Axio Scan.Z1

User instructions for the Axio Scan.Z1 in HSC Room 60

- Basics
- Advanced Profile Wizard

Basics

The normal operation of the Axio Scan.Z1 once you have a profile set up.

> These documents are to reinforce, not replace, hands on training on the microscope. All users MUST recieve training from Cell Imaging Staff prior to use of the instrument.

Start up

1. Turn on the power strip (Orange Dot #1) - wait 30 seconds.
2. Turn on the Axio Scan.Z1 (Orange Dot #2) - wait until the top left light is green.
3. If doing fluorescent imaging, turn on the X-Cite (Orange Dot #3).
4. Start the Zen software:
    1. As is starts, click on the Zen Slidescan (not Image Analysis).
    2. Watch for error messages.

Preparing your slides

Make sure that your slides are clean. Surface dirt will get in the way of focusing and imaging.

If you are going to use the Marker method for tissue detection, draw Sharpie lines on the cover slip that surround the tissues. Make sure the line closes and go over any faint lines. (The Marker method is most often used for fluorescent imaging when there is not strong visual contrast between the tissue and the slide.)

Loading slides

Slides are held in trays. The trays are loaded into the scanner. A single tray can hold 4 normal slides. The scanner can hold 25 trays.

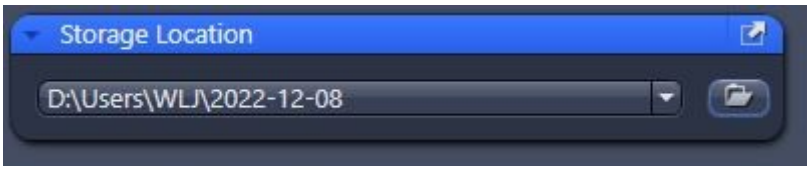
The trays have spring tensioned holders for each slide. While you can load a tray by hand, it is easier with the loading aide (the big black block).

1. Put the tray in the block and swing the lever back. This will open all of the slots.
2. Place your slides in place coverslip and label up.
3. Swing the lever forward. This should clamp your slides into the tray. Check that they are secure.

Loading trays into the scanner

1. Open the door, using the software or the button.
2. Swing open a tray holder.
3. Fill a holder with a tray. Cover slips / labels up and toward you.
4. Swing the tray holder back into the scanner.
5. Close the door, using the software or the button.

Storage location



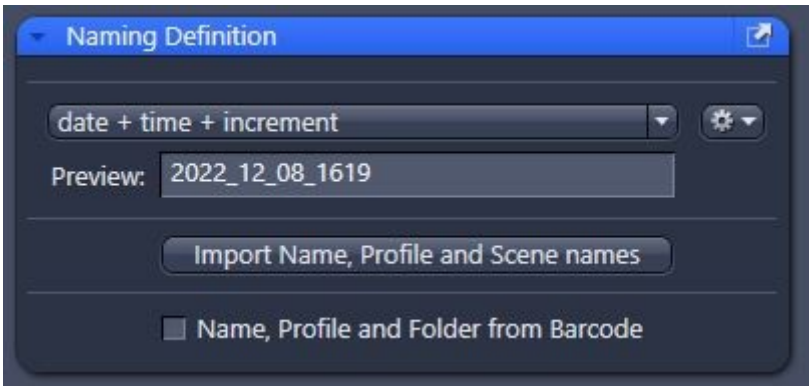
Open the Storage Location tool and select the file that your data will be stored in. The file folder will get you to a browser where you can select the folder and/or create the folder. Include your name or your lab name in the file path.

Double check that the correct path is in the storage location tool.

Data must be stored on the data drive. Not the C: drive, not the Desktop, not the Documents folder.

1. At the end of scanning, please copy your data to your portable drive.
2. If you could delete your old data at some point, that would be appreciated. However, we understand that you don't want to delete it until you have it backed up in your own system.
3. We attempt to provide space for about 6 months of data, and we will delete old data as space becomes limited. While we generally send out warnings, if we get desperate, we will delete older data first.
4. We do not guarantee against a hard drive failure. We do not back up the data drives on any of the microscopes.

Naming definition



Typically, the naming definition is "date + increment." This means that files will be named like:

2022_11_21_0123.czi

If you do want to change the definition, please change it back when you are done.

You can also define the name of each image manually after the Prescan in the Magazine tab view.

Scan profile

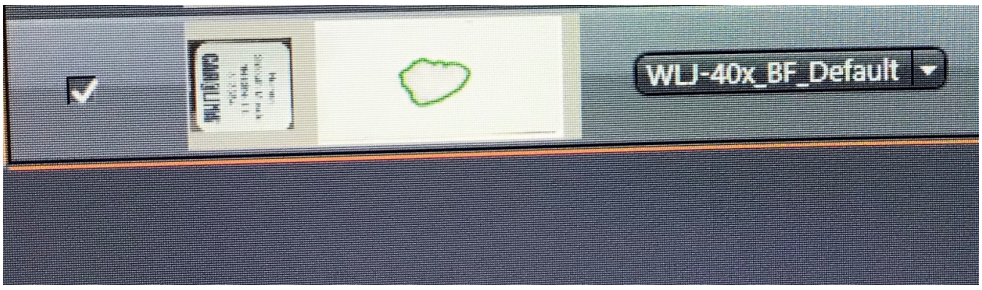
A scan profile is a set of parameters that determine how the scan will be done (e.g., what objectives, what channels,...). Typically, you will re-use your scan profile as is so that all of your imaging is consistent. You may want separate profiles for different sets of samples or it different magnifications are needed.

Select a default scan profile



If all of your slides will use the same scan profile, selecting the Default Scan Profile will apply that profile to all of your slides. The down arrow will show a list of all the scan profiles you can select.

Setting the profile for a single slide

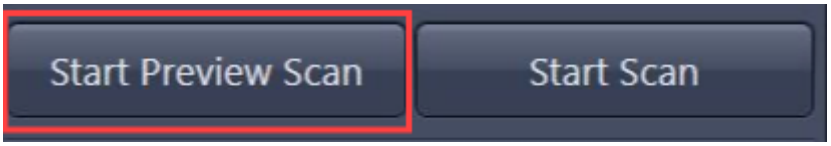


If some need a different profile, they can be set on a slide by slide basis in the Magazine view. The down arrow by the slides profile will show a list of all of the scan profiles you can select.

Modifying or creating a scan profile

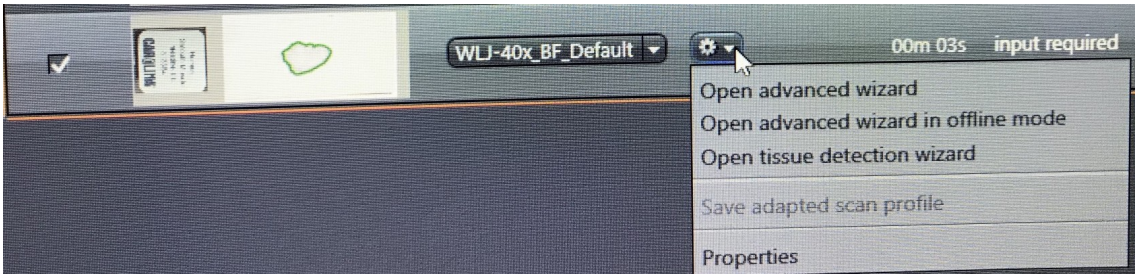
Profiles are created or modified with the Advanced Profile Wizard. That is outlined in a separate page "Profile Wizard."

Start preview scan



Pressing "Start Preview Scan" will take a picture of your slide that will be used to verify and/or modify the tissue selection. This scan will be done for all the slides that have been selected (have a check box to their left).

Tissue detection wizard



Once the preview is done (on at least one slide), Use the gear next to the preview to open the Tissue Detection Wizard. This is where you will:

1. Remove any spurious detections.
2. Improve or redo detections.
3. Optionally, connect tissues.

4. Click Next to move on to the next preview.
5. Click Finish when done with all the previews.

The tissue detection image

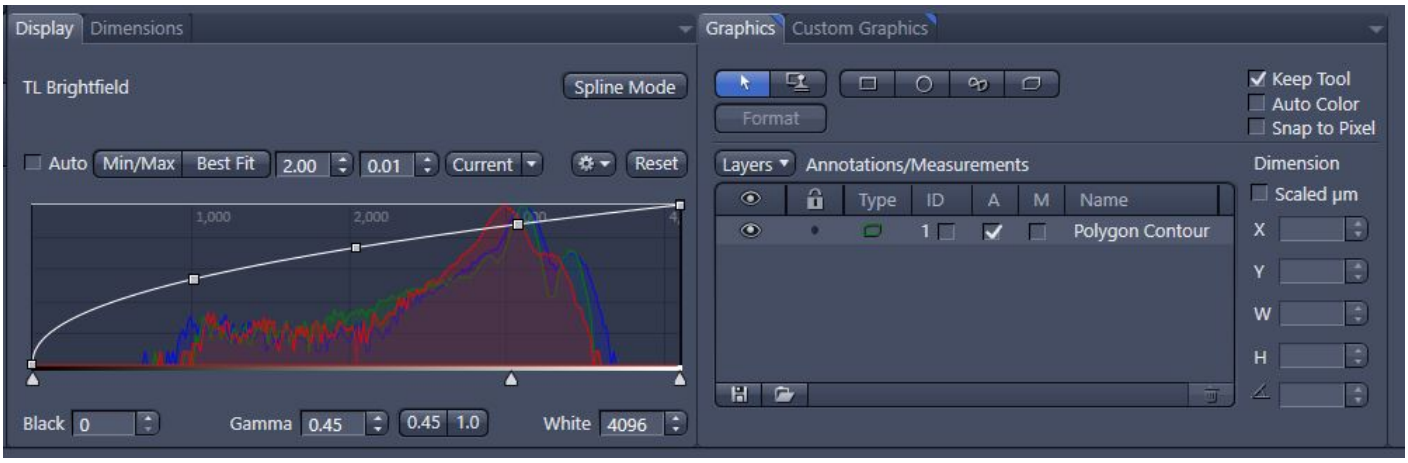


In the center of the screen will be the results of any automatic tissue detection. The green shapes are the detected tissues. If there are tan areas, they are areas that are used for collecting shading correction data. A red rectangle is the area of the slide that was used for tissue detection.

The mouse scroll wheel can be used to zoom in and out.

Editing the tissue detection

- The Display tab has the LUT and this can be manipulated to make it easier to see your tissues.
- The Graphics tab has the tools for working with the tissue detections.
  - The Arrow tool is the selection tool. Use this to select annotations on the image.
  - The four draw modes Rectangle, Circle, Spline and Polygon are used for drawing new annotations.
  - To remove an annotation, select it on the image or in the list and press "Delete" (not backspace) or use the Trash Can at the bottom of the list.
  - If a stray click selects the red rectangle and you can't edit annotations anymore, select one of the draw modes.
  - The Keep Tool check is useful if you are going to make a lot of the same operation (e.g., draw a bunch of rectangles). Just remember to go back to the Arrow when you want to get back to selections.



Connecting tissues (optional)

Each separate tissue will become an individual "scene" in your image file. This will result in the smallest files, especially if you eventually export your data as OME-Tiff. However, depending on how you analyze your images, it can be useful to connect your tissues so that there is a single "scene."

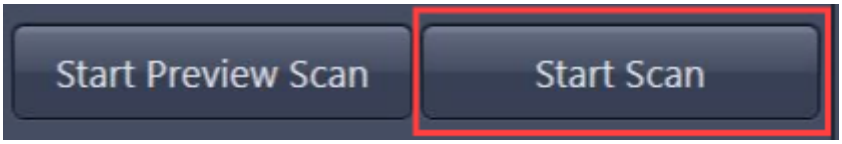
1. Use the polygon tool.
2. Left click to start and add points to your polygon. Right click to finish.
3. Draw thin (zero width) polygons that link tissues.
4. In the tissue list, select all the tissues.
5. In the view window, right click in a tissue and select "Merge."
6. Verify that all the tissues merged into 1 (check the list of detections). Sometimes, Zen gets confused and leaves some separated.

Previous, Next, Finish, Cancel

When you are done editing the tissue detection, press one of the 4 buttons:

- Previous: Go back to the previous slide
- Next: Move on to the next slide
- Finish: Once you are done with all the slides, click Finish.
- Cancel: If you feel like you need to start over, cancel without saving changes.

Scan



Before you press Scan

1. Make sure that all the appropriate slides are selected/de-selected (check box to the left of the preview). These are the slides that will be scanned.
2. Make sure that tissue detection is done for all the selected slides.

Press Start Scan

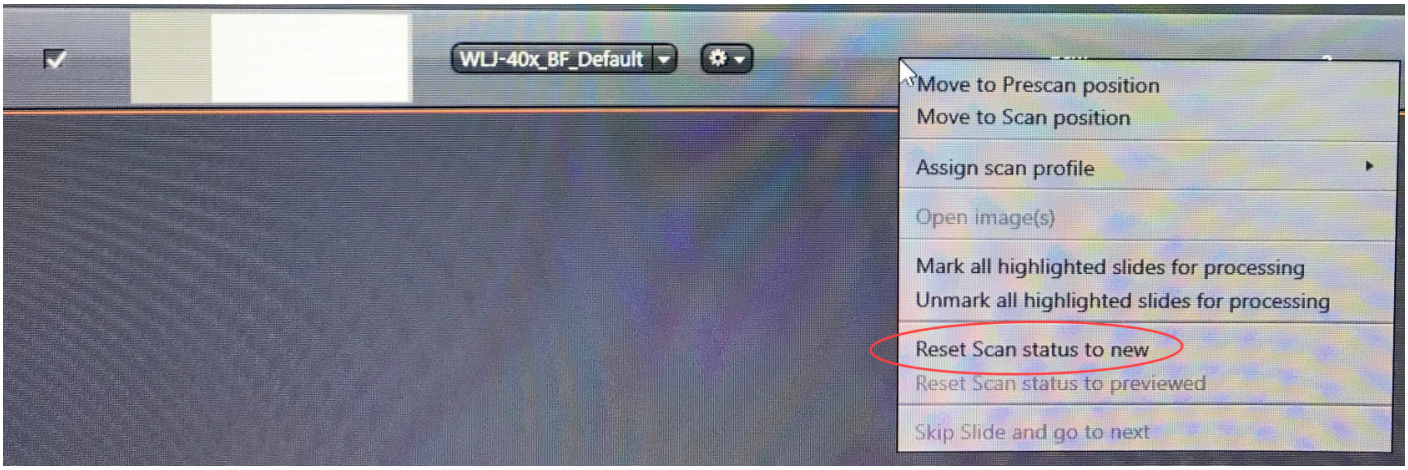
Each slide presents an estimated time for the scan. These are usually much more time than it will really take. Hour long estimates might be 15 minute scans.

Wait for the first image

When the first image is done, double click on it in the Magazine tab. This will bring the image up in its own tab in the viewer. Make sure that this one looks good before leaving. This is the time to stop the scan process and fix any problems.

To Rescan

Once a slide has been scanned, it will say "finished." In order to rescan it, first Right-Click in the gray part of the slide bar and select "reset scan status to new."

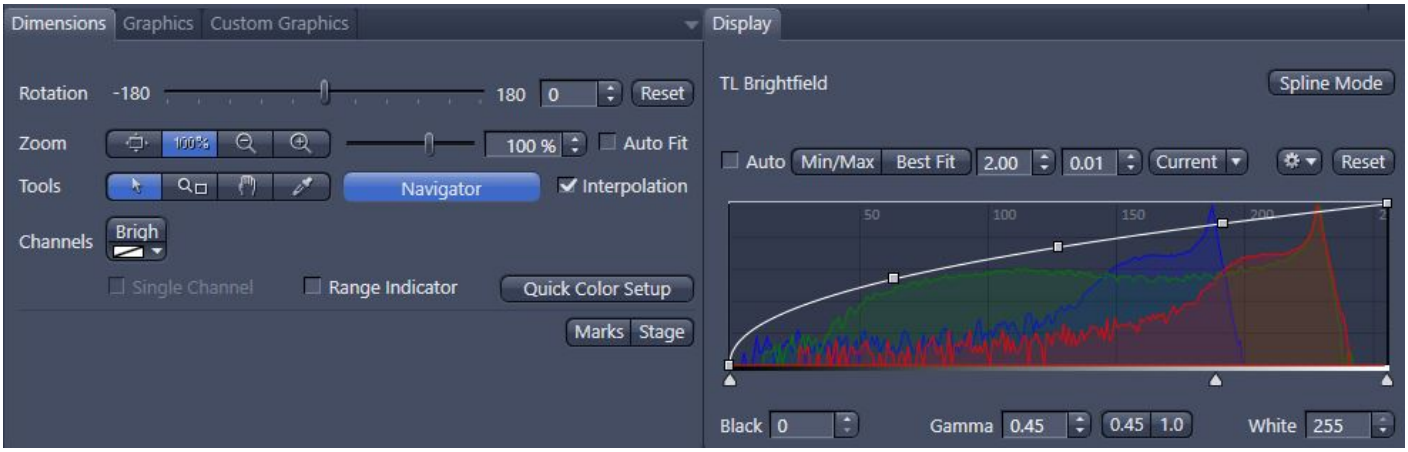


Inspect your images

Once your scans are all done, check them over. Especially look for focus problems. These may require adapting your scan profile to include more focus points.

Along the left side of the image view area are Image View Tabs. 2D is the normal view. Info will show the scan parameters.

At the bottom are the Dimensions and Display panes.



Dimensions pane:

Zoom

The zoom of the display can be set in the Zoom row or with the mouse scroll wheel. The current zoom will be displayed in the Zoom row.

Channels

If your image has multiple channels, you can select which channels are displayed by clicking them on and off.

Scenes

If your image has more than one scene (tissue detection), there will be a slider above the Rotation slider. This is where you will select what scene to display.

Navigator

Pressing the Navigator button will put up the Navigator window to the right of the display. You can use this to see the area of your scene that is being displayed (the blue rectangle). You can also grab the rectangle and move it to an new area.

Display pane:

In the display pane, you can manipulate the LUT for the current image. This does not affect your data, just the display.

Shut down

Check:

1. Slides are out.
2. Trays are back in place.
3. Door is closed.

Shut Down:

1. Exit the Zen software.
2. Turn off the X-Cite if it is on.
3. Turn off the AxioScan. Wait for the top left light to go out.
4. Turn off the power strip.
5. Copy your data to your portable drive.

Advanced Profile Wizard

A scan profile describes how the scan will be done (objectives, channels, exposures, etc). The Advanced Profile Wizard is one way to set up or modify a scan profile.

Brightfield vs Fluorescent Profiles

Brightfield profiles are simpler than Fluorescent profiles. The Advanced Profile Wizard goes through the "same" 6 steps, with differences. This document is going to assume Brightfield, with some notes on differences for fluorescent.

Select a Default Scan Profile.

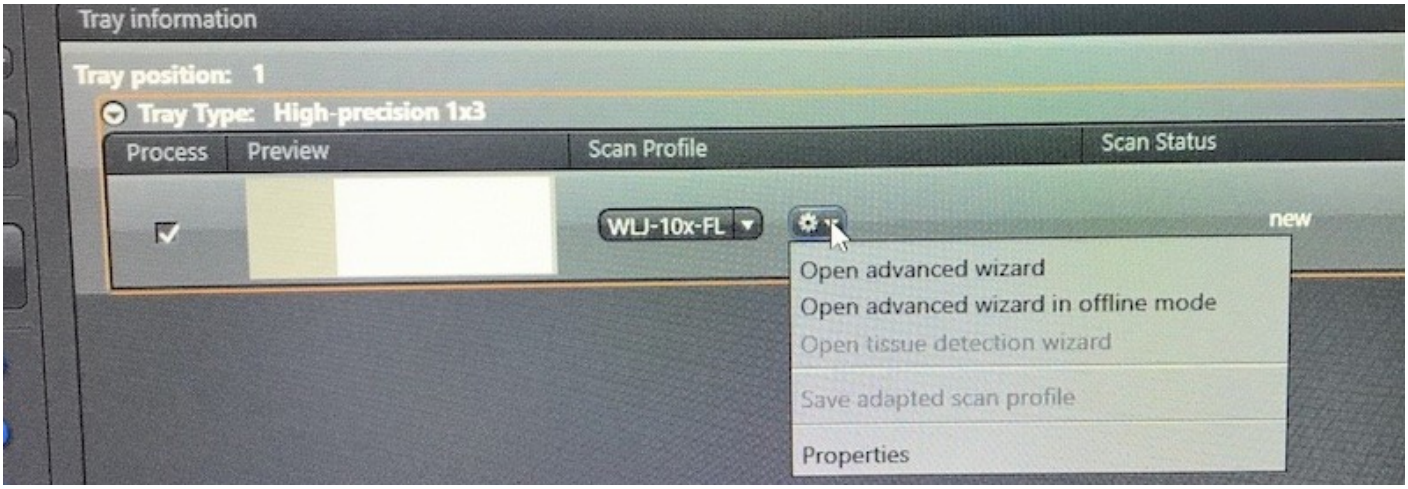


There is always a default scan profile. If you are going to adapt a profile, select the default profile from the drop down list. If you want to start from scratch, use the gear tool to start a new profile.

Load a slide into the scan position.

The Advanced Scan Profile Wizard really only works if there is a slide in the Pre-Scan position. The easiest way to do this is:

- Select one of the slides in the Magazine. Left click on the Gear Icon for that slide to select Advanced Scan Profile Wizard.



MTE1Njc0NS4yfDg4OTgwNC84ODk4MDQvTm90ZWJvb2svMzQ4NjY3Njg5MHwyOTM2MzUzLjE5OTk5C

From here, there are 6 steps in the wizard. The steps change depending on the type of scan you are setting up. For details, see the Cell Imaging staff, the instructional videos, or the online manual.

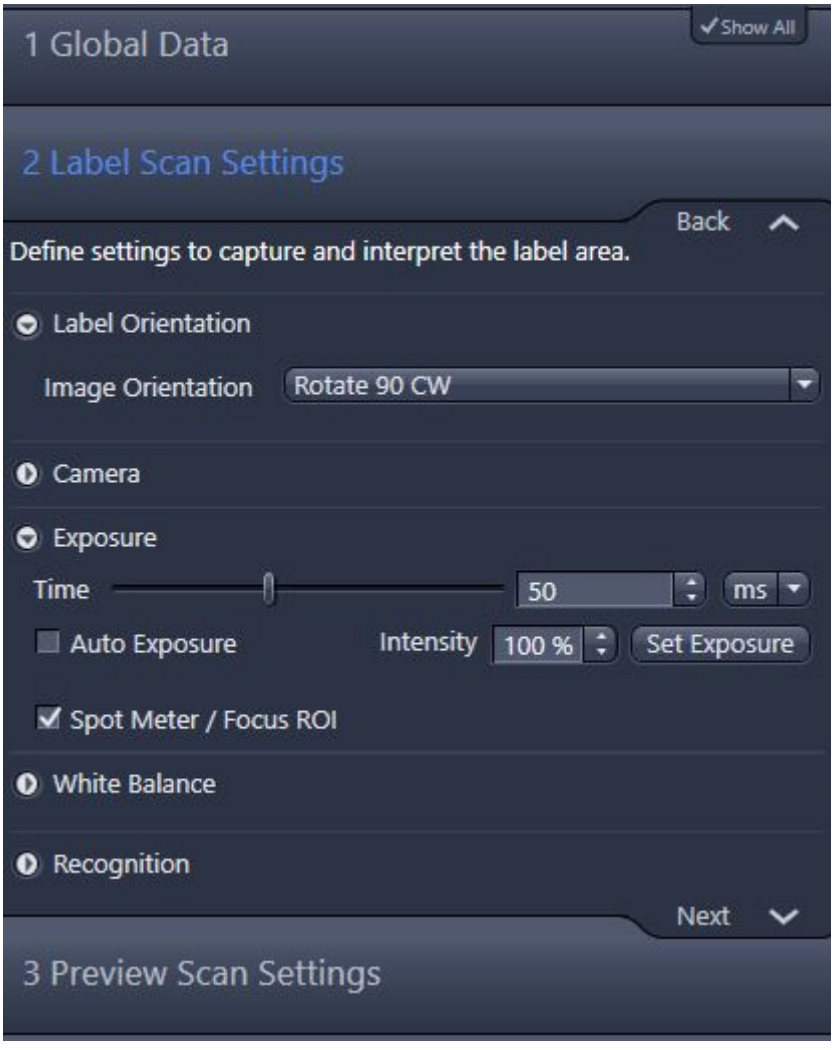
1: Global Data



1. Profile name: should be descriptive and should start with your name or initials (and/or lab name).
2. Description: optional
3. Debug Mode: This will add an overlay layer to your image with the focus points. This can be helpful in diagnosing why scans come out blurry.
4. Profile Type: Brightfield or Fluorescence
    1. This will affect the focus map and scan settings options.
5. Tissue Detection Mode:
    1. Automatic: Let Zen do the detection. If you run preview 1st, you can still edit the tissue selections.
    2. Manual: Hand draw regions. This can also be used to scan exactly the same area of each slide.
    3. Interactive: Pause a batch scan after the preview and wait for tissue selection editing.
6. Tissue Detection Recognition Type:
    1. Marker: you have drawn a region(s) on the slide with a Sharpie. This can be especially helpful for fluorescent samples.
    2. Tissue: This will use the contrast in the image to select the tissue.

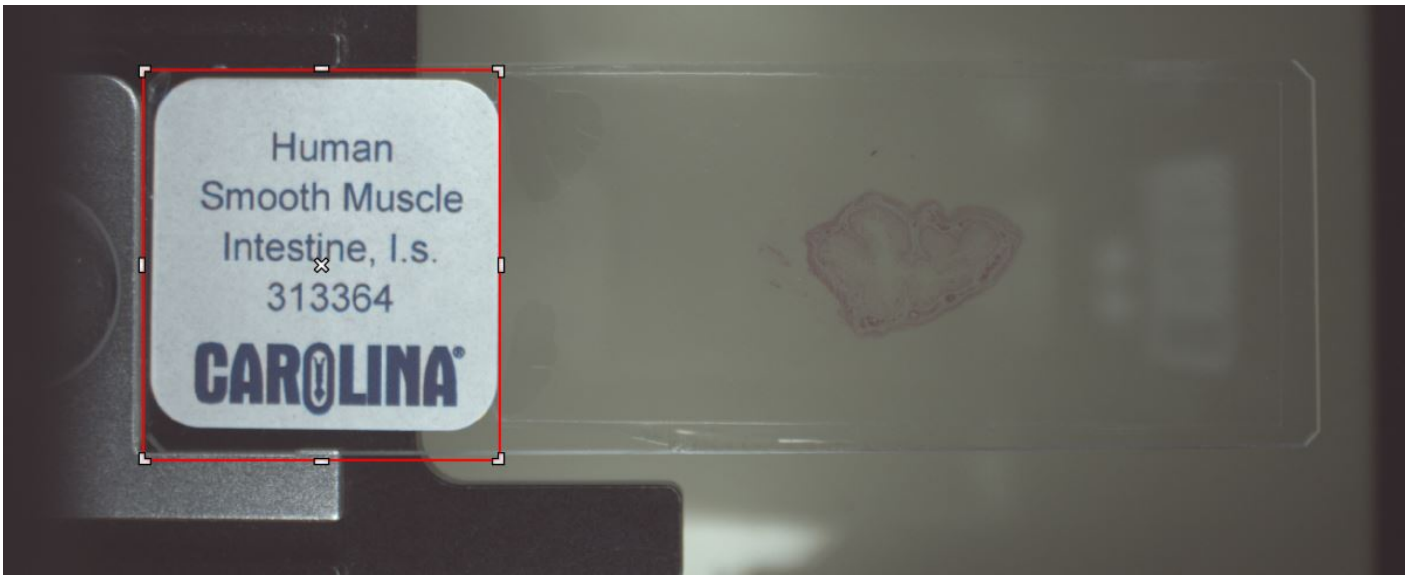
7. based on image from (i.e., tissue detection is based on an image from) (Only for Fluorescence?)
    1. Preview Cam: A quick preview. This is the normal mode and used if the tissue is clearly visible.
    2. Scan Cam: Creates an image with the scan camera and a selected objective. This will also do the Coarse focus map.
8. AutoFocus Contrast Type Coarse/Fine: (Only for Fluorescence)
    1. Channel: Will use one of the channels for the auto focus. Best, if there is a stain (e.g., DAPI) that is fairly evenly seen over the sample.
    2. RAC: Ring Aperture Contrast. This will use a phase contrast image to focus. Can be useful if fluorophores are sensitive to bleaching. It can also be faster. It seems useful up to 10x for the Coarse Focus map.

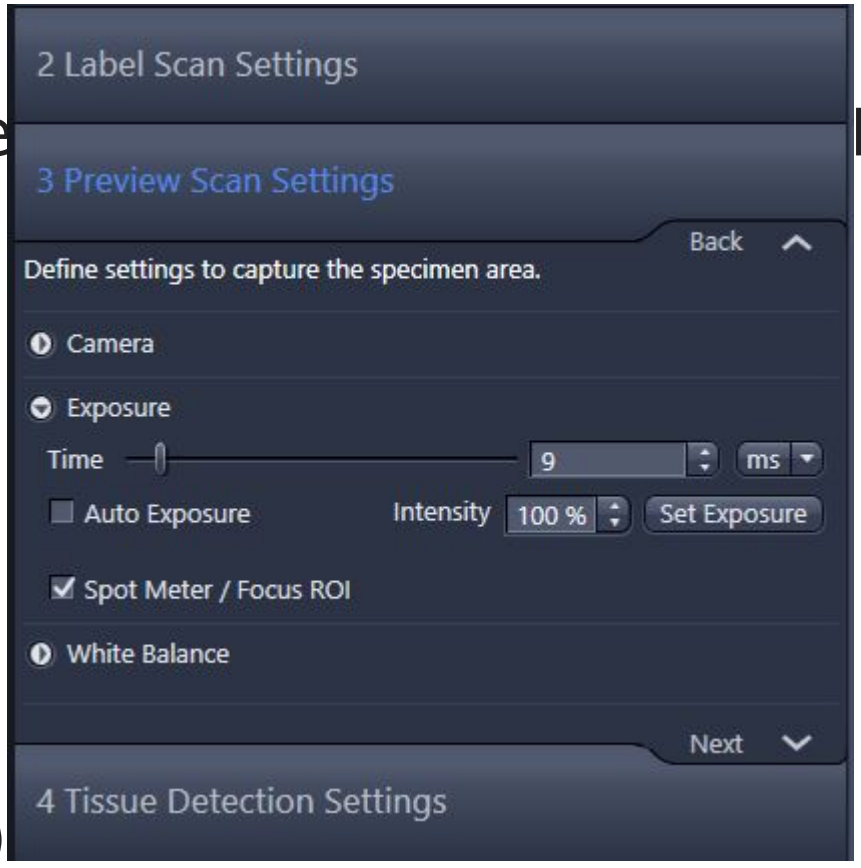
2: Label Scan Settings



Move the size the red box to surround your label. You may also want to set the orientation so that the label is right side up in the Info window. The rest of the settings are usually fine.

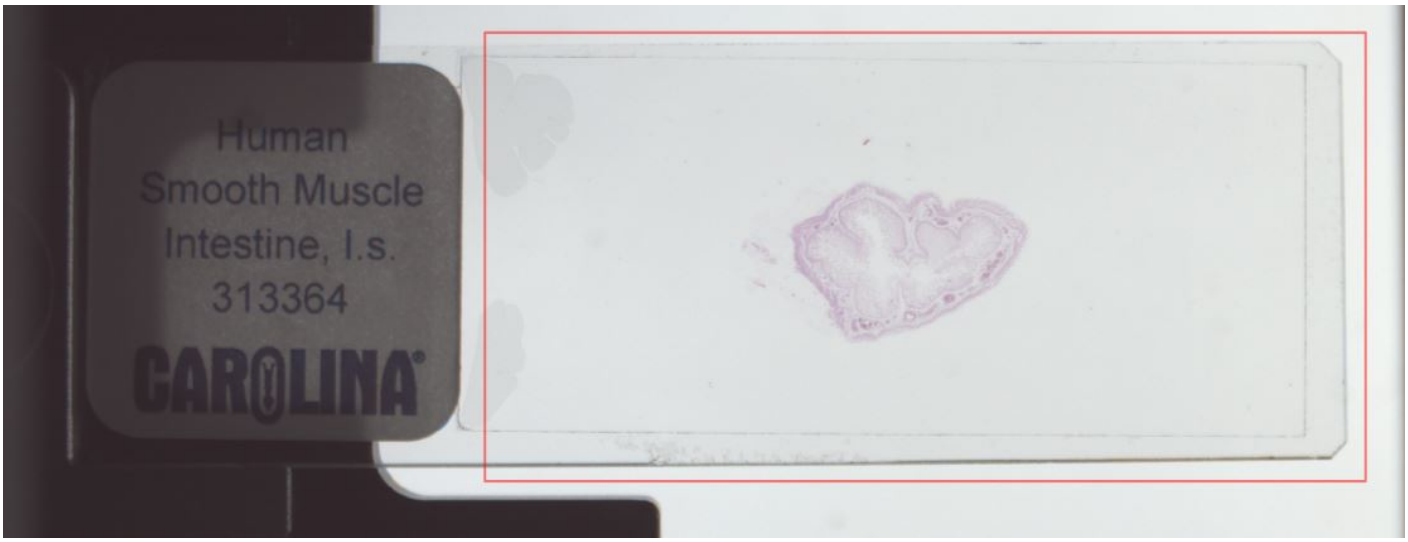
Note: For this scan, the Image Orientation should not have included a rotation.



3: Pre

Preview Scan)

Move and size the red box to cover the rest of the slide and include a little air around the slide. Don't include the label area. The rest of the settings are usually fine although you may want to use either Auto Exposure or Set Exposure.

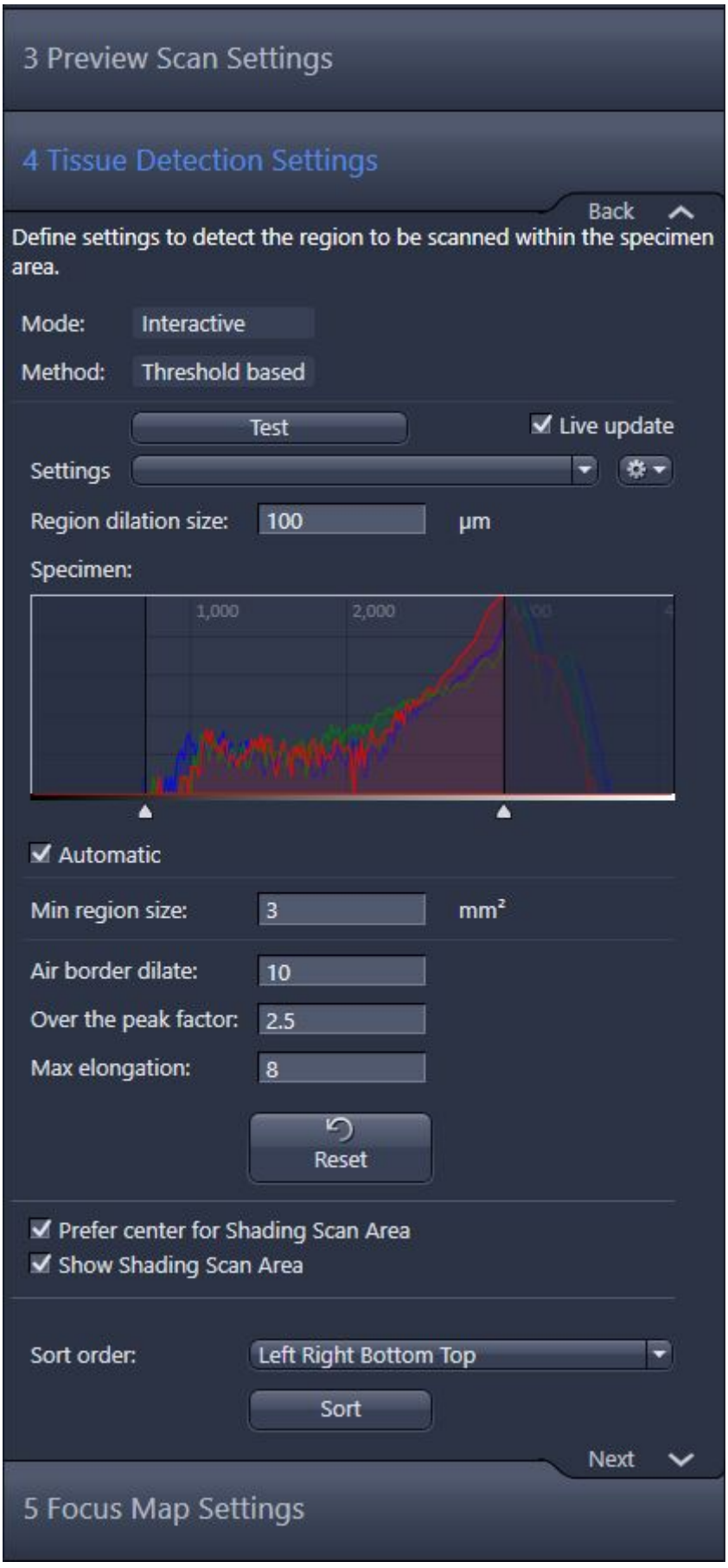


3B: Preview Scan Settings (With Pre-scan)

The Pre-Scan can be useful for automatic tissue detection in fluorescent samples. Because the pre-scan uses the coarse objective, it will take much longer than the preview scan. In setting up the pre-scan, you will also be setting up the Coarse focus map.

If you really want to do this, please consult the Cell Imaging staff and/or watch the video "Preview Scan with objective."

4: Tissue Detections Settings



- Automatic

The common method for automatic tissue detection is Threshold Based. The threshold can be set in this window. These thresholds will be used for all scans.

- Two check boxes - Brightfield Only - 20x and 40x only

The scanner can apply a shading correction that removes or reduces intensity variations in the stitched together image. For Brightfield, the shading information is collected in areas where there is no tissue. There are two checkboxes that appear in the tissue detection section that concern the shading correction.

1. Prefer center for Shading Scan Area
2. Show Shading Scan Area - I prefer to check this. Other prefer not to.

If the Show Shading Scan Area is checked, you will see odd little selections in brown on the slide. These are where Zen is going to get its shading correction. I prefer to see where these are being done.

The Region dilation size is the distance from the detection to the line. This can be especially useful when using the Marker method to adjust to how closely you draw your marker loop.



5: Focus Map Settings

4 Tissue Detection Settings

5 Focus Map Settings

Back

Define settings for the creation of the fine and coarse focus map.

Coarse | Fine

Camera

Exposure

Flash intensity: 192 %

Flash duration: 3 µs

Measure | Measure and White Balance

Post Processing

White Balance

Objective

Focus



Set Last 4301.00 µm

Range 400.00 µm

Slices 21

Interval 20.00 µm

Set First 3901.00 µm

Slice # 11

Position 4101.00 µm

Run Auto Focus

Use adaptive focus point distribution

Focus point distribution strategy set

Support Point Distribution Strategy	Parameter
Number of points	6

Sharpness measure set

Basic | Best | FFT | HG 2^8 | Contrast Power

Next

6 Scan Settings

If your sample and slide are perfectly flat, one Z value is good for your entire sample. The scanner would just have to find the focus at one point and be done. Assuming that things are not perfect, the scanner needs to focus at several points and interpolate between them. This is the focus map. The scanner will construct a Coarse map using a low magnification objective. This will be Coarse in the magnification and in the number of points needed. The Fine map should use the scan objective and will need more points.

You goal with all the settings is to get a good focus over your whole sample without spending too much time. It is a balance.

- Objective:

Check that the correct objective is being used for each Coarse and Fine. The coarse objective can be 2.5 or 5X (maybe 10X for 40X scan). The fine should be the same as your scan objective.

- Focus scan settings

To find the focus, a set of images will be taken at different Z. These will be analyzed to find the best focus (may be between the Z images taken). You can set the vertical range and the step size for the focus. In Brightfield Coarse, you would like the focus to be near the center of the range.

Smart focus will start in the center and look for the best focus without using the whole range. This will be faster, but it can occasionally cause problems.

- Focus point distribution strategy:

For Coarse, the standard is a fixed number of points and is often a fairly low number (4-6). This is a number per scene. If your slides have a lot of tissue and you have merged the scenes, you may want to increase the number.

For Fine, the standard is a density method (e.g., Onion Skin or Density). These methods also have a maximum number of points (again, per scene). To change the maximum, you have to double click on the method line and edit the pop-up window that pops-up near the top of the screen. Small numbers (12-24) are fine for smaller samples. Large samples that are merged into a single scene may require a large number of Fine focus points (50-100 or more) to stay in focus over the whole sample.

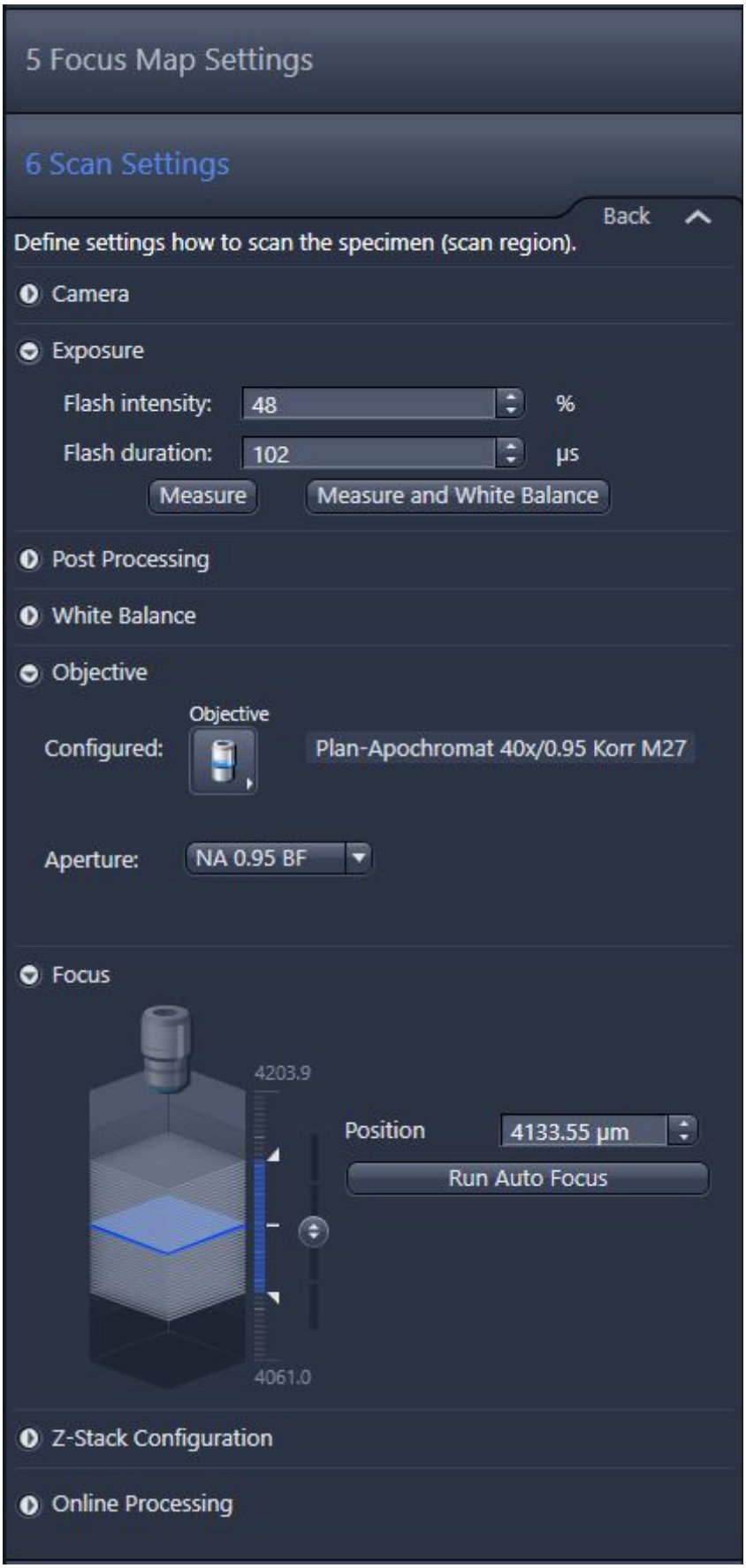
- Fluorescent Channel Settings

There are more focus map settings if you are using a fluorescent channel. You will need to set up the channel to be used for focus. Optimally, this channel has distinct features (sharp edges) and is present over the sample. After selecting the channel, check the light path, especially for the objective.

Then work on setting the exposure so that the histogram is 20-30% filled. You will need to get close on exposure before you can try to autofocus. Once focused, you may need to reset the exposure.

6: Scan Settings - Brightfield

MTE1Njc0NS4yfDg4OTgwNC84ODk4MDQv



- Objective

Open this tab to make sure that it is the objective you want!

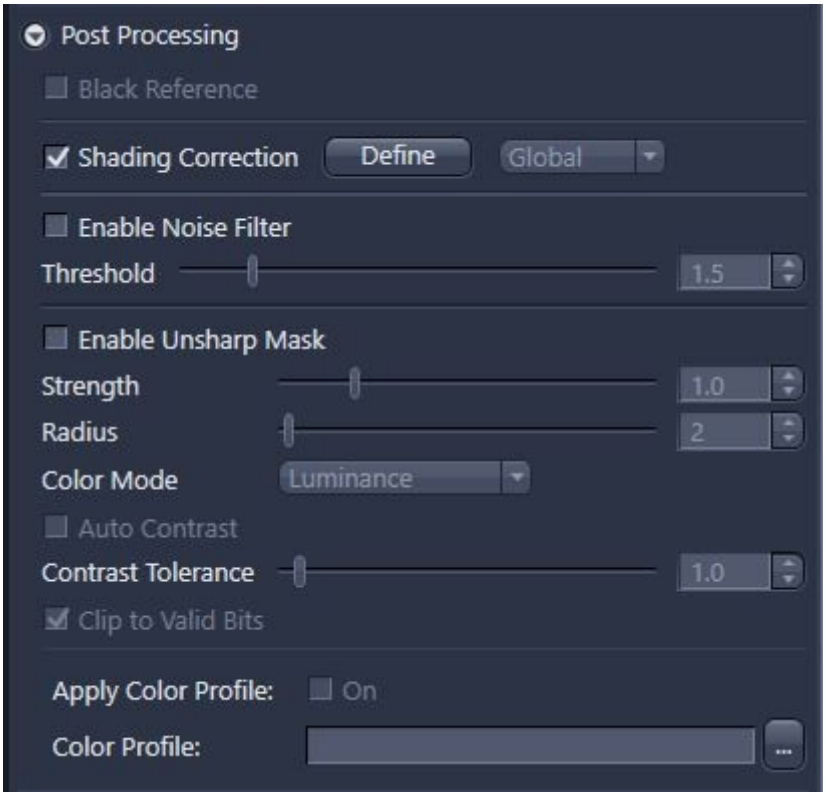
- Exposure

You can start with Measure and then adjust the flash intensity and flash duration to fill the histogram appropriately. For bright field, it should probably fill at least 2/3 of the histogram. For fluorescent, the target is typically 1/3.

- White Balance

Under the white balance section, I typically use "Pick." I will move to a blank part of the slide and click on a clear spot. The three color profiles should be co-centered.

- Post Processing

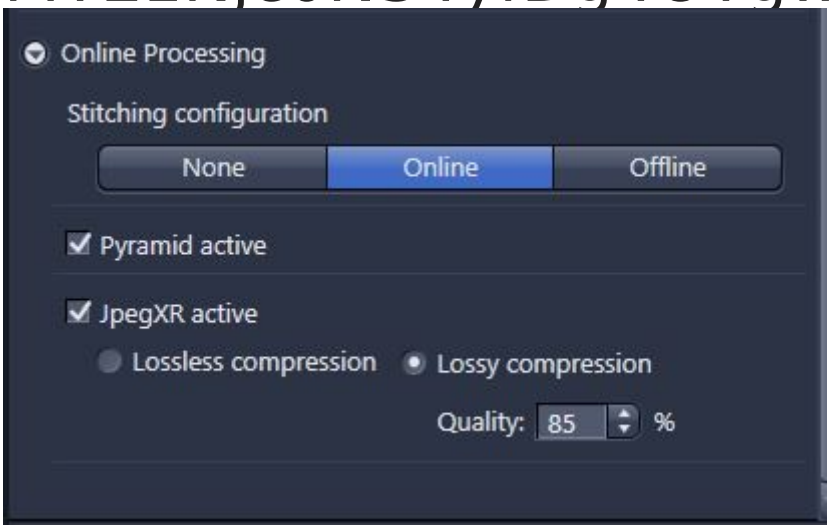


MTE1Njc0NS4yfDg4OTgwNC84ODk4MDQv

In the Post Processing, for brightfield, the shading correction should be checked.

- Online Processing

MTE1Njc0NS4yfDg4OTgwNC84ODk4MDQv



The typical setting for Online Processing is:

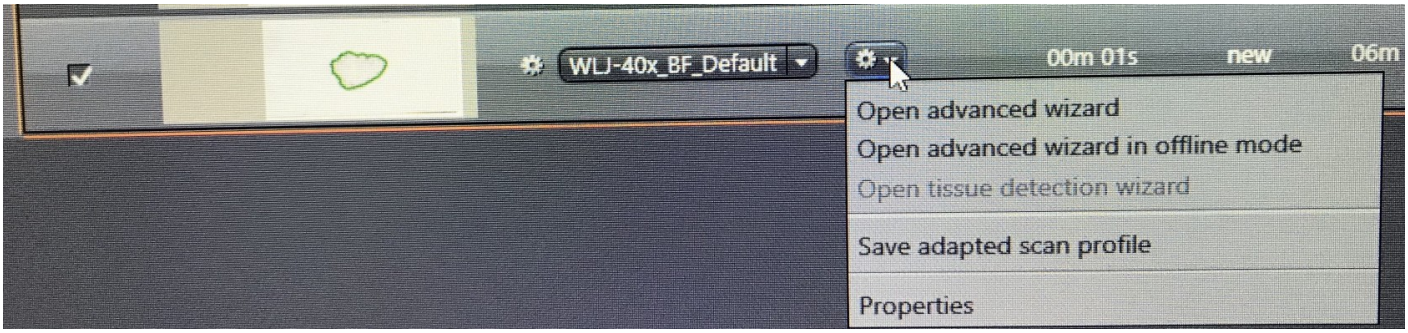
1. Stitching: Usually Online.
    1. Online is typically faster than offline, but if there are stitching errors, using Offline can fix this.
2. Pyramid Active: Yes (will speed up later viewing)
3. JpegXR active
    1. Lossy Compression
    2. 85%

Is your profile saved? Will your profile be used?

The Zen software does not give great feedback on this. It is easy to edit a profile, start a scan, and find out that the profile didn't get saved or perhaps even used.

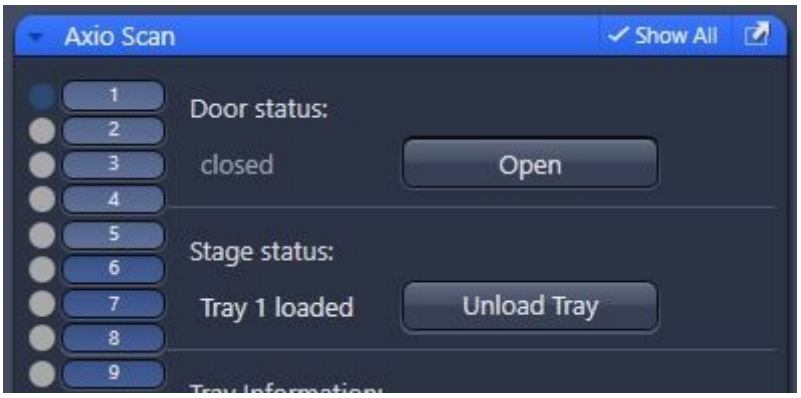
- Saving the modifications you have made:

Right after you have finished with the Wizard, go to the gear on the slide and select Save Adapted Profile. The default name starts with Adapted…. I suggest getting rid of all of that and saving it with a normal name (starting with your name or initials). You can even save it with the same name as your previous profile, overwriting the old one.



MTE1Njc0NS4yfDg4OTgwNC84ODk4MDQvTm90ZWJvb2svMzQ4NjY3Njg5MHwyOTM2MzUzLjE5OTk5C

- Making sure the profile will be used:



MTE1Njc0NS4yfDg4OTgwNC84ODk4MDQvTm90ZWJvb2svMzQ4NjY3Njg5MHwyOTM2MzUzLjE5OTk5C

That adapted profile will be used for the one slide that you set it up on. I know of two ways to get it used for the other slides.

1. Do a slide reset by unloading the tray (button below Open Door in the software). Open the Door. Swing the trays out, put them back and close the door. Now select the default profile you want to use.
2. When you save the profile, give it a new name. Now go slide by slide and make sure they have the new profile. I have tried setting the default to the new profile. It doesn't always reset the slide's profiles.