Zeiss LSM880

User manual

Cell Imaging & Cytometry

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1. Information

The Cell Imaging and Cytometry Core is responsible for maintenance of this microscope.
Each user must follow the CIC user policy.
More information: https://bioscience.fi/services/cell-imaging

- **Only authorized users may use the CIC instruments.**

- **Users must report any malfunction or problem to the CIC personnel.**

- **The user is responsible for removing their data from the hard drive and should do so immediately.**

- **Files older than 30 days are automatically wiped from the system without prior notice**

2. Before Imaging

Check your sample *with an ordinary fluorescence microscope*

It is a good practice to check the quality of your sample before making use of a more expensive instrument.

Clean your slides

Clean the remaining salt and mounting medium off the coverslip. Dirty coverslip compromises the image quality. You can use ethanol to aid the cleaning. Do not use the microscope lens tissue. Clean your slides beforehand in your own lab, as it is impractical to use microscopy time for cleaning.

Check the environment

Switch on the lights and check if the microscope environment is tidy.
If there are oil spills or other issues, please inform CIC personnel.
(microscopy@bioscience.fi)

Start the heating earlier if possible

When doing live cell imaging, it is good to switch on the heating at least two hours in advance. If someone is using the instrument, ask whether it is possible to switch it on. If no one is using the instrument, start the instrument and switch on the heating.

Cancellation

Cancellation must be done 24 hours before the reservation starts. However, if you suddenly cannot use your time, <u>inform the next user and cancel your reservation</u>.
If you are the last user of the day and cannot come, you are responsible for the instrument shutdown.

Unsure?

If you feel that you need support, please contact CIC personnel.

3. Working with immersion objectives

The immersion medium should match the objective.

The water objectives need water or oil with a refraction index of 1.334. For imaging fixed samples and short time experiments, MilliQ water is appropriate
For long experiments water immersion oil is more suitable as regular water evaporates over time. The water immersion bottle is marked with a **W**; the water-oil is very fluid and must be applied very carefully.

The oil objectives (63x & 100x) need oil with a refraction index of 1.518. This oil is more viscous than the water immersion oil.

The image will be suboptimal when incorrect oil is applied. Mixing immersion oils is prohibited.

A small drop of oil is enough, adding to much can make a mess and damage the instrument.

Start imaging with the objective in the lowest position. Then, focus the objective upwards until the oil touches the coverslip. Next, focus the sample visually through the eyepieces.

After imaging, wipe the oil off from the objective softly with a dry lens tissue. Then, finish the cleaning by wiping softly with a new lens tissue moistened with isopropanol.

Only lens tissue may touch the objective lens.

When changing the sample with the immersion objective, the objective must be lowered (inverted microscope) in between.
-Press **Load position** in the remote unit, in the software (Focus window) or on the top of the right focusing knob (closest, front most button). Objective moves down.
-Remove the sample and add oil if needed
-Place the new sample. If you are changing the objective in between, wipe the objective clean with a dry lens tissue. Otherwise, oil might spill into the microscope.
-Press **Work position** in the remote unit, software or on the top of the right focusing knob (second button). The objective moves upwards into the position it was before Load was pressed. It should be very close to the correct focus position.

4. Starting up and shutting down the instrument

Start up
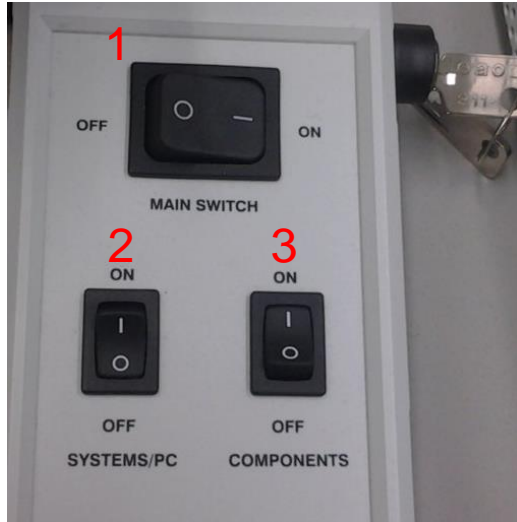
Switch lights on and check that the environment is clean.
If you see some issues, report them to CIC personnel.

1. **Main** on.

2. **Systems/PC** on. The computer starts.

3. **Components** on.

Shut down

If you are **NOT the last user** of the day

o   Clean the immersion objectives you used.

o   Leave the Argon laser to standby mode.

o   Remove your data.

If you **ARE the last user** of the day, **additionally**:

o   Switch off all lasers through the software (incl. Argon).

o   Bring the objective down.

o   Close the ZEN software.

o   Shut down the computer.

o   Switch off Systems/PC and Components.

o   Be sure that Argon cooling has finished (you will hear fan switch off).

o   Switch off Main.

o   Do not switch off anything else.

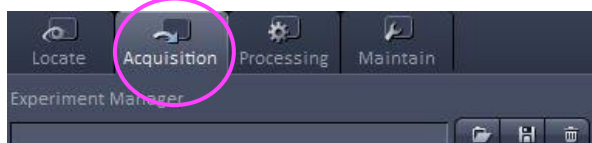# 5. Starting the Zen software

Start the ZEN software

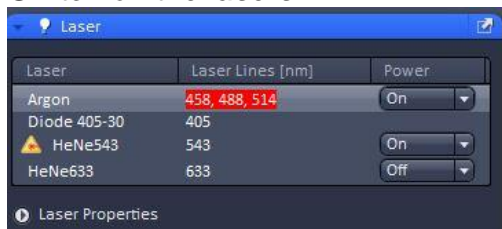Click the **Start System** button

Start-up will take a few minutes; a loading bar will be visible.

If there are any errors, contact CIC personnel
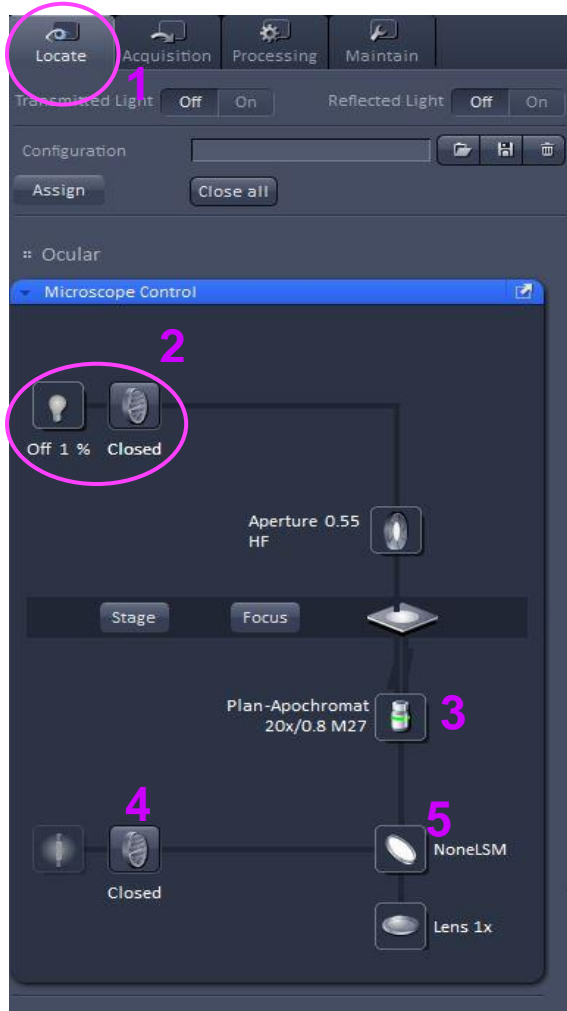
Choose Acquisition

Switch on the lasers

Switch on the lasers you need.
The Diode 405 is always on.

6. Using the fluorescence microscope

Visualizing the sample in ocular mode

! NOTE: the first user must manually open the RL shutter on the Zeiss touchpad!

1 Choose the **Locate** tab to view sample using the oculars.

2 Transmitted light turn on bulb and open the shutter.

3 Objective lenses:
- 10x/0.45 dry
- 20x/0.80 dry
- 40x/1.2 W/Gly/Si
- 63x/1.4 Oil
- 100x1.46 Oil

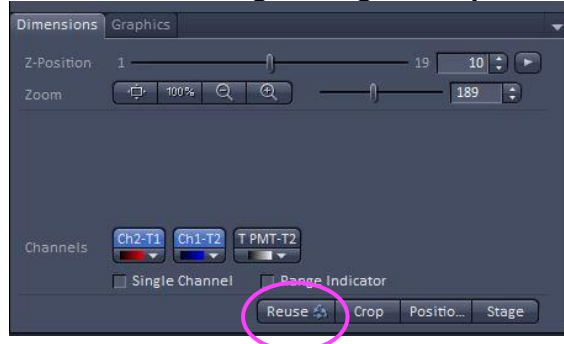**4 RL Reflected light** shutter (fluorescence)

5 Fluorescence filters:
- DAPI
- GFP
- RFP
- Analyzer filter
(for polarization imaging)
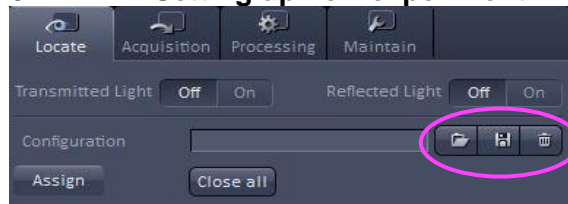- NoneLSM
(for confocal imaging)

Using the confocal microscope settings

6.1.1 Reusing settings from previous experiments

If you have earlier acquired images with desired light paths, open the image and use the
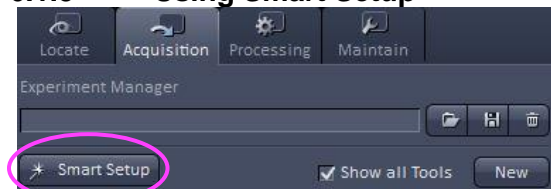**Reuse** button.
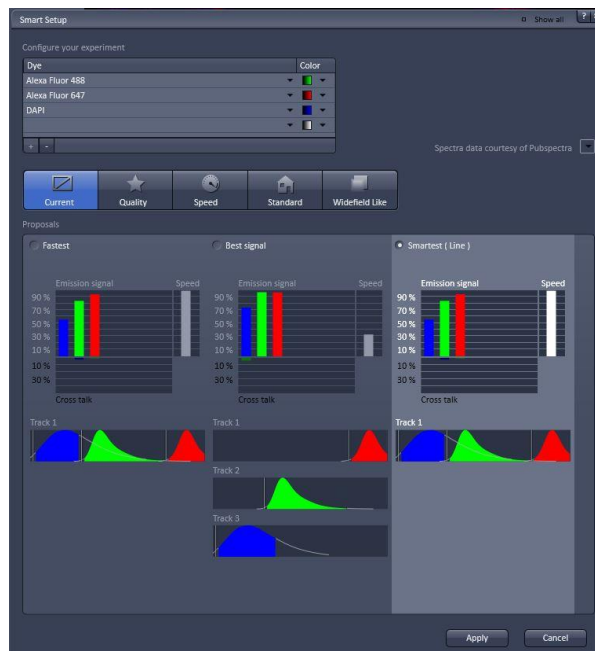
6.1.2 Setting up new experiment

To create new light paths, you can Use the Smart Setup or create them manually.

You can save a new imaging configuration you created or open an existing configuration

6.1.3 Using Smart Setup

The **Smart Setup** function allows you to insert your fluorophores and the software will provide light path suggestions.


Choose your fluorophore from the dropdown box

Fastest scans your labels with a single scan. This method gives most spectral cross talk/ bleed-through and should be avoided.

Best signal scans the light paths separately and is the most suitable option for everyday use.

Smartest (Line) reduces the number of scans and groups together light paths, which would produce least cross talk.

6.1.4 Manually create light paths
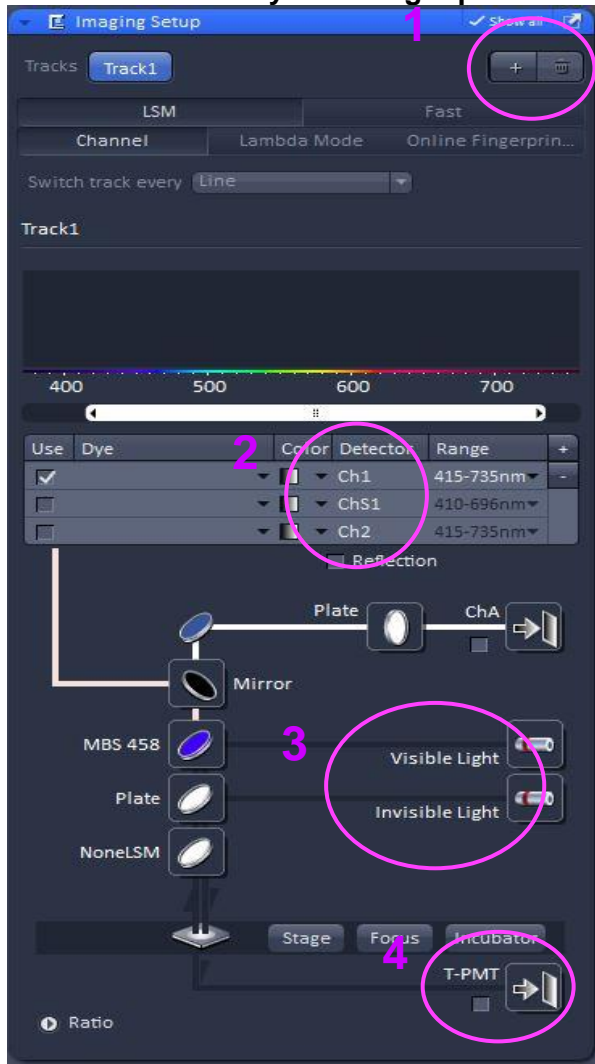
1 Click **+ or trash** button to add or delete track.

2 Ch1 is a standard photomultiplier tube (PMT).
ChS1 is a very sensitive detector, for very weak/sensitive samples.
**Ch2** is a cooled PMT, which is more (red-) sensitive than Ch1.

Once you select your fluorophore, an emission spectrum appears. You can adjust the slider, which will change the range of the emission filter.

3 Visible light includes Argon and both HeNe lasers.

Invisible light contains the 405nm diode.

4 T-PMT is used for collected transmitted light from lasers to create a widefield image.

6.1.5 Visualise sample on screen
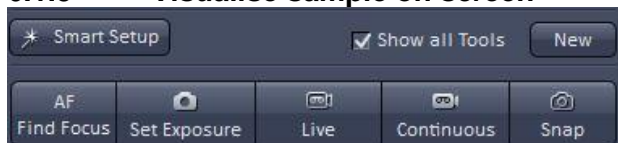
The buttons mentioned here are used to visualise your sample on screen. One you have selected 1 track (like in the adjust channels image) you can start optimizing the visualization of your sample.
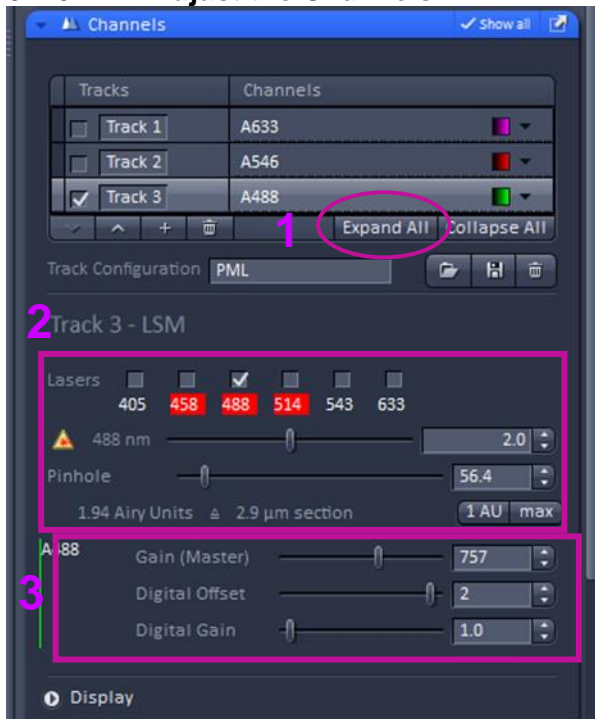
AF Find Focus and **Set Exposure** are automatic methods. They take extra time and are not necessarily optimal compared to manually doing so.

Live continuously scans the sample in a fast (maximum) speed.

Continuous scans continuously like Live, but with a final (Snap) image quality.

Snap takes a snapshot.

6.1.6   Adjust the Channels

1 Expand all shows all available tracks.

**2** Laser power and activated lasers can be adjusted.

Adjust pinhole to equalize the optical Section thicknesses in each channel. Do not equalize the Airy units.

Gain (Master) defines the PMT gain (brightness). Too much gain produces noise in the image.

Digital Offset adjusts the background intensity level. When using the range indicator, zero pixels are blue. Increasing the Digital Offset brings up the background and blue pixels disappear.

Digital Gain increases the PMT gain but can also create more noise.

6.1.7   Acquire images
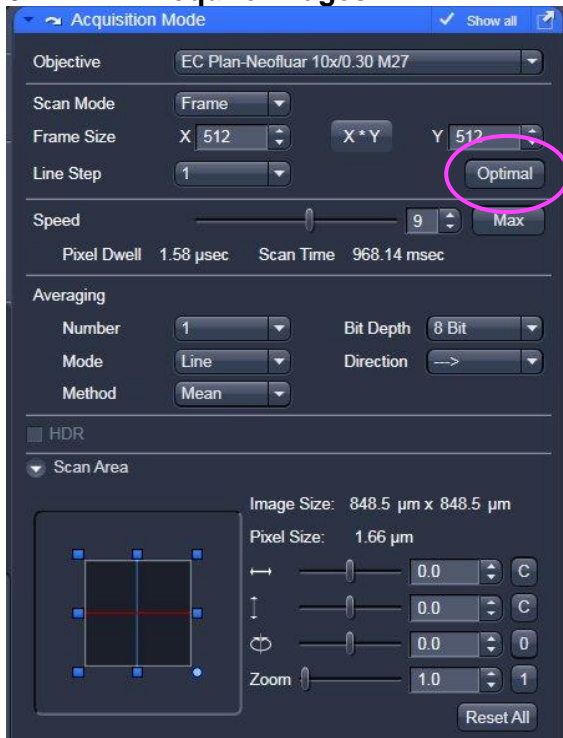
1 Objective can be chosen both here and in the Locate window.

2 Click **Optimal** for the maximum resolution for current objective and zoom level.

Increasing the **Line Step** value increases scanning speed as fewer lines are scanned.

Decreasing the **Speed** and increasing the **Averaging** increases signal-to-noise ratio with the cost of increased photobleaching and acquisition time.

Unidirectional scanning has more accuracy but a slower scan speed than bidirectional.

Scan Area defines the zoom level and angle.
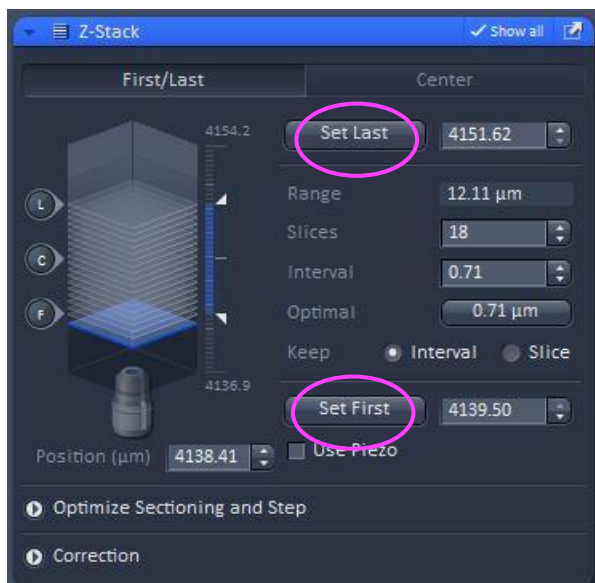
9

Experiments

Experiments are for a protocol more advanced than a single z slice.

They are activated by ticking desired boxes through the corresponding window.

Once parameters are selected they will open and new window, such as for z-stack.

Execute experiments through **Start Experiment** button

Z-stacks

During Live scan, mark the lowest and highest desired focus levels with **Set First** and **Set Last**.

Selecting **Optimal** will calculate the best interval between slices based on objective and lasers selected.

Alternatively, interval between Z-slices or number of Z-slices can be manually adjusted.

NOTE! Keeping interval spacing will alter the Z-range. Keeping slices will keep Z-range and alter intervals between Z-slices.
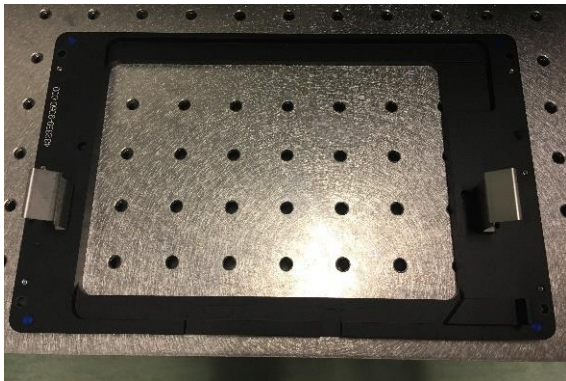
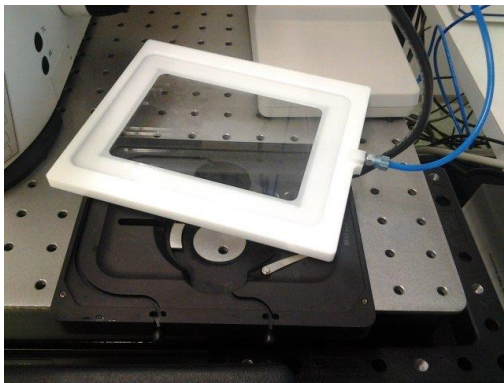Execute the procedure with the **Start Experimen**t button

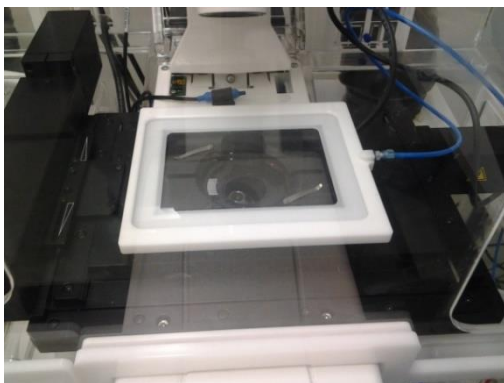# 7. Live Cell experiments
Live imaging equipment set up

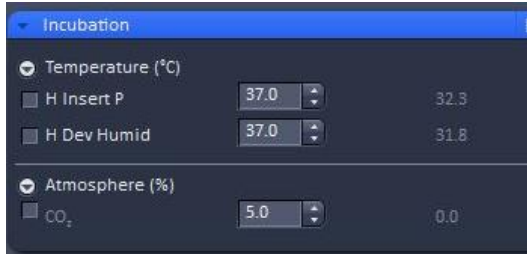Check that there is distilled water in the humidifier bottle.

Use this adapter when using the heating insert and CO_2 lid.

Place the heating insert onto the adapter mentioned above. Place your sample into the insert.

Place the CO_2 incubator lid onto the heating insert. Make sure the air holes on the lid are facing the sample.

Switch on/off the incubator and CO_2 through the software. Typically, 37°C and 5% CO_2 are used for normal cell imaging.

Setting up time-lapse

Setting up a time-lapse mean you can allow the software to run the experiment without the need to be at the microscope. You optimized your imaging set up and then you tell the software how often to capture and how long to wait between captures.
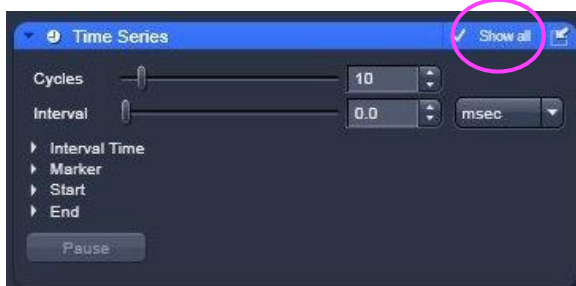
Time Series is activated in the Experiments (page 10)

Tick Show all to reveal more options

Cycles specifies the number of times you want to run your image capture set up

**Interval** specifies the gap of time between each captured image

The combination of cycles and interval will determine how long the experiment will run for.

8. AiryScan mode

1. The AiryScan module is **NOT** supported by Smart Setup. Light path settings must be configured manually.

2. Simultaneous imaging with the standard detectors is not possible.

3. While the AiryScan module may be configured to capture images in concert with the standard confocal detectors, this is only possible for sequential captures.
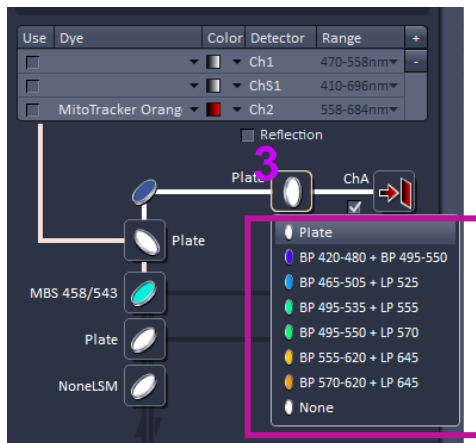
AiryScan channel setup

In the Channels setup, set the excitation laser power to 2% (see page 9)
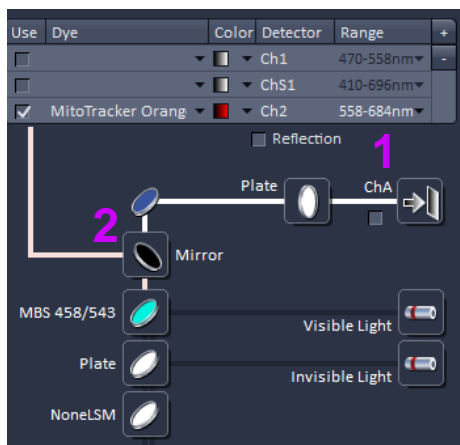
To capture image in AiryScan mode, select "**ChA**", as the imaging detector.

Selecting ChA turns off the default PMT selection and replaces the mirror in the Secondary Beam Splitter position (**2**) with a plate
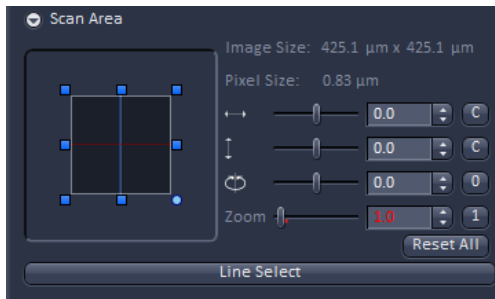

3 Select a fluorescence emission filter for the AiryScan channel. Most of these are dual pass filters; select the one most suitable for emission wavelengths of your fluorophore, and for rejection of unwanted emission.
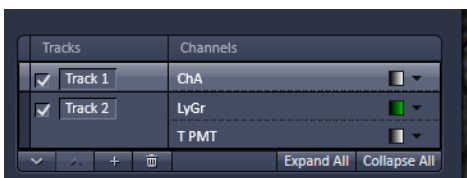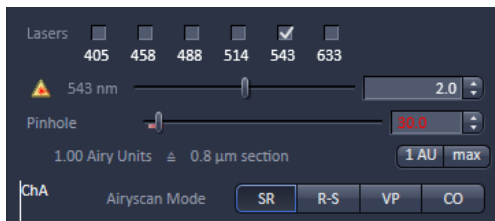

(Note! Once a channel is set to AiryScan mode, the channel name will change to **ChA** automatically

Adjust the pinhole size and zoom factor

Set the optical **zoom** to a level acceptable to the AiryScan module. The slider should **outside the red zone** and the zoom value should be in white text. The optimal zoom value is objective specific.

The pinhole value should exceed be **outside the red-zone**, minimum exceeding 1 AU.

Imaging with the AiryScan is done though the continuous mode rather than live.

AiryScan preview
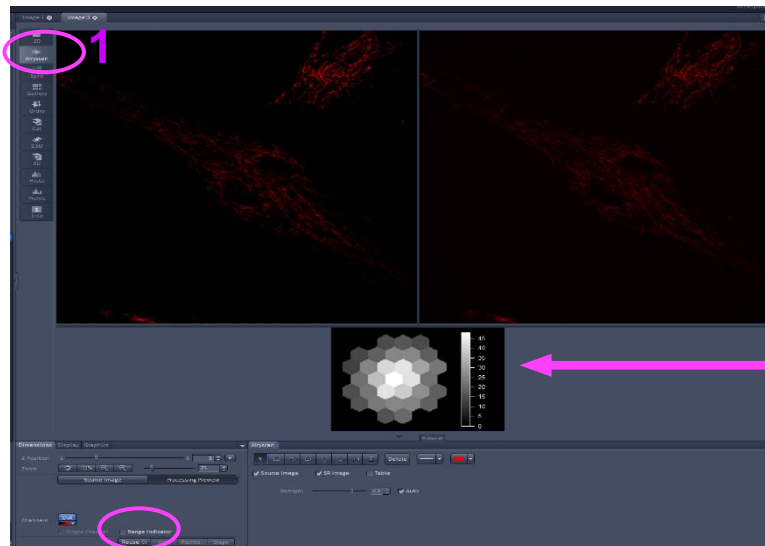
1. From the tabs on the top left side of the image display, select **Airyscan**.
You will have two windows. On the left a regular confocal imaging and on the right, the AiryScan image.

2. The hexagonal elements represent the AiryScan detectors. The brightest signal should be in the centre, falling off evenly toward the edges. If it remains markedly unbalanced, notify the CIC personnel.

3. Turn on the range indicator to ensure there are no saturated pixels.
(Note the AiryScan does not present an adjustment for offset (black level).)
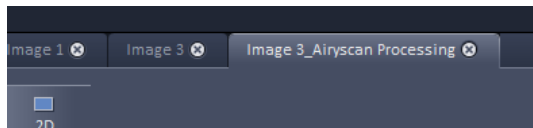
Processing AiryScan image
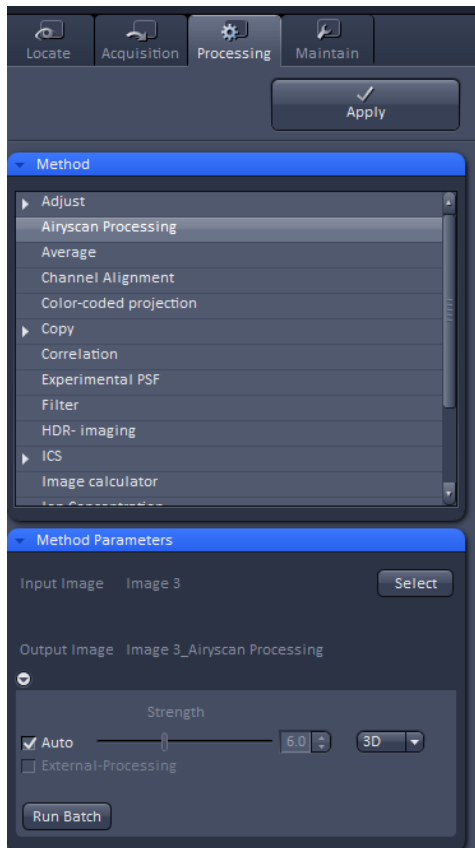
After taking the image in AiryScan mode, process the image in the **Processing tab** for AiryScan image.
1. Go to Processing tab

2. Select the image from the tab (See below)

3. Choose Airyscan Processing in **Method Parameters**

4. Click Apply

The processed image will be a new output file.

10. Troubleshooting
Do not see fluorescence in the Locate mode

Manually open the shutter in the remote control.

Errors during start-up require full restart.