

QuantCenter 2.2 User Guide

> August 6, 2018 Rev. 1

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- P1700483
- P1500394; PCT/HU2016/050039; EP 16840280.8.; US 15/756.370
- P0700409; US 2008/0306964
- P0800181; PCT/HU2009/000027; EP 09721248.4.
- P0600435; PCT/HU2007/000047; EP 2024772; US 12/301.781
- P0600177; PCT/HU2007/000018; EP 1.994.398; US 12/281.566
- P0900741; PCT/HU2010/000130; EP 10812989.1.
- P0900142; PCT/HU2010/000027; EP 10726183.6
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## **Character Types and Symbols**

Example	Abbreviation or term that is explained in section <u>Terms and Abbreviations</u> .
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 <b>F11</b> ) or the <b>Ctrl+O</b> 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.

## 1 Installation of QuantCenter 2.2 Bundled with CaseViewer 2.2

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

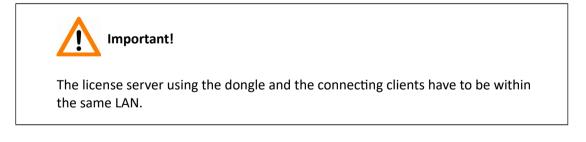
### **1.1** Prerequisites of the Installation

Prerequisites	Description
Hardware	Minimum:
	Intel 3,2 GHz i5 (Quad Core), 4GB RAM
Operating system	Microsoft Windows 7 Professional 64-bit, SP1
Display resolution	Recommended: 1280×720 or better
Disk space	CaseViewer 2.2 bundled with QuantCenter 2.2
	Minimum: 400MB

### 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.



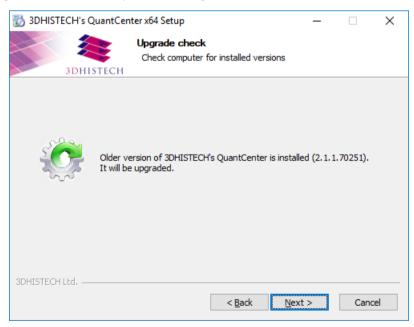
Contact 3DHISTECH support at support@3dhistech.com if you want to extend the license, as in this case some configuration of the license server and of the clients is required.

### **1.3** Installation of Application Modules

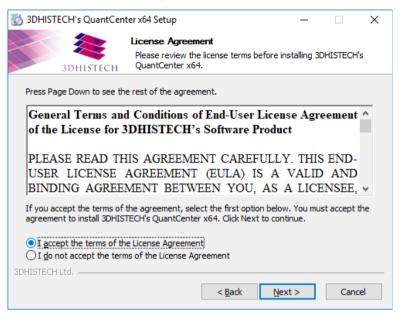
- 1. Install CaseViewer 2.2 first. Please refer to section 1 Installation of CaseViewer in CaseViewer 2.2 User's Guide.
- Launch the **3DHISTECH\_Apps\_2.2\_RTM.exe** file that you received on a 3DHISTECH installation disc or downloaded in a compressed file, then follow the instructions in the installation wizard.



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



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



5. Click **Install** to install missing prerequisite software. Installations run in the background.

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3DHISTECH	quisites of the ir	nstallation.		
Microsoft .NET Framework 4.5		Installed		
Microsoft Visual C++ 2013 Redistributable x64		Waiting for instal	I	
Microsoft ReportViewer 2010 SP1 Redistributab	le	Waiting for instal	I	
Install the required prerequisites to continue!				
3DHISTECH Ltd,				
	< <u>B</u> ack	<u>N</u> ext >	Canc	el

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

📸 3DHISTECH's QuantCenter x64 Setup		_		×
3DHISTECH Prerequisit Review the	<b>es</b> prerequisites of the in:	stallation.		
Microsoft .NET Framework 4.5		Installed		
Microsoft Visual C++ 2013 Redistributabl	e x64	Installed		
Microsoft ReportViewer 2010 SP1 Redistr	ibutable	Installed		
All of required prerequisites are installed	I. Click 'Next' to continu	e.		
3DHISTECH Ltd,	< <u>B</u> ack	<u>N</u> ext >	Canc	el

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

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	3DHISTECH	Checking for d	ongle driver			
		us of Aladdin HASP o Ingle driver: Waiting In	-			
	it, and do NO	tallation may t OT close the i			Cance	el

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



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

👸 3DHISTECH's QuantCer	ter x64 Setup	_		×
3DHISTECH	License Settings Set license mode			
Select a license file	-	B	rowse	
3DHISTECH Ltd. —	< <u>B</u> ack	ext >	Canc	el

10. 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.

📸 Open					×
← → × ↑ 📙 « 3DHIS	TEC	H > Viewer	~ Ū	Search Viewer	م
Organize 🔻 New folder				•==	
Intel	^	Name		Date modified	Туре
PerfLogs		Data		2014.10.17. 9:24	File folder
Program Files		SlideDriver_Redist_x86		2014.02.19. 9:38	File folder
Program Files (x86)		Users Guides		2014.10.17. 9:24	File folder
3DHISTECH	Т	MView.lic		2016.09.16. 14:50	LIC File
CaseCenterClient-re	E				
CaseViewer					
SlideAC-redist					
SlideDriver-redist					
TMA Control					
Viewer	¥	<			>
File <u>n</u> ame	: 1	/View.lic	~	License File(*.lic)	~
				<u>O</u> pen  ▼	Cancel

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

👸 3DHISTECH's QuantCer	nter x64 Setup	_		$\times$
3DHISTECH	License Settings Set license mode			
Extract: C:\WINDOWS\sy	stem32\opencv_ocl2410d.dll 100%	J		
Output folder: C:\WINDOWS\s Extract: C:\WINDOWS\s Output folder: C:\WINDO Extract: C:\WINDOWS\s Output folder: C:\WINDO Extract: C:\WINDOWS\s Output folder: C:\WINDOWS\s	ystem32\opencv_nonfree2410d.dll DWS\system32 ystem32\opencv_objdetect2410.dll DWS\system32 ystem32\opencv_objdetect2410d.dll. DWS\system32 ystem32\opencv_od2410.dll 100%	. 100% 100% %		~
3DHISTECH Ltd	< Back	Next >	Can	icel
	< Dack	Text >	Can	10.01

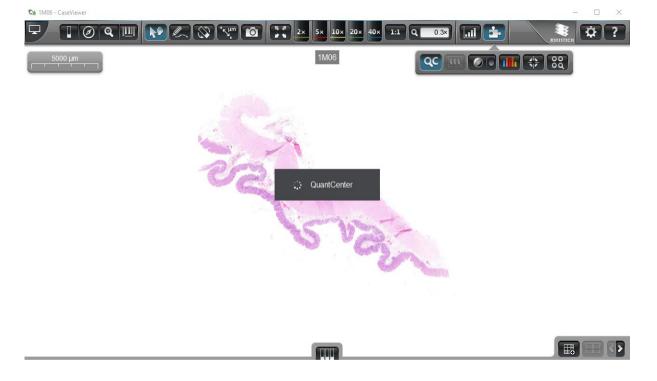
12. Click Finish to close installation wizard.



## 2 Operation and workflow

3DHISTECH QuantCenter is an embedded application for running quantification measurements on digital slides and is accessible from CaseViewer 2.2. 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 – 13. Plugins* in *CaseViewer 2.2 User's Guide*, and section *5 Running a Batch Process from CaseViewer* included in this guide.

## 2.1 Main application interface



Once QuantCenter is launched from CaseViewer, the splash screen is displayed.

QuantCenter 2.2 uses the CaseViewer slide canvas, and the visual result of each modification or adjustment (Quant measurements, preview, etc.) is applied to the opened slide in CaseViewer.



## 2.2 Quantification workflow

### 2.2.1 Creating measurements

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

Click 👥 to open the Quant menu from which the required Quant module can be selected.



Click to open a previously saved measurement 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.

### 2.3 Defining a Measurement scenario

With the help of **Scenario Builder**, a unique measurement profile, a so-called "scenario" can be defined by linking Quant algorithms (PatternQuant, HistoQuant, DensitoQuant, NuclearQuant, MembraneQuant, CellQuant, CISHQuant, and FISHQuant).

- 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 (see *sections 3.1* and *3.5*).

5. If a scenario is complete, specify the areas that will be included in the measurement (annotations / whole slide / current view). Before running the process make sure to set the desired selection on the panel.

When 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.

It is also available for you to import a profile for the selected Quant by clicking **Import profile...**, and also to export the set parameters as profile for a later use by selecting the **Export profile...** option.



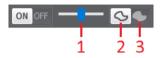
After the parameterization of a Quant is finished, **Mask** function can be activated on the preview image to show segmented objects related to the applied Quant application. To enable preview mask, click at the top of the selected form.



Once the preview mask is activated, additional options are available for you to select from. The rendering process of slide image with the applied mask is indicated by a processing indicator at the right side of the bar.



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.

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

Object Properties ×				
Name	Value			
Area	27,019			
Perimeter	23,356			
Shape factor	0,622			
Gray intensity	117,79			
Brown intensity from C/D	102,933			
Blue intensity from C/D	254,396			
Score	3			

### 2.4 Gallery (FISHQuant and CISHQuant)

**NOTE**: The Gallery function is available only if a FISHQuant or a CISHQuant process has been defined as the root element of a scenario.

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).

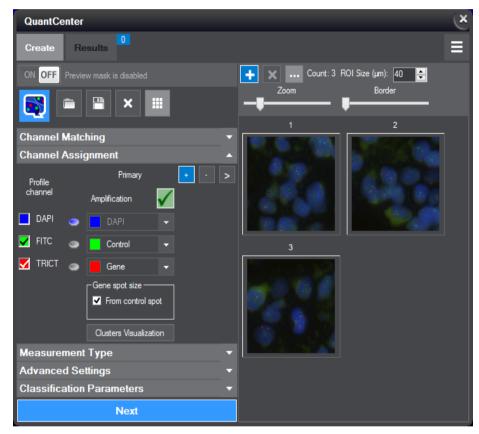
-	
0	, <b>‡</b> ,

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.

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, or you can use the **Ctrl+A** hotkey for selecting all the items in the gallery.



To run measurement process on the gallery items (handled as annotations), click **Next** first at the bottom of the panel, then select Galley teme. 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.

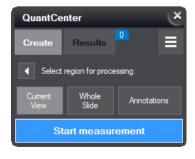
### 2.5 Running measurements

Once the scenario is built correctly and finished, the **Next** button at the bottom of the panel becomes active and the measurement process can be launched for the desired selection (**Current view**, **Whole slide**, and **Annotations**) by clicking this button.

QuantCenter	×
Create Results	Ξ
ON <b>OFF</b> Preview mask is disabled	
New cluster1	
New cluster2	)
Train	•
Untrain	
Contour:	) ≑
Dilation: U	) 🌻
Filters	•
Performance	•
Next	

**Current view** and **Whole slide** options are available for you to select by default. If there are annotations on the slide, these annotations are listed on the **Annotations** panel. At least one annotation must be selected from the annotations list. The measurement will be run on the selected annotation(s). **Separated annotations** option allows you to run measurement processes in the queue separately.

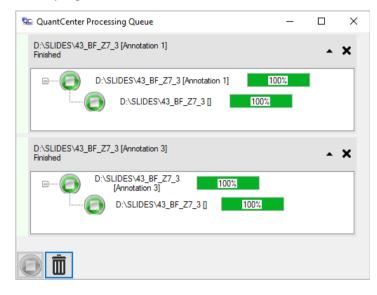
The **Gallery items** option is available only if you run FISHQuant or CISHQuant measurements and you have marked areas on the **Scenario Builder and Gallery** form (see *section 2.4* for more information).





To get back to Scenario Builder, click don the **Create** panel.

Upon running the measurement by clicking the **Start measurement** button, 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 subtton.



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 <sup>sc</sup> at the notification area of the system tray.

The measurement process is indicated by a 👶 processing indicator at the right side of the bar.

### 2.6 Measurement results

On the **Result** panel measurement data are shown. Segmented objects related to available measurement results can be displayed. To enable result rendering, click **m** at the top of the selected form. If result rendering is active, additional options are available for you.

**NOTE:** The **Result** button is active only if there is a displayable measurement on the slide. Measurement results generated with version 2.2 are not compatible with older software versions than 2.1, and cannot be opened.

QuantCenter		×
Create Results		
Scenario - Session - Quant I Ann	notation	W
a 2018.07.26. 14:14:57 (25s)		
	otation 1	
and 2018.07.26. 14:14:51 (5s)		
PattemQuant ROI		
Name	Value	Unit
Annotation area	2,62	mm2
Mask area	2,52	mm2
Total count	46092	pcs
Relative mask area	96,03	%
Object frequency	17591,75	pcs/mm2
New cluster1	159279,1	µm2
New cluster2	2356917,24	
New cluster1		µm2
	6,08	μm2 %
New cluster2	6,08 89,96	µm2
		μm2 %
		μm2 %
		μm2 %

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. The **Heatmap (4)** function offers you the visualization of stain positivity in the measured region by highlighting object density (for PatternQuant, HistoQuant, FISHQuant, and CISHQuant) and positivity (for NuclearQuant, MembraneQuant, and CellQuant).



The rendering process of slide image with the selected measurement(s) is indicated by a processing indicator at the right side of the bar.

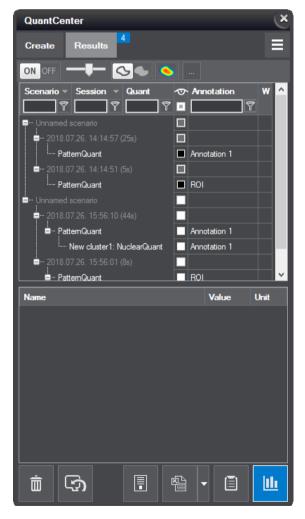
Click to display further data visualization options.



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.
- **Details** If filters are applied, pre-segmented area-related (PatternQuant and HistoQuant) measurement data can be displayed.

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 subtron. Filtered results can be ordered within each group by clicking at the right 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 a 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 or hit the **DEL** key. 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 under the Data Visualization window. For more information on Data Visualization, see *section 4*.

Click to reload the scenario based on which the measurement process has been initiated.

Click Liew form.

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.

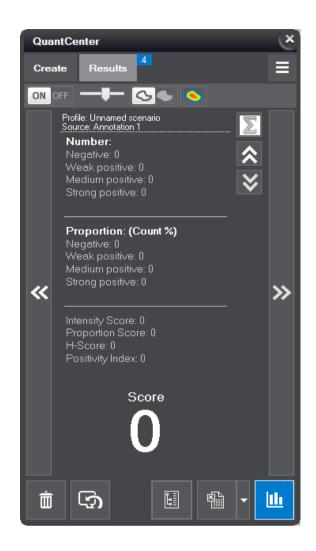
Click to display merged measurement data related to a specific Quant module of all the sessions of a scenario.

To delete measurements, click and or hit the **DEL** key.

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 4**.

To go back to Tree View form, click

### 2.7 QuantCenter menu

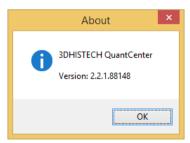
Click = at the top right corner of the QuantCenter window to open the menu from which the following options can be selected:



Select Preferences... to display Visualization and Measurement Data settings. Set Line thickness to define the thickness of masking line. Click Cleanup slide to erase all measurement data saved for the slide.



- The **Show Processing Queue...** option displays the QuantCenter Processing Queue window including all the processed measurements.
- Select User Guide (PDF) option to open the User Guide of the software.
- The **About...** option displays a window in which the name and the version number of the software is presented.



## 3 Available Quant applications

## 3.1 **PatternQuant**

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



Important!

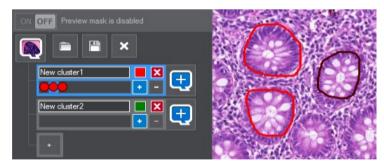
One cluster is displayed by default, but a group of clusters is available for you to create, and extend to a maximum of 5 clusters by clicking +.

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.

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. Select the required zoom level in the CaseViewer window by clicking the appropriate button or enter a custom value in the text box at the main menu bar.

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 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).

QuantCenter	(×	QuantCenter	×	QuantCenter	Ľ
Create Results		Create Results	≡	Create Results	≡
ON <b>OFF</b> Preview mask is disabled		ON OFF Preview mask is disabled		ON <b>OFF</b> Preview mask is disabled	
🁰 🛋 🖿 🗙		🁰 💼 🗎 🗙			
New cluster1		New cluster1		New cluster1	
			1		
				<b>-</b>	
Train	•	Train	-	Train	•
Train		Filters	A .	Filters	•
Contour: 20	÷	🦲 Size CopyToAll 🦲 Si	hape	Performance	•
Dilation: -	÷	Min 0 / Min 0 /			
Filters	•				
Performance	•	Max 10000000		Save objects to slide without connection	
Next		Performance	<b>•</b>	Next	
		Next			_

### 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.

QuantCenter X	18. 6. 3. 6
Create Results	
	S 40 / 200 - 9 16'
New cluster1	10 15A 0 6
	30 . 3V 800 - 80
New cluster2	
Train 🔺	Provide States and a state of the
Untrain	
Contour: 20 🚖	20 C 2 C 4 30 2 C
Dilation: 3 🚖	
Filters 🔹	
Performance •	
Next	000000000000000000000000000000000000000

- **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.
- Filter the result can be filtered based on Size and Shape either by typing values in the respective fields, or after clicking vou 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
   CopyToAl 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**.

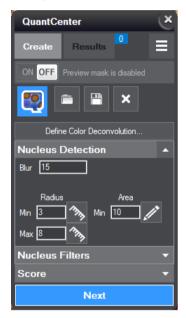
- Performance 3 options are available to select from (measurement time will increase according to the following order):
  - Save objects to slide disabled (Green)
  - Save objects to slide without connection (Orange)
  - Save objects to slide with connection (Red)

## 3.2 💽 NuclearQuant

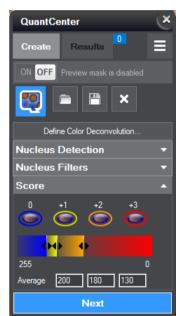
With the help of NuclearQuant image analyzing algorithm, cell nuclei can be detected and classified. It may be run as single (root) measurement, or embedded into PatternQuant or HistoQuant in which case NuclearQuant process will be run on areas segmented by the Quant into which it is embedded.

#### **NuclearQuant properties**

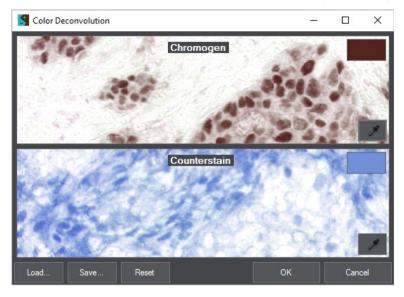
In this panel, the measurement parameters of NuclearQuant can be set.



QuantCenter X
Create Results
ON <b>OFF</b> Preview mask is disabled
🥞 🖹 ×
Define Color Deconvolution
Nucleus Detection -
Nucleus Filters
Intensity Min 60 💉
Contrast Min 60
Score 🔹
Next

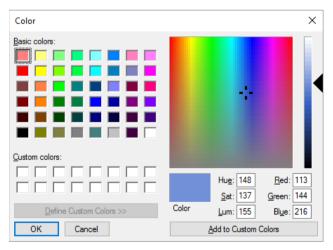


• **Define Color deconvolution**: the colors of positive and negative intensities can be defined in this window. The upper pane of the Color Deconvolution window represents objects of positive intensity (brown) and the lower pane represents objects of negative intensity (blue).

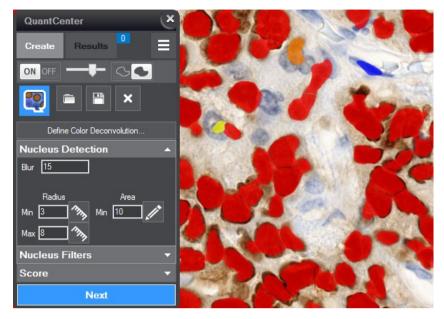


Representative colors can be set by either of the following two options:

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



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.

# 3.3 🚳 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

In this panel, the measurement parameters of MembraneQuant can be set.

QuantCe	enter		×
Create	Results		≡
ON OFF			
8		×	
Defin	e Color Deco	nvolution	
Membran	e Detecti	on	•
Blur 3	]		
Sensitivity	<b></b>	- 170 🖨	
L	.ow	High	
Membran	e Filters		•
Nucleus I	Detection		•
Nucleus I	Filters		•
Score			•
	Next		

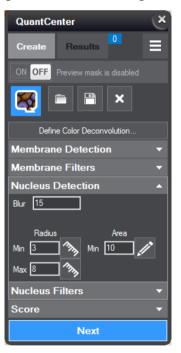
- **Define Color Deconvolution**: the type of Intensity can be set for MembraneQuant (positive) and nucleus detection (negative)
- Membrane Detection:
  - Blur: rough edges of the detected objects will be smoothened, therefore, besides decreasing noise-related image flaws the resulting image appear more pleasing to the eye
  - **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.

•

Membrane Filters: Filtering based on the set value ranges of Intensity and Area by entering minimum and maximum values.

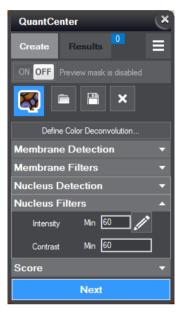
QuantCenter X
Create Results
ON <b>OFF</b> Preview mask is disabled
餐 🖻 🗎 🗙
Define Color Deconvolution
Membrane Detection 🔹
Membrane Filters
📄 Intensity 🦲 Area
Min 0 Min 40
Max 255 Max 300
Nucleus Detection 🔹
Nucleus Filters 🔹 🔻
Score 👻
Next

• Nucleus Detection: Settings for NuclearQuant algorithm used by MembraneQuant

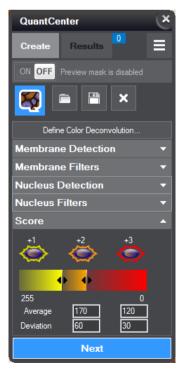


 Blur: rough edges of the detected objects will be smoothened, therefore, besides decreasing noise-related image flaws the resulting image appear more pleasing to the eye

- 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 .
- Nucleus 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.



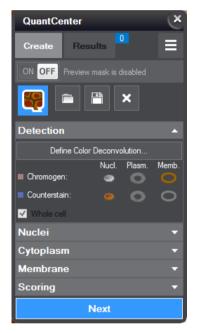
## 3.4 🖲 CellQuant

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

#### **Quant Properties**

In this panel, the 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 3.2* 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 automatically 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.

#### Nuclei

Nuclei		•
Size:		6,0 ≑
Intensity:	•	1 ≑
Smoothing:	<b>———</b>	30 🚔
Merge:	•	0 🚔
Contrast:	•	1 ≑

- 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.

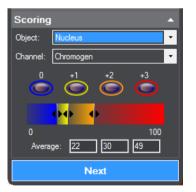
**Width:** the maximum width of cytoplasm (distance between the edge of nucleus and membrane)

 Contrast: the minimum contrast (intensity difference) between the nucleus and the background.

Cytoplasm

Membrane

#### Scoring



- Object: cell object selection for scoring
- **Channel:** select the channel for searching specified cell objects

Intensity: minimum intensity of membrane

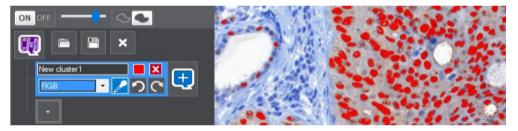
• Set intensity-related Average and Deviation values

## 3.5 🕕 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.

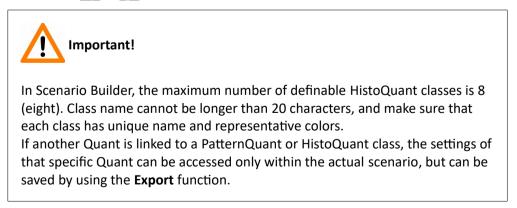


If a Brightfield slide is opened, you can select from **RGB** and **HSV** color models, for Fluorescent slides **Channeled** or **HSV** can be selected in order to define the color channels or color reproduction area from which the samples are taken.



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, click with the right mouse button. Steps of sampling can be cancelled or repeated by clicking the 🕥 or 💽 (undo/redo) buttons.



#### **HistoQuant Properties**

In this panel, the measurement parameters of HistoQuant can be set.



## 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 automatically matched with the channels of the slide). Channel matching is temporarily set and can be used to that specific measurement.

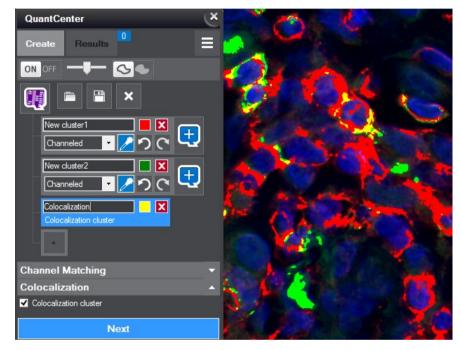
If saving an additional Quant that is linked to a Fluorescent slide, the program saves which channels of the slide are selected, and those will be presented as profile channels pairable with slide channels after reloading.

#### **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.



#### **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.

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.

#### 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.

#### Filters

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

#### Performance

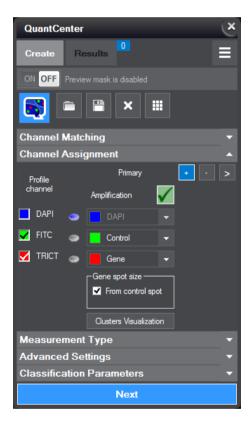
3 options are available to select from (measurement time will increase according to the following order):

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

## 3.6 💽 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**

#### **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.

🖳 Probe De	finition	×
New	Delete	
Aberration	Type Structural aberration: Translocation	•
	Kind Dual Color Dual Fusion	•
02		•
	Add Cancel	

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

💶 Probe Editor				×
Probe Template Filter				
Aberration Type	Locus specific			•
Kind	Amplification			•
Gene Matching —				
🕂 Nuclei_Channe	el	Name:	DAPI	
📕 Gene		Name:	Her2	
Control		Name:	Cep17	
Current Probe				
Name: LSI Her2_	neu_Cep17 DC	Save		Cancel

- 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 dropdown lists.

**NOTE**: When defining a secondary probe, you can only select **Numerical deviation** as **Aberration type**.

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 **secondary** 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.

Channel As	ssig	nment				
Profile channel		Primary LSI Her2_neu	<b>~</b>	Secondary Cep17 🖌	Spot threshold	Min. spot contrast
DAPI	•	DAPI	•	DAPI -		
😾 SpGreen	۲	Cep17	•	Cep17 -	auto 🔲 1 🚔	12 🚔
🛃 SpRed	۲	Her2	-		auto 🔽	12 🊔
		CGene spot size -				
		✓ From control s	oot			
		Clusters Visualisa	tion			

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

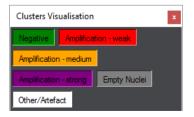
- The **Spot threshold** option is designed to set spot threshold value for the selected channel.
- Upon selecting the auto option to apply automated spot detection. The manual parameter setting for the detection of weaker spot signals is available in the Min. spot contrast value box.

#### 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

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.

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]

#### **Classification Parameters**

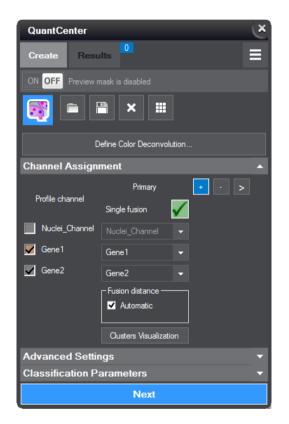
There are two functions that can be activated to assign limitation parameters for the classification.

- 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.

# 3.7 💽 CISHQuant

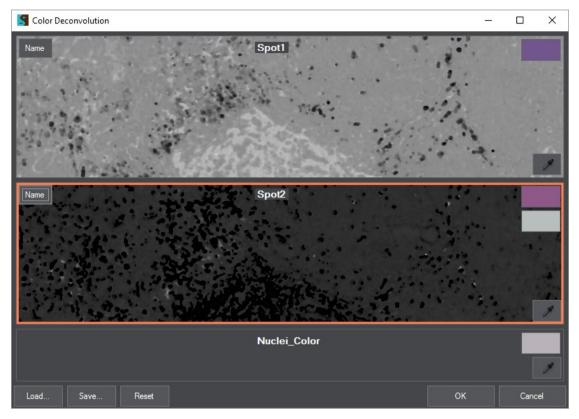
**CISHQuant** allows you to detect and separate CISH spots on the virtual slides digitized with Pannoramic microscopes. This CISH-based application performs *segmentation* and *measurements* on Pannoramic *digital slides* based on the colors of the gene spots and counter-stained nuclei. Based on the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

#### **CISHQuant Properties**



#### **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.

Channel Assignment						
Profile channel + Add Probe						
Nuclei_Channel						
🗹 Spot 1						
Spot 2						

**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.

😉 Probe Definition		×
New Delete		
Aberration Type Locus specific		•
Kind Amplification		•
01		•
	Add	Cancel

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

🗣 Probe Editor	×
Probe Template Filter	
Aberration Type Locus specific	▼
Kind Amplification	•
Gene Matching	
Nuclei_Channel	Name: Nuclei_Channel
Gene	Name: Spot1
Control	Name: Spot2
Current Probe	
Name: 01	Save Cancel

Channel Assign	ment			
Profile channel	Primary 01	✓	Spot threshold	+ · <
Nuclei_Channel	Nuclei_Channel	•		
🛃 Spot 1	Spot 1	•	auto 🔽	
Spot 2	Spot 2	•	auto 🔽	
	Gene spot size ——	nt		
	Clusters Visualisatio	n		

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.

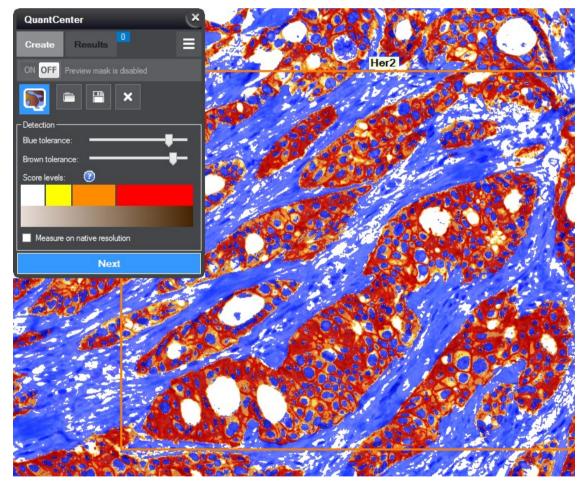
- Activate Show warning when Other/Artifact > function to allow the software to display a warning message in the tree as a tooltip, if the percentage of objects found in the Other/Artifact clusters exceeds the set value.
- The **Stop when object count** > option is responsible for terminating classification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

# 3.8 💽 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.

- 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.



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

Scenario v Session v Quant	÷	Annotation	W
DensitoQuant		Her2	
Unnamed scenario			
= 2017.02.15. 9:32:24 (2s)			
DensitoQuant		Her2	
Unnamed scenario			
= 2011.06.02.14-04-18.(N/A)			
Name		Value	Uni
No. Total non-background pixels		1666053	
No. Background pixels		282348	
No. Negative pixels		885069	
No. Weak-Positive pixels		50444	
No. Moderate-Positive pixels		243748	
No. Strong-Positive pixels		486792	
Mean Density of all pixels		23	
Mean Density of Negative pixels		12.95	
Mean Density of Weak-Positive pixels		11.31	
Mean Density of Moderate-Positive pixels		25.67	
Mean Density of Strong-Positive pixels		51.06	
Ratio of Negative pixels to total pixels		53	%
Ratio of Strong-Positive pixels to total pixels		29	%
Ratio of Moderate-Positive pixels to total pixels		15	%
Ratio of Weak-Positive pixels to total pixels		3	%
Ratio of all positive pixels to total pixels		46,8762998536061	%
H-Score		119.94312305791	

**NOTE**: Data Visualization function is not available for DensitoQuant.

## 4 Data Visualization



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.

### 4.1 Panels and functions

Upon clicking the button both on Measurement Card and Measurement Tree forms, 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.

🚹 Data Visua	lization										-
Table Repo	rt							1	2	3	
ID	✓ Area	Perimeter	Shape factor	Score	Gray intensity	Brown intensi	. Blue intensity ^		1000	S. Research	100
	44.85	28,97	0.67	1+	139,98	180,73	183,04				
	34,66	21,34	0,96		137,4	169,79	195,57			$\boldsymbol{\boldsymbol{\mathcal{C}}}$	12
	57,97	29,73	0.82		125,95	144.75	199,64	100 C 110	1000	Part of the later	
	40,15	23,21	0.94		154,71	204,61	176.34	5	6	7	
	43,28	30,25	0.59		130,85	153,21	198,45				1000
	41.32	23,99	0.9	2+	128,03	139,75	211.69	0.0			100
	49.74	27,75	0.81		128,25	163,55	186.51				
	22,72	16,7			133,57	156,97	203,38				80
	27,81	21,96	0.72		140,53	215,19	145,73	10024.00	<ul> <li>Mage</li> </ul>		N 1.4
10	28,4	23,29	0,66		149,39	193.02	180,22	9	10	11	1
	33,1	21,7	0.88	2+	125,95	139,37	209.92	1000	100000		100
12	36,43	22,11	0.94		134,75	156,49	198.32		5 23 4		
	29,57	20.97	0.85	2+	127,42	141,93	206.49	2.			
14	36.03	22,11	0.93		146,79	188,15	183,46	1000	2072 400	- Bar	Ref.
15	25,65	17,95		2+	126,02	132,7	213.02	13		15	
	39,17	26,13	0.72		127,07	148,14	200,86				
	42,69	26,65	0,76		136,8	172,71	188,61		1000		14
	29,57	20,93	0,85		121,62	135,32	207,92				14
19	22,52	20,42	0,68		137,3	166,22	191,39			10.1	180
20	60,12	29,78	0,85		134,04	158,99	198,34			10000	
21	CE 00	20.00	0.07	<b>n</b> .	103.01	100.40	207.00	17			

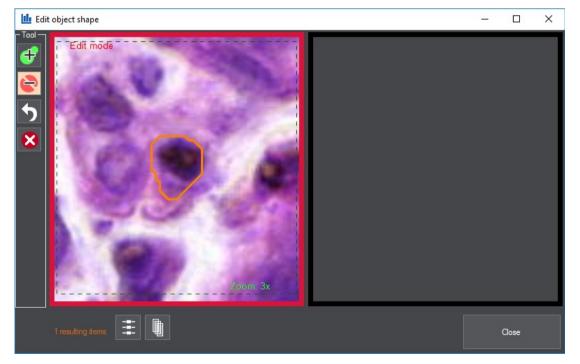
Each column can be ordered by clicking the header, so values will be listed in descending or increasing order. Upon clicking a list item, the image of the specific object is displayed on the right panel.

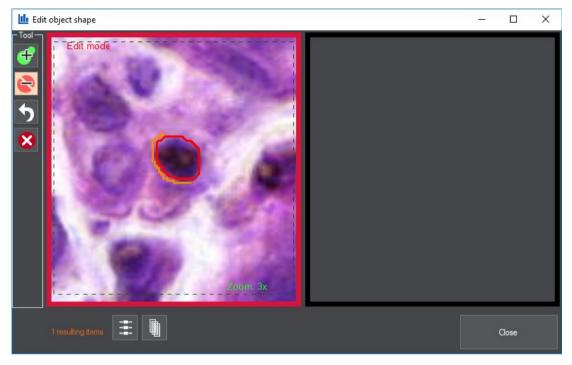
By keeping the **Ctrl** key pressed and clicking with the mouse, detected objects can be selected from the list and displayed in the right side of the window. Upon selecting any of the objects in the Gallery, its specific location on the slide is shown in the CaseViewer window. If more objects are selected, the area within these objects are located, is fitted in the slide view of CaseViewer.

🚺 Data Vis	alization						
Table Re	port						
ID	2 🕶 Area	Perimeter	Shape factor	Score 14	Gray intensity	Brown intensi	Blue intensity
196	32,12	20,45	0,96		103,51	95,1	230,48
							235,31
							233,7
							240,61
							232,69
672	31,33	21,34	0.86		97.68	91,14	233,98
							240,37
	57,97	29,73	0.82		125,95	144,75	199,64
	41,32	23,99	0.9		128,03	139,75	211,69
	33,1	21,7	0.88		125,95	139,37	209,92
	29,57	20,97	0.85	2+	127,42	141,93	206,49
							213,02
16	39,17	26,13	0.72		127,07	148,14	200,86
18	29,57	20,93	0.85		121,62	135,32	207,92
21	65,02	30,66	0.87		122,81	139,48	207,06
							212,14
29	31,92	20,34	0.97		122,57	134,4	212,57
							194,62
							213,63
38	29,18	19,57	0,96		123,86	135,03	213,3
10	11 CE	20.00	n 70	<u>э.</u>	110.0	100.05	210.11

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.

- 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.



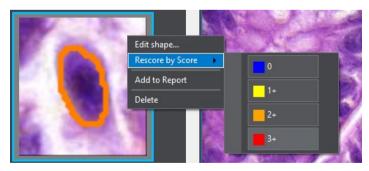


You can add area to nuclei by clicking the + button or cut nuclei (subract area from nuclei) by clicking –, then moving the mouse with holding the left mouse button down.

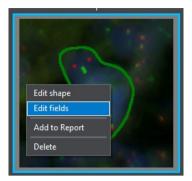
Click to undo changes made after the window was opened.

Click Cose (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



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



Le Edit Fields			×	
Editable fields				
Spot count in channel AF488	3	÷		
Spot count in channel AF594	0	÷		
Calculated fields				
Spot count in channel Gene in Probe LSI Her2_neu_Cep17	DC	0		
Spot count in channel Control in Probe LSI Her2_neu_Cep1	7 DC	3		
LSI Her2_neu_Cep17 DC cluster		Other/Artefact		
03 cluster	Other/Artefact			
1 cell(s)	ок		Cancel	

- 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.

It Data Visualization				- 🗆 ×
ButtonMatrix Piechart Cumulation table Histoplot Clustergram Report	23	24	27	28
All Negative Amplification Amplification Other/Antefact Normal Gene1-Norm	$\bigcirc$			$\bigcirc$
Gene 1-Norm	29	30	31	33
Gene2-Norm	8	-		
Gene 1-Polypl				
Gene1-Arue	34	35	36	37
Gene1-Anue				
Vertical axis Custerer LSI Her2_neu_Cep17 DC Gusterer 03 Use this clusterer	38	39	40	42
Custeres 837 of 929 (90,1%)	Zoom	Border		42 Save

ᅚ

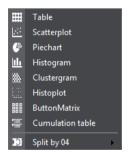
Cluster

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

.SI Her2_neu_Cep17 DC			
15	Name: 03		
	Normal		Γ
	Gene 1-Normal-Gene 2-Polyploidy		
	Gene1-Normal-Gene2-Anueploidy		
	Gene2-Normal-Gene1-Polyploidy		
	Gene2-Normal-Gene1-Anueploidy		
	Gene 1-Polyploidy-Gene 2-Polyploidy		
	Gene 1-Polyploidy-Gene 2-Anueploidy		
	Gene1-Anueploidy-Gene2-Polyploidy		
	Gene1-Anueploidy-Gene2-Anueploidy		

🛍 – Export data to an Excel file

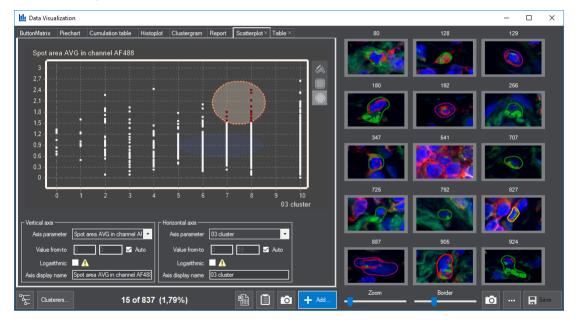
+ Add ... – Additional presentation forms can be activated after clicking this button:



#### • Table

ButtonMatrix	Piechart Cumulati	on table Histoplot	Clustergram	Report Scatterplot ×	Table ×					
ID	2 - Gene/Control	Area	Perimeter	Shape fa 1 🔻	LSI Her2_ne	03 cluster	Cell state	Spot count in	Spot area AV	^
145	2,17	67,05	34,47	0,71	Amplification - we	Gene 1-Anueploid			0,72	
157	1,17	34,78	24,79	0,71	Negative	Gene 1-Anueploid			0,67	
620	1,5	50,67	29,73	0,72	Negative	Gene 1-Anueploid		10	1,03	
268	0,2	87,67	38,77	0,73	Negative	Gene 1-Anueploid			1,09	
589		65,78	33,49	0,74	Amplification - we	Gene 1-Polyploidy			0,9	
56		54,71	30,5	0.74	Amplification - we	Gene 1-Polyploidy			0,79	
137	42	59,33	31,75	0,74	Amplification - str	Gene 1-Polyploidy			0,69	
744	0,5	59,89	31,83	0,74	Negative	Gene1-Anueploid			1,13	
142	0,38	81,2	37,04	0,74	Negative	Gene1-Anueploid			1,12	
783	3,91	80,38	36,84	0,74	Amplification - me	Gene1-Anueploid			0,82	
681	0,82	92	39,35	0,75	Negative	Gene 1-Polyploidy			0,75	
560	33	63,16	32,59	0,75	Amplification - str	Gene1-Anueploid			1,06	
482	2,75	68,55	33,71	0,76	Amplification - me	Gene1-Anueploid		12	0,68	
69	1,17	51,86	29,32	0,76	Negative	Gene 1-Anueploid		6	0,74	
619	1,4	97,23	39,83	0,77	Negative	Gene1-Anueploid			0,96	
246	1,33	48,96	28,2	0,77	Negative	Gene 1-Polyploidy			0,92	
828	1,25	52,31	29,14	0,77	Negative	Gene 1-Anueploid			1,09	
478		41,59	25,98	0,77	Negative	Gene 1-Anueploid			0.7	
654		44,1	26.7	0,78	Amplification - str	Gene 1-Anueploid			0,95	
807	1,6	22,42	19,03	0,78	Negative	Gene 1-Polyploidy			0,8	
170	0.12	90.21	20.10	0.70	Monativo	Gono1 Anucoloid	1	0	0.72	×

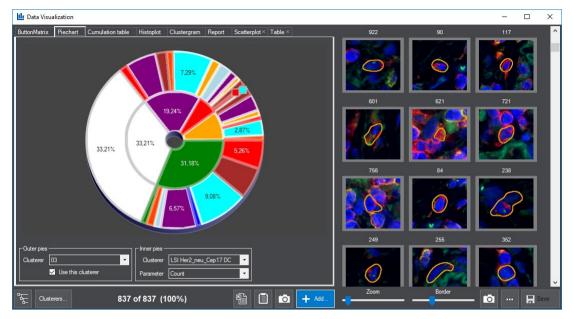
#### Scatterplot



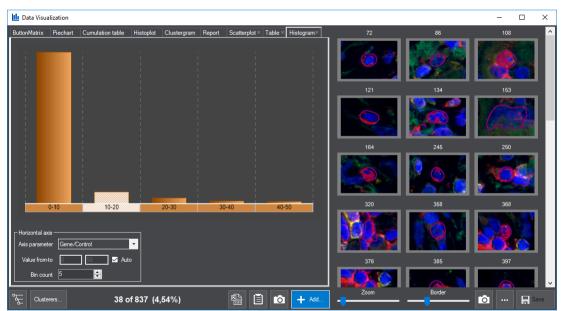
Cluster-related measurement data are displayed on an X-Y axis graph of the scatterplot (linear or logarithmic representations are both available). The axis parameter can be selected from the **Axis parameter** drop-down lists both for the **Vertical** and the **Horizontal** axis. If you deactivate the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



#### • 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.

Le Data Visualization			- 1	⊐ ×
ButtonMatrix Piechart Cumulation table Histoplot Clustergram Report Scatterplot × Table × Histogram ×	878	321	358	
88           88	554	397	517	
0         5         10         15         20         25         33         40         45         51           Other/Artefac         A         Amplification - strong         19.24%         19.24%	548	555 768	571	
Vertical axis         Horizontal axis           Clusterer         03         Clusterer         Clu	Zoom	Border	- 10	V Save

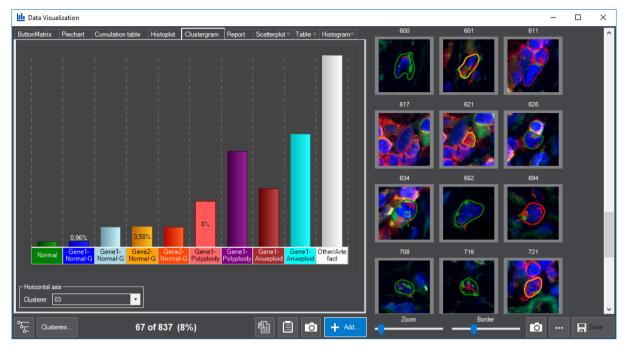
After right-clicking on the selected object displayed in the matrix can be deleted or moved to another cluster if needed. You can filter nuclei on the histoplot by drawing a circle around the objects.

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.

#### Clustergram



#### Cumulation table

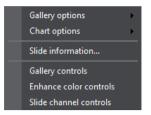
III Data Visualization							- 0	×
ButtonMatrix Piechart Cumulation table Histoplot Clustergram Report	Scatterplot × 1	Table × Hi	istogram×	327	498	574	694	
Name	Gene/Control	Percent	Count 🔺					
Negative (LSI Her2_neu_Cep17 DC)	0,93	31,18%	261					
🔓 Normal (03)		3,07%	8					
- Gene1-Normal-Gene2-Polyploidy (03)	0,35	3,07%	8	- ALCORE				
Gene1-Normal-Gene2-Anueploidy (03)	0,43	3,83%	10	787				
Gene2-Normal-Gene1-Anueploidy (03)	0,67	4,6%	12					
🖕 Gene1-Polyploidy-Gene2-Polyploidy (03)	1,02	16,86%	44					
	1,19	21,07%	55					
🖶 Gene1-Anueploidy-Gene2-Polyploidy (03)	0,78	18,39%	48					
- Gene1-Anueploidy-Gene2-Anueploidy (03)	0,95	29,12%	76					
Amplification - weak (LSI Her2_neu_Cep17 DC)	2,01	6,57%	55					
Gene1-Normal-Gene2-Anueploidy (03)		34,55%	19					
		12,73%	7					
Gene1-Polyploidy-Gene2-Polyploidy (03)		9,09%	5					
Gene1-Polyploidy-Gene2-Anueploidy (03)	2,01	21,82%	12					
Gene1-Anueploidy-Gene2-Polyploidy (03)	2,04	16,36%	9					
Gene1-Anueploidy-Gene2-Anueploidy (03)	2,1	5,45%	3					
Amplification - medium (LSI Her2_neu_Cep17 DC)	3.2	9,8%	82					
	3,44	10,98%	9					
Gene2-Normal-Gene1-Anueploidy (03)	2,93	8,54%	7					
	2.24	7 0.08	· · · · ·					
Secondary — Primary — Primary —								
Clusterer 03 Clusterer LSI Her2_neu_Cep17 DC								
Use this clusterer								
				Zoom	E	order		
Clusterers 5 of 837 (0,6%)		Ē	+ Add				<b>3</b> ···	Save



The complete list is copied to clipboard

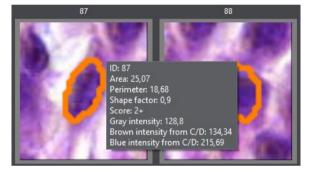
- Creates a snapshot of the selected item(s) that can be saved as a PNG/JPG/BMP/TIF file

- Further options are accessible by clicking this button:



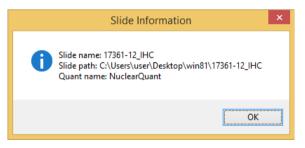
#### Gallery options

- 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



- 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 adjust Color balance	e			
۰	•		0,00	Þ
0	•		0,00	Þ
			1,00	Þ
Default Loade	i			

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).

Color R	r adju	st	Colo	r bal	ance						_										
G	·	'	'	'	'	1	•	'	'	'		'	'	'	'	'	'	'		·	١
в	<u> </u>	'	'	•	1	1	•	1	'	'		1	'	•	'	'		'	1	·	'
	ı	'	'	'	'	1	•	1	'	'	1	1	'	•	'	'	'	'	'	1	١
De	efault																				
	aull			Loa	ded																
	angri			Loa	ded																
	DAPI		F		ded	TF	RICT														
			F	Loa	ided	TF	RICT	1									•	1	0,01		Þ
			F		ided	TF	RICT	1									•		0,01		•
*			F			TF	RICT				-,-						<ul> <li></li> <li><td></td><td></td><td></td><td></td></li></ul>				
*	DAPI		F			TF	RICT									-	•		0,02		Þ

**h** \_ 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)

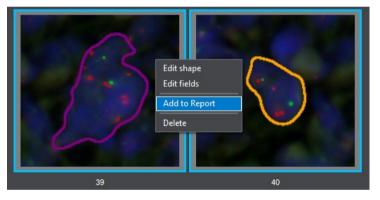
R Save
 − Saves data to slide

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.

LL Data Visualization	_		×
ButtonMatrix       Piechart       Cumulation table       Histoplot       Clustergram         Report       O       Pie chart       O       Clustergram         Histoplot       Export       Export         Generate Report       O       Pie chart       Clustergram			
Clusterers		Save	

**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.

III Data Visualization	- 🗆 X
ButtonMatrix       Piechart       Cumulation table       Histoplot       Clustergram       Report            • Pie chart         • Clustergram           • Clustergram           Clustergram           Clustergram             • Pie chart         • Clustergram           • Clustergram           Clustergram             • Histoplot           • Clustergram           Export             Generate Report           • Original State           • Dispersion Excel export	10 8 7 18
Custerers + Add	Zoom Border IS Save

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

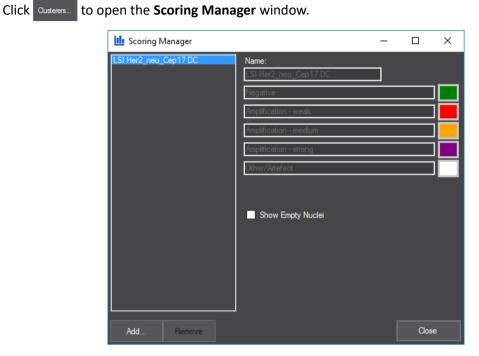
**NOTE**: The report can be generated only if you have added a Cumulation table to the DVT. Otherwise you receive a warning message informing you about this issue.

Error
Please create a 'CumulationTable' first to allow reporting!
ОК

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

	E23 🔹 🤄	$f_x$				×
	A	В	С	D	E	Ξ
1	Amplification - medium; Red-	Polyploidy-Green-Polyplo	idy			
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		Nucleus count			
13	SpRed - 10	10	3			
14						
15						
16						
17						
18	-					
19	Spots altogether					
20	Spot channel - spot number	SpGreen (spot number)		Nucleus count		
21	SpGreen - 04 ; SpRed - 10	4	10	3		
22						
23						*
14 -	Amplw.; Red(P)-G	reen(P) Amplm.; Re				
Kés	Z		100	)% ────		

### 4.2 Scoring and creating a Secondary Probe



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

💁 Probe Definition		×
New Delete		
Aberration Type Numerical deviation		•
Kind 2 Channels		•
03		•
		_
DAPI (Name: DAPI)	Dapi	-
Gene1 (Name:Gene1)	AF594	•
Gene2 (Name:Gene2)	AF488	•
Reset All 🗹 Show	Auto match	
	Add Cancel	

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

LSI Her2_neu_Cep17 DC	Name:
03	03
	Normal
	Gene1-Normal-Gene2-Polyploidy
	Gene 1-Normal-Gene 2-Anueploidy
	Gene2-Normal-Gene1-Polyploidy
	Gene2-Normal-Gene1-Anueploidy
	Gene 1-Polyploidy-Gene 2-Polyploidy
	Gene 1-Polyploidy-Gene 2-Anueploidy
	Gene 1-Anueploidy-Gene 2-Polyploidy
	Gene 1-Anueploidy-Gene 2-Anueploidy
	Other/Artefact
L	Show Empty Nuclei

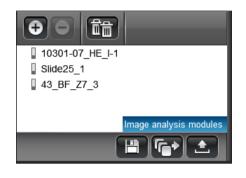


### 5 Running a Batch Process from CaseViewer

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 2.2 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 slides by clicking



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

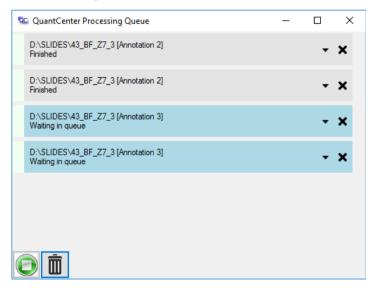
🚺 Image Analysis Batch Proc	essing				_		×	
Slides	Annotations           Whole slide           Every annotation	Scer Add Rem		+ -				
D:\SLIDES\43_BF_Z7_3 D:\SLIDES\MQ\Slide25 D:\SLIDES\NQ\10301-0	Annotation 1 Annotation 2 Annotation 3	Est Hei Ki6 Ki6 Ki6 P53	DC_ER-Her2 Estrogen Her2 Ki67 Ki67 profile Ki67_2 P53 Progesterone			Add		
Batch Processing List								
Slide	Annotation		Scenario			Remo	ve	
D:\SLIDES\43_BF_Z7_3 D:\SLIDES\43_BF_Z7_3	Annotation 1 Annotation 2		Her2					
D:\SLIDES\43_BF_Z7_3	Annotation 2		Estrogen Ki67 profile					
D:\SLIDES\43_BF_Z7_3	Annotation 2		Ki67					
D:\SLIDES\43_BF_Z7_3	Annotation 2		Her2					
Save cumulated XLS to:						Brow	wse	
Transposed XLS table								
				Start Batch	Processing	CI	ose	

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

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.

🚺 Open					×
$\leftarrow \rightarrow \cdot \uparrow$	Lo	cal Disk (C:) → ProgramData →	3DHISTECH > IA > MISPs	✓ Ö Search	MISPs ,
Organize 🔻 New	folde	er			::: • 🔟 ?
💻 This PC	^	Name	Date modified	Туре	Size
📃 Desktop		DC_ER-Her2	2015.11.18. 12:24	XML File	15 KB
🔮 Documents		🚺 Estrogen	2015.11.18. 12:24	XML File	15 KB
Downloads		🔀 Her2	2015.11.18. 12:24	XML File	15 KB
Music		🔟 Ki67 profile	2015.11.18. 12:24	XML File	11 KB
•		🔟 Ki67	2014.05.20. 13:23	XML File	15 KB
Pictures		🔟 Ki67_2	2015.11.18. 12:24	XML File	11 KB
Videos	л.	🔟 P53	2015.11.18. 12:24	XML File	19 KB
🏪 Local Disk (C:)		🔟 Progesterone	2015.11.18. 12:24	XML File	11 KB
🕳 Local Disk (D:)	~				
File <u>n</u> ame: QuantCenter scenario XML file					Center scenario XML files $  imes $
				<u></u>	pen Cancel

- 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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Α	
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н	
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