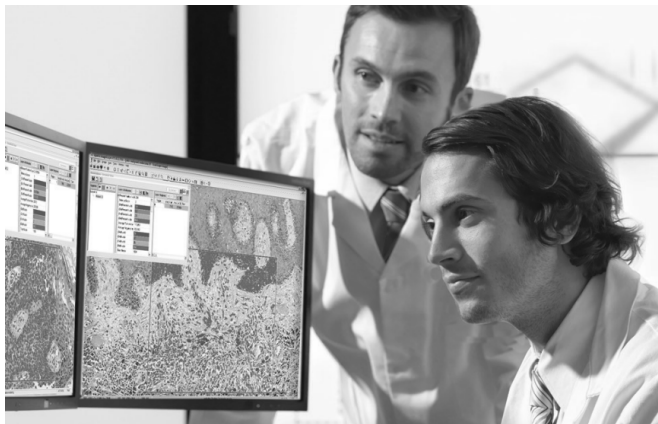The Pathology Company

Genie

User's Guide

For research use only. Not for use in diagnostic procedures.

Genie User's Guide

This document applies to eSlide Manager Release 12.3 and later.

Copyright Notice

Customer Resources

▸ For the latest information on Leica Biosystems Aperio ePathology products and services, please visit www.LeicaBiosystems.com/Aperio.

Disclaimers

▸ Use normal care in maintaining and using Aperio ePathology servers. Interrupting network connections or turning off servers while they are processing data (such as when they are analyzing eSlides or generating an audit report) can result in data loss.

▸ This manual is not a substitute for the detailed operator training provided by Leica Biosystems Imaging or for other advanced instruction. Leica Biosystems Imaging Field Representatives should be contacted immediately for assistance in the event of any instrument malfunction. Installation of hardware should only be performed by a certified Leica Biosystems Imaging Service Engineer.

▸ ImageServer is intended for use with eSlides created by scanning glass slides with the scanner. Educators will use Aperio ePathology software to view and modify eSlides in Composite WebSlide (CWS) format.

Patents

▸ Aperio ePathology products are protected by U.S. Patents: 6,711,283; 6,917,696; 7,035,478; 7,116,440; 7,257,268; 7,428,324; 7,457,446; 7,463,761; 7,502,519; 7,518,652; 7.602.524, 7,646,496; 7,738,688 and licensed under one or more of the following U.S. Patents: 6,101,265; 6,272,235; 6,522,774; 6,775,402; 6,396,941; 6,674,881; 6,226,392; 6,404,906; 6,674,884; and 6,466,690.

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Contents

1 Introduction to Genie

This chapter presents an overview of Genie, a major enhancement to the Aperio family of image analysis products. Genie is an interactive tool that "learns" from analyzing training slides, and then develops classifiers for locating and automatically annotating regions of interest in a large number of eSlides (digital slide images).

Intended Use

For research use only. Not for use in diagnostic procedures.

Origins and Background
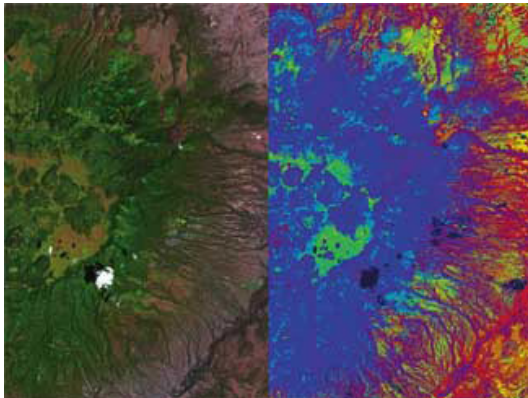
An acronym for **GEN**etic **I**magery **E**xploration, Genie was developed under the name Genie Pro at Los Alamos National Laboratories (LANL) for use in satellite imaging applications. Funded by the Department of Energy and Department of Defense, Genie Pro has been used to analyze damage caused by natural disasters such as wildfires, hurricanes, and earthquakes; to track environmental changes, and to monitor crop health. The figure below shows a NASA aerial photo (left) that has been enhanced in Genie Pro, making it much easier to analyze the visual data. By color coding layers containing the areas of interest (forests in blue, grasslands in green, scrub in yellow and bare ground in red), Genie Pro streamlines data analysis.

LANL recognized the value of the Genie Pro technology to a wide range of non-satellite imaging applications, including remote sensing, aerial imagery, and biomedical imagery and sought partners with expertise in those areas. LANL granted a license to Leica Biosystems Imaging for development of digital pathology applications. Leica Biosystems Imaging uses the shortened name *Genie* for its implementation of the technology.

What Can Genie Do For You?

The chapters that follow discuss how Genie works, but what is most important is what Genie does for you. Before Genie was available, analyzing an eSlide meant manually drawing annotations on the slide image to identify areas of interest (for example, areas containing tumor cells) and then running an algorithm to analyze those areas. Given the large number of eSlides that typically make up a project, this manual annotation process involved many hours of tedious labor and the possibility of human error.

Now we have introduced Genie into the process. A researcher trains Genie to recognize significant structures (such as tumor cells) to create a classifier. The Genie classifiers can be used by anyone who is using any Aperio algorithm to analyze an eSlide. By selecting a classifier when using an algorithm, pre-processing is done to automatically select areas of interest before the algorithm performs its analysis, improving the accuracy of the results and saving a great deal of time for the algorithm user.

Prerequisites

This section discusses Genie's prerequisites.

eSlide Manager

- ▸ eSlide Manager must be licensed for Genie.

- ▸ Genie_vx_vx.x.x.x.exe must be installed on the DSR (the server or computers connected to the server on which AAFClient is installed).

- ▸ Any algorithms that will make use of Genie classifiers must be installed on the DSR (the server or computers connected to the server on which AAFClient is installed).

Genie Classifier Developer

The person who develops Genie classifiers must:

- ▸ Be defined as an eSlide Manager user with a user role that permits use of the Genie data hierarchy and access to Genie projects, Genie training sets, and Genie templates.

- ▸ Have ImageScope installed on his or her workstation.

- ▸ Because this user needs to create new Genie algorithm macros, Genie_vx_vx.x.x.x.exe must be installed on his or her workstation. (Any other algorithms this user may need to create macros for also need to be installed on the workstation.)

Genie Classifier User

In order to use Genie classifiers while analyzing eSlides. the user must be defined as an eSlide Manager user with a user role that permits algorithm analysis. The user must also have ImageScope installed on his or her workstation. This user does not need access to the Genie data hierarchy, projects, or training sets.

The Genie Workflow

Genie is powerful, yet user friendly, and requires absolutely no programming expertise on the part of the user. The Genie workflow uses eSlide Manager and ImageScope.

The typical user who develops Genie classifiers is a pathologist or researcher who understands the structures, regions of interest and the nature of the problems that the Genie classifiers are created to identify or resolve. This Genie user trains and fine-tunes the training modules iteratively, verifying the accuracy of each iteration, until an algorithm is developed that finds the expected areas, structures, and cells of interest most correctly. The Genie classifier is then created from the training algorithm.

Developing Genie classifiers is not an activity that takes place on a daily basis. It is primarily intended to develop a classifier for automatically analyzing and cataloging a set of eSlides, based on similarities and distinguishing characteristics. Each time you need a new classifier to apply to a different type or class of eSlides, you begin at the beginning, creating a new Genie project and training set.

The typical user who uses the classifier is a technician whose basic function is to process scanned images. The process is simplified because instead of manually identifying structures on the eSlide to analyze, the user applies a Genie classifier that is appropriate for the eSlides being analyzed.
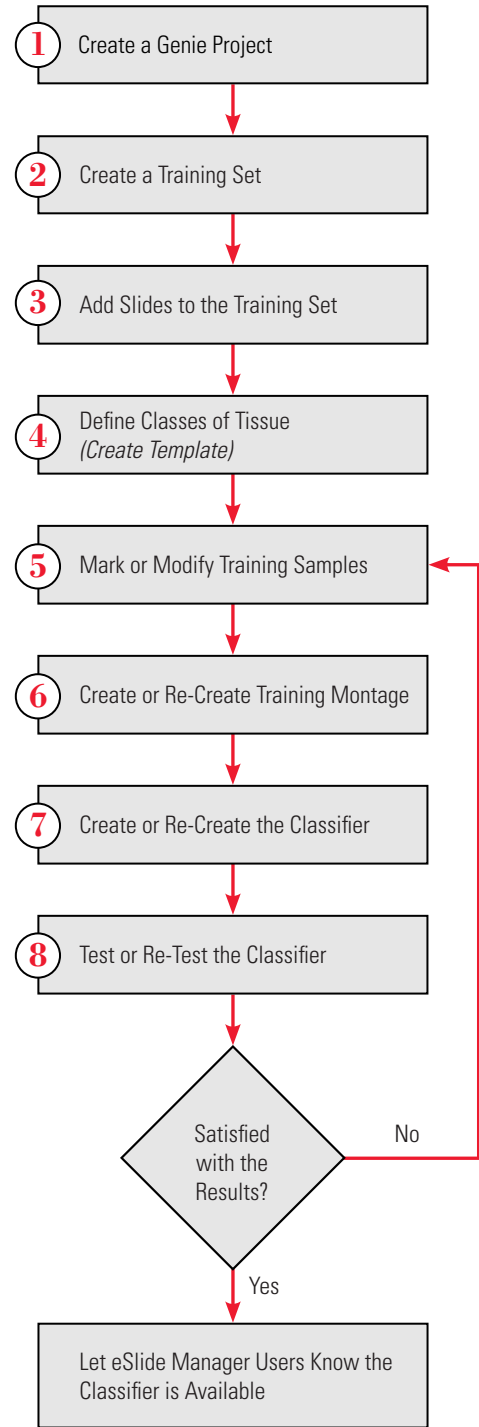
Developing the Genie Classifier

Genie "learns" by analyzing training data pixel by pixel, looking for variations in color, pattern, density, etc. In the first part of the Genie workflow, your role as a user is mainly to provide training data in the form of eSlides that you have annotated in ImageScope. Genie analyzes the training sets provided and develops a classifier that most correctly identifies the annotated regions in the eSlides. See *"Chapter 4: Creating a Genie Classifier" on page 21* for details.

Below is a brief illustration of the Genie workflow.

The classifier developer:

1	Logs into eSlide Manager in the Genie data hierarchy and opens a new Genie project.
2	Creates a training set that Genie will use to learn to identify tissue classes.
3	Prepares input for Genie by assembling existing eSlides that contain good examples of the structures to be identified. Adds the eSlides to the training set.
4	Defines classes of tissue to be identified by creating a template.
5	Annotates the eSlides in ImageScope to indicate examples of regions containing various targets of interest as well as examples of regions where targets are absent of the structure represented by that training set.
6	Creates a montage of images from eSlides in the set using Genie's Montage Maker.
7	Creates the classifier by running the training algorithm on the montage. The montage consists of all training regions marked in Step 5.
8	Tests the classifier. If performance is not satisfactory, goes back to step 5 to adjust the classifier.

1 Create a Genie Project

2 Create a Training Set

3 Add Slides to the Training Set

4 Define Classes of Tissue *(Create Template)*

5 Mark or Modify Training Samples

6 Create or Re-Create Training Montage

7 Create or Re-Create the Classifier

8 Test or Re-Test the Classifier

Satisfied with the Results?   No

Yes

Let eSlide Manager Users Know the Classifier is Available

For details on performing these steps, see *"Chapter 2: Navigating in Genie" on page 12* and *"Chapter 4: Creating a Genie Classifier" on page 21*.

Integration with Other Aperio ePathology Products

Genie works closely with other Aperio ePathology applications and in most cases, interaction among the programs is automatic and seamless. The interactions between Genie and the other Aperio products are described briefly below and in greater detail in the chapters that follow.

eSlide Manager

Genie is a licensed upgrade to eSlide Manager. After obtaining a valid license and installing the Genie algorithm, to access Genie you must first log into eSlide Manager. Access to Genie is restricted and is determined by the roles defined in eSlide Manager and assigned to a user by the eSlide Manager administrator.

ImageScope

ImageScope allows you to view eSlides and to annotate regions directly on an eSlide by using ImageScope's drawing tools.

2 Navigating in Genie

This chapter discusses the Genie components in the eSlide Manager user interface.

To develop Genie classifiers, an eSlide Manager user must have access to the data group containing the eSlides used in the training set as well as any data included in the Genie project. This user must also be assigned a user role that permits using Genie project and training sets. That user role should also permit creating Genie annotation templates and creating and modifying algorithm macros. These permissions are enabled by the _Genie user role which the eSlide Manager administrator can assign to Genie developers or can use as a base to initialize a new Genie user role. For details on user roles and data groups, see the *eSlide Manager Administrator's Guide*.

eSlide Manager is a digital pathology solution that coordinates all the information about the eSlides (body site, research project information, etc.) in an easy to use, web-based interface. Genie features appear on the eSlide Manager pages when a Genie license has been entered into eSlide Manager and when the Genie users are assigned user roles that allow access to Genie.

Logging into eSlide Manager

To log into eSlide Manager:

1. Open eSlide Manager in your Internet browser.

2. Log into eSlide Manager with the user name and password supplied by your eSlide Manager administrator.

   If you are logging in as the global eSlide Manager administrator, you may see a screen prompting you to select the data hierarchy to use.

   Or if you are not an administrator but have been assigned multiple user roles, you may see a screen from which to choose a user role; select the Genie user role.

   Select the Genie data hierarchy or user role to log into the Genie user interface of eSlide Manager.

The eSlide Manager Main Window

After you log into eSlide Manager and select Genie as the data hierarchy to use (if prompted), you'll see the main eSlide Manager window, shown below.
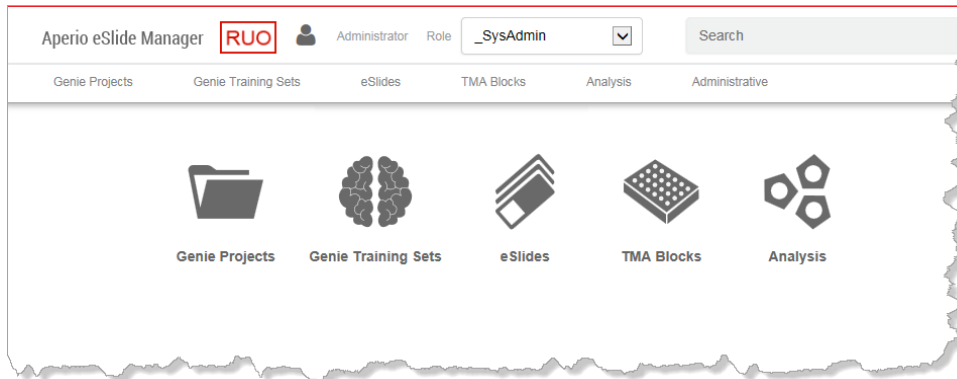
You can navigate among the Genie components either by clicking the links listed along the left side of the page, or by using the menu bar at the top of the page.

- ▶ **Genie Projects** – See all Genie projects in either list or folder format, search for a specific Genie project, or add a new project.

- ▶ **Genie Training Sets** – View training sets in list or folder format or search all training sets to locate a specific one.

- ▶ **eSlides** – List, view, search for and add eSlides.

- ▶ **Analysis** – View image analysis jobs, macros, or classifiers.

(Additional modules may be shown if eSlide Manager is licensed for them.)

The Administrative Menu

The Administrative menu contains different commands depending on your user permissions.

At the minimum, as a Genie developer you will be able to create and maintain Genie annotation templates.

If you have administrative rights, you can use this menu to customize the Genie pages in eSlide Manager (adding new data columns and changing data field names), adding/modifying stains and body sites, and adjusting system settings. For more information on the customizable settings available on this menu and how to use them, see the *eSlide Manager Administrator's Guide*.

Overview of Genie Pages

We will discuss the Genie pages in detail in future chapters, but here is an overview of the two main Genie pages: project detail page and the training set detail page.

To list all Genie projects, click **Genie Projects** on the main eSlide Manager page. The project list page appears.
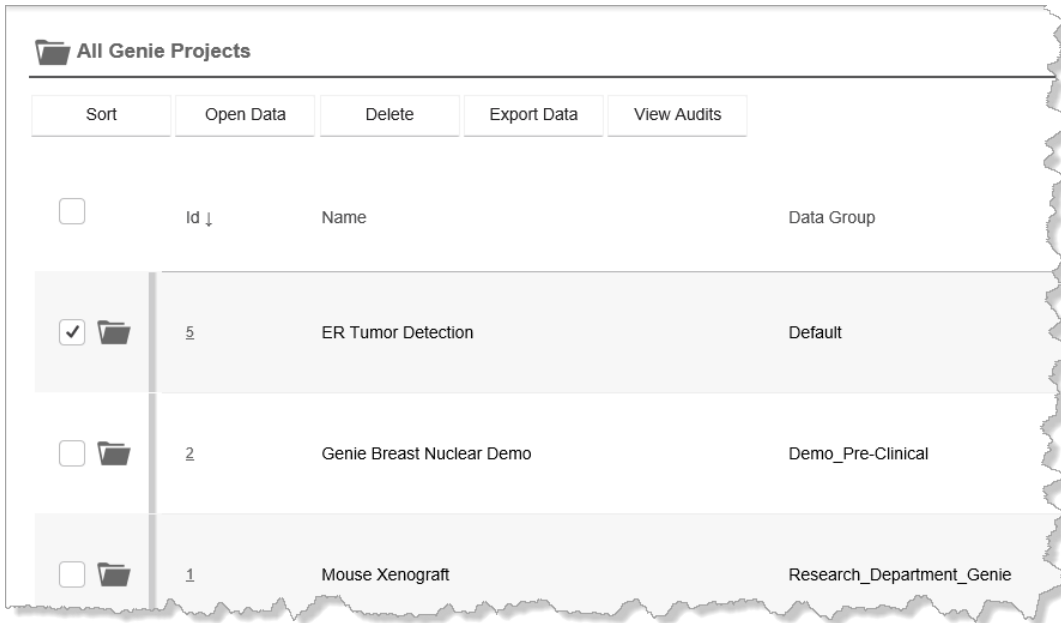
To see the details on a project, click the 📁 icon next to the project you are interested in.

The Genie Project Details page appears:
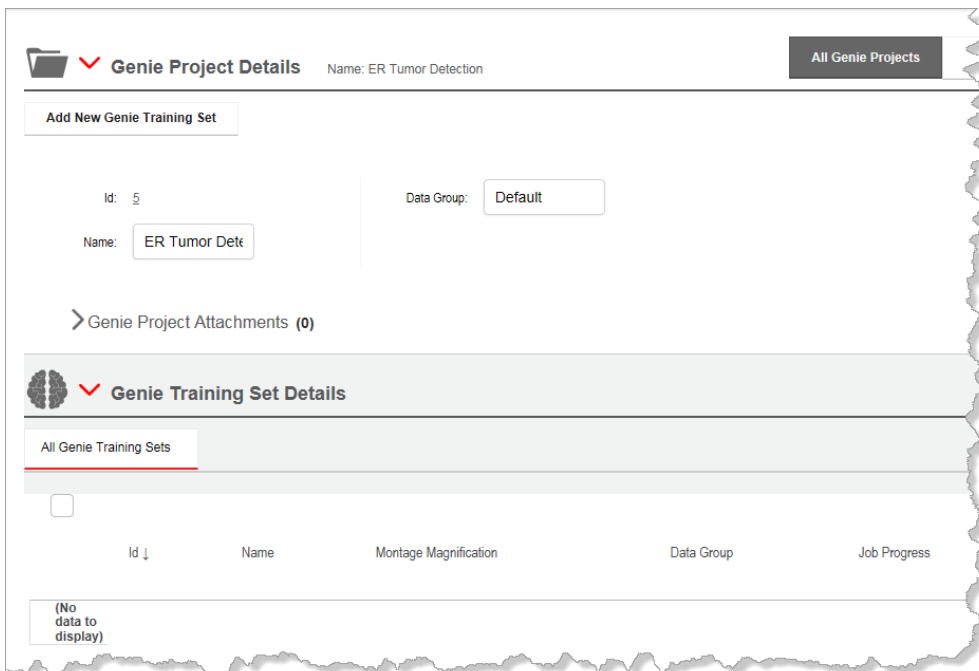
which contains information on the project, and the training sets and eSlides associated with them.

To list all Genie training sets, click **Genie Training Sets** on the main eSlide Manager page.

The training sets list page appears:

To see the details on the training set, click the ⬤ icon next to the training set you are interested in.

The Genie Training Set Details page appears:

which contains information on the project, training set details, including information on the montage and annotation templates, and a list of the eSlides in the training set.

Tips for Navigating the Genie Pages

▸ At any time you can return to the main eSlide Manager page by clicking the eSlide Manager logo at the top left of the page.

▸ You can use your browser's Back arrow to return to a previous page.

▸ Be sure to click the **Save** button on the page when you change text on the page.

▸ From any page in eSlide Manager you can access a Genie page by using the Genie menus at the top of the page.

For More Information

For general information on using eSlide Manager (such as searching, exporting data, customizing eSlide Manager, and so on), see:

- ▶ *eSlide Manager Operator's Guide*
- ▶ *eSlide Manager Administrator's Guide*

For information on using Aperio ePathology image algorithms, refer to the user guide that came with the specific algorithm you are using. Also, see:

- ▶ *Aperio Image Analysis User's Guide*

For information on using ImageScope to view and analyze eSlides, see:

- ▶ *ImageScope User's Guide*

3 Working with Genie

Before you begin creating your own Genie classifiers, please read this chapter which will introduce you to important concepts for understanding the training process.

Before starting right away with creating Genie classifiers, it is worthwhile to spend some time getting familiar with Genie and considering how it can help you solve the problem you are working on. It may be best to start with a simple application, even if is not the main application that you are intending to spend your time on. As with any new tool, you will learn many new things and gain proficiency with time. It is important to realize this and begin your learning process with something simple and move to more advanced projects as your understanding increases.

After you read this chapter, which discusses how to approach specific issues and gives some troubleshooting tips, we recommend going through the steps in the next chapter for a simple project so that you are familiar with the workflow for creating a classifier.

Start Simple

Introducing too much complexity at the beginning of your Genie development project will prove to be very confusing and frustrating. This is particularly true when you are first learning Genie, but even experienced algorithm developers will agree that working from the simple to the complex (introducing complexity only as required) is a good rule to follow and is the best overall approach.

▶ **Training Slides** – Don't worry about putting every sample image you can find in the training set—just add a few representative examples to begin with. You can always add more eSlides later.

▶ **Parameter Values** – Use the eSlide Manager/Genie default values at first. As you progress with the training process, you can consider changing these default values if the performance/accuracy of the training needs to be improved (see *"When to Add Complexity" on page 19*).

▶ **Classes** – Genie requires at least two classes, for example, "Tumor" and "Background." In this case, "Tumor" is the class that we are interested in and "Background" is everything else. It is not essential that there be a class for each type of tissue on the eSlide—this is a common misconception. If you are specifically interested in finding and measuring a number of different tissue types, then you will want a class for each type. The goal should be to have as few classes as possible, while achieving good performance.

▶ **Regions** – You must provide at least one region for each class. Regions should be only as large as necessary to capture the pattern of the tissue. Do not add redundant regions that appear similar. Redundant training data will only slow the training process and will not improve training accuracy. If a class is being used to identify more than one tissue type, such as "Background," then you will likely need to add at least one region of each type of tissue to be included in that class. Don't worry about giving an exhaustive set of regions in the beginning. It is better to train with a few regions in the beginning, adding more regions only as necessary.

▸ **Montage** – Start with regions from a single eSlide. As you train and test the classifier on that eSlide, you will reach a point where you are satisfied. When this occurs, move on to another eSlide and test the classifier. If you find areas on that eSlide where pixels are being misclassified, add regions from those areas to the proper classes, create a new montage, and continue the training process. Repeat this process until all of the eSlides in your training set indicate acceptable classifier performance.

The Montage

Creating a good montage is the key to generating a good classifier with Genie. Throughout the training process, you will modify the montage by adding/subtracting regions and classes until you have arrived at single montage that captures the variation inherent in your training set. Once you have successfully produced a good classifier, changes to the montage need only be made when classifier performance becomes unacceptable. This can happen when Genie encounters new structures in an eSlide that are not represented in the montage. Since Genie does not have an "I don't know what this is" class, Genie will put these new types of pixels into one of the prescribed classes, and more often than not, this will affect classes that you care about. When this happens, the new eSlide can be added to the training set and Genie re-trained to properly classify the newly found structures.

▸ **Montage Magnification** – When a training set is first created, the montage magnification is set to 5x. If the eSlides from which the regions are extracted were scanned at 20x, the resolution of the montage is reduced by a factor of 4. A region in the montage will have 16 times fewer pixels (4-squared) than the corresponding region in the parent image. This has two advantages: First, fewer training pixels means training will be faster; Second, applying the classifier to an eSlide will also be much faster, since the number of pixels to be processed will be reduced by a factor of 16.

The 5x montage magnification is well matched to other system components in terms of computational time. Increasing to 10x will cause a 4-fold increase in training and analysis times. *Montage magnification should only be increased if required to improve classifier performance.*

▸ **Remember to Update the Montage** – If you have added/deleted regions or classes, or changed the montage magnification value, you must create a new montage before continuing the training process. The montage does not automatically update when you change any of these items. If you fail to do this, training will continue on the old montage and will not incorporate your changes. There is currently nothing in the system to indicate that a new montage is required—it is up to you to ensure that you have a current montage.

Training

▸ **Accuracy** – The Genie Training algorithm makes iterative improvements to the classifier by comparing the predicted class with the actual class for the training pixels provided in the montage. The training accuracy of each class is first calculated; these class accuracies are then averaged together to obtain a mean training accuracy—a change to the classifier will only be made if it improves the mean training accuracy. This is important to understand, since it causes each class to be given equal weight, when considering potential improvements to the classifier.

▸ **Background Class(es)** – Since it is not possible to give a single class more weight than another, you may find that classes you don't care about are improving at the expense of the ones you do care about. One way to partially overcome this problem is to put all of the tissue types that you don't care about into a single class, like "Background." This keeps the number of classes you don't care about to a minimum, which helps increase the weight to the ones that are important to you. You may need more than one background class, but try to keep the number of these small.

▶ **Providing Adjacent Regions** – When providing regions for different classes, is it helpful to the training process to provide regions that share a common boundary. You can do this by drawing a region in the first class, then drawing the region in the second class so that one of its edges is next to the boundary of the region in the first class. Since many of the image processing operators used by Genie sense the neighborhood of the training pixels, this helps ensure that the boundaries between classes are being accurately taken into account.

▶ **Continuing the Training Process** – Each time you run the training macro on the montage image, a new output layer is created. These output layers provide a history of the changing accuracy and can be useful to review to see how fast improvements are being made. Whenever a new montage is created (by adding regions for example), these output layers are removed. However, training on the new montage will begin with the current classifier as a starting point. Only if the number of classes or the training algorithm parameters are changed (except for Number of Iterations), will the training begin from scratch.

When to Add Complexity

Complexity should only be introduced out of necessity. Simpler algorithms tend to be more robust and apply to a broader variety of circumstances. While more complex algorithms can provide superior performance, they tend to require more computational time and resources and often work well in a more narrowly defined set of circumstances. When training fails to achieve a high enough accuracy, adding more classes and/or increasing the number of features can help.

▶ **Adding More Classes**

• **Poor Specificity** – This is the case when you are getting too many false positives in a particular class. You should first try adding examples of these false positives to a background class (discussed above). If this does not work, remove them from the background class, define a new class, and add these regions to that class. It may be advantageous to have more than one background class in order to collect the various tissue structures that are not of interest.

• **Poor Sensitivity** – This is the case when you are getting too many false negatives in a particular class; that is, you are not picking a significant amount of tissue for a class. This can happen when the class contains a large amount of variation in color, texture, morphology, etc. Try to determine if the class can be divided into two or more logical parts. For example, if a number of regions in the "Tumor" class are false negatives and are being completely misclassified, add a "Tumor2" class and add these regions to that class. The two tumor classes can be combined in subsequent analysis to form the complete tumor class.

▶ **Training Parameters**

• **Adding More Features** – If you increase the number of classes, you may find it necessary to increase the "Max Number of Features" in order to achieve better training accuracy. The Genie classifier uses two stages in which feature planes are calculated; the first uses spatial/textural features, while the second uses morphological features. The number of features for each stage can be increased. The default number of features (8, 3) should be adequate for 3 or 4 classes. If you have more classes, try increasing the number of features proportionately.

• **Increasing Max Complexity** – The "Max Complexity" parameter for the Genie Training algorithm can be increased. Complexity is proportional to the amount of time required to calculate a feature. Increasing this parameter will allow for more complex algorithms—doing this will slow the training and classification processes, however.

Algorithm Macros

Developing a naming convention for your macros is critical. As an example, "HER2_Genie_Training" and "HER2_Genie_Classifier" would be useful to indicate that these macros were associated with the "HER2" Genie Project and the first is for training on the montage image, while the other is for applying the HER2 classifier to other eSlides. You may also wish to add other identifying information to the macro name to make it unique and easily recognizable.

It is important to remember that a training macro can only be run on the montage image, while the classification macro can be run on any eSlide. The system architecture does not enforce this requirement—it is up to you to select the correct macro when submitting an analysis job. This is where the naming of macros becomes important and it is important that you pay particular attention when selecting macros. If you run the training macro on an eSlide, the analysis job will fail. In this case, all you need to do is resubmit the job with the intended macro and things should proceed as intended. If you run a non-training macro on a montage, the job will finish and the result layer will contain results for the macro you ran. Just delete this layer if you don't want it and proceed with running the training macro on the montage.
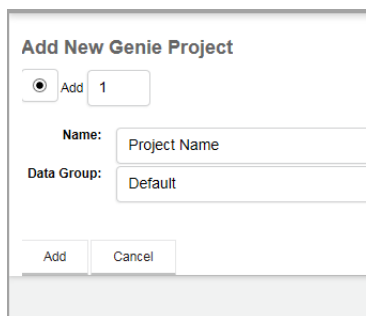
4 Creating a Genie Classifier

This chapter gives step by step instructions for creating a Genie classifier.

Step #1 - Create a Genie Project

The first step in creating a classifier is to create a new Genie project:

1. Log into eSlide Manager as a Genie developer as discussed in *"Logging into eSlide Manager" on page 12*.

2. On the **Genie Projects** menu, click the **Add Genie Projects** link. The Add New Genie Projects page appears:

    We recommend creating one Genie project, specifying its name and selecting the data group to which it belongs from the drop-down list, then clicking **Add**.

3. To open the project, click the ▣ icon on the Project list page. You see the Project Details page.
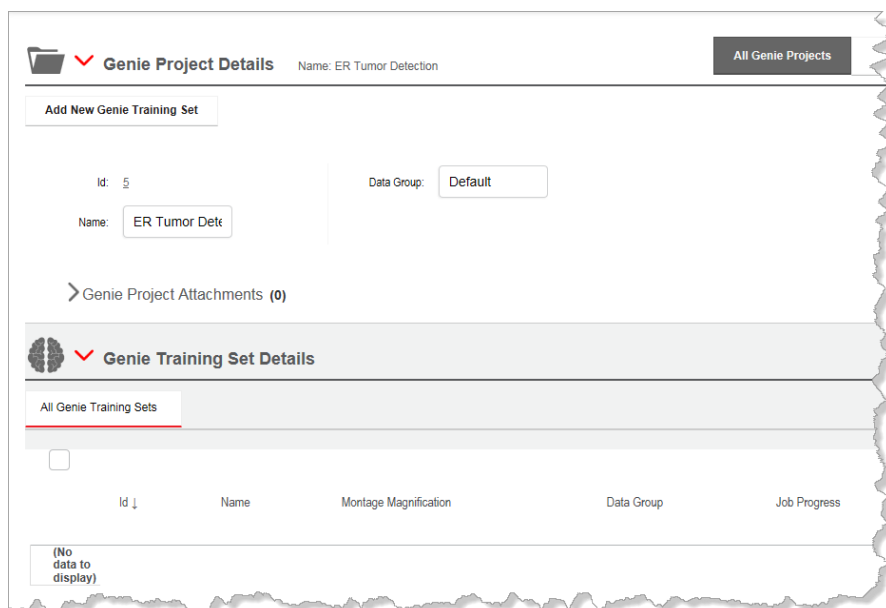
Tips on Using the Project Details Page

The Genie project details page summarizes basic information, allows you to navigate between projects, and to add attachments or training sets to a project.

▸ To navigate between Genie projects, click the **Previous Genie Project** or **Next Genie Project** links, or click **Genie Project List** to view all of them.

▸ If you want to modify the Name or Data Group fields, you may do so. Click **Save** to keep your changes or **Reset** to discard them.

▸ You can add documents such as project design documents by using the **Add Attachment** link in the Genie Project Attachment section.

Step #2 – Create a Training Set

The training set contains samples of the tissue you want to characterize; Genie analyzes the training set to learn about those tissue classes.

Decide what eSlides you want to use to train Genie. To do this, spend some time thinking about what characteristics you want to identify so you can create an effective classifier. It's not a good idea to pick eSlides that are too similar, as Genie won't learn enough from them. On the other hand, too much variation in your samples will make Genie's job harder and may decrease accuracy. For more information on making this decision, see *"Chapter 3: Working with Genie" on page 17*.

The number of eSlides depends on the complexity of what you are trying to identify, and the amount of variation you want to encompass.

To create a training set:

1. Create a Genie project as discussed in Step #1.

2. On the Genie Project Details page, click **Add New Genie Training Set** in the Genie Project Genie Training Sets

section. (Note that you may have multiple training sets for one project.)

The Add New Genie Training Sets page appears:

3. On the Add New Genie Training Sets page, enter the name of the training set you wish to add (for example, **IHC ER**), which will be the basis of the name of the classifier you are going to create.

4. Type a montage magnification value. The default value is 5.0, which is a good starting value. For information on why you would want to change the magnification value, see *"Chapter 3: Working with Genie" on page 17.*

5. Click **Add**.

> *The final classifier name will be based on the training set name you enter when you create the training set (for example, "IHC ER"). However, to ensure that classifiers have unique names, Genie prepends a numeric value to the front of the classifier name. For example, the training set IHC ER may create a classifier named "35_IHC ER."*

When you return to the Genie Project Details page in Genie, your newly added training set is listed.

Tips on Using Training Sets

To view a list of training sets that exist in Genie:
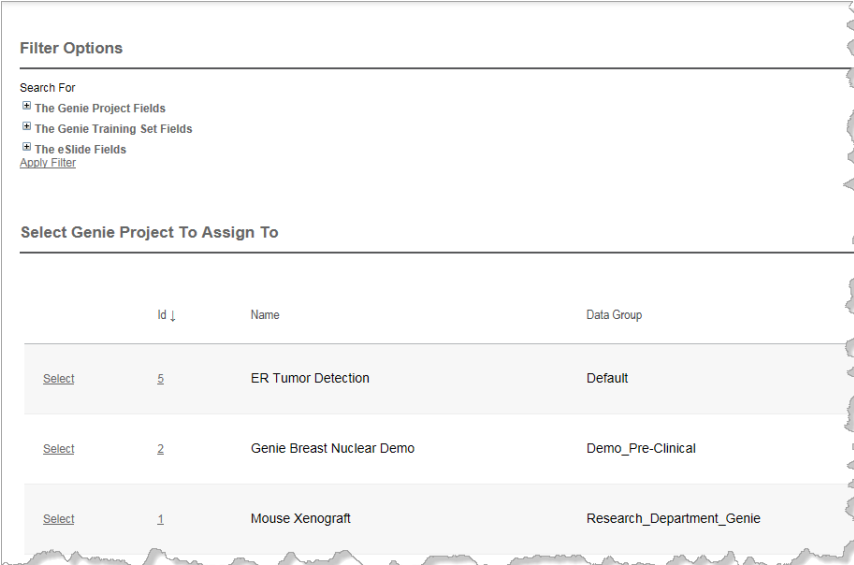
1. Go to the main eSlide Manager page and click **Genie Training Sets** to display a listing.

2. To display the training set details page for any of the training sets listed, select the check box beside the Training Set ID number and click **Open Data** or click the ⬤ icon next to the training set you are interested in.

To add an existing training set to a Genie project:

1. Open a training set as discussed above.

2. Click **Assign to Existing Genie Project**.

   Remember that a training set can have only one parent project. If you want to add a training set that has already been assigned to another Genie project, you will have to detach it from that project first.

3. Click **Assign** next to the Genie project you want to assign the training set to. (If you have multiple Genie projects, you can use the Filter Options to search for a specific project. See the *eSlide Manager Operator's Guide* for details on using the eSlide Manager search functions.)

You return to the Genie project detail page where you see the training set has been added.

Step #3 – Add Slides to the Training Set

As discussed in Step #2, choosing the eSlides for your training set is an important decision, and can affect the usefulness of the classifier you create, so be careful to choose eSlides that have good samples of the structures you are trying to identify.

To add eSlides to the training set:

1. Go to the Genie Training Sets menu at the top of the eSlide Manager page and select **All Genie Training Sets (As List)**. You see a list of training sets.

2. On the Genie Training Sets page, open a training set by clicking the ⬤ icon next to it. A details page appears:
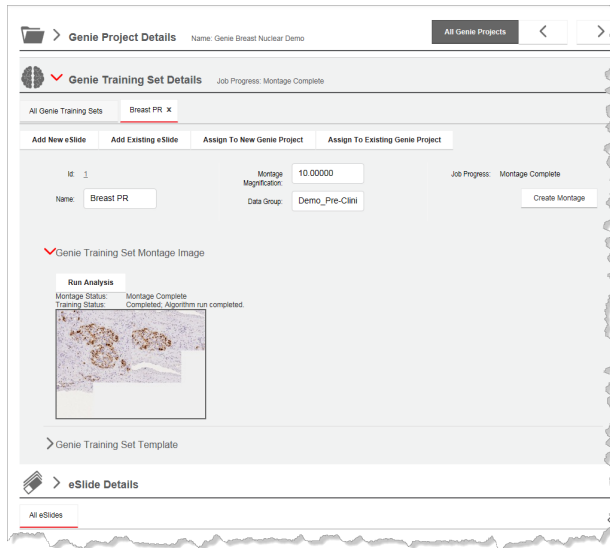
3. In the Genie Training Set eSlides section, click **Add New eSlide** to add an eSlide that does not yet exist (you can add its image later after the slide is scanned), or **Add Existing eSlide** to add an eSlide that has already been defined in eSlide Manager.

    If adding existing eSlides, you see the Add Unassigned eSlides to Genie Training Set page, which contains a list of all eSlides that can be added to this training set (that is, eSlides that are not already assigned to another training set):
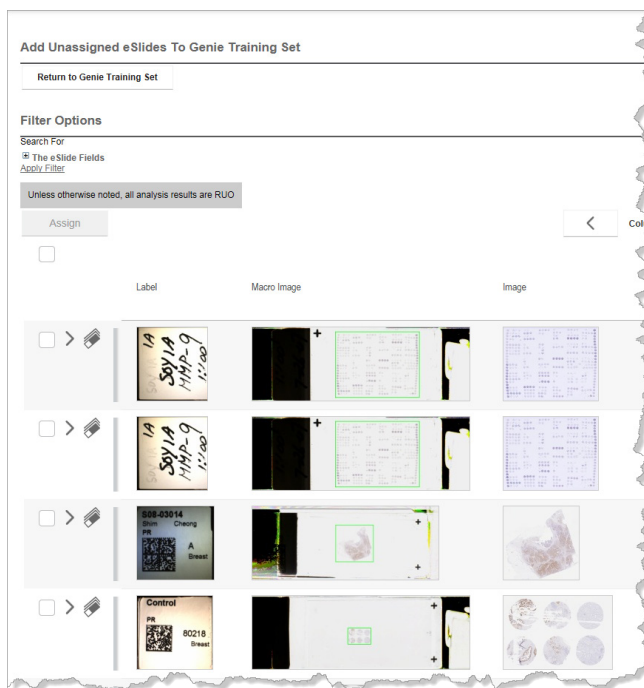
    Note the Filter Options section which allows you to search for eSlides that meet certain criteria (such as slides that are stained with an ER stain).

    Tip: If you want to use the same eSlide in multiple training sets, copy the eSlide using a different name so that it appears in eSlide Manager as a different eSlide.

4. Select the eSlides you want to use by selecting the check box next to each one, and then clicking **Assign** at the top of the eSlide list.

Step #4 – Define Classes of Tissue (Create a Template)

After setting up the training set, you need to define the classes of tissue structures you are going to find. This is done by building an annotation template that defines the classes.

Genie creates color-coded layers based on these classes, making it easy to distinguish the annotated regions. These layers are stored in the annotation template that is associated with the training set.

When you add eSlides to a training set, Genie applies the annotation template for the set to that eSlide. If you edit the definitions of the classes and corresponding layer properties in the annotation template for one eSlide in a training set, Genie modifies the corresponding annotation layers for all eSlides in the set. For example, if you add or delete classes or change the names or colors of associated layers, these annotation properties will be modified throughout the training set.

1. Open the training set. You can do this in a variety of ways:

    a. Go to the main eSlide Manager page and click **Genie Training Sets** then click next to the training set you want to open; or,

    b. Open a project and in the Genie Projects Genie Training Sets section click next to the training set assigned to that project.

2. On the training set details page, under the Genie Training Set Template section, click **Add Class**.

A new box appears in which you can type the name of the class (for example, **Tumor**):
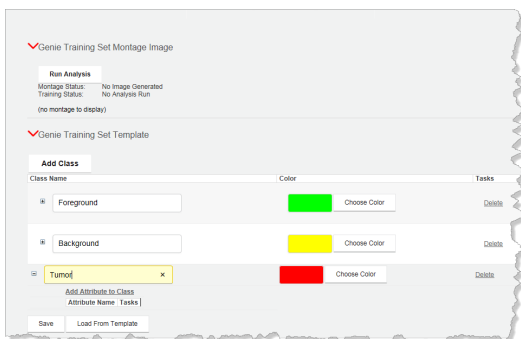
3. Click **Add Class** to add another class (for example, Background).

4. Click **Save**.

Tips on Creating Templates

‣ You can use the **Choose Color** button to select the color that will be used to display this class.

‣ You can also load settings from an existing template by using the **Load from Template** button. (See *"Creating a Template for Use with Multiple Training Sets"* below for information on creating a template that can be used with multiple training sets.)

‣ You can modify the template by adding and deleting layers, renaming them, changing their colors and adding attributes. (You can use attributes to add descriptive text to the class or to create fields into which text can be manually entered later.)

Creating a Template for Use with Multiple Training Sets

Instead of creating a template for each training set, you can create and save a template that can be applied to multiple training sets.

1. Go to the **Administrative** menu at the top of the eSlide Manager page and select **Genie Annotation Templates**.

2. Click **Add**. The Add Annotation Template page appears.

3. Enter a template name and click **Save**.

4. To define the classes in this template, click **Add** in the Template Layers section**.**

5. A new box appears in which you can type the name of the class.
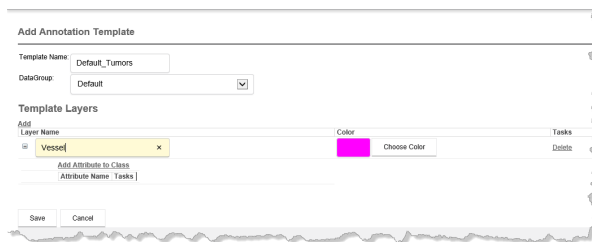
6. Keep clicking **Add** until you enter all the classes you want to use.

7. If you want to add attributes to the class, click **Add Attribute to Class**.

8. If you want to change the color with which tissue identified as belonging to the class will be displayed, click **Choose Color**.

9. When finished with the template, click **Save** and it appears on the Annotations Templates page.

To apply an annotation template to a training set:

1. Open a training set.

2. In the Genie Training Set Template section of the training set details page, click **Load from Template** and select the template you created from the popup window that appears.

> *If your Internet browser is set to open popup windows in a tab instead of a new window, the list of templates will open in a new tab—after you select the template, you may have a problem returning to the main eSlide Manager page. For this reason, we recommend you change your browser options to open a popup in a new window rather than in a tab.*

Step #5 – Mark Training Samples

For each of the eSlides in the training set, you need to draw annotations that identify the type of tissue you defined in Step #4, so that Genie can be trained on what tissue fits those definitions.

> *When you mark training regions on eSlides in the training set, remember that at least one region for each template class must be marked (not on each eSlide, but in the entire set of training eSlides).*

---

1. Open the training set and go to the Genie Training Set eSlides section.
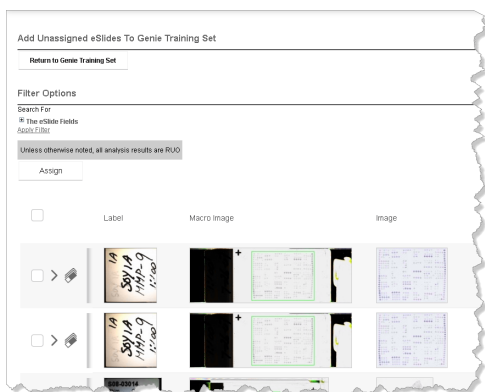
2. Click on the thumbnail image of the first eSlide to open it in ImageScope.

3. Change the eSlide magnification using the zoom slider to a magnification that makes it easier to view cell structures.

4. Go to the ImageScope **View** menu and select **Annotations** to see the template defined for this training set. For example:
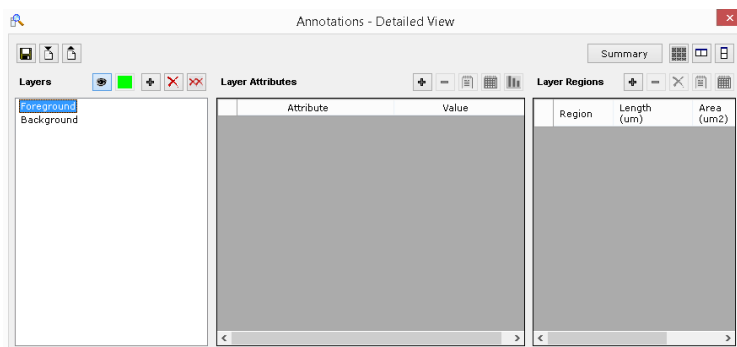
Every eSlide in this training set uses this same template.

5. Click on a named layer to select that type of tissue (for example, **Tissue**), and use the ImageScope ✎ pen tool to draw an annotation around a good example of that type of tissue. Do the same in several areas of the eSlide. Note that you can also use the rectangle and ellipsis drawing tools. You can also use the negative pen tool ✎ to draw an annotation around an area you wish to *exclude* from analysis—in this case, the area marked with the negative pen must be entirely surrounded by a region boundary. See the example below.

6. Click on another named layer and draw annotations for that type of tissue.

   The colors used for the annotations are the same as you previously defined in the template. In our example, the yellow region identifies background tissue. Note the use of the negative pen to draw an area to exclude from analysis (the dotted line within the rectangle below)

If the colors do not match the colors assigned in the template for that type of tissue, you may have clicked on the incorrect layer before drawing. If you need to delete an annotation, you can do so by clicking it to select it and pressing the **Delete** key on your keyboard.

7. Save the annotations by closing the image in ImageScope and clicking **Yes** when prompted to save the changes.

8. Open the next eSlide and annotate the tissue types. Repeat for each eSlide.

Step #6 – Create the Montage

The montage is a single image consisting of all of the training regions that have been drawn on the eSlides (see Step #5) and will be used by the Genie training algorithm to learn about tissue classifications.

To create a montage:

1. Open a training set.

2. In the Genie Training Set Details section of the page, click **Create Montage**. (The Montage Magnification box should already contain a value—lower magnifications can be used to make the training and classification run faster. You can experiment with this value later to see which values give acceptable performance.) If you change the magnification value here, it will *not* take effect until you click **Create Montage** to create a new montage.

   As the montage is created, you see text next to the **Run Analysis** link cycle through status messages until it says **Montage Complete**.

3. When processing is complete, the montage appears in the Genie Training Set Montage Image section:
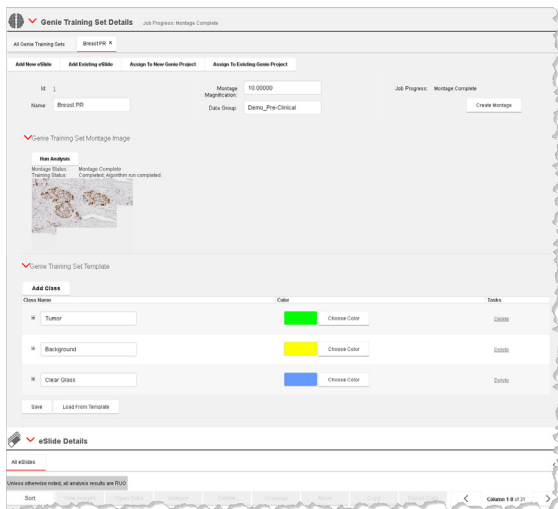
When you generate a montage, Genie creates an annotation layer that contains the class names and the colors used to color code the corresponding classes in the mark-up image.

Step #7 – Create the Classifier

Now that you have created the montage, you will run the Genie training algorithm on it to create the classifier.

> *If you change any of the regions in the eSlides in a training set (for example, adding or deleting regions) or change the montage magnification, you must re-create the montage for the changes to take effect in the montage.*

To see a larger version of the montage:

1. Click on the thumbnail image of the montage to see the image in ImageScope.

2. The montage shows the mark-up images in a semi-transparent layer. The mark-up layer uses the same color palette used to designate the classes and associated layers defined in the training set template.
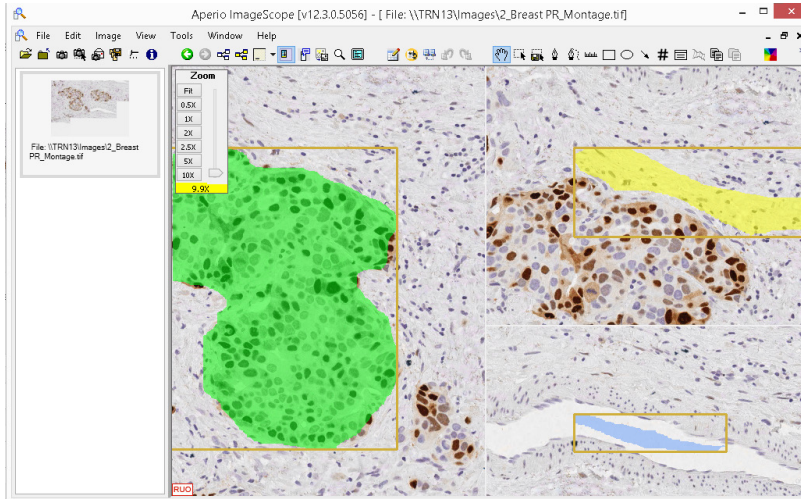
Create a Training Macro

The training macro is based on the Genie training algorithm and is used to analyze the training montage to create a Genie classifier. Note that you only use training macros to analyze training montages, never to analyze individual eSlides. The training macro is a general algorithm and can be used to analyze any training montage. The classifier that is created is a specific pre-processor that identifies specific tissue types based on the template you used to annotate the training set eSlides.

With the montage open in ImageScope:

1. Go to the **View** menu on the ImageScope menu bar and select **Analysis**. Click **Choose Algorithm**.
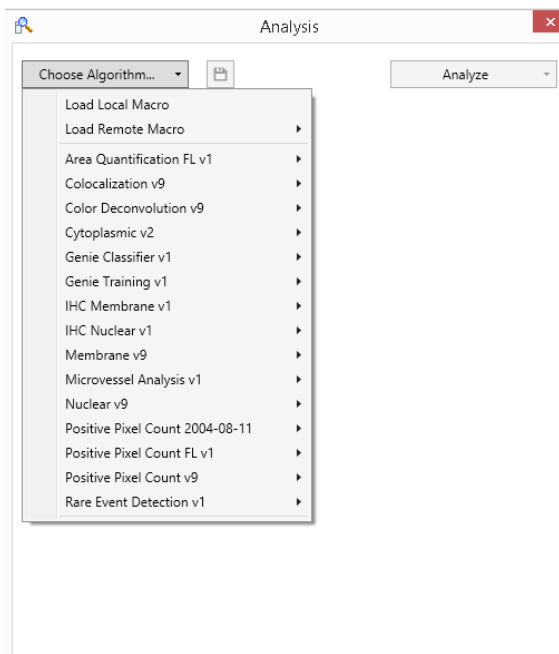
This window shows the list of available algorithms from which macros can be created.

2. Click **Load Remote Macro**, then select a Genie Training algorithm:
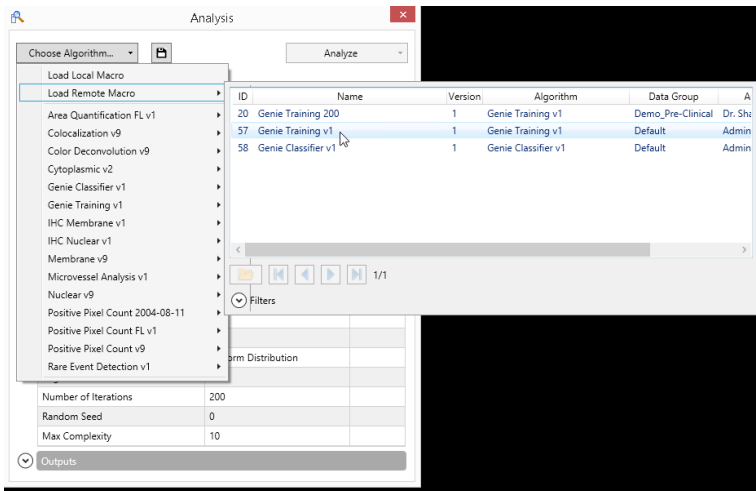
You now see the Genie Training algorithm parameters. For a quick reference to these parameters, see *"Appendix A: Genie Training and Classifier Algorithm Parameters" on page 45*.

In most cases, you can leave these parameters set to their default values, but one parameter that you might want to experiment with is the Number of Iterations parameter. The default value is 200. In adjusting this number, you need to balance the complexity of your problem and analysis time. Generally, the greater the number of iterations, the better the accuracy (and the longer the analysis time) but after a point increasing this number results in very little improvement.

Because Genie learns each time you run the training macro, running the training macro once with 1,000 iterations has the same effect as running it five times with 200 iterations. For more information on using the parameters to fine-tune your training, see *"Chapter 3: Working with Genie" on page 17*.
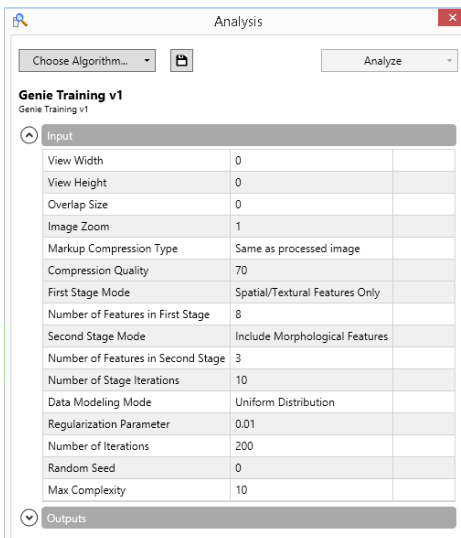
3. Click the Save icon to save the macro. (The training macro consists of all of the parameter settings for the training algorithm and is used to analyze the training montage.) *Don't* click **Run**—you will be using the **Run Analysis** link on the eSlide Manager Training Set Details page to analyze the montage with this macro.

4. In the save macro window, type a name such as **Genie Training - IHC**, select a data group, and click **OK**.

5. Close ImageScope.

Run the Training Analysis

1. In the Genie Training Set Montage Image section of the training set details page, click **Run Analysis**.
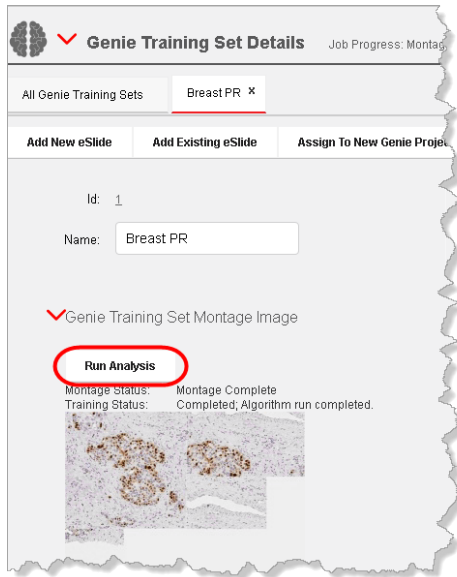
2. On the Analysis page, make sure the macro you saved above is shown in the Select Analysis Macro box. Also select:

   a. **Create mark-up image(s)**

   b. **Most Recent** in the Select Input Annotation Layer
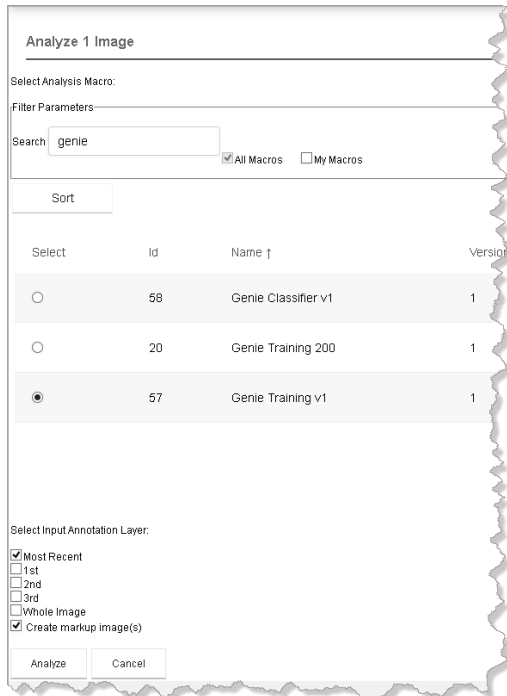
3. Click **Analyze.** This submits the analysis job and takes you to the Analysis Jobs page. Note that the analysis may take a while, depending on how many eSlides you included in the training set and the magnification set for the montage. When the analysis is finished, this page shows **Complete** in the Status column. Press F5 to refresh the page to see the latest analysis status.
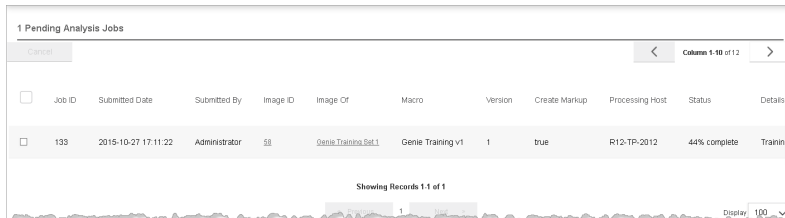
4. When the analysis is complete, open the training set.

5. Click on the thumbnail of the montage to open it in ImageScope. You see a result layer in the ImageScope Annotations window (Genie Training output) in addition to the original montage layer. Click the Genie Training output layer to see the training results:
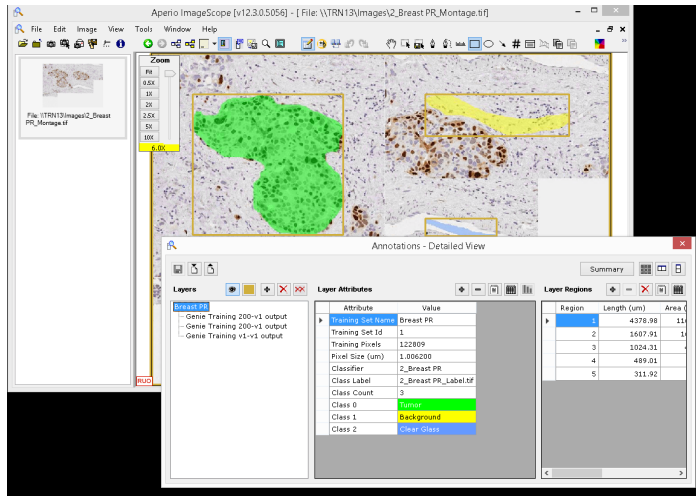
The Mean Training Accuracy value is the primary indicator of the effectiveness of the training analysis. You will not achieve perfection, 100%, but you can decide for yourself what percentage is acceptable for your project.

And you see the mark-up image in the main ImageScope window:
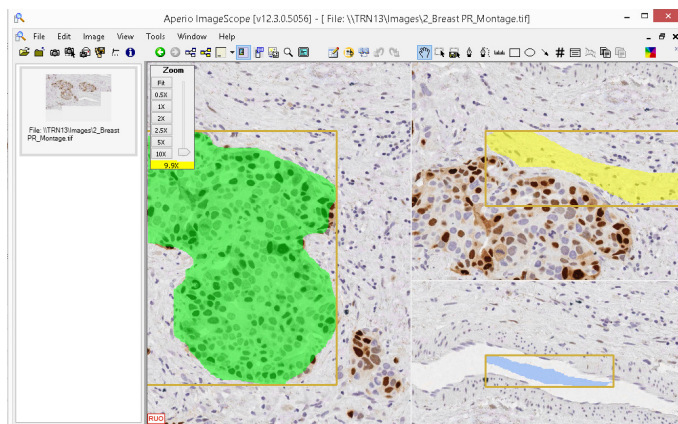
If the mark-up image in the ImageScope window shows smaller areas of color in the middle of another color, these represent errors in training. For example, if the tumor areas are green and background areas are yellow, small bits of green in background areas represent classification errors:
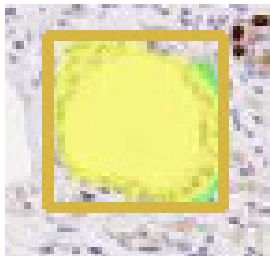
Running the training algorithm has created the Genie classifier, which we will test in the next step. See *"Chapter 3: Working with Genie" on page 17* for tips on maximizing training accuracy.

Viewing the Montage Regions in Context

You can use the ImageScope link feature to view the different regions in the montage in their original context in the training eSlide. On the original eSlide, you can redraw the region, delete it, or add additional regions to any of the class layers. Be sure to re-create the montage again if you change the regions to include these changes in the montage.

For details on using the ImageScope Link Manager, see the *ImageScope User's Guide*. Note that because Genie automatically creates the links for you in the montage, you do not have to use the Link Manager itself (the ⊞ icon on the ImageScope tool bar) to set up these links, but only need to use the forward link icon 🔗 to navigate through the existing links.

1. If not already open in ImageScope, click on the montage thumbnail image on the training set detail page to open it in ImageScope.

2. Click on the rectangular border around a region to select it. Or, select the region by clicking it in the Annotations window Layer Regions pane. When selected, the region border contains a black line:
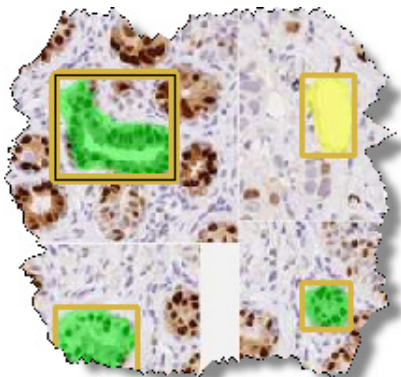
   Now click the **Next Link** icon on the ImageScope toolbar, 🔗. ImageScope opens the original eSlide on which the region was drawn and centers the annotation in the middle of the ImageScope window. (The black line in its boundary indicates which region is selected.)

If you have the filmstrip turned on, you can see that the montage and the eSlide are both open at the same time. (To turn on the filmstrip, go to the **View** menu and select **Filmstrip**.)

You can click on the montage in the filmstrip to go back to the montage, select another region, and click 🔗 to go to its parent eSlide.

Step #8 – Test the Classifier

Now that you have created the Genie classifier, you will want to test it to see how well it identifies the tissue classes.
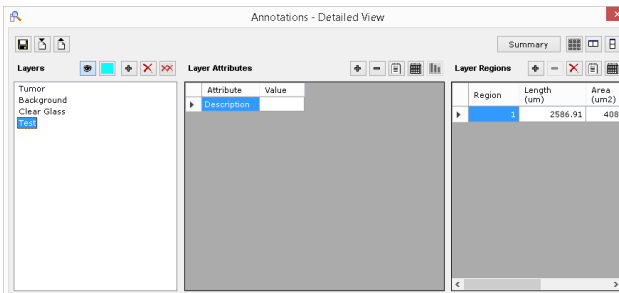
Create a Testing Region

1. Open the project or the training set and open one of the training set eSlides by clicking on its thumbnail in the eSlide list.

2. Go to the ImageScope **View** menu and select **Annotations**. You see the training set template in the Annotations window.

3. Create a new layer for testing purposes by clicking ✚ on the Layers pane of the Annotations window.
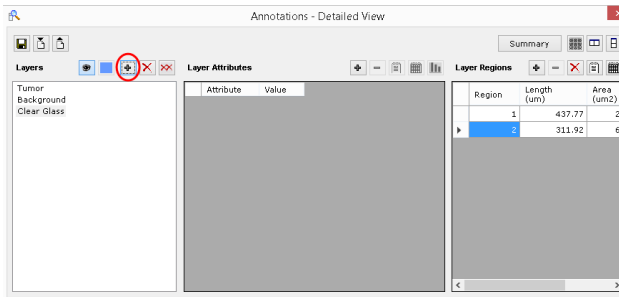
4. Click on the default layer name and change it to something that will remind you of its purpose, like **Test**.

5. Click on that layer in the Annotations window to select it and use the ImageScope rectangle tool to draw a testing region on the image which is larger than the original tissue annotations:
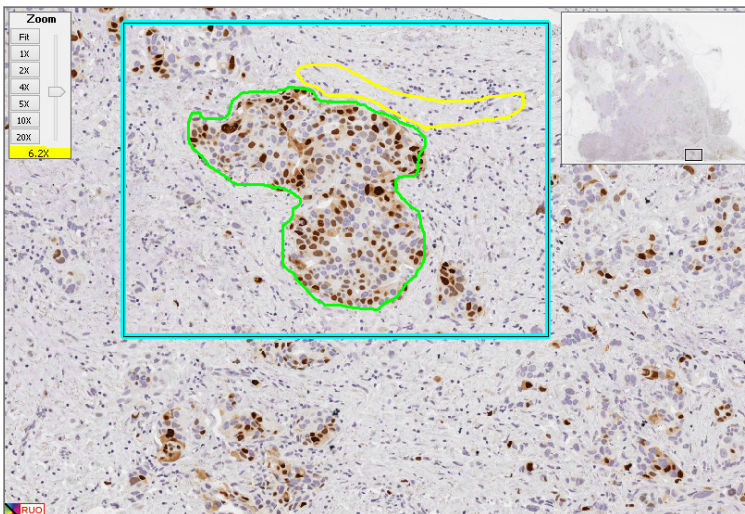

6. Save the new annotation by clicking 🖫 on the Layers pane in the Annotations window.

Create a Classification Macro

1. Go to the **View** menu on the ImageScope menu bar and select **Analysis**. The Analysis window appears:

2. Click **Choose Algorithm** on the Analysis window and click **Load Remote Macro** to see the list of macros on eSlide Manager.

3. Click the Genie Classifier algorithm macro to select it.
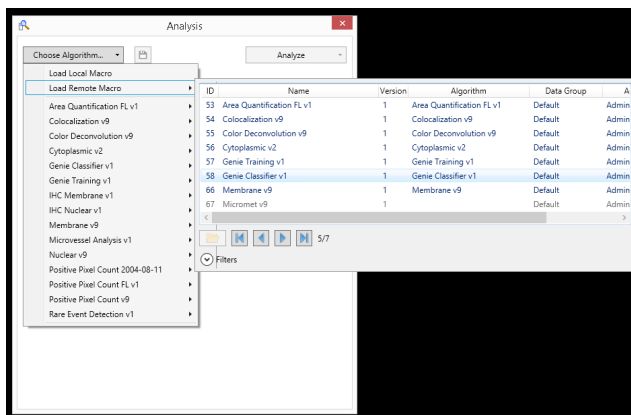
You now see the Genie Classifier algorithm parameters. For a quick reference to these parameters, see *"Appendix A: Genie Training and Classifier Algorithm Parameters" on page 45.*
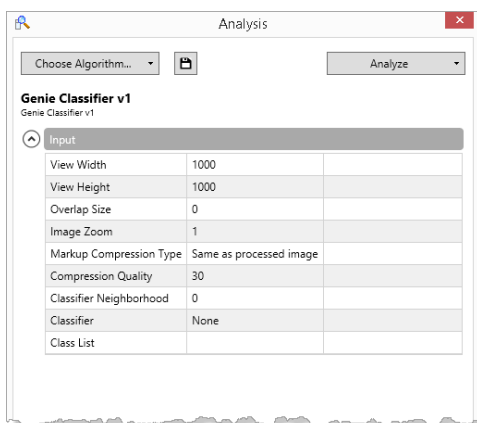
4. Select the classifier you want to use to create the macro from the Classifier drop-down list (this is the name we gave our training set). The list will contain every classifier you have developed on the eSlide Manager site.
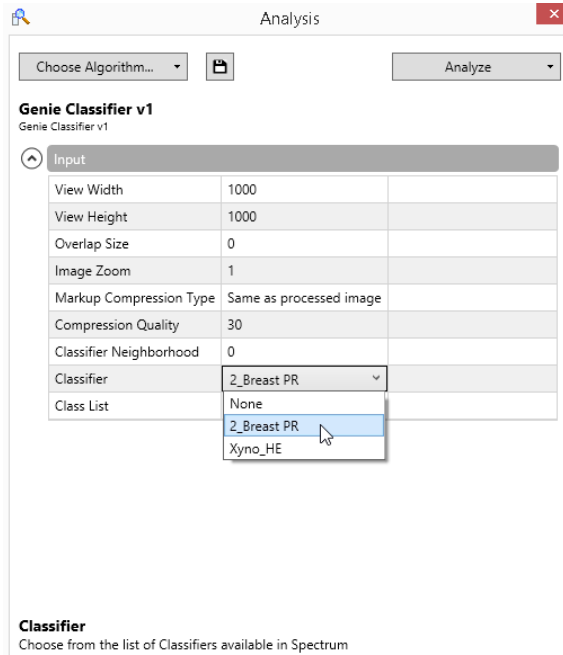
5. Click in the Class List box to see the classes you defined and select which classes you want to use for this macro.
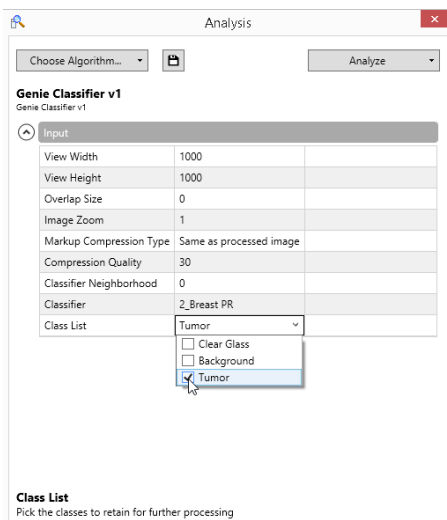
6. Click the Save icon.

7. In the save macro window, type a name that identifies the classifier you selected, such as IHC_ER and click **OK**.

Run the Classifier Macro

The classifier macro is tied to a specific classifier.

1. Open the eSlide on which you created the testing region.

2. Select the Test annotation.

3. Go to the ImageScope **View** menu and select **Analysis**.

4. On the Analysis window, click **Load Remote Macro** and select the macro you created above.

5. On the Analysis window, click **Analyze** and select **Analyze annotations**.

   When the analysis completes, the Annotations window shows analysis results in the Test layer you created, with the fractional content of each class as a percentage of the region of analysis. Also shown is a mark-up image in the main window that shows the color-coded tissue classes.

Improving Classification Performance

See *"Chapter 3: Working with Genie" on page 17* for tips on improving classification performance. Here are some general tips:

1. Add regions in the image where tissue classes were missed in the Genie Classifier output layer. Smaller areas of color in the middle of another color in the ImageScope mark-up image represent errors in training. For example, if the tumor areas are green and background areas are yellow, small bits of yellow in tumor areas represent classification errors.

2. Reduce the montage magnification to speed up the calculations and create a new montage image.

3. Run the Genie training algorithm macro.

4. Open the montage image and view the results.

5. Open the eSlide and analyze the same test region as before. Now you will see that the tissue class regions are much more filled in and the classifier is doing a better job at finding most of the tissue of that type.

Exporting and Importing Classifiers

Every classifier you create can be used by anyone on your eSlide Manager site who has permission to analyze images.

Classifiers you have developed on one eSlide Manager site can be exported and then imported on another eSlide Manager site. This allows you to share classifiers with other eSlide Manager sites.
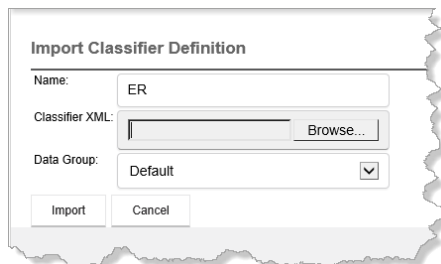
To Export a Classifier

1. Logged in as an eSlide Manager administrator, go to the **Analysis** menu and select **Macros**.

2. On the Analysis Macros page, choose a classifier in the Classifiers section and click **Export**.
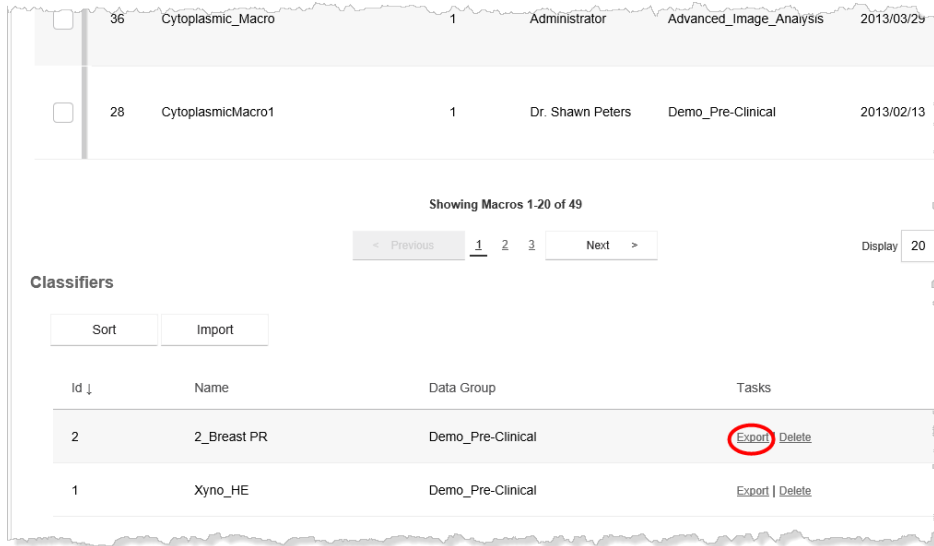
3. Your Internet browser displays its standard window for saving a file; save the file to your disk. The file is saved with a .GSF extension.

To Import a Classifier

1. Logged in as an eSlide Manager administrator, go to the **Analysis** menu and select **Macros**.

2. On the Analysis Macros page, click **Import** in the Classifiers section of the page. You see the Import Classifier Definition page:

3. Type a name for the classifier in the **Name** text box.

4. Click the **Browse** button to find the .GSF file that is the exported classifier.

5. Select the data group to put the classifier into.

6. Click **Import**.

Using the Classifier

When the developer has created the Genie classifier, the classifier is ready to be used by other eSlide Manager users. This is how the image analysis user uses a classifier:

1. Log into eSlide Manager as an administrator.

2. Open an eSlide from eSlide Manager in ImageScope.

3. Go to the **View** menu and select **Analysis** (or type Control G) to open the Analysis window:

4. Click **Choose Algorithm**.

5. Select an algorithm (for example, the Positive Pixel Count algorithm). You see the default settings.

6. Open the Inputs section of the algorithm parameters and select a Genie classifier in the Classifier box. You have available any of the Genie classifiers stored on this eSlide Manager site to use as a pre-processor to the algorithm for which you are creating the macro.
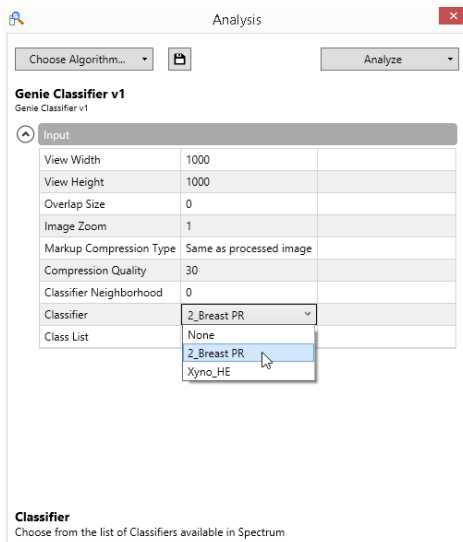
7. On the algorithm parameters window, modify the **Class List** parameter to select the classes to use for analysis:
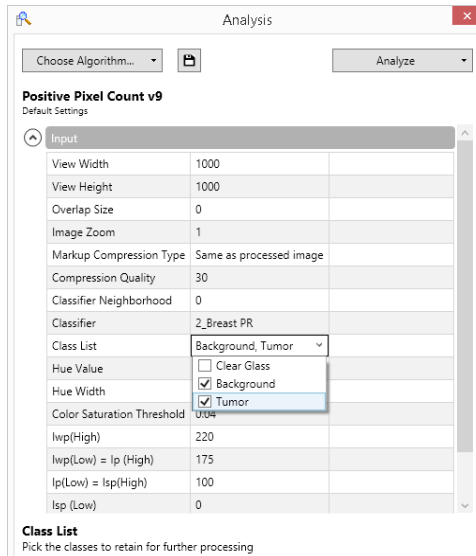
And then clear the check box next to any classes you don't want to use.

8. Save the macro with a unique name.

eSlide Manager users can now run the saved algorithm macro at any time, and the Genie classifier will be used to pre-identify areas on which to run the analysis.

Results of Using the Genie Classifier

After the algorithm user runs a macro that uses a Genie classifier, the mark-up image is the easiest way to see how the classifier has affected the analysis results.

The example below shows the portion of an eSlide that was selected for analysis.
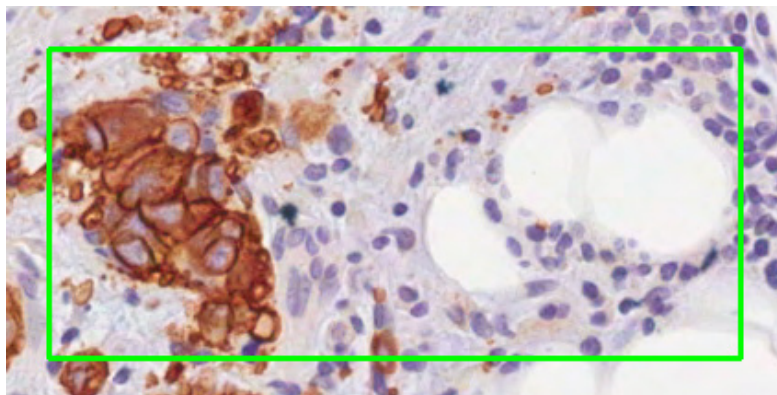
We then used the Positive Pixel Count algorithm, selecting a Genie classifier that identifies possible tumor cells. After running the algorithm, note the colored areas of the mark-up image.
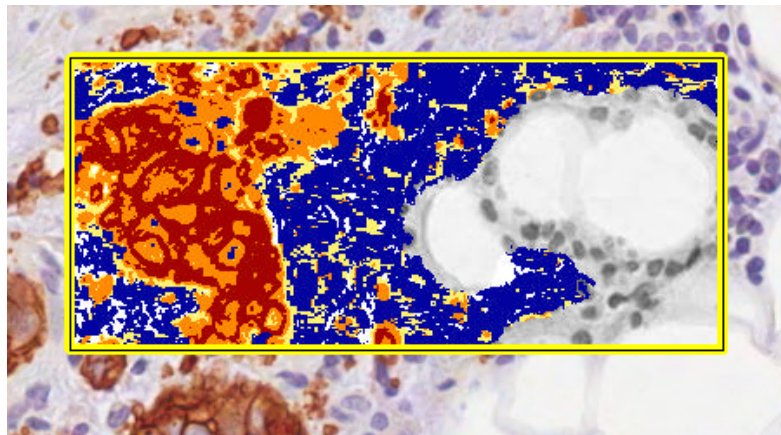
The colored areas indicate the tissue that was identified by the Genie classifier as tumor cells—therefore, only this area of the selected region was analyzed by the Positive Pixel Count algorithm. The colors correspond to the quantitative results returned by the algorithm (for example, the dark red pixels are those identified by Positive Pixel Count as strongly positive pixels).

The area that is in black and white indicates tissue that was not identified by the Genie classifier as tumor cells and so was not analyzed.

A Genie Training and Classifier Algorithm Parameters

This appendix contains quick reference information on the parameters you can supply to the Genie training and classifier algorithms during the process of creating a Genie classifier.

Genie Training Algorithm Parameters

The Genie Training Algorithm parameter window shows a number of items you can adjust to affect tissue identification:
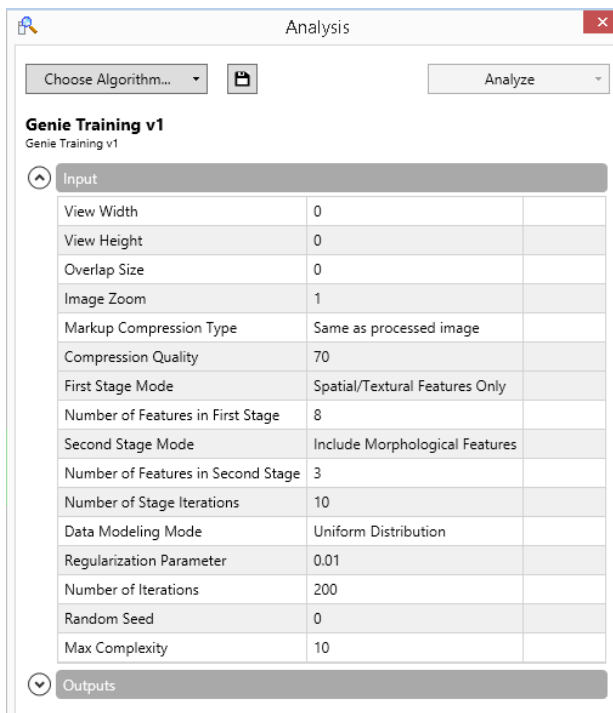
Introduction to Genie Training Architecture

Broadly speaking, Genie training is a randomly iterative process in which different image processing features are constantly generated and applied to create a classifier or to improve on an existing one. Different parameters can be adjusted to guide the training in a specific way. Because of the random nature of the training process, it is hard to quantify the effects of changing a parameter on the accuracy. Similar parameter changes can have contrasting effects because of training data or randomness. We suggest you use the default parameters for Genie training unless you understand the Genie architecture and parameter definitions. Please refer to the drawing below for an overview of Genie architecture.
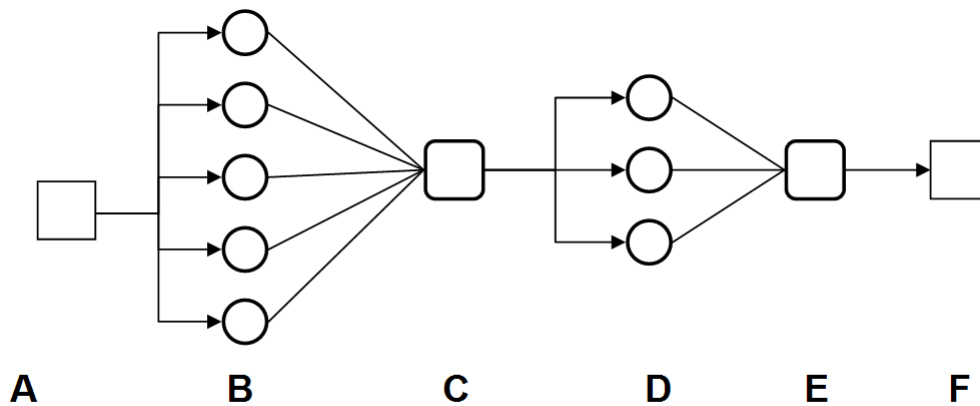
A	Input – Montage Image.
B	Evolving Spectral/Textural Features (1st Stage)
C	Mahalanobis Classifier Combines Spectral/Textural Features into a Preliminary Classification ì\Image. (1st Stage)
D	Evolving Morphological Features (2nd Stage)
E	Mahalanobis Classifier Combines Morphological Features into a Final Classification Image (2nd Stage)
F	Output – Final Classification Image/Result

Genie Training Parameter Definitions

The following parameters appear on the Genie Training Algorithm window:

▶ **View Width**, **View Height**, **Overlap Size**, and **Image Zoom** – Cannot be changed.

▶ **Mark-up Compression Type** – Sets the compression type for the algorithm analysis mark-up image. The default value is 0 – Same as processed image.

▶ **Compression Quality** – Higher compression quality makes the analysis take longer to process and results in a larger image file. This value does not apply to all compression types. The default value is 70.

▶ **First Stage Mode** – Choose a mode for the first training stage. The default is 0 – Spatial/Textural Features only (0). Other options are 1 – Raw Image Planes Only and 2 – Spatial/Textural Features + Raw Image Planes. Raw Image Planes are simply Red, Green and Blue channels of the Montage Image.

▶ **Number of Features in First Stage** – Number of feature attributes to be used in the first stage of the training. The default value is 8. A larger value may be useful for complex problems. Values larger than 12 are memory intensive and time-consuming.

▶ **Second Stage Mode** – Mode to be used for the second stage of training. The default is 1 – Include Morphological Features. Another option is 0 – No Morphological Features.

▶ **Number of Features in Second Stage** – Number of feature attributes to be used in the second stage of training. The default value is 3.

- ▶ **Number of Stage Iterations** – Number of iterations before optimizing the next stage. The default value is 10.

- ▶ **Data Modeling Mode** – Selects a mode for modeling the data as a Gaussian distribution. The default value is 1 – Uniform. Other options are: 0 – Independent Distribution, 2 – Semi-Uniform Distribution.

- ▶ **Regularization Parameter** – This value affects the stability of the training. The default value is 0.01.

- ▶ **Number of Iterations** – Total number of iterations the training will go through. The default value is 200.

- ▶ **Random Seed** – Sets the random number generator seed for the evolution of the training. The default value of zero allows the random number generator to set its seed from the current state of the host computer's clock. For repeatable results (classifiers), a fixed random seed can be set by choosing the same non-zero value (say 2).

- ▶ **Max Complexity** – Determines the complexity of a feature at each stage of the training. The default value is 10.

Genie Training Algorithm Output Parameters

Output parameters that can be selected or unselected for display on eSlide Manager pages are:

- ▶ **Total Training Pixels**
- ▶ **Correctly Classified Pixels**
- ▶ **Overall Training Accuracy**

All are selected by default.

Genie Classifier Algorithm Parameters

The Genie Classifier Algorithm parameter window shows a number of items you can adjust:
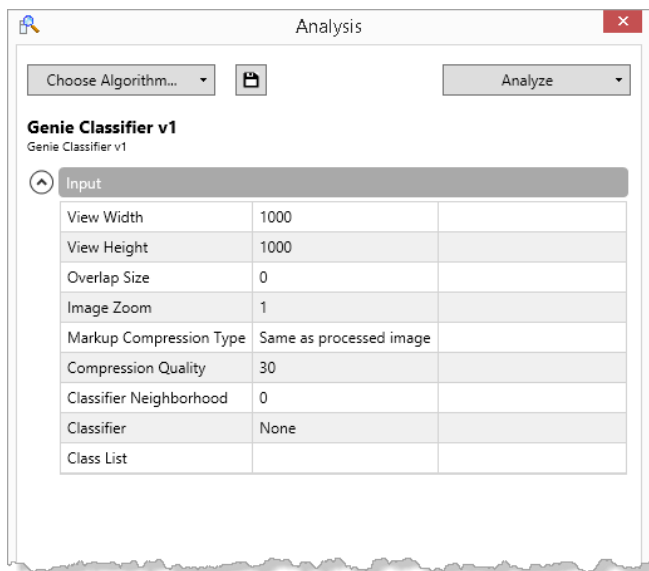
Genie Classifier Algorithm Input Parameters

- ▶ **View Width** – Width of processing box.
- ▶ **View Height** – Height of processing box.

▶ **Overlap Size** – Size of the overlap region for each view. The image is processed in blocks (views) and overlap is provided to ensure that regions are processed only once

▶ **Image Zoom** –1.0 (recommended) for processing of all pixels. Can be reduced to 0.5 for faster processing; however, the results may not be as accurate.

▶ **Markup Compression Type** – You can select among "Same as processed image," JPEG, or JPEG2000 for the markup image.

▶ **Compression Quality** – For the compressed markup image, you can select a compression quality of 0 to 95. Higher quality takes longer and yields larger files.

▶ **Classifier Neighborhood** – Size in microns of neighborhood to pad boundary of each view, as required by the classifier. This parameter is useful in removing edge artifacts between views. Once the user picks a classifier from the list, the appropriate Classifier Neighborhood value is automatically computed and populated. There is an upper threshold on Classifier Neighborhood (200 microns) for speed purposes, when it is automatically computed. Manual override is possible up to 500 microns. It is advised to set the Classifier Neighborhood manually only when the automatic value reaches 200 microns and visible edge artifacts are seen at view boundaries.

▶ **Classifier** – This parameter allows you to select from the list of classifiers that have been created on this eSlide Manager site.

▶ **Class List** – Click the ✐ edit icon on this line to choose the classes defined for the selected classifier.

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Symbols

▸ The following symbols may appear on your product label or in this user's guide:

Symbol	Description
[Manufacturer icon]	Manufacturer
[Date icon]	Date of manufacture (year - month - day)
EC REP	European Union Authorized Representative
IVD	In vitro diagnostic device
SN	Serial number
RH	Relative humidity range
[Thermometer icon]	Storage temperature range
[Waste disposal icon]	Electronic and electrical equipment waste disposal
[Exclamation triangle]	The exclamation point within an equilateral triangle is intended to alert you to the presence of important operating and maintenance (servicing) instructions. *Le point d'exclamation dans un triangle équilatéral vise à avertir l'utilisateur qu'il s'agit d'instructions d'utilisation et d'entretien importantes.*
[High voltage triangle] High voltage	The lightning flash with arrowhead symbol within an equilateral triangle is intended to alert you to the presence of uninsulated "dangerous voltage" within the product's enclosure that may be of sufficient magnitude to constitute a risk of electric shock to persons. *Le symbole de l'éclair avec la pointe de flèche dans un triangle équilatéral vise à avertir l'utilisateur que le boîtier du produit présente une « tension dangereuse » non isolée d'une amplitude suffisante pour constituer un risque d'électrocution.*
[Hot surface triangle]	The flat surface with waves symbol within an equilateral triangle is intended to alert you to the presence of hot surfaces which could cause burn damage. *Le symbole d'une surface plane et de vagues dans un triangle équilatéral vise à avertir l'utilisateur de la présence de surfaces chaudes qui peuvent causer des brûlures.*
[UV lamp triangle]	The UV lamp within an equilateral triangle is intended to alert you to the presence of UV light within the product's enclosure that may be of sufficient magnitude to constitute a risk to the operator. *La lampe UV dans un triangle équilatéral vise à avertir l'utilisateur de la présence de rayonnement UV dans le boîtier du produit qui peut être d'une amplitude suffisante pour constituer un risque pour l'utilisateur.*

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