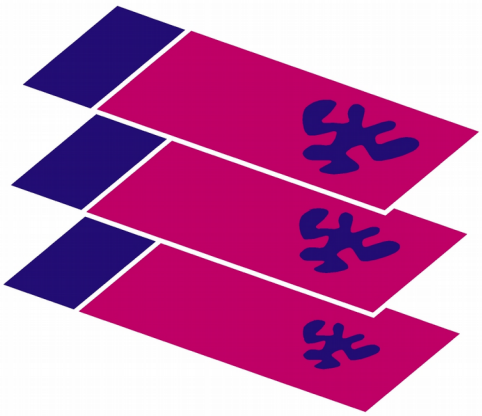

3DHISTECH

QuantCenter 2.1
User Guide

February 24, 2017
Rev. 1

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Character Types and Symbols

Example	Abbreviation or term that is explained in section Terms and Abbreviations.
Example	Words or characters that appear on the screen. These include field names, screen titles, pushbuttons and menu names, paths or options. Keys on the keyboard. For example, function keys (such as **F11**) or the **Ctrl+O** key combination.
Example	Cross-references to other documents or sections within this document.
Warning!	Indicates that you need an additional module to use the feature or that there are prerequisites for the task.
Important!	Contains an important piece of information or a recommendation. The application will work if you choose not to follow the recommendation but its performance might be less than optimal.
Tip!	Contains a suggestion about using the application in some other way or to some interesting purpose.

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1 Installation of QuantCenter 2.1 Bundled with CaseViewer 2.1

QuantCenter is not a stand-alone application. Because it runs under CaseViewer, you have to install CaseViewer before installing the application.

1.1 Prerequisites of the Installation

Prerequisites	Description
Hardware	**Minimum**: Intel 3,2 GHz i5 (Quad Core), 4GB RAM
Operating system	Microsoft Windows 7 Professional 64-bit, SP1 EN
Display resolution	**Recommended:** 1024×768 or better
Disk space	**CaseViewer 2.1** bundled with **QuantCenter 2.1** **Minimum:** 300MB

1.2 Licensing

You receive a product-specific license when purchasing any of the 3DHISTECH applications.

Floating license can be used for running applications within a LAN (Local Area Network) segment. The dongle key must be plugged into a computer that can be accessed any time (switched on and continuously operating) and on which CaseViewer has been installed.

Important!

The license server using the dongle and the connecting clients have to be within the same LAN.

Contact Thermo Fisher Scientific if you want to extend the license, as in this case some configuration of the license server and of the clients is required.

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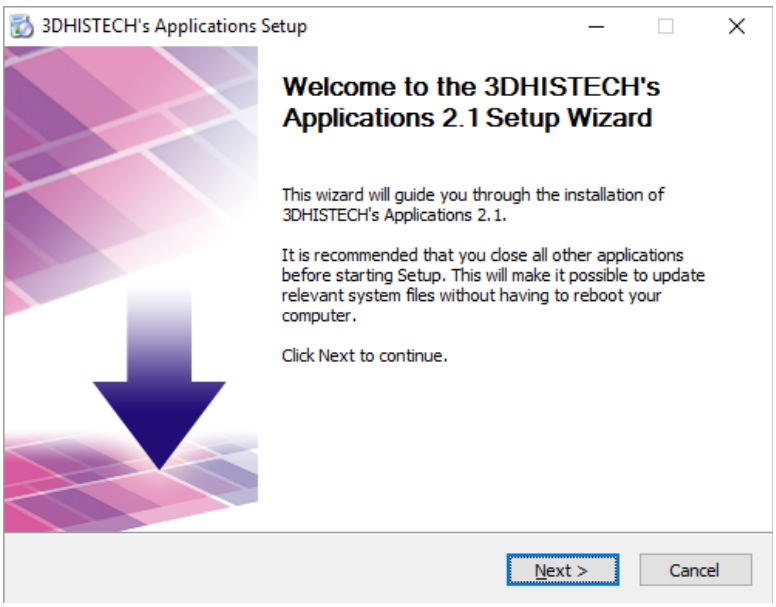
1.3 Installation of Application Modules

1. Install **CaseViewer 2.1** first. Please refer to section ***1 Installation of CaseViewer*** in ***CaseViewer 2.1 User's Guide***.

2. Launch the **3DHISTECH_Apps_2_1_RTM.exe** file that you received on a 3DHISTECH installation disc or downloaded in a compressed file, then follow the instructions in the installation wizard.



NOTE: If the wizard finds a previous version of the application, it has to be upgraded. See **step 12.**

3. Accept the End-User License Agreement, and click **Next**.

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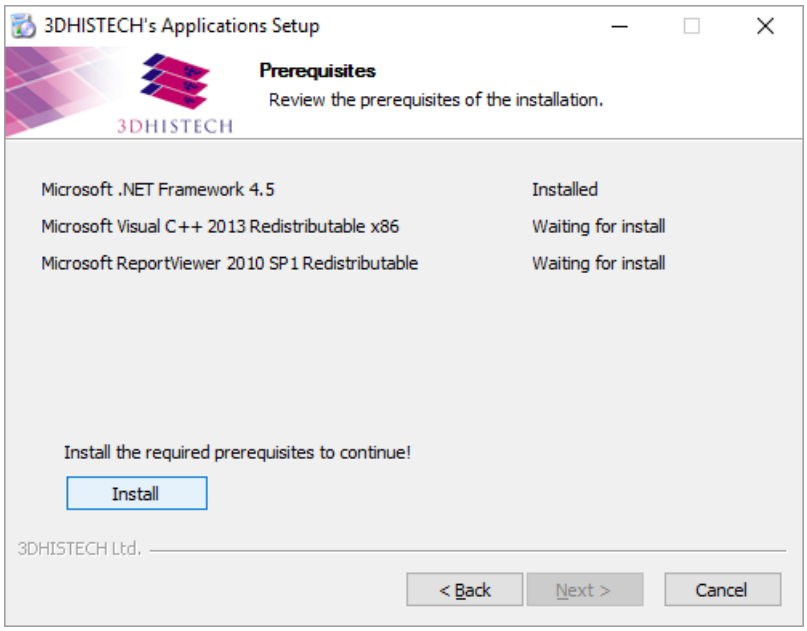
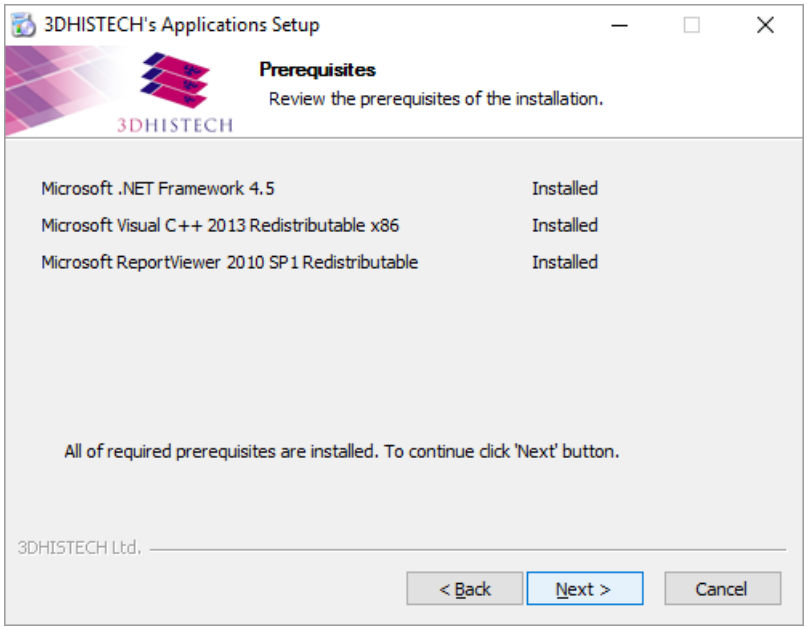
4. Click **Install** to install missing prerequisite software. Installation runs in the background.



5. When all the prerequisites have been installed, click **Install** to continue with the installation of the selected components of the 3DHISTECH application package.



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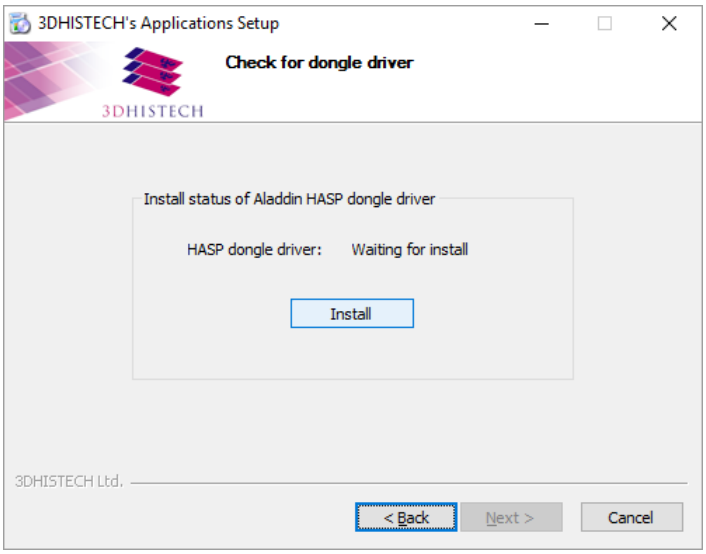
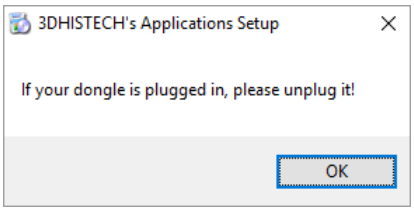
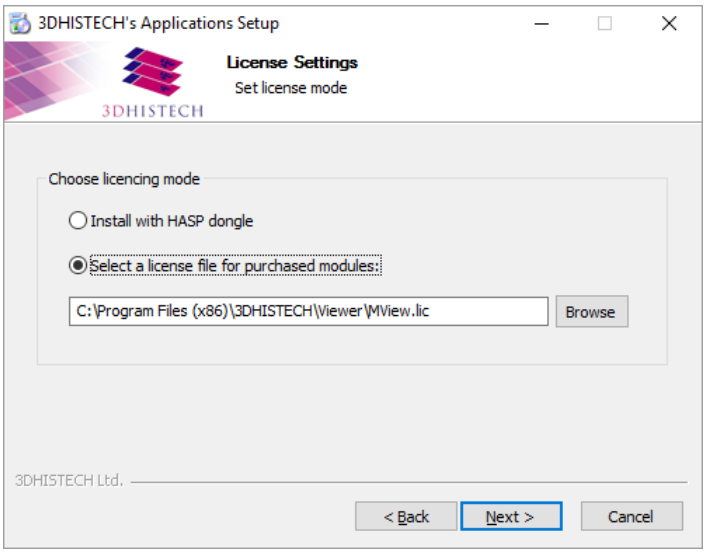
6. If the driver of the HASP dongle has not been installed yet, click **Install**.



7. First, remove the HASP dongle from the USB port, then click **OK**.



8. Set the license mode. If you have a dongle key attached, select **Install with HASP dongle**, otherwise select a license file.



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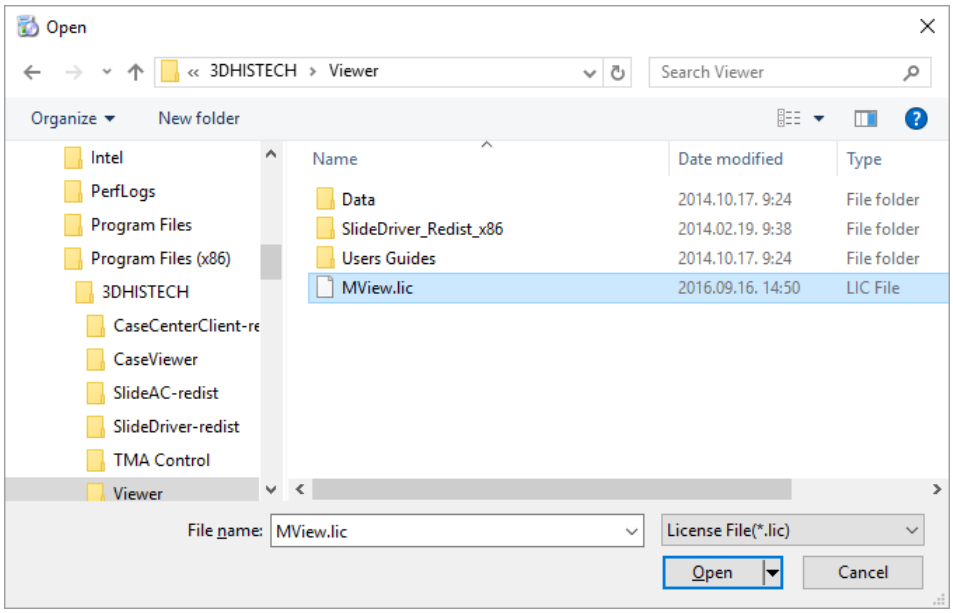
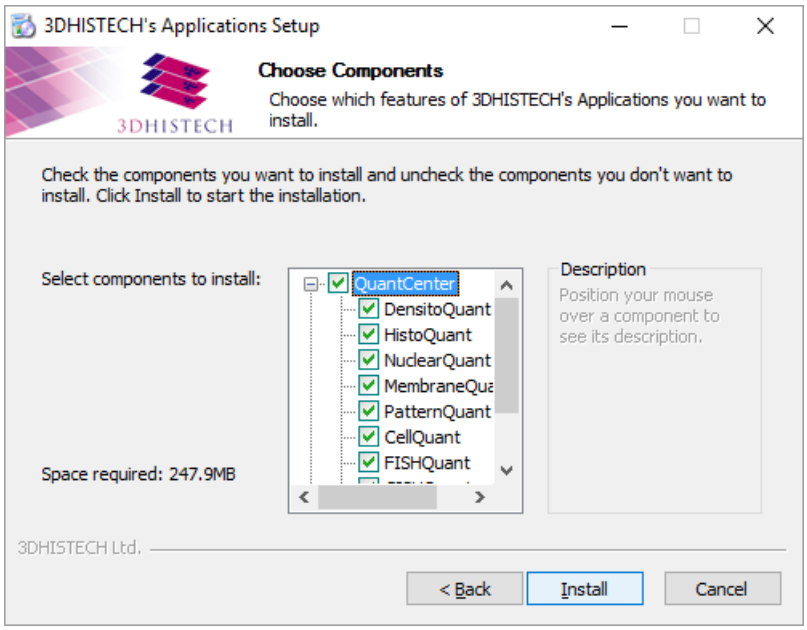
9. If you want to use a license file, click **Browse** to locate and open **MView.lic** in the containing folder. Select the file, then click **Open** to continue.



10. Select the components you want to install.



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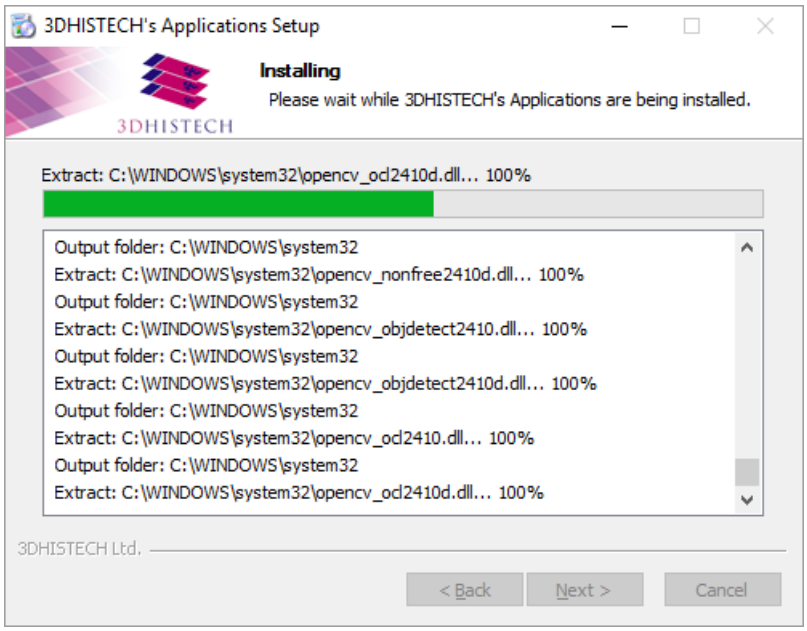
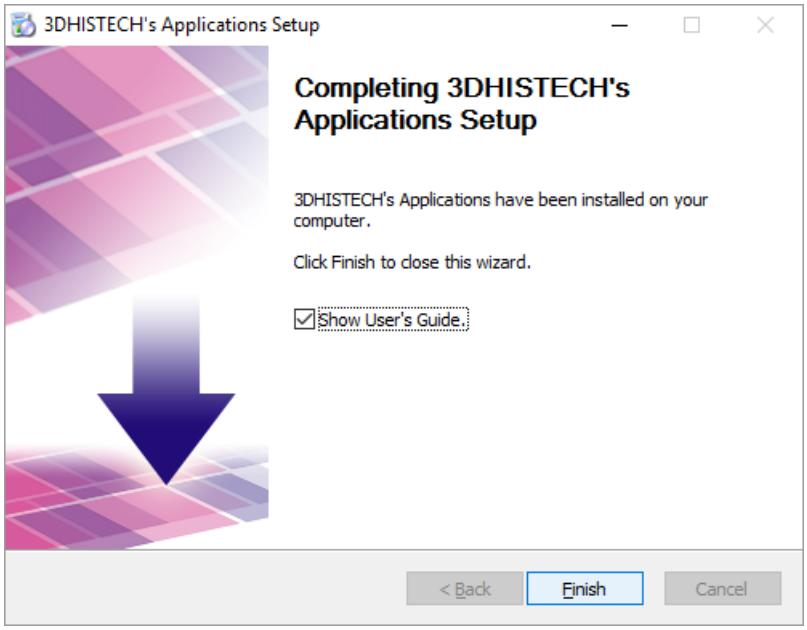
11. A progress bar and an information box is displayed, showing the status of the installation procedure.



12. Leave the **Show User's Guide** option checked to open the folder containing the digital copy of this User's Guide. Click **Finish** to close installation wizard.



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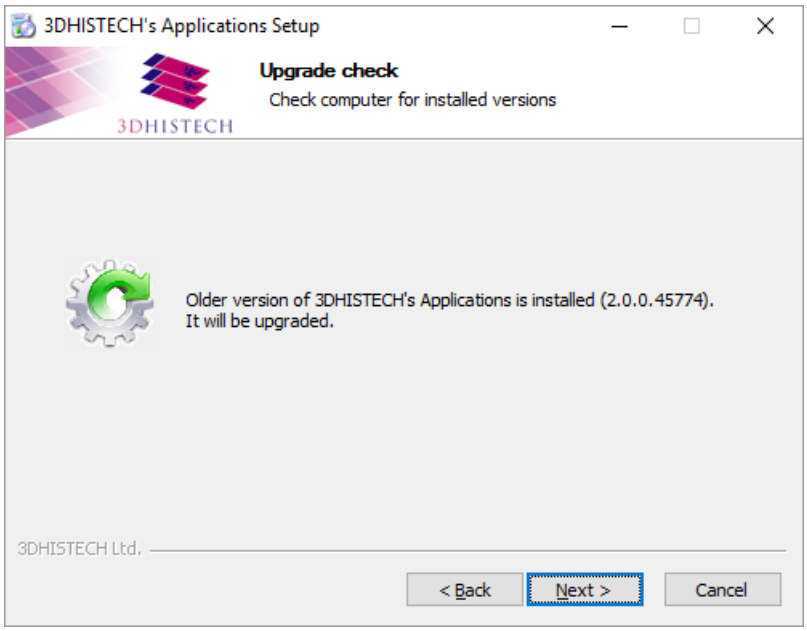
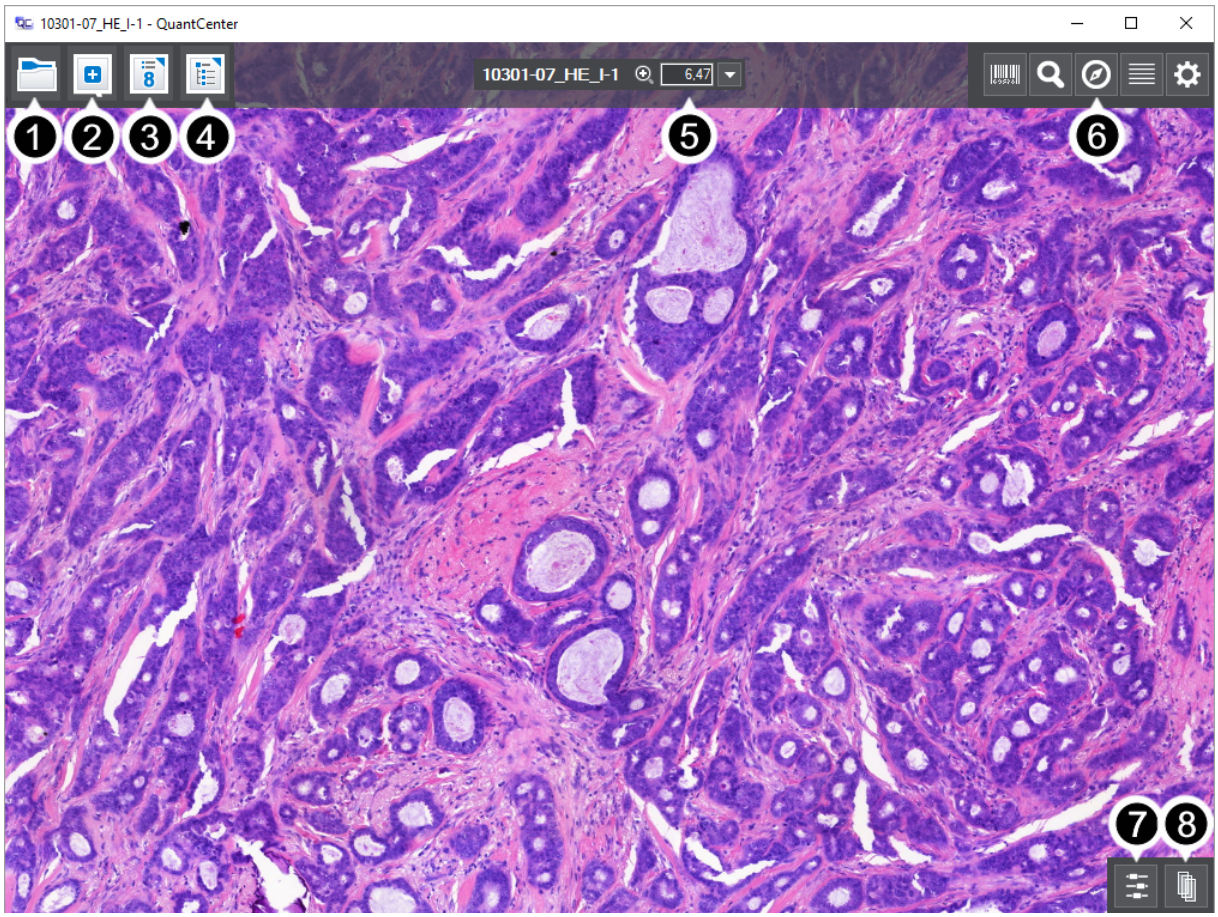
13. If there is an older version of the applications installed on your computer, it will be upgraded automatically after clicking **Next**.



2 About QuantCenter

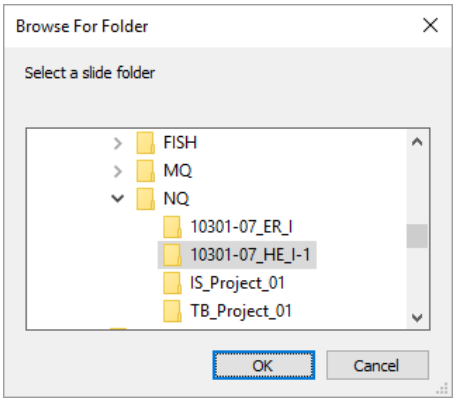
3DHISTECH QuantCenter is an embedded application for running quantification measurements on digital slides and is accessible from CaseViewer 2.1. For more information on how to open a slide in CaseViewer, and how to initiate measurement processs through opening the slide in QuantCenter, see sections ***2 Opening CaseViewer*** and ***3.2 Functions – 12. Plugins*** in ***CaseViewer 2.1 User's Guide***, and section ***6 Running a Batch Process from CaseViewer*** included in this guide.

2.1 Main application interface



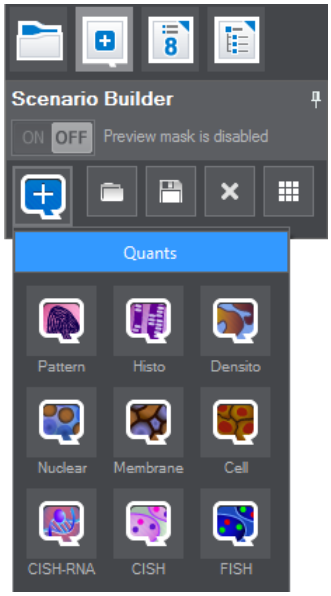
1. Open a slide

By clicking this button the Browse for Folder window is displayed. Browse for the directory in the window to open a slide.



2. Scenario Builder

With the help of this function by linking Quant algorithms (PatternQuant, HistoQuant, DensitoQuant, NuclearQuant, MembraneQuant, CellQuant, CISH-RNAQuant, CISHQuant, and FISHQuant), a unique measurement profile, a so called Scenario can be defined. On how to create a measurement profile, read section ***3 Scenario Builder***.



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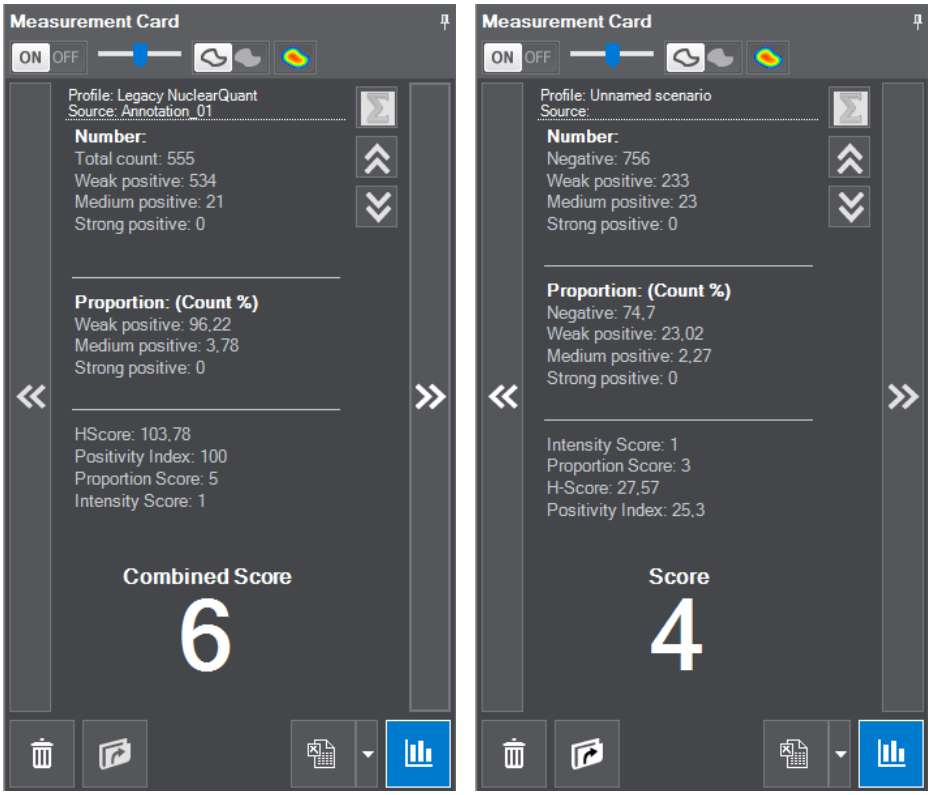
3. Measurement Card

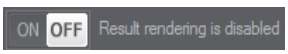
On the **Measurement Card** measurement data are shown.

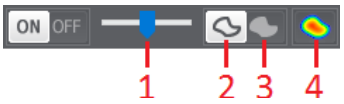
NOTE: This button is active only if there is a displayable measurement on the slide. Measurement results generated with version 2.1 are not compatible with previous software versions, and cannot be opened.

If you open a slide on which a measurement is available, then this form is displayed by default.



Upon clicking left/right arrows in the card view of the form, data of the selected profile (scenario) will be displayed. If there are more than one scenario sessions (measurements) on the slide, up/down arrows are responsible for loading measurement data of the selected session. Segmented spots related to available measurement results can be displayed. To enable result rendering, click ON at the top of the selected form. If result rendering is active, additional options are available for you. The transparency of the measurement result image can be set by dragging the slider (**1**) to a desired percentage value. **Outline** (**2**) or **Fill** (**3**) options can be selected as visualization type. **Object density visualization with heatmap** (**4**) offers you the visualization of objects within an annotation by highlighting dense area including high intensity pixels.





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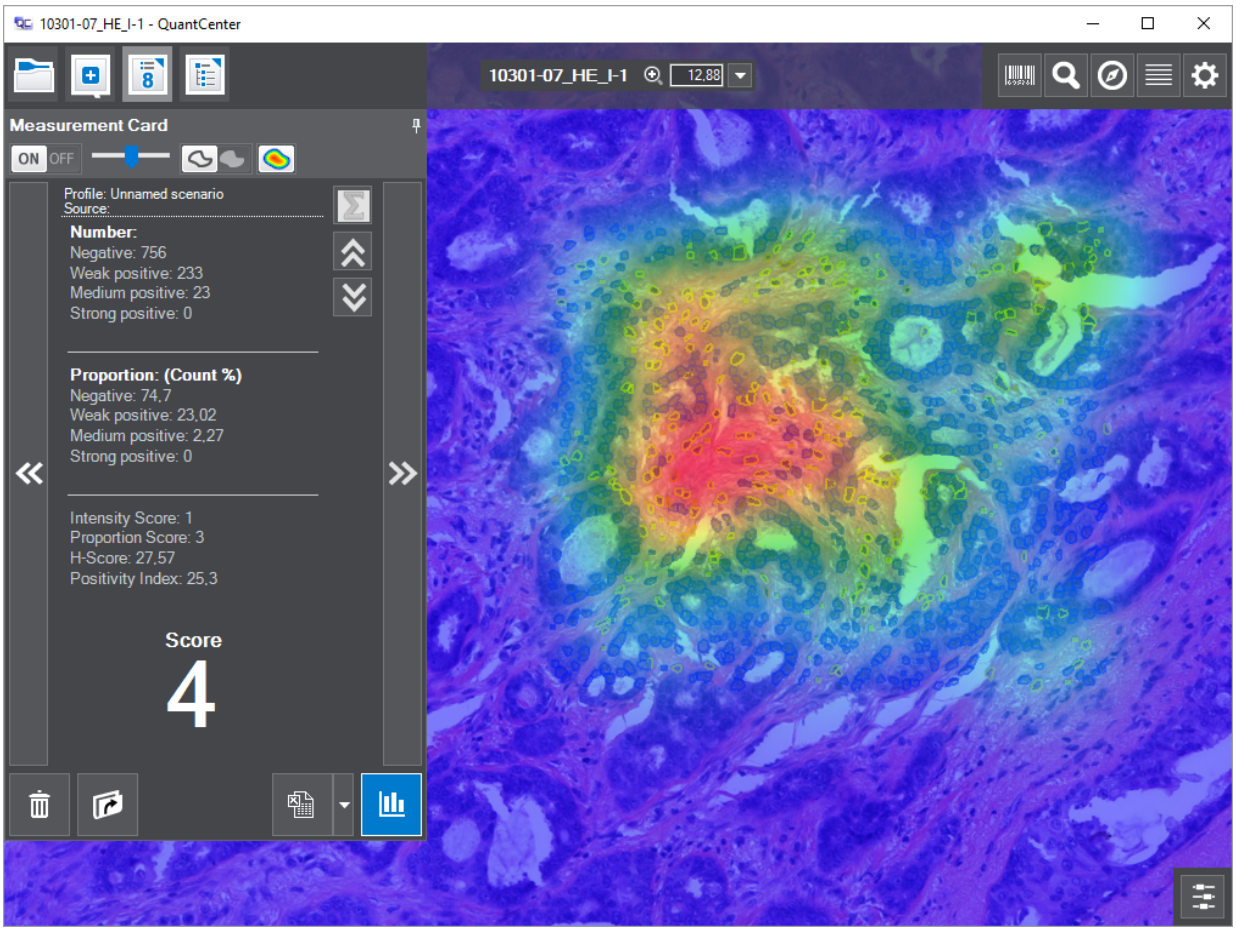

Click to display merged measurement data of all sessions of a scenario.

To delete measurements, click .

Click to reload the scenario based on which the measurements have been created, then the software switches to Scenario Builder form.

– Click this button to export measurement data as Excel worksheet. Click the down arrow at the right of this button and select **Transpose XLS table** to switch rows to columns or columns to rows when exporting measurement data.

NOTE: The selection of **Transpose XLS table** option is saved upon exiting, and will be used as default during export unless you deselect it.

– This button serves to open Data Visualization window in which spots are displayed. For more information on Data Visualization, see ***section 5***.

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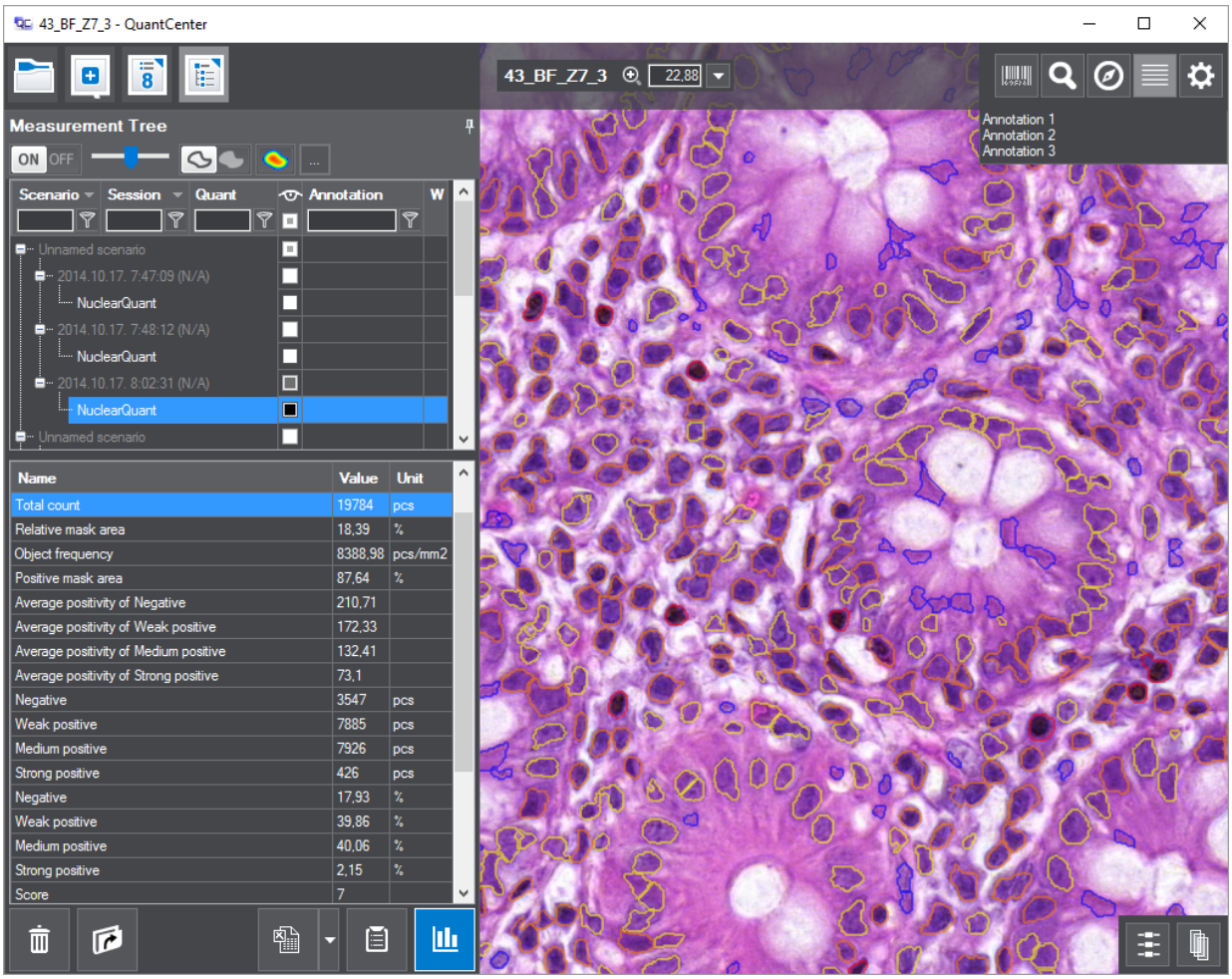
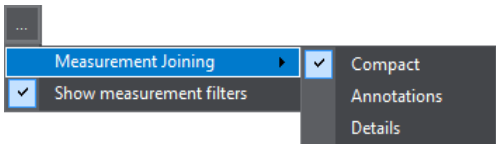
4. Measurement Tree

Click this button to display the detailed view of the form.



Click ... to display further data visualization options.



Measurement data are classified according to three different aspects selectable from the **Measurement Joining** list.

- **Compact** – Default view, shows combined measurements in a simplified form.
- **Annotation** – Can be selected if a simple (one-element) scenario has been created, and measurements were run on different annotations.
- **Detail** – If filters are applied and **Save objects to slide with connection** option is set under Performance, pre-segmented area-related (PatternQuant and HistoQuant) measurement data can be displayed.

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Filtering options can be enabled by selecting **Show measurement filters** option. If enabled, **Scenario**, **Session**, **Quant**, and **Annotation** data can be filtered after typing a query and clicking the button. Filtered results can be ordered within each group by clicking at the right side of a column header.

If clicking a Quant-related measurement on the tree (under a scenario, by date), the related measurement data is displayed on the bottom pane of the form. Summarized data can be listed if selecting identical Quants in the tree (for multiple selection within a scenario, press **Ctrl** / **Shift** key during selection; alternatively, select items with your mouse by holding the left mouse button pressed).

Click the checkbox of a scenario/session in the column to display detection results on the slide image, and by clicking the Quant name for which the measurement was created, data are displayed in the bottom pane of the form. The main checkbox activates the visualization of cumulative detection results on the slide image.

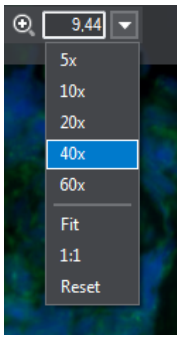
If you want to delete a measurement, first select the item on the tree, then click the button. For multiple selection press **Ctrl**/**Shift** while selecting the items.

NOTE: PatternQuant and HistoQuant measurement values displayed as percentages (related to area) represent values regarding the original annotation (FA).

Click to display the **Data Visualization** window. For more information on Data Visualization, see ***section 5***.

NOTE: CISH-RNAQuant and **CISHQuant** data cannot be displayed simultaneously.

5. Magnification



Type magnification value into the text box or click the arrow to set its value from the list of predefined values.

Click **Fit** to fit the actual view into the window, or **Reset** to both fit and set slide orientation to the default value (0 degree).

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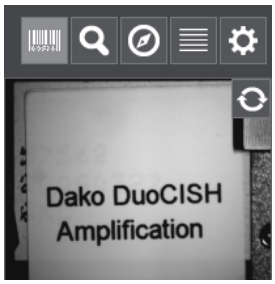
6. Assets

Barcode

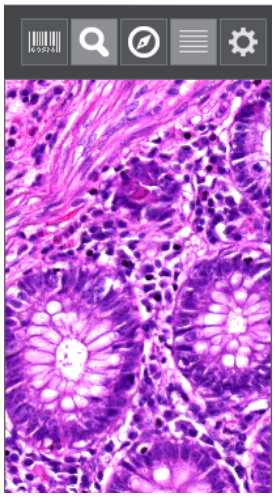


Active only if there is a barcode present by which the slide can be identified.

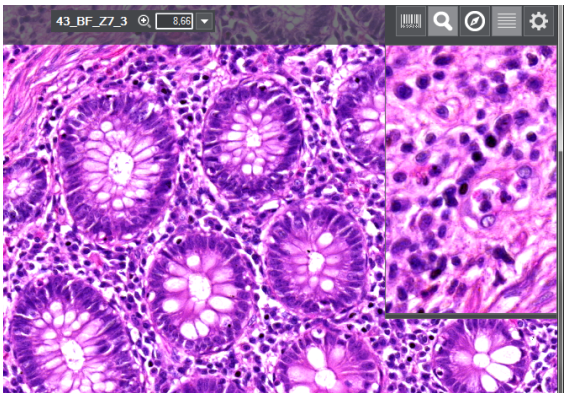
Click to display the barcode image.

Click the icon at the top right corner of this image to rotate image by 180° degrees.

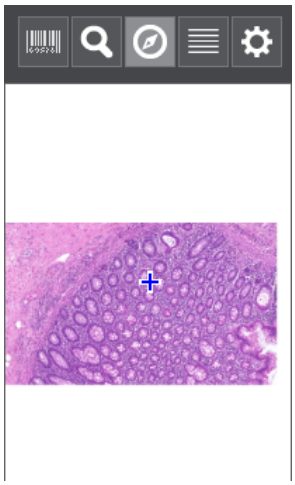
Magnifier



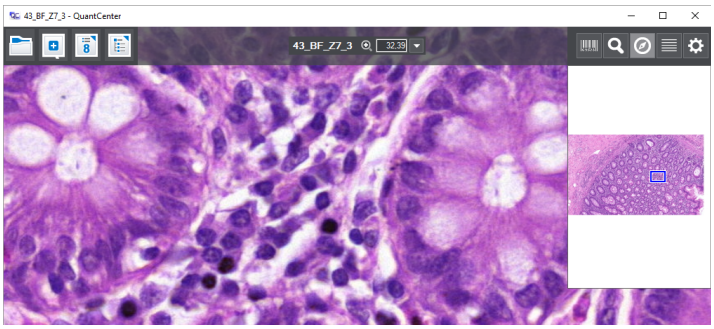
If activated, the area of the slide image (where the mouse cursor is actually located) viewed through the magnifier is two times as large as the actual set zoom level.



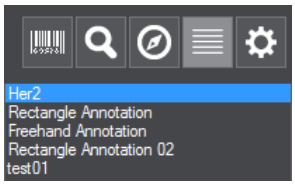
Preview



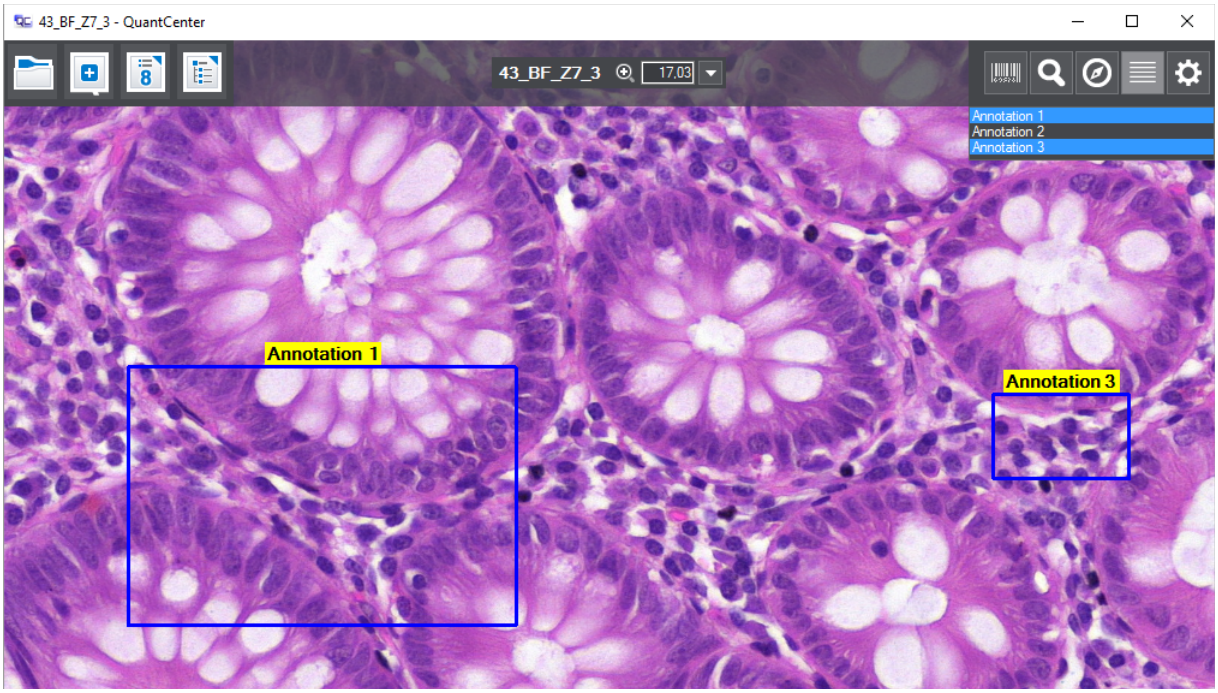
This function is not activated by default. This view helps to navigate on the slide. If lower magnification level is set, a tracer rectangle indicates the current location on the slide, as for higher magnification a cross-hair is displayed for the same purpose.



Annotation list



After a slide is opened, this list is displayed by default if there are annotations on the slide. Select multiple annotations by holding the **SHIFT** or **CTRL** key. The annotations you have selected are displayed on the slide image.



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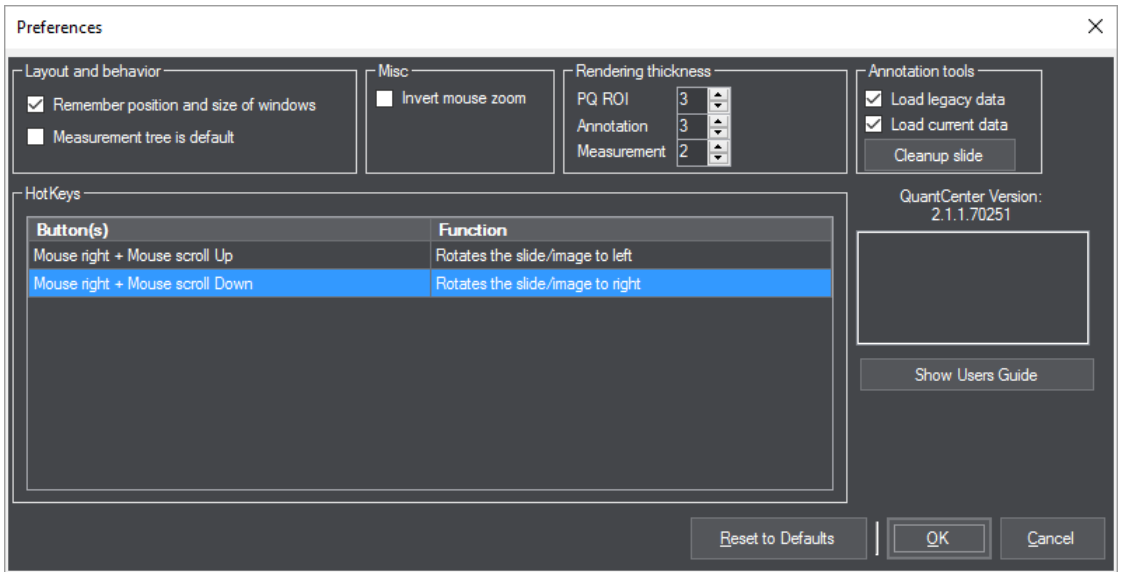
Preferences



Within the **Layout and behavior** section of the window the position of application windows can be set to fixed by activating the **Remember to position and size of windows** option, so that these windows will open at the same positions at the next software restart.

On slide open/reopen/reload if the **Measurement tree is default** option is selected the tree view of the Measurements, otherwise the Measurement Card will be displayed. If slide contains no previous measurements the Scenario Builder will open.

Zooming with mouse can be inverted in the **Misc** section of the window by selecting the **Invert mouse zoom** option.

Rendering thickness section includes setting the ROI thickness value in PatternQuant, the thickness of manual annotations selected from annotation list, and the thickness of displayed measurements.

Hotkeys for the slide rotation function are displayed in the **HotKeys** section.

In the **Preferences** window the actual build version is shown as well, and a button for displaying the User Guide for the software application.

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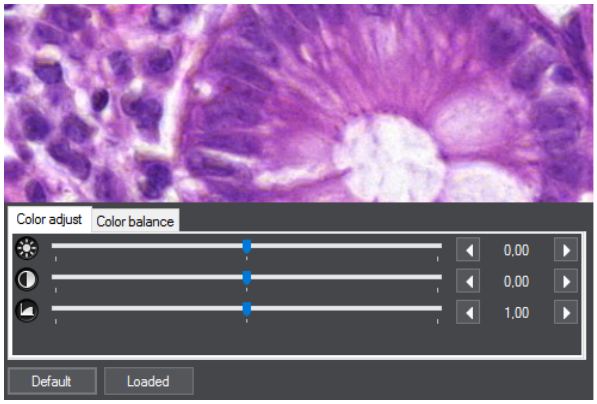
Click **Reset to Defaults** to set values to default:

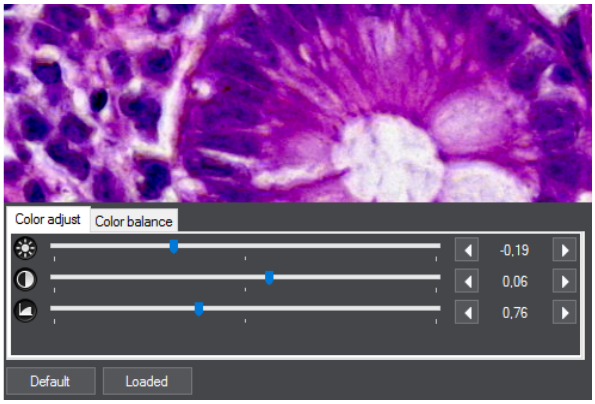
- **Remember to position and size of windows**: active
- **Measurement tree is default**: inactive
- **Invert mouse zoom**: inactive
- **Rendering thickness**: 3, 3, 2
- **Annotation tools**: both active

At the **Annotation tools** section of the window, the importing of data stored in MISP files can be activated upon clicking the **Load legacy data**, and the actual data of the slide can be imported if **Load current data** option is activated. Click **Cleanup slide** to delete all the measurement annotations of the slide.

7. Enhance Color

The color settings modified in CaseViewer can be turned on/off and further modified after clicking this button.



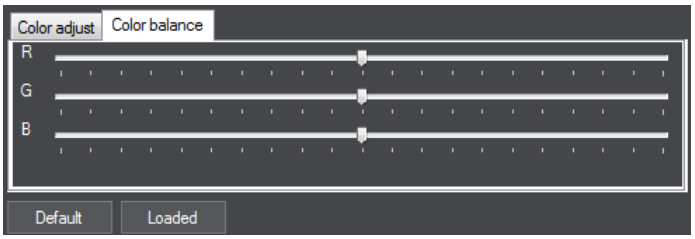


The looks of the button changes if default values have been modified, it turns from to
.

NOTE: Settings are saved to the slide immediately, measurement, masking, and preview are affected by the parameterization of this function.

Brightness, **Contrast**, and **Gamma** values can be adjusted on the **Color adjust** panel.

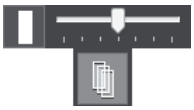
Color channels can be adjusted separately. Channels of a color model are represented in accordance with the type of slide (RGB color model for Brightfield slides, FL channels for Fluorescent slides).



Click **Default** to restore the default settings of the slide. Upon clicking **Loaded** the settings with which the slide was loaded will be applied.

8. Z-stack slider

If the slide was scanned in several focal planes during the digitization process, the resulting virtual slide is a so called Z-stack slide. By stacking of focal planes the slide image can be viewed in its depth, therefore a Z-stack slider is shown at the bottom right section of the window.



Activate Z-stack displaying option by clicking the slide button at the left of the slider bar. Thus, the display of each focal layers by moving the drag-bar below or above the 0 focal plane is enabled.

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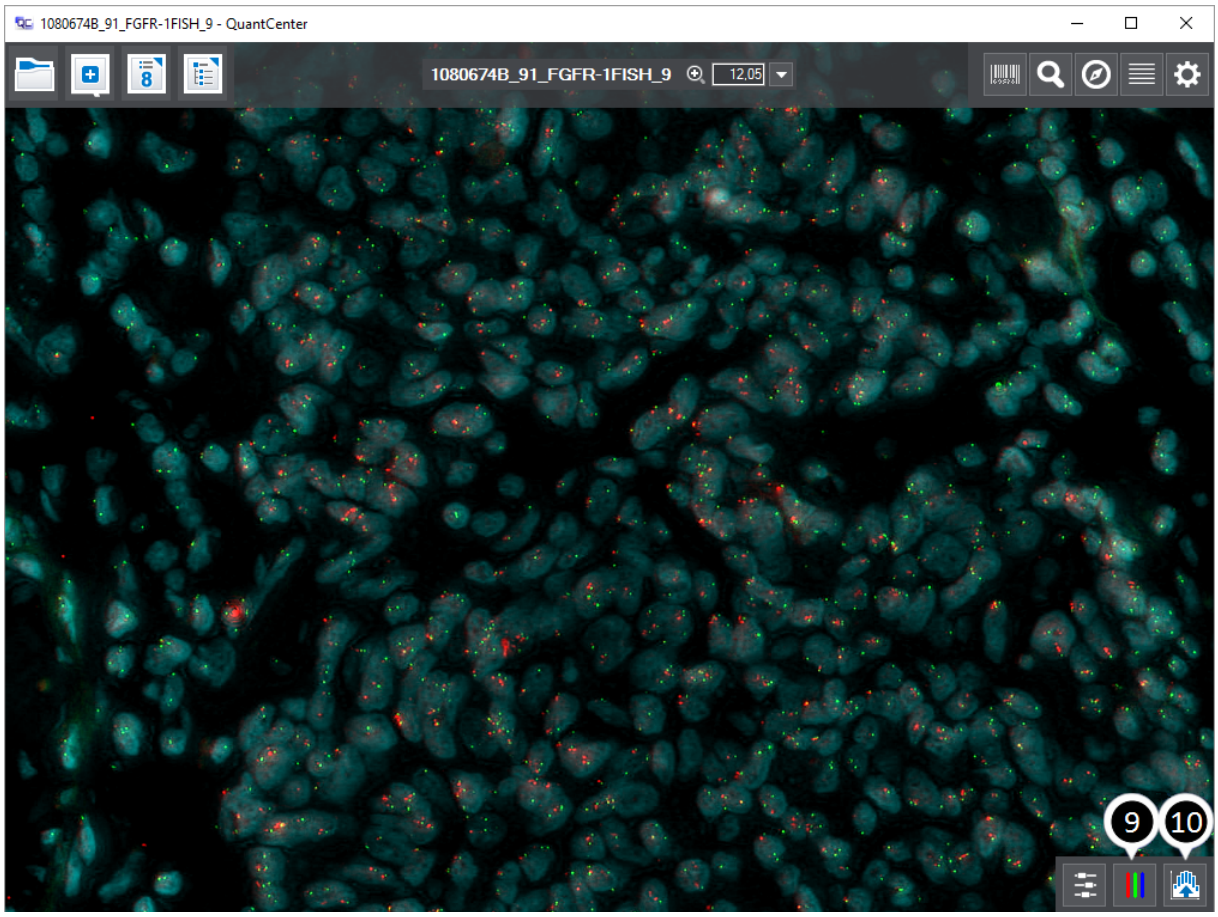
There are **two additional functions** present on the main window if a **Fluorescent slide** is opened.

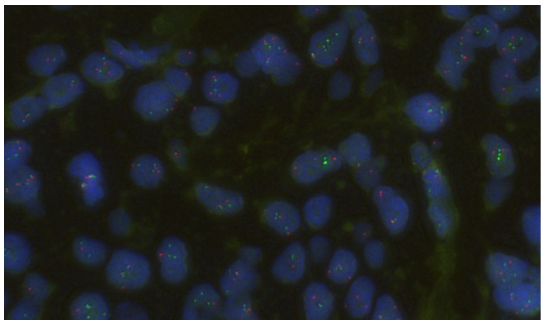


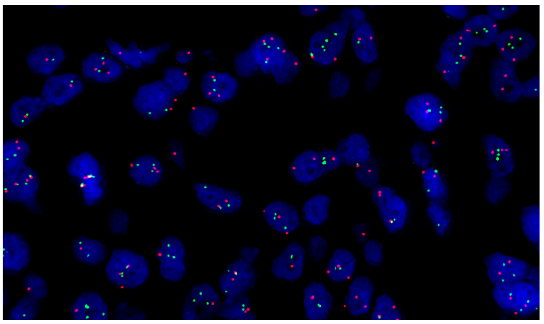
9. FL channels (Blue/Red/Green)

Click this button, then you can turn on/off channels by clicking the button of the relevant color channel.

10. AF Filtering

Autofluorescence filtering method can be applied on the FL slide by clicking this button only if **FISHQuant** is selected in Scenario Builder.





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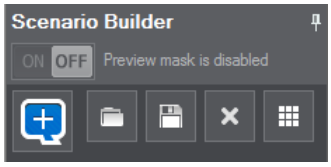
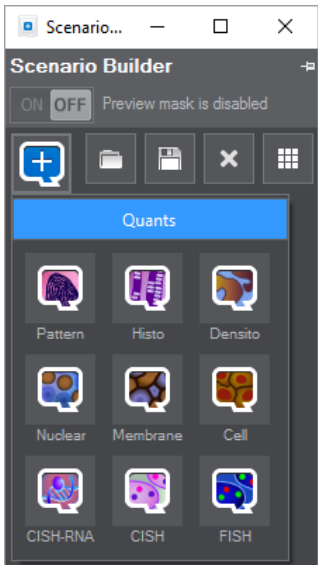
3 Scenario Builder

The main advantage of **Scenario Builder** is that the user is capable of defining a unique measurement scenario by creating a tree structure for the composition of measurements.



The **Scenario Builder** panel can be docked or separated as a window by clicking the button, and hidden by clicking the button at the main menu bar.



Click to open a previously saved scenario.

Click to save the actual scenario that has been created.

Upon clicking the whole scenario will be deleted, and only the root remains empty.

Click to open Gallery in which selected object can be collected. See section ***3.2 Gallery*** for more information.

3.1 Defining a Measurement scenario

1. Click the root element .
2. Select the icon of the desired Quant to start with as root.
3. The selected Quant is now added to the scenario.
4. If PatternQuant or HistoQuant is present in the measurement scenario, it must be trained first before running the measurement process: each PatternQuant/HistoQuant element must contain at least one selected area (ROI) within at least one cluster, then click **Train** in the Quant properties window to start the process after which the actual Quant becomes trained (see ***sections 4.1*** and ***4.5***).

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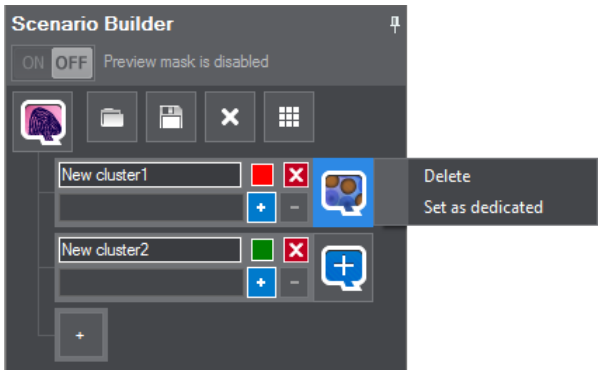
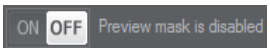
5. If a scenario is complete, then in the annotation selection panel, specify the areas that will be included in the measurement. Before running the process make sure to set the desired selection on the panel.

   For more information on running measurements, see ***section 3.3***.

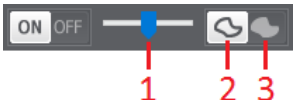
   After right-clicking the Quant button, you can delete that specific Quant item including selections, or you can select the **Set as dedicated** option by which the measurement of the dedicated Quant will be displayed on the **Measurement Card** form. If you do not set a Quant as dedicated manually, then the uppermost defined Quant will be set as dedicated by default.



After the parameterization of a Quant, **Mask** function can be activated on the preview image in each of the available forms (**Scenario Builder** and **Scenario Builder and Gallery**) to show segmented spots related to the Quant application selected from the **Scenario Builder**. To enable preview mask, click ON at the top of the selected form.



If preview mask is applied, additional options are available for you. The transparency of the mask image can be set by dragging the slider (**1**) to a desired percentage value. **Outline** (**2**) or **Fill** (**3**) options can be selected as mask type (only **Fill** option is available for you if preview mask results are generated by PatternQuant or HistoQuant).



If you create or select a class (click the Quant button on the tree of the scenario), its Quant-related properties in which measurement parameters can be set are displayed at the bottom of the Scenario Builder form.

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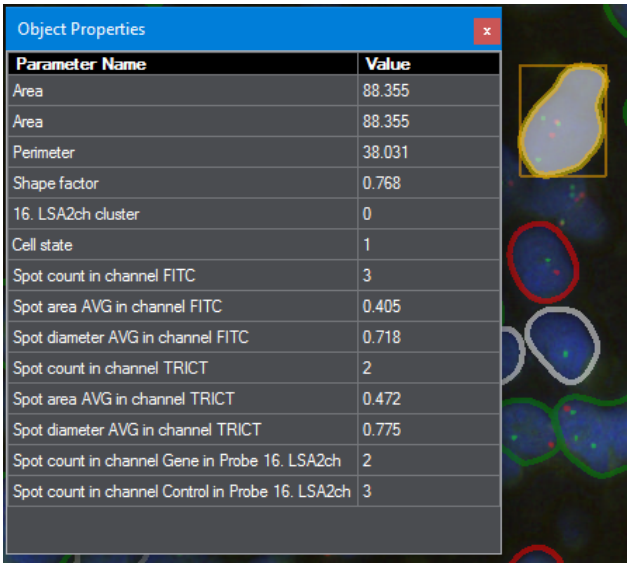
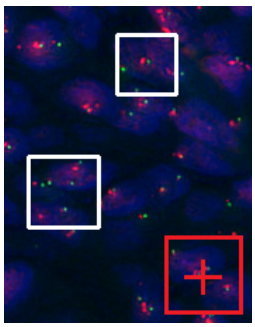
If a Quant is selected (except for PQ or HQ), and the **Mask** function is activated, a parameter table (Object Properties) is displayed upon right-clicking any of the segmented spots.



3.2 Gallery

Along with the Scenario Builder, desired areas of the slide can be imported into a Preview Gallery, or if a larger slide is loaded, the accuracy of segmentation/mask settings can be monitored by taking areas from farther locations of the slide.

After clicking the button, move the red marking box above the desired area of the slide, then click with the left mouse button. The size of the marker can be adjusted by entering/increasing or decreasing the actual value in the box, or with the mouse wheel by rolling it back or forth (value ranges from 1 to 100um).



If you want to move the slide so to navigate to a different location when selecting desired areas of the slide to be added to the Gallery, the following options are available for you:

Click with the right mouse button, then drag the image. Click again, then select slide area to be added as a Gallery item.

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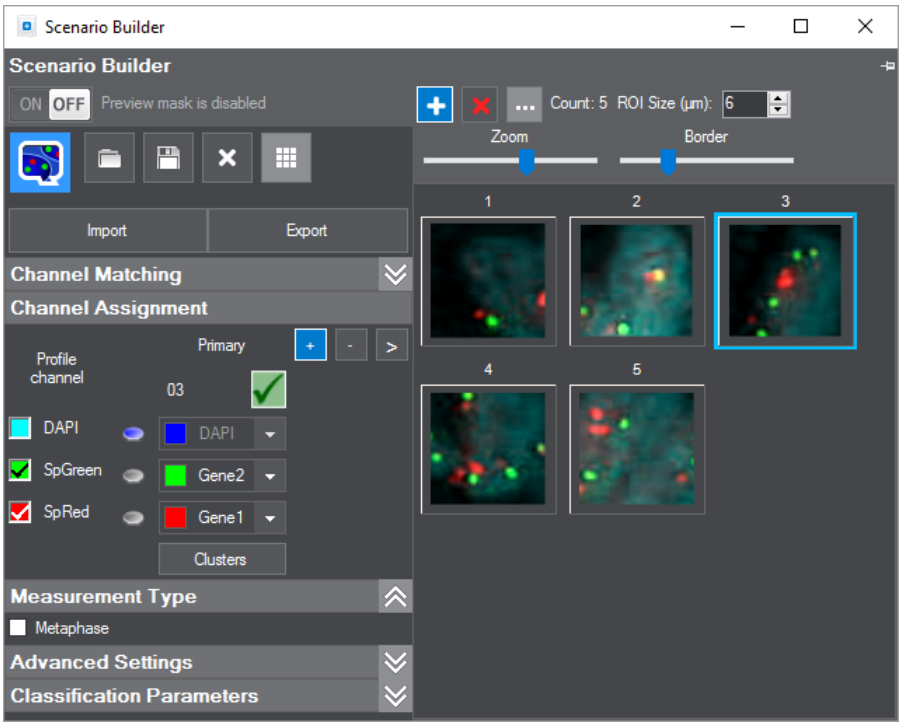
Drag slide image while keeping the **Crtl**, **Alt**, or **Shift** button pressed, then select slide area to be added as a Gallery item.

After clicking ... it is available for you to increase/decrease item size by dragging the **Zoom** slider to the appropriate direction. The **Border** slider is responsible for enlarging the border of items.

Click with the right mouse button if finished with adding items to the Gallery. In this window you can also delete selected gallery items by clicking ✕. For multiple selection hold the **Ctrl** key pressed while selecting items with the left mouse button.



To run measurement process on the gallery items (handled as annotations), first click ▼ under the Process panel, then select ▶ Gallery items from the list. A separate job is added to the list in the Processing window, then measurement results are displayed both on the measurement card and the slide.

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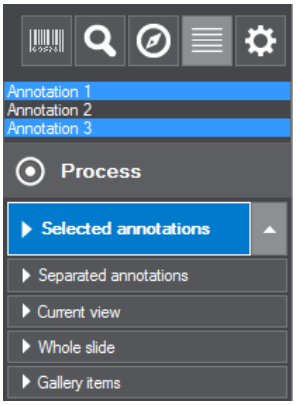
3.3 Running measurements

Under the **Process** panel, measurement process can be launched for the desired selection (**Selected annotations**, **Separated annotations**, **Current view**, **Whole slide**, and **Gallery items**).



Current view and **Whole slide** options are available for you to select by default.

Selected annotations option is displayed only if there are annotations on the slide, and at least one annotation is selected from the annotations list.

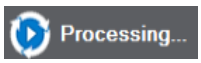
Separated annotations option allows you to run measurement processes in the queue separately.

The **Gallery items** option is available only if you have marked areas on the **Scenario Builder and Gallery** form (see ***section 3.2*** for more information).

If a scenario has been defined, the „*Scenario is not defined properly*" text will disappear from the Process panel, and so measurement on the selected item can be run.

The **Process** tab gives you feedback on the state of the actual processes, running or paused.

To run measurements, click the blue field below the **Process** tab (in which the name of the desired selection is displayed).

The tab switches to 

if measurements are being processed, and in the queue.

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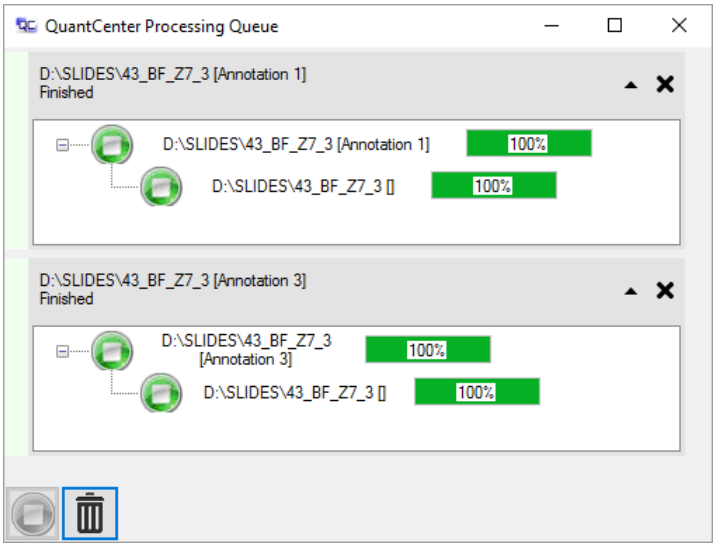
Upon clicking this tab the **QuantCenter Processing Queue** window is displayed in which the running process or processes are displayed. For the detailed view of the progress click the button.

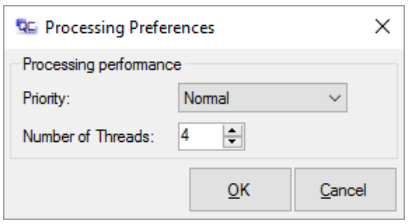


The **QuantCenter Processing Queue** window can be minimized (runs in the background) and moved to the notification area at the right side of the system tray.

NOTE: Measurements are processed according to the scenario, so if a scenario has not already been created (or being untrained), they cannot be processed.

Measurement processes that are running in the background can be visualized by clicking at the notification area of the system tray.

Right-click at the system tray, then select **Preferences** to display **Processing Preferences** window. It is available for you to select from **Low**, **Normal**, or **High** priority levels from the **Priority** drop-down list. **Number of threads** defines the number of used CPU cores for processing.



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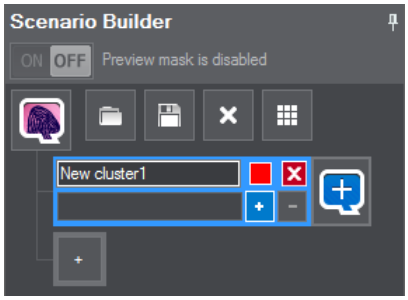
4 Available Quant applications

4.1 PatternQuant

This Quant module provides segmentation methods to decompose measurement area based on pattern and intensity.



One cluster is displayed by default, but a group of clusters is available for you to create, and extend to a maximum of 5 clusters by clicking .

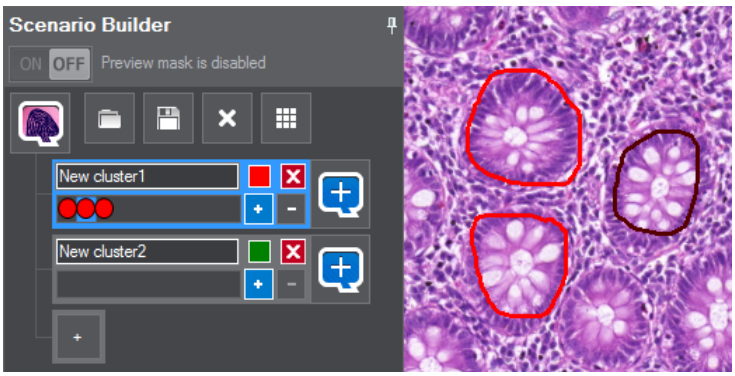
For each cluster, a unique name and color can be defined. Name is presented in the tree on the measurement form, and the area defined by the cluster is marked with the color on the slide.

Important!

Before defining areas on the slide make sure to zoom on the slide in an adequate amount to support accurate drawing around specific target areas. Desired magnification level can be selected from the **Magnification** drop-down list, and the location can be specified in the preview window by relocating the cross-hair with a simple click with the mouse.

Click first, then with the pen by using your mouse, define those areas on the slide that suits to the pattern of the specific cluster. Close selection border by releasing the left mouse button.

NOTE: It is advised to create more selections to get more accurate results. A maximum of 10 areas can be defined within a cluster.



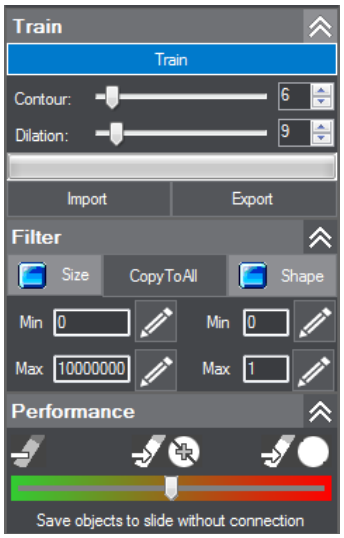
Click to delete a selected area; first click selection item on the list (alternatively, hold **Ctrl** while clicking the item to select more one by one, or **Shift** to make group selection) then deletion can be performed. Click to delete a cluster entirely.

Click at the right side of the cluster panel to append an additional Quant to the cluster. The same window is displayed as in the root view of the tree. The selected Quant algorithm will run on determined areas specified by the cluster. The measurement process will run only on those areas of the annotation that match the properties defined by the cluster.

It is available for you not only to embed other Quants, but another PatternQuant in the tree as well. The additional Quant algorithms can be run on the resulting areas of the previous defined Quant application.

NOTE: The number of linked PatternQuant measurements is limited: the software is restricted to run only two PatternQuant measurements that are linked together (including the root).

PatternQuant properties



- **Train** – click this button to launch the training process, at the end of which the software classifies slide areas based on all PatternQuant clusters and selected areas. After the training process is finished, the **Mask** function turns active, so the results are shown by coloring selected areas.

  NOTE: Parameters and settings on the **Train** section of the panel are Quant-specific, while other sections are class-specific.

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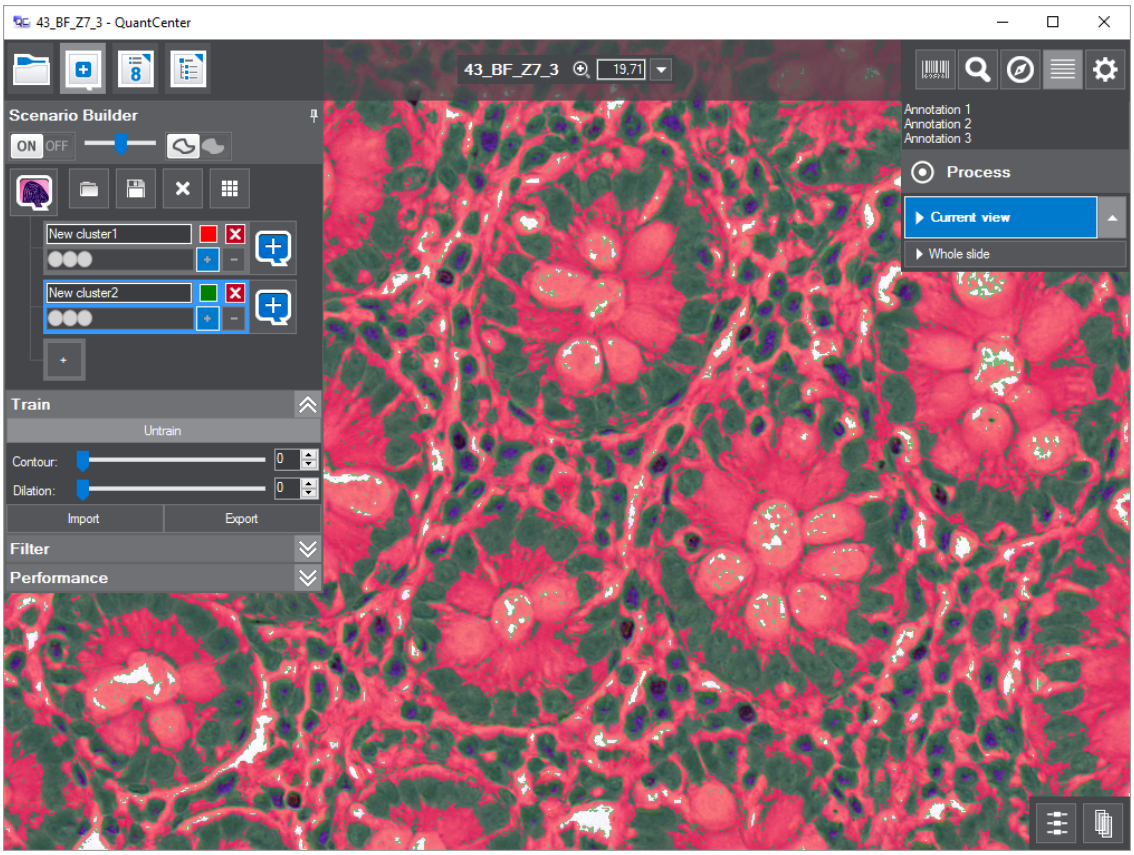

- **Contour** – the higher the set value is, the smoother the defined areas will be.
- **Dilation** – Mask boundaries can be set farther to surely include cells near the edge of the border.
- **Import** – Load PatternQuant measurement settings from an XML file.
- **Export** – Save measurement settings to XML.
- **Filter** – the result can be filtered based on **Size** and **Shape** either by typing values in the respective fields, or after clicking you may specify these values by measuring length or by selecting area on the slide image with the drawing function of the mouse. If you click CopyToAll each PatterQuant cluster will inherit the set values.

  NOTE: PatternQuant filter settings affect the measurement only, parameter modifications are not visualized on the preview image.

  NOTE: If filters are used, it is advised to set **Performance** to **Save objects to slide with connection**.

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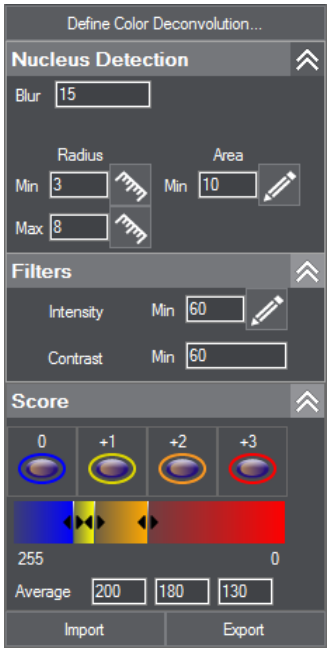
- **Performance** – 3 options are available to select from (measurement time will increase according to the following order):
  - **Save objects to slide disabled** (Green)
  - **Save objects to slide without connection** (Orange)
  - **Save objects to slide with connection** (Red)

4.2 NuclearQuant

With the help of NuclearQuant image analyzing algorithm, cell nuclei can be detected and classified. It may be run as single (root) measurement, or embedded into PatternQuant or HistoQuant in which case NuclearQuant process will be run on areas segmented by the Quant into which it is embedded.

NuclearQuant properties

After clicking the NuclearQuant button, Quant properties panel is displayed at the bottom left corner of the QuantCenter window in which measurement parameters of NuclearQuant can be set.



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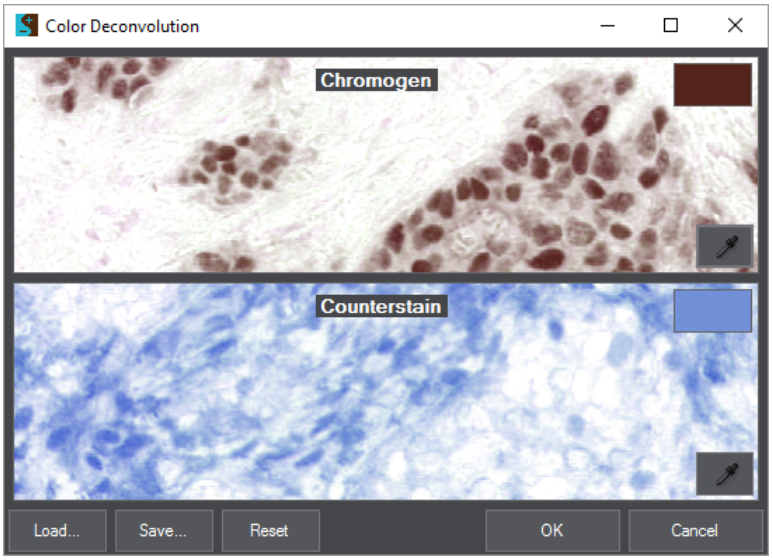
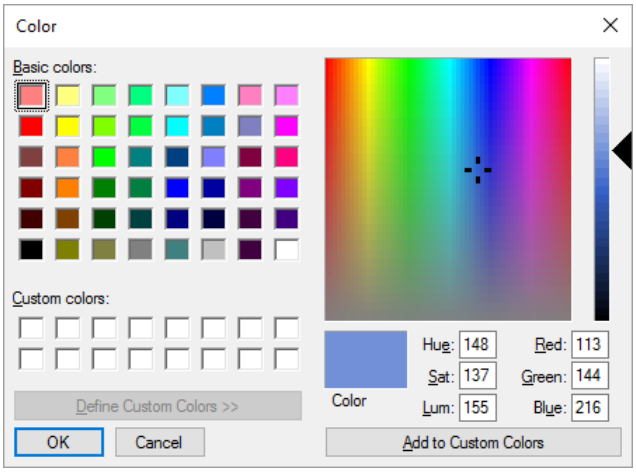
- **Define Color deconvolution**: the colors of positive and negative intensities can be defined in this window. The upper pane of the Color Deconvolution window represents objects of positive intensity (brown) and the lower pane represents objects of negative intensity (blue).



Representative colors can be set by either of the following two options:

- Click the pipette tool - - to pick color sample from the relevant area of the slide image in the QuantCenter window.

- Click the color boxes of Chromogen and Counterstain panes, then set the desired color in the Color window. Click **OK** when finished.



A Color Deconvolution profile can be loaded by clicking **Load** or saved by clicking **Save**. A profile is stored in a CCC file at the specified folder.

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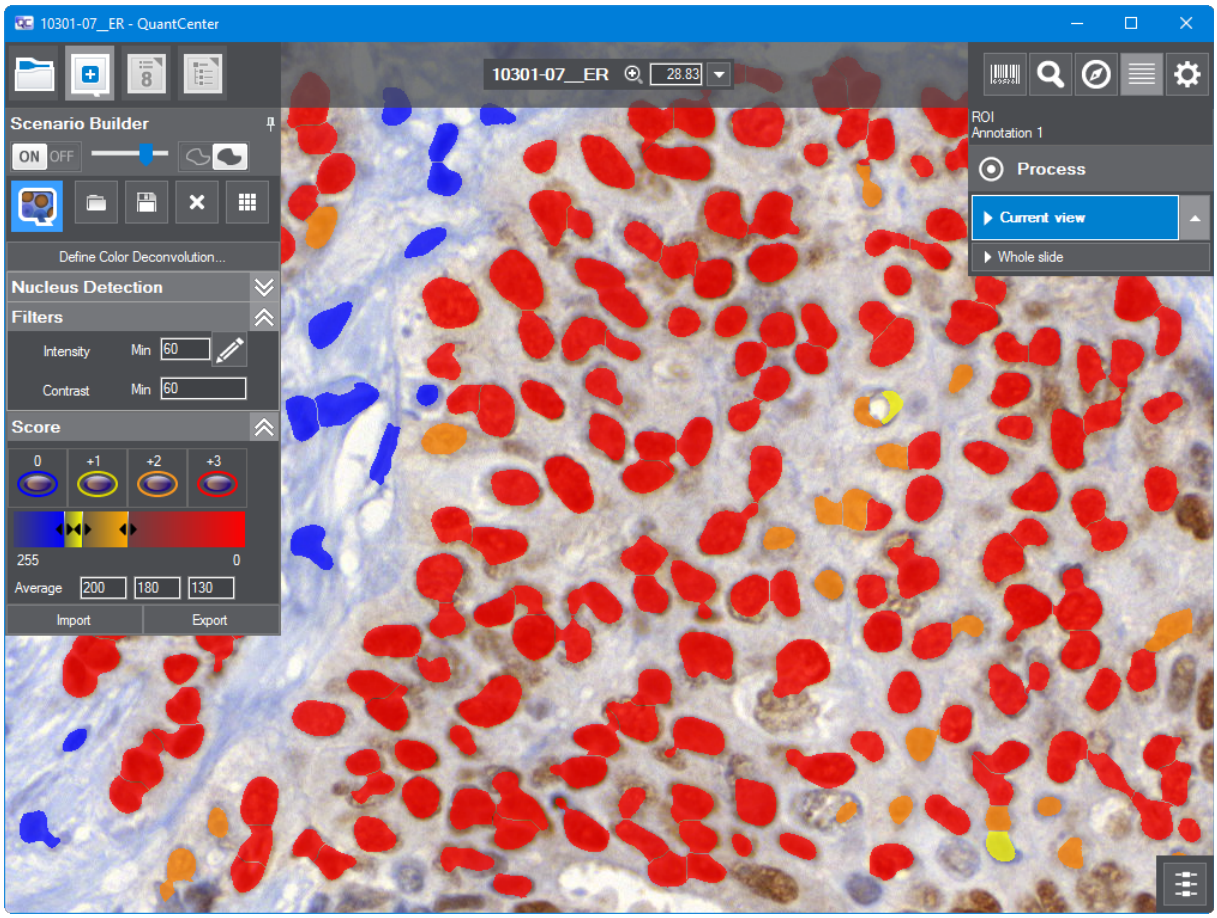
Detection results according to the parameterization are displayed on the slide image that is automatically updated whenever you modify a parameter value.



- **Nucleus Detection**: settings for nucleus detection
  - **Blur**: rough edges of the detected objects will be smoothened, therefore, besides decreasing noise-related flaws the resulting image appear more pleasing to the eye
  - Filtering based on **Radius** and **Area**. Set minimum and maximum values for Radius after clicking , and minimum area can be defined with the mouse by marking on the slide image after clicking .
- **Filters**: Set color **Intensity** (by entering value into the box, or by marking on the slide image after clicking), and **Contrast** minimum values.
- **Score**: Scoring based on intensity. Ranges can be modified by dragging the dividers, or by defining **Average** values in the relevant boxes.
- **Import**: Import an existing NuclearQuant profile (MISP file)
- **Export:** Export the specified NuclearQuant profile to a MISP file

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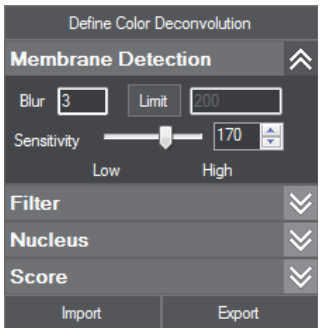
4.3 MembraneQuant

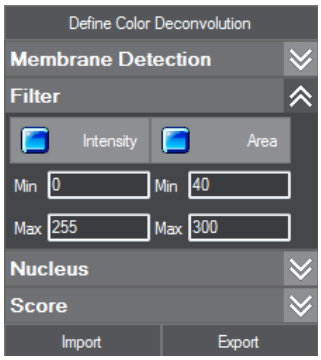
MembraneQuant is responsible for membrane detection and classification, furthermore it is capable of nuclei detection (since it cannot identify negative membranes, NuclearQuant is needed for this procedure).

MembraneQuant properties

After selecting the Quant button, the Quant Properties window is displayed at the bottom left corner of the QuantCenter window in which measurement parameters of MembraneQuant can be set.



- **Define Color Deconvolution**: the type of Intensity can be set for MembraneQuant (positive) and nucleus detection (negative)
- **Membrane Detection**:
  - **Blur**: rough edges of the detected objects will be smoothened, therefore, besides decreasing noise-related image flaws the resulting image appear more pleasing to the eye
  - **Limit**: the running of the algorithm stops if the detected membrane count reaches the set limit value. Click Limit to activate this option.
  - **Sensitivity**: sensitivity of the algorithm can be set either by dragging the slider bar, by entering a value to the box, or by clicking the arrows.
- **Filter**: Filtering based on the set value ranges of **Intensity** and **Area** by entering minimum and maximum values.



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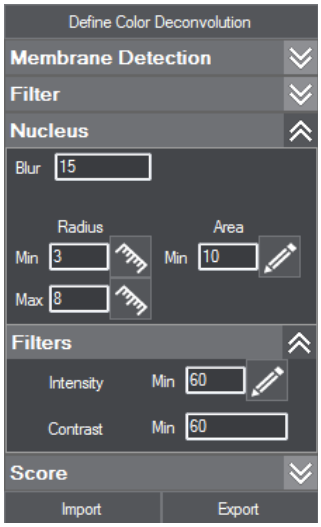
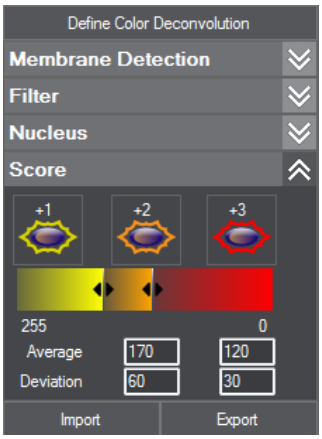
- **Nucleus**: Settings for NuclearQuant algorithm used by MembraneQuant



  - **Blur**: rough edges of the detected objects will be smoothened, therefore, besides decreasing noise-related image flaws the resulting image appear more pleasing to the eye
  - Filtering based on **Radius** and **Area**. Set minimum and maximum values for Radius after clicking , and minimum area can be defined with the mouse by marking on the slide image after clicking .
  - **Filters**: **Intensity** and **Contrast** minimum values related to Nucleus settings. Set color **Intensity** (by entering value into the box, or by marking on the slide image after clicking), and **Contrast** minimum values.

- **Score**: Scoring is based on intensity-related **Average** and **Deviation** values. Ranges can be modified by dragging the dividers, or by defining **Average** / **Deviation** values in their relevant boxes.



- **Import**: Import an existing MembraneQuant profile (MISP file)
- **Export:** Export the specified MembraneQuant profile to a MISP file

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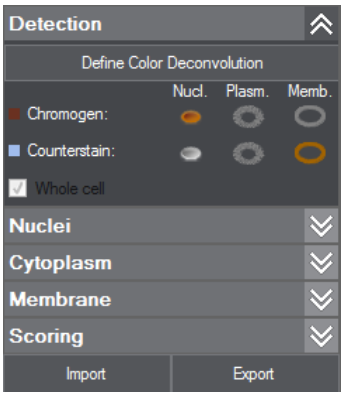
4.4 CellQuant

CellQuant is a module that can be adapted for nuclear-, cytoplasmic-, and membrane-localized IHC-stained biomarker identification based on intensity.

Quant Properties

After selecting the Quant button, the Quant Properties window is displayed at the bottom left corner of the QuantCenter window in which measurement parameters of CellQuant can be set.

Detection



- **Define Color Deconvolution:** the colors of positive and negative intensities based on the staining protocol (brightfield slides) can be defined in this window.

  Definition procedure is the same as detailed in ***section 4.2*** under NuclearQuant.

- **Channel matching:** Specify the intensities of objects (nucleus, plasm, membrane). Only one object can be selected for an intensity type (i. e. only one selection can be activated in a row).

> **Important!**
>
> If you link an additional Quant to the actual scenario, and you specify channels and run measurements, after saving/exporting scenario or Quant, pairing will not be restored after reloading the scenario or Quant (when reloading, channels stored in the profile will be matched with the channels of the slide). Channel matching is temporarily set and can be used to that specific measurement. If saving an additional Quant that is linked to a Fluorescent slide, the program saves which channels of the slide are selected, and those will be presented as profile channels pairable with slide channels after reloading.

- **Whole cell**: if selected, the algorithm (based on estimation) detects those objects that are not selected to be detected. If the selection box is not marked, only those objects will be displayed and measured that are being searched for.

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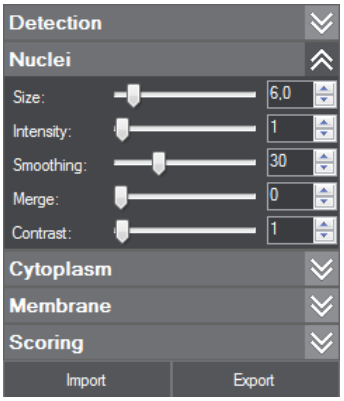
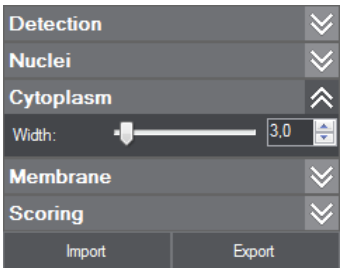
Nuclei



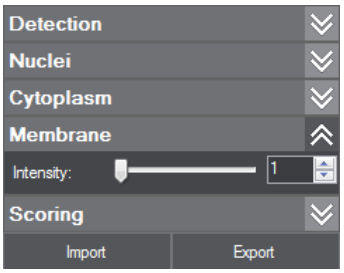
- **Size**: average size of nuclei
- **Intensity**: minimum intensity of nuclei
- **Smoothing**: smoothing the contour of nuclei
- **Merge:** if the center area of nucleus is brighter (because of the type of the applied staining – e.g. Ki67), set the percentage value of filling these holes in the nucleus. 0 means no filling, 100 stands for filling in full. This option is useful for preventing objects from becoming over-segmented.
- **Contrast:** the minimum contrast (intensity difference) between the nucleus and the background.

Cytoplasm



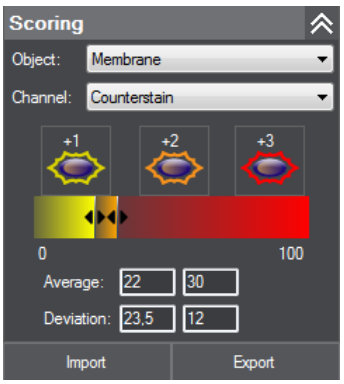
- **Width:** the maximum width of cytoplasm (distance between the edge of nucleus and membrane)

Membrane



- **Intensity:** minimum intensity of membrane

Scoring



- **Object:** cell object selection for scoring
- **Channel:** select the channel for searching specified cell objects
- Set intensity-related **Average** and **Deviation** values

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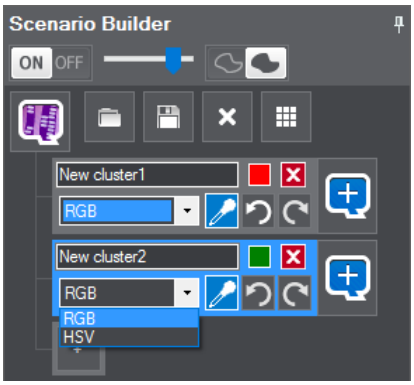
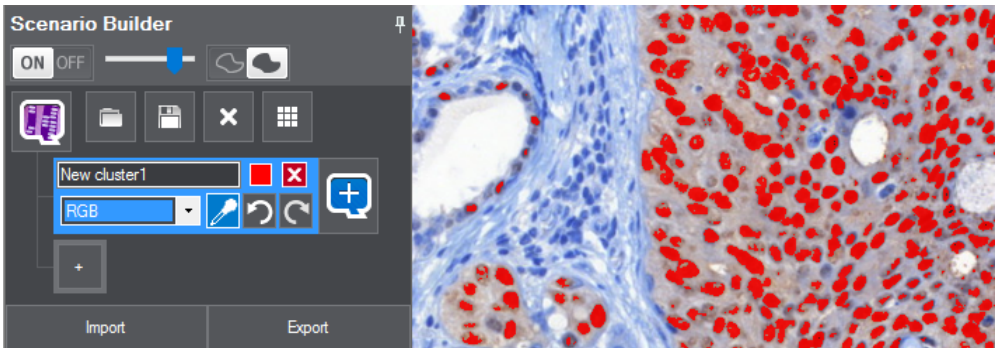
4.5 HistoQuant

HistoQuant is a quantification algorithm that can be used for performing segmentation and measurements on a Pannoramic digital slide based on the colors of cell compartments.



If a Brightfield slide is opened, you can select from **RGB** and **HSV** color models, for Fluorescent slides **Channeled** or **HSV** can be selected in order to define the color channels or color reproduction area from which the samples are taken.



Color samples can be taken after clicking the pipette tool - -, then move the mouse (this case, the pipette cursor) over the desired area of the slide, and click with the left mouse button.

To finish sampling, either hit **ESC** or click with the right mouse button. Steps of sampling can be cancelled or repeated by clicking the or (undo/redo) buttons.

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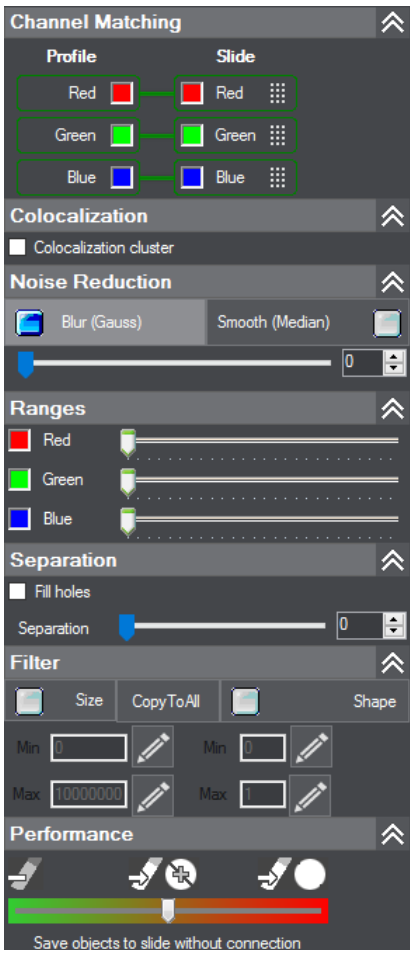
Important!

In Scenario Builder, the maximum number of definable HistoQuant classes is 8 (eight). Class name cannot be longer than 20 characters, and make sure that each class has unique name and representative colors.

If another Quant is linked to a PatternQuant or HistoQuant class, the settings of that specific Quant can be accessed only within the actual scenario, but can be saved by using **Export** located on the Properties panel of that specific Quant, and can be imported when adding an identical Quant during creating another scenario.

You can import XML data after clicking **Import** or export HistoQuant class properties into an XML file when clicking **Export**.

HistoQuant Properties



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Important!

If you link an additional Quant to the actual scenario, and you specify channels and run measurements, after saving/exporting scenario or Quant, pairing will not be restored after reloading the scenario or Quant (when reloading, channels stored in the profile will be matched with the channels of the slide). Channel matching is temporarily set and can be used to that specific measurement.

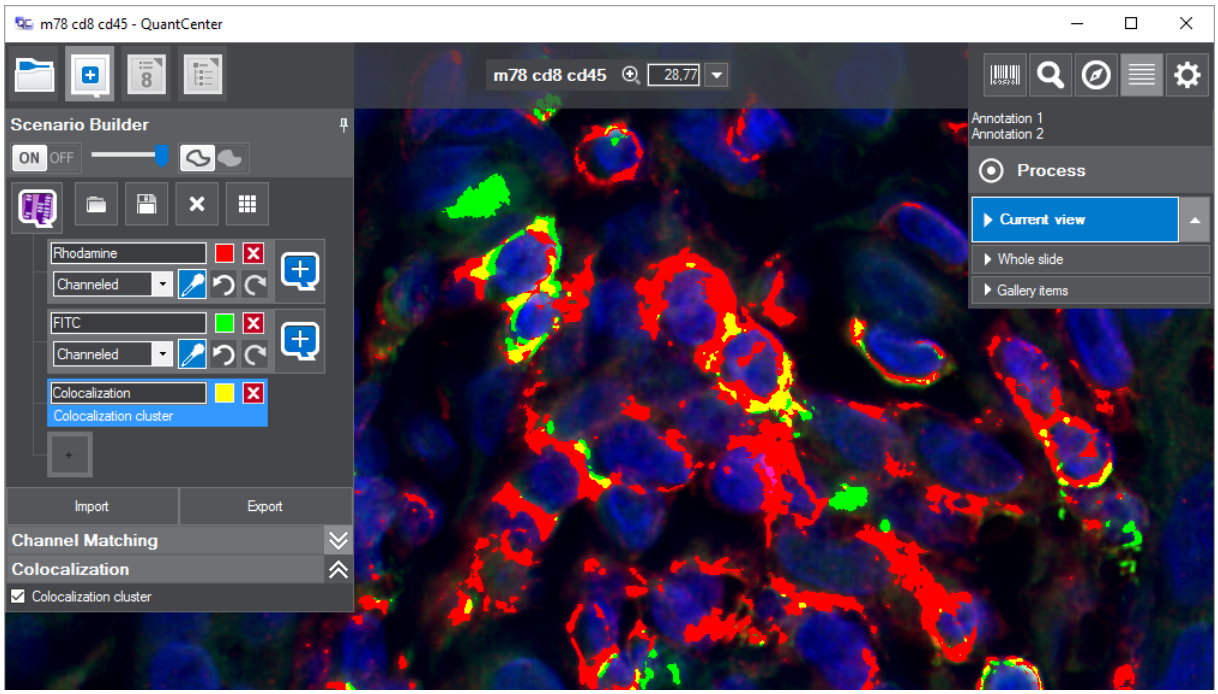
If saving an additional Quant that is linked to a Fluorescent slide, the program saves which channels of the slide are selected, and those will be presented as profile channels pairable with slide channels after reloading.

Channel Matching

This panel includes channel representation of the profile and the slide. When opening a slide for which channel matching is ambiguous, only this panel will be opened. Measurements can be run only if all the channels of the profile are conjugated with slide channels. Slide channel tabs include a dotted area showing that those channels are moveable. The pairing between a profile channel and the relevant slide channel is marked with a green line.

Colocalization

If a slide is opened, **Colocalization cluster** option can be activated to investigate stain colocalization, thus enabling a third, separate disjunct cluster to include colocalized objects.



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Noise Reduction

To perform noise reduction on the slide image (to make it easier for HistoQuant algorithm to detect objects by colors that is crucial for detecting nuclei or cell compartments), Gauss or Median filters are available for you to select on this panel. Gauss reduces the noise and blurs the image. Median is a simple but fast filtering method, it uses the median of adjacent pixel values.

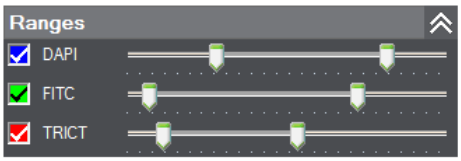
NOTE: If high noise reduction values are set, it is advised to activate the Gauss blur, as it is less sensitive for differences resulting from ROI shifts, zoom variations, etc.

Ranges

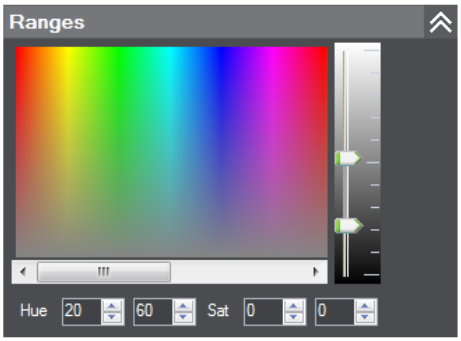
On this panel channel adjustments can be performed both for RGB (BF), Channeled (FL) and HSV (both) color models with the help of channel sliders. A tick mark is then visible in the checkbox of the relevant channel. If clicking the slider, a value box is displayed in which the desired value can be set or entered.

RGB Channels (BF)

Channeled (FL)



HSV Channels (BF/FL)



As for Fluorescent slides the number of channels is variable according to the defined channels set for digitization, so the relevant number of sliders for each channel are displayed in the Range panel if **Channeled** is selected for a cluster.

When **HSV** (Hue-Saturation Value) mode is selected from the drop-down menu, a color scale and a brightness slider is displayed in the panel, and during sampling a specific area can be defined on the scale that is marked with a white border. This area is both resizable and movable, therefore the selection is modifiable. Below the scale, four numeric fields are displayed, representing **Hue** and **Saturation** values. The combined slider at the right side of the color scale is responsible for adjusting brightness values within a range.

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Separation

Two functions are available on this panel:

- **Fill holes** – Areas belonging together will be joined.
- **Separation** – If objects are too close to each other, they might be detected as one. If the value is 0 there will be no separation, and if value is set to 10, the area of the specific class is cut into several pieces.

Filter

Shape- and size-related filtering methods can be activated and set on this panel similar to PatternQuant.

Performance

3 options are available to select from (measurement time will increase according to the following order):

- **Save objects to slide disabled** (Green)
- **Save objects to slide without connection** (Orange)
- **Save objects to slide with connection** (Red)

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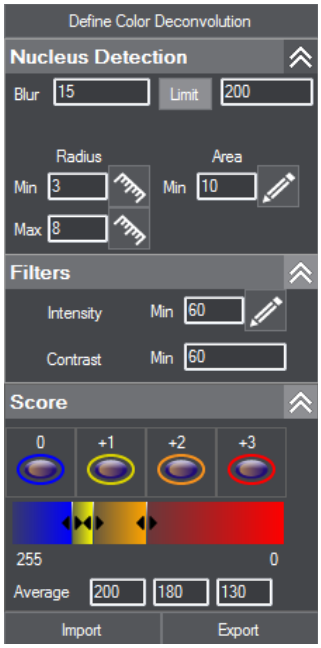
4.6 CISH-RNAQuant

CISH-RNAQuant allows you to detect and separate CISH spots on the virtual slides (treated with CISH probes that bind strongly to complementary DNA or RNA strands) digitized with Pannoramic microscopes.

CISH-RNAQuant Properties

After selecting the Quant button, Quant properties window is displayed at the bottom left corner of the QuantCenter window in which measurement parameters of **CISH-RNAQuant** can be set.



Define Color Deconvolution

The representative colors of Chromogen and Counterstain can be defined in the Color Deconvolution window.

The upper pane of the Color Deconvolution window represents objects of positive intensity (brown) and the intensity of negative objects (blue). First, click into the pane of the desired intensity to be set, and by using the pipette tool take a color sample from the relevant area of the slide image in the QuantCenter window.

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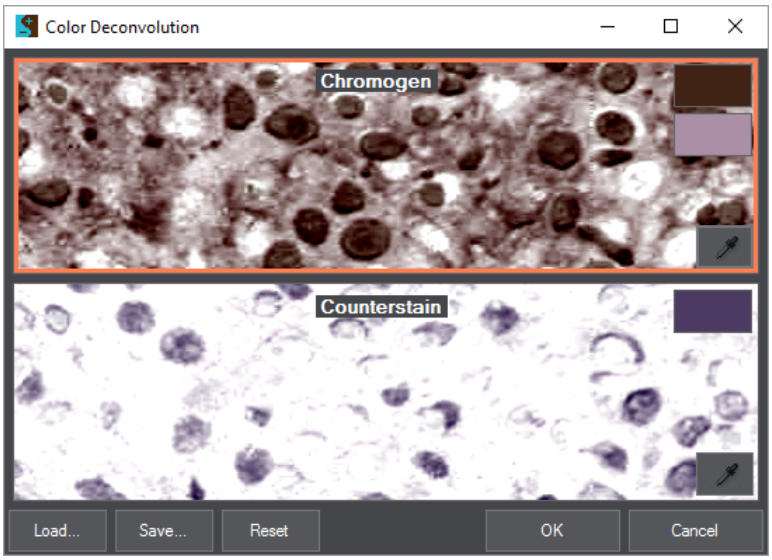

A Color Deconvolution profile can be loaded by clicking **Load** or saved by clicking **Save**. A profile is stored in a CCC file at the specified folder.

Nucleus Detection

This section includes settings for nucleus detection.

- **Blur**: rough edges of the detected objects will be smoothened, therefore, besides decreasing noise-related flaws the resulting image appear more pleasing to the eye
- **Limit**: the running of the algorithm stops if the detected nuclei count reaches the set limit value
- Filtering based on **Radius** and **Area** parameter values

Filters

Set minimum values of **Intensity** and **Contrast**.

Score

Set scoring ranges based on intensity.

Import

Import an existing **CISH-RNAQuant** profile.

Export

Export the specified **CISH-RNAQuant** profile.

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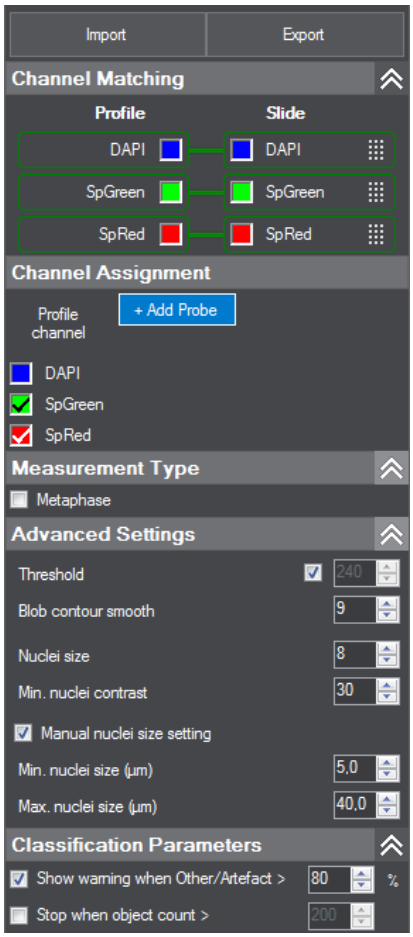
4.7 FISHQuant

FISHQuant is an image analysis application, consisting of an automatic *segmentation* and a thresholding setup section. The information needed for the algorithm is not saved on the slide but in an XML file, which can be used later to evaluate other *annotations* on the same slide or on other slides.

NOTE: Since **FISHQuant** allows you to make measurements on the slides, and digital image processing can cause loss of quality, always make sure that the measurements are acceptable to you.

FISHQuant Properties



You can import XML data after clicking **Import**, and export **FISHQuant** class properties into an XML file when clicking **Export**.

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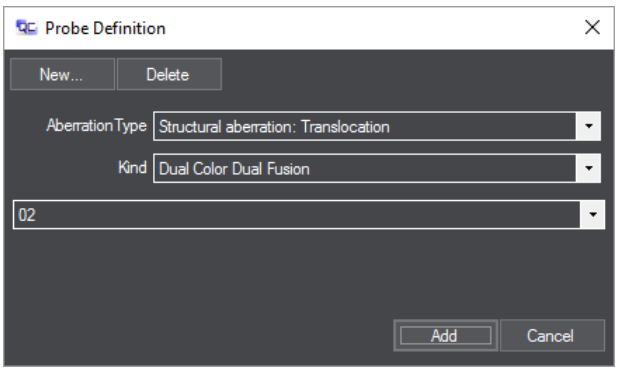
Channel Matching

This panel includes channel representation of the profile and the slide. When opening a slide for which channel matching is ambiguous, only this panel will be opened. Measurements can be run only if all the channels of the profile are conjugated with slide channels.

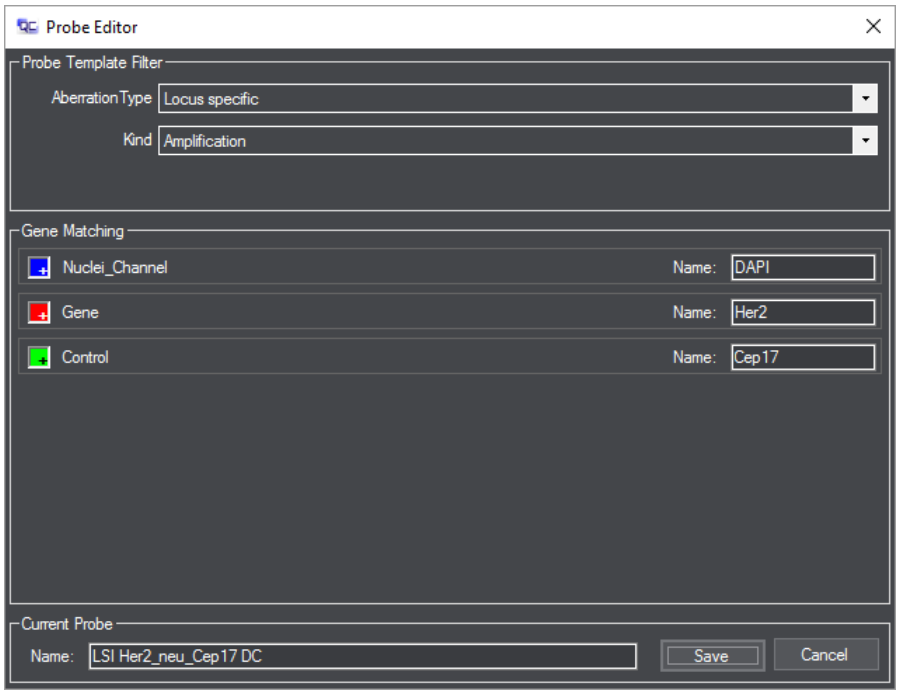
Slide channel tabs include a dotted area showing that those channels are movable. The pairing between a profile channel and the relevant slide channel is marked with a green line.

Channel Assignment

Under *Profile channel*, the color channels of the slide are listed. Click + Add Probe to define a primary probe. In the appearing Probe Definition window, set **Aberration Type**, **Kind**, then select the probe from the bottom drop-down list. Click New to create a new probe, or click Delete to delete the selected aberration type.



If creating a new probe, the following window is displayed:



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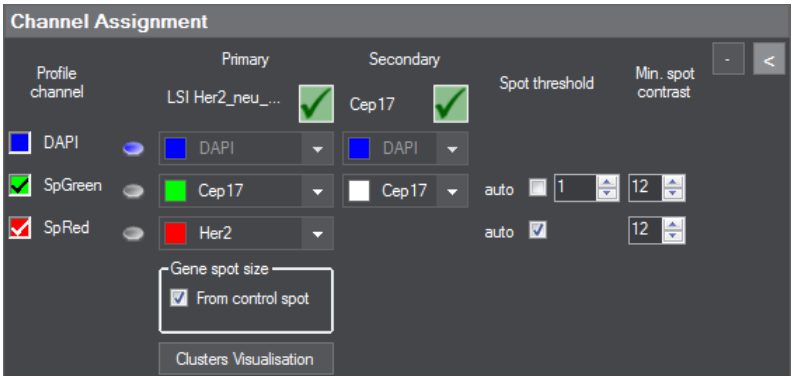
- Set **Aberration Type** and **Kind**, then fill in the fields of Gene Matching section.
- Add name to the probe, then click Save.
- In the Probe Definition window, click **Add** to set the created probe as *Primary*.
- To define a secondary probe, click + first, then define parameters from the drop-down lists.

Probes will be presented as *Primary* and *Secondary* above the probe name. If you want to delete a probe, first click the probe name, then the - button.

- By clicking the grey spot next a channel name, the desired channel can be selected as nuclei channel. If DAPI FISH slide is opened, the tick marks in the color boxes of the related channel, the detected signals on the displayed slide image can be switched on/off.



Click > to display further, spot-detection-related settings.

- The **Spot threshold** option is designed to set spot threshold value for the selected channel.
- Upon selecting the **auto** option to apply automated spot detection. The manual parameter setting for the detection of weaker spot signals is available in the **Min. spot contrast** value box.

Gene spot size

Upon selecting an amplification probe on the **Probe editor** panel, this option is displayed below the probe. Select one of the following options:

- **From control spot** – breaks down the spot cloud of the highly amplified genes with the average size of the control spot
- **Manual** – set a parameter value manually between 1.00 and 10.00 microns

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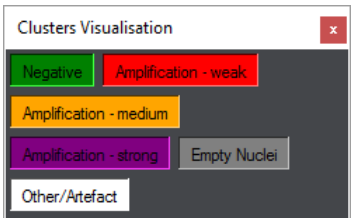
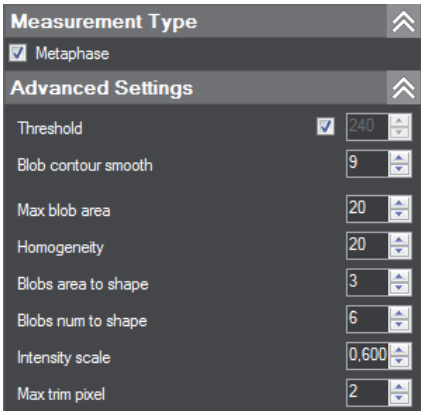
Click **Clusters Visualization** to display the names and colors of the defined clusters of the primary probe. Clusters can be switched on/off in order to make them displayed/hidden on the mask image.



Measurement Type



Two types of segmentation algorithm are available for you to select from under this panel. By default, an automated nuclei segmentation algorithm parameters for the evaluation of tissue samples (optimally, the maximum thickness of a section is 3-4 micrometers) are listed under the Advance Settings panel. The parameters you can modify are the following:

- **Threshold**

  Color intensity threshold for setting the least intensive pixels of the detected nuclei (value ranges from 0 to 255). Increment this value to find less intensive nuclei. Tick the check-box for automatic mode.

- **Blob contour smooth**

  Circumference smoothing parameter (value ranges from 1 to 15, and only odd values are accepted) which defines the length that is taken to account when smoothing the circumferences of the detected nuclei. Increment this value to get smoother outlined nuclei.

- **Nuclei size**

  The size of nuclei to be detected can be set here.

- **Min. nulcei contrast**

  The minimum contrast value of spots can be set here.

- **Manual nuclei size setting**

  If activated, minimum and maximum size values can be set as input data for segmentation algorithm.

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If **Metaphase** option is selected, nuclei segmentation algorithm is activated (the related parameters are displayed in the **Advanced Settings** panel) for the evaluation of separate cells, cell cultures, and cytological specimens.

NOTE: If **Metaphase** option is activated, secondary probe cannot be defined.

Advanced Settings

- **Threshold**

  Color intensity threshold for setting the least intensive pixels of the detected nuclei (value ranges from 0 to 255). Increment this value to find less intensive nuclei. Tick the check-box for automatic mode.

- **Blob contour smooth**

  Circumference smoothing parameter (value ranges from 1 to 15, only odd values are accepted). Defines the length that is taken to account when Smoothing the circumferences of the detected nuclei. Increment this value to get smoother outlined nuclei.

- **Max blob area**

  Nuclei size relative measure (value ranges from 0 to 50). Sets the size of the detected objects, that are not going to be split. Decrement this value to allow splitting of smaller objects.

- **Homogeneity**

  Parameter of intensity difference (the difference between the most and the least intensive pixels). Decrementing this value allows nuclei to be formed on more homogeneous areas. Value range: [0,255]

- **Blobs area to shape**

  Object convexity measure. Average area of nucleus circumference concavities. Decrement this value to detect more circular nuclei. Value range: [0,20]

- **Blobs num to shape**

  Object convexity measure. Number of allowed nucleus circumference concavities. Decrement this value to detect more circular nuclei. Value range: [0,100]

- **Intensity scale**

  Average intensity related measure. Uniformly dim objects will not be split. Increment this value to allow splitting of less intensive nuclei. Value range: [0,1]

- **Max trim pixel**

  Iteration count of erosion operator. If multiple objects are connected with thin structures, they will be split. Increment this value to allow splitting through thicker connecting segments. Value range: [0,10]

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Classification Parameters

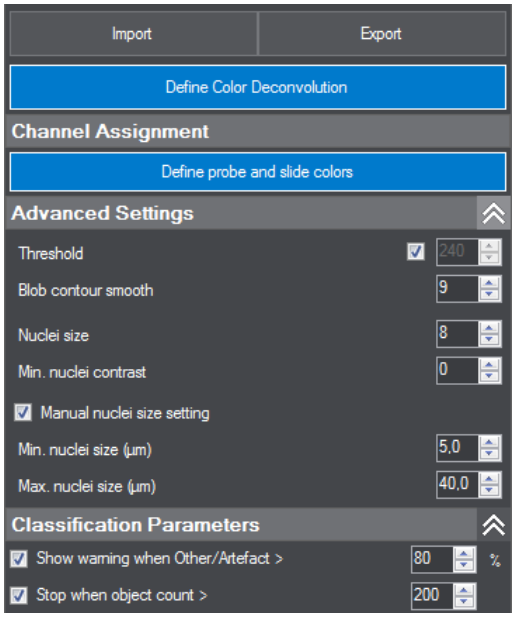
There are two functions that can be activated to assign limitation parameters for the classification.

- Activate **Show warning when Other/Artifact >** function to allow the software to display a warning message in the tree as a tooltip, if the percentage of objects found in the Other/Artifact clusters exceeds the set value.
- The **Stop when object count >** option is responsible for terminating classification if object count reaches the set maximum number of nuclei identified in negative and positive clusters.

4.8 CISHQuant

CISHQuant allows you to detect and separate CISH spots on the virtual slides digitized with Pannoramic microscopes. This CISH-based application performs *segmentation* and *measurement*s on Pannoramic *digital slide*s based on the colors of the gene spots and counter-stained nuclei. Based on the applied CISH probe, it can classify the detected objects.

CISHQuant Properties



You can import XML data after clicking **Import**, and export **CISHQuant** class properties into an XML file when clicking **Export**.

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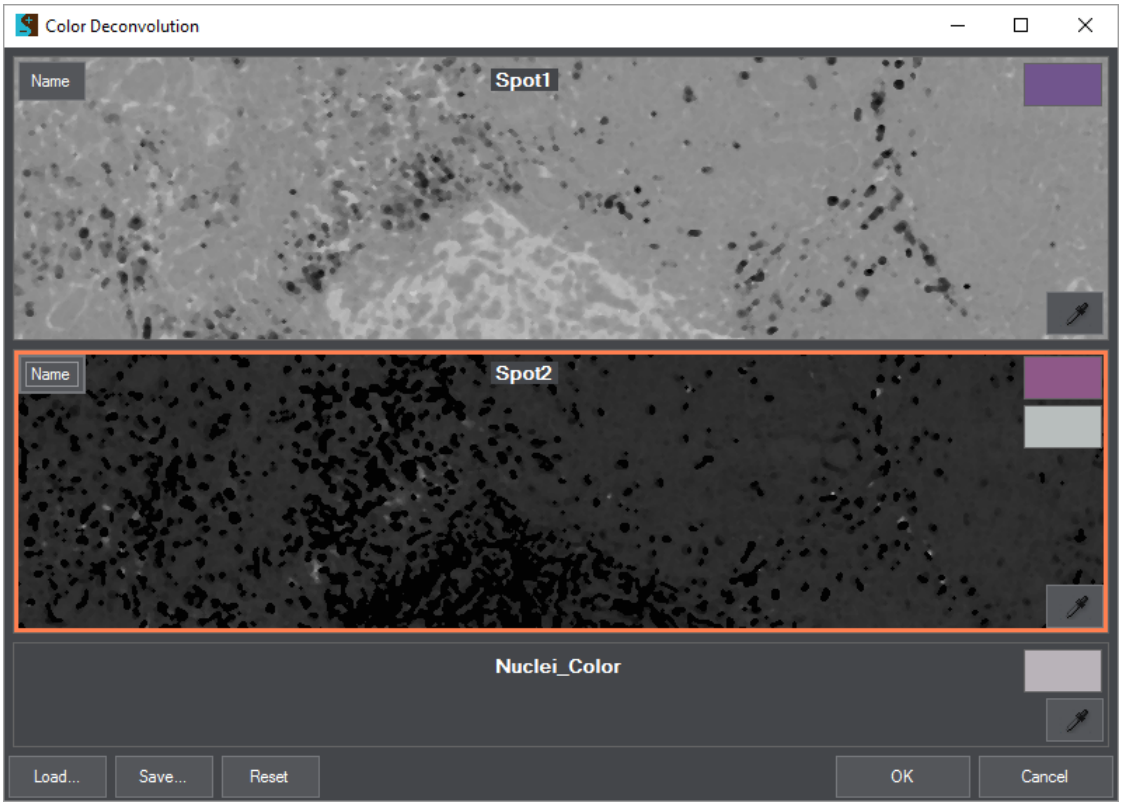
Define Color Deconvolution

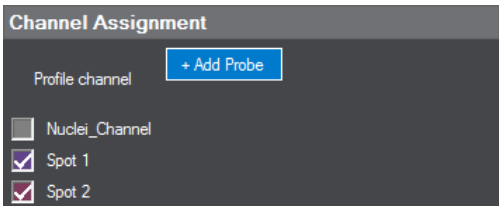
The representative colors of Nuclei, Spot channel 1, and Spot channel 2 can be defined in the Color Deconvolution window.



Use the pipette tool for setting the proper color for each channel (set a typical color of imperfections or impurities on the slide for the first channel, that will be removed before running the segmentation algorithm, then set colors of the two spot channel). Channel names can be edited by clicking the **Name** buttons. Click **Load** to import an existing Color Deconvolution profile and **Save** to export settings into a profile. Click **Reset** to undo all changes made to color selection since the window was opened, or **Cancel** to quit from the window, then **OK** to continue with probe definition.

Channel Assignment

Click **Define Probe and slide colors** to define a probe. If you have loaded a Color Deconvolution profile, click + Add Probe.



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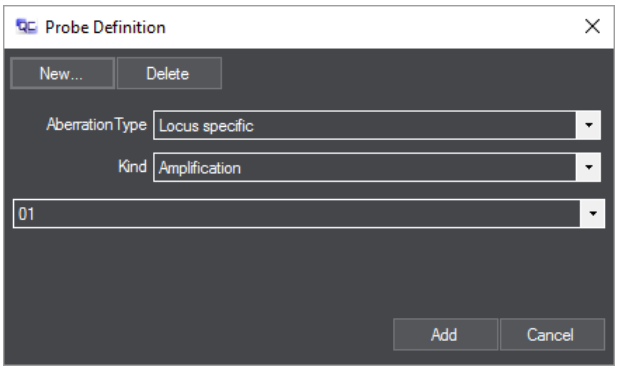
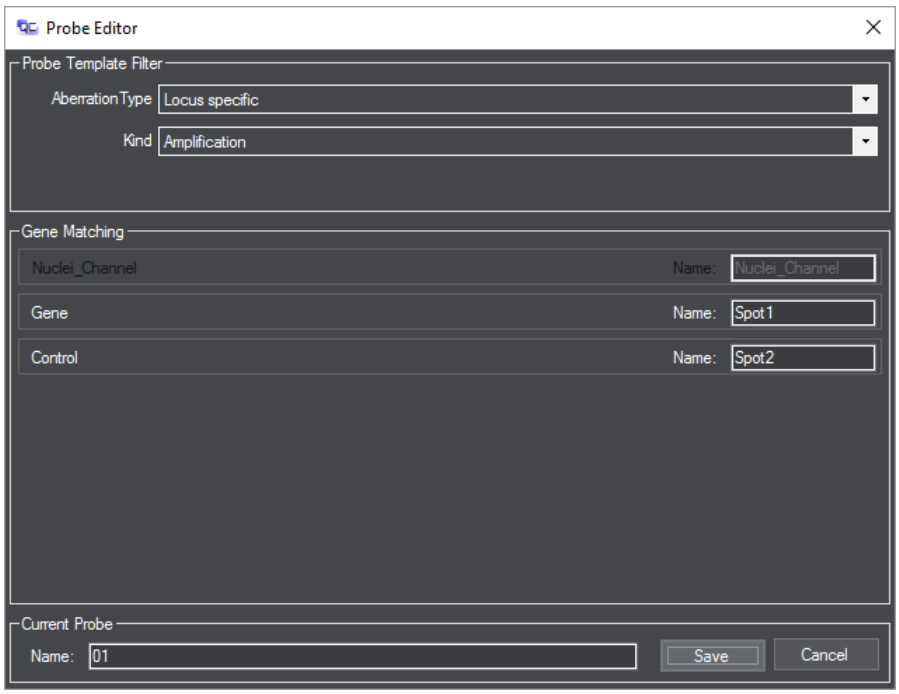
NOTE: After clicking **OK** in the Color Deconvolution window, the Probe Definition window is automatically displayed.

In the **Probe Definition** window, set **Aberration Type**, **Kind**, and probe from the relevant drop-down lists, delete probe by clicking **Delete**, or create new by clicking the **New** button.



If you want to create a new probe, click **New** first, then the Probe Editor window is displayed.



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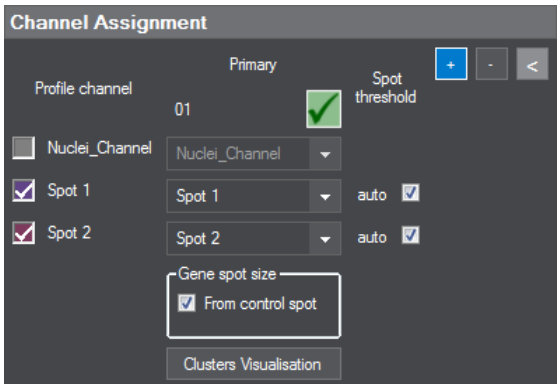
The primary probe created is shown in the **Channel Assignment** section of the panel. You can add a secondary probe by clicking and remove a probe by selecting it first, then clicking



Advanced Settings

This section of the panel includes parameters of the measurement that can be modified if necessary.

- **Threshold**

  Color intensity threshold for setting the least intensive pixels of the detected nuclei (value ranges from 0 to 255). Increment this value to find less intensive nuclei. Tick the check-box for automatic mode.

- **Blob contour smooth**

  Circumference smoothing parameter (value ranges from 1 to 15, only odd values are accepted) which defines the length that is taken to account when smoothing the circumferences of the detected nuclei. Increment this value to get smoother outlined nuclei.

- **Nuclei size**

  The size of nuclei to be detected can be set here.

- **Min. nulcei contrast**

  The minimum contrast value of spots can be set here.

- **Manual nuclei size setting**

  If activated, minimum and maximum size values can be set as input data for segmentation algorithm.

Classification Parameters

There are two functions that can be activated to assign limitation parameters for the classification.

- Activate **Show warning when Other/Artifact >** function to allow the software to display a warning message in the tree as a tooltip, if the percentage of objects found in the Other/Artifact clusters exceeds the set value.
- The **Stop when object count >** option is responsible for terminating classification if object count reaches the set maximum number of nuclei identified in negative and positive clusters.

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4.9 DensitoQuant

DensitoQuant offers a framework to configure, perform and visualize immunohistochemical measurements on slides, and measures the density of immunostain based on the colors of cell compartments from immunohistochemical stains.

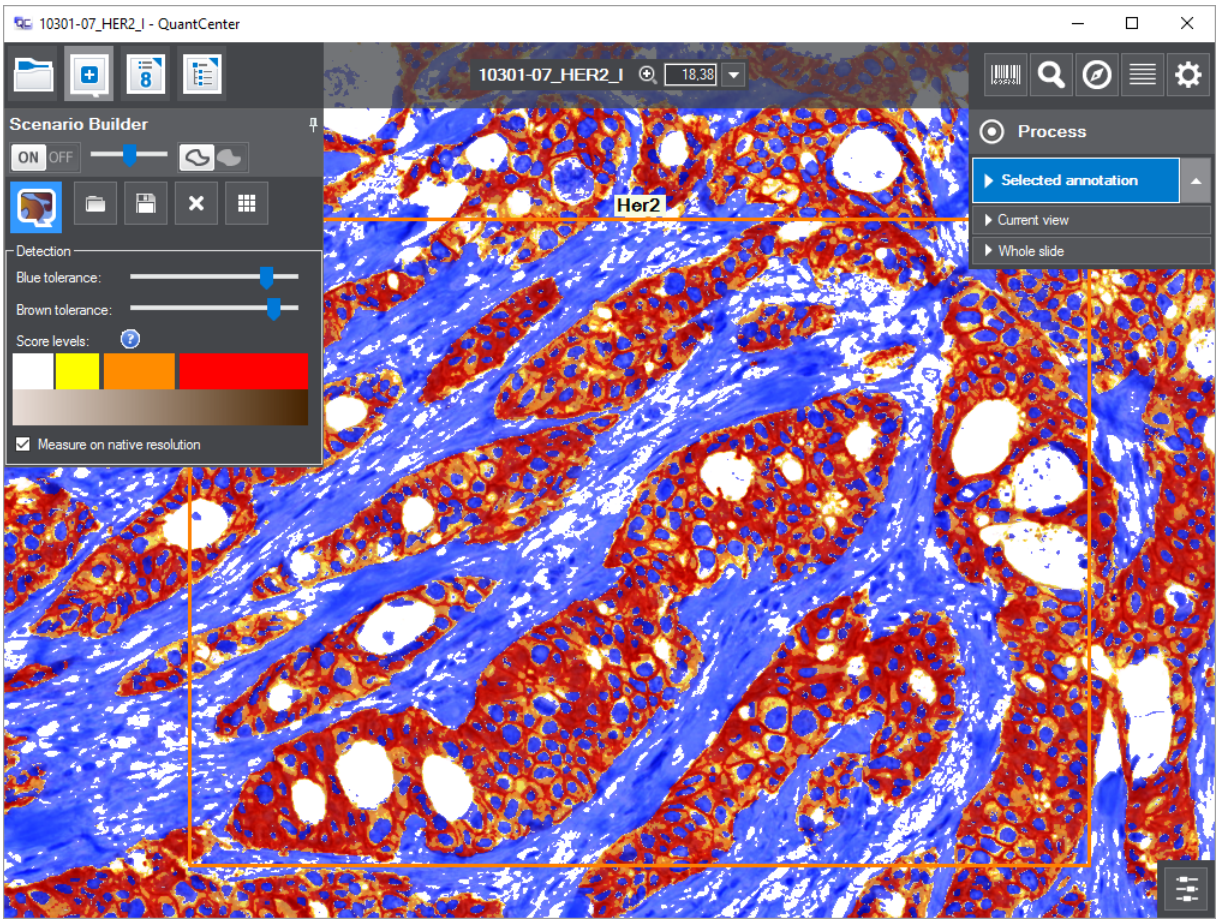
NOTE: DensitoQuant cannot be linked under PatternQuant nor HistoQuant, as it eliminates filtering.

1. Set **Detection** parameters **Blue tolerance** and **Brown tolerance**.

2. Activate **Measure on native resolution** if you want to run measurements at 1:1 magnification.

3. Select the annotations you want to run measurement on and run measurement process. The **Measurement Card** form is displayed after the process is finished.

On the **Scenario Builder** form turn **Mask** on to advert the shape of input annotation, and adjust parameters if necessary.



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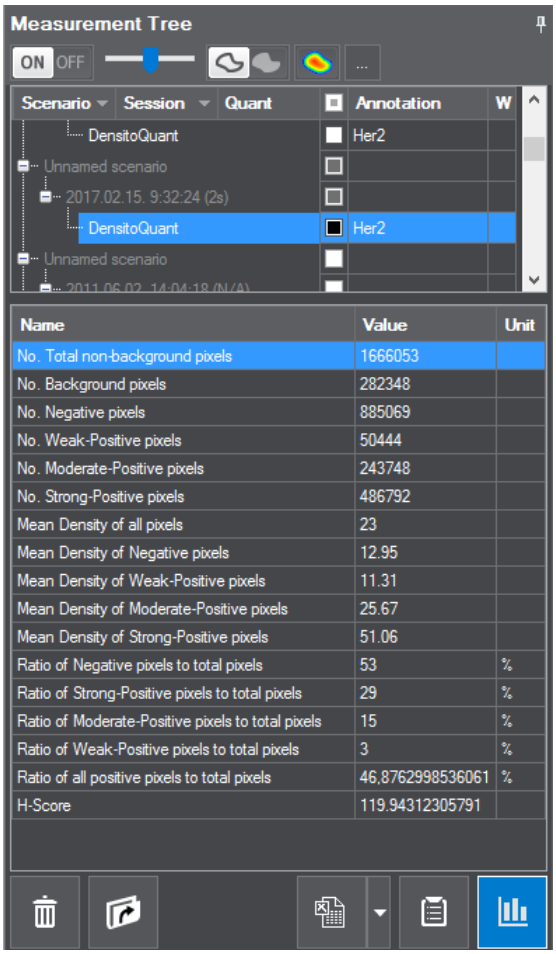
For detailed measurement results, activate **Measurement Tree** form first, then simply click the desired annotation name to show the related measurement data.



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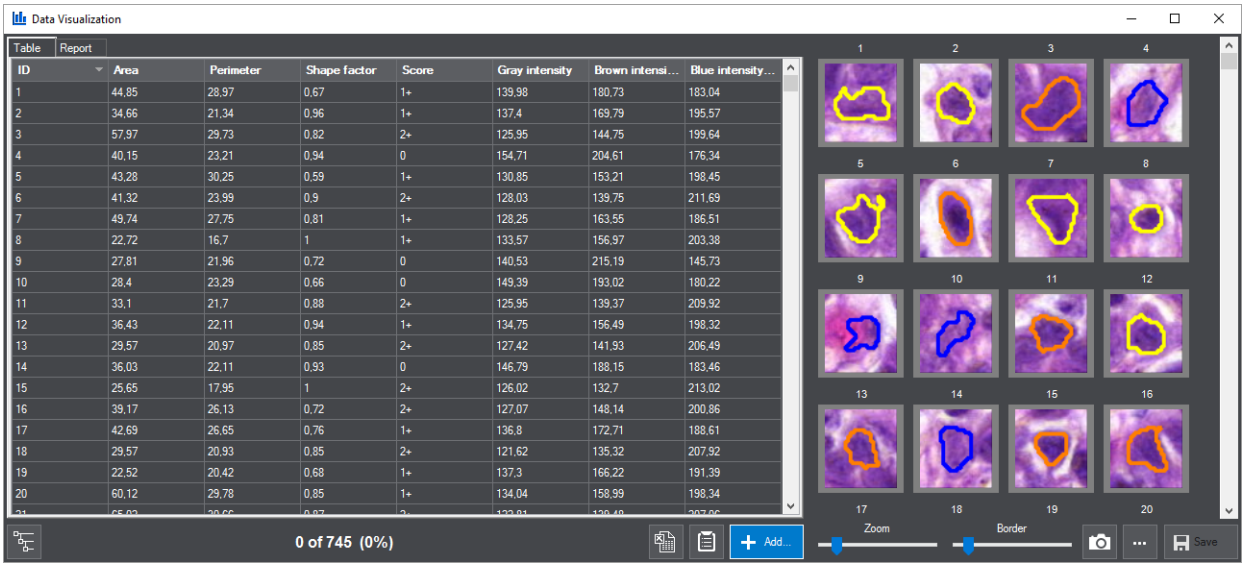
5 Data Visualization

> **Important!**
>
> For the perfect visualization of the application make sure that the display is set to **Smaller – 100% (default)** under **Control Panel\Appearance and Personalization\ Display** menu in Windows. Running the application using different display settings results in unexpected behavior of visual apprearance.

5.1 Panels and functions

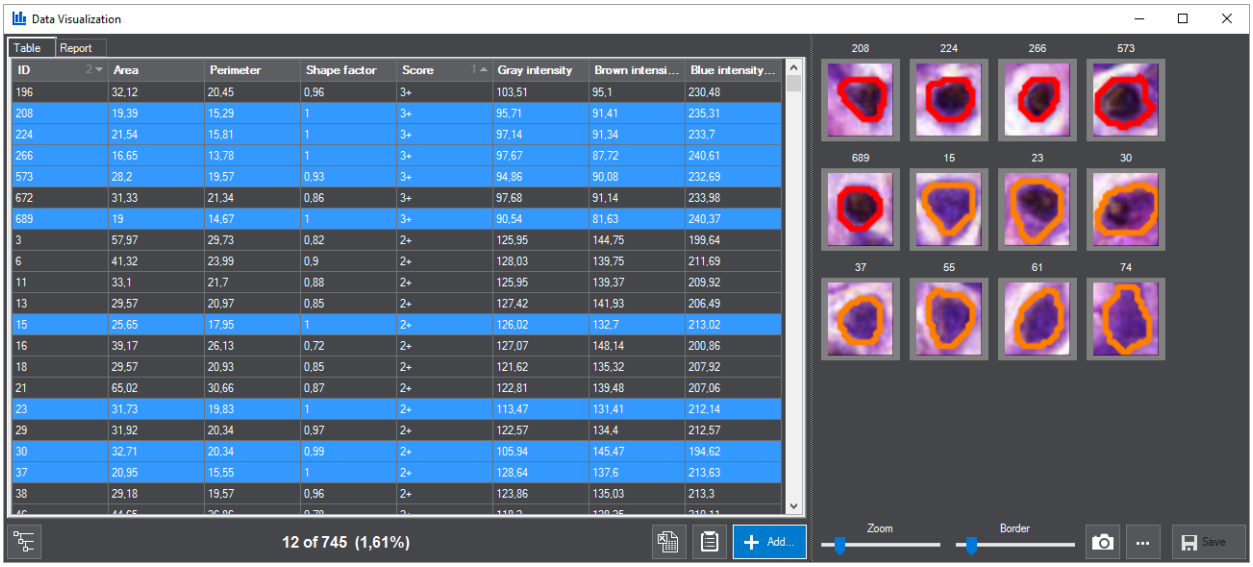
Upon clicking the button both on Measurement Card and Measurement Tree forms (and also on the tool bar, if available), the Data Visualization window can be displayed.

Upon opening the Data Visualization window, the **Table** view is shown by default (except for FISHQuant and CISHQuant) in which detected objects and their detailed data related to the measurement are listed.



Each column can be ordered by clicking the header, so values will be listed in descending or increasing order. Upon clicking a list item, the image of the specific object is displayed on the right panel.

By keeping the **Ctrl** key pressed and clicking with the mouse, detected objects can be selected from the list and displayed in the right side of the window.

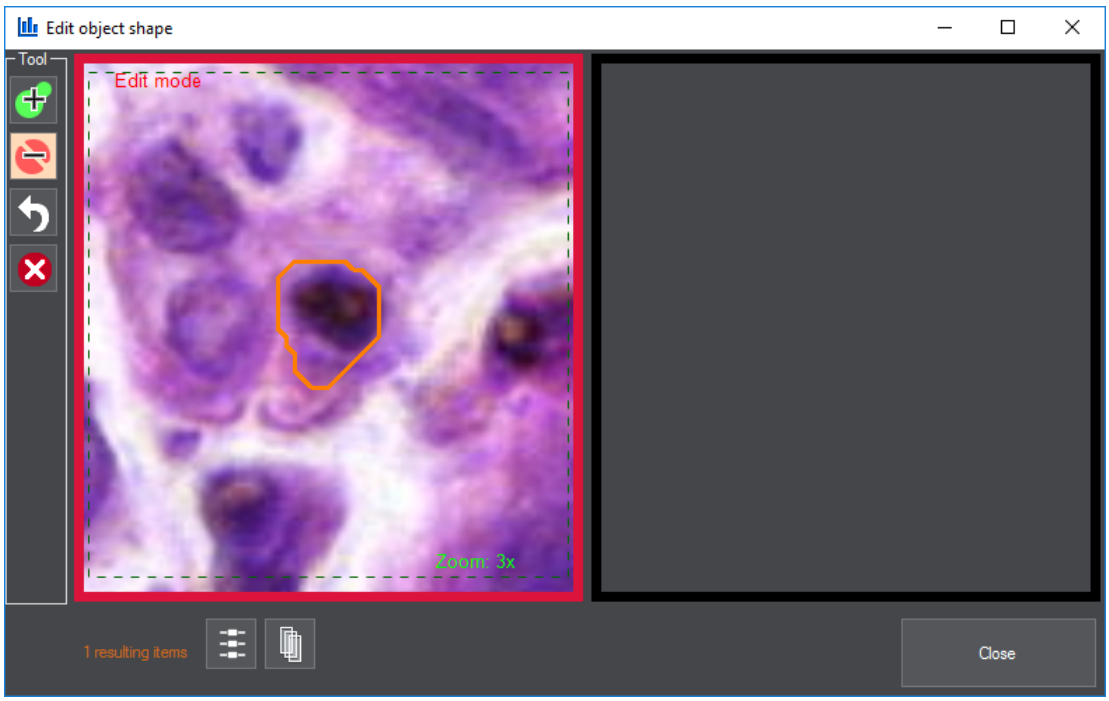


The visualization of gallery objects can be adjusted by dragging the Border and Zoom sliders. Item size can be increased/decreased by dragging the **Zoom** slider to the appropriate direction. The **Border** slider is responsible for enlarging the border of items.

To turn on masking controls, select **Gallery controls** after clicking ⋯.

Upon right-clicking an object in the gallery, you can select from the following options:

- **Edit shape** – Use this tool to alter masking boundary of detected objects or separate two or more objects that have been detected incorrectly as one.



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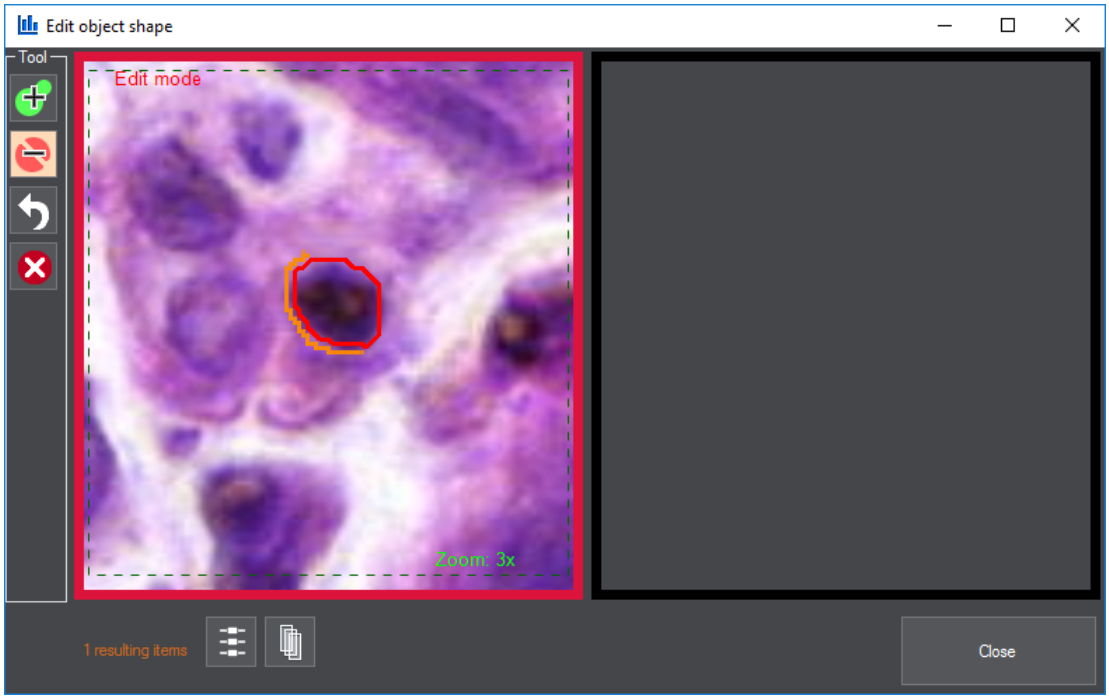
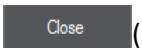
You can add area to nuclei by clicking the **+** button or cut nuclei (subract area from nuclei) by clicking **–**, then moving the mouse with holding the left mouse button down.

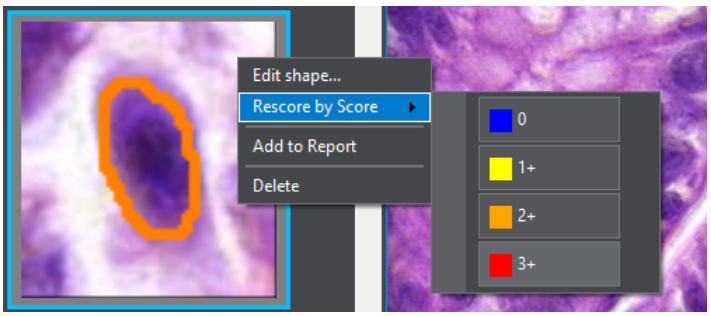


Click to undo changes made after the window was opened.

Click 

(or right-click on the slide area) to apply changes, then the images of the modified object and its neighboring objects are displayed on the right pane of the window. After double clicking on them, the shape of these objects can be further modified.

- **Rescore by Score** – Set a different score for an object



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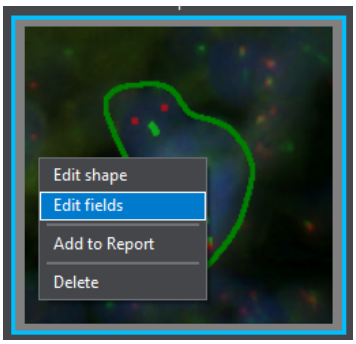
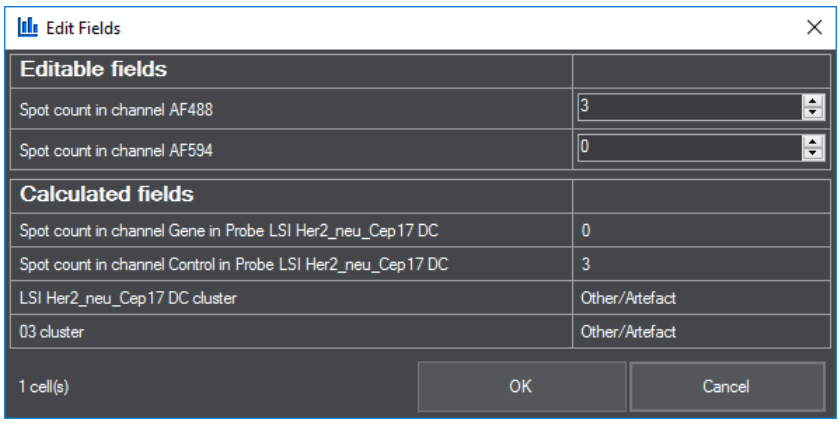
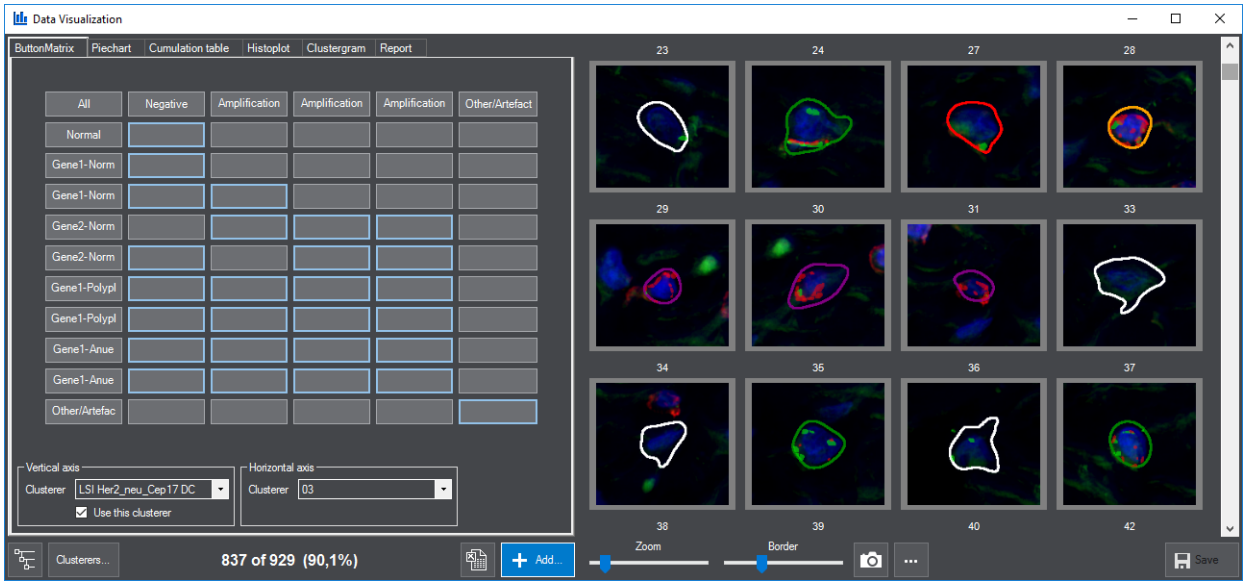
- **Edit fields** – Spot counts in channels for the selected objects can be modified in the **Edit Fields** window (available only for Fluorescent slides).





- **Add to Report** – Add object to Report
- **Delete** – Remove object from the gallery

If you have opened the **Data Visualisation** window for FISHQuant or CISHQuant, the **ButtonMatrix** panel is displayed by default, in which the active clusters are displayed in the matrix.



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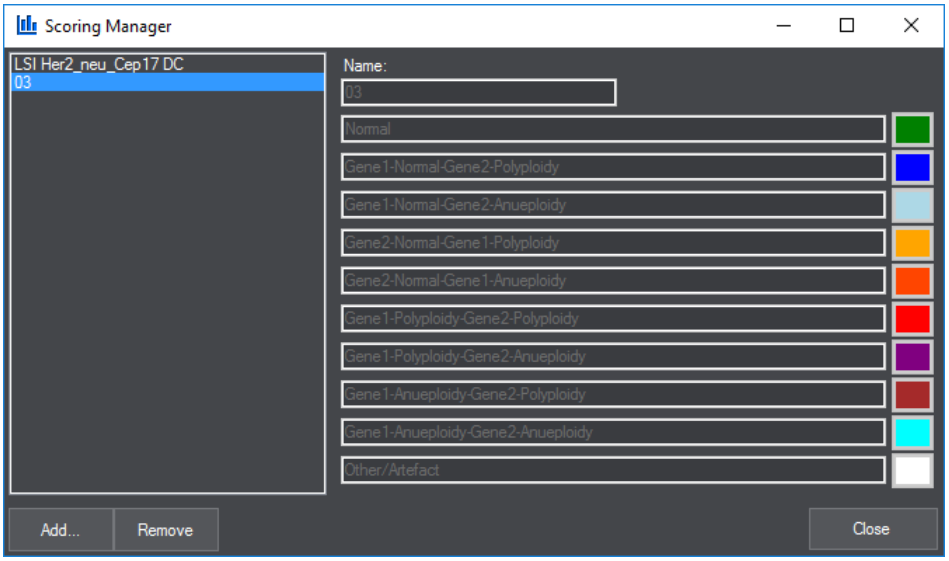
At the bottom of the window you can find buttons by which additional functions can be activated:

– Tree view of panels

– Opens Scoring manager



– Export data to an Excel fájl

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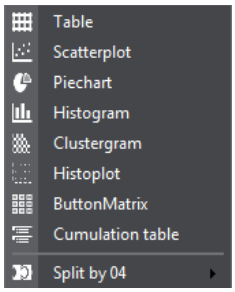
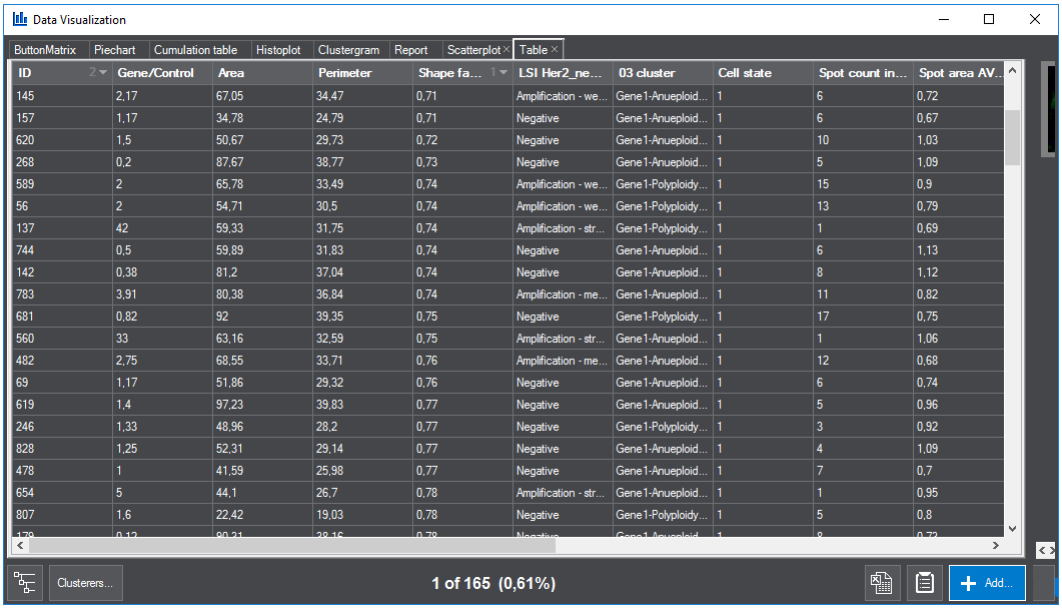
– Additional presentation forms can be activated after clicking this button:

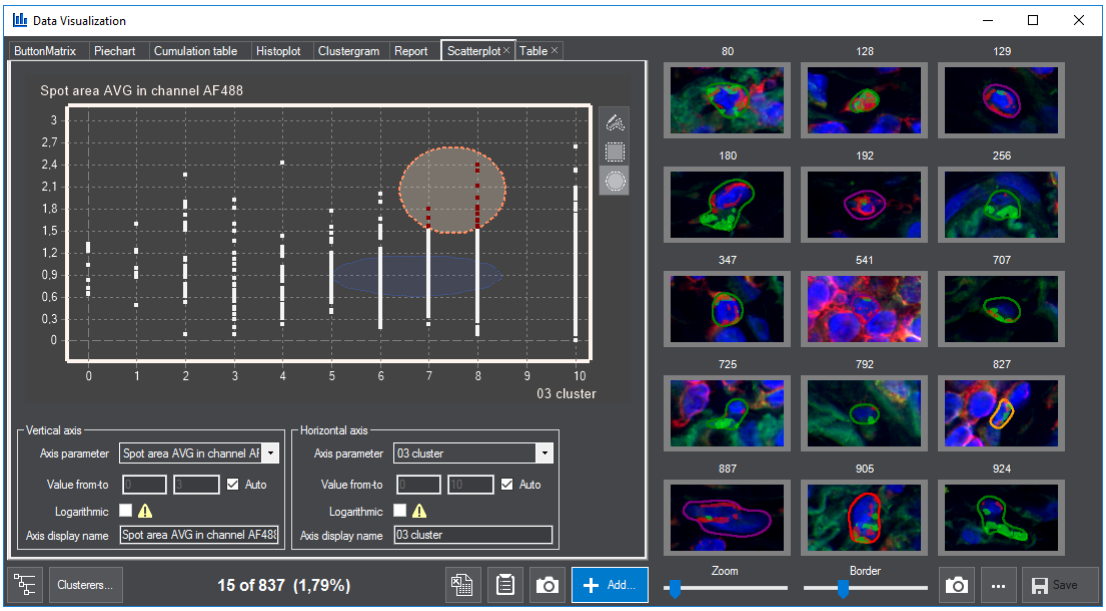


- **Table**



ID	Gene/Control	Area	Perimeter	Shape fa...	LSI Her2_ne...	03 cluster	Cell state	Spot count in...	Spot area AV...
145	2,17	67,05	34,47	0,71	Amplification - we...	Gene1-Anueploid...	1	6	0,72
157	1,17	34,78	24,79	0,71	Negative	Gene1-Anueploid...	1	6	0,67
620	1,5	50,67	29,73	0,72	Negative	Gene1-Anueploid...	1	10	1,03
268	0,2	87,67	38,77	0,73	Negative	Gene1-Anueploid...	1	5	1,09
589	2	65,78	33,49	0,74	Amplification - we...	Gene1-Polyploidy...	1	15	0,9
56	2	54,71	30,5	0,74	Amplification - we...	Gene1-Polyploidy...	1	13	0,79
137	42	59,33	31,75	0,74	Amplification - str...	Gene1-Polyploidy...	1	1	0,69
744	0,5	59,89	31,83	0,74	Negative	Gene1-Anueploid...	1	6	1,13
142	0,38	81,2	37,04	0,74	Negative	Gene1-Anueploid...	1	8	1,12
783	3,91	80,38	36,84	0,74	Amplification - me...	Gene1-Anueploid...	1	11	0,82
681	0,82	92	39,35	0,75	Negative	Gene1-Polyploidy...	1	17	0,75
560	33	63,16	32,59	0,75	Amplification - str...	Gene1-Anueploid...	1	1	1,06
482	2,75	68,55	33,71	0,76	Amplification - me...	Gene1-Anueploid...	1	12	0,68
69	1,17	51,86	29,32	0,76	Negative	Gene1-Anueploid...	1	6	0,74
619	1,4	97,23	39,83	0,77	Negative	Gene1-Anueploid...	1	5	0,96
246	1,33	48,96	28,2	0,77	Negative	Gene1-Polyploidy...	1	3	0,92
828	1,25	52,31	29,14	0,77	Negative	Gene1-Anueploid...	1	4	1,09
478	1	41,59	25,98	0,77	Negative	Gene1-Anueploid...	1	7	0,7
654	5	44,1	26,7	0,78	Amplification - str...	Gene1-Anueploid...	1	1	0,95
807	1,6	22,42	19,03	0,78	Negative	Gene1-Polyploidy...	1	5	0,8

- **Scatterplot**



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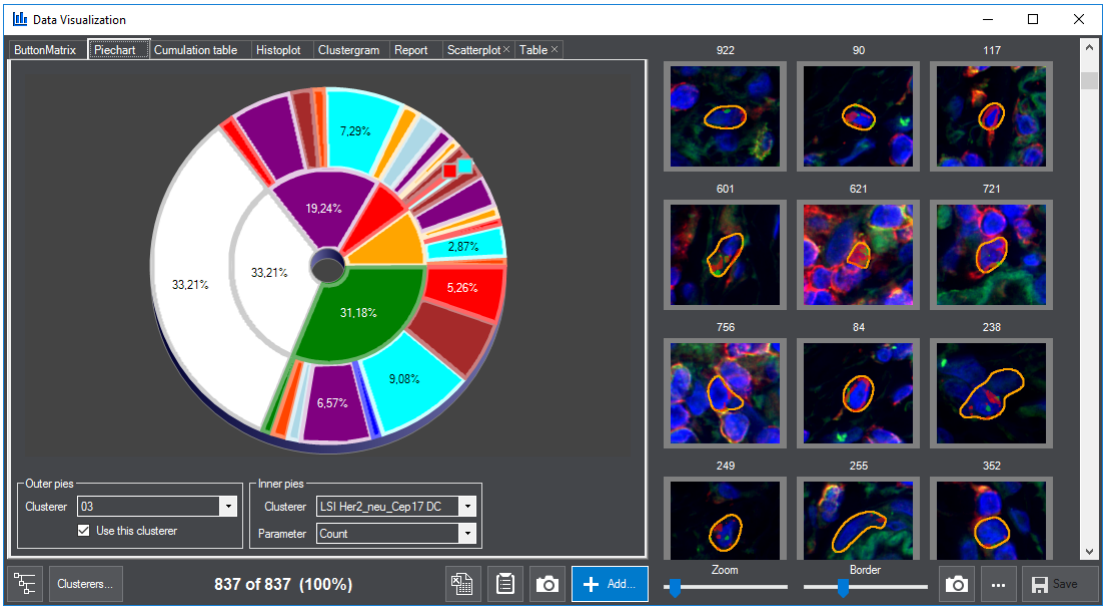
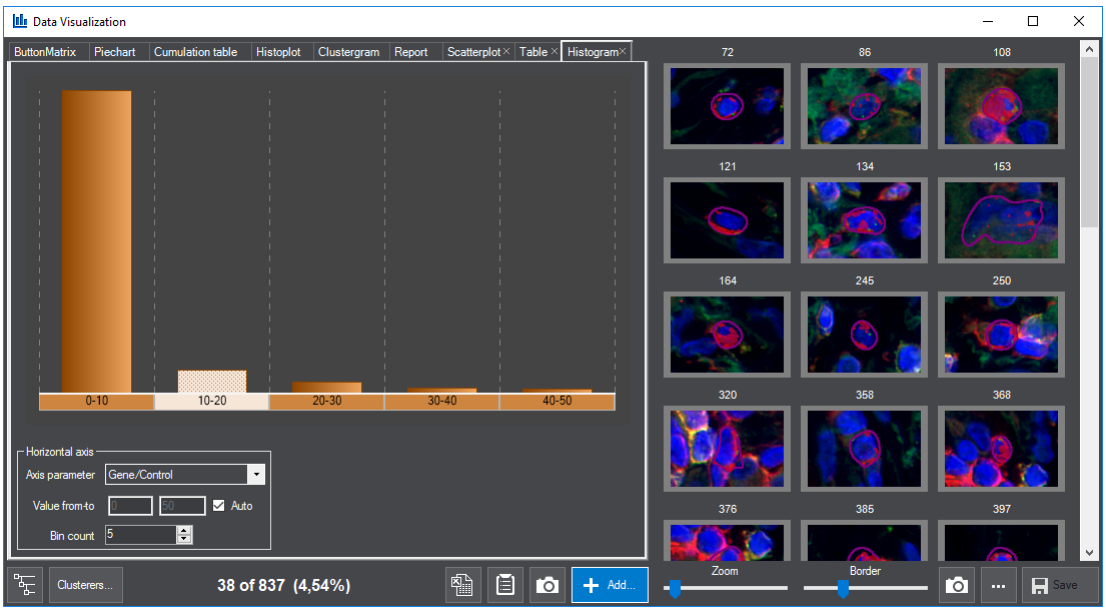
Cluster-related measurement data are displayed on an X-Y axis graph of the scatterplot (linear or logarithmic representations are both available). The axis parameter can be selected from the **Axis parameter** drop-down lists both for the **Vertical** and the **Horizontal** axis. If you deactivate the **Auto** function, value ranges of X and Y axis can be modified.

- **Piechart**

  Another option for graphic representation of cluster-related measurements is the Piechart, by which percentage values of clusters are shown.



- **Histogram**



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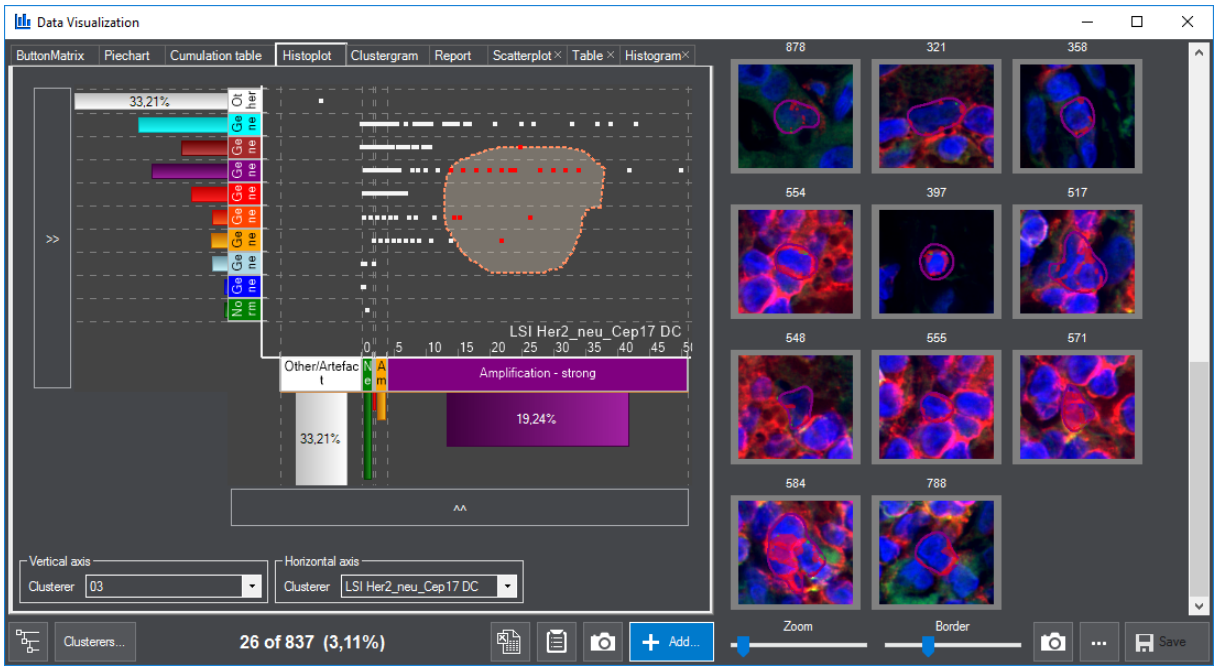
- **Histoplot**

  Activated clusters are displayed on this diagram. Items of selected columns are included in the gallery. Cluster editing/deletion is available only if the probe is amplified, therefore the axis is not editable if other types of probe have been defined.

  Cluster column and row width can be modified by dragging the divider, or right-click to enter new value.



  After right-clicking on the selected object displayed in the matrix can be deleted or moved to another cluster if needed. You can filter nuclei on the histoplot by drawing a circle around the objects.

  On the X axis of the Histoplot, right-click the clusters to select from the following options:

- Change name
- Change color
- Create new cluster here
- Remove this cluster

  NOTE: Any of the clusters can be removed, but at least one cluster must be present in the matrix. Cluster editing/deletion is available only if the probe is amplified, therefore the axis is not editable if other types of probe have been defined.

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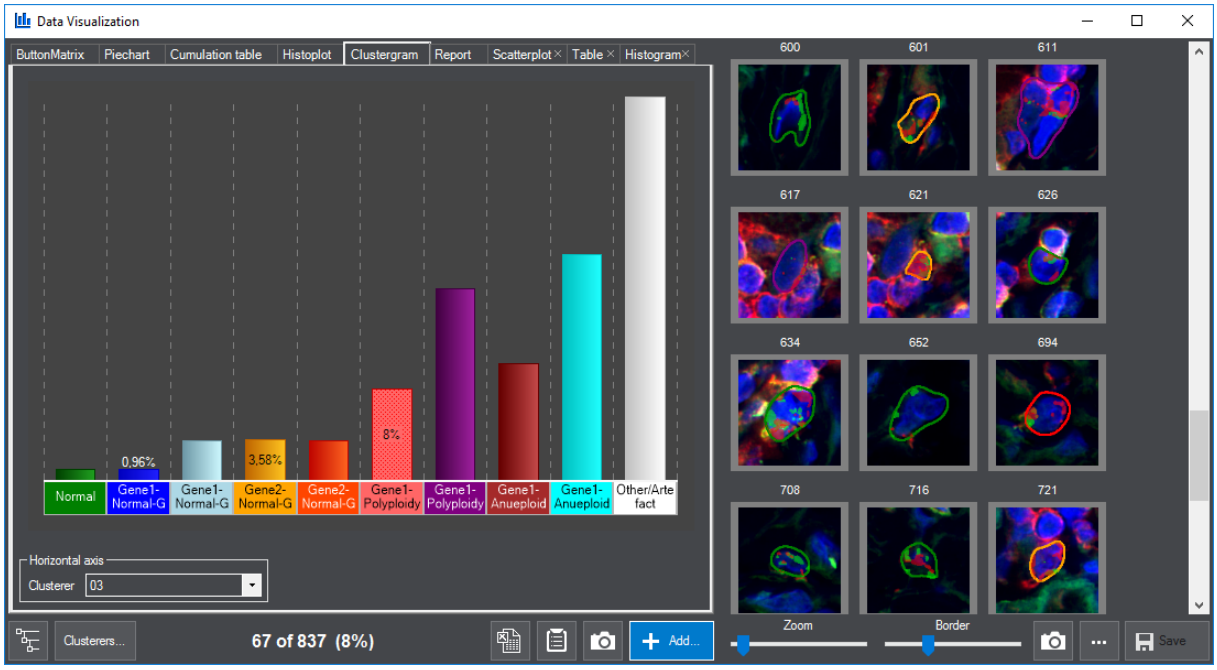
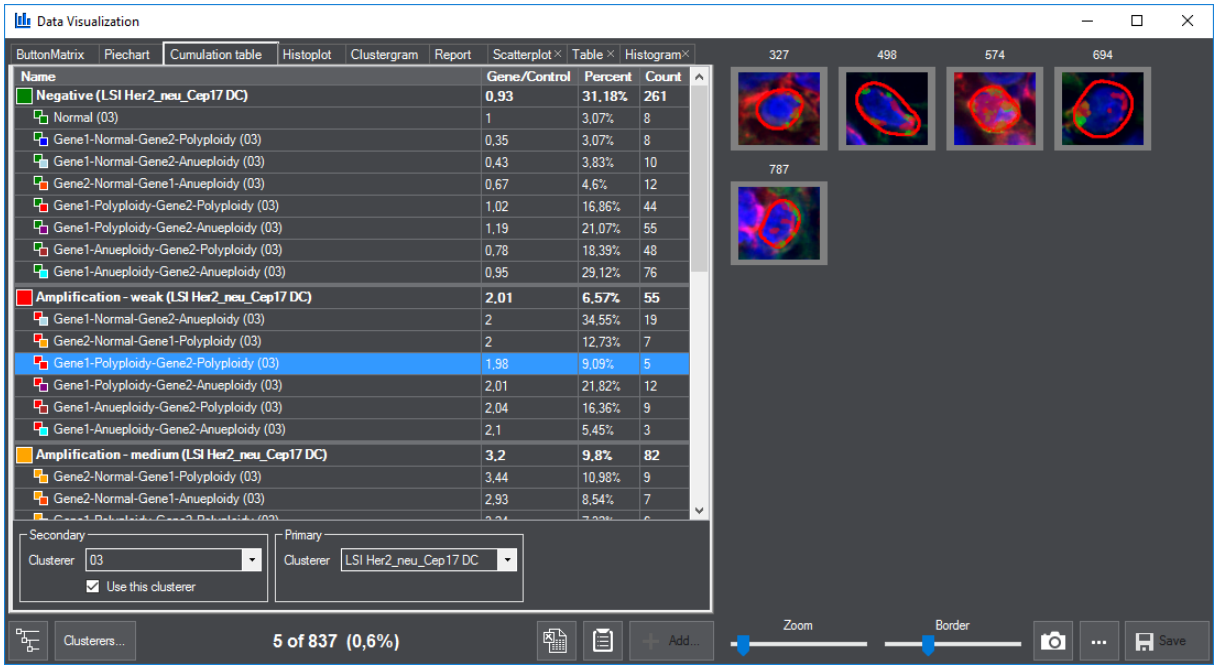
- **Clustergram**



- **Cumulation table**



– The complete list is copied to clipboard

– Creates a snapshot of the selected item(s) that can be saved as a PNG/JPG/BMP/TIF file

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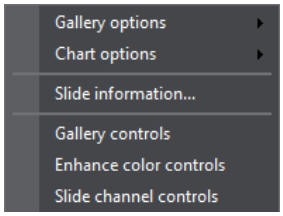
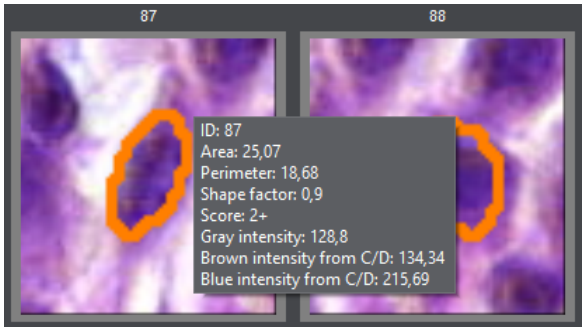
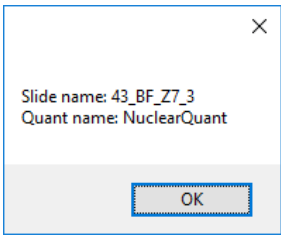
– Further options are accessible by clicking this button:



- **Gallery options**
  - Fit objects to same size – Fits objects into their frames in the gallery
  - Single click selection mode – Multiple selection is available for you upon activating this function (alternatively, hold the **Ctrl** key while clicking on an item with the mouse)
  - Show information tooltip on items – Object data are displayed in a tooltip when pointing the mouse cursor over an item if activated



- **Chart options**
  - Show grid
  - Show text
- **Slide information** – Slide name and the name of the applied Quant is displayed in a window.



- **Gallery controls – Object rendering**, **Outline** and **Fill** masking modes

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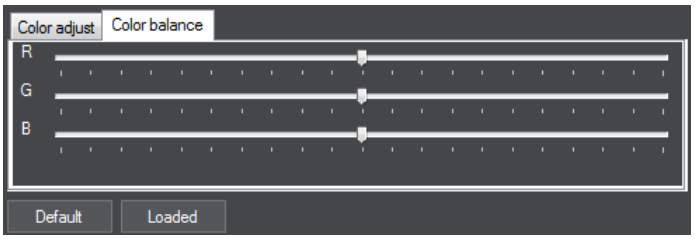
- **Enhance color controls** – A **Color adjust** and **Color Balance** window is displayed to modify values if necessary
- **Slide channel controls** – Fluorescent channel buttons are displayed
- **Z-stack controls**

– **Brightness**, **Contrast**, and **Gamma** values can be adjusted on the **Color adjust** panel

Color channels can be adjusted separately. Channels of a color model are represented in accordance with the type of slide (RGB color model for Brightfield slides, FL channels for Fluorescent slides).



– Click this button to activate Z-stack displaying function, then drag the slider bar left or right to switch between the focal planes of a Z-stack slide

– FL channel buttons can be displayed, channels can be turned off/on separately (available for Fluorescent slides only)

– Saves data to slide

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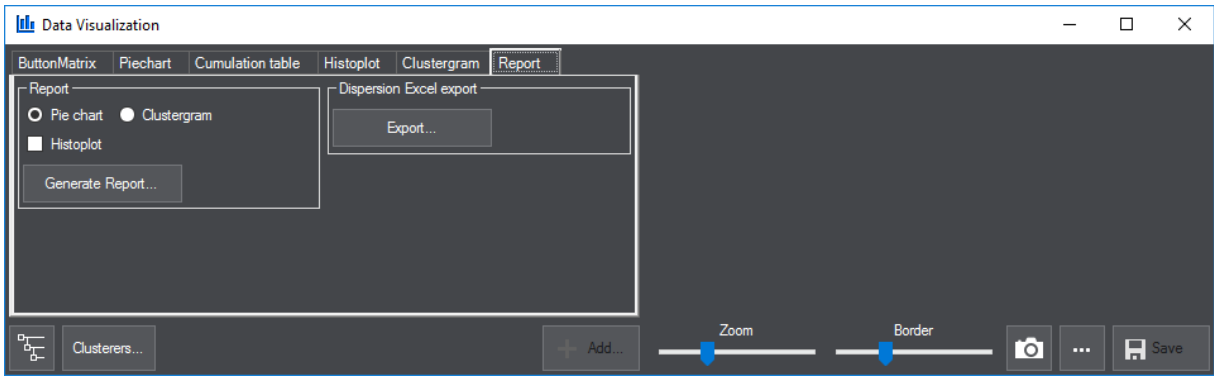
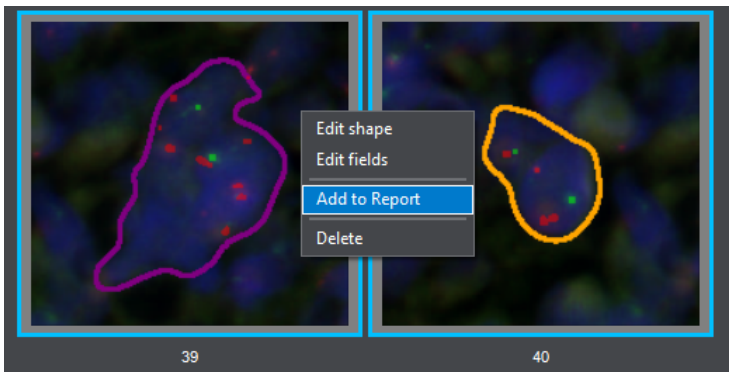
The **Report** panel offers you a reporting tool by which the selected gallery items and their data supplemented by additional diagrams (Piechart, Clustergram, and Histoplot) can be presented on the Report form.

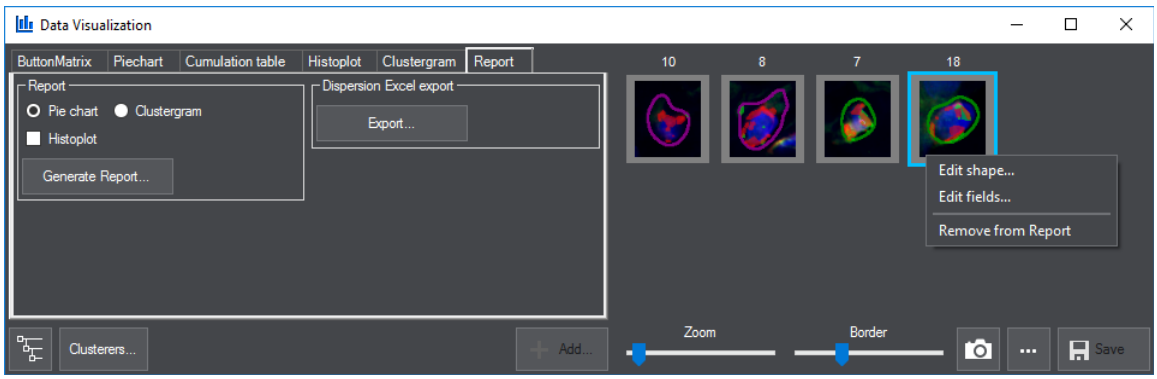


NOTE: Results of secondary measurements (numeric deviation) are visualized by a Histoplot (function can be activated only if a secondary measurement is created).

Items can be added to the Report by right clicking one of the selected gallery items, then selecting the **Add to Report** option from the menu.



Selected items on the **Report** form can be deleted if right-clicking on the item(s) you want to exclude from the report, then selecting the **Remove from Report** option from the menu.



If you have finalized the settings of the Report, click Generate Report to create the report on the selected gallery items.

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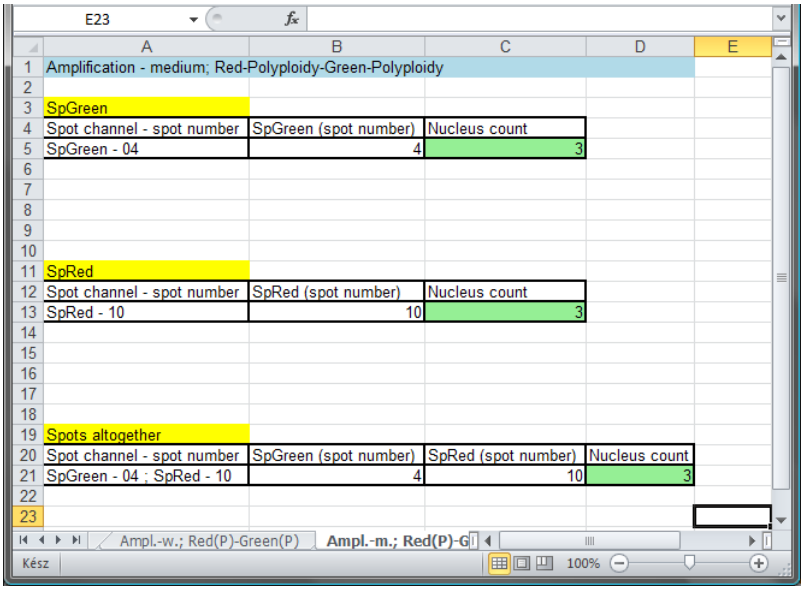
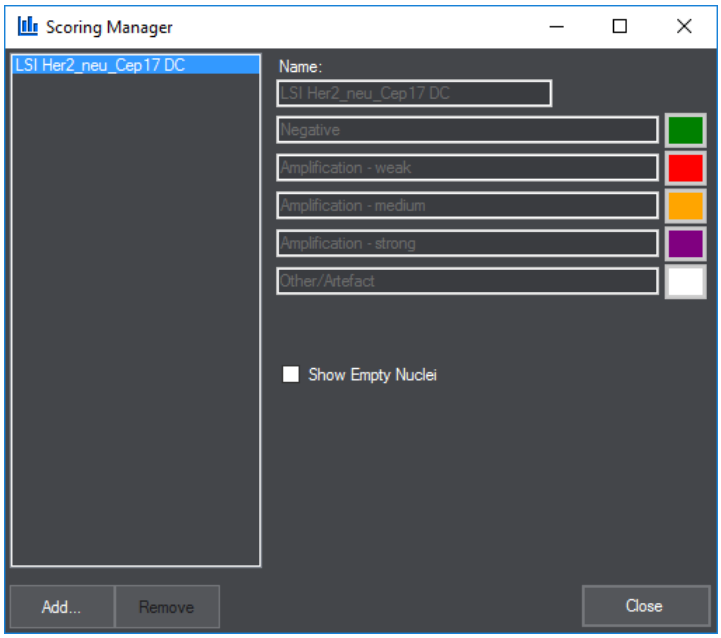
Upon clicking Export in the Dispersion Excel export field, spot number-related data of specific clusters can be exported to an Excel file.



	A	B	C	D	E
1	Amplification - medium; Red-Polyploidy-Green-Polyploidy				
2					
3	SpGreen				
4	Spot channel - spot number	SpGreen (spot number)	Nucleus count		
5	SpGreen - 04	4	3		
6					
7					
8					
9					
10					
11	SpRed				
12	Spot channel - spot number	SpRed (spot number)	Nucleus count		
13	SpRed - 10	10	3		
14					
15					
16					
17					
18					
19	Spots altogether				
20	Spot channel - spot number	SpGreen (spot number)	SpRed (spot number)	Nucleus count	
21	SpGreen - 04 ; SpRed - 10	4	10	3	
22					
23					

5.2 Scoring and creating a Secondary Probe

Click Clusterers... to open the **Scoring Manager** window.



Upon clicking Add..., then selecting **Secondary probe**, the probe of secondary measurement can be created.

In the Probe Definition window, set parameters, channels, then click Add.

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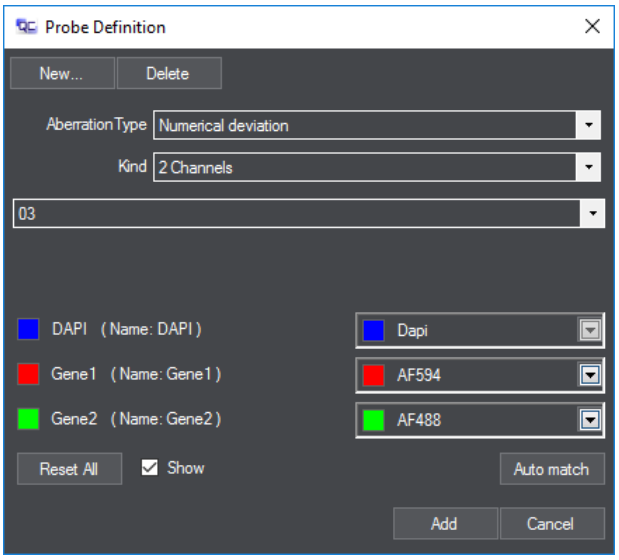
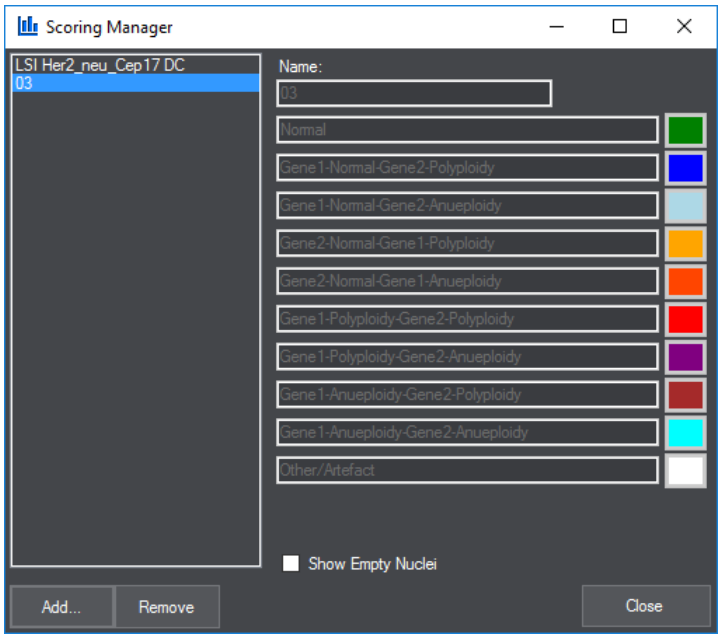


The clusters of the created secondary probe are presented with their colors in the Scoring Manager window. If you want to remove a secondary probe, select the probe name from the list first, then click Remove.



Click Close when finished.

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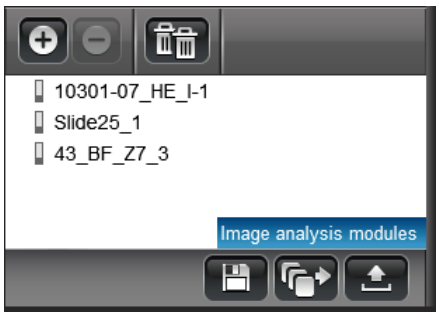
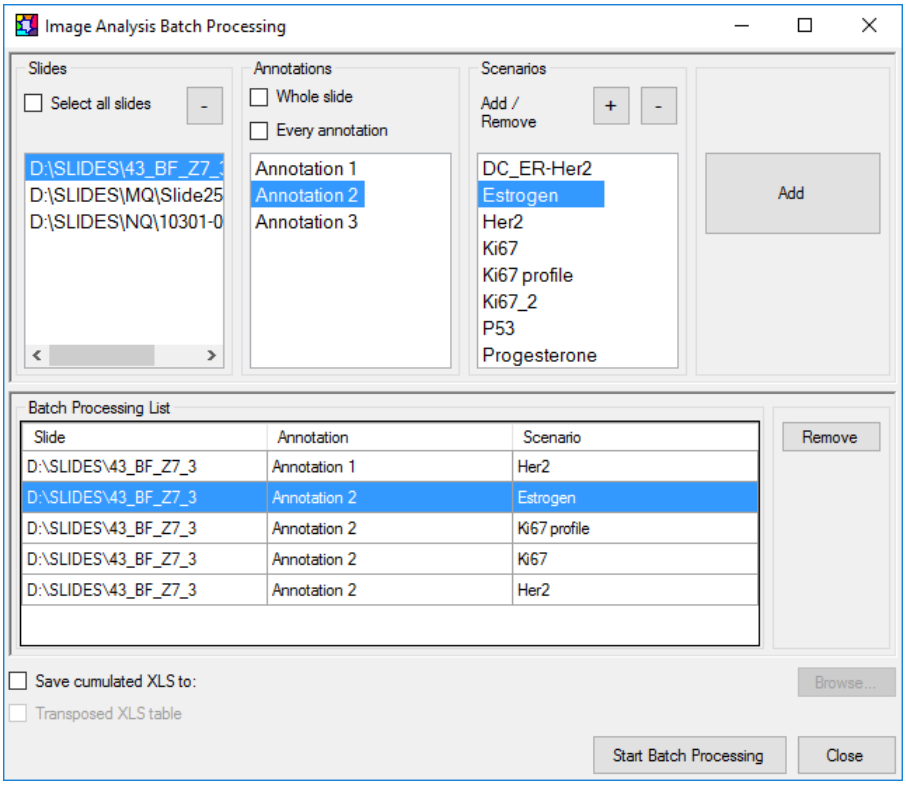
6 Running a Batch Process from CaseViewer

CaseViewer allows you to batch process a set of slides. You can find the batch processing panel at the bottom section of the tree-view panel on the left side of the CaseViewer main window. Please, refer to ***CaseViewer 2.1 User's Guide*** for more information.

1. In CaseViewer, select a slide or folder in the tree-view panel, then click the button to add them to the list. Remove a slide from the list by clicking the button, or remove all the selected slides by clicking .



2. Click the button when finished, then the Image Analysis Batch Processing window is displayed.



3. Select the slides you want to work with and specify Annotations and Scenarios for which they should be analyzed.

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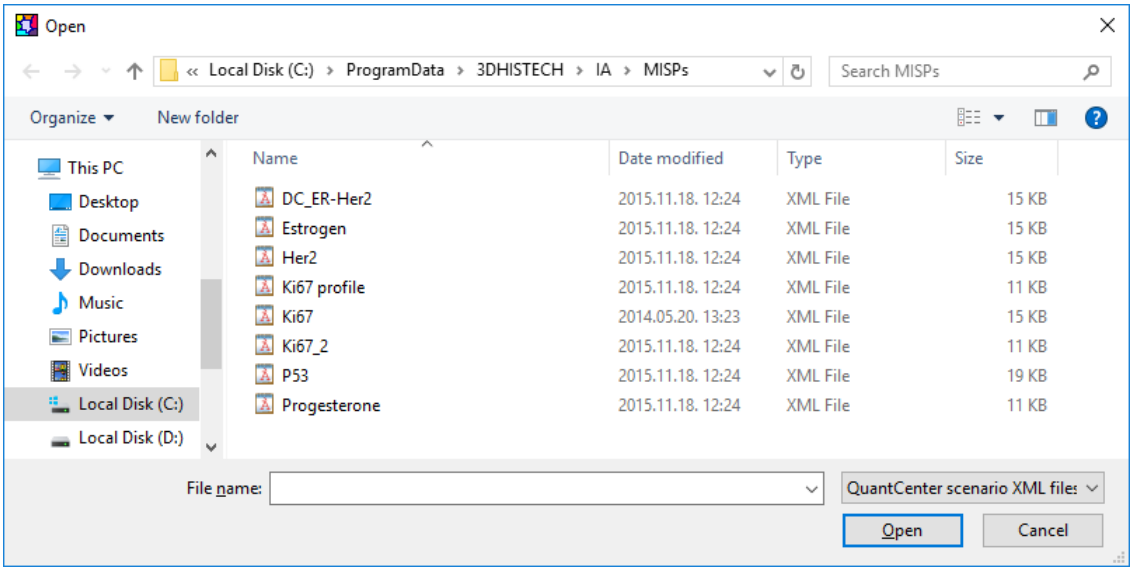
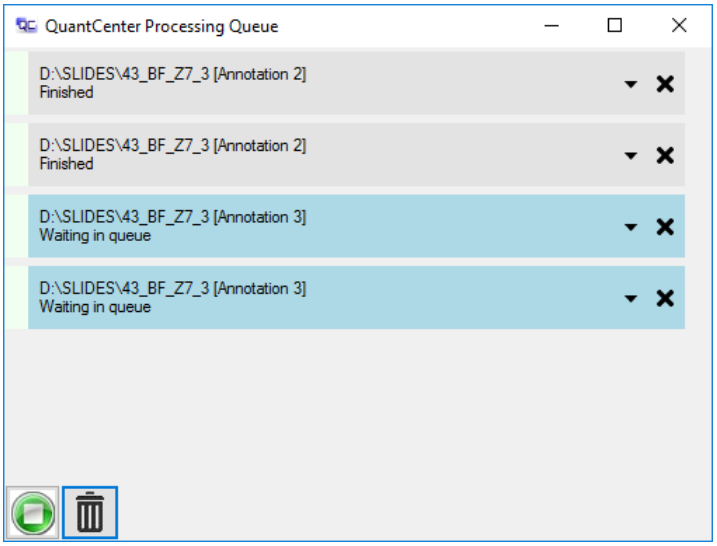
4. Click + to add more scenario XML files to the list of **Scenarios**. Select the file from the designated folder, then click **Open** to add them.



5. Click the **Add** button to add the slide to the **Batch Processing List**.

6. Tick **Save cumulated XLS to:** if you want to save a cumulated data sheet to a specified location. Click **Browse** to locate a folder. If you have already saved a cumulated XLS file, it is available for you to save a **Transposed XLS table** by ticking this option.

7. Click **Start Batch Processing** when finished. In QuantCenter, processes are displayed in the **QuantCenter Processing Queue** window.



NOTE: Scenarios that can be used during batch processing are listed from the following folder by default: *C:\ProgramData\3DHISTECH\IA\MISPs.*

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