# Airy Scan (i880)

Imaging Setup

1. Set up excitation lasers and main dichroic (MBS) as you would normally do under confocal mode.

2. Activate the airy scan detector by ticking the ChA checkbox in the beam path.

3. You can activate as many tracks (up to 4) as you have fluorophores.

4. For each track, set the secondary dichroic so that the required wavelengths are directed towards ChA, or choose Plate.

5. Select an emission bandpass filter according to the fluorophores you need to detect.

6. If all tracks use the same dichroics and filters, set the Switch Track configuration to *Every Line*.

7. If the dichroics are different in each track, set the Switch Track configuration to *Every Frame*.
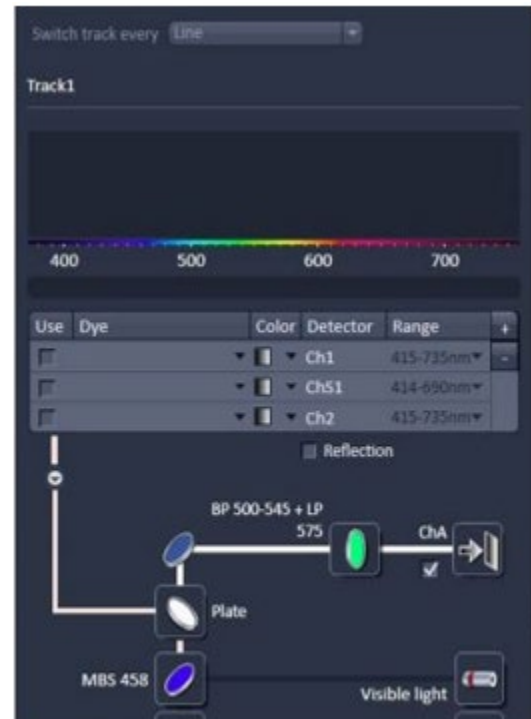
Light Path Configuration: Excitation laser, Dichroic, Emission filter, Ch A (airy scan) detector

Acquisition (Scanning Parameters)

1. Use a high-NA objective – 63x/1.4 oil.
2. Center scan area, set zoom to 1.8 or higher (past red mark)
3. Set frame size to optimal by pressing the "Optimal" button or use 1024x1024 or larger.
4. Set speed to Max, Averaging to 1, bit depth to 12 or 16.
5. Center scan area, set zoom to 1.8 or higher (past red mark)

Channel Settings

1. Select *SR* mode. Set PH to a value just above the red mark. For separate tracks, set PH to get similar optical section thickness.

2. Set detection *Gain.*

3. Start with *Laser* power set to ~0.5%, then increase slowly to get desired signal level.

   Avoid Overexposure! *If you have several tracks, adjust the Digital Gain for individual adjustment of tracks.*
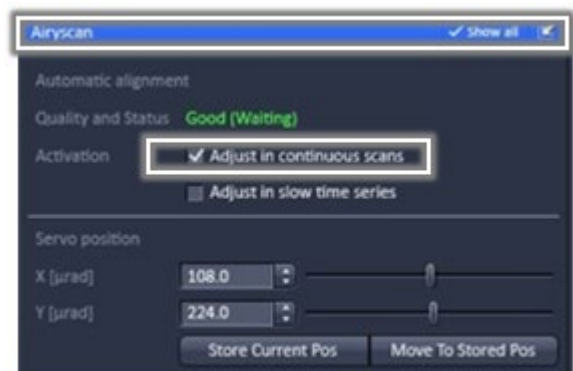
Alignment (SR mode)

1. Before image acquisition, go to the *Maintain* tab and open *Airy Scan.*

2. Check *Adjust in continuous scans.*

3. Undock this tab, bring to the lower part of screen, and go back to *Acquisition* tab.

4. Focus at the top of your sample (close to coverslip) and find brightest signal, then scan.

5. Click *Airy scan* tab to the left of the image.

6. On the *Airyscan* tab below the image, check "Detector View" to see a representation of detector elements.

7. Run a *Continuous* scan.

8. Monitor status of detector alignment.

Tool for Airy Scan Image Display

- *Unknown (Waiting)* no alignment done or possible. Make sure airy scan detector is activated and gain is set high enough to detect emission light.

- Bad (Waiting/Adjusting): Automatic alignment is in progress; in red text.

- Waiting: system is waiting for position analysis feedback

- *Adjusting*: Adjustment is performed

- *Good (Waiting/Adjusting)*: Automatic alignment is in progress; in green text

Tool for Automatic Alignment

9. For image acquisition, the status should indicate *Good (Waiting)*.

   • The brightest element in the detector view should be in the center with a drop of signal towards the periphery.

10. If status is "*Bad*", adjust frame size or zoom.

11. If auto adjustment does not work:

    • Use the sliders in the Servo position to manually adjust the detector in x and y until the brightest element is centered.

    • Note down the original values before changing them.
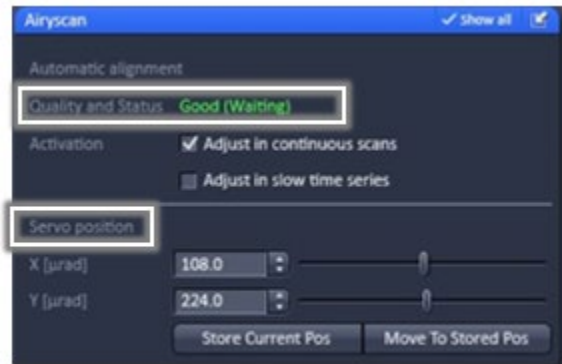
Airy Scan Alignment Status

Airy Scan View (Image Acquisition)

1. After alignment, start image acquisition.

2. Airyscan view displays the source image (left) and an online processing SR image (right) side by side.

3. In the Airyscan tab, you may use the *Super Resolution* slider to get the maximum resolution increase in SR mode. When Auto is unchecked, moving the *slider to the right increases the processing filter strength and results in higher resolution.* However, if the SR processing filter is too strong, dot shaped artefacts will occur. Slightly decrease the SR filter strength to get an optimal result. Typically, the position is 0.3 to 0.5 units higher than the Auto default value.

Manual Adjustment of Super Resolution
Processing Filter

Multidimensional Acquisition

• For z stack acquisition, you may set Switch Track to Every z position.

• Tile scans are not possible with Airy scan.

Image Processing

1. After image acquisition, go to the **Processing** tab, and choose the *Airyscan Processing* function in the method list.

2. Click on the open image you want to process and *press Select*. Choose 2D for a 2-dimensional image, or 3D for a z series.

3. Activate the *Auto* checkbox for automatic processing.

4. Hit the *Apply* button. Processing will start and may take a few minutes. A new image will open with the result of the auto processing.

5. Save the processed image if desired. Always save the original unprocessed image.