TISSUEGNOSTICS

PRECISION THAT INSPIRES

TissueFAXS Viewer

USER MANUAL

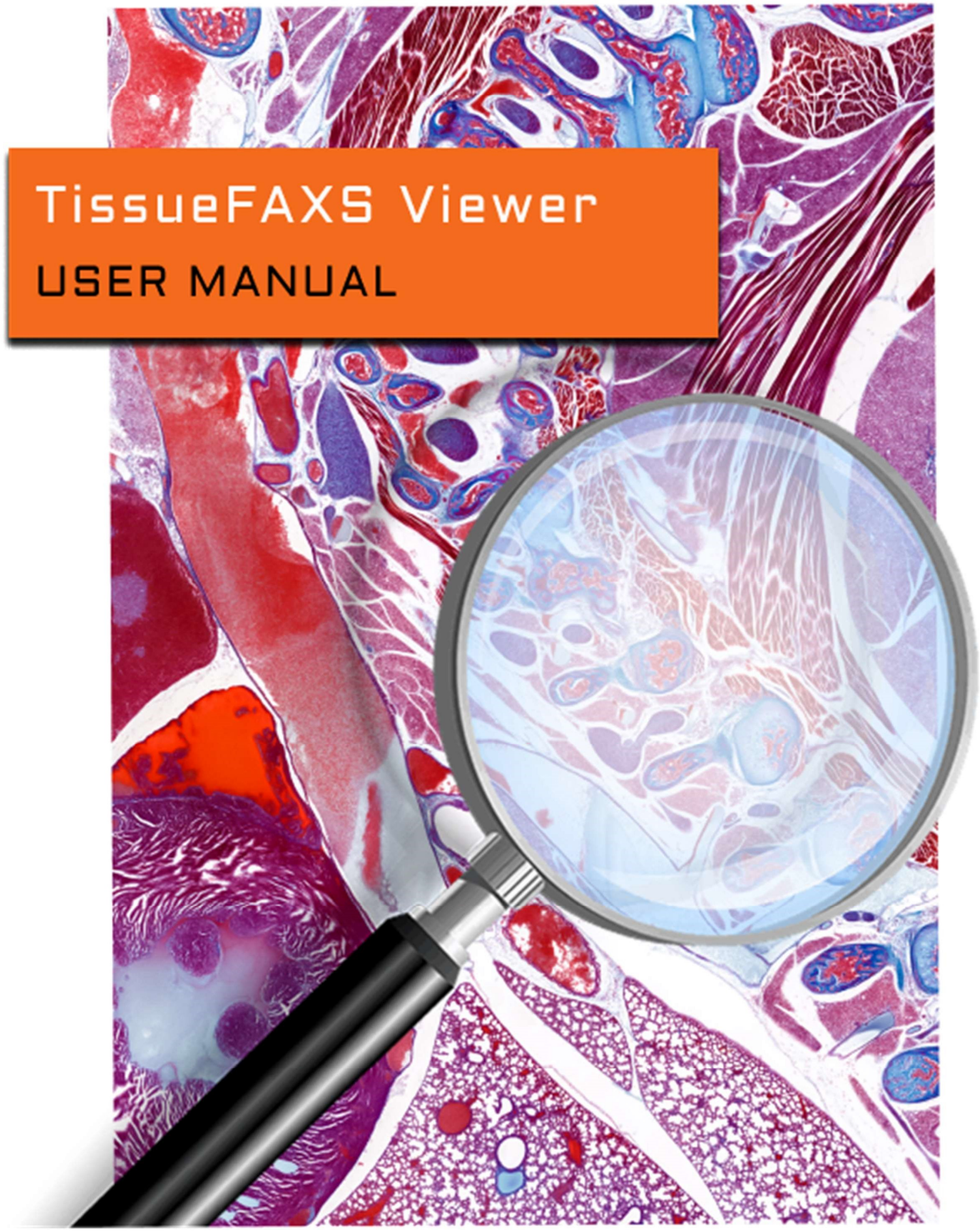
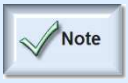


Table of Contents

1. 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 projects acquired with the acquisition software **TissueFAXS**.

- **Please always make sure you are using TissueFAXS Viewer with projects acquired with the same TissueFAXS version!**
- For example, for TissueFAXS Viewer V7.1 you will only use projects acquired with TissueFAXS V7.1.



- **Some options in TissueFAXS Viewer are only available in Edit Mode (see Chapter 8.3).**

1.1. Purpose

The purpose of this document is to guide the user through the features of **TissueFAXS Viewer** software.

2. Login

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

Figure 1 - TissueFAXS Viewer Splash Screen

3. Overview

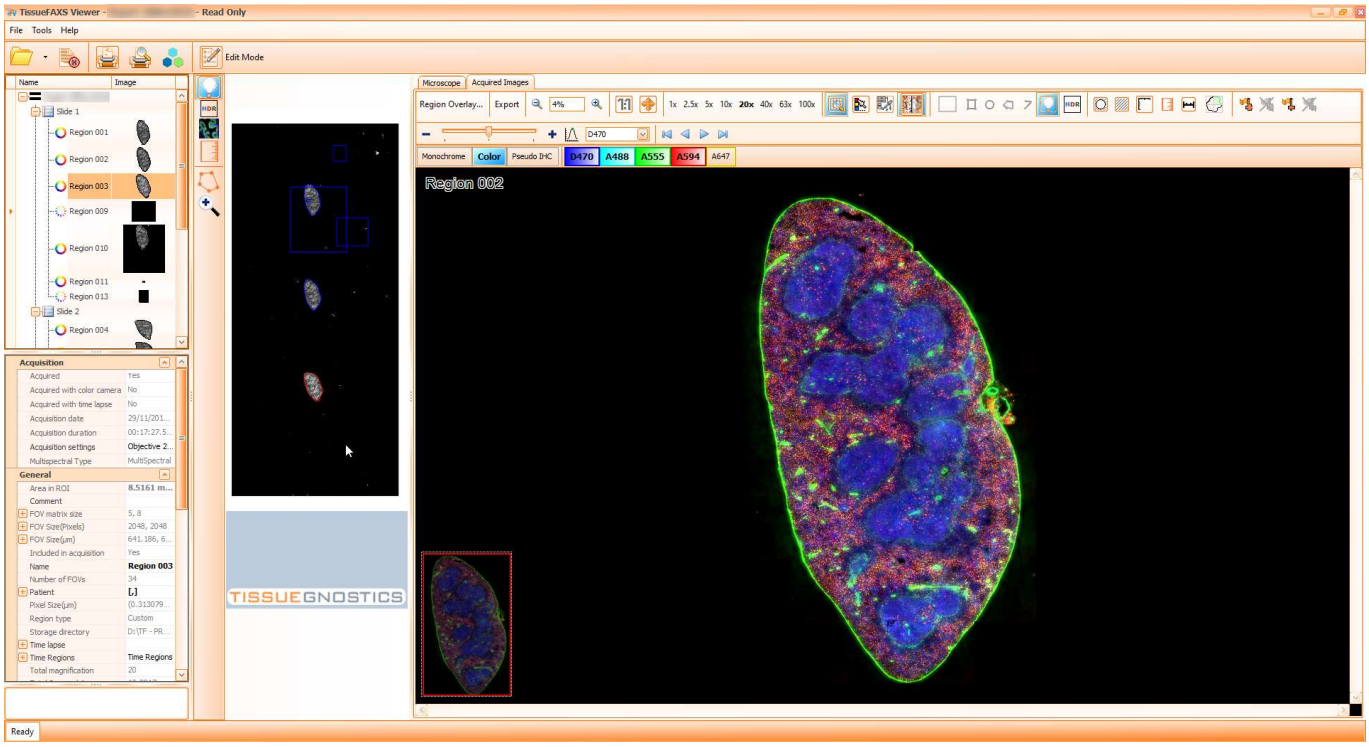


Figure 2 - TissueFAXS Viewer overview

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.

TissueFAXS Viewer has a section where you can see listed all the slides and their regions with their properties, an overview of the selected slide, a Microscope tab and an Acquired Images tab.

In the chapters below, you can find detailed all the features of the application.

4. Slides Section: experiment tree-like representation

All the slides and regions from the currently opened experiment are listed here. You can expand or collapse them depending on your needs.

If you right-click on any item, a dropdown menu is available:

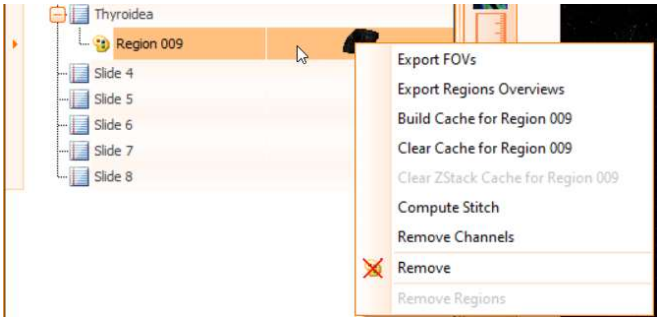


Figure 3 – Dropdown menu

- **Export FOVs**: you can export on your computer hard drive all the FOVs of the selected item;
- **Export Regions Overviews**: you can export on your computer hard drive the region overview(s) of the selected item;
- **Build Cache**: builds cache for selected item;
- **Clear Cache**: clears cache for selected item;
- **Build ZStack Cache**: builds ZStack cache for selected item;
- **Clear ZStack Cache** (for ZStack projects): clears ZStack cache for selected item;
- **Compute Stitch**: computes stitch for selected item;
- **Remove Channels** (for multi-channel experiments): removes channels for selected item. Select the channels you want to remove, then press Remove. You can select all the items from the list, unselect all and invert selection. Also, you can expand or collapse entire channels list.

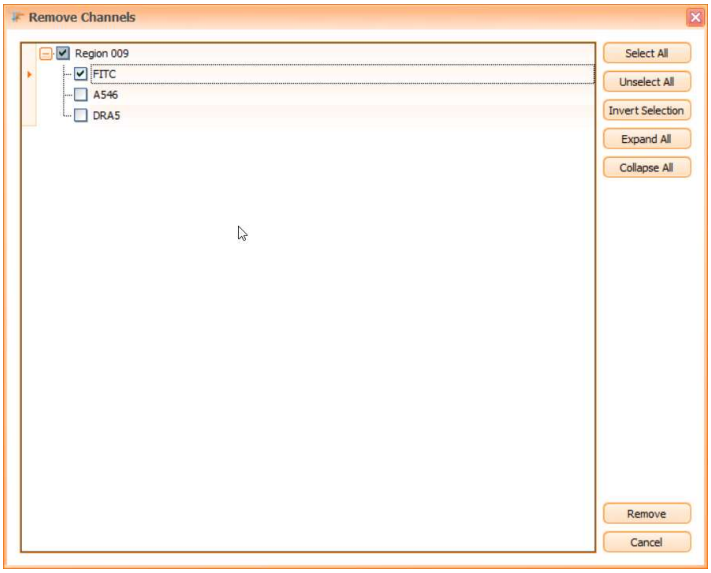
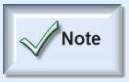


Figure 4 – Removing channels

- **Remove**: removes selected item
- **Remove Regions**: removes all currently selected items.



- All above mentioned options are only available in Edit Mode.

Under the tree-like experiment representation, you will find a section including all the features of the selected item.

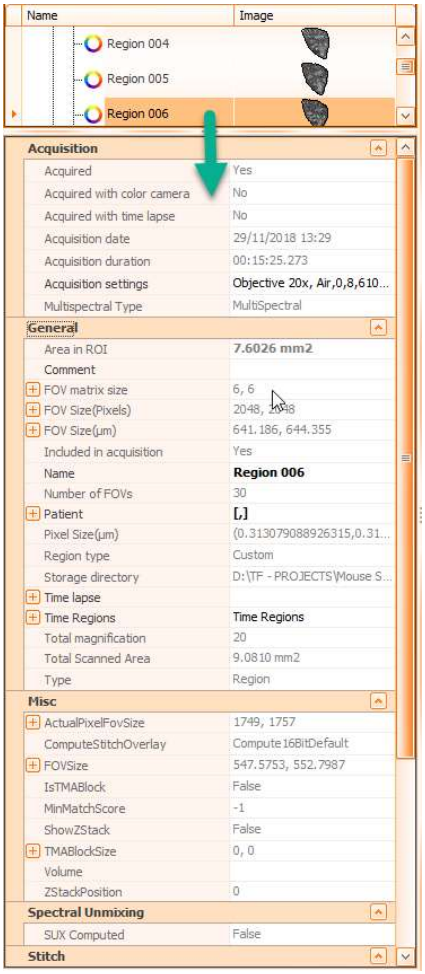


Figure 5 – Selected items' properties

Acquisition details

- Acquisition status;
- Camera type;
- The item is/is not acquired with time lapse;
- Acquisition date;
- Acquisition duration;
- Acquisition settings;
- Multispectral type.

General info

- Area in ROI: area for the cropped shape (including only acquired fields of view);
- The comment indicates sample related data;
- The item consists of a virtual matrix containing a number of Fields of View;
- The FOV matrix size (Width and Height);
- FOV size (pixels);

- FOV size (µm);
- The item is/is not included in acquisition;
- The item name;
- Data concerning the patient;
- Pixel size (µm);
- Region type;
- The storage directory is shown here;
- Time lapse: number of runs and time lapse are shown here;
- Time regions;
- The total magnification;
- Total scanned area;
- Type of item.

Spectral Unmixing

- SUX computed.

Stitch Details

- Overlap size in pixels;
- Stitch status;
- Stitch rectangle size (pixels);
- Stitch rectangle size (µm);
- Support stitch.

ZStack Details

- ZStack acquisition status;
- Extended Focus;
- Step size above;
- Step size below;
- Steps above;
- Steps below.

5. Slide Overview

The selected slide will appear in the Slide Overview section.

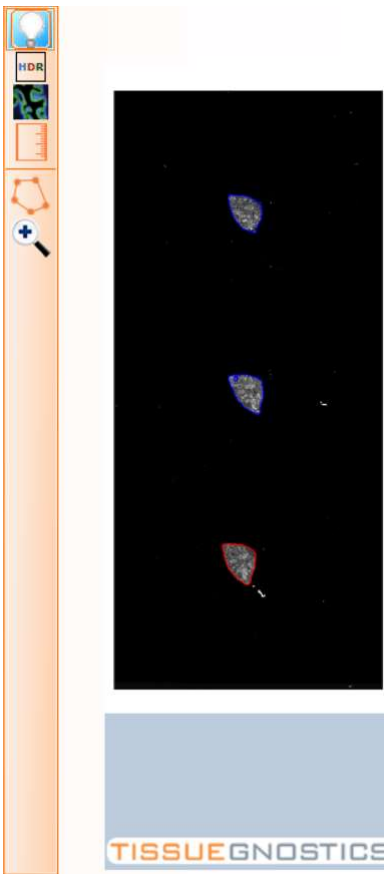


Figure 6 – Slide overview

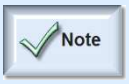
If you right-click on the image, you can export on your computer hard drive the slide's image.

The following options are available:

- **Illumination Correction (BF)/Shading Correction (FL)**

Occasionally, on the acquired images, some shades may appear. They can be caused by imperfections of any component of the lightpath, specks/impurities on the camera/objective.

TissueFAXS allows you fixing such shading problems by using the **Illumination/Shading Correction** function.



- This operation is available for **brightfield** experiments (Illumination correction) and for fluorescence (Shading correction).

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

- **HDR**

Enables HDR viewing type.

- **Slide Overlay**

Located in the toolbar, the **Slide Overlay** button () will allow you to view your preview image for each reflector/channel (in **fluorescence** experiments). Check the desired reflector in the panel shown below in order to obtain the desired preview.

For more details regarding the Overlay feature please consult ***TissueFAXS User Manual***.

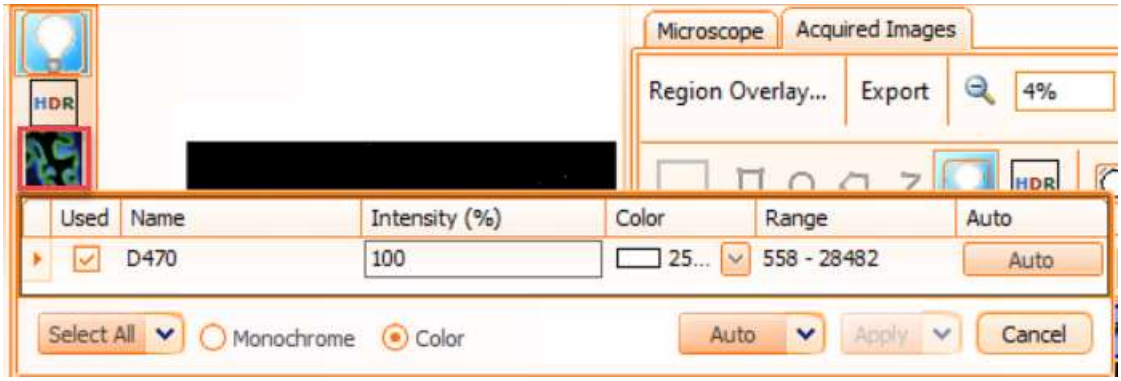


Figure 7 - Slide Overlay Dialog

- ## Scale Bar

The **Scale bar** is represented by a segment that indicates the scale of the image.

It has two adjustable attributes:

- The **color**;

- The **location** list with four values:

- ## Measure

This function is used to measure the distance between two points (on the sample) specified by the user (by clicking on the start point, then on the end point).

The distance and the unit of measure are displayed on the measured image.

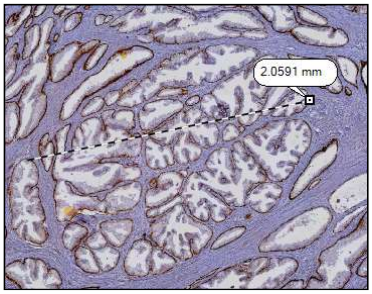


Figure 8 - Measured image example

Note

- There are two adjustable attributes, the color and the measure unit. The default color is **aquamarine** and the default measure unit is **millimeter**.

- ## Custom selection

Custom Selection button () allows the user to freely draw a selection over the sample in order to select the desired group of ROIs. This feature helps avoiding multiple clicks for one-by-one selection of the desired items.

6. Microscope tab

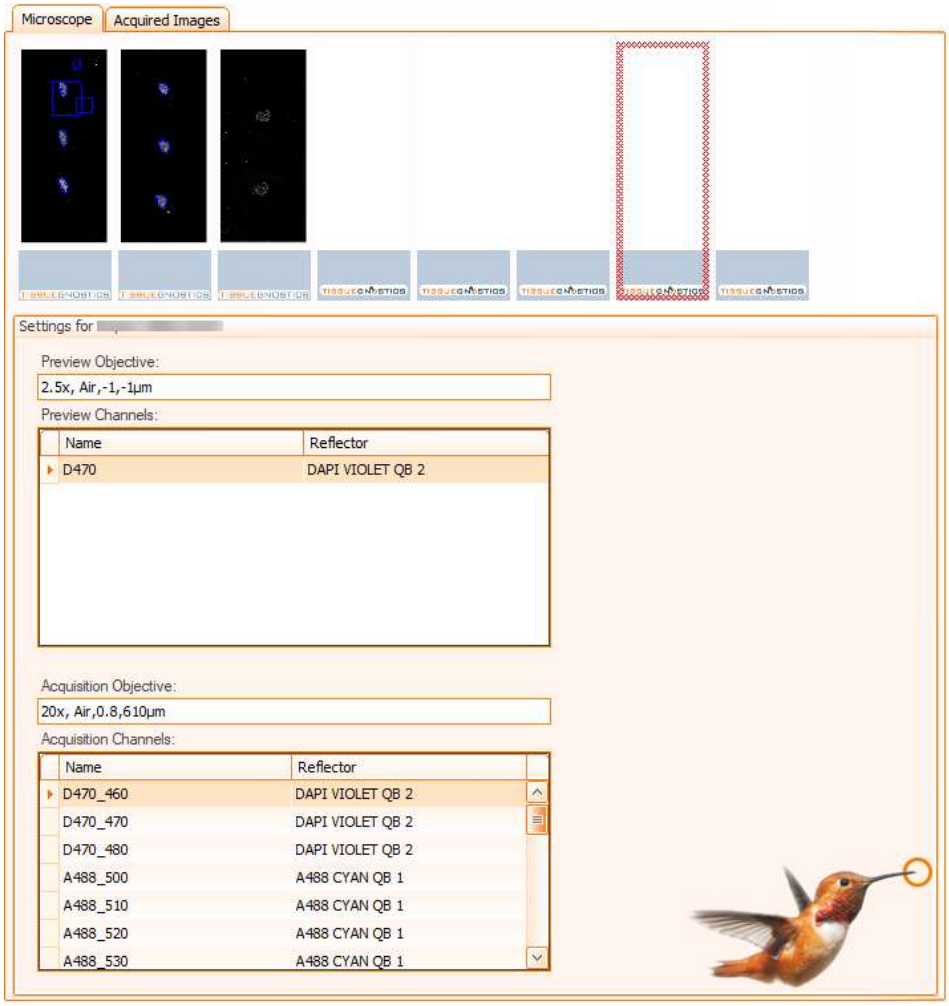


Figure 9 - Microscope tab

In the Microscope tab you will see all the previewed slides with their regions, as they appear on the stage.

If you right-click on any of these slides (

), you can **export** its image on your computer hard drive.

The following settings information is available:

- Preview objective;
- Preview channels;
- Acquisition objective;
- Acquisition channels.

7. Acquired Images tab: 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 regions, double-click on the region from the experiment editor (on the left side of the main window). The region viewer panel, which was empty before, will be updated with the region's image. The **Region Viewer** is shown in the next screenshot:

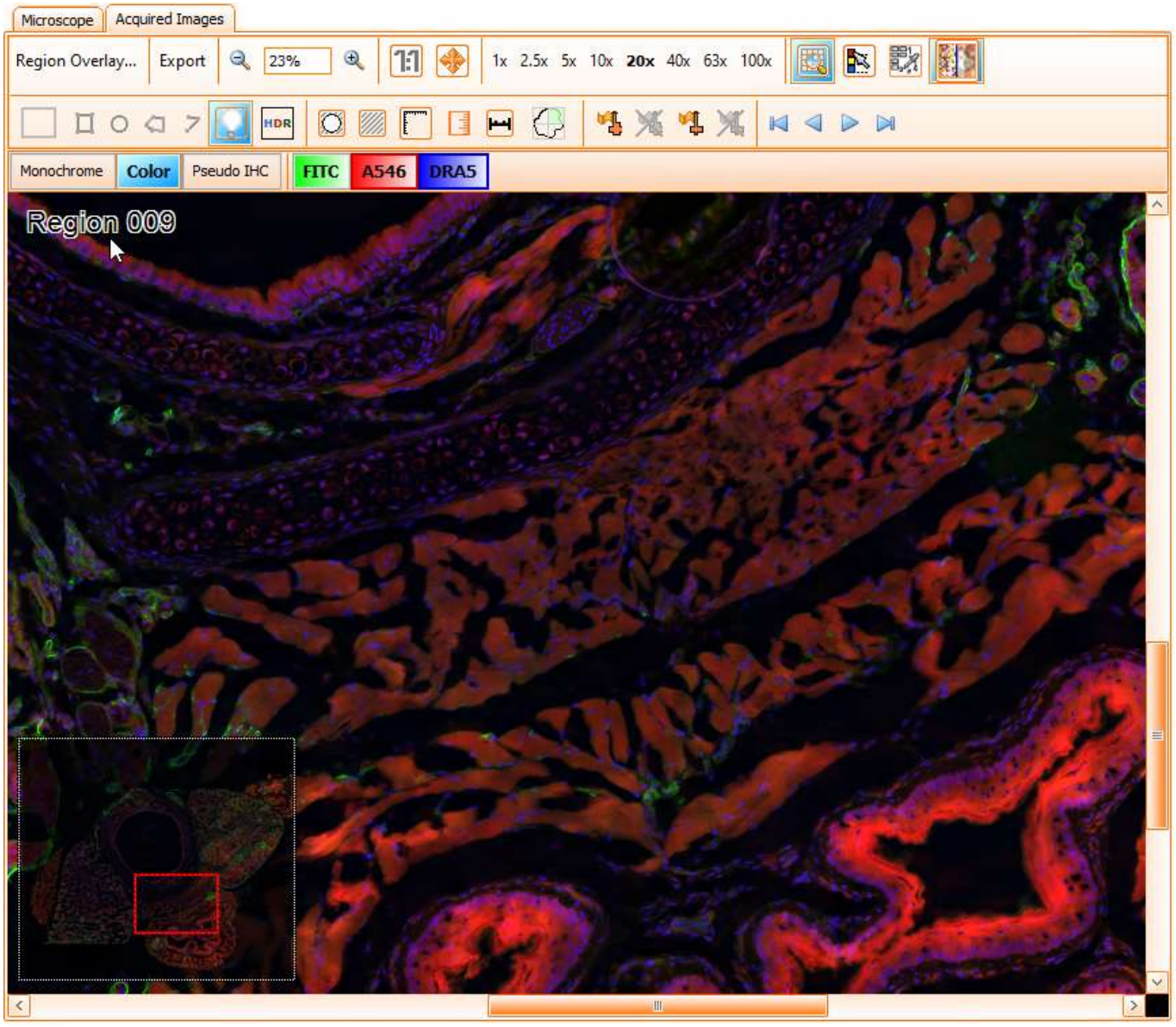


Figure 10 - Acquired Images tab

The toolbar contains the following buttons:

- Region Overlay (for fluorescence experiments);
- Z stack (if acquisition was made with Z stack);
- Export: **Fields of View**, **Region Overview**;
- Plugins;
- Zoom in;
- Zoom out;
- Objective magnification zoom buttons (ex. 1x, 25.5x)
- Map: shows/hides region map;
- Add subregions and tissue areas belonging to categories;
- Subregions: **Show Defined Regions**, **All Categories**, **Manage Categories**;
- Crop (it marks the images that have been acquired outside the defined region);
- Gridlines: default value is **Off**;
- Scalebar: default value is **Off**;

- Focus Points;
- Measure;
- Annotations;
- Illumination Correction/Fluorescence Shading;
- HDR;
- Reacquire Flag;
- Add Flag (it adds Fields of View to the region);
- Remove Flag (it removes Fields of View from the region);
- Time lapse acquisition button (if it is the case);
- Enable Post-processing;
- Smoothing Image;
- Pixel Inspector;
- Go to First/Previous/Next/Last Region navigation buttons;
- Show RGB.

7.1. Region Overlay

In **fluorescence** experiments, the 

button is enabled. Clicking on it yields a new window, as shown below:

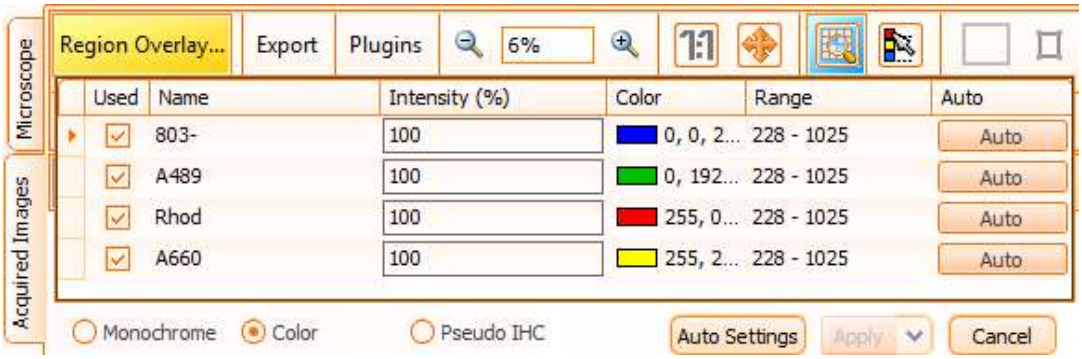


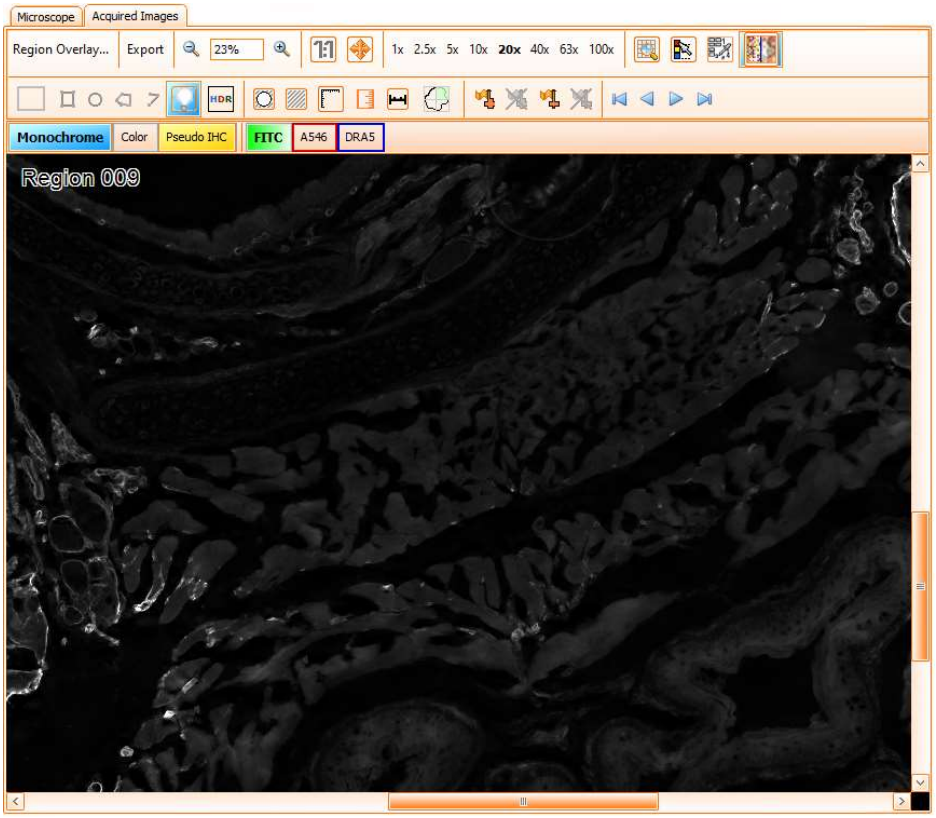
Figure 11 - Adjusting channel intensity and color

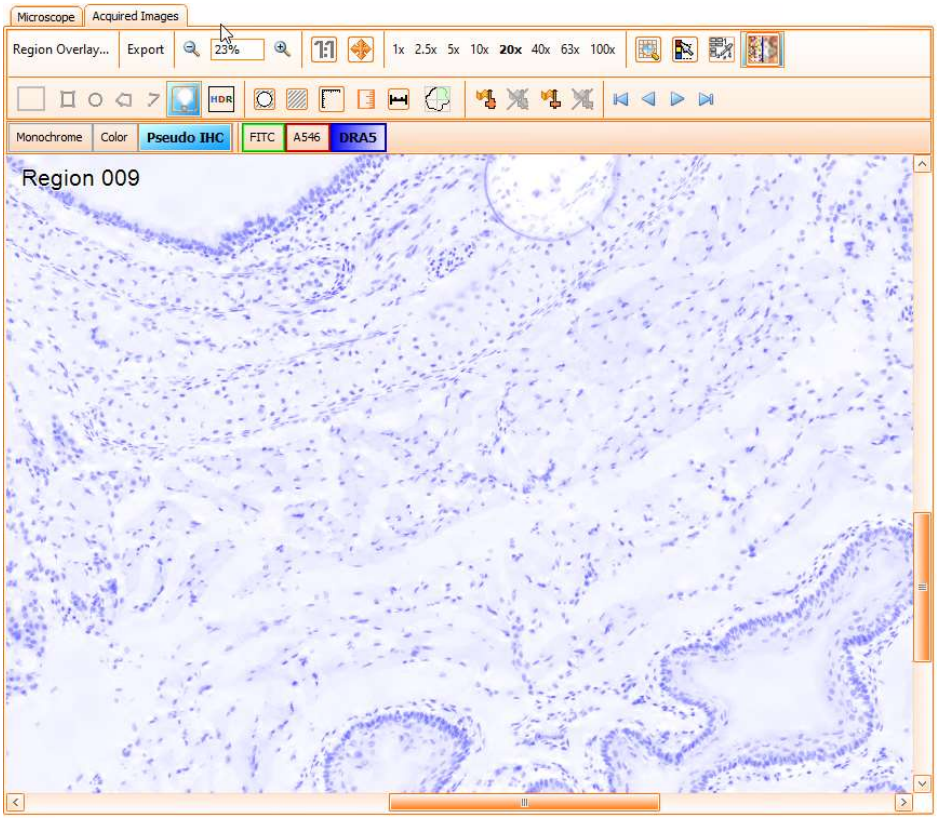
This window allows you to choose which channels to view in your acquired image. Here, you can adjust the color, light intensity, dynamic range (only for channels acquired with 16bit) for each channel. If more than one channel is selected, clicking **Apply** will yield an overlay image, which is composed of the selected channels according to the set algorithms.

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

There are more 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**: 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.


Microscope
Acquired Images
Region Overlay...
Export
23%
1x 2.5x 5x 10x 20x 40x 63x 100x
HDR
Monochrome
Color
Pseudo IHC
FITC
A546
DRA5
Region 009


Microscope
Acquired Images
Region Overlay...
Export
23%
1x 2.5x 5x 10x 20x 40x 63x 100x
HDR
Monochrome
Color
Pseudo IHC
FITC
A546
DRA5
Region 009

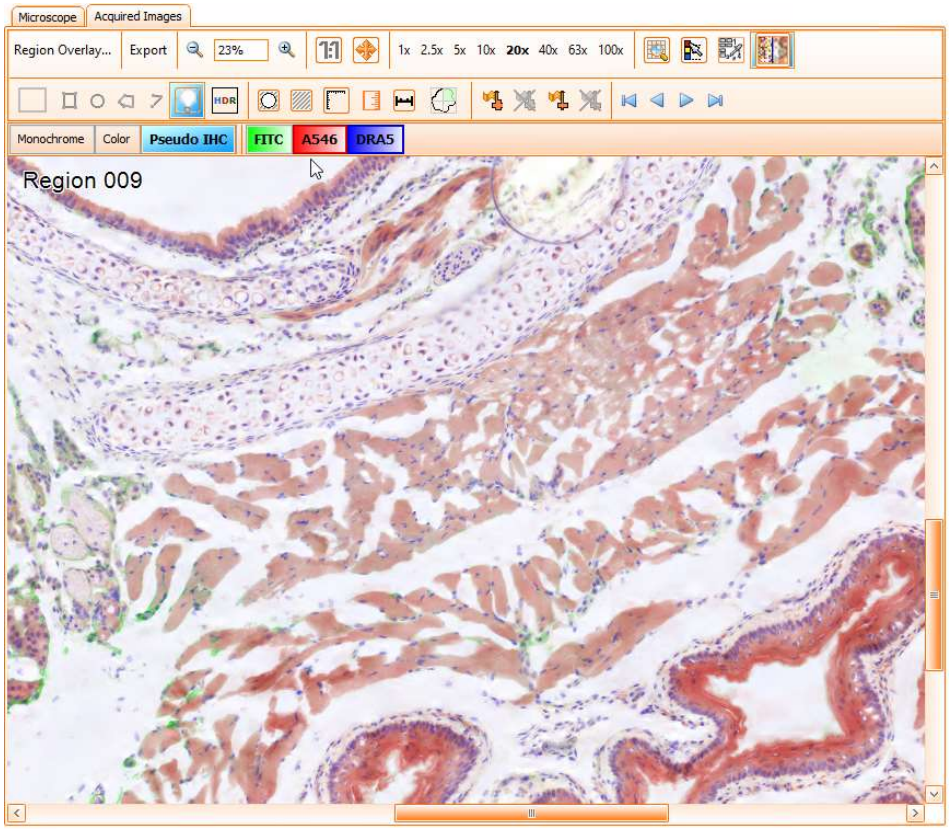


Figure 12 - Example of pseudo IHC visualization

Easy visualization for overlay images

For fluorescence projects, in the **Region Viewer/Acquired Images** toolbar there are **buttons** for all the existing channels for easy overlay changes.

In the **multispectral** experiments, the acquired images display in the viewer toolbar two particular features: *wavelength track bar* and *dropdown box for selecting desired reflector*.

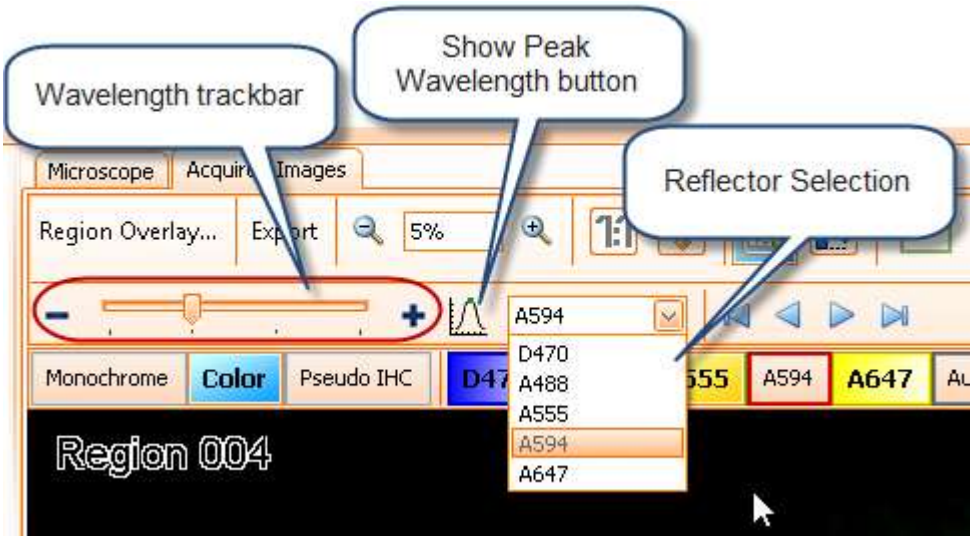


Figure 13 – Wavelength step and wavelength range features

The **track bar** controls the wavelength displayed for the selected channel in the associated dropdown.

Show Peak Wavelength: by pressing this button the image will be displayed with 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 (on the track bar).

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

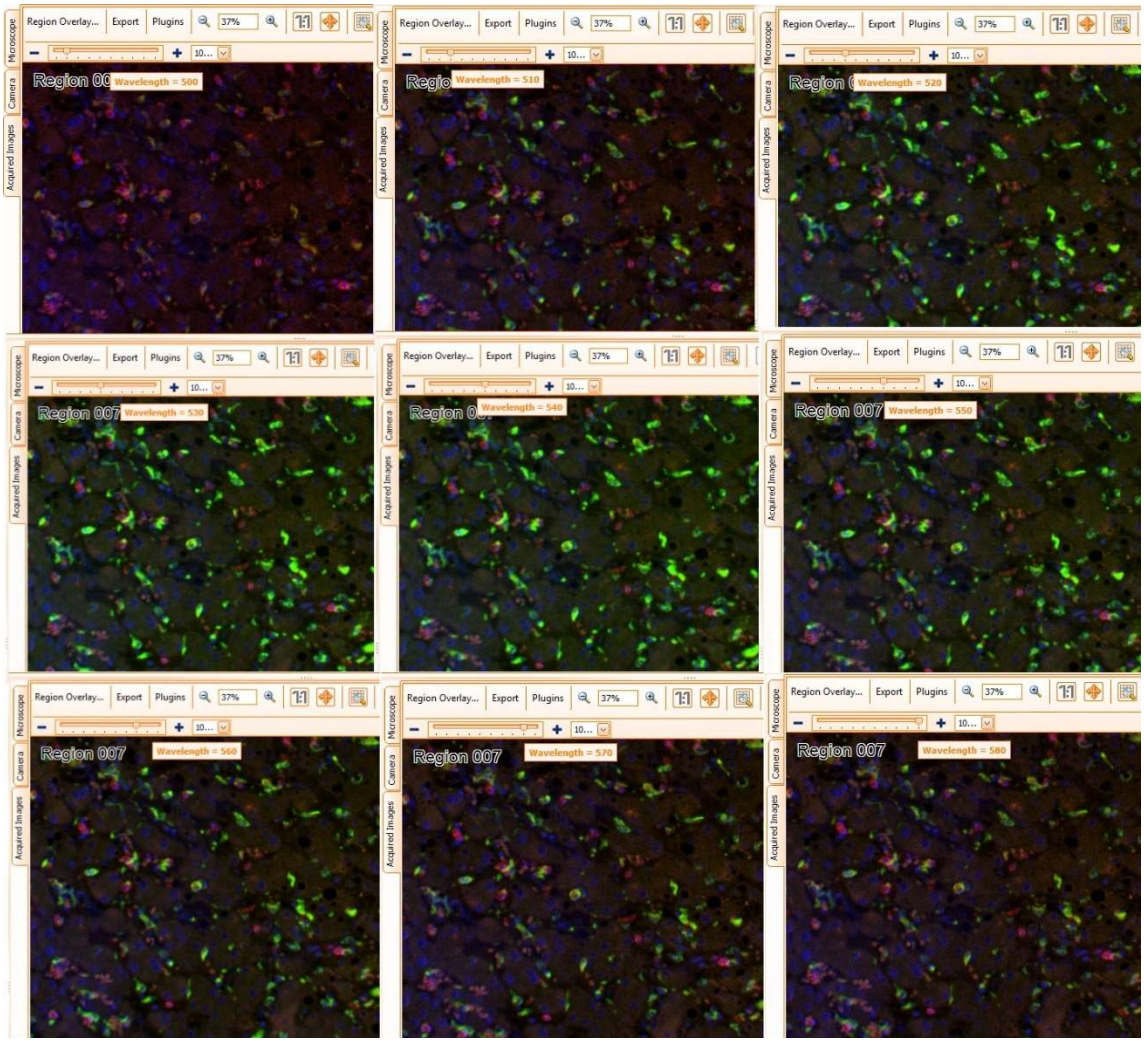


Figure 14 – Lambda stack for Alexa Fluor overlay images: from 500 to 580 with step 10

Note

- In Monochrome Mode the reflector dropdown list is disabled.

In **brightfield** experiments, the **Region Overlay…** button is disabled because there is only one image for each Field of View.

Zoom in and **Zoom out** buttons allow zooming the image. Another option would be to type the desired zoom value in the zoom editor. Keep in mind, however, that the Region Viewer is designed

to give the user an overview of the entire region and therefore is limited in giving detailed magnified images.

After a region is acquired, the user can double press the left mouse button on the region to open the **Acquired Image** tab and to see the acquired image split into Fields of View.

7.2. Export

7.2.1 Fields of View

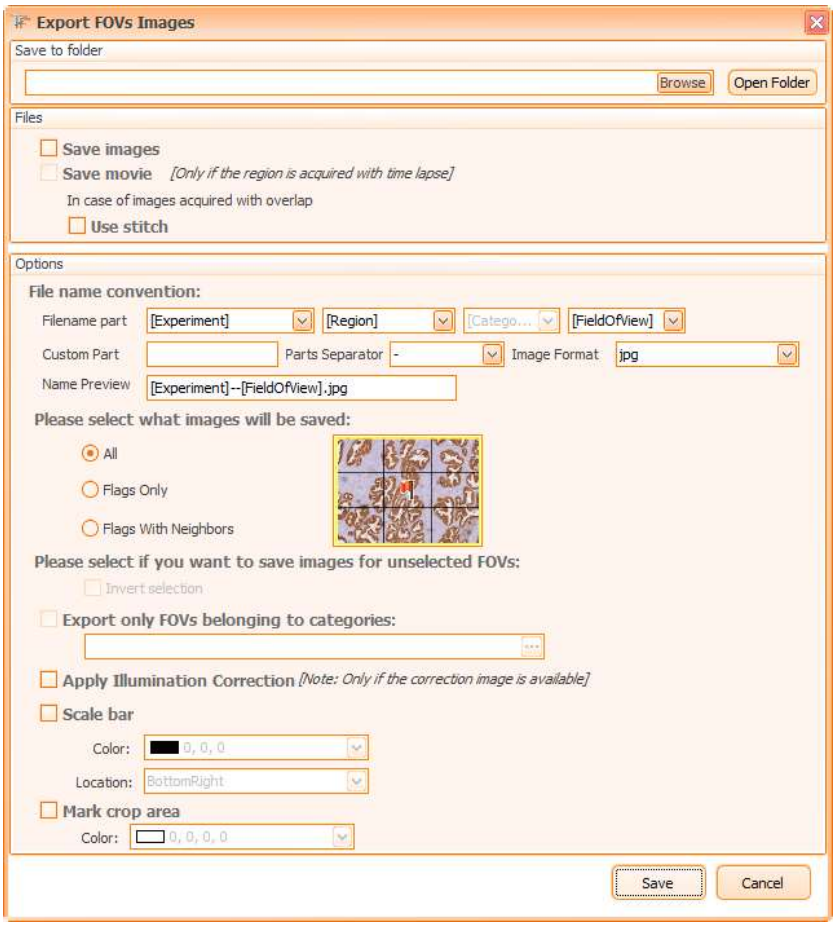


Figure 15 – Export FOVs Images dialog (brightfield)

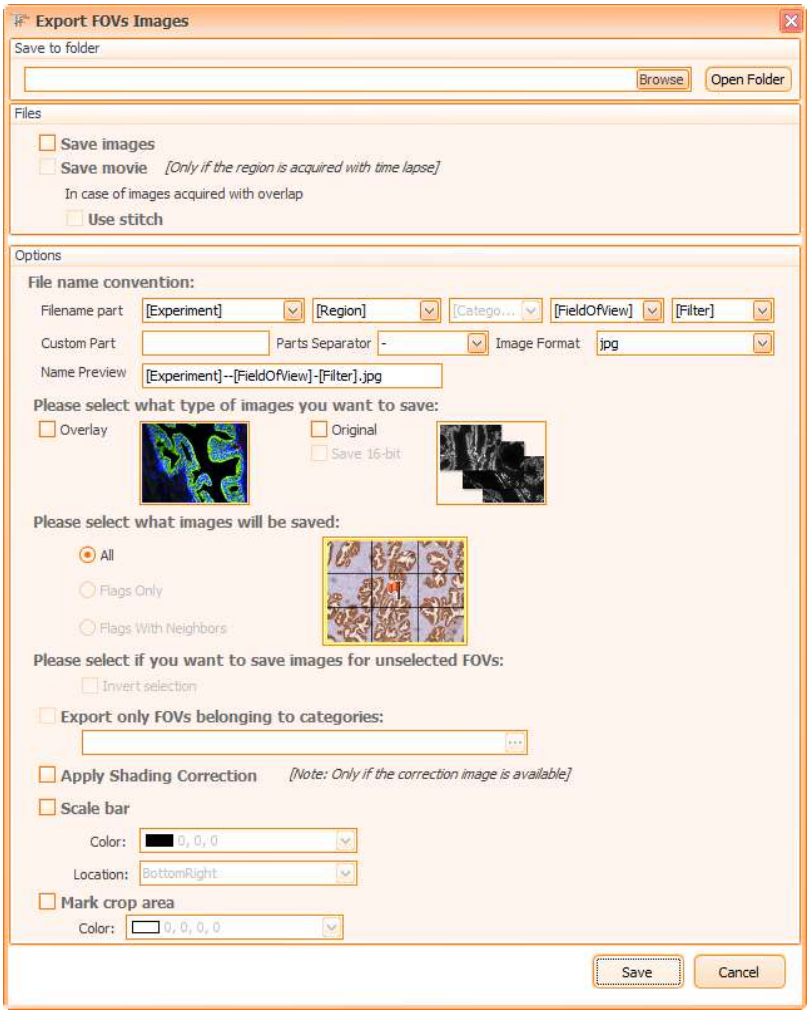


Figure 16 – Export FOVs Images dialog (fluorescence)

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.
  - **Save 16 bit**: enabled when **Original** option is checked and if *tif* is used as extension. The FOV images will be exported in 16 bit format for each fluorescence channel.
- 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 ().

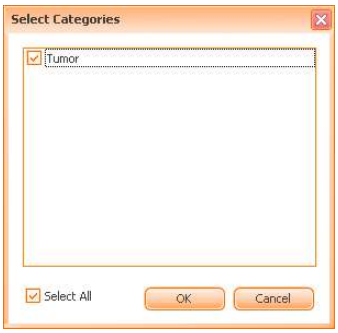
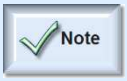


Figure 17 – Export FOVs: Categories included



- 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**: please see **Chapter 8.2.2** of the current manual.
- **Apply Shading Correction (FL)/ Apply Illumination Correction (BF)**: the correction image will be applied to exported images if the correction image is available.
- **Dots per inch**: here you can set the resolution for the images to be exported.

7.2.2 Region Overview

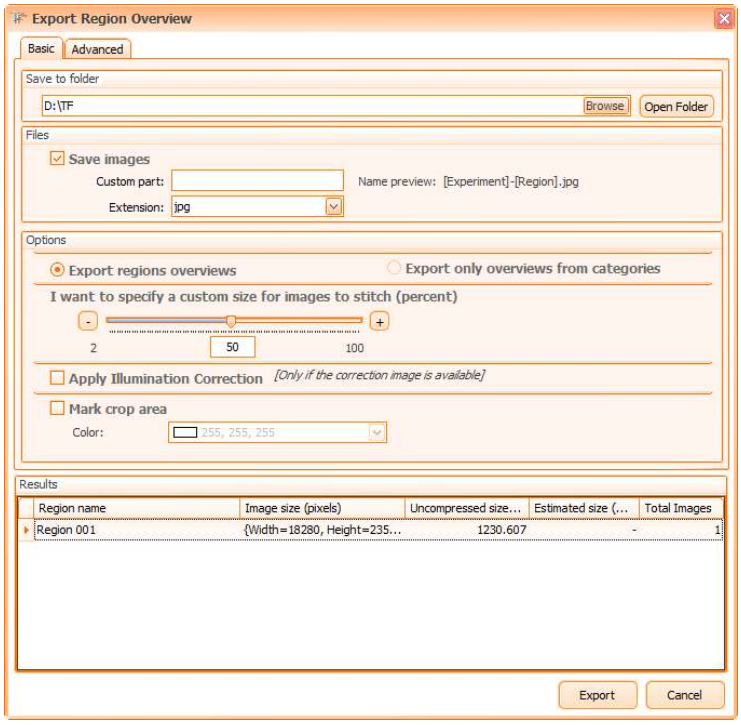


Figure 18 – Export Region Overview dialog: Basic (Brightfield)

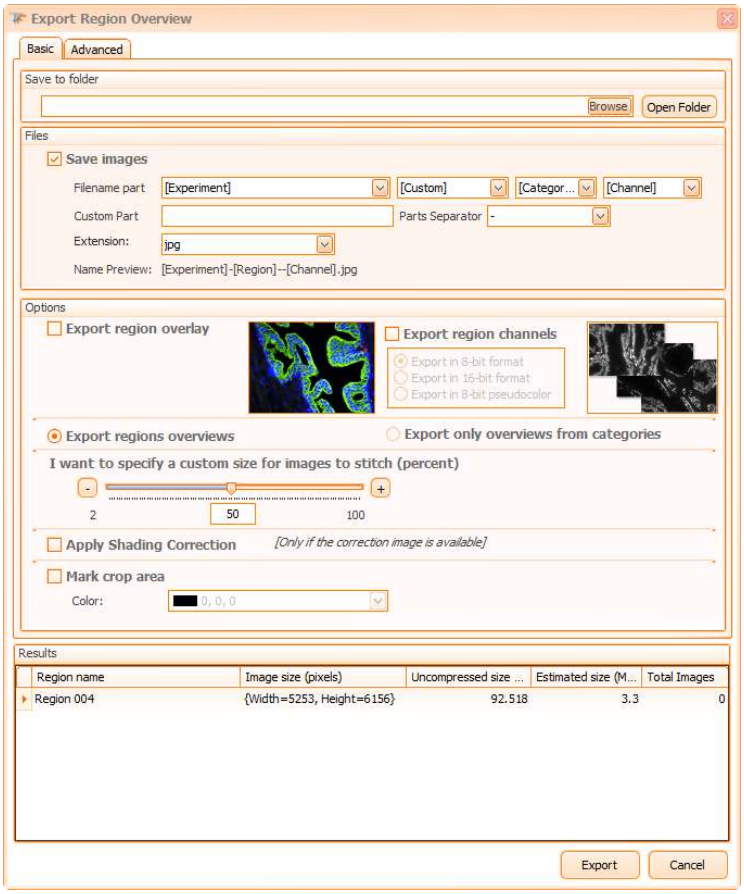


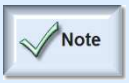
Figure 19 – Export Region Overview dialog: Basic (Fluorescence)

7.2.3 Basic

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 Viewer 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**:

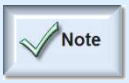
- Select the **storage folder**;
- Select **files** to be exported:
  - Images
  - Movies (option available only if the region was acquired with time lapse)
  -
- Enter **Custom Part**: type desired custom part for the name of the exported item.



- 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 (see **Chapter 7.2.1**). The custom part is defined by the user.

- Select the file **extension**: choose exported item format (also available: *Tiled Tiff (OME metadata)* in order to export bigger images);

- If choosing as extension the *Tiled Tiff*, the items from the **Options for Tiled Tiff** (OME metadata) 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.
  -



Tiled Tiff contain OME- TIFF (OME stands for Open Microscopy Environment) metadata embedded in exported TIFF images provide the following details:

- Objective information
- Pixel size
- Channel information
- ZStack information

  -
- **Export channels as single Tiff files**: when **Export Region Channels** is checked, all channels will be exported within the same Tiff as Tiff pages.
- Select the **type of images** you want to save:
  - **Overlay**: the image as it appears in the One FOV Viewer.
  - **Region Channels**: the images for each channel as acquired.

Save 16bit: you can also export 16bit images, if acquisition was made with 16bit. Only channel images can be 16 bit type. For the fluorescence regions you can export the region overlay and also images from each channel separately, with the option of exporting them 16bit also. This feature is enabled when previously selecting Tiled Tiff from the Extension dropdown.

Export 8-bit or **8-bit pseudocolor.**

- **Export region overviews**
- **Export only overviews from 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.
- **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.
- **Apply Shading Correction**: this option is enabled only for **Fluorescence** experiments. The correction image will be applied to exported images if the correction image is available.
- **Mark Crop Area**: it marks the contour of the region on the final image; you can select its color.

7.2.4 Advanced

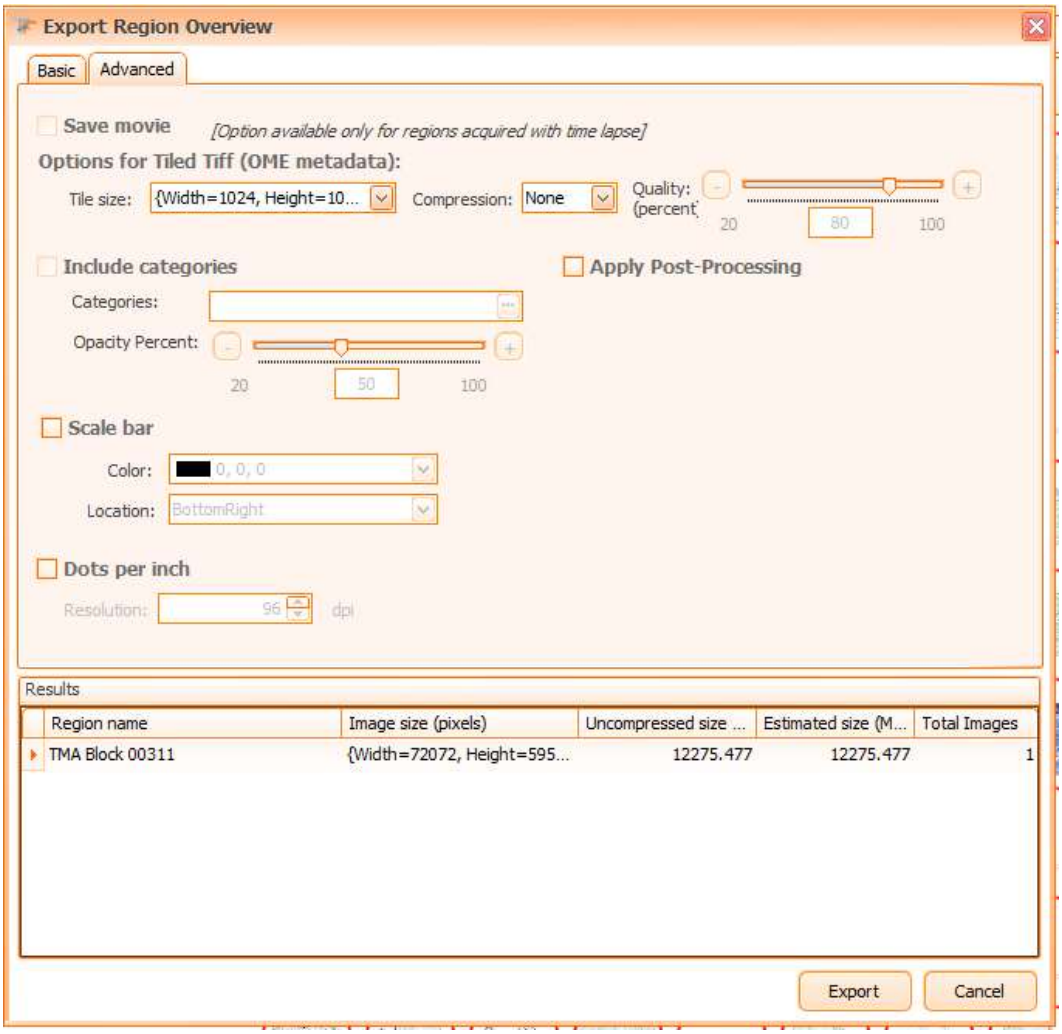


Figure 20 – Export Region Overview dialog: Advanced

- **Scale bar**: please see **Chapter 8.2.2** of the current manual;
- **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.

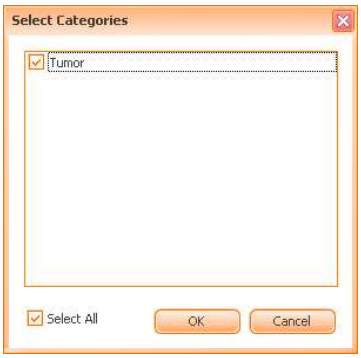


Figure 21 – Export Region Overview: Categories included

- **Opacity** of the categories: adjust the **Opacity** parameter, in order to choose the transparency of the tissue area name that appears on the region.

Note

- Restrictions regarding the images to be exported:
- For all export formats (excluding *Tiled Tiff*) the image size must not exceed 80000000 pixels.

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:

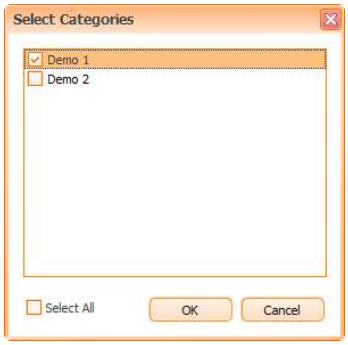


Figure 22 – Export subregions: Select categories

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.

7.2.5 Export Slides Images

To export the image of a slide, right click on the slide and press **Export Slides Images**.

Figure 23 – Export Slides Images (a)

Export Slides Images dialog will open.

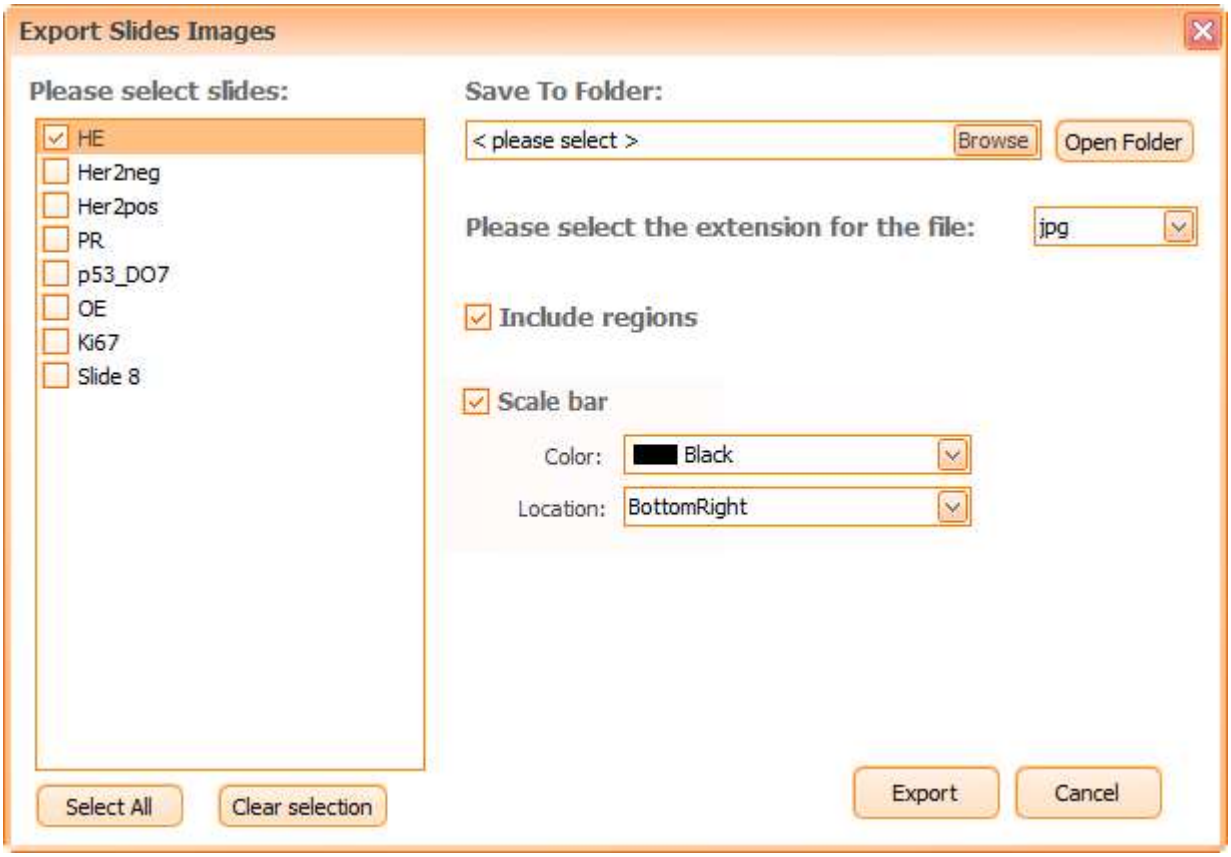


Figure 24 – Export Slides Images (b)

Select desired slides, a storage folder for the outcome of the export and select a file format.

Also, you have to mention if you wish or not to include existing regions and the scalebar on your exported image. When you are done with these settings, press **Export** button to finalize the export.

7.3. Zoom in/out

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

7.4. View Original Size

View Original Size displays original size of the image.

7.5. View Best Fit

View Best Fit displays the entire region.

7.6. Map

The Map shows/hides region map.

7.7. Subregions, annotations

Figure 25 – Subregions options

TissueFAXS Viewer is able to display subregions previously defined in **TissueFAXS**.

Subregions are normal regions defined within the TissueFAXS Region Viewer, having the same properties as the regions from the Slide Preview. The advantage of the subregions is to ensure higher flexibility and more detailed analysis on a more detailed area of tissue.

7.7.1 Categories

Sometimes, you may want to emphasize certain small areas on your region that could contain high interest information for your research. 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 (e.g. tumor, non-tumor) you may create a **category** in order to highlight that particular area on the image.

Using categories might help you in order to perform a precise analysis.

Add categories

In order to add categories, go to **Region Viewer Toolbar** → **Subregions** → **Manage Categories…** A dialog will appear (containing already added categories, if existing) where you can add new categories, giving each one a name (mandatory) and assigning a color. The list of categories is per project, not per region.

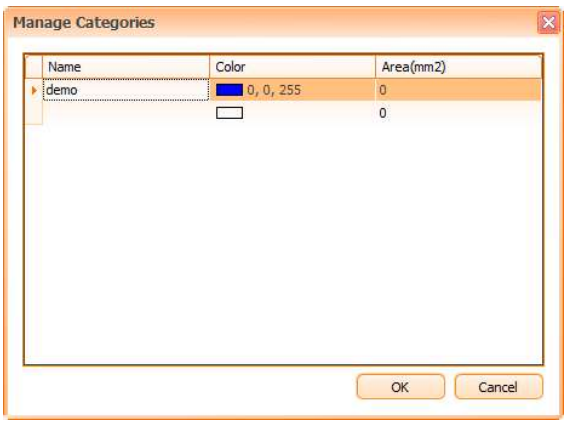


Figure 26 - Add categories dialog

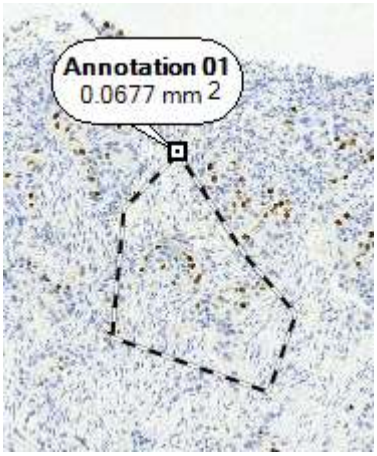


Figure 27 - Region Viewer: example of categories

You may also use the categories when you are about to export images.

Remove categories

In order to perform this action, go to **Region Viewer Toolbar** → **Subregions** → **Manage Categories…** and select the category you want to remove, then press the **Delete** key from your keyboard.

Apply categories on regions

To create these areas, go to **Region Viewer Toolbar** → **Subregions**, then select only the desired category.

Now, choose the area shape from the toolbar.

Figure 28 - Toolbar: categories shapes

Then add the desired area on the viewer using the mouse, just like for any normal region.

Once created, these areas cannot be modified, only removed. To remove the areas, right click inside the desired area and then choose **Remove [*area name*] …**

View / hide categories on regions

To be able to view all tissue areas belonging to a certain category, check only that particular category in **Region Viewer Toolbar → Subregions**.

If you want to see all existing categories, check **All Categories** option from **Region Viewer Toolbar → Subregions**.

To hide the tissue areas belonging to a category, simply uncheck that particular category in **Region Viewer Toolbar → Subregions**.

Export tissue areas belonging to categories

- When exporting the **Region Overview**, you have the possibility to choose an option that also exports the categories.
- When exporting the **FOVs images**, you have the possibility to choose an option that only exports the images belonging to the respective category.

7.7.2 Annotations

Press the **Subregions** (Subregions) button in order to access the **Subregions** contextual menu.

The following options will be available:

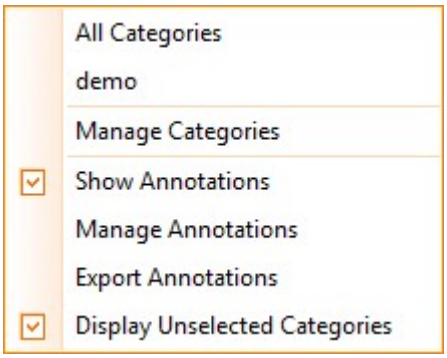


Figure 29 - Annotations contextual menu

Show Annotations

To add annotations, go to **Region Viewer Toolbar → Subregions →Show Annotations** (only **Show Annotations** should appear checked).

Now, choose the annotation shape from the toolbar. Then add the desired shape on the viewer using the mouse, just like for any normal region.

To be able to see all annotations you have added, simply check **Show Annotation** option from the Region Viewer toolbar.

PolyLine Measurements

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

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

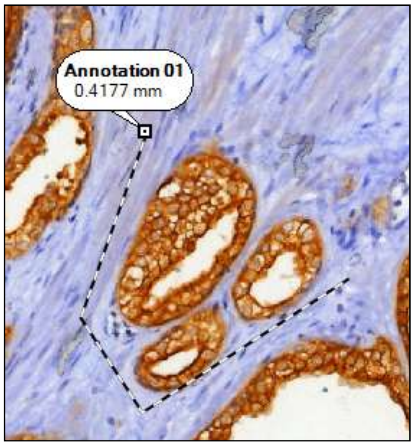


Figure 30 - Drawing PolyLine

Manage Annotations

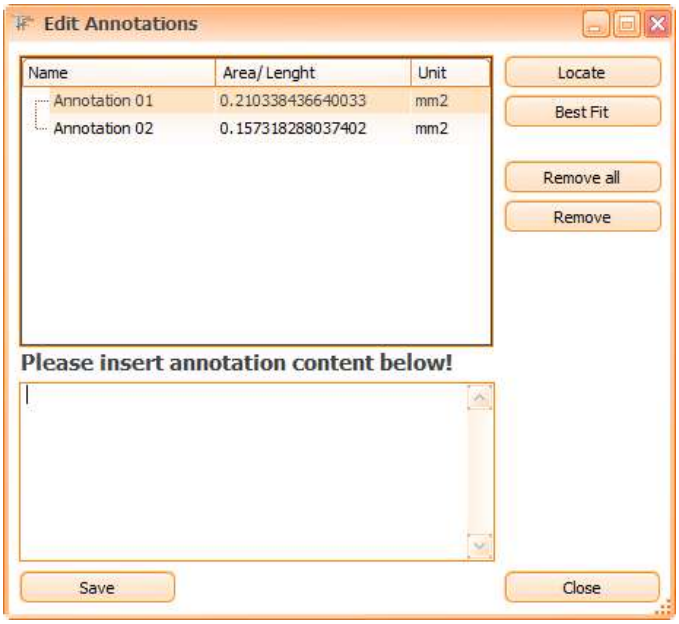


Figure 31 - Edit Annotations dialog

- **Locate**: by pressing this button, the selected annotation will be located on the sample and displayed at the current size of the sample.
- **Best Fit:** by pressing this button, the selected annotation will be located on the sample and displayed at the current size of the region viewer.
- **Remove**: removes the selected annotation.
- **Remove all**: removes all existing annotations.
- **Please insert annotation content below**: write desired content 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 you must use the little black framed square on the contour of the annotation. If pressed, this little square will display an edit box, where you can operate the following actions:

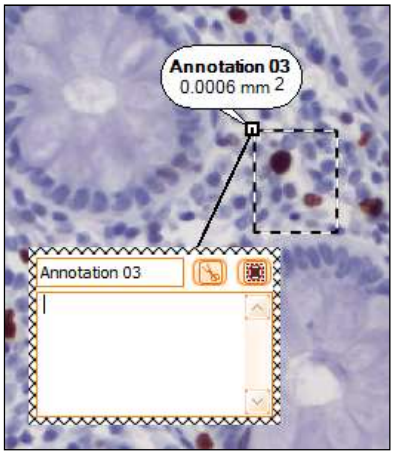


Figure 32 - Edit annotation directly on the sample

- Edit the name of the annotation: write the desired name in the upper-left corner of the box, then press **Enter** key to save the changes;
- Write a comment in the space below the name, you can also erase the existing comments (all the changes concerning the notes are automatically saved);
- Delete the annotation by pressing button;
- Close the edit box by pressing button.

7.8. Crop

It marks the images that have been acquired outside the defined region.

7.9. Gridlines

Default value is Off.

7.10. Scalebar

Default value is Off.

7.11. Measure

You can measure anything you want on the sample.

7.12. Illumination Correction (BF Experiments)

Occasionally, on the acquired images, some shades may appear. They can be caused by imperfections of any component of the lightpath, specks/impurities on the camera/objective.

TissueFAXS Viewer allows you fixing such shading problems by using the **Illumination Correction** function. In this chapter, you will find how to use this feature.

Note - This operation is available only for **brightfield** experiments.

You can access the **Illumination Correction** menu by pressing the **Illumination Correction** button () from the **Region Viewer** control.

To apply illumination correction, a *correction image* is required (mandatory).

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

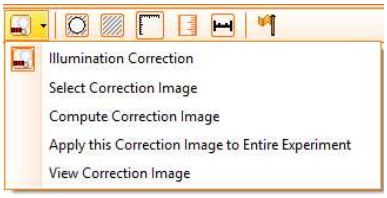
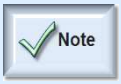


Figure 33 - Illumination Correction menu

The **Illumination Correction menu** contains the following items:

- **Illumination correction**: choose this option to automatically apply the correction image to your region.
- **Compute Correction Image**: If there is no correction image available, you can compute a correction image, using the already acquired images; this is the solution when a correction image is not available and reacquiring the correction image with the same settings/hardware as the region is not possible anymore.
- **Select correction image**: by choosing this option, you will be displayed a dialog where you can choose from the listed regions (containing correction image) the one you want as correction image.



- The region that appears in *red* in this dialog is the currently opened region.
- These options are available only in Edit Mode.

After selecting the respective image, you can effectively apply the illumination correction.

Figure 34 – Select Correction Image

- **Apply this correction image to the entire experiment**: the correction image of the current region from the region viewer will be applied to all regions of an opened experiment.

7.13. Shading Correction (FL experiments)

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

TissueFAXS Viewer allows you fixing such shading problems in fluorescence experiments by using the **FL Shading Correction** function. In this chapter, you will find how to use this feature.

You can access the **FL Shading Correction** menu by pressing the **FL Shading Correction** button () from the **Region Viewer/Acquired Images** control.

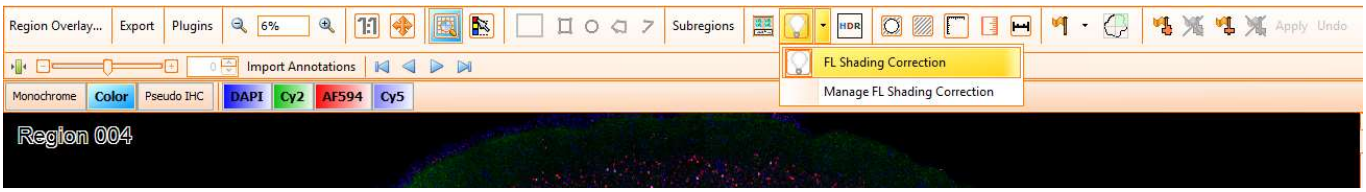


Figure 35 - FL Shading Correction button

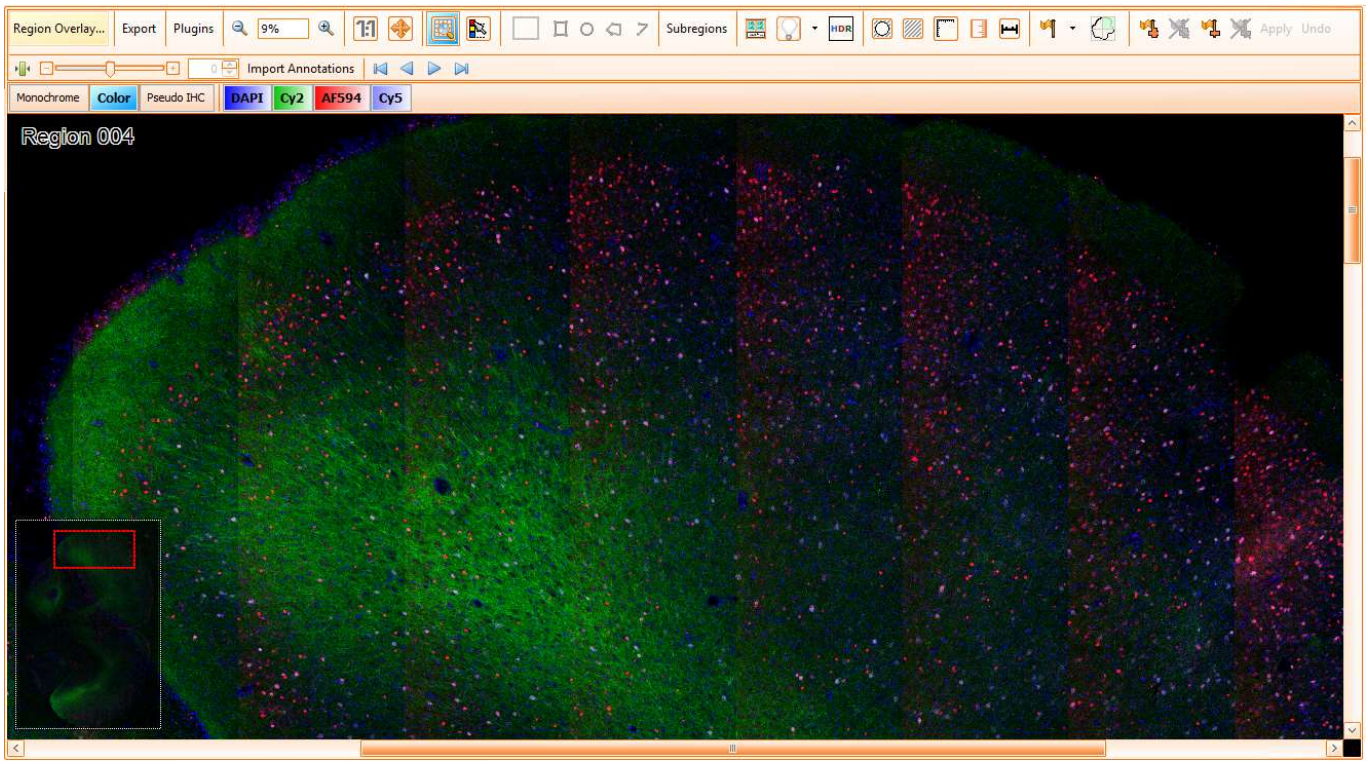


Figure 36 - BEFORE FL Shading Correction

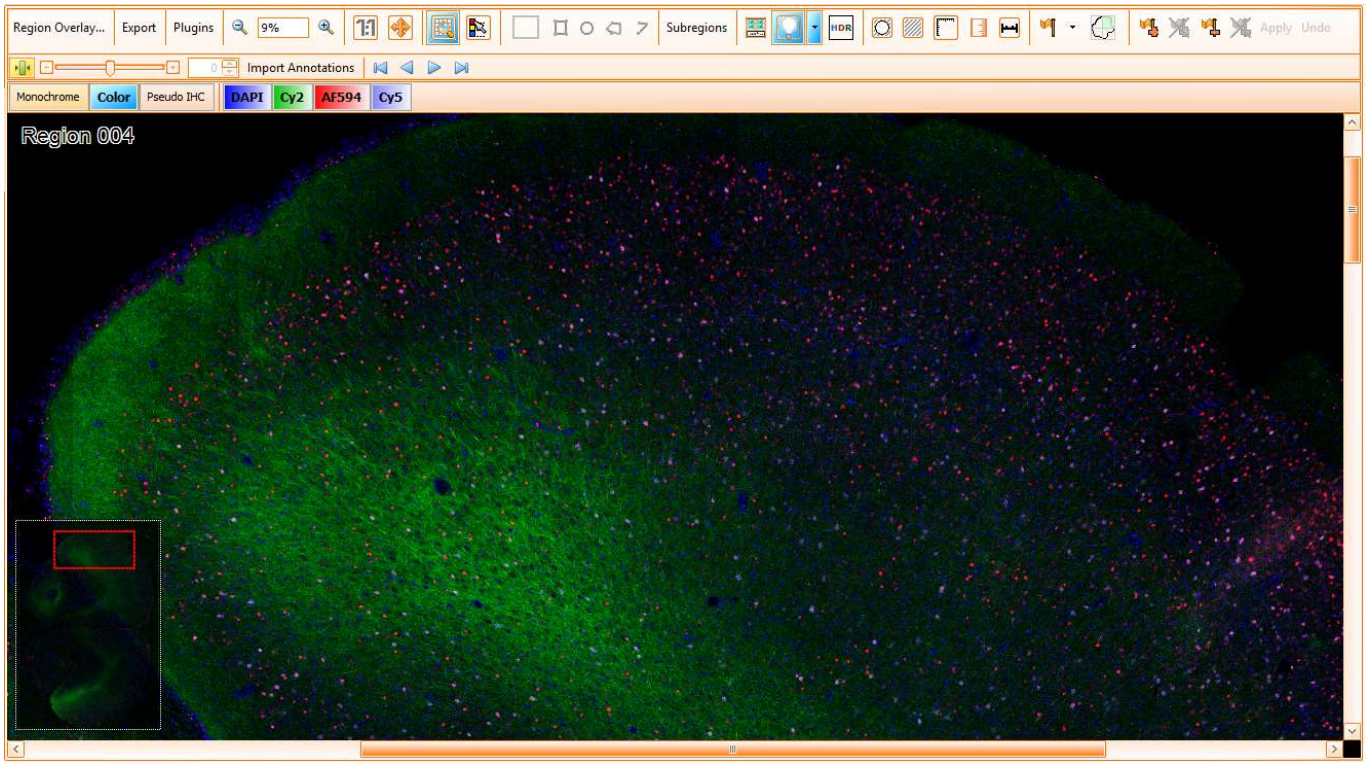


Figure 37 - AFTER FL Shading Correction

To apply shading correction, a *correction image* is required (mandatory).

The **correction image** is an image computed in order to store information about the shades in the light path. By applying this image to a certain region, the shades will be eliminated 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.

Note

- **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 we see "shading effects", which come from the tissue itself, different preparation and/or fixation methods. These effects might appear in some areas while they are not visible on other areas or other samples and 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 any shading correction.

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

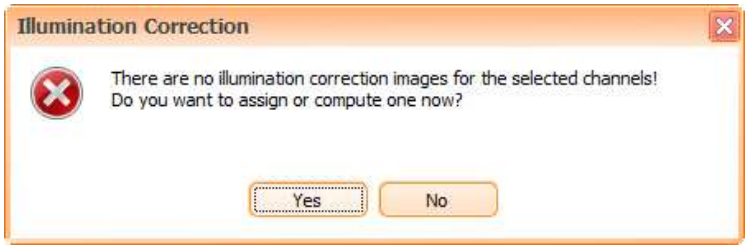


Figure 38 - Illumination Correction: no illumination correction images

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

You can compute the correction image for:

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

7.13.1 Propagate Shading Correction

Shading reference image can be propagated only to user specified channels.

This option can be found here: **Region viewer** -> **Shading correction** -> **Propagate Shading Correction**.

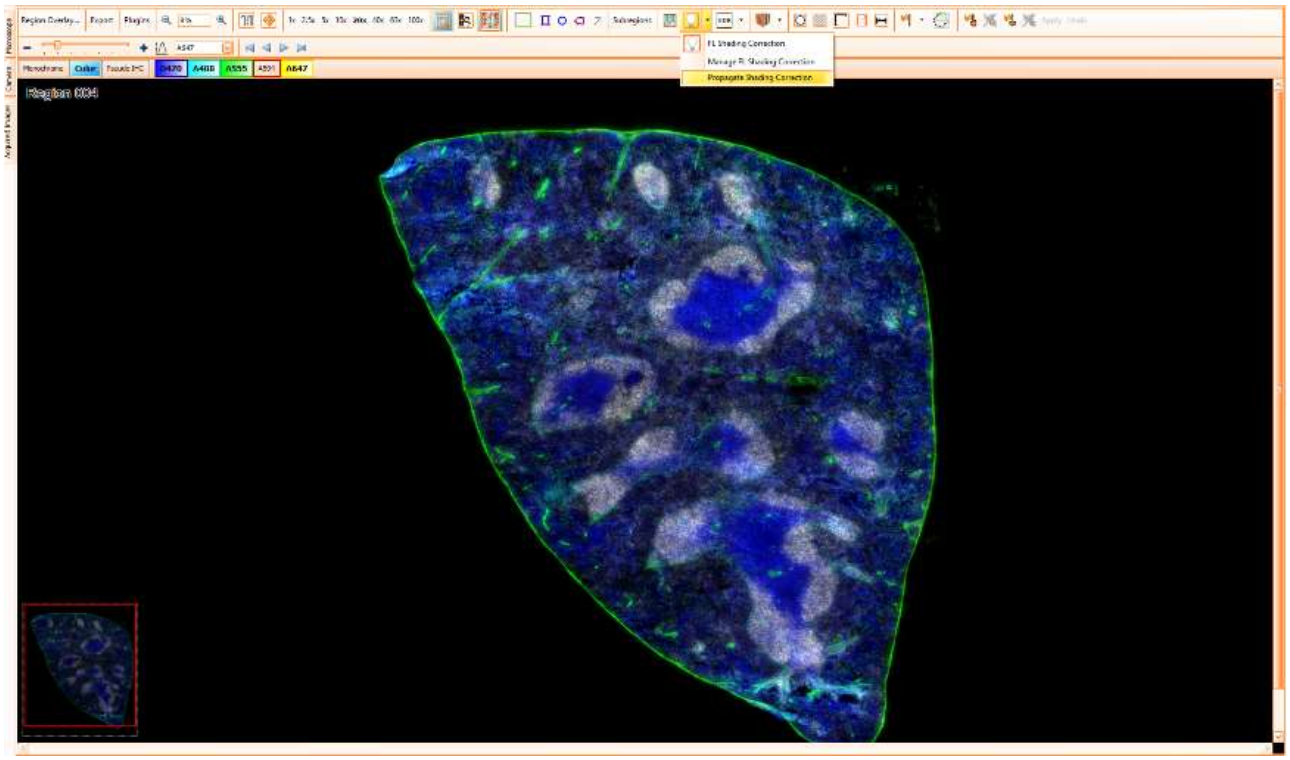


Figure 39 – Propagate Image Correction

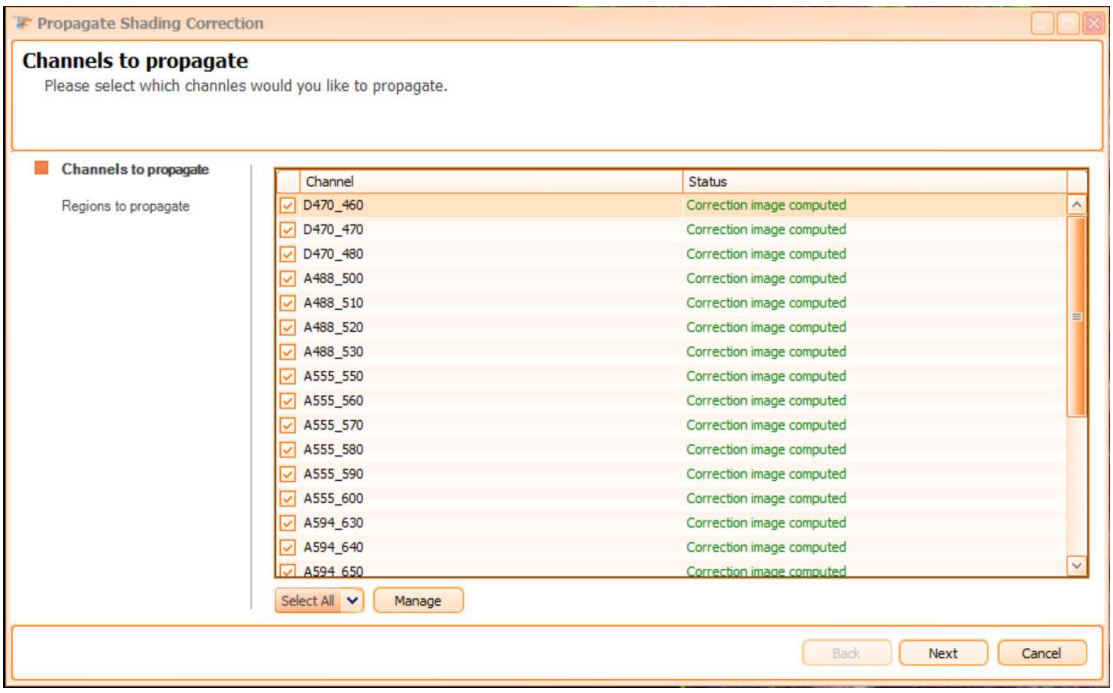


Figure 40 – Channels to propagate

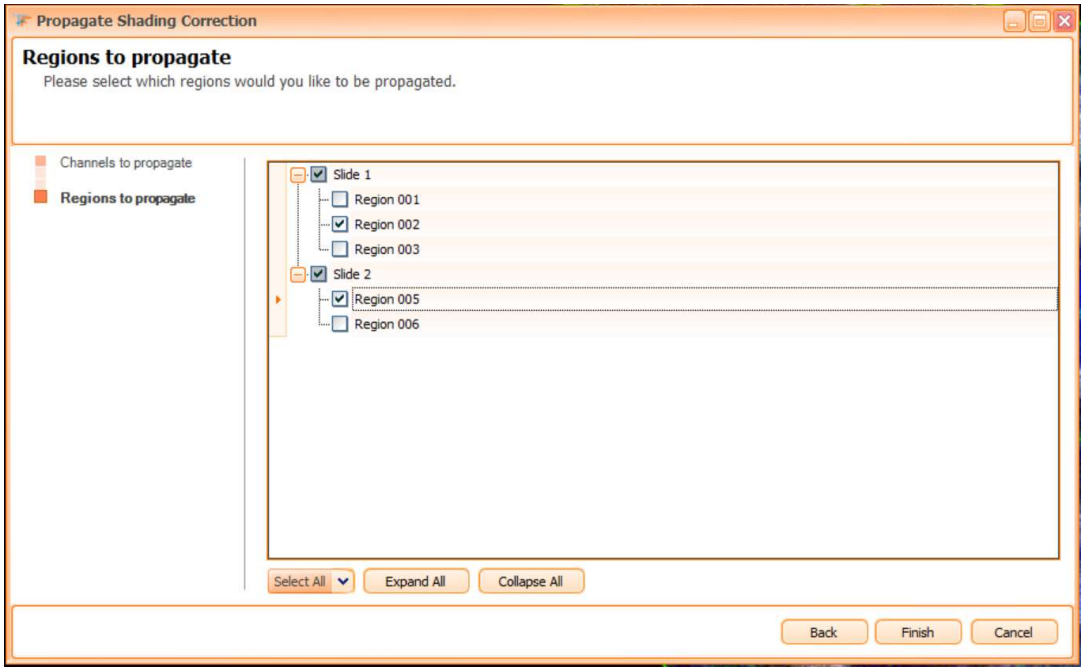


Figure 41 – Regions to propagate

7.13.2 Managing FL Correction Images

As the management of correction images can prove really helpful to the user, **TissueFAXS** has a management panel for the existing correction images.

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

For each channel you will see the name, the status, the status for Z-stack and the intensity range of the respective channel.

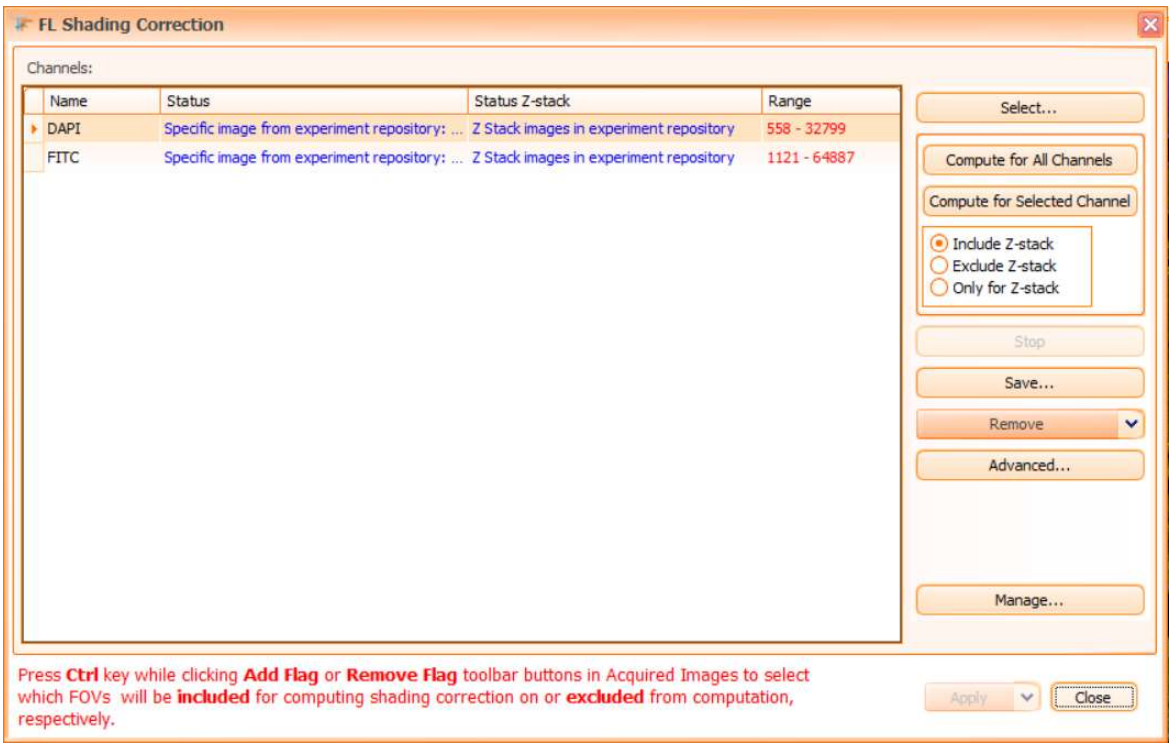


Figure 42 - FL Shading Correction : selecting correction images

Note

- When computing correction image, if overexposed areas are present, this will affect the correction image. You can use Flag feature in order to select what images you want to keep for computing shading correction.

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

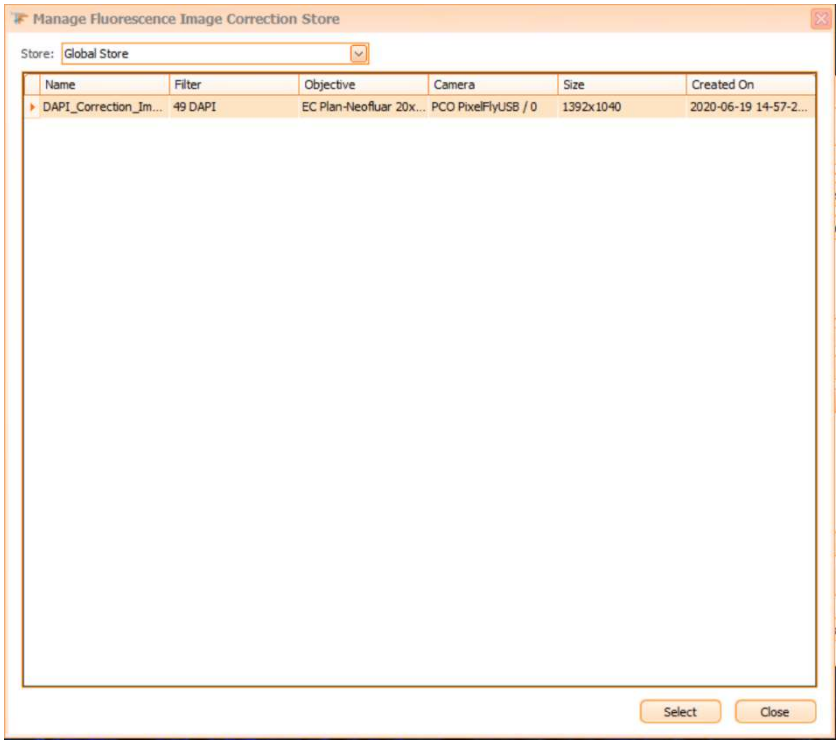


Figure 43 - Managing Fluorescence Image Correction Store

Firstly, you should select the type of store where the correction images are: in a global store or an 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.

Note

- Global Store can be accessed to save or load images only if TissueFAXS Viewer was opened as Administrator!

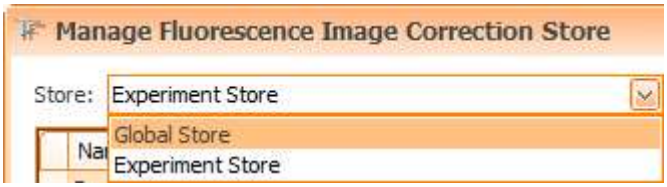


Figure 44 - Image Correction storage

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

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

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

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

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

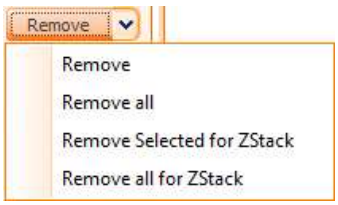


Figure 45 - Removing correction image options

In the end, the user has to select where to **apply** the correction image/images :

-To current **region** ;

- To current **slide** ;

- To current **experiment**.

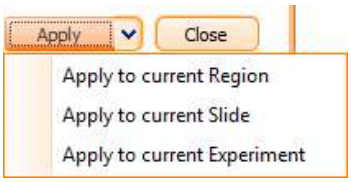


Figure 46 - Options when applying shading correction

Stop : when computing correction image for large regions, you can choose to stop the process if you consider this is too time consuming.

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.

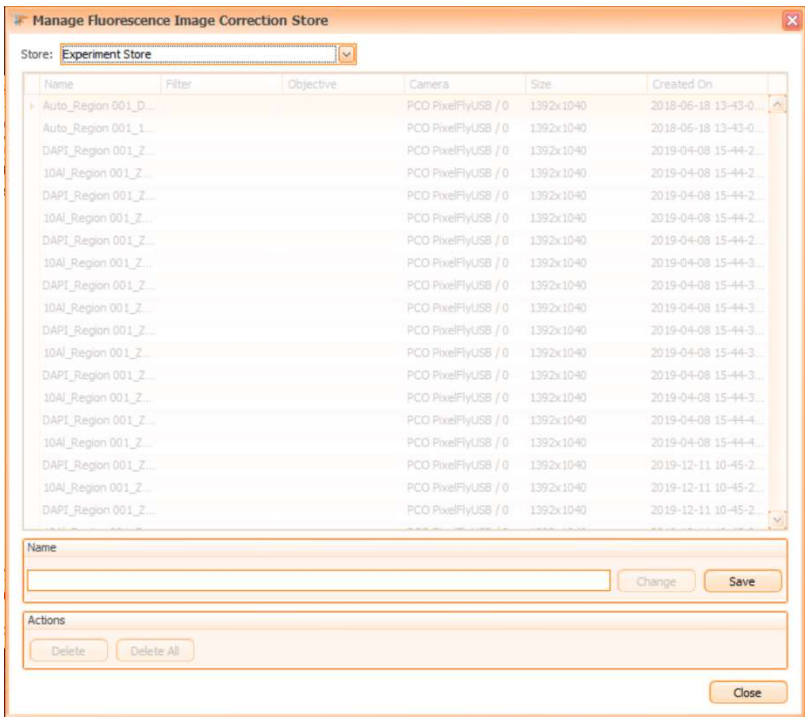


Figure 47 - Saving correction images

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

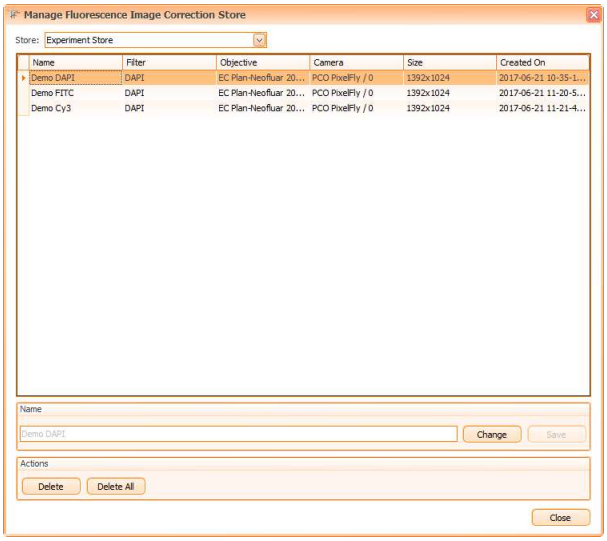


Figure 48 - Selecting correction images

The name of the existent correction images can be edited by pressing **Change** button and, after the modification has been done, **Save** button should be pressed.

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

7.14. Flags

When pressing the Flag button, you can begin marking desired FOVs on the sample.

If right-clicking on any marked FOV, the following options become available:

- Set/Clear Export Flag
- Clear All Export Flags: for current region, for whole slide or for the entire experiment
- Save Displayed Image: save displayed image or displayed image with data
- Copy Displayed Image: copy displayed image or displayed image with data

Figure 49 - Flag options

7.15. Show RGB

When enabled, this option will display the RGB value on the live image.

7.16. Spectral Unmixing

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

Figure 50 - Configure spectral unmixing button

Configure Spectral Unmixing dialog will open.

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

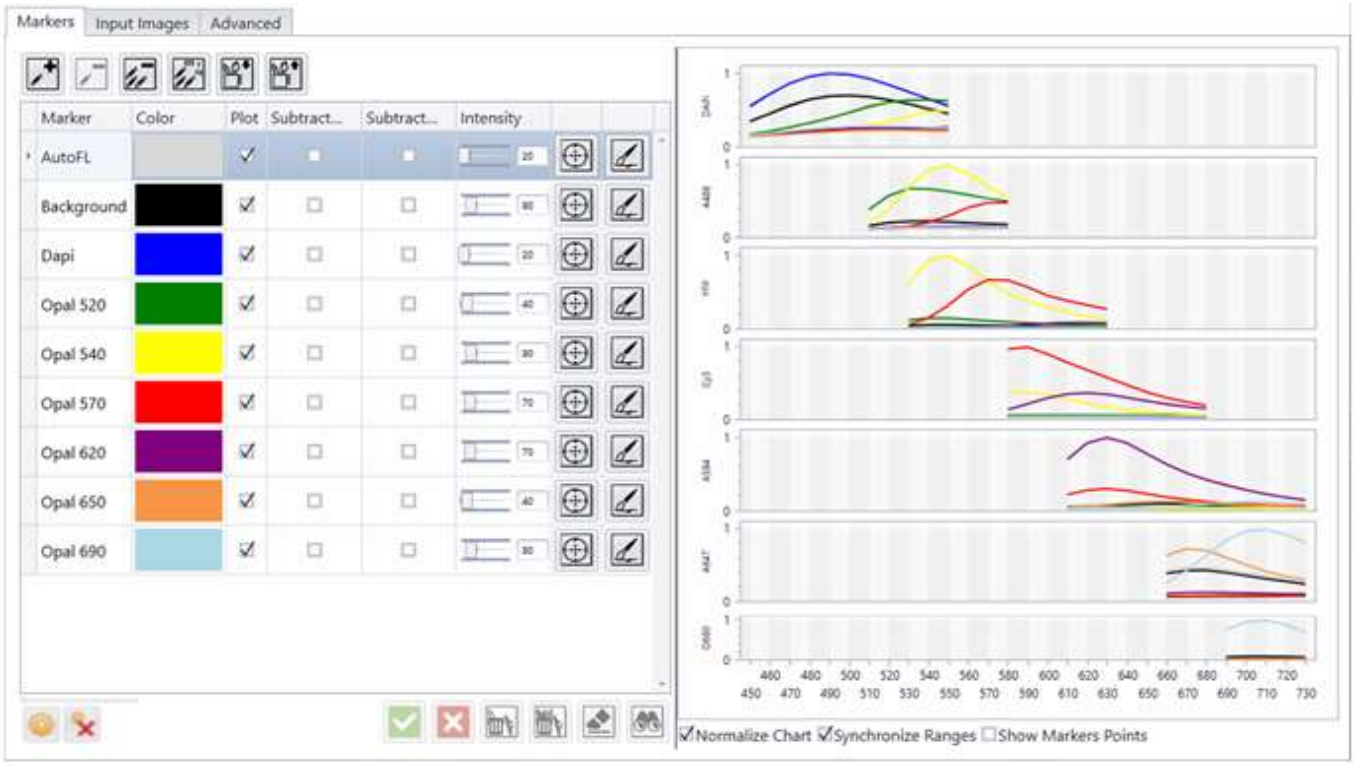


Figure 51 - Configure Spectral Unmixing panel

Reference Marker Definition

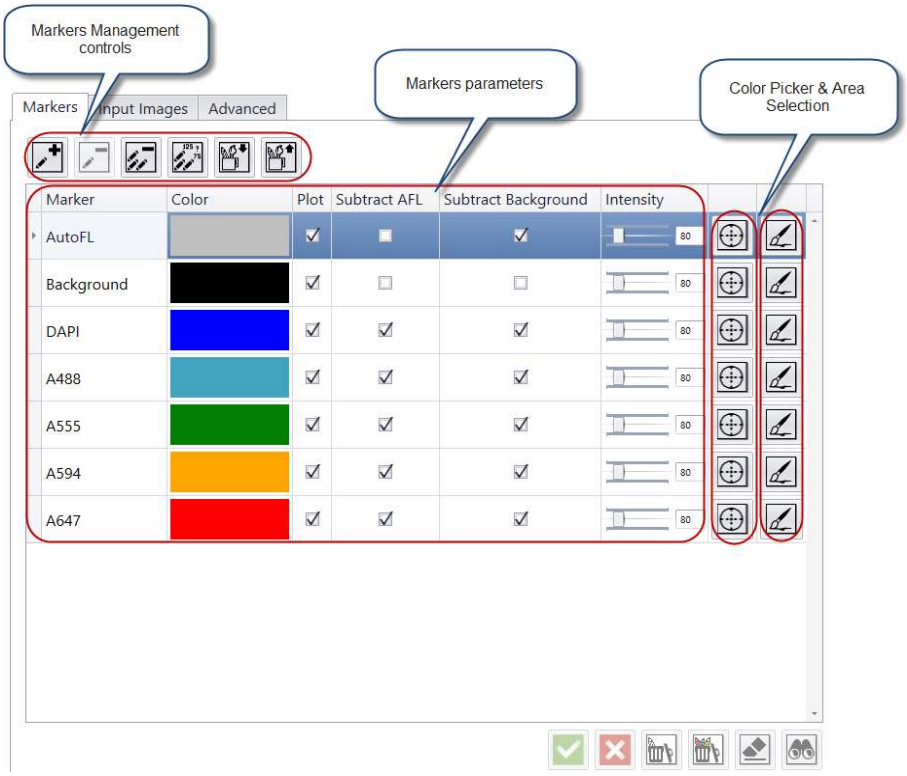


Figure 52 - Marker Section

Markers management

To manage markers use the following controls:

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

For each marker you can set the following parameters:

- **Marker name:** define marker name.
- **Color:** define marker color.
- **Plot:** enables plot display.
- **Subtract AFL:** this parameter works like a flag which specifies the fact that 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 **Import from DB File** option, you will import markers from another database (for example StrataQuest database). Before importing from another database, you can choose to remove current database by using **Clear Current Database**.

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

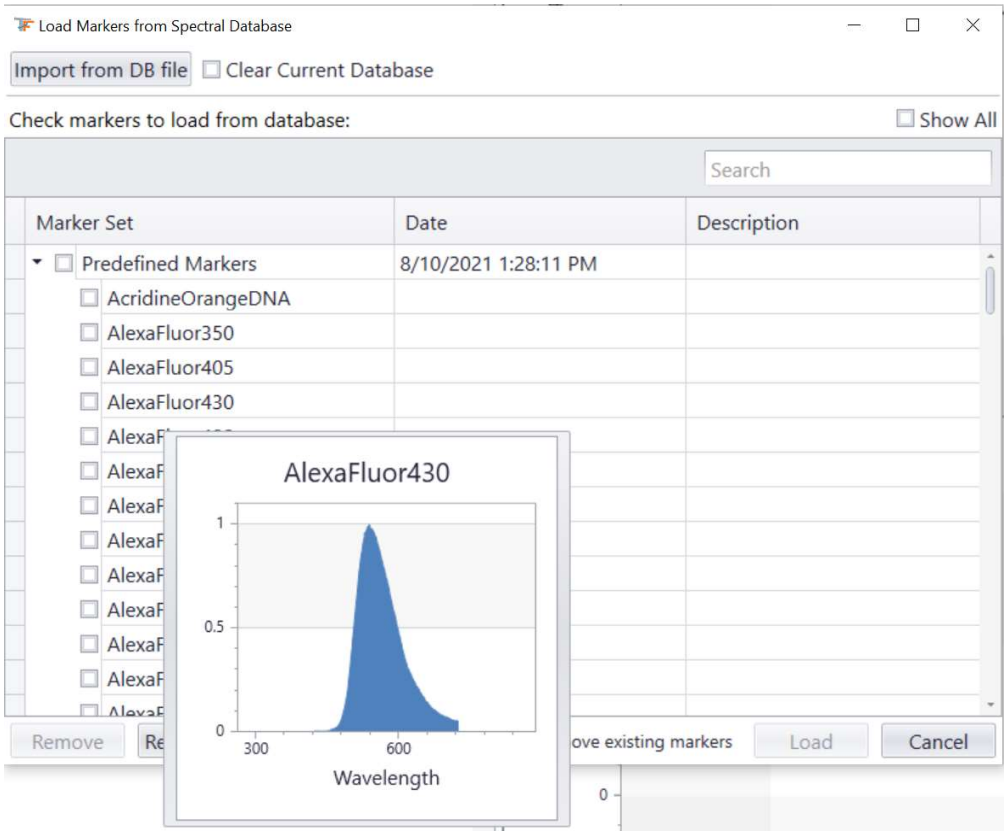


Figure 53 – Choosing markers to load from database

StrataQuest offers a comprehensive list of predefined standard markers covering the most used ones. The search option on the top right side of the window makes the selection easier.

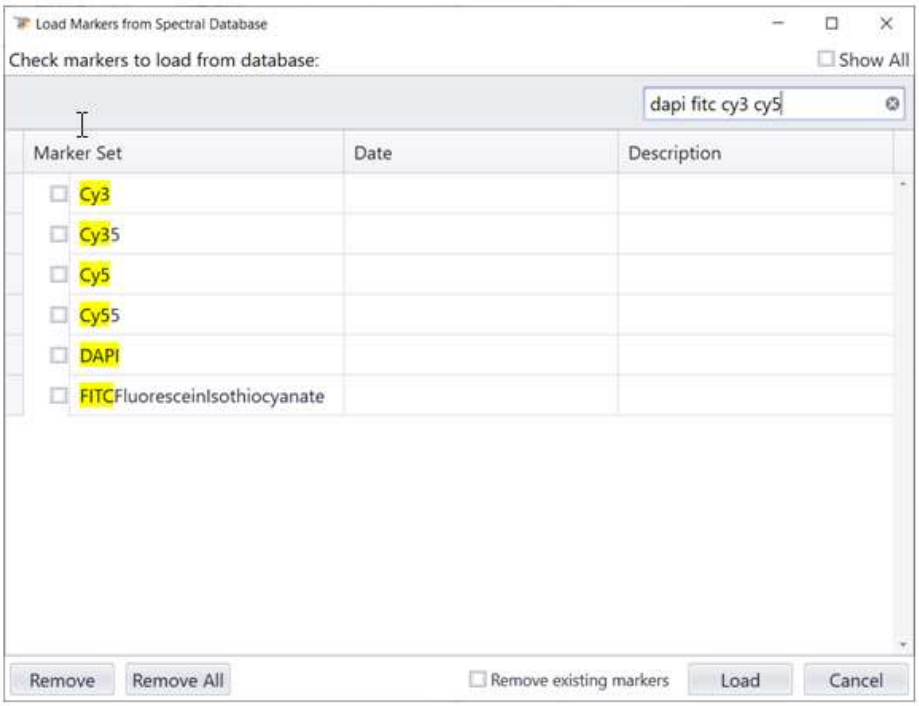


Figure 54 – Predefined markers

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 trigger 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

In this tab it is possible to select the images you want to use as input. Input images (or the Lambda stack) represent a list with all images used in the unmixing process.

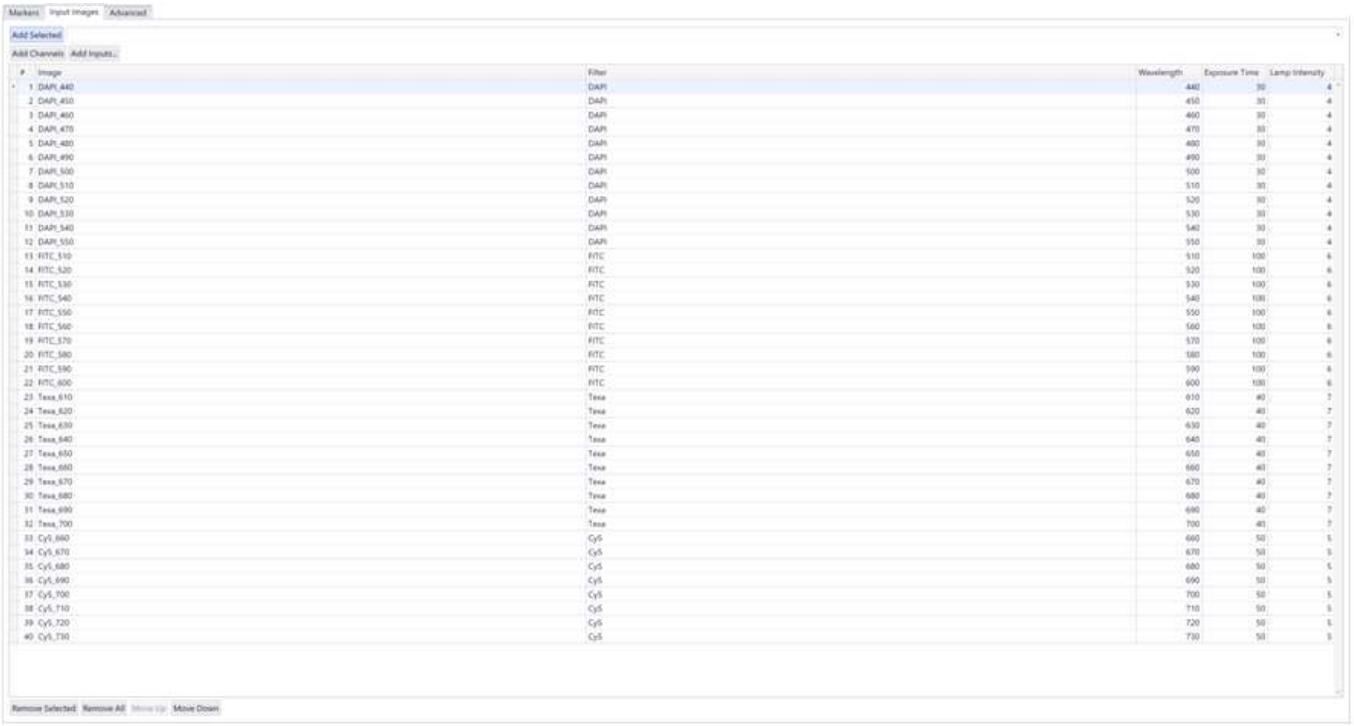


Figure 55 - Spectral Unmixing: Input Images

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

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

Note: 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 possibility 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 defined markers components.
- **Generate Colored Images**: if enabled, it will generate 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**: specify the method used to normalize the reference markers values - maximum on filter or maximum on marker. Normalization is only for the plot.
- **Fluorescence mode**: switch between fluorescent / brightfield mode.
- **Apply Shading Correction**: enables shading correction on the input image.

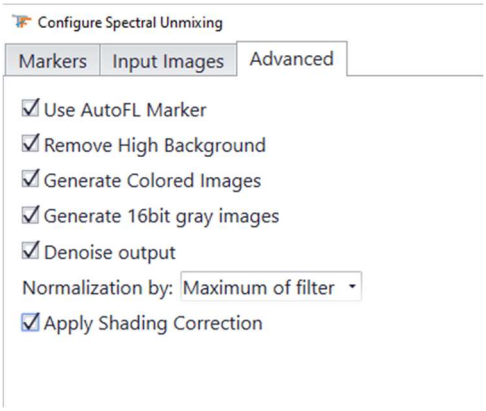


Figure 56 - Spectral Unmixing Engine: Advanced

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

Based on your 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. If you want to stop the run process, press **Clear Spectral Unmixing** ( ).

Plot

After applying the selection for color picker/area selection, you can visualize the plot.

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**: if selected, the values will be displayed on the plots as points.

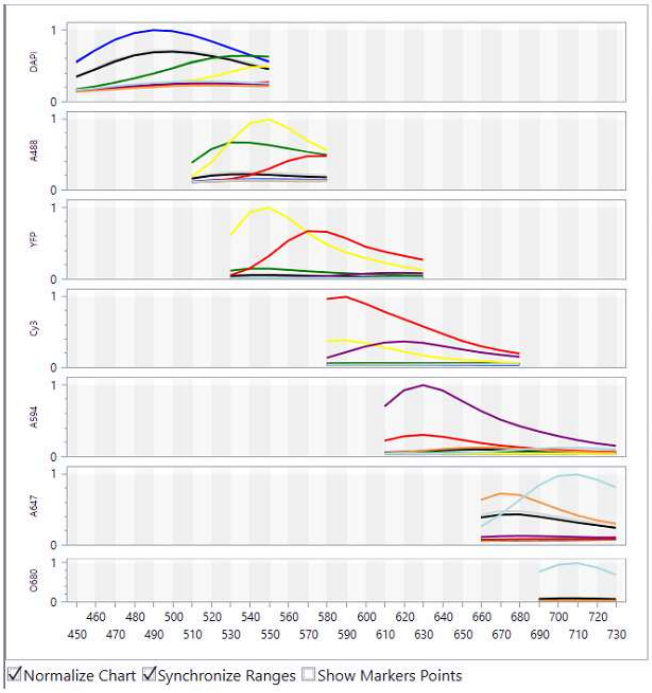


Figure 57 - Spectral Unmixing Plot

Propagating and running spectral unmixing

To run **Spectral Unmixing** you have more options:

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

Figure 58 - Main toolbar: Run spectral unmixing button

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

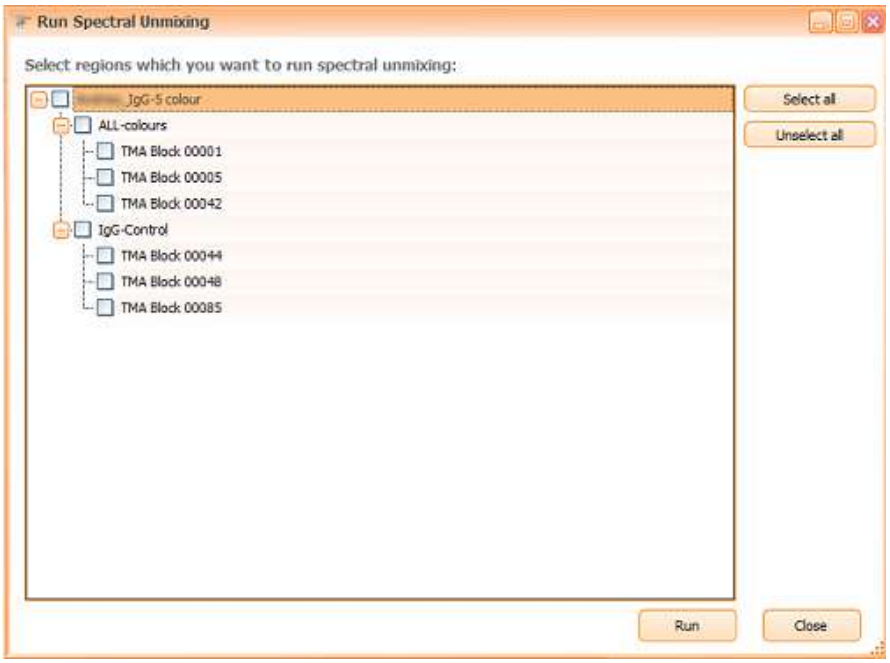


Figure 59 - Run Spectral Unmixing panel

You can select the items for running spectral unmixing:

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

When you are done with the selection, press **Run**.

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

Figure 60 - Region viewer toolbar: Run spectral unmixing button

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

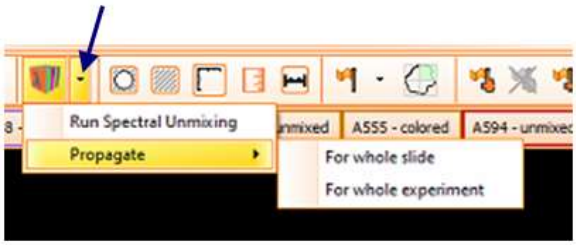


Figure 61 - Propagate and run spectral unmixing

- **Run Spectral Unmixing**: runs spectral unmixing for current region.
- **Propagate:**
    - **For whole slide**: all the current settings for spectral unmixing will be propagated to the whole selected slide.
    - **For whole experiment**: all the current settings for spectral unmixing will be propagated to the entire experiment.

3. Run Spectral unmixing using the contextual menu

Running spectral unmixing can be also accessed from the **contextual menu** of a region or a slide, like shown in the images below:

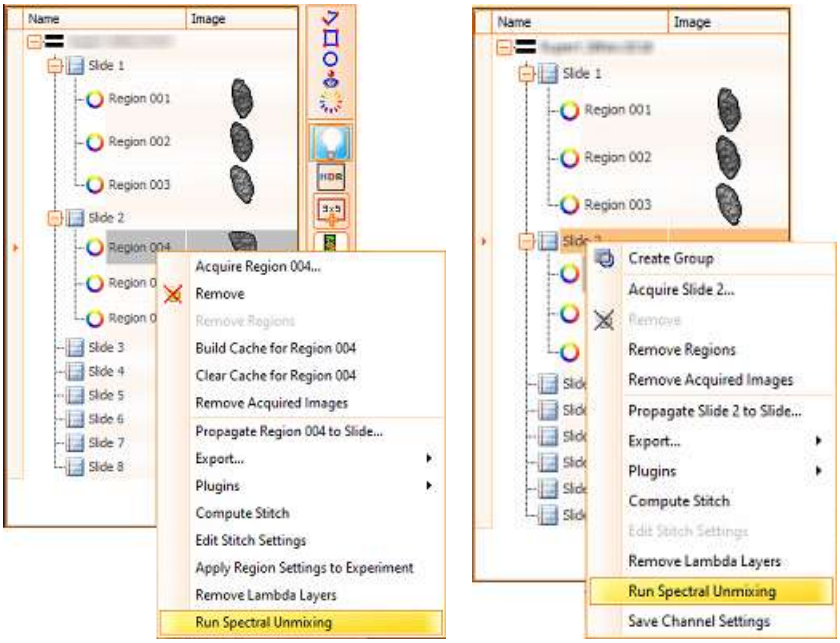


Figure 62 - Running spectral unmixing for a slide or a region using contextual menu

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

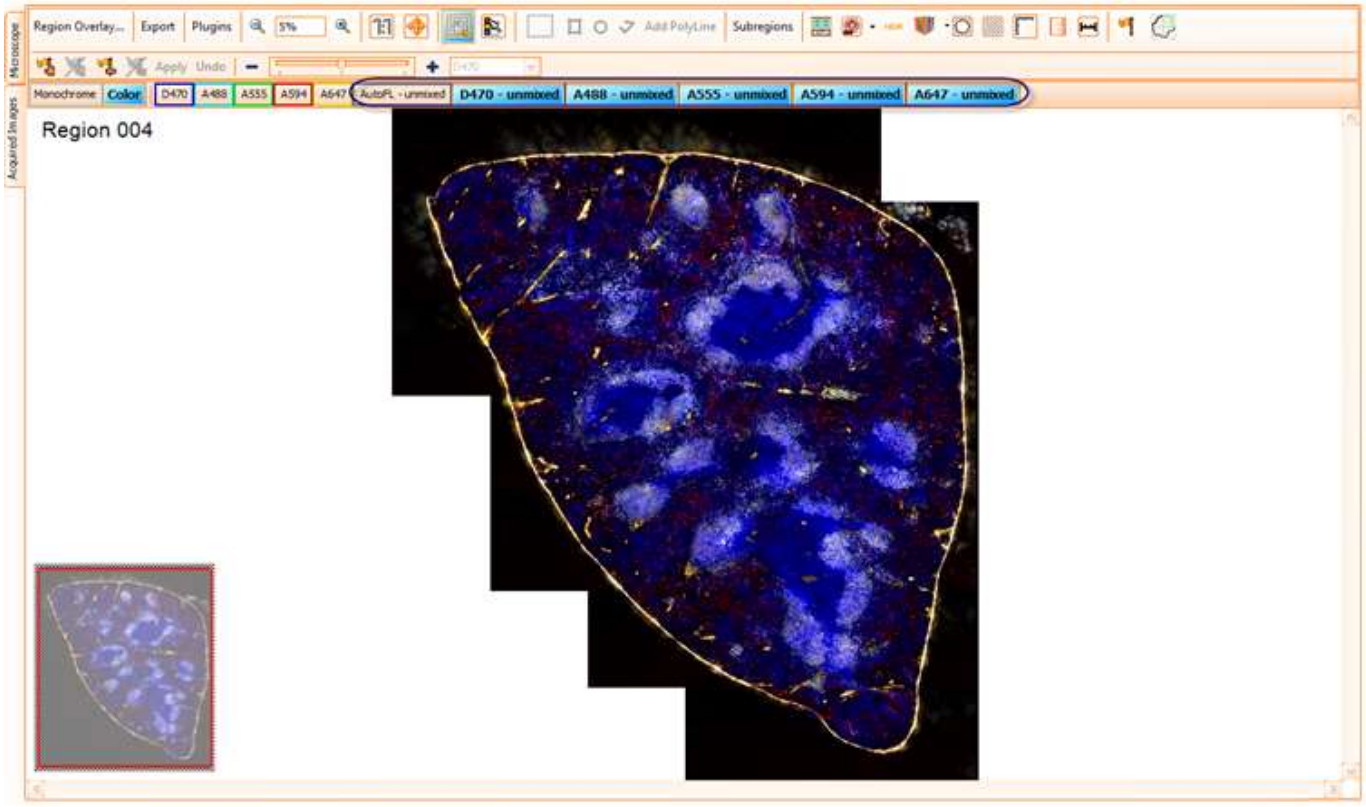


Figure 63 - Spectral unmixing: output image

7.17. Pixel Inspector

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

To access it, press **Pixel Inspector** button (). Once **Pixel Inspector** dialog opens, you will have to go on the sample and select the desired pixel using the color picker.

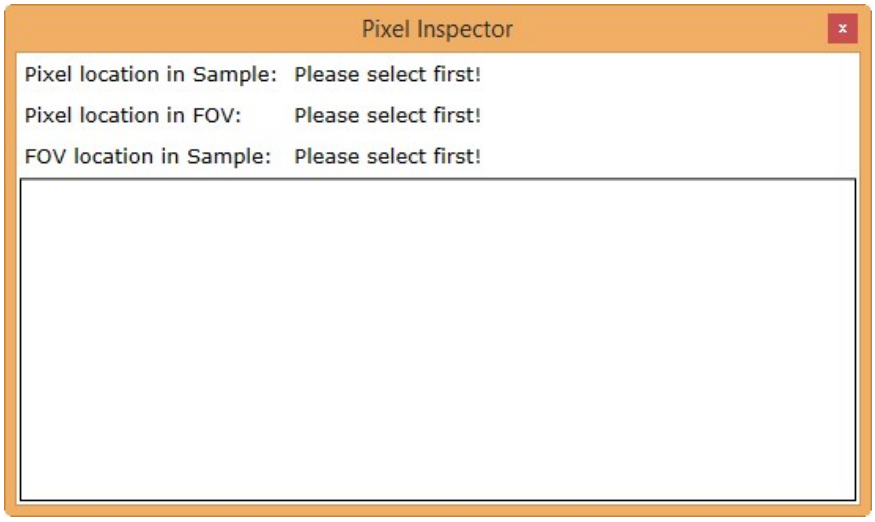


Figure 64 - Pixel Inspector before selection

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

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

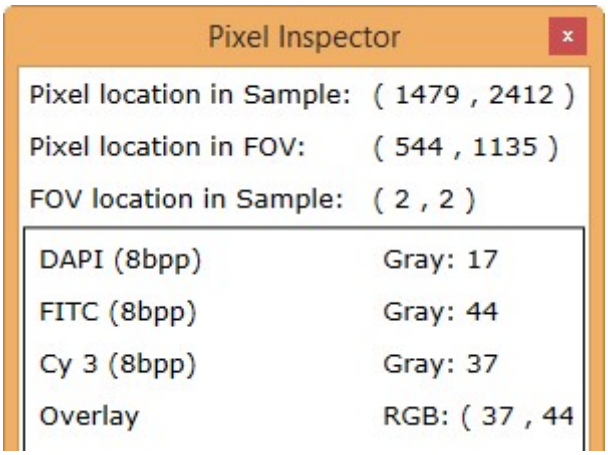


Figure 65 - Pixel Inspector after selection

7.18. Smoothing Image

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

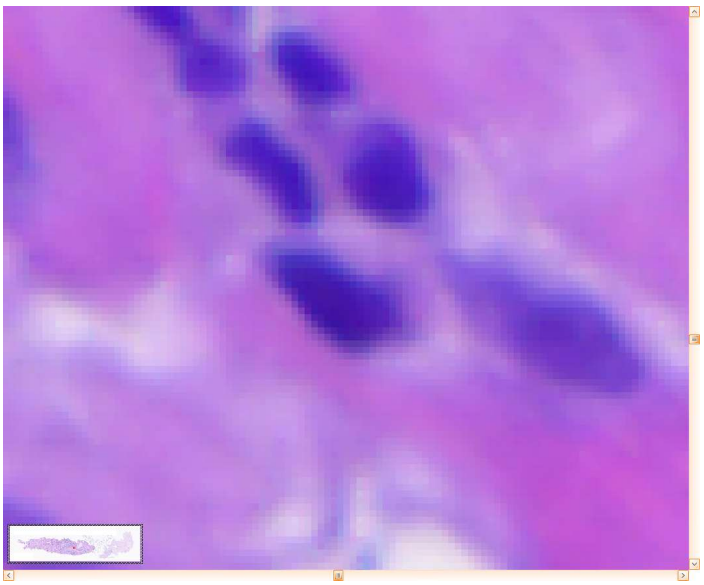


Figure 66 - Smoothen OFF

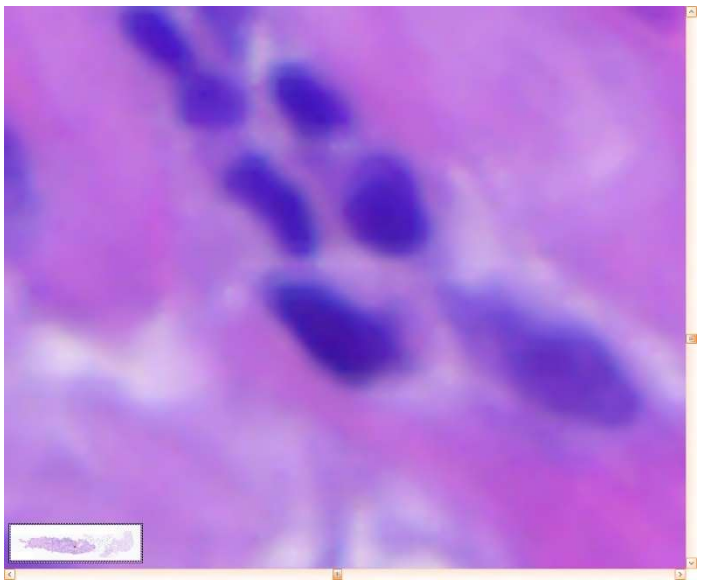


Figure 67 - Smoothen ON

7.19. HDR (available in Edit Mode)

TissueFAXS Viewer gives you the possibility to visualize samples as **HDR** images.

In other words, you can use a non-linear display to improve the dynamic range of the images, enhancing details in shadows (areas with weak signal) and highlights (areas with strong signal).

Use the **HDR** button from the sample viewer toolbar to activate **HDR** visualization mode.

7.20. Post Processing (available only for BF experiments in Edit Mode)

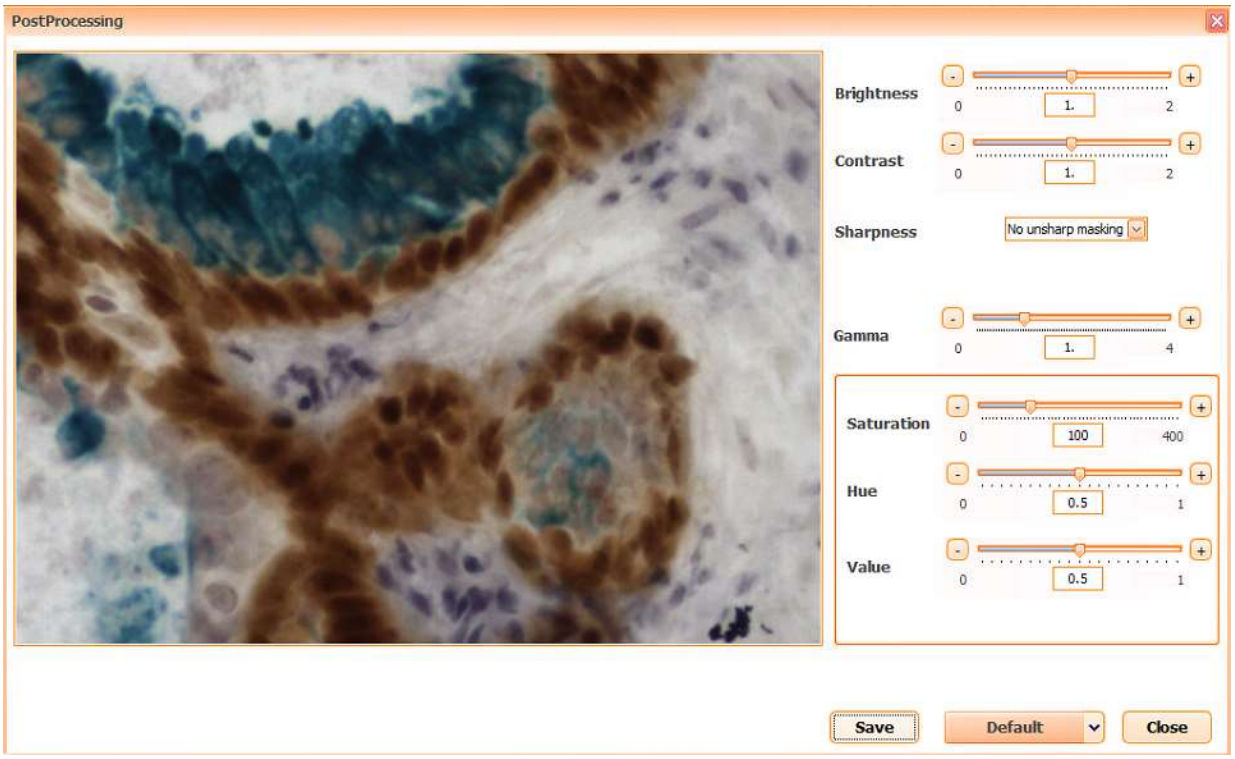


Figure 68 - Post Processing form

In Post Processing panel, you can adjust your images by using image processing dedicated **parameters**:

- 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**.

Note - Post Processing feature is only available in Edit Mode.

8. Toolbar

You can easily use the features of **TissueFAXS Viewer** by accessing its toolbar.

The most frequently used features – **Open**, **Close**, **Print**, **Print Preview** - are reunited in a quick toolbar.

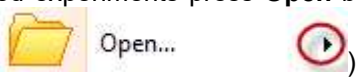
Figure 69 - TissueFAXS Viewer toolbar

8.1. File

Figure 70 – File menu

8.1.1 Open

To browse for an existing experiment press **Open** button () from the toolbar or press **Open…** from the **File** menu ().

To access the recently used experiments press **Open** button arrow () from the toolbar or press **Open…** arrow from the **File** menu ():

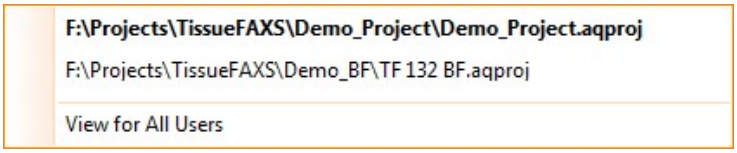


Figure 71 - Recent Experiments list

From this list, choose the desired experiment and left click on its name to open it.

Currently opened experiment name appears bold on the list.

If you choose **View for All Users** option, **Recent Experiments** dialog will open. Here you can see the previously opened experiments by other users and you can open any experiment by double clicking on it.

8.1.2 Print/Print Preview

Printing is only possible for an opened experiment. First, a dialog appears and then items to be printed must be selected (acquired items, all items).

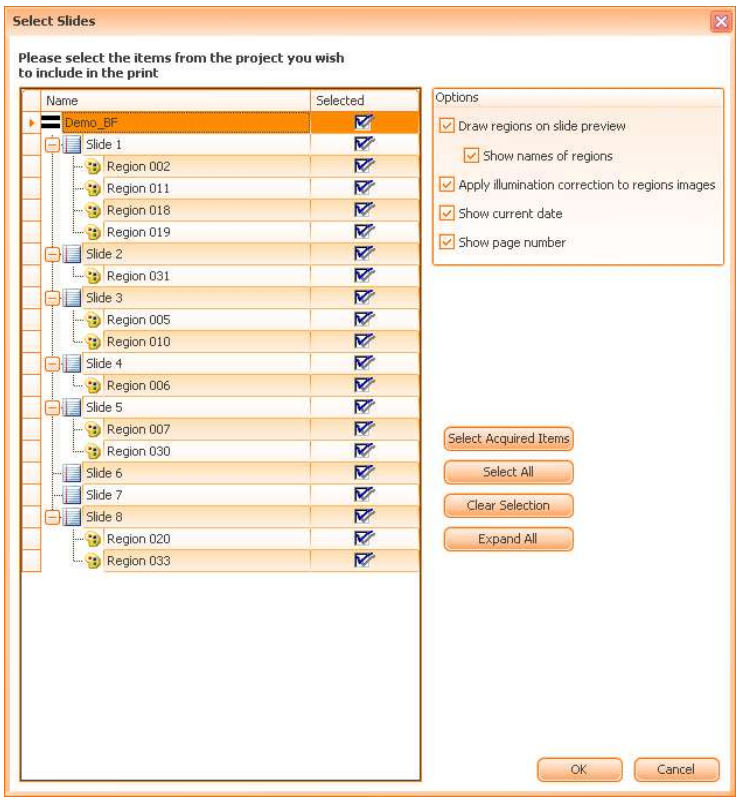


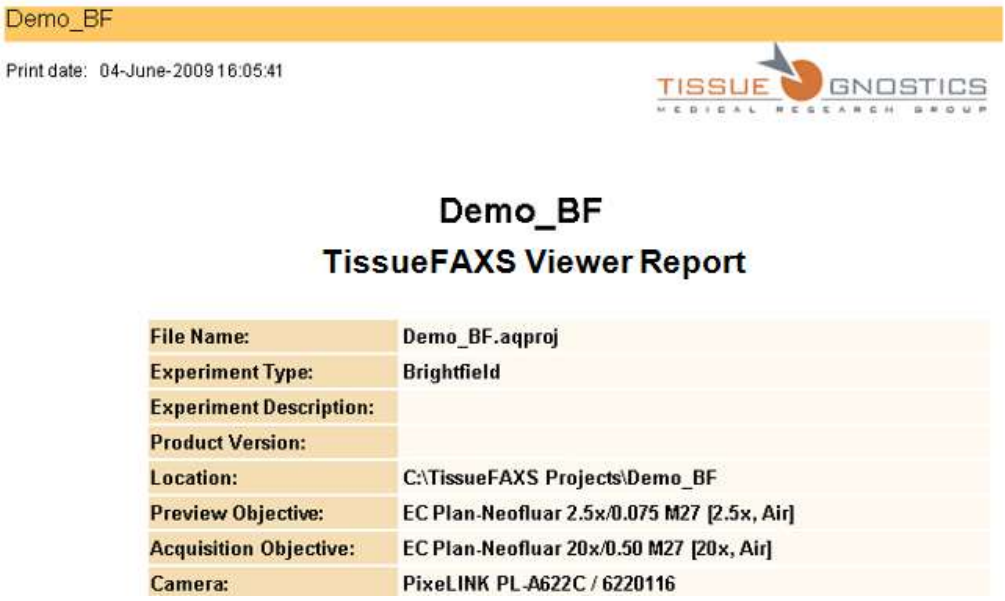
Figure 72 – Print dialog: choosing items to print

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: this option is available only if the first option is selected;
- Apply illumination correction to regions: 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 Items

- If you choose the **Print** option, a dialog will appear that permits to choose the printer.
- If you choose the **Print Preview** option, you will be able to see a preview report.



Demo_BF

Print date: 04-June-2009 16:05:41

TISSUE GNOSTICS
MEDICAL RESEARCH GROUP

Demo_BF

TissueFAXS Viewer 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

Figure 73 – Preview report example: first page

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: 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: 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.

8.1.3 Properties

The **Properties** button (), if pressed, will show a dialog that displays the most important properties of the currently opened project:

  - Name
  - Type (brightfield or fluorescence)
  - Comments (if any)
  - Storage Directory
  - Camera

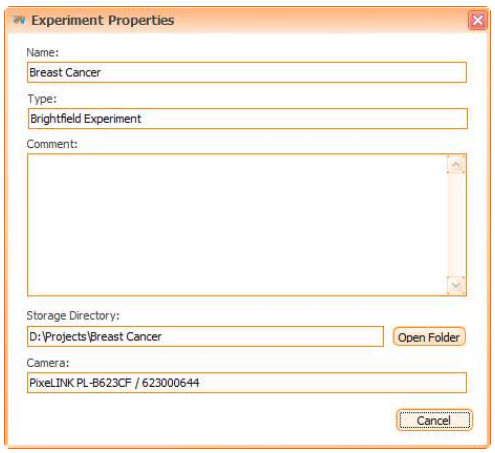


Figure 74 - Experiment properties dialog

8.1.4 Close

This button () closes currently opened project.

8.1.5 Exit

This button () closes TissueFAXS Viewer application.

8.2. Tools

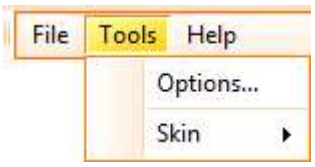


Figure 75 – Tools menu

8.2.1 Options

When using **TissueFAXS Viewer**, as in any other software application, you might occasionally encounter different kinds of errors, or you might simply need support and answers to your **TissueFAXS Viewer** related questions. The **Support** section was designed to help you in these situations.

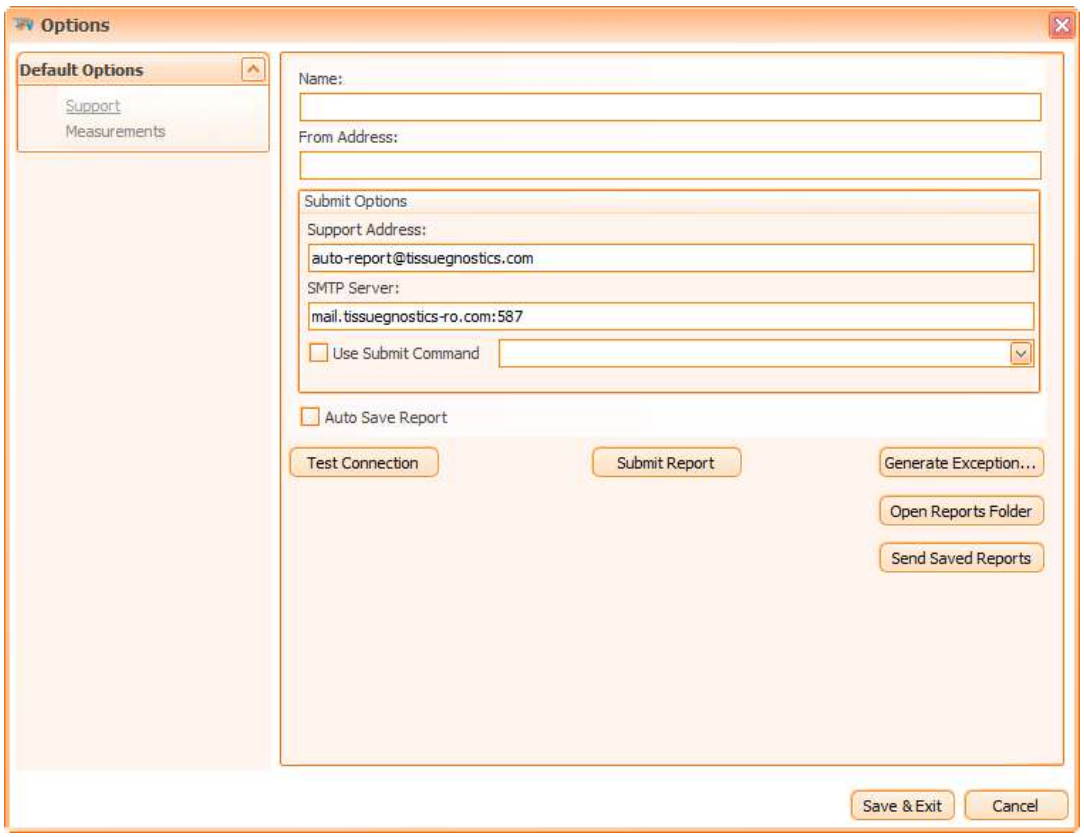


Figure 76 - Options: Support

Enter the following **information for dynamic error reporting**:

- The real name of the user who bought the software (e.g.: institution name);
- The email address that will be set as sender;
- The email address of Tissue Gnostics support;
- The available SMTP server/port to be used when sending the error report. (Contact your network administrator for details).

Use Submit Command: check this in order to access an external executable file that will send the files to a server.

Auto Save Report: check this option in order to save the log files on the hard drive, no matter if the report is sent or not.

Test Connection is used to see if the connection to SMTP server works.

Submit Report will help you send a mail containing your possible questions or problems regarding **TissueFAXS**. Beside the information you type in the form, the email automatically includes the log files, the running processes, and the configuration files of the application. To effectively send the mail, press the **Send Report** button.

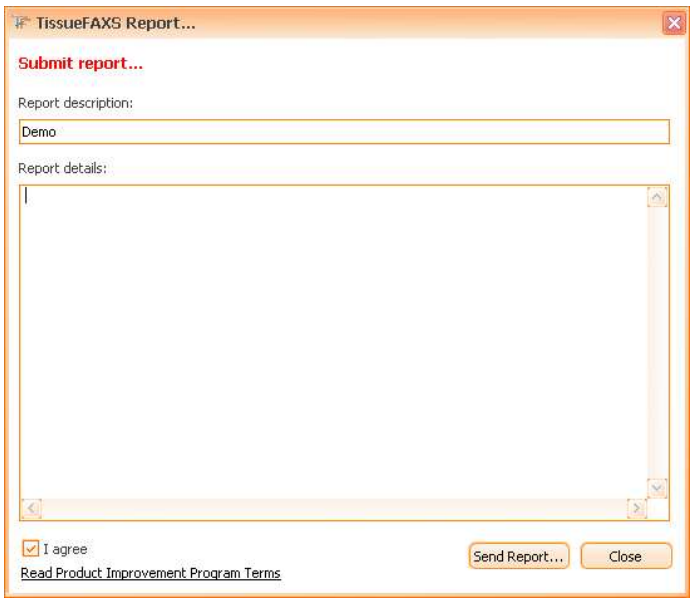


Figure 77 - Submit Report dialog

Generate exception is used to simulate an error in order to test if sending emails with the specified settings works.

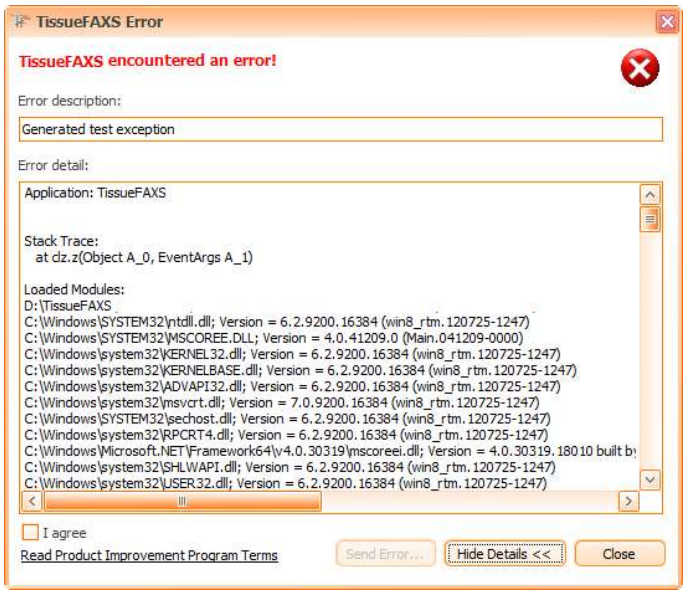


Figure 78 - Error dialog

- **Error description**: here you can see a short description of the error.
- **Error detail**: here you can find detailed data related to the error.

Open Reports Folder: if a report is not sent, it will be automatically stored in a local folder; press **Open Reports Folder** to open that folder.

Send Saved Reports: press this button to send all unsent reports, if any. After effectively sending saved reports, they will be automatically deleted from the local folder where they were stored.

8.2.2 Measurements

In the **Measurements** dialog you can manage the display options for the scale bar and measure function.

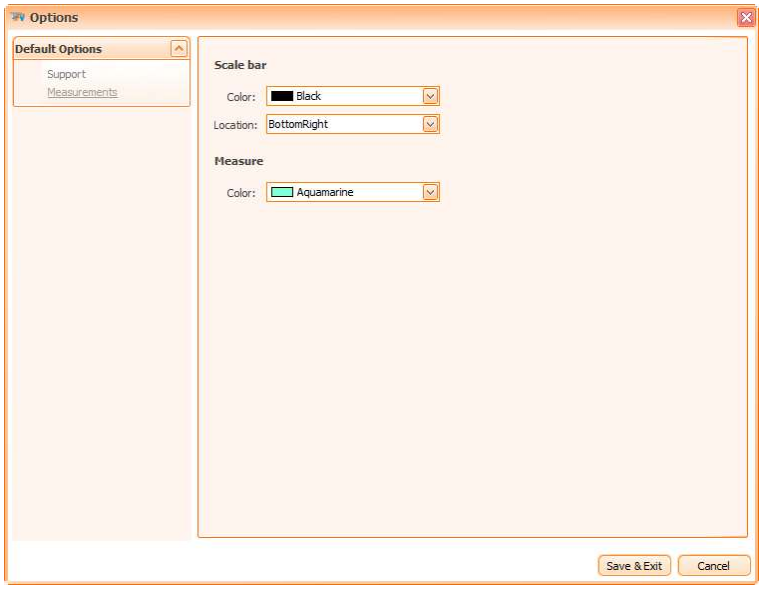


Figure 79 - Options: Measurements

Scale bar

The **Scale bar** icon looks like this:  .

The **Scale bar** is represented by a segment that indicates the scale of the image.

It has two adjustable attributes:

- The **color**;
- The **location** list with four values:

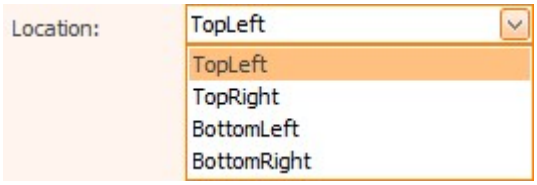
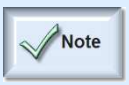


Figure 80 - Scale bar location combo box



- The default values are **Black** (for color) and **Bottom Right** (for location).

The scale bar can be found on:

- Live Image;
- Slide Preview;
- Region Viewer;
- One Image Viewer;
- Exported Images.

Measure

The **Measure** icon looks like this:  .

This function is used to measure the distance between two points (on the sample) specified by the user (by clicking on the start point, then on the end point).

The distance and the unit of measure are displayed on the measured image.

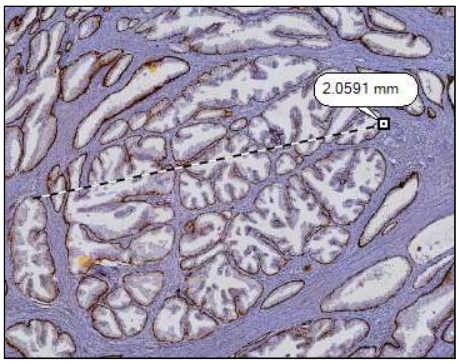


Figure 81 - Measured image example

Note - There are two adjustable attributes, the color and the measure unit. The default color is **aquamarine** and the default measure unit is **millimeter**.

This function is available on:

- Live Image;
- Slide Preview;
- Region Viewer;
- One Image Viewer.

RGB

Here you can select the color used to display information about **RGB** or **Grey value** for a pixel on the camera live image.

8.3. 3D Viewer

How to access 3D Viewer

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

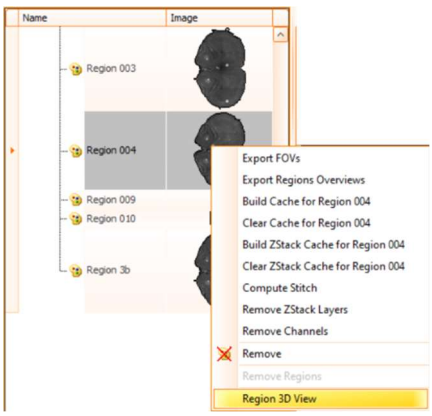


Figure 82 – Accessing 3D Viewer

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 and selecting **Build Z Stack Cache**.

The selected region will open in full 3D Viewer mode, as shown below:

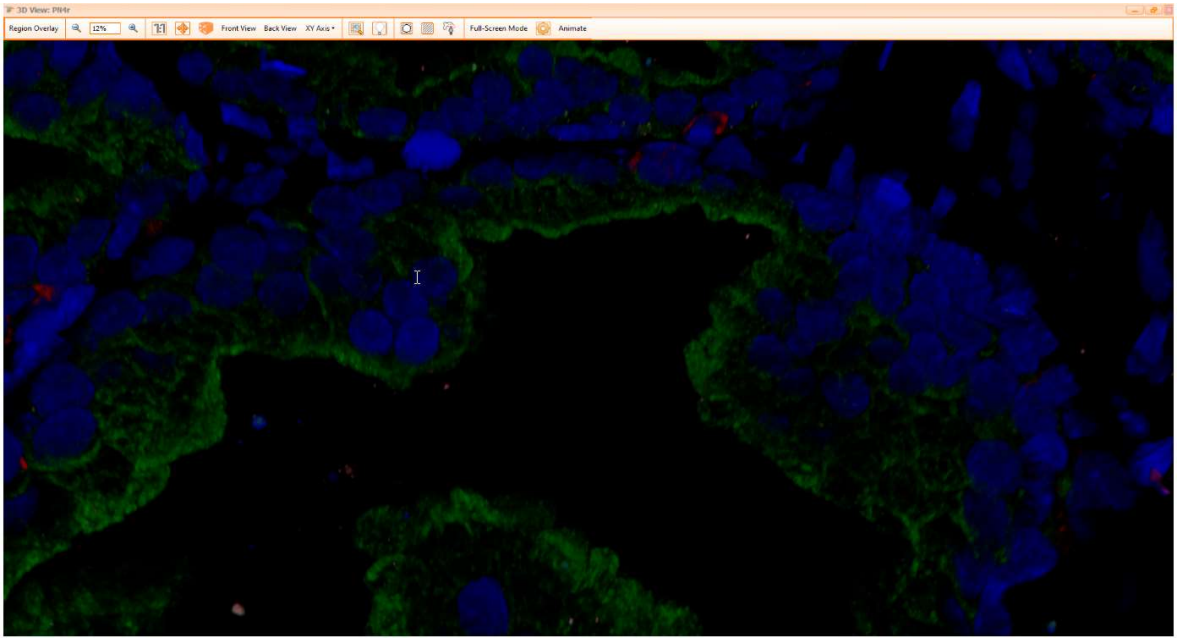


Figure 83 – 3D Viewer 1

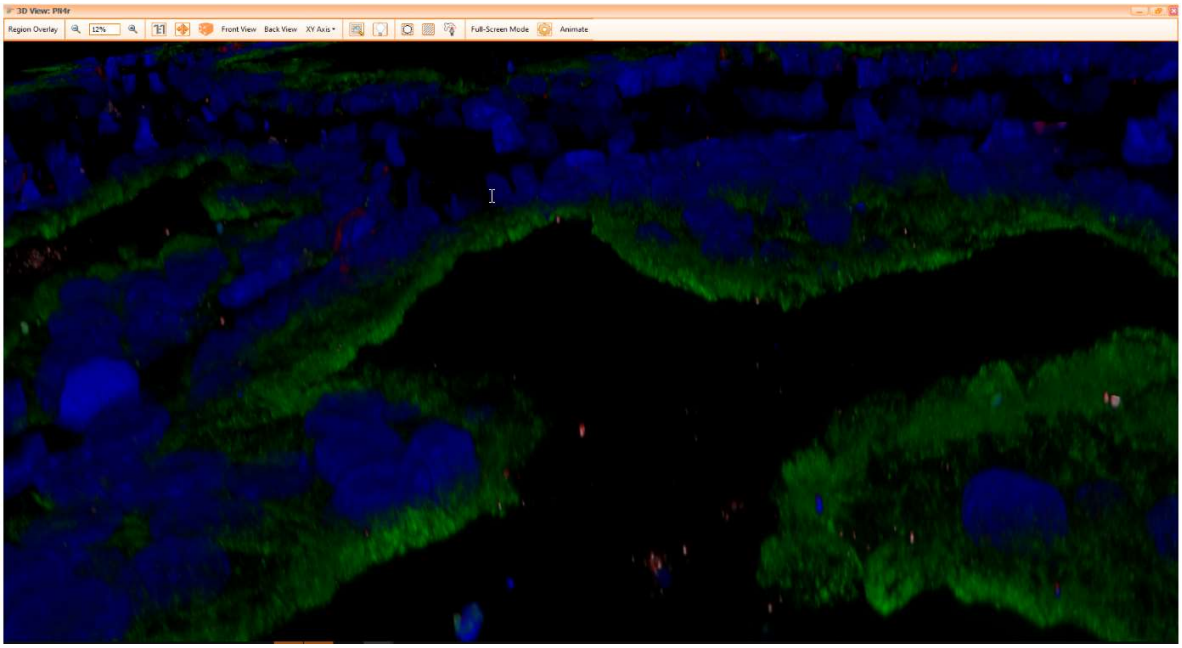


Figure 84 – 3D Viewer 2

3D Viewer tools

1. **Region Overlay**: overlay feature in 3D Viewer helps selecting the channels to be displayed and adjust their values (intensity, color, range).

2. **Zoom in/Zoom out.**
3. **Display 1:1 image.**
4. **Display image in Best fit mode**.
5. **Reset to original view**: resets image to its original view, in other words the image will look like being freshly opened in 3D Viewer.
6. **Front View**: shows the front of the opened image.
7. **Back View**: shows the back of the opened image.
8. **XY Axis**: select the type of rotation you want in your 3D visualization:

- Rotation XY axis
- Rotation X axis
- Rotation Y axis

9. **Show Map**: shoes/hides image map.
10. **Shading Correction**: enables/disables shading correction.
11. **Show crop**: displays cropped image.
12. **Show grid**: shows the grid that separates the FOVs composing the image.
13. **Show categories (annotations)**: annotations, if any, will be shown.

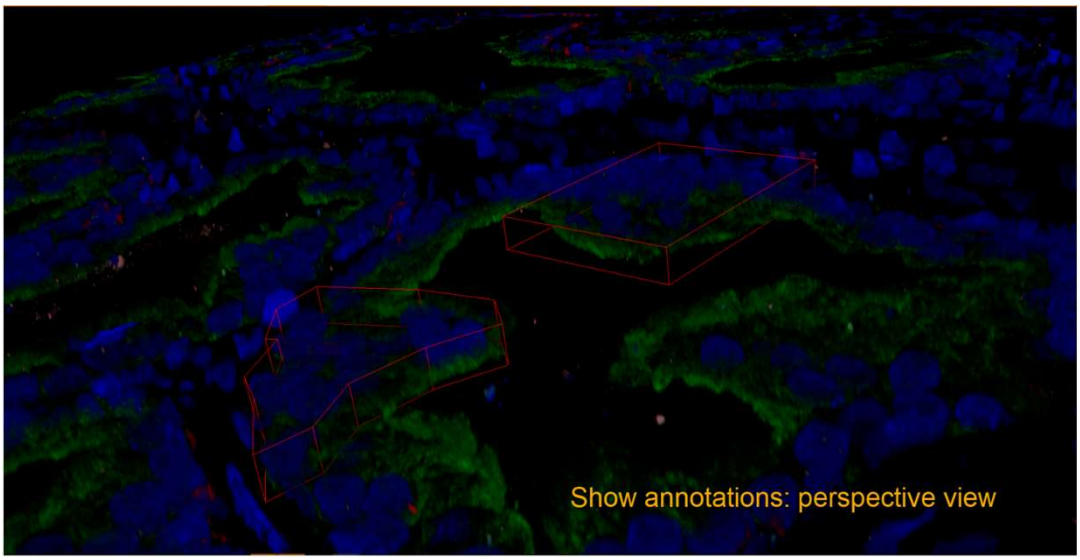


Figure 85 – 3D Viewer: perspective view on annotations

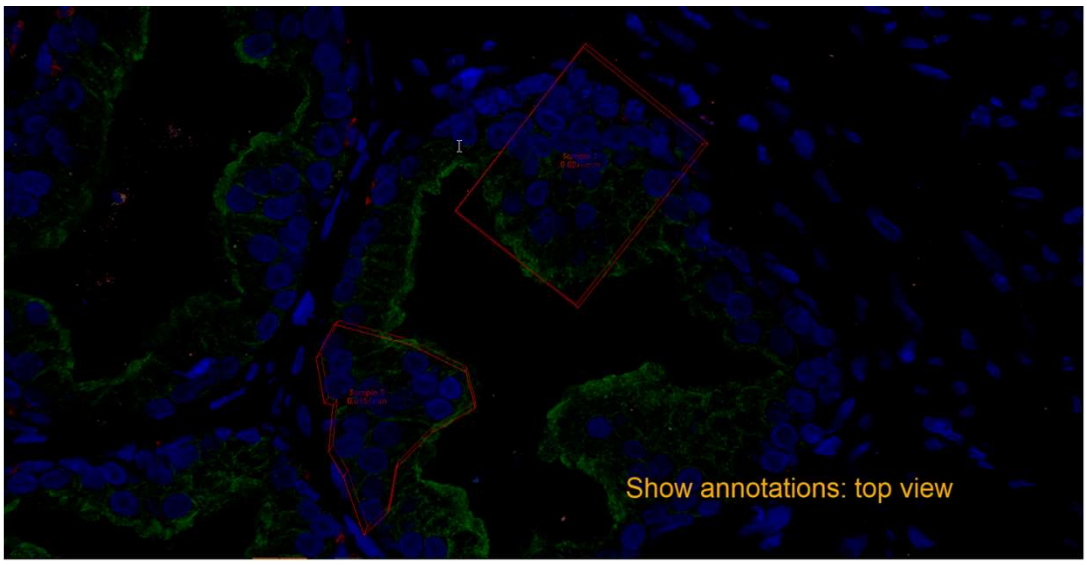


Figure 86 – 3D Viewer: top view on annotations

- Select a region/annotation 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/annotation by hovering the mouse over the edge of the shape.

14. **Full screen mode** displays image in full screen mode.

15. **3D View Options**: the advanced settings for 3D Viewer help you obtain an optimal view, getting the most of the graphical performance of your computer.

- Quality: select Highest or High if you have a performant graphic card, if not – select Medium or Low.

- Sample Distance: represents the step made by a raytracing ray in the volume. The more points you have, a more detailed volume you will get. For an optimal quality, 1 point per voxel is recommended (one sample per voxel). For low quality graphic cards, you can rise the sample distance: some quality loss will occur in the image, but the oval performance will be improved.

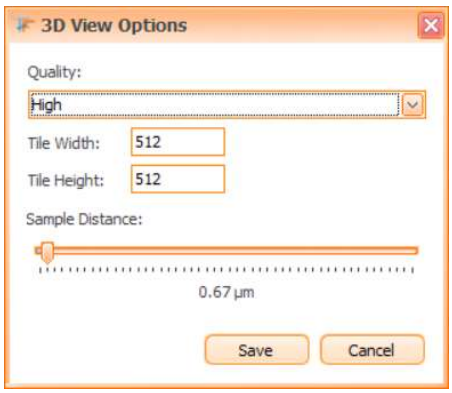


Figure 87 – 3D Viewer advanced settings

16. **Animate**

TissueFAXS 3D Viewer allows creating and storing animation sequences.

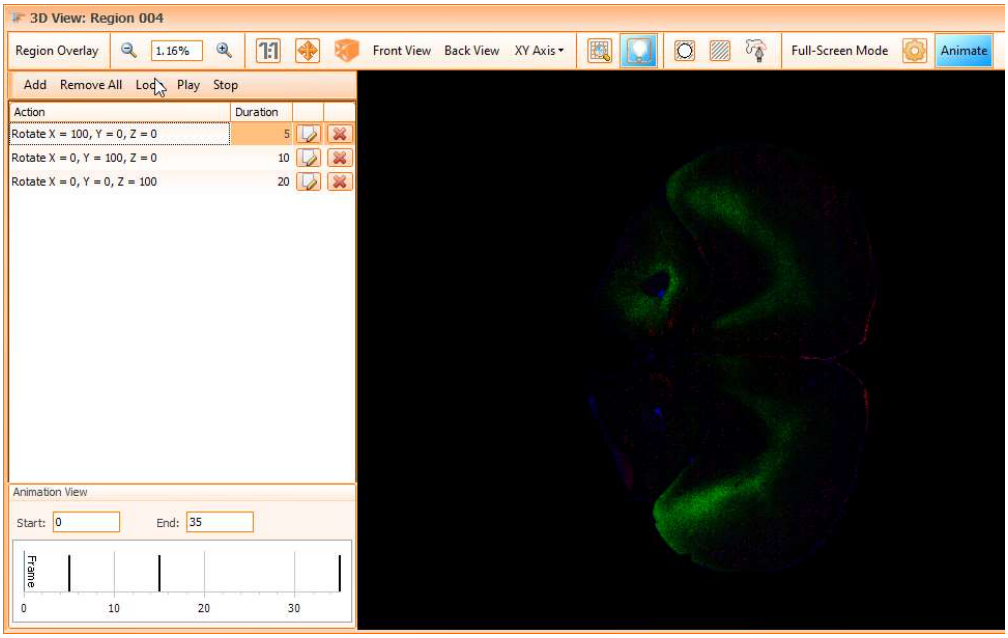


Figure 88 – 3D Viewer Animate feature

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.

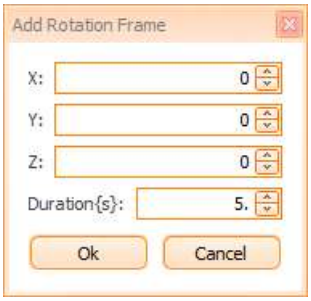


Figure 89 – Adding rotation frame for animation

To edit an animation, press  . To delete an animation, press  . 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.

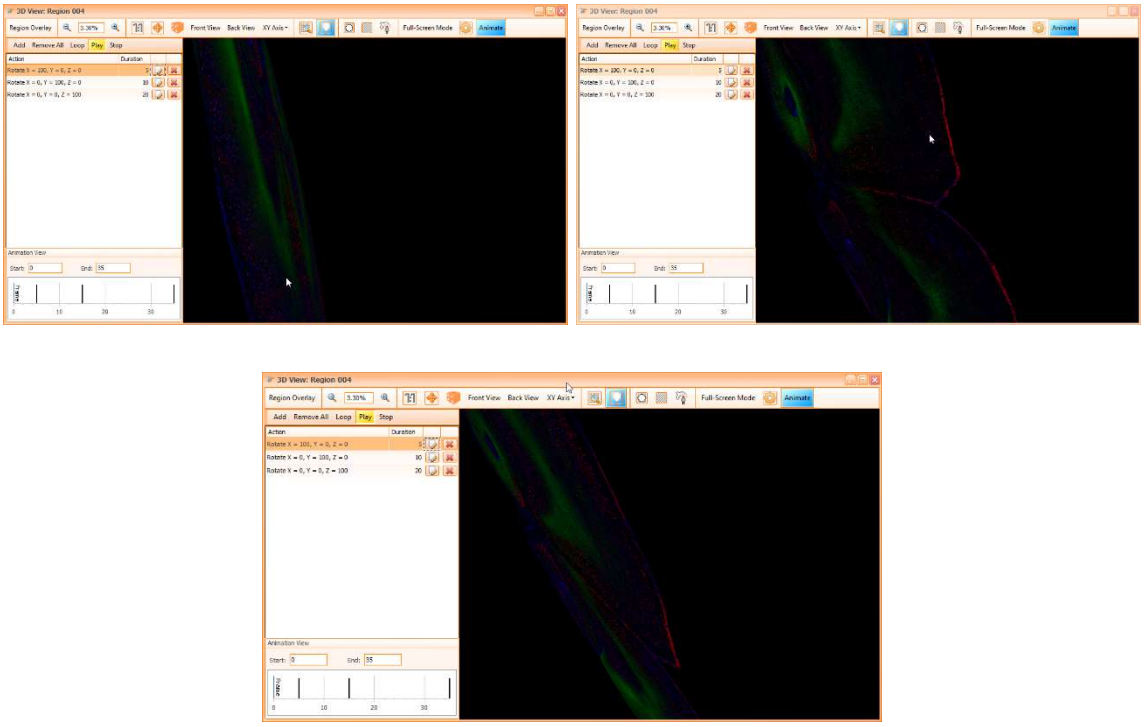


Figure 90 – Examples of frames of animations running

Operations Shortcuts in 3D Viewer

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.

8.4. Edit Mode

This feature allows editing current experiment.

If the project you want to open was created with a TissueFAXS version previous to the TissueFAXS Viewer version you are using, that project will be opened in read only mode.

To be able to use some features of the TissueFAXS Viewer, you have to select Edit Mode and upgrade the experiment. You will be notified with a pop-up message.

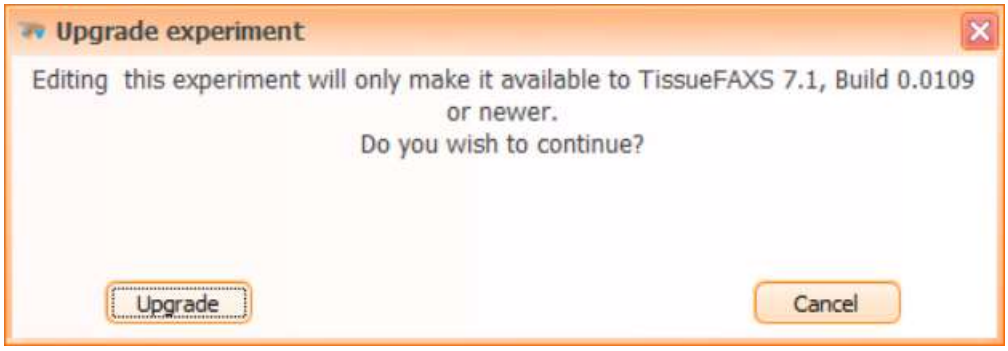


Figure 91 – Upgrading experiment

8.5. Help

Figure 92 - Help menu

About... this option will display a splash screen containing the main information about the **TissueFAXS** version in use.

***Human Protein Atlas*:** opens Human Protein Atlas.

Figure 93 - About TissueFAXS Viewer splash screen