Zeiss Axio Scan

Switch on:

1. Switch on Zeiss Axio scan (botton on the left from corner). **Do not use the main switch on the power cord extender**
2. If you need to use fluorescence plug in the cable for ORCA camera
3. Switch on the PC. Login: **zeiss** password: **AxioScan.Z1**
4. Start Zenn blue software. **User zeiss, password zeiss**
5. If using only bright field disregard the warning about ORCA camera
6. Insert the slides in the trays.
7. Do not use damaged slides!
8. Machine can work with up to 100 slides (25 trays with 4 slides each).
9. Trays are on the left in the drawer. Trays can be loaded manually one at a time or four at a time.
10. Open the chamber (press the OPEN/CLOSE button on the machine or in the software). Insert the trays in.
11. Close the chamber (press OPEN/CLOSE button)
12. Software recognizes the inserted trays and slides and numbers them.
13. Choose a profile
14. **DO NOT USE SCAN PROFILE WIZARD AND SMART PROFILE SELECT**
15. Naming definition: either use standard or use excel template from the desktop
16. Store location: save files first locally and then transfer to data transfer server only.
17. To start: press scan preview
18. For bright field profiles: the ROIs are automatically selected. To adjust, press the wheel next to the profile name and select “tissue detection wizard”.
19. FLOU: If you use fluorescent samples: the preview is done with Bright field and the sample areas are not recognized. To draw ROIs press the wheel next to the profile name and select “tissue detection wizard”.
20. Focus Reference channel is usually DAPI.
21. Channel settings: it can be up to 5 wavelength used: 385nm, 430nm, 475nm, 555nm, 630nm, 735nm.
22. Press Finish
23. Start scan.
24. Images are saved in *.czi format. It can be exported into tif later. Use analysis workstation to work with images

Finish:

1. Unload the tray, wait for the green light on the tray number
2. Open the chamber (press OPEN/CLOSE button), take out the samples
3. Close the chamber (press OPEN/CLOSE button)
4. Close the software
5. Switch off the machine (left front button)
6. Unplug the ORCA camera plug behind the machine if the camera was on

Specifications:

LED: 385nm, 430nm, 475nmn, 555nm, 630nm and 735nm.

Plus Brightfield option.

Objectives:

5X/0.25 Air, 10X/0.45 Air, 20X/0.8 Air and 40X/0.95 Air.

Additional:

- The machine can to be loaded during an active scan. To do this, **do not press Stop** (it will abort the active scan) but press "unmark all", so the current slide will be completed and then the trays can be reloaded.
- After an abort by Stop, the aborted slide must be reset. To do this, click on the slide with the right mouse button and select previewed.
- Start preview: Slide + label are photographed

Create and edit profiles

- Select storage location (NEVER save to C, either select the internal computer memory D or save directly to one of the servers (best: S, will be mirrored again by server T).

- For both brightfield and fluorescence, default profiles are already set up, matched to the machine illumination and objectives. New profiles should be created based on these. They are called MASTER…

- Never USE SCAN PROFILE WIZARD AND SMART PROFILE SELECT below the profile selection! These profiles are not adapted to the hardware!

- In principle, a different profile can be assigned to each slide. It is also possible to switch between fluorescence and brightfield. The order of the scans can be changed by simply moving the trays in the view.

Focus map setting: sets the number of autofocus points

1. Coarse focus with the 5x lens: basically one focus point is enough (run autofocus)
2. Fine focus with scan lens: default = focus points are selected depending on the size of the object. Here, experience/knowledge of the nature of the specimen is important (more focus points for wavy specimens, fewer for stacks, otherwise the neighborhood relationships of the individual tiles will not be correct later).
3. Scan settings: define Z-stack and projection
4. Move through the object and define the limits of the stack
5. EDF active, so Z stacks are combined in one file directly. Methods: Variance, Contrast, wavelet (you have to try out which one makes sense for the is useful for the respective preparation) → finish

6. Save profile: tab next to the preview slide - save adapted scan profile
7. Wizard with limited functions that are available during a running scan.
8. Tissue detection wizard: Here individual ROIs can be selected for the scan.

Post processing:

1. File format: CZI - can only be opened with the program Zen (light) and Slideview, is a data pyramid = image is available in defined enlargements (1:1, 50% 25% etc...), intermediate enlargements are always zoomed and accordingly blurrier, but can also be recorded via a snapshot tool (pushbutton, or loudspeaker button on the workstations)
2. Open the configurator (CTRL N, or options - documents - show navigator) to easily set zoom levels.
3. To capture an image, press the button "create image from view" in the upper left corner, save as with options
4. Insert scale bar: Window under the image, second tab "Graphics". 3rd button, right click on scale bar opens display options, burn in via export: burn in graphics possible
5. Image export (with many options), basically also possible as batch.
6. Select ROI: Right click on image - create subset of ROI
7. also possible and often more accurate: offline stitching (as calculated from the center →four instead of two sides for comparison)