Slidescanner - Zeiss Z1 (Schlieren) - Part2: Setting Up a Fluorescence Scan Profile

How to define a fluorescence profile in the AxioScan.Z1 slide scanner located at Schlieren Campus, Wagistrasse 12 , WAD H127 (325).

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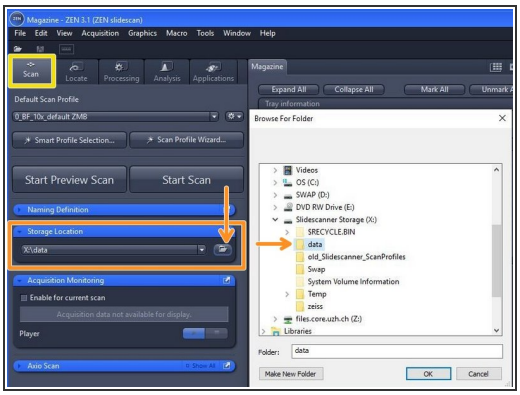
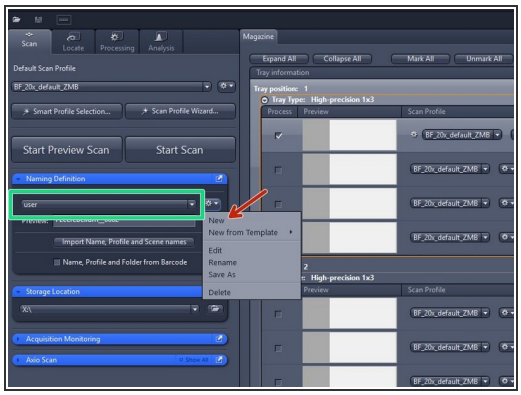
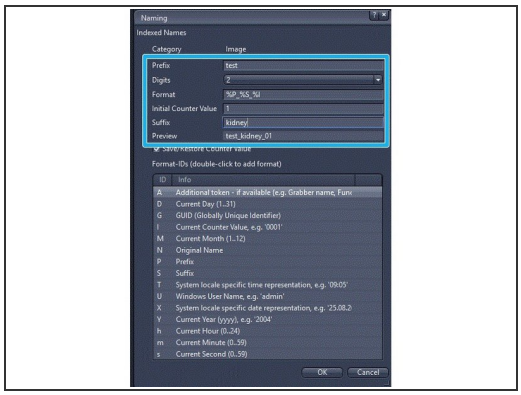
INTRODUCTION

This guide provides instructions on how to set up a fluorescence scan profile. Note that slides have to be loaded in order to set up a profile (see "Start up" guide). Please refer to the manuals on the ZMB homepage if you need more detailed information.

Step 1 — Define Storage Location and Naming



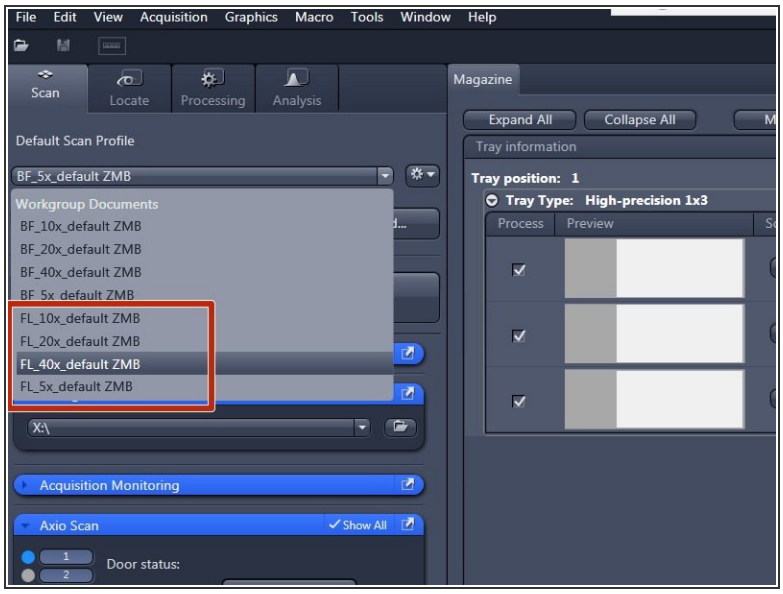


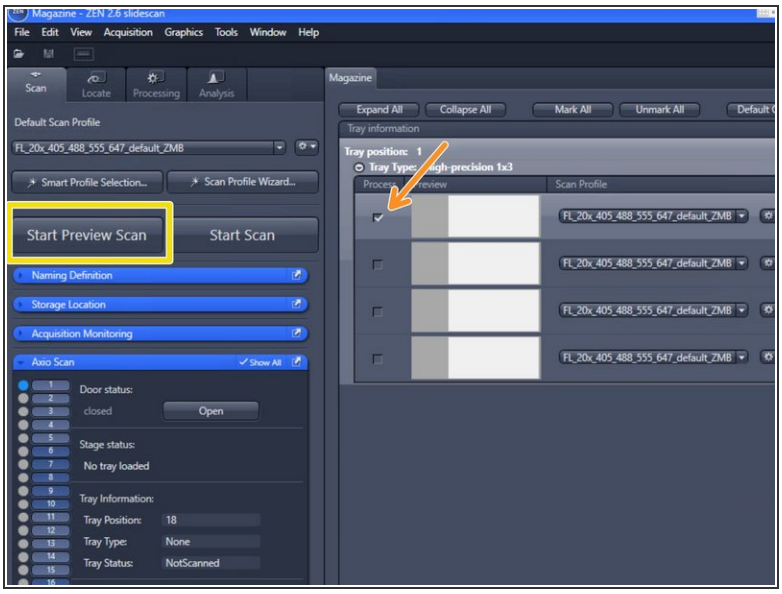


- Select the "Scan" menu.
- Verify that the saving location is on the network drive with the letter **X:**\ . Otherwise click on "Browse" and select " This PC \ SlidescannerStorage (X:)\data" as a storage location.
- Define the automatic naming of your slides:
  - Select "Edit".
  - Define a name for your naming template and save it.
  - Define your files name in the "Naming" window.

ⓘ The import of slide names and the automatic assignment of scan profiles to individual slides is possible via a comma-separated file (.csv). See corresponding guide.

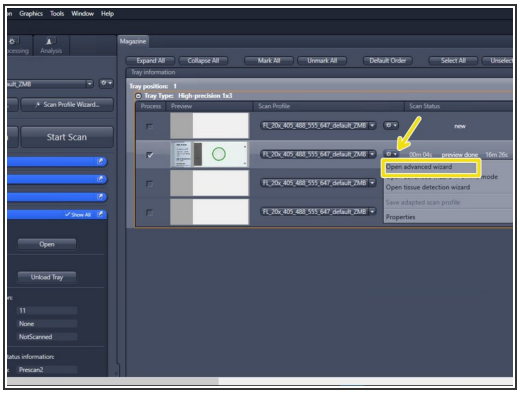
Step 2 — Select a default scan profile and capture a preview

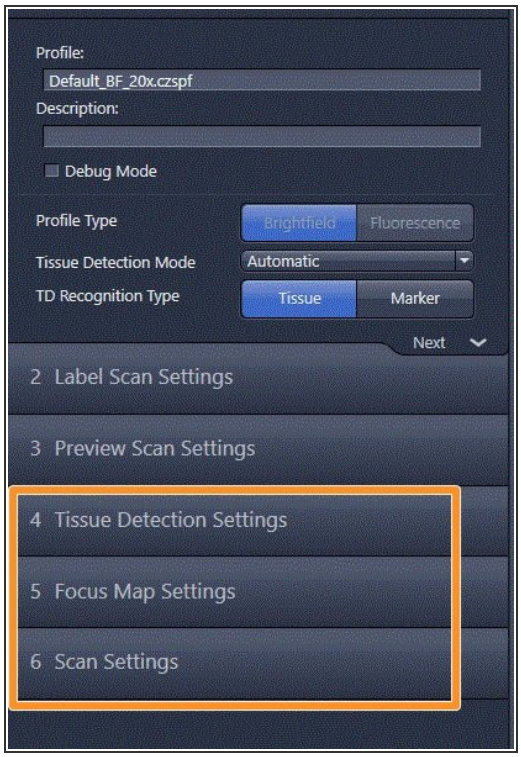


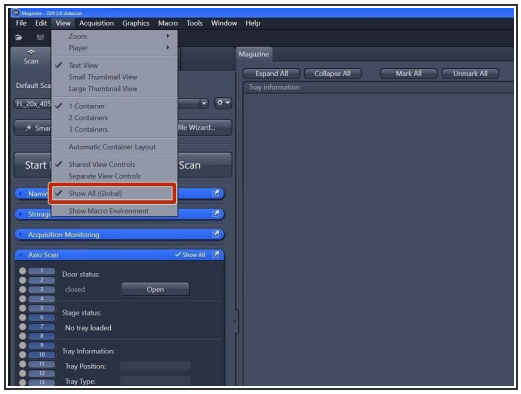


- ⓘ Start with one of the ZMB default profiles (they are already optimized for imaging with a particular objective).
- Expand the "Default Scan Profiles" menu and select a "FL default ZMB" profile according to the objective you wish to use.
- Mark one representative slide of your experiment to adapt the settings of the profile .
- Capture a preview image by clicking on "Start Preview Scan".

Step 3 — Edit a scan profile in the advanced wizard

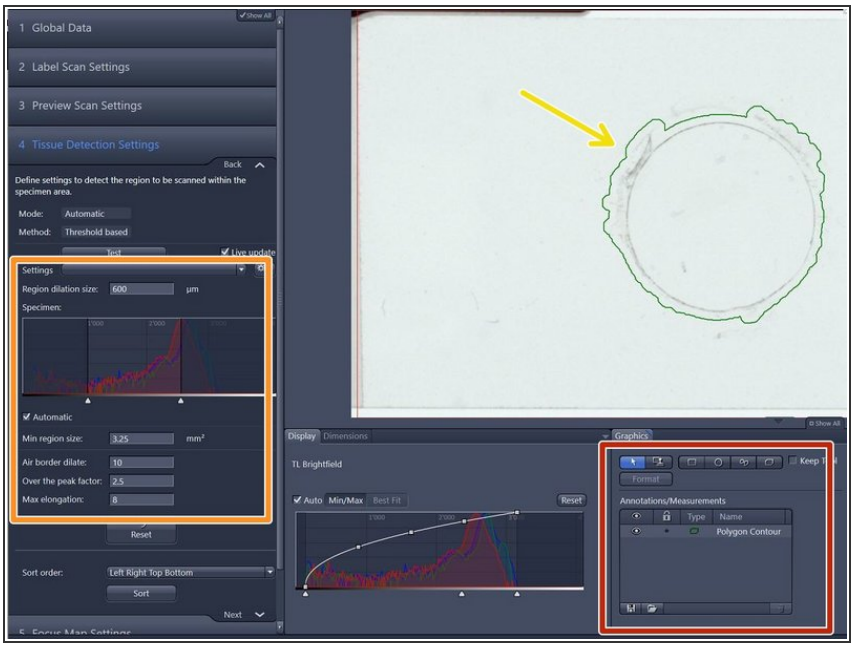






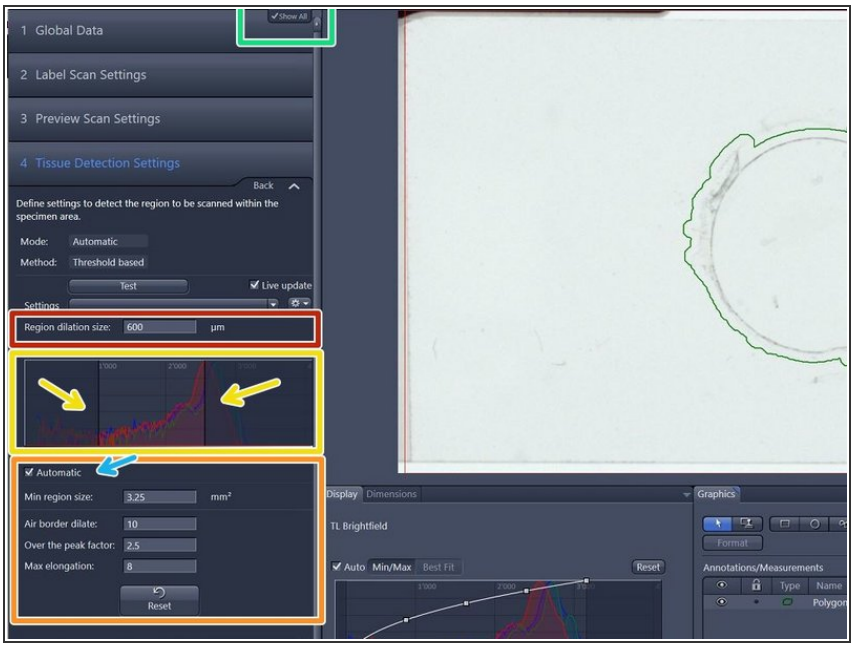
- Select "Open advanced wizard" to adjust scan parameters.
  - ⓘ The advanced wizard is composed of 6 steps to adjust scan parameters. Step 1-3 are already optimized for standard slides.
  - How to optimize step 4-6 will be explained in the next steps of the guide.
- Make sure that "Show All (Global)" is activated under the tab "View".

Step 4 — Tissue Detection Settings



- General information:
  - The objects surrounded by a green line are the selected region of interests (ROIs) for scanning.
  - Automatic tissue detection is possible based on the preview histogram and the parameters in the "Tissue Detection Settings" dialog window if there is enough contrast in the sample.
  - Manual tissue selection might be necessary for samples with low contrast e.g. fluorescence samples.

Step 5 — Automatic Tissue Detection



- Deactivate "Show All" and activate it again in order to see all options for the automatic tissue detection. (This is a software bug).
- Modify upper and lower threshold of the "Specimen histogram" to select the ROIs according to intensity values.

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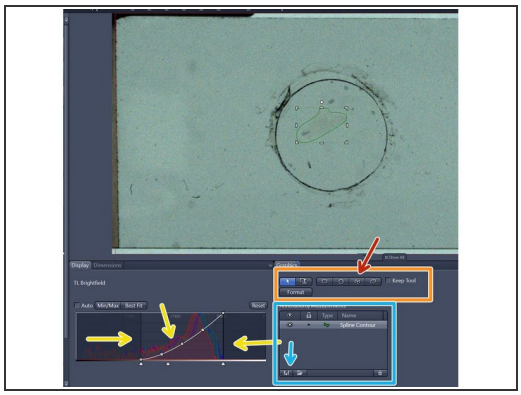
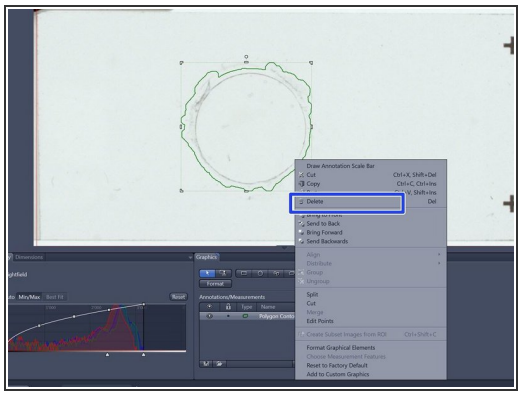
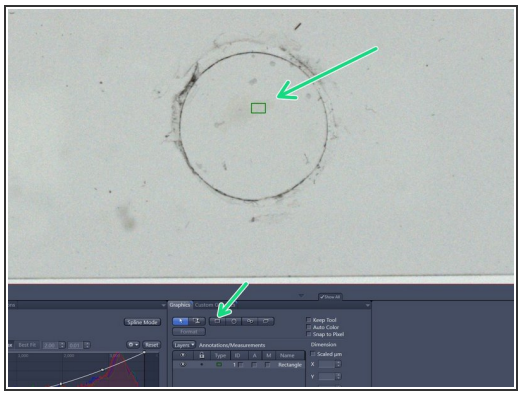
- If the "Automatic" checkbox is activated the upper threshold will be determined automatically based on the "Over the peak factor" (a measure for the expected contrast between the glass and tissue).

- Filter out artifacts with the help of following parameters:
  - "Min region size" : defines the minimum area a ROI should have.
  - "Air border dilate": defines the expected distance of the object from borders of the preview scan region.
  - "Max elongation" can be adjusted to exclude elongated artifacts.
- Define a spacing of the border around the detected tissue by adjusting the "Region dilation size" (normally adjusted between 150-200).

Step 6 — Manual Tissue Detection

ⓘ If signals are faint it might be necessary to select the ROIs manually with the help of different drawing tools.

- Adjust the display curve of the histogram for maximal contrast.
- Draw the wanted regions manually with the tools in the "Graphics" tab.
  - Tip: Draw the outline of the scanning area with the "Draw Spline Contour" tool.
    - ⓘ Make sure that there is not too much empty space within your ROI since this could lead to focusing issues.
- The defined ROIs are listed in the Graphics tab. They can be saved and later be applied to other slides.
- Regions that should not be scanned can be deleted manually.
- Select a small ROI if you want to test new settings.

Step 7 — Focus Map Settings

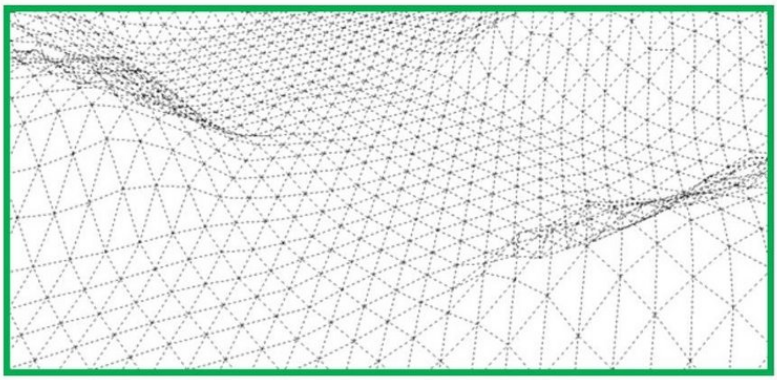
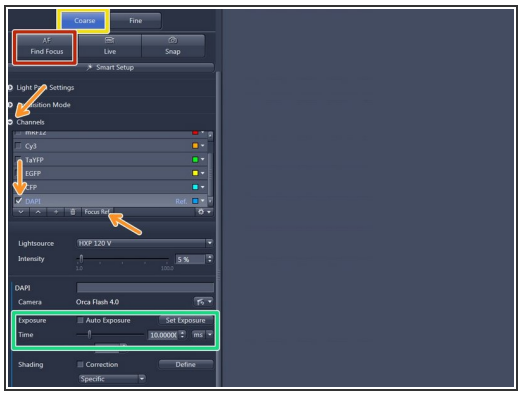


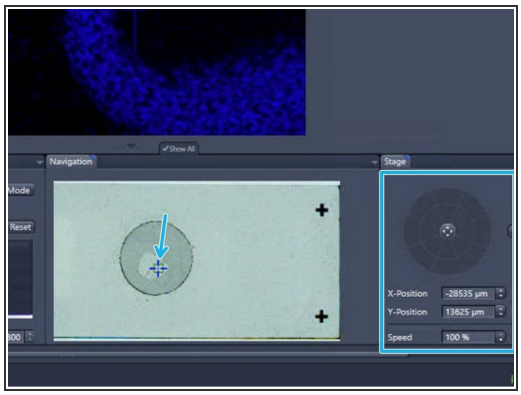
Figure 14 Representation of a focus map

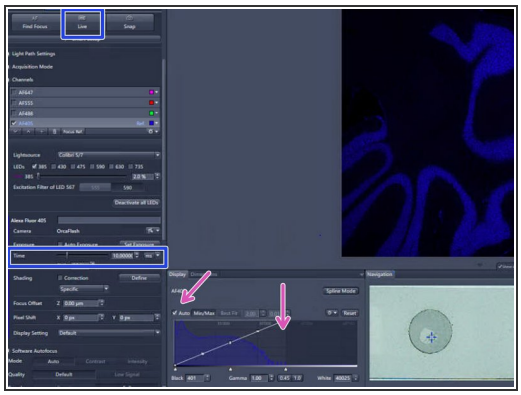
- General information:
  - ⓘ The focus at each position of the sample is determined automatically by the slidescanner based on a focus map.
  - ⓘ The focus map is created in two steps: In a first step a coarse focus map is created with a low magnifying lens like the 5x objective. In a next step the focus map will be refined by a fine focus, that is performed with the imaging lens.

⚠ Appropriate focus map settings are important to ensure good focus quality throughout the specimen.

Step 8 — Define Coarse Focus Settings

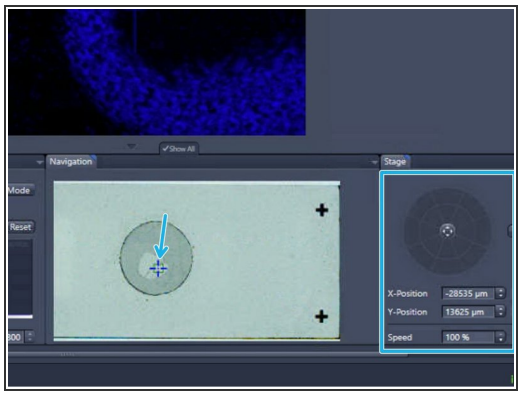


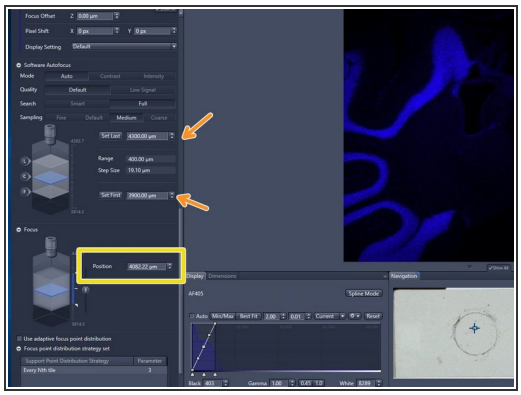


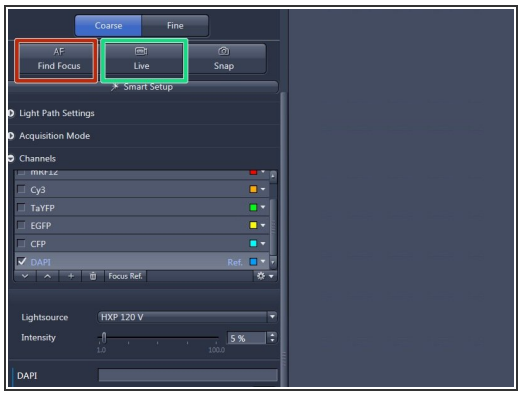


- Make sure that "Coarse" is active .
- Expand the "Channels" drop down menu and set the reference channel for the autofocus. Per default AF405 (DAPI) is selected.
- Start with the given exposure time e.g. 10 ms for relatively bright samples.
- The initial x-y position is per default in the center of the ROI. To move the sample click into he slide view shown in the "Navigation" tab or use the virtual joystick to navigate more precisely.
- Click on "Find Focus"
- Look at the histogram to determine the appropriate exposure:
  - Check "Auto" above the histogram. Make sure the histogram shows **at least 2` 000 intensity values** but no saturation.
  - Adapt the exposure time if necessary. Click on "LIVE" to see the changes.

Step 9 — Test Coarse Focus at Different Positions







- Move to different locations in the sample to test the "Coarse Focus".

ⓘ If the focus quality is fine at different positions of the sample, move to the next step of the guide.

- If the focus was not determined properly try to search manually for the focal plane:
  - Click on "LIVE" in order to switch on the camera.
  - Focus manually: click on the arrows of the "Focus Position" display or click into the number field and move up/down with the mouse wheel.
  - Adapt the borders of the capture range so that the focal plane is roughly in the middle.
  - Click on "Find Focus" to test the new autofocus settings.

Step 10 — Define Focus Point Distribution Strategy

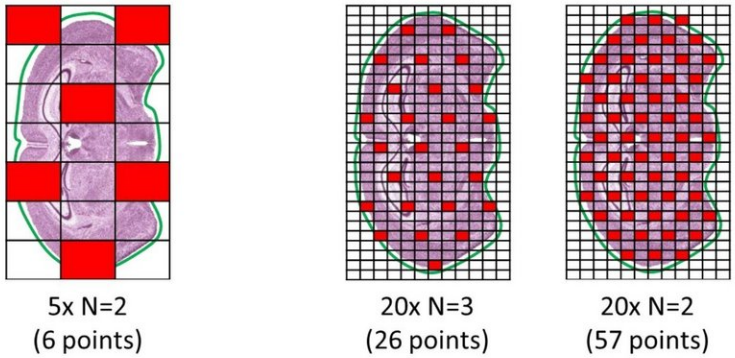
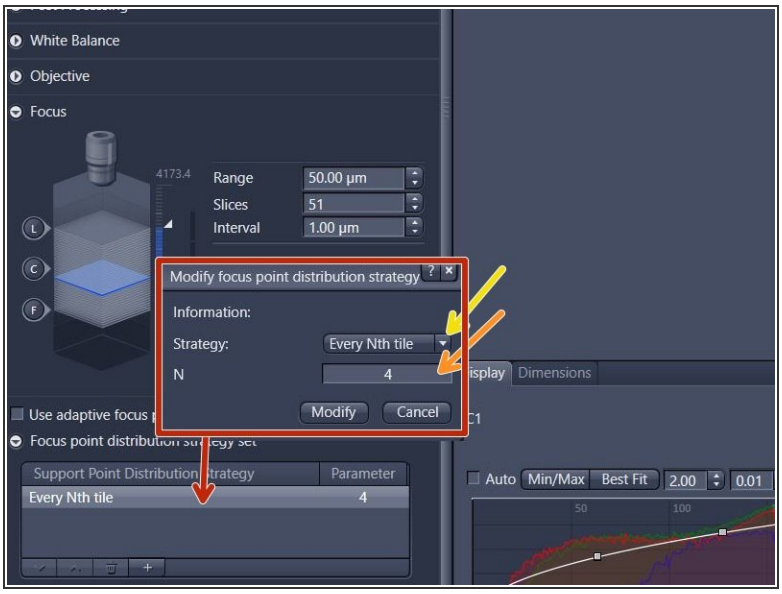


Figure 25 Course and fine point distribution using **every Nth tile**



ⓘ The "Focus Point Distribution Strategy" defines how and how often the autofocus will be executed.

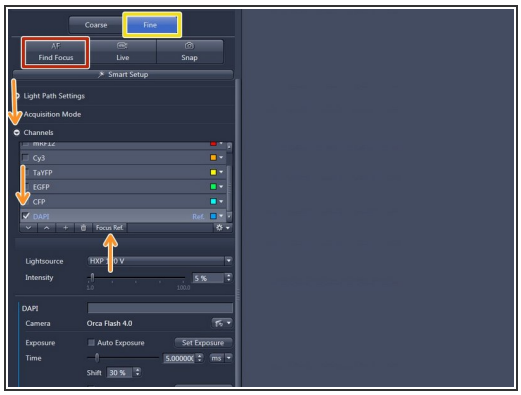
The default settings work normally fine for standard tissue samples with a medium size. For very small, very large or uneven samples, the default settings may have to be adjusted for increased precision or speed:

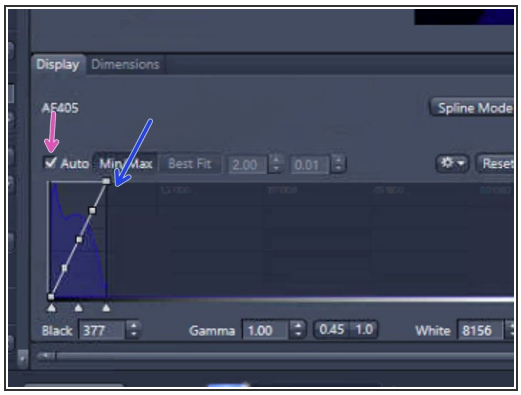
- To change settings:
  - Double-click on the entry of the table view and change parameters in the new window. Click on "Modify" to apply the changes.
    - For **uneven samples**, it might be necessary to increase the number of focus points. Note: for the "Every Nth Tile" strategy the value "N" has to be decreased in this case. Be aware that a higher accuracy of the focus map comes at the cost of a longer scanning time.
    - For **very small** or **large** tissue sections, selecting another focus strategy might be useful. Change the "Strategy" set via the dropdown menu. Tipp: Select "Center of Gravity" for very small sections or "Onion Skin" for large specimens.

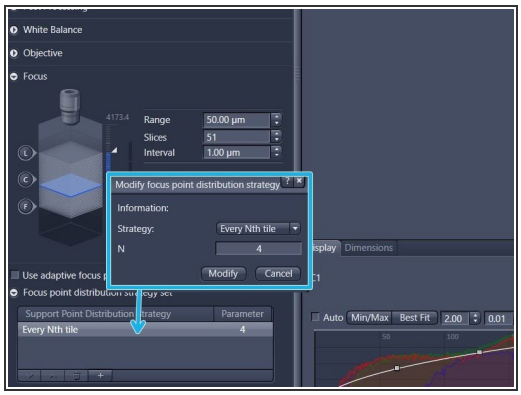
⚠ The number of focus points should not exceed 400. Restrict the number of focus points for very large specimen by selecting the "Onion Skin"strategy with a maximum of 400 focus points.

ⓘ For more information consult the AxioScan Z.1. Application Guide or ask the ZMB staff.

Step 11 — Define Fine Focus Settings

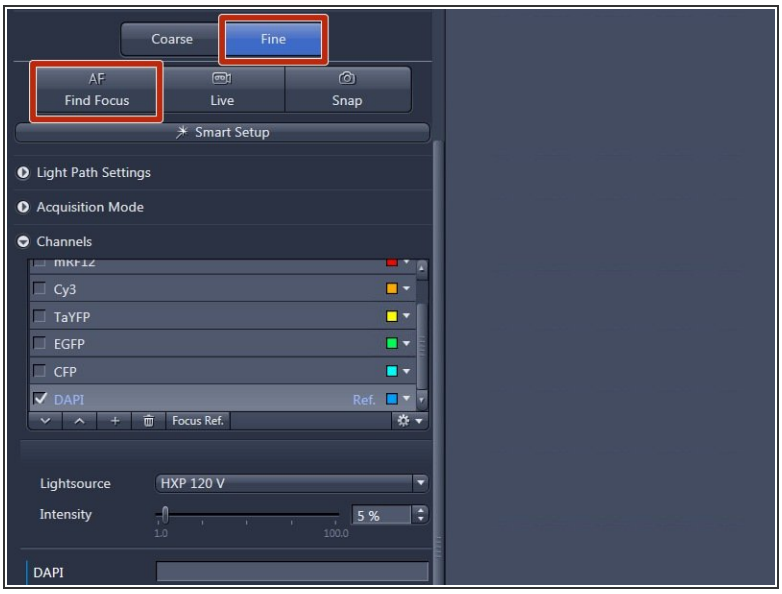


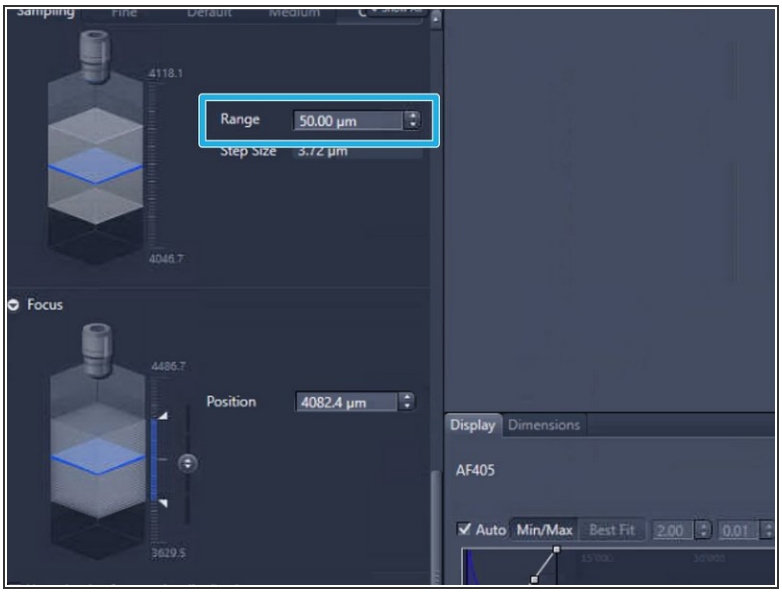




- Select "Fine"
- Expand the "Channels" drop down menu and set the reference channel for the Fine autofocus. Per default AF405 (DAPI) is selected.
- Start with the given exposure time for relatively bright samples.
- Click on "Find Focus".
- Investigate the histogram to determine the appropriate exposure:
  - Check "Auto" above the histogram.
  - Make sure the histogram shows **at least 2'000 intensity values** and no saturation. Adapt the exposure time if necessary.
- Define the Focus point distribution as described in the previous step. Note: the default settings are a good starting point.

Step 12 — Test Fine Focus at Different Positions



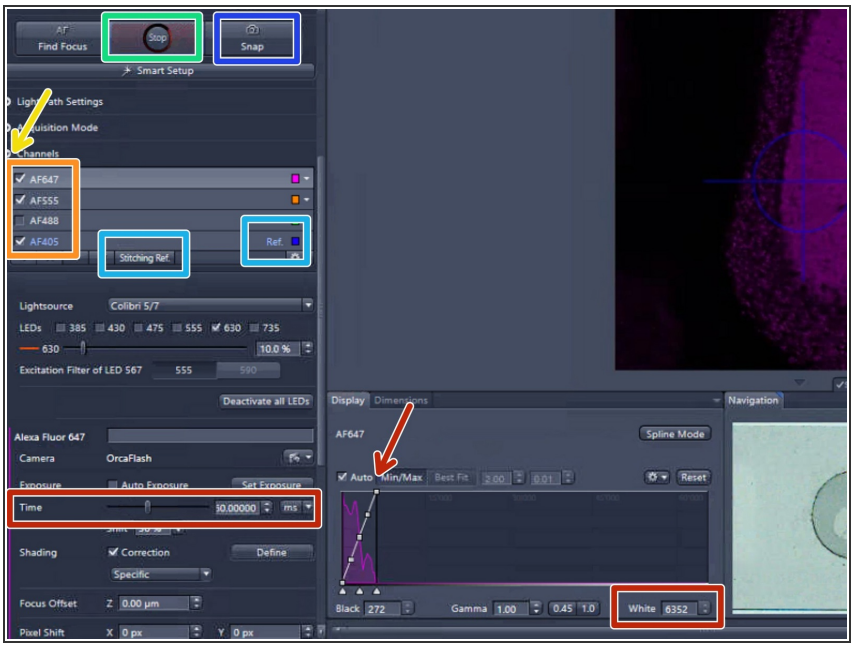


- Select "Fine" and press "Find Focus" .
- If the focus could not be found increase the focus "Range" and try again.

  ⚠ The maximum fine focus range should not exeed 100 um.
- Test the "Fine Focus" at different locations on the sample.

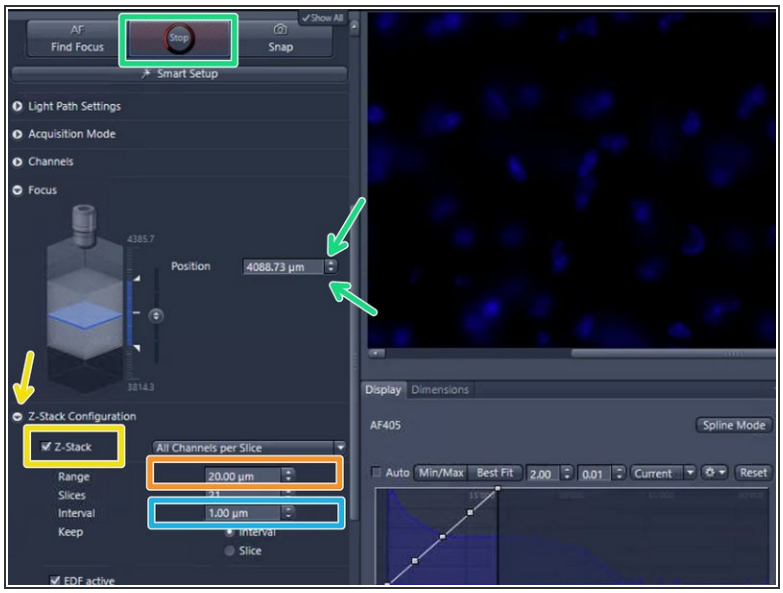
ⓘ The fine focus can only be succesful if the coarse focus was applied before.

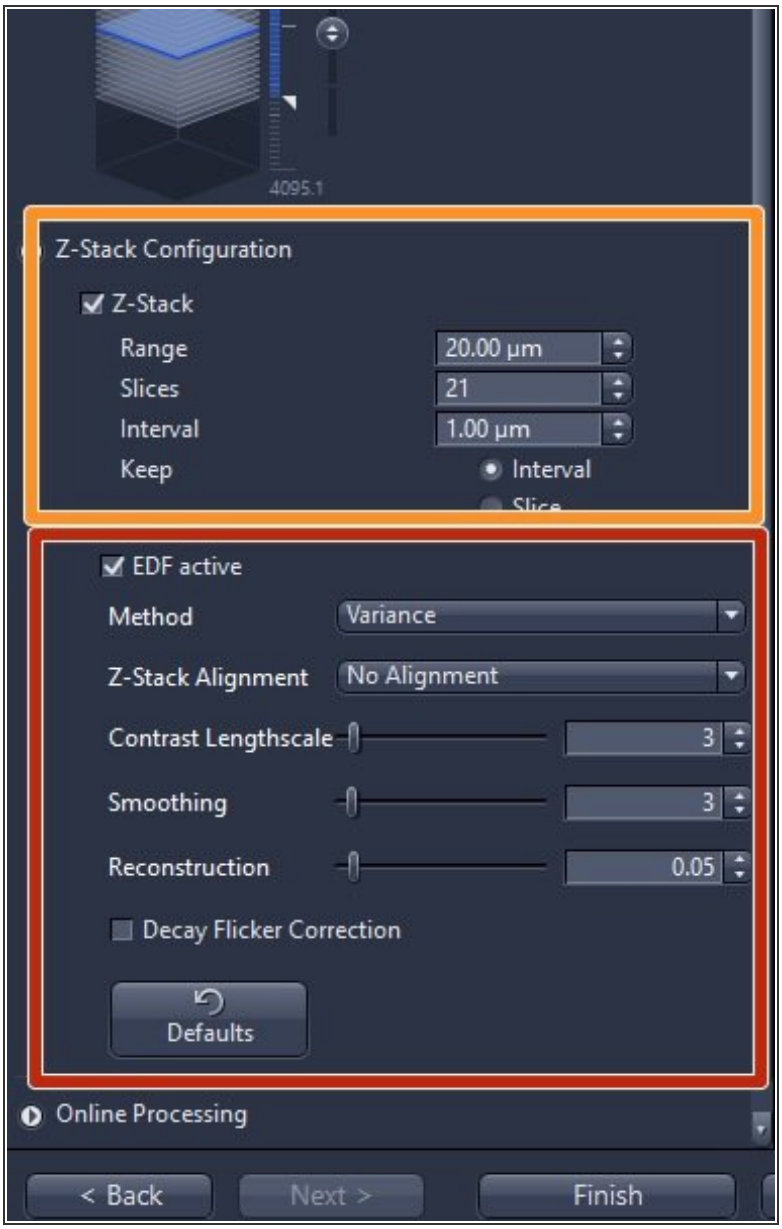
Step 13 — Define Scan Settings



- Expand the "Channels" drop down menu.
- Activate the channels you need and set up an appropriate exposure time for each channel:
  - Highlight a channel.
  - Click on "Live" .
  - Set up the exposure time for each channel making sure you observe **at least 5000 intensity values** (check histogram).
- Set the reference channel for stitching: Per default AF405 (DAPI) is selected as reference channel. If another reference channel is needed, highlight the appropriat channel and click on "Stitching Ref.".
  - ⓘ An appropriate stitching channel is one in which the dye covers the specimen evenly.
- Optional: Click on "Snap" to capture a single image with all the channels.

Step 14 — Define a Z-Stack (Optional)





ⓘ Define a z-stack if the tissue is not flat or very thick. By default the z-stack function is disabled.

- Tick "Z-Stack".
- Set the "Range".
  - By clicking on the arrows of the "Focus Position" display or into the number field and moving up/down with the mouse wheel you can focus through your specimen in order to determine an appropriate z-range. Make sure that the camera is on "Live".

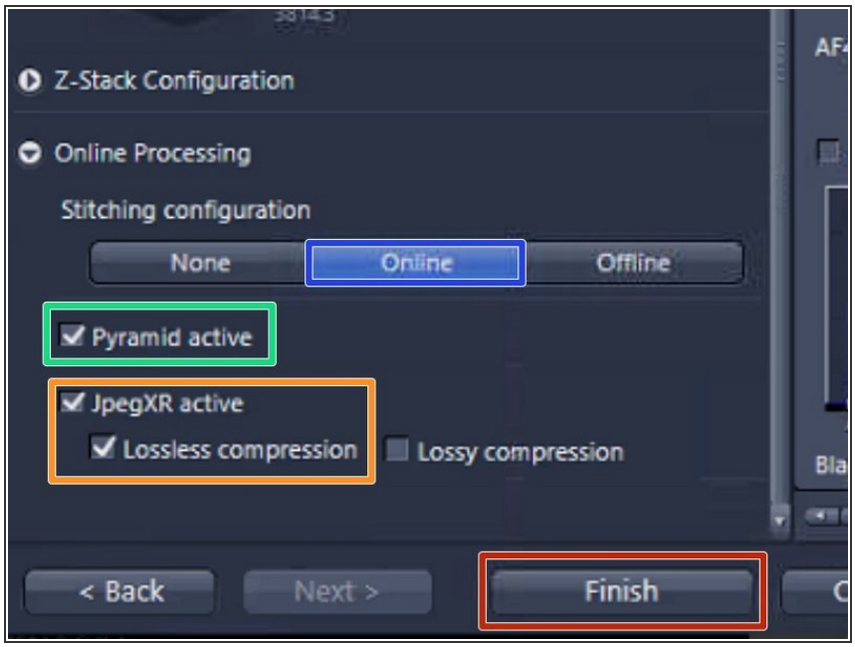
    ⚠ The maximum z-range should be below 300 um for the 5x, 10x and 20x lens and below 50 um for the 40x lens to avoid damage of the objective and / or slide.

- Set the z-interval depending on the imaging objective and desired z-resolution e.g. 5 um for the 5x lens, 2 um for 10x lens, 1 um for 20x lens and 0.5 um for 40x lens.

- Check "EDF active" (Extended depth of focus) if you would like to get a 2D projection of your z-stack. Select either "Variance" or "Maximum intensity projection".

ⓘ We strongly recommend to use the predefined standard parameters for each method.

Step 15 — Online Processing



ⓘ The "Online Processing" expander is the final step of the wizard. For online stitching and imaging with the default format nothing has to be changed here.

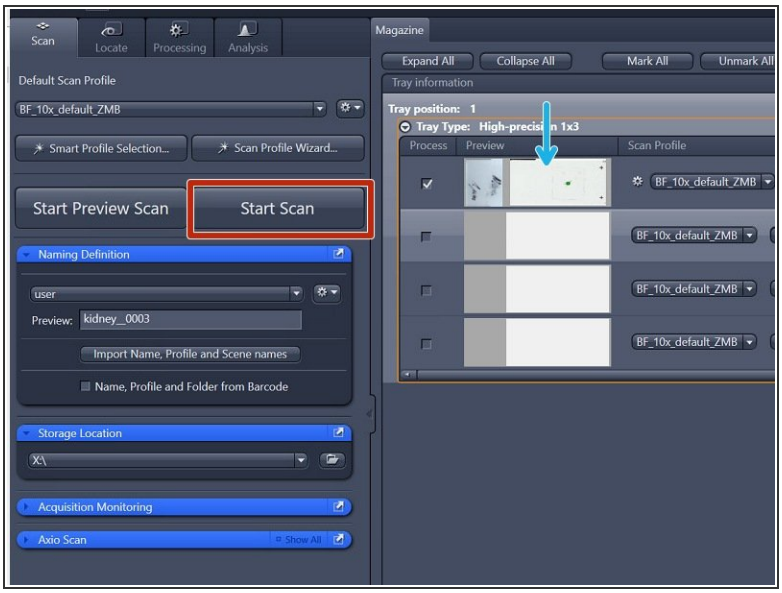
- "Online" stitching is the default setting and means that the acquired tiles are already stitched during the scan process.

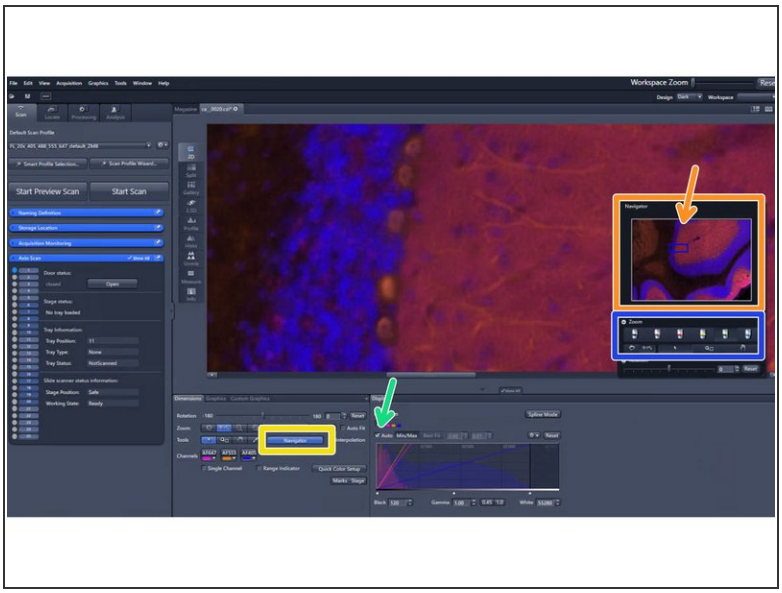
- "Pyramid active" should always be acitvated as this will speed up the viewing of the images afterwards.

- The files will be saved in the Zeiss file format (.czi). A lossless compression is selected per default. In order to reduce the file size "lossy compression" with a quality of 85% could be selected at the expense of a lower image quality.

- Press "Finish"

Step 16 — Scan a Test Region and Investigate the Result



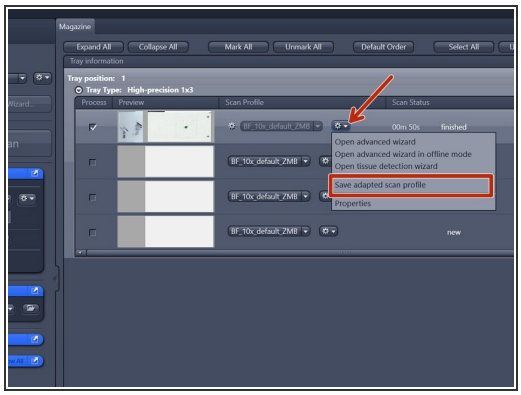


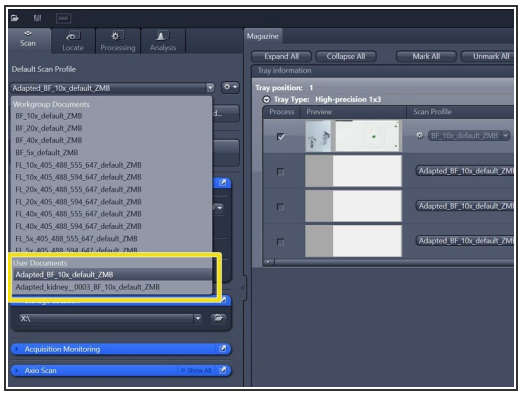
ⓘ Once all parameters are adjusted, test the adapted scan profile on a small ROI. Note: a small ROI has to be manually defined in the "Tissue Detection Settings" step.

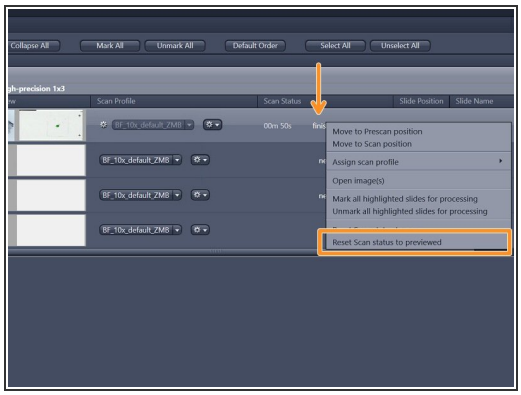
- Press "Start Scan" .
- Visualize the resulting image by double-clicking on the slide's preview image after the scan process has been completed.
- Check "Auto" above the histogram to optimize the visualization of the image.
- Click on "Navigator"
  - You can now navigate in the small window.
  - Select the imaging lens to visualize the data with an appropriate zoom factor.

ⓘ Navigation is also possible with the computer mouse: zoom in / out with the mouse wheel and left click on the image and drag it to move around.

Step 17 — Save an Adapted Scan Profile





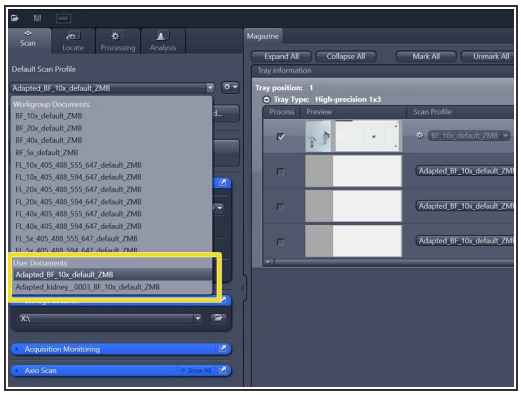


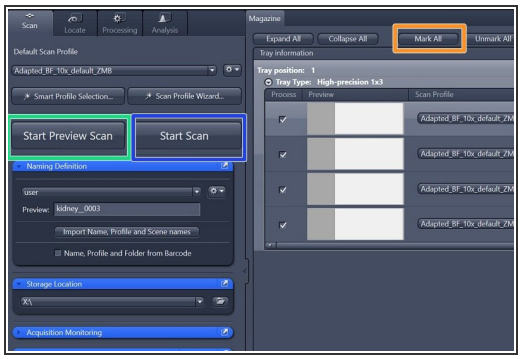
- If you are happy with the scan result, select "Save adapted scan profile" and save your profile under:

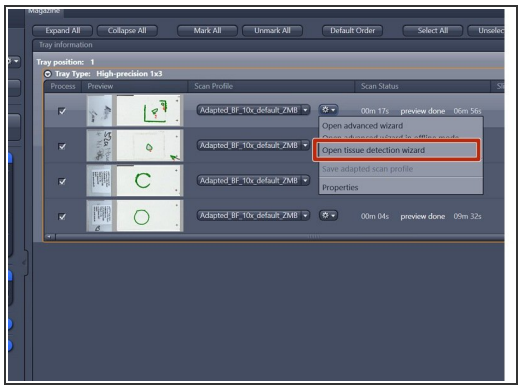
"C:\Users\%userprofile%\Documents\Carl Zeiss\ZEN\Documents\Scan Profiles\"

- The saved profile will now be available in your personal "Default Scan Profiles" list and can be applied to other slides.
- If you are not satisfied with the resulting image, right-click into the scan status "finished"and select "Reset Scan Status to previewed"
  - Go again to "Open advanced wizard" and adjust the parameters.

Step 18 — Scan all your Slides

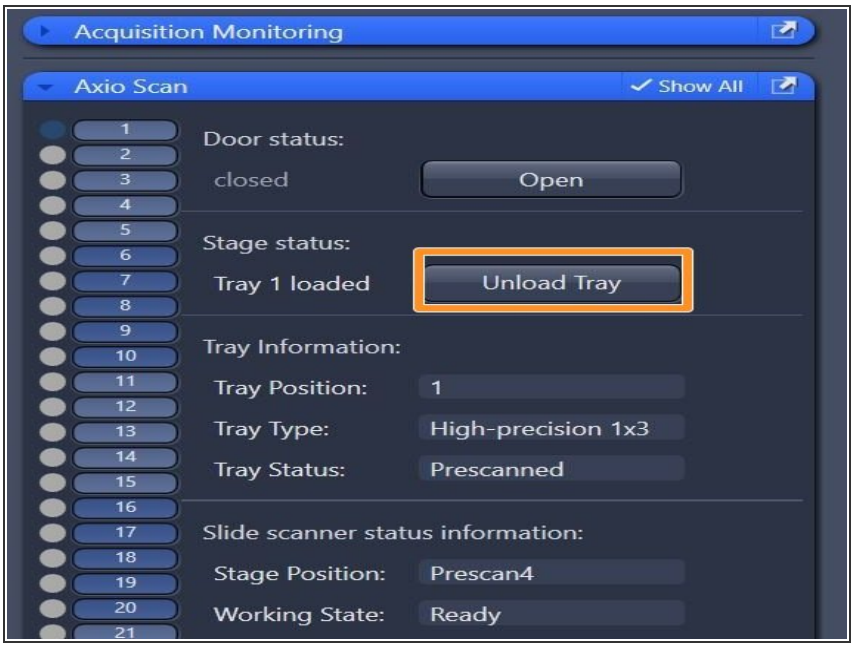






- Select your saved profile under "Default Scan Profiles" > "User Documents".
  - ⓘ The selected profile will be assigned to all slides with the status "new" .
- Select all the slides that should be imaged: Click on "Mark All" or select the slides manually.
- Click on "Start Preview Scan".
- After all previews have been captured select "Open tissue detection wizard" and edit the selected ROIs if necessary.
- In order to scan all your slides click on "Start Scan".

Step 19 — After the Scan: Unload your Slides and Transfer the Data



- Make sure that all trays are unloaded and take out your slides.
- Close the software.

⚠ After the software has been closed, you have to initiate the transfer of your data via This guide.

- If no other user booked the system after you for that day, switch off the whole system according to the "Shut down" guide . Otherwise just log off.

ⓘ To visualize alidescanner datasets without prior file conversion to .tif, the software **Zen blue**, the open source tool **QuPath** or **Imaris** can be used. This software tools are available on the ZMB virtual machines.

ⓘ To export Zeiss AxioScan .czi files to .tif we recommend to use ZEN blue. See corresponding "Export .czi files to .tif " guide.