TISSUEGNOSTICS
PRECISION THAT INSPIRES

V8.0

TissueFAXS Viewer

User Manual

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2.Introduction

TissueFAXS Viewer is a free software tool offered by **TissueGnostics**.

As its name says, it is a viewer-type application and it allows you to visualize and validate projects acquired with **TissueFAXS** acquisition software.

Note: Please always make sure you are using TissueFAXS Viewer with projects acquired with the same TissueFAXS version!

3. Getting Started with TissueFAXS Viewer

Double click on the **TissueFAXS Viewer** icon on your computer or on the **Launch TissueFAXS Viewer** button in **TissueFAXS**. A splash screen will be displayed until the user interface is initialized.

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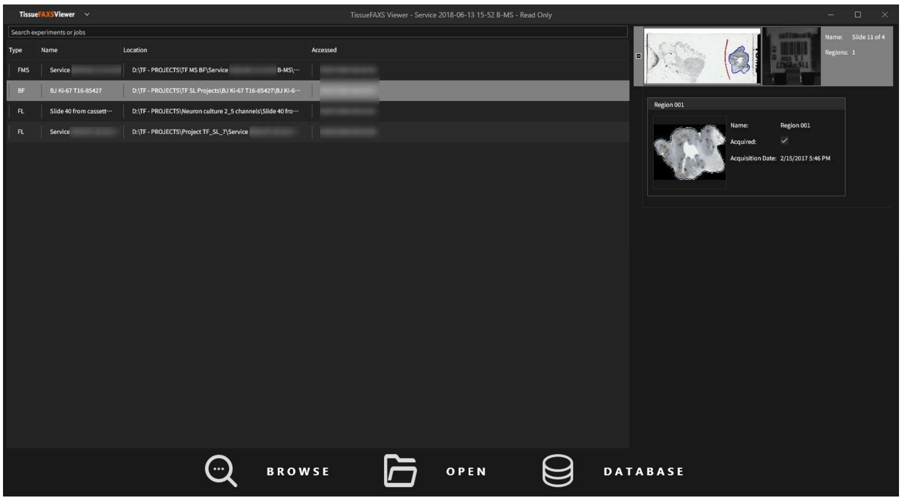
TissueFAXS Viewer Start panel will open.

Open Existing Project



TissueFAXS Viewer - Service 2018-06-13 15-52 B-MS - Read Only

Search experiments or jobs

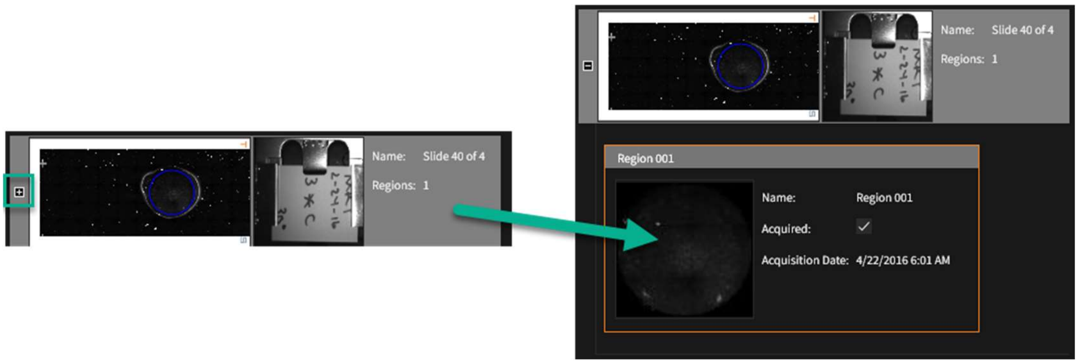
Type	Name	Location	Accessed
FMS	Service [redacted]	D:\TF - PROJECTS\TF MS BF\Service [redacted] B-MS\···	[redacted]
BF	BJ Ki-67 T16-85427	D:\TF - PROJECTS\TF SL Projects\BJ Ki-67 T16-85427\BJ Ki-6···	[redacted]
FL	Slide 40 from cassett···	D:\TF - PROJECTS\Neuron culture 2_5 channels\Slide 40 fro···	[redacted]
FL	Service [redacted]	D:\TF - PROJECTS\Project TF_SL_7\Service [redacted]	[redacted]

Name: Slide 11 of 4
Regions: 1

Region 001
Name: Region 001
Acquired: ✓
Acquisition Date: 2/15/2017 5:46 PM

- You can either open a project from the list (if you already have previously opened projects in the list) or browse on your computer for a project.

- For each selected project from the list, a preview and a set of details are available on the right side. Also, you can see the type of experiment (FL, BF, FL MS, JOB), its location and the last time it was accessed.
- For each region present in the project, you can press **+** button to see details:



Name: Slide 40 of 4
Regions: 1

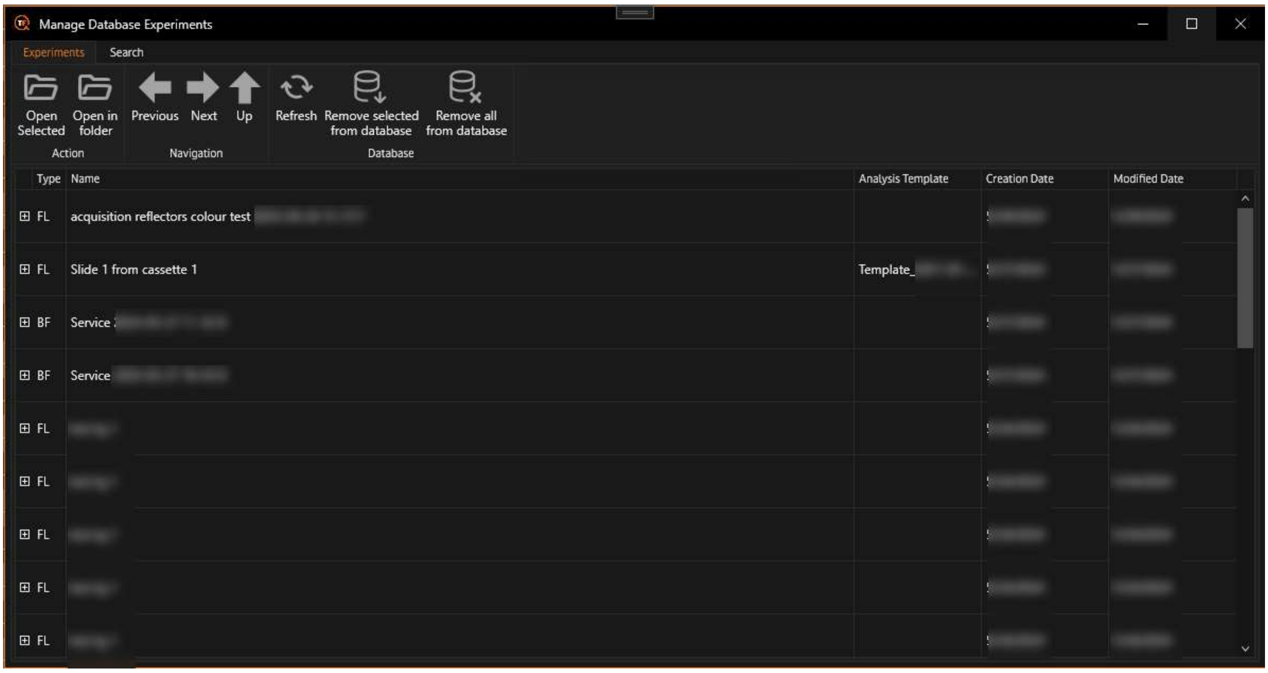
Region 001

Name: Region 001
Acquired: ✓
Acquisition Date: 4/22/2016 6:01 AM

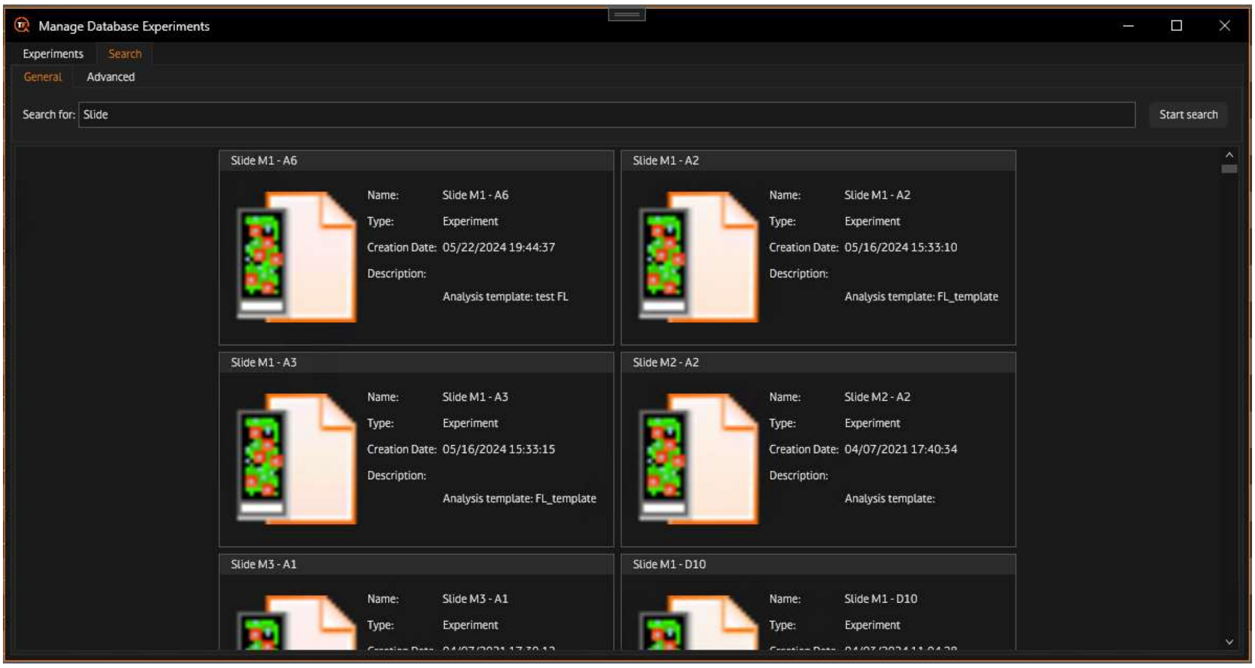
- Use the **Search** feature in order to find a certain project in the list.

Use **Back** button to go back to **Start-up** screen.

Open from Database



Type	Name	Analysis Template	Creation Date	Modified Date
FL	acquisition reflectors colour test [redacted]		[redacted]	[redacted]
FL	Slide 1 from cassette 1	Template_[redacted]	[redacted]	[redacted]
BF	Service [redacted]		[redacted]	[redacted]
BF	Service [redacted]		[redacted]	[redacted]
FL	[redacted]		[redacted]	[redacted]
FL	[redacted]		[redacted]	[redacted]
FL	[redacted]		[redacted]	[redacted]
FL	[redacted]		[redacted]	[redacted]
FL	[redacted]		[redacted]	[redacted]



Search for: Slide

Slide M1 - A6
Name: Slide M1 - A6
Type: Experiment
Creation Date: 05/22/2024 19:44:37
Description:
Analysis template: test FL

Slide M1 - A2
Name: Slide M1 - A2
Type: Experiment
Creation Date: 05/16/2024 15:33:10
Description:
Analysis template: FL_template

Slide M1 - A3
Name: Slide M1 - A3
Type: Experiment
Creation Date: 05/16/2024 15:33:15
Description:
Analysis template: FL_template

Slide M2 - A2
Name: Slide M2 - A2
Type: Experiment
Creation Date: 04/07/2021 17:40:34
Description:
Analysis template:

Slide M3 - A1
Name: Slide M3 - A1
Type: Experiment

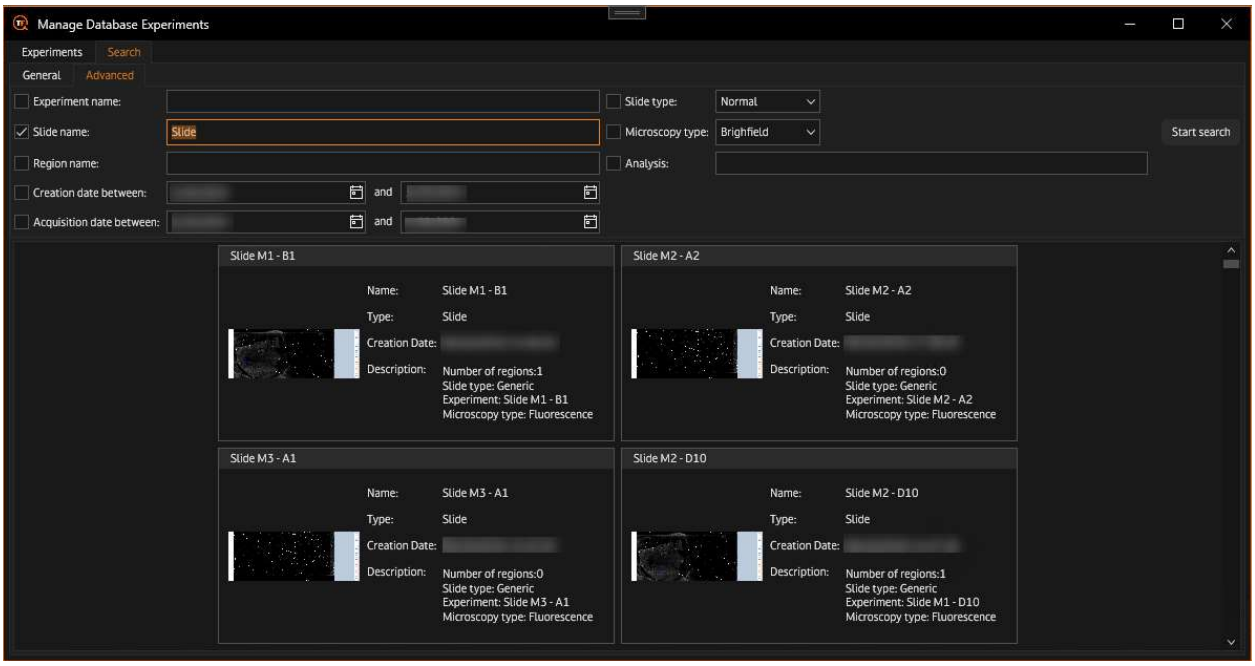
Slide M1 - D10
Name: Slide M1 - D10
Type: Experiment

In **Manage Database Experiments** dialog, double click on a slide to open the corresponding TissueFAXS project or expand a job to see its details.

A user can also perform an advanced search in the database to find a specific project in a job.

Additional search types include:

- **General search**: enter the exact name of the slide in the upper field, then press **Find**: The entered job will be displayed.
- **Advanced search**: uses search criteria to find a specific slide – slide name, experiment name, creation/acquisition date, slide type, and microscopy type. After filling in one or more criteria, press the **Start search** button to see results.



☐ Experiment name:
☑ Slide name: Slide
☐ Region name:
☐ Creation date between: [redacted] and [redacted]
☐ Acquisition date between: [redacted] and [redacted]
☐ Slide type: Normal
☐ Microscopy type: Brighfield
☐ Analysis:

Slide M1 - B1
Name: Slide M1 - B1
Type: Slide
Creation Date: [redacted]
Description: Number of regions:1
Slide type: Generic
Experiment: Slide M1 - B1
Microscopy type: Fluorescence

Slide M2 - A2
Name: Slide M2 - A2
Type: Slide
Creation Date: [redacted]
Description: Number of regions:0
Slide type: Generic
Experiment: Slide M2 - A2
Microscopy type: Fluorescence

Slide M3 - A1
Name: Slide M3 - A1
Type: Slide
Creation Date: [redacted]
Description: Number of regions:0
Slide type: Generic
Experiment: Slide M3 - A1
Microscopy type: Fluorescence

Slide M2 - D10
Name: Slide M2 - D10
Type: Slide
Creation Date: [redacted]
Description: Number of regions:1
Slide type: Generic
Experiment: Slide M1 - D10
Microscopy type: Fluorescence

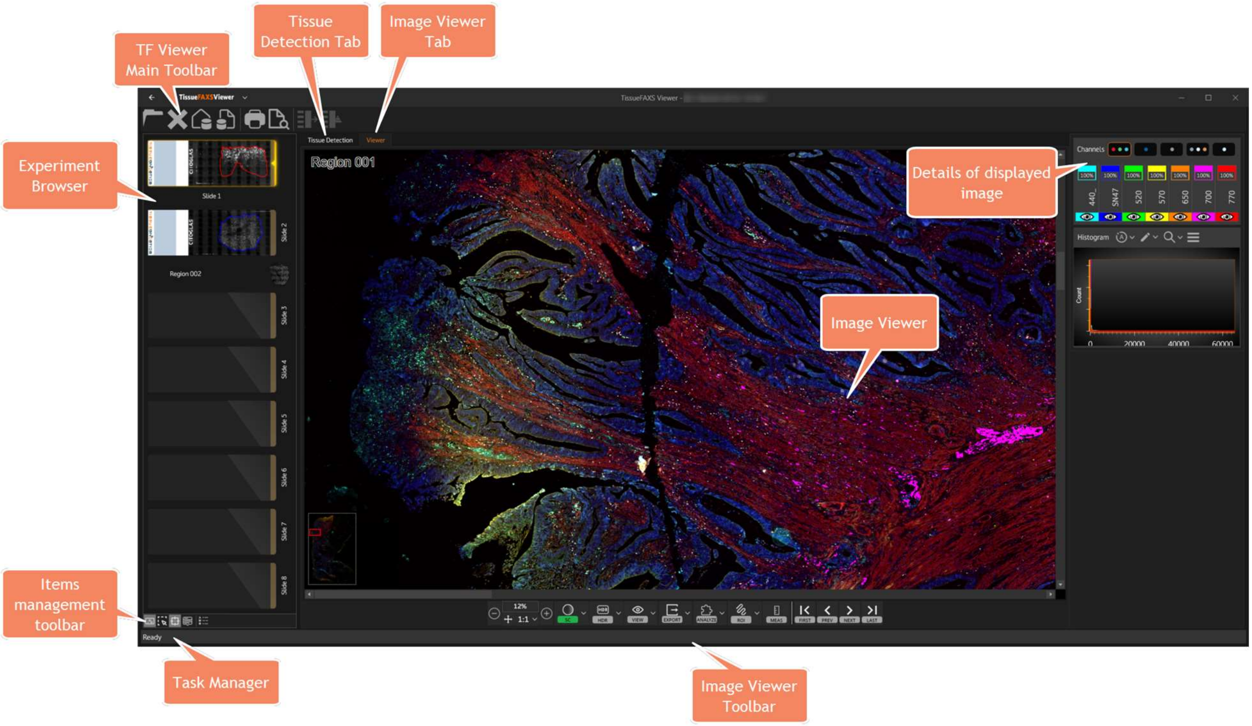
4. Application Overview

TissueFAXS Viewer is a viewer-type application. Its user interface revolves around the idea of ***viewing*** the essential details of an experiment and to gain as much information at a glance. ***Printing*** an experiment in **TissueFAXS Viewer** is also very at hand.

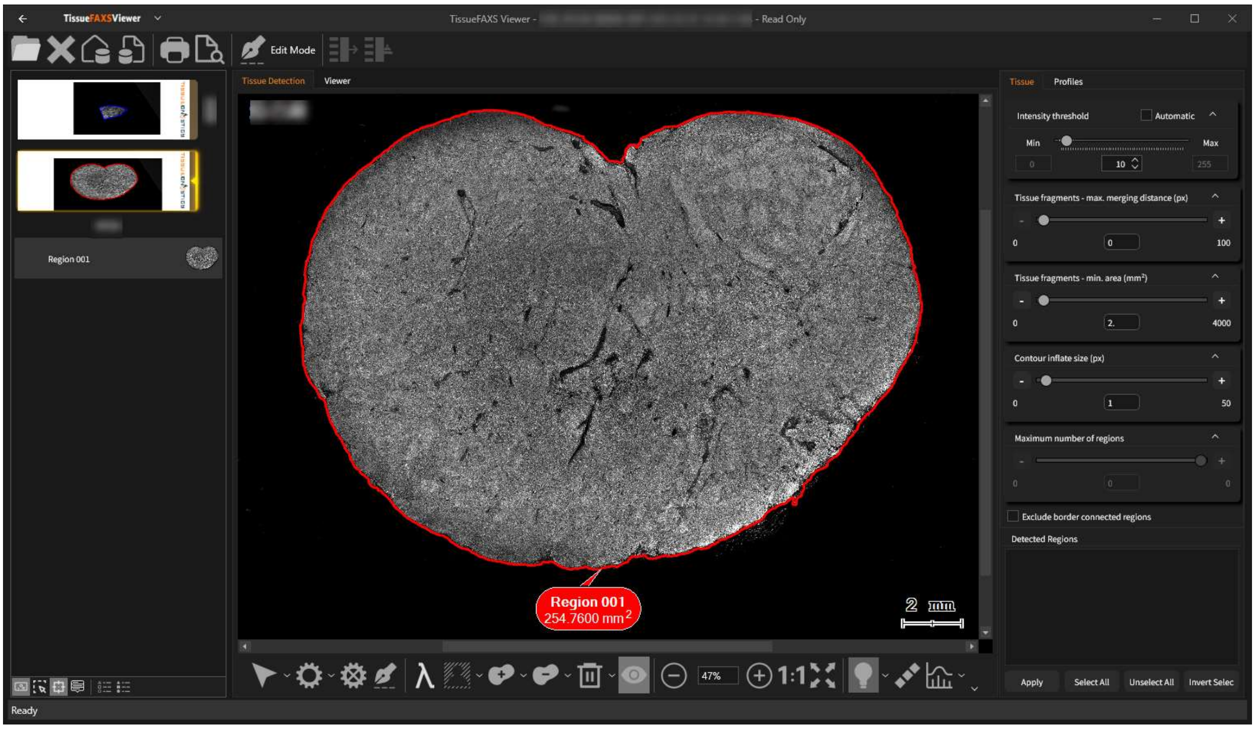
This free application can also be a great tool when using it in synchronous mode with **TissueFAXS**.

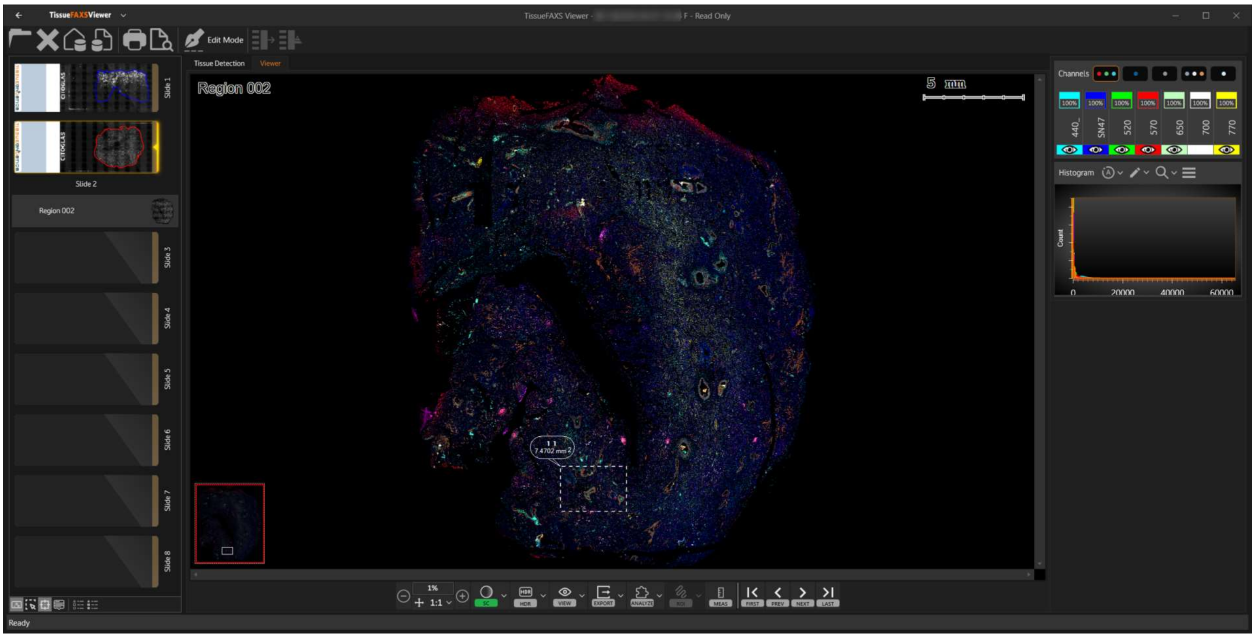
Note: **TissueFAXS Viewer** on Slide Loader systems has an extra section where you can see listed all the job slides and their regions with their properties

It also contains a **Scan** tab to be used when the application validates **TissueFAXS** slides in synchronous mode.



In the image below, check **Tissue** tab:





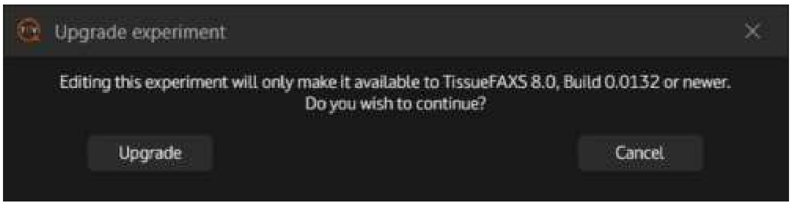
Read-Only Mode and Edit Mode

When you open a project created with a **TissueFAXS** version older than the version of your **TissueFAXS Viewer**, the project will open in **Read-Only** mode.

This means you will be able only to view the project, but not change it.

To be able to modify the project, such as adding regions, press **Edit Mode** button.

The following message will appear:



Upgrade experiment

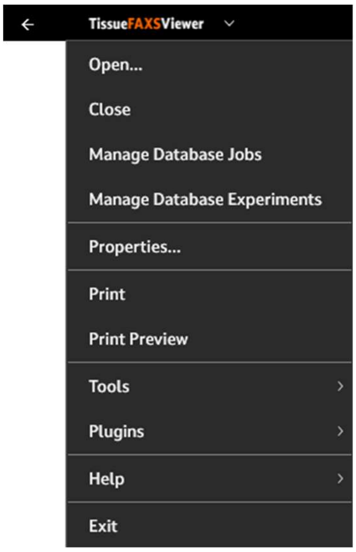
Editing this experiment will only make it available to TissueFAXS 8.0, Build 0.0132 or newer.
Do you wish to continue?

This means that once you convert the experiment to the newer version of software, you will not be able to open it with previous versions.

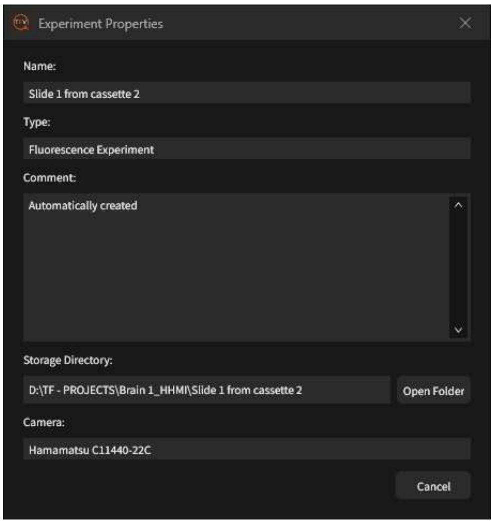
Note: If pressing **Upgrade**, TissueFAXS Viewer will automatically create a backup for your project.

5. Start Menu

Start Menu can be accessed from TissueFAXS Viewer **Start** button.



- **Open**: opens a project (see Login and Start);
- **Close**: closes current project;
- **Manage Database Jobs**: see Operating jobs in TissueFAXS SL Viewer;
- **Manage Database Experiments**: see Login and Start;
- **Properties**: opens **Experiment Properties** window, where you can see experiments main properties - name, type, storage, acquisition camera;



Name: Slide 1 from cassette 2

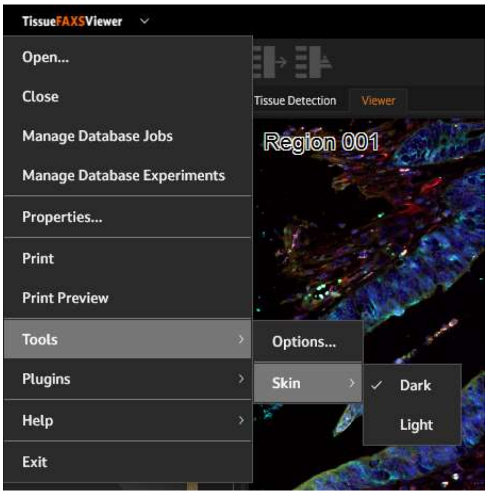
Type: Fluorescence Experiment

Comment: Automatically created

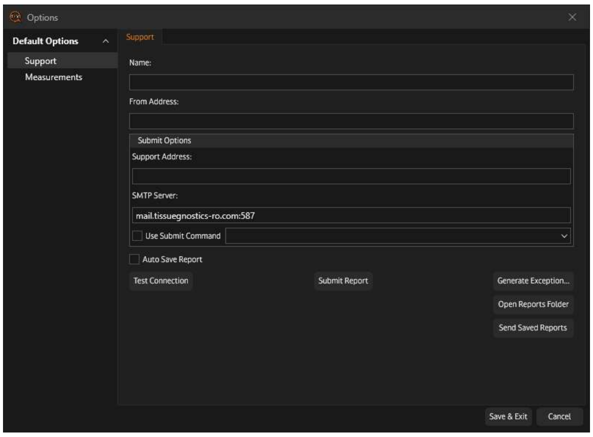
Storage Directory: D:\TF - PROJECTS\Brain 1_HHMI\Slide 1 from cassette 2

Camera: Hamamatsu C11440-22C

- **Print/Print Preview**: see Print;
- **Tools**



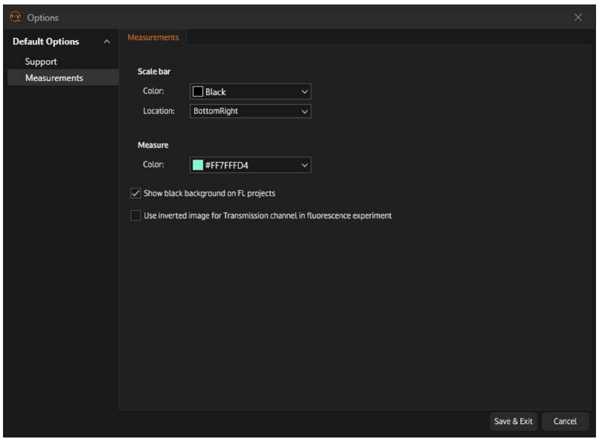
- **Options**: opens **Options** panel. In **Support**, you can send an email in order to ask for assistance to TissueGnostics support team. In **Measurements**, select various properties/display modalities for elements in the viewer.



SMTP Server: mail.tissuegnostics-ro.com:587

☐ Use Submit Command

☐ Auto Save Report



Scale bar

Color: Black

Location: BottomRight

Measure

Color: #FF7FFFD4

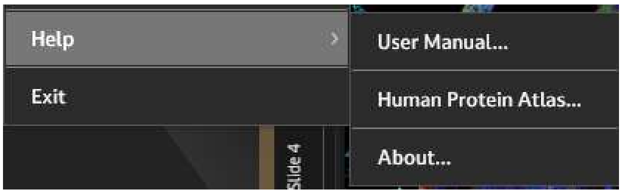
☑ Show black background on FL projects

☐ Use inverted image for Transmission channel in fluorescence experiment

- **Skin**: select Dark mode or Light Mode for the application interface;

- **Plugins**: use this menu to launch TissueGnostics analysis software;

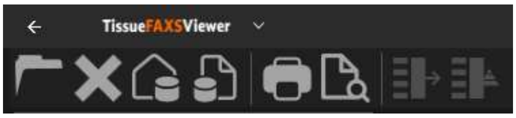
- **Help**: access TissueFAXS Viewer User manual, Human Protein Atlas or details about current TissueFAXS Viewer version.



- **Exit**: closes TissueFAXS Viewer.

6. Main Toolbar

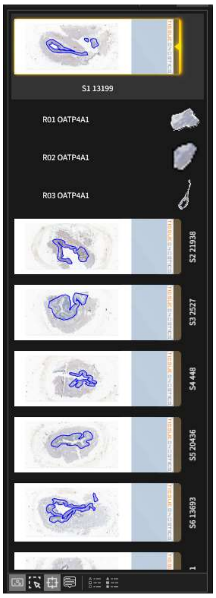
TissueFAXS Viewer **Main Toolbar** contains a set of essential controls, as shown below:



- **Open**: opens a project (see Login and Start);
- **Close**: closes current project;
- **Manage Database Jobs**: see Operating jobs in TissueFAXS SL Viewer;
- **Manage Database Experiments**: see Login and Start;
- **Print/Print Preview:** see Print.

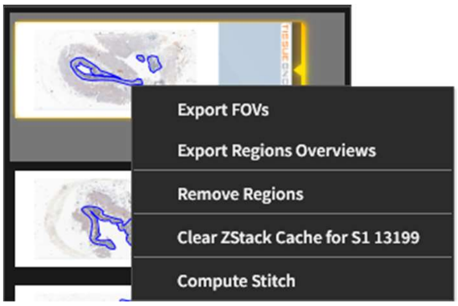
7. Project Browser

Project Browser shows experiments (when a job is opened), the slides from the experiment and its regions/TMA blocks.



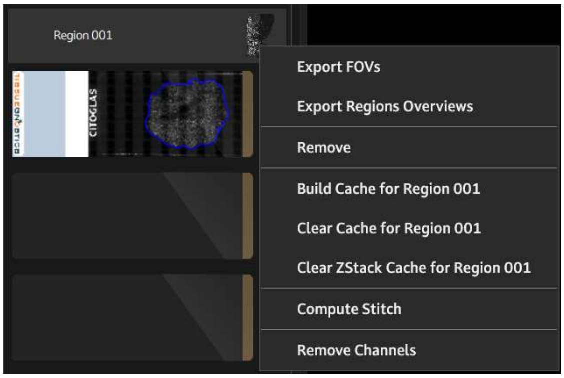
If you right-click on an item or on a multiple selection of items, a contextual menu will appear.

For slides



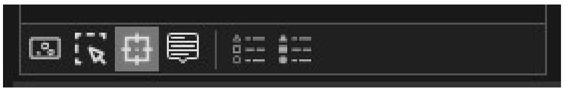
- **Export FOVs**: export FOVs for selected sample;
- **Export Regions Overviews**: export Regions Overviews for selected sample;
- **Remove Regions**: removes existing regions for selected sample;
- **Clear ZStack Cache for Slide 1**: erases ZStack cache for selected slide;
- **Compute Stitch**: computes stitch for selected slide;
- **Remove Channels**: removes channels of selected slide.

For ROIs



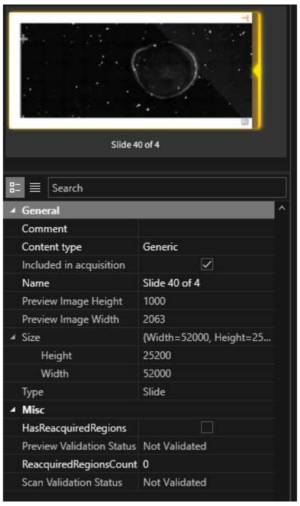
- **Export FOVs**: export FOVs for selected region;
- **Export Regions Overviews**: export Regions Overviews for selected region;
- **Remove**: removes selected region;
- **Build Cache for Region**: builds cache for selected region;
- **Clear Cache for Region**: removes cache for selected region;
- **Clear ZStack Cache for Region**: erases ZStack cache for selected region;
- **Compute Stitch**: computes stitch for selected slide;
- **Remove Channels**: removes channels of selected slide.

Project Browser Toolbar



- **Display Regions on Slide**: shows detection contours of existing regions on slide;

- **Display Only Selected Region on Slide**: shows detection contour of selected region on slide;
- **Show Stage Position Target**: displays stage position target on slide;
- **Show Properties**: shows selected item's properties. These properties vary depending on the type of item.



General	
Comment	
Content type	Generic
Included in acquisition	☑
Name	Slide 40 of 4
Preview Image Height	1000
Preview Image Width	2063
Size	{Width=52000, Height=25...
Height	25200
Width	52000
Type	Slide
Misc	
HasReacquiredRegions	☐
Preview Validation Status	Not Validated
ReacquiredRegionsCount	0
Scan Validation Status	Not Validated

- **Show Slide Loader Legend/Show Legend**: the legend helps you check in a glimpse the status of the items from the Project Browser.

Legend
Selected Slide
Unselected Slide
Validated Slide
Reacquired Slide
Slide under Objective
Region in Acquisition
Reacquired Region
λ Multispectral Region

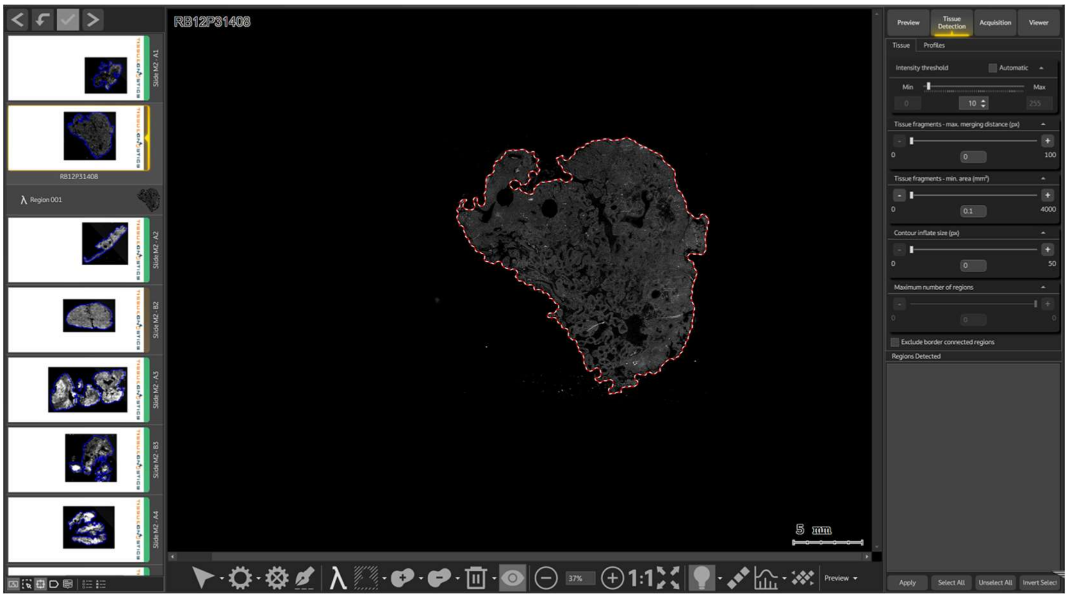
8. Tissue Detection

Automatic Tissue Detection

In TissueFAXS Viewer, tissue detection is available to create regions that will furtherly be scanned in TissueFAXS. Also, it helps validating slides in parallel with the running slide preview phase in TissueFAXS.

TissueFAXS Viewer will automatically autodetect regions using the default parameters.

Note: If a region was previously added to the experiment, the detection will not run automatically, you have to press **Run** button.

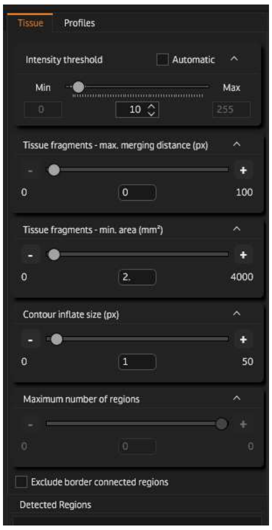


Manual Tissue Detection

If the automatic results of the detection need to be refined, go to **Tissue Detection** tab and manually adjust the settings until you obtain a good detection.

There is a set of parameters that can be adjusted to refine the results of automatic detection.

Tip: To begin a detection, set all parameters to "**0**". Then, one by one, starting with the first parameter, begin increasing the values until you obtain a neat detection.



Intensity threshold ☐ Automatic
Min 0 | 10 | Max 255

Tissue fragments - max. merging distance (px)
0 | 0 | 100

Tissue fragments - min. area (mm²)
0 | 2. | 4000

Contour inflate size (px)
0 | 1 | 50

Maximum number of regions
0 | 0 | 0

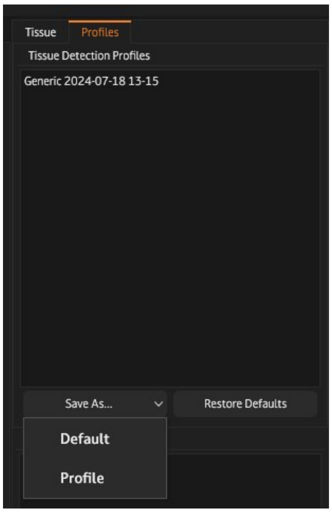
☐ Exclude border connected regions

Detected Regions

- **Automatic**: If **Automatic** is checked, the **Intensity Threshold** will be automatically computed on the gray image in the range selected by the user. The determined value will be shown in the edit box in the middle. A value can be entered by disabling **Automatic** and inputting the desired value in the edit box. Use the **Gray** indicator on the gray image to determine the pixel intensity in the image.
- **Intensity Threshold**: sets the threshold for intensity;
- **Tissue Fragments - max. merging distance**: try to increase this parameter if the region is split into multiple small subregions. The result will be a single region containing smaller subregions;
- **Tissue Fragments - min. area**: is the smallest area a tissue region can have. All regions with a smaller area will not be considered;
- **Contour Inflate Size**: the initially detected region will be inflated with a specified number of pixels, in order to include the edges of the tissue and a small surrounding area;
- **Maximum number of regions**: sets a maximum number of regions to be detected;
- **Exclude border connected regions**: regions touching the border are excluded from detection.

Detection Profiles

For an easier workflow, you can create detection profiles that work like detection templates.



Profile options:

- **Default**: allows the current set of parameter values to be stored for future detection;
- **Restore Defaults**: restores all the parameters to the last saved default parameters;
- **Profile**: saves the current parameter settings for future use;
- **Load Profile**: loads a previously saved profile from the existing list (by double clicking on it).

Tissue Detection contextual menu: see **Chapter** Tissue Detection Contextual Menu.

Tissue Detection Toolbar

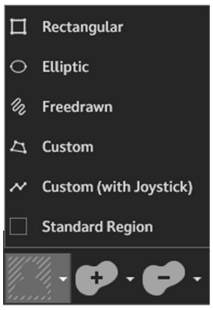
Tissue detection specific controls are located within a **toolbar** in the lower area of the viewer:

- **Select**: selects a rectangular detection area on the tissue. To remove the selection, press **Clear Selection**.

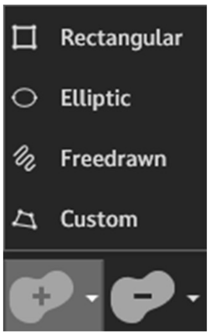
- **Run**: runs detection on selected area. If no selection is made, the detection will run for all the slide. From the arrow menu you can disable **Auto Tissue Detection**.

- **Clear**: clears all detected regions from the defined selection. If there is no defined selection, it will clear the detected regions from the full slide preview.

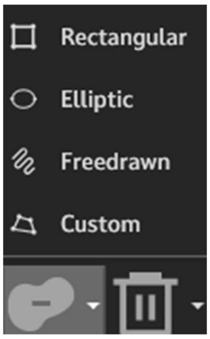
- **Edit**: enables/disables region editing mode. For details see the end of this chapter.
- **Multi-Spectral Mode On/Off**: if Multispectral mode is **On**, all created regions will be multispectral. If it is **Off**, all the new regions will be regular fluorescence regions.
- **User Defined Regions**: enables various shaped regions drawn by the user that will be included in the detection.



- **Add Extension Areas**: enables rectangular/elliptic/free-drawn areas drawn by the user in addition to the detection algorithm results (for example - TissueFAXS has automatically detected a region and you want to manually expand it).

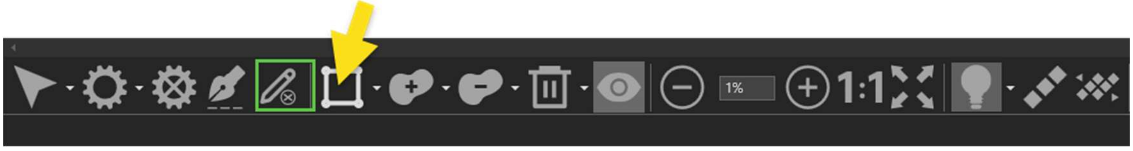


- **Add Excluded Areas**: enables rectangular/elliptic/free-drawn areas drawn by the user that will NOT be included in the automatic detection of regions.

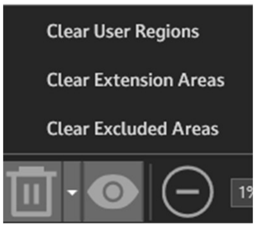


- **Exit Drawing Mode:** when in drawing mode for any types of regions, you can exit by pressing this button or by pressing **Esc** key on the keyboard.

<u>**Note**</u>: When in drawing mode, tissue detection contextual menu is not available.

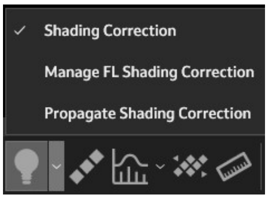


- **Clear All:** clears all the items added by the user.



Clear User Regions
Clear Extension Areas
Clear Excluded Areas

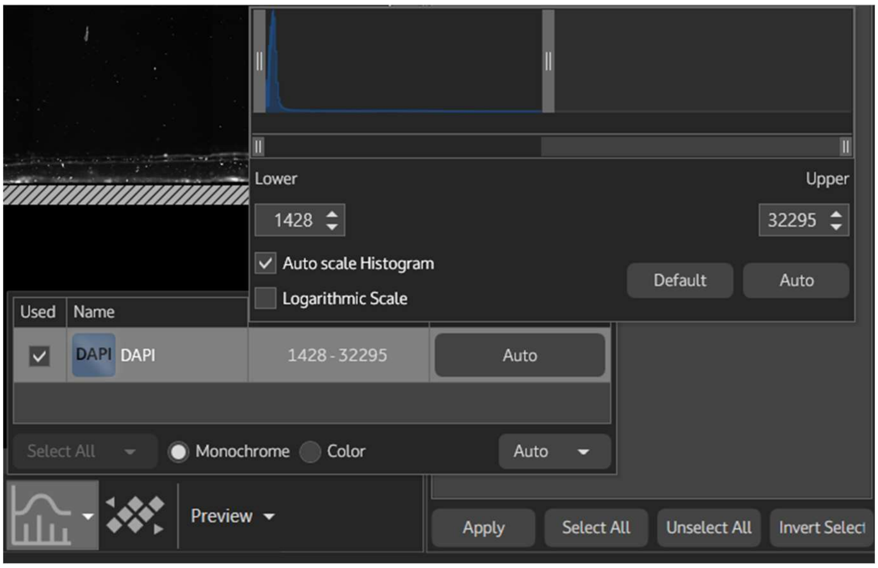
- **Show All:** shows/hides all regions, extensions and excluded areas.
- **Zoom in/Zoom out**
- **Best Fit**
- **Original**
- **Illumination Correction** (please see **Chapter** Illumination/Shading Correction).



✓ Shading Correction
Manage FL Shading Correction
Propagate Shading Correction

Note: Illumination correction affects the detection results as detection is done on the displayed image.

- **Show RGB color**
- **Slide Overlay (**see **Chapter** Overlay).



Lower: 1428
Upper: 32295
☑ Auto scale Histogram
☐ Logarithmic Scale
Default | Auto

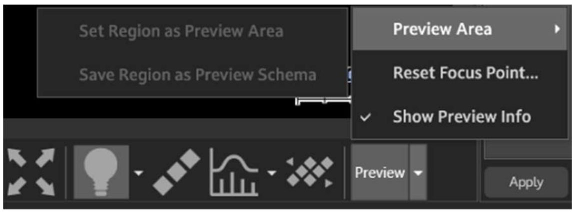
Used	Name		
☑	DAPI DAPI	1428 - 32295	Auto

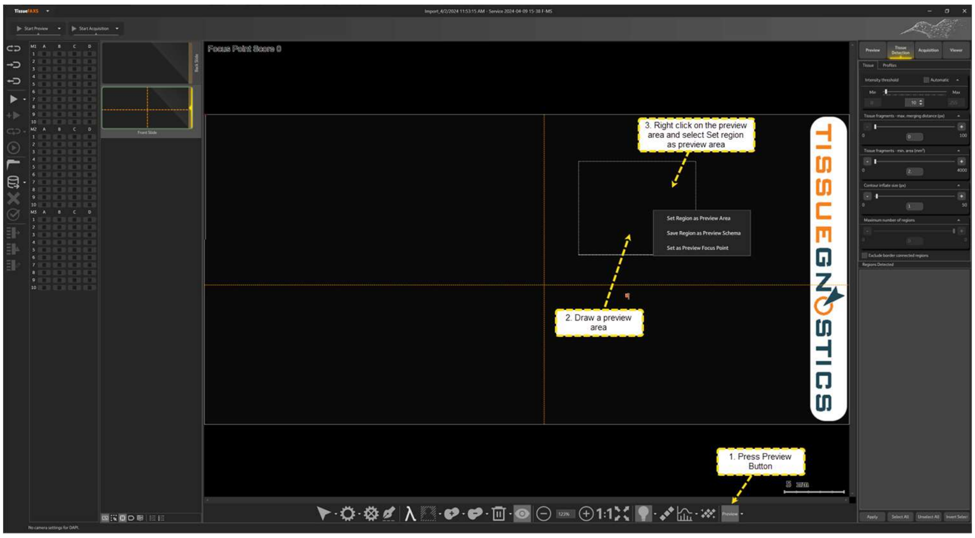
Select All ● Monochrome ○ Color Auto
Preview
Apply | Select All | Unselect All | Invert Select

- **Turn on/off gray image**

- **Preview**

You can use the default preview schema or define a new one from the Tissue Detection **Preview** button, as shown below.





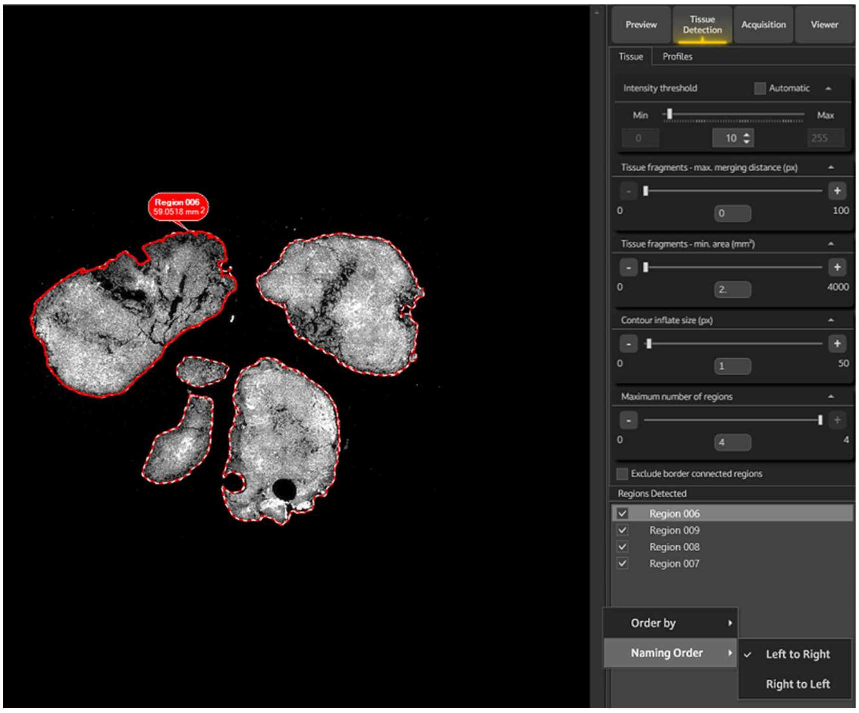
3. Right click on the preview area and select Set region as preview area

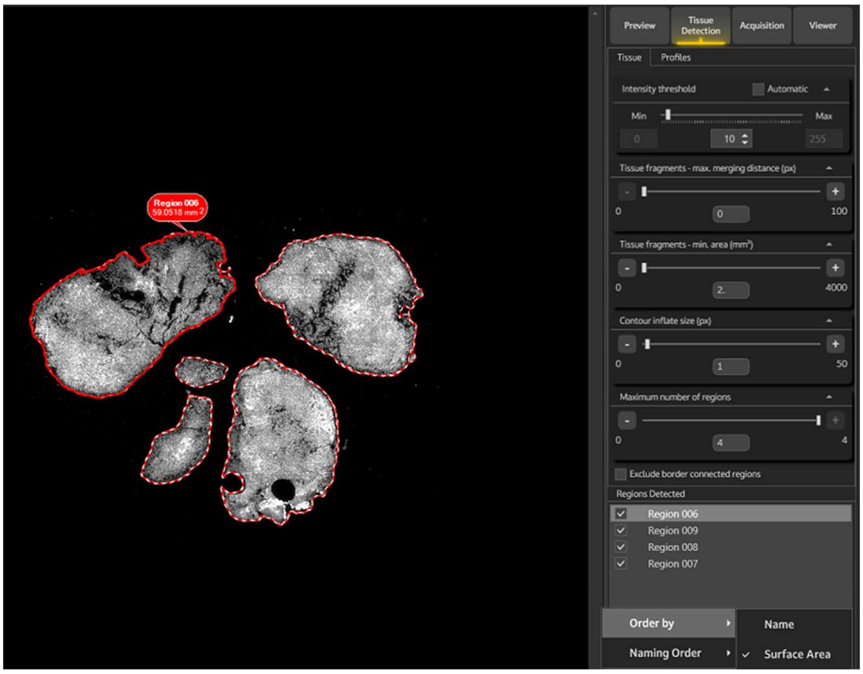
2. Draw a preview area

1. Press Preview Button

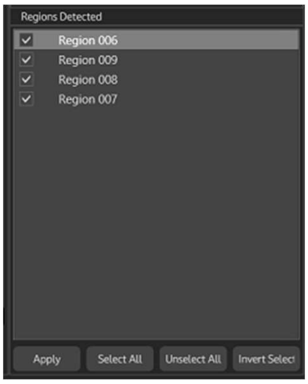
Detected ROIs manager

- The region resulted from the tissue detection will be shown in a list. Check the ones you want to acquire, then press **Apply**.
- Detected regions can be ordered by name or by surface area.
- The naming order can be made from left to right or from right to left.

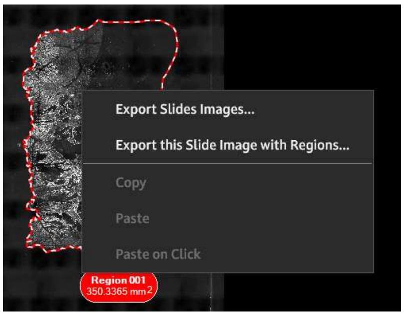




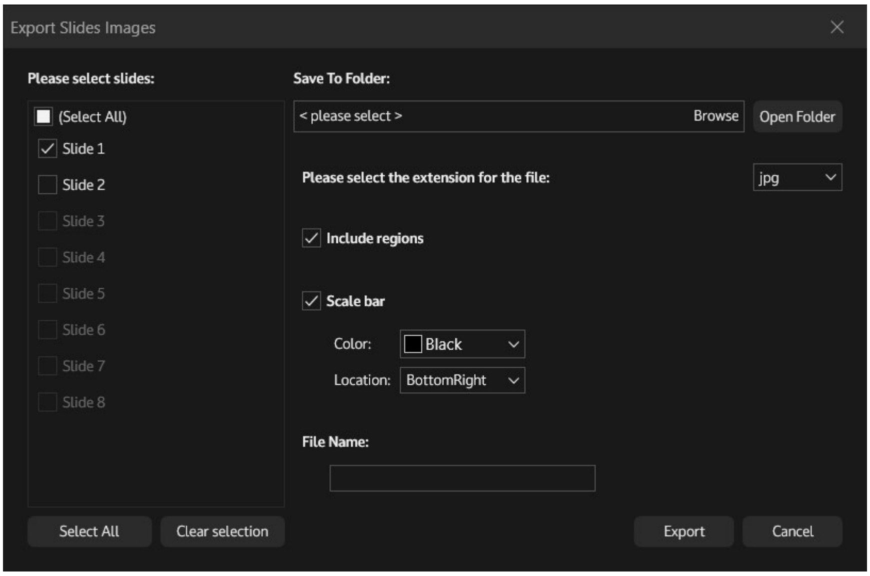
- For the detected regions, you have a set of selection controls: **Select All, Unselect All, Invert Selection**.



Slide Contextual Menu in Image Viewer



- **Export Slides Images**: select slides you need to import, then choose a location for the export. Also, you can choose to include in the export the regions (if any) and the scalebar;


Export Slides Images

Please select slides:
☐ (Select All)
☑ Slide 1
☐ Slide 2
☐ Slide 3
☐ Slide 4
☐ Slide 5
☐ Slide 6
☐ Slide 7
☐ Slide 8

Save To Folder: < please select >

Please select the extension for the file: jpg

☑ Include regions

☑ Scale bar

Color: Black

Location: BottomRight

File Name:

- **Export this Slide Image with Regions**: you can export just the image of the current slide with its regions, if any;
- **Copy/Paste**
- **Paste on Click**

Edit ROIs

To edit a region, press **Edit** button from the **Image Viewer** toolbar, then choose one of the options described below:

Any region can be modified (resized, moved), as long as no images have been acquired for them.

- **Moving** a ROI requires 2 steps:

1. Select the region by right clicking an inner point of its shape;
2. Use the arrows from the keyboard to move the region to desired location.

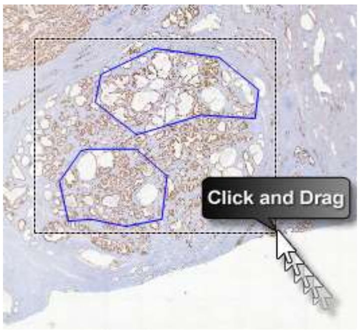
- **Resize or reshape ROI**:

Each region has special points that can be dragged for resizing or changing its shape, depending on the region type:

- For **Custom Regions**, select one by one the points that you want to move (they appear marked by a little blue highlight). All desired points must be modified one by one to reshape the region, by dragging them with the mouse in the desired direction.
- For **Rectangular Regions**, the entire rectangle is resized when the selected point is moved inwards or outwards (it appears marked by a little blue highlight).
- **Circular Regions** are resized by selecting a point on a region's contour and dragging inwards or outwards (it appears marked by by a little blue highlight).
- **Copy and Paste ROI**

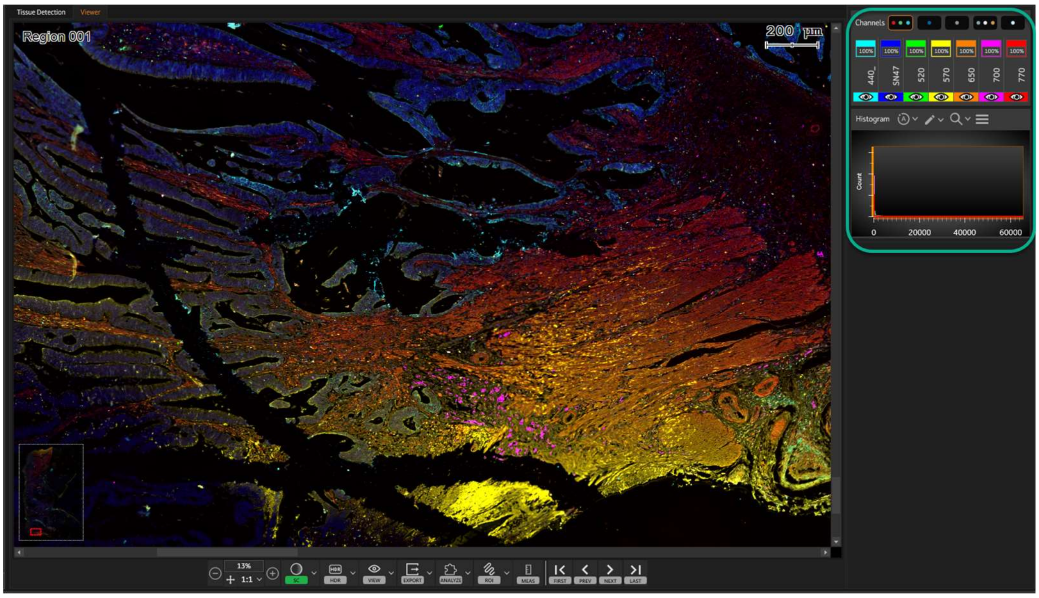
- **Copy Region**: this operation can be done by choosing the **Copy** option from the region contextual menu or by using the Ctrl+C combination from the keyboard.
- **Paste Region**: this operation can be done by choosing the **Paste** option from the region contextual menu or by using the Ctrl+V combination from the keyboard.

It is possible to select multiple regions to be copied, by using the mouse selection.



9. Viewer Options Tab

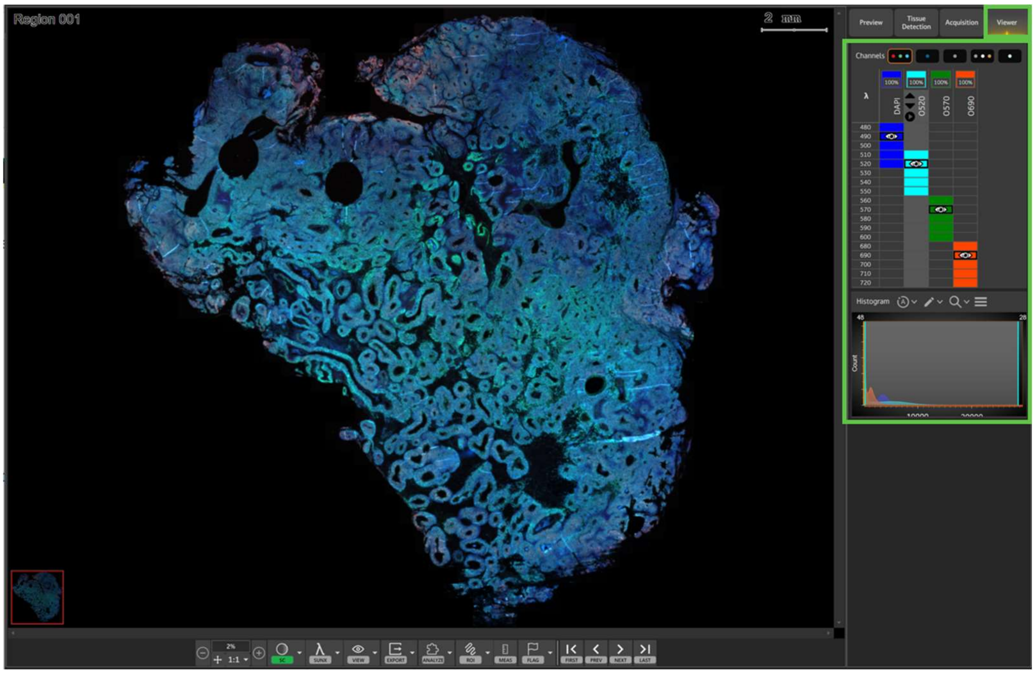
In the **Image Viewer** options section, you can find details related to selected image.



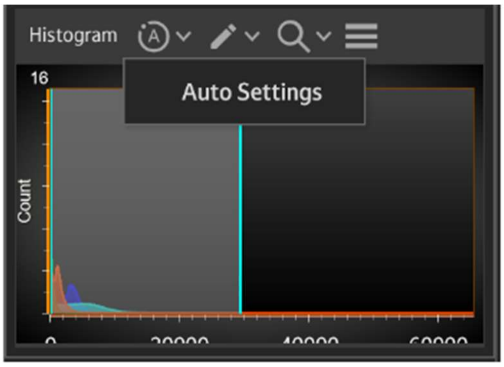
ROI Overlay

This feature allows choosing which channels to view in the acquired image.

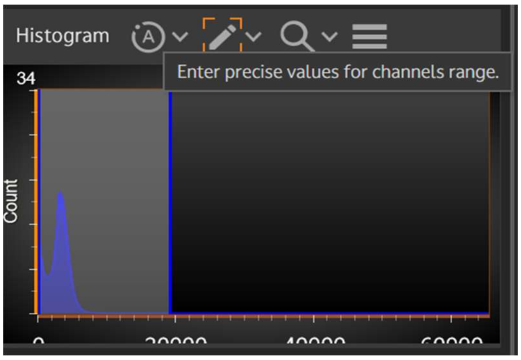
Here, adjustments can be made to the color, light intensity, and dynamic range (only for channels acquired with 16bit) for each channel.

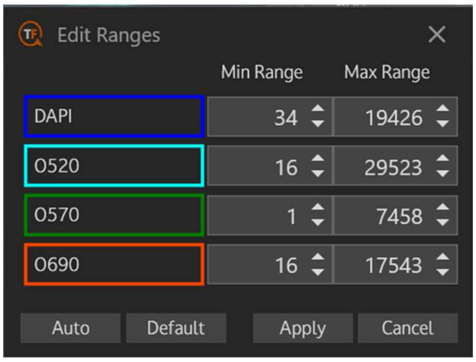


Pressing **Auto** will automatically compute the proper dynamic range settings.

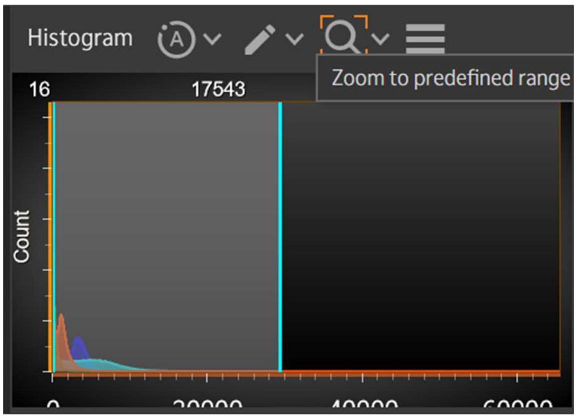


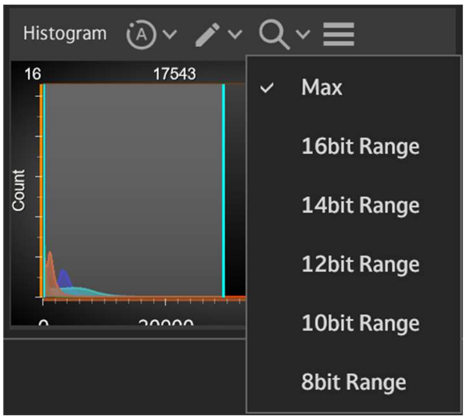
The **Dynamic range** can also be set manually, by manually entering values, as shown below.

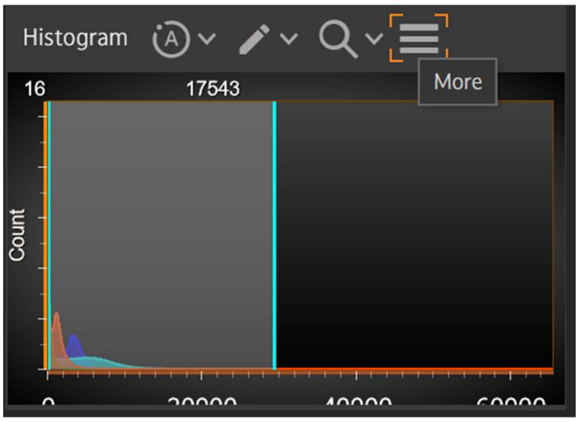


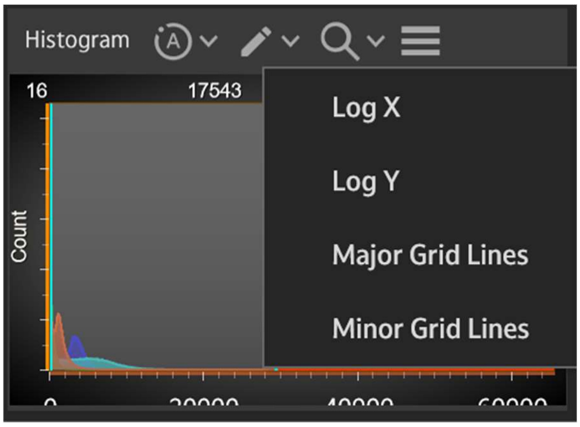


	Min Range	Max Range
DAPI	34	19426
0520	16	29523
0570	1	7458
0690	16	17543







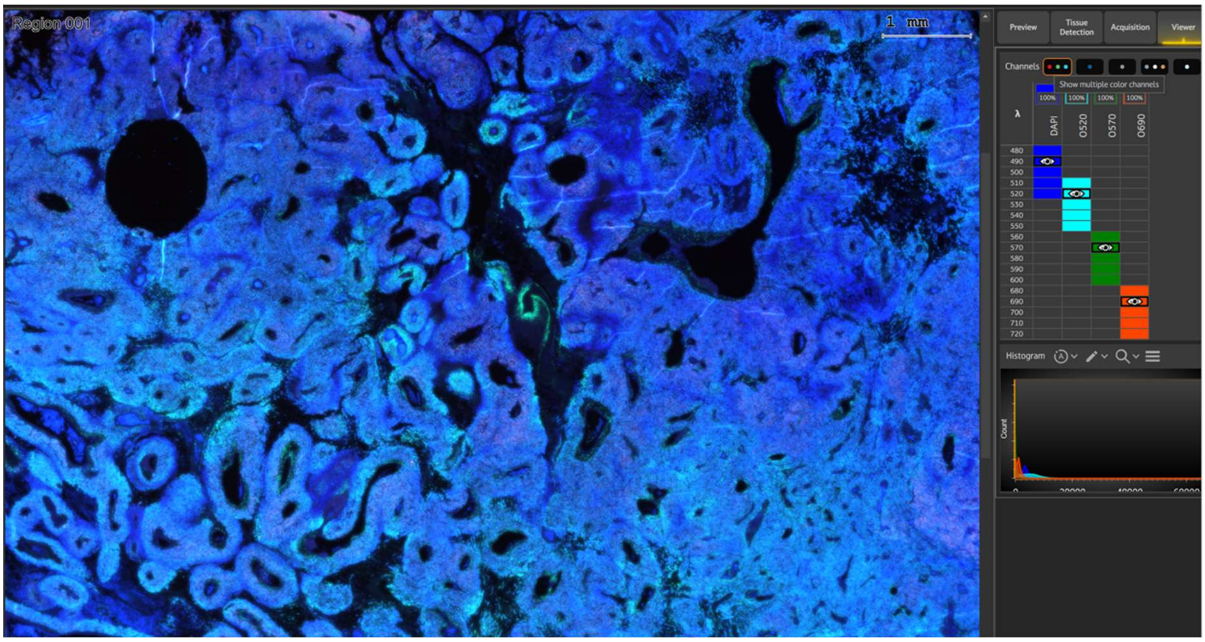


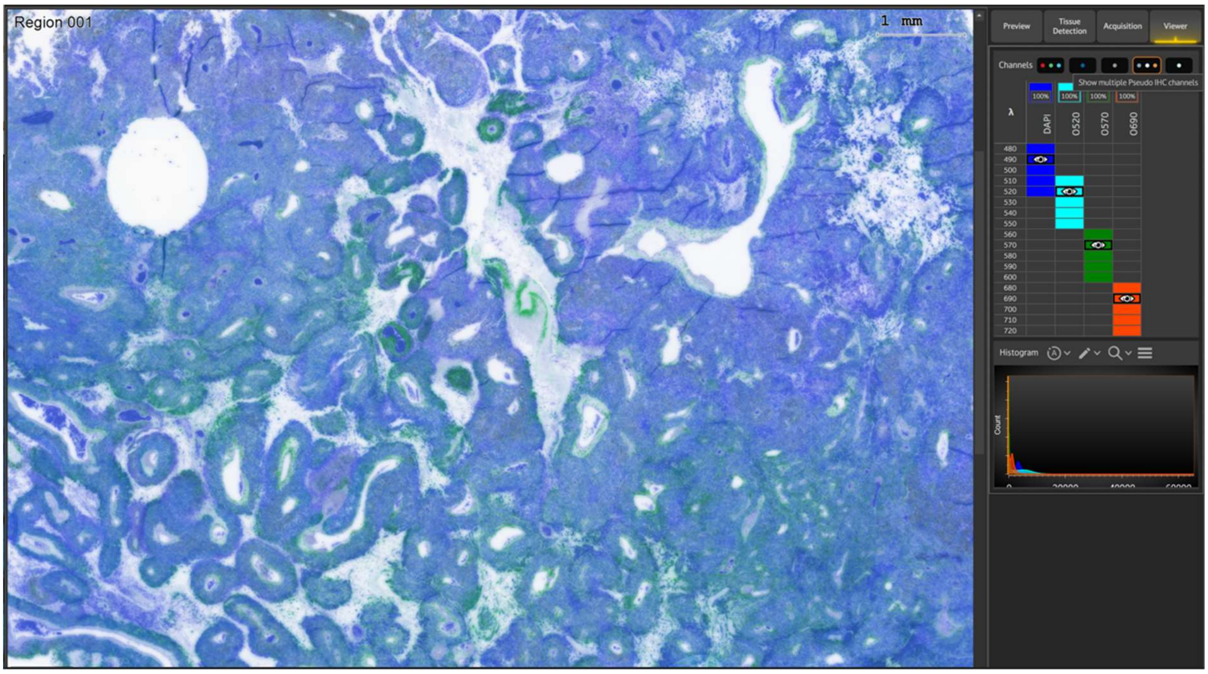
There are two ways of visualizing acquired images: **Monochrome** and **Color** mode.

- **Monochrome Mode**: will display a single channel at once, ignoring the color set for the respective channel;
- **Color Mode**: will display an overlay image.

Pseudo IHC Mode:

The Pseudo IHC view mode takes a monochrome image and converts it to a 24bpp color IHC-like image. In other words, a user can visualize individual channel fluorescent images as converted in brightfield images. The purpose of this conversion is an easier visual evaluation of morphological details.

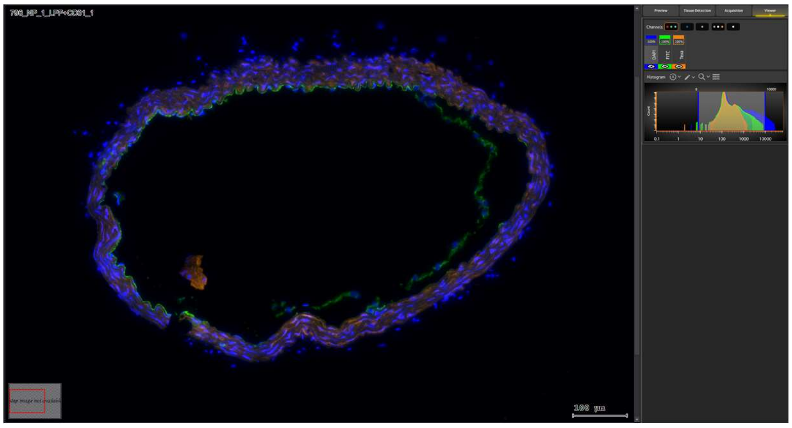


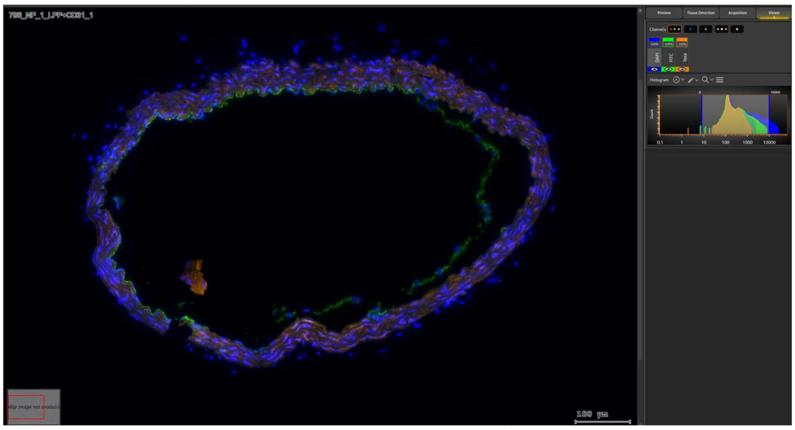


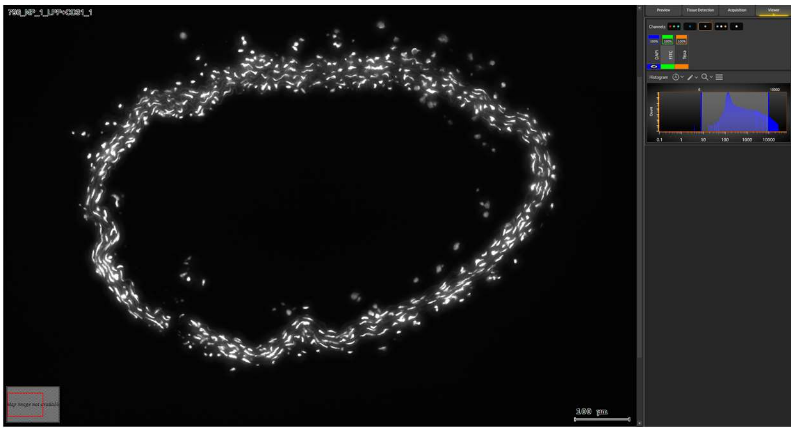
Easy visualization for overlay images

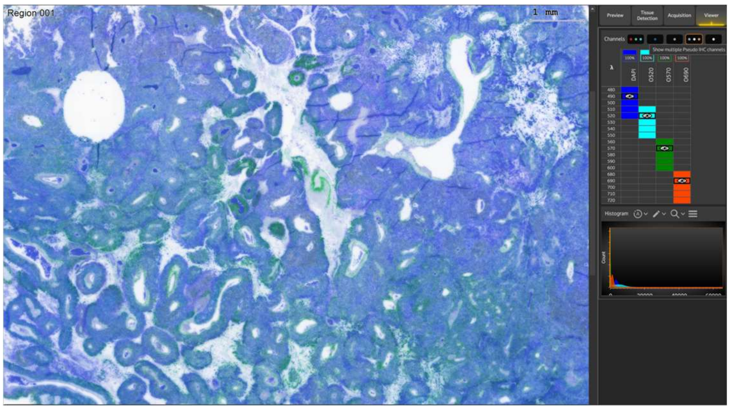
For fluorescence projects, the **Region Viewer** displays visualization buttons (the eye shaped button on each channel) for an easy selection of the channels.

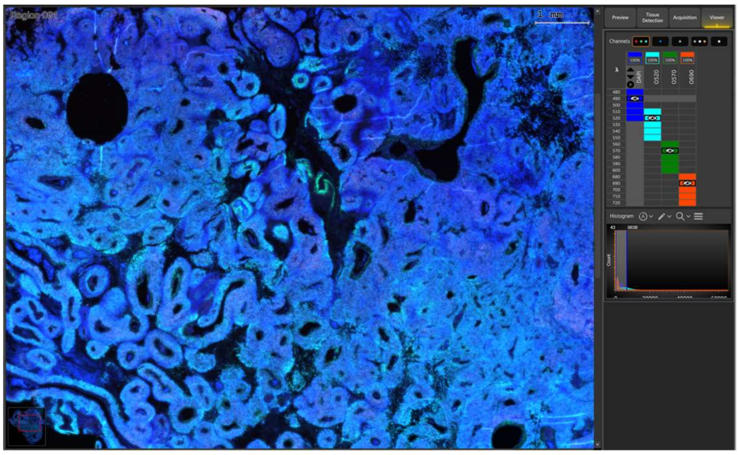
Below, you can see fluorescence and multispectral images. Please notice the differences between the channel selection.

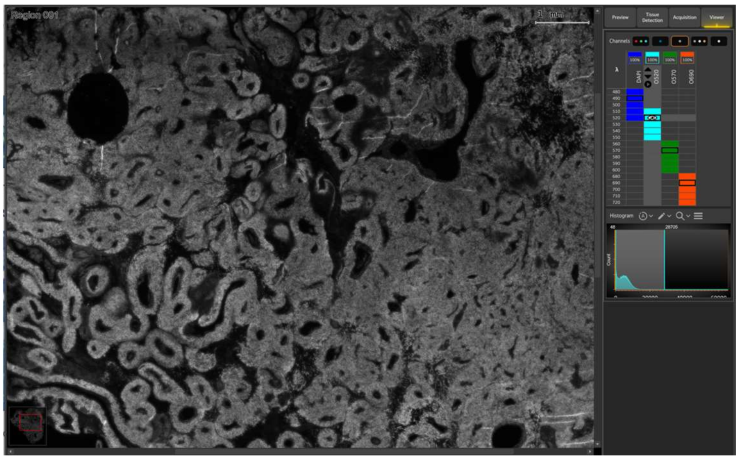

Preview
Tissue Detection
Acquisition
Viewer
Channels
DAPI
FITC
Histogram
Count
0.1
1
10
100
1000
10000
100 µm


Preview
Tissue Detection
Acquisition
Viewer
Channels
DAPI
FITC
Histogram
Count
0.1
1
10
100
1000
10000
100 µm


Preview
Tissue Detection
Acquisition
Viewer
Channels
DAPI
FITC
Histogram
Count
0.1
1
10
100
1000
10000
100 µm

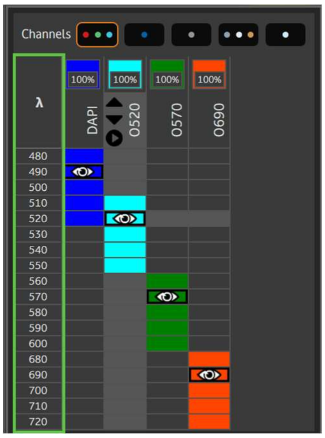

Region 001
1 mm
Preview
Tissue Detection
Acquisition
Viewer
Channels
Show multiple Pseudo IHC channels
100%
100%
100%
100%
λ
DAPI
O520
O570
O690
480
490
500
510
520
530
540
550
560
570
580
590
600
680
690
700
710
720
Histogram
Count





In **multispectral** experiments, acquired images display a specific feature: *wavelength selection channel*.

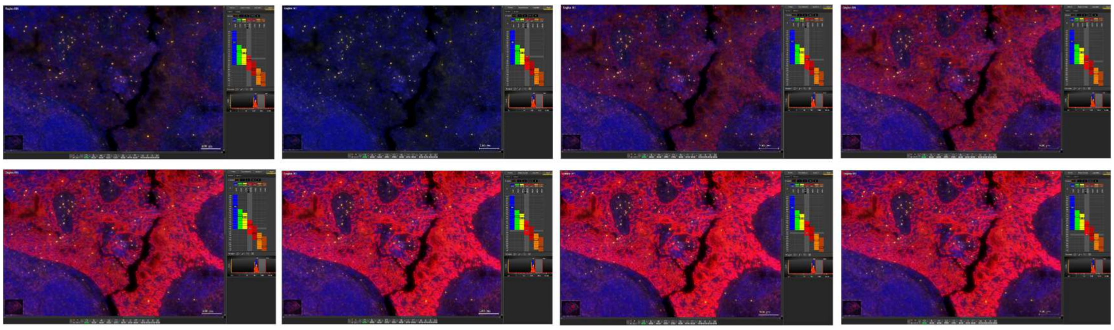
The **Eye button** controls the wavelength displayed for the selected channel.



Show Peak Wavelength: the image selected with a black rectangle will display the wavelength value giving the best signal for the selected channel.

The image that will be displayed in the **Region Viewer/Acquired Images** is an overlay (if **Color Mode** is selected) between the channels checked in the **Overlay** section for each selected wavelength.

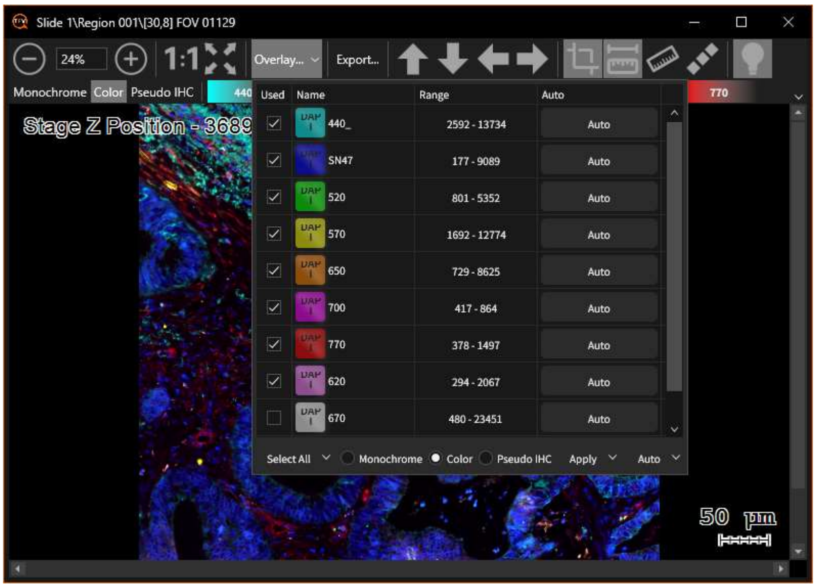
In the example below, you can see how the overlay image changes depending on the selected **Lambda** stack image (in this example the step is set to value “10”) for a single reflector.



Note: In monochrome mode, only one channel will be selected.

Field of View Overlay

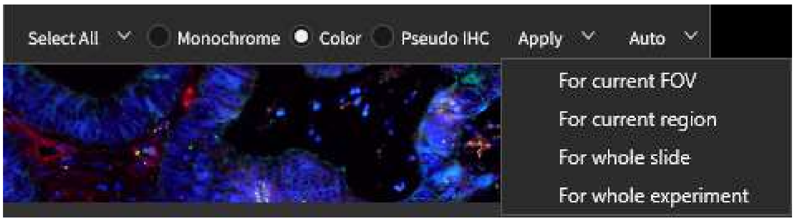
The **field of view (FOV) Overlay** displays a panel for adjusting channels intensities and colors.



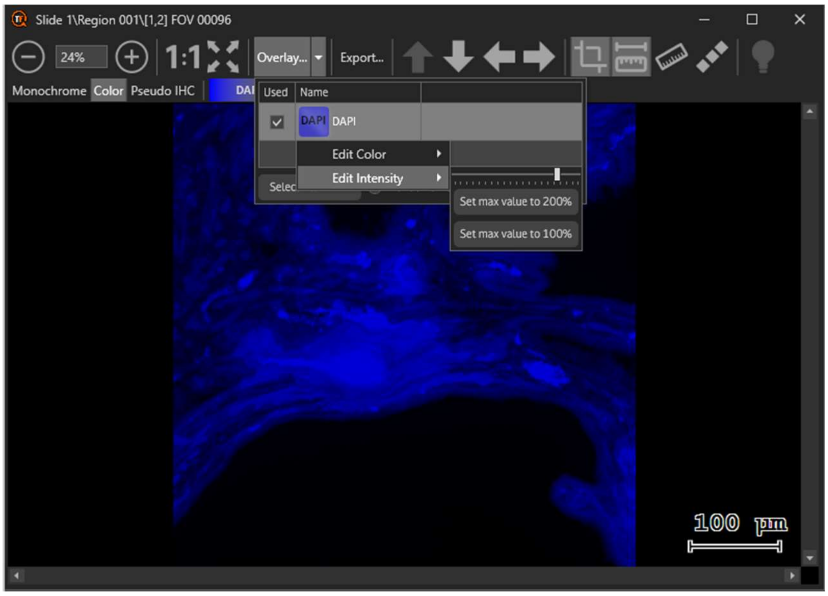
Slide 1\Region 001\[30,8] FOV 01129

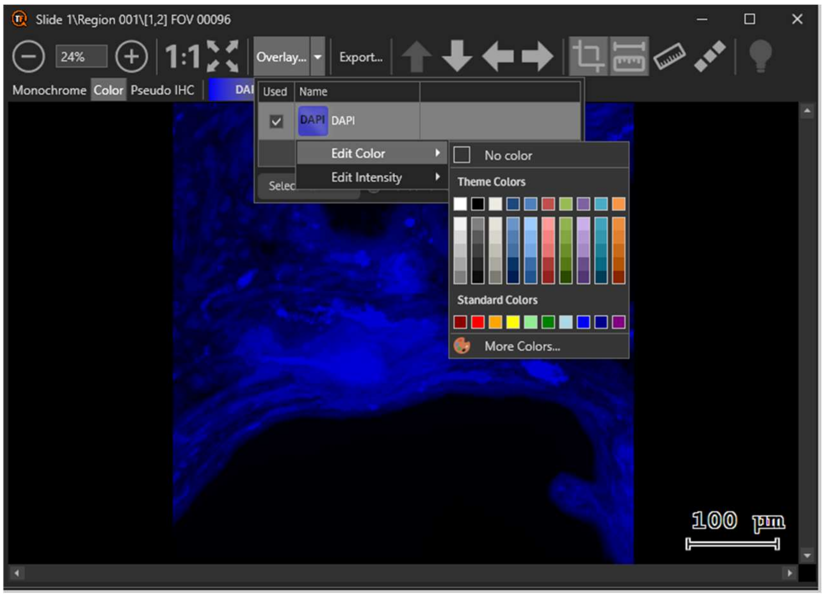
Used	Name	Range	Auto
☑	440_	2592 - 13734	Auto
☑	SN47	177 - 9089	Auto
☑	520	801 - 5352	Auto
☑	570	1692 - 12774	Auto
☑	650	729 - 8625	Auto
☑	700	417 - 864	Auto
☑	770	378 - 1497	Auto
☑	620	294 - 2067	Auto
☐	670	480 - 23451	Auto

Apply button has multiple options:



The user can also adjust the **intensity** and color for each channel by typing the desired value in the corresponding field.



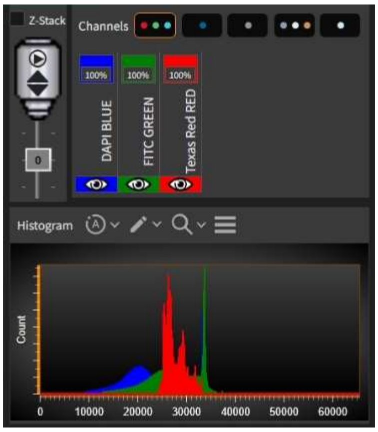


Any modification done in the **Overlay** window can be seen in real time.

Z-Stack

Z Stack represents a set of images that are acquired above and below the optimal focus position. By combining these images, a final FOV image will result, containing a greater level of detail than any of the initial Z Stack.

You can navigate through different levels of Z-Stack.

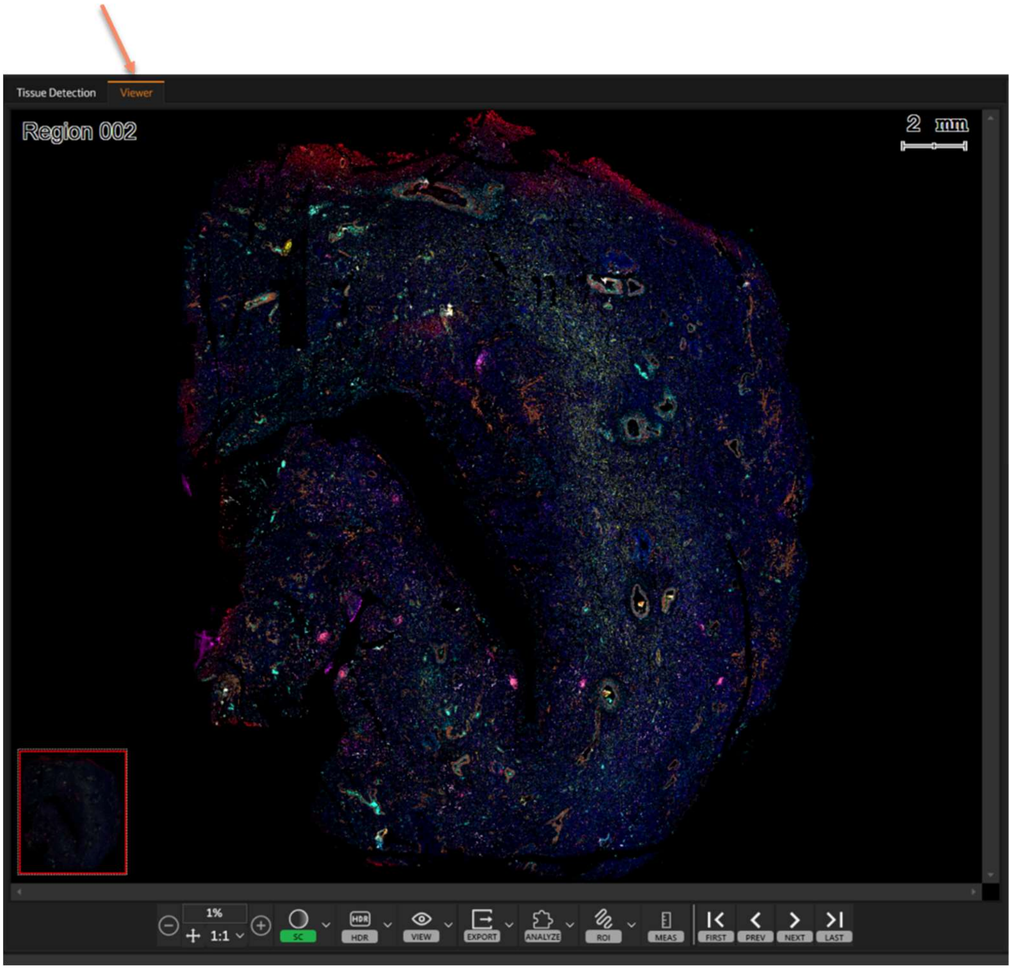


10. Image Viewer

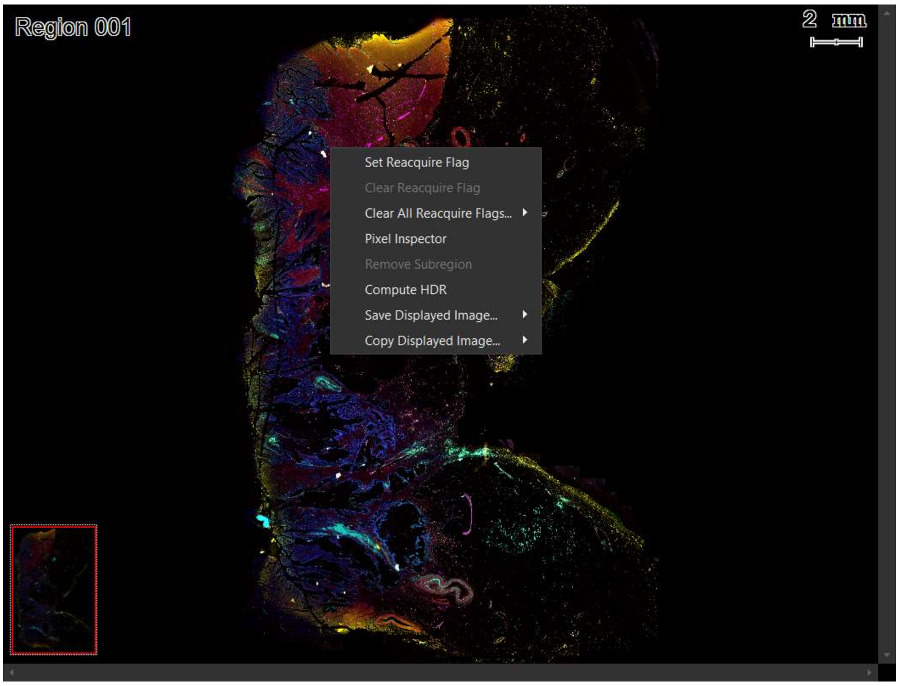
The image visualization tool in TissueFAXS Viewer is called **region viewer**.

A region is an array of FOVs disposed in a matrix structure. Each FOV has its own position within the matrix, which represents the number of rows and columns. The FOV Matrix Size property of the region represents the number of rows and columns.

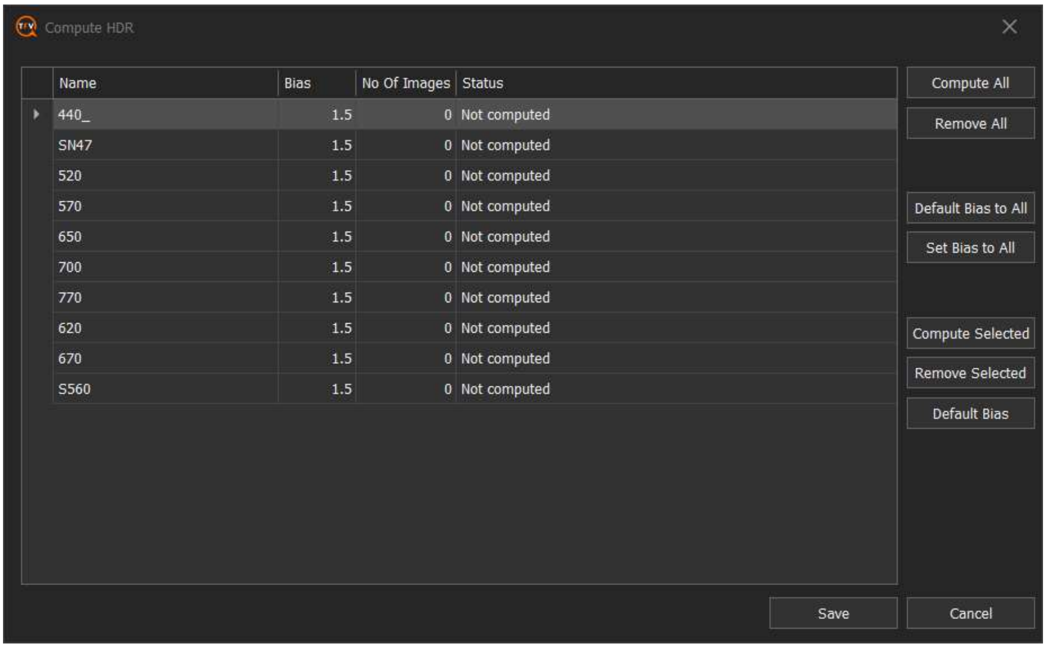
To view acquired items, double-click on the item from the project browser (on the left side of the main window).



Region Contextual Menu in Image Viewer



- **Set Reacquire Flag**
- **Clear Reacquire Flag**
- **Clear All Reacquire Flags**
- **Pixel Inspector**: activates Pixel Inspector feature (see Tools and Viewing Options);
- **Remove Subregion**: clears existing subregions, if any;
- **Compute HDR**: computes HDR for the channels;



Name	Bias	No Of Images	Status
440_	1.5	0	Not computed
SN47	1.5	0	Not computed
520	1.5	0	Not computed
570	1.5	0	Not computed
650	1.5	0	Not computed
700	1.5	0	Not computed
770	1.5	0	Not computed
620	1.5	0	Not computed
670	1.5	0	Not computed
S560	1.5	0	Not computed

Bias operations

The Bias parameter can be used to control the overall intensity increase in the generated image.

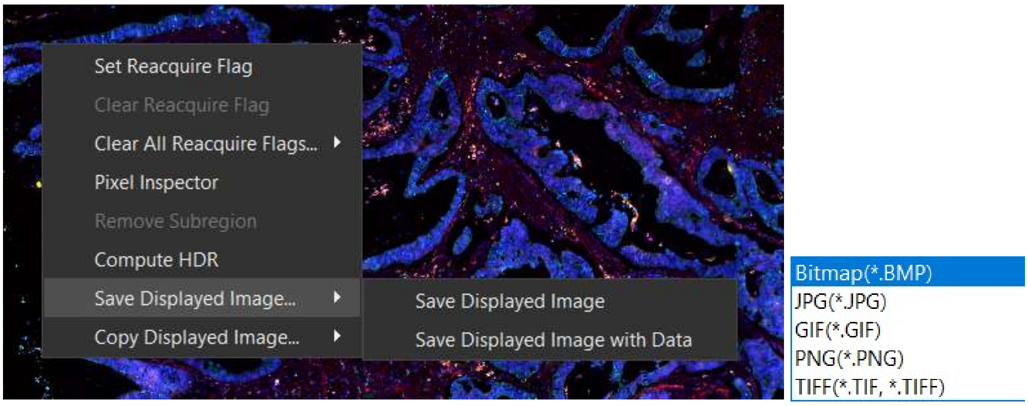
A lower value will generate a bigger amplification for lower intensities. If the HDR image tends to be over-saturated, the Bias value should be increased.

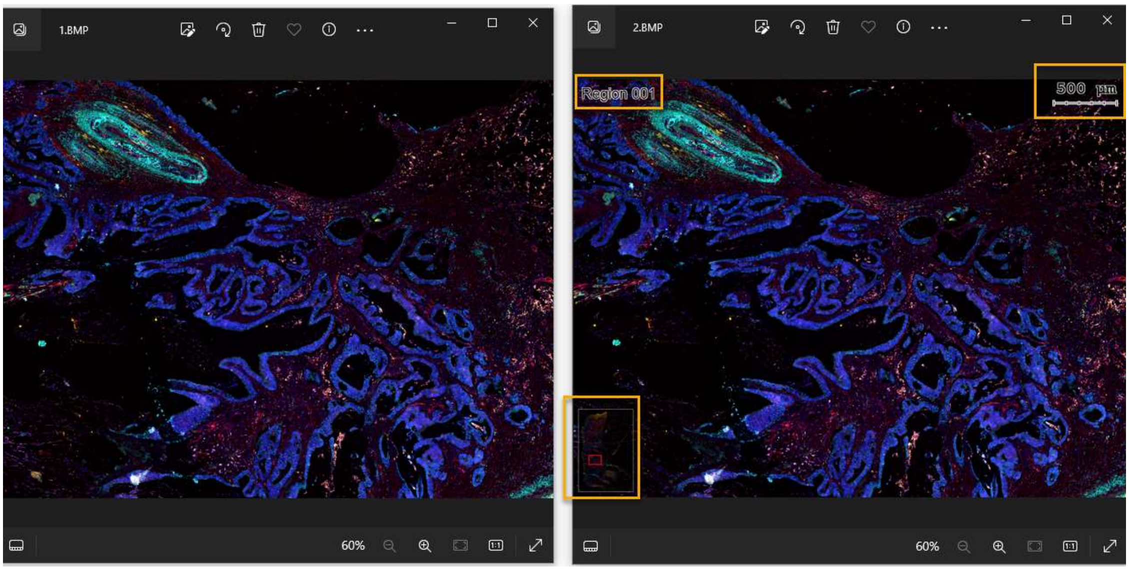
- **Default Bias to All**: apply default bias value to all channels;

- **Set Bias to All**: set same bias value to all channels;

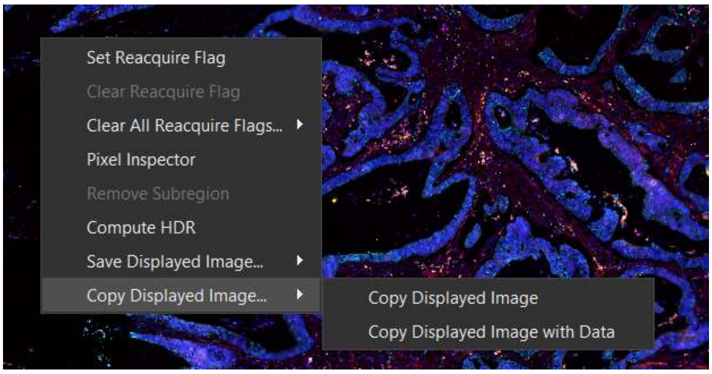
- **Default Bias**: choose the default bias value.

- **Save Displayed Image**: saves the image currently displayed in the Image Viewer in various graphical formats. You can choose to save the image with or without data (name, map, scalebar etc.)



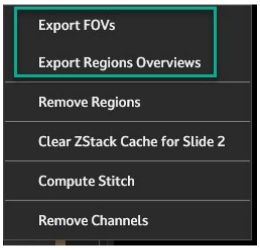


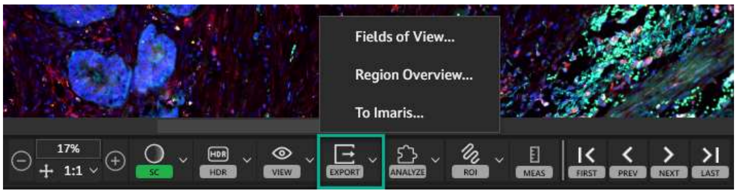
- **Copy Displayed Image:** copies currently displayed image from the image viewer, with or without data.



10.1. Export

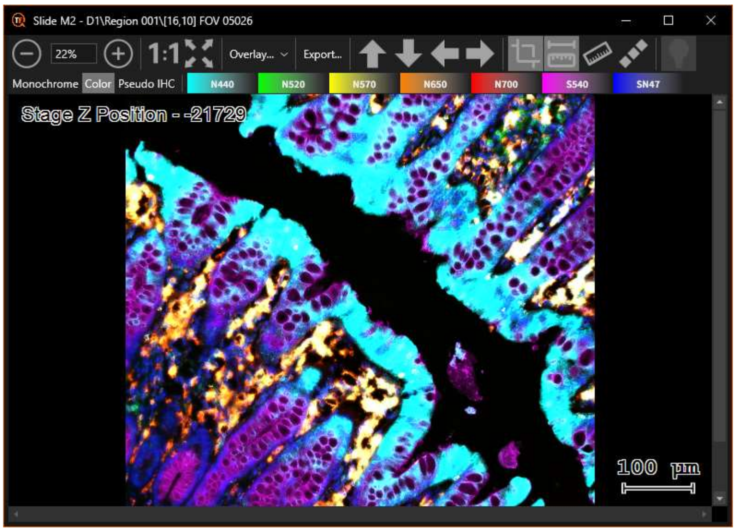
The main export options are located in region's contextual menu and in the **Export** section from the **Image Viewer** toolbar.



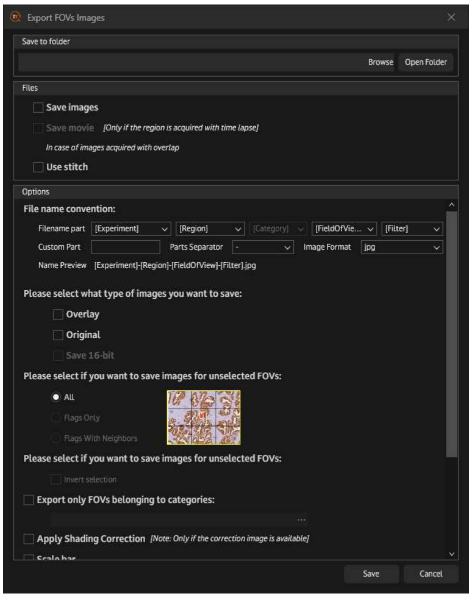


10.1.1. Fields of View

Double click on a FOV to open it, then press **Export** from the FOV Viewer Toolbar.



Export FOV's Images panel will open.



☐ Save images
☐ Save movie *[Only if the region is acquired with time lapse]*
In case of images acquired with overlap
☐ Use stitch

File name convention:
Filename part [Experiment] [Region] [Category] [FieldOfVie... [Filter]
Custom Part
Parts Separator -
Image Format jpg
Name Preview [Experiment]-[Region]-[FieldOfView]-[Filter].jpg

Please select what type of images you want to save:
☐ Overlay
☐ Original
☐ Save 16-bit

Please select if you want to save images for unselected FOVs:
● All
○ Flags Only
○ Flags With Neighbors

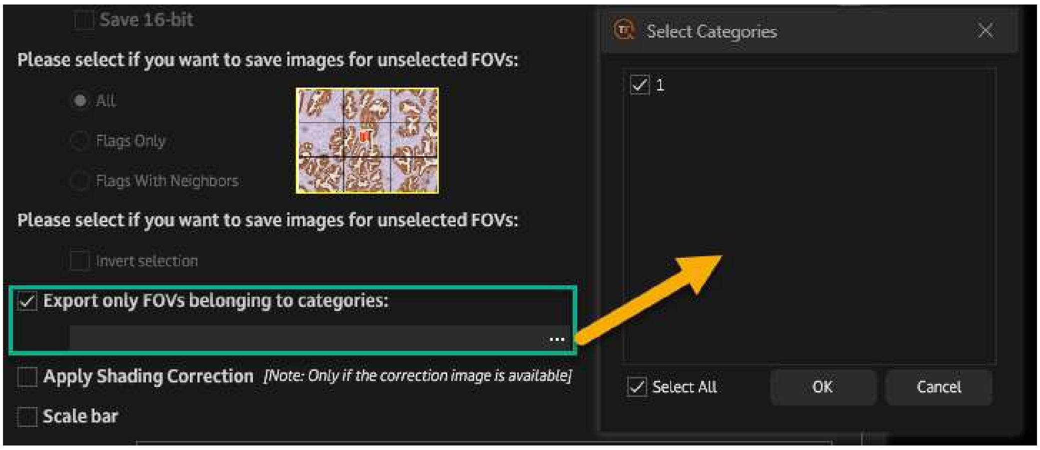
Please select if you want to save images for unselected FOVs:
☐ Invert selection

☐ Export only FOVs belonging to categories:

☐ Apply Shading Correction *[Note: Only if the correction image is available]*
☐ Scale bar

TissueFAXS Viewer allows you configure the export as you desire, by choosing exactly the contents that is of interest to you. In the dialog displayed above, you can adjust the following **settings**:

- Select the **storage folder**;
- Select **files** to be exported:
- Images
- Movies (option available only if the region was acquired with time lapse)
- Use stitch
- Select the **file name convention** (Name Parts, Custom Part, Parts, Image Format, Name Preview);
- Select the **type of images** you want to save:
- **Overlay –** The FOV images will be composed from all the channels as currently specified in region viewer.
- **Original** – The FOV images will be exported separately for each channel as they were acquired.
- Select **what images will be saved**:
- **All**;
- **Flags Only** and **Flags with Neighbors** (these options are available only if you have at least one flag set).
- **Invert selection** – Only the FOVs not marked with flag or neighbors of the flagged FOVs (applies if **Flags with Neighbors** is selected) are exported;
- **Export only FOVs belonging to categories**: in this case, only the FOVs that belong to the selected categories are exported, by pressing the browse button.



☐ Save 16-bit

Please select if you want to save images for unselected FOVs:

● All
○ Flags Only
○ Flags With Neighbors

Please select if you want to save images for unselected FOVs:

☐ Invert selection

☑ Export only FOVs belonging to categories:

☐ Apply Shading Correction *[Note: Only if the correction image is available]*

☐ Scale bar

Select Categories

☑ 1

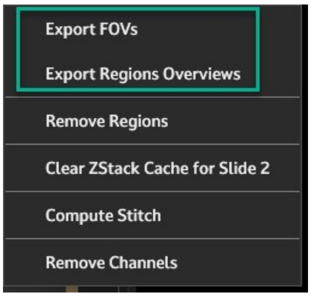
☑ Select All

<u>**Note**</u>: In the selection list, only those categories will be listed that contain tissue areas on their overview image.

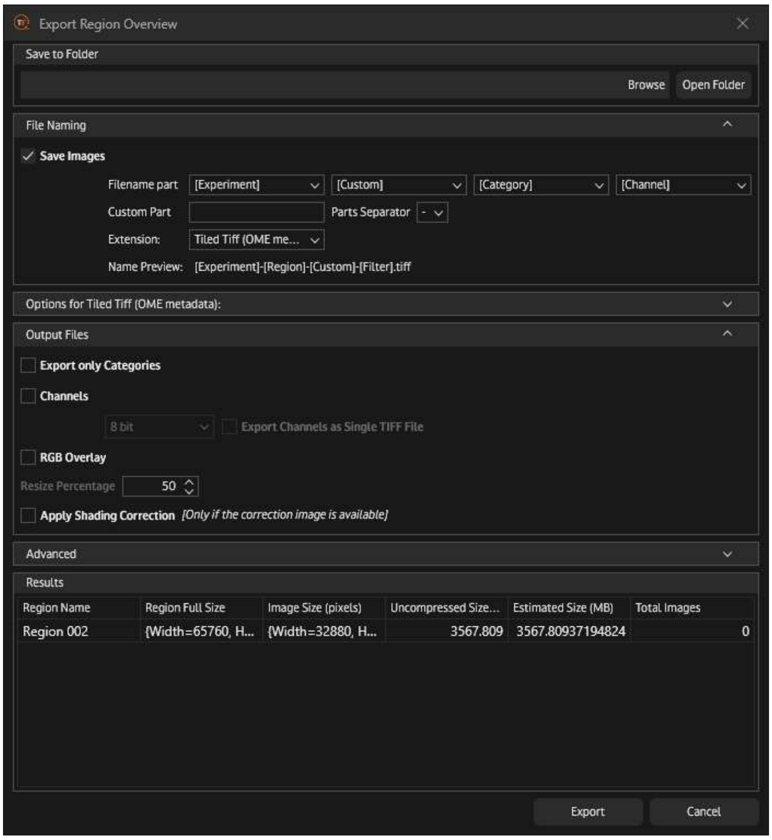
- **Mark Crop Area**: it marks the contour of the region for FOVs near the region border; you can also select its color;
- Scale bar
- **Apply Illumination Correction**: this option is enabled only for **Brightfield** experiments. The correction image will be applied to exported images if the correction image is available.

10.1.2. Region Overview

Choose **Export Regions Overviews** from any acquired region contextual menu.



The following dialog will appear:



☑ Save Images

Filename part: [Experiment] | [Custom] | [Category] | [Channel]

Custom Part:

Parts Separator: -

Extension: Tiled Tiff (OME me...

Name Preview: [Experiment]-[Region]-[Custom]-[Filter].tiff

☐ Export only Categories

☐ Channels

8 bit ☐ Export Channels as Single TIFF File

☐ RGB Overlay

Resize Percentage: 50

☐ Apply Shading Correction *[Only if the correction image is available]*

Region Name	Region Full Size	Image Size (pixels)	Uncompressed Size...	Estimated Size (MB)	Total Images
Region 002	{Width=65760, H...	{Width=32880, H...	3567.809	3567.80937194824	0

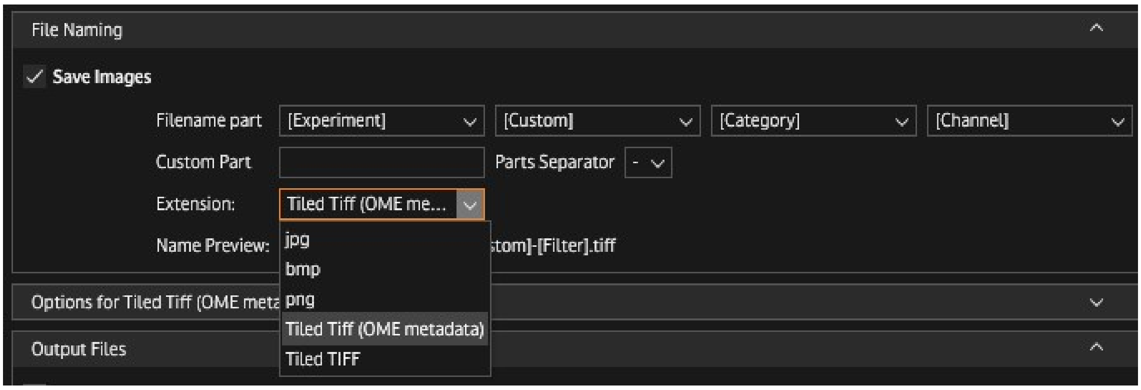
Some information is displayed for each region: **name, size, uncompressed size, estimated size**. If one of the regions is too large, the respective region will be highlighted in **red**.

TissueFAXS allows configuring the export as desired, by choosing exactly the contents of interests. In the dialog displayed above, the user can adjust the following settings:

- Select the **storage folder**;
- Select **files** to be exported:
- Images
- Movies (option available only if the region was acquired with time lapse)
- Enter **Custom Part**: type desired custom part for the name of the exported item.

Note: The file name to be exported consists of a default part and a custom part. The default part consists of items from the File Name Convention. The custom part is defined by the user.

- Select the file **extension**: choose exported item format (also available: *.tiff with multiple tiles* in order to export bigger images);



☑ Save Images

Filename part: [Experiment] [Custom] [Category] [Channel]

Custom Part:

Parts Separator: -

Extension: Tiled Tiff (OME me...

Name Preview: …stom]-[Filter].tiff

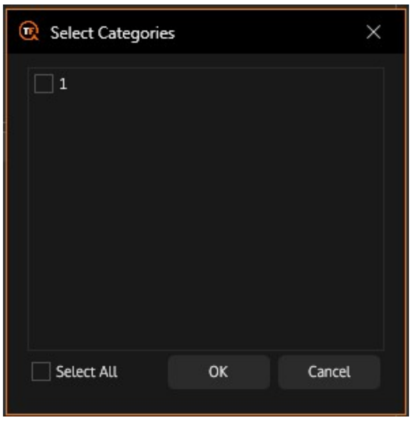
If choosing as extension the *tiff with multiple tiles*, the items from the **Options for tiff with multiple files** will be enabled:

- **Tile size**: the dimension of an image (tile) composing the tiff (there are three predefined options);
- **Compression**: there are two options – **None** and ***jpeg***.
- **Quality**: the quality of the compression can be adjusted using the slider.

Note: If choosing as extension the tiff with multiple tiles, the following options will not be available anymore: Mark Crop Area, Scale bar, Include Categories.

- **I want to stitch thumbnails**: choose this to export a low resolution of the region;
- **I want to specify a custom size for images to stitch**: choose the desired size of the resulting export image. Be aware that, for larger images, there is a memory restriction that comes from the operating system and hence, this operation may not work if a system is low of memory;
- **Mark Crop Area**: it marks the contour of the region on the final image; you can select its color;
- Scale bar
- **Include Categories**: in this case, the selected categories will appear on the final image.

Here the user can choose the categories to be included in the region overview image, by pressing the browse button (...).



Note: In the selection list, only those categories will be listed that contain regions on their overview image.

- **Opacity** of the categories: adjust the **Opacity** parameter, in order to choose the transparency of the tissue area name that appears on the region.
- **Apply Illumination Correction**: this option is enabled only for **Brightfield** experiments. The correction image will be applied to exported images if the correction image is available.

Notes:

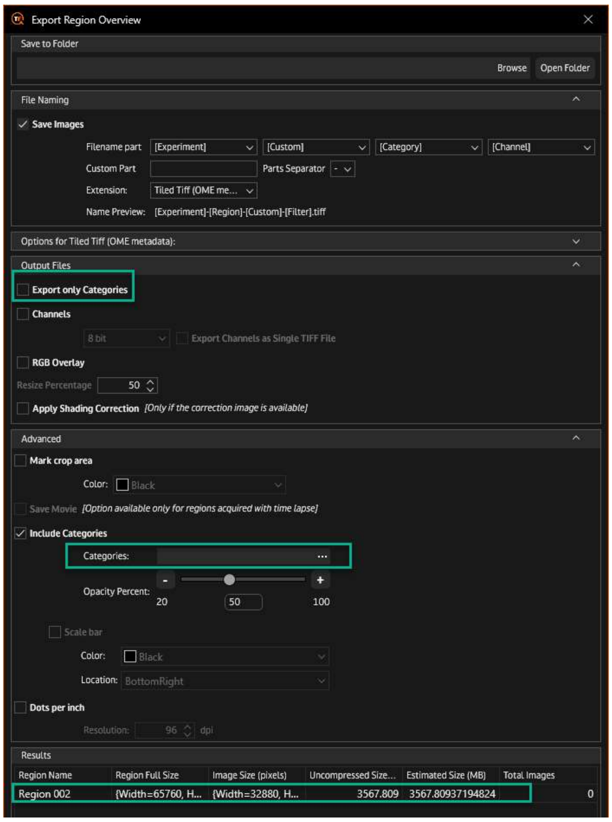
Restrictions regarding the images to be exported:

- For all export formats (excluding *tiff with multiple tiles*) the image size must not exceed 80000000 pixels.
- For *tiff with multiple tiles* the uncompressed size must not exceed SL0 Mb.

Export Overviews from Categories

To export categories, first check **Export only overviews from categories**. You should also select the types of categories to be exported, by pressing the ... button. The following dialog will appear, listing the available categories:

In the lower part of the **Export Region Overview** dialog you will now see a section including all the overviews that will be exported. For each overview the name and the image size is displayed.


Export Region Overview

Save to Folder

File Naming

☑ Save Images

Filename part: [Experiment] [Custom] [Category] [Channel]
Custom Part:
Parts Separator: -
Extension: Tiled Tiff (OME me...
Name Preview: [Experiment]-[Region]-[Custom]-[Filter].tiff

Options for Tiled Tiff (OME metadata):

Output Files

☐ Export only Categories
☐ Channels
8 bit ☐ Export Channels as Single TIFF File
☐ RGB Overlay
Resize Percentage: 50
☐ Apply Shading Correction *[Only if the correction image is available]*

Advanced

☐ Mark crop area
Color: Black
☐ Save Movie *[Option available only for regions acquired with time lapse]*
☑ Include Categories
Categories: ...
Opacity Percent: 20 50 100
☐ Scale bar
Color: Black
Location: BottomRight
☐ Dots per inch
Resolution: 96 dpi

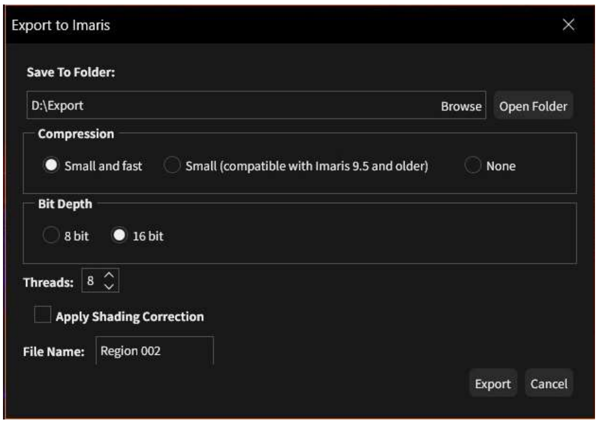
Results

Region Name	Region Full Size	Image Size (pixels)	Uncompressed Size...	Estimated Size (MB)	Total Images
Region 002	{Width=65760, H...	{Width=32880, H...	3567.809	3567.80937194824	0

10.1.3. Export to Imaris

TissueFAXS provides the option to export to *ims* file format which is mandatory for importing in **Imaris** software from *Oxford Instruments*.

Export to Imaris is available only for fluorescence experiments for the regions acquired with Z-stack.


Export to Imaris

Save To Folder: D:\Export

Compression: ● Small and fast ○ Small (compatible with Imaris 9.5 and older) ○ None

Bit Depth: ○ 8 bit ● 16 bit

Threads: 8

☐ Apply Shading Correction

File Name: Region 002

10.1.4. Batch Command Tool

Batch Command Tool is a TissueFAXS Viewer feature allowing automatically batch exporting a large number of region overviews from different projects.

The export will be made for any acquired region as OME TIFF with 16bit channels in multipage mode by default.

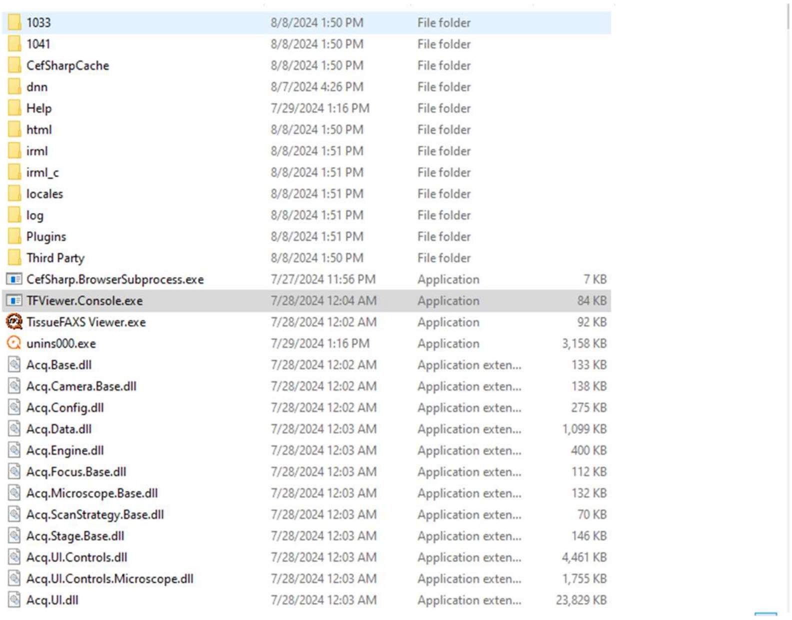
1. Creating Input /Output Folders (or select from existing folders)

The first step when using **Batch Command Tool** is creating on your computer two folders:

- An **Input** folder, where you will store your experiments/jobs;
- An **Output** folder, where you will store the output of the export, as such resulted regions overviews.

Note: The **Output** folder will inherit the folder structure from the Input folder.

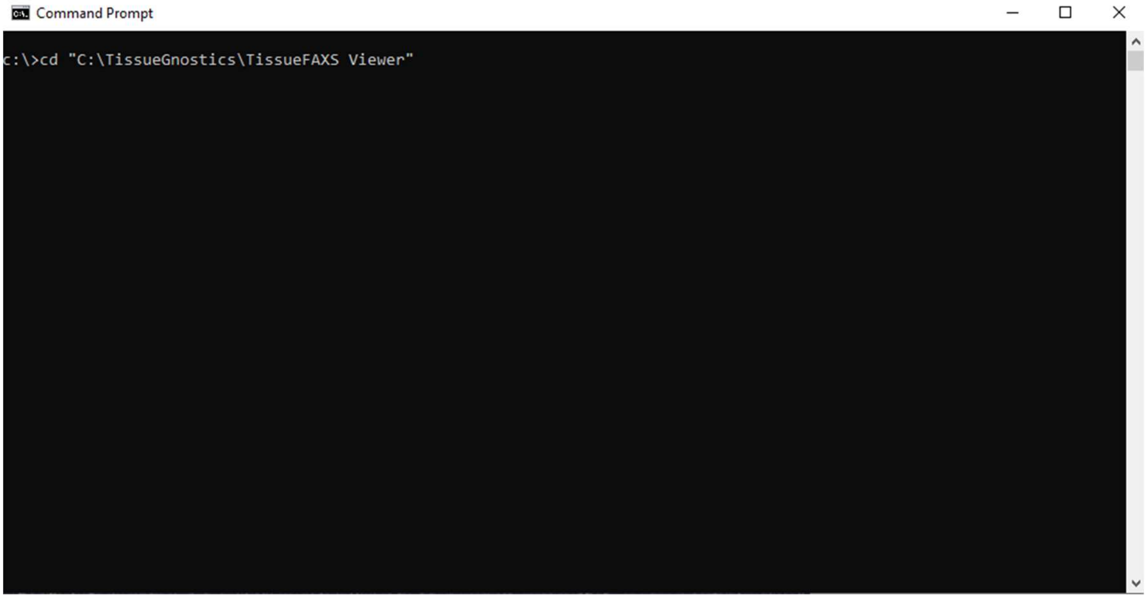
2. Using TFViewer.Console.exe



1033	8/8/2024 1:50 PM	File folder	
1041	8/8/2024 1:50 PM	File folder	
CefSharpCache	8/8/2024 1:50 PM	File folder	
dnn	8/7/2024 4:26 PM	File folder	
Help	7/29/2024 1:16 PM	File folder	
html	8/8/2024 1:50 PM	File folder	
irml	8/8/2024 1:51 PM	File folder	
irml_c	8/8/2024 1:51 PM	File folder	
locales	8/8/2024 1:51 PM	File folder	
log	8/8/2024 1:51 PM	File folder	
Plugins	8/8/2024 1:51 PM	File folder	
Third Party	8/8/2024 1:50 PM	File folder	
CefSharp.BrowserSubprocess.exe	7/27/2024 11:56 PM	Application	7 KB
TFViewer.Console.exe	7/28/2024 12:04 AM	Application	84 KB
TissueFAXS Viewer.exe	7/28/2024 12:02 AM	Application	92 KB
unins000.exe	7/29/2024 1:16 PM	Application	3,158 KB
Acq.Base.dll	7/28/2024 12:02 AM	Application exten...	133 KB
Acq.Camera.Base.dll	7/28/2024 12:02 AM	Application exten...	138 KB
Acq.Config.dll	7/28/2024 12:02 AM	Application exten...	275 KB
Acq.Data.dll	7/28/2024 12:03 AM	Application exten...	1,099 KB
Acq.Engine.dll	7/28/2024 12:03 AM	Application exten...	400 KB
Acq.Focus.Base.dll	7/28/2024 12:03 AM	Application exten...	112 KB
Acq.Microscope.Base.dll	7/28/2024 12:03 AM	Application exten...	132 KB
Acq.ScanStrategy.Base.dll	7/28/2024 12:03 AM	Application exten...	70 KB
Acq.Stage.Base.dll	7/28/2024 12:03 AM	Application exten...	146 KB
Acq.UI.Controls.dll	7/28/2024 12:03 AM	Application exten...	4,461 KB
Acq.UI.Controls.Microscope.dll	7/28/2024 12:03 AM	Application exten...	1,755 KB
Acq.UI.dll	7/28/2024 12:03 AM	Application exten...	23,829 KB

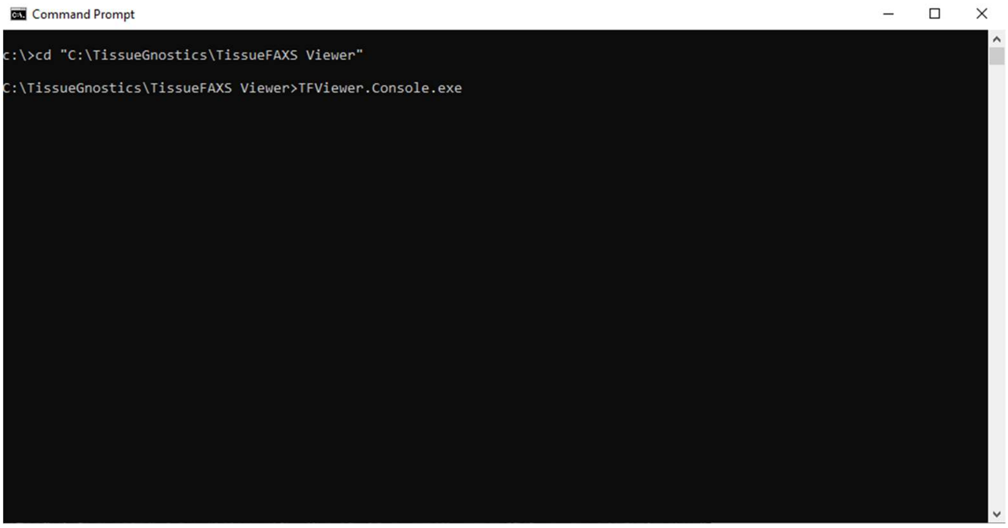
3. Now open Command Prompt window.

Go to **TissueFAXS Viewer** installation folder.



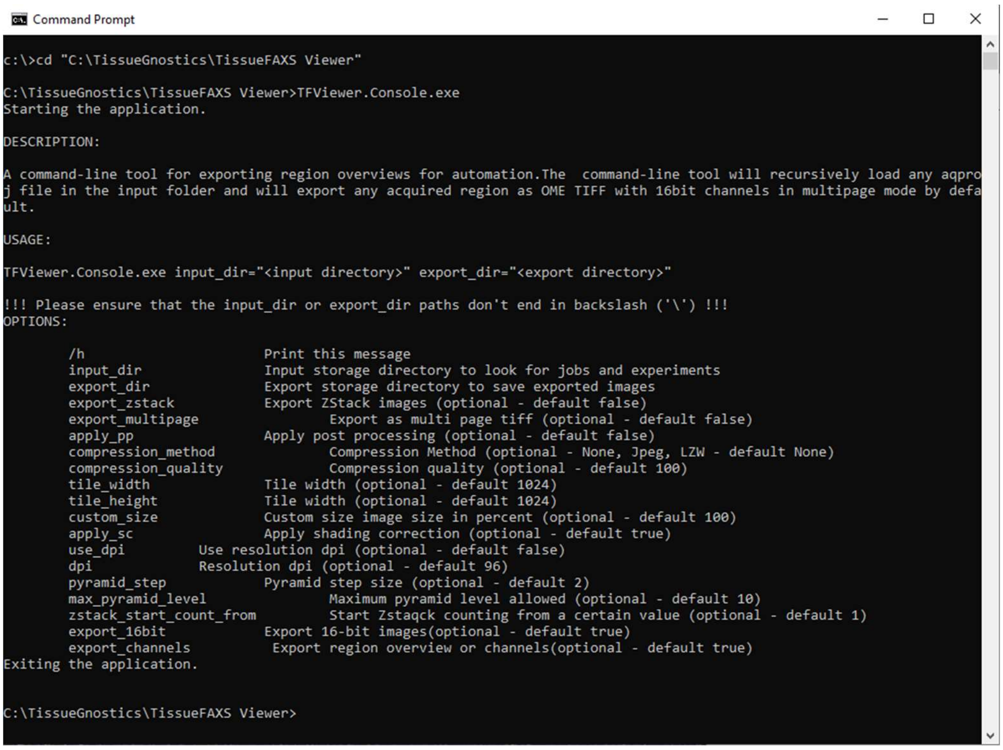
```
c:\>cd "C:\TissueGnostics\TissueFAXS Viewer"
```

4. Run TFViever.Console.exe



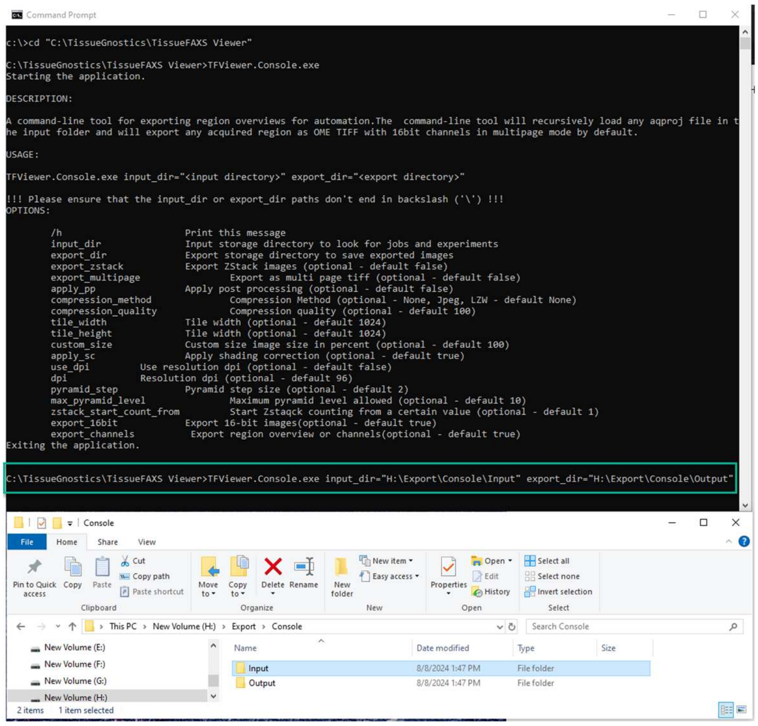
```
c:\>cd "C:\TissueGnostics\TissueFAXS Viewer"

C:\TissueGnostics\TissueFAXS Viewer>TFViewer.Console.exe
```

5. Tool Commands Description



```
c:\>cd "C:\TissueGnostics\TissueFAXS Viewer"

C:\TissueGnostics\TissueFAXS Viewer>TFViewer.Console.exe
Starting the application.

DESCRIPTION:

A command-line tool for exporting region overviews for automation.The  command-line tool will recursively load any aqpro
j file in the input folder and will export any acquired region as OME TIFF with 16bit channels in multipage mode by defa
ult.

USAGE:

TFViewer.Console.exe input_dir="<input directory>" export_dir="<export directory>"

!!! Please ensure that the input_dir or export_dir paths don't end in backslash ('\') !!!
OPTIONS:

        /h                      Print this message
        input_dir               Input storage directory to look for jobs and experiments
        export_dir              Export storage directory to save exported images
        export_zstack           Export ZStack images (optional - default false)
        export_multipage                Export as multi page tiff (optional - default false)
        apply_pp                Apply post processing (optional - default false)
        compression_method              Compression Method (optional - None, Jpeg, LZW - default None)
        compression_quality             Compression quality (optional - default 100)
        tile_width              Tile width (optional - default 1024)
        tile_height             Tile width (optional - default 1024)
        custom_size             Custom size image size in percent (optional - default 100)
        apply_sc                Apply shading correction (optional - default true)
        use_dpi         Use resolution dpi (optional - default false)
        dpi             Resolution dpi (optional - default 96)
        pyramid_step            Pyramid step size (optional - default 2)
        max_pyramid_level               Maximum pyramid level allowed (optional - default 10)
        zstack_start_count_from         Start Zstaqck counting from a certain value (optional - default 1)
        export_16bit            Export 16-bit images(optional - default true)
        export_channels          Export region overview or channels(optional - default true)
Exiting the application.


C:\TissueGnostics\TissueFAXS Viewer>
```

6. Command Example



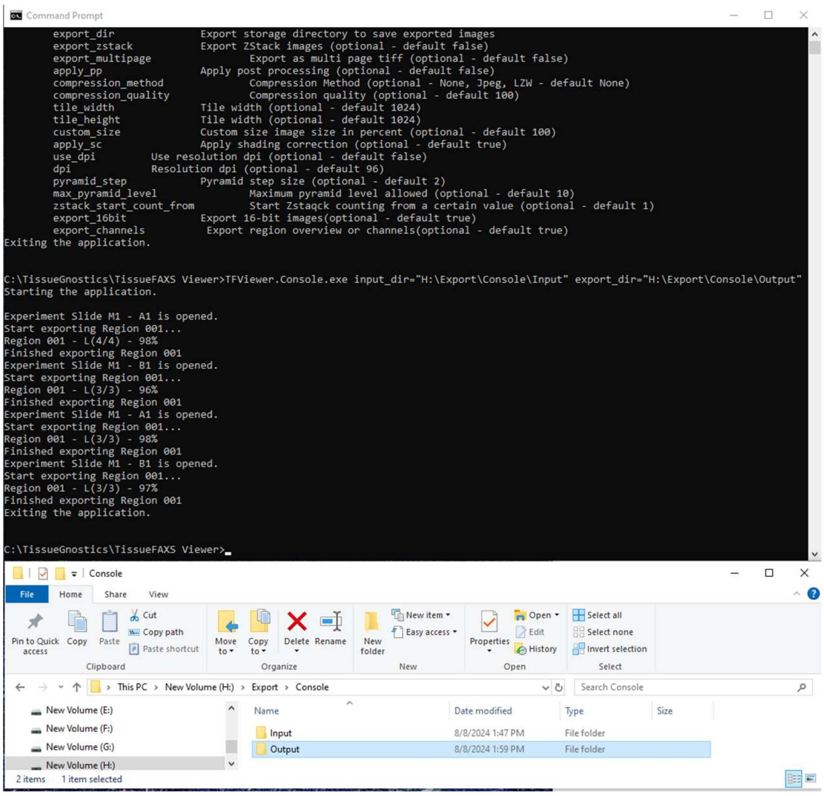
```
c:\>cd "C:\TissueGnostics\TissueFAXS Viewer"

C:\TissueGnostics\TissueFAXS Viewer>TFViewer.Console.exe
Starting the application.

DESCRIPTION:

A command-line tool for exporting region overviews for automation.The  command-line tool will recursively load any aqproj file in t
he input folder and will export any acquired region as OME TIFF with 16bit channels in multipage mode by default.

USAGE:

TFViewer.Console.exe input_dir="<input directory>" export_dir="<export directory>"

!!! Please ensure that the input_dir or export_dir paths don't end in backslash ('\') !!!
OPTIONS:

        /h                      Print this message
        input_dir               Input storage directory to look for jobs and experiments
        export_dir              Export storage directory to save exported images
        export_zstack           Export ZStack images (optional - default false)
        export_multipage                Export as multi page tiff (optional - default false)
        apply_pp                Apply post processing (optional - default false)
        compression_method              Compression Method (optional - None, Jpeg, LZW - default None)
        compression_quality             Compression quality (optional - default 100)
        tile_width              Tile width (optional - default 1024)
        tile_height             Tile width (optional - default 1024)
        custom_size             Custom size image size in percent (optional - default 100)
        apply_sc                Apply shading correction (optional - default true)
        use_dpi         Use resolution dpi (optional - default false)
        dpi             Resolution dpi (optional - default 96)
        pyramid_step            Pyramid step size (optional - default 2)
        max_pyramid_level               Maximum pyramid level allowed (optional - default 10)
        zstack_start_count_from         Start Zstaqck counting from a certain value (optional - default 1)
        export_16bit            Export 16-bit images(optional - default true)
        export_channels          Export region overview or channels(optional - default true)
Exiting the application.

C:\TissueGnostics\TissueFAXS Viewer>TFViewer.Console.exe input_dir="H:\Export\Console\Input" export_dir="H:\Export\Console\Output"
```

This PC › New Volume (H:) › Export › Console

Name	Date modified	Type	Size
Input	8/8/2024 1:47 PM	File folder	
Output	8/8/2024 1:47 PM	File folder	

2 items   1 item selected

7. Initiate automatic batch export for regions



```
        export_dir              Export storage directory to save exported images
        export_zstack           Export ZStack images (optional - default false)
        export_multipage                Export as multi page tiff (optional - default false)
        apply_pp                Apply post processing (optional - default false)
        compression_method              Compression Method (optional - None, Jpeg, LZW - default None)
        compression_quality             Compression quality (optional - default 100)
        tile_width              Tile width (optional - default 1024)
        tile_height             Tile width (optional - default 1024)
        custom_size             Custom size image size in percent (optional - default 100)
        apply_sc                Apply shading correction (optional - default true)
        use_dpi         Use resolution dpi (optional - default false)
        dpi             Resolution dpi (optional - default 96)
        pyramid_step            Pyramid step size (optional - default 2)
        max_pyramid_level               Maximum pyramid level allowed (optional - default 10)
        zstack_start_count_from         Start Zstaqck counting from a certain value (optional - default 1)
        export_16bit            Export 16-bit images(optional - default true)
        export_channels          Export region overview or channels(optional - default true)
Exiting the application.


C:\TissueGnostics\TissueFAXS Viewer>TFViewer.Console.exe input_dir="H:\Export\Console\Input" export_dir="H:\Export\Console\Output"
Starting the application.

Experiment Slide M1 - A1 is opened.
Start exporting Region 001...
Region 001 - L(4/4) - 98%
Finished exporting Region 001
Experiment Slide M1 - B1 is opened.
Start exporting Region 001...
Region 001 - L(3/3) - 96%
Finished exporting Region 001
Experiment Slide M1 - A1 is opened.
Start exporting Region 001...
Region 001 - L(3/3) - 98%
Finished exporting Region 001
Experiment Slide M1 - B1 is opened.
Start exporting Region 001...
Region 001 - L(3/3) - 97%
Finished exporting Region 001
Exiting the application.


C:\TissueGnostics\TissueFAXS Viewer>_
```

This PC › New Volume (H:) › Export › Console

Name	Date modified	Type	Size
Input	8/8/2024 1:47 PM	File folder	
Output	8/8/2024 1:59 PM	File folder	

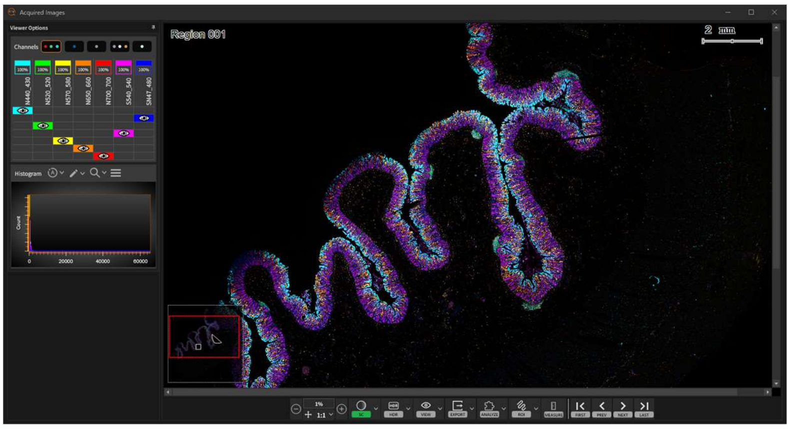
2 items   1 item selected

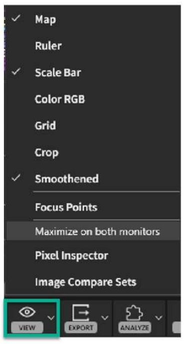
8. Check exported region overviews in Output folder.

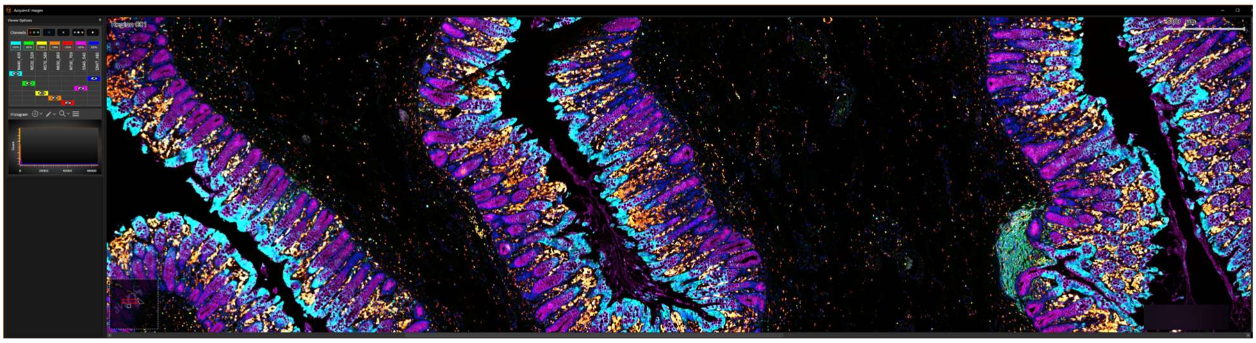
10.2. Tools and Viewing Options

The image visualization tool in TissueFAXS is called **region viewer**.

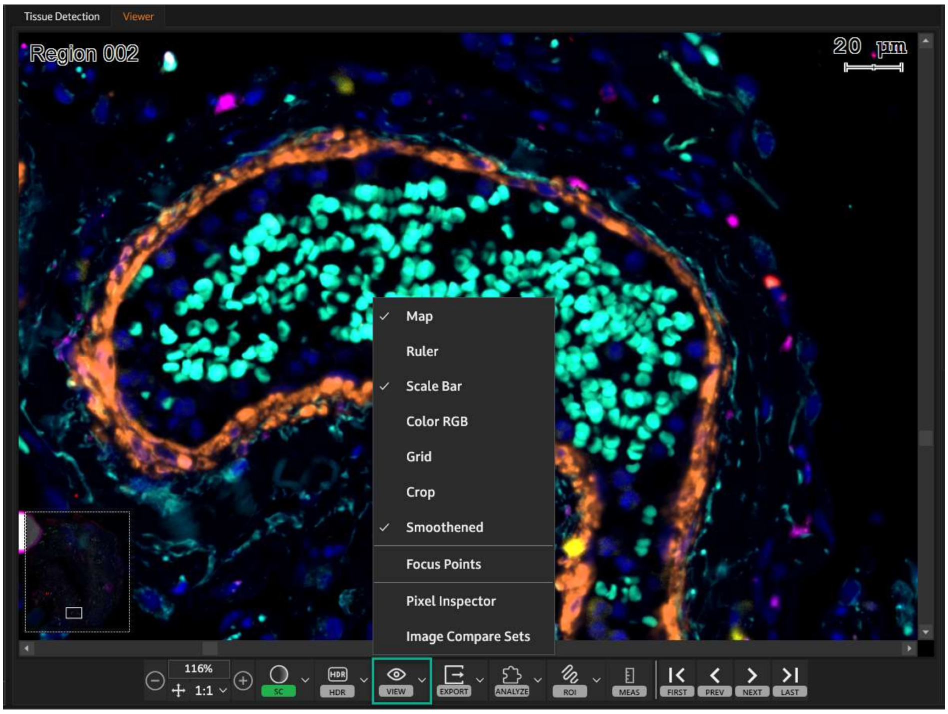
A double click on the **Viewer** tab opens the viewer in a separate window, where **Maximize on both monitors** option can be found in the **View** menu (if the system has two monitors).







Double click on any acquired region to open it in region viewer.



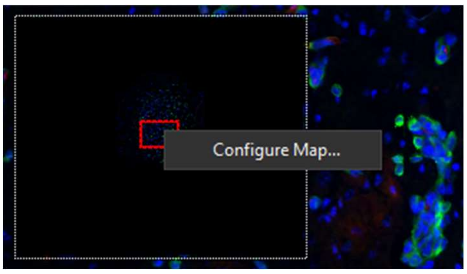
By pressing **View** button, a set of visualization tools will become available.

1. Map

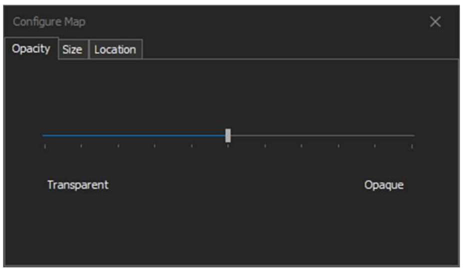
The map displays the entire sample. The part of the sample within the red frame is shown in the Region Viewer window.

The map has a set of parameters: size, opacity, location. These parameters are saved by the application.

To change the default settings, right click on the map and choose Configure Map... from the displayed menu.

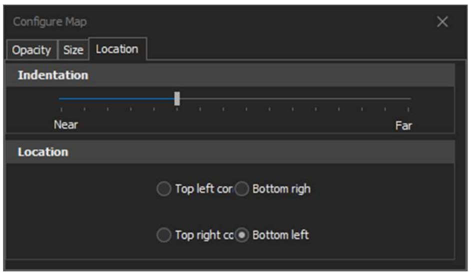


- **Opacity** - allows changing the degree of transparency for the map by using a slider.



- **Size** - allows changing the size of the map. By default, the value of this parameter is “Medium”.

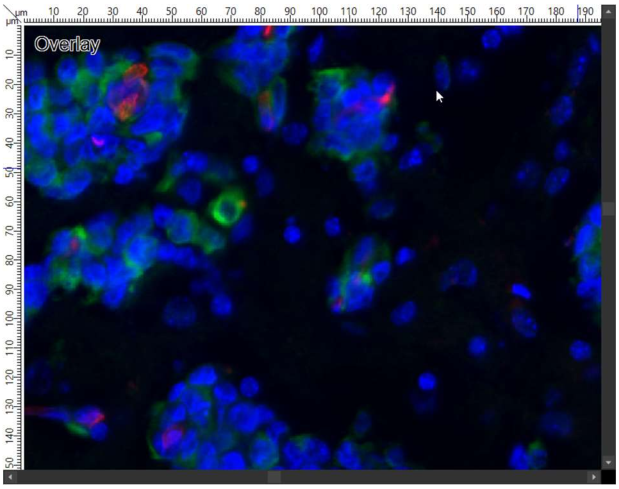
- **Location** - allows changing the corner of the Region Viewer in which the map should be displayed (top left, top right, bottom left, bottom right) and the indentation from the closest border. By default, the map is displayed in the bottom left corner.



2. Ruler

The Ruler shows the physical dimensions of a current viewing size.

A physical dimension is expressed in metrical units. The Ruler values are computed using the experiment's FOV size.

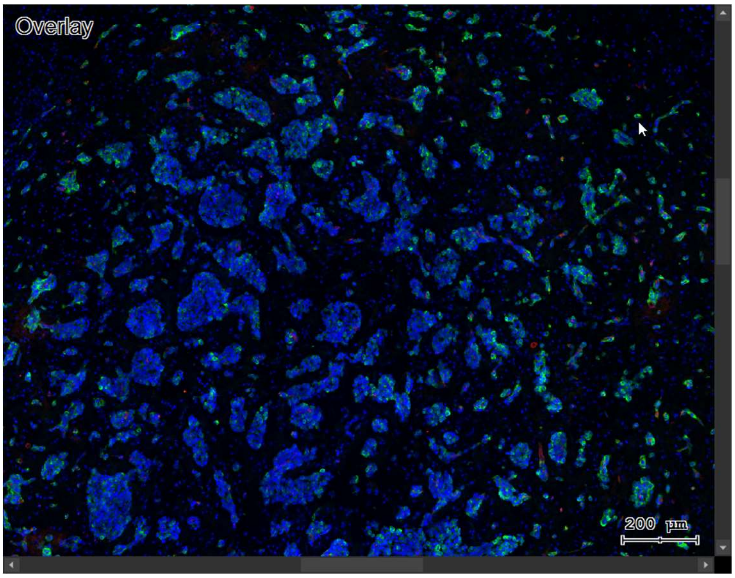


Notes:

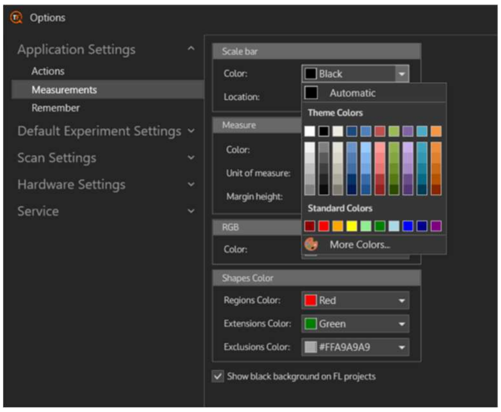
- Depending on the zoom level, the Ruler will display different divisions.
- If the user has pressed the Ruler button, StrataQuest will remember it next time it runs.

3. Scale Bar

The **Scale Bar** is a graphical element which shows a sample scale graphically.



To change the color of the Scale Bar and its location on the viewer, go to **TissueFAXS Viewer** -> **Tools** -> **Options**:



Color: Black

Regions Color: Red

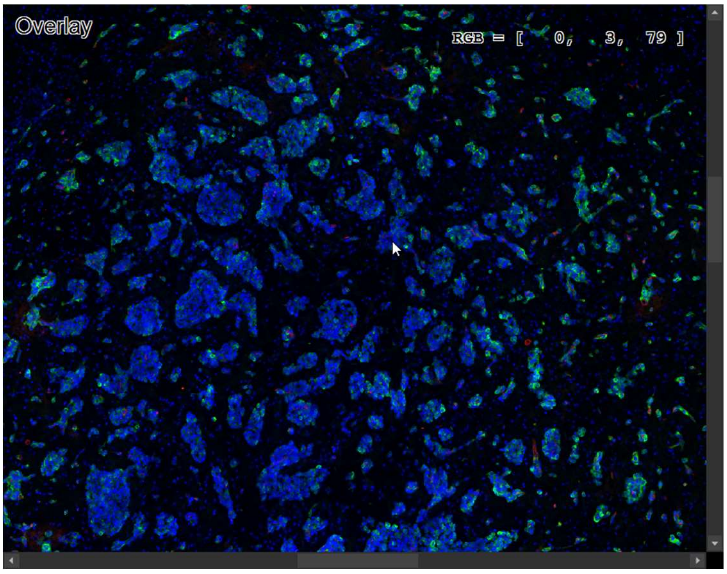
Extensions Color: Green

Exclusions Color: #FFA9A9A9

☑ Show black background on FL projects

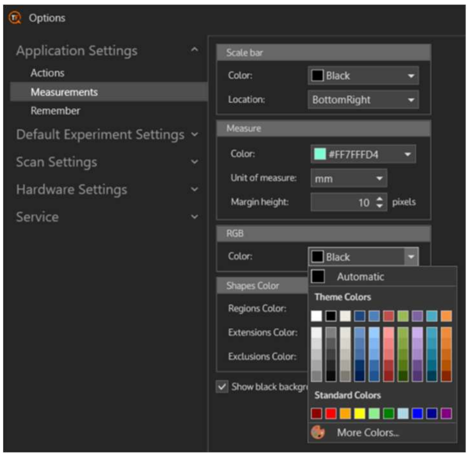
4. Color RGB

The **Show Color RGB** button shows the color at the current position of the mouse.



RGB = [0, 3, 79]

To change the color of the **RGB** and its location on the viewer, go to **TissueFAXS Viewer**-> **Tools** -> **Options**:



Scale bar
Color: Black
Location: BottomRight

Measure
Color: #FF7FFFD4
Unit of measure: mm
Margin height: 10 pixels

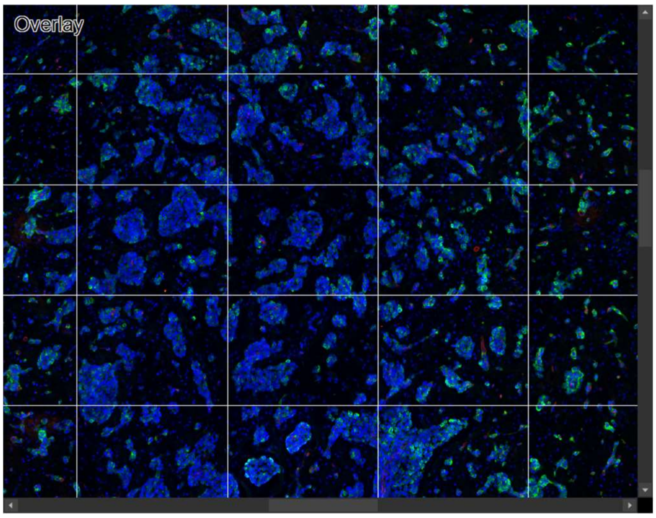
RGB
Color: Black

Shapes Color
Regions Color:
Extensions Color:
Exclusions Color:

☑ Show black backgr

5. Grid

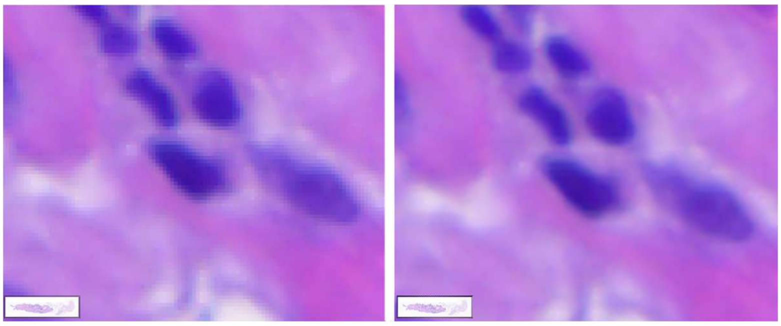
Show grid button shows/hides gridlines between FOVs.



6. Crop: displays the shape of the region in the viewer.

7. Smoothen Image button, found in the main viewer toolbar, is enabled by default in **TissueFAXS Viewer**.

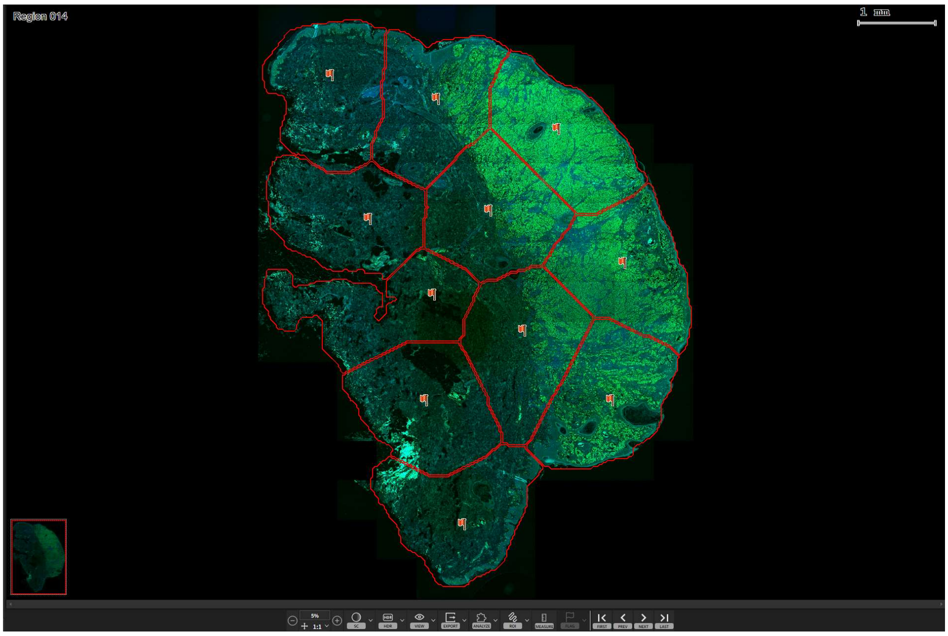
It will smoothen the image from the viewer for a better general visualization.



It can be disabled at any time.

8. Focus Points

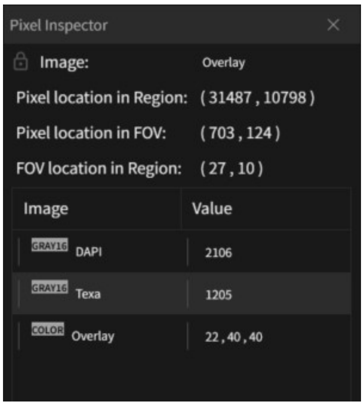
This feature displays within region viewer a map with the focus points, and the area where the focus was made.



9. Pixel Inspector

Pixel Inspector is a tool that allows you to visualize information about a pixel selected within the tissue.

When **Pixel Inspector** dialog opens, you will have to go on the sample and select the desired pixel using the color picker.



Pixel Inspector

Image: Overlay

Pixel location in Region: (31487 , 10798)

Pixel location in FOV: (703 , 124)

FOV location in Region: (27 , 10)

Image	Value
GRAY16 DAPI	2106
GRAY16 Texa	1205
COLOR Overlay	22 , 40 , 40

Once the selection is done, you will be able to see the following data:

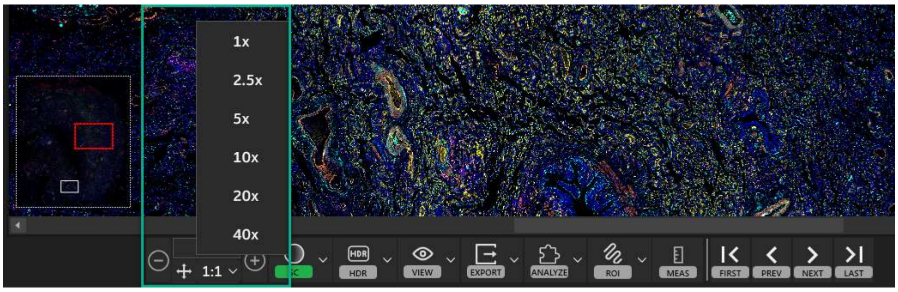
- Pixel location in region
- Pixel location in FOV
- FOV location in region
- Data regarding the channels

To close pixel inspector, close the window or uncheck the menu entry.

Notes:

- If the user has pressed the **Scale bar** button, **TissueFAXS** will remember it next time it runs.
- If the user changes the FOV size in the **Project Properties** the values displayed by the scale bar will also change.
- If the user has pressed the **Show Color RGB** button, **TissueFAXS** will remember it next time it runs.

10.2.1. Visualization Options



Zoom in and **Zoom out** buttons allow zooming the image. Another option would be to type the desired zoom value in the zoom editor.

View Original Size: displays image at its original size.

Best Fit: displays image in best fit mode.

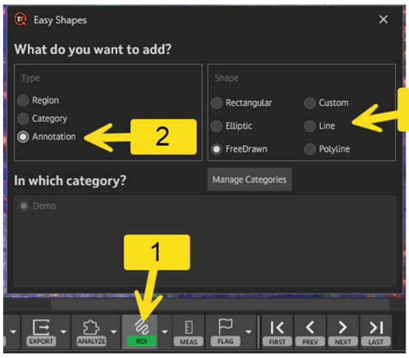
10.3. Annotations and Categories

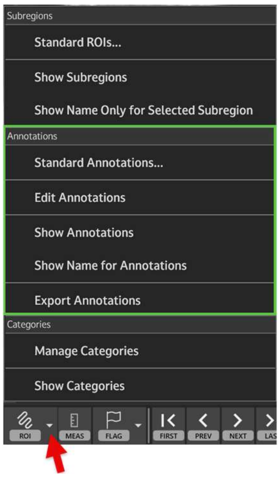
Annotation

An annotation is a tissue section that can be defined within a region. The annotation cannot be acquired, but it has a set of metadata (notes, area, etc.).

To create an annotation, press **ROI** button from **Region Viewer Toolbar**, then, in the **Easy Shapes** panel, select **Annotation**.

Then, select a shape for the annotation you will draw.





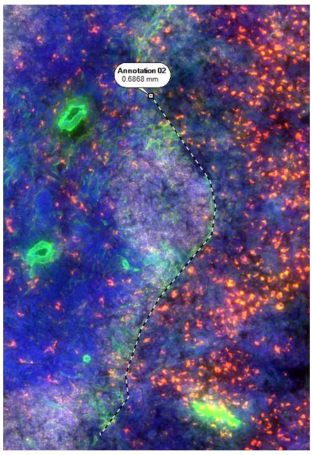
Now, add desired shape on the viewer using the mouse, as with any normal region (see **Chapter Generic ROIs**).

To be able to see all added annotations, check the **Show Annotation** option from the **ROI** button menu.

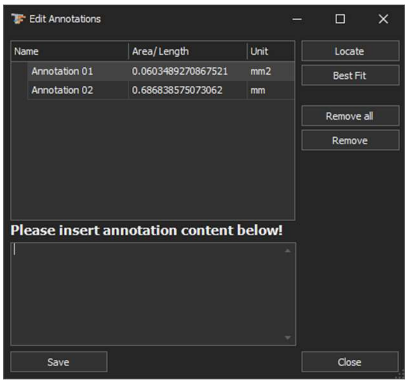
PolyLine Measurements

This feature allows drawing a line shape in order to measure a certain area.

Press the **PolyLine** button and draw the desired shape on the sample.



Manage Annotations



Edit Annotations

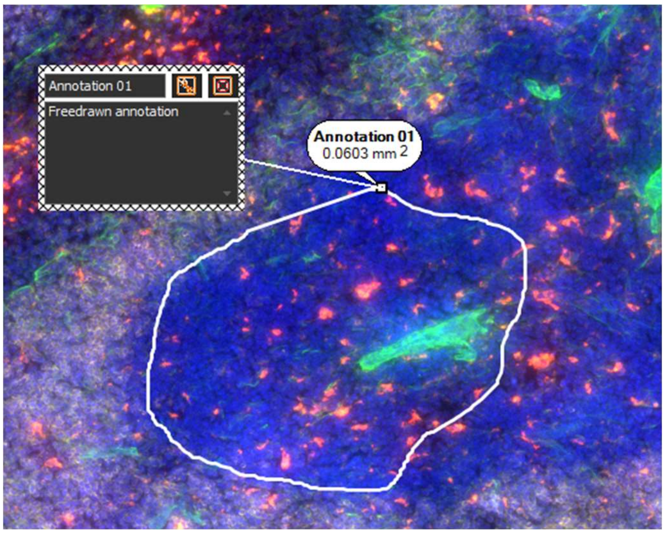
Name	Area/ Length	Unit
Annotation 01	0.0603489270867521	mm2
Annotation 02	0.686838575073062	mm

Please insert annotation content below!

- **Locate**: selected annotation will be located on the sample and displayed at the current size of the sample.
- **Best Fit:** selected annotation will be located on the sample and displayed at the current size of the region viewer.
- **Remove**: removes selected annotation.
- **Remove all**: removes all existing annotations.
- **Please insert annotation content below**: write some info for the respective annotation.

Edit annotation directly on the sample

An annotation can also be edited directly on the image viewer: it can be renamed, its content can be changed, or it can be removed. To edit an annotation, press the little black framed square on the contour of the annotation. When pressed, this little square displays an edit box, which enables the following actions:


Annotation 01
Freedrawn annotation

- Edit the name of the annotation: write the desired name in the upper-left corner of the box and press the **Enter** key to save the changes;
- Write a comment in the space below the name. Existing comments can also be erased (changes to the notes are saved automatically);
- Delete the annotation by pressing the button;
- Close the edit box by pressing the button.

Export Annotations (see **Chapter Export and Printing**).

Categories

Sometimes, you may want to emphasize certain small areas on a region that could contain high-interest research information. These areas can be exported for analysis or they can be used as a highlight tool.

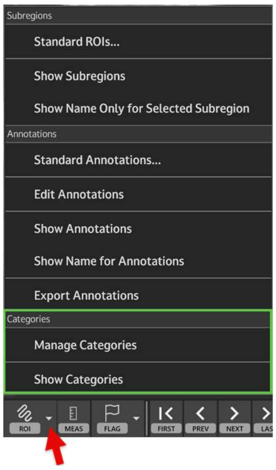
For instance, a tissue may contain both tumor areas and normal adjacent tissue (non-tumor) areas. For each type of area (i.e., tumor and non-tumor), a **category** can be created to highlight that particular area on the image.

Using **categories** can assist in performing a more precise analysis.

The advantage of categories is to ensure higher flexibility and more thorough analysis of a specific area of tissue.

- **Adding Categories**

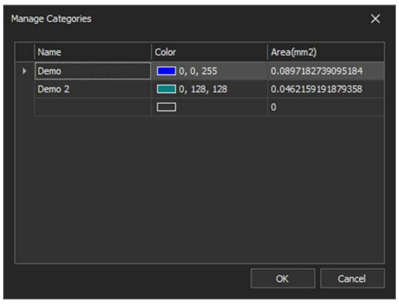
To add categories, go to **Region Viewer Toolbar** → **ROI** → **Easy Shapes** → **Manage Categories…** or **Region Viewer Toolbar → ROI → Manage Categories…** A dialog will appear (containing any previously added categories), where new categories can be added. Each one requires a name and an assigned a color. The list of categories is per project, not per region.



To draw categories, press **ROI** button from **Region Viewer Toolbar**, then, in the **Easy Shapes** panel, select **Category**.

Manage Categories button and the category list will be enabled.

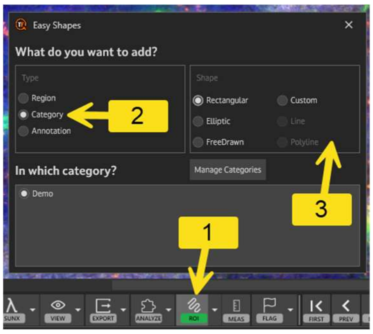
At this point, you can add categories, remove or edit them.



Name	Color	Area(mm2)
Demo	0, 0, 255	0.0897182739095184
Demo 2	0, 128, 128	0.0462159191879358
		0

From existing list, select a category where the new shapes will be added.

Then, choose a shape to be included in the category.



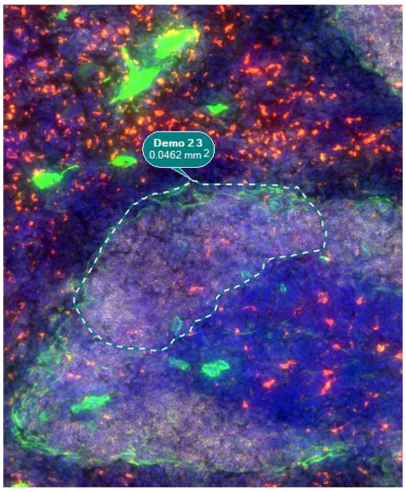
Easy Shapes

What do you want to add?

Type
○ Region
● Category
○ Annotation

Shape
● Rectangular
○ Custom
○ Elliptic
○ Line
○ FreeDrawn
○ Polyline

In which category?
● Demo



Now, add desired shape on the viewer using the mouse, as with any normal region (see **Chapter** **Generic ROIs**).

To be able to see all added categories, check the **Show Categories** option from the **ROI** button menu.

To **remove** categories: go to **Region Viewer Toolbar → ROI→ Easy Shapes→ Manage Categories…,** select the category to be removed, then press the **Delete** key on the keyboard.

- **Export**: Categories can also be used when exporting images. See **Chapter** **Export and Printing** for further details.
- **Transforming regions from category shapes into subregions for reacquisition**

If you need to reacquire the tissue area from a category, it is possible to transform it into a **Defined Region** and then perform reacquisition.

In the region viewer, right click on the border of a category and from the contextual menu choose the **Transform into Defined Region** option. The category will transform into a **Defined Region**, disappear from the region viewer, and be visible in the experiment editor (so it can be reacquired).

- When exporting the **Region Overview**, there is an option to also export the categories.
- When exporting the **FOV images, there is an option to only export images belonging to the respective category.**

10.4. Illumination/Shading Correction

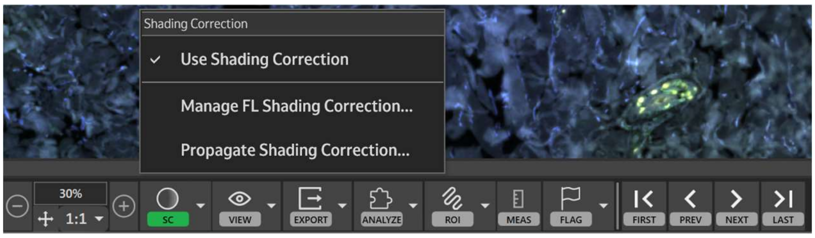
About image illumination/shading correction

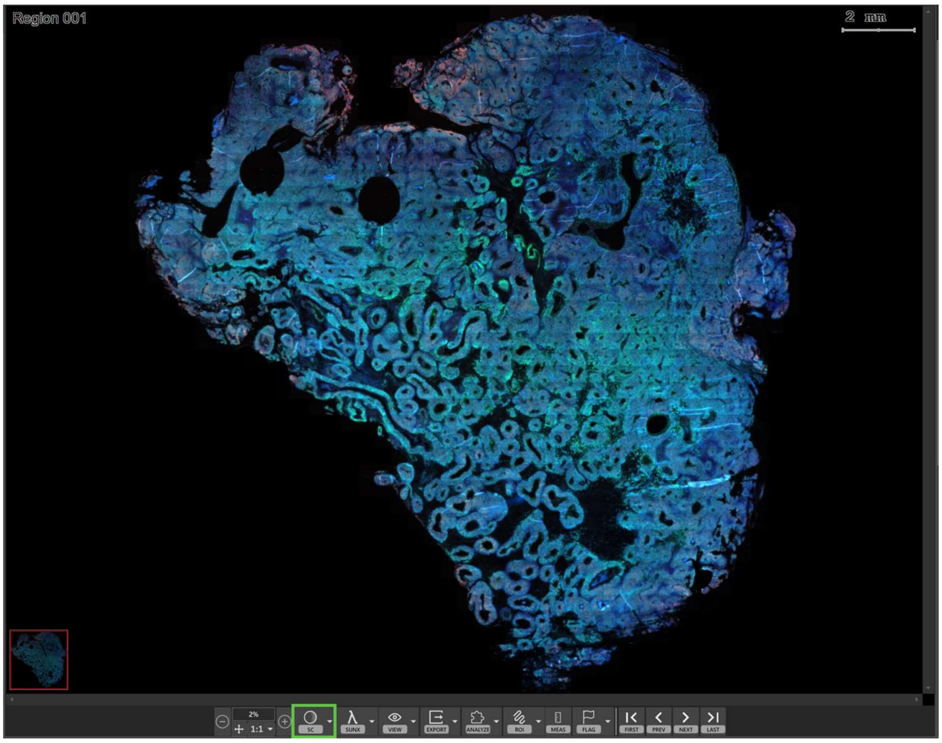
Occasionally, some shades may appear on the acquired images. They can be caused by imperfections of any component of the light path (e.g., FL Lamp, filters, etc.). That is, specks/impurities on the camera/objective.

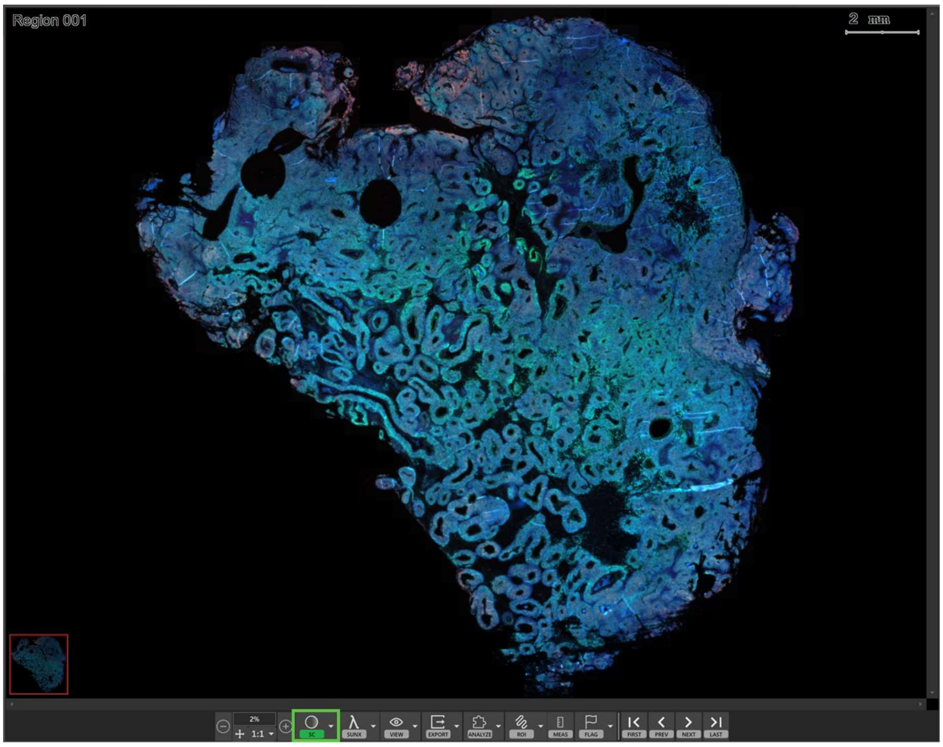
TissueFAXS can correct these shading problems in fluorescence experiments by using the **FL Shading Correction** function.

For BF experiments, the feature is called **Illumination Correction**.

The **FL Shading Correction** menu can be accessed by pressing the **FL Shading Correction** button from the **Region Viewer/Acquired Images** control.







A *correction image* is necessary to apply shading correction.

The **correction image** is an image generated to store information about the shades in the light path. By applying this image to a certain region, the shades will be removed and the images will be uniformly illuminated.

The shading reference image can be applied to z-stacks as well.

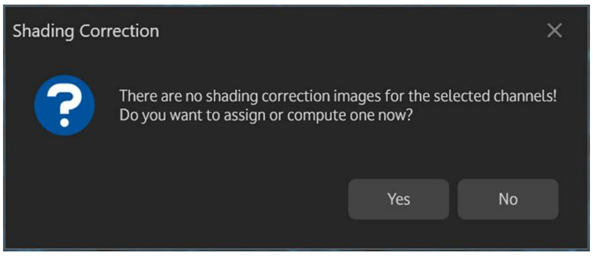
The shading reference is specific for each channel, including confocal channels.

Notes:

- **16-bit mode** available for **FL Shading Correction**.

- The shading reference corrects shading that comes from the light path - *it cannot correct optical aberrations* that come from the sample itself. In some samples, there are "shading effects," which come from the tissue, different preparation, or fixation methods. These effects might appear in some areas while not visible in other areas or in other samples. This is not considered shading. Such effects are optical aberrations that have their origin in different optical properties of the sample. They will not be corrected by the shading correction operation.

If no correction image is present, the following message will pop out:



There are no shading correction images for the selected channels!
Do you want to assign or compute one now?

To compute a correction image, press **Yes** in the above message or choose **Illumination Correction** to access the **FL Shading Correction** panel.

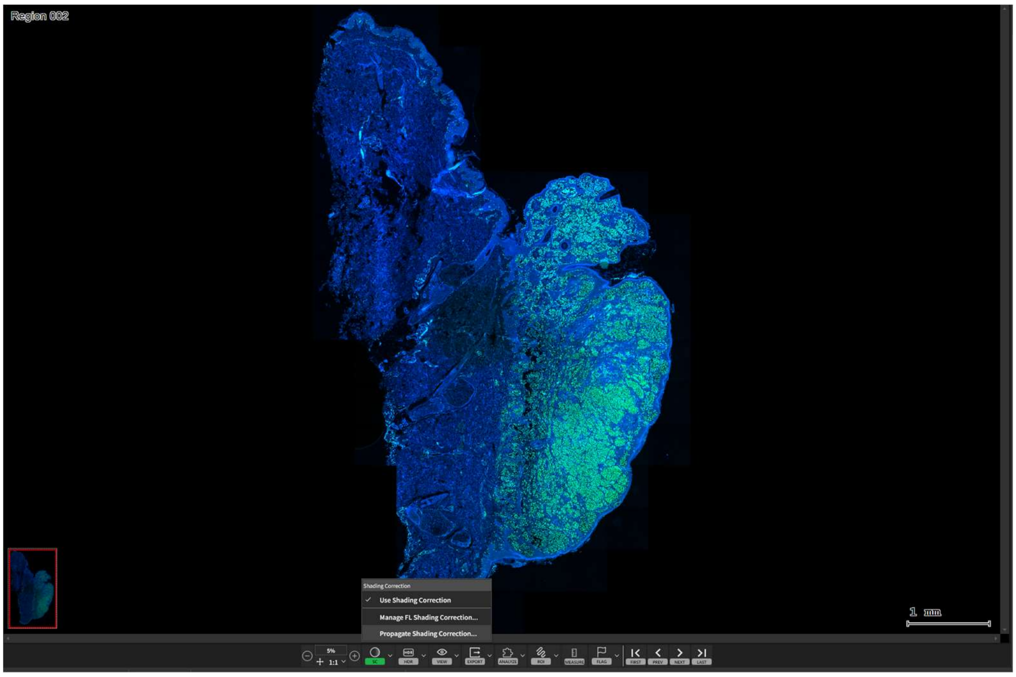
A correction image can be computed for:

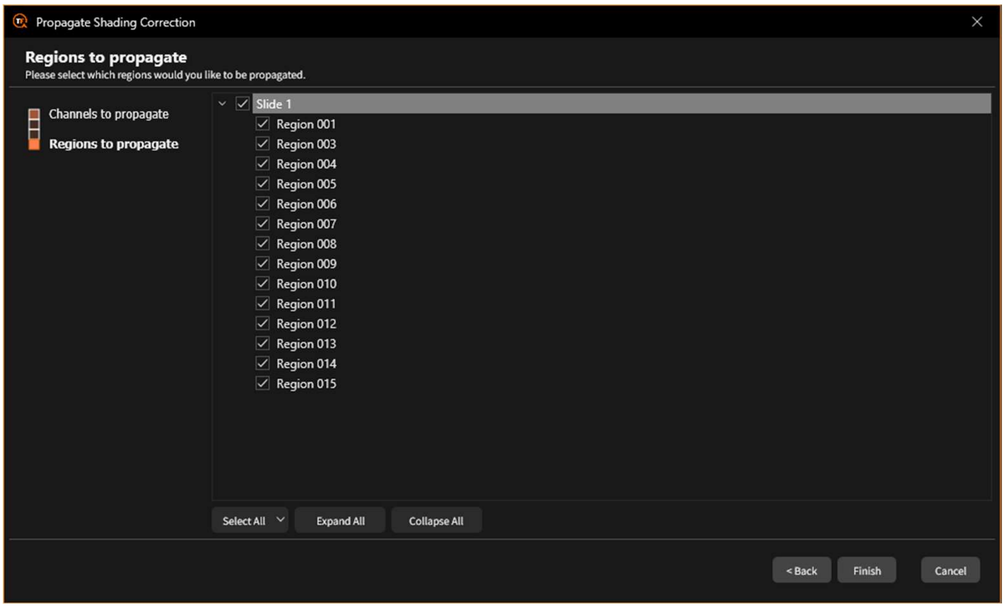
- All existing channels;
- Only for selected channels;
- If the project was acquired with Z stack, the correction images can be computed for selected channels for Z stack slices (each slice will have its own correction images).

Propagate Shading Correction

A shading reference image can be propagated only to user-specified channels.

This option is located at: **Region viewer** -> **Shading correction** -> **Propagate Shading Correction**.





Propagate Shading Correction

Regions to propagate

Please select which regions would you like to be propagated.

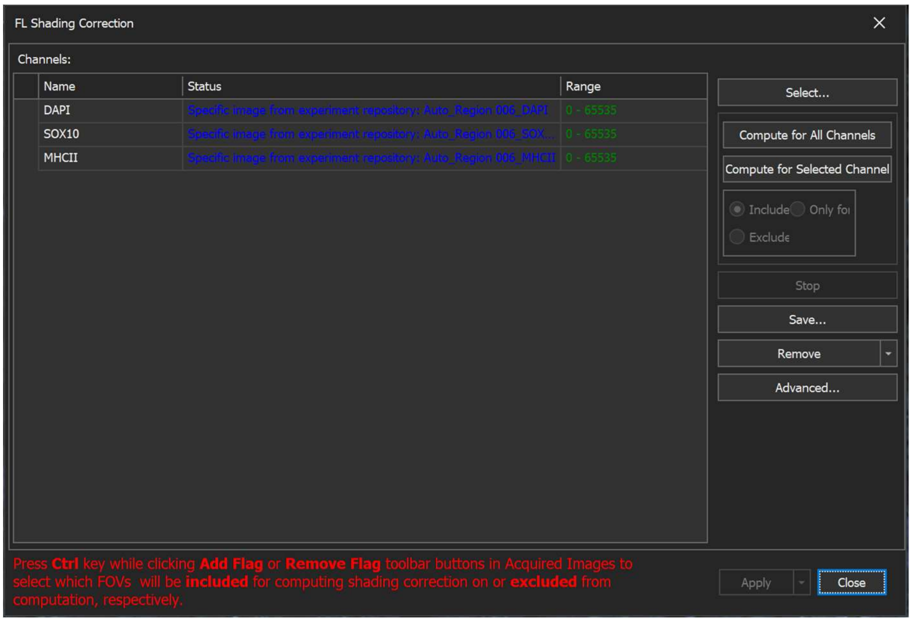
- ☑ Slide 1
  - ☑ Region 001
  - ☑ Region 003
  - ☑ Region 004
  - ☑ Region 005
  - ☑ Region 006
  - ☑ Region 007
  - ☑ Region 008
  - ☑ Region 009
  - ☑ Region 010
  - ☑ Region 011
  - ☑ Region 012
  - ☑ Region 013
  - ☑ Region 014
  - ☑ Region 015

Managing FL Correction Images

TissueFAXS provides a management panel for existing correction images.

To manage the correction images, choose the **Manage FL Shading Correction** option and then press the **Manage…** button. The **FL Shading Correction** panel will open.

The name, status, status for Z-stack, and intensity range will be displayed for each channel.



FL Shading Correction

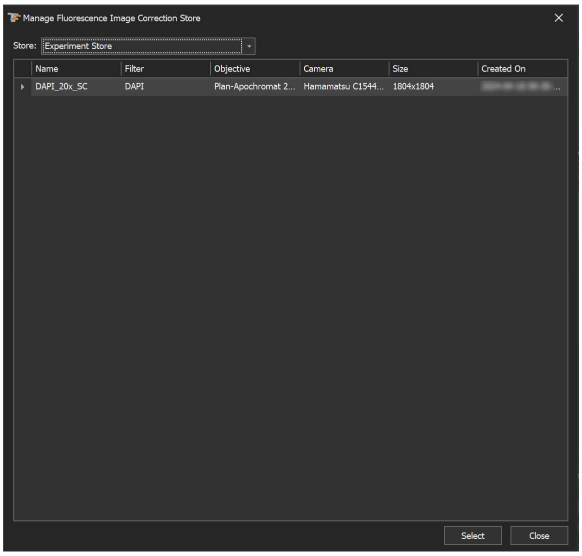
Channels:

Name	Status	Range
DAPI	Specific image from experiment repository: Auto_Region 006_DAPI	0 - 65535
SOX10	Specific image from experiment repository: Auto_Region 006_SOX...	0 - 65535
MHCII	Specific image from experiment repository: Auto_Region 006_MHCII	0 - 65535

Press **Ctrl** key while clicking **Add Flag** or **Remove Flag** toolbar buttons in Acquired Images to select which FOVs will be **included** for computing shading correction on or **excluded** from computation, respectively.

Note: The correction image will be affected if overexposed areas are present when computing correction image. The **Flag** feature can be used in order to select which images to keep for computing shading correction.

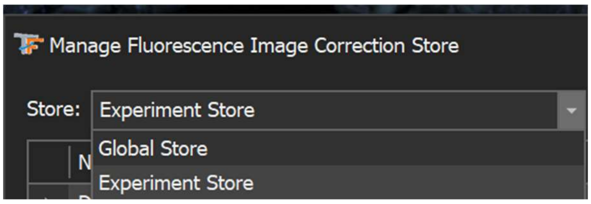
Press the **Select** button to open **Manage Fluorescence Image Correction Store**.



Store: Experiment Store

Name	Filter	Objective	Camera	Size	Created On
DAPI_20x_SC	DAPI	Plan-Apochromat 2...	Hamamatsu C1544...	1804x1804	[redacted]...

First, select the type of store where the correction images are located: a global store or experiment store. The **global store** will make the images available to all the experiments, while the **experiment store** will only make the images available for the current experiment.



Compute for All Channels: computes correction image for all channels;

Compute for Selected Channel: computes correction image only for a selected channel;

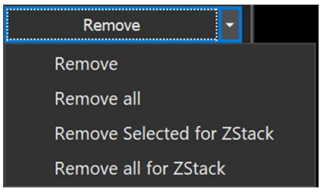
Include Z-Stack: computes a correction image for all the images that include Z-Stack;

Exclude Z-Stack: computes a correction image for all the images that exclude Z-Stack;

Only for Z-Stack: computes a correction image only for Z-Stack images.

You can **remove** the images as follows:

- **Remove**: will remove selected correction image;
- **Remove all**: will remove all the existing correction images;
- **Remove Selected for ZStack**: will remove selected image only for ZStack;
- **Remove all for ZStack**: will remove all the correction images only for ZStack.

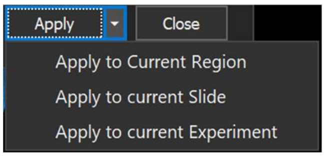


In the end, the user must select where to **apply** the correction image(s):

-To the current **region**;

- To the current **slide**;

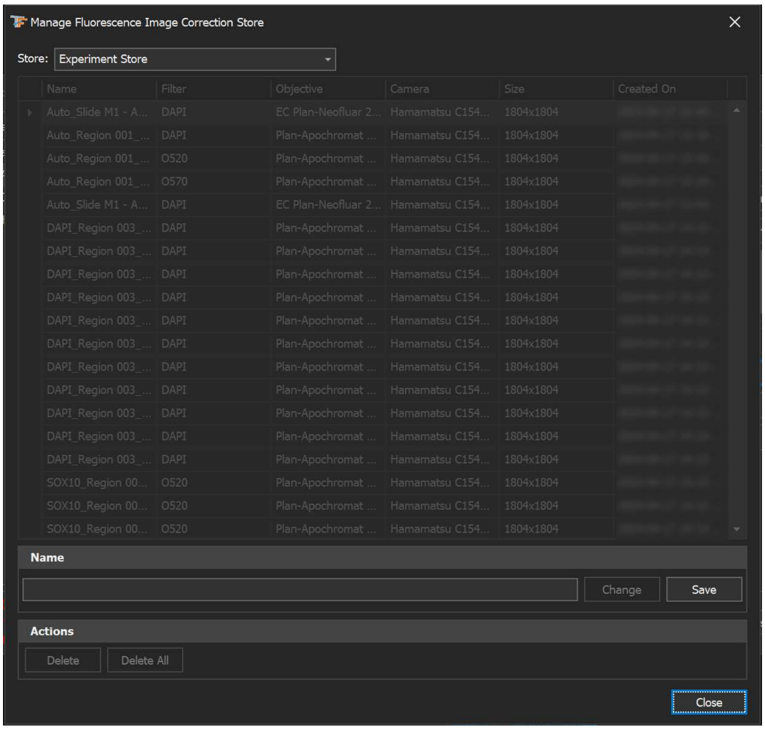
- To the current **experiment**.



Stop: will halt the process of computing correction images for large regions.

Save: there are two ways of saving correction images:

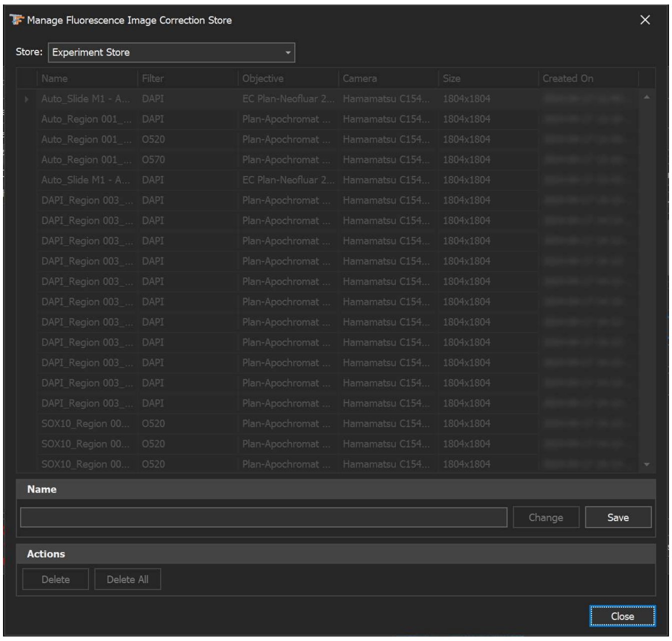
- In **Experiment store**: the correction images saved here will be used only for the current experiment;
- In **Global**: the correction images saved here can be used in any other experiment.



Store: Experiment Store

Name	Filter	Objective	Camera	Size	Created On
Auto_Slide M1 - A...	DAPI	EC Plan-Neofluar 2...	Hamamatsu C154...	1804x1804	[redacted]
Auto_Region 001_...	DAPI	Plan-Apochromat ...	Hamamatsu C154...	1804x1804	[redacted]
Auto_Region 001_...	O520	Plan-Apochromat ...	Hamamatsu C154...	1804x1804	[redacted]
Auto_Region 001_...	O570	Plan-Apochromat ...	Hamamatsu C154...	1804x1804	[redacted]
Auto_Slide M1 - A...	DAPI	EC Plan-Neofluar 2...	Hamamatsu C154...	1804x1804	[redacted]
DAPI_Region 003_...	DAPI	Plan-Apochromat ...	Hamamatsu C154...	1804x1804	[redacted]
DAPI_Region 003_...	DAPI	Plan-Apochromat ...	Hamamatsu C154...	1804x1804	[redacted]
DAPI_Region 003_...	DAPI	Plan-Apochromat ...	Hamamatsu C154...	1804x1804	[redacted]
DAPI_Region 003_...	DAPI	Plan-Apochromat ...	Hamamatsu C154...	1804x1804	[redacted]
DAPI_Region 003_...	DAPI	Plan-Apochromat ...	Hamamatsu C154...	1804x1804	[redacted]
DAPI_Region 003_...	DAPI	Plan-Apochromat ...	Hamamatsu C154...	1804x1804	[redacted]
DAPI_Region 003_...	DAPI	Plan-Apochromat ...	Hamamatsu C154...	1804x1804	[redacted]
DAPI_Region 003_...	DAPI	Plan-Apochromat ...	Hamamatsu C154...	1804x1804	[redacted]
DAPI_Region 003_...	DAPI	Plan-Apochromat ...	Hamamatsu C154...	1804x1804	[redacted]
DAPI_Region 003_...	DAPI	Plan-Apochromat ...	Hamamatsu C154...	1804x1804	[redacted]
DAPI_Region 003_...	DAPI	Plan-Apochromat ...	Hamamatsu C154...	1804x1804	[redacted]
SOX10_Region 00...	O520	Plan-Apochromat ...	Hamamatsu C154...	1804x1804	[redacted]
SOX10_Region 00...	O520	Plan-Apochromat ...	Hamamatsu C154...	1804x1804	[redacted]
SOX10_Region 00...	O520	Plan-Apochromat ...	Hamamatsu C154...	1804x1804	[redacted]

If you need to make changes to the correction images store, press **Manage**. The **Correction Image Store Manager** will open.



Manage Fluorescence Image Correction Store

Store: Experiment Store

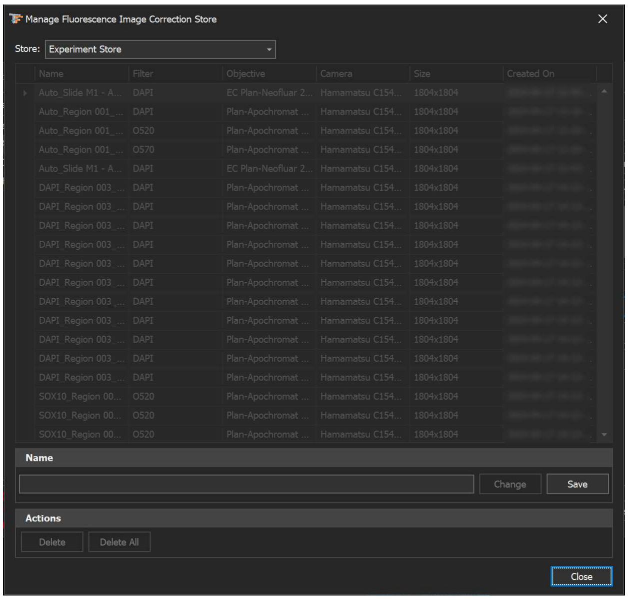
Name	Filter	Objective	Camera	Size	Created On
Auto_Slide M1 - A...	DAPI	EC Plan-Neofluar 2...	Hamamatsu C154...	1804x1804	[redacted]
Auto_Region 001_...	DAPI	Plan-Apochromat ...	Hamamatsu C154...	1804x1804	[redacted]
Auto_Region 001_...	O520	Plan-Apochromat ...	Hamamatsu C154...	1804x1804	[redacted]
Auto_Region 001_...	O570	Plan-Apochromat ...	Hamamatsu C154...	1804x1804	[redacted]
Auto_Slide M1 - A...	DAPI	EC Plan-Neofluar 2...	Hamamatsu C154...	1804x1804	[redacted]
DAPI_Region 003_...	DAPI	Plan-Apochromat ...	Hamamatsu C154...	1804x1804	[redacted]
DAPI_Region 003_...	DAPI	Plan-Apochromat ...	Hamamatsu C154...	1804x1804	[redacted]
DAPI_Region 003_...	DAPI	Plan-Apochromat ...	Hamamatsu C154...	1804x1804	[redacted]
DAPI_Region 003_...	DAPI	Plan-Apochromat ...	Hamamatsu C154...	1804x1804	[redacted]
DAPI_Region 003_...	DAPI	Plan-Apochromat ...	Hamamatsu C154...	1804x1804	[redacted]
DAPI_Region 003_...	DAPI	Plan-Apochromat ...	Hamamatsu C154...	1804x1804	[redacted]
DAPI_Region 003_...	DAPI	Plan-Apochromat ...	Hamamatsu C154...	1804x1804	[redacted]
DAPI_Region 003_...	DAPI	Plan-Apochromat ...	Hamamatsu C154...	1804x1804	[redacted]
DAPI_Region 003_...	DAPI	Plan-Apochromat ...	Hamamatsu C154...	1804x1804	[redacted]
DAPI_Region 003_...	DAPI	Plan-Apochromat ...	Hamamatsu C154...	1804x1804	[redacted]
DAPI_Region 003_...	DAPI	Plan-Apochromat ...	Hamamatsu C154...	1804x1804	[redacted]
SOX10_Region 00...	O520	Plan-Apochromat ...	Hamamatsu C154...	1804x1804	[redacted]
SOX10_Region 00...	O520	Plan-Apochromat ...	Hamamatsu C154...	1804x1804	[redacted]
SOX10_Region 00...	O520	Plan-Apochromat ...	Hamamatsu C154...	1804x1804	[redacted]

Press the **Change** button to edit the names of a correction image, then press **Save**.

The selected correction image can be deleted by pressing **Delete**. If pressing **Delete All**, then all the correction images from the respective storage will be deleted.

FL Experiment Shading Settings

In the **FL Experiment Shading Settings** from the main toolbar of the application, you can select the images you want to use for the experiment. The correction images selected here will be used during the acquisition process.

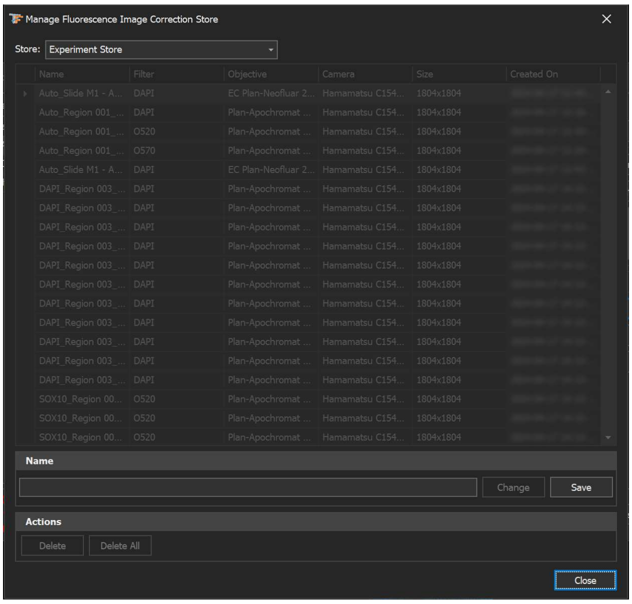


Manage Fluorescence Image Correction Store

Store: Experiment Store

Name	Filter	Objective	Camera	Size	Created On
Auto_Slide M1 - A...	DAPI	EC Plan-Neofluar 2...	Hamamatsu C154...	1804x1804	[redacted]
Auto_Region 001_...	DAPI	Plan-Apochromat ...	Hamamatsu C154...	1804x1804	[redacted]
Auto_Region 001_...	O520	Plan-Apochromat ...	Hamamatsu C154...	1804x1804	[redacted]
Auto_Region 001_...	O570	Plan-Apochromat ...	Hamamatsu C154...	1804x1804	[redacted]
Auto_Slide M1 - A...	DAPI	EC Plan-Neofluar 2...	Hamamatsu C154...	1804x1804	[redacted]
DAPI_Region 003_...	DAPI	Plan-Apochromat ...	Hamamatsu C154...	1804x1804	[redacted]
DAPI_Region 003_...	DAPI	Plan-Apochromat ...	Hamamatsu C154...	1804x1804	[redacted]
DAPI_Region 003_...	DAPI	Plan-Apochromat ...	Hamamatsu C154...	1804x1804	[redacted]
DAPI_Region 003_...	DAPI	Plan-Apochromat ...	Hamamatsu C154...	1804x1804	[redacted]
DAPI_Region 003_...	DAPI	Plan-Apochromat ...	Hamamatsu C154...	1804x1804	[redacted]
DAPI_Region 003_...	DAPI	Plan-Apochromat ...	Hamamatsu C154...	1804x1804	[redacted]
DAPI_Region 003_...	DAPI	Plan-Apochromat ...	Hamamatsu C154...	1804x1804	[redacted]
DAPI_Region 003_...	DAPI	Plan-Apochromat ...	Hamamatsu C154...	1804x1804	[redacted]
DAPI_Region 003_...	DAPI	Plan-Apochromat ...	Hamamatsu C154...	1804x1804	[redacted]
DAPI_Region 003_...	DAPI	Plan-Apochromat ...	Hamamatsu C154...	1804x1804	[redacted]
DAPI_Region 003_...	DAPI	Plan-Apochromat ...	Hamamatsu C154...	1804x1804	[redacted]
SOX10_Region 00...	O520	Plan-Apochromat ...	Hamamatsu C154...	1804x1804	[redacted]
SOX10_Region 00...	O520	Plan-Apochromat ...	Hamamatsu C154...	1804x1804	[redacted]
SOX10_Region 00...	O520	Plan-Apochromat ...	Hamamatsu C154...	1804x1804	[redacted]

Press the **Select** button to open a store and pick a correction image for the desired preview/acquisition channel.



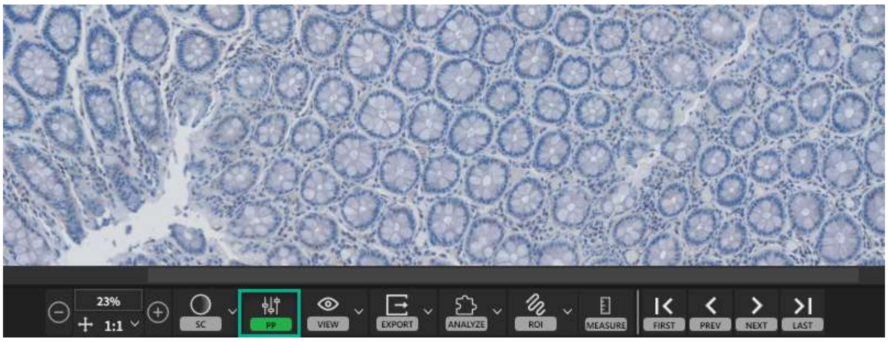
Manage Fluorescence Image Correction Store

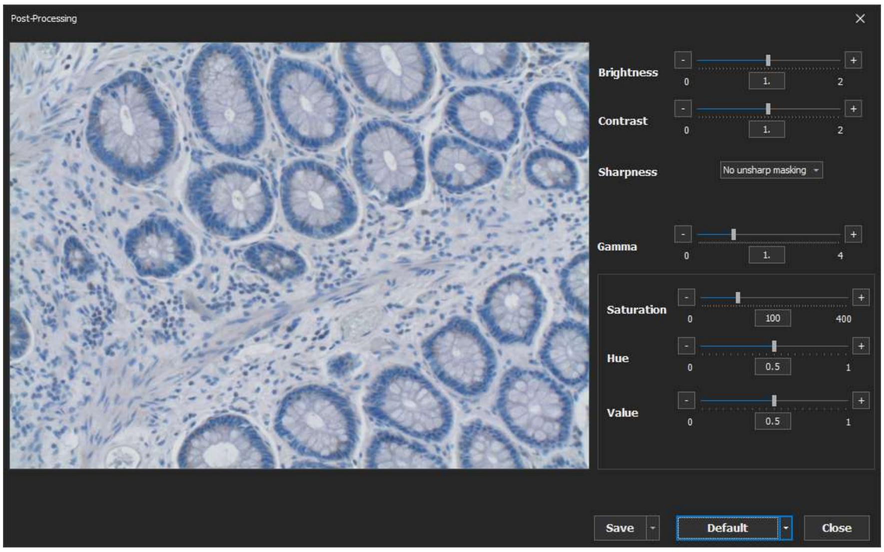
Store: Experiment Store

Name	Filter	Objective	Camera	Size	Created On
Auto_Slide M1 - A...	DAPI	EC Plan-Neofluar 2...	Hamamatsu C154...	1804x1804	[redacted]
Auto_Region 001_...	DAPI	Plan-Apochromat ...	Hamamatsu C154...	1804x1804	[redacted]
Auto_Region 001_...	O520	Plan-Apochromat ...	Hamamatsu C154...	1804x1804	[redacted]
Auto_Region 001_...	O570	Plan-Apochromat ...	Hamamatsu C154...	1804x1804	[redacted]
Auto_Slide M1 - A...	DAPI	EC Plan-Neofluar 2...	Hamamatsu C154...	1804x1804	[redacted]
DAPI_Region 003_...	DAPI	Plan-Apochromat ...	Hamamatsu C154...	1804x1804	[redacted]
DAPI_Region 003_...	DAPI	Plan-Apochromat ...	Hamamatsu C154...	1804x1804	[redacted]
DAPI_Region 003_...	DAPI	Plan-Apochromat ...	Hamamatsu C154...	1804x1804	[redacted]
DAPI_Region 003_...	DAPI	Plan-Apochromat ...	Hamamatsu C154...	1804x1804	[redacted]
DAPI_Region 003_...	DAPI	Plan-Apochromat ...	Hamamatsu C154...	1804x1804	[redacted]
DAPI_Region 003_...	DAPI	Plan-Apochromat ...	Hamamatsu C154...	1804x1804	[redacted]
DAPI_Region 003_...	DAPI	Plan-Apochromat ...	Hamamatsu C154...	1804x1804	[redacted]
DAPI_Region 003_...	DAPI	Plan-Apochromat ...	Hamamatsu C154...	1804x1804	[redacted]
DAPI_Region 003_...	DAPI	Plan-Apochromat ...	Hamamatsu C154...	1804x1804	[redacted]
DAPI_Region 003_...	DAPI	Plan-Apochromat ...	Hamamatsu C154...	1804x1804	[redacted]
DAPI_Region 003_...	DAPI	Plan-Apochromat ...	Hamamatsu C154...	1804x1804	[redacted]
SOX10_Region 00...	O520	Plan-Apochromat ...	Hamamatsu C154...	1804x1804	[redacted]
SOX10_Region 00...	O520	Plan-Apochromat ...	Hamamatsu C154...	1804x1804	[redacted]
SOX10_Region 00...	O520	Plan-Apochromat ...	Hamamatsu C154...	1804x1804	[redacted]

10.5. Post-Processing

In **Post-Processing** panel, you can adjust your images by using image processing dedicated **parameters**. Post-Processing is available only for BF experiments.





Post-Processing

Brightness: 1. (0 – 2)
Contrast: 1. (0 – 2)
Sharpness: No unsharp masking
Gamma: 1. (0 – 4)
Saturation: 100 (0 – 400)
Hue: 0.5 (0 – 1)
Value: 0.5 (0 – 1)

- Brightness
- Contrast

- Sharpness
- Gamma
- Saturation
- Hue
- Value

After modifying the parameters above, you have two **reset** options (available by pressing **Default** button):

- **Reset to default values**: original values of parameters will be restored;
- **Default from current came**ra: default values of current camera will be applied.

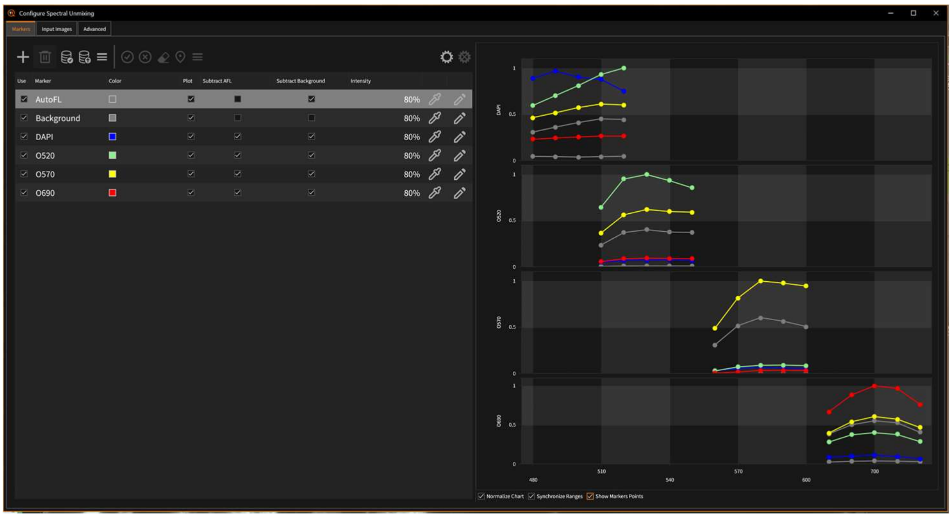
When you are done, press **Save**.

10.6. Spectral Unmixing

To configure the **Spectral Unmixing** settings, press the **Configure Spectral Unmixing** button from the region viewer:

Configure Spectral Unmixing dialog will open.

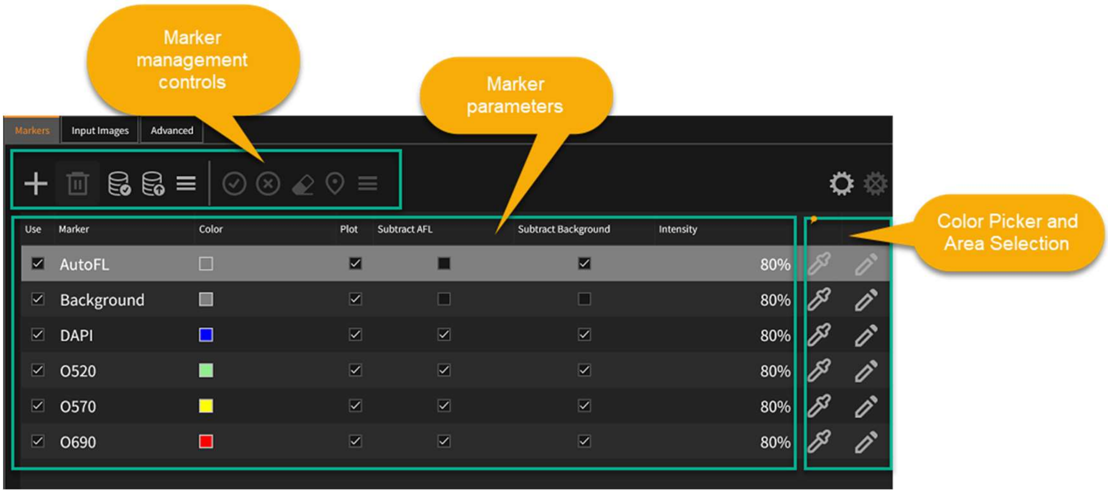
It has three sections: **Markers**, **Input Images**, and **Advanced**.



Use	Marker	Color	Plot	Subtract AFL	Subtract Background	Intensity
☑	AutoFL	☐	☑	■	☑	80%
☑	Background	▨	☑	☐	☐	80%
☑	DAPI	🟦	☑	☑	☑	80%
☑	O520	🟩	☑	☑	☑	80%
☑	O570	🟨	☑	☑	☑	80%
☑	O690	🟥	☑	☑	☑	80%

☑ Normalize Chart ☑ Synchronize Ranges ☑ Show Markers Points

Reference Marker Definition



Marker management controls

Marker parameters

Color Picker and Area Selection

Use	Marker	Color	Plot	Subtract AFL	Subtract Background	Intensity
☑	AutoFL	☐	☑	■	☑	80%
☑	Background	▨	☑	☐	☐	80%
☑	DAPI	🟦	☑	☑	☑	80%
☑	O520	🟩	☑	☑	☑	80%
☑	O570	🟨	☑	☑	☑	80%
☑	O690	🟥	☑	☑	☑	80%

Markers management

To manage markers, use the following controls:

- **Add Markers**: adds a new marker to the list of markers.
- **Remove Marker**: deletes a specified marker from the list of markers.
- **Remove All Markers**: deletes all markers from the list of markers.
- **View Values**: displays the numeric values for all markers present in the list of markers.
- **Save Markers to Spectral Database**: saves the reference values of a specified marker into the database.

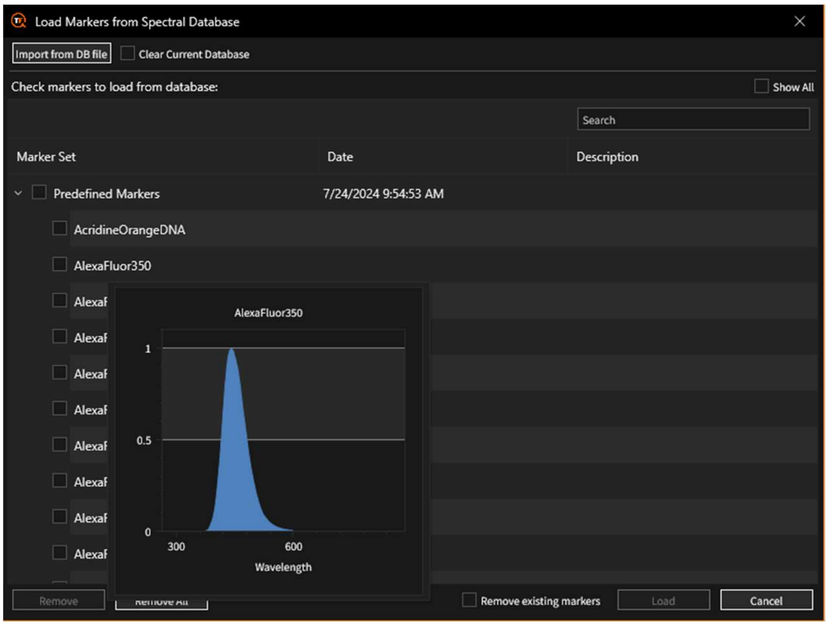
The following parameters can be set for each marker:

- **Marker name:** defines the marker name.
- **Color:** defines the marker color.
- **Plot:** enables plot display.
- **Subtract AFL:** this parameter works like a flag, which specifies that the auto FL component will be subtracted from the marker.

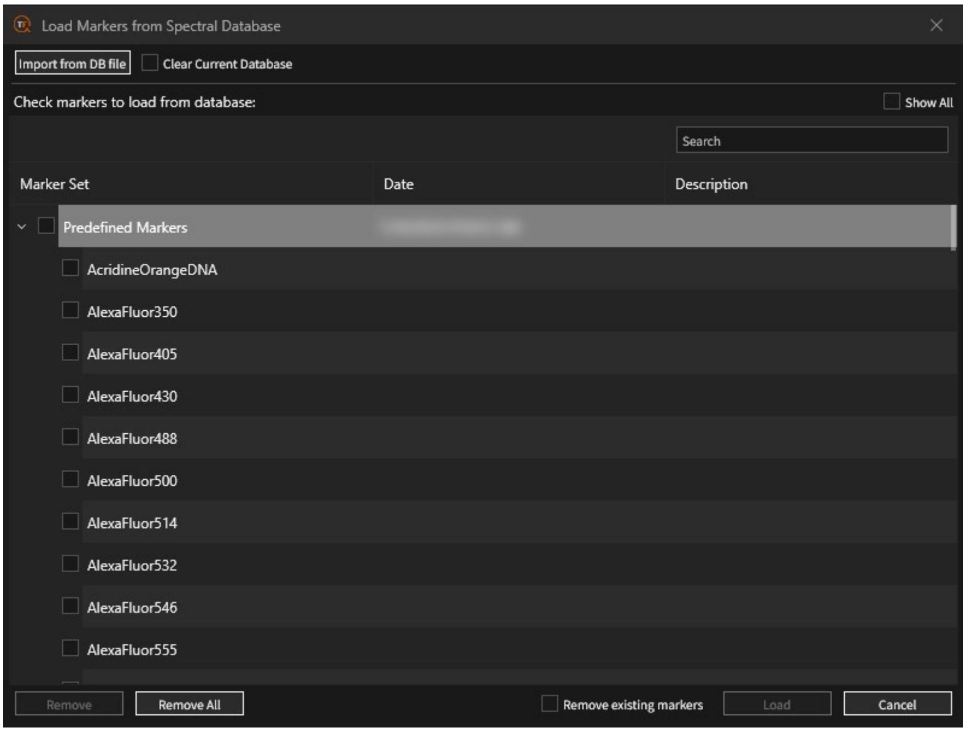
Load from Spectral Database

Previously saved markers or predefined standard markers are available for loading from the spectral database for usage. If using the **Import from DB File** option, markers will be imported from another database (for example StrataQuest database). Before importing from another database, the current database can be removed by using **Clear Current Database**.

Hoovering the mouse on the marker’s name will open a small window showing the marker’s spectrum.



There is a list of the most used predefined standard markers. The search option on the top right side of the window makes the selection easier.



Once the selection is made, the loading process is finalized by pressing the **Load** button.

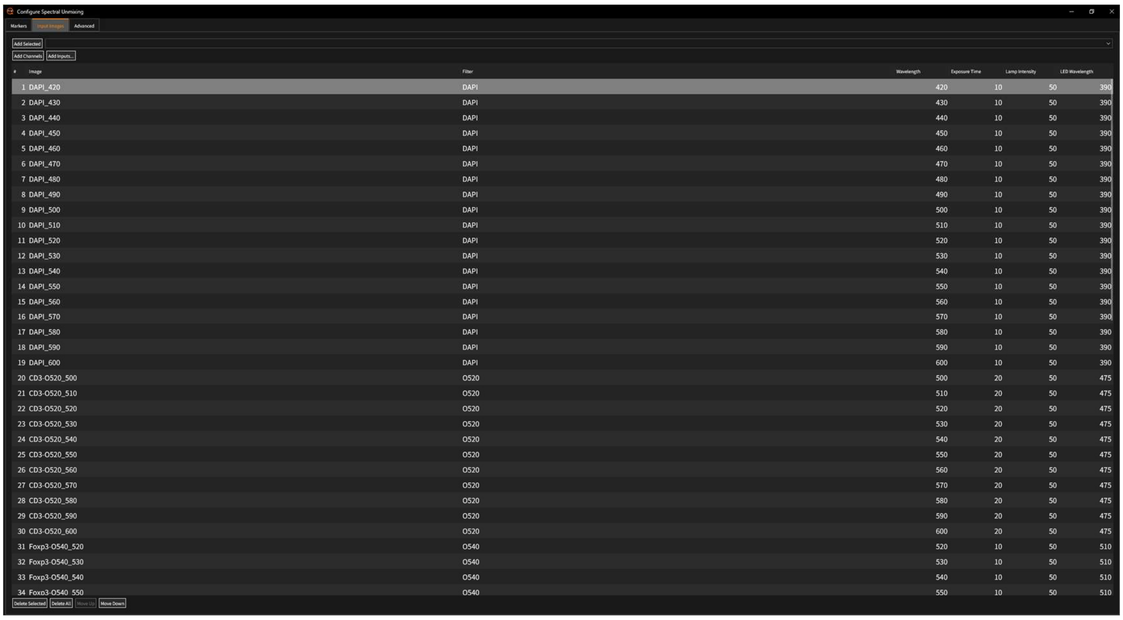
Color Picking and Area Selection

For a proper definition of the reference spectrum of a specified marker, it is recommended to use a single marker stained sample. Otherwise, it is possible to select an unwanted mixture of two or more markers, which will generate the result (of that particular mixture selected spectrum) to be a mixture also.

- **Color picking** – the user selects a single pair of coordinates (x, y). These coordinates are used to collect all the reference values of the marker, from the images corresponding to the wavelengths defined within the Lambda stack.
- **Area selection** – the user draws a mask using a brush, meaning a collection of coordinates (x, y). All (x, y) positions indicated by the drawn mask will be used to generate the reference values of the marker, from the images corresponding to the wavelengths defined within the Lambda stack.

Input Images

Input images can be selected in this tab. Input images (or the Lambda stack) represent a list with all images used in the unmixing process.



#	Image	Filter	Wavelength	Exposure Time	Lamp Intensity	LED Wavelength
1	DAPI_420	DAPI	420	10	50	390
2	DAPI_430	DAPI	430	10	50	390
3	DAPI_440	DAPI	440	10	50	390
4	DAPI_450	DAPI	450	10	50	390
5	DAPI_460	DAPI	460	10	50	390
6	DAPI_470	DAPI	470	10	50	390
7	DAPI_480	DAPI	480	10	50	390
8	DAPI_490	DAPI	490	10	50	390
9	DAPI_500	DAPI	500	10	50	390
10	DAPI_510	DAPI	510	10	50	390
11	DAPI_520	DAPI	520	10	50	390
12	DAPI_530	DAPI	530	10	50	390
13	DAPI_540	DAPI	540	10	50	390
14	DAPI_550	DAPI	550	10	50	390
15	DAPI_560	DAPI	560	10	50	390
16	DAPI_570	DAPI	570	10	50	390
17	DAPI_580	DAPI	580	10	50	390
18	DAPI_590	DAPI	590	10	50	390
19	DAPI_600	DAPI	600	10	50	390
20	CD3-O520_500	O520	500	20	50	475
21	CD3-O520_510	O520	510	20	50	475
22	CD3-O520_520	O520	520	20	50	475
23	CD3-O520_530	O520	530	20	50	475
24	CD3-O520_540	O520	540	20	50	475
25	CD3-O520_550	O520	550	20	50	475
26	CD3-O520_560	O520	560	20	50	475
27	CD3-O520_570	O520	570	20	50	475
28	CD3-O520_580	O520	580	20	50	475
29	CD3-O520_590	O520	590	20	50	475
30	CD3-O520_600	O520	600	20	50	475
31	Foxp3-O540_520	O540	520	10	50	510
32	Foxp3-O540_530	O540	530	10	50	510
33	Foxp3-O540_540	O540	540	10	50	510
34	Foxp3-O540_550	O540	550	10	50	510

The following **operations** are available to manage the input images list when defining an input:

- **Add Selected**: selects an image to add to the Spectral Unmixing input images list
- **Add Channels**: adds all original wavelengths for each channel to the input
- **Add Inputs**: adds more images to your input at the same time (batch)
- **Remove Selected**: removes selected images
- **Remove All**: removes all images
- **Move Up** / **Move Down**: controls change the position of the selected image (+1, -1)

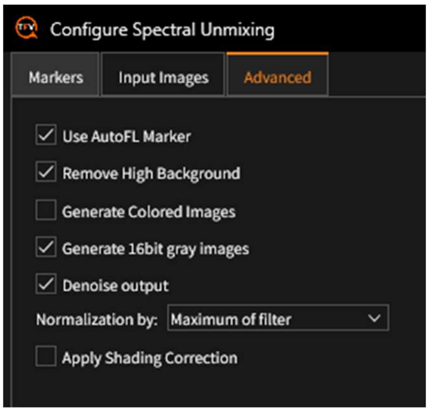
<u>**Note:**</u> All available input images are gray images acquired with 8 bit or 16bit.

Advanced

The **Advanced** tab contains the following settings:

- **Use AutoFL Marker:** enables auto fluorescence. It behaves like a marker in the unmixing process.

- **Remove High Background**: offers the ability to define the background. Usually, in 16-bit images, the background is never 0-value. Any value above 0 is considered signal and will be decomposed into the defined marker's components.
- **Generate Colored Images**: generates colored images for each unmixed marker. The color used is the one associated with each marker.
- **Generate 16bit gray images**: The grayscale images generated for each unmixed marker will be on 16-bits.
- **Denoise output**: A small filter is applied on the unmixed images to remove noise.
- **Normalization by**: specifies the method used to normalize the reference marker's values - maximum on filter or maximum on marker. Normalization is only for the plot.
- **Fluorescence mode**: switches between fluorescent / brightfield mode.
- **Apply Shading Correction**: enables shading correction on the input image.



☑ Use AutoFL Marker
☑ Remove High Background
☐ Generate Colored Images
☑ Generate 16bit gray images
☑ Denoise output
Normalization by: Maximum of filter
☐ Apply Shading Correction

After finishing the configuration for Spectral Unmixing, press **Analyze** button.

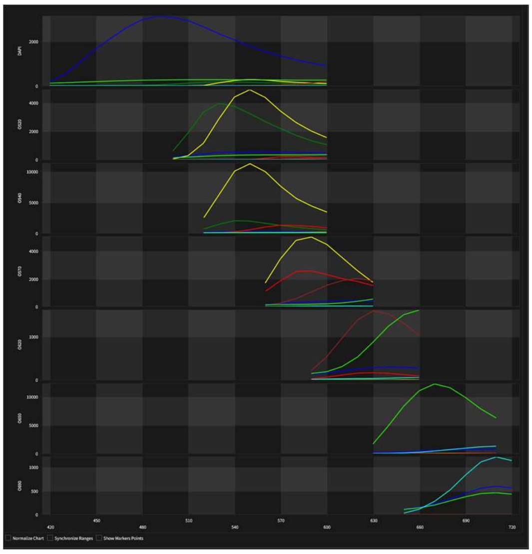
Based on the input, a set of images will be generated: the images for all individual channels used as input and a mixed image with all the channels superposed. To stop the run process, press **Clear Spectral Unmixing**.

Plot

The plot can be visualized after applying the selection for color picker/area selection.

The following settings are available:

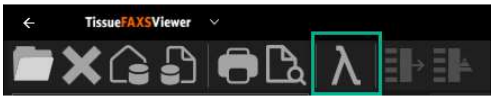
- **Normalize Chart**: if selected, normalized values will be used in the plot, using the marker values. If not selected, the raw values will be used.
- **Synchronize Ranges**: if selected, all the plots will have the same range on y axis.
- **Show Marker points**: displays the values on the plots as points.



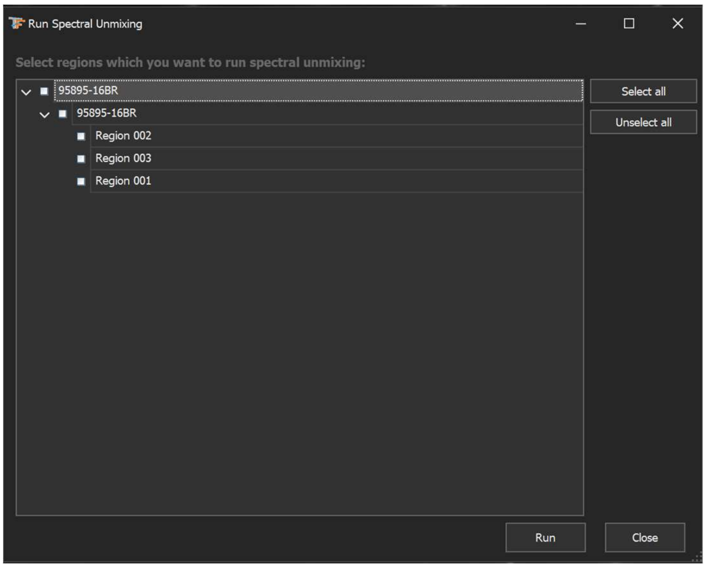
Propagating and running spectral unmixing

To run **Spectral Unmixing,** there are more options:

1. **Run Spectral Unmixing from main toolbar**



If pressing the **Run spectral unmixing** button from main toolbar, the **Run Spectral Unmixing** panel will open:



Run Spectral Unmixing

Select regions which you want to run spectral unmixing:

- ■ 95895-16BR
  - ■ 95895-16BR
    - ■ Region 002
    - ■ Region 003
    - ■ Region 001

Items for running spectral unmixing can be selected:

- by manually selecting the sample and the regions;
- by using the **Select All** option in order to run spectral unmixing for all the listed items.

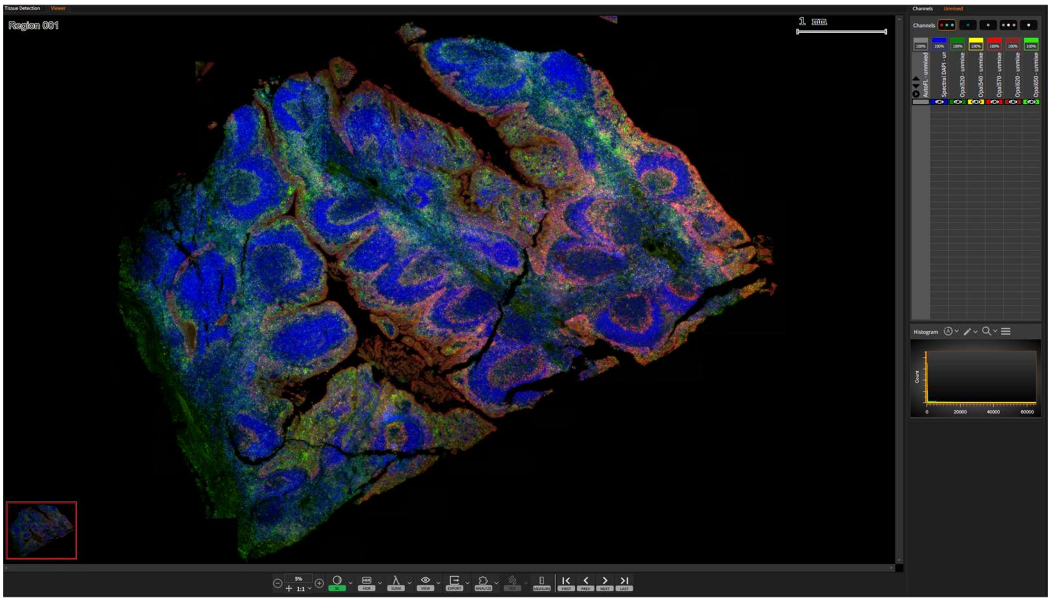
When the selection is done, press **Run**.

2. **Run Spectral Unmixing from Region Viewer toolbar**

If pressing the arrow near the **Configure spectral unmixing** button from the region viewer, two options will appear:

- **Run for current region**: runs spectral unmixing for the current region.
- **Run for whole slide**: all the current settings for spectral unmixing will be propagated to the whole selected slide.
- **Run for whole experiment**: all the current settings for spectral unmixing will be propagated to the entire experiment.
- **Run for whole job** (for TissueFAXS Loader projects): all the current settings for spectral unmixing will be propagated to the entire experiment.

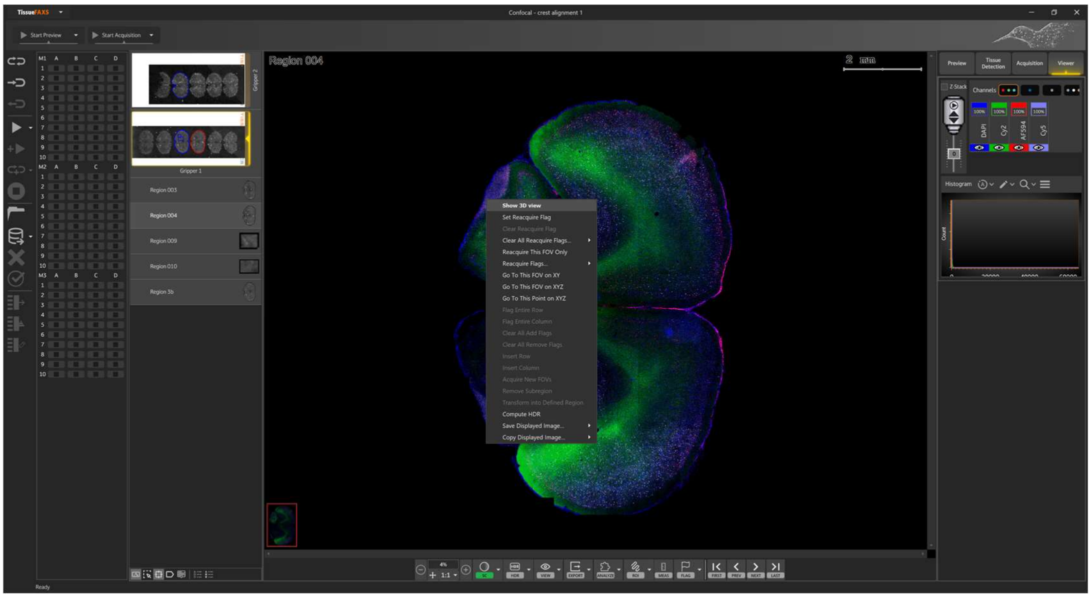
After running the algorithm, the unmixed images will become available in the toolbar as buttons.

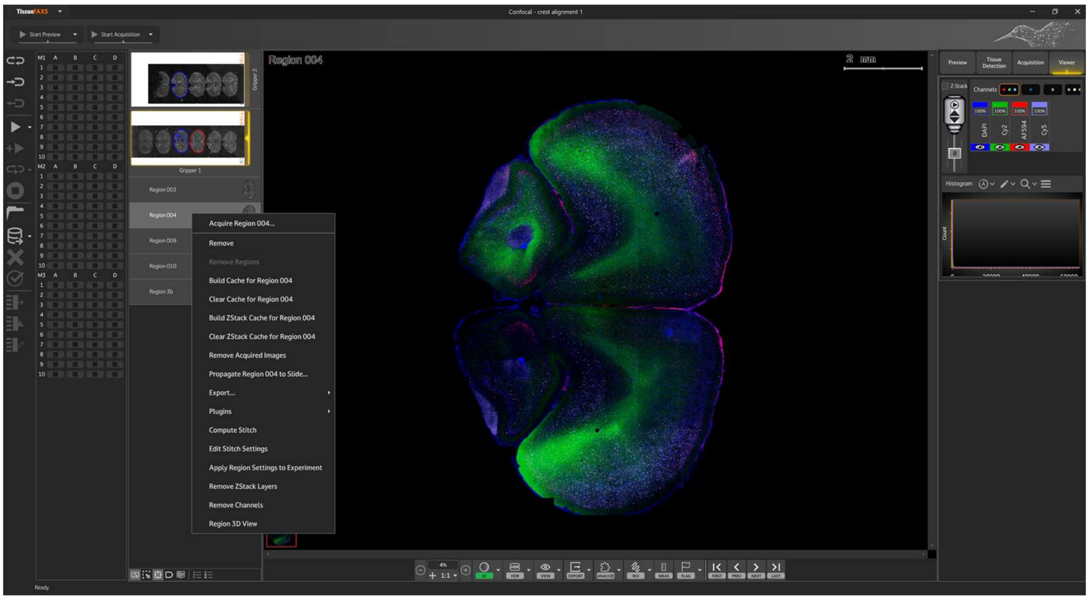


10.7. 3D Viewer

A region can be visualized in **3D Viewer** if it has been acquired with Z-stack.

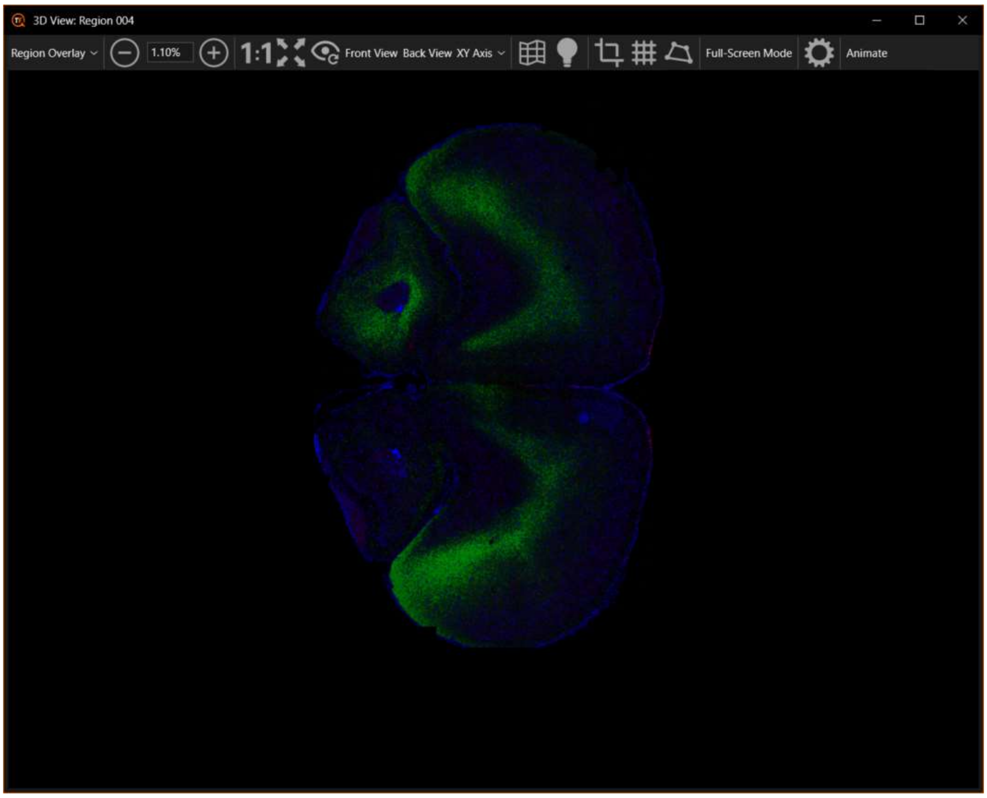
To access the **3D Viewer** feature, right click on a region and select **Region 3D View** from the contextual menu.

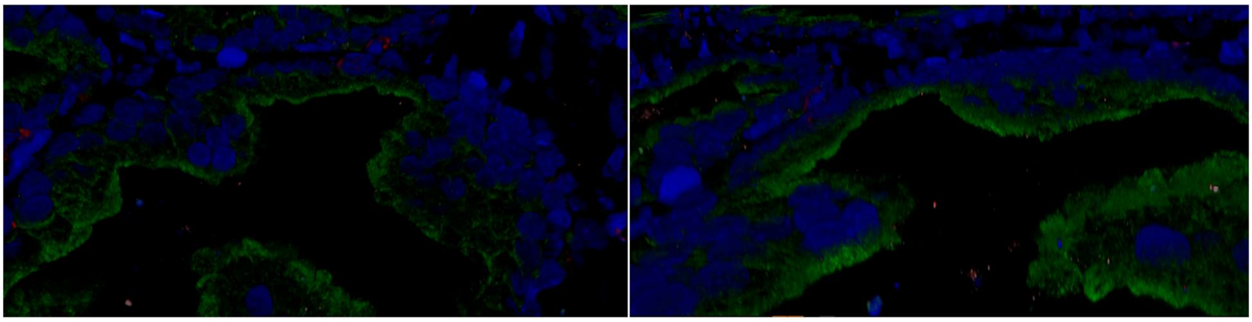




Note: Before accessing the 3D Viewer feature, the **Z Stack cache** needs to be built. This can be done by right clicking on a region and, from the contextual menu, selecting **Build Z Stack Cache**.

The selected region will open in full 3D Viewer mode:



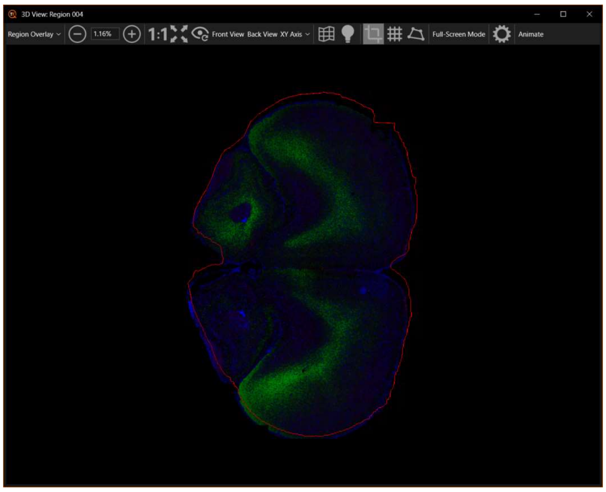


Full screen mode displays image in full screen mode.

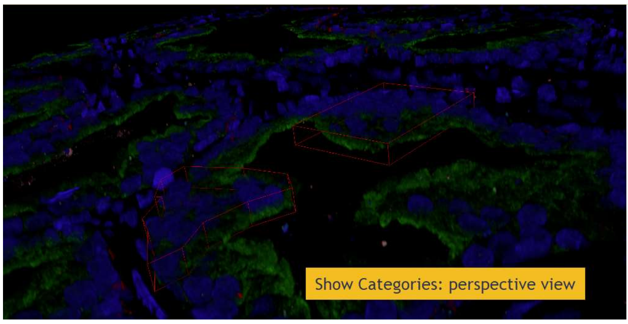
3D Viewer Toolbar

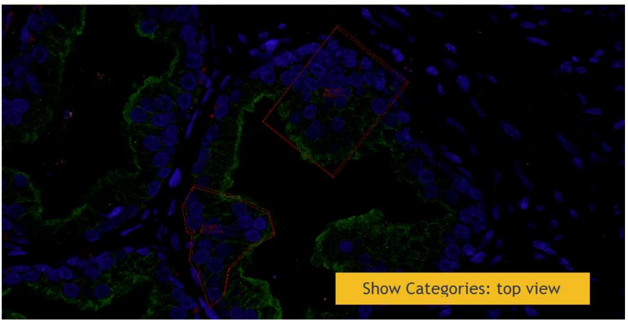
- **Region Overlay**: overlay feature in 3D Viewer helps selecting the channels to be displayed and adjust their values (intensity, color, range). See also Chapter Viewer Options;
- **Zoom in/Zoom Out;**
- **Original View;**
- **Best Fit View;**
- **Reset to Original View**: resets image to its original view, in other words the image will look like being freshly opened in 3D Viewer;
- **View Type: Front/Back/XY Axis**: choose to see the front/back of the opened image and select the rotation axis;
- **Map**: shows map; also see Chapter Images and Viewing Options;

- **Shading Correction**: activates shading correction; also see Chapter Illumination/Shading Correction;
- **Show Crop**: shows region's contour;



- **Show Grid**: shows the grid that separates the FOVs composing the image; also see Chapter Images and Viewing Options;
- **Show Categories**: shows categories (if any) in 3D mode. For more details see Chapter Categories;



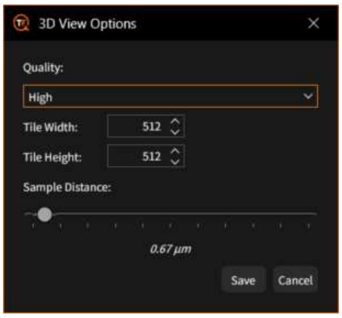


To handle 3D categories in the viewer:

- Select a region/category by using the mouse to click over the edge of the shape. To make multiple selections at once, hold down the CTRL key and click the edges of the shapes to be selected.

- You can highlight a region/category by hovering the mouse over the edge of the shape.

- **Full Screen Mode**: enters full screen mode. To exit, press Esc key;
- **Settings**: the advanced settings for 3D Viewer help you obtain an optimal view, getting the most of the graphical performance of your computer;



Quality: High

Tile Width: 512

Tile Height: 512

Sample Distance: 0.67 µm

- Set the **quality** of the 3D image. Lower quality will generate a fast rendering, higher quality will generate a slower rendering but a better image. Select Highest or High if you have a performant graphic card, if not – select Medium or Low;

- Set **tile** width and height values;

- Set **Sample Distance**: it represents the step made by a raycasting ray in the volume. The more points you have, the more detailed volume you will get. For optimal quality, 1 point per voxel is recommended (one sample per voxel). For low-quality graphic cards, you can increase the sample distance: some quality loss will occur in the image, but the overall performance will improve.

- **Animate:** TissueFAXS 3D Viewer allows creating and storing animation sequences.

- To create an animation, press **Add**. Enter values for X, Y and Z planes, and also a duration for the animation (in seconds). Press **Ok** to create the animation.

Action	Duration	Edit	Remove
Rotate X = 300, Y = 3...	60		✕
Rotate X = 300, Y = 3...	20		✕

X: 0

Y: 0

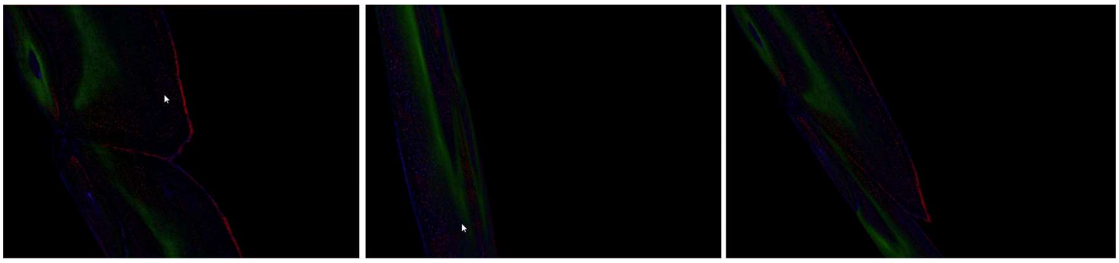
Z: 0

Duration[s]: 5

☐ Add reverse rotation

Start:

End: 80



- To edit an animation, press **Edit**.

- To delete an animation, press **Remove**. To delete all animations from the list, press **Remove All**.

- Press **Loop** to run the animations continuously.

- Press **Play** to run once.

- Press **Stop** to end current rendering.

- **Animation View** is a graphical overview of the “playlist” of existing animations. You can select at what time the animation starts and ends. You can also have a good visual understanding of how many animations you have and also their duration.

Operations Shortcuts

The following shortcuts are available for more dynamic interactions with an image in the 3D viewer:

- Mouse Right Click → Rotates camera around the focal point;

- Mouse Left Click → Zooms in on a selection;

- Shift + Mouse Left Click → Zooms in on a selection (the center is the view on the selection);

- Ctrl + Mouse Left Click → Zooms in on a selection (the center is the view on the selection);

- Mouse Wheel → Zoom in and out;

- Shift + Right Click → Zoom in and out;

- Mouse Wheel Click → Pans the region;

- R key → reset to original view;

- B key → view best fit;

- F key → Fly to point (animation that zooms in to mouse pointer, for single channel regions);

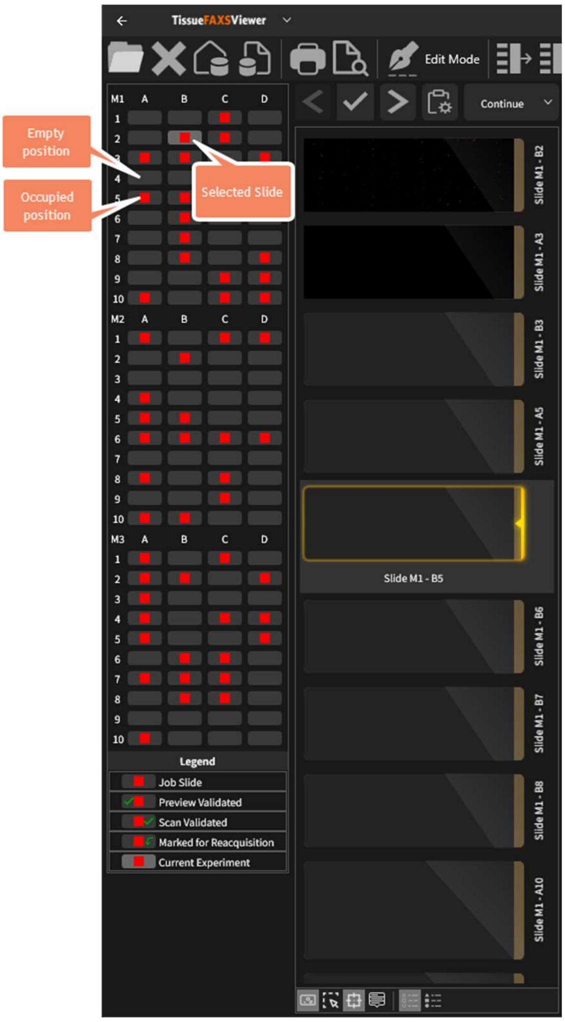
Note: Given a position x, and a movement of the camera’s current focal point to x, the movement is animated over the number of frames specified.

- Esc → exit full screen.

11. Operating jobs in TissueFAXS SL Viewer

11.1. Manage jobs

For **Slide Loader projects**, **TissueFAXS Viewer** will display a dedicated **job section**. This section displays all the slide loader positions. The empty positions look clear; the occupied positions are shown as tissue slides.



- **Legend**: shows the status of the slides.

Legend
🟥 Job Slide
✓🟥 Preview Validated
🟥✓ Scan Validated
🟥↺ Marked for Reacquisition
🟥 Current Experiment

- **Job Slide**: is the slide included in acquisition;
- **Preview Validated**: is the slide with validated preview;
- **Scan Validated**: is the slide with validated scan;

- **Marked for Reacquisition**: marks which slide will be included in reacquisition;
- **Current Experiment:** marks the currently opened experiment.

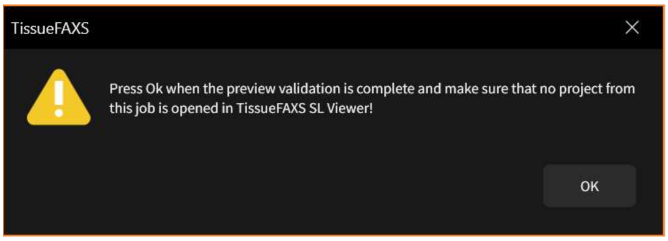
11.2. Validating slides in synchronous mode

When **TissueFAXS** and **TissueFAXS Viewer** work in synchronous mode, the workflow covers the steps below:

- Start a job in **TissueFAXS**.
- Open that job in **TissueFAXS Viewer**, by using **Validate Job** option or **Launch TissueFAXS SL Viewer** option followed by the manual opening of the job.
- Once a slide is previewed in **TissueFAXS**, it becomes available for validation in **TissueFAXS Viewer**.

Notes:

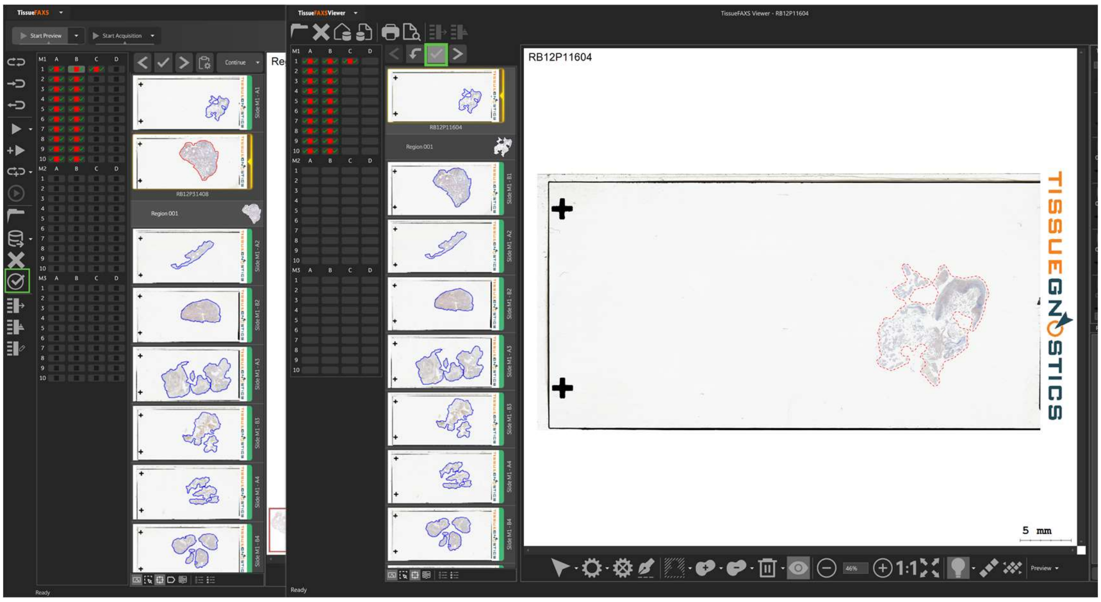
- To have the full content of a job available in **TissueFAXS Viewer** the user should press **Refresh Job** button.
- After **TissueFAXS** has finished the preview for all the slides, the validation started in **TissueFAXS Viewer** can be finalized in **TissueFAXS.** There will be a warning message for the user reminding him to close the job and its experiments in **TissueFAXS Viewer.**



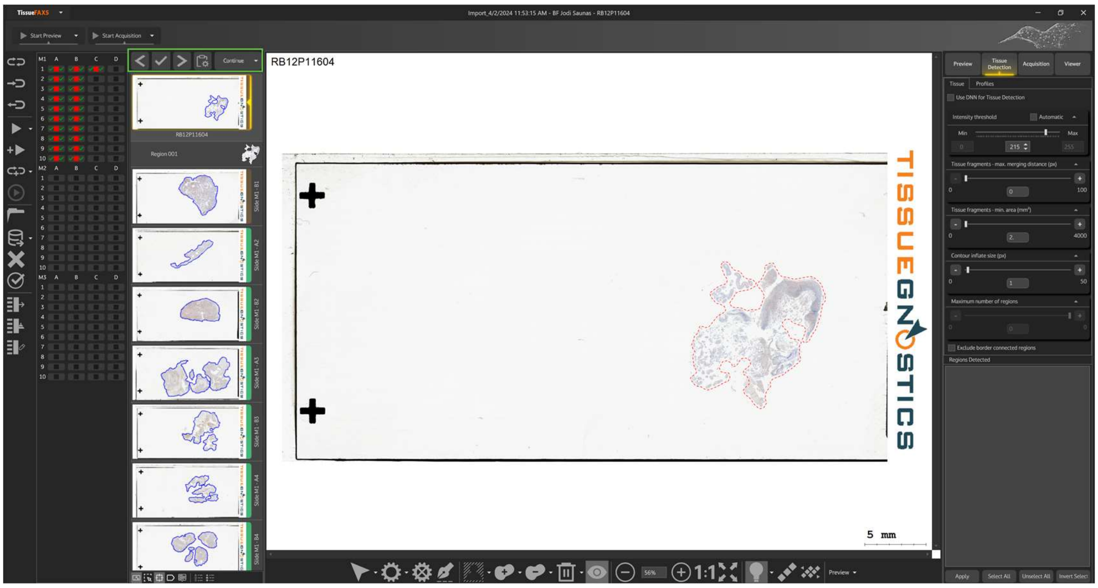
Press Ok when the preview validation is complete and make sure that no project from this job is opened in TissueFAXS SL Viewer!

You can automatically open the job for validation in **TissueFAXS Viewer** by using **Validate** button.

During the preview acquisition, you can define regions (by automatic tissue detection or manually) and validate each slide by using **TissueFAXS Viewer**.

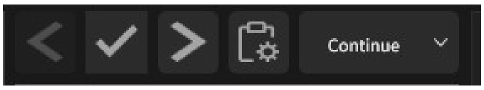


After **TissueFAXS** ends the preview (only for the **Preview All/ Scan all workflow**), you can start validating your slides.



You can navigate through slides and validate them using the validation controls.

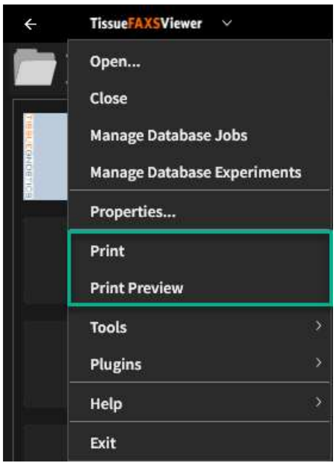
There is a toolbar with more buttons for moving in between slides and performing validation process:



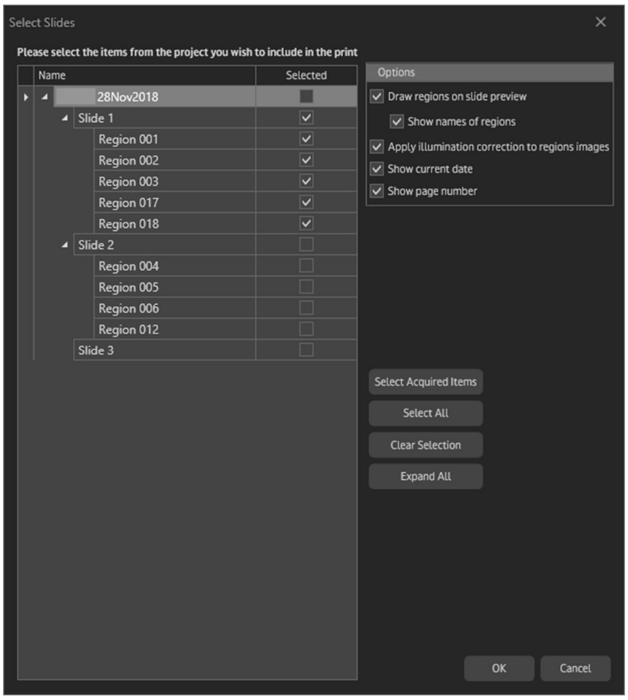
- **Move to previous slide**
- **Validate slide**: validates current slide
- **Move to next slide**

12. Print

From **TissueFAXS Viewer Start** menu, you can open **Print/Print Preview** feature.



Printing is only possible for an opened experiment. When the dialog appears, select the items (acquired items, all items, etc.).



Please select the items from the project you wish to include in the print

Name	Selected
[redacted] 28Nov2018	■
Slide 1	☑
Region 001	☑
Region 002	☑
Region 003	☑
Region 017	☑
Region 018	☑
Slide 2	☐
Region 004	☐
Region 005	☐
Region 006	☐
Region 012	☐
Slide 3	☐

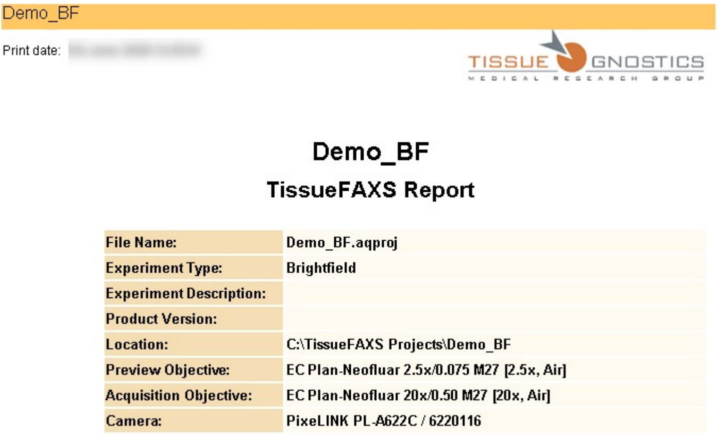
Options
- ☑ Draw regions on slide preview
  - ☑ Show names of regions
- ☑ Apply illumination correction to regions images
- ☑ Show current date
- ☑ Show page number

The default selection for this dialog is the selected slide in the slide viewer. It also offers a set of **options**:

- Draw regions on slide preview: The preview image may also contain region shapes;
- Show names of regions: is available only if the first option is selected;
- Apply illumination correction to regions: is available only for **Brightfield** experiments, the correction image will be applied to exported images if the correction image is available;
- Show current date: the print date is visible on each page of the report;
- Show page number: the page number is visible on each page of the report.

Print Options

- Selecting the **Print** option will bring up a dialog where the printer can be chosen.
- Selecting the **Print Preview** option will generate a preview report.



Demo_BF

Print date: [redacted]

Demo_BF

TissueFAXS Report

File Name:	Demo_BF.aqproj
Experiment Type:	Brightfield
Experiment Description:	
Product Version:	
Location:	C:\TissueFAXS Projects\Demo_BF
Preview Objective:	EC Plan-Neofluar 2.5x/0.075 M27 [2.5x, Air]
Acquisition Objective:	EC Plan-Neofluar 20x/0.50 M27 [20x, Air]
Camera:	PixeLINK PL-A622C / 6220116

The **preview report** contains the following:

- Experiment Name;
- File Name;
- Experiment Type;
- Experiment Description;
- Product Version: the **TissueFAXS** version used to print this info;
- Location: the location of the experiment;
- Preview Objective: the objective lens used for the preview operation;
- Acquisition Objective: the objective lens used for acquisition;
- Camera: the camera used for this experiment;
- Each Slide selected in the list:

- Slide Name;

- Slide Image;

- Content Type;

- Comments: the comments referring to this slide;

- Objective: the objective lens used for preview.

- Slide Preview Channels: is a table that contains the channel list for the current slide and some properties for each channel in the list:

- Checked: this flag indicates if the current channel is used for overlay;

- Name: the channel name;

- Intensity: the channel intensity;

- Color: the channel color.

- Region list for each generic slide:

- Region Name;

- Region Image;

- Comments: the comments referring to current region;

- Acquired: this flag indicates if current region is acquired or not. Two possible values are present: **Yes** or **No**;

- Path: the path for current region files;

- Objective: the objective used for acquire current region;

- Rows: the number of rows for region;

- Columns: the number of columns for region;

- FOV’s Count-: the number of FOVs items;

- Patient Name: the patient name;

- Patient Reference number: the individual reference number;

- Time Lapse (if acquired with time lapse);

- Number of Runs (if acquired with time lapse);

- Time between Runs (if acquired with time lapse).

- Regions Channels: is a table that contains the channels list for current region and some properties for each channel in the list:

- Checked: this flag indicates if the current channel is used for overlay;

- Name: the channel name;

- Intensity: the channel intensity;

- Color: the channel color.

- TMA Blocks list for each TMA slide:

- TMA Block Name;

- TMA Block Image;

- Comments: the comments referring to current TMA block;

- Acquired: this flag indicates if current TMA block is acquired or not. Two possible values are present: **Yes** or **No**;

- Objective: the objective lens used for acquisition of the current region;

- Rows: the number of rows for the current region;

- Columns: the number of columns for the current region.

- TMA Spots Count-: the number of spot items.