphic element out of the image.

Copy

Copies the selected graphic element.

Insert

Inserts the copied graphic element into the image.

Delete

Deletes the selected graphic element.

Select All

Selects all graphic elements drawn into the image.

Display

Here you can manage display settings.

Selection Region

Here you can draw a new selection region into the image.

View menu

Zoom

Here you can configure various zoom settings.

Player

Here you can navigate through Z-stack or time lapse images.

Document Bar Display Options section

Text View	Displays the text of a file in the document bar.
Small Preview Image	Displays a small preview image of a file in the document bar.
Large Preview Image	Displays a large preview image of a file in the document bar.

Image Area Display Options section

1 Container	Displays one image container in the image area.
2 Containers	Displays two image containers in the image area.
3 Containers	Displays three image containers in the image area.

Automatic Container Layout

Uses the predefined container layout.

Viewing Options for All Containers

Applies the viewing options to all containers.

Viewing Options per Container

Applies the viewing options to the selected container.

Show All (Global)

Activates **Show All** mode globally.

Acquisition menu

Live

Switches to live mode (**Shift+F2**).

Snap

Executes a snap command (**F2**).

Exposure

Starts an exposure time measurement.

White Balance

Performs an automatic white balance.

Find Focus

Starts an autofocus search.

Graphics menu

Here you will find tools that can be used to draw graphic elements into images and edit graphic elements. The tools are arranged in groups with other related tools. These groups can be identified by separation lines. Additional explanations relating to graphic elements, and in particular to formatting changes, can be found in the help text for the Graphics tab [→ 286].

Selection Mode menu item

Using this menu command you can return to Selection mode if you had previously selected a different tool. Alternatively, you can press Alt+F1 or ESC.

If the mouse pointer is already in this mode, this menu command is inactive.

Tool Group

In this group you will find all the tools used to draw in graphic elements. Please also note the options for individual tools. These are indicated by an arrow on the right-hand side. Most tools are only active if an image has been opened.

Draw In Selection Region

Using the left mouse button, drag out a rectangle of the desired size in the image concerned. You can draw in as many regions as you wish. Holding down the Ctrl key and mouse button, now drag out the region content outside the region and release the Ctrl key and mouse button there: a new image of the content of the region is created.

Text

Using this tool you can insert free text into the image. The following options are available:

- Click in the image once and immediately enter the text. The text box grows dynamically with the text. As soon as you reach the image edge, the text is automatically wrapped onto the next line.
- Click in the image and drag out a rectangle of the desired size before releasing the mouse button: the text is now entered inside the text box. If you reach the end of the text box, its height is automatically adjusted to the text.

To change text that you have entered, double-click inside the text. The text is now shown against a white background and you are able to make changes. To confirm the entry, click once in the image outside the text box.

Scale Bar

If you select this menu command, a horizontal scale bar is automatically inserted at the bottom right edge of the image. If you execute the command a second time, a scale bar is inserted in the top right corner. If you execute it a third time, a scale bar is inserted in the top left corner. Each subsequent execution of the command inserts an additional scale bar into the image in a slightly offset position.

The scale bar also shows the unit in text form.

The length of the scale bar is automatically adjusted to the image width so that roughly 5% of the image width is used. You can, however, change the length by grabbing a corner point with the mouse and dragging it accordingly. Lengths can only be set in fixed steps.

To align the scale bar vertically, grab a corner point and move it upwards before releasing the mouse button.

Scale bars cannot contain any additional measurement data or texts.

Line

Using this tool you can draw a line with an indication of the length into the image. Select the command, click once in the image to define the start point and once more to define the end point. The line is then drawn in with a text annotation showing the length measurement.

To change a drawn-in line, grab one of the end points and drag it to the desired location. As soon as you release the button, the new measured value is displayed.

To add additional free text to a line, double-click on the line and enter the text in the text field.

Additional key commands

Various key commands are available to you that can make drawing easier:

- **Shift key**: If you hold down the Shift key, the line is only drawn in at fixed angle increments of 15°.
- **Alt key**: If you hold down the Alt key, the first click is not used as the start point, but as the center point of the line.
- **Ctrl** key: If you hold down the Ctrl key, the current direction in which the line is drawn in is retained.

Arrow

Using this tool you can draw an arrow into the image. Select the command, click once in the image to define the start point and once more to define the end point. The arrow is then drawn in. The arrow symbol indicates the end point.

To change a drawn-in arrow, grab one of the end points and drag it to the desired location.

To add free text to an arrow, double-click on the arrow and enter the text in the text field.

Arrows cannot contain any measurement data.

Additional key commands

Various key commands are available to you that can make drawing easier:

- **Shift key**: If you hold down the Shift key, the line is only drawn in at fixed angle increments of 15°.
- **Alt key**: If you hold down the Alt key, the first click is not used as the start point, but as the center point of the line.
- **Ctrl** key: If you hold down the Ctrl key, the current direction in which the arrow is drawn in is retained.

Rectangle (Parallel)

Using this tool you can draw a simple rectangle into the image. The first click defines the start point and the second click the end point. The rectangle is initially always drawn in parallel to the horizontal edge of the image. It can, however, be rotated later.

A rectangle automatically displays the **Area** measured values and the **Average Gray Value Intensities** in the image. In the case of multichannel images these are also shown for each channel.

To change a rectangle, grab one of the corner or line points and drag it to the desired location. As soon as you release the button, the new measured values are displayed.

To add additional free text to a rectangle, double-click on the rectangle and enter the text in the text field.

Meta data on the acquisition procedure can also be assigned to a rectangle from the image; please see --- FEHLENDER LINK ---.

Besides the standard measurement data, a rectangle can also display a range of other measured values; please see --- FEHLENDER LINK ---.

Additional key commands

Various key commands are available to you that can make drawing easier:

- **Shift key**: If you hold down the Shift key, a square is drawn instead of a rectangle.
- **Alt key**: If you hold down the Alt key, the first click is not used as the start point, but as the center point of the rectangle.

See also

Features section [➙ 181]

Circle (Diameter)

Using this tool you can draw a circle into the image. The circle is inserted based on the diameter that you draw in. The first click defines the start point and the second click the end point. The line with an arrow at either end defines the diameter.

A circle automatically displays the **Area** measured values and the **Average Gray Value Intensities** in the image. In the case of multichannel images these are also shown for each channel.

To change a circle, grab one of the end or line points and drag it to the desired location. As soon as you release the button, the new measured values are displayed.

To add additional free text to a circle, double-click on the circle and enter the text in the text field.

Meta data on the acquisition procedure can also be assigned to a circle from the image; please see .

Besides the standard measurement data, a circle can also display a range of other measured values; please see .

Additional key commands

A key command is available to you that can make drawing easier:

- **Shift key**: If you hold down the Shift key, the circle is generated horizontally from left to right. The diameter line is then parallel to the horizontal edge of the image.

Contour (Spline)

Using this tool you can draw a contour into the image. The corner points of the contour are always rounded. Two drawing methods are available to you:

- Click in the image to define the start point. Then hold down the mouse button and outline the desired structure in the image. When the mouse pointer gets near to the start point, release the mouse button. As soon as the mouse pointer icon changes, click once and the contour is closed.

- Alternatively, after defining the start point you can also click in the image to define additional corner points. Right-click to close the contour.

As soon as the contour has been closed, the **Area** measured values and the **Average Gray Value Intensities** are displayed in the image. In the case of multichannel images these are also shown for each channel.

To change the size of a **contour (spline)** as a whole, grab one of the corner or line points and move it. All elements of the contour are changed in a uniformly proportional way. As soon as you release the button, the new measured values are displayed.

To add additional free text to a contour, double-click on the graphic element and enter the text in the text field.

Meta data on the acquisition procedure can also be assigned to a contour from the image; please see --- FEHLENDER LINK ---.

Besides the standard measurement data, a contour can also display a range of other measured values; please see --- FEHLENDER LINK ---.

Contours consist of a series of points that are connected by a line. To edit these points, use the shortcut menu (right mouse button).

Contour (Spline) shortcut menu

For a general description of the shortcut menu for graphic elements, please see --- FEHLENDER LINK ---.

To edit the points of a contour, you should select these first. Now right-click on the contour. In the shortcut menu you will find the following commands:

- Split: Draw a separation line through the contour in the form of a free curve. End the drawing of the separation line by right-clicking. The contour is now separated along the separation line.
- Separate: Draw in a separation region in the form of a free contour and end this by right-clicking. The region covered by the separation region is removed from the contour and you are left with two smaller contours.
- Connect: Select at least two contours and draw in a connecting contour (end by right-clicking). The regions of the selected contours and the image regions covered by the newly drawn-in contour are connected to form a single contour.

It is also possible to edit the points of the contour. To do this, select the **Edit Points** command from the shortcut menu. You can now grab and move individual points. The shortcut menu also contains the following additional commands:

- Add Point (only active if the mouse is **not** currently positioned over an existing point): adds an additional point to the line.
- Delete Point (only active if the mouse is currently positioned over an existing point): deletes the current point from the contour.

To exit editing mode, select the command **End Editing of Points** from the shortcut menu or click once in the image outside the edited contour.

Profile

Draws a gray value profile into the image along a line. Click in the image to define the start and end point. In the case of multichannel images a separate profile line is displayed for each channel.

To change a drawn-in line profile, grab one of the end points and drag it to the desired location.

To add additional free text to a line profile, double-click on the line profile and enter the text in the text field.

Additional key commands

Various key commands are available to you that can make drawing easier:

- **Shift key**: If you hold down the Shift key, the line is only drawn in at fixed angle increments of 45°.
- **Alt key**: If you hold down the Alt key, the first click is not used as the start point, but as the center point of the line.
- **Ctrl** key: If you hold down the Ctrl key, the current direction in which the profile is drawn in is retained.

Rectangle – Profile

This tool draws in a rectangle, across the width of which (i.e. in a vertical direction in relation to the base line) a profile of the average gray values is displayed.

With your first click you define the start point, with your second click the end point and with your third click the width of the rectangle. If you drag out the rectangle from left to right, the profile lines are drawn from bottom to top. If you drag out the rectangle from right to left, the profile lines are drawn from top to bottom.

To change the rectangle profile, grab one of the end or line points and drag it to the desired location. As soon as you release the button, the profile lines are recalculated.

To add free text to a rectangle profile, double-click on the rectangle and enter the text in the text field.

Additional key commands

Various key commands are available to you that can make drawing easier:

- **Shift key**: If you hold down the Shift key, the base line is drawn in at fixed angle increments of 15°.
- **Alt key**: If you hold down the Alt key, the first click is not used as the start point, but as the center point of the base line.

Grid

Displays a grid in the image area. To display the grid, click on the Grid menu item.

Formatting a grid

➢ An image is being displayed in the image area.

➢ A grid is being displayed in the image area.

1. Right-click on the grid in the image area.
   ⇨ The Graphic Elements shortcut menu opens.
2. Click on the Format menu item.

⇨ The Format Grid dialog window opens.

Frequently Used Annotations

Frequently used annotations are graphic elements of the rectangle type that are linked to fixed meta information from the image. Annotations are inserted into the image starting from the top left and then one below the other.

All frequently used annotations can be moved to positions of your choice. To change the size of the text box, grab one of the end or line points and drag it out to the desired size.

The following annotations are available as frequently used annotations:

Relative Time menu item

Adds a time stamp to the image. This time indicator is in the format HH:MM:SS:MS (00:00:00:000) and is set to zero for the time point or Z position currently selected using the sliders under **Dimensions**.

Only the digits that actually contain data are displayed. This means that hours are hidden, for example, if the experiment only lasted 10 minutes.

In the case of multichannel images separate values are displayed for each channel in the mixed color view. Only the first channel is set to zero.

You can insert as many Relative Time annotations into the image as you wish.

You can also add free text to an annotation. To do this, double-click on the rectangle and enter the text in the text field. Make sure that the text [a] is not changed.

Acquisition Time menu item

Adds the absolute acquisition date/time to the image. The format corresponds to the Windows system settings and cannot be changed.

To change an annotation, grab one of the end or line points and drag it to the desired location.

You can also add free text to an annotation. To do this, double-click on the rectangle and enter the text in the text field. Make sure that the text [a] is not changed.

Relative Focus Position menu item

Adds a focus position to the image as an annotation. This focus position is set to zero for the focus position currently selected using the slider for the Z **dimension**.

You can insert as many Relative Focus Position annotations into the image as you wish.

You can also add free text to an annotation. To do this, double-click on the rectangle and enter the text in the text field. Make sure that the text [a] is not changed.

Focus Position menu item

Adds the absolute focus position to the image as an annotation. This focus position corresponds to the focus position of the microscope that applied at the time of acquisition.

You can also add free text to an annotation. To do this, double-click on the rectangle and enter the text in the text field. Make sure that the text [a] is not changed.

Exposure Time menu item

Adds the exposure time that applied during acquisition to the image as an annotation. This exposure time corresponds to the camera setting at the time acquisition was triggered.

In the case of multichannel images separate values are displayed for each channel in the mixed color view.

You can also add free text to an annotation. To do this, double-click on the rectangle and enter the text in the text field. Make sure that the text [a] is not changed.

Channel Name menu item

Adds the channel names used during acquisition to the image as an annotation.

In the case of multichannel images separate names are displayed for each channel in the mixed color view.

You can also add free text to an annotation. To do this, double-click on the rectangle and enter the text in the text field. Make sure that the text [a] is not changed.

more ... menu item

Besides the preconfigured frequently used annotations, it is also possible to add annotation rectangles containing other meta data. To do this, click on the **more ...** entry. The **Select Meta Data** dialog opens.

Select Meta Data dialog window

Meta Data Used

This list shows the meta information that you have already used.

Search For Meta Data

In this search field you can search for any meta information. If you are looking for camera data, for example, simply enter the term **Camera**. The Available Meta Data list is now filtered and only shows meta information containing the term **Camera**.

Please note: As a rule the terms are only available in English. Please therefore use English search terms.

Available Meta Data

This list contains all the meta data saved in the image with a brief description and an indication of the internal path.

To apply a selection, click on the **Apply** button. To close the dialog without making a selection, click on **Cancel**.

Distance

The functions grouped under this menu item are only available if you have installed the Measurement module. .

To use the functions, select the Distance menu item and select the desired command from the window that opens.

Length menu item

Using the **Length** measurement tool you can measure the distance between two points.

With your first click you define the start point, with your second click the end point and with your third click the distance between the measurement line and the measurement points. The length is displayed in scaled units.

To change the **Length** measurement tool, grab one of the end or line points and drag it to the desired location. As soon as you release the button, the measured value is recalculated.

To add free text to the **Length** measurement tool, double-click on the graphic element and enter the text in the text field.

Distance menu item

Using the **Distance** measurement tool you can measure the distance between two parallel lines.

With your first click you define the start point and with your second click the end point of the first line. With your third click you define both the distance between the second and first measurement line and the distance between the dimension line and the measurement lines.

The length is displayed in scaled units.

To change the **Distance** measurement tool, grab one of the end or line points and drag it to the desired location. As soon as you release the button, the measured value is recalculated.

It is not possible to add free text to the **Distance** measurement tool.

Additional key commands

- **Alt key**: If you hold down the Alt key, the center point of the line is used as the anchor point when the length of the measurement line is changed.

Curve (Spline) menu item

Using the **Curve (Spline)** tool you can measure the length of any line drawn into the image. The line runs through all points that you have set in the active window and is flattened using an interpolated curve ("spline").

Two drawing methods are available to you:

- Click in the image to define the start point. Then hold down the mouse button and follow the desired structure in the image. Right-click to end the measurement.
- Alternatively, after defining the start point you can also click repeatedly in the image to define additional points. Right-click to end the measurement.

The length is displayed in scaled units.

To change the size of a **curve (spline)**, grab one of the corner or line points and move it. All elements of the curve are changed in a uniformly proportional way. As soon as you release the button, the new measured values are displayed.

To add free text to a **curve (spline)**, double-click on the **curve (spline)** and enter the text in the text field.

To edit the points of a **curve (spline)**, use the shortcut menu (right mouse button).

Curve (Spline) shortcut menu

For a general description of the shortcut menu for graphic elements, please see --- FEHLENDER LINK ---.

To edit the points of a curve, you should select these first. Now right-click on the curve. In the shortcut menu you will find the following commands:

- Split: Draw a separation line through the curve in the form of a free curve. End the drawing of the separation line by right-clicking. The curve is now separated along the separation line.
- Separate: Draw in a separation region in the form of a free contour and end this by right-clicking. The region covered by the separation region is removed from the curve and you are left with two smaller curves.

It is also possible to edit the points of the curve. To do this, select the **Edit Points** command from the shortcut menu. You can now grab and move individual points. The shortcut menu also contains the following additional commands:

- Add Point (only active if the mouse is **not** currently positioned over an existing point): adds an additional point to the line.
- Delete Point (only active if the mouse is currently positioned over an existing point): deletes the current point from the contour.

To exit editing mode, select the command **End Editing of Points** from the shortcut menu or click once in the image outside the edited contour.

Active Curve menu item

Using the **Active Curve** tool you can measure the length of a line drawn into the image. The line is of the **polygon** type and defined by individual points. It is automatically adapted to structures in the image.

Two drawing methods are available to you:

- Click in the image to define the start point. Then hold down the mouse button and follow the desired structure in the image. If the local contrast is sufficient, the line automatically jumps to the correct position. Right-click to end the measurement.
- Alternatively, after defining the start point you can also click repeatedly in the image to define additional points. In this case the active search for local contrasts is not used. Right-click to end the measurement.

The length is displayed in scaled units.

To change the size of an **active curve**, grab one of the corner or line points and move it. All elements of the curve are changed in a uniformly proportional way. As soon as you release the button, the new measured values are displayed.

To add free text to an **active curve**, double-click on the **active curve** and enter the text in the text field.

To edit individual points of an **active curve**, use the shortcut menu (right mouse button).

Active Curve shortcut menu

For a general description of the shortcut menu for graphic elements, please see --- FEHLENDER LINK ---.

To edit the points of an **active curve**, you should select these first. Now right-click on the curve. In the shortcut menu you will find the following commands:

- Split: Draw a separation line through the curve in the form of a free curve. End the drawing of the separation line by right-clicking. The curve is now separated along the separation line.
- Separate: Draw in a separation region in the form of a free contour and end this by right-clicking. The region covered by the separation region is removed from the curve and you are left with two smaller curves.

It is also possible to edit the points of the curve. To do this, select the **Edit Points** command from the shortcut menu. You can now grab and move individual points. The shortcut menu also contains the following additional commands:

- Add Point (only active if the mouse is **not** currently positioned over an existing point): adds an additional point to the line.
- Delete Point (only active if the mouse is currently positioned over an existing point): deletes the current point from the contour.

To exit editing mode, select the command **End Editing of Points** from the shortcut menu or click once in the image outside the edited contour.

Curve (Polygon) menu item

Using the **Curve (Polygon)** tool you can measure the length of any line drawn into the image. The line runs directly through all the points you have set in the active window.

Two drawing methods are available to you:

- Click in the image to define the start point. Then hold down the mouse button and follow the desired structure in the image. Right-click to end the measurement.
- Alternatively, after defining the start point you can also click repeatedly in the image to define additional points. Right-click to end the measurement.

The length is displayed in scaled units.

To change the size of a **curve (polygon)**, grab one of the corner or line points and move it. All elements of the curve are changed in a uniformly proportional way. As soon as you release the button, the new measured values are displayed.

To add free text to a **curve (polygon)**, double-click on the **curve (polygon)** and enter the text in the text field.

To edit the points of a **curve (polygon)**, use the shortcut menu (right mouse button).

Curve (Polygon) shortcut menu

For a general description of the shortcut menu for graphic elements, please see --- FEHLENDER LINK ---.

To edit the points of a curve, you should select these first. Now right-click on the curve. In the shortcut menu you will find the following commands:

- Split: Draw a separation line through the curve in the form of a free curve. End the drawing of the separation line by right-clicking. The curve is now separated along the separation line.
- Separate: Draw in a separation region in the form of a free contour and end this by right-clicking. The region covered by the separation region is removed from the curve and you are left with two smaller curves.

It is also possible to edit the points of the curve. To do this, select the **Edit Points** command from the shortcut menu. You can now grab and move individual points. The shortcut menu also contains the following additional commands:

- Add Point (only active if the mouse is **not** currently positioned over an existing point): adds an additional point to the line.
- Delete Point (only active if the mouse is currently positioned over an existing point): deletes the current point from the contour.

To exit editing mode, select the command **End Editing of Points** from the shortcut menu or click once in the image outside the edited contour.

Caliper

To measure a distance perpendicular to a base line, first drag out the base line by defining the start and end point with a click of the mouse. Then define the length perpendicular to the base line by dragging with the mouse.

Complete the measurement by clicking with the mouse.

The distance is measured in scaled units.

To change the Distance tool, grab one of the end or line points and drag it to the desired position. As soon as you release the button, the measured value is recalculated. You can also move the measurement line independently of the base line by grabbing it with the mouse and moving it to the desired location in the image.

Additional key commands

- **Alt key**: If you hold down the Alt key, the center point of the line is used as the anchor point when the length of the measurement line is changed.

Several Distances

The functions grouped under this menu item are only available if you have installed the Measurement module.

To use the functions, select the Multiple Distance menu item and select the desired command from the window that opens.

Multiple Distance menu item

Using this tool you can measure several distances between parallel lines in a single measurement procedure. The first mouse click defines the start point of the base line and the second its end point. Each subsequent click generates a new line of the same length which you can position anywhere you wish. With each click, the distance from the new line to the base line is measured again.

Right-click to end the multiple measurement.

The distances are measured in scaled units.

To move the entire graphic element, hold the mouse over the element. As soon as the mouse pointer changes to the  icon, you can move the graphic element as a whole.

You can subsequently move the position of the individual measurement lines: To do this, click close to the line point  and drag the line to the desired position. To change the rotation, move the mouse to an adjustment handle (pointer icon ) and rotate the element.

Multiple Caliper menu item

Using this tool you can measure several distances in a single measurement procedure. The first mouse click defines the start point of the base line from which the measurement is made and the second its end point. Each subsequent click generates a new line perpendicular to the base line, the end point and position of which you can determine with a click of the mouse. A new measurement line is generated with each additional click. Right-click to end the measurement.

The distances from the base line to the end point of the measurement lines are measured in scaled units.

To move the entire graphic element, hold the mouse over the element. As soon as the mouse pointer changes to the  icon, you can move the graphic element as a whole.

You can subsequently move the position of the individual measurement lines: To do this, click close to the line point  and drag the line to the desired position. To change the length of the measurement lines, move the mouse to an adjustment handle (pointer icon ) and change the length.

Region

The functions grouped under this menu item are only available if you have installed the Measurement module.

To use the functions, select the **Region** menu item and select the desired command from the window that opens.

Contour (Polygon) menu item

Using this tool you can draw a contour of the polygon type into the image, i.e. the lines are not rounded as they are in the case of Contour (Spline). Two drawing methods are available to you:

- Click in the image to define the start point. Then hold down the mouse button and outline the desired structure in the image. When the mouse pointer gets near to the start point, release the mouse button. As soon as the mouse pointer icon changes, click once and the contour is closed.
- Alternatively, after defining the start point you can also click in the image to define additional corner points. Right-click to close the contour.

As soon as the contour has been closed, the **Area** measured values and the **Average Gray Value Intensities** are displayed in the image. In the case of multichannel images these are also shown for each channel.

To change the size of a **contour (spline)** as a whole, grab one of the corner or line points and move it. All elements of the contour are changed in a uniformly proportional way. As soon as you release the button, the new measured values are displayed.

To add additional free text to a contour, double-click on the graphic element and enter the text in the text field.

Meta data on the acquisition procedure can also be assigned to a contour from the image; please see --- FEHLENDER LINK ---.

Besides the standard measurement data, a contour can also display a range of other measured values; please see --- FEHLENDER LINK ---.

Contours consist of a series of points that are connected by a line. To edit these points, use the shortcut menu (right mouse button).

Contour (Polygon) shortcut menu

For a general description of the shortcut menu for graphic elements, please see --- FEHLENDER LINK ---.

To edit the points of a contour, you should select these first. Now right-click on the contour. In the shortcut menu you will find the following commands:

- Split: Draw a separation line through the contour in the form of a free curve. End the drawing of the separation line by right-clicking. The contour is now separated along the separation line.
- Separate: Draw in a separation region in the form of a free contour and end this by right-clicking. The region covered by the separation region is removed from the contour and you are left with two smaller contours.
- Connect: Select at least two contours and draw in a connecting contour (end by right-clicking). The regions of the selected contours and the image regions covered by the newly drawn-in contour are connected to form a single contour.

It is also possible to edit the points of the contour. To do this, select the **Edit Points** command from the shortcut menu. You can now grab and move individual points. The shortcut menu also contains the following additional commands:

- Add Point (only active if the mouse is **not** currently positioned over an existing point): adds an additional point to the line.
- Delete Point (only active if the mouse is currently positioned over an existing point): deletes the current point from the contour.

To exit editing mode, select the command **End Editing of Points** from the shortcut menu or click once in the image outside the edited contour.

Active Contour menu item

Using this tool you can draw a contour of the polygon type into the image. It is automatically adapted to structures in the image.

Two drawing methods are available to you:

- Click in the image to define the start point. Then hold down the mouse button and outline the desired structure in the image. If the local contrast is sufficient, the line jumps automatically to the correct position. Right-click to end the measurement.
- Alternatively, after defining the start point you can also click in the image to define additional corner points. In this case the active search for local contrasts is not used. Right-click to close the contour.

As soon as the contour has been closed, the **Area** measured values and the **Average Gray Value Intensities** are displayed in the image. In the case of multichannel images these are also shown for each channel.

To change the size of a **contour** as a whole, grab one of the corner or line points and move it. All elements of the contour are changed in a uniformly proportional way. As soon as you release the button, the new measured values are displayed. In this case the contour is not adapted to structures in the image.

To add additional free text to a contour, double-click on the graphic element and enter the text in the text field.

Meta data on the acquisition procedure can also be assigned to a contour from the image; please see --- FEHLENDER LINK ---.

Besides the standard measurement data, a contour can also display a range of other measured values; please see --- FEHLENDER LINK ---.

Contours consist of a series of points that are connected by a line. To edit these points, use the shortcut menu (right mouse button).

Active Contour shortcut menu

For a general description of the shortcut menu for graphic elements, please see --- FEHLENDER LINK ---.

To edit the points of a contour, you should select these first. Now right-click on the contour. In the shortcut menu you will find the following commands:

- Split: Draw a separation line through the contour in the form of a free curve. End the drawing of the separation line by right-clicking. The contour is now separated along the separation line.
- Separate: Draw in a separation region in the form of a free contour and end this by right-clicking. The region covered by the separation region is removed from the contour and you are left with two smaller contours.
- Connect: Select at least two contours and draw in a connecting contour (end by right-clicking). The regions of the selected contours and the image regions covered by the newly drawn-in contour are connected to form a single contour.

It is also possible to edit the points of the contour. To do this, select the **Edit Points** command from the shortcut menu. You can now grab and move individual points. The shortcut menu also contains the following additional commands:

- Add Point (only active if the mouse is **not** currently positioned over an existing point): adds an additional point to the line.
- Delete Point (only active if the mouse is currently positioned over an existing point): deletes the current point from the contour.

To exit editing mode, select the command **End Editing of Points** from the shortcut menu or click once in the image outside the edited contour.

Contour with Holes menu item

Using this tool you can draw a contour of the polygon type into the image. In a second step, you can draw in regions within the contour that are excluded from the measurement. Two drawing methods are available to you:

- Click in the image to define the start point. Then hold down the mouse button and outline the desired structure in the image. When the mouse pointer gets near to the start point, release the mouse button. As soon as the mouse pointer icon changes, click once and the contour is closed.
- Alternatively, after defining the start point you can also click in the image to define additional corner points. Right-click to close the contour.
- Move the mouse to the starting point and click. The contour is now closed.
- You can now draw and close additional contours inside the contour you have just drawn. As soon as these have been closed, they are displayed with hatchings.
- End the drawing by double-clicking outside the region.

The **Area** measured values and the **Average Gray Value Intensities** are now displayed in the image. In the case of multichannel images these are also shown for each channel. The hatched regions are excluded from the measurements.

To change the size of a **contour** as a whole, grab one of the corner or line points and move it. All elements of the contour are changed in a uniformly proportional way. As soon as you release the button, the new measured values are displayed.

You can also select the hatched contours on the inside using the mouse and change their size and position.

Besides the standard measurement data, a contour can also display a range of other measured values; please see --- FEHLENDER LINK ---.

Contours consist of a series of points that are connected by a line. To edit these points, use the shortcut menu (right mouse button).

Contour with Holes shortcut menu

For a general description of the shortcut menu for graphic elements, please see --- FEHLENDER LINK ---.

Please note: You can only change the individual points of the surrounding contour.

To edit the points of a contour, you should select these first. Now right-click on the contour. In the shortcut menu you will find the following commands:

- Split: Draw a separation line through the contour in the form of a free curve. End the drawing of the separation line by right-clicking. The contour is now separated along the separation line.
- Separate: Draw in a separation region in the form of a free contour and end this by right-clicking. The region covered by the separation region is removed from the contour and you are left with two smaller contours.
- Connect: Select at least two contours and draw in a connecting contour (end by right-clicking). The regions of the selected contours and the image regions covered by the newly drawn-in contour are connected to form a single contour.

It is also possible to edit the points of the contour. To do this, select the **Edit Points** command from the shortcut menu. You can now grab and move individual points. The shortcut menu also contains the following additional commands:

- Add Point (only active if the mouse is **not** currently positioned over an existing point): adds an additional point to the line.
- Delete Point (only active if the mouse is currently positioned over an existing point): deletes the current point from the contour.

To exit editing mode, select the command **End Editing of Points** from the shortcut menu or click once in the image outside the edited contour.

Rectangle (Oblique) menu item

Using this tool you can draw a rectangle into the image along any defined base line. The first click defines the start point of the base line and the second click its end point. The size of the resulting rectangle is defined with the third mouse click.

A rectangle automatically displays the **Area** measured values and the **Average Gray Value Intensities** in the image. In the case of multichannel images these are also shown for each channel.

To change a rectangle, grab one of the corner or line points and drag it to the desired location. As soon as you release the button, the new measured values are displayed.

To add additional free text to a rectangle, double-click on the rectangle and enter the text in the text field.

Meta data on the acquisition procedure can also be assigned to a rectangle from the image; please see --- FEHLENDER LINK ---.

Besides the standard measurement data, a rectangle can also display a range of other measured values; please see --- FEHLENDER LINK ---.

Additional key commands

Various key commands are available to you that can make drawing easier:

- **Shift key**: If you hold down the shift key, the base line is drawn in at fixed angle increments of 15° and a square is drawn rather than a rectangle.
- **Alt key**: If you hold down the Alt key, the first click is not used as the start point, but as the center point of the rectangle.

Burn in Annotations menu item

Using this function you can copy annotations and measurements from the graphics plane into the pixel plane of the image and create a new image. Annotations and measurements are then "burnt" into the image permanently. In the resulting image you no longer have the option of changing or deleting annotations and measurements in order to restore the regions of the original that have been overwritten.

Show Bounding Boxes menu item

With this menu command, the external bounding box is displayed in green for each element. This is particularly useful if you have created graphic elements without a line color.

Hide Bounding Boxes menu item

With this menu command, external bounding boxes around graphic elements are hidden again.

Move to Foreground menu item

Brings selected graphic elements to the foreground and lays them over other graphic elements.

Move to Background menu item

Moves selected graphic elements to the background so that they are positioned behind other graphic elements.

One Plane Forwards menu item

Brings selected graphic elements one plane further forwards and lays them over the next graphic element in the plane sequence.

One Plane Backwards menu item

Moves selected graphic elements one plane further backwards. The previous graphic element in the plane sequence is consequently moved to the foreground.

Extras menu

Diagnosis menu item

Opens the dialog window...

Kitchen Clock menu item

Opens the dialog window...

Dosimeter menu item

Opens the dialog window...

Dye Editor menu item

Opens the dialog window...

Expansion Management menu item

Opens the Expansion Management dialog window.

Module Management menu item

Opens the Module Management [→ 41] dialog window.

Calibration Management menu item

Opens the dialog window...

Customize Tool Bar menu item

Opens the Customize Tool Bar [→ 42] dialog window.

Scaling menu item

Opens the Scaling [→ 42] dialog window.

Sample Carrier Templates menu item

Opens the dialog window...

Options menu item

Opens the Options [→ 44] dialog window.

Extras menu dialog windows

Module Management dialog window

Here you can select which of the modules for which you have a license are activated.

Available Products section

Here you can see the available licenses. Click on the relevant button to select a version.

Included Modules list

In this list you can activate the modules that are included in your ZEN product. To activate a module, activate the checkbox in front of the module in question.

Optional Modules list

In this list you can activate the modules that you have licensed as an option for your ZEN product. To activate a module, activate the checkbox in front of the module in question.

Optional Hardware list

In this list you can activate the control software for the devices that you have licensed for your ZEN product.

Select All button

Activates all licensed modules.

Deselect All button

Deactivates all licensed modules.

Save Module Information button

Saves the current selection of modules in a file.

Customize Tool Bar dialog window

Here you can add menu commands to the tool bar as buttons.

Available Buttons list

Here you will find the individual commands that you can add to the **tool bar**.

button

Adds a selected menu command to the tool bar. It then appears in the **Selected Buttons** list.

Selected buttons list

The menu commands that you have added to the tool bar are listed here. You can select commands here in order to sort them, for example.

button

Deletes a selected menu command.

button

Moves a selected menu command up a position.

button

Moves a selected menu command down a position.

Separator button

Inserts a vertical separator bar into the tool bar after the current position.

Close button

Closes the dialog window.

Scaling dialog window

Here you can specify how your images are scaled.

Active Scaling section

The scaling that is currently active is displayed in the Active Scaling section.

Automatic Scaling checkbox

Activated: Determines the scaling from the configuration set for the microscope and camera.

Unit of Measurement dropdown list

Select the desired unit of measurement for the scaling from the dropdown list.

The following units are available for selection:

- Meter
- Centimeter
- Millimeter
- Micrometer
- Nanometer
- Inch
- Mil

Available Scalings section

Available Scalings dropdown list

Here you can select an existing scaling.

button

Clicking on the Options button opens a shortcut menu with the following entries:

Activate Scaling Activates the scaling selected in the **Available Scalings** dropdown list so that it is applied to all images acquired from that moment.

Assign Scaling to Image The scaling selected in the **Available Scalings** dropdown list is assigned to the current image.

Import Opens the **Import Scaling** dialog window. Here you can select the scaling file that you want to import.

Export Opens the **Export Scaling** dialog window to export the scaling selected in the **Available Scalings** dropdown list. Here you can select the folder in which you want the exported scaling file to be saved and specify a file name.

Delete The scaling selected in the **Available Scalings** dropdown list is deleted.

Create New Scaling section

Interactive Calibration button

Starts the Scaling Wizard [→ 44].

Scaling Wizard

Here you can create a new scaling. To do this, draw a reference line with a predefined length in the current image. An image of a scale bar is best suited for this.

You can draw in two types of reference line:

Simple Reference Line Draw a line along a distance with a known length.

Parallel Reference Lines Draw two parallel lines along a distance with a known length. The two parallel lines allow errors in the parallel axis resulting from the drawing of the lines to be corrected. A third, corrected line is drawn in automatically from which the scaling is determined.

Determine Lines Automatically checkbox

Activated: Automatically detects individual lines of the scale bar in the image close to the interactively defined distance. Using this method the centers of the lines are determined exactly, increasing the precision of the scaling.

Length input field

Enter the length of the line you have drawn in in the spin box/input field.

Unit dropdown list

Select the scaling unit from the dropdown list.

Name input field

Here you can enter the name for the scaling file that has been created.

Save button

Saves the scaling file that has been created under the specified name.

Options dialog window

Here you can configure the settings for general software options.

Software Options

General

Language section

Select Automatically checkbox

Activated: Automatically selects the user language of the operating system as the user language for the software.

Language Selection dropdown list

Select the language from the dropdown list in which ZEN (blue edition) will be run next time it is started.

Start Options

Start Screen section

Activate Start Screen checkbox

Activated: Displays the start screen.

Configuration section

Load Last Files Used checkbox

Activated: When the software is started, loads all files that were open when you last exited the system.

Name

Here you can specify how images are named.

Category dropdown list

Select the category of images from the dropdown list for which you want to define the naming. The following categories are available:

New Images	Images that are created using File menu | New [→ 18].
Individual Image	Images that are created by clicking on the **Snap** button.
Continuous Acquisition	Images that are created by clicking on the **Continuous** button.
Experiment	Images that are created by clicking on the **Start Experiment** button.
Film	Images that are created by the Movie Recorder.
Result of Image Processing	Images that are created by means of an image processing function.
Result of Batch Processing	Images that are created by means of a batch processing function.

Prefix input field

Here you can enter a name prefix.

Digits dropdown list

Here you can select how many digits you want the counter used to have.

Format input field

Here you can specify what information is appended to the prefix.

To adopt an entry directly into the input field, double-click on the entry in the **Format IDs** list.

Current Counter Value input field

Displays the current status of the counter. The counter value is saved during a session.

If you exit the software, the counter value will be reset to 1.

Preview display field

Displays the name that will be allocated next for the current category.

Save/Load Counter Value checkbox

Activated: Saves the counter values for the individual categories. If the software is restarted, the values are reloaded.

Format IDs list

Here you will see a list containing all the formatting options.

Save

Locate/Camera section

Save Automatically checkbox

Activated: Automatically saves images that are acquired on the **Locate** (Camera) tab using the **Snap** button.

Do Not Open Document checkbox

Only active if the **Save Automatically** checkbox is activated.

Activated: The automatically saved images are closed immediately after acquisition.

Image Format dropdown list

Select the image format from the dropdown list that will be used when an image is saved automatically.

Folder for Automatic Save input field

Here you can specify the folder in which the images are saved automatically.

Browse button

Opens the **Browse For Folder** dialog window. Here you can select the folder in which the images are saved automatically.

Documents

Settings for New Windows section

Show Ruler checkbox

Activated: Displays the ruler at the top and right-hand edge of the image.

Fit Automatically to Window checkbox

Activated: Automatically adjusts the zoom factor of the image so that the entire image is visible.

Interpolation for Image Display checkbox

Activated: Displays pixels in interpolated form.

Logarithmic Scaling in Histogram checkbox

Activated: Displays the frequency distribution in the histogram in a logarithmic scaling.

Acquisition

Live Window section

Close Live Window After Snap checkbox

Activated: Closes the live window after acquisition.

Action After Snap dropdown list

Select an action from the dropdown list to be performed after an image is acquired using the **Snap** button. The following options are available:

Create New Document Creates a new document each time.

Automatic mode As long as the dimension of the acquired image does not change, a newly acquired image is always saved in the current document. Previous images are therefore lost.

User

Here you can enter your own details. These are then written into the image during acquisition. They are also used in reports.

User Information section

First Name Here you can enter your first name.

Last Name Here you can enter your last name.

E-mail Here you can enter your e-mail address.

Telephone Here you can enter your telephone number.

Fax Here you can enter your fax number.

Company Information section

Name Here you can enter the company name.

Address Here you can enter the company address.

Zip Code, Town/City Here you can enter the zip code and town/city of the company.

Country Here you can enter the country.

Logo Here you can add a company logo to your user profile. This can then be displayed in a report, for example. To add a logo, click on the **Add** button.

Tables

Window menu

Help menu

2.3.3 Tool bar

Tool bar

Here you can gain quick access to important functions, e.g. saving or opening files. You can adapt the tool bar to your personal requirements in the **Extras** menu | **Customize Tool Bar**.

2.3.4 Status Area

Here you will see important information on the system status:

Scaling Group

Displays which lateral scaling is currently being used. From here you can also create a new scaling or start the scaling wizard.

System Information:

Always shows the latest, currently active process that the system is performing.

Progress Bar

Displays the progress of the currently active process. Each new process added supersedes older processes that are still active. If you click on the icon, a window opens in which all processes currently running are listed chronologically. You can stop a process that is running using the **Stop** button.

Performance Indicators

In this group you will see an overview of the performance of individual computer components:

- **Free RAM** indicates how much physical memory is still available;
- **Free HD** indicates how much space is still available on the hard drive on which the next image is to be acquired (see Extras/Options/Save);
- **CPU** indicates the usage of the Central Processing Unit.
- The **status bar** provides an overall assessment of the system usage.

Frame Rate

Indicates the current frame rate in frames per second (fps) with which the active camera is producing new images. Please note that from a speed of approx. 100 frames per second this value can no longer be determined correctly in every case.

Pixel Value

Displays the gray value in the image at the current position of the mouse pointer. In the case of multichannel images the gray value/channel is displayed for up to 4 channels.

Position:

Displays the X/Y position (in pixel coordinates) of the mouse pointer in the image.

Information (i)

If you click on the icon, a window opens with a List of System Messages [→ 49].

Folder

Displays the location where new images are automatically saved. This path can be changed in the Extras menu | Options | Save.

User

Shows the Windows user name of the user who is logged in.

Time

Displays the current Windows system time.

List of System Messages

Important system messages are collected here. There are three categories:

Information

System information that arises during normal operation. This system information does not lead to an interruption of the workflow. The information window is not displayed automatically.

Warnings

Information that requires input from the user, e.g. a prompt to change a mechanical microscope component. This information leads to the information window being shown briefly. It closes again after a few seconds, however.

Errors

Error messages indicate a malfunction by the system. In this case the information window opens and remains open. The system requires input from the user in order to continue.

Information

Hundreds of messages can accumulate in the course of a session. A maximum of 300 messages are displayed. To display messages for a certain category, activate or deactivate the corresponding checkboxes.

3 Left Tool Area

3.1 Locate tab

On the **Locate** tab you can enter all the settings for your microscope and camera. For example, you can configure buttons here that will allow you to access your preferred hardware settings quickly. You can also control acquisition, define the acquisition light path and camera settings, and acquire films of your experiments.

3.1.1 Transmitted Light/Reflected Light

Transmitted Light button

Off Closes the shutter for the transmitted light source on a motorized microscope.

On Opens the shutter for the transmitted light source on a motorized microscope.

Reflected Light button

Off Closes the shutter for the reflected light source on a motorized microscope.

On Opens the shutter for the reflected light source on a motorized microscope.

3.1.2 Favorites

Configure button

Opens the Configure Favorites dialog window.

3.1.3 Acquisition buttons

The **Acquisition** buttons are used to prepare the microscope and camera and to acquire your images. The functions of the buttons are explained in the following section:

Find Focus button

Starts an autofocus search using the current settings from the **Focus Devices** tool. Here the system settings are used that apply to the channel set as the reference channel in the **Channels** tool. Immediately after the search the **Snap** function is executed and an image of all activated ("checked") channels is acquired.

Set Exposure button

Starts an exposure time measurement for all activated ("checked") channels in the **Channels** tool. Here the system settings are used that apply to the relevant channels in the experiment. Immediately after the measurement the **Snap** function is executed and an image of all activated ("checked") channels is acquired.

Live button

Opens a live window and starts a fast live camera image. Here the system settings (e.g. filter and illumination) are used that apply to the channel currently selected in the **Channels** tool. No pseudo colors from the channel setting are applied to the live image, however. If several channels are available, but none have been selected, the first channel of the experiment is used.

Continuous button

Starts a series of **"Snaps"** for all activated ("checked") channels in the **Channels** tool. Here the system settings are used that apply to the relevant channels in the experiment. In contrast to a live image, the precise camera setting that has been set in the **Acquisition Mode** tool is used. Pseudo colors and other settings, e.g. exposure times, from the channel setting are also applied. Other image dimensions (Z-stacks, tiles, time series, etc.) are not taken into account in **Continuous** mode. The result at the end of this mode is a single, acquired image that can be saved.

Snap button

Acquires an image with all activated ("checked") channels in the **Channels** tool. Here the system settings are used that apply to the relevant channels in the experiment. Pseudo colors and other settings, e.g. exposure times, from the channel setting are also applied.

Stop button

Only active if one of the acquisition buttons has been clicked. In this case the relevant button changes into an animated **Stop** symbol.

Stops the function of the relevant acquisition button.

3.1.4 Active Camera

Link Cameras checkbox

Only active if you have connected two structurally identical cameras to your system.

Activated: Acquires images using two cameras in parallel. This is often the case with 2-channel images for ratio measurements or FRET measurements.

Active Camera dropdown list

Shows the active camera. If you have connected several cameras, you can select the desired camera here.

3.1.5 Light Path tool

In the **Light Path** tool you can configure the light path of components and the microscope for your experiment. The configuration of your system (see MicroToolBox) is shown here in the form of a graphical display. The graphical display of the light path shows all the components on the path from the light source to the specimen and from there to the camera or eyepiece. The icons correspond to the components that are installed on your system.

Please bear the following points in mind when working with the Light Path tool:

- To activate/deactivate a setting, left-click on the relevant icon.
- Icons with an arrow in the bottom right corner contain dialog windows that allow you to configure additional settings. To open the dialog windows, left-click on the corresponding icon.
- Icons with a hand in the bottom left corner indicate components that have to be operated manually.

The following functions are only visible if the **Show All** mode is activated:

Interactive button

Activated: The **Light Path** tool is operated interactively via the icons.

Settings Editor button

Activated: The **Settings Editor** is visible. The icons of the components used in the **Reflected/ Transmitted Light Path** section are grayed out. To use the desired component in the light path, activate the relevant checkbox above the icon.

Settings Editor

Here you can select from existing hardware settings or adopt the settings from the hardware being used into the software. You can also transfer settings from the software to the hardware that you are using.

Reflected/Transmitted Light Path

In the graphical display of the reflected/transmitted light path you will see various optical components, such as the shutter, diaphragms, filters and beam splitters, depending on the microscope you are using. All the available components must be configured in advance in the MTB (MicroToolBox). The settings for the components can be configured via the icons.

Information

If you are not using any motorized components, you will have to make the relevant adjustments manually.

Microscope Manager

To open the Microscope Manager [→ 54] dialog window, left-click on the Microscope Manager icon.

Reflected Light/Transmitted Light Changer

If your microscope has a halogen lamp for both reflected and transmitted light illumination, here you can select whether you want to control the halogen lamp for reflected light illumination or the halogen lamp for transmitted light illumination.

Shutter

To set the shutter to **Open** or **Closed**, left-click on the shutter icon. The status is displayed in text form above the icon.

Aperture Diaphragm

To open the **Aperture Diaphragm** dialog window, left-click on the aperture diaphragm icon.

Enter the diaphragm opening (0% to 100%) using the slider or spin box/input field.

Filter Wheel 1

To open the **Filter Wheel** dialog window, left-click on the filter wheel icon.

Here you can enter the first neutral density filter (e.g. 0.4%, 6%, 100%, 100%) that you require.

Filter Wheel 2

To open the **Filter Wheel** dialog window, left-click on the filter wheel icon.

Here you can enter the second neutral density filter (e.g. 12%, 25%, 50%, 100%) that you require.

Condenser

The condenser is only available in the **Transmitted Light** path.

To open the **Condenser** dialog window, left-click on the icon.

Select the contrast method from the dropdown list (e.g. brightfield, darkfield, phase contrast ring 1, 2, 3, DIC I, II, III).

Lens checkbox

Activated: Swings in the auxiliary lens.

Enter the condenser aperture using the **N.A.** slider or the spin box/input field.

Only visible if the **Show All** mode is activated.

Various condenser settings can be saved in the list to allow you to restore them quickly.

Luminous Field Diaphragm

To open the **Luminous Field Diaphragm** dialog window, left-click on the icon.

Enter the diaphragm opening (0% to 100%) using the slider or spin box/input field.

Reflector Turret

To open the **Reflector Turret** dialog window, left-click on the reflector turret icon.

The filter cubes for the reflected light techniques can be found in thelist. Enter the desired filter cube here (e.g. brightfield or fluorescence filter cube). The filter cube's beam splitter directs the light through the objective and onto the sample.

Only visible if the **Show All** mode is activated.

The wavelength ranges of the fluorescence filters and beam splitters are also shown in the list.

Nosepiece

To open the **Nosepiece** dialog window, left-click on the nosepiece icon.

Select the desired **Objective** from the list.

Only visible if the **Show All** mode is activated.

In addition to the primary magnification, all the other details for each objective are also displayed in the list.

Specimen Stage

In the Specimen Stage section you will find the options for Stage Control [→ 55] and Focus Control [→ 56]. This section is highlighted in dark gray in the graphical display.

TV/Eyepiece Switch

To open the **TV/Eyepiece Switch** dialog window, left-click on the **TV/Eyepiece Switch** icon.

Select from the list whether you want to direct the light to the camera only (**100% Camera**), to the camera and the eyepiece (**30% Eyepiece/70% Camera**) or to the eyepiece only (**100% Eyepiece**).

Eyepiece

Above the **Eyepiece** icon the total magnification of the selected beam path with all activated intermediate magnifications is displayed. To direct the light path fully to the eyepiece, left-click on the eyepiece icon.

Camera

The selected camera is shown above the **Camera** icon.

Microscope Manager

Contrast Manager

Select the setting for the contrast mode from the **Mode** dropdown list.

Off The Contrast Manager is not used. All settings must be made manually or via a settings file.

On Demand The function of the Contrast Manager is activated via the touchscreen on the microscope.

Keep Contrast If core components (e.g. condenser, reflector, shutter) for a certain contrast technique are changed, dependent components are also changed accordingly.

Select one of the available methods for the contrast mode from the **Method** dropdown list.

Light Manager

Activated checkbox

Activated: Activates the Light Manager. The **Mode** dropdown list in the Light Manager is active.

Select a setting for adjusting the brightness of the light from the Mode dropdown list.

Objective Adjusts the brightness of the light via the lamp voltage. The color temperature changes accordingly.

Conventional Adjusts the brightness on the basis of the available filter wheels. The color temperature is retained. Only if the brightness adjustment cannot be achieved via the filter wheels does adjustment take place via the lamp voltage.

Glare Protection checkbox

Activated: Activates glare protection. Glare protection prevents light from passing through the eyepiece and dazzling the user, for example when reflector positions are changed. This is mainly achieved by closing the reflected or transmitted light shutter. If no shutters are installed, the lamp voltage is adjusted.

Parfocal Correction checkbox

Activated: Activates parfocal correction.

Loading/Working Position Link checkbox

Activated: Loads the loading/working position link.

Stage Control

To open the **Stage Control** dialog window, left-click on the **Stage** button.

In the **Stage Control** dialog window you can use the software to move stages equipped with a motorized X/Y-drive.

Stage control using navigation circle

CAUTION

Danger of crushing by stage!

1. Remove your fingers or any objects from the danger area before using the navigation circle to move the stage.

In the dialog window you will see a navigation circle with a number of segments.

1. Move the mouse pointer over a segment of the circle.
   ⇨ The corresponding segment is highlighted.
2. To move the stage, hold down the left mouse button.

⇨ The corresponding segment is highlighted in blue. The stage keeps moving while the left mouse button is held down. It stops when you release the left mouse button again.

Alternatively, you can control the stage using the navigation button at the center of the navigation circle.

1. Move the mouse pointer over the navigation button.
   ⇨ The navigation button is highlighted.
2. Hold down the left mouse button and move the mouse in the desired direction.

⇨ The stage moves in the direction you have specified. When you release the left mouse button the stage stops.

Information

If you control the stage using the inner segments of the circle, the travel speed is slow. If you control it using the outer segments of the circle, it travels at a faster speed. To double the travel speed, right-click on the navigation button. All segments of the circle and the navigation button are highlighted in red.

Stage control through entry of absolute coordinates

CAUTION! Danger of crushing by stage! If you enter absolute coordinates, the stage automatically moves to the entered position. Remove your fingers or any objects from the danger area before entering absolute coordinates. To stop the stage movement, click on the Stop button.

Enter the absolute position in the **X/Y-Position** spin boxes/input fields under the navigation circle. Confirm each entry you make with the Enter key. The stage then moves automatically to the coordinates you have entered. The position of the stage is shown in the display fields to the right of the spin boxes/input fields.

Stop button

Stops the stage movement.

X/Y-Position section

Set Zero Point button

Sets the current position as the new zero point for the X/Y-coordinates.

Calibrate button

CAUTION! Danger of crushing by stage! If you perform an automatic stage calibration, the stage moves automatically to the end positions in the X/Y-direction. Remove your fingers or any objects from the danger area before performing automatic calibration. To stop calibration, click on the Stop button.

Performs an automatic stage calibration. For this the stage moves to the limit switches to determine the zero points in the X and Y-direction and then returns to its starting position, which is now defined with its absolute coordinates.

Focus Control

To open the **Focus Control** dialog window, left-click on the **Focus** button.

In the **Focus Control** dialog window you can use the software to control stages equipped with a motorized Z-drive.

Stage control using navigation bar

CAUTION! Danger of crushing by stage! Remove your fingers or any objects from the danger area before using the navigation bar to move the stage.

In the dialog window you will see a navigation bar with a number of segments.

1. Move the mouse pointer over a segment.

⇨ The corresponding segment is highlighted.

2. To move the stage in the Z-direction, hold down the left mouse button.

⇨ The corresponding segment is highlighted in blue. The stage keeps moving while the left mouse button is held down. It stops when you release the left mouse button again.

Alternatively, you can control the stage using the  navigation button at the center of the navigation bar.

1. Move the mouse pointer over the navigation button.

⇨ The navigation button is highlighted.

2. Hold down the left mouse button and move the mouse up or down.

⇨ The stage moves in the direction you have specified. When you release the left mouse button the stage stops.

Information

If you control the stage using the inner segments, the stage moves slowly. If you control it using the outer segments, it travels at a faster speed. To double the travel speed, right-click on the navigation button. All segments and the navigation button are highlighted in red.

Stage control through entry of absolute coordinates

CAUTION! Danger of crushing by stage! If you enter absolute coordinates, the stage automatically moves to the entered position. Remove your fingers or any objects from the danger area before entering absolute coordinates. To stop the stage movement, click on the Stop button.

Enter the absolute position in the **X/Y-Position** spin boxes/input fields under the navigation bar. Confirm each entry you make with the Enter key. The stage then moves automatically to the coordinate you have entered. The position of the stage is shown in the display fields to the right of the spin boxes/input fields.

Stop button

Stops the stage movement.

The following functions are only visible if the **Show All** mode is activated:

Positioning Increment spin box/input field

Here you can enter the increment for the individual travel steps.

Home button

Moves the stage to the defined start position.

Work button

Moves the stage to the last focus range set.

Load button

Lowers the stage by a predefined fixed amount, depending on the microscope. This makes it easier to change the sample, especially in the case of immersion objectives with a short working distance.

Measure section

Distance display field

Shows the Z-distance between the starting position and the current stage position.

Z-Position section

Set Zero Point button

Sets the current position as the new zero point for the Z-coordinates.

Calibrate button

CAUTION! Danger of crushing by stage! If you perform an automatic stage calibration, the stage moves automatically to the end positions in the Z-direction. Remove your fingers or any objects from the danger area before performing automatic calibration. To stop calibration, click on the Stop button.

Performs an automatic stage calibration. For this the stage moves to the lower limit switch to determine the absolute zero point in the Z-direction and then returns to its starting position, which is now defined with its absolute coordinate. It is recommended that you remove the specimen from the stage to do this.

3.1.6 Camera tool

In the **Camera** tool you can configure all the settings for the selected camera.

Settings section

In the **Settings** section you can manage your camera settings.

Default button

Resets all camera settings in the Camera tool to the factory default settings. These settings can also be selected from the dropdown list of available camera settings to the right of the **Default** button. To do this, select the **Original Settings** entry.

button

Opens the **Options** shortcut menu.

Reload button

Undoes the changes you have made to a loaded setting and restores the original status of the loaded setting.

Options

New menu item

Creates a new camera setting. Enter a name for the camera setting in the input field. To save the camera setting, click on the **Diskette** icon to the right of the input field.

Rename menu item

Renames the current camera setting. Enter another name for the camera setting in the input field. To save the camera setting, click on the **Diskette** icon to the right of the input field.

Save menu item

Saves the current camera setting.

Save As menu item

Saves the current camera setting under a new name. Enter a new name in the input field. To save the camera setting, click on the **Diskette** icon to the right of the input field.

Import menu item

Imports an existing camera setting.

Export menu item

Exports the selected camera setting.

Delete menu item

Deletes the selected camera setting.

Exposure Time section

In the **Exposure Time** section you can set the desired exposure time for your image. You can have the exposure time calculated automatically using the **Measurement** button or set it manually using the **Time** slider or the spin box/input field.

Time slider

Set the exposure time here or enter the desired value in the spin box/input field. Select the units of time (**ms**, **s**) from the dropdown list to the right of the spin box/input field.

Automatic Exposure checkbox

Activated: Calculates the exposure time automatically before each image is acquired.

Measurement button

Starts a one-off system measurement of the exposure time, which is used for all subsequent images.

The following functions are only visible if the **Show All** mode is activated:

Auto Exposure Intensity spin box/input field

Here you can set the actual exposure time to between 5% and 200% of the measured value. This is helpful in the case of fluorescence images to prevent overexposure or to make signals stand out more clearly. You can change the value by increments using the **arrow buttons** or you can enter the desired value directly using the keyboard. To do this, you must activate the number already present using the cursor. Complete the entry by using the Tab or Enter key.

Spot Measurement/Focus ROI checkbox

Here you can use the cursor to set a region in the live image to which the exposure time measurement will be restricted. The same region is also used as the region for the autofocus setting.

White Balance section

Only active if a color camera has been selected.

Auto button

Sets the white balance automatically.

Select button

Select a point in the image as a reference point for the white balance.

3200K button

Sets the color temperature to 3200K.

5500K button

Sets the color temperature to 5500K.

Display Color Channels checkbox

Only visible if the **Show All** mode is activated.

Activated: Displays 3 sliders to allow each color channel to be set precisely.

Color Temperature slider

Here you can set the color temperature.

Saturation slider

Only visible if the **Show All** mode is activated.

Here you can set the saturation.

Default button

Resets all settings to the default values.

Operating Mode section

Only visible if the **Show All** mode is activated.

Color Mode button

RGB Sets the color mode to RGB.

B/W Sets the color mode to B/W.

Live Mode dropdown list

Here you can select the speed of the live mode. You can choose between **Slow**, **Medium** and **Fast**.

Resolution dropdown list

Here you can select the camera resolution.

Binning dropdown list

Here you can select the binning mode for the camera.

NIR Mode checkbox

When the checkbox is active you can use the camera in the near infrared range.

Area section

In the **Area** section you can define a particular area that you want to be acquired.

After clicking on the **Update Overview** button, in the window on the left you will see a **Preview** of your sample under the camera. The **Pixel Size** below the preview window indicates the size of a pixel depending on the scaling set. The blue frame indicates the selected area. The entire preview window is highlighted by default. To change the area, move the mouse pointer over the blue frame. The mouse pointer will appear as a double-headed arrow. If you hold down the mouse button and move the mouse, you can change the size of the frame and in this way highlight a certain area on the sample. To move the area, position the mouse pointer inside the frame. The mouse pointer will appear as a four-headed arrow. If you hold down the mouse button and move the mouse, the frame can be moved.

Maximize button

Maximizes the frame to the maximum size in the preview window.

Center button

Positions a frame that has been changed precisely at the center of the image.

Size input field

Here you can enter the size of the frame that you want. Depending on the camera type available, select from the predefined sizes in the dropdown list (1388x1040, 1024x1024, 512x512, 256x256, 128x128). Alternatively you also have the option of entering the **Size** in X and Y, and the top left **Start** position. You can change the values by increments using the **arrow buttons** next to the input windows or you can enter the desired values directly using the keyboard. To do this, you must activate the number already present using the cursor. Complete the entry by using the Tab or Enter key.

Start input field

Here you can enter the start position for the frame, measured from the top left corner.

Update Overview button

Adopts the image from the camera into the preview window.

Post-Processing section

Depending on which camera you are using, some functions are not visible. The most important functions are explained in the section below.

Black Reference checkbox

Only visible if the **Show All** mode is activated.

Only active if you have defined a black reference via the **Define** button.

Activated: Applies the measured black reference to the image.

Deactivated: The measured black reference is not used. The reference image is retained.

Define button

Automatically defines the black reference. To do this a reference image is acquired, from which the black reference is measured. The light path to the camera must be closed and the lamp must be switched on for the measurement. The measurement lasts for several seconds. The **Black Reference** checkbox is then activated automatically.

Shading Correction checkbox

Only active if you have defined a shading correction via the **Define** button.

Activated: Applies the measured shading correction to the image.

Deactivated: The measured shading correction is not used. The reference image is retained.

Define button

Automatically defines the shading correction. To do this you will acquire a reference image for the shading correction. An empty image without structures is required to measure the shading image. Move the slide to an empty position on the sample with no artifacts in the image. The **Shading Correction** checkbox is activated automatically after the definition.

Activate Noise Filter checkbox

Activated: The **Limit** slider and the spin box/input field are active.

Enter the limit for the noise filter using the slider or spin box/input field.

Unsharp Masking checkbox

Activated: Emphasizes edges in the image more clearly.

Strength slider

Enter the strength of the filtering using the slider or spin box/input field.

The following functions are only visible if the **Show All** mode is activated:

Radius slider

Enter the radius of the filtering using the slider or spin box/input field.

Color Mode dropdown list

Here you can select the desired color mode. You can choose between **RGB** or **Luminance**.

Autocontrast checkbox

Activated: The **Contrast Tolerance** slider and spin box/input field are active.

Enter the contrast tolerance (0-20) using the slider or spin box/input field.

Limit to Valid Bit Number checkbox

Activated: Limits the setting option for the **Contrast Tolerance** function to a valid bit number. Diese

Trigger Control section

Only visible if the **Show All** mode is activated.

To show the section in full, click on the  button.

Trigger Output

As soon as this output is activated, you can select the polarity with which you want this signal to be generated.

Active During Snap checkbox

When this checkbox is activated, the shutter pulse is only generated during acquisition. If you want a trigger signal to be generated both during the live image and during acquisition, both checkboxes must be activated simultaneously.

Active During Live checkbox

When this checkbox is activated, the shutter pulse is only generated during the live image. If you want a trigger signal to be generated both during the live image and during acquisition, both checkboxes must be activated simultaneously.

Control Signal dropdown list

Active High means that the **Control Signal** jumps from 0V to 5V when the camera's exposure begins and returns to 0V following exposure.

Active Low means that the **Control Signal** jumps from 5V to 0V when the camera's exposure begins and returns to 5V following exposure.

Configure this behavior in accordance with the requirements of the device that you have connected.

Delay When Opening input field

Due to the inertia of the masses being moved, a mechanical shutter needs a certain amount of time to change from the closed to the open position after the control signal has been generated. To ensure the sensor does not record this transitional state when it is exposed, which would lead to uneven exposure, it is possible to delay the start of actual acquisition. Enter a value in accordance with the data for the device that you have connected. The delay time can be set from 0 to 819 ms.

Trigger Input

If the camera that you are using has a control input, you can also trigger acquisition by means of an external control pulse.

Active During Snap checkbox

When this checkbox is activated, acquisition after a snap command is only triggered following the input of the control signal.

Control Signal dropdown list

Active High means that the **Control Signal** jumps from 0V to 5V when the camera's exposure begins and returns to 0V following exposure.

Active Low means that the **Control Signal** jumps from 5V to 0V when the camera's exposure begins and returns to 5V following exposure.

Configure this behavior in accordance with the requirements of the device that you have connected.

Model-Dependent section

Only visible if the **Show All** mode is activated.

To show the section in full, click on the  button.

Orientation dropdown list

Here you can select the orientation of the camera image. This allows you to adjust the camera image to the properties of the different camera ports. The following selection options are available:

- **Original**
- **Horizontal Flip**
- **Vertical Flip**
- **-90° Rotation**
- **+90° Rotation**
- **180° Rotation**
- **-45° Flip**
- **+45° Flip**

Default button

Resets all settings to the default values.

3.1.7 Software Autofocus tool

Quality section

Basic Selects the simple and fast algorithm to calculate the software autofocus.

Best Selects a more complex, optimized algorithm to calculate the software autofocus.

Region Coverage section

Smart Sets a part of the travel range as the region for determining the autofocus.

Full Sets the full travel range as the region for determining the autofocus.

Increment section

Coarse Uses a large Z-distance between the individual focus images that are used to calculate the best focus position.

Optimal Uses a medium Z-distance between the individual focus images that are used to calculate the best focus position.

Fine Uses a small Z-distance between the individual focus images that are used to calculate the best focus position.

Autofocus Search Range section

Only visible if the **Show All** mode is activated.

Relative Range button

Activated: The software autofocus is calculated over a relative range.

Automatic Range checkbox

Activated: Calculates the range for the autofocus search automatically depending on the objective set.

Range spin box/input field

Only active if the **Automatic Range** checkbox is not activated.

Enter a range here that you want to be used for the autofocus search.

Increment display field

Shows the distance between the individual focus images set in the **Increment** section.

Fixed Range button

Activated: The software autofocus is calculated over a fixed range.

Last button

Defines the current Z-position as the end point for the software autofocus. Alternatively, you can enter the desired value in the spin box/input field to the left of the button.

First button

Defines the current Z-position as the start point for the software autofocus. Alternatively, you can enter the desired value in the spin box/input field to the left of the button.

Autofocus ROI section

To show the section in full, click on the button.

Spot Measurement/Focus ROI checkbox

Activated: Spot measurement is used to calculate the software autofocus.

3.1.8 Movie Recorder tool

In the **Movie Recorder** tool you can acquire image sequences in the form of videos using the camera's fastest burst mode.

Start button

Starts acquisition. The button changes into the **Pause** button. The animated **Stop** button appears in the window above the button.

Pause button

Pauses acquisition. The button changes into the **Continue** button.

Continue button

Continues acquisition if it has been paused. The button changes into the **Pause** button.

Stop button

Stops acquisition. Save the acquired movies either in the internal CZI format or as a series of individual images via **File** menu | **Export** or as an AVI file via **File** | **Export Film**.

3.1.9 Locate tab dialog windows

Configure Favorites dialog window

In the **Configure Favorites** dialog window you can define up to 20 new buttons to give you quick access to your preferred camera and hardware settings.

Favorites section

If you have not yet defined any buttons, you will see an empty list here. To create a new button, click on the  button.

Name input field

Here you can enter a name for the button.

Hardware Settings display field

Shows the selected hardware settings.

Camera Settings display field

Shows the selected camera settings.

Color dropdown list

To change the color of the button, click on the colored rectangle. Select a color of your choice from the list.

Text Color checkbox

Activated: Uses the selected color as the text color.

Hardware Settings section

Here you will see all the hardware settings that are saved on your hard drive. Select the hardware setting that you want to use as a favorite with the current button.

Camera Settings section

Here you will see all the camera settings that are saved on your hard drive. Select the camera setting that you want to use with the current button.

3.2 Acquisition tab

On the **Acquisition** tab you can configure and control your acquisition experiments.

Settings that you configure in the top part of the tab have an effect on settings in the bottom part of the tab.

Example:

Settings that you configure in the **Acquisition Parameters** tool group in the **Channels** tool also apply to the acquisition of all images that you configure in the **Multidimensional Acquisition** tool group in the **Z-Stack**, **Tiles** or **Time Series** tools.

Information

The content of the **Acquisition** tab changes depending on the technical possibilities offered by your imaging system and the options that you activate or deactivate.

3.2.1 Experiment Manager

In the **Experiment Manager** you can load and save your experiments, control acquisition and decide which tools will appear in the tool groups.

Experiment Selection dropdown list

Here you can select saved experiments. If you make changes to an experiment, the name of the experiment is marked with an asterisk (*). If you close the application without saving a changed ("asterisked") experiment, you will be asked whether you want to save the changes.

button

Opens the Options [→ 69] shortcut menu.

Smart Setup button

Opens the Smart Setup [→ 102] dialog window.

Show All Tools checkbox

If the checkbox is activated,

- the **System Settings** tool group is shown with the **Light Path Settings** tool.
- the **Experiment Designer** tool is also shown in the **Multidimensional Acquisition** tool group.
- the Acquisition Sequence [→ 72] dropdown list is also shown under the acquisition dimensions. From the list you can select various sequences for **Time, Regions, Tiles, Channels or Z-stacks.**

Acquisition buttons

Information

The Acquisition buttons on the **Acquisition** tab differ from the Acquisition buttons on the **Locate** tab. The buttons on the Locate tab relate to an individual image. The buttons on the Acquisition tab relate to a multidimensional image with at least one channel.

Find Focus button

Only visible if a motorized focus drive has been configured in the MTB (MicroToolBox).

Starts an autofocus search using the settings from the **Focus Devices** tool.The autofocus search is performed for the selected reference channel in the **Channels** tool.

Information

An image is only acquired automatically after the search if the **Live** button is deactivated.

Set Exposure button Starts the exposure. The exposure time that has been predefined for each activated channel in the **Channels** tool is used.

Information

An image is only acquired automatically after the exposure time measurement if the **Live** button is deactivated.

Live button Opens the live window and displays a fast live camera image. The settings (e.g. filters and illumination) that have been predefined for each activated channel in the **Channels** tool are used. No pseudo colors from the channel setting are applied to the live image, however. If several channels are available, but none have been selected, the first channel of the experiment is used.

Continuous button Starts a series of acquisitions.

The precise camera setting that has been set in the **Acquisition Mode** tool is used. Pseudo colors and other settings, e.g. exposure times, from the channel setting are also applied. Other image dimensions (Z-stacks, tiles, time series, etc.) are not taken into account in **Continuous** mode. If you click on the **Stop** button, an image is acquired automatically.

Snap button Acquires an image. The settings that have been predefined for each activated channel in the **Channels** tool are used. The pseudo colors from the relevant channel setting are applied.

Stop button Only active if one of the Acquisition buttons has been clicked. In this case the relevant button changes into an animated **Stop** symbol.

Start Experiment button

Only active if settings have been configured on the **Acquisition** tab (e.g. additional acquisition dimensions added during selection of dimensions).

Starts the acquisition of the experiment.

Information

If you have not added any acquisition dimensions other than channels, the **Start Experiment** button will not be active. To start acquisition, click on the **Snap** button.

Options

In the **Options** shortcut menu you can create new experiments and rename, save, import, export or delete existing experiments.

New menu item

Creates a new, empty experiment. Enter a name for the experiment.

Rename menu item

Enter a new name for the experiment.

Save menu item

Saves a changed ("asterisked") experiment under the current name.

Save As menu item

Saves the current experiment under a new name. Enter a new name for the experiment.

Import menu item

Imports an existing experiment. The experiment is shown in the **Experiment Selection** drop-down list.

Export menu item

Exports the current experiment.

Delete menu item

Deletes the current experiment.

New From menu item

The **New From** menu item only appears if there is a valid acquisition experiment in **.czexp** format in the **\ZEN\Templates\Experiment Setups** folder.

Creates a copy of the selected experiment with a new name. You can use the experiment immediately or change it. The default experiment cannot be deleted or changed.

Acquisition Dimensions

In the **Acquisition Dimensions** section you can activate the acquisition dimensions (Z-stack, tiles, panorama, time series) required in your experiment. The corresponding tool only appears in the Multidimensional Acquisition [➙ 87] tool group once an acquisition dimension has been activated. The field to the right of the acquisition dimension shows how extensive the acquisition will be (e.g. number of sections or number of tile images).

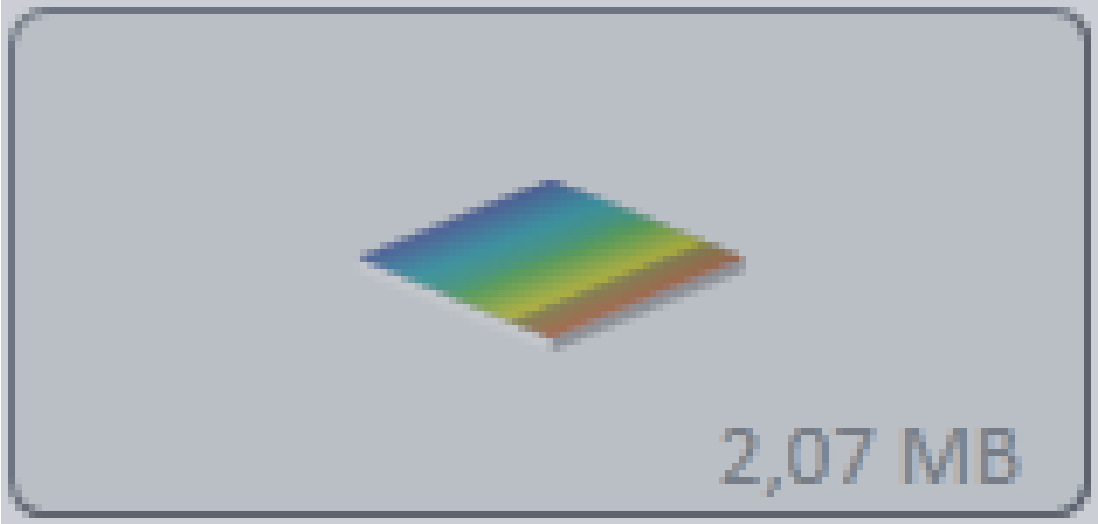
Experiment Preview

The Experiment Preview shows you the acquisition dimensions of your current experiment.

Example 1

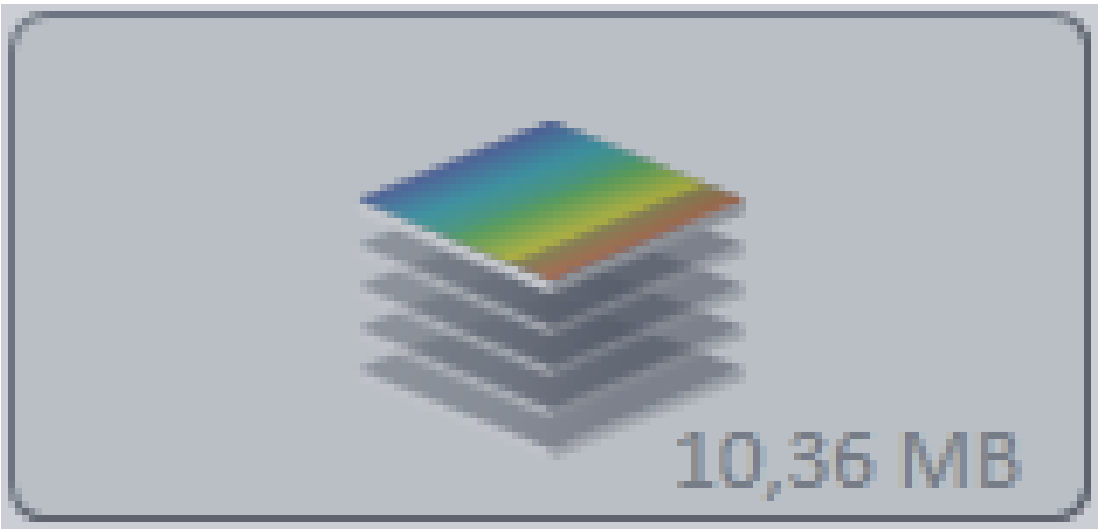
No channel is active or no fluorescence channels have been configured. No other experiment dimensions have been activated.

Example 2



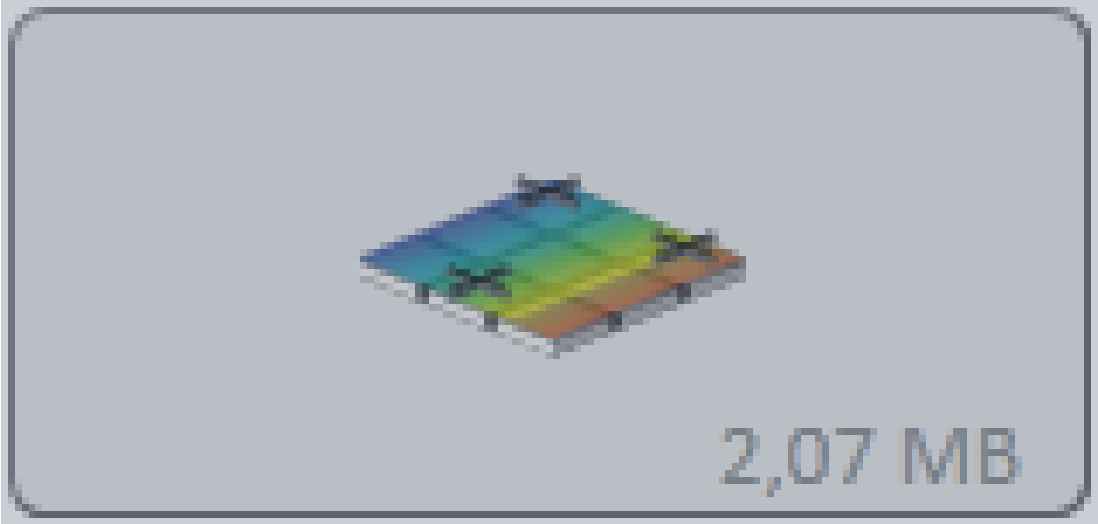
A fluorescence channel has been configured in the current experiment. No other experiment dimensions have been activated. The value in the bottom right corner shows the calculated size of the image file on the hard drive.

Example 3



At least one fluorescence channel has been configured in the current experiment, as well as a Z-stack. The number of visible planes does not reflect the actual number of planes in the experiment. The value in the bottom right corner shows the calculated size of the Z-stack on the hard drive.

Example 4



At least one fluorescence channel has been configured in the current experiment, as well as a tile acquisition or a multiposition experiment. The number of positions displayed does not reflect the actual number of tile images in the experiment. The value in the bottom right corner shows the calculated size of the complete acquisition on the hard drive.

Acquisition Sequence

Acquisition Sequence dropdown list

Only active if the **Show All Tools** checkbox is activated.

The available selection options (*default settings) in the list depend on the acquisition dimensions selected.

Acquisition dimensions	Selection option	Function
Channels	"All Channels"	Acquires all configured channels one after the other.
Channels Z-stacks	"All planes per channel" (*)	Acquires the complete Z-stack for a channel and then processes the next channel.
	"All channels per plane"	Acquires all channels for each Z-plane and then locates the next plane of the Z-stack.
Channels Z-stacks Tiles/Positions	"All planes per channel" (*)	Acquires the complete Z-stack at a tile/position for each channel and then processes the next channel. Once all Z-stacks at a tile/position have been acquired, the next one is located.

Acquisition dimensions	Selection option	Function
	"All channels per plane"	Acquires all channels at a tile/position for each Z-plane before locating the next plane of the Z-stack. Once all Z-planes and channels at a tile/position have been acquired, the next one is located.
- Channels - Z-stacks - Time series	"All planes per channel" (*)	Acquires the complete Z-stack for each channel for a time point and then processes the Z-stack of the next channel.
	"All channels per plane"	Acquires all channels for each Z-plane for a time point before locating the next plane of the Z-stack.
- Channels - Tiles/Positions - Time series	"All tiles per time point"	Acquires images for all channels as well as images at all positions of the experiment for each time point and then waits for the next time point.
	"Time series per tile"	Acquires the complete time series at a position with all channels, before doing the same at the next position. Connected tile images are, however, acquired in full for each time point.
- Channels - Z-stacks - Tiles/Positions - Time series	"Time-Regions-Tiles-Channels-Z" (*)	Acquires images for all channels as well as images at all positions of the experiment for each time point and then waits for the next time point. The complete Z-stack is acquired at every position for each channel and the next channel is then processed.

Acquisition dimensions	Selection option	Function
	"Time-Regions-Tiles-Z-Channels"	Acquires images for all channels as well as images at all positions of the experiment for each time point and then waits for the next time point. Acquires all channels for each Z-plane and then locates the next plane of the Z-stack.
	"Regions-Time-Tiles-Channels-Z"	Acquires the complete time series at a position with all channels, before doing the same at the next position. Acquires the complete Z-stack for each channel and then processes the next channel.
	"Regions-Time-Tiles-Z-Channels"	Acquires the complete time series at a position with all channels, before doing the same at the next position. Connected tile images are, however, acquired in full for each time point. Acquires all channels for each Z-plane and then locates the next plane of the Z-stack.

3.2.2 System Settings tool group

In the **System Settings** tool group you will find the **Light Path Settings** tool. This is only displayed if the **Show All Tools** checkbox is activated in the **Experiment Manager**.

Light Path Settings tool

In the **Light Path Settings** tool you can view and change the hardware settings used in the experiment. You will see a graphical display of the acquisition light path with various icons. The arrangement of the icons represents the typical set-up of the various microscope components on your system.

Information

Please note that the **Light Path** tool can be found on the **Locate** tab. In some cases this has a similar appearance and similar control elements. Its function differs, however, from the **Light Path Settings** tool described here.

Before Experiment/After Experiment section

Here you can edit the hardware settings that should be applied before and after the experiment.

Before Experiment list item

Shows the name of the hardware setting that will be applied immediately before the experiment.

After Experiment list item

Shows the name of the hardware setting that will be applied immediately after the experiment.

buttons

Open the **Options** shortcut menus for the relevant hardware settings.

Go! buttons

Apply the selected hardware settings.

Previous/Next buttons

The **Previous/Next** buttons allow you to navigate through the various hardware settings.

Options

Change Hardware Settings

Opens a dialog window in which you can change the relevant hardware setting.

Remove Hardware Setting

Removes the existing hardware setting.

Use Current Device Status

Adopts the current device status.

Hardware Settings List

The shortcut menu shows a list of the existing hardware settings.

Saved Hardware Settings

The shortcut menu shows a list of the saved hardware settings.

Select File

Opens the **Import Hardware Settings** dialog window. Select a ZIS hardware settings file (*.czhws).

Export Hardware Settings

Exports the current hardware setting.

Acquisition Light Path section

In the graphical display of the acquisition light path you can see the typical set-up of all the microscope components on your system. The associated hardware settings are shown above the icons and can be changed here. To change the relevant hardware settings, left-click on the icons. In the shortcut menus you will see numerous selection and setting options for adjusting your settings.

Information

Any change you make is automatically adopted and written to the corresponding hardware setting of the experiment. If you want to undo these changes, do not save the experiment. Instead, reload the experiment in the **Experiment Manager**.

If you change the hardware settings in the **Acquisition Light Path** section, please bear the following points in mind:

- Components with an activated checkbox are adopted into the hardware settings of the experiment and subsequently applied in the experiment. These components are displayed with a colored icon.
- Components with a deactivated checkbox are not adopted into the hardware settings of the experiment and are not subsequently applied in the experiment. These components are displayed with a grayed-out icon.
- Components with a filled-in checkbox and a triangle underneath are only partially adopted into the hardware settings of the experiment and subsequently applied in the experiment. To show the sub-components, click on the triangle under the checkbox. To adopt the sub-components into the hardware settings of the experiment and subsequently apply them in the experiment, activate the relevant checkboxes for the sub-components.

Hardware Setting section

Only visible if the **Show All** mode is activated.

Remove button

Removes hardware settings that are not being used from your experiment.

Information

If you frequently change an experiment, e.g. delete channels and add them again, unused hardware settings build up. Only remove settings if you will no longer need them in the future.

List of Settings Available in Experiment

Shows all the hardware settings available in the experiment. You can add further hardware settings to your experiment using the  button. Use the  button to delete the selected hardware setting.

3.2.3 Acquisition Parameter tool group

Acquisition Mode tool

In the **Acquisition Mode** tool you can set the various microscope camera parameters that you want to apply for the entire experiment. Microscope camera settings that are configured in the **Acquisition Mode** tool apply to all channels or tracks acquired using this camera.

Information

If you have created an experiment using the **Experiment Designer** tool, the settings in the **Acquisition Mode** tool only apply to the relevant experiment block and may differ in the next block.

In terms of its content and appearance, the **Acquisition Mode** tool is largely dependent on which camera(s) or devices are available on your system.

Example 1

If only one microscope camera is configured on your system, the **Acquisition Mode** tool on the **Acquisition tab** scarcely differs from the **Camera** tool on the **Locate tab**. In this case the exposure time settings are not available in the **Acquisition Mode** tool.

Example 2

If additional devices are configured on your system, e.g. a **VivaTome** or **ApoTome**, corresponding sections are available in the **Acquisition Mode** tool.

Selected Camera section

Only visible if at least two cameras are connected to the system and these are both being used in the current experiment.

Here you can select the camera that you want to configure in the **Acquisition Mode** tool. All settings then apply to this camera only.

Information

Dual camera experiments, which use two identical camera types, form an exception here. If two cameras of the same type are used within a track, the camera settings can no longer be changed independently of one another in the **Acquisition Mode** tool; instead they are synchronized.

Settings From Locate Tab section

Get button

Transfers the settings from the **Camera** tool on the **Locate** tab to the **Acquisition Mode** tool.

Default button

Resets all settings to the default settings.

White Balance section

Only active if a color camera has been selected.

Auto button

Sets the white balance automatically.

Select button

Select a point in the image as a reference point for the white balance.

3200K button

Sets the color temperature to 3200K.

5500K button

Sets the color temperature to 5500K.

Display Color Channels checkbox

Only visible if the **Show All** mode is activated.

Activated: Displays 3 sliders to allow each color channel to be set precisely.

Color Temperature slider

Here you can set the color temperature.

Saturation slider

Only visible if the **Show All** mode is activated.

Here you can set the saturation.

Default button

Resets all settings to the default values.

Operating Mode section

Only visible if the **Show All** mode is activated.

Color Mode button

RGB Sets the color mode to RGB.

B/W Sets the color mode to B/W.

Live Mode dropdown list

Here you can select the speed of the live mode. You can choose between **Slow**, **Medium** and **Fast**.

Resolution dropdown list

Here you can select the camera resolution.

Binning dropdown list

Here you can select the binning mode for the camera.

NIR Mode checkbox

When the checkbox is active you can use the camera in the near infrared range.

Area section

In the **Area** section you can define a particular area that you want to be acquired.

After clicking on the **Update Overview** button, in the window on the left you will see a **Preview** of your sample under the camera. The **Pixel Size** below the preview window indicates the size of a pixel depending on the scaling set. The blue frame indicates the selected area. The entire preview window is highlighted by default. To change the area, move the mouse pointer over the blue frame. The mouse pointer will appear as a double-headed arrow. If you hold down the mouse button and move the mouse, you can change the size of the frame and in this way highlight a certain area on the sample. To move the area, position the mouse pointer inside the frame. The mouse pointer will appear as a four-headed arrow. If you hold down the mouse button and move the mouse, the frame can be moved.

Maximize button

Maximizes the frame to the maximum size in the preview window.

Center button

Positions a frame that has been changed precisely at the center of the image.

Size input field

Here you can enter the size of the frame that you want. Depending on the camera type available, select from the predefined sizes in the dropdown list (1388x1040, 1024x1024, 512x512, 256x256, 128x128). Alternatively you also have the option of entering the **Size** in X and Y, and the top left **Start** position. You can change the values by increments using the

arrow buttons next to the input windows or you can enter the desired values directly using the keyboard. To do this, you must activate the number already present using the cursor. Complete the entry by using the Tab or Enter key.

Start input field

Here you can enter the start position for the frame, measured from the top left corner.

Update Overview button

Adopts the image from the camera into the preview window.

Post-Processing section

Depending on which camera you are using, some functions are not visible. The most important functions are explained in the section below.

Black Reference checkbox

Only visible if the **Show All** mode is activated.

Only active if you have defined a black reference via the **Define** button.

Activated: Applies the measured black reference to the image.

Deactivated: The measured black reference is not used. The reference image is retained.

Define button

Automatically defines the black reference. To do this a reference image is acquired, from which the black reference is measured. The light path to the camera must be closed and the lamp must be switched on for the measurement. The measurement lasts for several seconds. The **Black Reference** checkbox is then activated automatically.

Shading Correction checkbox

Only active if you have defined a shading correction via the **Define** button.

Activated: Applies the measured shading correction to the image.

Deactivated: The measured shading correction is not used. The reference image is retained.

Define button

Automatically defines the shading correction. To do this you will acquire a reference image for the shading correction. An empty image without structures is required to measure the shading image. Move the slide to an empty position on the sample with no artifacts in the image. The **Shading Correction** checkbox is activated automatically after the definition.

Activate Noise Filter checkbox

Activated: The **Limit** slider and the spin box/input field are active.

Enter the limit for the noise filter using the slider or spin box/input field.

Unsharp Masking checkbox

Activated: Emphasizes edges in the image more clearly.

Strength slider

Enter the strength of the filtering using the slider or spin box/input field.

The following functions are only visible if the **Show All** mode is activated:

Radius slider

Enter the radius of the filtering using the slider or spin box/input field.

Color Mode dropdown list

Here you can select the desired color mode. You can choose between **RGB** or **Luminance**.

Autocontrast checkbox

Activated: The **Contrast Tolerance** slider and spin box/input field are active.

Enter the contrast tolerance (0-20) using the slider or spin box/input field.

Limit to Valid Bit Number checkbox

Activated: Limits the setting option for the **Contrast Tolerance** function to a valid bit number. Diese

Trigger Control section

Only visible if the **Show All** mode is activated.

To show the section in full, click on the  button.

Trigger Output

As soon as this output is activated, you can select the polarity with which you want this signal to be generated.

Active During Snap checkbox

When this checkbox is activated, the shutter pulse is only generated during acquisition. If you want a trigger signal to be generated both during the live image and during acquisition, both checkboxes must be activated simultaneously.

Active During Live checkbox

When this checkbox is activated, the shutter pulse is only generated during the live image. If you want a trigger signal to be generated both during the live image and during acquisition, both checkboxes must be activated simultaneously.

Control Signal dropdown list

Active High means that the **Control Signal** jumps from 0V to 5V when the camera's exposure begins and returns to 0V following exposure.

Active Low means that the **Control Signal** jumps from 5V to 0V when the camera's exposure begins and returns to 5V following exposure.

Configure this behavior in accordance with the requirements of the device that you have connected.

Delay When Opening input field

Due to the inertia of the masses being moved, a mechanical shutter needs a certain amount of time to change from the closed to the open position after the control signal has been generated. To ensure the sensor does not record this transitional state when it is exposed, which would lead to uneven exposure, it is possible to delay the start of actual acquisition. Enter a value in accordance with the data for the device that you have connected. The delay time can be set from 0 to 819 ms.

Trigger Input

If the camera that you are using has a control input, you can also trigger acquisition by means of an external control pulse.

Active During Snap checkbox

When this checkbox is activated, acquisition after a snap command is only triggered following the input of the control signal.

Control Signal dropdown list

Active High means that the **Control Signal** jumps from 0V to 5V when the camera's exposure begins and returns to 0V following exposure.

Active Low means that the **Control Signal** jumps from 5V to 0V when the camera's exposure begins and returns to 5V following exposure.

Configure this behavior in accordance with the requirements of the device that you have connected.

Model-Dependent section

Only visible if the **Show All** mode is activated.

To show the section in full, click on the button.

Orientation dropdown list

Here you can select the orientation of the camera image. This allows you to adjust the camera image to the properties of the different camera ports. The following selection options are available:

- **Original**
- **Horizontal Flip**
- **Vertical Flip**
- **-90° Rotation**
- **+90° Rotation**
- **180° Rotation**
- **-45° Flip**
- **+45° Flip**

Default button

Resets all settings to the default values.

Other Devices section

VivaTome section

Only visible if you have connected a VivaTome to your system.

Functions...

ApoTome section

Only visible if you have connected an ApoTome to your system.

Functions...

Fast Acquisition section

In this section, 3 modes are available for defining how acquisition is performed.

Interactive mode

Acquisition is comparatively slow. You can intervene manually at certain points during acquisition.

Triggered mode

Fast acquisition via the hardware.

Compromise mode

The Compromise mode is activated automatically if only individual hardware components, but not the whole system, are compatible with the Triggered mode for acquiring an experiment.

Validate button

To establish whether the system is able to perform an experiment in Triggered mode, click on the **Validate** button.

Channels tool

In the **Channels** tool you can configure channels for acquisition. The tool offers you the option of entering the hardware settings for acquisition manually or performing the configuration automatically.

Channels list

If you have not yet defined a channel or all channels have been deleted, you will only see an empty list here. To add a channel, click on the button. The other sections will only be visible once you have added one or more channels.

Please bear the following points in mind for the Channels list:

- The selected channel is highlighted by a light gray bar.
- The reference channel is highlighted by a blue font color.
- The preview color for the channel is shown on the right in the list. To change the preview color for the channel, click on the colored rectangle with the arrow icon and select an alternative color from the shortcut menu. The preview color is also shown in the sections for channel-specific hardware settings as a thin line on the left-hand side.

button

Opens the Add Dye or Contrast Technique [→ 104] dialog window.

Information

If you select a dye or contrast technique in the **Add Dye or Contrast Technique** dialog, a suggestion for the hardware settings for the acquisition of this channel is made automatically. If no suggestion can be made, a channel without hardware settings is added. You will then see a corresponding indication in the status area of the program interface.

button

Only active if you have added a channel to the list.

Selects the channel below the selected channel.

button

Only active if you have added a channel to the list.

Selects the channel above the selected channel.

button

Only active if you have added a channel to the list.

Deletes the selected channel.

button

Opens the Options [→ 85] shortcut menu.

Options

New menu item

Opens the Add Dye or Contrast Technique [→ 104] dialog window.

Copy menu item

Creates a copy of the selected channels.

Rename menu item

Opens a dialog that allows you to change the name of the dye or contrast technique for the selected channel.

Reset Color menu item

Resets the setting for the channel's preview color to the original color.

Select All menu item

Selects all channels in the list.

Delete menu item

Deletes the selected channel.

Delete All menu item

Deletes all existing channels.

Compare menu item

Opens the **Compare Channels** dialog window. In this dialog window all selected channels are displayed in a horizontal arrangement. This view allows you to compare the selected channels more easily.

Channel-specific settings

The settings always relate to the channel you have selected in the **Channels** list.

To show the settings for all channels, click on the button | **Select All** in the **Channels** list.

Light Source section

In the **Light Source** section you can select the available light sources and enter the corresponding settings. Here you can adjust the parameters of the light sources without having to save these in the hardware settings. You can therefore adjust the intensity of the laser lines or LEDs, for example, immediately before starting acquisition.

If your system is equipped with a TIRF slider, the TIRF angle and type of illumination can also be set here.

Light Source dropdown list

Select the light source here.

If you select the **Use Hardware Setting** entry, the settings for the light sources disappear. The light source parameters from the hardware settings are used instead for the acquisition of the channel.

Camera Settings section

Dye Name input field

In the input field after the selected dye you can enter an additional name.

Camera dropdown list

Select the desired camera for the channel from the dropdown list.

Time slider

Enter the exposure time for the camera using the **Time** slider or spin box/input field. Select the unit of time from the dropdown list to the right of the spin box/input field.

Range spin box/input field

Only visible if the **Show All** mode is activated.

Here you can enter the range of the camera's dynamic range that is utilized.

Auto checkbox

Activated: Automatically determines the camera's exposure time for the channel. The value set manually is ignored.

Measure button

Starts an exposure time measurement for the channel. After the measurement the value is adopted as the exposure time setting.

The following functions are only visible if the **Show All** mode is activated:

Focus Offset spin box/input field

Here you can enter the focus offset from the channel to the Z-position of the reference channel or to the current position.

Display dropdown list

Here you can select an existing predefined display setting. You must have previously saved a predefined display setting in *.CZDSP format.

Set button

Adopts the selected predefined display setting. The values are shown in the display fields.

Remove button

Deletes the selected predefined display setting.

Z-Stack Mode dropdown list

Only visible if the **Z-Stack** checkbox is activated in the **Experiment Manager**.

Selection option	Function
Yes	Acquires a Z-stack as defined in the **Z-Stack** tool.
No	Does not acquire a Z-stack, only the central focal plane.
No, fill with central plane	Does not acquire a Z-stack, only the central focal plane, and copies this to all Z-planes of the image in this channel.
No, fill with black	Does not acquire a Z-stack, only the central focal plane. All the other Z-planes of the image are filled with black images.

3.2.4 Multidimensional Acquisition tool group

See also

Experiment Manager [→ 68]

Experiment Designer tool

Information

The **Experiment Designer** tool is only available if the **Show All Tools** checkbox is activated in the **Experiment Manager**.

In the **Experiment Designer** tool you can create experiments for multidimensional acquisition. The experiments can consist of any number of components. A component is referred to as an **experiment block**. Each experiment block has a distinct number, which is shown above the block. Special actions that influence the course of an experiment are performed by means of a **special block**. In the **Show All** mode you can define **repetitions** and specify the number of image files.

Activate Experiment Designer Mode section

Activate Experiment Designer Mode checkbox

Activated: The Experiment Designer Mode is active. The entire experiment is saved in the Experiment Manager. If you now click on the **Start Experiment** button in the **Experiment Manager**, all active blocks are run.

Deactivated: The Experiment Designer Mode is deactivated. Only the most recently selected experiment block is saved.

Experiment Block section

Add New button

Creates a new, empty experiment block.

Copy button

Creates an exact copy of the selected block.

Delete button

Deletes the selected block from your experiment.

Add Special Block section

Pause button

Adds the **Pause** special block. This block pauses the experiment for a predefined period. Set the length of the pause using the slider or selection field to the right of the button. Change the unit of time using the dropdown menu to the right of the selection field.

Wait button

Adds the **Wait** special block. This block displays a user-defined message and interrupts the experiment until the message is confirmed with OK. Enter the desired message into the **input field** to the right of the button.

Apply button

Adds the Apply special block. This block applies a change to the hardware.

Async checkbox

Activated: Runs the next experiment block immediately and irrespective of the duration of the hardware change.

Deactivated: Only runs the next experiment block once the hardware setting has been applied.

button

Opens the **Options** shortcut menu.

Go! button

Applies the selected hardware setting immediately.

Options

Change Hardware Settings

Opens a dialog window in which you can change the relevant hardware setting.

Remove Hardware Setting

Removes the existing hardware setting.

Use Current Device Status

Adopts the current device status.

Hardware Settings List

The shortcut menu shows a list of the existing hardware settings.

Saved Hardware Settings

The shortcut menu shows a list of the saved hardware settings.

Select File

Opens the **Import Hardware Settings** dialog window. Select a ZIS hardware settings file (*.czhws).

Export Hardware Settings

Exports the current hardware setting.

One Image File Per Acquisition Block section

One Image File Per Acquisition Block checkbox

Only visible if the **Show All** mode is activated.

Activated: One image file is created per acquisition block.

Deactivated: A single image file is created for all images of the active experiment.

Repetitions section

Only visible if the **Show All** mode is activated.

To show the section in full, click on the  button.

In the **Repetitions** section you can specify which experiment blocks should be repeated during the course of the experiment. You can define as many repetitions as you like for each experiment. An experiment block may only appear once within the repetitions defined.

Information

If you define several repetitions, the following conditions must be met:

- Repetitions must form a complete unit
- One repetition may not be placed within another

If these conditions are not met, the repetition cannot be performed. In this case a yellow warning symbol appears under the **Active** field.

Number field

Enter the number of repetitions that you want to be performed in the spin box/input field.

Starting Block field

Enter the number of the starting block in the spin box/input field.

End Block field

Enter the number of the end block in the spin box/input field.

Active field

If the checkbox after the repetition in question is activated, this repetition is performed in the experiment.

Plus button

Adds a new repetition to the experiment.

Wastepaper Bin button

Deletes the selected repetition.

Z-Stack tool

In the **Z-Stack** tool you can configure acquisitions that comprise several Z-planes of your sample. You can set all the parameters manually or have configuration performed automatically.

Information

The Z-Stack tool is only available if you have activated the **Z-Stack** checkbox in the **Experiment Manager** | **Acquisition Dimensions**.

Automatic Configuration

Automatic configuration is performed in the **Z-Stack Autoconfiguration** section at the bottom of the tool. The following parameters are set automatically:

- Z-position of the central plane

- Distance between the individual planes
- Number of section planes

Information

Before you perform automatic configuration, the current focus position must be at the center of the sample. The camera's current field of view must always be at a position on the sample that shows a signal in the selected channel.

Information

Automatic Z-stack configuration only works with microscopes and systems that do not use an optical sectioning technique. If you use an **ApoTome**, **VivaTome**, **Spinning Disc (CSU)** or another technique for generating optical sections, the Z-stack must be configured manually.

Configure Z-Stack button

Automatically configures the Z-stack using the current sample.

Manual Configuration

Information

Z-stack images are always acquired from bottom to top automatically, irrespective of whether you have defined the top or bottom Z-plane of your stack as the first Z-plane. This acquisition sequence increases the accuracy of the Z-positioning.

First/Last button

Only visible if the **Show All** mode is activated.

Activates the **First/Last** acquisition mode.

Activate the **First/Last** button if you want to specify manually which Z-plane is acquired first and which is acquired last.

Center button

Activates the **Center** acquisition mode.

Activate the **Center** button if you want to specify the central Z-plane manually.

Only visible if the **Show All** mode is activated.

Z-Stack Preview

The graphic in the left tool area represents the configured Z-stack. In the case of inverse microscopes the objective appears in stylized form at the bottom of the Z-stack. In the case of upright systems it appears at the top.

The blue plane indicates the current section plane. The round **L**, **C** and **F** buttons refer to the corresponding planes (**L** = Last, **C** = Center, **F** = First). To change the current Z-position, click on the relevant buttons. The blue plane then jumps to the desired position.

The values at the top and bottom of the measurement scale on the right-hand side of the graphic indicate the distance to the center of the Z-stack.

The **Position** display field below the graphic indicates the Z-position at which the section plane is located. Here you can navigate precisely to the relevant Z-positions.

The **Section** display field below the graphic indicates the number of the section in question.

First/Last acquisition mode

Last button

Sets the position for the last Z-plane. You can also enter the value in the input field to the right of the button.

Range display field

Displays the range of the configured Z-stack from the last to the first section plane.

Sections input field

Here you can enter the number of section planes that you want.

Interval input field

Here you can enter the desired distance between the section planes.

Optimal button

This button shows the distance calculated for the channels set and the current microscope according to the Nyquist criterion. If you click on this button, this value is automatically adopted into the **Interval** input field.

Keep radio button

Interval Keeps the set interval between the section planes constant if you change configuration parameters in the Z-Stack tool.

Section Keeps the set number of section planes constant.

First button

Sets the position for the first Z-plane. You can also enter the value in the input field to the right of the button.

Center acquisition mode

Range display field

Displays the range of the configured Z-stack from the last to the first section plane.

Sections input field

Here you can enter the number of section planes that you want.

Interval input field

Here you can enter the desired distance between the section planes.

Optimal button

This button shows the distance calculated for the channels set and the current microscope according to the Nyquist criterion. If you click on this button, this value is automatically adopted into the **Interval** input field.

Keep radio button

Interval Keeps the set interval between the section planes constant if you change configuration parameters in the Z-Stack tool.

Section Keeps the set number of section planes constant.

Center button

Sets the position for the central Z-plane. You can also enter the value in the input field to the right of the button.

Offset input field

Here you can enter an offset value if you want to shift the acquisition of the Z-stack relative to the focus position.

Tiles tool

In the **Tiles** tool you can configure the acquisition of images that consist of several image fields (tile regions) and positions. If an image is made up of several tile regions, we also talk of a mosaic image. Here you can enter the positions, the number of tiles and other specifications relating to the size, area and distribution of the images on the sample carrier.

Information

The **Tiles** tool is only available if you have activated the **Tiles** checkbox in the **Experiment Manager** | **Acquisition Dimensions**. This checkbox is only visible if you have a corresponding license and a motorized microscope stage has been detected.

See also

Acquisition Dimensions [→ 70]

Advanced Setup section

Advanced Setup button

Opens the Tiles Advanced Setup View [→ 274] in the **center screen area** for the **Tiles** tool.

Add Tile Region section

Contour section

Only visible if the **Show All** mode is activated.

Defines the outline of the tile region that you are adding. To add a rectangular tile region, click on the **Rectangle** button. To add a circular tile region, click on the **Circle** button.

Tiles button

Selects the number of tile regions as a reference for the size of the tile region. Enter the number of tile regions in the **X/Y** spin boxes/input fields. If you are adding a circular tile region, enter the number of tile regions in the **Diameter** spin box/input field.

Size button

Selects the size as a reference for the size of the tile region. Enter the size of the tiles in the **X/Y** spin boxes/input fields. If you are adding a circular tile region, enter the diameter of the tile regions in the **Diameter** spin box/input field.

 button

Adds the tile region to the **Tile Regions** list and activates it for acquisition.

Added tiles are displayed in the form of red grids in the stage view. Sie können die Gitter beliebig

Add Position section

X-Position display field

Displays the X coordinate of the current position.

Y-Position display field

Displays the Y coordinate of the current position.

 button

Adds the current position to the **Positions** list and activates it for acquisition.

Tile Regions and Positions section

Tile Regions tab

Tile Regions list

Displays the added tile regions. The list contains the following columns:

Checkbox Activates the relevant list entry for acquisition.

Name Displays the name of the tile region.

Contour Displays the contour of the tile region.

Lock Indicates whether the tile region can be changed.

Category Displays the category of the tile region.

Tiles Displays the number of tile regions.

Size Displays the size of the tile region.

Z Displays the Z-position of the tile region.

 **button**

Deletes the selected list entry.

Lock button

Locks the selected list entry to prevent editing.

Positions tab

Individual Positions button

Displays the individual positions you have added in the **Individual Positions** list.

Position Array button

Displays the position arrays you have added in the list.

Individual Positions list

Checkbox Activates the relevant list entry for acquisition.

Name Displays the name of the individual position.

Contour Displays the contour of the individual position.

Category Displays the category of the position.

X Displays the X-position of the position.

Y Displays the Y-position of the position.

Z Displays the Z-position of the position.

 **button**

Deletes the selected list entry.

Lock button

Locks the selected list entry to prevent editing.

Position Arrays list

Checkbox Activates the relevant list entry for acquisition.

Name Displays the name of the individual position.

Contour Displays the contour of the individual position.

Category Displays the category of the position.

Positions Displays the number of positions in a position array.

Size Displays the size of a position array.

 **button**

Deletes the selected list entry.

Lock button

Locks the selected list entry to prevent editing.

Positions of Selected Array list

The individual positions of the selected array are displayed here. The description of the table columns corresponds to that of the **Individual Positions** list.

Options section

Only visible if the **Show All** mode is activated.

Here you can determine the acquisition and travel behavior during the experiment. Changes in this section of the tool affect all elements, mosaic acquisitions, positions and position arrays.

Tile Overlap spin box/input field

Enter the overlap of individual image tiles of a mosaic image here. The settings are applied exclusively to mosaic images.

Scan Movement

Comb button Acquires the mosaic image from one travel direction following a comb pattern. This scan movement is more precise.

Meander button Acquires the mosaic image from both travel directions following a meander pattern. This scan movement is faster.

Optimize Stage Movement checkbox

Activated: Optimizes the movement of the stage. Short paths are used between tile regions and positions.

Information

If you activate the checkbox, individual positions and mosaic images are not located in the sequence in which they were originally defined. The path is automatically adapted to the location of the individual mosaic images and positions. If you add or remove tile regions or positions, the sequence of acquisition therefore also changes.

Keep Tile Number Constant With New Scaling checkbox

Activated: Keeps the tile number constant if a new scaling is applied.

Split Scenes into Individual Files checkbox

Activated: Splits the scenes into individual files.

Sample Carrier section

To show the section in full, click on the button.

Sample Carrier display field

Displays the selected or calibrated sample carrier.

Select button

Opens the Select Sample Carrier Template dialog window. Here you can select the sample carrier template.

Calibrate button

Opens the Sample Carrier Calibration Wizard. Here you can calibrate the sample carrier.

Move Focus Drive to Loading Position Between Containers checkbox

Activated: Moves the focus drive to the loading position during the movement to another container of the sample carrier (e.g. a well or slide). This prevents possible damage.

Focus Area section

To show the section in full, click on the button.

Reference Points list

X column Displays the X coordinate of the focus reference point.

Y column Displays the Y coordinate of the focus reference point.

Z column Displays the Z coordinate of the focus reference point.

button

Deletes the selected list entry.

Lock button

Locks the selected list entry to prevent editing.

Interpolation Level dropdown list

Here you can select a method for the interpolation of the focus reference area.

Panorama tool

In the **Panorama** tool you will see information on the microscope stage and the status of panorama images. Panorama images are controlled directly in the Panorama View [→ 281].

Information

The **Panorama** tool is only available if you have activated the **Panorama** checkbox in the **Experiment Manager** | **Acquisition Dimensions**.

See also

- Acquisition Dimensions [→ 70]

Time Series tool

In the **Time Series** tool you can configure acquisitions that allow you to acquire an image series consisting of a number of time points. Here you can enter, for example, the acquisition interval, the length of the experiment and other specifications to control the experiment.

Information

The Time Series tool is only available if the **Time Series** checkbox has been activated in the **Experiment Manager** | **Acquisition Dimensions**. This checkbox is only available if you have the corresponding license.

Duration section

Here you can define the duration of your experiment. You can either specify the number of time points (in cycles) or the duration (in milliseconds, seconds, minutes, hours or days).

Slider

Enter the number of time points or the duration using the slider or spin box/input field.

Dropdown list

Select the desired unit for the duration from the dropdown list. If you select the **Cycles** entry, specify the number of time points using the slider or spin box/input field.

As Long As Possible checkbox

Activated: Uses the entire memory space on your hard drive. The duration of acquisition is only limited by the maximum amount of memory space available.

Information

If the **As Long As Possible** checkbox is activated, the acquisition of the time series continues until only 10% of the memory space remains. This can impair other programs. To guarantee optimum system performance, make sufficient memory space available before acquisition.

Interval section

Here you can define the interval from individual image to individual image in an image series. You can specify the interval to set the gap between individual time points (in milliseconds, minutes, hours or days).

Slider

Enter the value for the interval using the slider or spin box/input field.

Dropdown list

Select the desired unit for the interval from the dropdown list.

As Quick As Possible checkbox

Activated: Defines the shortest possible value for the interval.

Measure Speed button

Checks whether the experiment can be performed using the interval set. If the interval is too small, the shortest possible value is defined automatically for the interval.

Information

The shortest possible interval is calculated by performing a blind experiment. The camera exposure time, number of steps of a Z-stack and the number of acquisition channels are taken into consideration in the calculation. Depending on the number of Z-stacks and channels and whether long exposure times have been set, it may take some time to calculate the shortest time interval!

Start/Stop/Pause section

Here you can define the **Start**, **Stop** and **Pause conditions** for your experiment. Select the **requirements for the relevant conditions** from the corresponding dropdown lists.

Conditions

Start Starts the experiment if the requirements are met.

Stop Stops the experiment if the requirements are met.

Start Pause Pauses the experiment if the requirements are met.

End Pause Ends the pause if the requirements are met.

Requirements

Manual The experiment is started immediately using the **Start Experiment** button in the **Experiment Manager**.

Time The experiment is started, stopped or paused at the entered time. Die Vorgabe wird erst nach der Experimentausführung berücksichtigt. Enter the desired time in the spin box/input field to the right of the dropdown list.

Delay The experiment is only started, stopped or paused once the length of time entered has passed.

On Trigger The experiment is started, stopped or paused once a TTL signal has been received.

Information

If you define times as start, stop and pause conditions, these apply once for the entire experiment. This also applies to experiments that use the **Experiment Designer**.

Buttons section

To show the section in full, click on the  button.

Here you can add and configure **buttons** that can be used to execute certain actions during your experiment.

To add a new button, click on the  button.

To configure a button, left-click on the button of an existing button. A dialog window will open in which you can configure various settings.

Name input field

Here you can enter a name for the button.

Description input field

Here you can enter a description for the button.

Color checkbox

Activated: Shows a colored line at the left edge of the button.

Color Selection

Opens the **Color Selection** dialog window. Here you can select a color for the line at the left edge of the button.

Action dropdown list

Here you can select one of the following actions. This action will be executed when you click on the button.

None

Set Interval The **Interval** function is executed.

As Quick As Possible Trigger

Hardware Setting A hardware setting is applied. To select a hardware setting, click on the button.

Delete Button button

Deletes the selected button.

Experiment Information tool

In the **Experiment Information** tool you can find out various details about your experiment, e.g. the memory requirement of the experiment or its duration.

Display field	Function
Memory space	Indicates the calculated memory space that the experiment will take up on your hard drive. All the activated blocks of an experiment created using the **Experiment Designer** are taken into account.
Duration (Theoretical)	The system adds together all the exposure times arising during acquisition in the experiment and indicates this value. In the case of time series the intervals set are also taken into account. The actual acquisition duration will always turn out longer, however, as switching times for components (diaphragms, reflectors) and positioning times (Z-plane, stage position) also come into play.
Maximum acquisition rate	If the **Time Series** acquisition dimension is activated in the **Experiment Manager**, you can measure the maximum possible frame rate of the system in the **Time Series** tool. In that case the frame rate is shown here. Otherwise "not available" is displayed. After any change is made to the experiment the frame rate must be determined again in the **Time Series** tool.

Display field	Function
Time Required (Last Experiment)	If you have already run the current experiment before on the system, the duration actually required for it is displayed here. This information disappears again if you change the experiment.
Tile Size	Shows the X/Y dimensions of your experiment. In the case of a single position this value is identical to the size of the camera field.

3.2.5 Acquisition dialog window

Smart Setup dialog window

The **Smart Setup** dialog window offers you support when configuring multichannel acquisition. You can select the fluorescent dyes and contrast techniques that you want to include in your experiment from a database. **Smart Setup** takes the configuration of your microscope hardware and the properties of the selected dyes into account. Based on this information it makes one or more suggestions for acquisition. You can adopt these into your experiment as required and make further changes to them there.

Information

If there are several cameras on your system, select the camera for which you want a suggestion to be made from the dropdown list.

Smart Setup configures the motorized components of your system for the acquisition of multichannel images. It does not, however, influence any camera parameters (e.g. exposure time or resolution) and also does not change any parameters of other acquisition dimensions (e.g. Z-stack, time series or multiposition acquisitions).

Apply button

Adopts the suggestion displayed as the current acquisition experiment. The suggestion overwrites existing experiments on the **Acquisition** tab.

Cancel button

Ends **Smart Setup**. The suggestions are not adopted into the experiment.

Configure Experiment section

In the **Configure Experiment** section you can add up to four reflected light fluorescence channels and one transmitted light contrast technique to your experiment. The added dyes or the contrast technique are shown in the **Contrast Technique/Dye** list.

button

Opens the Add Dye or Contrast Technique [→ 104] dialog window.

Suggestions section

In this section the suggestions made by **Smart Setup** are displayed graphically. The number of suggestions (max. 3) depends on the microscope hardware being used, the selected dyes and the contrast technique.

Information

If Smart Setup is unable to make a suggestion, acquisition using the selected dyes or contrast technique and the current microscope hardware is not possible. Select other dyes or another contrast technique or configure your acquisition experiment using the **Acquisition Mode** tool and the **Channels** tool.

Best Signal radio button

Activated: Selects the suggestion that results in the best signal strength.

Fastest Acquisition radio button

Activated: Selects the suggestion that results in the fastest acquisition speed.

Best Compromise radio button

Activated: Selects the suggestion that results in the best compromise between signal strength and acquisition speed.

Emission Signal, Speed and Crosstalk display field

Emission Signal A filled, colored bar in the **Emission Signal** display field shows the relative emission signal to be expected for the corresponding channel. The channel color corresponds to the color of the selected dye in the **Configure Experiment** section.

Speed A gray bar in the **Speed** display field represents the approximate acquisition speed that can be expected. This is the time required for the movement of microscope hardware during multichannel acquisition. Camera exposure times or parameters of other acquisition dimensions are not taken into account here.

Crosstalk A hatched bar in the **Crosstalk** display field shows the relative crosstalk originating from one or more dyes of other channels that can be expected.

Information

The bars in the graphs only show relative values. The actual strength of the emission signal and the crosstalk in the image can deviate substantially from this estimate, as Smart Setup has no knowledge of the strength with which the sample has been dyed with the individual dye components.

Tracks display field

Only visible if the **Show Excitation** and **Show Emission** checkboxes are activated.

The various tracks are labeled with **T1**, **T2**, etc. and the various channels with **C1**, **C2**, etc. The white lines show the excitation and emission spectra of the dyes schematically. The spectra are filled in color in the places that will be acquired by the acquisition configuration suggested by **Smart Setup**. Transmitted light channels are displayed as a filled white field.

Show Excitation checkbox

Shows the excitation spectrum of the selected dyes in the **Tracks** display field.

Show Emission checkbox

Shows the emission spectrum of the selected dyes in the **Tracks** display field.

Add Dye or Contrast Technique dialog window

In the **Add Dye or Contrast Technique** dialog window you can add dyes and contrast techniques to your experiment. The dyes in the database contain important information that is saved in the image document (e.g. spectral characteristics). This information can be used later during image processing (e.g. deconvolution).

Information

You can add additional dyes to the database via the **Extras** menu | **Dye Editor**.

Most Recently Selected list

Shows the six most recently selected dyes and contrast techniques in a list. This ensures that you have quick access to the dyes or contrast techniques that you frequently use.

Search input field

Enter the name or initial letters of the dye or contrast technique that you want to search for. The search results are displayed immediately in the **Dye Database** list or the **Contrast Techniques** list. If no search filter is active, the lists of dyes or contrast techniques are arranged in alphabetical order.

Information

If you cannot find a certain dye, try using a related dye name or a general name.

Dye Database list

The available fluorescent dyes are displayed here. In the left column you will see the name of the dye. The right column contains its color and main emission wavelength. Add a channel without any spectral or other information by selecting the "Custom" entry.

Information

The "Custom" entry adds a channel to your experiment without any additional information. This means that the resulting image cannot be used for certain processing operations. To create an entry with self-defined characteristics, use the **Dye Editor**.

Contrast Techniques list

The available contrast techniques are displayed here.

3.3 Processing tab

On the **Processing** tab you will find all the functions you need for image processing using ZEN (blue edition).

Single button

Activates Single Processing mode.

Batch button

Activates Batch Processing mode [➙ 180].

Information

In this mode only a limited selection of processing methods is available.

Apply button

Only visible in **Single** Processing mode.

Applies the selected method to the input image.

Run button

Only visible in **Batch** Processing mode.

Applies the selected method to the list of images.

3.3.1 Single Processing mode

In Single Processing mode you apply a selected processing method, with the relevant method and image parameters, to a single image.

Method Selection tool group

Method tool

Last Used list

Shows a list of the last processing methods used. Click on an entry in this list to activate the processing method.

Search input field

Enter the name or initial letters of the processing method you are searching for.

Deconvolution methods

Deconvolution (Default Values) method

This method allows you to use 4 different algorithms for deconvolution without setting any parameters. Simply select the desired algorithm in the **Parameter** tool and click on the **Apply** button.

See also

- Deconvolution (Default Values) parameters [→ 114]

Deconvolution (Configurable) method

This method allows you to use and individually configure 4 different algorithms for deconvolution.

See also

- Deconvolution (Configurable) parameters [→ 114]

Adjust methods

Color Balance method

This method allows you to adjust the weighting of the individual color channels of a true color image.

See also

- Color Balance parameters [→ 123]

Hue/Saturation/Brightness method

This method allows you to adjust the hue, saturation and brightness of a true color image.

White Balance method

This method allows you to adjust the white balance of an image.

Color Temperature method

This method allows you to adjust the color temperature of a true color image.

Brightness/Contrast/Gamma method

This method allows you to adjust the brightness, contrast and gamma value of an image.

Information

Unlike the adjustments that can be made on the **Display** tab, here the pixel values of the image are changed.

Z-Stack Correction method

This method allows you to improve the quality of Z-stack images that have been affected by bleaching effects during acquisition.

Shading Correction method

This method allows you to improve images in which the quality has been impaired by uneven illumination or vignetting.

Geometric Transformation methods

Align Z-Stack method

This method allows you to bring the individual planes of a Z-stack image into line if these are not positioned precisely one above the other. This is the case, for example, when you acquire Z-stacks at an oblique angle using a stereomicroscope.

See also

- Align Z-Stack parameters [→ 127]

Align Tiles method

This method allows you to align the individual tiles of a tile image with one another automatically and correctly.

See also

- Align Tiles parameters [→ 128]

Rotate method

This method allows you to rotate an image around a freely positionable rotation axis and by a defined angle.

See also

- Rotation parameters [→ 128]

Mirror method

This method allows you to flip an image horizontally or vertically. In the case of multidimensional images, such as Z-stack or time lapse images, you can also use the mirror method to reverse the sequence of the relevant dimension.

See also

- Flip parameters [→ 130]

Resample method

This method allows you to change the size of an image in every dimension. You can either enlarge or reduce the image size.

See also

- Change Image Size parameters [→ 131]

Shift method

This method allows you to shift the content of an image in the direction of the 3 axes X, Y and Z.

See also

- Shift parameters [→ 132]

Image Sharpness methods

Extended Focus method

Using this method you can combine the sharp regions from the individual sections of a Z-stack image to form a single image. This enables you to display a considerably larger depth of field than is possible on a microscope.

See also

- Extended Focus parameters [→ 133]

Edge Enhancement method

This method enhances the edges of individual regions in an image. It corrects the halo effect and only affects edges.

See also

- Edge Enhancement parameters [→ 133]

Contour Enhancement method

This method allows you to enhance contours in an image and emphasize regions in which gray values change. The function is suitable for visually emphasizing fine structures in an image.

See also

- Contour Enhancement parameters [→ 134]

Unsharp Masking method

Using this method you can increase the impression of sharpness in an image and consequently obtain an image display that is richer in detail. The function allows contrasts at small structures and edges to be enhanced in a targeted way.

See also

- Unsharp Masking parameters [→ 135]

Image Smoothing methods

Median method

This method allows you to reduce noise in an image. Each pixel is replaced by the median of its neighbors. The size of the area of the neighboring pixels considered is defined by a quadratic filter matrix. The modified pixel is the central pixel of the filter matrix. The median is the middle value of the gray values of the pixel and its neighbors sorted in ascending order.

See also

- Median parameters [→ 136]

Sigma method

This method allows you to reduce noise in an image. Each pixel is replaced by the average of its neighbors. The size of the area of the neighboring pixels considered is defined by a quadratic filter matrix. The modified pixel is the central pixel of the filter matrix. To calculate the average, only the gray values that lie within a defined range (+/- sigma) around the gray value of the central pixel are taken into consideration. As a result, fine object structures are not blurred; only the gray levels in image regions that belong together are adjusted.

See also

- Sigma parameters [→ 137]

Low Pass method

This method allows you to reduce noise in an image. Each pixel is replaced by the average of its neighbors. The size of the area of the neighboring pixels considered is defined by a quadratic filter matrix. The modified pixel is the central pixel of the filter matrix.

See also

- Low Pass parameters [→ 138]

Binomial Filter method

This method allows you to reduce noise in an image. Each pixel is replaced by a weighted average of its neighbors. The weighting factors are calculated from the binomial coefficients in accordance with the filter size. The binomial filter is very similar to a Gaussian filter [→ 110] in its effect.

See also

- Binomial Filter parameters [→ 139]

Gauss method

This method allows you to reduce noise in an image. Each pixel is replaced by a weighted average of its neighbors. The neighboring pixels are weighted in accordance with a two-dimensional Gauss bell curve.

See also

- Gaussian parameters [→ 139]

Single Pixel Filter method

This method allows you to remove single pixel phenomena, such as those that occur in the case of clocking induced charge with EMCCDs and as radio telegraph signal noise with CMOS sensors. It can also be used to remove hot pixels.

See also

- Single Pixel parameters [→ 140]

Time Series Processing methods

Extras methods

Copy Annotations

This method allows you to copy all the annotations of one image into another image.

Copy Image

This method allows you to create a copy of an image.

Change Pixel Type

This method allows you to change the pixel type of an image. This can be useful if you want to compare or combine images that have different pixel types.

See also

- Change Pixel Type parameters [→ 140]

Create Image Subset

This method allows you to extract parts from one image and use these to create a new image. You can select these parts freely from the individual dimensions of the image.

See also

- Create Image Subset parameters [→ 141]

Fuse Image Subset

This method allows you to insert an image subset back into the original image. Its contents are replaced by the contents of the image subset. Using this method you can process a previously created image subset using image processing functions and copy the result back into the original image.

See also

- Create Image Subset [→ 111]
- Insert Image Subset parameters [→ 146]

Create Gray Scale image

This method allows you to create a gray scale image.

See also

- Create Gray Scale Wedge parameters [→ 146]

Image Calculator

This method allows you to apply arithmetic operations to images in the form of a calculator.

You can process a single image or combine two images.

All operations are performed pixel by pixel.

See also

Image Calculator parameters [→ 147]

Add Channels

This method allows you to combine two input images that have different channels but otherwise have the same dimension (Z-stack, time series, tile, scene). An image is produced that contains all the channels of the input images.

If the two input images differ from one another in the dimensions Z-stack, time series, tiles or scene, input image 1 and input image 2 are copied into the output image as two separate blocks.

Generate Pyramid

This method allows you to calculate a resolution pyramid for a tile image. Using the resolution pyramid you can navigate extremely efficiently even in very large tile images and display individual regions in the image window.

See also

Calculate Image Pyramid parameters [→ 148]

Calculate Histogram

This method calculates a histogram distribution for selected measurement parameters of a measurement data table.

See also

Calculate Histogram parameters [→ 149]

Export/Import methods

Image Export method

Using the **Image Export** method you can export images into various file types so that you can continue to use them in other programs. Multidimensional images (multichannel, Z-stack, time lapse, tile images) are exported as individual images.

See also

Image Export parameters [→ 151]

Film Export method

Using the **Film Export** function you can export multidimensional images (time lapse, Z-stack, multichannel images) into various file types in the form of film sequences so that you can continue to use them in other programs.

See also

- Film Export parameters [→ 158]

OME TIFF Export method

Using the **OME TIFF Export** function you can export your images into OME (Open Microscopy Environment) TIFF format so that you can continue to use them in other programs. The images are then available as a multipage TIFF file.

See also

- OME TIFF Export parameters [→ 165]

ZVI Export method

Using the **ZVI Export** function you can export your images into ZVI format so that you can continue to use them in AxioVision.

See also

- ZVI Export parameters [→ 170]

Image Import method

Using the **Image Import** function you can create a multidimensional image (multichannel, Z-stack, time lapse, tile, position image) from individual images. The individual images may be in any of the external formats supported by ZEN (e.g. .bmp, .jpg, .tiff). The resulting image can then be saved in CZI format and processed further using the functions available in ZEN.

See also

- Image Import Parameters [→ 171]

Method Parameters tool group

Parameters tool

Only visible if you have selected a processing method.

Deconvolution parameters

Deconvolution (Default Values) parameters

Simple button, very fast (Nearest Neighbor)

Executes the fast Nearest Neighbor method using default parameters. To use it, click on this button and then on **Apply**.

Better button, fast (Inverse Filter)

Executes the Regularized Inverse Filter algorithm for image enhancement. To use it, click on this button and then on **Apply**.

Good button, medium speed (Fast Iterative)

Executes the Fast Iterative restoration method. To use it, click on this button and then on **Apply**.

Excellent button, slow (Iterative)

Executes the Constrained Iterative quantitative restoration method. To use it, click on this button and then on **Apply**.

Deconvolution (Configurable) parameters

Two tabs are available for detailed configuration:

On the **Deconvolution** tab you can select the desired algorithm and define the precise settings for it.

On the **PSF Settings** tab you can see and change all key parameters for generating a theoretically calculated Point Spread Function ("PSF").

Deconvolution tab

Algorithm dropdown list

Here you can select the algorithm that you want to use. The following algorithms are available:

- Nearest Neighbor
- Regularized Inverse Filter
- Fast Iterative
- Constrained Iterative

Information

Expert knowledge is required for some of the settings. If you are in doubt, leave the settings unchanged.

Channel-Specific Settings checkbox

Activated: Applies the settings on a channel-specific basis. This allows you to set parameters in a targeted way for each channel. You will see a separate, colored tab for each of the channels.

Deactivated: Applies the settings to all channels of a multichannel image.

Normalization section

Normalization dropdown list

Here you can specify how the data of the resulting image are handled:

Clip Sets negative values to 0 (black).

If the values exceed the maximum possible gray value of 65636 when the calculation is performed, they are limited to 65636 (pixel is 100% white).

Automatic Normalizes the output image automatically.

In this case the lowest value is 0 and the highest value is the maximum possible gray value in the image (gray value of 65636). The maximum available gray value range is always utilized fully in the resulting image.

Adjust Strength section

Adjust Strength checkbox

If you have selected the **Nearest Neighbor** algorithm, the checkbox is always activated.

Information

If you have selected the **Fast Iterative** algorithm, the checkbox is also always activated. Using the slider you can then enter the number of iterations used directly, as, in contrast to the other methods, no regularization is performed.

Activated: Enter the degree of restoration using the slider.

To achieve strong contrast enhancement, move the slider towards **Strong**.

To achieve less contrast enhancement, move the slider towards **Weak**.

If the setting is too strong, image noise may be intensified and other artifacts, such as "ringing", may appear.

Deactivated: Determines the restoration strength for optimum image quality automatically.

The restoration strength is inversely proportional to the strength of so-called regularization. This is determined automatically with the help of Generalized Cross Validation (GCV).

Z-Stack Correction section

To show the section in full, click on the button.

Lamp Flicker checkbox

Activated: Analyzes the total brightness of each Z-plane. In the event of non-constant deviations in the total brightness between neighboring planes, a compensation factor is taken into account.

Activate this function if you have acquired your images using an old fluorescent lamp that exhibits strong fluctuations in brightness.

Bleaching Correction checkbox

Activated: Corrects bleaching of the sample during acquisition of the Z-stack.

This function should only be activated for widefield images. Use it if your sample undergoes strong bleaching during acquisition.

Background Correction checkbox

Activated: Analyzes the background component in the image and removes it before the DCV calculation.

This can prevent background noise being intensified during DCV.

Advanced Settings section

Only visible if the **Show All** mode is activated.

This section is only visible if you have selected the **Inverse Filter**, **Iterative (Fast)** or **Iterative** algorithm.

To show the section in full, click on the button.

Depending on which algorithm you have selected, different advanced setting options are available. The relevant settings are described in the following sections for each algorithm:

Advanced settings for Inverse Filter algorithm

Regularization

Here you can select which frequencies in the image are taken into account during regularization:

Zero Order Regularization based on G-difference, modeled on Tikhonov, but accelerated.

First Order Regularization based on Good's roughness. Under certain circumstances, more details are extracted from noisy data. May be better suited to the processing of confocal data sets.

Advanced settings for Fast Iterative algorithm

Likelihood

Here you can decide which likelihood calculation you want to work with:

Poisson (Meinel) The calculation according to Meinel works with one fold per iteration and converges significantly faster, normally in 4-5 iterations. This method can also produce artifacts, however.

Poisson (Richardson-Lucy) The calculation according to Richardson-Lucy, on the other hand, normally requires hundreds of iterations and therefore takes considerably longer. This method is, however, somewhat more robust.

Regularization

For the **Poisson (Meinel)** calculation it is also possible to perform **zero order** (G-difference) regularization here as an option. This means, however, that the calculation will take considerably longer and the main advantage of the greater speed of Meinel is lost.

Optimization

Numerical Gradient If you select this option, an attempt is made to determine the trend of the iterations in advance and extrapolate this to the entire calculation. This can significantly speed up the calculation.

First Estimate

Input Image The input image is used for the first estimate of the target structure (default).

Last Output Image The result of the last calculation is used to estimate the next calculation. This can speed up a calculation that is repeated using slightly different parameters.

Average of Input Image No estimate is made. This is the most rigid application of deconvolution, but can take a long time.

Maximum Number of Iterations

Here you can indicate the maximum permitted number of iterations that you want. In the case of Richardson-Lucy, you should allow significantly more iterations here.

Quality Threshold

Defines the quality level at which you want the calculation to be stopped. The percentage describes the difference in enhancement between the last and next-to-last iteration compared with the greatest difference since the start of the calculation. 1% is the default value. Lowering this can bring about small improvements in quality.

Advanced settings for Iterative algorithm

Likelihood

Here you can decide which likelihood calculation you want to work with:

- Calculation according to Poisson
- Calculation according to Gauss

Regularization

Here you can enter which frequencies in the image are taken into account during regularization:

Zero Order Regularization based on G-difference, modeled on Tikhonov, but accelerated;

First Order Regularization based on Good's roughness. Under certain circumstances, more details are extracted from noisy data. This regularization can sometimes produce better results for the processing of confocal data sets;

Second Order Regularization according to Tikhonov-Miller. Here higher frequencies are penalized more than in the case of Good's roughness.

Optimization

Analytical (Newton Raphson) Here an attempt is made to optimize the iterations analytically. This option is usually faster but may also be somewhat less precise.

Line Search Searches rigorously and comprehensively for the minimum. It is therefore more robust, but the calculation takes longer.

First Estimate

Input Image The input image is used for the first estimate of the target structure (default).

Last Output Image The result of the last calculation is used to estimate the next calculation. This can speed up a calculation that is repeated using slightly different parameters.

Average of Input Image No estimate is made for the next iteration. This is the most rigid application of deconvolution, but can take a long time.

Maximum Number of Iterations

Here you can indicate the maximum permitted number of iterations that you want.

Quality Threshold

Defines the quality level at which you want the calculation to be stopped. The percentage describes the difference in enhancement between the last and next-to-last iteration compared with the greatest difference since the start of the calculation. 1% is the default value. Lowering this can bring about small improvements in quality.

Diagnosis section

Only visible if the **Show All** mode is activated.

This section is only visible if you have selected the **Fast Iterative** or **Iterative** algorithm.

To show the section in full, click on the button.

The course of the calculation is displayed here in a graphical display. Various parameters are shown and the aim of the calculation is to minimize these. This display allows you to observe directly how the iterative method affects the available data.

PSF Settings tab

All key parameters for generating a theoretically calculated Point Spread Function ("PSF") are displayed here.

Information

Ordinarily, images that have been acquired using **ZEN** (of the *.czi type) automatically contain all microscope parameters, meaning that you do not have to configure any settings on this page. Most parameters are therefore grayed out in the display. It is possible, however, that as a result of an incorrect microscope configuration values may not be present or may be incorrect. You can change these here. The correction of spherical aberration can also be set here.

Microscope Parameters section

The most important microscope parameters for PSF generation that are not channel-specific are displayed in this section.

Microscope dropdown list

Displays which type of microscope has been used. There are two options: conventional microscope (also known as a widefield microscope) and confocal microscope, for which the additional pinhole diameter parameter applies.

NA Obj. input field

Displays the numerical aperture of the objective.

Immersion input field

Displays the refractive index of the immersion medium. Please note that this can never be smaller than the numerical aperture of the objective. You can make a selection from typical immersion media in the dropdown list next to the input field.

Lateral Scaling input field

Displays the geometric scaling in the X/Y direction.

Axial Scaling input field

Displays the geometric scaling in the Z direction.

Change button

To change the input fields that are normally grayed out, click on the **Change** button. The input fields and dropdown lists are now active.

The text on the button changes to **Reset**. To restore the original values saved in the image, click on the **Reset** button.

Information

If you enter incorrect values, this can lead to incorrect calculations. If the values here are obviously wrong or values are missing, check the configuration of your microscope system.

Advanced Settings section

Only visible if the **Show All** mode is activated.

To show the section in full, click on the  button.

Phase Ring dropdown list

If you have acquired a fluorescence image using a phase contrast objective, the phase ring present in the objective is entered here. This setting has significant effects on the theoretical Point Spread Function ("PSF").

Origin dropdown list

There are two models for calculating the PSF:

Scalar Theory The wave vectors of the light are interpreted as electrical field = intensity and simply added. This method is fast and is sufficient in most cases (default setting).

Vectorial Theory The wave vectors are added geometrically. However, the calculation takes considerably longer.

Z-Stack text field

This field can only be changed if it was not possible to define this parameter during acquisition, e.g. because the microscope type was unknown. It describes the direction in which the Z-stack was acquired:

Downwards The Z-stack "grows" away from the front lens of the objective.

Upwards The Z-stack "grows" towards the objective. This setting only applies if you are working with a correction of the spherical aberration.

Aberration Correction section

Only visible if the **Show All** mode is activated.

Here you can select whether you want spherical aberration to be taken into account and corrected during the calculation of the PSF. As with the other PSF parameters, most values are extracted automatically from the information about the microscope that is saved with the image during acquisition. The input option is therefore inactive. To make changes manually, click on the **Change** button.

Use Correction checkbox

Activated: Uses the correction function. All options are active and can be edited.

Embedding Medium dropdown list

Here you can select the embedding medium used from the list.

Refractive Index input field

Displays the refractive index of the selected embedding medium. Enter the appropriate refractive index if you are using a different embedding medium.

Manufacturer

Displays the manufacturer, if known.

Distance to Cover Slip

Displays the distance of the acquired structure from the side of the cover slip facing the embedding medium. Half the height of the Z-stack is assumed as the initial value for the distance from the cover slip. The value can be corrected if this distance is known. If possible, this distance should be measured.

Cover Slip Thickness input field and dropdown list

Commercially available cover slips are divided into different groups depending on their thickness (0, 1, 1.5 and 2), which you can select from the dropdown list. Cover slips of the 1.5 type have an average thickness of 170 µm. In some cases, however, the actual values can vary greatly depending on the manufacturer. For best results the use of cover slips with a guaranteed thickness of 170 µm is recommended. Values that deviate from this can be entered directly in the **input field**.

Cover Slip Index input field and dropdown list

Select the material that the cover slip is made of from the dropdown list. The corresponding refractive index is displayed in the input field next to it.

Working Distance input field

Displays the working distance of the objective (i.e. the distance between the front lens and the side of the cover slip facing the objective). The working distance of the objective is determined automatically from the objective information, provided that the objective was selected correctly in the MTB 2004 Configuration program. You can, however, also enter the value manually.

Change/Reset button

Only active if the **Use Correction** checkbox is activated.

To reset the values, click on the **Reset** button.

Channel-Specific section

In this section you will find all settings that are channel-specific. This means that they may be configured differently for each channel.

Illumination

Displays the excitation wavelength for the channel dye [in nm]. The maximum is assumed. The color field corresponds to the wavelength (as far as possible).

Detection

Displays the maximum of the emission wavelength for the channel dye. The color field corresponds to the wavelength (as far as possible).

Lateral Sampling Rate

Depends on the geometric pixel scaling in the X/Y direction and displays the extent of the oversampling according to the Nyquist criterion. The value should be close to 2 or greater in order to achieve good results during DCV. As, in the case of widefield microscopes, this value is generally determined by the objective, the camera adapter used and the camera itself, it can only be influenced by the use of an Optovar.

Axial Sampling Rate

Depends on the geometric pixel scaling in the Z direction and displays the extent of the oversampling according to the Nyquist criterion. The value should be at least 2 or greater in order to achieve good results during DCV. This value is determined by the increment of the focus drive during acquisition of Z-stacks and can therefore be changed easily.

Pinhole input field

Only available if a confocal microscope has been entered under the microscope parameters.

Displays the size of the confocal pinhole in Airy units (AU).

NA Cond. input field

Only visible if the microscope is a **Conventional Microscope** and **Transmitted Light** has been selected as the illumination type.

Displays the numerical aperture of the condenser with which transmitted-light acquisition was performed.

Microscope Info section

Displays advanced microscope information that influences the form of the PSF in a channel-dependent way:

Illumination dropdown list

Here you can select the illumination method with which the data set has been acquired. In the event that a **Conventional Microscope** has been entered under the microscope parameters, the following options are available here: **Epifluorescence**, **Multiphoton Excitation** and **Transmitted Light**. In the case of confocal microscopes, **Epifluorescence** is the only option.

Imaging

Displays whether the imaging was incoherent (**Conventional Microscope**) or coherent (**Laser Scanning Microscope**).

Lateral Resolution

Displays the lateral resolution of the calculated PSF.

Axial Resolution

Displays the **full width half maximum** as a measure of the axial resolution of the PSF.

PSF View section

This tool shows you the PSF that is calculated for a channel based on the current settings. If you select the **Update Automatically** checkbox, all changes made to the PSF parameters are applied immediately to the PSF view. This makes it possible to check quickly whether the settings made meet your expectations.

Adjust parameters

Color Balance parameters

Channel-specific checkbox

Only visible if your input image is a multichannel image.

If the checkbox is activated, you can adjust the parameters for each channel individually.

Adjustment Range dropdown list

Here you can select the adjustment range for the color balance. There are 3 ranges available:

Dark Tones The settings relate to tones in the dark color range.

Mid The settings relate to tones in the mid color range.

Light The settings relate to tones in the light color range.

Cyan/Red slider

Enter the desired balance between cyan and red tones using the **Cyan/Red** slider or spin box/input field.

Yellow/Blue slider

Enter the desired balance between yellow and blue tones using the **Yellow/Blue** slider or spin box/input field.

Magenta/Green slider

Enter the desired balance between magenta and green tones using the **Magenta/Green** slider or spin box/input field.

Default Values button

Resets all settings to the default values.

See also

- Color Balance method [→ 106]

Color Tone/Saturation/Brightness parameters

Channel-specific checkbox

Only visible if your input image is a multichannel image.

If the checkbox is activated, you can adjust the parameters for each channel individually.

Color Tone slider

Enter the desired shift in the color tone using the **Color Tone** slider or spin box/input field.

The value of the shift represents an angle on the color wheel. The values -180 and +180 therefore have an identical effect. Negative angles shift the color tone towards blue and positive ones shift it towards red.

Saturation slider

Enter the desired saturation using the **Saturation** slider or spin box/input field.

Saturation describes how intense the color of a pixel is. "Chromatic" is the maximum saturation, while "achromatic" describes colors that do not leave a color impression.

Brightness slider

Enter the desired brightness using the **Brightness** slider or spin box/input field.

Brightness describes how light or dark a pixel appears. The greatest difference is between black and white or between violet and yellow.

Default Values button

Resets all settings to the default values.

White Balance parameters

Channel-specific checkbox

Only visible if your input image is a multichannel image.

If the checkbox is activated, you can adjust the parameters for each channel individually.

Automatic checkbox

If the checkbox is activated, the white point is calculated automatically from the image data.

Select button

Only visible if the **Automatic** checkbox is deactivated.

To define the white point, click on the **Select** button. The mouse pointer then changes to a selection arrow. Use the selection arrow to click on a white region of your input image. The coordinates and color values of the selected white point are displayed under the button.

Temperature Difference slider

Enter the difference that will be added on to the newly calculated color values using the **Temperature Difference** slider or spin box/input field. Negative values reduce the color temperature, while positive values increase it. A value of 1 corresponds to 10 kelvin.

Default Values button

Resets all settings to the default values.

Color Temperature parameters

Temperature Difference slider

Enter the difference that will be added on to the newly calculated color values using the **Temperature Difference** slider or spin box/input field. Negative values reduce the color temperature, while positive values increase it. A value of 1 corresponds to 10 kelvin.

Default Values button

Resets all settings to the default values.

Brightness/Contrast/Gamma parameters

Channel-specific checkbox

Only visible if your input image is a multichannel image.

If the checkbox is activated, you can adjust the parameters for each channel individually.

Brightness slider

Enter the desired brightness using the **Brightness** slider or spin box/input field.

Changing the brightness means that each gray or color value is increased or decreased by the same value. The difference between the biggest and smallest gray or color value in the image remains the same, however.

Contrast slider

Enter the desired contrast using the **Contrast** slider or spin box/input field.

Changing the contrast means that the gray or color values are multiplied by a factor. The difference between the biggest and smallest gray or color value changes.

Gamma slider

Enter the desired gamma value using the **Gamma** slider or spin box/input field.

Changing the gamma value means that the gray or color values are multiplied by individual factors.

Default Values button

Resets all settings to the default values.

Z-Stack Correction parameters

Third Dimension dropdown list

Only visible if the **Show All** mode is activated.

Here you can select how you want the function to work in the case of multidimensional images.

2D Sections Each section of a Z-stack image/each time point of a time lapse image is processed as an individual 2D image.

Z, T or C Special parameters are available to you for the selected dimension.

Channel-specific checkbox

Only visible if your input image is a multichannel image.

If the checkbox is activated, you can adjust the parameters for each channel individually.

Correction Mode dropdown list

Here you can select the desired correction mode.

Bleaching The **Bleaching** correction mode compensates for the bleaching effect.

Flicker The **Flicker** correction mode compensates for the flicker of the lamp voltage.

Background The **Background** correction mode reduces background noise.

Default Values button

Resets all settings to the default values.

Shading Correction parameters

Automatic checkbox

If the checkbox is activated, the function automatically calculates the reference image for shading correction from the input image.

Channel-specific checkbox

Only visible if your input image is a multichannel image.

If the checkbox is activated, you can adjust the parameters for each channel individually.

Mode dropdown list

Here you can select the desired correction mode.

Additive Influences the brightness of the image.

Multiplicative Influences the contrast of the image.

Offset slider

Enter the gray value that will be added on to the newly calculated gray values using the **Offset** slider or spin box/input field. If this results in negative values, these are set to 0. Values that exceed the maximum gray value are set to the maximum gray value.

Default Values button

Resets all settings to the default values.

Geometric Transformation parameters

Align Z-Stack parameters

Third Dimension dropdown list

Only visible if the **Show All** mode is activated.

Here you can select how you want the function to work in the case of multidimensional images.

2D Sections Each section of a Z-stack image/each time point of a time lapse image is processed as an individual 2D image.

Z, T or C Special parameters are available to you for the selected dimension.

Quality dropdown list

Here you can select the quality level that you want the function to work with.

The quality values have the following meanings:

Low Highest speed with low image quality.

Medium High speed with medium image quality.

High Low speed with high image quality.

Very high Lowest speed with best image quality.

Method dropdown list

Here you can select the method to be used to align the Z-stack image.

The following methods are available and are also offered as a combination:

Shift The neighboring sections of the Z-stack image are shifted in relation to each other in the X and Y direction.

Rotation The neighboring sections of the Z-stack image are rotated in relation to each other.

Iso Scaling The magnification is adjusted from section to section.

Skew Scaling The magnification is adjusted from section to section.

Affin The neighboring sections of the Z-stack image are shifted in the X and Y direction, rotated and the magnification is adjusted from section to section.

Interpolation dropdown list

Here you can select how you want interpolation to be performed if a pixel is calculated from several individual pixels.

Nearest Neighbor The output pixel is given the gray value of the input pixel that is closest to it.

Linear The output pixel is given the gray value resulting from the linear combination of the input pixels closest to it.

Cubic The output pixel is given the gray value resulting from a polynomial function of the input pixels closest to it.

Default Values button

Resets all settings to the default values.

Align Tiles parameters

Rotation parameters

Third Dimension dropdown list

Only visible if the **Show All** mode is activated.

Here you can select how you want the function to work in the case of multidimensional images.

2D Sections Each section of a Z-stack image/each time point of a time lapse image is processed as an individual 2D image.

Z, T or C Special parameters are available to you for the selected dimension.

Adjust Size checkbox

Only visible if the **Show All** mode is activated.

Activated: The size of the output image is adjusted in such a way that the entire input image is also visible after the rotation.

Deactivated: The output image is the same size as the input image. Depending on the image size and rotation angle, parts of the input image may not be visible in the output image.

Channel-specific checkbox

Only visible if your input image is a multichannel image.

If the checkbox is activated, you can adjust the parameters for each channel individually.

Interpolation dropdown list

Here you can select how you want interpolation to be performed if a pixel is calculated from several individual pixels.

Nearest Neighbor The output pixel is given the gray value of the input pixel that is closest to it.

Linear The output pixel is given the gray value resulting from the linear combination of the input pixels closest to it.

Cubic The output pixel is given the gray value resulting from a polynomial function of the input pixels closest to it.

Angle slider

Enter the angle by which you want the input image to be rotated using the slider or spin box/input field. Positive angles rotate the image clockwise.

The following parameters are only visible if the **2D Sections** value is selected in the **Third Dimension** dropdown list:

Angle X slider

Enter the angle by which you want the input image to be rotated on the X axis using the slider or spin box/input field.

Angle Y slider

Enter the angle by which you want the input image to be rotated on the Y axis using the slider or spin box/input field.

Angle Z slider

Enter the angle by which you want the input image to be rotated on the Z axis using the slider or spin box/input field.

The following parameters are only visible if the **Adjust Size** checkbox is deactivated:

Center X slider

Enter the X coordinate of the center of the rotation using the slider or spin box/input field.

The value 0 means that the image is rotated around its center point. Negative values mean that the center of the rotation in the image is shifted to the left in relation to the image's center point. Positive values shift the center to the right.

Center Y slider

Enter the Y coordinate of the center of the rotation using the slider or spin box/input field.

The value 0 means that the image is rotated around its center point. Negative values mean that the center of the rotation in the image is shifted downwards in relation to the image's center point. Positive values shift the center upwards.

Center Z slider

Enter the Z coordinate of the center of the rotation using the slider or spin box/input field.

The value 0 means that the image is rotated around its center point. Negative values mean that the center of the rotation in the image is shifted forwards in relation to the image's center point. Positive values shift the center backwards.

Default Values button

Resets all settings to the default values.

Flip parameters

Third Dimension dropdown list

Only visible if the **Show All** mode is activated.

Here you can select how you want the function to work in the case of multidimensional images.

2D Sections Each section of a Z-stack image/each time point of a time lapse image is processed as an individual 2D image.

Z, T or C Special parameters are available to you for the selected dimension.

Channel-specific checkbox

Only visible if your input image is a multichannel image.

If the checkbox is activated, you can adjust the parameters for each channel individually.

Mode dropdown list

Here you can select the direction of the flip.

Horizontal Flips the image horizontally.

Vertical Flips the image vertically.

T , Z Only visible if your input image is a multichannel image.

The sequence of the sections (Z) or time points (T) is reversed.

Default Values button

Resets all settings to the default values.

Change Image Size parameters

Third Dimension dropdown list

Only visible if the **Show All** mode is activated.

Here you can select how you want the function to work in the case of multidimensional images.

2D Sections Each section of a Z-stack image/each time point of a time lapse image is processed as an individual 2D image.

Z, T or C Special parameters are available to you for the selected dimension.

Adjust Size checkbox

Only visible if the **Show All** mode is activated.

Activated: The size of the output image is adjusted in such a way that the entire input image is also visible after the rotation.

Deactivated: The output image is the same size as the input image. Depending on the image size and rotation angle, parts of the input image may not be visible in the output image.

Channel-specific checkbox

Only visible if your input image is a multichannel image.

If the checkbox is activated, you can adjust the parameters for each channel individually.

Interpolation dropdown list

Here you can select how you want interpolation to be performed if a pixel is calculated from several individual pixels.

Nearest Neighbor The output pixel is given the gray value of the input pixel that is closest to it.

Linear The output pixel is given the gray value resulting from the linear combination of the input pixels closest to it.

Cubic The output pixel is given the gray value resulting from a polynomial function of the input pixels closest to it.

Scaling in X slider

Enter the desired scaling for X using the slider or spin box/input field.

Scaling in Y slider

Enter the desired scaling for Y using the slider or spin box/input field.

Scaling in Z slider

Enter the desired scaling for Z using the slider or spin box/input field.

The following parameters are only visible if the **Adjust Size** checkbox is deactivated:

Shift in X slider

Enter the shift in the X direction using the slider or spin box/input field.

Shift in Y slider

Enter the shift in the Y direction using the slider or spin box/input field.

Shift in Z slider

Enter the shift in the Z direction using the slider or spin box/input field.

Default Values button

Resets all settings to the default values.

Shift parameters

Third Dimension dropdown list

Only visible if the **Show All** mode is activated.

Here you can select how you want the function to work in the case of multidimensional images.

2D Sections Each section of a Z-stack image/each time point of a time lapse image is processed as an individual 2D image.

Z, T or C Special parameters are available to you for the selected dimension.

Channel-specific checkbox

Only visible if your input image is a multichannel image.

If the checkbox is activated, you can adjust the parameters for each channel individually.

Shift in X slider

Enter the shift in the X direction using the slider or spin box/input field.

Shift in Y slider

Enter the shift in the Y direction using the slider or spin box/input field.

Shift in Z slider

Enter the shift in the Z direction using the slider or spin box/input field.

Shift in T slider

Enter the shift in the time points using the slider or spin box/input field.

Default Values button

Resets all settings to the default values.

Image Sharpness parameters

Extended Focus parameters

Z-Stack Alignment dropdown list

Here you can select whether you want the Z-stack image to be aligned before the calculation and with what quality level.

None The Z-stack image is not aligned before the calculation. You should select this setting if the Z-stack image has not been acquired using a stereomicroscope.

Normal High speed with normal image quality.

High Low speed with high image quality.

Very high Lowest speed with best image quality.

Default Values button

Resets all settings to the default values.

Edge Enhancement parameters

Channel-specific checkbox

Only visible if your input image is a multichannel image.

If the checkbox is activated, you can adjust the parameters for each channel individually.

Threshold Value slider

Enter the threshold value for edge detection using the slider or spin box/input field. The threshold value should correspond roughly to the gray value difference between objects and the background.

Size slider

Enter the size of the edge detection filter using the slider or spin box/input field. The value should correspond to the size of the transition area between objects and the background.

Default Values button

Resets all settings to the default values.

Contour Enhancement parameters

Third Dimension dropdown list

Only visible if the **Show All** mode is activated.

Here you can select how you want the function to work in the case of multidimensional images.

2D Sections Each section of a Z-stack image/each time point of a time lapse image is processed as an individual 2D image.

Z, T or C Special parameters are available to you for the selected dimension.

Channel-specific checkbox

Only visible if your input image is a multichannel image.

If the checkbox is activated, you can adjust the parameters for each channel individually.

Strength slider

Enter the factor for increasing edge enhancement using the slider or spin box/input field.

Normalization dropdown list

Here you can select how the gray/color values that exceed or fall short of the value range should be dealt with.

Clip Automatically sets the gray levels that exceed or fall short of the predefined gray value range to the lowest or highest gray value (black or white). The effect corresponds to underexposure or overexposure. In certain circumstances some information may therefore be lost.

Automatic Normalizes the gray values automatically to the available gray value range.

Wrap If the result is larger than the maximum gray value of the image, the maximum gray value + 1 is deducted from this value.

Shift and Clip Normalizes the output to the value "gray value + maximum gray value/2".

Absolute Converts negative gray levels into positive values.

Default Values button

Resets all settings to the default values.

Unsharp Masking parameters

Channel-specific checkbox

Only visible if your input image is a multichannel image.

If the checkbox is activated, you can adjust the parameters for each channel individually.

Strength slider

Enter the strength of the Unsharp Masking using the slider or spin box/input field. The higher the value selected, the greater the extent to which small structures are enhanced.

The following parameters are only visible if the **Show All** mode is activated:

Radius slider

Enter the radius using the slider or spin box/input field. **Radius** defines the width of structures that you want to appear sharper. A small radius enhances smaller details. If the radius is too big, halo effects can occur at edges.

Color Mode dropdown list

Select the desired color mode from the dropdown list.

RGB Calculates the sharpness for each color channel individually. The color saturation and the color of structures may be changed and color noise may occur.

Luminance Only calculates the sharpness on the basis of the brightness signal detected. This mode does not show any color noise and changes the color saturation accordingly.

Boundary dropdown list

Here you can select a setting from the dropdown list for calculating the boundary between the sharpened image regions.

It is only effective if the value for the **Lower Threshold Value** parameter is not equal to 0 or the value for the **Upper Threshold Value** parameter is not equal to 100.

None No adjustment takes place.

Binary The transition follows the threshold values.

Linear Calculates a linear course.

Lower Threshold Value slider

Enter the lower threshold value using the slider or spin box/input field. This determines the lower limit from which existing contrast structures are changed.

Upper Threshold Value slider

Enter the upper threshold value using the slider or spin box/input field. This prevents the existing strong contrasts in the image from being increased further unnecessarily.

Adjust Bit Depth checkbox

Activated: The value range of the gray/color values of the output image is adjusted to the value range of the input image.

Default Values button

Resets all settings to the default values.

Image Smoothing parameters

Median parameters

Third Dimension dropdown list

Only visible if the **Show All** mode is activated.

Here you can select how you want the function to work in the case of multidimensional images.

2D Sections Each section of a Z-stack image/each time point of a time lapse image is processed as an individual 2D image.

Z, T or C Special parameters are available to you for the selected dimension.

Channel-specific checkbox

Only visible if your input image is a multichannel image.

If the checkbox is activated, you can adjust the parameters for each channel individually.

Filter Size slider

Enter the size of the filter matrix in the X and Y direction using the slider or spin box/input field.

The following parameters are only visible if the **Show All** mode is activated:

Filter Size in X slider

Enter the size of the filter matrix in the X direction using the slider or spin box/input field.

Filter Size in Y slider

Enter the size of the filter matrix in the Y direction using the slider or spin box/input field.

Filter Size in Z slider

Enter the size of the filter matrix in the Z direction using the slider or spin box/input field.

Default Values button

Resets all settings to the default values.

Sigma parameters

Third Dimension dropdown list

Only visible if the **Show All** mode is activated.

Here you can select how you want the function to work in the case of multidimensional images.

2D Sections Each section of a Z-stack image/each time point of a time lapse image is processed as an individual 2D image.

Z, T or C Special parameters are available to you for the selected dimension.

Channel-specific checkbox

Only visible if your input image is a multichannel image.

If the checkbox is activated, you can adjust the parameters for each channel individually.

Sigma slider

Enter the sigma value using the slider or spin box/input field.

Filter Size slider

Enter the size of the filter matrix in the X and Y direction using the slider or spin box/input field.

The following parameters are only visible if the **Show All** mode is activated:

Filter Size in X slider

Enter the size of the filter matrix in the X direction using the slider or spin box/input field.

Filter Size in Y slider

Enter the size of the filter matrix in the Y direction using the slider or spin box/input field.

Filter Size in Z slider

Enter the size of the filter matrix in the Z direction using the slider or spin box/input field.

Default Values button

Resets all settings to the default values.

Low Pass parameters

Third Dimension dropdown list

Only visible if the **Show All** mode is activated.

Here you can select how you want the function to work in the case of multidimensional images.

2D Sections Each section of a Z-stack image/each time point of a time lapse image is processed as an individual 2D image.

Z, T or C Special parameters are available to you for the selected dimension.

Channel-specific checkbox

Only visible if your input image is a multichannel image.

If the checkbox is activated, you can adjust the parameters for each channel individually.

Repetitions slider

Enter the number of repetitions using the slider or spin box/input field. The function can be applied several times in succession to the result of the filtering. This intensifies the effect accordingly.

Filter Size slider

Enter the size of the filter matrix in the X and Y direction using the slider or spin box/input field.

The following parameters are only visible if the **Show All** mode is activated:

Filter Size in X slider

Enter the size of the filter matrix in the X direction using the slider or spin box/input field.

Filter Size in Y slider

Enter the size of the filter matrix in the Y direction using the slider or spin box/input field.

Filter Size in Z slider

Enter the size of the filter matrix in the Z direction using the slider or spin box/input field.

Default Values button

Resets all settings to the default values.

Binomial Filter parameters

Third Dimension dropdown list

Only visible if the **Show All** mode is activated.

Here you can select how you want the function to work in the case of multidimensional images.

2D Sections Each section of a Z-stack image/each time point of a time lapse image is processed as an individual 2D image.

Z, T or C Special parameters are available to you for the selected dimension.

Filter Size slider

Enter the size of the filter matrix using the slider or spin box/input field.

Default Values button

Resets all settings to the default values.

Gaussian parameters

Third Dimension dropdown list

Only visible if the **Show All** mode is activated.

Here you can select how you want the function to work in the case of multidimensional images.

2D Sections Each section of a Z-stack image/each time point of a time lapse image is processed as an individual 2D image.

Z, T or C Special parameters are available to you for the selected dimension.

Channel-specific checkbox

Only visible if your input image is a multichannel image.

If the checkbox is activated, you can adjust the parameters for each channel individually.

Sigma slider

Enter the sigma value using the slider or spin box/input field.

Sigma in X slider

Enter the sigma value in the X direction using the slider or spin box/input field.

Sigma in Y slider

Enter the sigma value in the Y direction using the slider or spin box/input field.

Sigma in Z slider

Enter the sigma value in the Z direction using the slider or spin box/input field.

Default Values button

Resets all settings to the default values.

Single Pixel parameters

Channel-specific checkbox

Only visible if your input image is a multichannel image.

If the checkbox is activated, you can adjust the parameters for each channel individually.

Threshold Value slider

Enter the threshold value using the slider or spin box/input field.

Default Values button

Resets all settings to the default values.

Time Series Processing parameters

Extras parameters

Change Pixel Type parameters

Pixel Format dropdown list

Select the desired pixel type from the dropdown list.

Monochrome 8 Bit	The output image is a monochrome image, the whole-number gray values of which can lie in the range from 0 to 255.
Monochrome 16 Bit	The output image is a monochrome image, the whole-number gray values of which can lie in the range from 0 to 65535.
Monochrome Real	The output image is a monochrome image with real numbers as pixel values.
Monochrome Complex	The output image is a monochrome image with complex numbers (real part and imaginary part) as pixel values. Such images are generally created by means of transformation into the Fourier space.

Color 24 Bit The output image is a color image, the whole-number color values of which in the red, green and blue channels can lie in the range from 0 to 255.

Color 48 Bit The output image is a color image, the whole-number color values of which in the red, green and blue channels can lie in the range from 0 to 65535.

Color Real The output image is a color image with real numbers as color values in the red, green and blue channels.

Color Complex The output image is a color image with complex numbers (real part and imaginary part) in the red, green and blue channels. Such images are generally created by means of transformation into the Fourier space.

Default Values button

Resets all settings to the default values.

Create Image Subset parameters

Default Values button

Resets all settings to the default values.

Information

Each of the sections described below is only visible if the corresponding dimension is present in the input image.

Channels section

To show the section in full, click on the  button.

In the **Channels** section you can select which channels of the input image you want to be used. All channels are selected by default. To deselect a channel, click on the relevant channel button.

 **button**

Adopts the values from the current display. These values can also be found on the Dimensions tab.

Z-Position section

To show the section in full, click on the  button.

Z-Stack dropdown list

You can select which sections of the input image you want to be used from the dropdown list.

All Selects all sections of the input image.

Select Individual Selects an individual section.

Select Range Selects a continuous range of sections.

Select Several Selects several continuous ranges and individual sections.

Select Individual slider

Enter the section that you want to select using the slider or spin box/input field.

 **button**

Adopts the values from the current display. These values can also be found on the Dimensions tab.

Select Range slider

Enter the first and last section that you want to select using the sliders or spin boxes/input fields.

Interval checkbox

Activated: Interval mode is active. The Interval spin box/input field appears.

Interval spin box/input field

Enter the desired interval here. If you enter the value 2, for example, only every 2nd value from the range is considered.

Select Several input field

Enter one or more sections that you want to select in the input field. To do this, enter the first section, followed by a minus sign, and then the last section. If you want to define an interval, after the last section enter a colon and then the interval. The entry "2-10:2" means that every second section is selected from section 2 to section 10.

Enter a comma after the first section if you want to define another section. You can also select individual sections separated by commas. By entering "2-10:2,14-18,20,23", you select every second section from section 2 to section 10, followed by sections 14 to 18, as well as sections 20 and 23.

Time section

To show the section in full, click on the  button.

Time Points dropdown list

You can select which time points of the input image you want to be used from the **Time Points** dropdown list. The following selection options are available:

All Selects all time points of the input image.

Select Individual Selects an individual time point.

Select Range Selects a continuous range of time points.

Select Several Selects several continuous ranges and individual time points.

Select Individual slider

Enter the time point that you want to select using the slider or spin box/input field.

 **button**

Adopts the values from the current display. These values can also be found on the Dimensions tab.

Select Range slider

Enter the first and last time point that you want to select using the sliders or spin boxes/input fields.

Interval checkbox

Activated: Interval mode is active. The Interval spin box/input field appears.

Interval spin box/input field

Enter the desired interval here. If you enter the value 2, for example, only every 2nd value from the range is considered.

Select Several input field

Enter one or more time points that you want to select in the input field. To do this, enter the first time point, followed by a minus sign, and then the last time point. If you want to define an interval, after the last time point enter a colon and then the interval. The entry "2-10:2" means that every second time point is selected from time point 2 to time point 10.

Enter a comma after the first time point if you want to define another time point. You can also select individual time points separated by commas. By entering "2-10:2,14-18, 20, 23", you select every second time point from time point 2 to time point 10, followed by time points 14 to 18, as well as time points 20 and 23.

Scene section

To show the section in full, click on the  button.

Scene dropdown list

You can select which scenes of the input image you want to be used from the **Scene** dropdown list.

All Selects all scenes of the input image.

Select Individual Selects an individual scene.

Select Range Selects a continuous range of scenes.

Select Several Selects several continuous scenes and individual scenes.

Select Individual slider

Enter the scene that you want to select using the slider or spin box/input field.

 **button**

Adopts the values from the current display. These values can also be found on the Dimensions tab.

Select Range slider

Enter the first and last scene that you want to select using the sliders or spin boxes/input fields.

Interval checkbox

Activated: Interval mode is active. The Interval spin box/input field appears.

Interval spin box/input field

Enter the desired interval here. If you enter the value 2, for example, only every 2nd value from the range is considered.

Select Several input field

Enter one or more scenes that you want to select in the input field. To do this, enter the first scene, followed by a minus sign, and then the last scene. If you want to define an interval, after the last scene enter a colon and then the interval. The entry "2-10:2" means that every second scene is selected from scene 2 to scene 10.

Enter a comma after the first scene if you want to define another scene. You can also select individual scenes separated by commas. By entering "2-10:2,14-18, 20, 23", you select every second scene from scene 2 to scene 10, followed by scenes 14 to 18, as well as scenes 20 and 23.

Block section

To show the section in full, click on the  button.

Block dropdown list

You can select which blocks of the input image you want to be used from the **Block** dropdown list. The following selection options are available:

All Selects all blocks of the input image.

Select Individual Selects an individual block.

Select Range Selects a continuous block of time points.

Select Several Selects several continuous blocks and individual blocks.

Select Individual slider

Enter the block that you want to select using the slider or spin box/input field.

 **button**

Adopts the values from the current display. These values can also be found on the Dimensions tab.

Select Range slider

Enter the first and last block that you want to select using the sliders or spin boxes/input fields.

Interval checkbox

Activated: Interval mode is active. The Interval spin box/input field appears.

Interval spin box/input field

Enter the desired interval here. If you enter the value 2, for example, only every 2nd value from the range is considered.

Select Several input field

Enter one or more blocks that you want to select in the input field. To do this, enter the first block, followed by a minus sign, and then the last block. If you want to define an interval, after the last block enter a colon and then the interval. The entry "2-10:2" means that every second block is selected from block 2 to block 10.

Enter a comma after the first block if you want to define another block. You can also select individual blocks separated by commas. By entering "2-10:2,14-18,20,23", you select every second block from block 2 to block 10, followed by blocks 14 to 18, as well as blocks 20 and 23.

Region section

To show the section in full, click on the  button.

Region dropdown list

You can select which sections of the input image you want to be used from the **Region** dropdown list.

All The entire image is selected.

Region In the input image, drag out the rectangular region that you want to be used using the mouse.

X spin box/input field

Enter the X coordinate of the starting point of the region in the spin box/input field.

Y spin box/input field

Enter the Y coordinate of the starting point of the region in the spin box/input field.

W spin box/input field

Enter the width of the region in the spin box/input field.

H spin box/input field

Enter the height of the region in the spin box/input field.

Insert Image Subset parameters

Subset input field

The **Subset** input field contains the description of how the input image was created as a subset.

For each dimension (channels, Z-stack, time series), as well as for the defined frame, details are provided of the ranges that were selected to create the image subset.

The entry "Z(1-8:2)|T(2-7)" means that the image subset is composed of sections 1,3,5,7 at time points 2 to 7 of the original image.

Create Gray Scale Wedge parameters

Model dropdown list

You can select the desired model for the gray scale wedge from the dropdown list.

Homogeneous	All pixels in the image have an identical gray/color value
2D Gray Scale Wedge (Vertical)	The gray scale wedge runs from top to bottom and starts with the gray value selected in the **Min. Gray Value** parameter.
2D Gray Scale Wedge (Horizontal)	The gray scale wedge runs from left to right and starts with the gray value selected in the **Min. Gray Value** parameter.

Width slider

Enter the desired width of the output image in pixels using the slider or spin box/input field.

Height slider

Enter the desired height of the output image in pixels using the slider or spin box/input field.

Min. Gray Value slider

Enter the lowest gray value of the gray scale wedge using the slider or spin box/input field.

Max. Gray Value slider

Enter the highest gray value of the gray scale wedge using the slider or spin box/input field.

Pixel Format dropdown list

Select the desired pixel type from the dropdown list.

Monochrome 8 Bit The output image is a monochrome image, the whole-number gray values of which can lie in the range from 0 to 255.

Monochrome 16 Bit The output image is a monochrome image, the whole-number gray values of which can lie in the range from 0 to 65535.

Color 24 Bit The output image is a color image, the whole-number color values of which in the red, green and blue channels can lie in the range from 0 to 255.

Color 48 Bit The output image is a color image, the whole-number color values of which in the red, green and blue channels can lie in the range from 0 to 65535.

Default Values button

Resets all settings to the default values.

Image Calculator parameters

Channel Input 1 dropdown list

Select from the dropdown list whether you want to use an individual channel or all channels of the first input image for the calculation.

Channel Input 2 dropdown list

Select from the dropdown list whether you want to use an individual channel or all channels of the second input image for the calculation.

First Images checkbox

Activated: For the second input image uses only the first time points of a time lapse image for the calculation. This allows you, for example, to normalize a time lapse image to the intensity values of the first time points.

First Images spin box/input field

Only visible if the **First Images** checkbox is activated.

Enter the number of images that you want to be used for the calculation using the spin box/input field.

Formula input field

Enter the calculation formula here using the keyboard and numeric keypad. Use "S1" as a placeholder for the first input image and "S2" for the second input image.

Input 1 button

Inserts the placeholder for the first input image into the **Formula** input field at the current cursor position.

Input 2 button

Inserts the placeholder for the second input image into the **Formula** input field at the current cursor position.

Absolute Intensities radio button

Activate the **Absolute Intensities** radio button if input image 1 and input image 2 have the same pixel type.

Normalize Intensities 0..1 radio button

Activate the **Normalize Intensities** radio button if input image 1 and input image 2 have different pixel types. To allow such images to be combined, the intensity values of the two images are normalized to the value range from 0 to 1 before the calculation.

Operators button

Opens a list of all available operators. Here you can select the operator that you want. If you double-click on a list entry, it is inserted into the **Formula** input field at the current cursor position.

Delete button

Deletes the contents of the Formula input field.

Undo button

Undoes the last entry in the **Formula** input field.

Calculate Image Pyramid parameters

Only visible if the **Show All** mode is activated.

Remove Pyramid checkbox

Activated: Removes the resolution pyramid from the image.

Calculate Histogram parameters

Columns input field

In the **Columns** input field enter the column number of the measurement parameter for which you want to calculate the histogram.

The  button opens the **Column Selection** dialog window.

Class Boundaries dropdown list

Select how you want the class boundaries of the calculated histogram to be determined from the dropdown list.

The following calculation options are available:

- **>=,…,>**: A numerical value falls into the histogram class if it is greater than or equal to the lower class boundary and less than the upper class boundary.
- **>,…,=<**: A numerical value falls into the histogram class if it is greater than the lower class boundary and less than or equal to the upper class boundary.

Automatically Determine Class Boundaries checkbox

Activate the checkbox if you want the class boundaries to be calculated automatically from the data. The value range from the lowest to the highest data value is divided into as many classes of equal width as you have set in the **Class Number** input field.

Logarithmic checkbox

Activate the Logarithmic checkbox if you want the width of calculated class boundaries to be distributed logarithmically.

You can only activate this checkbox if the **Automatically Determine Class Boundaries** checkbox has also been activated.

Class Number input field

Here you can enter how many classes you want to be calculated.

Mode dropdown list

Here you can select how you want the values of the histogram to be calculated.

The following types of calculation are available:

Number The histogram indicates how many data sets fall into the relevant class; it therefore contains the frequency of the values in the class concerned.

Cumulative Number In each class the histogram contains the total number for that class and the preceding class. Class 1 contains the number of values for class 1, class 2 contains the sum of the values from class 1 and class 2, class 3 contains the sum of the values from class 2 and class 3, etc.

Percent The histogram indicates what percentage of the data sets fall into the relevant class; it therefore contains the percentage share of the values in the class concerned.

Cumulative Percent In each class the histogram contains the total percentages of the preceding classes. Class 1 contains the percentage for class 1, class 2 contains the sum of the percentages from class 1 and class 2, class 3 contains the sum of the percentages from class 2 and class 3, etc. The last class therefore contains 100%.

Sum The histogram contains the sum of the numerical values of the data sets that fall into the relevant class; the values of the data sets that fall into the class concerned are therefore added together.

Cumulative Sum In each class the histogram contains the total sums of the preceding classes. Class 1 contains the sum of the numerical values from class 1, class 2 contains the sum of the numerical values from class 1 and class 2, class 3 contains the sum of the numerical values from class 2 and class 3, etc. The last class therefore contains the sum of all individual values.

Sum Percentages The histogram indicates the percentage share of the total numerical values in the relevant class.

Cumulative Sum Percentages In each class the histogram contains the total percentages of the preceding classes. Class 1 contains the percentage of the total numerical values from class 1, class 2 contains the sum of the percentages of the total numerical values from class 1 and class 2, class 3 contains the sum of the percentages of the total numerical values from class 2 and class 3, etc. The last class therefore contains 100%.

Column Selection dialog window

Here you can select the columns of the table for which you want the histogram to be calculated.

Direct Selection

Activate the checkboxes of the columns that you want to select.

Search For Text input field

In the input field you can enter one or more character strings that should appear in the column heading.

All radio button

Select this option if each character string has to appear in the column heading in order for it to be selected.

Any radio button

Select this option if only one of the character strings has to appear in the column heading in order for it to be selected.

Without the Words input field

Here you can enter one or more character strings that must not appear in the column heading.

Run button

Selects the column headings in accordance with the conditions for the text search.

Deselect All button

Resets all activated checkboxes.

Select All button

Activates all checkboxes.

OK button

Adopts the selection.

Cancel button

Exits the dialog window without making a selection.

Export/Import parameters

Image Export parameters

Default Values button

Resets all settings to the default values.

See also

- Image Export method [→ 112]

File Type section

File Type dropdown list

Select the desired file type from the dropdown list.

Various setting options are available to you depending on the file type selected (*.jpg, *.bmp, *.tiff, *.png, *.hdp).

Quality slider

Only available for the file types *.jpg, *.hdp.

Enter the image quality using the slider or spin box/input field to influence the size of the file. Although low values result in very small files, image quality may be considerably reduced.

Size slider

Enter the image size in percent using the slider or spin box/input field.

Convert to 8 Bit checkbox

Only available for the file types *.tiff, *.png, *.hdp.

Activated: Converts a 16 bit gray level image into an 8 bit gray level image, or a 48 bit color image into a 24 bit color image.

Compression dropdown list

Only available for the file type *.tiff.

Select the compression method for reducing the data volume from the dropdown list.

LZW Performs lossless compression in accordance with the Lempel-Ziv-Welch algorithm (LZW).

None Retains the data volume of the original image. No compression is performed.

ZIP Performs lossless compression in accordance with the ZIP method.

Image Data section

The following functions are only visible if the **Show All** mode is activated:

Original Data checkbox

Activated: Exports the image with the original channel colors and the original display characteristic curve.

Apply Display Characteristic Curve and Channel Color checkbox

Activated: Exports the image with the changed channel color and display characteristic curve settings. These settings are applied to the pixel values of the exported images. They are particularly important if you want to use dark images with a dynamic range of more than 8 bits in other programs.

Information

At least one of the two checkboxes must be activated. If both checkboxes are activated, you can export the original images and the images with the changed settings in a single step.

Burn In Graphic checkbox

Activated: Burns the graphic elements into the image. The pixels under the graphic element (e.g. scale bars) are overwritten. The burnt-in graphic elements cannot be subsequently modified.

Multichannel Image checkbox

Only visible for multichannel images.

Activated: Exports the pseudo color image of all selected channels.

Individual Channels checkbox

Only visible for multichannel images.

Activated: Exports the individual colored images of all selected channels.

Information

At least one of the three checkboxes must be activated. If the **Multichannel Image** and **Individual Channels** checkboxes are activated, you can export the individual colored images and the pseudo color image in a single step.

Dimensions section

The following functions are only visible if the **Show All** mode is activated:

Use All Dimensions radio button

Select this option if you want to export all dimensions without changing them.

Define Subset radio button

Select this option if you only want to export individual dimensions or subsets of individual dimensions.

Information

Each of the sections described below is only visible if the corresponding dimension is present in the input image.

Channels section

To show the section in full, click on the button.

In the **Channels** section you can select which channels of the input image you want to be used. All channels are selected by default. To deselect a channel, click on the relevant channel button.

 **button**

Adopts the values from the current display. These values can also be found on the Dimensions tab.

Z-Position section

To show the section in full, click on the button.

Z-Stack dropdown list

You can select which sections of the input image you want to be used from the dropdown list.

All Selects all sections of the input image.

Select Individual Selects an individual section.

Select Range Selects a continuous range of sections.

Select Several Selects several continuous ranges and individual sections.

Select Individual slider

Enter the section that you want to select using the slider or spin box/input field.

 **button**

Adopts the values from the current display. These values can also be found on the Dimensions tab.

Select Range slider

Enter the first and last section that you want to select using the sliders or spin boxes/input fields.

Interval checkbox

Activated: Interval mode is active. The Interval spin box/input field appears.

Interval spin box/input field

Enter the desired interval here. If you enter the value 2, for example, only every 2nd value from the range is considered.

Select Several input field

Enter one or more sections that you want to select in the input field. To do this, enter the first section, followed by a minus sign, and then the last section. If you want to define an interval, after the last section enter a colon and then the interval. The entry "2-10:2" means that every second section is selected from section 2 to section 10.

Enter a comma after the first section if you want to define another section. You can also select individual sections separated by commas. By entering "2-10:2,14-18,20,23", you select every second section from section 2 to section 10, followed by sections 14 to 18, as well as sections 20 and 23.

Time section

To show the section in full, click on the  button.

Time Points dropdown list

You can select which time points of the input image you want to be used from the **Time Points** dropdown list. The following selection options are available:

All Selects all time points of the input image.

Select Individual Selects an individual time point.

Select Range Selects a continuous range of time points.

Select Several Selects several continuous ranges and individual time points.

Select Individual slider

Enter the time point that you want to select using the slider or spin box/input field.

 **button**

Adopts the values from the current display. These values can also be found on the Dimensions tab.

Select Range slider

Enter the first and last time point that you want to select using the sliders or spin boxes/input fields.

Interval checkbox

Activated: Interval mode is active. The Interval spin box/input field appears.

Interval spin box/input field

Enter the desired interval here. If you enter the value 2, for example, only every 2nd value from the range is considered.

Select Several input field

Enter one or more time points that you want to select in the input field. To do this, enter the first time point, followed by a minus sign, and then the last time point. If you want to define an interval, after the last time point enter a colon and then the interval. The entry "2-10:2" means that every second time point is selected from time point 2 to time point 10.

Enter a comma after the first time point if you want to define another time point. You can also select individual time points separated by commas. By entering "2-10:2,14-18, 20, 23", you select every second time point from time point 2 to time point 10, followed by time points 14 to 18, as well as time points 20 and 23.

Scene section

To show the section in full, click on the  button.

Scene dropdown list

You can select which scenes of the input image you want to be used from the **Scene** dropdown list.

All Selects all scenes of the input image.

Select Individual Selects an individual scene.

Select Range Selects a continuous range of scenes.

Select Several Selects several continuous scenes and individual scenes.

Select Individual slider

Enter the scene that you want to select using the slider or spin box/input field.

 **button**

Adopts the values from the current display. These values can also be found on the Dimensions tab.

Select Range slider

Enter the first and last scene that you want to select using the sliders or spin boxes/input fields.

Interval checkbox

Activated: Interval mode is active. The Interval spin box/input field appears.

Interval spin box/input field

Enter the desired interval here. If you enter the value 2, for example, only every 2nd value from the range is considered.

Select Several input field

Enter one or more scenes that you want to select in the input field. To do this, enter the first scene, followed by a minus sign, and then the last scene. If you want to define an interval, after the last scene enter a colon and then the interval. The entry "2-10:2" means that every second scene is selected from scene 2 to scene 10.

Enter a comma after the first scene if you want to define another scene. You can also select individual scenes separated by commas. By entering "2-10:2,14-18, 20, 23", you select every second scene from scene 2 to scene 10, followed by scenes 14 to 18, as well as scenes 20 and 23.

Region section

To show the section in full, click on the  button.

Region dropdown list

You can select which sections of the input image you want to be used from the **Region** dropdown list.

All The entire image is selected.

Region In the current image, drag out the rectangular region that you want to be used using the mouse.

X spin box/input field

Enter the X coordinate of the starting point of the region in the spin box/input field.

Y spin box/input field

Enter the Y coordinate of the starting point of the region in the spin box/input field.

W spin box/input field

Enter the width of the region in the spin box/input field.

H spin box/input field

Enter the height of the region in the spin box/input field.

Tiles section

To show the section in full, click on the  button.

Use Existing Tiles radio button

Activated: Exports the image with an unchanged number of tiles and unchanged overlap.

New Tiles radio button

Configure the column, row and overlap settings using the spin boxes/input fields if you want to change the number of tiles and the overlap.

Columns spin box/input field

Only active if the **New Tiles** radio button is activated.

Enter the number of columns using the spin box/input field.

Rows spin box/input field

Only active if the **New Tiles** radio button is activated.

Enter the number of rows using the spin box/input field.

Overlap spin box/input field

Only active if the **New Tiles** radio button is activated.

Enter the percentage for the overlap using the spin box/input field.

Export To section

Export To display field

To open the **Browse For Folder** dialog window, click on the  button to the right of the display field. Here you can select the folder to which you want to save the image that is being exported. The path of the export folder is displayed automatically in the display field.

The following functions are only visible if the **Show All** mode is activated:

Create Subfolder checkbox

Activated: Creates a separate folder with the name of the input image.

Create XML Files checkbox

Activated: Creates two XML files containing the meta information relating to the input image.

- **Meta.xml** contains all additional information relating to the input image (e.g. hardware settings, dimensions).
- **Info.xml** only contains additional information relating to the exported individual images (e.g. names, dimensions, sizes).

Create ZIP Archive checkbox

Activated: Creates a ZIP file in which all exported individual images, including the XML files, are saved.

Prefix input field

Here you can edit the prefix specified or enter a new name. The name of the original image is specified by default.

Film Export parameters

Default Values button

Resets all settings to the default values.

See also

Film Export method [→ 113]

File Type section

File Type dropdown list

Select the desired file type from the dropdown list.

Various setting options are available to you depending on the file type selected (*.avi,*.wmf).

Format dropdown list

Select the desired format from the dropdown list.

Original Size Not available for the file type AVI (DV).

Uses the height and width of the input image and sets the frame rate to 5 frames per second.

User-Defined Not available for the file type AVI (DV).

Enter the values in the Width, Height and Frame Rate input fields.

720x576/25fps (PAL 576p/25) Uses the PAL (Phase Alternating Line) video resolution with 25 frames per second.

720x480/29.97fps (NTSC) Uses the NTSC (National Television Systems Committee) video resolution with 29.97 frames per second.

1280x720/50fps (HD 720p/50) Not available for the file types AVI (DV) and AVI (MS-Video 1).

Uses the HD (High Definition 720) video resolution with 50 frames per second.

1920x1080/25fps (HD 1080p/25) Not available for the file types AVI (DV) and AVI (MS-Video 1).

Uses the HD (High Definition 1080) video resolution with 25 frames per second.

1920x1080/29.97fps (HD 1080p/29.97) Not available for the file types AVI (DV) and AVI (MS-Video 1).

Uses the HD (High Definition 1080) video resolution with 29.97 frames per second.

Width input field

Only active if you have selected the **User-Defined** entry in the **Format** dropdown list.

Here you can indicate the width of the image in pixels (px).

Height input field

Only active if you have selected the **User-Defined** entry in the **Format** dropdown list.

Here you can indicate the height of the image in pixels (px).

Frame Rate input field

Only active if you have selected the **User-Defined** entry in the **Format** dropdown list.

Here you can enter the frame rate in frames per second (fps).

Quality slider

Only visible if you have selected the **AVI (M-JPEG compression)** entry in the **File Type** dropdown list.

Enter the image quality using the slider or spin box/input field to influence the size of the file. Although low values result in very small files, image quality may be considerably reduced.

Image Data section

The following functions are only visible if the **Show All** mode is activated:

Burn In Graphic checkbox

Activated: Burns the graphic elements into the image. The pixels under the graphic element (e.g. scale bars) are overwritten. The burnt-in graphic elements cannot be subsequently modified.

Multichannel Image checkbox

Only visible for multichannel images.

Activated: Exports the pseudo color image of all selected channels.

Individual Channels checkbox

Only visible for multichannel images.

Activated: Exports the individual colored images of all selected channels.

Information

At least one of the three checkboxes must be activated. If the **Multichannel Image** and **Individual Channels** checkboxes are activated, you can export the individual colored images and the pseudo color image in a single step.

Adjust section

The following functions are only visible if the **Show All** mode is activated:

Adjust dropdown list

Select the desired type of adjustment from the dropdown list.

Adjust Adjusts the image to the selected resolution. The original aspect ratio is retained.

Adjust and Clip Adjusts the image to the selected resolution and clips it. The original aspect ratio is not retained.

Adjust and Stretch Stretches the image to the selected resolution. The original aspect ratio is not retained.

Clip Clips the image to the selected resolution. The original aspect ratio is retained.

Sequence section

The following functions are only visible if the **Show All** mode is activated:

In the list enter the sequence of the dimensions in which you want the films to be created.

 **button**

Shifts the selected dimension up a line.

 **button**

Shifts the selected dimension down a line.

Image Sequence section

Image Sequence dropdown list

From the dropdown list, select how you want the images to be assigned.

Fixed Duration Enter the time per image in seconds using the spin box/input field. The total length is displayed in the Total Film Length text field.

1 Image per Time Point Assigns one image to one time point of the film.

Number of Images display field

Indicates the number of images in the input image.

Total Film Length display field

Indicates the total length of the resulting film, depending on the selected image sequence and the time.

Dimensions section

The following functions are only visible if the **Show All** mode is activated:

Use All Dimensions radio button

Select this option if you want to export all dimensions without changing them.

Define Subset radio button

Select this option if you only want to export individual dimensions or subsets of individual dimensions.

Information

Each of the sections described below is only visible if the corresponding dimension is present in the input image.

Channels section

To show the section in full, click on the button.

In the **Channels** section you can select which channels of the input image you want to be used. All channels are selected by default. To deselect a channel, click on the relevant channel button.

button

Adopts the values from the current display. These values can also be found on the Dimensions tab.

Z-Position section

To show the section in full, click on the button.

Z-Stack dropdown list

You can select which sections of the input image you want to be used from the dropdown list.

All Selects all sections of the input image.

Select Individual Selects an individual section.

Select Range Selects a continuous range of sections.

Select Several Selects several continuous ranges and individual sections.

Select Individual slider

Enter the section that you want to select using the slider or spin box/input field.

 **button**

Adopts the values from the current display. These values can also be found on the Dimensions tab.

Select Range slider

Enter the first and last section that you want to select using the sliders or spin boxes/input fields.

Interval checkbox

Activated: Interval mode is active. The Interval spin box/input field appears.

Interval spin box/input field

Enter the desired interval here. If you enter the value 2, for example, only every 2nd value from the range is considered.

Select Several input field

Enter one or more sections that you want to select in the input field. To do this, enter the first section, followed by a minus sign, and then the last section. If you want to define an interval, after the last section enter a colon and then the interval. The entry "2-10:2" means that every second section is selected from section 2 to section 10.

Enter a comma after the first section if you want to define another section. You can also select individual sections separated by commas. By entering "2-10:2,14-18,20,23", you select every second section from section 2 to section 10, followed by sections 14 to 18, as well as sections 20 and 23.

Time section

To show the section in full, click on the  button.

Time Points dropdown list

You can select which time points of the input image you want to be used from the **Time Points** dropdown list. The following selection options are available:

All Selects all time points of the input image.

Select Individual Selects an individual time point.

Select Range Selects a continuous range of time points.

Select Several Selects several continuous ranges and individual time points.

Select Individual slider

Enter the time point that you want to select using the slider or spin box/input field.

 **button**

Adopts the values from the current display. These values can also be found on the Dimensions tab.

Select Range slider

Enter the first and last time point that you want to select using the sliders or spin boxes/input fields.

Interval checkbox

Activated: Interval mode is active. The Interval spin box/input field appears.

Interval spin box/input field

Enter the desired interval here. If you enter the value 2, for example, only every 2nd value from the range is considered.

Select Several input field

Enter one or more time points that you want to select in the input field. To do this, enter the first time point, followed by a minus sign, and then the last time point. If you want to define an interval, after the last time point enter a colon and then the interval. The entry "2-10:2" means that every second time point is selected from time point 2 to time point 10.

Enter a comma after the first time point if you want to define another time point. You can also select individual time points separated by commas. By entering "2-10:2,14-18, 20, 23", you select every second time point from time point 2 to time point 10, followed by time points 14 to 18, as well as time points 20 and 23.

Scene section

To show the section in full, click on the  button.

Scene dropdown list

You can select which scenes of the input image you want to be used from the **Scene** dropdown list.

All Selects all scenes of the input image.

Select Individual Selects an individual scene.

Select Range Selects a continuous range of scenes.

Select Several Selects several continuous scenes and individual scenes.

Select Individual slider

Enter the scene that you want to select using the slider or spin box/input field.

 **button**

Adopts the values from the current display. These values can also be found on the Dimensions tab.

Select Range slider

Enter the first and last scene that you want to select using the sliders or spin boxes/input fields.

Interval checkbox

Activated: Interval mode is active. The Interval spin box/input field appears.

Interval spin box/input field

Enter the desired interval here. If you enter the value 2, for example, only every 2nd value from the range is considered.

Select Several input field

Enter one or more scenes that you want to select in the input field. To do this, enter the first scene, followed by a minus sign, and then the last scene. If you want to define an interval, after the last scene enter a colon and then the interval. The entry "2-10:2" means that every second scene is selected from scene 2 to scene 10.

Enter a comma after the first scene if you want to define another scene. You can also select individual scenes separated by commas. By entering "2-10:2,14-18, 20, 23", you select every second scene from scene 2 to scene 10, followed by scenes 14 to 18, as well as scenes 20 and 23.

Region section

To show the section in full, click on the  button.

Region dropdown list

You can select which sections of the input image you want to be used from the **Region** dropdown list.

All The entire image is selected.

Region In the current image, drag out the rectangular region that you want to be used using the mouse.

X spin box/input field

Enter the X coordinate of the starting point of the region in the spin box/input field.

Y spin box/input field

Enter the Y coordinate of the starting point of the region in the spin box/input field.

W spin box/input field

Enter the width of the region in the spin box/input field.

H spin box/input field

Enter the height of the region in the spin box/input field.

Export To section

Export To display field

To open the **Browse For Folder** dialog window, click on the  button to the right of the display field. Here you can select the folder to which you want to save the image that is being exported. The path of the export folder is displayed automatically in the display field.

Prefix input field

Here you can edit the prefix specified or enter a new name. The name of the original image is specified by default.

OME TIFF Export parameters

Default Values button

Resets all settings to the default values.

See also

OME TIFF Export method [➝ 113]

Image Format section

Size slider

Enter the image size in percent using the slider or spin box/input field.

BigTIFF checkbox

Only visible if the **Show All** mode is activated.

Activated: Creates a BigTIFF image that can be bigger than 4 gigabytes and uses 64-bit offset format.

Image Data section

The following functions are only visible if the **Show All** mode is activated:

Original Data radio button

Activated: Exports the image with the original channel colors and the original display characteristic curve.

Apply Display Characteristic Curve and Channel Color radio button

Activated: Exports the image with the changed channel color and display characteristic curve settings. These settings are applied to the pixel values of the exported images. They are particularly important if you want to use dark images with a dynamic range of more than 8 bits in other programs.

Burn In Graphic checkbox

Activated: Burns the graphic elements into the image. The pixels under the graphic element (e.g. scale bars) are overwritten. The burnt-in graphic elements cannot be subsequently modified.

Dimensions section

The following functions are only visible if the **Show All** mode is activated:

Use All Dimensions radio button

Select this option if you want to export all dimensions without changing them.

Define Subset radio button

Select this option if you only want to export individual dimensions or subsets of individual dimensions.

Information

Each of the sections described below is only visible if the corresponding dimension is present in the input image.

Channels section

To show the section in full, click on the  button.

In the **Channels** section you can select which channels of the input image you want to be used. All channels are selected by default. To deselect a channel, click on the relevant channel button.

 **button**

Adopts the values from the current display. These values can also be found on the Dimensions tab.

Z-Position section

To show the section in full, click on the  button.

Z-Stack dropdown list

You can select which sections of the input image you want to be used from the dropdown list.

All Selects all sections of the input image.

Select Individual Selects an individual section.

Select Range Selects a continuous range of sections.

Select Several Selects several continuous ranges and individual sections.

Select Individual slider

Enter the section that you want to select using the slider or spin box/input field.

 **button**

Adopts the values from the current display. These values can also be found on the Dimensions tab.

Select Range slider

Enter the first and last section that you want to select using the sliders or spin boxes/input fields.

Interval checkbox

Activated: Interval mode is active. The Interval spin box/input field appears.

Interval spin box/input field

Enter the desired interval here. If you enter the value 2, for example, only every 2nd value from the range is considered.

Select Several input field

Enter one or more sections that you want to select in the input field. To do this, enter the first section, followed by a minus sign, and then the last section. If you want to define an interval, after the last section enter a colon and then the interval. The entry "2-10:2" means that every second section is selected from section 2 to section 10.

Enter a comma after the first section if you want to define another section. You can also select individual sections separated by commas. By entering "2-10:2,14-18,20,23", you select every second section from section 2 to section 10, followed by sections 14 to 18, as well as sections 20 and 23.

Time section

To show the section in full, click on the  button.

Time Points dropdown list

You can select which time points of the input image you want to be used from the **Time Points** dropdown list. The following selection options are available:

All Selects all time points of the input image.

Select Individual Selects an individual time point.

Select Range Selects a continuous range of time points.

Select Several Selects several continuous ranges and individual time points.

Select Individual slider

Enter the time point that you want to select using the slider or spin box/input field.

 **button**

Adopts the values from the current display. These values can also be found on the Dimensions tab.

Select Range slider

Enter the first and last time point that you want to select using the sliders or spin boxes/input fields.

Interval checkbox

Activated: Interval mode is active. The Interval spin box/input field appears.

Interval spin box/input field

Enter the desired interval here. If you enter the value 2, for example, only every 2nd value from the range is considered.

Select Several input field

Enter one or more time points that you want to select in the input field. To do this, enter the first time point, followed by a minus sign, and then the last time point. If you want to define an interval, after the last time point enter a colon and then the interval. The entry "2-10:2" means that every second time point is selected from time point 2 to time point 10.

Enter a comma after the first time point if you want to define another time point. You can also select individual time points separated by commas. By entering "2-10:2,14-18, 20, 23", you select every second time point from time point 2 to time point 10, followed by time points 14 to 18, as well as time points 20 and 23.

Scene section

To show the section in full, click on the  button.

Scene dropdown list

You can select which scenes of the input image you want to be used from the **Scene** drop-down list.

All Selects all scenes of the input image.

Select Individual Selects an individual scene.

Select Range Selects a continuous range of scenes.

Select Several Selects several continuous scenes and individual scenes.

Select Individual slider

Enter the scene that you want to select using the slider or spin box/input field.

 **button**

Adopts the values from the current display. These values can also be found on the Dimensions tab.

Select Range slider

Enter the first and last scene that you want to select using the sliders or spin boxes/input fields.

Interval checkbox

Activated: Interval mode is active. The Interval spin box/input field appears.

Interval spin box/input field

Enter the desired interval here. If you enter the value 2, for example, only every 2nd value from the range is considered.

Select Several input field

Enter one or more scenes that you want to select in the input field. To do this, enter the first scene, followed by a minus sign, and then the last scene. If you want to define an interval, after the last scene enter a colon and then the interval. The entry "2-10:2" means that every second scene is selected from scene 2 to scene 10.

Enter a comma after the first scene if you want to define another scene. You can also select individual scenes separated by commas. By entering "2-10:2,14-18, 20, 23", you select every second scene from scene 2 to scene 10, followed by scenes 14 to 18, as well as scenes 20 and 23.

Region section

To show the section in full, click on the button.

Region dropdown list

You can select which sections of the input image you want to be used from the **Region** dropdown list.

All The entire image is selected.

Region In the current image, drag out the rectangular region that you want to be used using the mouse.

X spin box/input field

Enter the X coordinate of the starting point of the region in the spin box/input field.

Y spin box/input field

Enter the Y coordinate of the starting point of the region in the spin box/input field.

W spin box/input field

Enter the width of the region in the spin box/input field.

H spin box/input field

Enter the height of the region in the spin box/input field.

Export To section

Export To display field

To open the **Browse For Folder** dialog window, click on the button to the right of the display field. Here you can select the folder to which you want to save the image that is being exported. The path of the export folder is displayed automatically in the display field.

ZVI Export parameters

Export To display field

To open the **Browse For Folder** dialog window, click on the button to the right of the display field. Here you can select the folder to which you want to save the image that is being exported. The path of the export folder is displayed automatically in the display field.

Prefix input field

Here you can edit the prefix specified or enter a new name. The name of the original image is specified by default.

Default Values button

Resets all settings to the default values.

See also

- ZVI Export method [➝ 113]

Image Import Parameters

In the **Image Import Parameters** tool you can enter all the settings you need to import multidimensional images. To select the desired dimension, activate the checkbox in front of the relevant **Section**. At least one dimension must be selected. You can enter the settings for each dimension in the individual **Sections**.

You will find the settings for the import mode, import folder and for identifying your import images at the bottom of the tool.

Default Values button

Resets all settings to the default values.

Automatic button

Allows you to import all the images that are available in an import folder automatically.

Sequential button

Allows you to import an image sequence in a certain order (e.g. image dimensions that are encoded by means of numbers in the image name).

Import From display field

Displays the path of the import folder. To select a folder, click on the  button to the right of the display field. The names of the images are displayed in the **File Name** list below the display field.

Folder radio button

Only visible if the **Sequential** button is activated.

To select a folder, click on the  button to the right of the display field. The names of the images are displayed in the **File Name** list below the display field.

Multipage TIFF Image radio button

Only visible if the **Sequential** button is activated.

To select a multipage TIFF image, click on the  button to the right of the display field. The names of the images are displayed in the **File Name** list below the display field.

See also

- Image Import method [→ 113]

Multichannel Image section

In the **Multichannel Image** section you can enter all the settings you need to import multichannel images.

Multichannel Image checkbox

Activates the settings to import multichannel images. The **C** dimension (for multichannel images) is displayed automatically in the list in the **Specify Name** section.

No. column

Displays the channel number.

Dye column

Click on the dropdown list to select a dye. If the desired dye is not available, select the **Empty** entry.

Color column

Displays the pseudo color for the dye. To change the color, click on the color field. Select the desired color from the list.

Name column

Here you can enter a name for the dye. If you have selected a dye from the dropdown list, the name of the dye will appear automatically in the input field.

 button

Shifts the selected channel down a line.

 button

Shifts the selected channel up a line.

 button

Adds a new channel.

 **button**

Deletes the selected channel.

button

Opens the **Options** shortcut menu.

- **Add**
  Adds a new channel at the end of the list.
- **Copy**
  Copies the selected channel.
- **Reset Color**
  Resets the changed color of the selected channel to the default setting.
- **Delete**
  Deletes the selected channel.
- **Delete All**
  Deletes all channels and resets the first channel to the default settings.

Use Channel Name as Name checkbox

Only visible if the **Show All** mode is activated.

Uses the name specified in the **Name** column to identify the channel. The channel name will appear in the **Preview** display field in the **Specify Name** section.This deactivates the **Name**, **Start Index** and **Increment** columns in the list.

Z-Stack section

In the **Z-Stack** section you can enter all the settings you need to import Z-stack images.

Z-Stack checkbox

Activates the settings to import Z-stack images. The **Z** dimension (for Z-stacks) is displayed automatically in the list in the **Specify Name** section.

Interval spin box/input field

Here you can enter the value in µm for the distance between the individual Z-sections. The total height of the Z-stack is calculated automatically from this value and the number of sections.

Sections spin box/input field

Here you can enter the number of sections.

The following functions are only visible if the **Show All** mode is activated:

Interval radio button

Activated: The **Interval** spin box/input field and the **Range** display field are visible.

Here you can enter the value in µm for the distance between the individual Z-sections. The total height of the Z-stack is calculated automatically from this value and the number of sections and displayed in the **Range** display field.

Range radio button

Activated: The **Range** spin box/input field and the **Interval** display field are visible.

Here you can enter the total height of the Z-stack in µm. The distance between the individual Z-stacks is calculated automatically from this value and the number of sections and displayed in the **Interval** display field.

Advanced Parameters checkbox

Activates additional parameters that are necessary for further processing of the imported image (e.g. for deconvolution).

Magnification dropdown list

Select the objective magnification that was used for acquisition from the dropdown list.

Immersion dropdown list

Select the immersion medium that was used for acquisition from the dropdown list.

Aperture spin box/input field

Here you can enter the value of the numerical aperture of the objective that was used for acquisition.

Time Series section

In the **Time Series** section you can enter all the settings you need to import time lapse images.

Time Series checkbox

Activates the settings to import time lapse images. The **T** dimension (for time series) is displayed automatically in the list in the **Specify Name** section.

Interval spin box/input field

Here you can enter the value for the **Interval** between the individual time points. Select the unit of time from the dropdown list to the right of the spin box/input field.

Time Points spin box/input field

Here you can enter the number of time points.

The duration of the entire time series is calculated automatically from the interval and time points values.

The following functions are only visible if the **Show All** mode is activated:

Interval radio button

Activated: The **Interval** and **Time Points** spin boxes/input fields and the **Duration** display field are visible.

Here you can enter the value for the interval between the individual time points. Select the unit of time from the dropdown list to the right of the spin box/input field. Enter the number of time points in the **Time Points** spin box/input field.

The duration of the entire time series is calculated automatically from the interval and time points values and displayed in the **Duration** display field.

Duration radio button

Activated: The **Duration and Time Points** spin boxes/input fields and the **Interval** display field are visible.

Here you can enter the value for the duration of the entire time series. Select the unit of time from the dropdown list to the right of the spin box/input field. Enter the number of time points in the **Time Points** spin box/input field.

The interval between the individual time points is calculated automatically from the duration and time points values and displayed in the **Interval** display field.

Tiles section

Tiles checkbox

Activates the settings to import tile images. The **M** dimension (for MosaiX/tiles) is displayed automatically in the list in the **Specify Name** section.

Columns spin box/input field

Here you can enter the number of columns of the tile image.

Overlap spin box/input field

Here you can enter the percentage by which the tiles overlap.

Rows spin box/input field

Here you can enter the number of rows of the tile image.

Meander radio button

Select this option if the images to be imported were acquired in the Meander acquisition/travel mode.

Comb radio button

Select this option if the images to be imported were acquired in the Comb acquisition/travel mode.

Positions section

In the **Positions** section you can enter all the settings you need to import scenes.

Positions checkbox

Activates the settings to import images of individual scenes/motorized stage positions. The **S** dimension (for Scene) is displayed automatically in the **Specify Name** field.

No. column

Displays the position number.

X column

Enter the X coordinate of the position in the spin box/input field.

Y column

Enter the Y coordinate of the position in the spin box/input field.

button

Shifts the selected position down a line.

button

Shifts the selected position up a line.

button

Adds a new position.

button

Deletes the selected position.

button

Opens the **Options** shortcut menu.

- **Add**
  Adds a new position.
- **Delete**
  Deletes the selected position.
- **Delete All**
  Deletes all positions with the exception of the first position.

Scaling section

To show the section in full, click on the button.

Lateral Scaling (X) spin box/input field

Here you can enter the geometric scaling value for the X dimension of the image to be imported. Select the unit for the scaling value from the dropdown list to the right of the spin box/input field.

The following functions are only visible if the **Show All** mode is activated:

Define button

Enter the desired values in the **Lateral Scaling (X)** spin box/input field and in the **Lateral Scaling (Y)** spin box/input field. Select the unit for the scaling value from the dropdown list to the right of each spin box/input field.

Use Current button

Uses the geometric scaling currently selected and displays the values for **Lateral Scaling (X)** and **Lateral Scaling (Y)** with the corresponding unit in the relevant display field.

Specify Name section

In the **Specify Name** section you can enter all the settings you need to identify your images.

Prefix input field

Not available if the **Sequential** button is activated and the **Multipage TIFF Image** radio button has been selected.

Here you can edit the prefix specified or enter a new name. The name of the original image is specified by default.

Suffix input field

Not available if the **Sequential** button is activated and the **Multipage TIFF Image** radio button has been selected.

Here you have the option of entering a suffix.

Name column

In the input field you can enter the name of the relevant dimension from the original image name. A capital letter is displayed by default for the image dimension selected in each case (e.g. **C** for Channel, **T** for Time).

Start Index column

Enter the number of the first image in the spin box/input field.

End Index column

Only available if the **Sequential** button is activated.

Enter the number of the last image in the spin box/input field.

Increment column

Enter the increment in the spin box/input field. This allows you to specify whether you want every image or every xth image to be imported.

Preview display field

Shows the full name of the image (prefix, dimensions, suffix).

Check Consistency button

Not available if the **Sequential** button is activated and the **Multipage TIFF Image** radio button has been selected.

Checks the consistency of the selected settings and marks these in the **File Name** list.

Image Parameters tool group

Input tool

Input preview window

Only visible if you have selected a processing method.

Displays the input image. The image name appears in the title bar of the **Input** preview window.

Information

Two input images are required for the **Image Calculator**, **Add Channels** and **Shading Correction** methods.

No input image is required for the **Create Gray Scale Wedge** and **Image Import** methods.

A measurement data table is required as input for the **Calculate Histogram** method.

Select input image

➢ You have opened at least one image.

1. Left-click in the **Input** preview window.
   ⇨ The list of available images opens.
2. Select the appropriate image from the list.

⇨ You have selected an input image.

Block slider

Only visible if your images contain blocks.

Enter the number of the block that you want to process using the slider or spin box/input field.

The following functions are only visible if the **Show All** mode is activated:

Set Input Automatically checkbox

Activated: Automatically takes the current image as the input image.

Preview Window Only checkbox

Only visible if a preview is available for the processing method.

Activated: Only applies the processing method in the area of the preview window in **Preview** mode. To apply the processing method, click on the **Apply** button on the **Processing** tab.

Switch to Output Image radio button

Activated: Switches to the output image in the **center screen area** after you have applied the processing method.

Don't Switch radio button

Activated: Does not switch to the output image in the **center screen area** after you have applied the processing method. The current image is displayed in the **center screen area**.

Output tool

Information

The **Output** tool is not available for the **Calculate Histogram**, **Calculate Image Pyramid**, **Align Tiles**, **Image Export**, **Film Export**, **OME TIFF Export** or **ZVI Export** methods.

A diagram is produced as the output for the **Calculate Histogram** method.

Output preview window

Only visible if you have selected a processing method.

Displays the output image. The image name appears in the title bar of the **Output** window.

Select output image

➢ You have selected an input image.

➢ You have clicked the **Apply** button on the **Processing** tab.

➢ You have selected the **Overwrite** radio button in the **Output** tool.

1. Left-click in the Output preview window.
   ⇨ The list of available images opens.
2. Select the appropriate image from the list.

⇨ You have selected an output image.

Individual Name input field

Only active if you have selected the **In New Document** radio button.

Enter a name for the output image.

Overwrite radio button

Only visible if the **Show All** mode is activated.

Activated: Overwrites the existing output image when you apply the processing method.

Select this radio button if you want to try out different parameter settings, but only want to create one output image.

In New Document radio button

Only visible if the **Show All** mode is activated.

Activated: Creates a new output image when you apply the processing method.

Select this radio button if you want to create an output image for each of the different parameter settings. The name of the processing method is used by default together with the prefix IP and a sequential number.

Name button

Opens the **Name** dialog window. Here you can define the default setting for the name of the output image. The name of the processing method is used by default together with the prefix IP and a sequential number.

Preview button

Information

The **Preview** button is not available for the **Copy Annotation**, **Copy Image**, **Create Gray Scale Wedge**, **Add Channels**, **Align Channels**, **Align Z-Stack**, **Orthogonal Projection** or **Image Import** methods.

Switches to the Preview mode [➙ 227].

3.3.2 Batch Processing mode

In Batch Processing mode you apply a selected processing method, with the relevant method and image parameters, to the list of images.

3.4 Analysis tab

On the Analysis tab you will find all the functions you need for image analysis using ZEN (blue edition).

3.4.1 Interactive Measurement tool

Features section

Feature Selection dropdown list

In the Feature Selection dropdown list you can select and load previously saved feature definitions. If you have made changes to a feature definition, the name of the feature selection is marked with an asterisk (*). If you close the application without saving a changed ("asterisked") feature selection, you will be asked whether you want to save the changes.

button

Opens the Options [→ 69] shortcut menu.

Define button

Opens the Feature Selection [→ 182] dialog.

Feature Subset section

Only visible if the **Show All** mode is activated.

Feature Subset dropdown list

Here you can select and load previously saved definitions of subsets. If you have made changes to a subset definition, the name of the feature subset is marked with an asterisk (*). If you close the application without saving a changed ("asterisked") feature subset, you will be asked whether you want to save the changes.

button

Opens the Options [→ 69] shortcut menu.

Define button

Opens the Define Feature Subset [→ 184] dialog.

Measurement Procedure section

Only visible if the **Show All** mode is activated.

Measurement Procedure dropdown list

Here you can select and load previously saved measurement procedures. If you have made changes to a measurement procedure, the name of the measurement procedure is marked with an asterisk (*). If you close the application without saving a changed ("asterisked") measurement procedure, you will be asked whether you want to save the changes.

button

Opens the Options [→ 69] shortcut menu.

Define button

Opens the Define Interactive Measurement Procedure [→ 185] dialog.

Start button

Starts the selected interactive measurement procedure [→ 186].

Create Measurement Data Table button

Creates a measurement data table. This contains the measurement data from the **Measure** view of the current image.

Interactive Measurement dialog windows

Feature Selection dialog window

Here you can specify which features are measured with the available graphic elements. This selection is adopted into the current feature definition. The feature definition is then marked ("asterisked") as having been changed.

Available Elements section

In this section you can specify for each available graphic element which features you want to be measured. The graphic elements are ordered by type. The following types are available for selection:

Regions (2D)	Here you will find all the graphic elements that define a closed region.
Single Distances	Here you will find all the graphic elements with which you can measure a single distance.
Multiple Distances	Here you will find all the graphic elements with which you can measure several distances at once.
Angle	Here you will find the graphic elements with which you can measure an angle.
Point	Here you will find the graphic elements with which you can perform measurements at a pixel.
Events	Here you will find the graphic elements with which you can count various events in an image.

Selected Features section

The features that you have selected for each individual graphic element are listed in this section.

Display checkbox

Activate the **Display** checkbox for each feature to display the value of the measured feature in the graphics plane of the image.

Features section

All the features that you can measure with the graphic element activated in the **Available Elements** section are listed in this section.

Search for Feature input field

Here you can enter parts of the name of the feature that you are looking for. The features in which the entered character string occurs are listed.

Features dropdown list

From the dropdown list, select a type of feature according to which you want the features to be filtered.

The following feature types are available for selection:

All	If this is selected all features are listed.
Geometric Features	If this is selected all geometric features are listed.
Intensity Features	If this is selected all features that analyze intensity values are listed.
Image Features	If this is selected all features that contain meta information about the measured image are listed.
Position Features/Unscaled Position Features	If this is selected all features that describe the position are listed.

+ button

Click on the + button to select a feature for the measurement.

Wastepaper Bin button

Click on the button to delete the selected feature.

Upwards button

Click on the button to move the selected feature a position higher.

Downwards button

Click on the button to move the selected feature a position lower.

OK button

Click on the **OK** button to end the feature selection.

Cancel button

Click on the **Cancel** button to cancel the feature selection.

Define Feature Subset dialog window

Here you can specify which features are available in the **Feature Selection** dialog window. These features are adopted into the current subset definition. The subset definition is then marked ("asterisked") as having been changed.

Features section

All the features that you can measure with the graphic element activated in the **Available Elements** section are listed in this section.

Search for Feature input field

Here you can enter parts of the name of the feature that you are looking for. The features in which the entered character string occurs are listed.

Features dropdown list

From the dropdown list, select a type of feature according to which you want the features to be filtered.

The following feature types are available for selection:

All If this is selected all features are listed.

Geometric Features If this is selected all geometric features are listed.

Intensity Features If this is selected all features that analyze intensity values are listed.

Image Features If this is selected all features that contain meta information about the measured image are listed.

Position Features/Unscaled Position Features If this is selected all features that describe the position are listed.

Checkbox

There is a checkbox in front of the name of each of the listed features. Activate the checkbox in front of the features that you want to be offered in the **Feature Selection** dialog.

Shortcut menu

Right-click in the Features section. Select **Select All Features** to activate all checkboxes. Select **Deselect All Features** to deactivate all checkboxes.

OK button

Click on the **OK** button to end the subset definition.

Cancel button

Click on the **Cancel** button to cancel the subset definition.

Define Interactive Measurement Procedure dialog window

Here you can define an interactive measurement procedure. You can specify the order in which you want the individual graphic elements to be drawn in and which measurement parameters you want to have calculated for them. The definition is adopted into the measurement procedure currently selected. The measurement procedure is then marked ("asterisked") as having been changed.

Available Elements section

In this section you can specify for each available graphic element which features you want to be measured. The graphic elements are ordered by type. The following types are available for selection:

Regions (2D) Here you will find all the graphic elements that define a closed region.

Single Distances Here you will find all the graphic elements with which you can measure a single distance.

Multiple Distances Here you will find all the graphic elements with which you can measure several distances at once.

Angle Here you will find the graphic elements with which you can measure an angle.

Point Here you will find the graphic elements with which you can perform measurements at a pixel.

Events Here you will find the graphic elements with which you can count various events in an image.

Double-click on a graphic element to select it and adopt it into the **Order of the Elements** section.

Order of the Elements list

This list displays the selected graphic elements in the order in which they will be drawn in during the measurement, from top to bottom. To display the value of the measured feature in the image's graphics plane, activate the corresponding checkbox of the graphic elements.

Wastepaper Bin button

Click on the button to delete the selected feature.

Upwards button

Click on the button to move the selected feature a position higher.

Downwards button

Click on the button to move the selected feature a position lower.

Features section

All the features that you can measure with the graphic element activated in the **Available Elements** section are listed in this section.

Search for Feature input field

Here you can enter parts of the name of the feature that you are looking for. The features in which the entered character string occurs are listed.

Features dropdown list

From the dropdown list, select a type of feature according to which you want the features to be filtered.

The following feature types are available for selection:

All	If this is selected all features are listed.
Geometric Features	If this is selected all geometric features are listed.
Intensity Features	If this is selected all features that analyze intensity values are listed.
Image Features	If this is selected all features that contain meta information about the measured image are listed.
Position Features/Unscaled Position Features	If this is selected all features that describe the position are listed.

+ button

Click on the + button to select a feature for the measurement.

Interactive Measurement Procedure dialog window

In this dialog you can run a predefined interactive measurement procedure.

Start button

Click on the **Start** button to begin drawing in the graphic elements in the image.

Pause button

Click on the **Pause** button to interrupt the measurement procedure. This allows you to modify graphic elements that have already been drawn in, for example.

Continue button

If you have interrupted the measurement procedure using the **Pause** button, click on the **Continue** button to continue measuring.

End button

Click on the **End** button to end the current measurement procedure at a position of your choice. To restart the measurement procedure at the first graphic element, click on the **Start** button again.

Graphic Elements section

In this section you will see the graphic elements of the current measurement procedure in the order that you have defined.

Measurement Data section

In this section you will see the graphic elements of the current measurement procedure in the order that you have defined.

OK button

Click on the **OK** button to end the interactive measurement procedure.

Cancel button

Click on the **Cancel** button to cancel the interactive measurement procedure. In this case the graphic elements that have been drawn in are not adopted into the image.

3.4.2 Image Analysis tool

Image Analysis Wizard section

Start Image Analysis Wizard button

To define a new analysis program or to change an existing program, click on the **Start Image Analysis Wizard** button.

See also

The Image Analysis Wizard [➙ 188]

Programs section

Program dropdown list

In the **Program** dropdown list you can select and load previously saved analysis programs.

Run Interactively button

Runs the selected analysis program with all the interactive steps.

Run Without Interruption button

Runs the selected analysis program without interruption.

Steps that you have marked as interactive in the **Image Analysis Wizard** are run with the values set in the analysis program. The program does **not** stop to allow you to change these interactively.

The Image Analysis Wizard

Step 1 - Program

In this step you can create new analysis programs. You can also rename, copy and delete existing analysis programs here.

Program dropdown list

Here you can select and load saved analysis programs. If you make changes to an analysis program, the name of the analysis program is marked with an asterisk (*). If you close the wizard without saving a changed ("asterisked") analysis program, you will be asked whether you want to save the changes.

Options button

Opens the Options shortcut menu.

Next button

Moves on to the next step of the wizard.

Cancel button

Cancels the analysis program.

Options shortcut menu

Here you can create new analysis programs and rename, save or delete existing analysis programs.

New menu item

Creates a new analysis program. Enter a name for the analysis program.

Rename menu item

Enter a new name for the analysis program.

Save menu item

Saves a changed ("asterisked") analysis program under the current name.

Save As menu item

Saves the current analysis program under a new name. Enter a new name for the analysis program.

Delete menu item

Deletes the current analysis program.

Step 2 - Classes

In this step you can define the classes into which the measured objects in the image are divided.

Back button

Moves back to the previous step of the wizard.

Classes list

The defined classes are listed here. If you create a new measurement program, a class is created automatically. Each class consists of two entries. The first entry concerns all the objects belonging to the class. The second entry represents an individual object.

Add Class button

Adds a new class to the list.

Delete Class button

Deletes the selected class from the list.

Name input field

Here you can enter a name for the selected class in the **Classes** list.

Channel selection field

If you create a measurement program for a multichannel image, in this selection field you can select a channel for the selected class in the **Classes** list.

Color button

Opens the **Color Selection** dialog window.Here you can select a color that you want to assign to the selected class.

Next button

Moves on to the next step of the wizard.

Cancel button

Cancels the analysis program.

Step 3 - Measurement Frames

In this step you can define one or more measurement frames. These allow you to specify how objects are dealt with at the edge of the image.

Back button

Moves back to the previous step of the wizard.

Interactive checkbox

Activated: The measurement frame definition can be changed interactively while the measurement program is running.

Tool bar

Selection Use this to select measurement frames that have already been drawn in. To select a measurement frame, click inside it. To select several measurement frames, hold down the **Ctrl** key and click inside the desired measurement frames. Once you have selected a measurement frame, you can change its size.

Rectangle Use this to create a rectangle as a measurement frame in the current image.

Circle Use this to create a circle as a measurement frame in the current image.

Contour Use this to create a contour as a measurement frame in the current image.

button

Deletes all drawn-in measurement frames in the current image.

Mode dropdown list

Here you can select how you want the measurement frame to be applied. The following modes are available:

In and On the Frame All objects are measured that are lying completely within the measurement frame, are touching it or are intersected by it.

Inside the Frame Only those objects are measured that are lying completely within the measurement frame. Objects that are touching the frame or are intersected by it are not measured.

Cut at Frame All objects are measured that are lying completely within the measurement frame. Objects that are intersected by the measurement frame are measured precisely up to the measurement frame.

Maximize Circle checkbox

Only active if you have defined precisely one circle.

Activated: Maximizes the drawn-in circle to the full image size and centers it. In the case of rectangular images the circle is adjusted to the shorter side.

The following fields are only active if you have selected a drawn-in graphic element:

Left spin box/input field

Here you can enter the start point for the frame on the X axis in pixels.

Top spin box/input field

Here you can enter the start point for the frame on the Y axis in pixels.

Width spin box/input field

Here you can enter the width of the measurement frame in pixels.

Height spin box/input field

Here you can enter the height of the measurement frame in pixels.

Next button

Moves on to the next step of the wizard.

Cancel button

Cancels the analysis program.

Step 4 - Threshold Values

In this step you can enter the threshold values for the segmentation of the objects that you want to measure.

Back button

Moves back to the previous step of the wizard.

Run checkbox

Activated: Sets the defined threshold values when the measurement program is run.

Interactive checkbox

Activated: The defined threshold values can be changed while the measurement program is running.

Classes list

Here you can select the class for which you want to define the threshold values.

Next button

Moves on to the next step of the wizard.

Cancel button

Cancels the analysis program.

See also

- Low Pass method [→ 110]
- Gauss method [→ 110]
- Median method [→ 109]
- Edge Enhancement method [→ 109]
- Unsharp Masking method [→ 109]

Smooth section

Smoothing dropdown list

Here you can select how you want to smooth the image before the threshold values are set. The following methods are available:

None The image is not smoothed.

Low Pass Applies the Low Pass Method [→ 110].

Gaussian Applies the Gaussian Method [→ 110].

Median Applies the Median Method [→ 109].

Size/Sigma slider

Enter the size of the filter matrix in the X and Y direction or the sigma value using the slider or spin box/input field.

Sharpen section

Image Sharpness dropdown list

Here you can select how you want to improve the sharpness of the image before the threshold values are set. The following methods are available:

None The sharpness of the image is not changed.

Edge Enhancement Applies the Edge Enhancement Method [→ 109].

Unsharp Masking Applies the Unsharp Masking Method [→ 109].

Threshold Value slider

Enter the threshold value for edge detection using the slider or spin box/input field. The threshold value should correspond roughly to the gray value difference between objects and the background.

Size slider

Enter the size of the edge detection filter using the slider or spin box/input field. The value should correspond to the size of the transition area between objects and the background.

Strength slider

Enter the strength of the Unsharp Masking using the slider or spin box/input field. The higher the value selected, the greater the extent to which small structures are enhanced.

Minimum Area section

Minimum Area slider

Using the slider or spin box/input field, enter the minimum area in pixels that an object must have in order to be segmented.

Threshold section

Here you can define the threshold values for the selected class in the **Classes** list.

Reset button

Resets all threshold value settings.

Undo button

Undoes the last change made to the threshold values.

Redo button

Restores the last undone change to the threshold values.

Color Model buttons

Only visible if the image is a color image.

RGB In RGB Mode [→ 195] you can define the threshold values for the red, green and blue color channels.

HLS In HLS Mode [→ 196] you can define the threshold values for hue, saturation and lightness.

Histogram

In the histogram you can change the lower and upper threshold value for the activated value. Drag the lower or upper adjustment handle or shift the entire highlighted area between the lower and upper threshold value.

Threshold Value Definition button

Click Click in the image on the regions that you want to define as objects.

Automatic The threshold values are determined automatically.

The following parameters are only visible if **Click** has been selected for threshold value definition:

Click Behavior buttons

+ button Click on this button to expand the currently segmented regions by the gray values/colors of the objects subsequently clicked on.

- button Click on this button to reduce the currently segmented regions by the gray values/colors of the objects subsequently clicked on.

Tolerance slider

Using the slider or spin box/input field, enter the tolerance range by which the gray/color value read out when you click is expanded to define the threshold value.

Neighborhood slider

Using the slider or spin box/input field, enter a neighborhood range around the pixel clicked on. The threshold value is calculated from the average of the gray/color values in this neighborhood range.

The following parameter is only visible if **Automatic** has been selected for threshold value definition:

Method dropdown list

Select the method from the dropdown list that you want to use for the automatic calculation of the threshold values.

Information

After the automatic calculation of the threshold values you can further modify the threshold values found interactively by selecting **Click** for threshold value definition.

The following methods are available:

Otsu The threshold value is calculated according to the Otsu method.

Most Frequent Gray Value The threshold value is the gray/color value that occurs most frequently in the image.

Iso Data The threshold value lies in the middle between two maximums in the histogram.

Triangular Threshold Value

3 Sigma Threshold Value The threshold value is calculated from the sum of the average and three times the sigma value of the histogram distribution.

RGB mode

Red Color Channel threshold values

Red button

Click on the red  button to activate the red channel in the Expander **histogram**.

Lower (Red Channel) input field

Enter the lower threshold value for the red channel using the spin box/input field.

Upper (Red Channel) input field

Enter the upper threshold value for the red channel using the spin box/input field.

Invert (Red Channel) button

Click on the **Invert** button to swap the **Lower** and **Upper** values for the red channel.

All (Red Channel) button

Click on the **All** button to set the **Lower** value to 0 and the **Upper** value to the maximum possible gray value for the red channel.

Green Color Channel threshold values

Green button

Click on the green  button to activate the green channel in the Expander **histogram**.

Lower (Green Channel) input field

Enter the lower threshold value for the green channel using the spin box/input field.

Upper (Green Channel) input field

Enter the upper threshold value for the green channel using the spin box/input field.

Invert (Green Channel) button

Click on the **Invert** button to swap the **Lower** and **Upper** values for the green channel.

All (Green Channel) button

Click on the **All** button to set the **Lower** value to 0 and the **Upper** value to the maximum possible gray value for the green channel.

Blue Color Channel threshold values

Blue button

Click on the blue  button to activate the blue channel in the Expander **histogram**.

Lower (Blue Channel) input field

Enter the lower threshold value for the blue channel using the spin box/input field.

Upper (Blue Channel) input field

Enter the upper threshold value for the blue channel using the spin box/input field.

Invert (Blue Channel) button

Click on the **Invert** button to swap the **Lower** and **Upper** values for the blue channel.

All (Blue Channel) button

Click on the **All** button to set the **Lower** value to 0 and the **Upper** value to the maximum possible gray value for the blue channel.

HLS mode

Hue threshold values

H button

Click on the 

button to activate the hue in the Expander **histogram**.

Lower (Hue) input field

Enter the lower threshold value for the hue using the spin box/input field.

Upper (Hue) input field

Enter the upper threshold value for the hue using the spin box/input field.

Invert (Hue) button

Click on the **Invert** button to swap the **Lower** and **Upper** values for the hue.

All (Hue) button

Click on the **All** button to set the **Lower** value to 0 and the **Upper** value to the maximum possible value for the hue.

Lightness threshold values

L button

Click on the  button to activate the lightness in the Expander **histogram**.

Lower (Lightness) input field

Enter the lower threshold value for the lightness using the spin box/input field.

Upper (Lightness) input field

Enter the upper threshold value for the lightness using the spin box/input field.

Invert (Lightness) button

Click on the **Invert** button to swap the **Lower** and **Upper** values for the lightness.

All (Lightness) button

Click on the **All** button to set the **Lower** value to 0 and the **Upper** value to the maximum possible value for the lightness.

Saturation threshold values

S button

Click on the  button to activate the saturation in the Expander **histogram**.

Lower (Saturation) input field

Enter the lower threshold value for the saturation using the spin box/input field.

Upper (Saturation) input field

Enter the upper threshold value for the saturation using the spin box/input field.

Invert (Saturation) button

Click on the **Invert** button to swap the **Lower** and **Upper** values for the saturation.

All (Saturation) button

Click on the **All** button to set the **Lower** value to 0 and the **Upper** value to the maximum possible value for the saturation.

Fill section

Fill Holes checkbox

Activated: Fills holes in segmented objects.

Separate section

Separate dropdown list

Here you can select whether you want to process the image further after segmentation. Objects that are touching one another can be separated using different methods.

None Objects are not separated.

Watersheds Using this method you can separate objects that are roughly the same shape. This method may however result in the splitting of elongated objects.

Morphology This method separates objects by first reducing and then enlarging them, making sure that once objects have been separated they do not merge together again.

Number slider

Using the slider or spin box/input field, enter how often the method is applied successively to the result at the location of the separation.

Step 5 - Condition

In this step you can define the conditions under which you want an object to be measured.

Back button

Moves back to the previous step of the wizard.

Run checkbox

Activated: Uses the measurement conditions when the measurement program is run.

Interactive checkbox

Activated: The measurement conditions can be changed while the measurement program is running.

Classes list

Here you can select the class for which you want to define the conditions.

Select button

Opens the Conditions Editor [→ 199] dialog window.

Conditions list

If you have defined one or more blocks with conditions in the **Conditions Editor** dialog window, here you can select the block for which you want to set the condition. To do this, click on the relevant block and then on the objects in the image that you want to measure.

Undo button

Undoes the last change made to the condition.

Redo button

Restores the last undone change to the condition.

Reset button

Resets all settings for the conditions.

Next button

Moves on to the next step of the wizard.

Cancel button

Cancels the analysis program.

Conditions Editor dialog window

Here you can add features to a block with measurement conditions.

Search for Feature input field

Here you can enter parts of the name of the feature that you are looking for. The features in which the entered character string occurs are listed.

Features dropdown list

From the dropdown list, select a type of feature according to which you want the features to be filtered.

The following feature types are available for selection:

All	If this is selected all features are listed.
Geometric Features	If this is selected all geometric features are listed.
Intensity Features	If this is selected all features that analyze intensity values are listed.
Image Features	If this is selected all features that contain meta information about the measured image are listed.
Position Features/Unscaled Position Features	If this is selected all features that describe the position are listed.

+ button

Click on the + button to select a feature for the measurement.

Selected Features for the Condition list

In this list, the features that you have selected for the condition are displayed block by block. All features in a block are "And"-linked for the condition, i.e. an object is only measured if the values of each individual feature fall within the defined range.

Add button

Adds an "Or" block. If several "Or" blocks have been defined, an object is measured if it meets the condition in at least one block.

Empty button

Deletes all features in an "Or" block.

Remove button

Deletes the selected "Or" block.

Remove All button

Deletes all "Or" blocks.

Step 6 - Interactive Segmentation

In this step you can postprocess the segmented objects interactively.

Back button

Moves back to the previous step of the wizard.

Interactive checkbox

Activated: The segmented objects can be postprocessed interactively while the measurement program is running.

Classes list

Here you can select the class whose objects you want to process.

Remove button

Using this button you can remove parts of an object. Holding down the left mouse button, outline the parts of the object that you want to remove. Right-click to remove these parts of the object.

Separate button

Use this button to separate connected objects. Holding down the left mouse button, draw in the separation line between the objects. Right-click to separate the objects.

Connect button

Use this button to connect objects. Holding down the left mouse button, outline the parts of the object that you want to connect. Right-click to connect the objects.

Delete button

Click on the **Delete** button to delete an object by subsequently clicking on it.

Tool bar

Selection Switches the mouse pointer to Selection mode.

Rectangle

Use this button to add a rectangular object or cut a rectangular region from an object.

Circle

Use this button to add a circular object or cut a circular region from an object.

Draw Contour

Use this button to add an object or cut a region from an object.

Contour (Spline)

Use this button to add an object or cut a region from an object.

Active Contour

Use this button to add an object or cut a region from an object.

Delete

Deletes all objects.

Keep checkbox

Activated: The selected graphic element remains selected until you deselect it.

Fill Hole button

Fills a hole. To fill a hole, left-click on the hole.

Region Growth button

Activates the Region Growth mode. Then click on objects in the image that you want to segment. The object "grows" around the point that has been clicked on.

Intensity slider

Enter a tolerance value for the intensity using the slider or spin box/input field. The tolerance value specifies how much the intensity of a pixel may deviate from the average intensity of the object in order to still "grow" to become part of the object.

Color slider

Only active if your input image is a color image.

Enter a tolerance value for the color using the slider or spin box/input field. The tolerance value specifies how much the color value of a pixel may deviate from the average color value of the object in order to still "grow" to become part of the object.

Undo button

Undoes the last action.

Redo button

Restores the last undone action.

Next button

Moves on to the next step of the wizard.

Cancel button

Cancels the analysis program.

Step 7 - Measurement Features

In this step you can select the measurement features that you want to measure.

Back button

Moves back to the previous step of the wizard.

Classes list

Here you can select the class for which you want to define measurement features. For each class there are two entries for which you can define features. The first entry concerns all the objects belonging to the class. The second entry represents an individual object.

Field Features section

Only visible if a "parent" class is active.

The list shows all Field Features that are measured for the current class.

Object Features section

Only visible if a "child" class is active.

The list shows all Object Features that are measured for the current class.

Annotations section

Only visible if a "child" class is active.

The list shows all annotations that are drawn in for the current class.

Select button

Opens the Feature Selection [→ 203] dialog window for the current class.

Copy button

Copies the selected features of the current class to all other classes.

Next button

Moves on to the next step of the wizard.

Cancel button

Cancels the analysis program.

Feature Selection dialog window

Here you can specify which features you want to measure.

Selected Features section

The features that you have selected are listed in this section.

Wastepaper Bin button

Click on the button to delete the selected feature.

Upwards button

Click on the button to move the selected feature a position higher.

Downwards button

Click on the button to move the selected feature a position lower.

Search for Feature input field

Here you can enter parts of the name of the feature that you are looking for. The features in which the entered character string occurs are listed.

Features dropdown list

From the dropdown list, select a type of feature according to which you want the features to be filtered.

The following feature types are available for selection:

All If this is selected all features are listed.

Geometric Features If this is selected all geometric features are listed.

Intensity Features If this is selected all features that analyze intensity values are listed.

Image Features If this is selected all features that contain meta information about the measured image are listed.

Position Features/Unscaled Position Features If this is selected all features that describe the position are listed.

+ button

Click on the + button to select a feature for the measurement.

OK button

Click on the **OK** button to end the feature selection.

Cancel button

Click on the **Cancel** button to cancel the feature selection.

Step 8 - Measure

In this step you will see the result of the measurement. The measured image is displayed in the Analysis View [→ 273].

Back button

Moves back to the previous step of the wizard.

Classes list

Here you can select the class for which you want to see the measured features. For each class there are two entries: The "parent" class, which shows the features for all objects together, and the "child" class, which shows the features for each individual object.

End button

Saves the analysis program created and ends the wizard.

Cancel button

Cancels the analysis program.

3.4.3 Features

Geometric features

Number of Inner Parts

Number of "holes" and objects within these holes for the measured object.

Diameter

Diameter of circle with equal area

It is assumed that the measured area of an object is the area of a circle. This area is then used to calculate back to the corresponding diameter.

Formula: 2 * √(Area/π)

Ellipse Major Half-Axis

Length of the major axis of an ellipse with the same geometric moment of inertia as the object. The moment of inertia is calculated in relation to the object's center of gravity.

Ellipse Minor Half-Axis

Length of the minor axis of an ellipse with the same geometric moment of inertia as the object. The moment of inertia is calculated in relation to the object's center of gravity.

Ellipse Angle

Angle of the major axis of the ellipse

The major axis of an ellipse with the same geometric moment of inertia as the current object is calculated in accordance with the **Ellipse Major Half-Axis** parameter. Then the angle in relation to the X axis is determined. The angle is always indicated in a counterclockwise direction.

Fiber Length

Length of a fiber-like region

To calculate the fiber length, a structure that actually resembles a fiber is required. The calculation does not determine the distance between a start and end point. You can check the calculation by means of the shape factor, for example.

Formula: (PerimF - √ ($PerimF^2$ – 16* AreaF)) / 4

Feret Maximum

Maximum feret of a region

The maximum feret is calculated on the basis of distance measurements. Two straight lines are placed against the object, like a caliper, at 32 **angle positions**. The corresponding distance is measured for each angle position. The maximum value calculated in this way is the maximum feret.

Feret Maximum Angle

Angle of the maximum feret of a region in relation to the X axis

The maximum feret is calculated in accordance with the description for **Feret Maximum**. The angle of the maximum feret in relation to the X axis is then determined. The angle is always indicated in a counterclockwise direction.

Feret Minimum

Minimum feret of a region

The minimum feret is calculated on the basis of distance measurements. Two straight lines are placed against the object, like a caliper, at 32 angle positions. The corresponding distance is measured for each angle position. The minimum value calculated in this way is the minimum feret.

Feret Minimum Angle

Angle of the minimum feret of a region in relation to the X axis

The minimum feret is calculated in accordance with the description for **Feret Minimum**. The angle of the minimum feret in relation to the X axis is then determined. The angle is always indicated in a counterclockwise direction.

Feret Vertical to Maximum

Feret Ratio

Ratio of the ferets

The ratio of Feret Minimum to Feret Maximum is calculated. This ratio allows statements to be made about the shape of the measured objects. Smaller values for the feret ratio mean that elongated objects are present. Values close to 1 mean that compact or circular objects are present, as Feret Minimum and Feret Maximum then have very similar values.

Area

Area of an object

Area of an object without any holes it may contain. The areas of the holes are not included in the measurement.

Area Filled

Area of the filled object

Area of an object including any holes it may contain.

Area Convex

Area of the convex shell of an object

A convex polygonal chain is placed around the current object. The filled area of the resulting object is then measured.

Shape Factor Circle

Shape factor of an object

Describes the shape of an object on the basis of its circularity. A perfect circle is given the value 1. The more elongated the object is, the smaller the shape factor. The calculation is performed on the basis of the Area Filled and Perimeter Crofton parameters.

Formula: $(4 * \pi * \text{Area Filled}) / \text{Perimeter Convex}^2$

Object Class

Class number of the object

Object Class Name

Class name of the object

Compactness

Compactness of an object

Describes the compactness of an object on the basis of its circularity. A perfect circle without holes is given the value 1. The calculation is performed on the basis of the Area and Perimeter Convex parameters.

Formula: $(4 * \pi * \text{Area}) / (\text{Perimeter Convex})^2$

Convexity

Convexity of an object

Describes the convexity of an object. For a convex object the Convexity parameter has the value 1.

Formula: (Perimeter Convex / Perimeter)^2

Circularity

Circularity of an object

Describes the circularity of an object based on the Area and Feret Maximum parameters. A perfect circle has the circularity √1.

Formula: √(4 * Area) / (π * Feret Maximum^2)

Radius

It is assumed that the measured area of an object is the area of a circle. This area is then used to calculate back to the corresponding radius.

Formula: √(Area / π)

Bound Width

Horizontal feret of an object

Indicates the width (size in X direction) of a bounding box for an object. The box is drawn in parallel to the X and Y axis.

Bound Height

Vertical feret of a region

Indicates the height (size in Y direction) of a bounding box for an object. The box is drawn in parallel to the X and Y axis.

Object ID

Unique ID of an object within the measurement mask

The Object ID parameter indicates the original number of an object within a class. After segmentation and subsequent binary image processing, a unique number is assigned to each object within the class. You can then exclude individual objects from the measurement by using measurement conditions or setting a measurement frame. These objects are not de-

leted, however. They are simply not considered for the measurement. This means that there is a difference between the number of objects present and the number of measured objects. The Object ID parameter indicates the number of an object in the list of objects present.

Object Index

Serial number of the measured object

Indicates the number of a measured object within a class. After segmentation and subsequent binary image processing, a unique number is assigned to each object within the class. You can then exclude individual objects from the measurement by using measurement conditions or setting a measurement frame. These objects are not deleted, however. They are simply not considered for the measurement. This means that there is a difference between the number of objects present and the number of measured objects. Only the objects that have actually been measured are listed in the resulting data table, however. The Object Index can be used to guarantee unambiguous assignment between measured objects in the image and the individual rows of a data table.

Roundness

Roundness of an object

Describes the roundness of an object based on the Area and Feret Maximum parameters. A perfect circle has the roundness 1.

Formula: $(4 * \text{Area}) / (\pi * \text{Feret Maximum}^2)$

Perimeter

Perimeter of an object

The perimeter of an object is calculated. If the measured object contains holes, the total perimeter is calculated, including the perimeters of the hole structures.

Number

Number of measured objects

Indicates the number of measured objects within the class.

Field Area Percent

Total area of all regions in relation to the area of the measurement frame

Sum of the areas of all objects. For each object the Area parameter is measured without any holes the object may contain and all the individual areas are added together. The areas of the holes are not included in the measurement.

Formula: 100 * (∑ Area) / Area of the Measurement Frame

Field Area

Area of all regions

Sum of the areas of all regions. For each region the Area parameter is measured without any holes the region may contain and all the individual areas are added together. The areas of the holes are not included in the measurement.

Field Area Unscaled

Area of all regions in pixels

Sum of the areas of all regions in pixels. For each region the Area parameter is measured without any holes the region may contain and all the individual areas are added together. The areas of the holes are not included in the measurement.

Intensity features

Intensity Spread

Spread of the intensity values of an object

The intensity values of an object are analyzed and the difference between the maximum and minimum value is displayed.

If this parameter is measured in a color image, the intensity values of the individual color channels of the color image are converted into an intensity value in accordance with the formula (R+G+B) / 3 and the corresponding parameter is then displayed.

Maximum Intensity

Highest intensity value of an object

The intensity values of an object are analyzed and the maximum value is displayed. This is the brightest point of an object.

If this parameter is measured in a color image, the intensity values of the individual color channels of the color image are converted into an intensity value in accordance with the formula (R+G+B) / 3 and the corresponding parameter is then displayed.

Minimum Intensity

Lowest intensity value of an object

The intensity values of an object are analyzed and the minimum value is displayed. This is the darkest point of an object.

If this parameter is measured in a color image, the intensity values of the individual color channels of the color image are converted into an intensity value in accordance with the formula (R+G+B) / 3 and the corresponding parameter is then displayed.

Average Intensity

Average of all intensity values of an object

The intensity values of an object are analyzed and the average of all intensity values is displayed.

If this parameter is measured in a color image, the intensity values of the individual color channels of the color image are converted into an intensity value in accordance with the formula (R+G+B) / 3 and the corresponding parameter is then displayed.

Standard Deviation of Intensity

Standard deviation of all intensity values of an object

The intensity values of an object are analyzed and the standard deviation of all intensity values is displayed.

If this parameter is measured in a color image, the intensity values of the individual color channels of the color image are converted into an intensity value in accordance with the formula (R+G+B) / 3 and the corresponding parameter is then displayed.

Maximum Intensity (Field)

Highest intensity value in all objects

The intensity values of all measured objects are analyzed and the maximum value is determined. This is the brightest point of all objects.

If this parameter is measured in a color image, the intensity values of the individual color channels of the color image are converted into an intensity value in accordance with the formula (R+G+B) / 3 and the corresponding parameter is then displayed.

Minimum Intensity (Field)

Lowest intensity value in all objects

The intensity values of all measured objects are analyzed and the minimum value is determined. This is the darkest point of all objects.

If this parameter is measured in a color image, the intensity values of the individual color channels of the color image are converted into an intensity value in accordance with the formula (R+G+B) / 3 and the corresponding parameter is then displayed.

Average Intensity (Field)

Average of all intensity values in all objects

The intensity values of all measured objects are analyzed and the average is determined.

If this parameter is measured in a color image, the intensity values of the individual color channels of the color image are converted into an intensity value in accordance with the formula (R+G+B) / 3 and the corresponding parameter is then displayed.

Standard Deviation of Intensity (Field)

Standard deviation of all intensity values in all objects

The intensity values of all measured objects are analyzed and the standard deviation is determined.

If this parameter is measured in a color image, the intensity values of the individual color channels of the color image are converted into an intensity value in accordance with the formula (R+G+B) / 3 and the corresponding parameter is then displayed.

Image features

Acquisition Time

Acquisition time of the image

Date and time when the image was acquired.

Relative Acquisition Time

Relative acquisition time of the image

Relative acquisition time of an individual image within a multidimensional image. The time difference in relation to the first image acquired is indicated. This value is not calculated for 2D images. If the parameter is nevertheless measured, the corresponding field in the data table does not contain a value.

Relative Acquisition Time 1 to 4

Relative acquisition time of the image in relation to time point 1, 2, 3, or 4

Relative acquisition time of an individual image within a multidimensional image. The time difference in relation to the image acquired at the set time point (1, 2, 3, or 4) is indicated. This value is not calculated for 2D images. If the parameter is nevertheless measured, the corresponding field in the data table does not contain a value.

Width

Width of the image

Indicates the width of the image in scaled units.

Width Unscaled

Width of the image

Indicates the width of the image in pixels.

Focus Position

Focus position

Indicates the position of the microscope's focus drive at the time of acquisition.

Focus Depth

Depth of field of the image

Indicates the depth of field of an image. (Only with images that have been acquired using a SteREO microscope.)

Height

Height of the image

Indicates the height of the image in scaled units.

Height Unscaled

Height of the image

Indicates the height of the image in pixels.

Channel Index

Channel index of the image

Displays the channel number of a channel in a multichannel fluorescence image.

Position Index

Position index of the image

Displays the position number of a position within the position list.

Scene Index

Scene index of the image

Indicates the unique number of the scene in an image. Scene refers to a continuous object on a slide that contains several objects for analysis.

Z Index

Z-plane index of the image

Displays the current Z position of a Z-stack image.

Time Index

Time index of the image

Displays the number of the time point of a time lapse image.

Channel Name

Name of the channel

Displays the name of the fluorescence channel in a multichannel image.

Scaling X

Indicates the scaling of the image in the X direction.

Scaling Y

Indicates the scaling of the image in the Y direction.

Date Saved

Date on which image was saved

Displays the date on which the image was last saved.

Stage Position X

Stage position X

Displays the X position of the stage at the time of acquisition. An encoded or motorized scanning stage is required to calculate this parameter.

Stage Position Y

Stage position Y

Displays the Y position of the stage at the time of acquisition. An encoded or motorized scanning stage is required to calculate this parameter.

Image Name

Name of the image

Displays the file name of the image without folder information.

Position features

Bound Left

Indicates the X coordinate of the left edge of a bounding box for a region. The box is drawn in parallel to the X and Y axis.

Bound Top

Indicates the Y coordinate of the top edge of a bounding box for a region. The box is drawn in parallel to the X and Y axis.

Bound Right

Indicates the X coordinate of the right edge of a bounding box for a region. The box is drawn in parallel to the X and Y axis.

Bound Bottom

Indicates the Y coordinate of the bottom edge of a bounding box for a region. The box is drawn in parallel to the X and Y axis.

Center of Gravity X

Geometric center of gravity of a region, X coordinate

X coordinate of the geometric center of gravity of a region. Depending on the shape of the object, this point may also lie outside a region.

Center of Gravity Y

Geometric center of gravity of a region, Y coordinate

Y coordinate of the geometric center of gravity of a region. Depending on the shape of the object, this point may also lie outside a region.

Unscaled geometric features

Diameter Unscaled

Diameter of circle with equal area in pixels

It is assumed that the measured area of an object is the area of a circle. This area is then used to calculate back to the corresponding diameter.

Formula: $2 * \sqrt{(\text{Area in Pixels}/\pi)}$

Ellipse Major Half-Axis Unscaled

Length in pixels of the major axis of an ellipse with the same geometric moment of inertia as the object. The moment of inertia is calculated in relation to the object's center of gravity.

Ellipse Minor Half-Axis Unscaled

Length in pixels of the minor axis of an ellipse with the same geometric moment of inertia as the object. The moment of inertia is calculated in relation to the object's center of gravity.

Ellipse Angle Unscaled

Angle of the major axis of the ellipse

The major axis of an ellipse with the same geometric moment of inertia as the current object is calculated in accordance with the **Ellipse Major Half-Axis Unscaled** parameter. Then the angle in relation to the X axis is determined. The angle is always indicated in a counterclockwise direction.

Fiber Length Unscaled

Length of a fiber-like region in pixels

To calculate the fiber length, a structure that actually resembles a fiber is required. The calculation does not determine the distance between a start and end point. You can perform a check by means of the shape factor, for example.

Formula: (PerimF (in Pixels) - √ (PerimF (in Pixels) ^{2} – 16* AreaF(in Pixels))) / 4

Area Filled Unscaled

Area of the filled object in pixels

Area of an object including any holes it may contain.

Area Unscaled

Area of an object in pixels

Area of an object without any holes it may contain. The areas of the holes are not included in the measurement.

Radius Unscaled

Radius of circle with equal area in pixels

It is assumed that the measured area of an object is the area of a circle. This area is then used to calculate back to the corresponding radius.

Formula: √(Area in Pixels/π)

Bound Width Unscaled

Horizontal feret of an object in pixels

Indicates the width (size in X direction) of a bounding box for an object. The box is drawn in parallel to the X and Y axis.

Bound Height Unscaled

Vertical feret of a region in pixels

Indicates the height (size in Y direction) of a bounding box for an object. The box is drawn in parallel to the X and Y axis.

Perimeter Unscaled

Perimeter of an object in pixels

The perimeter of an object is calculated. If the measured object contains holes, the total perimeter is calculated, including the perimeters of the hole structures.

Unscaled position features

Acp X Unscaled

The first pixel in the first line of a region, X coordinate

To identify measurement objects, the image is scanned from top left to bottom right. The so-called ACP point is the first point identified for a new object. The AcpX parameter indicates the X coordinate of this point in pixels.

Acp Y Unscaled

The first pixel in the first line of a region, Y coordinate

To identify measurement objects, the image is scanned from top left to bottom right. The so-called ACP point is the first point identified for a new object. The AcpY parameter indicates the Y coordinate of this point in pixels.

Bound Left Unscaled

Indicates the X coordinate of the left edge of a bounding box for a region in pixels. The box is drawn in parallel to the X and Y axis.

Bound Top Unscaled

Indicates the Y coordinate of the top edge of a bounding box for a region in pixels. The box is drawn in parallel to the X and Y axis.

Bound Right Unscaled

Indicates the X coordinate of the right edge of a bounding box for a region in pixels. The box is drawn in parallel to the X and Y axis.

Bound Bottom Unscaled

Indicates the Y coordinate of the bottom edge of a bounding box for a region in pixels. The box is drawn in parallel to the X and Y axis.

Center of Gravity X Unscaled

Geometric center of gravity of a region in pixels, X coordinate

X coordinate of the geometric center of gravity of a region in pixels. Depending on the shape of the object, this point may also lie outside a region.

Center of Gravity Y Unscaled

Geometric center of gravity of a region in pixels, Y coordinate

X coordinate of the geometric center of gravity of a region in pixels. Depending on the shape of the object, this point may also lie outside a region.

3.5 Reporting tab

On the **Reporting** tab you can create reports. If you have an image open and click on the **Reporting** tab, a **Report Preview** is automatically generated. You can create the report directly, change the template in the **Report** tool or link other images/tables.

Information

All reports in ZEN are based on templates which are then filled with data. The appearance and content of the report are defined in the template. The creation of templates is not included as a feature in ZEN.

3.5.1 Report buttons

New Report button

Only visible if the **Report Preview** is not active.

Opens the **Report Preview**. The appearance of the report is determined by the template, which you can select in the Report [→ 221] tool.

Information

If you have an image open and click on the **Reporting** tab, the **Report Preview** opens automatically. If the Report Preview is active, the **New Report** button changes into the **Close** button.

Close button

Only visible if the Report Preview is active.

Closes the Report Preview without creating a report.

Print button

Only active if a report has been created.

Opens the Print Preview [→ 223] dialog window. Here you can configure the print settings.

Save button

Only active if a report has been created, but not yet saved.

Saves the report. If the report has not yet been saved, the **Save As** dialog window opens. The report is saved in *.czr (Carl Zeiss Report) format.

Export button

Only active if a report has been created.

Exports the current report into your file system. The *.pdf or *.xps formats are available for the export.

Information

All reports in ZEN are based on templates which are then filled with data. The appearance and content of the report are defined in the templates.

See also

- Report preview [→ 299]

3.5.2 Report tool

In the **Report** tool you can select various templates for your report. In addition you can see important information about your report.

Select Template dropdown list

Here you can select the desired template for the report that is to be created.

Information

If the **Report Preview** is active, you can change the template for the report here. You will see the change directly in the Report Preview.

Description

Displays a description of the report template.

Created By

Displays who created the report template.

Created On

Displays when the report template was created.

button

Opens the Options [→ 222] shortcut menu.

See also

- Report preview [→ 299]

Report Management section

To show the section in full, click on the button.

Name input field

Only active in the **Report Preview**.

Here you can enter a name for the report. This is no longer possible for a report that has already been created.

If you have already created a report, here you will see a display field showing the name of the report.

Information

If you do not give the report its own name in the **Report Management** section, it will be allocated a name by default. You can change this default setting in the **Extras** menu | **Options** | **Name**.

Description input field

Only active in the **Report Preview**.

Here you can enter a description for the report. This is no longer possible for a report that has already been created.

Created By display field

Displays who created the report.

Information

The user data are automatically adopted from the **Extras** menu | **Options** | **User**.

Created On display field

Displays the date on which the report was created.

Options

In the **Options** shortcut menu you can open or save reports and import or export report templates.

Open menu item

Opens an existing report.

Save menu item

Saves the current report. The name is adopted from the **Report Management** section | **Name**.

Save As menu item

Saves the report under a new name. Enter a new name for the report.

Import Template menu item

Imports an existing report template to ZEN. The template is then shown in the **Select Template** dropdown list.

Export Template menu item

Exports the current report template to back it up or share it with other users.

See also

Report Management section [➙ 221]

3.5.3 Report dialog window

Print Preview dialog window

Printer section

Here you can select the printer that you want to use.

Properties button

Opens a dialog window containing the printer properties. Here you can configure advanced settings. This dialog window is dependent on the printer.

Format section

Here you can select the page format. The following page formats are available:

Format	Width in mm	Height in mm
A 3	210	297
A 4	148	210
A 5	105	148
Letter	216	279
Legal	216	356
Executive	184	267
Ledger	279	432
JIS B4	257	346
JIS B5	182	257

Width display field

Displays the width of the page.

Height display field

Displays the height of the page.

All Pages radio button

Activated: Prints all pages of the report.

Pages radio button

Activated: Prints a certain number of pages of the report.

In the input field to the right of the radio button, enter the pages that you want to print. **Example:**

The entry **1-3; 5** prints pages 1,2,3 and 5.

Pages per Sheet dropdown list

Here you can select how many pages are printed per sheet.

Copies spin box/input field

Here you can enter the number of copies that you want to print.

Collate checkbox

Only active if you have chosen to print several copies.

Activated: Sorts the pages of each copy printed.

Fit to Page radio button

Activated: Adjusts the size of the report or image to the size of the page.

Original Size radio button

Activated: Uses the original size of the report or image.

Adjust radio button

Activated: Adjusts the size of the report or image to the factor set in the spin box/input field to the right of the **Adjust** radio button.

Here you can set the desired enlargement/reduction factor for the report or image. A factor of 100% corresponds to the **Fit to Page** option.

Print button

Prints the report using the options set.

 **button**

Displays the next page.

 **button**

Displays the previous page.

Adjust dropdown list

Here you can select the zoom factor with which the page view is displayed in this dialog window.

4 Center Screen Area

4.1 Image views

4.1.1 General image views

These image views are available with any image. Depending on the type of image in question, the **general control elements** may have additional or more limited functions.

2D View

The **2D view** is the default view for images in ZEN (blue edition). It allows you to view your images in two dimensions. In the **2D view** the general control elements are available to you. To open the view's shortcut menu, right-click in the image area.

See also

General view controls [➙ 282]

2D View Navigator window

Here you can set the zoom factor for the image area. In the Navigator window you can see a preview of the current image. The blue frame indicates the visible region of interest.

Information

If you are viewing a multidimensional image, you can use the **Navigator** to navigate through the available dimensions. These are represented by thin lines before or after the preview image. To navigate through the dimensions, left-click on the thin lines or use the mouse wheel.

In the case of time lapse images the time points present are displayed to the left and right of the preview image. To navigate through the time lapse images, click on the areas to the left or right of the preview image.

Enlarging the image area

➢ You have activated the **Navigator** button on the **Dimensions** tab. The button is highlighted in blue.

➢ You can see the **Navigator window** in the image area.

1. Move the mouse pointer over the blue frame in the Navigator window.
   ⇨ The mouse pointer will appear as a double-headed arrow.
2. Hold down the left mouse button and move the mouse.

⇨ The size of the frame changes and the image area is enlarged.

Moving the region of interest

➢ You have enlarged the image area.

➢ You have activated the **Navigator** button on the **Dimensions** tab. The button is highlighted in blue.

➢ In the Navigator window you can see the full image and the region of interest. The region of interest is indicated by a blue frame.

1. To move the region of interest, move the mouse pointer inside the blue frame.
   ⇨ The mouse pointer will appear as a four-headed arrow.
2. Hold down the left mouse button and move the mouse.
   ⇨ The region of interest moves.
3. Release the left mouse button again.

⇨ You have moved the region of interest with the help of the Navigator. Alternatively, it is also possible to move the region of interest using the **Move** tool on the **Dimensions** tab.

Enlarging the Navigator window

➢ The Navigator window is open in the image area.

1. Move the mouse pointer to the adjustment handle in the bottom right corner of the Navigator window.
   ⇨ The mouse pointer will appear as a double-headed arrow.
2. Hold down the left mouse button and move the mouse.

⇨ The size of the Navigator window changes.

Moving the Navigator window

➢ The Navigator window is open.

➢ The image area has been enlarged.

1. Move the mouse pointer inside the blue frame.
   ⇨ The mouse pointer will appear as a four-headed arrow.
2. Hold down the left mouse button and move the mouse.

⇨ You can position the Navigator window freely within the image.

2D View shortcut menu

Full-Screen mode (F11)

Switches to full-screen mode. To exit full-screen mode, press **F11** again or **ESC**.

Zoom Group

Here you have access to the main zoom functions (Dimensions tab | Zoom section [➞ 283]).

Show Ruler

Shows rulers at the top and left edge of the image.

Show Navigator

Shows the **2D view** Navigator window.

Spot Measurement/Focus Region

This function is only active in the live image or during **Continuous** mode.

Shows a region in which the exposure time is measured and the software autofocus is focused.

Show Graphic Elements

This function is activated by default.

Shows graphic elements that have been drawn into the image, e.g. annotations or scale bars.

Show Bleaching Regions

This function is only visible with FRAP images.

Shows graphic elements that were used during acquisition for bleaching (FRAP).

Copy Display Settings

Copies the display settings from an image (Display tab [→ 295]).

Insert Display Settings

Inserts copied display settings into an image (Display tab [→ 295]).

Selection Region

Draw In Selection Region Draw a certain region that particularly interests you into the image. You can draw several regions into an image.

Create Image From Selection Region Creates new image documents from the selection regions you have drawn in. All dimensions of the image are taken into account here.

Insert

Inserts a graphic element into the current image from the clipboard.

Preview mode

Here you can see a preview of the result of a processing method.

Preview window

Region Shows the result in the preview window in a region at the center of the input image.

Right Image Half Shows the result in the preview window in the right half of the input image.

Full Image Shows the result in the preview window in the full size of the input image.

Right Shows the result in the preview window to the right of the input image.

Freely positioning the preview window

➢ Preview mode has been activated.

➢ You have selected the **Region**, **Right Image Half** or **Full Image** preview window.

1. Move the mouse pointer inside the preview window.
   ⇨ The mouse pointer will appear as a four-headed arrow.
2. Left-click on the preview window and hold down the left mouse button.
3. Move the mouse.
4. Release the left mouse button again at the desired position.

⇨ The preview window can be positioned freely. The result of the processing method is overlaid in the input image.

Adjusting the size of the preview window

➢ Preview mode has been activated.

➢ You have selected the **Region** or **Right Image Half** preview window.

1. Move the mouse pointer to the adjustment handle in the bottom right corner of the preview window.
   ⇨ The mouse pointer will appear as a double-headed arrow.
2. Left-click on the adjustment handle and hold down the left mouse button.
3. Move the mouse.
4. Release the left mouse button again once the desired size has been reached.

⇨ The size of the preview window can be changed. The result of the processing method is overlaid in the input image.

Gallery View

In the **Gallery view** you can see an overview of your multidimensional images. The individual images of the images concerned are presented in a gallery. It is possible to show any combination of dimensions, e.g. channels against time.

When you view images for the first time in the **Gallery view**, they are displayed as follows:

Multichannel images All the channels present in an image are shown, including the mixed color image.

Time lapse images All the time points present in an image are shown.

Z-stack images All Z-planes are shown.

Multichannel & time lapse images All the time points present in an image are shown. All channels are shown as a mixed color image.

Multichannel & Z-stack images All Z-planes are shown. All channels are shown as a mixed color image.

Time lapse & Z-stack images All Z-planes are shown.

Time lapse, Z-stack & multichannel images All Z-planes are shown. All channels are shown as a mixed color image.

See also

General view controls [→ 282]

View specific controls

Gallery Tools tab

Here you can specify which dimension you want to be displayed on which axis of the Gallery view. To do this, click on the corresponding button on the tool bar.

Displayed Dimensions section

Each of the buttons is only visible if the current image contains the corresponding dimension.

Channels button Shows the channels present as individual images.

Z-Stack button Shows the Z-planes present as individual images.

Time button Shows the time points present as individual images.

Chann.&Z (Channels and Z-Stack) button Shows the channels present in relation to the Z-stack images present.

Chann.&Time (Channels and Time Lapse) button Shows the channels present in relation to the time lapse images present.

Z&Time (Z-Stack and Time Lapse) button Shows the Z-stack images present in relation to the time lapse images present.

X Axis section Only visible if the **Show All** mode is activated.

Use the buttons to select which dimension is shown on the X axis.

Y Axis section Only visible if the **Show All** mode is activated.

Use the buttons to select which dimension is shown on the Y axis.

Show Dimension Details checkbox

Activated: Inserts annotations into each individual image that provide information on the time point or Z-plane.

Invert X and Y Axis checkbox

This checkbox is only available if the **Show All** mode is not activated.

Only active if two dimensions are shown in relation to each other (Chann.&Z, Chann.&Time, Z&Time).

Activated: Inverts the X and Y axis of the **Gallery view**.

Show Mixed Channel Image checkbox

Only visible for multichannel images. Only active if the channels present are shown.

Activated: Shows the mixed image of all channels in addition to the individual images.

The following functions are only visible if the **Show All** mode is activated:

Create Image From section

Here you can create images from the Gallery view. Select the type of image that you want to create from the dropdown list.

Current View Creates an image of the current Gallery view.

Selection Creates an image from the images that have been selected in the current view.

The resulting image contains all the information of the input image; the pixel data are not changed.

Selection Range Creates an image from a defined selection range.

If this entry is selected, sliders for the selected dimensions appear (Start, End and Interval). Use the sliders to set the selection range you want.

Create button

Creates an image from the Gallery view.

Including section

Select a dimension that is not currently displayed from the dropdown list. This dimension is taken into account when the image is created.

The resulting image is always a 24 bit RGB color image. The pixel data of the original image are changed.

Burn In Annotations checkbox

Burns the annotations present into the image that is created.

Gallery Display tab

Only visible if the **Show All** mode is activated.

Dimension Details Formatting section

Here you can define the font and the style, color, position and size of the text for the dimension details that are shown.

Distance and Background section

Here you can set the background color of the **Gallery view** and the distance between the individual images (from 1-10 pixels).

2.5D View

In the **2.5D view** intensity values in a two-dimensional image are converted into a height map. Here the highest intensity values are represented by the greatest extension in the Z-direction. Overall this results in a so-called 2.5D or pseudo-3D image.

Information

If you are viewing a multichannel image, you can have the intensity values of the individual channels displayed. To do this, activate or deactivate the desired channels on the **Dimensions** tab.

See also

General view controls [→ 282]

2.5D View tool bars

The tool bars are arranged to the left of and underneath the image area. You can use the tools to control the display of the 2.5D volumes in the image area.

Left tool bar

Top thumb wheel

Enlarges or reduces the image area.

Tool bar

Rotate Use this to rotate the 2.5D volume in any way you wish within the space. This is the default mode when you switch to **2.5D view** for the first time.

Zoom Use this to increase the zoom factor of the image area.

Move Use this to move the 2.5D volume laterally.

Bottom thumb wheel

Rotates the 2.5D volume around the horizontal (X) axis.

Bottom tool bar

Left thumb wheel

Use this to rotate the 2.5D volume around the vertical (Y) axis.

Tool bar

Bounding Box Use this to show or hide a bounding box around the 2.5D volume.

Show X/Y Axis Use this to show or hide the X/Y axis.

Show Z Axis

Use this to show or hide the Z axis.

Start View Use this to switch back to the start view. A top view of the 2.5D volume is displayed. Lateral movements and the zoom factor are adjusted so that the 2.5D volume can be seen at the center of the image area.

Right thumb wheel

Use this to compress the 2.5D volume on the (Z) axis perpendicular to the screen plane.

View specific controls

2.5D Display tab

On the **2.5D Display** tab you have 4 options for displaying your 2.5D image.

Profiles display

Displays the relief divided into a number of profiles with an equal distance.

Set the number of profiles using the **Fineness** slider.

Grid display

Displays the relief overlaid with a grid. This view supports gray levels only.

Make the grid more closely or more coarsely meshed using the **Fineness** slider.

Blocks display

Displays the relief by means of discrete, upwardly protruding columns of differing heights.

Surface display

Displays the relief as a continuous, flowing landscape.

Make the surface coarser or finer using the **Fineness** slider.

Invert Z checkbox

Activated: Displays the lowest intensity values by means of the greatest extension in the Z direction.

Use this function for images that contain many large, bright regions.

Apply Palette checkbox

Activated: Overlays the relief with the pseudo colors that have been set on the **Dimensions** tab.

Show Sides checkbox

Only available in the **Surface** display.

Activated: Closes the sides of the relief.

Show Planes checkbox

Activated: Shows two blue, transparent planes in the 2.5D volume.

Set the position of the planes using the **X/Y** sliders.

Extract Image section

To save an individual image in the current view, click on the **Save As** button.

Series tab

On the **Series** tab you can create a series of images in the 2.5D view. These series can be played back later as a video clip, for example.

Render Series dropdown list

Here you can select the desired series mode:

Rotate Around X	Here you can define the start/stop angle and the rotation direction around the X axis.
Rotate Around Z	Here you can define the start/stop angle and the rotation direction around the Z axis.
Start/Stop	Here you can define the angle and zoom settings for the start and end position of your series. The intermediate positions are interpolated evenly.
Position List	Here you can define any number of positions. The positions can each have completely different rotation, zoom and illumination settings.
Over Time	Here you can define the start time point and end time point for a series. All other settings (rotation, zoom, etc.) remain unchanged.

Apply button

Creates a series image with the current settings.

Preview section

To obtain a preview of the series, click on the **Play** button. To end the preview, click on the **Stop** button.

Number of Images section

Select the number of individual images in the series from the dropdown list.

2.5D Display Options tab

Only visible if the **Show All** mode is activated.

Shape and Position section

Angle X Enter the rotation angle in the X direction with a precision of 1 degree using the slider or spin box/input field.

Angle Y Enter the rotation angle in the Y direction with a precision of 1 degree using the slider or spin box/input field.

Z Scaling Enter the Z scaling using the slider or spin box/input field.

Lighting Parameters section

Ambient Reduces or increases the intensity of the ambient lighting in the 2.5D view.

Reflection Reduces or increases the proportion of the ambient light reflected on the relief.

Shine Reduces or increases the effect of the ambient light shining on the relief.

Light Intensity Reduces or increases the intensity of the lighting in the 2.5D view. A small distance means a circular light source at the center, while a large distance illuminates the scene evenly.

Reset button

Resets all settings to the default values.

Histo View

The **Histo** (Histogram) view shows you the gray value histogram of your image. In the right image area you can see your current image and in the left image area you can see the **Histogram window**. At the side you will also find four **data tables**:

- In the first table from the left you will find all the **raw data** for each channel.
- In the second table from the left you will find all the **limits** for each channel of the image next to the image name.
- In the third table from the left you will find the **statistical values** for the gray value distribution, e.g. average, standard deviation, minimum and maximum value.
- The fourth table shows the measured values of measurements in the histogram.

See also

General view controls [→ 282]

View specific controls

Histo Definition tab

Tool bar

Using the tools you can add certain measurement regions to your image. The histogram window shows you the gray value histogram for the relevant region.

Selection Changes the mouse pointer to Selection mode. You can use this to select graphic elements in the image.

Copy Use this to copy the last selected element and insert it at another position in the image.

Rectangle Use this to insert a rectangular measurement region.

Circle Use this to insert a circular measurement region.

Freehand Use this to insert a measurement region with a shape of your choice. The line is closed automatically.

Polygon Use this to insert a polygonal measurement region in the original image.

Keep checkbox

Activated: Keeps the last selected tool active.

Color checkbox

Only visible if the **Show All** mode is activated.

Activated: Highlights each drawn-in measurement region with a random color.

Histogram Measurements section

Normal button Switches the Profile window back to the view display.

X Measurement button Use this to perform a measurement of a region in the X direction in the relevant histogram. To adopt the value into the measurement data table, click on the **Insert Values** button.

Reset button

Empties the measurement data table below the original image.

Insert Values button

Adds the current measurement in the histogram window to the measurement data table below the original image.

Histo. Table section

Only visible if the **Show All** mode is activated.

Select the type of gray value distribution from the dropdown list. The following types are available:

- Frequency distribution
- Sum above
- Sum below

Relative Frequency checkbox

Only visible if the **Show All** mode is activated.

Activated: Displays the histogram according to the relative frequency of the gray values in percent.

Using the relevant sliders, you can enter the precise number of classes, class size and upper/lower threshold value.

Class Number slider

Enter the number of classes using the slider or spin box/input field.

Class Size slider

Only visible if the **Show All** mode is activated.

Enter the class size using the slider or spin box/input field.

Log. Scale checkbox

Only visible if the **Show All** mode is activated.

Activated: Switches from the linear to a logarithmic class width. The class size is calculated automatically.

Lower Threshold Value slider

Enter the lower threshold value for the gray value distribution using the slider or spin box/input field. All regions in the image with gray values below the lower threshold value are overlaid in blue and all those with gray values above the upper threshold value are overlaid in red.

Without Black checkbox

Activated: Automatically subtracts the lowest value of the gray distribution. The settings for the lower threshold value are deactivated.

Upper Threshold Value slider

Enter the upper threshold value for the gray value distribution using the slider or spin box/input field. All regions in the image with gray values below the lower threshold value are overlaid in blue and all those with gray values above the upper threshold value are overlaid in red.

Without Black checkbox

Activated: Automatically subtracts the highest value of the gray distribution. The settings for the upper threshold value are deactivated.

Show Threshold Values checkbox

Only visible if the **Show All** mode is activated.

Activated: Shows the threshold values as colored overlays in the original image.

Histo View tab

Here you can configure the display for the Histo view.

Show section

Statistical Values checkbox

Activated: Shows the table containing the statistical values in the image area.

Measurement Data Table checkbox

Activated: Shows the measurement data table below the original image.

Frequency Distribution checkbox

Only visible if the **Show All** mode is activated.

Activated: Shows the table containing the raw data for each channel.

Image checkbox

Only visible if the **Show All** mode is activated.

Activated: Shows the original image in the image area.

Channel section

Here you can activate or deactivate the histograms for each channel.

X/Y Axis section

Only visible if the **Show All** mode is activated.

Here you can determine the limits for the **X axis** and **Y axis**.

Auto button Sets the limits for the axes automatically.

Norm button Normalizes the histogram display to the maximum values of the distribution.

Fixed button Enter the min/max values for the histogram display in the **Min**/**Max** spin boxes/input fields.

Data Table section

Click on the **Create** button to create a data table from all the measured values displayed. To save the table, click on the **Save As** button.

New Image From section

Here you can create a new image document. Select the type of image from the dropdown list. To save the image, click on the **Save As** button.

Profile View

In the **Profile** view you can create intensity profiles of certain regions in your image. In the right image area you can see your image. In the left image area you can see the **Profile window**. The raw data for each channel are displayed in the **Profile table** below the **Profile window**. The measured values of measurements in the profile are shown in the **measurement data table** below the original image.

Information

To create an intensity profile of a certain region, select a tool on the **Profile Definition** tab. Use this to highlight a region in your image. An intensity profile of the region is generated automatically and displayed in the **Profile window**. To enlarge the view in the Profile window, drag out a rectangular frame using the left mouse button in the Profile window. The selected region is displayed in enlarged form. Right-click to return to the original view.

See also

- General view controls [→ 282]

View specific controls

Profile Definition tab

Tool bar

Using the tools you can add certain measurement lines to your image. The intensity profile of each line is shown in the Profile window.

Selection Changes the mouse pointer to Selection mode. You can use this to select graphic elements in the image.

Copy Use this to copy the last selected element and insert it at another position in the image.

Arrow Use this to insert a measurement line in the original image. The measurement is shown in the **Profile window** in the direction of the arrow.

Polygon Use this to insert a polygonal measurement line in the original image.

Freehand Use this to insert a measurement line with a shape of your choice.

Rectangle Use this to insert a rectangular measurement region.

Keep checkbox

Activated: Keeps the last selected tool active.

Color checkbox

Only visible if the **Show All** mode is activated.

Activated: Highlights each drawn-in measurement line with a random color.

Line Width spin box/input field

Only visible if the **Show All** mode is activated.

Here you can enter the line width of the measurement line.

Display Profile in Image checkbox

Activated: Also displays the profile of a measurement line in the original image.

Profile Measurements section

Normal button Switches the Profile window back to the view display.

Measurement button Use this to perform a point measurement in the profile. To adopt the value into the measurement data table, click on the **Insert Values** button.

X Measurement button Use this to perform a measurement of a region in the X direction in the profile. . To adopt the value into the measurement data table, click on the **Insert Values** button.

Y Measurement button Use this to perform a measurement of a region in the Y direction in the relevant profile. To adopt the value into the measurement data table, click on the **Insert Values** button.

Empty Table button

Empties the measurement data table below the original image.

Insert Values button

Adds the current measurement in the Profile window to the measurement data table below the original image.

Grid Distance section

Only visible if the **Show All** mode is activated.

Enter the grid distance of the measurement line using the slider or spin box/input field.

Profile View tab

Here you can configure the display for the Profile view.

Show section

Profile Table checkbox

Activated: Shows the profile table.

Measurement Data Table checkbox

Activated: Shows the measurement data table below the original image.

Image checkbox

Only visible if the **Show All** mode is activated.

Activated: Shows the original image in the image area.

Channel section

Here you can activate or deactivate the profiles for each channel.

X/Y Axis section

Only visible if the **Show All** mode is activated.

Here you can determine the limits for the **X axis** and **Y axis**.

Auto button Sets the limits for the axes automatically.

Norm button Normalizes the profile display to the maximum values of the distribution.

Fixed button Enter the min/max values for the profile display in the **Min**/**Max** spin boxes/input fields.

Data Table section

Click on the **Create** button to create a data table from all the measured values displayed. To save the table, click on the **Save As** button.

New Image From section

Here you can create a new image document. Select the type of image from the dropdown list. To save the image, click on the **Save As** button.

Measure View

In this view measured values from images are displayed in a table. The table is only visible if there are annotations/measured values in the image. To highlight the row of the table containing the measured values of a graphic element, click on a graphic element in the image. To highlight a graphic element in the image, click on the measured value in the row of the table.

See also

General view controls [→ 282]

View specific controls

Measure tab

Here you can specify how to draw the graphic elements for measurements into an image and how the measurement data are displayed. You can also add user-specific features to individual graphic elements.

Graphic Elements section

Only visible if the current image is a multidimensional image.

Here you can decide, for multidimensional images, whether to draw a graphic element "globally" into all channels, Z-positions, time points, etc., or whether to draw in separate elements for the view currently displayed.

Channel checkbox

Activated: Activates the Single Channel mode. Only draws graphic elements into the channel currently displayed.

Time checkbox

Activated: Only draws graphic elements into the time point currently displayed.

Z-Position checkbox

Activated: Only draws graphic elements into the Z-position currently displayed.

Copy in All Following checkbox

Activated: Draws a new graphic element into the view currently displayed and into all subsequent time points or Z-positions.

New Feature section

Here you can add a defined feature to the selected graphic element.

Name input field

Here you can enter the name you want to give the feature.

Value input field

Here you can enter the value that you want the feature to have for the current graphic element.

Unit input field

Here you can enter the unit you want for the feature.

Add button

Adds the feature. The measurement data table is expanded to include this feature.

Remove button

Removes the selected feature.

Measurement Data section

Here you can specify how you want the measured values for the drawn-in graphic elements to be displayed.

Table button

Displays the measured values in a row of a table. As you can specify the features individually for each graphic element, the number of columns containing measured values may differ from graphic element to graphic element (i.e. from row to row).

List button

Displays each measured value in a separate row. The measurement data table then has the following defined columns:

Name Name of the graphic element (e.g. line)

Feature Name of the feature (e.g. distance)

Value Value of the feature

Unit Unit of the feature (e.g. µm)

Current View button

Only displays the measured values of the current view.

All Views button

Displays all measured values contained in the image.

Create Measurement Data Table button

Creates a measurement data table from the measured values displayed. The table may be saved as a separate document.

Info View

The **Info View** allows you to display extensive information about your image. Using the buttons in each of the sections you can show additional fields in the sections or hide fields that are currently showing. To show or hide individual sections, click on the button to the left of the headings for each of the sections.

Information

The **Info View** only shows the fields that actually contain data. Using the buttons in each of the sections you can show additional fields. To do this, activate the corresponding check-boxes in the shortcut menu.

General section

Title input field

Here you can enter a title for your image.

Description input field

Here you can enter a description for your image.

Comment input field

Here you can enter a comment.

Keywords input field

Here you can enter keywords for your image.

Rating input field

Here you can enter a rating for your image. To enter a rating, click on the star icons.

File section

Name display field

Displays the file name of the image.

File Type display field

Displays the file type of the image.

Path display field

Displays the location where the image is saved in your file system.

File Size display field

Displays the file size of the image.

Created On display field

Displays when the image was created.

Last Changed display field

Displays when the image was last changed.

User Name display field

Displays the name of the user. You can enter the user name in the **Extras** menu | **Options** | User Management [➙ 47].

Image Dimensions section

Time Series

Displays how many time points the image contains. The value in brackets shows the full duration of acquisition.

Z-Stack

Displays how many Z-planes the image contains. The value in brackets shows the full size of the Z-stack.

Channels

Displays how many channels the image contains.

Tiles

Displays how many individual images (tiles) the image is composed of.

Scaling (per Pixel)

Displays the scaling per pixel.

Edit button

Opens the Edit Scaling [➞ 247] dialog window.

Image Size (Pixels)

Displays the image size in pixels. The first number indicates the horizontal dimension and the second the vertical dimension.

Image Size (Scaled)

Displays the scaled image size. The first number indicates the horizontal dimension and the second the vertical dimension.

Image Format

Displays the bit depth. This depends on the camera settings.

Stage Position

Displays the stage position. Within the image this is the center point. In the case of tile images this is the center point of the first tile.

Scanning mode

Displays the scanning mode. This can either be the image field, an image line or a pixel.

Zoom

Displays the zoom factor. The value 1 corresponds to the standardized image field of all confocal systems.

Rotation

Displays the rotation of the image field around the optical axis.

Crop Offset

Displays the shift of the scanned region from the center of the image.

Pixel Time

Displays for how long the emission signal is collected per pixel. This is the so-called integration time.

Line Time

Displays how long the system needs to scan an image line.

Image Field Time

Displays how long the system needs to scan the image field displayed in X and Y in full.

Averaging

Displays the number of individual measurements per image or line. The average of the individual measurements produces the pixel intensity values for the image.

Acquisition section

Microscope

Displays which microscope was used to acquire the image.

Objective

Displays which objective was used to acquire the image.

Optovar

Displays which Optovar was used to acquire the image.

Reflector

Displays which reflector cube was used to acquire the image.

Beam Splitter

Displays which beam splitter was used to acquire the channel.

Emission Wavelength

Displays the main emission wavelength of the channel or dye used.

Excitation Wavelength

Displays the main excitation wavelength of the channel or dye used.

Contrast Method

Displays the contrast technique. In transmitted light this is the condenser setting, while in reflected light it corresponds to the selected reflector cube.

Lamp Intensity

Displays the lamp intensity with which the image was acquired.

Confocal Diaphragm

Displays the diameter of the confocal diaphragm.

Percent Laser Power

Displays the percentage of laser power used for acquisition.

Laser Blanking

Blanking of the laser during scanner movement without acquisition.

Bleaching Laser Power

Displays the laser power used for bleaching.

Channel Name

Displays the name of the channel.

Channel Description

Here you can enter a description of the channel. Describe the exact use of the channel or what can be seen in this channel.

Dye

Displays the name of the dye.

Channel Color

Displays the pseudo color allocated to the channel.

Camera

Displays which camera was used to acquire the image.

Camera Adapter

Displays which camera adapter was used to acquire the image.

EM Gain

Displays the factor by which the camera signal was increased.

Exposure Time

Displays the exposure time with which the image was acquired.

Depth of Field

Displays the depth of field. This is calculated according to the following formula:

Depth of field = (2 * n * λ) / (NA)² = (2 * refractive index * emission wavelength) / (numerical aperture)²

Section Thickness

Displays the thickness of the optical section.

Binning

Displays whether binning was applied during acquisition and how much.

Detector

Displays which detector was used for acquisition.

Detector Gain

Displays the gain setting of the detector for acquisition.

Digital Detector Gain

Displays the digital gain of the detector during acquisition.

Detector Offset

Displays the offset settings of the detector during acquisition.

Information

In the case of multichannel images the channel-dependent information is saved in a table. Here the sorting of the individual information fields may differ.

Info View dialog window

Edit Scaling dialog window

The **Edit Scaling** dialog window is divided up into table form. The columns contain the **Scaling Factor** and **Scaling Unit** and the rows the dimensions.

Scaling Factor column

Enter the desired scaling factor in the spin boxes/input fields.

Scaling Unit column

Select the desired scaling unit from the dropdown list. The metric units **Meter**, **Centimeter**, **Millimeter**, **Micrometer** and **Nanometer** are available as options, as well as the imperial units **Inch** and **Mil**.

Row X

Shows the scaling in the horizontal direction.

Row Y

Shows the scaling in the vertical direction.

Row Z

Shows the scaling in the 3rd dimension. This is usually the focus direction.

Information

Row **Z** for the third dimension is only displayed if the image has a third dimension.

OK button

Adopts the settings into the current image.

Cancel button

Closes the dialog window without adopting the settings.

4.1.2 Specific image views

These image views are only visible if the image has corresponding features. The **3D view**, for example, is only visible for Z-stack images.

Channels View

Only visible for multichannel images.

In the **Channels** view you will see the channels of a multichannel image. The channels are displayed side by side, in the channel colors that have been assigned to them. You will also see the mixed image view in which all the channels are overlaid.

Information

By double-clicking on a channel image, you can switch quickly to the **2D** view.

Double-clicking on the image in the **2D** view switches you back to the **Channels** view. If you double-click on one of the displayed channels, only this channel will be shown in 2D View. If you double-click on the merge view, it will be shown in 2D View.

See also

2D View [→ 225]

General view controls [→ 282]

Ortho View

Only visible for Z-stack images.

In the **Ortho** (orthogonal section) view you can analyze your Z-stack images. Here, in addition to the top view (X/Y axis), you will also see the section views of the X/Z axes (top) and Y/Z axes (right).

See also

General view controls [→ 282]

View specific controls

Ortho Display tab

Section Lines section

Enter the positions (pixel values) for the section lines using the **X**/**Y**/**Z** sliders or spin boxes/input fields.

Information

Alternatively you can also adjust the positions directly in the image area. To adjust the positions, move the mouse over a section line in the image. Hold down the left mouse button and move the mouse.

To position the relevant slider at the center of the view, click on the **Center** button.

Line Thickness section

Only visible if the **Show All** mode is activated.

Enter the thickness of the section lines in pixels using the sliders or spin boxes/input fields. This results in a maximum intensity projection being displayed over the selected pixel width.

Line Opacity slider

Only visible if the **Show All** mode is activated.

Here you can enter the degree of opacity of the section lines from 0% (invisible) to 100% (completely opaque).

Maximum Intensity Projection checkbox

Activated: Displays a maximum intensity projection (MIP) across all planes for all 3 views. The section lines are hidden and the control elements that are not relevant in this view are deactivated.

3D Distance Measurement checkbox

Only visible if the **Show All** mode is activated.

Activated: Activates the 3D distance measurement. The **Set Start** and **Set End** buttons, the coordinate displays and the **Distance** display window are visible. To set a starting point for the measurement, navigate the cutlines to the desired starting point and click on the **Set Start** button. The **Set End** button will become active. To set an end point for the measurement, navigate the cutlines to the desired end point and click on the **Set End** button. The pixel coordinates of the measurement points are displayed next to the buttons. The measured distance is displayed in the **Distance** display field.

New Image section

Here you can create a new image document. Select the desired view from the dropdown list (only in **Show All** mode). To save the image, click on the **Save As** button.

Information

The resulting image contains the image data in the same dynamics (bit depth) as the original image and consists of the same number of channels (in the case of multichannel images) or time points (in the case of time lapse images) as the original image, but only contains the Z-plane currently displayed.

3D View

Only visible for Z-stack images.

In the **3D** view you can display your Z-stack fluorescence images three-dimensionally.

See also

General view controls [→ 282]

3D View tool bars

The tool bars are arranged to the left and right of the image area and underneath it. You can use the tools to control the display of the 3D volumes in the image area.

Left tool bar

Top thumb wheel

Enlarges or reduces the image area.

Bottom thumb wheel

Rotates the 3D volume around the horizontal (X) axis.

Tool bar

Arrow Use this to select end points of measurement tools that have been drawn into the 3D volume (**Measure** tab). You can then edit the position of the end points.

Editing end points of measurement tools

➢ The **Arrow** tool has been selected.

1. Move the mouse pointer over a blue-highlighted end point of a measurement tool.
   ⇨ The mouse pointer appears in the form of cross hairs.
2. Left-click on the end point and hold down the left mouse button.
   ⇨ The end point is selected and highlighted in yellow.
3. Move the end point to another position. When the mouse pointer appears in the form of cross hairs again you can release the left mouse button.

⇨ This position is then adopted as the new end point.

Rotate Use this to rotate the 3D volume in any way you wish within the space. This is the default mode when you switch to **3D view** for the first time.

Rotating the 3D volume

➢ The **Rotate** tool has been selected.

1. Move the mouse pointer inside the image area.
2. Hold down the left mouse button and move the mouse.

⇨ The 3D volume is rotated according to the movements of the mouse.

Zoom Use this to increase or reduce the zoom factor of the image area.

Increasing/reducing the zoom factor of the image area

➢ The **Zoom** tool has been selected.

1. Move the mouse pointer inside the image area.
   ⇨ The mouse pointer appears in the form of a magnifying glass.
2. Hold down the left mouse button.
3. Move the mouse up.

⇨ The zoom factor of the image area is increased.

1. Move the mouse down.

⇨ The zoom factor of the image area is reduced.

Alternatively, hold down the **Ctrl** key and the left mouse button and then move the mouse.

Move Use this to move the 3D volume laterally.

Moving the 3D volume

➢ The **Move** tool has been selected.

1. Move the mouse pointer inside the image area.
   ⇨ The mouse pointer appears in the form of a hand.
2. Move the mouse in a direction of your choice.

⇨ The 3D volume is moved.

Alternatively, hold down the **Shift** key and the left mouse button and then move the mouse.

Fly Use this to fly through the 3D volume.

Flying through the 3D volume

➢ The **Fly** tool has been selected.

1. Move the mouse pointer inside the image area.
2. Hold down the left mouse button.

⇨ The view flies through the 3D volume. If you move the mouse during this process, you can influence the trajectory. Try it out and see.

Bottom tool bar

Left thumb wheel

Use this to rotate the 3D volume around the vertical (Y) axis.

Right thumb wheel

Use this to rotate the 3D volume around the (Z) axis perpendicular to the screen plane.

Tool bar

Start View Use this to switch back to the start view from any view. A top view of the 3D volume is displayed. Lateral movements and the zoom factor are adjusted so that the 3D volume can be seen at the center of the image area.

Show Measurements Use this to show or hide drawn-in measurements. If measurements are being shown, a table of the measurements present in the image appears at the right edge.

Bounding Box Use this to show or hide a bounding box around the 3D volume.

Highlight Coordinate Axes in Color Use this to highlight the coordinate axes.

- X axis = red
- Y axis = blue
- Z axis = green

Show Scaling Use this to show the scaling on each axis.

Animation Mode Use this to set the 3D volume in continuous motion.

Animating the 3D volume

➢ The Animation Mode tool has been selected.

1. Move the mouse inside the image area.
2. Hold down the left mouse button and move the mouse.
3. Release the left mouse button again.

⇨ The 3D volume rotates continuously in the direction in which you moved the mouse. If you move the mouse quickly, the 3D volume rotates quickly. If you move the mouse slowly, the 3D volume rotates slowly.

⇨ To stop the animation, left-click again in the image area.

Right tool bar

X/Y clipping plane (blue) Use this to show or hide the X/Y clipping plane.

X/Z clipping plane (green) Use this to show or hide the X/Z clipping plane.

Y/Z clipping plane (red) Use this to show or hide the Y/Z clipping plane.

Snapshot Use this to create a 2D image of the current view. The image is a 24 bit color image. All annotations are burnt in automatically.

Plus Use this to add the current view to a position list as a new position. With the help of position lists you can have your view calculated as a series of individual images. This series can then be exported as a film, for example.

Play Only active if a position list containing at least two saved positions exists. Use this to play back a preview of the series that is calculated. To stop the preview, click on the button again.

View specific controls

3D tab

Here you can specify which view mode you want to use to display the 3D volume. There are 5 view modes available. To activate the desired view mode, click on the relevant button.

View Mode buttons

Shadow button Activates Shadow view mode .

Transparency button Activates Transparency view mode .

Maximum button Activates Maximum view mode .

Surface button Activates Surface view mode .

Mixed button Activates Mixed view mode .

Fine/Fast slider

Enter the level of detail with which you want the view to be calculated here.

Information

If you select the **Fine** setting, all the information present in the image is used to achieve the best possible display. The calculation time can increase accordingly.

If you select the **Fast** setting, the image data are significantly reduced before the calculation. The calculation is fast, but only a very coarse 3D display of the volume is achieved.

Color-Code Depth checkbox

Only active if **Transparency**, **Maximum** or **Mixed** mode is activated.

Activated: Replaces the channel colors of the volume with a rainbow color table. This is also shown as a palette with an indication of the depth (in scaled units).

Calculation Method dropdown list

Only visible if the **Show All** mode is activated.

This dropdown list is not available in **Mixed mode**.

Accelerated This list entry is only available in **Transparency**, **Maximum** and **Surface** modes.

Performs the calculation using the graphics card. The display is faster and you have more setting options.

Restricted This list entry is only available in **Transparency**, **Maximum** and **Surface** modes.

Performs the calculation using the computer's CPU only. The setting options are restricted here.

Detailed This list entry is only available in **Surface** mode.

Die Beschreibung dieses Listeneintrags wird nachgereicht.

Free View This list entry is only available in **Shadow mode**.

Allows you to position the 3D volume freely.

Front This list entry is only available in **Shadow mode**.

Shows the 3D volume from the front.

Back This list entry is only available in **Shadow mode**.

Shows the 3D volume from the back.

Clipping Planes buttons

Only visible if the **Show All** mode is activated.

Only visible if **Transparency**, **Maximum**, **Surface** or **Mixed** view mode is activated.

Using the three buttons you can activate or deactivate clipping planes in the 3D volume.

If you right-click on one of the buttons, a shortcut menu opens. Here you can choose whether you want the front, back or both sides of the 3D volume to be clipped. You can also specify how the clipping plane is displayed.

Under each button is a slider. You can use this to move the relevant clipping plane within the volume.

Wedge checkbox

Only visible if the **Show All** mode is activated.

Activated: Activates two texture planes. Only the sector between the planes is cut out. You can select which planes you want to be used for the wedge function from the dropdown list. The selection is also visible in the relevant buttons.

Saved dropdown list

Only visible if the **Show All** mode is activated.

Here you can select saved 3D settings.

button

Only visible if the **Show All** mode is activated.

Opens a shortcut menu with the following options:

New Creates a new settings file that is given a name automatically and has the file extension *.cz3dr. The settings file can be found in the user path under \My Documents\Carl Zeiss\ZEN \Documents\3D render settings

Delete Deletes the selected settings file from the hard drive.

Rename Renames the selected settings file. Enter a new name in the input field and confirm with **OK**.

Save As Saves the selected settings file under a different name.

Import Imports a *.cz3dr file and applies it to the current image.

Export Exports a *.cz3dr file to a different location.

Create Image button

Creates a new image from the current view. This image is a 24 bit RGB color image. All graphic elements, such as annotations, are burnt in.

Appearance tab

Here you can define the appearance of the 3D volume. On the tabs available on this tab, select the setting that you want to change (e.g. Transparency). Depending on which view mode you have activated, different control elements are available on the tabs.

Transparency tab

Channel Selection

Here you can select the channel of a multichannel image for which you want to set the transparency.

Threshold Value slider

Here you can set the lower threshold value in percent of the gray levels displayed. With this setting you specify the gray value range for the relevant channel that you want to be included in the rendered image.

Ramp Width slider

Here you can set the extent of the transition from completely transparent to completely opaque (0-100 percent).

Maximum slider

Here you can enter the level of opacity (0-100 percent).

Histogram Display

Here the settings that you enter using the sliders are displayed schematically. The X axis represents the gray level values and the Y axis the opacity. You can also change the position of the curve using the mouse.

Reset button

Resets all entries to the original values.

Surface tab

Only visible if **Shadow**, **Surface** or **Mixed** view mode is activated.

Roughness slider

Only visible if **Shadow** view mode is activated.

Enter the roughness of the surface of the 3D volume using the slider or spin box/input field. This changes the plasticity of the display.

Information

If pixels enter the saturation range, artifacts may occur. In this case enter a lower roughness value.

The following sliders are only visible if **Surface** or **Mixed** view mode is activated:

Threshold Value slider

Here you can set the lower threshold value in percent of the gray levels displayed. With this setting you specify the gray value range for the relevant channel that you want to be included in the rendered image.

Diffuse Light slider

Here you can set the basic brightness on a scale from 0 to 100%.

Directional Light slider

Here you can set the directional brightness from 0 to 100%. This value influences the differences between bright and dark structures.

Shine slider

Here you can set the surface shine.

Reset button

Resets all entries to the original values.

Channels tab

Only visible if **Mixed** view mode is activated.

Here you can specify how Transparency and Surface are mixed. In the case of multichannel images you can also configure these settings differently for each channel.

Activate the corresponding checkboxes for Transparency and Surface in the list.

Background tab

Background Color section

Here you can set the background color for the 3D view. To do this, click on the color field and select the desired color.

Background Distance section

Only visible if **Shadow** view mode is activated.

Enter the distance of the background from the 3D volume using the slider or spin box/input field.

Shadow checkbox

Only visible if **Shadow** view mode is activated.

Activated: Displays the shadow cast by the 3D volume.

Light tab

Brightness slider

Here you can enter the brightness of the light source.

Distance slider

Only visible if **Surface** or **Mixed** view mode is activated.

Here you can enter the distance of the light source from the 3D volume.

Azimuth slider

Here you can enter the angle of the light source above the virtual horizon.

Extension slider

Here you can enter the light source's horizontal angle of incidence.

Light Source Display

As an alternative to the slider or input field, you can set the Azimuth and Extension together by using the mouse to move the point within the light source display.

Illuminated Interiors checkbox

Activated: Illuminates the interior of a surface-rendered volume.

Colored Interiors checkbox

Activated: Illuminates the interior of a surface-rendered volume in color. It is easier to differentiate the inside from the outside if this option is activated. In this case the illumination is colored blue.

Reset button

Resets all entries to the original values.

Projection tab

Projection Angle slider

Here you can set the projection angle at which you want to view the scene freely between 0° and 80°. The effect of this on the perspective display is as if you are viewing the 3D image through a telephoto or wide-angle lens.

Z Extension slider

Here you can set the extension of the volume in the Z direction (value range 10% - 600%).

Stereo Image checkbox

Activated: Displays the 3D volume as anaglyphs. You can choose between the **Red/Green** or **Red/Cyan** radio button.

Camera Distance slider

Here you can enter the distance between the two virtual cameras (0-20%).

Parallax slider

Here you can enter the degree of movement that is necessary to bring the two camera images back into line (-100 to +100%).

Reset button

Resets all entries to the original values.

Clipping Planes tab

Only visible if the **Show All** mode is activated.

Here you can edit clipping planes. To select a clipping plane, click on the relevant button. The editing functions which you can use to modify the selected clipping plane become visible below the buttons.

X/Y button

Activates the editing functions for the X/Y clipping plane.

X/Z button

Activates the editing functions for the X/Z clipping plane.

Y/Z button

Activates the editing functions for the Y/Z clipping plane.

Activate Clipping Planes checkbox

Activated: Automatically inserts all 3 clipping planes into the 3D volume. Activates the editing functions for each clipping plane.

Information

On the **Clipping Planes** tab you can edit the clipping planes. On the **3D** tab you can activate or deactivate the relevant clipping planes in the 3D volume.

Reset button

Resets all entries to the original values.

The following functions are only visible if the **Activate Clipping Plane** checkbox is activated and a clipping plane has been selected:

Activate checkbox

Activated: Activates the selected clipping plane in the 3D volume. Each plane is positioned at the center of the 3D volume and aligned orthogonally (in the X/Y, X/Z, Y/Z direction).

Change the display of the selected clipping plane using the dropdown list to the right of the checkbox. The following settings are available:

Invisible	The plane is invisible.
Colored	The plane is displayed in color. The frame color is used with 50% transparency here.
Black/White	The data above the threshold value that are touched by the clipping plane are displayed in binary form as a white area. Black pixels are non-transparent.
Transparent	The data that are touched by the clipping plane are displayed as they are in **Transparent** view mode, but in 2 dimensions. The ramp for the transparency is linear here. Black pixels are transparent.
Textured	The data that are touched by the clipping plane are displayed in textured form. Black pixels are transparent.
Finely Textured	The display appears as it does with the **Textured** setting, but with full resolution. Black pixels are transparent.
Opaque	The display appears as it does with the **Transparent** setting. Black pixels do not let any light through, however, meaning that the render data behind them are not displayed.

Frame checkbox

Activated: Displays the frame of the selected clipping plane. Enter the frame color via the color field.

Clip Front checkbox

Activated: Clips the front of the 3D volume.

Clip Back checkbox

Activated: Clips the back of the 3D volume.

Clip Transparency checkbox

Only active if **Mixed** view mode is activated.

Activated: In addition to the surface data, also clips the transparency data.

Clip Surfaces section

Only visible if **Surface** or **Mixed** view mode is activated.

Here you can enter which channel you want to be clipped using the channel buttons.

Orientation Sliders section

Position Here you can enter the position of the selected clipping plane.

<X (X Angle) Here you can enter the X angle for the selected clipping plane.

<Y (Y Angle) Here you can enter the Y angle for the selected clipping plane.

Reset Orientation button

Resets the selected clipping plane to the original position.

Series tab

Here you can create render series of individual views, which you can later view and export as a film.

The tab contains different control elements depending on the series type. The following elements are the same, however, for all types: **Saving of Settings**, **Selection of Settings**, **Apply** button, **Number of Individual Images** input field and **Fixed Resolution** checkbox.

Settings according to series type

It is possible to create 5 different types of series:

- Rotation around a fixed X, Y or Z axis, indicating the start and end point
- Definition of a start and end point
- Definition of a list of positions

Select the type of series that you want to create from the **Series Type** dropdown list.

Rotation control elements

The X rotation, Y rotation and Z rotation series types all have the same control elements and differ only in the axis around which the rotation is calculated.

The preview function is not available for these types of series.

Panorama/Partial Panorama selection field

Select the 360° panorama if you want to generate a complete rotation.

If you select a partial panorama, you can specify the starting angle and stopping angle that you want to be used to create the series. To do this, enter the desired values in the input fields.

The direction of rotation with which you want the series to be created can be specified using the two buttons showing the rotation icons.

Angle Definition control element

When you are configuring a partial panorama, the desired angles can also be indicated easily using the circular control element: Grab the white start/stop points with the mouse and position these accordingly on the circle. The number of individual images is also displayed here.

Use the **Apply** button to create the series.

Start/Stop control elements

If you have selected Start/Stop as the series type, 4 sliders will appear with input fields for the individual parameters to define the starting and stopping position.

Starting Position

Select the **Starting Position** option.

You can position the volume as required using the mouse. The geometric parameters are displayed in the input fields. You can also indicate the angle for X, Y or Z and the zoom factor directly using the slider or value field. All changes are displayed immediately in the image area.

Stopping Position

Select the **Stopping Position** option.

You can position the volume as required using the mouse. The geometric parameters are displayed in the input fields. You can also indicate the angle for X, Y or Z and the zoom factor directly using the slider or value field. All changes are displayed immediately in the image area.

Use the **Apply** button to create the series.

Position List control elements

With the Position List series type you can save any number of views of the volume within the space as positions in a position list and in this way generate an animation around changing rotation axes or with changing zoom factors.

The positions are displayed in a list view.

Append/Insert buttons

You can add a current position by clicking on the **Append** button. If you want to insert a new position between two existing positions, click on the **Insert** button.

Position List

Each position is displayed in the list with its X, Y, Z angle and zoom level. Using the control elements at the bottom of the list you can change the order of the positions (arrow buttons), cut positions (scissors icon) and insert them again at another position (insert icon) or delete positions (wastepaper bin icon).

If you want to delete all positions, click on the **Empty List** button.

Parameters checkbox

You can determine which of the following parameters you want to be taken into consideration when the series is calculated. To do this, select the corresponding option in the checkbox:

- Light: Illumination parameters are considered
- Transparency: Various transparency settings (not in Surface mode)
- Background: Color and distance of the background
- Time Series (only for time lapse images)
- Viewing Angle
- Planes (not in Shadow mode)
- Surfaces (only in Surface and Mixed mode)

Saving of Settings

Saving of Settings

Clicking on the **Options** dropdown button opens a shortcut menu with the following options:

- **New**: Creates a new **settings file**. This file can be found in the user's local document path (e.g. \My Documents\Carl Zeiss\ZEN\Documents, in a corresponding subfolder).
- **Delete**: Deletes the settings file currently selected in the **Saved** dropdown list from the hard drive.
- **Rename**: Opens a dialog to rename the currently selected settings file.
- **Save As** ... : Saves a copy of the currently selected settings file under a different name.

- **Save**: Saves changes to a currently selected settings file.
- **Import**: Opens settings files that have been saved at a location other than the default location using an **Open File** dialog.
- **Export**: Saves settings files at a location of your choice using a **Save File** dialog.

Settings Selection

Select Saved Settings

Via a dropdown list you can select a settings file. This is then used when you click on the **Apply** button.

Preview

To obtain a preview of the series to be created, click on the **Play** button. To stop the preview, click on the **Stop** button.

Apply button

Apply button

The series is calculated when you click on the **Apply** button.

Number of Individual Images input field

Number of Individual Images input field

Here you can indicate the number of individual images that you want the series to consist of after the calculation. The more individual images that you specify here, the more fluidly the scene transitions will be displayed later. Click on the dropdown button to select predefined values from a list.

Fixed Resolution checkbox

As a rule, the image series is calculated using the current screen resolution. If you want to set a different format for the series, select the **Fixed Resolution** checkbox. In the input fields that are now visible you can enter the width x height in pixels with which you want the series to be created.

Measure tab

Only visible if the **Show All** mode is activated.

Here you can perform interactive measurements in the 3D volume. No measurements are possible in Shadow view mode. The measurement tools and measured values are displayed in the 3D volume and can be changed there. The measured values can also be displayed in the form of a table and processed further in other programs.

Tool bar

Using the tools you can perform interactive measurements in the 3D volume. The following tools are available:

Selection Changes the mouse pointer to Selection mode. Use this to select measurements in the 3D volume in order to change them.

Line Use this to measure the length of a line in µm. Click once on the starting point and hold down the mouse button. Then drag the mouse to the end point and release the mouse button again. The measurement is complete. The result of the measurement is displayed in the list to the right of the image area.

Angle Use this to measure the angle between two connected legs. First define the starting point. Then use the mouse to drag the first leg to the desired first end point. Define the second leg by clicking on the second end point. The angle measurement ends with a display of the angle measured (in degrees). The result of the measurement is displayed in the list to the right of the image area.

Curve (Polygon) Use this to measure along a line with any number of segments. Click from corner point to corner point. Complete the measurement by right-clicking. The result of the measurement is displayed in the list to the right of the image area.

Information

All measurement tools are also displayed on the **3D Graphics** tab. You can make changes to the format and name there.

Keep Tool checkbox

Activated: Keeps the selected tool active.

Change Color checkbox

Activated: Automatically changes the color of the drawn-in tool.

Display Measurements checkbox

Activated: Shows the list of measured values to the right of the image area. The following settings are also activated:

In Foreground checkbox

Activated: Brings all drawn-in measurement tools into the foreground, even if these are in fact obscured by image structures.

Display Values section

In Image checkbox

Activated: Displays the measured values in the 3D volume.

As List checkbox

Activated: Displays the measured values in the measurement data table.

Delete Selected button

Only active if a measurement tool has been selected in the 3D volume.

Deletes selected measurement tools from the 3D volume.

Delete All button

Deletes all measurement tools from the 3D volume.

3D Graphics tab

Only visible if the **Show All** mode is activated.

Tool bar

Using the tools you can perform interactive measurements in the 3D volume. The following tools are available:

Selection Changes the mouse pointer to Selection mode. Use this to select measurements in the 3D volume in order to change them.

Line Use this to measure the length of a line in µm. Click once on the starting point and hold down the mouse button. Then drag the mouse to the end point and release the mouse button again. The measurement is complete. The result of the measurement is displayed in the list to the right of the image area.

Angle Use this to measure the angle between two connected legs. First define the starting point. Then use the mouse to drag the first leg to the desired first end point. Define the second leg by clicking on the second end point. The angle measurement ends with a display of the angle measured (in degrees). The result of the measurement is displayed in the list to the right of the image area.

Curve (Polygon) Use this to measure along a line with any number of segments. Click from corner point to corner point. Complete the measurement by right-clicking. The result of the measurement is displayed in the list to the right of the image area.

Information

All measurement tools are also displayed on the **3D Graphics** tab. You can make changes to the format and name there.

Measurement Tools list

All the measurement tools contained in the 3D volume are displayed here. The list contains the following columns:

Eye icon Here you can select whether or not a measurement tool is displayed in the image. If you click in the title field of the column, the setting is made simultaneously for all entries.

Lock No function!

Type Displays the tool type. If you click on the icon, you can change the color of the tool.

ID Displays the unique identification number of the measurement tool.

A No function

M checkbox **Activated:** Displays the measurement data in the image. If you click in the title field of the column, the setting is made simultaneously for all entries.

Name Displays the name of the tool. To change the name, double-click on the entry. Then enter a new name. Confirm the entry with the Enter key.

Renderer tab

Only visible if the **Show All** mode is activated.

Here you will find special settings that do not normally have to be changed.

Fly Mode section In the **Flight Speed** input field you can enter the speed (value range 1-1000%) that you want to be used for Fly mode.

Appearance section

Draw Edge Triangles checkbox

Using this option you can specify whether smooth areas are displayed in Surface mode. If this option is not activated, these areas have the appearance of openings through which you can look into the rendered volume.

2D Textures checkbox

Activated: Uses 2D contours instead of 3D textures.

Texture Opacity input field:

Changes the opacity of all textures.

Texture Change Angle input field

Here you can enter the angle at which the next collection of 2D textures is loaded (value range 0-90°).

Renderer section

OpenGL Mode dropdown list

In the event of problems with the graphics card, the OpenGL calculations can be performed by the CPU. Select the **Software** entry from the dropdown list to use this option.

Use Palette Textures checkbox

Activated: Uses palette textures. This is only possible with older graphics cards.

Use Intensity Alpha Textures checkbox

Activated: Uses a particularly memory-efficient texture type for single-channel images.

Use Display Lists checkbox

Activated: Uses display lists for precalculation. This can speed up the display.

Hardware-Accelerated Series checkbox

Activated: Speeds up the calculation of render series. Deactivate this function in the event of problems with the creation of series.

Limit Memory checkbox

Activated: Limits the maximum graphics card memory used.

Reset button

Resets all entries to the original values.

Colocalization View

Only visible for multichannel fluorescence images.

In the **Colocal.** (colocalization) view, you can analyze the extent of colocalization quantitatively in two fluorescence channels. The view consists of two main areas: the **X/Y scatter plot** on the left and the actual image (2 channels are displayed) in the right image area. Using the **Colocalization** tab, you can also display the **Colocalization table** in the lower image area.

Information

The channels that you are comparing with one another are displayed in the image area in the form of a color overlay. The channel color of the image is used here. If the images have more than 2 channels, you can add additional channels on the **Dimensions** tab. This temporary selection is deactivated, however, when you select the channels to be compared on the **Colocalization** tab.

See also

General view controls [→ 282]

X/Y Scatter Plot

The pixel intensities of two channels are plotted against one another in the diagram and each pixel pair with the same X/Y image coordinates is displayed as a point. The frequency with which pixels of a certain brightness occur is visualized by means of a color palette that is displayed at the bottom of the diagram. The relative value range lies between 0-255.

The vertical and horizontal axes show the gray value range that applies for the relevant channel.

The diagram is overlaid with two lines that subdivide it into 4 quadrants, numbered from 1-4. Using the mouse you can position the lines freely and therefore adjust the threshold values to the data.

The quadrants have the following meanings:

- 1: Non-colocalizing pixels from channel 1
- 2: Non-colocalizing pixels from channel 2
- 3: Colocalizing pixels
- 4: Background

Colocalization table

Only visible if the **Table** checkbox is activated on the **Colocalization** tab.

For each quadrant of the scatter plot there is a correspondingly labeled row in the table. The **Global** row contains the values for the entire image. The table contains columns for the following measured values:

Region

Once a region has been selected it has a number assigned to it. This number appears in the image and in the table.

Quadrant

Indicates the measured values for the four quadrants of the scatter plot.

Pixel Number

Shows the total number of pixels of each quadrant. The sum of all pixels in this column for all 4 quadrants corresponds to the product of the height x width of the original image.

Area (µm²)

Area = number of pixels x scaling factor for X/Y
If there is no scaling for the original image, the following applies: 1 pixel = 1 µm.

Relative Area (%)

Relative area = area of quadrant/total area

Pearson's Correlation Coefficient

Provides information on the intensity distribution within the colocalization region.
Value range: -1 to 1.

1: All pixels are on a straight line in the scatter plot from bottom left to top right (if, for example, you have used the same channel twice for the colocalization, you will find the value 1 in this column).

0: The pixels in the scatter plot are distributed in a cloud without a preferred direction.

-1: The pixels do not overlap. The scatter plot stretches from top left to bottom right. This situation can be described as negative colocalization and means "exclusion".

The calculation formula is as follows:

GV: Gray Value; AV: Average Gray Value; C: Channel

Manders' Correlation Coefficient

Insensitive to differences in the signal intensity between the two channels and bleaching.

Value range: 0 to 1

The calculation formula is as follows:

$$\frac{\sum Grauwert\,K1 \cdot Grauwert\,K2}{\sqrt{\sum Grauwert\,K1^2 \cdot \sum Grauwert\,K2^2}}$$

C: Channel

Coloc. Coefficient 1

This coefficient indicates the relative number of colocalized pixels in channel 1 in relation to the total number of pixels above the threshold value:

$$\frac{\sum Pixels\,Kanal\,1_{kolokalisiert}}{\sum Pixels\,Kanal\,1_{gesamt}}$$

The values range between 0 and 1, with 0 indicating no colocalization and 1 indicating full colocalization.

Numerator = Number of pixels in quadrant 3

Denominator = Number of pixels in quadrant 3 + number of pixels in quadrant 1

Coloc. Coefficient 2

This coefficient indicates the relative number of colocalized pixels in channel 2 in relation to the total number of pixels above the threshold value:

$$\frac{\sum Pixels\,Kanal\,2_{kolokalisiert}}{\sum Pixels\,Kanal\,2_{gesamt}}$$

The values range between 0 and 1, with 0 indicating no colocalization and 1 indicating full colocalization.

Numerator = Number of pixels in quadrant 3

Denominator = Number of pixels in quadrant 3 + number of pixels in quadrant 2

CC (weighted) 1

Weighted correlation coefficient channel 1. Calculated like the simple colocalization coefficient, but using the sum of the gray value intensity rather than the number of pixels.

$$\frac{\sum SummeGrauwerteKanal_{kolokalisiert}}{\sum SummeGrauwerteKanal_{gesamt}}$$

The values range between 0 and 1, with 0 indicating no colocalization and 1 indicating full colocalization.

Numerator = Sum of intensity of all pixels in quadrant 3

Denominator = Sum of intensity of all pixels above the threshold value

CC (weighted) 2

Weighted correlation coefficient channel 2. Calculated like the simple colocalization coefficient, but using the sum of the gray value intensity rather than the number of pixels.

The values range between 0 and 1, with 0 indicating no colocalization and 1 indicating full colocalization.

Numerator = Sum of intensity of all pixels in quadrant 3

Denominator = Sum of intensity of all pixels above the threshold value

Average Intensity 1

The sum of all gray values from channel 1, divided by the total number of pixels in this channel:

$$\frac{\sum Grauwerte\,Kanal\,1}{Fläche Kanal\,1}$$

Average Intensity 2

The sum of all gray values from channel 2, divided by the total number of pixels in this channel:

$$\frac{\sum Grauwerte\,Kanal\,2}{Fläche Kanal\,2}$$

Standard Deviation 1

Displays the standard deviation of the gray values in channel 1:

Standard Deviation 2

Displays the standard deviation of the gray values in channel 2:

Z Index

Displays the Z index for Z-stack images.

T Index

Displays the time index for time lapse images.

Relative Time

Displays the time of acquisition for all dimensions of a multidimensional image, beginning at 0h:00min:00sec:00msec.

Relative Focus

Displays the relative focus position at which an image has been acquired.

View specific controls

Colocalization tab

Here you will find all the control elements that you need to perform a colocalization analysis.

Tool Bar section

Use the tools to draw regions into the image in which you want the analysis to be performed. A description of the tools can be found on the Graphics tab [→ 286].

Once a region has been drawn in, it is automatically treated as an active region. The scatter plot shows the pixel value frequencies for this region.

The **Colocalization table** displays the data for the entire image and for the selected region. To select several regions, hold down the **Ctrl** key and click on the desired regions.

Apart from drawing regions into the image, you can also draw them into the **X/Y scatter plot**. If you have used the function in the Masking section [→ 273], only those pixels that are framed by a region in the scatter plot are taken into consideration. This means that you can correlate interesting point clouds quickly with the corresponding pixels in the image.

If you have drawn regions into the scatter plot, the ROI (region of interest) button will also appear in the tool bar. As long as this button is activated (highlighted in blue), you can select, move and change the regions in the scatter plot using the **Selection** tool. If you want to change the quadrant lines again, you will need to deselect the ROI button beforehand.

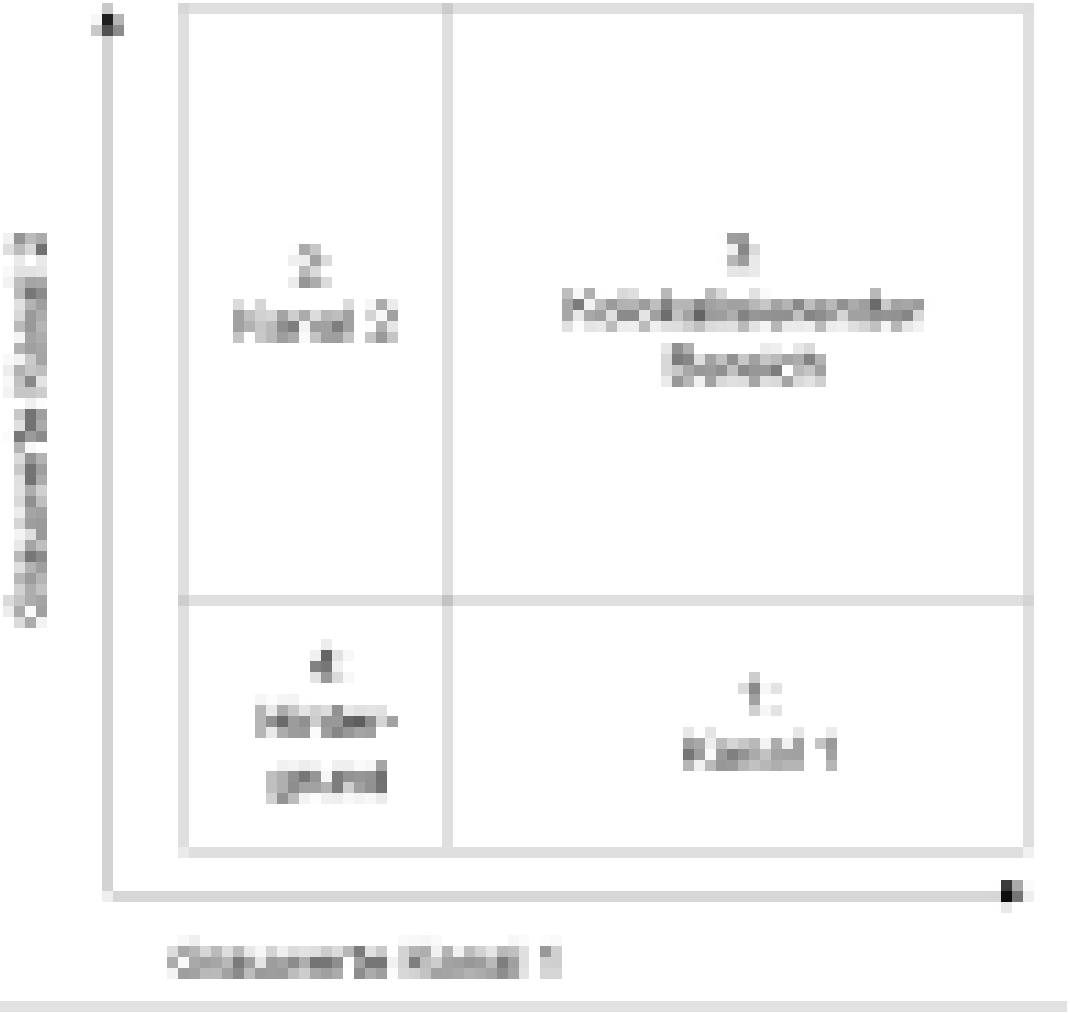
Channels and Scatter Plot Control section

Channels dropdown lists

Here you can select which channels of a multichannel image are compared with one another. Select a channel for both the horizontal and vertical diagram axis from the **Channels** dropdown list. The first and second channel are always selected by default. As soon as you have made a selection, all other channels are automatically removed from the image display. You can, however, add other channels temporarily on the **Dimensions** tab.

Threshold sliders

Using the two Threshold sliders and the two spin boxes/input fields, you can set the threshold value (in gray levels) for both channels.



Range dropdown lists

Only visible if the **Show All** mode is activated.

Here you can define the gray value range that the diagram axes will display. **Auto** is selected here by default, which means that the range is automatically set to the brightest pixel in the image. You can, however, enter a fixed gray value range between 256 (8 bits) and 65535 (16 bits). If the image is a time lapse or Z-stack image, provided Auto has been selected, you can select the dimension for which you want the gray value range to be automatically determined from another dropdown list. In this way you can easily determine a valid diagram setting for an entire time series, for example, without having to analyze each individual time point.

Costes button

Calculates the optimal threshold value according to Costes et al.

Masking section

Channels buttons

Here you can mask pixels in the image according to which one of the four quadrants they belong to. The numbers on the buttons correspond to the numbering of the quadrants in the **X/Y scatter plot**. The color selection window is accessed by clicking on the **color field**. Using the **Opacity** slider you can determine the degree of transparency of the masking.

Cut button

Only active if a quadrant has been masked.

Creates a new image exclusively containing the masked pixels.

Create section

Diagram button

Creates a new image document from the X/Y scatter plot. In the case of time series or Z-stacks the dimensions are also created automatically.

The following functions are only visible if the **Show All** mode is activated:

Table button

Creates a new table document. The document contains all measurement data from the co-localization analysis. All dimensions, such as T and Z, are also taken into account. This table can be saved as a *.csv document for further processing in other programs.

Table checkbox

Activated: Displays the colocalization table in the image area.

Analysis View

Only visible if an interactive measurement has been performed.

In the **Analysis** view the image from the interactive measurement and the table containing the measurement results are displayed.

Information

To highlight the row of the table containing the measured values of an object, click on a measured object in the image.

To highlight the corresponding measured object in the image, click on a row in the table.

See also

General view controls [→ 282]

View specific controls

Analysis tab

On the Analysis tab you can define how the measured objects are displayed in an image.

Show Objects checkbox

Activated: Displays the measured objects in the graphics plane.

Fill checkbox

Activated: Displays the objects in filled form.

Deactivated: Displays only the contours of the objects.

Opacity slider

Here you can set the opacity with which the measured objects are displayed in the graphics plane.

Delete Measurement Data button

Deletes all objects and measurement data from the image.

Show All Classes checkbox

Activated: Displays the objects of all classes.

Deactivated: Displays the objects of the selected class.

Create Measurement Data Tables button

Creates two measurement data tables. One measurement data table contains the field features for all classes and the other the object features for all classes.

Classes section

In the Classes section select the class whose measurement features you want to be displayed in the measurement data table. For each class there are two entries: the first entry concerns all the objects belonging to the class (field features) and the second represents an individual object (object features).

Tiles Advanced Setup View

Here you can configure advanced settings for the acquisition of mosaic images. In the image area you can see the stage view [➙ 275]. Additional settings and tools relating to tile regions or positions can be found under specific control elements [➙ 276].

See also

- General view controls [➙ 282]

Stage View

The image area shows the full travel range of the microscope stage, along with the current stage position, the graphical display of sample carriers and your acquired mosaic images. You can control the stage view using the arrow icons at the edges of the image area. The view can be enlarged, reduced or moved using the general control elements.

Navigator frame

The current stage position is shown as a tile outlined in blue, the Navigator frame. To move the frame, double-click on the position to which you want to move it.

The Navigator frame can also be used to control acquisition. If you click on one of the frame's blue arrow icons, an image is acquired. The Navigator frame is moved one frame width in the relevant direction. You can create mosaic images of your sample easily in this way.

Positions

Positions are represented in the stage view by a plus symbol.

Tiles

Tiles are represented in the stage view by a red grid.

Tools window

Here you can obtain an overview of the position of your sample as well as of your mosaic definitions, positions and position arrays.

Center at Stage Position button

Centers the view at the current stage position.

Preview Scan

To show the section in full, click on the  button.

Delete Existing Preview Images checkbox

Activated: Deletes all existing preview images when the next preview is acquired.

Objective dropdown list

Here you can select an objective. To acquire an overview of all active mosaic images and positions, switch to an objective with a low magnification. Set the channel exposure time and start the acquisition of the overview image.

Start Preview Scan button

Starts the preview scan to acquire the overview images.

View specific controls

Carrier tab

Here you can see a graphical preview of the sample carrier being used. Please note the following features of the display:

- sample carriers that are filled with tile regions only are displayed in orange.
- sample carriers that are filled with positions only are displayed in yellow.
- sample carriers that are filled with tile regions and positions are displayed in a mixture of orange and yellow.

Tile Region Setup tab

Here you can select which setup you want to be used for the settings of the tile regions. Three setups with different setting options are available:

Contour Setup

Here you can define the tile regions by means of the contour.

Contour section

The following tools are available:

Selection Use this to select an element in the stage view.

Rectangle Use this to draw in a rectangular tile region.

Ellipse Use this to draw in an elliptical tile region.

Polygon Use this to draw in a polygonal tile region.

Keep Tool checkbox

Activated: Keeps the selected tool active.

Anchor section

Only visible if the **Show All** mode is activated.

Anchor Top Left The anchor of the defined shape is at the top left.

Anchor Centered The anchor of the defined shape is centered.

Anchor Bottom Right The anchor of the defined shape is at the bottom right.

Predefined Setup

Here you can define the tile regions by means of the number or size.

Tools section

Selection Use this to select an element in the stage view.

Add Tile Region Adds the current tile definition in the image area.

Keep Tool checkbox

Activated: Keeps the selected tool active.

Contour section

Only visible if the **Show All** mode is activated.

Defines the outline of the tile region that you are adding. To add a rectangular tile region, click on the **Rectangle** button. To add a circular tile region, click on the **Circle** button.

Tiles button

Selects the number of tile regions as a reference for the size of the tile region. Enter the number of tile regions in the **X/Y** spin boxes/input fields. If you are adding a circular tile region, enter the number of tile regions in the **Diameter** spin box/input field.

Size button

Selects the size as a reference for the size of the tile region. Enter the size of the tiles in the **X/Y** spin boxes/input fields. If you are adding a circular tile region, enter the diameter of the tile regions in the **Diameter** spin box/input field.

 **button**

Adds the tile region to the **Tile Regions** list and activates it for acquisition.

Anchor section

Only visible if the **Show All** mode is activated.

Anchor Top Left The anchor of the defined shape is at the top left.

Anchor Centered The anchor of the defined shape is centered.

Anchor Bottom Right The anchor of the defined shape is at the bottom right.

Carrier Setup

Here you can define the tile regions automatically by means of the fill factor of the sample carrier.

Create button

Only active if you have selected a container on the **Carrier** tab or in the **stage view**.

Automatically creates the tile regions with the set fill factor in the selected container of the sample carrier.

Remove button

Removes all tile regions in the selected container.

Fill Factor spin box/input field

Here you can enter the fill factor used to fill the selected container.

Tile Region Properties tab

Here you can adjust the properties of the selected tile region. The settings are only active if you have selected a tile region.

Use For Acquisition checkbox

Activated: Uses the selected tile region for acquisition.

Name input field

Here you can enter a name for the selected tile region.

The following functions are only visible if the **Show All** mode is activated:

Size spin boxes/input fields

Here you can enter the size of the tile region in the X/Y/Z dimension.

Tiles spin box/input field

Here you can enter the number of tiles in the X/Y dimension.

Anchor Position spin box/input field

Here you can enter the anchor position of the selected tile region.

Set Current Z button

Sets the Z dimension at the current Z position of the stage.

Category dropdown list

Here you can select various categories of tile regions. You can create, edit, load or delete your own categories here.

Position Setup tab

Here you can select which setup you want to be used for the settings of the positions. Three setups with different setting options are available:

Location Setup

Here you can define the positions by means of the location. You can add various positions in the stage view using the mouse.

Tools section

Selection Use this to select an element in the stage view.

Cross Hairs Use this to add positions in the stage view.

Keep Tool checkbox

Activated: Keeps the selected tool active.

X/Y Position display fields

Display the current stage position.

button

Adds a new position at the current stage position.

Array Setup

Here you can define the positions by means of position arrays. You can add various contours for position arrays in the stage view.

Information

Position arrays are groups made up of a number of individual positions. Typically, position arrays contain several hundred individual positions. They make your work easier if you work with regular or evenly distributed samples.

Array Contour section

The following tools are available:

Selection Use this to select an element in the stage view.

Rectangle Use this to draw in a rectangular position array.

Ellipse Use this to draw in an elliptical position array.

Keep Tool checkbox

Activated: Keeps the selected tool active.

Distribution Bias section

Only visible if the **Show All** mode is activated.

Enter the distribution of the positions within the position array using the slider.

Random checkbox

Activated: Distributes the positions randomly within the position array.

Distribute By section

Number button Enter the number of positions within a position array in the spin box/input field under the **Number** button.

Percent button Enter the percentage share of positions within a position array in the spin box/input field under the **Percent** button.

Carrier Setup

Here you can define the positions automatically by means of the relevant sample carrier. To do this, select a sample carrier via the shortcut menu or on the **Carrier** tab.

Create button

Only active if you have selected a container on the **Carrier** tab or in the **stage view**.

Automatically creates the positions in the selected container of the sample carrier. Enter the distribution and number of positions in the **Distribution Bias** and **Distribute By** sections.

Remove button

Removes all positions in the selected container.

Distribution Bias section

Only visible if the **Show All** mode is activated.

Enter the distribution of the positions within the container using the slider.

Random checkbox

Activated: Distributes the positions randomly within the container.

Distribute By section

Number button Enter the number of positions within a container in the spin box/input field under the **Number** button.

Percent button Enter the percentage share of positions within a container in the spin box/input field under the **Percent** button.

Position Properties tab

Here you can adjust the properties of the selected positions. The settings are only active if you have selected a position.

Use For Acquisition checkbox

Activated: Uses the selected position for acquisition.

Deactivated: Deletes the selected position from the experiment

Name input field

Here you can enter a name for the selected position.

The following functions are only visible if the **Show All** mode is activated:

X/Y/Z spin boxes/input fields

Here you can enter the position of the selected position in the X/Y/Z dimension.

Set Current Z button

Sets the Z dimension at the current Z position of the stage.

Category dropdown list

Here you can select various categories of positions. You can create, edit, load or delete your own categories here.

Panorama View

In the **Panorama** view you can see the complete overview of the microscope stage. The tools that allow you to control acquisition are arranged in the tools window in the bottom right corner of the image. There you can control the exposure time, determine the focus position and acquire an individual or Z-stack image.

See also

General view controls [→ 282]

Stage View

In the image area the full travel range of the microscope stage is displayed. You can control the stage view using the arrow icons at the edges of the image area. The view can be enlarged, reduced or moved using the general control elements.

Navigator frame

The current stage position is shown as a tile outlined in blue, the Navigator frame. In the Navigator frame you can see the camera's live image.

To move the frame, double-click on the position on the microscope stage to which you want to move it.

To acquire images, use the **Acquisition** buttons in the **Tools window.**

Tools window

Center at Live Navigator button

Centers the stage view at the current position of the Navigator frame.

Acquisition button

Acquisition buttons [→ 50]

Acquire Tile Image button

Acquires a tile image. This comprises all activated channels as well as Z-stacks. Following acquisition a tile image is placed in the corresponding location in the stage view.

4.1.3 General view controls

Dimensions tab

On the **Dimensions** tab you can configure the settings for how the image will be displayed on the screen. You can select the size of the display and call up information about the content of the image. In the case of multidimensional images you can select here which dimension is displayed.

Slider section

Depending on how many dimensions your image contains, up to 4 sliders are available in this section. Using the **sliders** you can set the position that you want for each dimension available in the image. You will see the current position number in the **input field** to the right of the relevant slider. You can also enter the position number directly here.

The  buttons to the right of the input fields enable you to play back the dimension in question automatically. This takes place at a rate of 5 images per second by default. You can change the speed on the Player [➙ 298] tab.

Depending on the available dimensions, the following sliders may be visible:

Z-Position Only visible in the case of Z-stack images.

Here you can enter the desired Z-position.

Time Only visible in the case of time lapse images.

Here you can enter the desired time point.

Scene Only visible in the case of images of different positions.

Here you can enter the desired scene. If you deactivate the **Scene** checkbox, all scenes are displayed as an overview.

Block Only visible if you have used the **Experiment Designer**.

Here you can enter the desired experiment block.

Total Time Only visible if you have used the **Experiment Designer**.

Here you can enter the duration across all blocks.

Information

For images with more than 3 dimensions a scrollbar is displayed which you can use to access the other sliders.

See also

- Experiment Designer tool [➙ 87]

Zoom section

The bar in the **Zoom** section contains tools that you can use to adjust the size of the image region displayed.

Fit to Window Automatically sets a zoom factor at which the entire image can be displayed visibly on the screen.

Normal View (100%) Shows the image without increasing or decreasing the zoom factor. One pixel of the image corresponds to one pixel on your screen.

Decrease Zoom (F8) Decreases the zoom factor.

Increase Zoom (F7) Increases the zoom factor.

Zoom slider Here you can set the display size steplessly. The desired zoom factor can be entered in the spin box/input field in percent.

Auto Fit checkbox **Activated:** Adjusts the display size automatically and continuously to the size of the window.

Information

If you hold down the **Ctrl** key, you can zoom in and out of the image using the mouse wheel.

Tools section

The bar in the **Tools** section contains other tools that you can use to interact with the image. A selected tool appears in blue.

Selection mode Changes the mouse pointer to Selection mode. The mouse pointer appears in the form of the default icon.

Zoom Rectangle Changes the mouse pointer to Zoom Rectangle mode. The mouse pointer appears in the form of cross hairs. Hold down the left mouse button and drag out a selection rectangle. When you release the left mouse button, the region within the rectangle is displayed in enlarged form.

Move Changes the mouse pointer to Move mode. The mouse pointer appears in the form of a hand. Click inside the enlarged image region and hold down the left mouse button. You can now move the image region.

Information

If you have a mouse with a mouse wheel, you can also use this to move enlarged image regions. Position the mouse pointer in Selection mode inside the enlarged image region. Hold down the mouse wheel. The mouse pointer will then appear as a hand icon. You can now move the image region. When you release the mouse wheel, the mouse pointer changes back to the default icon.

Inner Zoom Only visible in Gallery view.

This function allows you to set a new zoom level for all images simultaneously using the mouse wheel. The size of the **Gallery View** does not change here. This allows you to limit the view to one interesting image region. Use the **Move** tool to move the view.

Show Pixel Values Changes the mouse pointer to Show Pixel Values mode. If you move the mouse pointer into the image region, a vertical arrow and a display field will appear. The pixel values of the position to which the arrow is pointing are displayed in the display field. In the first line of the display field the X/Y coordinates are shown. The second line shows the X/Y coordinates in scaled units. In the other lines the gray values for each channel are shown.

Navigator button Opens the **Navigator** dialog window in the image region. There you will see an overview of your image and you can navigate to different positions using a rectangular window.

Interpolation checkbox **Activated:** The pixel elements of the image are shown in an interpolated display. This makes it possible to avoid the pixelated display of small or greatly enlarged images.

Deactivated: The pixel elements of the image are displayed on the screen as they are. This function is activated by default, but can be deactivated under Options [→ 46] if required.

See also

- 2D View Navigator window [→ 225]

Channels section

The bar in the **Channels** section contains all the channels that you are using in your image. Here you can switch the display of channels in images on or off and change the channel colors (pseudo color assignment).

Channel buttons

A button is displayed for each channel. Each button has two functions:

1. The channel name is displayed in the top section. To switch a channel off/on again, left-click on this section of the button.
2. The bottom section of the button shows the channel color. The display changes depending on the status of the button:
   - When switched off, you will see a colored line below the button.
   - When switched on, you will see a color field with a dropdown list below the button. Clicking on the dropdown list opens the Color Selection [→ 299] dialog window.

Information

For images with 8 or more channels, the **Channel buttons** are reduced in size. In this case it is no longer possible to change the color channel by channel.

Single-Channel Mode checkbox

Activated: Only a single channel is displayed.

Show Colors checkbox

Only visible in the Channels view.

Activated: Displays the individual channels of multichannel images without the assignment of pseudo colors.

Saturation Display checkbox

Activated: Changes the display to the Single-Channel Mode. The channel is displayed in monochrome. At the same time you will see regions in which the camera sensor is saturated (displayed in red) and regions in which the pixel values = 0 (displayed in blue). This function helps you to set your camera's exposure time so that saturation of the detector is avoided.

Information

With camera systems it is not normally possible to achieve pixel values of 0. The blue indicator is therefore not normally displayed.

Fast Color Selection button

Opens a dialog that allows you to select a color quickly for all channels of a multichannel image. The following options can be set:

- **No Color**: All channels are displayed without a pseudo color
- **Gray**: All channels are displayed in monochrome (this applies in particular to multichannel images that have been acquired using color cameras)
- **BGR**: Channel 1: blue, channel 2: green, channel 3: red, no color assigned to any other channels
- **GRB**: Channel 1: green, channel 2: red, channel 3: blue, no color assigned to any other channels
- **RGB**: Channel 1: red, channel 2: green, channel 3: blue, no color assigned to any other channels
- **From LUT**: Colors for all channels are selected using a reference look-up table. The LUT is divided up into as many sections as there are channels, with the channel color being used at the separation point. You can select the reference LUT using the **Reference LUT...** button
- **User-defined**: The colors defined by the user are restored
- **Dye**: The color of the dye used during the experiment is restored

Reuse button

Only visible if you have loaded an image in *.CZI image format. This is the only image format that is compatible with the **Reuse** function.

Reproduces the acquisition conditions for the image.

The function only works correctly if the system configuration at the time of acquisition is identical to the system configuration at the time when you execute the function.

Removing components (e.g. filter cubes, LEDs, cameras, etc.) can result in an experiment being created incorrectly. It is therefore essential that you check after executing the Reuse function whether the configuration of the experiment is in line with your expectations.

Information

Clicking on the **Reuse** button overwrites the current experiment without a prompt and marks it as having been modified. This can be seen from the appearance of an asterisk after the file name. If you want to keep the experiment in its previous form, you must save the modified experiment with a new file name under **Experiment Manager** | **Options** | **Save As**.

If you acquire images and save them in *.CZI image format, the following acquisition conditions are saved together with the image:

- Information on the type and status of your imaging system
- Time of acquisition
- Parameters set in the software.

Graphics tab

On the **Graphics** tab you can select various tools and use these to draw graphic elements into your images. You can also obtain an overview here of the graphic elements that you are using in your image.

Tool Bar section

A selection of standard tools which you work with on a regular basis are available to you here. You can adapt this tool bar to your requirements. Other tools can be found in the **Graphics** menu | Tool Group [➙ 23].

Selection

Use this to select the graphic elements in the image area. If you are currently in another mode, you can switch back to the Selection mode using this button.

Stamp

Use this to create an identical copy of the last graphic element drawn in. To exit this mode, either switch back to the Selection mode or press the ESC key.

Selection Region

Use this to draw a rectangular selection region into the image.

Text

Use this to enter text into the image.

Scale Bar

Inserts a scale bar into the bottom right corner of the image.

Arrow

Use this to draw in an arrow.

Rectangle

Use this to draw in a rectangle that is always parallel to the edges of the image.

Circle

Use this to draw in a circle.

Contour (Spline)

Use this to draw in a freely selectable contour. Close this contour by right-clicking. Corners are always rounded with this tool.

Keep checkbox

Activated: Keeps the current tool active. This allows you to draw in a number of the same graphic elements one after the other.

Color checkbox

Only visible if the **Show All** mode is activated.

Activated: Uses a new color for each graphic element drawn in.

Pixel-Precise checkbox

Only visible if the **Show All** mode is activated.

Activated: Draws in the graphic elements with pixel precision. If this option is not active, the graphic elements are drawn into the graphics plane independently of the actual pixel resolution.

Modify button

Opens the Modify Tools [➙ 289] dialog window.

Planes section

Only visible if the **Show All** mode is activated.

Here you can specify which planes are active and visible in the image. To open the shortcut menu, click on the **Planes** button.

Active Plane menu item

Here you can specify which graphics plane is active in the image. The other planes are visible but blocked.

Automatic **Activated:** Sets the active plane automatically. This is the default setting.

Selection **Activated:** Sets the **Selection** plane as the active plane. This plane contains graphic elements such as **Selection Area**, **Grid**, etc.

Annotation/ Measurement **Activated:** Sets the **Annotation/Measurement** plane as the active plane. This plane contains graphic elements such as **Annotations, Measurement Tools**, etc.

Show/Hide Plane menu item

Here you can specify which plane is visible in the image. The other planes are not visible.

Selection **Activated:** Displays the **Selection** plane in the image.

Annotation/ Measurement

Activated: Displays the **Annotation/Measurement** plane in the image.

Graphic Elements list

Here you will see the graphic elements that you are using in your image. You can also control the behavior of the graphic elements here, e.g. block or hide them. You can format each graphic element as you wish.

To format graphic elements, double-click on the list entry for the graphic element in question or call up the corresponding shortcut menu directly at the graphic element in the image. Select the **Format** entry. The Format Graphic Element [→ 291] dialog window opens. There you can format the graphic element in question according to your requirements.

Information

In the list you will only see the graphic elements relating to the active graphics plane. To change the active graphics plane, click on the **Planes** button. This button is only visible in **Show All** mode. Select the graphics plane that you want to display via the **Active Plane** shortcut menu.

The columns of the list contain the following entries:

Eye icon Shows or hides a graphic element.

Lock Locks a graphic element to prevent changes being made.

Type Displays the icon for the tool type. To format a graphic element, double-click on the icon. The Format Graphic Elements dialog then opens.

ID Only visible if the **Show All** mode is activated.

Displays the ID for the graphic element. To do this, activate the checkbox at the corresponding list entry.

A Only visible if the **Show All** mode is activated.

Displays annotations for a graphic element. To do this, activate the checkbox at the corresponding list entry.

M Only visible if the **Show All** mode is activated.

Displays measurement data for a graphic element. To do this, activate the checkbox at the corresponding list entry.

Name Displays the name of the graphic element. To change the name, double-click in the Name field. Then enter the text of your choice.

Information

If you want to change a setting for all the graphic elements in the list, click in the title bar of the relevant column.

Diskette button

Saves the selected graphic element.

Folder button

Loads an existing graphic element.

Delete button

Deletes the selected graphic element.

Dimensions section

Only visible if the **Show All** mode is activated.

The coordinates and dimensions of the selected graphic element (standard unit = pixels) are displayed in the spin boxes/input fields:

X spin box/input field

Displays the X coordinate of the center point of a graphic element.

Y spin box/input field

Displays the Y coordinate of the center point of a graphic element.

W spin box/input field

Displays the width of a graphic element.

H spin box/input field

Displays the height of a graphic element.

Angle

Displays the rotation angle of a graphic element. In the case of the Angle graphic element the measured angle is displayed here.

Unit checkbox

Activated: The unit of the coordinates changes to µm.

Graphics dialog window

Modify Tools dialog window

Here you can select up to 7 tools or frequently used annotations that you want to appear in the tool bar. You can therefore add the tools that you use frequently.

User Tool Bar list

The tools or annotations that you select from the **Tools** list or the **Frequently Used Annotations** list appear here. These tools or annotations will then also appear below the tool bar on the **Graphics** tab.

Search input field

Here you can search for certain tools or annotations. Enter the name or initial letters of the tool or annotation that you are looking for. The search results will appear in the **Tools** list or the **Annotations** list.

Tools list

Here you can select the tools that you use frequently and want to add to the tool bar.

Frequently Used Annotations list

Here you can select the annotations that you use frequently and want to add to the tool bar.

Last Used

This list contains the tools that you have added to the tool bar. Only the first seven tools appear in the tool bar. You can change the order of the tools using the arrow buttons at the bottom of the list. To delete a selected tool, click on the **Wastepaper Bin** button.

Search

To find a certain tool, enter a search text into the input field. Only those tools that contain this text are then displayed in the Tools list.

Tools

This list contains all the tools that are available in ZEN. Use the scroll bar if the tool you want is not displayed, or use the text search.

Information

To ensure that you see all tools, the text search must be empty.

To add a tool to the **Last Used** list, click on the  button. If you want to reset the default formatting of a tool back to its original setting, click on the **Reset Selected** button. You can also reset the formats of all tools using the **Restore All** button.

Frequently Used Annotations

Here you can make a selection from 6 preconfigured, frequently used annotations. If you want to reset the default formatting of the frequently used annotations back to the original setting, click on the **Reset Annotations** button.

Planes

Graphic elements are organized in planes. Depending on the plane type, graphic elements may have different properties. The following plane types are possible:

- Annotation/Measurement: most graphic elements belong to this plane type.

- Selection: special planes that contain only selection regions and other short-term selection elements. Changes to the selection plane are not saved when the image is closed.
- Acquisition/Bleaching: graphic elements in this plane were used during acquisition, e.g. in bleaching experiments. These graphic elements cannot be changed.

In the **Planes** dropdown list, under **Active Plane**, you can select which plane is active in the image. Other planes are accordingly displayed as inactive. The **Automatic** command ensures that planes are activated automatically as soon as a corresponding graphic element is selected. This is the default setting.

Using the Show/Hide Planes setting, you can specify which planes you want to be displayed. If you deselect a plane, the corresponding graphic elements are not displayed.

Format Graphic Element dialog window

Here you can format the relevant graphic element. You can adjust the line thickness, text or opacity.

Zoom with the Image checkbox

Activated: Relates the line thickness and font size to the pixels in the image.

Line section

Color field

Here you can change the line color. To change the line color, click on the color field. The Color Selection [→ 295] dialog window opens.

Line Thickness

Here you can change the line thickness. Enter the desired value for the line thickness (in pixels) in the spin box/input field to the right of the color field.

Line Style dropdown list

Here you can change the style of the line.

None The line is not displayed.

Continuous The line is an unbroken line.

Dashed The line is made up of dashes.

Dotted The line is made up of dots.

Line Start/Line End dropdown list

Here you can change the style of the start/end of the line. The following styles are available:

- Flat
- Small bar
- Large bar
- Open arrow

- Closed arrow

Text section

Font button

Opens the **Font Selection** dialog window. Here you can change the font, font size, font color and character style used for text.

To change the **font**, click on the **Font** dropdown list. Select the desired font from the list.

To change the **font size**, enter the desired font size (in points) using the slider or spin box/ input field.

To change the **font color**, click on the color field to the right of the **Font** dropdown list. The Color Selection [→ 295] dialog window opens.

To change the **character style**, click on the corresponding buttons. The following character styles are available:

- Bold
- Italic
- Underline
- Strikethrough
- Normal
- Uppercase
- Small Caps

Align Horizontally dropdown list

Here you can select the horizontal alignment of the text (Left, Center, Right).

Align Vertically dropdown list

Here you can select the vertical alignment of the text (Top, Center, Bottom).

Fill section

Here you can change the way in which closed graphic elements (including text) are filled.

Color field

Here you can change the fill color. To change the fill color, click on the color field. The Color Selection [→ 295] dialog window opens.

Fill Style dropdown list

Here you can change the fill style.

Opacity section

Opacity slider

Enter the fill opacity using the slider or spin box/input field (values range from 0-100%).

Annotations section

Annotations dropdown list

Here you can select annotations that you want to assign to the graphic element. Annotations are meta data such as the acquisition time or acquisition duration. For certain annotations you can select additional settings, e.g. the unit of time, from the dropdown list to the right of the **Annotations** dropdown list.

The most frequently used annotations are available here by default.

Adding additional annotations

1. Click on the last entry in the list **(...)**.
   ⇨ The **Select Meta Data** dialog window opens.
2. Select an annotation that you want to add to the list from the **Available Meta Data** list.
3. Click on the **Apply** button.

⇨ The entry is adopted into the **Annotations** dropdown list.

Format Grid dialog window

Here you can define how you want the grid to be displayed in the graphics plane.

Mode radio button

Here you can select whether you want the grid to be anchored at the center or top left of the image.

button

Loads a saved grid setting.

button

Saves the current grid setting.

Horizontal Lines checkbox

Activated: Displays horizontal lines in the grid.

Vertical Lines checkbox

Activated: Displays vertical lines in the grid.

Circles checkbox

Activated: Displays concentric circles in the grid.

Cross checkbox

Activated: Displays a cross in the grid.

Unit dropdown list

Here you can select the unit in which the distances of the lines and circles are indicated.

Synchronize checkbox

Activated: Automatically keeps the distances for horizontal and vertical lines the same.

Width slider

Enter the desired width for the grid distance using the slider or spin box/input field.

Height slider

Enter the desired height for the grid distance using the slider or spin box/input field.

Columns slider

Enter the desired number of grid columns using the slider or spin box/input field.

Rows slider

Enter the desired number of grid rows using the slider or spin box/input field.

Circles slider

Enter the desired number of circles using the slider or spin box/input field.

Grid, Cross, Circle Color Selection

Opens the Color Selection dialog window [→ 295].

Line Thickness input field

Enter the line thickness for **Grid**, **Cross** or **Circle** using the spin box/input field.

Line Style dropdown list

Here you can select the style of the line for **Grid**, **Cross** or **Circle**. You can select from the following line styles:

- None
- Continuous

- Dashed
- Dotted

Set As Default button

Sets the current settings for the grid as the default settings.

Reset button

Resets the default settings to their status on delivery.

Color Selection dialog window

Color Selection dialog window

Here you can select the desired color for the graphic element. In the bottom section of the dialog window you will see 2 buttons that offer you different methods for selecting colors. The selected button is highlighted in blue. To change the method, click on the corresponding button.

Color button

Here you can select the desired color from 18 standard colors. The selected color is displayed in a color field above the **Color** button.

Custom button

Here you can define up to 16 RGB colors of your choice.

Display tab

On the **Display** tab you can adjust your image display to your screen. This function is particularly important if you want to display images with a very high dynamic range on the screen.

Channel Selection

Here you can determine the channel of a multichannel image for which you want to adjust the display on the screen. To select all channels, click on the **All** button.To select a certain channel, click on the corresponding color field. Hovering the mouse pointer over a color field displays the relevant channel name.

If the image consists of more than 29 channels, a scrollbar will be displayed which you can use to switch to the desired channel.

Please note: In the case of images containing only one channel the selection fields are hidden.

Spline Mode button

Clicking on this button allows you to add up to 8 points to the display characteristic curve. You can then bend the curve around these points. To do this, click on the desired section of the display curve and move it as required. Clicking on the display curve again adds another

point. You can delete points by moving them along the display curve until they lie on top of another point. In this way, even in difficult situations you can adjust the display curve so that all important image regions can be displayed well.

Auto checkbox

The image display is adjusted automatically for each image. This is particularly useful in the case of a **live image**, in **Continuous** mode or if you play back a time lapse image that contains changing brightnesses. Certain settings are no longer available in this mode.

Min/Max button

Adjusts the display characteristic curve so that the darkest pixel is black and the brightest pixel is white in the display.

BestFit button

Adjusts the display characteristic curve so that 0.1% of the darkest pixels contained in the image are black and 0.1% of the brightest pixels are white in the display.

Spin boxes/input fields

Only visible if the **Show All** mode is activated.

In the two spin boxes/input fields to the right of the **BestFit** button you can adjust the black/white values from 0.1% to values from 0 to 90% according to your requirements.

Select Dimension dropdown list

Only visible if the **Show All** mode is activated.

If your images contain time series, Z-stacks or both, here you can select the aspect of an image for which the display settings should be applied. The following options are available:

Current Adjusts the display for the current image and keeps this setting for all other time points or Z-planes.

All T Collects the intensity values from all time points and adjusts the display according to the brightest and darkest pixels within the entire time series.

All Z Collects the intensity values from all Z-planes and adjusts the display according to the brightest and darkest pixels within the entire Z-stack.

All T+Z Collects the intensity values from all Z-planes and time points and adjusts the display according to the brightest and darkest pixels within the entire Z+T series.

Please note: With all settings other than **Current** there may be several seconds of calculation time until the setting is applied, depending on the number of time points/Z-planes.

button

Here you can copy display settings to the clipboard, insert them into other images from there or save and reload settings. This allows you to apply identical display settings to several images in order to produce comparable display conditions.

Reset button

Restores the default settings.

Display Histogram section

The display histogram shows the brightness distribution of the pixels that are present from all channels simultaneously. The Y axis represents the relative frequency and the X axis indicates the brightness. A curve showing the corresponding distribution, the so-called display characteristic curve, is displayed for each channel.

If you want to adjust an individual channel, activate this via the relevant color field under **Channel Selection**. Alternatively you can also click on the corresponding distribution curve directly in the histogram.

Display characteristic curve

Each channel has a display characteristic curve. Using the display characteristic curve you can set the limit for the black value (left) and the limit for the white value (right). This allows you to influence the contrast in the image. Move the mouse pointer over the corresponding adjustment handles at the bottom edge of the display histogram or to the small rectangles on the display characteristic curve. Hold down the left mouse button and move the adjustment handles or rectangles as required.

The curvature of the display characteristic curve influences the gamma value. To change the curvature, move the mouse pointer to the second or fourth small rectangle on the display characteristic curve. Hold down the left mouse button and move the rectangles up or down. The setting is used immediately for the display. Using the middle rectangle you can move the whole display curve. This changes the brightness of the image.

The following functions are only visible if the **Show All** mode is activated:

Black spin box/input field

Displays the gray value currently set up to which all pixels are shown as black. You can also enter the value that you want directly here.

Gamma spin box/input field

Displays the gamma value currently set. You can also enter the value that you want here.

0.45 button

Sets a gamma value of 0.45. This is the recommended setting for most color images.

1.0 button

Sets a linear display characteristic curve with a gamma value of 1.0.

White spin box/input field

Displays the gray value currently set from which all pixels are shown as white. You can also enter the value that you want directly here.

Player tab

Only visible if the **Show All** mode is activated.

Using the functions on this tab you can play back multidimensional images. The functions largely correspond to the functions for playing back films.

Player buttons

The following control elements are available:

Plays back the image series forwards. The dimensions are played back one after the other in the sequence specified.

Stops the play-back of the image series.

Plays back the image series backwards.

Plays back the image series forwards and backwards alternately.

Jumps to the start of the image series.

Jumps to the end of the image series.

Speed slider

Here you can enter the speed at which an image series is played back. The set speed is displayed in images/second in the spin box/input field. You can also enter the desired speed directly in the spin box/input field. The maximum play-back speed is 25 images/second.

Follow Acquisition checkbox

Activated: Always displays the last acquired image during an ongoing acquisition procedure, as well as the slider for the corresponding dimension.

Dimensions slider

A slider is displayed here for each of the dimensions available in a multidimensional image. The following sliders are possible:

- Z-Position
- Time
- Scene
- Block

Dimensions are only shown if they are also available in the image. If there are several dimensions, you can determine by means of the associated checkboxes whether you want these to be taken into account during the play-back or whether the current position should be retained. Each slider offers as many steps as there are individual positions in the specified dimension.

The sliders each have two adjustment handles, which can be used to define the start and end point. The image series is played back within these points. You can define here which range you want to play back from a longer time series. When you move the adjustment handle, the corresponding image is also displayed.

A third adjustment handle indicates the current position and cannot be controlled directly.

4.1.4 Image views dialogue windows

4.2 Report views

4.2.1 Report preview

Here you will see a preview of your report. You can select other templates here or insert images or tables into the report using drag & drop. To insert images or tables, the report template must contain corresponding placeholders to accommodate them.

Information

To display several images in one image placeholder, select the desired images in the list of open images and documents (**right tool area**). Hold down the **Ctrl** or **Shift** key and the left mouse button and drag the images over the placeholder with the mouse. As soon as you release the left mouse button, the images will appear in the placeholder.

It is not possible to display several tables in a single table placeholder.

Create button

Creates the report and closes the report preview.

Reject button

Closes the report preview.

Navigation area

The **Navigation** area to the left of the preview window displays all pages of the report as thumbnails. This allows you to move easily between the pages.

button

Displays the next page.

button

Displays the previous page.

Information

You can make changes to the report while the **report preview** is open. Once the report has been created this is no longer possible.

See also

- Report tool [→ 221]
- Report buttons [→ 220]
- General control elements [→ 301]

4.2.2 Report view

The report that has been created is displayed here.

Navigation area

The **Navigation** area displays all pages of the report as thumbnails. This allows you to move easily between the pages.

button

Displays the previous page.

button

Displays the next page.

Information

You can no longer make changes to the report in the **report view**. This is only possible in the **report preview**.

See also

- Report tool [➙ 221]
- Report buttons [➙ 220]
- General control elements [➙ 301]

4.2.3 General control elements

General tab

Zoom section

The bar in the **Zoom** section contains tools that you can use to adjust the size of the report displayed.

The following tools are available to you here:

Fit to Window Automatically sets a zoom factor at which the entire report can be displayed visibly on the screen.

Normal View (100%) Shows the report without increasing or decreasing the zoom factor. One centimeter of the report corresponds to one centimeter on your screen.

Decrease Zoom (F8) Decreases the zoom factor.

Increase Zoom (F7) Increases the zoom factor.

Zoom slider Here you can set the display size steplessly. The desired zoom factor can be entered in the input field in percent.

Adjust checkbox **Activated:** Adjusts the display size automatically and continuously to the size of the window.

Information

If you hold down the **Ctrl** key, you can zoom in and out of the report using the mouse wheel.

Tools section

The bar in the **Tools** section contains other tools that you can use to interact with the report view. A selected tool appears in blue.

The following tools are available to you here:

Selection mode Changes the mouse pointer to Selection mode. The mouse pointer appears in the form of the default icon.

Zoom Rectangle Changes the mouse pointer to Zoom Rectangle mode. The mouse pointer appears in the form of cross hairs. Hold down the left mouse button and drag out a selection rectangle. When you release the left mouse button, the region within the rectangle is displayed in enlarged form.

Move Changes the mouse pointer to Move mode. The mouse pointer appears in the form of a hand. Click inside the enlarged region and hold down the left mouse button. You can now move the region.

Information

If you have a mouse with a mouse wheel, you can also use this to move enlarged regions. Position the mouse pointer in Selection mode inside the enlarged region. Hold down the mouse wheel. The mouse pointer will then appear as a hand icon. You can now move the region. When you release the mouse wheel, the mouse pointer changes back to the default icon.

Glossary

Bleaching Correction

The characteristics of a widefield fluorescence microscope are based on the assumption that all Z-planes have the same total brightness, irrespective of the focus position. Use is made of bleaching correction by applying a correction factor to each Z-plane. However, this assumption does not apply to techniques that result in the generation of optical sections, such as confocal images.

Clipping planes

The purpose of clipping planes is to cut open the calculated 3D image so that elements on the inside can be visualized. Clipping planes can cut the volume in such a way that either the front, back or both sides of the volume data are no longer visible. In addition, the clipping plane itself can be given various textures. This is a very important modeling option for analyzing 3D data.

Colocalization

Acquiring fluorescence images in several channels makes it possible to visualize the relationship between biological structures. A combined display of two channels in color overlay mode makes it easier to assert whether the components are "colocalized", i.e. whether they are located at the same position. Conventionally, two fluorescence channels are displayed in the form of a color-coded overlay. The most common form is the red/green overlay. Regions in which both fluorescent dyes are present at the same place are displayed in yellow. It is not possible, however, to make quantitative statements concerning the extent of colocalization on the basis of this display. At best, a qualitative statement is possible with regard to whether or not two dyes are colocalizing. The Colocalization module is able to fill this gap and presents the user with a tool that enables colocalization to be determined quantitatively. Principle: It is always the colocalization of two channels that is analyzed. Colocalization results from the pixel-by-pixel comparison of intensities for each channel.

Constrained Iterative

The best image quality is achieved using the iterative maximum likelihood algorithm (see Schaefer et al.: "Generalized approach for accelerated maximum likelihood based image restoration applied to three-dimensional fluorescence microscopy", J. of Microscopy, Vol. 204, Pt 2, November 2001, pp. 99ff.). This algorithm is able to calculate light from various focal planes back to its place of origin. Consequently, with this method it is possible to derive the 3D structure from fluorescence images with the correct brightness distribution and to visualize optical sections. It is also possible for missing information to be partially restored from neighboring voxels. The spatial resolution can be increased without artifacts up to a theoretical limit (one voxel). It is essential for Z-stacks to have been acquired in accordance with Nyquist. Acquiring sufficient planes above and below the structure of interest is also imperative for achieving good results. As this is a complex mathematical method, the calculation can take longer, depending on the image size and the PC being used.

Costes

Costes et al. (Biophysical Journal, 2004, vol. 86, pp 3993-4003) have published a statistical method with the help of which an attempt is made to determine an optimal colocalization threshold automatically. This takes place by initially maximizing the threshold for both channels and then gradually reducing it. With each step Pearson's Correlation Coefficient is determined for all pixels below the set value. These steps are repeated until the Pearson value is minimized (ideally a value of 0 for perfectly colocalizing channels). See the publication for further details. This method has been implemented in Colocalization. Clicking on Auto ini-

tiates the above iterative process, which, depending on the sample, can take several seconds. The threshold now set corresponds to the confidence criterion calculated. This method works very well with large, diffusely stained structures such as nucleoplasm or diffuse cytoplasmic structures. Under certain circumstances it does not function so well for small structures (e.g. nuclear speckles or vesicular structures), particularly in the case of widefield images, where the signal to background ratio is not as good as it is with methods that involve the generation of optical sections (e.g. LSM, TIRF or ApoTome). The Regions button becomes active as soon as a region is inserted into the scatter plot. It remains active as long as regions are selected or moved there. Activating and deactivating the button makes it possible to switch between threshold selection using the mouse and the selection/moving of selected regions in the scatter plot image. If regions are defined in the scatter plot, the corresponding data appear in the table in addition to the overall image.

Display characteristic curve

The display characteristic curve allows you to define the range of the gray value histogram of an image that you want to display on the screen. The limit on the left defines the gray value up to which all pixels are displayed as pure black (black value), while the limit on the right defines the gray value from which all pixels are displayed as pure white (white value). The curvature of the curve defines the so-called gamma value.

Dynamic range

The dynamic range describes the number of brightness gradations that a camera or another detector is able to distinguish. Modern, scientific digital CCD cameras, for example, have a dynamic range of up to 2^16 gray levels. In this case we talk of 16 bit cameras.

Fast Iterative

The "Fast Iterative" method is an iterative restoration method that uses only one iteration per convolution step (see Meinel, E. S.: Origins of linear and nonlinear recursive restoration algorithms. J.Opt.Soc.Am, 3 (6), 1986, 787-799). No regularization is used in this case. Due to the fast processing and convergence after just a few iterations, this method is suitable in particular for the processing of larger time lapse images. The results of the method can quickly lead to good results and remove most of the out-of-focus light. They do not, however, create quantitative brightness conditions in the image. If undersampled images are present, artifact formation may also result.

Field Feature

A field feature is calculated for all segmented objects of a class. The geometric or intensity parameters of all objects of the class, e.g. the area or the average intensity, are added together. In addition, all objects can be counted, for example, or the area of the objects in relation to the total image area can be calculated as a percentage.

Gamma value

The gamma value makes it possible to correct the display of images on computer screens which do not allow the linear display of gray value curves. By changing the gamma value you can emphasize certain intensity ranges within your image when it is displayed on the screen. A value <1 emphasizes the ranges of medium pixel intensity (medium gray values), while a value >1 emphasizes the dark and bright pixel intensities and therefore increases the contrast. The recommended settings are 0.8 for fluorescence images, 1.2 for phase contrast or DIC images and 0.45 for true color images. Please bear in mind that a "correct" gamma value setting depends on numerous parameters, such as screen settings, ambient brightness, etc., and a universal setting cannot therefore be given.

Gaussian Distribution

The emission of fluorescent light in fact follows a Poisson distribution. If, however, detector noise predominates during imaging, or the image data are only just above the camera noise and therefore very dark, a normal distribution according to Gauss tends to apply to such images.

Generalized Cross Validation (GCV)

Regularization, which lessens the influence of noise during restoration, is normally controlled by a parameter that in most cases is determined heuristically via trial and error. The "generalized cross validation" (GCV) method makes it possible to estimate this parameter even under the complex conditions of Poisson maximum likelihood minimization.

Halo effect

Image display

A maximum range of 256 gray levels (black and white image) or 16 million colors can normally be displayed on a screen. Modern digital cameras capture a much larger range: black and white cameras up to 65536 values and color cameras theoretically up to (65536)3 colors (281 billion). The display of these gray values/ colors therefore needs to be adjusted for the monitor by the user. For this adjustment an upper and a lower gray/color value are defined. All gray/color values between these limits are displayed on the monitor within the 256 gray values/16 million colors that can be represented.

Lamp Flicker

This phenomenon mainly occurs if fluorescent arc lamps are operated for a long period of time. Under certain circumstances alternating darker and brighter layers can then appear in the Z direction in Z-stacks. This effect may prevent 3D deconvolution from being usefully applied, for example.

Maximum mode

In the case of a maximum intensity projection, only the pixels with the highest intensity are displayed along the observation axis. This view is well suited to the two-dimensional display of three-dimensional images, e.g. in publications, one reason being that a maximum transparency effect is only visible in this mode.

Mixed mode

In Mixed mode, a volume can be displayed in both Surface mode and Transparency mode. In the case of multichannel images, for example, structures inside a cell, such as FISH signals or nucleoli, can be displayed in Surface mode and the cytoplasm around these structures can be displayed transparently in another channel. This means that even highly complex spatial relationships can be shown convincingly.

Nearest Neighbor

The Nearest Neighbor method uses the simplest and fastest algorithm (Castleman, K.R., Digital Image Processing, Prentice-Hall, 1979). Its function is based on subtraction of the out-of-focus information in each plane of a stack, taking the neighboring sections above and below the corrected Z-plane into account. This method is applied sequentially to each plane of the entire 3D stack. It allows you to enhance contrast quickly, even if image stacks have not been put together optimally.

Nyquist Criterion

The Nyquist criterion states that a signal must be detected with at least double precision in order to reliably acquire all the frequencies in the signal. In the case of images acquired with coarser resolution, undesired effects such as aliasing may otherwise result. For the deconvolution of microscope images, this means, in practical terms, that images should be acquired with a pixel resolution that is at least double the optical resolution, both in the lateral and axial direction.

Object Feature

An object feature is calculated for an individual segmented object. It describes a geometric or intensity property of the object, e.g. its area or its average intensity.

Point Spread Function ("PSF")

All optoelectronic effects that influence the creation of a microscope image can be described mathematically in the form of the point spread function (PSF). If the PSF is known, deconvolution can be used to largely remove the negative effects from microscope images. There are three possible ways to determine the PSF: theoretically through knowledge of the key optical parameters, experimentally through measurement using fluorescent beads of a known diameter, or blindly using a method that works with less prior knowledge. In ZEN the theoretical model according to Lanni and Gibson has been implemented, which also models asymmetries like those that can arise due to spherical aberrations (see S. F. Gibson, F. Lanni, "Experimental test of an analytical model of aberration in an oil-immersion objective lens used in three-dimensional light microscopy", J. Opt. Soc. Am. A, vol. 8, no. 10, pp. 1601-1613, October 1991).

Poisson Distribution

The emission of photons by fluorochromes follows a statistical distribution, known as a Poisson distribution. This is the preferred model taken as the basis for the deconvolution calculation. It applies if the predominant proportion of image noise is caused by shot noise ("salt and pepper noise"). This assumption applies to images that have been acquired using good, low-noise detectors, the dynamic range of which has been utilized to a certain extent.

Pseudo color assignment

In fluorescence microscopy, pseudo color assignment describes the assignment of any artificially selected color to the channel of a multichannel fluorescence image. As it is mostly monochrome cameras (which produce black and white images rather than "true colors") that are used in this area of application, we talk of pseudo coloring.

Regularization

Working with real microscope images that are affected by noise leads to considerable difficulties with the practical application of deconvolution methods, which is why regularization (e.g. according to Tikhonov-Miller-Phillips) is essential. Regularization is a method that lessens the influence of noise by means of various penalty terms. Stronger regularization leads to weaker restoration and weaker regularization to stronger restoration, although in this case noise is also intensified.

Regularized Inverse Filter

The inverse filter is a genuine 3D method and generally achieves better results than the Nearest Neighbor algorithm. It essentially involves dividing the Fourier transformation ("FT") of the volume by the FT of the PSF, which can be performed very quickly. In the real space this corresponds to deconvolution. In addition, a statistical method ("General Cross Validation – GCV") is applied, which determines the noise component of the image and automatically sets the restoration strength to the optimum level in line with this. This process is also known as regularization. The method is very well suited to the processing of several image stacks in order to preselect images for the application of the iterative "high-end" method. Z-stacks must, however, have been acquired at the correct (Nyquist) distance. The additional acquisition of Z-planes above and below the structure of interest is recommended.

Render Series

To display a 3D volume on the screen, each image must be recalculated. This takes time and, in the case of large images, cannot be done interactively. You can, however, have a series of individual images calculated which

represent the animation that you want. Such an image series can be displayed considerably faster and more fluidly than is possible interactively on the screen as, in this case, the views no longer have to be rerendered. Furthermore, an image series like this lends itself extremely well to being exported as a film.

Shadow mode

In Shadow mode the structures in the image are illuminated by means of a virtual light source. The image stack is viewed from above, as if through the microscope's eyepiece, and a shadow is projected onto a virtual base (in the image background). This gives the data a reference in relation to the space, which makes visualization easier. The impression of a three-dimensional structure is created from the combination of light being reflected and opacity (degree of impenetrability to light) and the casting of a shadow.

Spherical Aberration

Every objective requires the use of a defined immersion medium to deliver the best optical resolution. In microscopy practice, particularly in the area of biosciences, it is not always possible, however, to embed the sample in a medium with the correct refractive index. When light enters the embedding medium with the wrong refractive index this results in "spherical aberration". The PSF becomes more asymmetrical the further away from the cover slip it is measured. In practical terms this becomes noticeable in the form of an increasing loss of brightness as the distance from the cover slip increases. It is possible to compensate for spherical aberration either by using objectives with correction rings or objectives that have been calculated for certain embedding media (e.g. aqueous solutions). Within certain limits, however, spherical aberration can also be compensated for during deconvolution, by taking the parameters responsible for this effect into consideration when calculating the theoretical PSF. For further details see S. F. Gibson, F. Lanni, "Experimental test of an analytical model of aberration in an oil-immersion objective lens used in three-dimensional light microscopy", J. Opt. Soc. Am. A, vol. 8, no. 10, pp. 1601-1613, October 1991.

Surface mode

The two modes previously described display the data with soft transitions or with a transparent character, depending on the setting. In Surface mode, the program calculates solid surfaces ("isosurfaces") from the gray values, which emphasizes particularly flat structures (e.g. cell walls of plant cells). This display can be used if you want to draw attention to certain structures, while other, internal structures are hidden.

Threshold

Which threshold is the correct one is a question that is frequently asked. Unfortunately it is not possible to give a definitive answer to this question, particularly because this often depends on the problem and the properties of the sample. Generally speaking it can only be said that the best approach is to determine the thresholds using appropriate control samples, e.g. samples without colocalization as a negative control and samples with biologically relevant colocalization as a positive control. Thresholds determined in this way can, under certain circumstances, be transferred to the sample of interest.

Transparency mode

In Transparency mode a three-dimensional image is calculated with a transparency effect. At least two 2D texture stacks are calculated for this from different views, which are used depending on the position angle. In contrast to Shadow mode, in this case the scene is illuminated from behind by diffuse, white light. Using the setting options, in this mode you can "mix" several channels with one another and also make information visible inside a structure. This view is therefore particularly well suited to visualizing the spatial relationship between structures within the image.