Microscopy Shared Resource Facility
Icahn School of Medicine at Mount Sinai

Zeiss LSM 880 Protocol

NOTE: Be sure to use a no. 1.5 coverslip and the 30° oil for all Airyscan imaging. You cannot combine Airyscan imaging with regular confocal or tile imaging.

1) Open the Zen (black) software

☞ Start Zen 2 Black 

- Click Start System

- During the System Startup, the incubation system may produce an error message in the lower left hand corner.

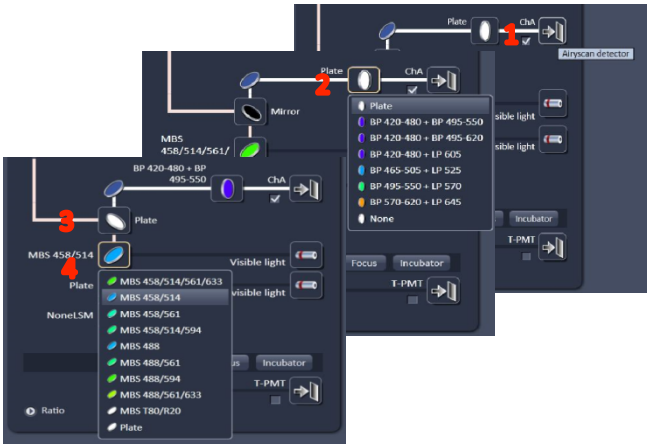
☞ Check sample using Locate tab in software.

☞ Switch to Acquisition tab when sample is in focus.

2) Airyscan setup

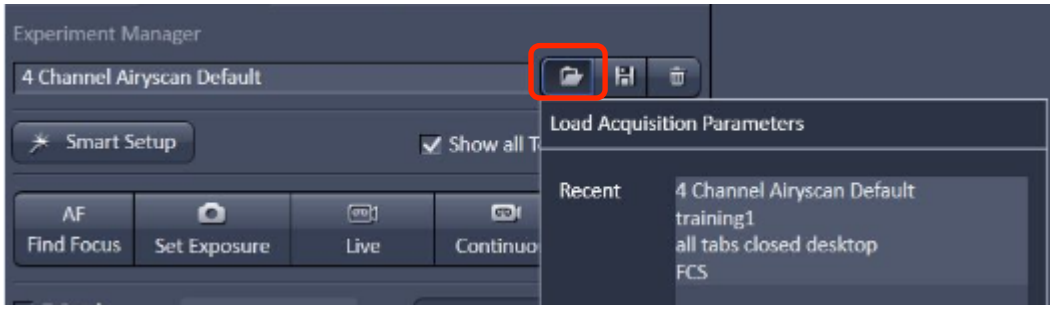
☞ Set up the Airyscan light path

- Manual Setup:
  - Open the Image Setup Window
  - Adjust the Airyscan beam path
    - (a) Turn on Ch. A
    - (b) Replace the mirror with a plate
    - (c) Select the dual bandpass filter
    - (d) Select the beam splitter 1
    - (e) Turn on the appropriate laser



- Recommended Settings:
  (a) DAPI: Ch. A_ BP 420-480 + BP 495-550_ Plate_MBS 488/561/633_405 laser
  (b) FITC/GFP: Ch. A_ BP 420-480 + BP 495-550_ Plate_MBS 488/561/633_488 laser
  (c) Texas Red/TRITC: Ch. A_BP495-550 + LP 570_SBS SP 615_ MBS 488/561/633_561 laser
  (d) Far Red: Ch.A_BP 570-620 + LP 645_SBS LP 660_ MBS 488/561/633_633 laser

- Automated Setup:
  - Within the Experiment Manager saved configurations, select " 4 Channel Airyscan Configuration"



☞ Set your parameters for Airyscan detector alignment in the **Acquisition Mode Window:**
- Format : 512 x 512
- Speed: Max
- Bit Depth: 16
- Zoom factor: > 1.8

☞ Within the **Channels Window: '**
- Make sure that the pinhole is at least 2.5 AU (or greater than the auto red zone)
- Make sure the SR mode is selected.

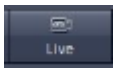
3) Detector alignment

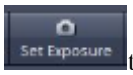
☞ Open the **Maintenance Tab** (to the left of processing)
- Open and undock the **Airyscan window**. Leave this window open until alignment is complete
- Under **Auto Alignment**, check the box for adjusting in continuous scan mode.

☞ Select a single laser line in the Channels Window.

☞ Use Continuous Mode to visualize sample.

- Click the Airyscan visualization tab. Check option for "Detector View" in the display options to use the schematic for alignment.
- Adjust gain and offset settings as usual.
- Check Airyscan window status. The scan should go through the following sequence:
  - Unknown (waiting)
  - Bad
  - Adjusting
  - Good

☞ If the sequence does not show within the first few minutes in the continuous mode, manually change the detector alignment in the Airyscan window. Once the detector is aligned, you can use 

or 

to set exposure times.

4) Acquisition of Airyscan Image

☞ Be sure to **check all fluorophores** in your specimen.

☞ Change the parameters in the Acquisition Mode Window

(i) * Click Optimal (this option adjusts laser wavelength to the numerical aperture, and zoom factors)

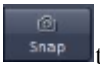
1. Select Optimal only after all channels have been adjusted.
2. Select Optimal without the DAPI channel selected

(ii) Frame Size: Optimal

(iii) Speed: Optimal

(iv) Averaging: 2

(v) Bit Depth: 16

- Click **"Stop"** (the same icon as "Live")
- When all exposure times are set, Click 

to acquire the image.
- If you are in the Airyscan mode when you click Snap, you will see the 2D raw confocal image on the left and the 2D Airyscan image on the right.

5) Processing Airyscan Image

☞ Within the Airyscan tab, check the Auto option to add the automatic noise filter.

- You may have to raise the filter approximately 0.3 above the automatic setting.
- Too much filtering will result in a patterned image.

☞ After you have adjusted the filter, go to the Processing tab.

- Click Airyscan Processing under methods.

- Select the image you want processed.
- Click Apply.