TISSUEGNOSTICS

PRECISION THAT INSPIRES

TISSUEFAXS

LIST OF FEATURES

TissueFAXS SLIDELOADER

General Workflow

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1. Introduction

1.1. Purpose

The purpose of this document is to give the user a concise and systematic presentation of the workflow in order to perform the preview in ***TissueFAXS SL*** *experiments.*

2. Login

In order to login to **TissueFAXS 200 Confocal**, please enter a username into the **User Name** field and type the proper password into the **Password** field.

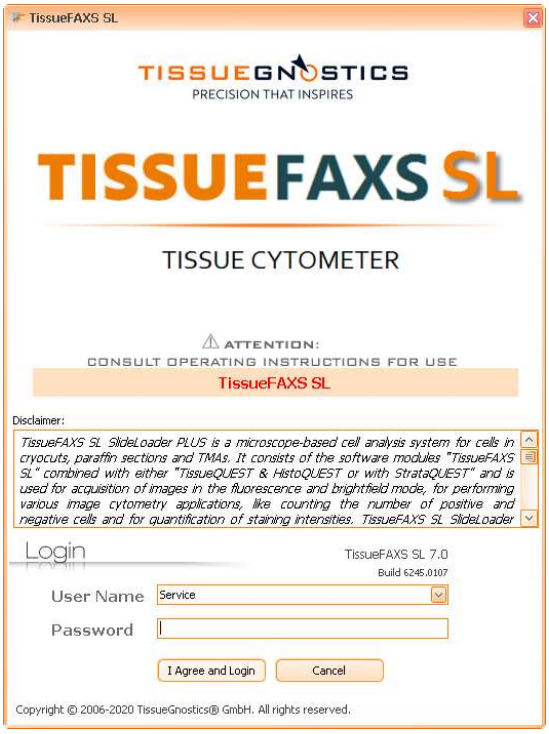


Figure 1 - Login Panel

3. Calibrate devices

Calibrate Stage

To start an image acquisition, please choose to carefully calibrate the stage when starting the application.

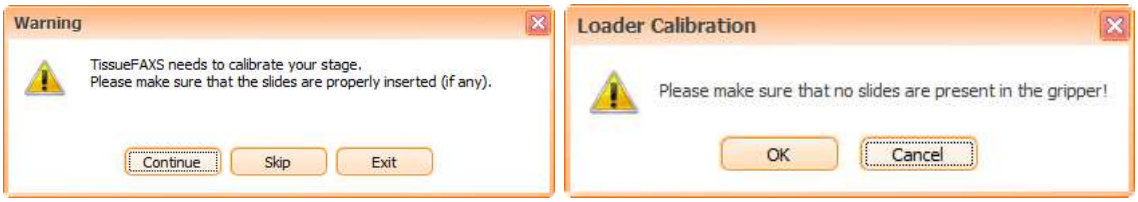


Figure 2 - Stage Calibration warning messages

CAUTION

- **If slides are present in the gripper before starting the calibration process, they will be broken!**
- **In case any slides are broken during a loader operation, please make sure to remove all glass pieces from the loader and the gripper in order to prevent other damage!**

4. Settings for preview

Before considering a job acquisition, don't forget you need a template that stores all the necessary settings for preview and acquisition (objectives, cameras, reflectors etc.)

Please create new template to store the information described below.

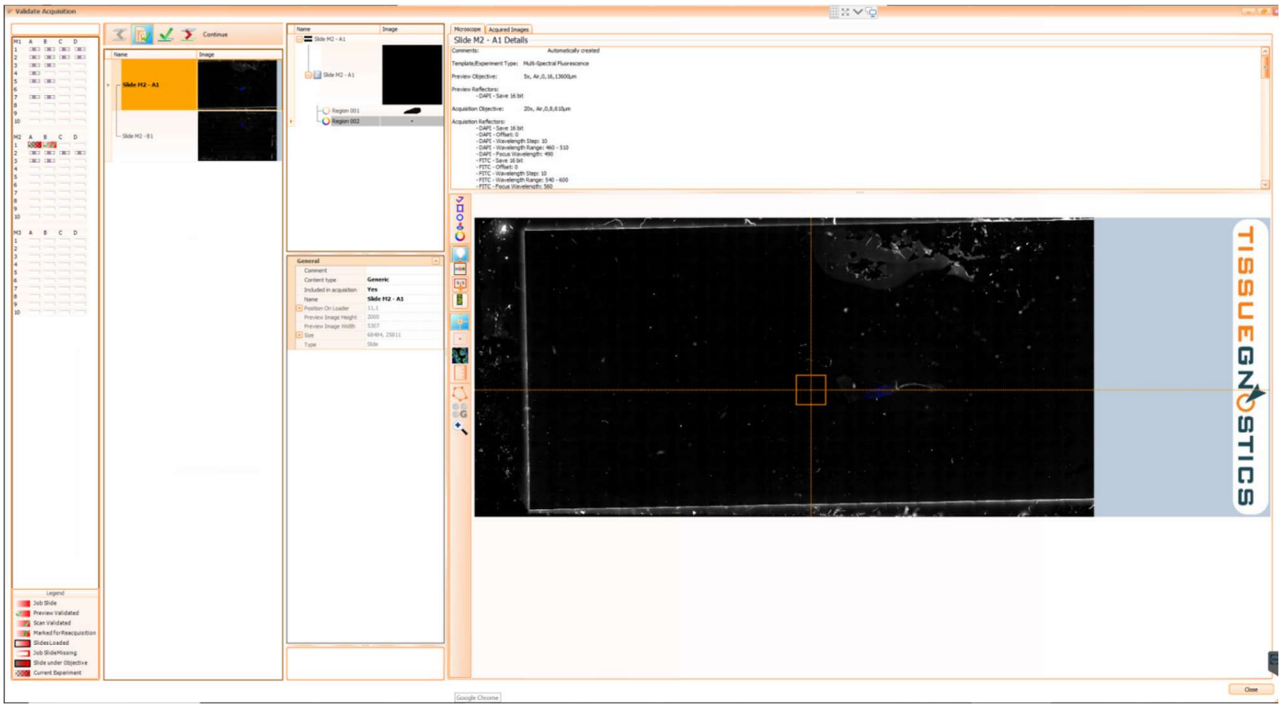
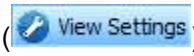


Figure 3 - Validate Acquisition

Preview Settings can be accessed by clicking on the **Preview** tab in the upper left corner of the main **TissueFAXS** window. If the **Preview** tab is not visible, you can make it visible by pressing the **View Settings** button (

) from **Home** tab.

Figure 4 - Preview tab

Select objective for preview

Choose the desired objective from the **Preview Objective** dropdown list of the **Preview** tab.

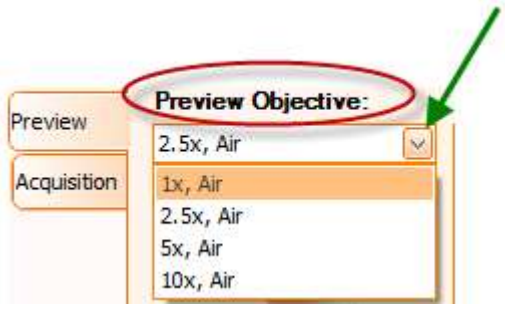


Figure 5 - Preview Objective dropdown list

Select channels for preview

To add or remove channels, press the **Add/Remove Channels…** button (Add/Remove Channels…). In the dropdown list that appears, the already selected channels will appear checked.

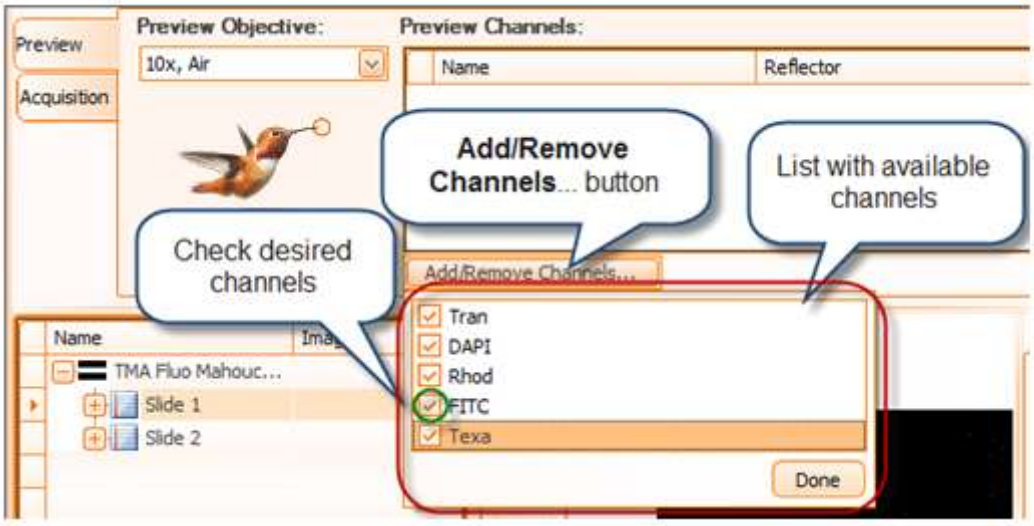


Figure 6 - Preview Settings panel: Channel list

Adjust camera settings for each channel

To edit the camera settings for a channel, click the **View** button next to it.

You can change the names of the channels by clicking on each in the settings field.

Note

- To adjust the camera settings for any channel, you must have a camera present in your project. Otherwise, the **View** button (View) for each channel will not be visible.

You can select more than one channel for the preview operation because each slide can be scanned multiple times (once for each channel in the list, that will yield the final overlay image). For each channel you may adjust the camera settings (light intensity, exposure time, colors, etc.) by pressing the **View** button next to each reflector in the list. Make your adjustments in the new window that appears and press **Save**. When all your channel settings are saved, you are ready to acquire your preview image.

It might be an option to make the preview for fluorescent experiments in darkfield illumination.

Adjust preview area

A region can be set as a preview area for future preview operations in the current experiment.

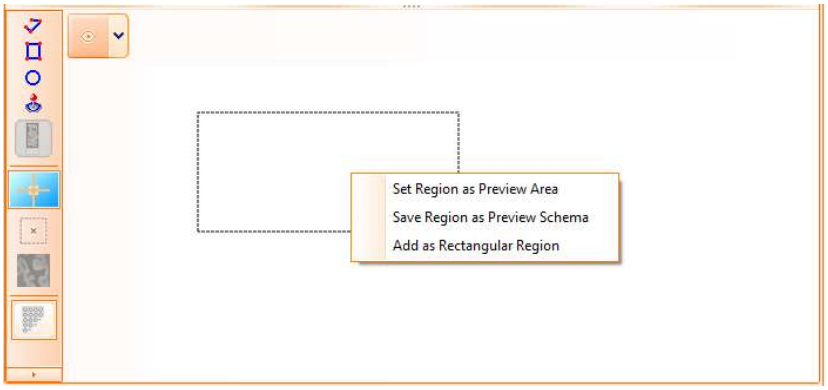


Figure 7 - Set new preview area context menu

If the preview area chosen in the experiment setup needs to be adjusted, a rectangle can be drawn on the slide, clicking the left mouse button to accept a drawn rectangle. Right-clicking within the selection will allow doing the following:

- **Set region as preview area** - set current region as preview area for current experiment. This will affect all subsequent slides in this experiment.
- **Save region as preview schema** - saves current region as preview area for future use and set it as preview area for current experiment. You can give a name to this preview scheme and store it (to use it again in future experiments).

Show Preview Info

There are two possibilities to see where the preview will be performed on the slide:

- By pressing **Show preview info** button ( ) from the slide editor.
- By right-clicking on the slide, then – from the contextual menu - choosing **Preview** → **Show preview info**.

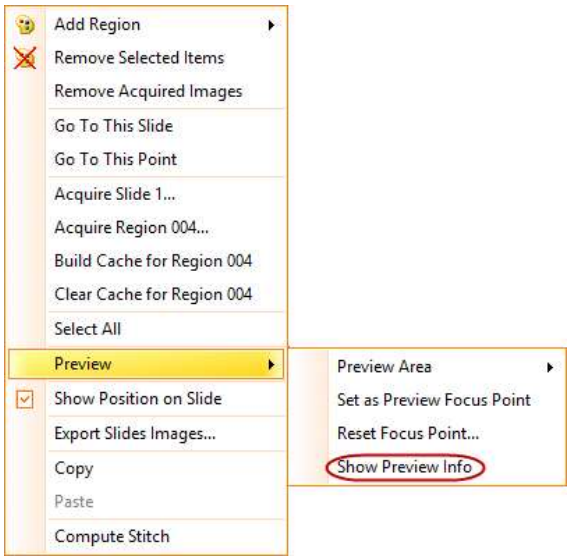


Figure 8 – Show preview info

In both cases, the preview info will be displayed on the slide:

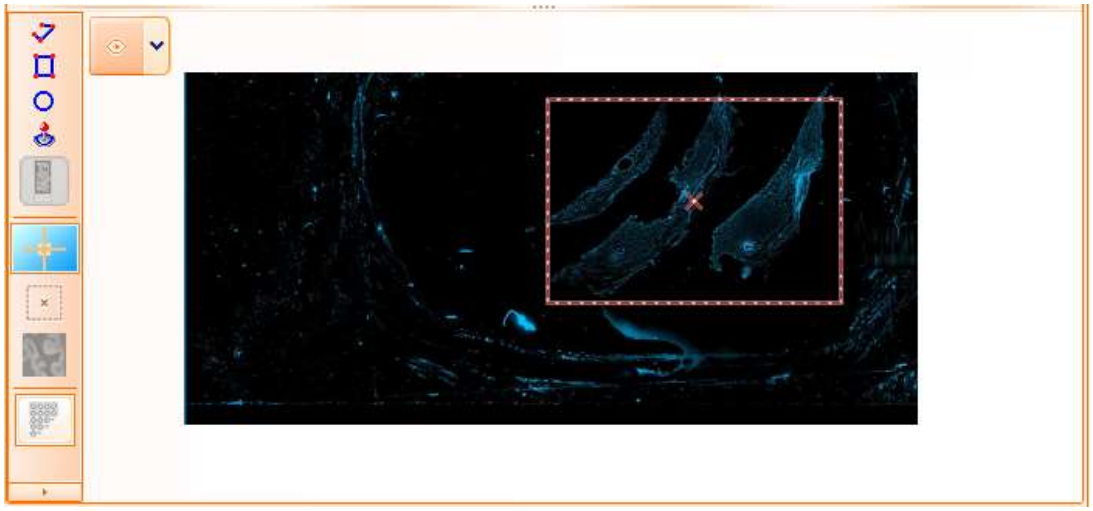


Figure 9 - Preview info on the slide

Set / reset focus point

To change the focus position, right-click on the slide in the desired place and then choose **Preview → Set as preview focus point**. The point where the right-click was performed becomes the new preview focus point.

To **reset the focus point**, choose **Preview → Reset Focus Point**: you will come back to the default focus position (the center of the preview area).

The "Preview" button

You will be ready to capture a preview image of your slide when you have set up your experiment and adjusted your camera settings for your preview objective. To initiate preview, press the **Preview** button () located within your selected slide panel.

To preview another other slides too, click on the drop-down menu of the **Preview** button for more preview options.

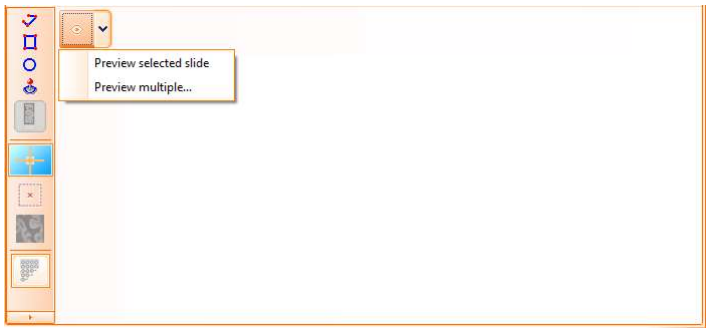


Figure 10 - Preview options panel

Preview multiple slides (for 2-Slides Project)

To preview **multiple slides**, click on the drop-down menu of the **Preview** button for more preview options.

When **Preview Multiple** is chosen, the following dialog will appear:

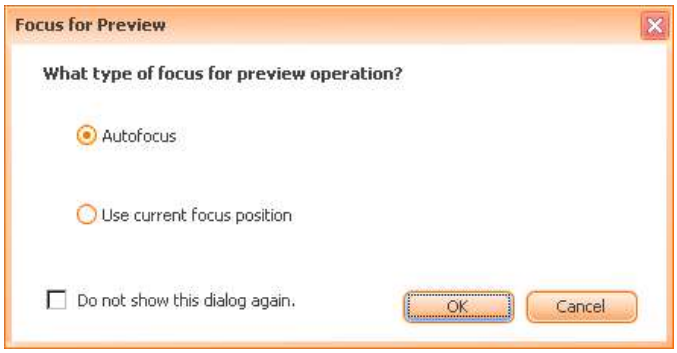


Figure 11 - Focus for Preview dialog

Select your focus method then press **OK**. In the next window, select the slides you wish to acquire.

TIP

- Autofocus method will use the method defined in **Options → Focus**. If you check **Do not show this dialog again,** your current choice will be remembered for future use and you will not be prompted again. This selection can be reset from **Tools → Options → Remember**.

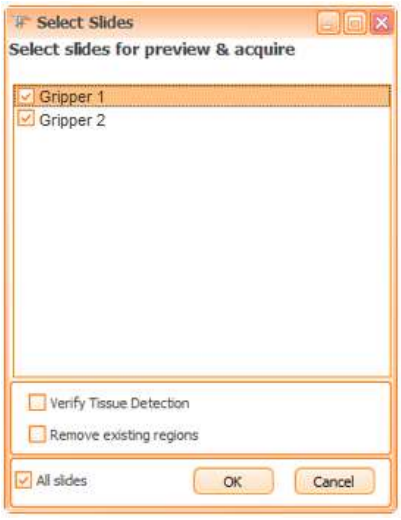


Figure 12 - Slides selection checkbox

Region Overlay

In fluorescence experiments, the **Region Overlay** button is enabled. Clicking on it yields a new window, as shown below:

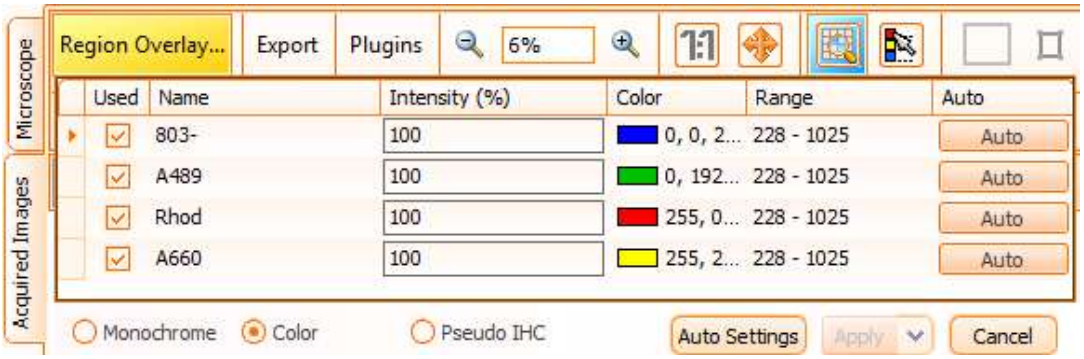


Figure 13 - Adjusting channel intensity and color

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.

When **exporting** images, you can choose the **Overlay** option from the export panels: the images will be composed from all the channels as currently specified in region viewer.

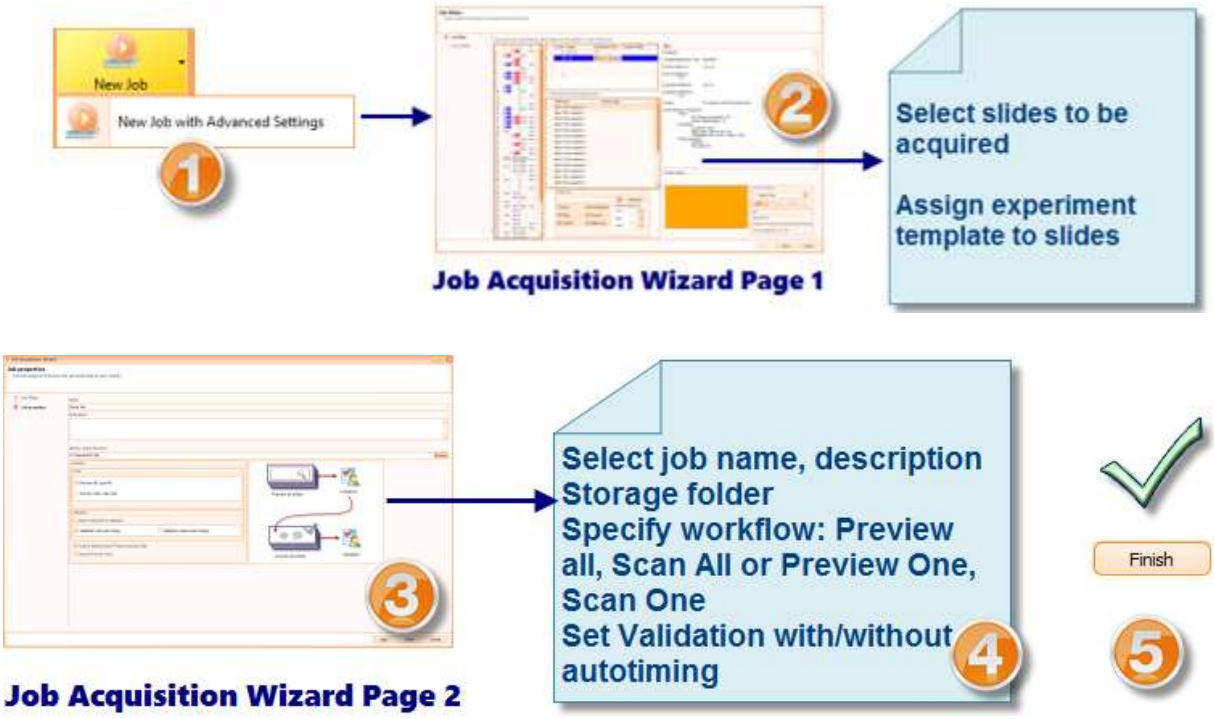
5. New job

5.1. Create new fluorescence acquisition template

To be able to create and acquire a job, you must first create a template. Use **New Template** button to open a wizard where you will choose fluorescence as experiment type, then you will select desired settings, as shown in the image below.

5.2. Create Job

Once you have a template, you may proceed to job creation: choose **New Job with Advanced Settings** from **New Job** button, and the **Job Acquisition Wizard** will open. Follow the steps shown below to complete job creation.



5.3. Validate Preview

After **TissueFAXS SL** will end the preview (only for the Preview all Scan all workflow), **Validate Preview** form fill open. If you are pleased with the automatic tissue detection you can let the application initiate the acquisition process.

If you consider the automatic tissue detection can be improved, manually adjust the parameters until you obtain desired region(s) – please see **Chapter 5.4**. Then you have to manually validate the preview for the slide(s) to start acquisition.

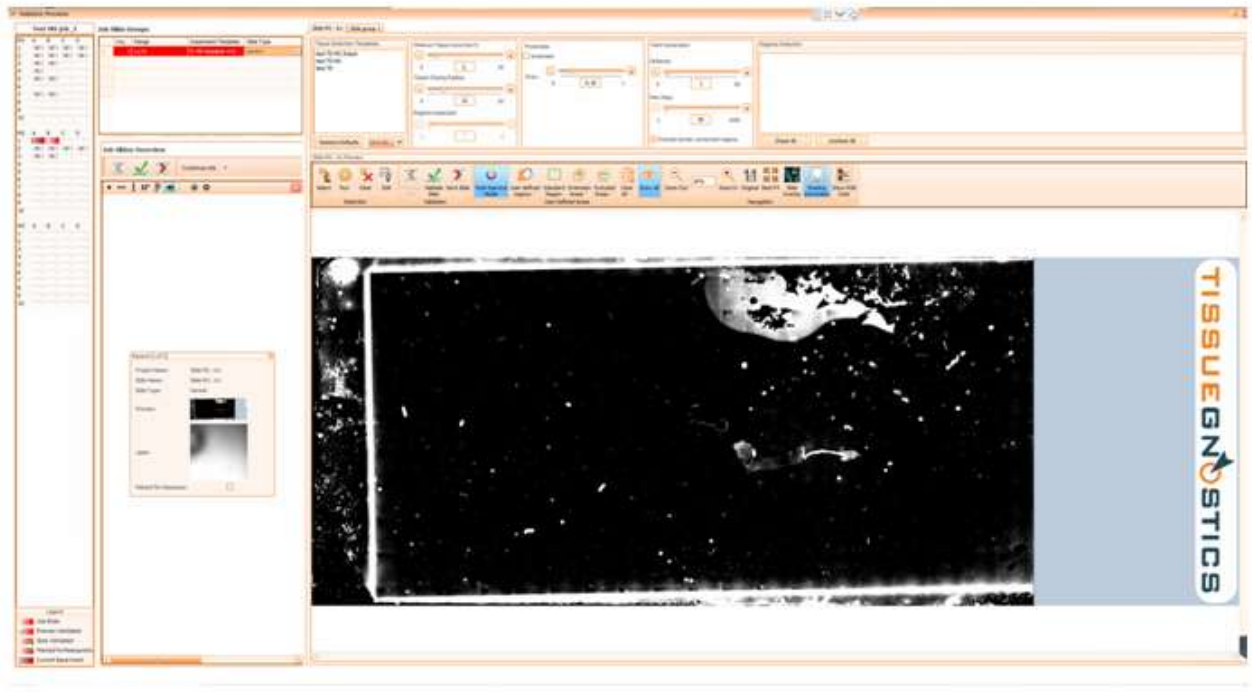


Figure 14 - Job Acquisition: Validate Preview

5.4. Tissue Detection

In the Preview Validation phase, **TissueFAXS SL**is able to auto-detect the tissue regions on the slide. This is a great tool provided by **TissueFAXS** that can help you save time and improve the accuracy of the tissue detection.

If you take no action within a previously determined amount of time, **TissueFAXS SL**will automatically detect regions and proceed to acquisition. To avoid automatic detection, press **Stop** button or interact in any other way with the validation window.

Figure 15 - Counter for the auto-timing validation

In order to detect generic tissue samples, please make sure that the **Content Type** property of the slide is set to **Generic**.

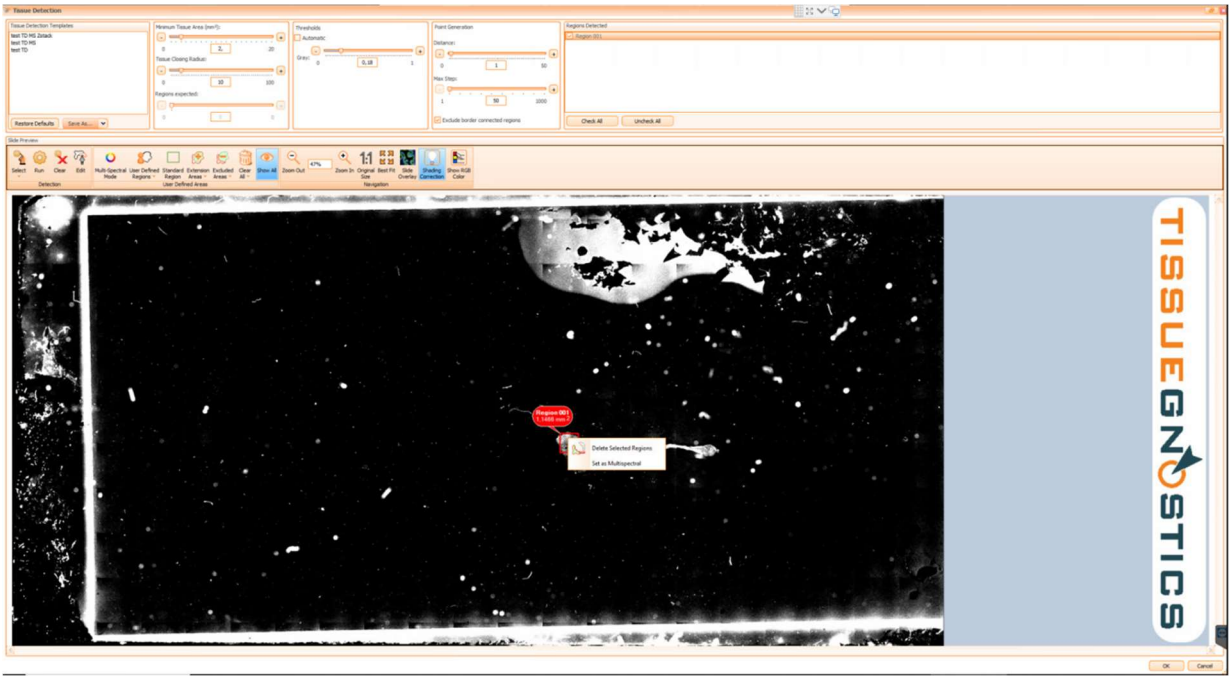


Figure 16 - Tissue Detection panel

By default, the detection is run on the entire preview image. If you want to refine the results, you can run the detection on a smaller area:

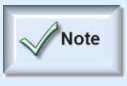
- select the desired area by drawing it on the displayed image
- click on **Run Selection**

If you want to clear regions from the selected area, press the **Clear Selection** button.

For the **2-Slide projects**, to access tissue detection press **Detect Tissue** button () from the slide editor toolbar.

5.5. Validate Acquisition

Once the scan is done, **TissueFAXS SL** will prompt you to validate the incoming acquisition, through **Validate Acquisition** form.



- If the user marked some slides to be reacquired, TissueFAXS will not start the process automatically, the user must manually choose to reacquire.

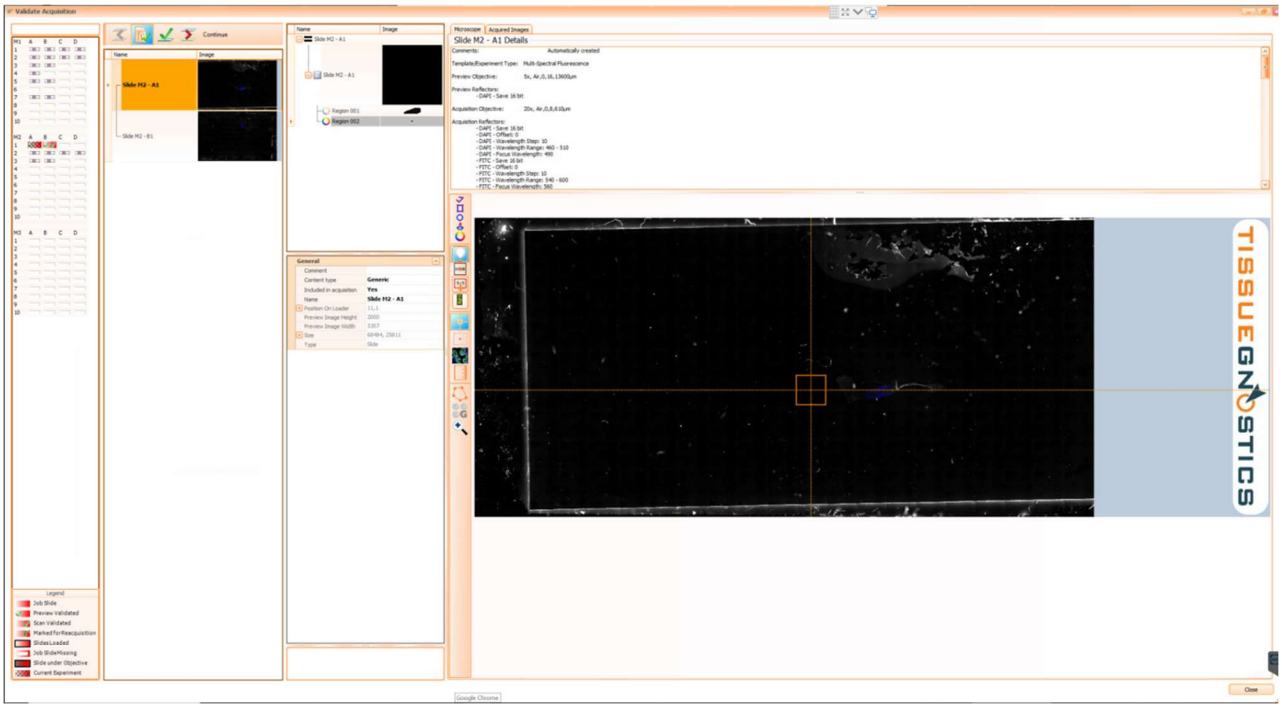


Figure 17 - Job Acquisition: Validate Acquisition

6. Acquisition Settings

Acquisition settings can be accessed by clicking on the **Acquisition** tab in the upper left corner of the main **TissueFAXS** window. If the **Acquisition** tab is not visible, you can make it visible by pressing the **Settings** button from the application's toolbar.

Figure 18 - Acquisition tab

- Acquisition settings are similar to the preview settings, except for the fact that they act when acquiring regions.
- The list of objectives which may be used for acquisition is not limited (as for the preview).
- An important difference is that you can select the channel for auto focus in the acquisition workflow.

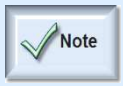
Focus Channel

The **Focus Channel** is the channel for which **TissueFAXS** will perform the auto focus in fluorescence experiments. The focus for **Focus Channel** will be memorized by the application and then used in acquiring the other channels.

The default **Focus Channel** in **TissueFAXS** fluorescence experiments is DAPI. If DAPI is absent from the experiment, **TissueFAXS** will automatically set as **Focus Channel** the first channel listed in the **Acquisition Channel** section.

Channels

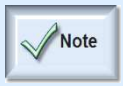
For Fluorescence Experiments, choose the desired reflectors (including transmission).



- After choosing the reflectors, you must readjust the camera settings for each reflector.
- The name of the channel is editable.

Objective Lens

Select the desired objective from **Acquisition** → **Acquisition Objective**.



- After choosing the acquisition objective, you must readjust the camera settings for each combination of acquisition objective and acquisition channel.

Camera Settings

TissueFAXS 200 supports the following cameras for Fluorescent imaging:

- PCO USB cameras
- Hamamatsu Orca Flash 4
- Andor Zyla

To edit camera settings press **View** button.

You may also want to use settings from **camera profiles** (if you have previously created any) instead of making new settings for the camera.

Additionally, **TissueFAXS** stores TL lamp intensity value (for Transmission) and attenuator settings value (for Fluorescence channels) per channel.

Camera Profile

A **Camera Profile** represents a set of camera related settings that you can save for further use, in order make available different combinations of settings for the acquisition process. Press Camera Profile button (

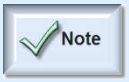
) to access the options:

Figure 19 - Profile Options menu

- **Save**: the current camera settings can be saved in order to make them available for further use. You must specify a name and a short description for each profile. A default name is already generated. It contains the camera name and type, the objective magnification and the reflector selected on microscope; you can also add your own information to the already existing name.

If "**Save as default channel profile**" is checked then all new experiments that will have 20x as acquisition objective will automatically load this profile for acquisition.

If pressed, **Acquire Correction Image** button will acquire the correction image for the acquired images.



- **Acquire Correction Image** button is only available for Transmission channel.

- **Load**: an existing camera profile can be loaded for a camera. You must select any profile you want from the existing list.
- **Delete**: if an existing camera profile is no longer needed, it can be deleted by selecting it and then pressing the **Delete** button.

7. Reacquisition

TissueFAXS SL comes with a **Reacquire Job** option which allows the user to select desired slides for reacquisition.

To proceed to reacquisition, add new regions or flag FOVs from existing regions to desired slides, then mark the slides for reacquisition () by accessing slide's contextual menu or the scan validation window, then press **Reacquire Job** button (). The following types of reacquisition are available:

- **Entire Job**: reacquires entire job.
- **Only Marked Slides** - reacquires slides you have previously selected for reacquisition.
- **Entire Job with New settings** - **TissueFAXS** supports restarting jobs with validated previews using different acquisition settings. This is useful in case you need to change acquisition settings after canceling job acquisition or checking acquisition results in **Validate Acquisition** phase.

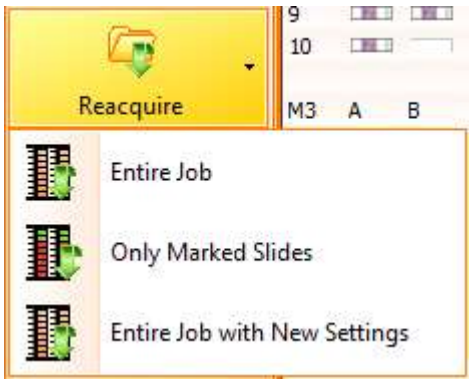


Figure 20 - Reacquire Job options

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