19 Module Panorama (ZEN lite)

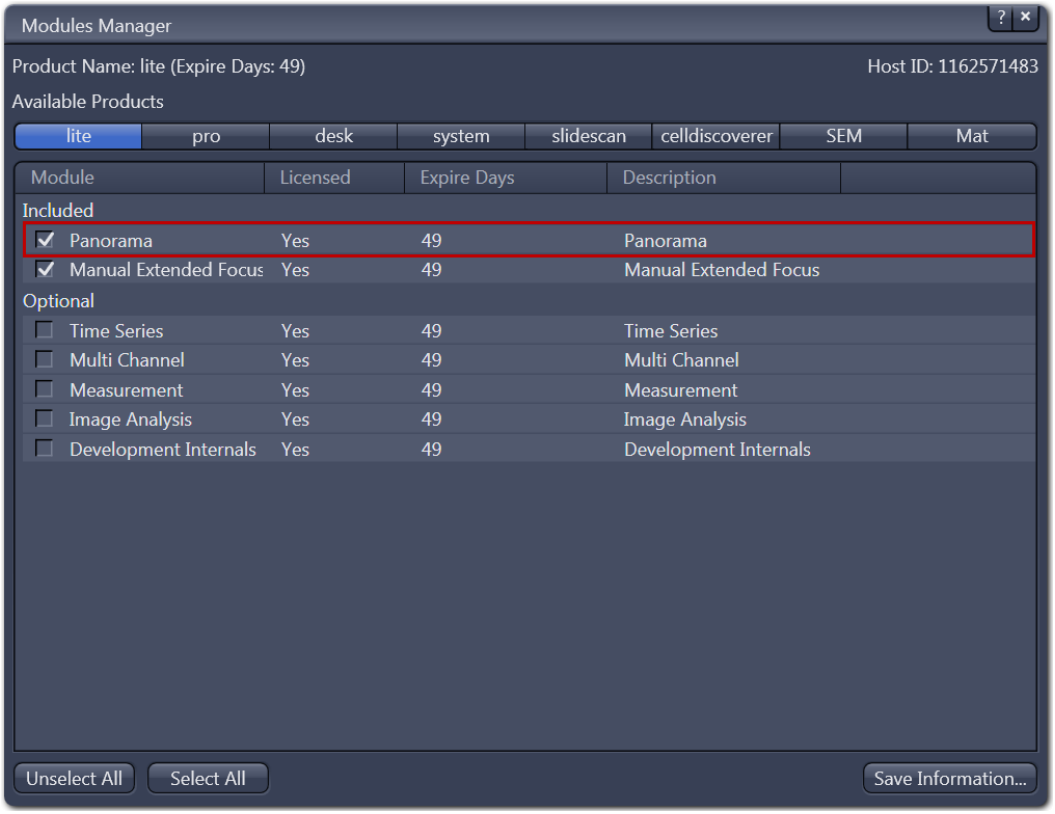
19.1 Introduction

By using the **Panorama** module for **ZEN lite** you can create overview images of large areas of your sample.

Prerequisites

For the interactive panorama acquisition following prerequisites are necessary:

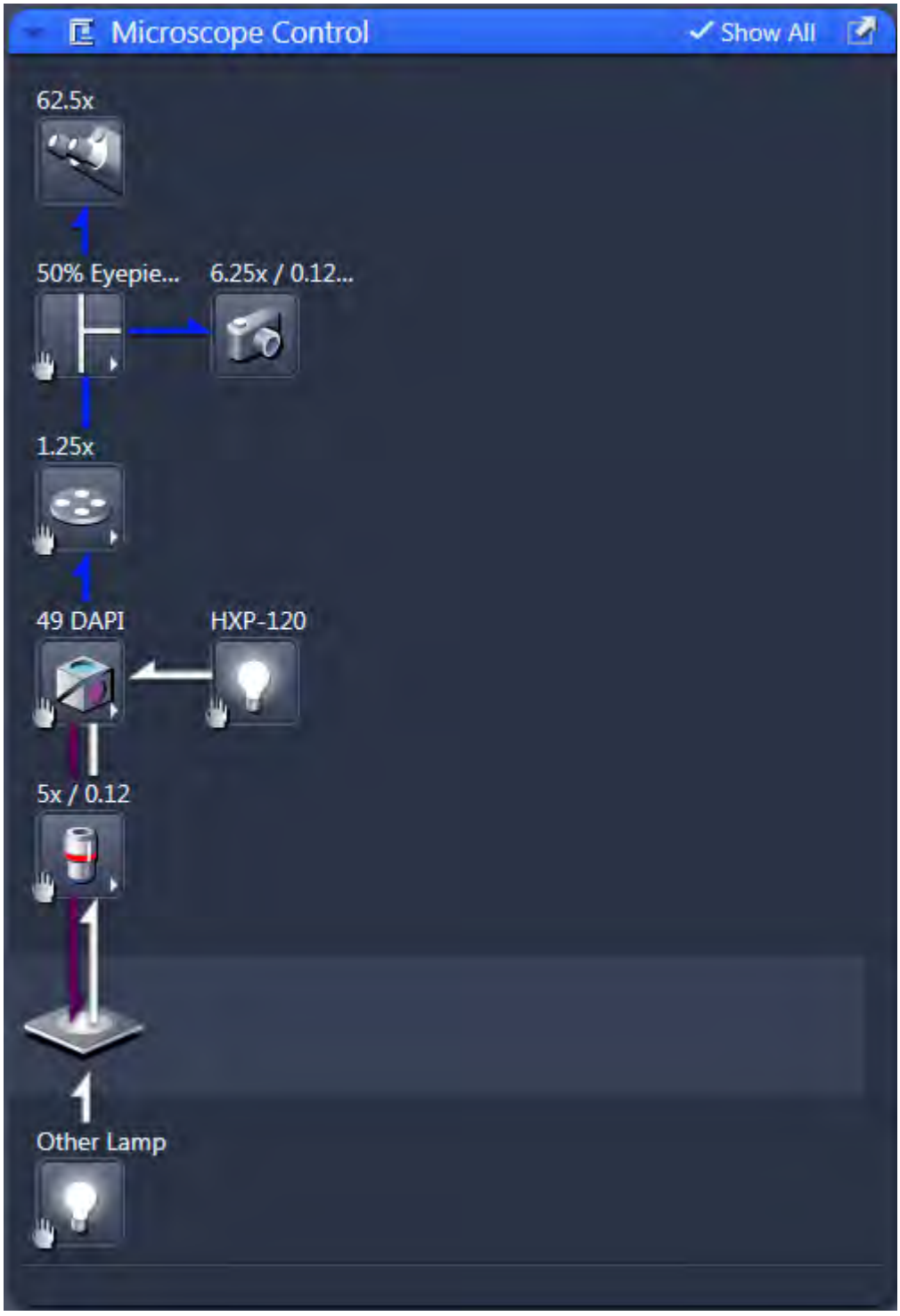
- All available microscope components in the **MTB** (MicroToolBox) have to defined correctly.
- The **Panorama** module is activated in the menu **Tools** | **Modules Manager**.



19.2 Prerequisites

Prerequisite ✓ You are on the **Locate** tab.

1. In the **Lightpath** tool set the components that you want to use for the acquisition.



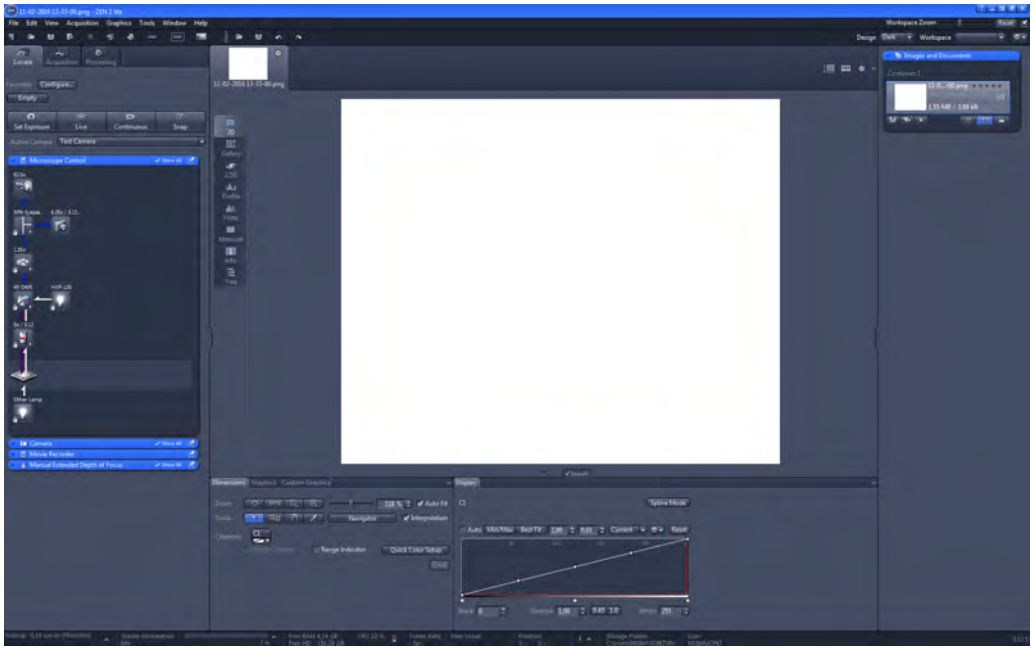
   → All these settings will be stored as metadata of the image.
   → Additionally the optical components will be used to automatically determine the pixel size for the scaling.
2. Click on the **Live** button.
   → Now you see the cameras live image of your sample in the **Center Screen Area**.
3. Click on the **Set Exposure** button.
   → The exposure time will be calculated automatically.
4. Alternatively you can set the camera parameters manually in the **Camera** tool.
5. Focus on your sample now.

You have completed the prerequisites for a panorama experiment.

19.3 Acquiring a Reference Image

Before starting the experiment itself, we recommend to acquire a reference image for the shading correction first. This image will be used later for processing the panorama image.

1. Move the sample to an empty field.
2. Click on the **Snap** button to acquire this position as reference image.



3. Save the image and let it opened in the background.

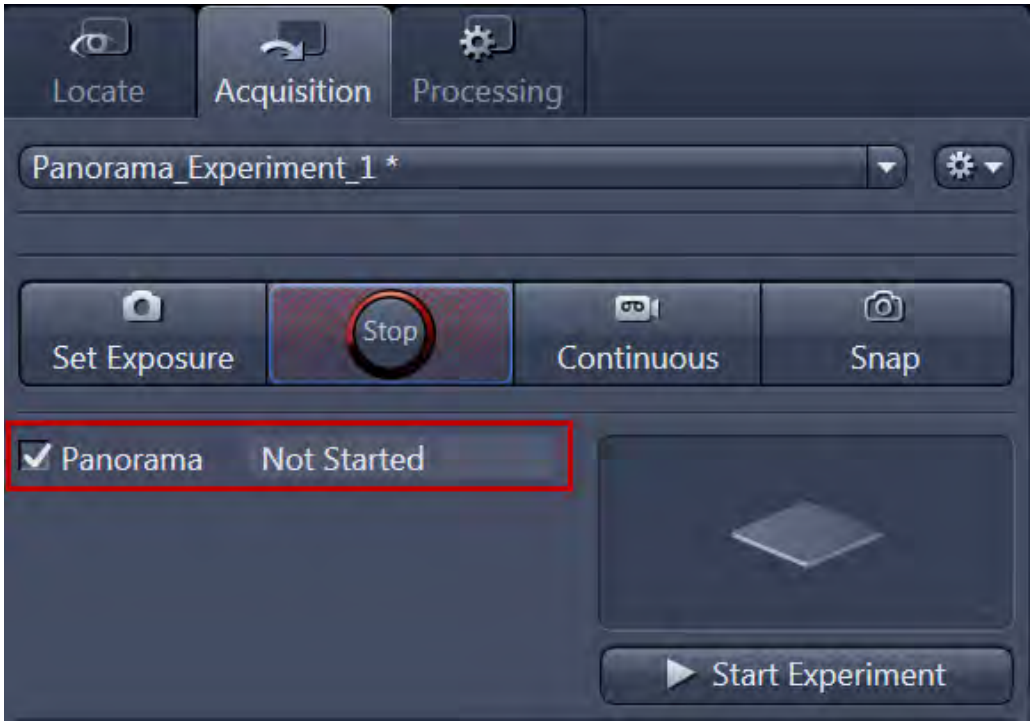
You have successfully acquired a reference image for the shading correction.

19.4 Settings for the Panorama Experiment

Prerequisite

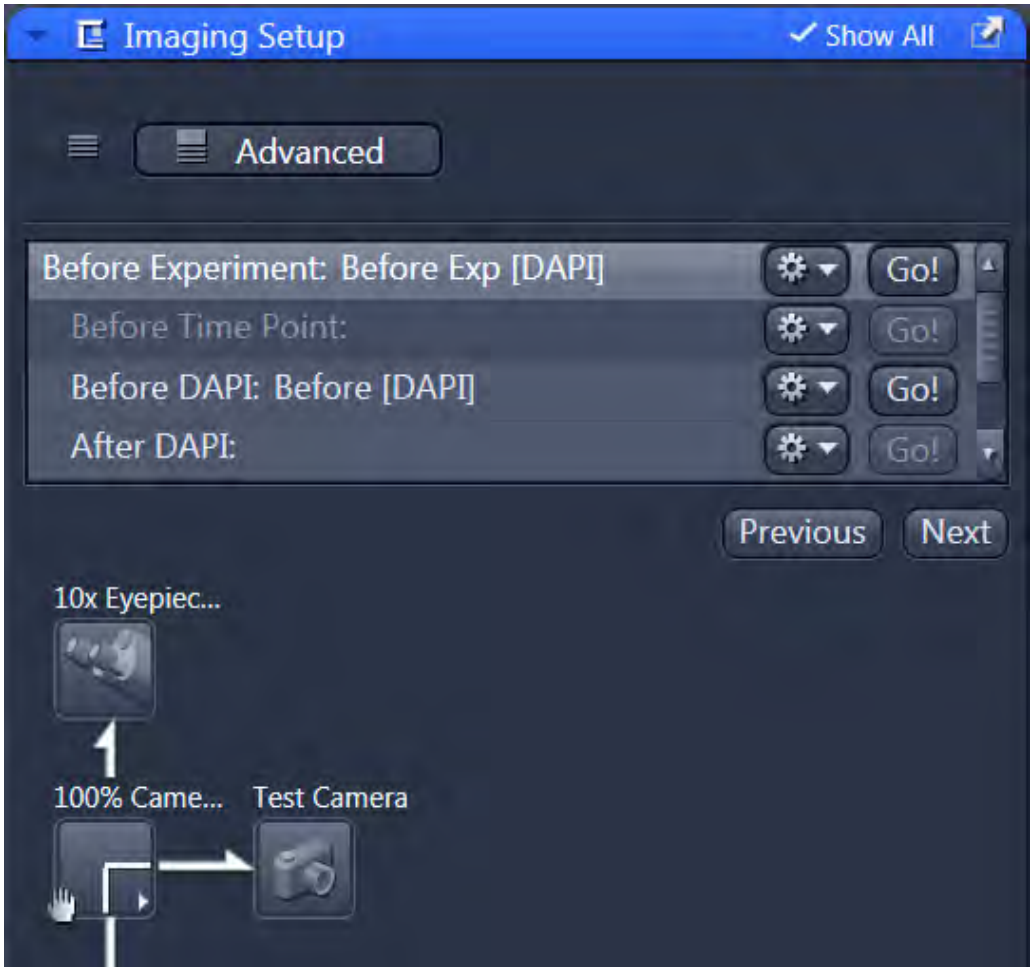
- ✓ You are on the **Acquisition** tab.
- ✓ You have started the live mode via the **Live** button.

1. Activate the **Panorama** checkbox.



2. Move the sample to the desired start field of the panorama image to be acquired.
3. Check the defined exposure time again if necessary.

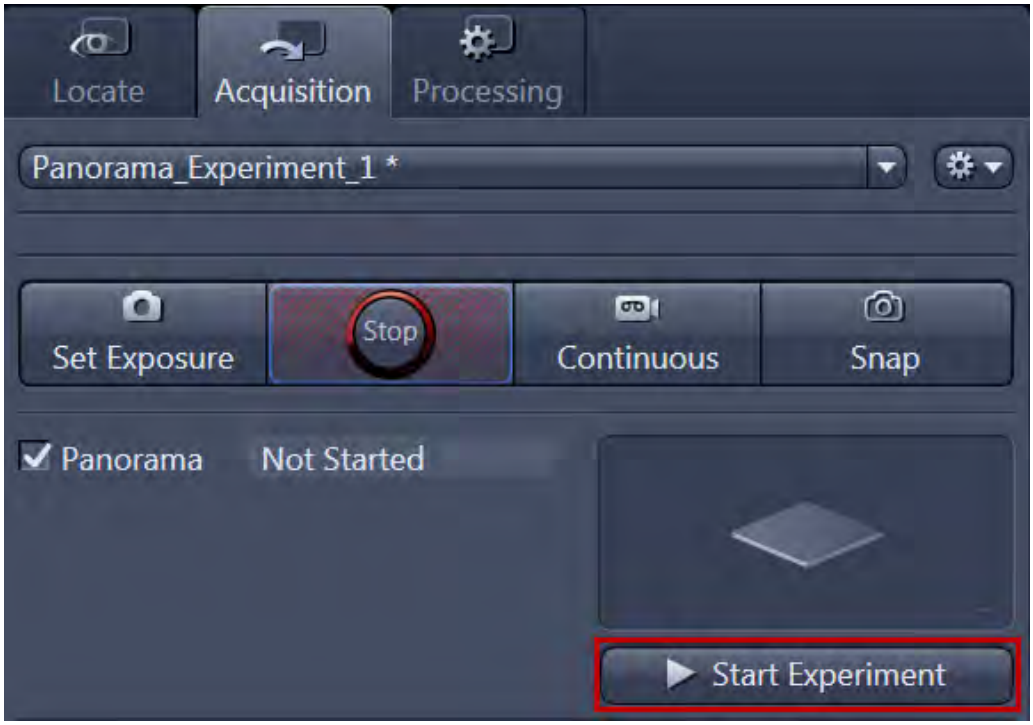
4. In the **Imaging Setup** tool, you can check the settings before/after the experiment.



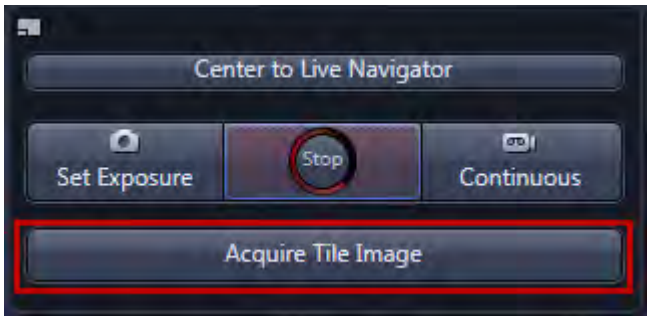
5. In the **Acquisition Mode** tool, click the **Get** button to transfer the active camera settings into the experiment.
   As an alternative you can define your experiment settings here as well.
6. In the **Panorama** tool you can adjust several options for automatic or manual stitching, if desired.
7. Finally save your experiment with a suitable name in the **Experiment Manager**.

19.5 Acquiring the Panorama Image

1. Click on the **Start Experiment** button to start the Panorama acquisition.

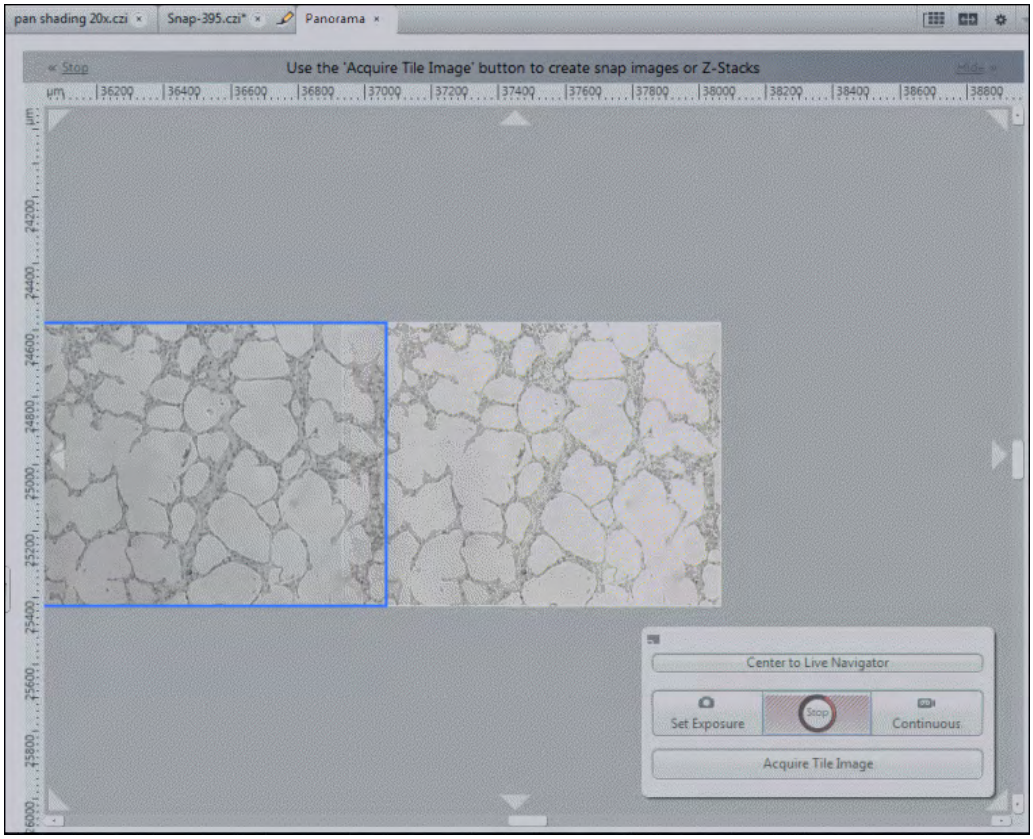


   → You will see the reduced display of the start image in the **Center Screen Area**.
   → The displayed image is a live image. You still can change position and focus.
2. Click on the **Acquire Tile Image** button in the **Center Screen Area** to acquire the first tile image.



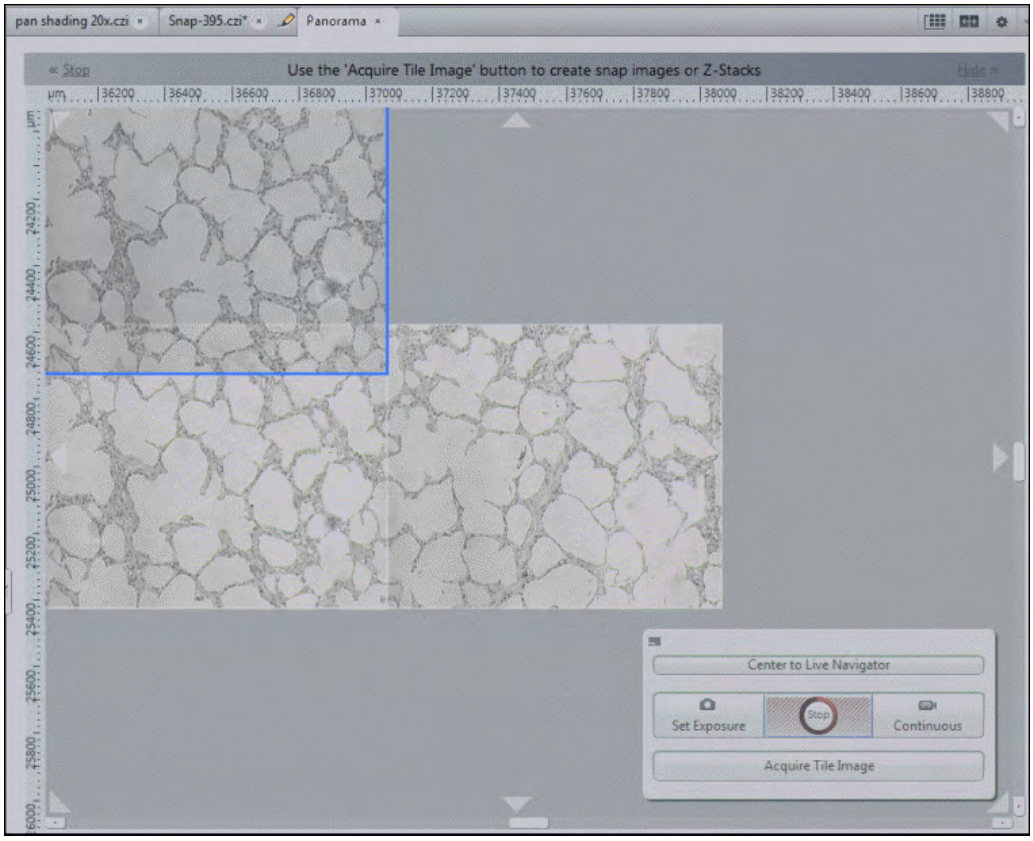
   → The image will be acquired and stored. The live image is still active as an overlay to the stored image.

3. Move the blue frame with the active live image in the desired direction aside the stored image by using the mouse.

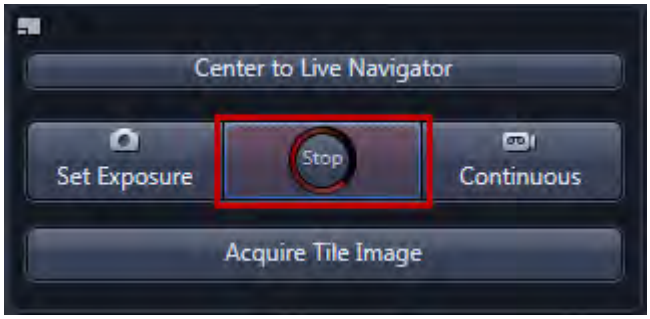


4. Now move the sample to the corresponding neighbor position using the microscope stage.
   → Try to position the structures in the overlap region as good as possible.
5. Click again on the **Acquire Tile Image** button to acquire this tile to the image.

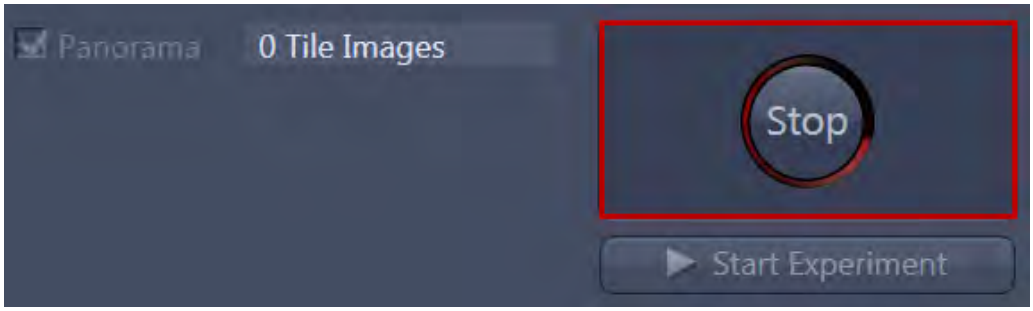
6. Move the blue frame to the next position.



7. Continue these steps until you acquired the desired panorama image of your sample.
8. After acquiring the last tile image click on the **Stop** button to close the live mode.



9. Finally end the experiment via the **Stop** button on the **Acquisition tab**.



→ As a result you now see the recorded panorama image in a new image container.

10. Save the image as a raw image (*.**czi**).

You have successfully acquired and stored a panorama image.

Info

- You can adapt the size of image and surrounding area with the zoom keys *F7* and *F8* to your needs.
- Keep a sufficient overlap area of the live with the stored image.
- In the case of errors during the following processing steps you therefore always have access to the original image data.

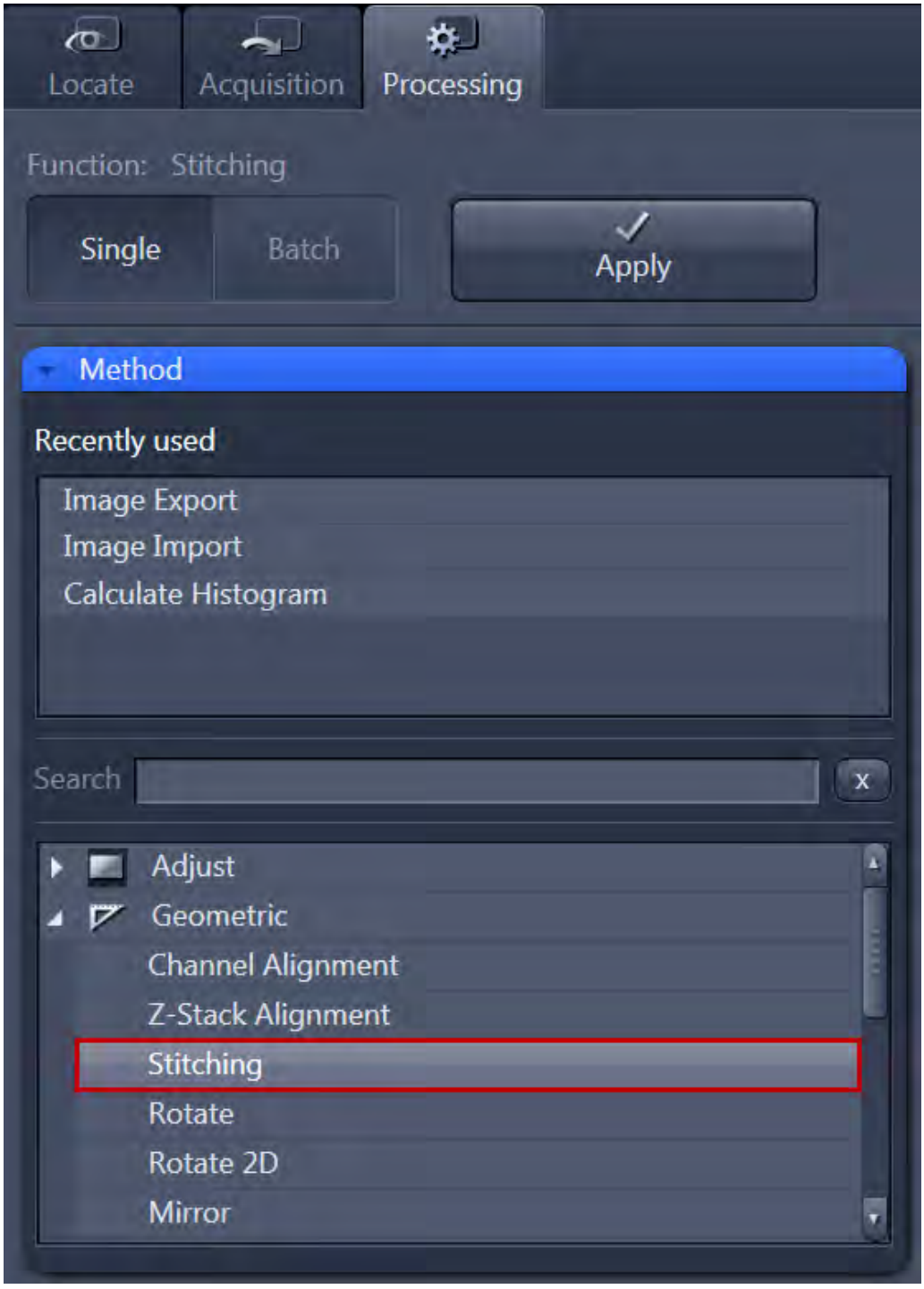
19.6 Processing the Panorama Image

The next chapters will show you how to process panorama images with the **Stitching** processing function. Using this method you can correct an offset between the tile images. We will show you the different settings and make a comparison of the output images. So you can see which settings will give you the best result.

Prerequisites

Prerequisite ✓ You are on the **Processing** tab.

1. Open the **Method** tool and select in the group **Geometric** the **Stitching** function.

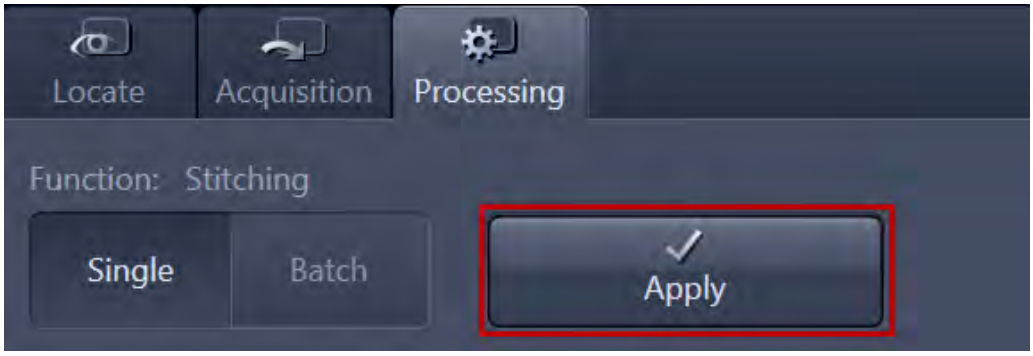


The following instructions will all base on this selection and show the different settings and results of this function.

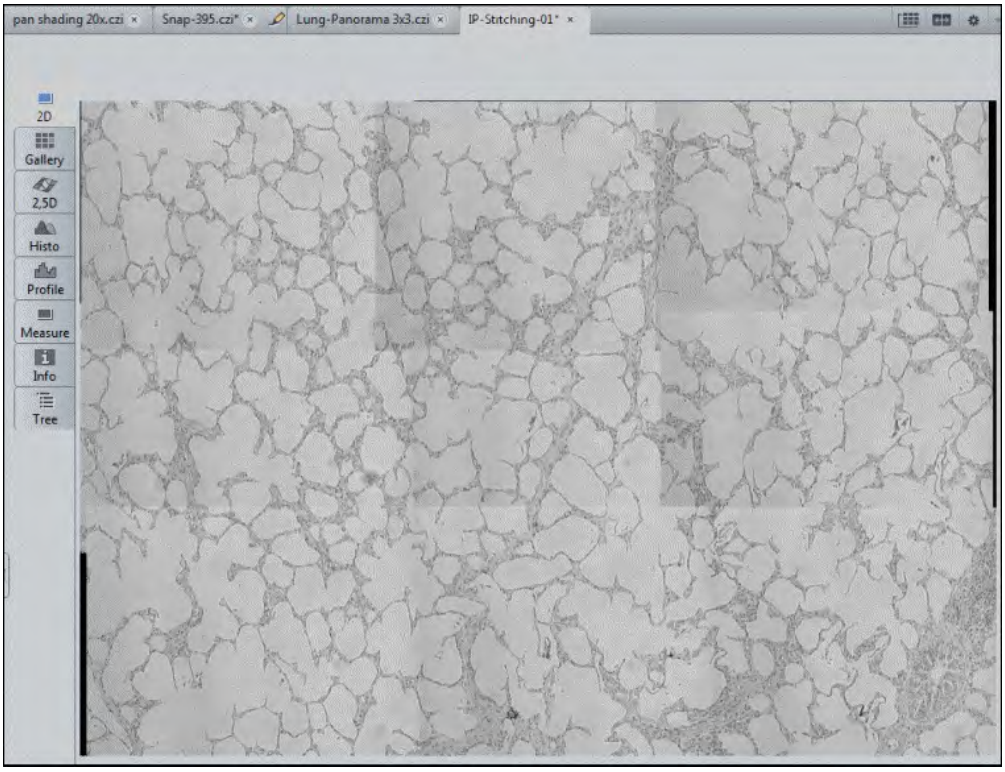
19.6.1 Stitching (Defaults)

1. In the **Method tool** open the **Geometric** group.
2. Select the **Stitching** function.

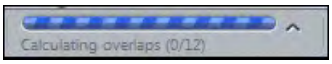
3. Click on the **Apply** button in the **Processing** tab to start the processing.



→ The stitching process will take a while depending on the image size.



→ In the **Status bar** you can see the work in progress.



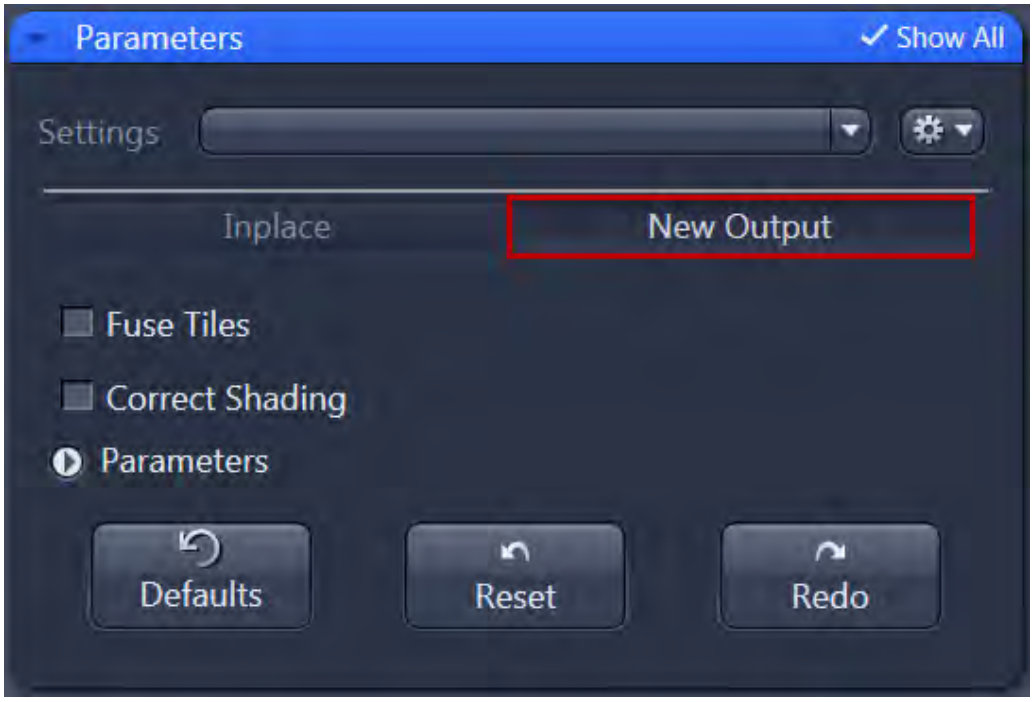
You have successfully used the **Stitching function**. As you still can see shadows and edges in the output image we will show you how to use the function receiving better results in the following chapters.

19.6.2 Stitching with Shading Correction

If your tile images contain a certain background shading you can correct this if you have acquired a reference image for the shading correction. This image has to be opened in the **Center Screen Area**.

1. Select in the tile image for the stitching in the **Input** tool as first input.

2. In the **Parameters** tool select the **New Output** button.



   → This will keep the original image and create a new output image.
3. Activate the **Correct Shading** checkbox.
4. Select the **Reference** entry from the dropdown list.
   → This will let you select your reference image which is opened in Center Screen Area.´
5. Now as a second input image select the reference image for the shading correction in the **Input** tool.
6. Click on the **Apply** button to start the processing.

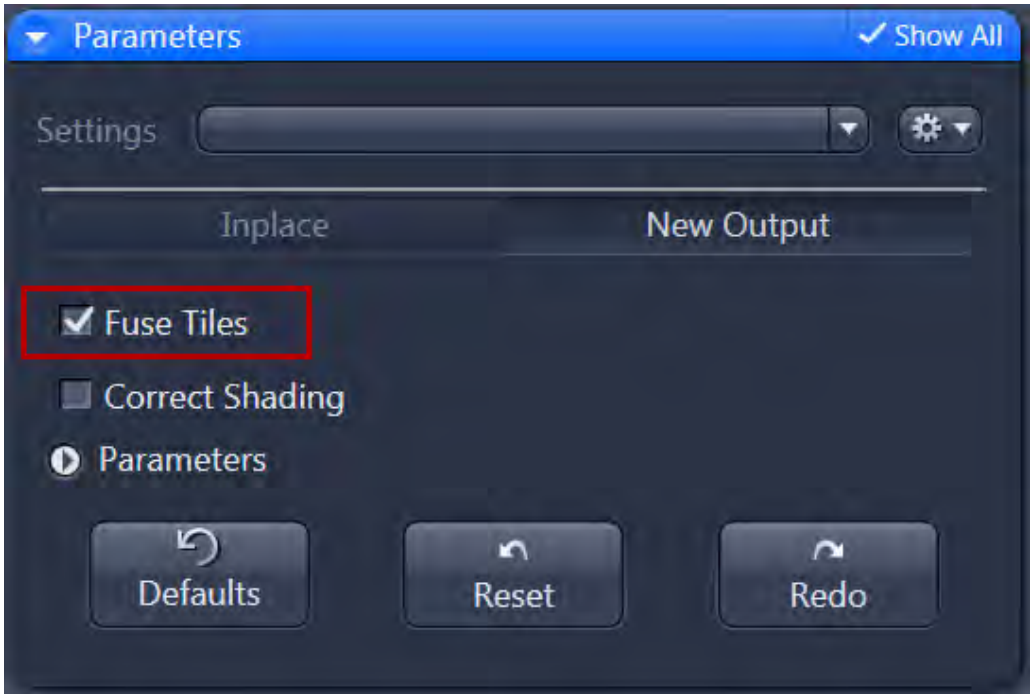
As a result you will get a stitched panorama image without any shading influences. The next chapter will show you how to get rid of the edges which are still visible between the tiles.

19.6.3 Stitching with Fuse Tiles

With very low shading content in the image you have an alternative method to homogenize the image transitions between the single tiles.

1. Under **Image Parameters** in the **Input** tool select the tile image for the stitching.
2. In the **Parameters** tool, click on the **New Output** button.

3. Activate the **Fuse Tiles** checkbox.



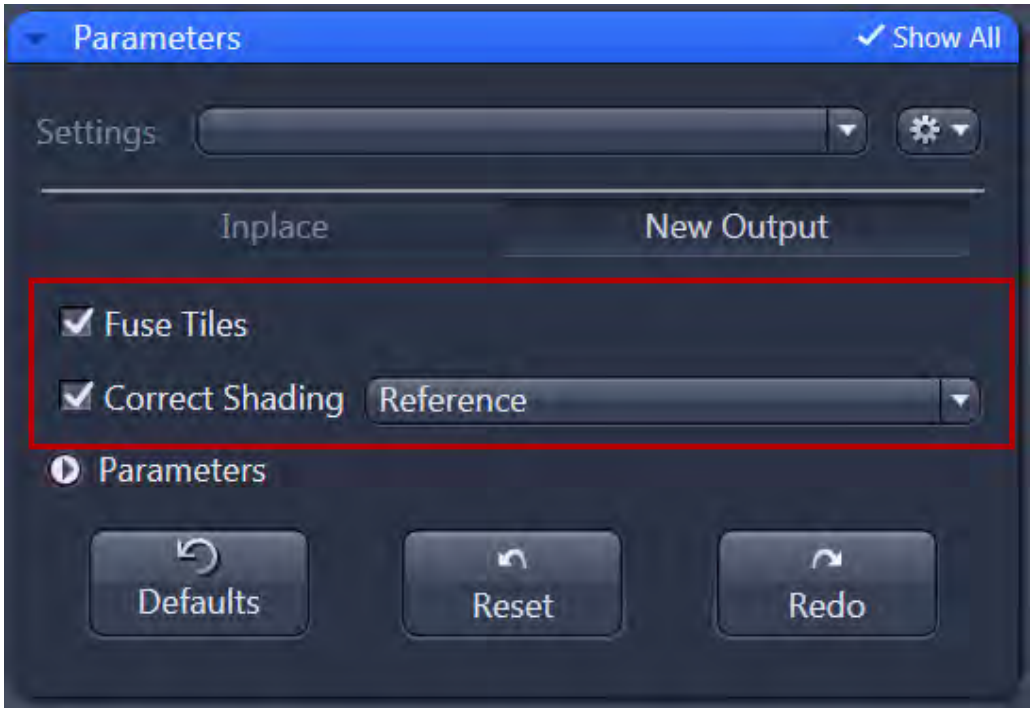
4. Click on the **Apply** button to start the processing.

This function will smoothly average all edge regions of the tiles via their grey values in order to avoid sharp edges. With high background shading this function alone will not be sufficient.

19.6.4 Stitching with Fuse Tiles and Shading Correction

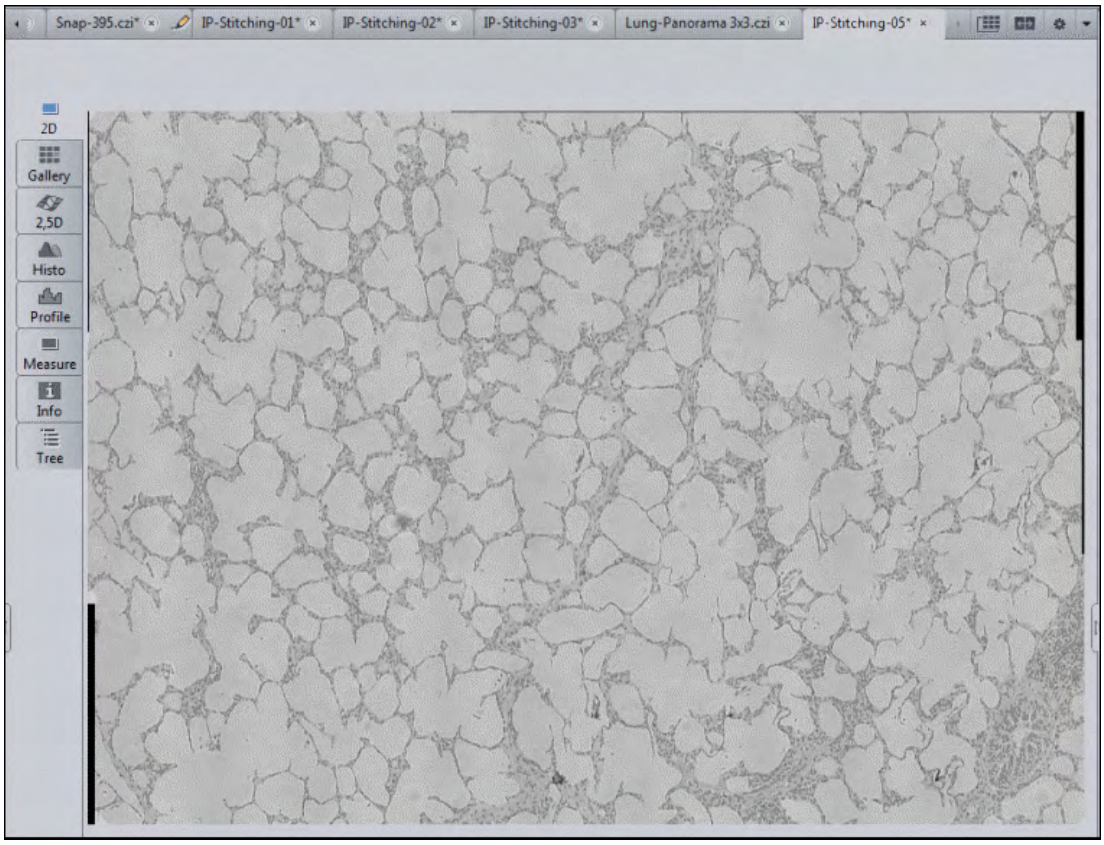
For extreme cases you have the possibility to combine both transition corrections.

1. In the **Parameters** tool click on the **New Output** button.
2. Activate the checkboxes **Correct Shading** and **Fuse Tiles.**



3. Under **Image Parameters** in the **Input** tool select the tile image for the stitching and the reference image for the shading correction.
4. Click on the **Apply** button.

Both settings will be applied to the image. With this method you will receive a perfectly stitched image with no visible transition areas between the tiles any more.

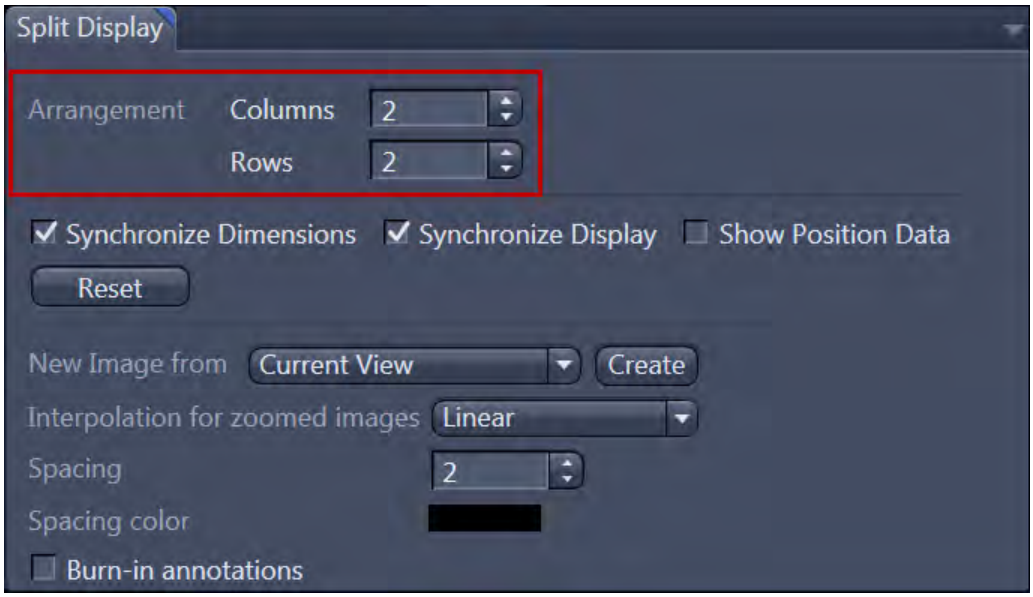


19.6.5 Image Comparison via Split Display

You can create a Multi Image to compare the different results of your processed images via the **Splitter-Mode.**

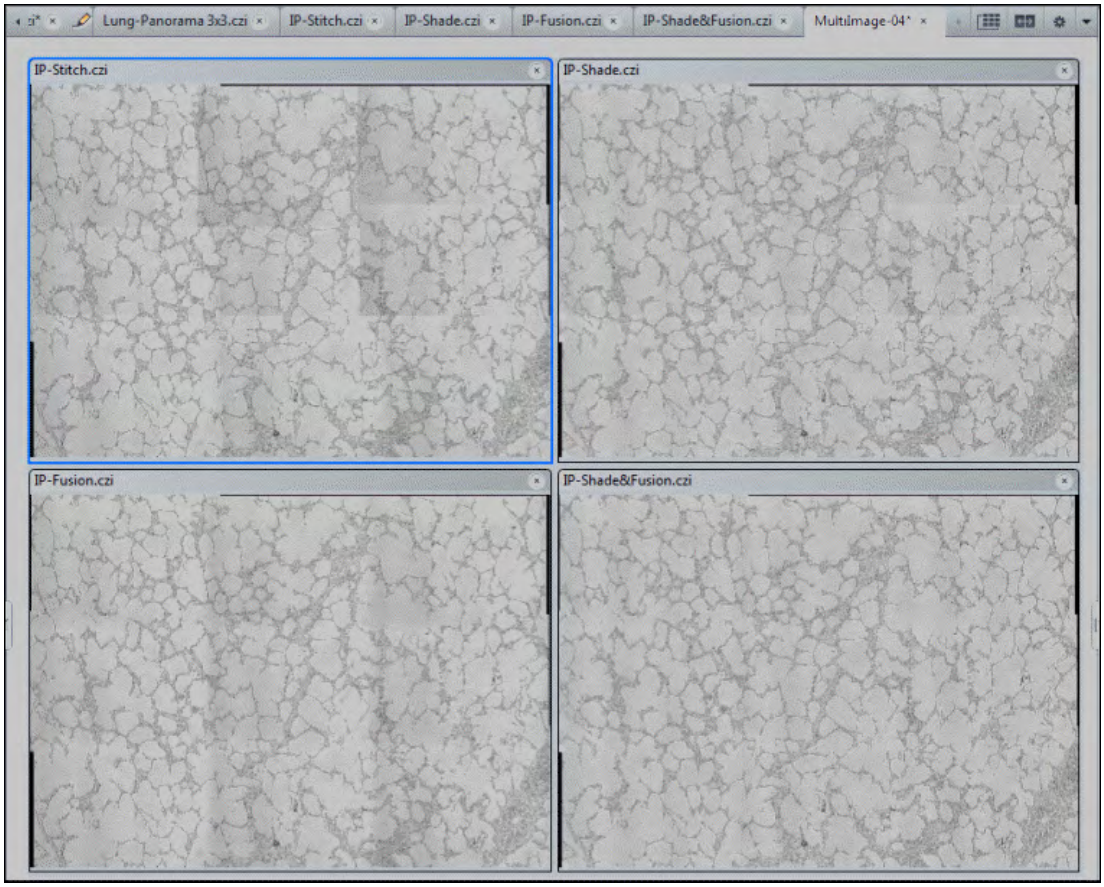
1. To compare different images, you can select the **Split Display** via the **Create New Multi Image**  button.

2. On the **Split Display** tab you can define how many images shall be displayed in X- and Y-direction aside each other and how they shall be synchronized, e.g. 2 **Columns** and 2 **Rows**.



3. Move each of the different panorama images via drag&drop from the **Right Tool Area** | **Images and Documents gallery** to an empty frame in **Center Screen Area**.

In our example we show the transition areas of the tile image as **raw image** (top left), as **fused tile image** (top right), as **shading corrected tile image** (bottom left) and finally the combination of **shading correction and fused tiles** (bottom right). In this last image no transitions between the tiles are visible any more.



19.7 Functions & Reference

19.7.1 Panorama Tool

Here you can adjust settings for the panorama acquisition.

After you have acquired the single tile images simply activate the **Perform stitching after the experiment** checkbox. If you want to stitch the images manually, click on the **Perform Stitching** button, after you have finished the experiment.

The panorama acquisition itself is performed interactively via in the **Panorama** view. If you click on '**Start Experiment**' the Panorama view will be opend and you can start to acquire the single tile images.

See also

Panorama View [▶ 521]

19.7.2 Panorama View

In this view you see the representation of the microscope stage. The **Live** image from the camera (blue frame) is automatically shown in the middle of the image area. Furthermore a tool window is displayed, that allows you to control the image acquisition, e.g. perform auto exposure or acquire an individual tile image.

See also

General View Options [▶ 866]

19.7.2.1 Stage View

In the image area the full travel range of the microscope stage is displayed. You can control the stage view using the arrow icons at the edges of the image area. The view can be enlarged, reduced or moved using the general control elements.

Navigator frame

The current stage position is shown as a tile outlined in blue, the Navigator frame. In the Navigator frame you can see the camera's live image.

To move the frame, double-click on the position on the microscope stage to which you want to move it.

To acquire images, use the **Acquisition** buttons in the **Tools window.**

See also

Tools window [▶ 521]

19.7.2.2 Tools window

The tool window for panorama view is normally visible in the lower right corner of the center screen area. It becomes active, if you move the cursor over it. You can use it to set acquisition parameters and acquire tile images for your panorama image.

Parameter	Description
Center to Live Navigator	Centers the stage view at the current position of the Navigator frame.
Action Buttons	With the three action buttons (**Live**, **Set Exposure**, **Continuous**) you're able to control acquisition parameters like you are used to do it on the **Acquisition** tab.
Acquire Tile Image	Acquires a tile image. This comprises all activated channels as well as Z-stacks. After the acquisition the tile image is placed in the corresponding location in the stage view.