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.
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.)
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
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).

Axioscan – Fluorescence

Global changes

Local changes

We will describe here changed to local settings.
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
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 fluorescence 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.
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 µm from the focus position to calculate the numbers for ‘Set First’ and ‘Set Last’
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\textsuperscript{nd}/5\textsuperscript{th} tile).
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 µm.

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 specifications:
- Excitation: 387/485/559/649
- Emission: 440/521/607/700
- LEDs: 365/470/555/625
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).
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.
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 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.
`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`.