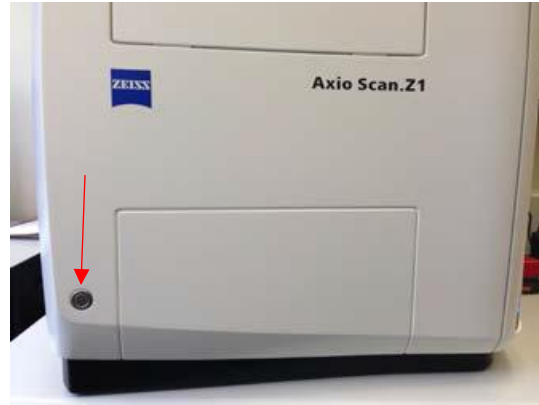# Research

Axio Scan.Z1 Operating Manual

Axio Scan.Z1

For questions please e-mail james.schwartz@uky.edu

1. Turn on hardware
   a. Turn on PC
   b. Turn on slide scanner switch on the back and on the front

2. Login to PPMS with your UK credentials
3. Start Zen Blue after the slide scanner is turned on and the display light in the top left corner is green

4. Click on "ZEN slidescan" in the Zen Blue software
5. Load your slides into the trays

   Use this device in order to easily open tray's frame. Push first the holder in front of it and then the one on the left side. Place your slides face up with the label in between the holder, then press the silver button to open the device.

"Open" the slide scanner in order to insert trays. Please take extra care when you select a free slot. A slot is free when there is no light (grey).

Note: occupied slots are highlighted on slide scanner in blue, green, orange, red or white.
• Blue = tray loaded and no slide preview taken. Slide status is "new".

- Green = tray loaded and slide status is either "preview" or "finished".
- Orange = an error occurred during scanning or tray loading.
- Red = probably a tray is stacked inside the slide scanner.
- White = tray is inserted but empty.

6. Storage Location- Create a folder on the DATA (D:) drive for yourself or your lab. Make sure this folder is selected under "Storage Location".
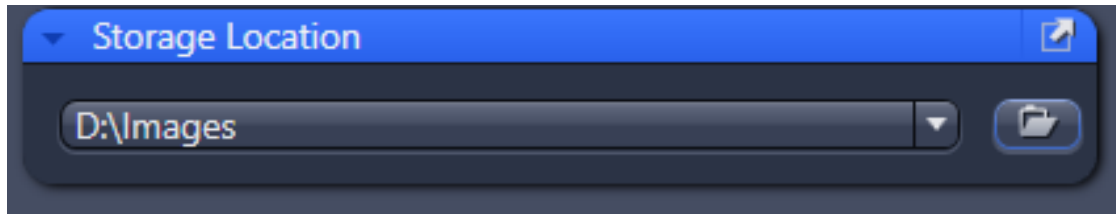
7. Edit "Naming Definition" to fit your needs. You can also leave it so that the file is automatically named for you.

8. Mark slides- All slides are displayed in the Magazine tab that appears automatically when slides are loaded into Axio Scan.Z1. "Unmark all" slides in order to avoid compromising other experiments.
   If you don't have a personalized scan profile yet, select only 1 slide in order to test a basic profile and adjust the desired parameters.
   If you already have a personalized scan profile look for it in "Default Scan Profile", this will automatically change profile for all slides with "new" status. You can select all your slides either by "Mark all" or by selecting trays and right click with the mouse to select "Mark all slides of highlighted tray for processing".

9. Default Scan Profiles- Scan profiles are named by the objective used and type of scan they perform. For instance, 10x_BF_Default indicates the 10x objective is used in a brightfield scan. 20x_FL_Default indicates the 20x objective is used in a fluorescent scan. Users will never need to use the Shading_Creation profiles, and should avoid selecting them.

10. Custom Scan Profiles- Scan profiles can be customized to fit specific types of experiments. The brightfield (BF) profiles generally do not need to be adapted to specific experiments, and the default brightfield profiles should work for all brightfield imaging. The fluorescent (FL) profiles will need to be customized to include the specific fluorescent channels needed for your experiment.

    Editing Fluorescence Scan Profiles-
   It is recommended that users not change parameters under "Global Data".

    Label Scan Settings allows you to adjust the size of the window displayed in red that defines the slide label image size and position. As not all glass slides have the same label size, you might want to check if the predefined size corresponds to your needs. This image is always saved as a separate file called "pt1" and is very useful since allows you to easily recognize your slides in absence of detailed information within the file name.

Preview Scan Settings allows you to adjust the size of the window displayed in red that defines the size of the region where the tissue should be detected. As sometimes it is useful to reduce this area in order to improve the automatic tissue recognition on the border of the slide, you might want to check if the predefined size corresponds to your needs.
This image can be optionally saved as a separate file called "pt2", which is useful for people that need to know the exact order in which sections are scanned on the slide (e.g. serial sections).

Tissue Detection Settings allows you to adjust how tissue is detected on the slide. Fluorescently labeled tissue is sometime difficult to detect automatically. Try to finely tune the specimen histogram range in order to get tissue recognition as accurate as possible. In some cases you will have to manually delete/add ROIs.

Users will generally not need to adjust focus map settings. DAPI is typically recommended as the channel to use for both coarse and fine focus. If your sample has absolutely no signal in DAPI you may use a different channel for focus reference. Click the "AF Find Focus" button (1) in both the Coarse and Fine Focus tab. After finding focus the software should automatically take a snap shot. Use this snap shot to ensure that the sample is in focus (in both Coarse and Fine). If the autofocus repeatedly fails to find the correct focal point you can focus manually by holding the "Ctrl" key and scrolling with the mouse wheel. You can also change the exposure at this time, if needed. To change the exposure simply select the channel you are using as a reference (DAPI, in the vast majority of samples) and click set exposure (2). The final step of the Focus Map settings is to define the Focus Point Distribution. We recommend you not change the strategy, however if your sample does not look focused after a scan you may increase the number of points.

Scan Settings is the final tab you will need to complete editing a scan profile. Under scan settings you will select the fluorescent channels you will use in your scan under "Channels". After selecting the required channels go through each of them and set the exposure for each channel by clicking "Set Exposure" as in the image above. After the channels are
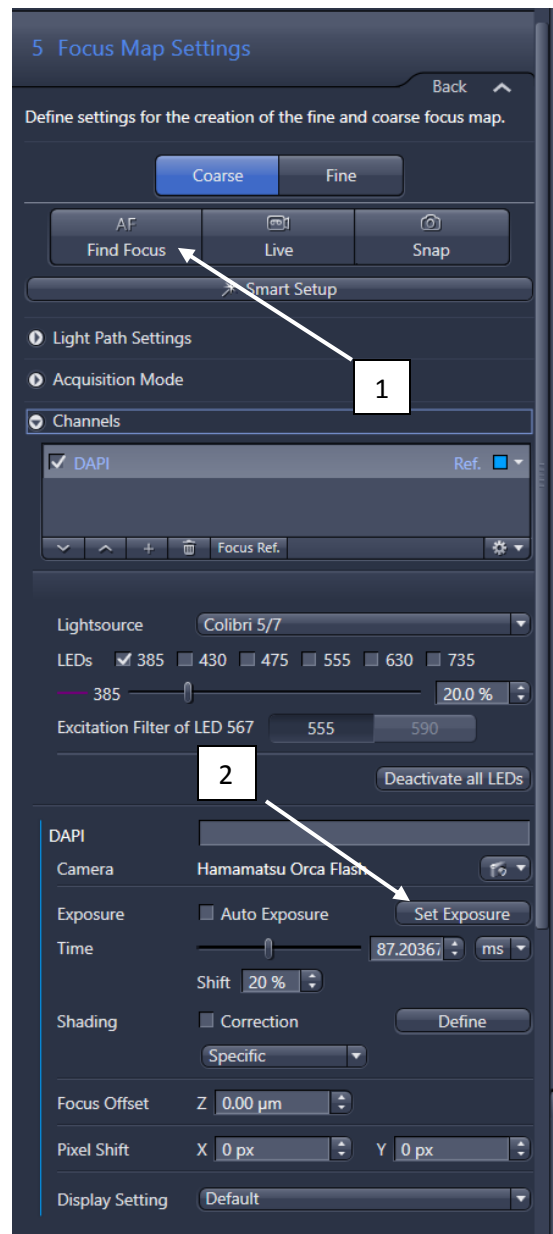
selected and the individual exposures are set, you can click "AF Find Focus" which will focus and give you a snapshot of your sample using all the selected channels. After that you have the option of enabling and setting up a Z-Stack. The final step is to set up the online processing. For best results, we suggest selecting offline stitching, with fuse active, pyramid active and JpegXR active with a Lossy compression of 85%.

After this, you can click "Finish". You can then either test your custom profile by scanning a slide, or save it by clicking the gear wheel (shown below) and selecting "Save Adapted Scan Profile". Name it by putting your last name as the first word of the title, and leaving the objective and scan type (20x_FL, etc) and then give it some other identifier such as the date of the type of sample you will use this profile for. An example would be "Schwartz_20x_FL_braintissue". **Never overwrite the default (10x_FL_Default, 20x_FL_Default, 40x_FL_Default, etc.) profiles, you must give your adapted scan profiles a different name.**

11. Scanning- To scan you will load your slides into the trays, then load the trays into empty slots in the AxioScan. Select the scan profile you will want to use (or create a profile) for each slide. Make sure the box is checked next to all the samples you wish to scan. Click "Start Preview". This will start a preview scan for all the samples you have selected. After the preview scan is completed you can either double click on the first sample you want to view or go to "Open Tissue Detection Wizard". This will allow you to select your ROIs on your slides. After you have selected your ROIs you can either start the scan, using your selected profile, or set up your focus map and/or scan settings (as described above in step 10). To start the scan, click the "Start Scan" button. The AxioScan will continue scanning until all slides that have been previewed and marked are completed. Be sure to unmark any samples you do not wish to scan.

12. After the scan is completed you can double click on the preview image under "Magazine" to open the full scan in a new tab. Under the "Dimensions" tab you can either view the ROIs individually as "scenes" or you can view the entire slide by unchecking the "scene" box. Under the "Display" tab you have a look-up table and several options to change how you view the image (best fit, min/max, etc.). You can also add a scale bar to your image by right clicking on the image and selecting either a "floating" scale bar that will update as you zoom in and out of your image, or you can draw an annotation scale bar. There are also several processing options in the "Processing" tab. If you make any changes to your scanned image, be sure to save it to your folder before closing the window.