nSolver 4.0 Quick Start Guide

This quick-start guide is designed to direct you through the steps of importing, processing, exporting, and analyzing your nCounter data in nSolver version 4.0 and later. While this guide is appropriate for all analyte types and combinations, more detailed information may be needed and is available in the **nSolver 4.0 User Manual.** *All About SNV Analysis, All About Fusion Analysis, and All About PlexSet Analysis* guides are also available.
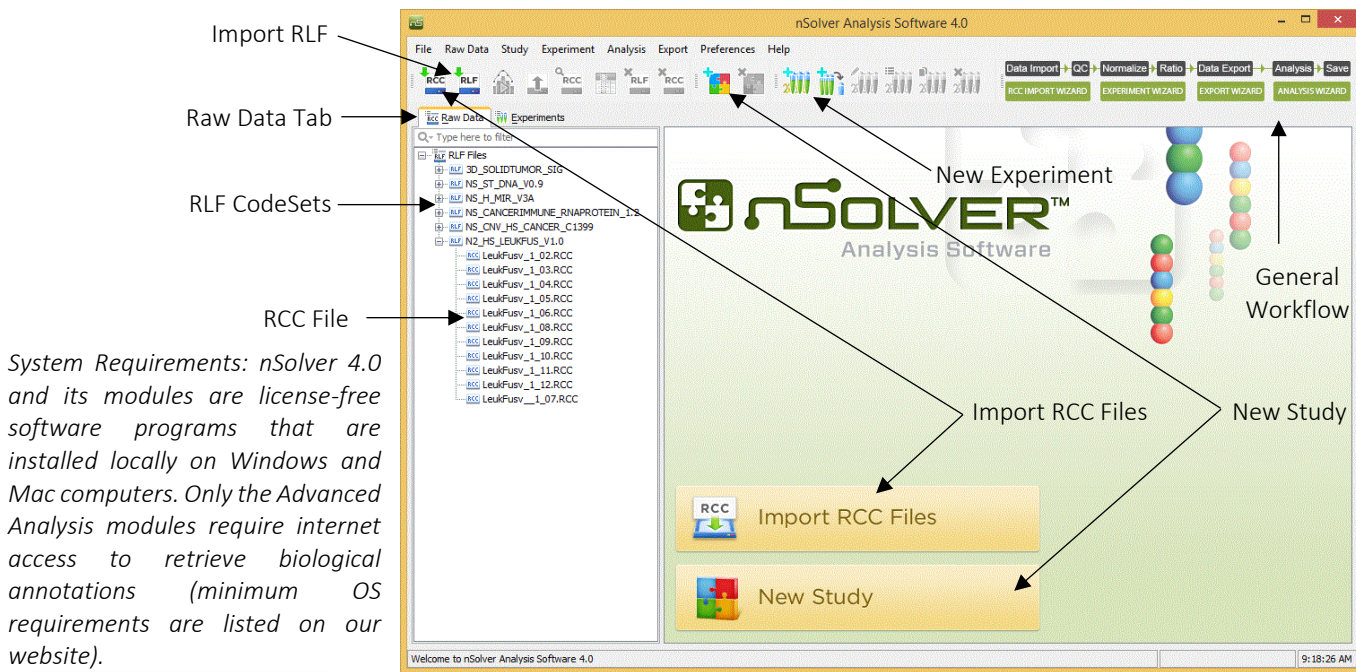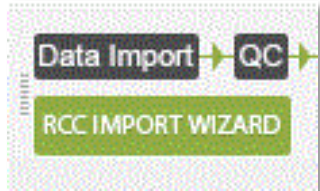

Figure 1: nSolver dashboard - raw data tab

System Requirements: nSolver 4.0 and its modules are license-free software programs that are installed locally on Windows and Mac computers. Only the Advanced Analysis modules require internet access to retrieve biological annotations (minimum OS requirements are listed on our website).

Data Import

Open your data folder and unzip RCC data files using right-click and **Extract All**. *Note: Most operating systems have built-in unzipping functions but a freeware program (e.g. 7-Zip) can achieve the same thing.*

Open **nSolver 4.0** and select **Import RLF,** then **Import RCC Files**. Follow the prompts in each process to navigate to your unzipped data folder and select files. Select **Next**. See QC for next steps. For SNV, Fusion, and PlexSet data, refer to the respective *All About…* guides.

QC

Choose the RLF, then the QC parameters (see Figure 2). If hidden, select the double arrow at the right side of the screen to reveal the System QC parameters.

- The **Imaging QC** is a measure of the percentage of requested fields of view successfully scanned in each cartridge lane.
- The **Binding Density QC** is a measure of reporter probe density on the cartridge surface within each sample lane.
- The **Positive Control Linearity QC** is a measure of correlation between the counts observed for the Positive ERCC probes and the concentrations of the spike-in synthetic target nucleic acids.
- The **Positive Control Limit of Detection QC** indicates whether the counts for the POS_E control probe and target sequence, spiked in at 0.5fM (assumed to be the system's limit of detection) are significantly above the counts of the Negative control probes.

QC (continued)

Additional QC parameters will appear, depending on the analyte types detected in your data. nSolver displays and applies the QC parameters recommended by NanoString; it is usually not necessary to adjust the default settings. Select **Import**.
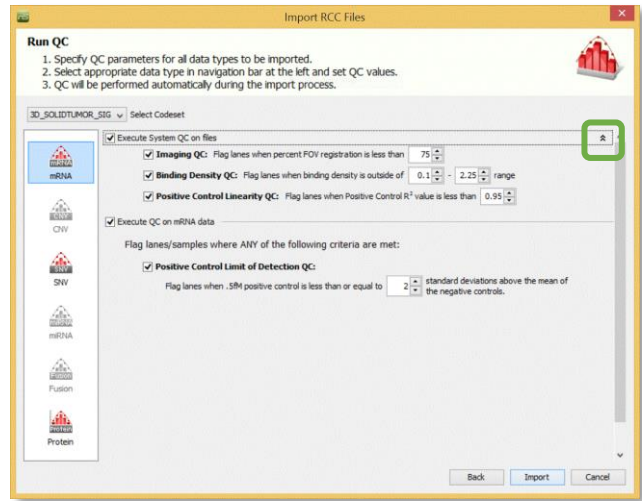

Figure 2: QC window

Exploring Raw Data

Your RCC data files will now be stored under the corresponding RLF CodeSet on the **Raw Data tab** (see Figure 1). Selecting the RLF name allows you to view all RCC files in a table format. Scroll to check for QC flags (see Figure 3). Selecting samples and clicking the **Table** button allows you to review the raw data in more detail. Examine the data to ensure that counts of POS/NEG controls and Housekeeping/Endogenous genes meet expectations, especially for samples with QC flags.

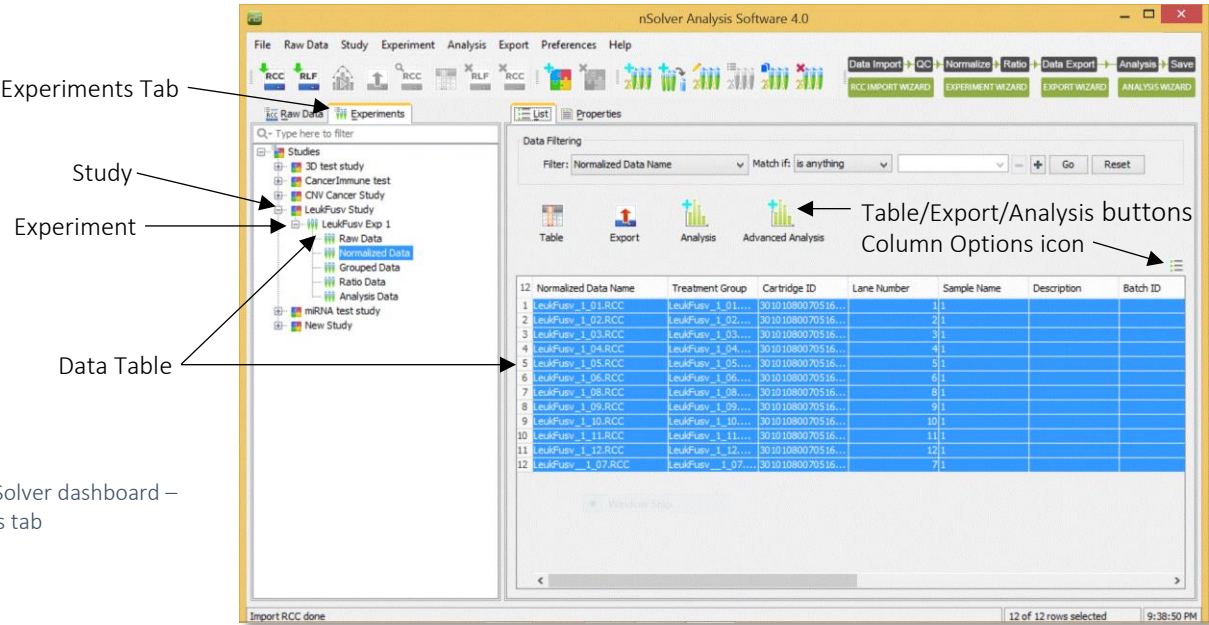Figure 3: Table with QC flags

Figure 4: nSolver dashboard – experiments tab

Creating Experiments

Within nSolver, any studies and experiments you create will be visible on the **Experiments tab** (see Figure 4).

A **Study** is an organizational folder used to store experiments; select the **New Study** button to create a study.

An **Experiment** is a collection of samples that have been analyzed together to allow comparisons between samples or samples grouped in conditions; select the **New Experiment** button to create an experiment under your study of choice. Follow the prompts to select the samples to include in your experiment.

nanoString™
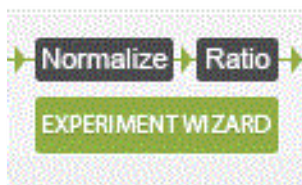
Creating Experiments (continued)

Annotations to define sample groups should be assigned for experiments in which fold-change estimates and their statistical significance will be studied. These annotations can also be used in Advanced Analysis (see Figure 5).

The recommended method for adjusting for **Background** noise in data will appear by default for most analyte types. Confirm/select an option below (see wizard steps) and select **Next**.
- o   No background calculation (option clicked off or greyed out).
- o   Background thresholding, which uses a user-defined threshold count value; all raw counts below this value will be adjusted to it. This is recommended over subtraction.
- o   Background subtraction, which can be calculated by using the blank lane (if loaded) counts, by assigning a defined value (any negative value after background correction will be set to 1), or by taking the mean/geometric mean/median/max of the Negative Control counts.

Figure 5: Annotations

Normalize

Normalization can be accomplished by using the geometric mean of the Positive Control counts and by selecting normalization genes in the CodeSet Content. The settings recommended for most analyte types will appear by default. For SNV, Fusion, and PlexSet data, refer to the respective *All About…* guides. Review defaults, set preferences, and select **Next**.

Ratio

Fold Changes (**Ratios**) can be calculated by specifying the sample(s) that represent the baseline of your experiment. *All pairwise ratios* compares all groups to one another, while *Partitioning by* allows you to choose a group as the reference. Use the **Calculate False Discovery Rate** box (if active) to calculate FDR; output for this will be in the Ratio Table. Select **Next**, confirm the ratios you wish to calculate, and select **Finish**.

Data Export

Your experiment will now be visible under your study on the **Experiments tab**. Expand the navigation tree. Select the desired data table, highlight samples of interest in the central window, and use the **Table** button to examine your data or the **Export** button to export results. Highlight an experiment and select the **Report** button (not shown) to run a report.

- o   The **Raw Data** table contains unprocessed data for all samples in this experiment.
- o   The **Normalized Data** table contains the processed data for all samples. Samples with unusually low counts for POS controls or Housekeeping genes may receive Normalization flags, which can be seen by scrolling to the far right in the central window. Paying particular attention to any flagged samples, review this data to ensure that counts of POS/NEG controls and Housekeeping/Endogenous genes meet expectations (for SNV, Fusion, or PlexSet data, refer to the respective *All About…* guide).
- o   The **Grouped Data** table contains the geometric mean of expression levels for all samples from each group (as defined by the sample annotations).
- o   The **Ratio Data** table contains the fold-change results, as well as any statistical inferences surrounding those estimates. You may need to view hidden columns of data by right clicking any column header or using the column options icon to view all columns.
- o   The **Analysis Data** table contains any analyses you have run.
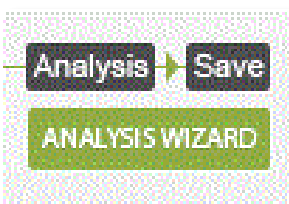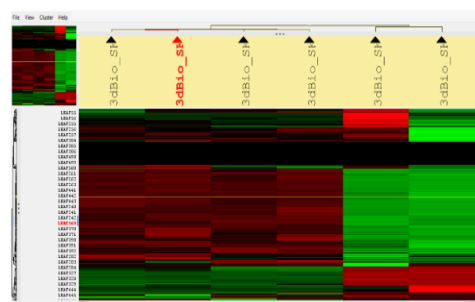
Analysis & Advanced Analysis
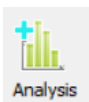
Highlight your data table and select **Analysis**. Select the plot desired (see Figure 6), then select **Next**. Select the samples, then the probes you would like included in your analysis and select **Next/Finish**. If creating a heatmap, you will be asked to set **Clustering Parameters**. Once your data is plotted, you can fine tune the settings.

- o **File** allows you to save and print the plot image.

- o The tables to the left of the **Violin**, **Box**, and **Histogram** plots allow you to select the probes you would like included in the plots.

- o The **Heatmap** is interactive and provides several customization options.

- o The options to the left of the **Scatter Plot** allow you to select the sample(s) you would like included in the plot, as well as the color designations of the data points. Additional customization (such as the axis and legend settings) is possible in **Settings**.
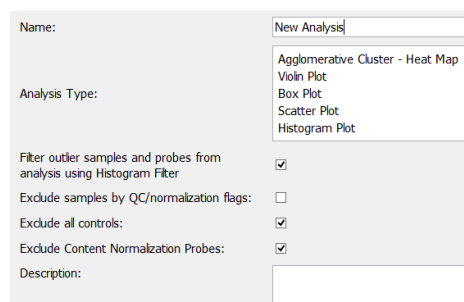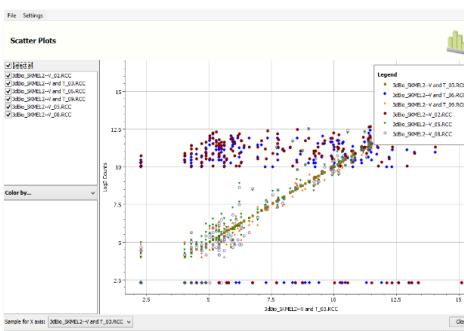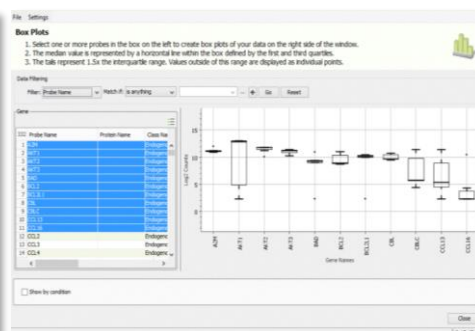

Analysis Wizard menu

Heat Map
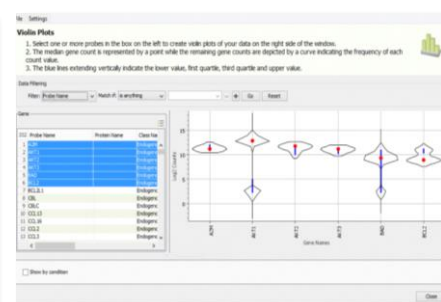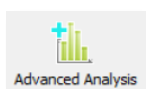

Violin Plot

Scatter Plot

Box Plot

Histogram

Figure 6: Visualizations available in basic Analysis in nSolver 4.0

Advanced Analysis is available for raw and normalized SNV, Fusion, mRNA, and protein data (not for miRNA, CNV, Plex^2, or PlexSet assays) and uses the R program. This plug-in gives you more options and flexibility in statistically analyzing and visualizing your data. You can choose between a **Quick** and **Custom Analysis.**

Help

For troubleshooting and/or guidance through your analysis, contact support@nanostring.com

nanoString™