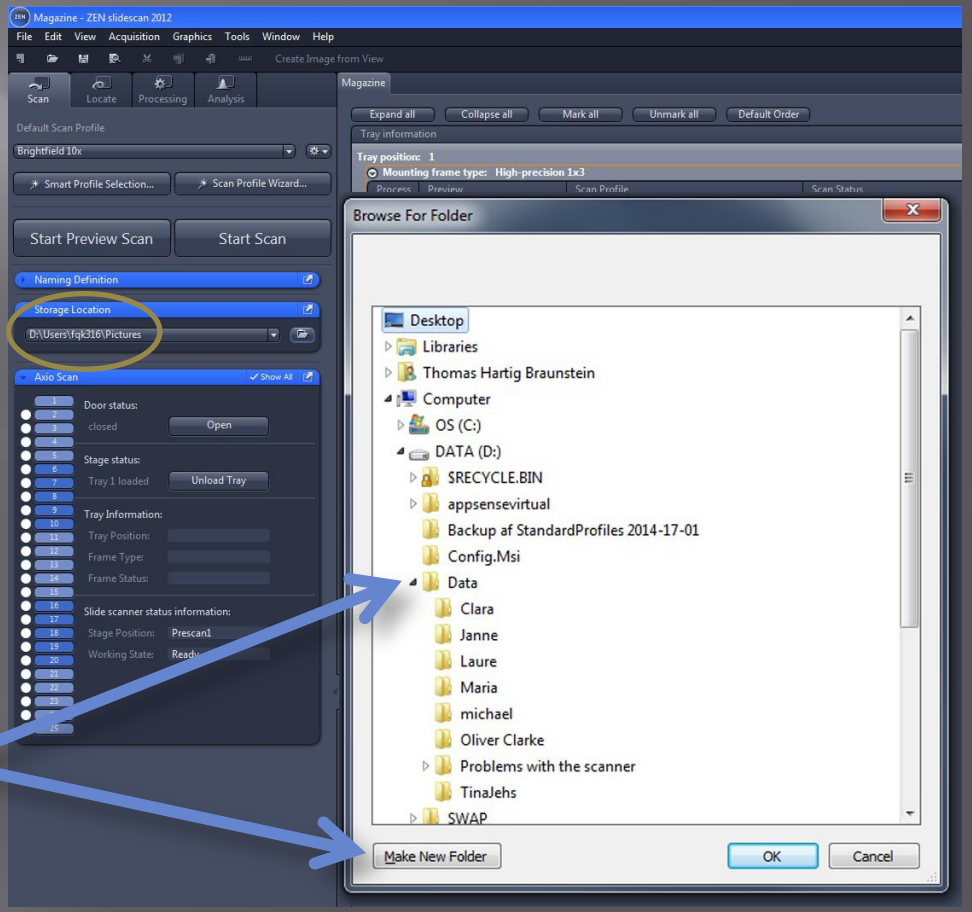
Axioscan - Startup

1. Turn on the Axioscan on (button to the left) and turn on the computer

2. Log on and start the ZEN Blue software from the desktop

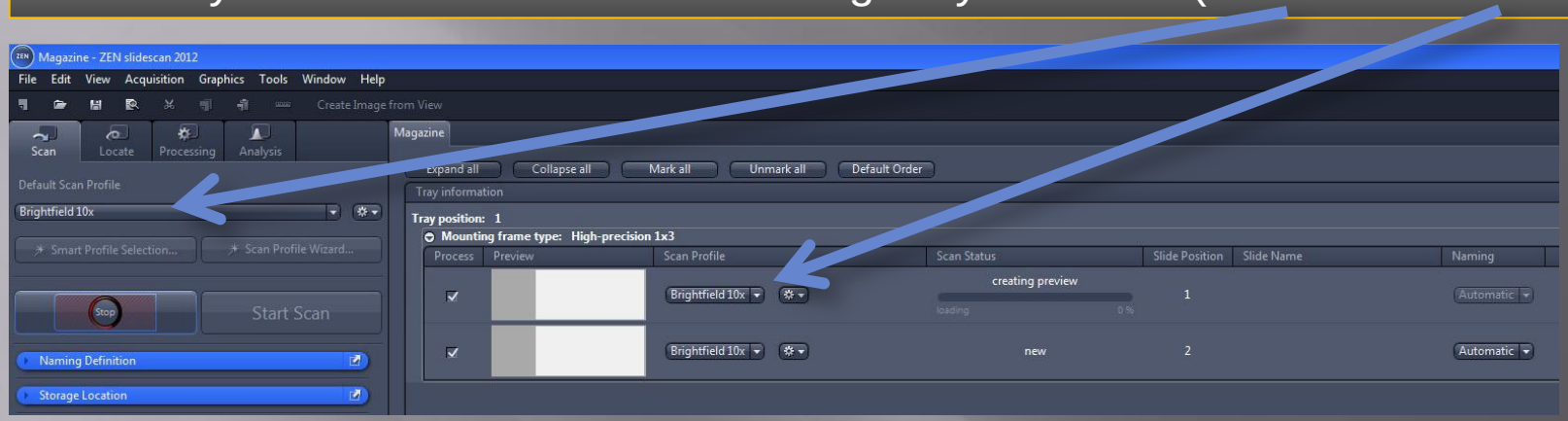
3. Press ZEN slidescan and ‘Start System’

4. Start by changing the Storage location to D:\Data\’Your Name’ and remember to move your data to your network drive RIGHT after your session has finished. Data on the D:\ will be deleted regularly.



Axioscan – Brightfield

1. Insert your slides and choose a setting for your slides (all slides/individually)



Different standard profiles are available:

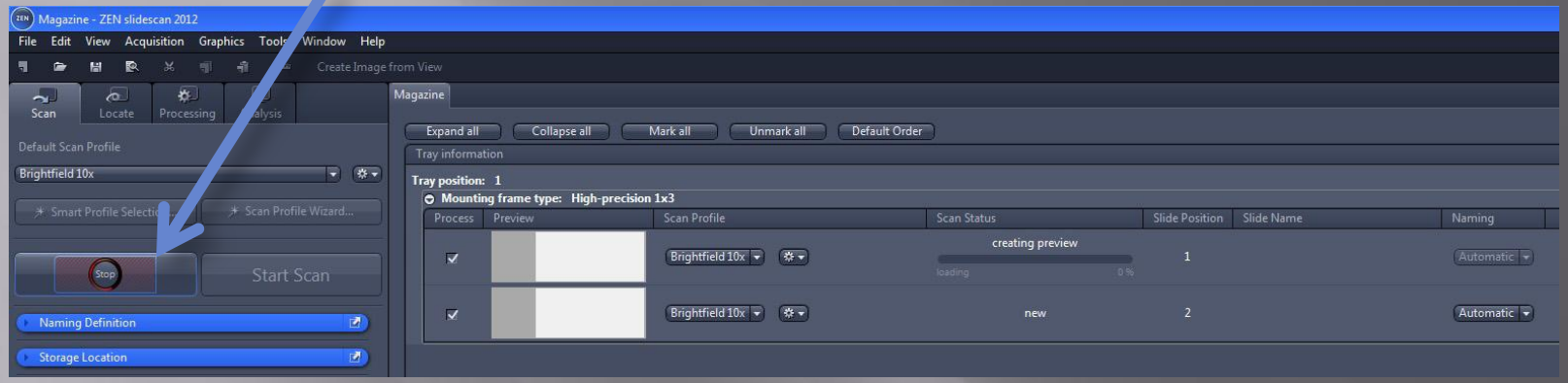
Brightfield 10x, 20x and 40x and Fluorescence 10x, 20x and 40x.
These settings can be adapted to your sample but should be saved under a new name for later use.

Standard settings are written over, each time the system is restarted. It ensures that all users always have access to the standard settings.

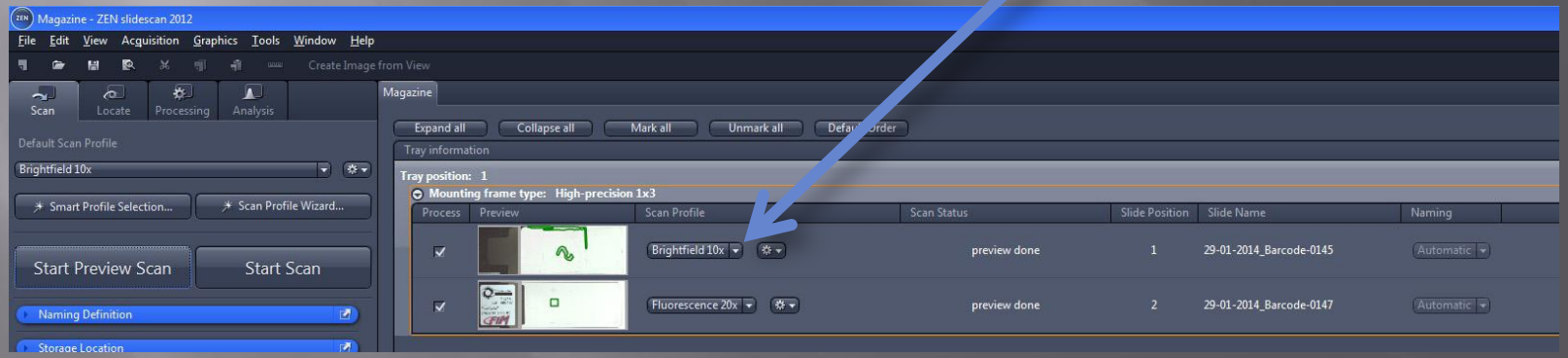
(See ‘Notes’ at the end of this manual for instructions on loading and unloading trays, global and local changes and adaptation of scan profiles.)

Axioscan – Brightfield

2. Press ‘Start Preview Scan’



3. For each slide, you may choose a different scan profile (Brightfield 10x, 20x or 40x)



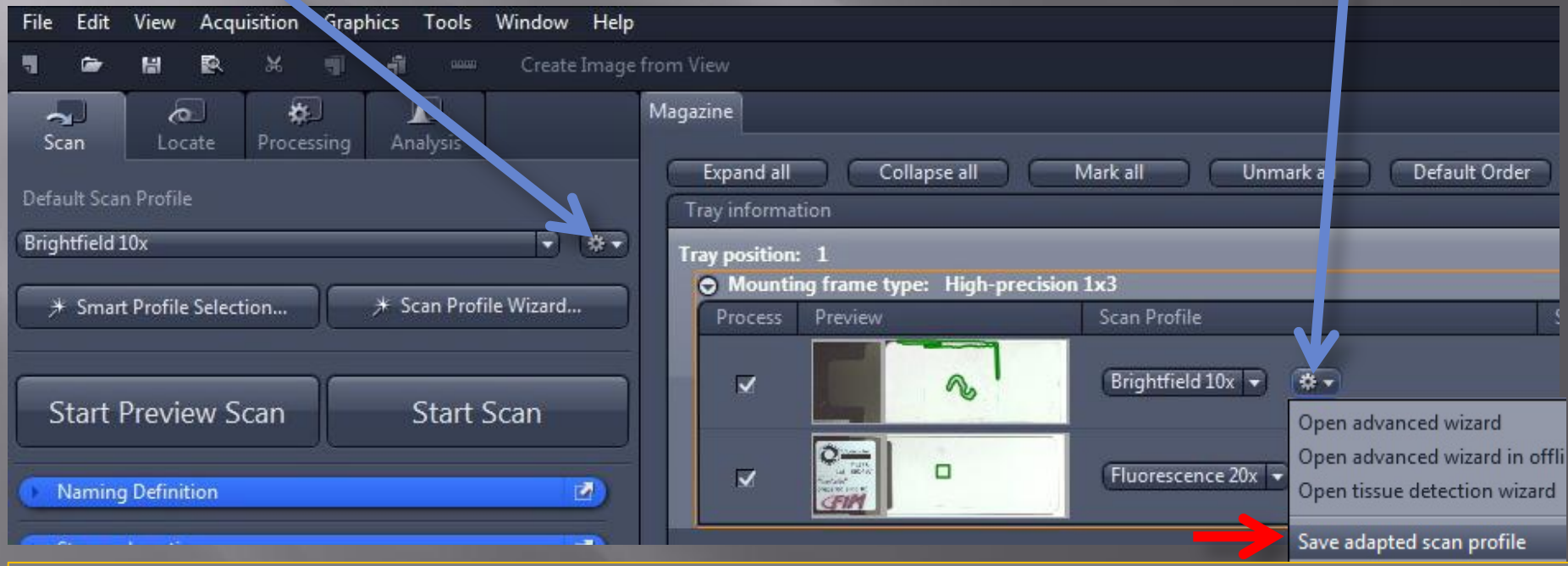
4. If you change the profile for a slide, you have to do a new Preview Scan. Before you can do a new Preview scan, you have to set the ‘Scan status’ to new. Do so by right-clicking

Axioscan – Brightfield

When you make changes to the settings, they may be either local or global changes.
Global changes affect all slides for which this scan profile is selected.
Local changes affect only one slide.
If you want a local change to become global, you can use the option ‘Save adapted scan profile’ (shown below – red arrow).

Global changes

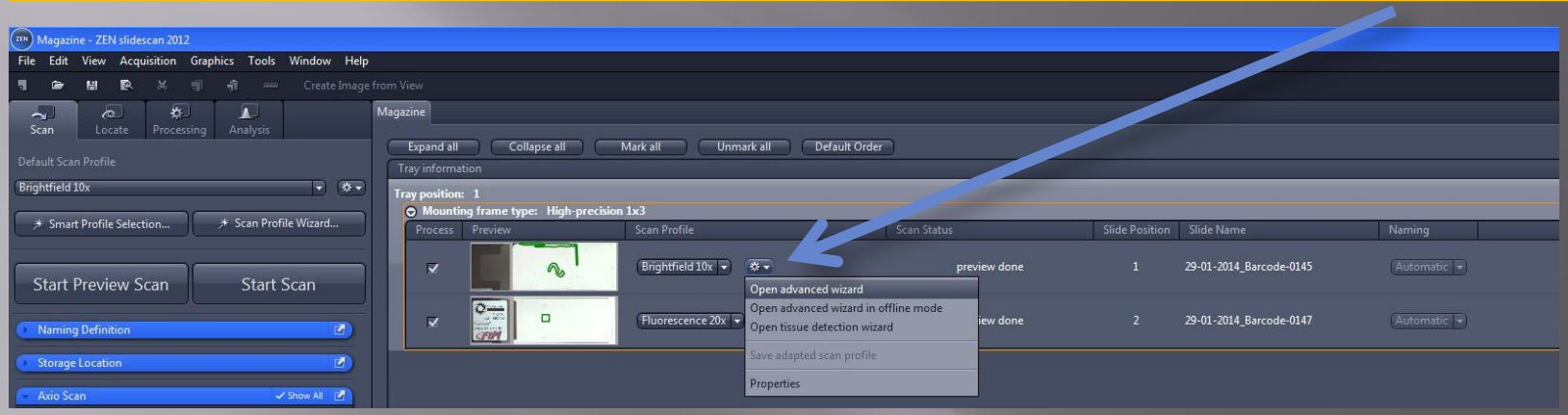
Local changes

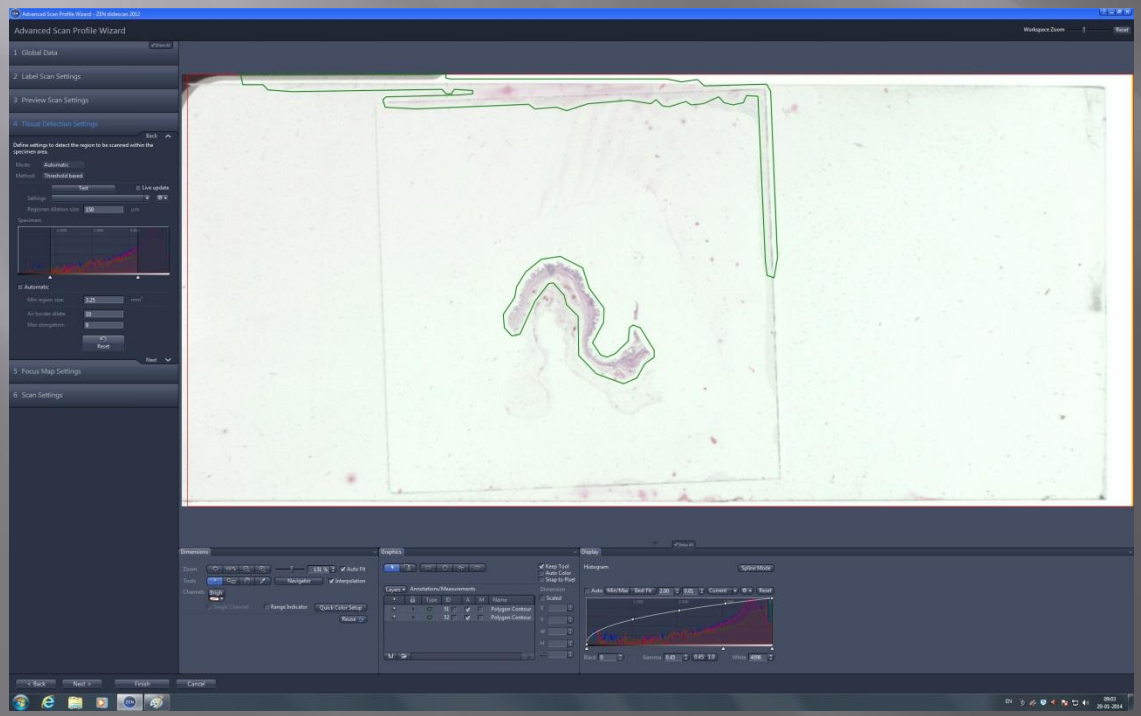


We will describe here changes to local settings.

Axioscan – Brightfield

5. In order to check focus and scan area for the slide, press ‘Open advanced wizard’

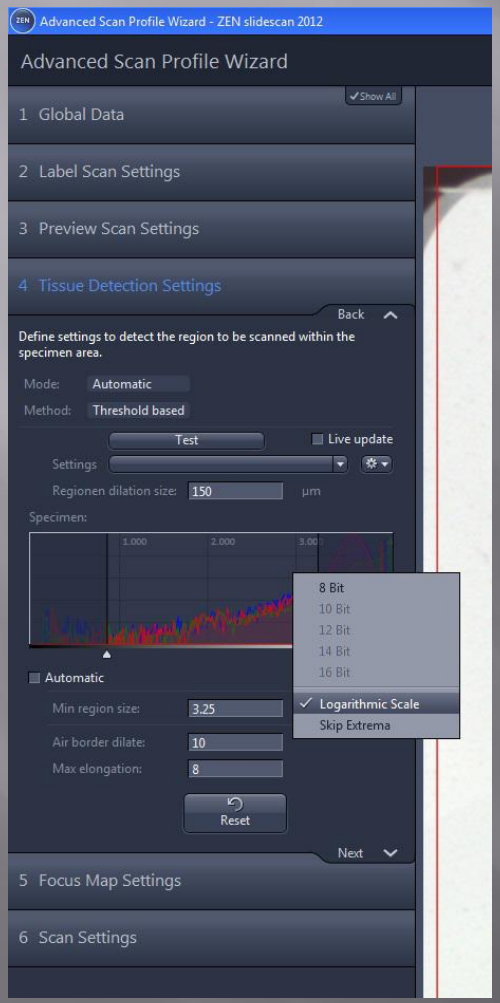




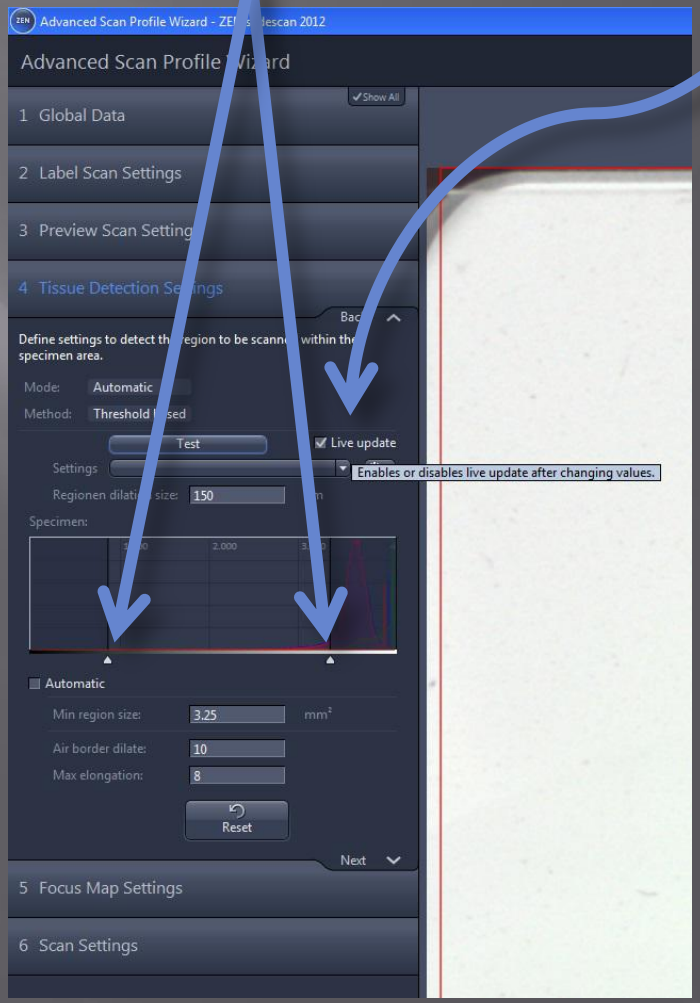
6. The green areas show the scanning areas detected by the software. These areas may be changed manually (redrawn) or by setting the detection boundaries differently. We will continue to set the boundaries automatically.

Axioscan – Brightfield

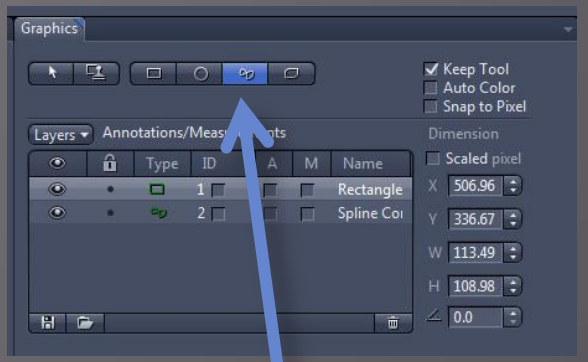
7. For automatic detection, right click in the ‘Specimen’ field and click ‘Logarithmic Scale’ off.



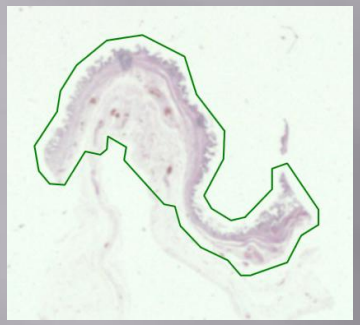
8. Press ‘Live update’ and change the boundaries for the tissue detection to see the effect.



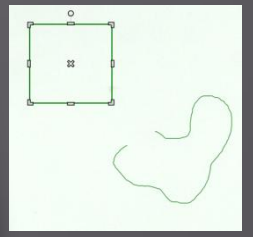
Axioscan – Brightfield



9.If the line is too close to the edge of the tissue, the 'Region dilation size' should be increased (see 8). Standard setting is 150.



10. When the settings are perfect you should have your sample delineated like the example to the left



11. (If neccessary, the outline may be drawn using the spline tool)

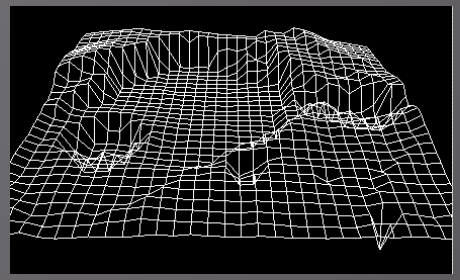
12. Press 'Next' to start defining Coarse and Fine focussing

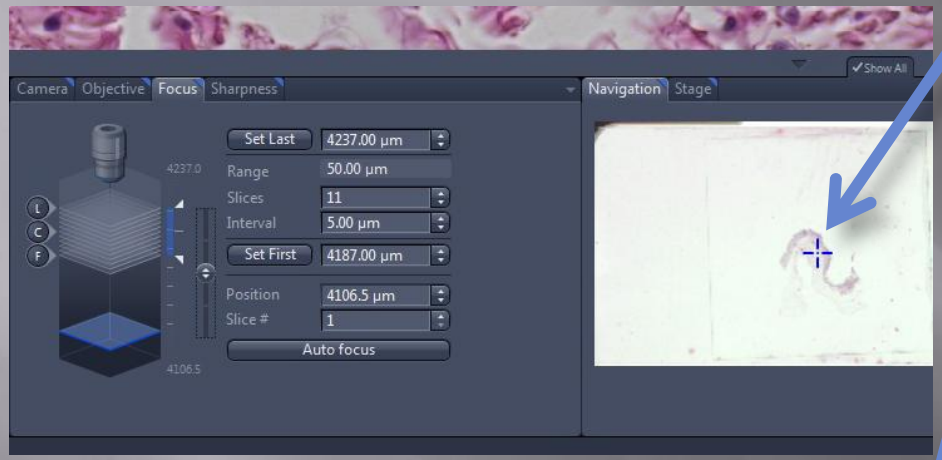
Coarse focussing. One may compare the knots of a coarse fishing net seen to the left as points where the Coarse focussing will find the focus depths

Fine focussing. The same goes for Fine focussing. The fine focussing should focus in between the Coarse focus points to make a perfect focus map for the scan procedure

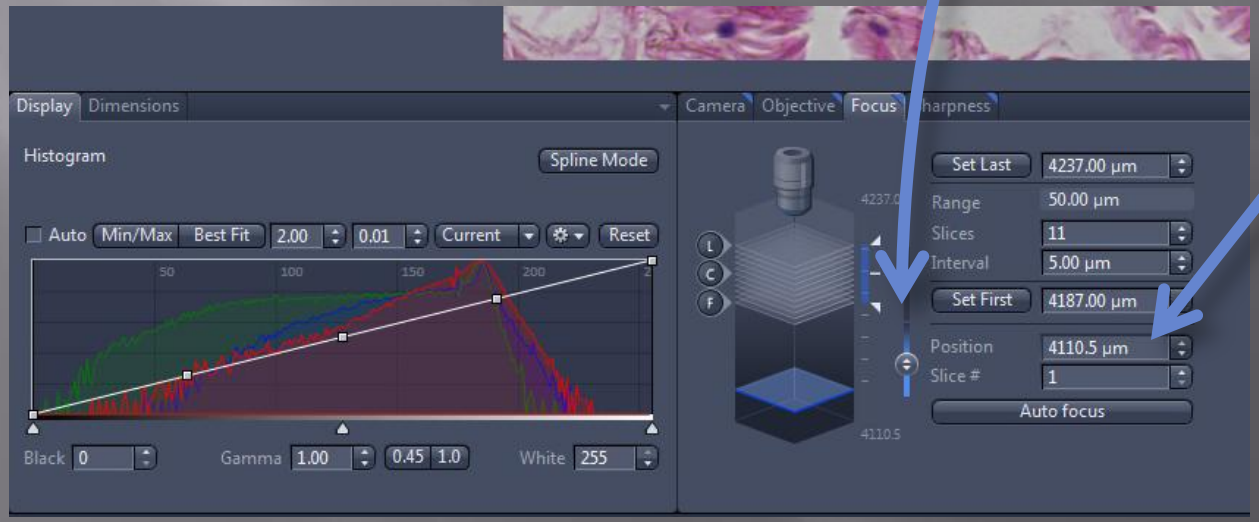
Axioscan – Brightfield

13. Coarse focussing: A topographical map of the focus depths like the map shown to the right is performed. Move to the area that will be part of the scanned area.





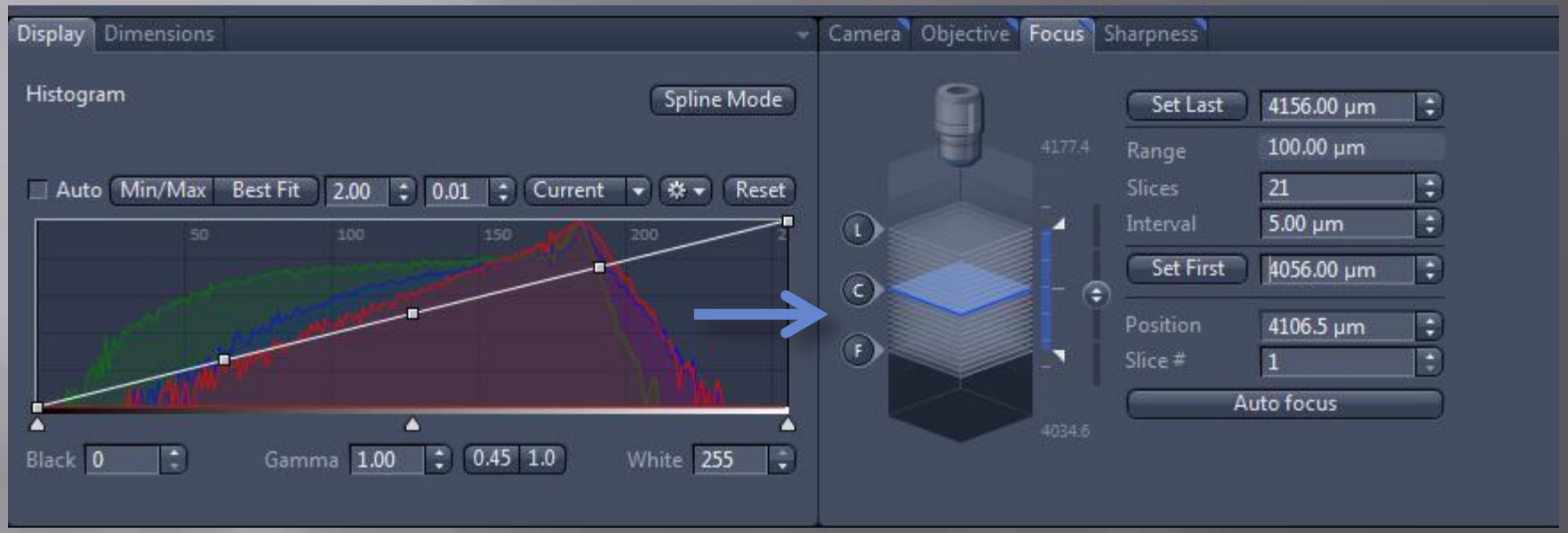
14. Press ‘Auto focus’ and observe that your specimen comes into focus. If it is not able to focus, use the slider to bring it into focus yourself.

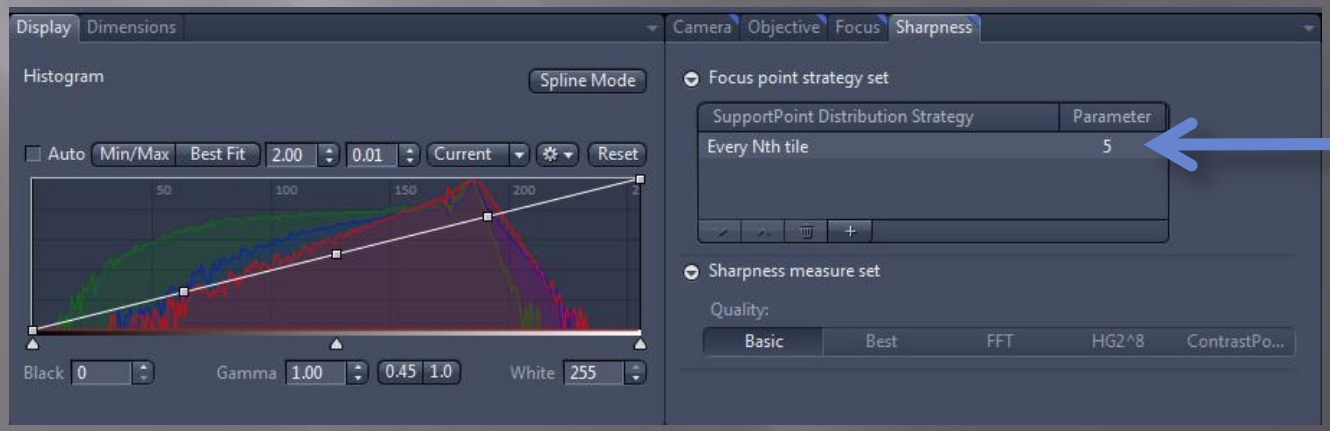


From the ‘Position’ value add and subtract 50 and type it in the ‘Set First’ and ‘Set Last’ fields, respectively. (You may use 25 instead of 50 for faster focus mapping)

Axioscan – Brightfield

15. If you did your calculation correctly you should have a picture where your blue focussed optical section lies in the middle of the stack, as seen below.

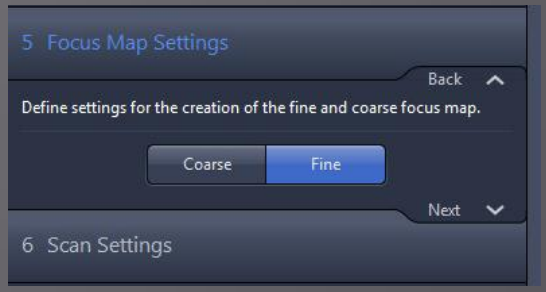


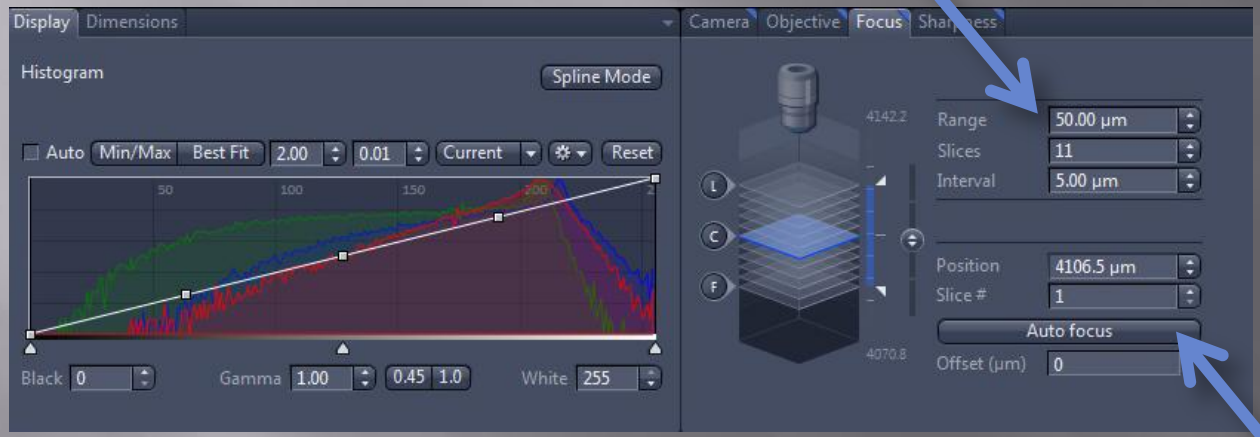


16. Under Sharpness, change the number in Parameter to 2 for small sections and 5 for larger sections (focusing will be performed every 2nd/5th tile).

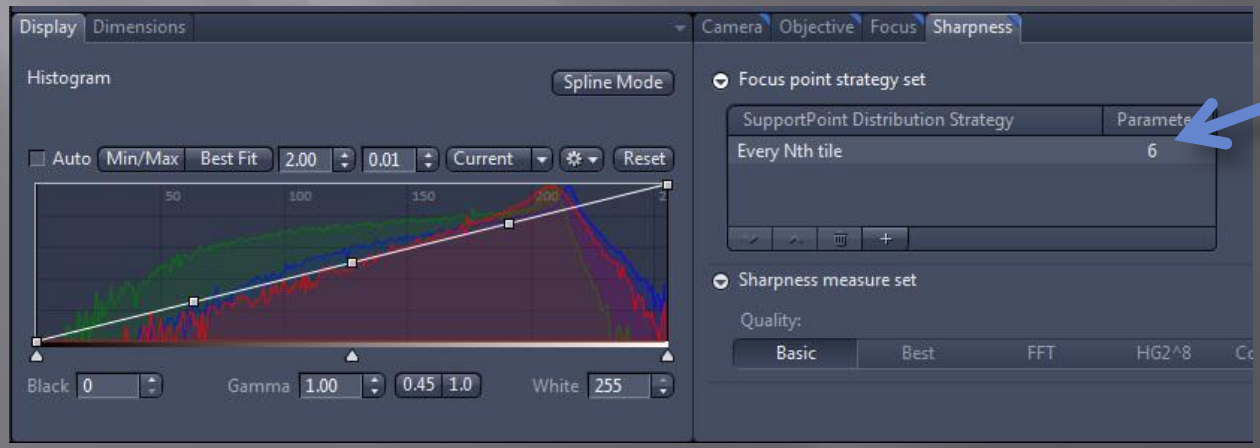
Axioscan – Brightfield

17. It is very important that you first focus with coarse focusing, before you go to Fine focussing, without changing position. Under ‘Fine’, ensure that the range is set to 50 µm.



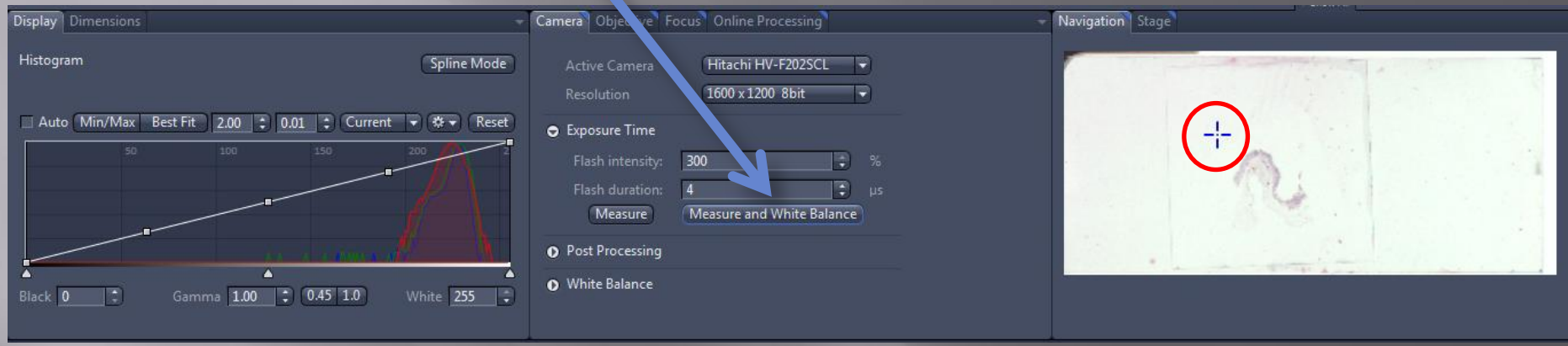


18. Check that the Axioscan can find the focus by pressing ‘Auto focus’. Set Fine focussing ‘Parameter’ to one higher than for the Coarse focussing. Press ‘Next’.

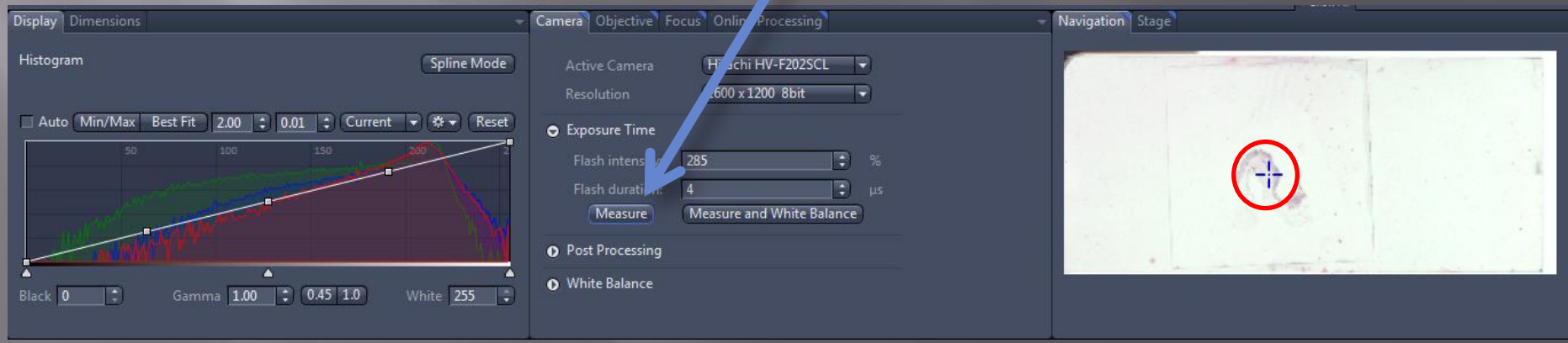


Axioscan – Brightfield

20. *Scan settings:* Under ‘Camera’, point to a place outside the scanning area and press ‘Measure and White Balance’

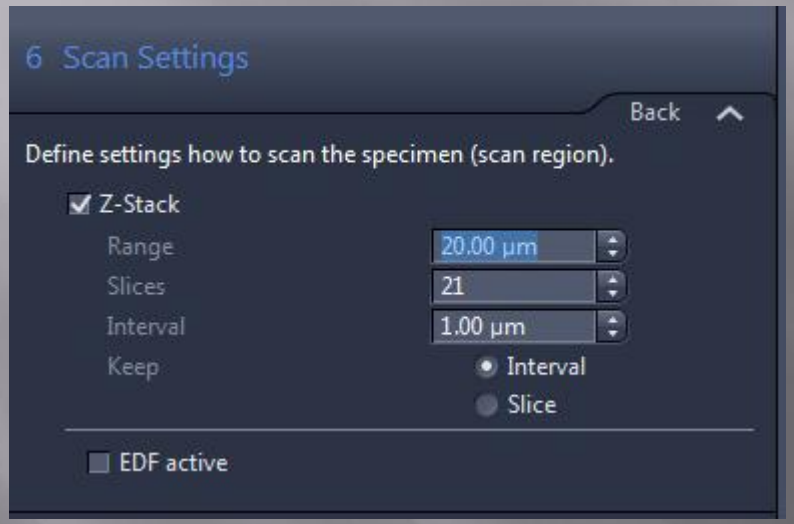


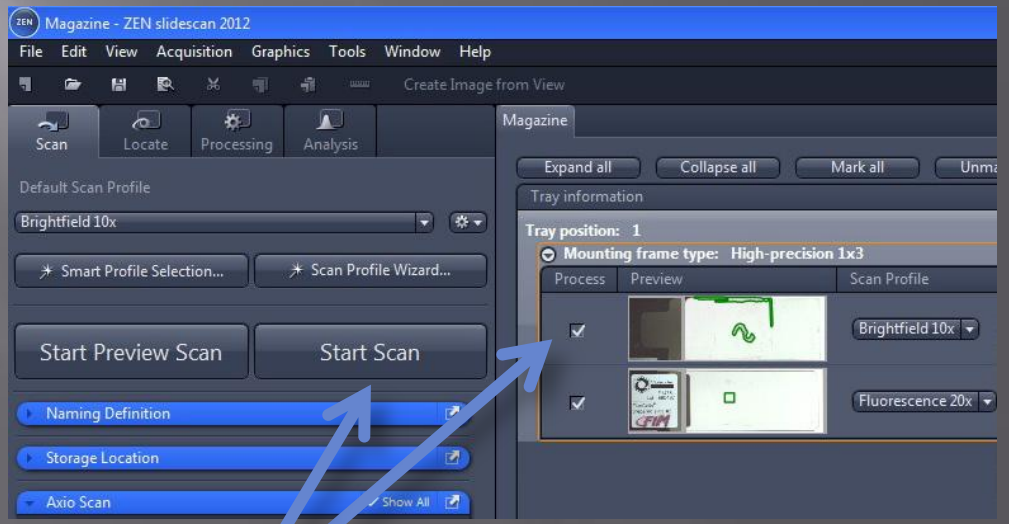
21. Now point to the section and press ‘Measure’ to get the right illumination



Axioscan – Brightfield

20. Scan settings: If desired, you may choose to scan a Z-stack to have the opportunity to change the focus plane afterwards (on your computer). This takes up more space and increases scan time. Therefore, limit the range to the thickness of your section. You can also tick off the EDF (Enhanced Depth Focus): a single image plane is created using the regions in focus from the image stack. This option saves space (not time).





21. Press ‘Finish’. These changes are local and they will be applied only to the slide next to the modified ’Scan Profile’. Press ‘Start Scan’. See next page for applying these changes globally.

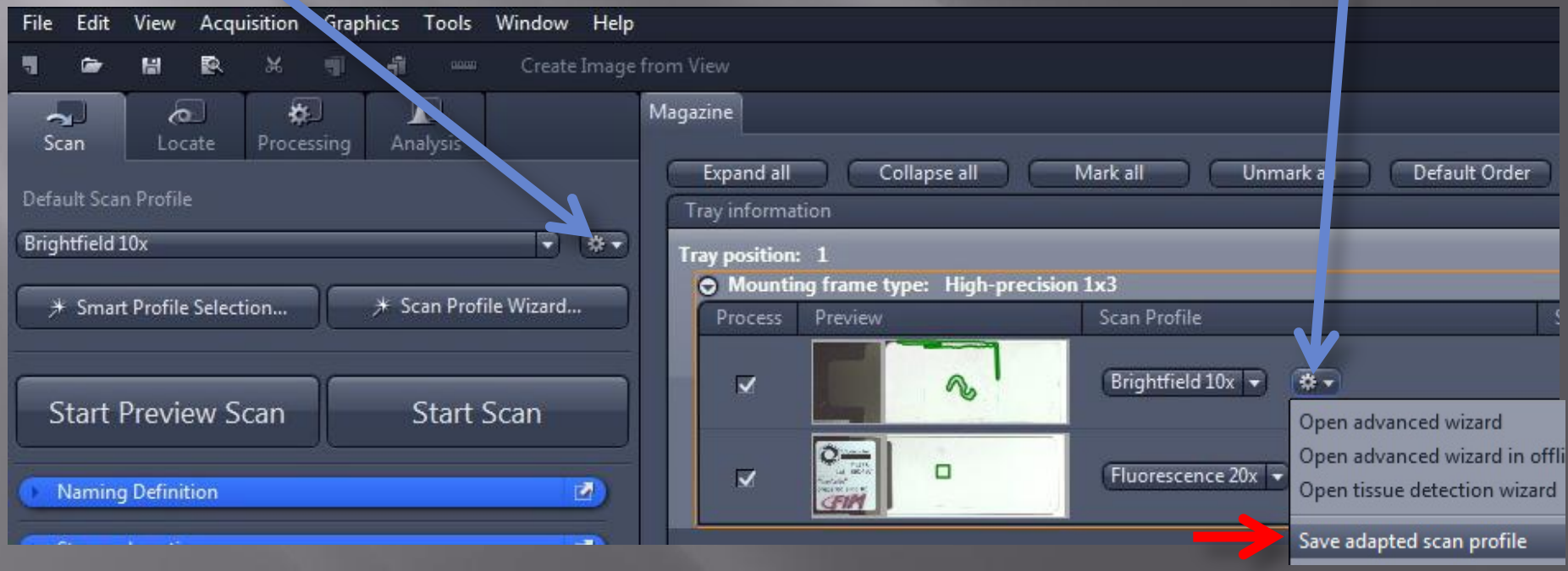
Axioscan – Notes

A few notes: If you modify one of the standard settings (i.e. Brightfield 10x, 20x or 40x, Fluorescence 10x, 20x and 40x) and you want to save these changes for later use, save them under a new name before changing them. The standard settings are written over every time the system starts up.

When you make changes to the settings, they may be either local or global changes. If you make a local change, that you would like to make global, you can use the option ‘Save adapted scan profile’ (shown below – red arrow).

Global changes

Local changes



Axioscan – Notes

Global or Local settings?

Global: In some cases you want to scan a lot of similar slides. In that case it is most convenient to adapt a global Scan Profile for all the slides. To do this follow the instructions above. To ensure that all slides will be focussed and scanned perfectly you may want to increase focussing depths (eg. From 100 to 200 µm) when designing a global Scan Profile.

Local: When you have a mix of slides, different stains and/or section thicknesses, you may want to adapt the Scan Profile for each of the slides individually to ensure the best result. In that case you will have to go through the slides manually, to optimize the focussing, white balance etc.

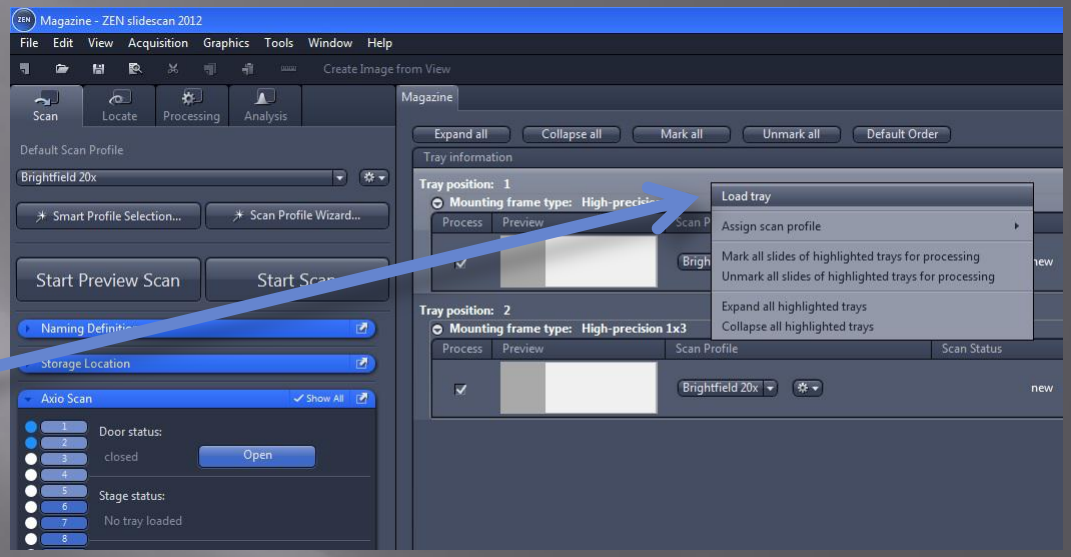
Axioscan – Notes

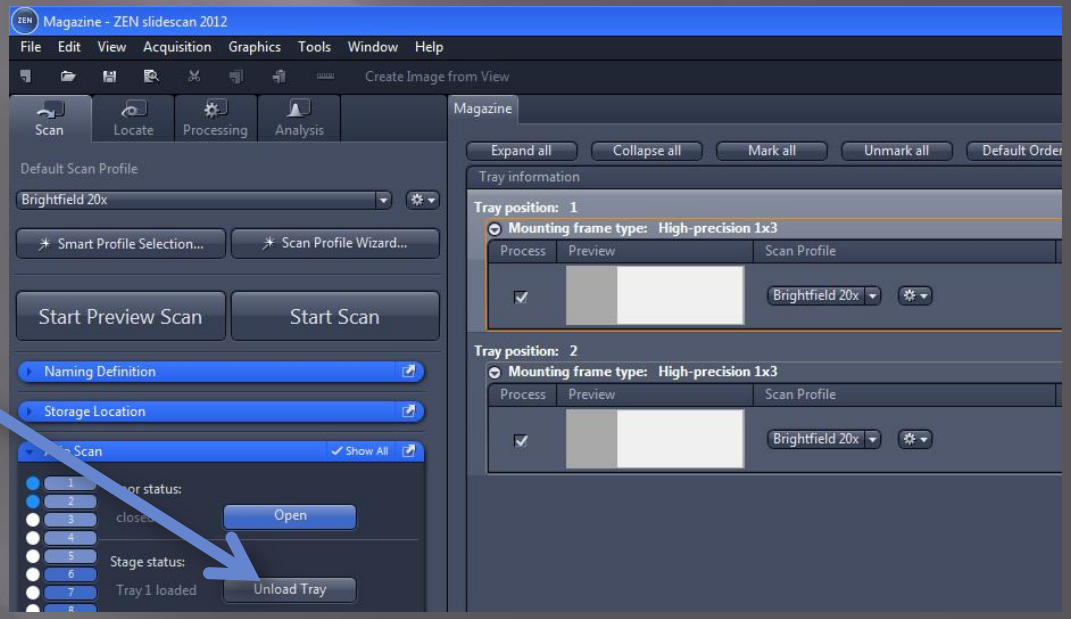
Loading trays:

Before you can change advanced settings for a slide, the tray needs to be loaded. To load a tray, right-click on the tray header and select ‘Load tray’

Unloading trays:

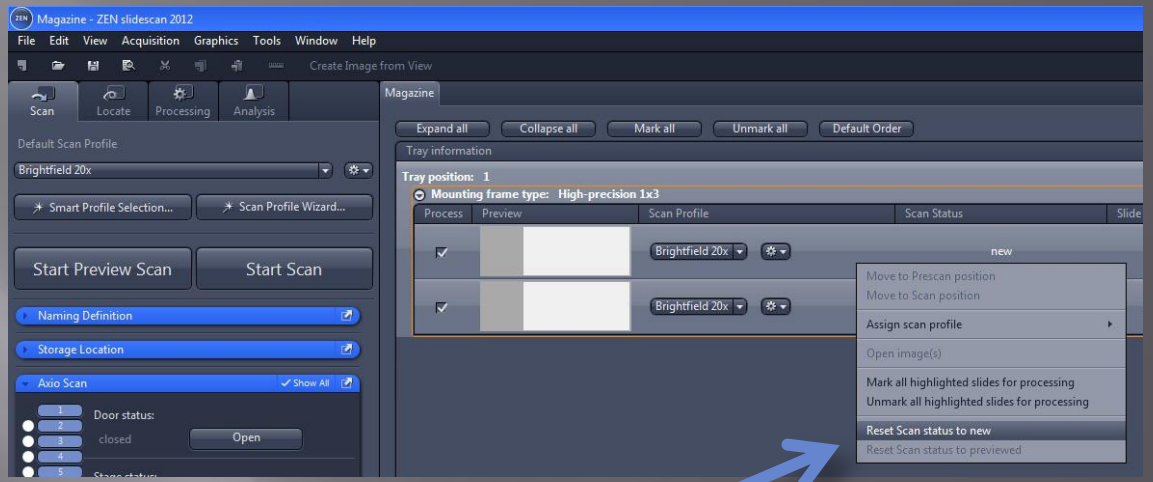
If you need to take out your slides you first have to unload the tray. Do so by pressing the ‘Unload Tray’ button.

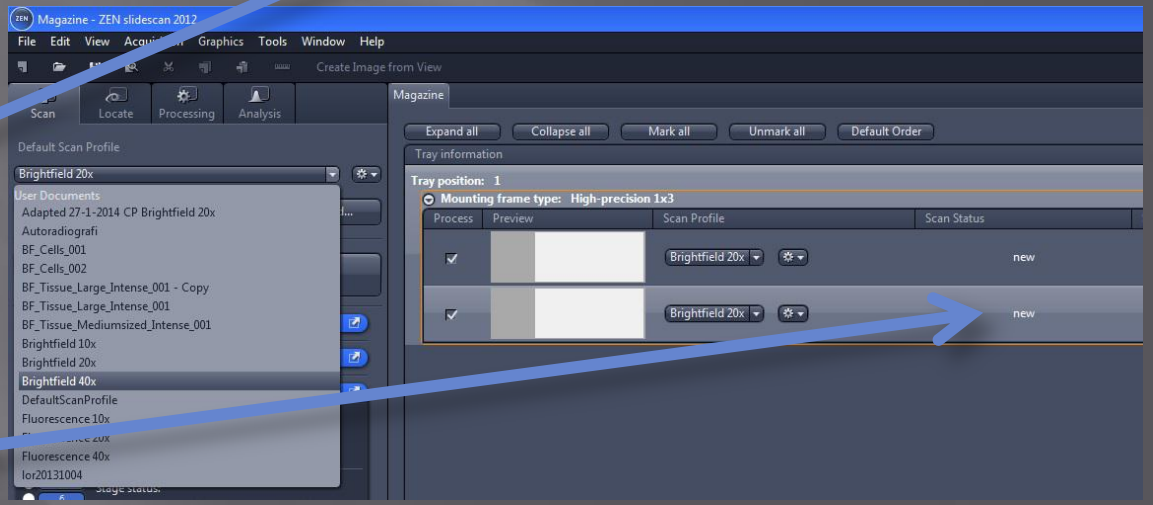




Axioscan – Notes

‘Reset scan status to new’: If you have changed a Scan Profile globally you need to ‘Reset scan status to new’ and then change back to the Scan Profile you changed, *before the changes will take effect*. You can do this for all the slides in a tray by highlighting one and pressing ‘Alt + A’ or by holding down shift while clicking on each of the slides you want to reset to new. Right-click and choose ‘Reset Scan status to new’. After this, choose the Scan Profile you want from the menu under ‘Default Scan Profile’– this will affect all slides with Scan Status ‘new’.





In case of problem, contact

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