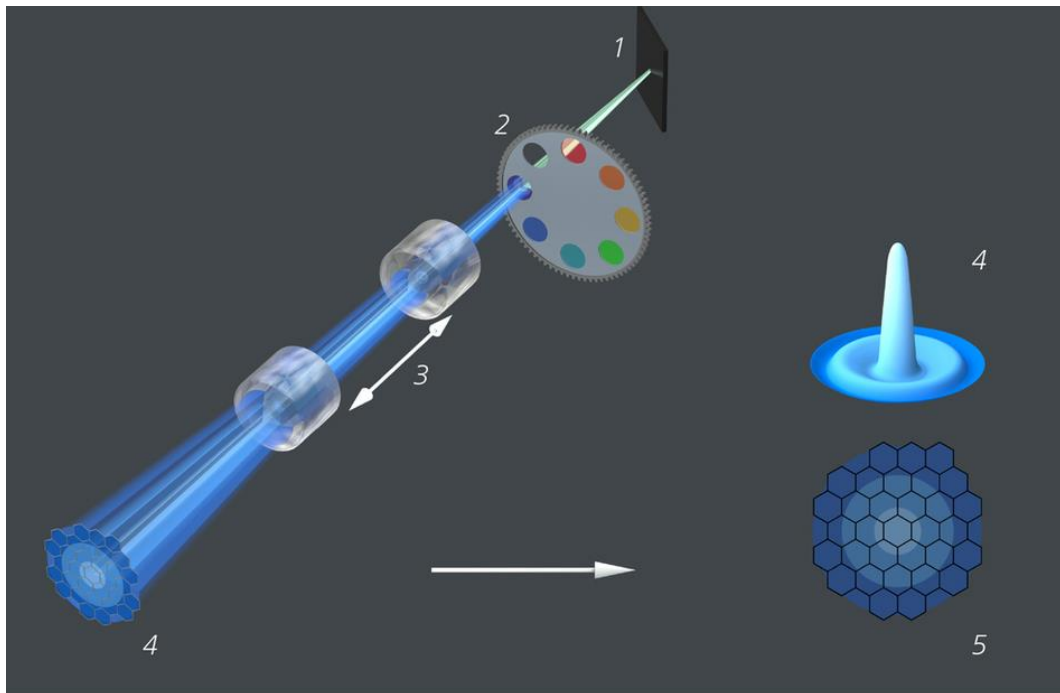
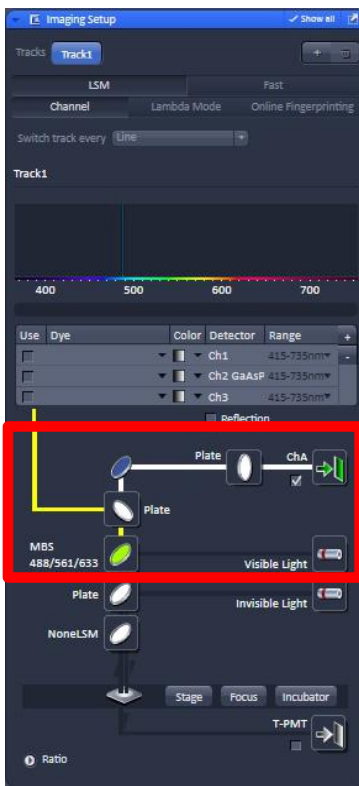


### Airyscan Beam Path



- 1. Mirror
- 2. Emission filters
- 3. Zoom optics

- 4. Airy disk
- 5. Airyscan detector



### 1. Activation of the Airyscan

The Airy Scan improves the Signal to Noise Ratio given by any objective. Nevertheless, improvement of resolution up to 1.7 times higher than confocal imaging can only be achieved using the 63 x objective.

Before starting, examine your sample visually. Do not skip that step.

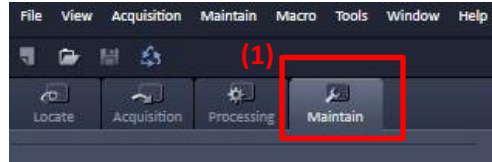
- 1.1 . Activate Airyscan channel (ChA) in „Imaging Setup“
- 1.2. Check that a Plate is moved in between Airyscan and the other detectors
- 1.3. Make sure that the right MBS (Main Beam Splitter) is activated according to your laser line.
- 1.4. Choose the right bandpass filter in front of the detector that suits your fluorophore emission.

## 2. Alignment Step

The Detector/Signal alignment is done automatically in „Continuous“ Mode.

The sample should be focused. If your sample is too dim, the alignment can be done with a bright test sample.

2.1. Select the ‚Maintain‘ tab (1)



2.2. Locate the Airyscan menu on the left side of the screen and either pull it to the middle of the screen or click on the arrow symbol ↗ in its top right corner (2) to pop it out & keep it visible after switching back to the ‚Acquisition‘ tab.

2.3. Make sure all 3 checkboxes are activated (3)

2.4. Once back in ‚Acquisition‘ mode, set frame size to a low value, like 256x256

2.5. Set scan speed to Maximum

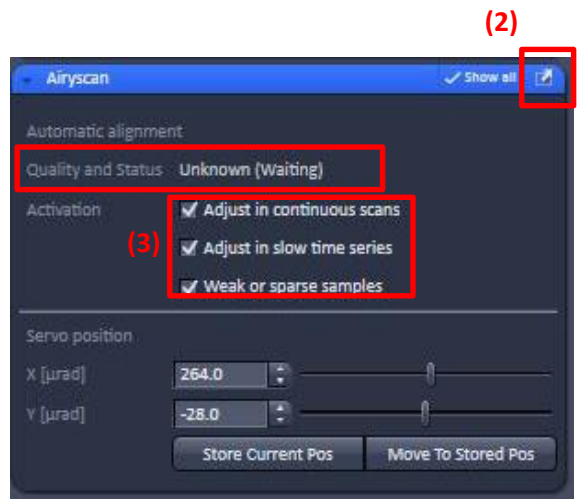
2.6. Use a zoom factor of at least 2

2.7. Set gain between 700 and 800

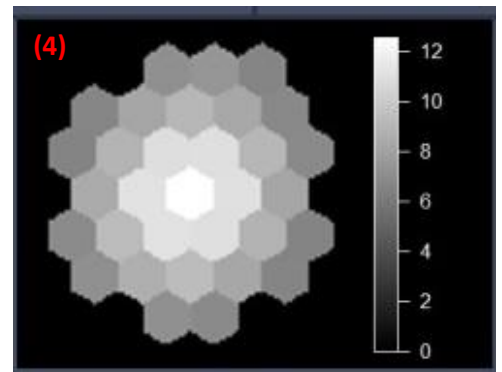
2.8. Set laser power to an intensity where you are expecting a signal (compare with confocal)

2.9. Press „Continuous“ adjust laser power and Gain until you find a good (centered) illumination pattern in detector view, see (4).

2.10. Remain in continuous mode until detector is fully aligned: in the ‚Airyscan‘ window, the text next to ‚Quality and status‘ will change from ‚Unknown (waiting)‘ to ‚Good (waiting)‘ (5)



Align the detector for every sample: alignment is sample, focus and temperature dependent. Don't start acquiring unless the detector is aligned (‚Good‘)



## 3. Taking Images

3.1. Display grey values of your „Preview image“ in „Live“ mode, fill your colormap up to 1/3rd or 1/4th of the maximum 255 grey values (8 bit) by adjusting Laser Power and Gain

3.2. Best is to put the Gain optimal between 700 and 750 and then adjust the Laserpower until a decent number of grey values is displayed.

3.3. Z-stack, and timelapse are set like in confocal imaging

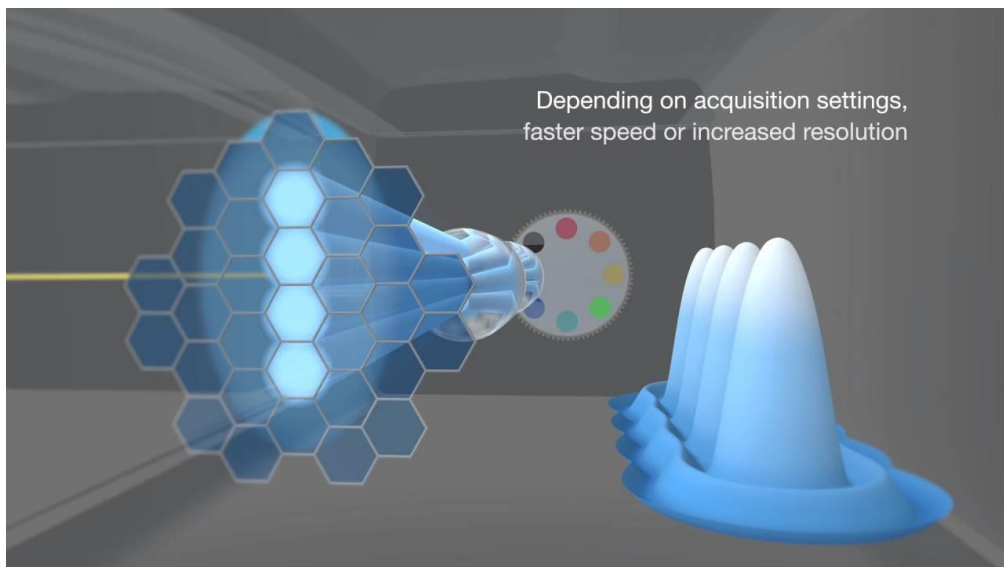
3.3. before taking images set the pixel number to „optimal“, make sure Scan Speed is set to maximal.

## 4. Image Processing

All Airyscan Images have to be processed in order to get 16 bit high resolution images

- 4.1. Go to main menu „Processing“ and choose „Airyscan Processing“
- 4.2. Select your image, press „apply“
- 4.3. For further optimization of the image quality untick „Auto“ and choose your own Wiener Filter value
- 4.4. Save the processed Image

## Fast Airyscan



In Fast Airyscan an Oval Aperture leads to an elongated Laser Beam. 4 pixels are scanned at the same time which results in a higher Scan Speed and less Photobleaching. Only 16 of the 32 Airyscan Detector Elements are read out. Up to 30 fps are possible

## 5. Activation and Imaging with the Fast Airyscan option

5.1. Activate the Airyscan Detector like it is described in Step (1.) and choose „Fast“ option in „Imaging Setup“

5.2. Sample in (SR) Superresolution, (Opt) Optimal, Flex or (FS) Fast mode. Frame sizes are changing accordingly. The speed slider offers additional flexibility for imaging speed.

5.3. Due to the beamshaping some excitation light is lost. Therefore Laser Power Value is divided in actual laserpower and resulting laser power reaching the sample.

5.4. Imaging Z-stack and timelapse works like in confocal imaging.

5.5 Image Procassing of Fast Airyscan Images like in 4.



Airy scan experiments generate **larger files** (up to 32 values per pixel). After postprocessing, **these volumes can be significantly reduced. Save only what's necessary.** In most cases the raw data is not needed again. **Move your files to the file server as soon as you finish your experiments.** The Facility is not responsible of the files collected by users and computers will be cleaned regularly.