

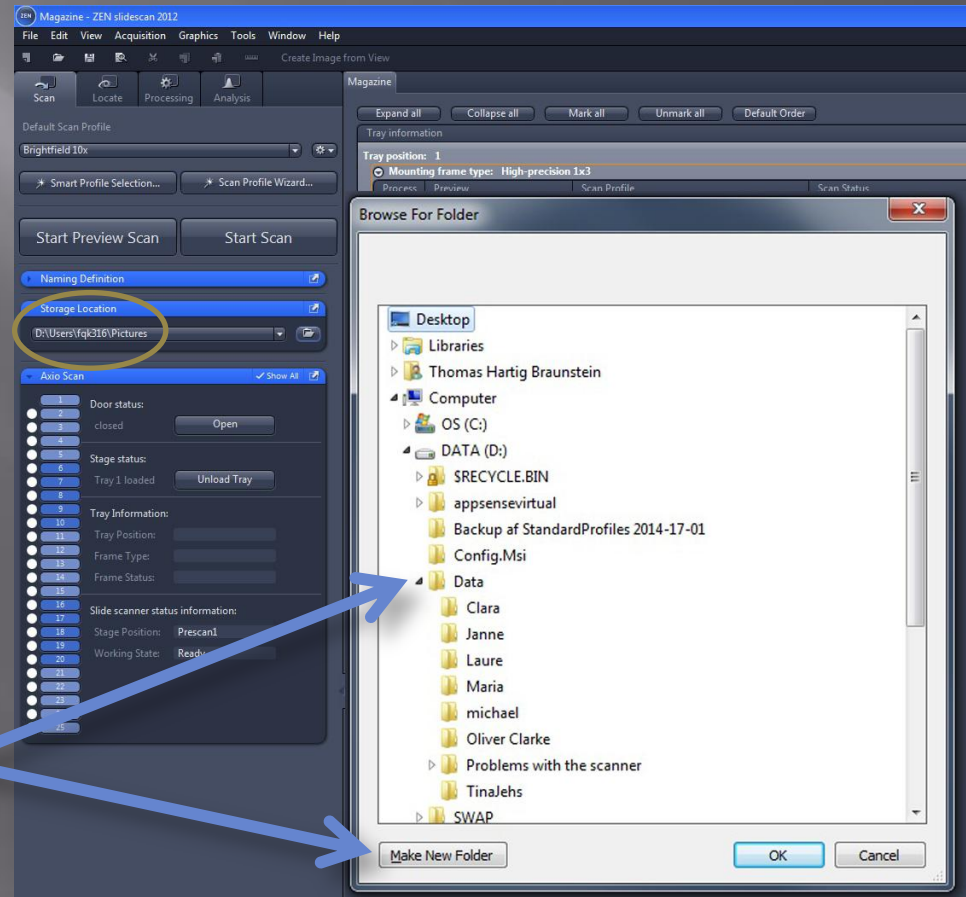
# Axioscan - Startup



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

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

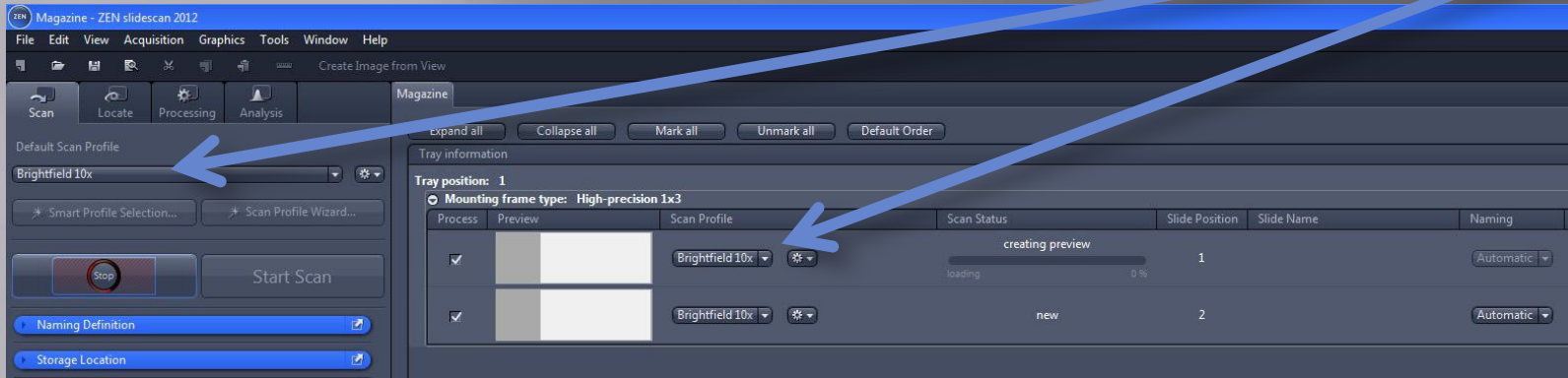
3. Press ZEN slidescan and 'Start System'



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 – Fluorescence

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.

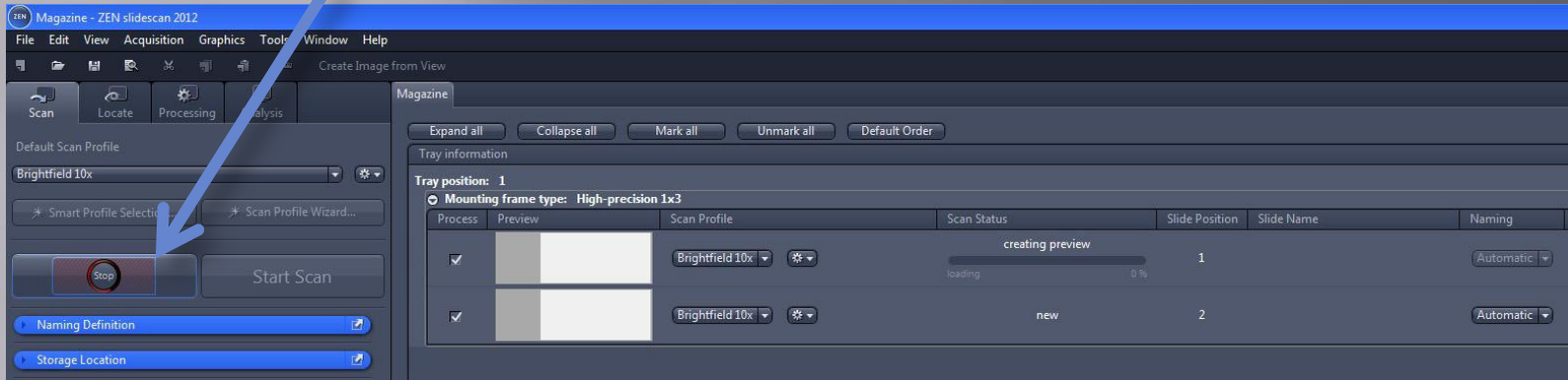
These settings can be adapted to your sample but should be saved under a new name for later use.

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

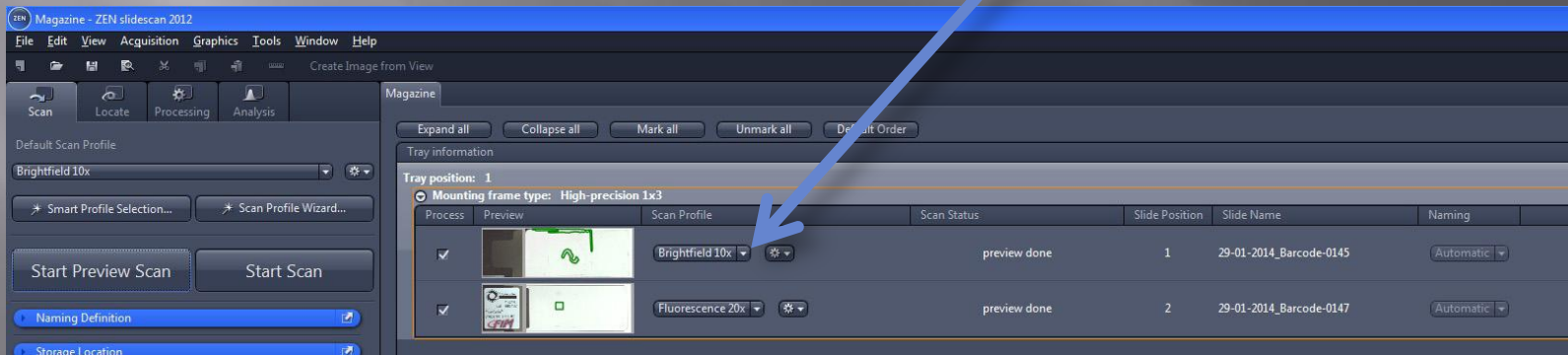
(See 'Notes' at the end of this manual for instructions on loading and unloading trays, global and local changes and adaptation of scan profiles.)

# Axioscan – Fluorescence

## 2. Press 'Start Preview Scan'



## 3. For each slide, you may choose a different scan profile (Fluorescence 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 – Fluorescence

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

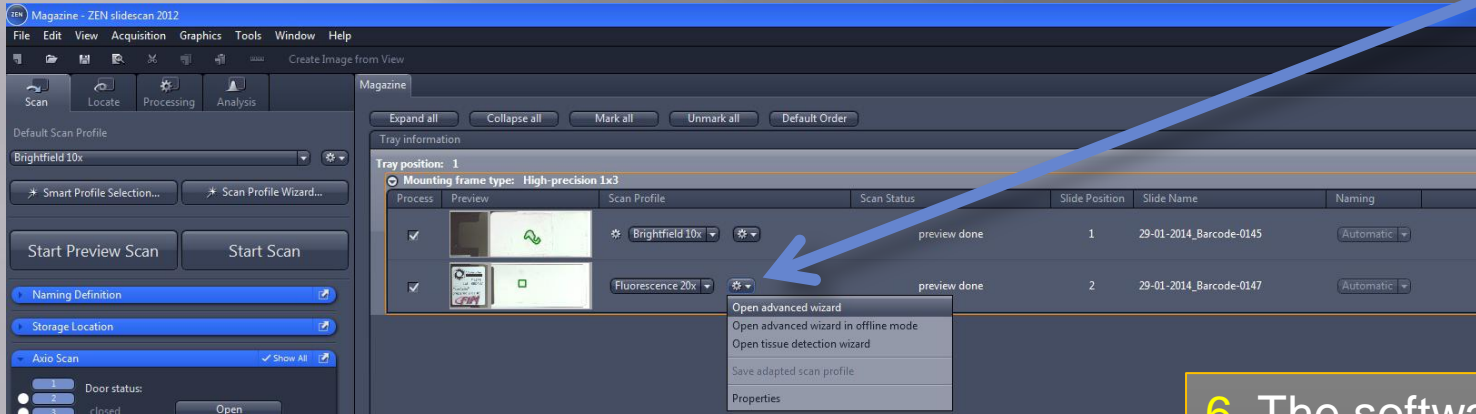
Local changes

The screenshot displays the Axioscan software interface. The menu bar includes File, Edit, View, Acquisition, Graphics, Tools, Window, and Help. The toolbar contains icons for Scan, Locate, Processing, and Analysis. The 'Default Scan Profile' section shows 'Brightfield 10x' selected, with a gear icon for settings. Below this are buttons for 'Smart Profile Selection...' and 'Scan Profile Wizard...'. The 'Start Preview Scan' and 'Start Scan' buttons are also visible. The 'Tray information' section shows 'Tray position: 1' and 'Mounting frame type: High-precision 1x3'. A table lists two slides with their respective scan profiles: 'Brightfield 10x' and 'Fluorescence 20x'. A red arrow points to the gear icon for the 'Fluorescence 20x' profile, which has opened a context menu with options: 'Open advanced wizard', 'Open advanced wizard in offline mode', 'Open tissue detection wizard', and 'Save adapted scan profile'.

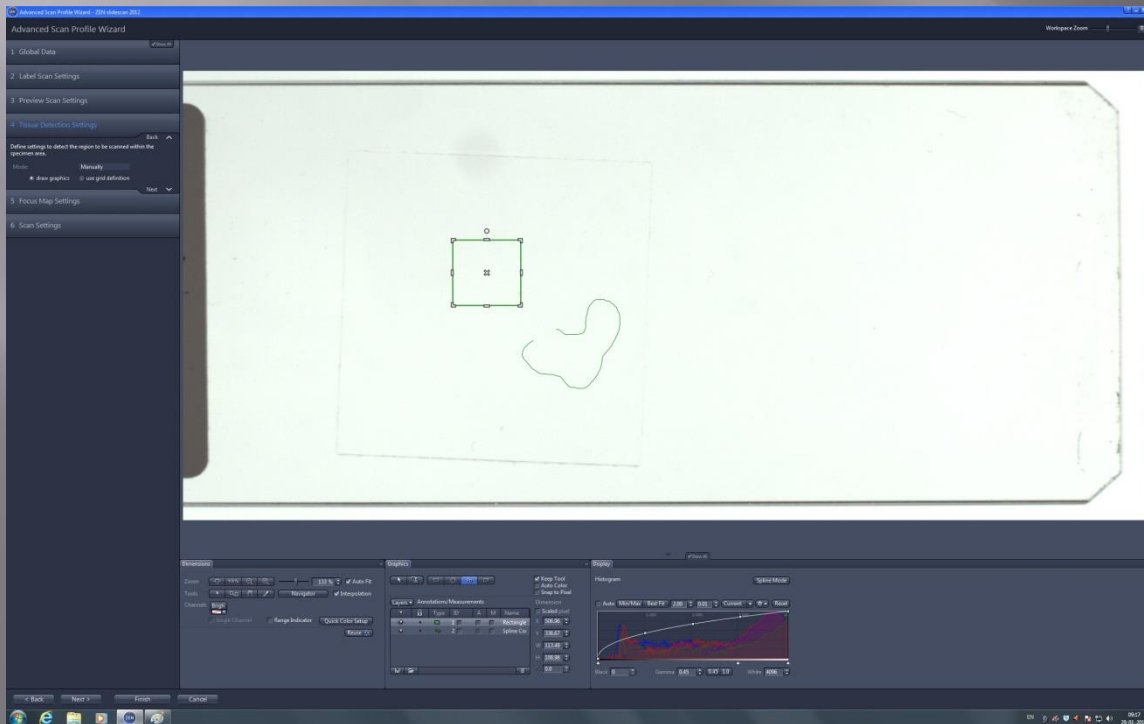
We will describe here changed to local settings.

# Axioscan – Fluorescence

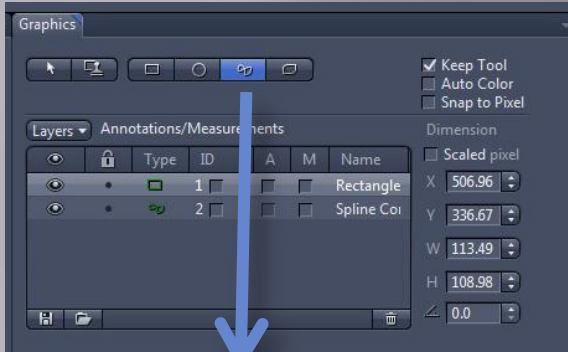
5. In order to check focus and scan area for the slide, press 'Open advanced wizard'



6. The software can't find fluorescently labelled areas (no staining in bright light). The square green area shows the scanning area preset by the software. The line shows a handdrawn area. To choose the area yourself, choose the spline tool (highlighted in blue below the area), hold down left mouse button to draw and right-click to close the line.

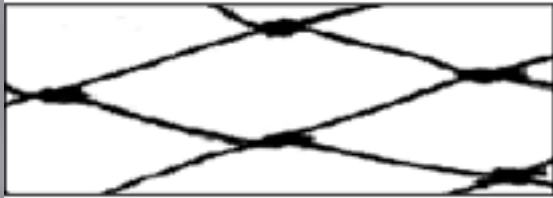
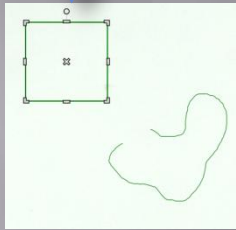


# Axioscan – Fluorescence



7. There is also a possibility to draw directly on the slide with a black marker and let the software recognize this area automatically. Or if you have a tissue section the areas of interest may be detected using RAC (Ring Aperture Contrast). To use these options talk to the staff.

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



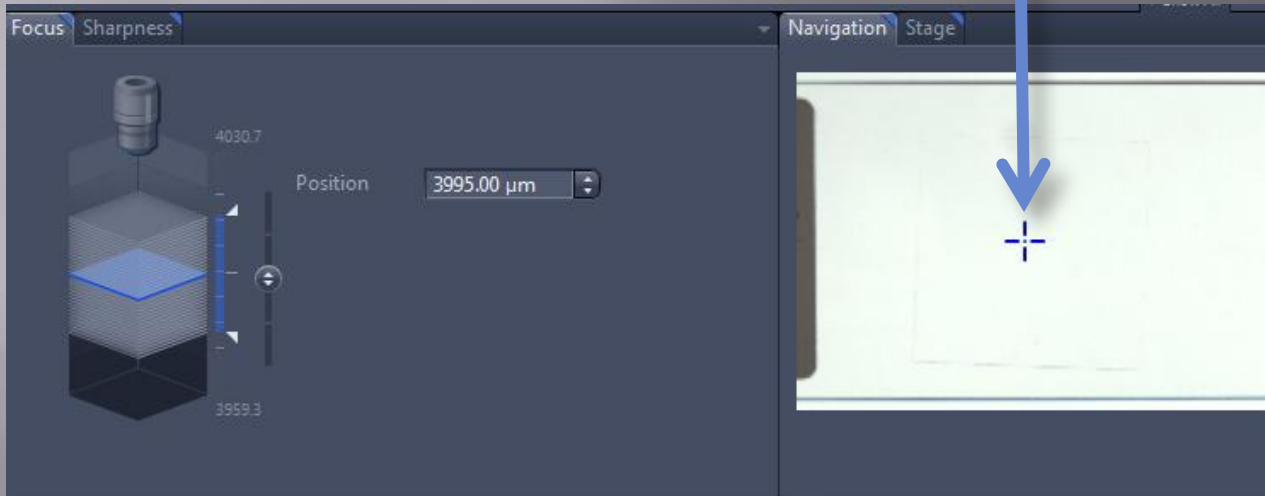
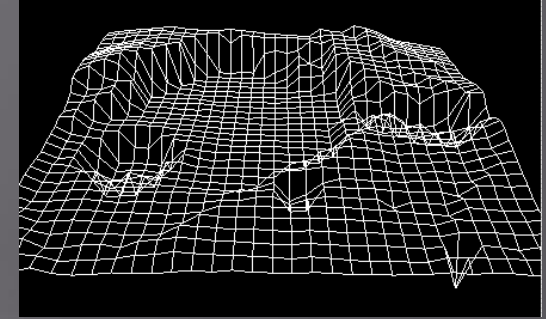
**Coarse focussing.** One may compare the knots of a coarse fishing net seen to the left as points where the Coarse focussing will find the focus depths



**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 – Fluorescence

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



10. The best way to perform a coarse and fine focus map is by using nucleic stain (like DAPI og Hoechst). This stain is normally very bright and need very short exposure time. The standard fluorecence profiles are all set up to use nucleic stains for focussing maps. Contact the staff if you don't have nucleic stain in your fluorescent samples and need to set up another focussing channel.

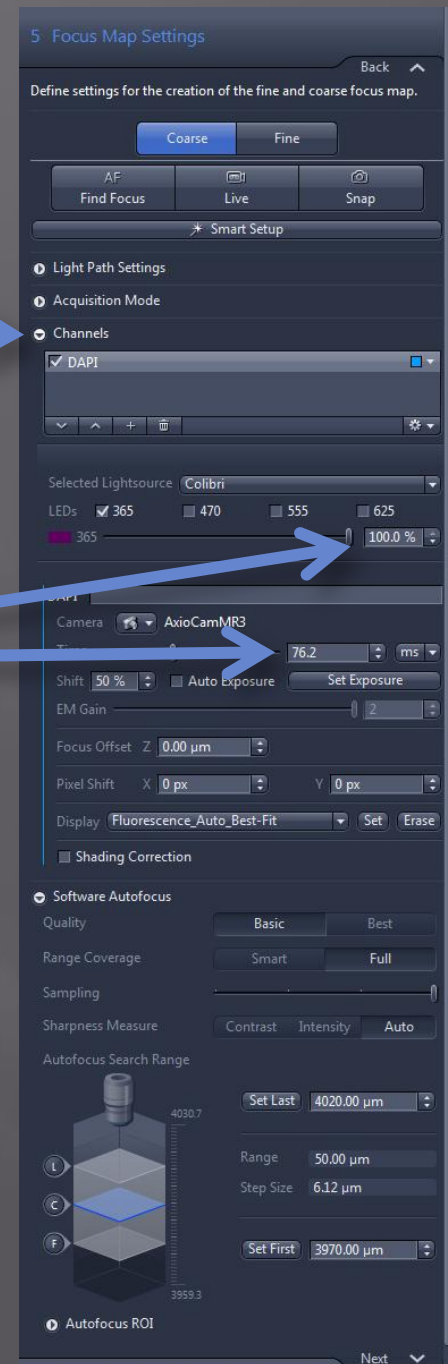
# Axioscan – Fluorescence

11. If you need another channel than the nucleic stains DAPI or Hoechst for focussing you may set it up by opening the channel tab.

After that press the 'Smart Setup' button (see below) above to choose another focussing channel.

Once you have the correct focussing channel, set the LED to 100% and the exposure to app. 100 ms.

Now press 'Snap', the rightmost top-button to see if you are anywhere near the correct focus.





# Axioscan – Fluorescence

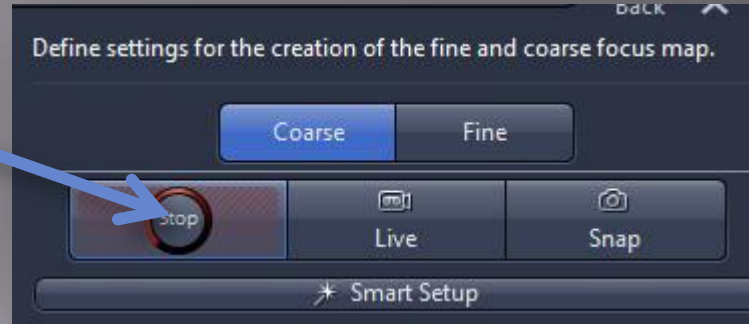
12. You can now press 'Auto Focus' and 'Live' to see if the scanner is able to find the correct focus automatically. If the focus lies outside the predefined search area (like in the example below), use the focussing slider to find the correct focus yourself.

Now add and subtract 25  $\mu\text{m}$  from the focus position to calculate the numbers for 'Set First' and 'Set Last'

The screenshot displays the software interface for the Axioscan microscope. The left panel, titled 'Software Autofocus', includes settings for Quality (Basic/Best), Range Coverage (Smart/Full), Sampling, Sharpness Measure (Contrast/Intensity/Auto), and Autofocus Search Range. The search range is defined by 'Set Last' (4059.00  $\mu\text{m}$ ) and 'Set First' (4009.00  $\mu\text{m}$ ), with a Range of 50.00  $\mu\text{m}$  and Step Size of 6.12  $\mu\text{m}$ . A 3D model of a sample is shown with a blue plane indicating the current focus position. The right panel, titled 'Focus Sharpness', shows a 'Position' of 4034.58  $\mu\text{m}$ . A histogram is displayed in the center, showing the intensity profile of the sample. The histogram has a 'Spline Mode' button and a 'Reset' button. The histogram shows a peak at approximately 1000  $\mu\text{m}$  and a secondary peak at approximately 3000  $\mu\text{m}$ . The histogram also has 'Black' (0), 'Gamma' (1.00), and 'White' (4096) settings. A blue arrow points from the text 'Now add and subtract 25  $\mu\text{m}$  from the focus position to calculate the numbers for 'Set First' and 'Set Last' to the 'Set Last' and 'Set First' fields in the Autofocus Search Range panel. Another blue arrow points from the same text to the 'Position' field in the Focus Sharpness panel. A third blue arrow points from the 'Position' field to the focus slider in the Focus Sharpness panel. A fourth blue arrow points from the 'Position' field to the 'Set Last' field in the Autofocus Search Range panel.

# Axioscan – Fluorescence

13. You should now test if the autofocus finds the correct focus and that it ends of in the middle of the stack. 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.



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

Autofocus Search Range

Set Last: 4059.00  $\mu\text{m}$

Range: 50.00  $\mu\text{m}$

Step Size: 6.12  $\mu\text{m}$

Set First: 4009.00  $\mu\text{m}$

Autofocus ROI

Next

6 Scan Settings

Display Dimensions

Histogram

Spline Mode

Auto Min/Max Best Fit 2.00 0.01 Current \* Reset

Black 0 Gamma 1.00 0.45 1.0 White 4096

Focus Sharpness

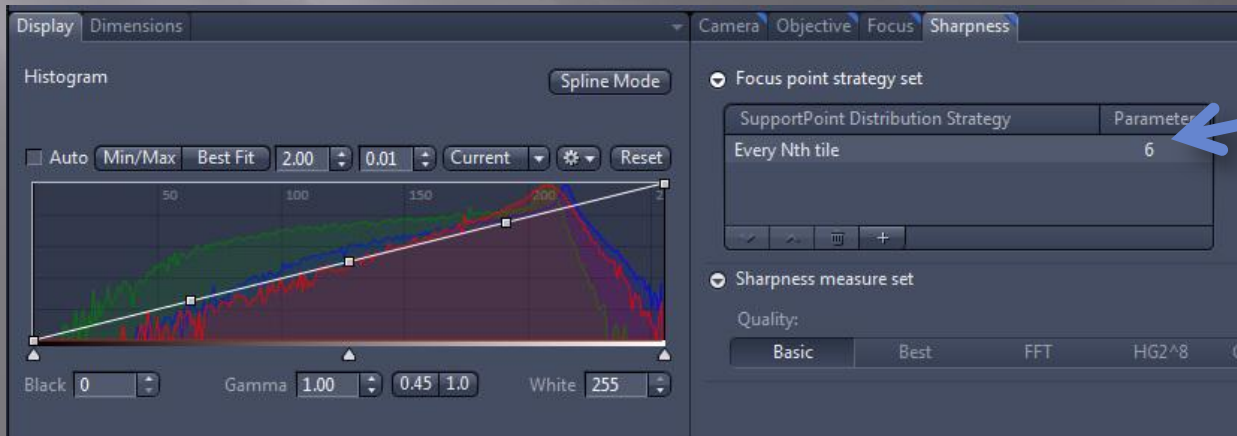
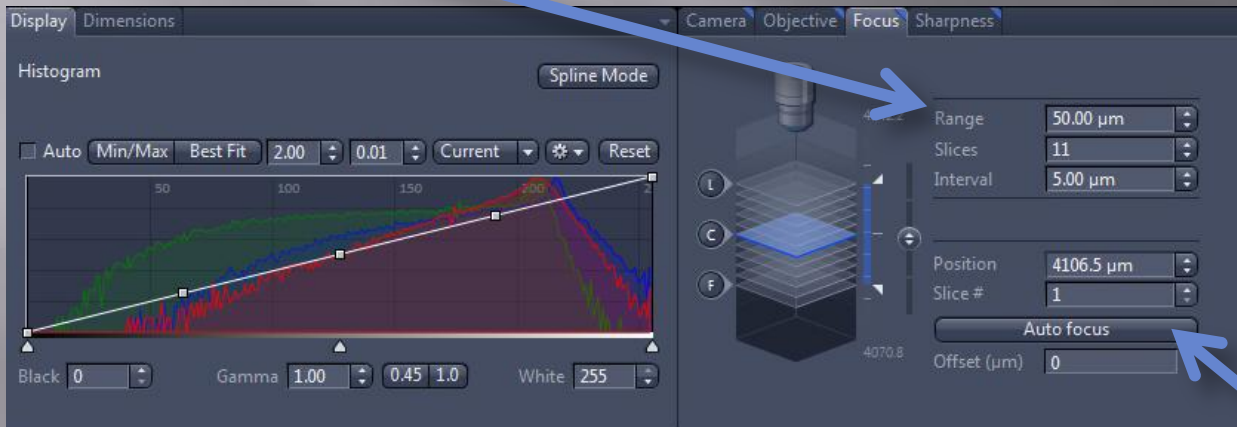
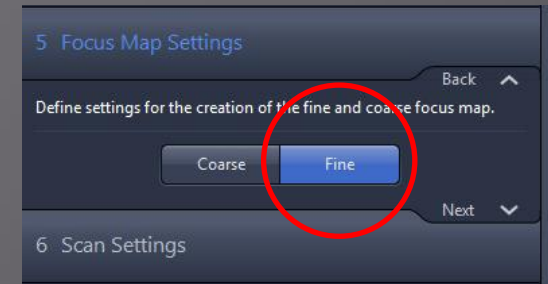
Focus point strategy set

SupportPoint Distribution Strategy	Parameter
Every Nth tile	5

A blue arrow points from the text in block 13 to the 'Autofocus ROI' section. Another blue arrow points from the text in block 14 to the 'Parameter' value of 5 in the sharpness settings table.

# Axioscan – Fluorescence

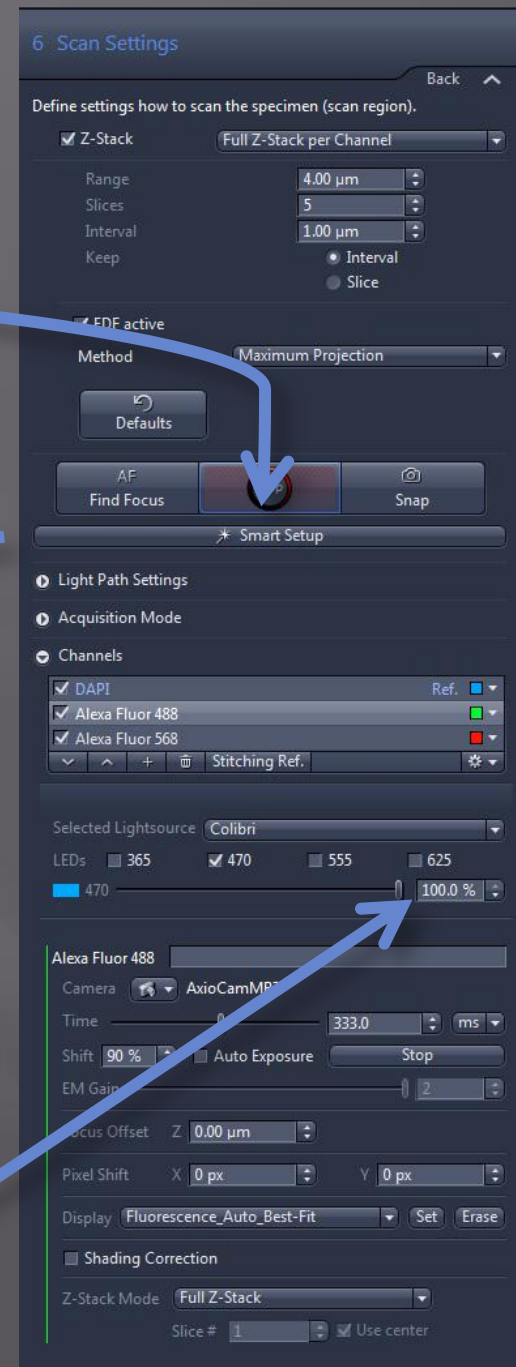
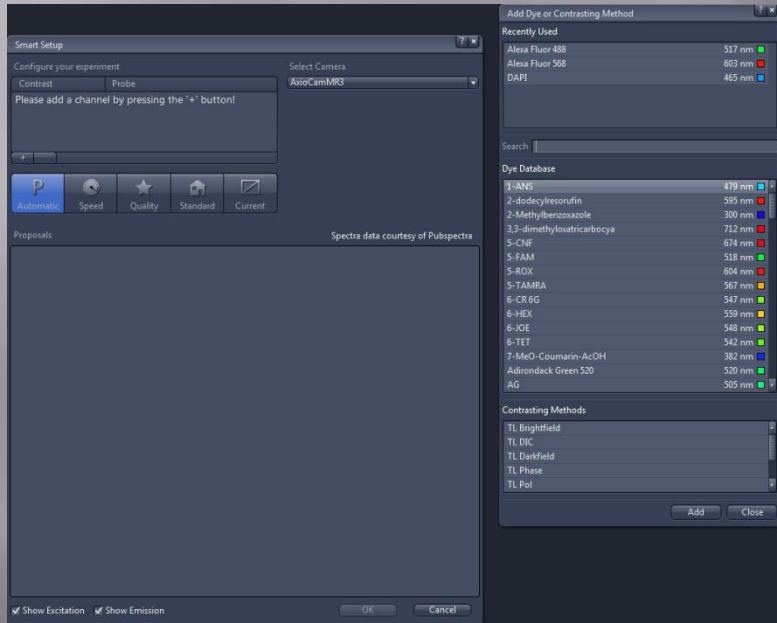
15. It is very important that you first focus with coarse focusing and that the focus is in the middle of the stack before you go to Fine focussing, without changing position. Under 'Fine', ensure that the range is set to 25-50  $\mu\text{m}$ .



18. Check that the Axioscan can find the focus by pressing 'Auto focus' – press 'Live' to see the result. Set Fine focussing 'Parameter' to one higher than the number used in Coarse focussing. Press 'Next'.

# Axioscan – Fluorescence

19. *Scan settings:* The default setting is three standard colors. If you need something different than this you may use the 'Smart Setup'. The 'Smart Setup' will allow you to choose the exact right fluorophores and the best suited filters.



Once you have chosen your fluorophores ensure that they are all set to 100% in intensity, to minimize the exposure time. See next page for advice on filters and scanning.

# Axioscan – Fluorescence

20. 'Light Path Settings': If you have a triple/quadro staining with (blue, green and red (+far red)) you should keep the same filter in place and only change wavelengths of the LEDs to make the scanning fast. This is how this standard setting is set up and in this case you don't need to make changes. The quadro filter (No. 25) is shown to the right (specifications in the software are not correct). However, sometimes the quadro filter is not suitable for your fluorophores and separate filters are needed. This has a great impact on scanning time and should be carefully considered before starting the scan. If the 'Smart Setup' is used this normally results in a setup with separate filters for all fluorophores (slow).

Please ask Staff if you are in doubt about filters and wavelengths.

Quadro filter

Quadro filter specifications:

Excitation	387/485/559/649
Emission	440/521/607/700
LEDs	365/470/555/625

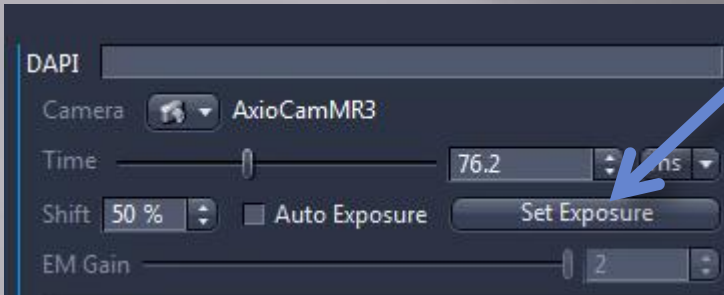
The screenshot shows the 'Light Path Settings' window in a software application. At the top, there are several 'Go!' buttons for different stages: 'Before Experiment: HardwareBefore', 'Before DAPI: Before [DAPI]', 'After DAPI: After [DAPI]', 'Before Alexa Fluor 488: Before [AF480]', 'After Alexa Fluor 488: After [AF480]', 'Before Alexa Fluor 568: Before [AF568]', 'After Alexa Fluor 568: After [AF568]', and 'After Experiment: HardwareAfter'. Below these are 'Previous' and 'Next' buttons. The main area shows a tree view of the light path, including 'AxioCamM...', 'Camera Ch...', 'Hitachi HV...', '25 DAPI / ...', 'Colibri/Coli...', '10x Motorized Reflector Changer', '20x Transm.', and 'Close'. A blue arrow points to the '25 DAPI / FITC / TexasRed' filter configuration. The configuration table is as follows:

01 DAPI	BP 359 - 371	BS 395	LP 397
38 HE Green Fluorescent Prot	BP 450 - 490	BS 495	BP 500 - 550
43 HE DsRed	BP 538 - 562	BS 570	BP 570 - 640
50 Cy 5	BP 625 - 655	BS 660	BP 665 - 715
25 DAPI / FITC / TexasRed	BP 392 - 409	BS 410	BP 450 - 470
	BP 489 - 501	BS 505	BP 515 - 545
	BP 558 - 582	BS 585	BP 600 - 655
none			
none			
none			
none			

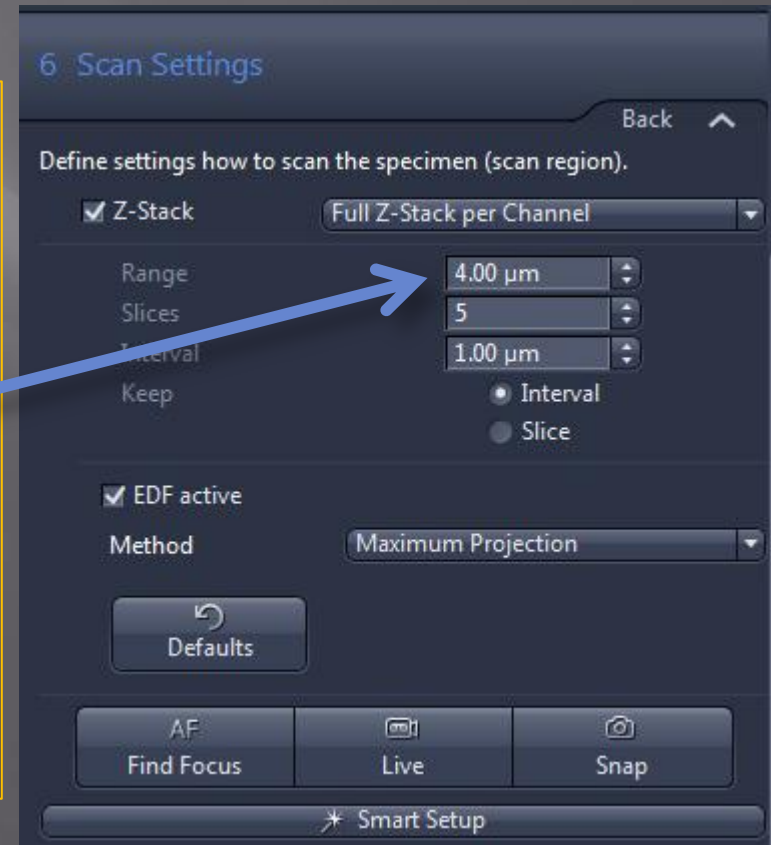
At the bottom, there are 'Experiment Settings Pool' and 'Clear' buttons, and a 'Multiple C' section with 'All' and 'Auto' options.

# Axioscan – Fluorescence

21. Open the 'Channels' tab: For each of the channels you should now press 'Set Exposure'

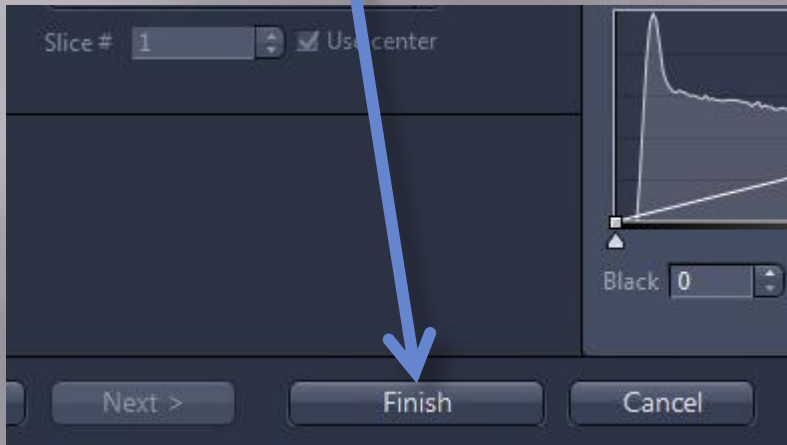


21. You should now decide if you want a Z-stack, which will give you the opportunity of changing the focus from the software after scanning. Please note that it will prolong the scanning duration and will take up more space. Therefore, limit the range to the thickness of your section. An alternative is to tick off the 'EDF active' (Enhanced Depth Focus): a single image plane is created using the regions in focus from the image stack. This option saves space (not time).



# Axioscan – Fluorescence

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



**22.** If you only need this profile for the slide you changed the settings for now simply press 'Scan'.

If you need the profile for several slides, see next page for instructions on how to make these settings available to more slides.

# 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

The screenshot displays the Axioscan software interface. On the left, the 'Default Scan Profile' is set to 'Brightfield 10x'. A blue arrow points from the 'Global changes' label to the gear icon next to this profile. In the center, the 'Magazine' section shows a table of scan profiles. The first row is for 'High-precision 1x3' with a 'Brightfield 10x' profile. A blue arrow points from the 'Local changes' label to the gear icon next to this profile. A red arrow points from the 'Save adapted scan profile' option in the dropdown menu to the 'Save adapted scan profile' option in the menu.

Process	Preview	Scan Profile
<input checked="" type="checkbox"/>		Brightfield 10x
<input checked="" type="checkbox"/>		Fluorescence 20x

- Open advanced wizard
- Open advanced wizard in offline mode
- Open tissue detection wizard
- Save adapted scan profile



# 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  $\mu\text{m}$ ) 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.

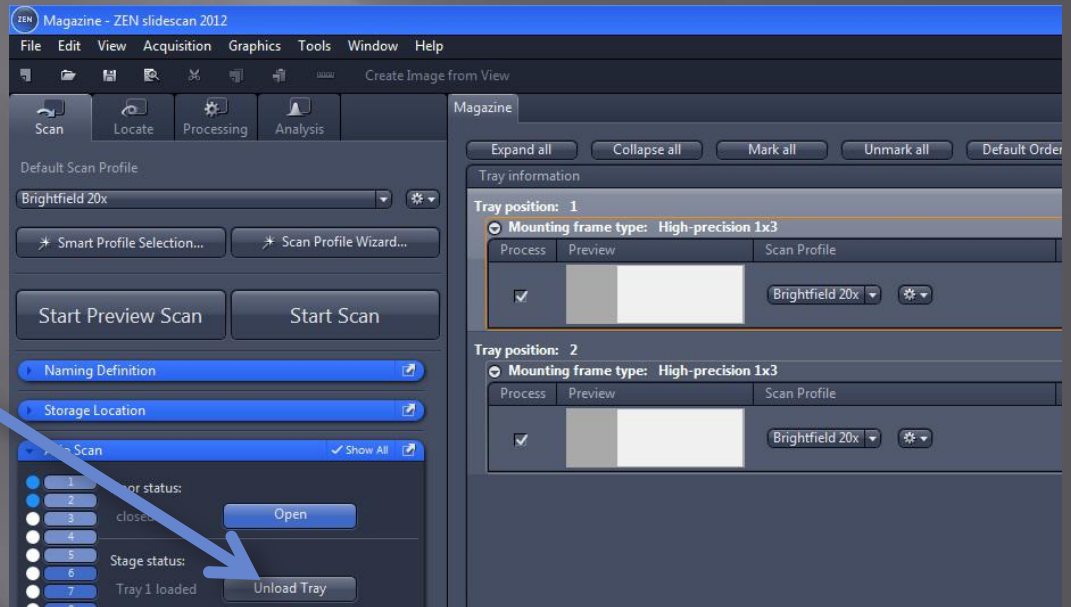
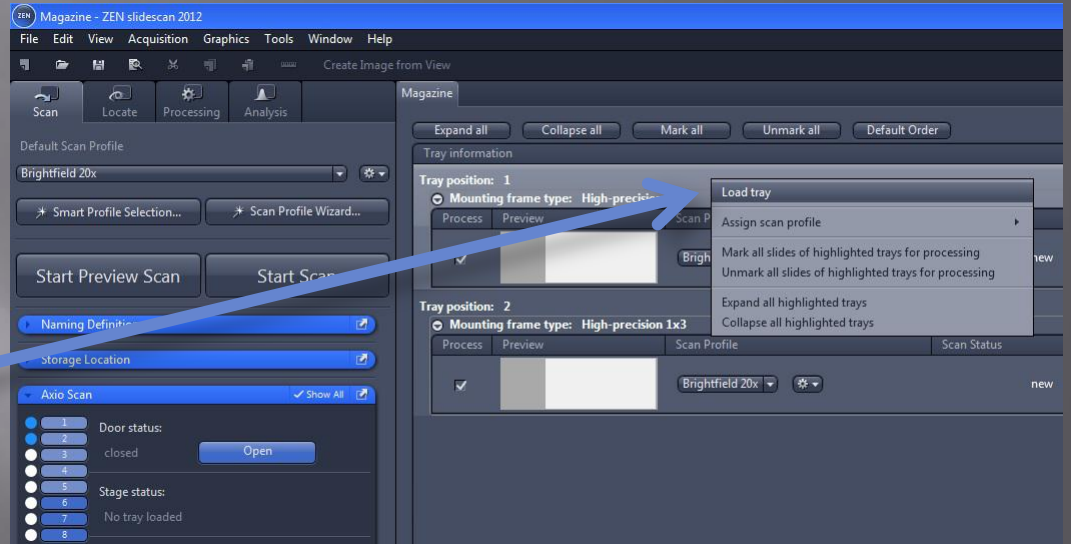
# Axioscan – Notes

## 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'.

