

Zeiss LSM780

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

2.1 *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.

2.2 *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.

2.3 *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)

2.4 *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.

2.5 *Cancellation*

Cancellation must be done 24 hours before the reservation starts. However, if you suddenly cannot use your time, inform the next user and cancel your reservation.

If you are the last user of the day and cannot come, you are responsible for the instrument shutdown.

2.6 *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 40x and 63x objectives need water or oil with a refraction index of 1.334. For fixed samples and short time experiments, MilliQ water is appropriate
- For long experiments water immersion oil is more suitable. The water immersion bottle is marked with a **W**; the water-oil is very fluid and must be applied very carefully.
- The 100x objective needs 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. Do not mix immersion oils.
- A small drop of oil is enough, as too much will make a mess, damaging 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 will move up into the position before Load was pressed. It should be very close to the correct focus position.

4. Starting up and shutting down the instrument

4.1 Start up

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

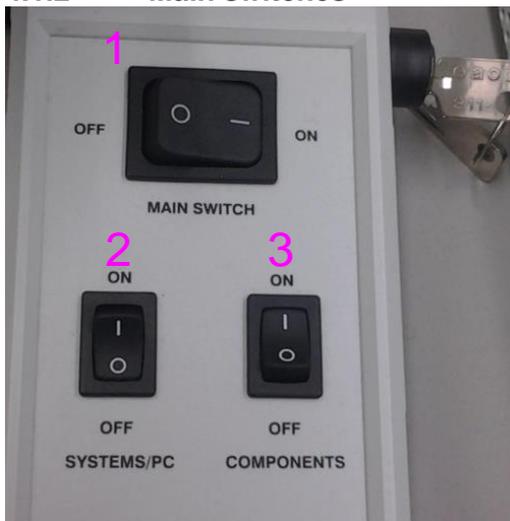
4.1.1 Definite focus



If needed, plug in and switch on definite Focus.

If definite focus remains off Zen will give an error on start up.

4.1.2 Main switches



1. **Main** on.
2. **Systems/PC** on. The computer starts.
3. **Components** on.

4.1.3 Argon laser



Turn the key on the Lasos control unit from 0 to 1.

Switch the Argon stand-by switch from Idle to Run.

The warm-up process will take several minutes. The green LED will lit when the laser is ready to use.

Note: the Argon laser will emit light also when in **Idle** but the light intensity is much weaker.

Do not touch the light control knob (the laser current selector). However, if the red LED is on, turn the knob counter-clockwise until the red light goes off.

4.2 Shut down

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

- Clean the immersion objectives you used.
- Leave the Argon laser to standby mode.
- Remove your data.

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

- Switch off all lasers through the software (incl. Argon).
- Bring objective down.
- Close the ZEN software.
- Shut down the computer.
- Switch off Systems/PC and Components.
- Be sure that Argon cooling has finished (you will hear fan switch off).
- Switch off Main.

5. Starting the Zen software

5.1 Start the ZEN software



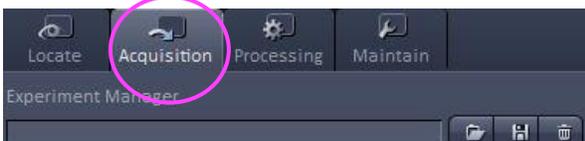
Click the **Start System** button



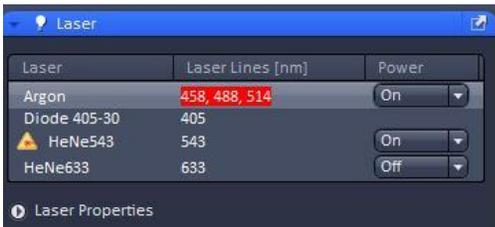
Start-up will take a few minutes; a loading bar will be visible.

If there are any errors, contact CIC personnel

Choose *Acquisition*



Switch on the *lasers*

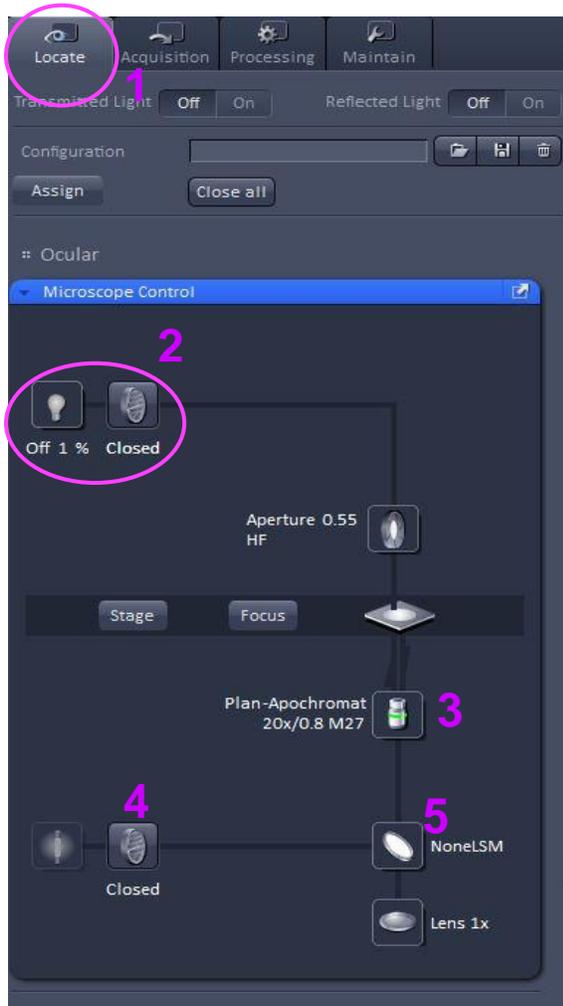


Switch on the lasers you need.
The Diode 405 is always on.

6. Using the fluorescence microscope

6.1 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 Water
- 63x/1.2 Water
- 100x1.4 Oil

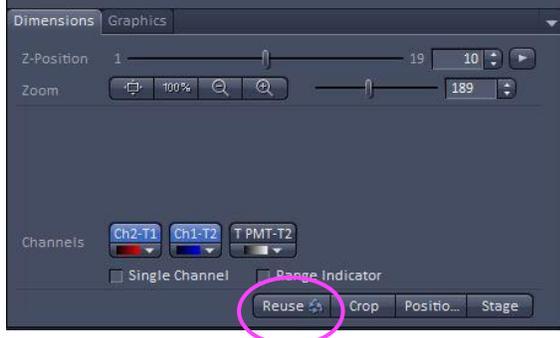
4 RL Reflected light shutter (fluorescence)

5 Fluorescence filters:

- DAPI
- GFP
- RFP
- Analyser filter (Polarization imaging)
- NoneLSM (Confocal imaging)

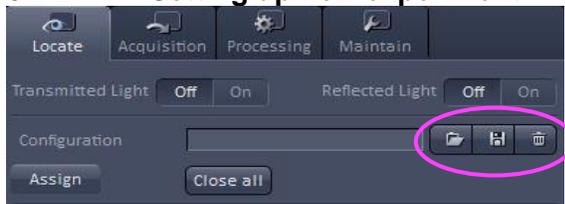
6.2 Using the confocal microscope settings

6.2.1 Reusing settings from previous experiments



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

6.2.2 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

6.2.3 Using Smart Setup



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



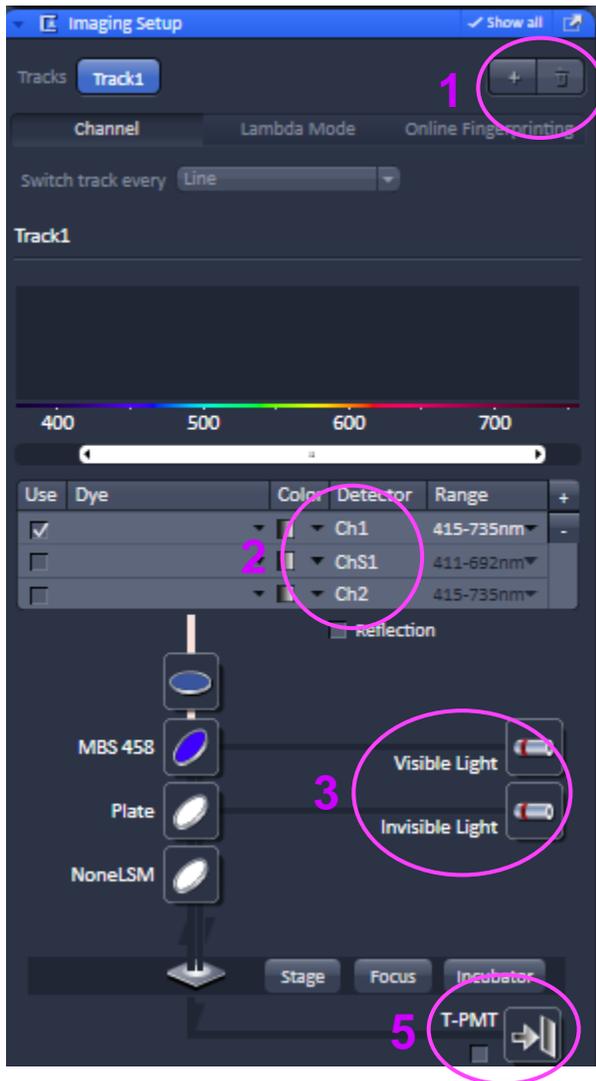
Choose your fluorophore from the dropdown box

Fastest scans your labels with a single scan. This gives the 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.

6.2.4 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**.

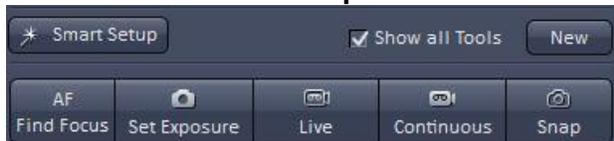
3 **Visible light** includes Argon and both HeNe lasers.

Invisible light contains the 405nm diode.

4 You must select the correct mirror to reflect the laser line toward the sample (arrow)

5 **T-PMT** is used for collected transmitted light from lasers to create a wide field image.

6.2.5 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.

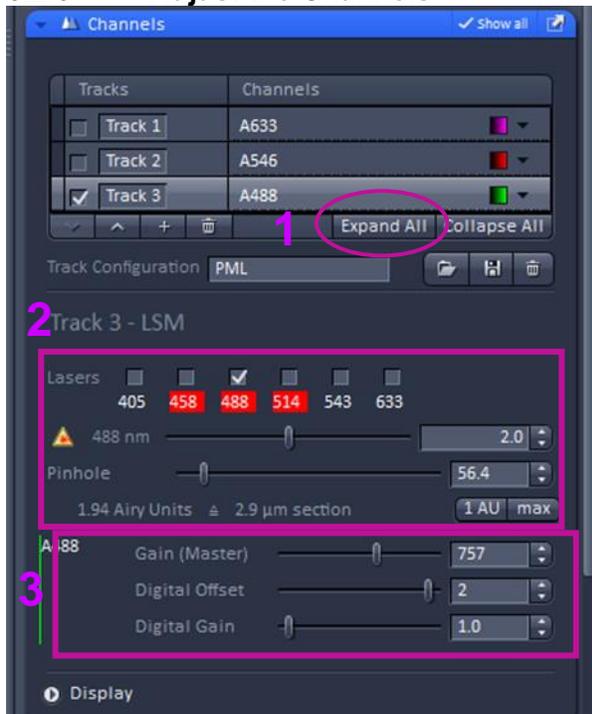
AF Find Focus and **Set Exposure** are automatic methods. They take extra time and are not necessarily optimal compared to manually doing so.

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.

6.2.6 Adjust the Channels



1 Expand all shows all available tracks.

2 Laser power and activated lasers can be adjusted.

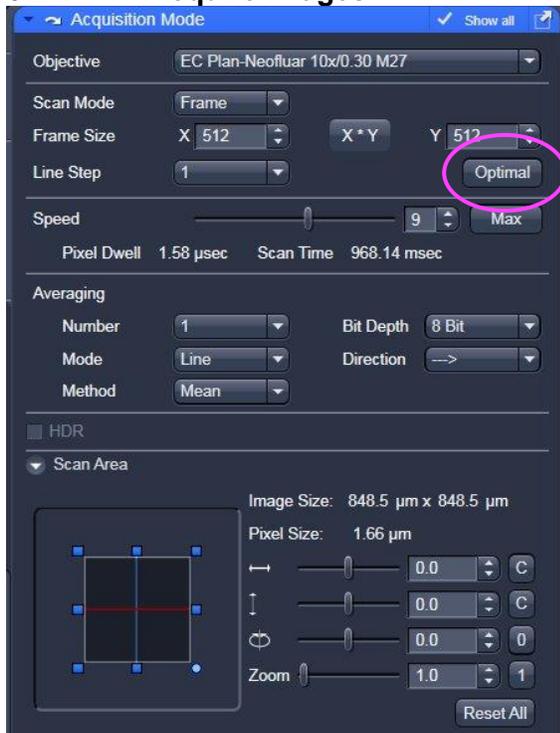
Adjust pinhole to equalize the optical Section thicknesses in each channel.
Do not equalize the Airy units.

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.

6.2.7 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 photo bleaching and acquisition time.

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

Scan Area defines the zoom level and angle.

6.3 Experiments



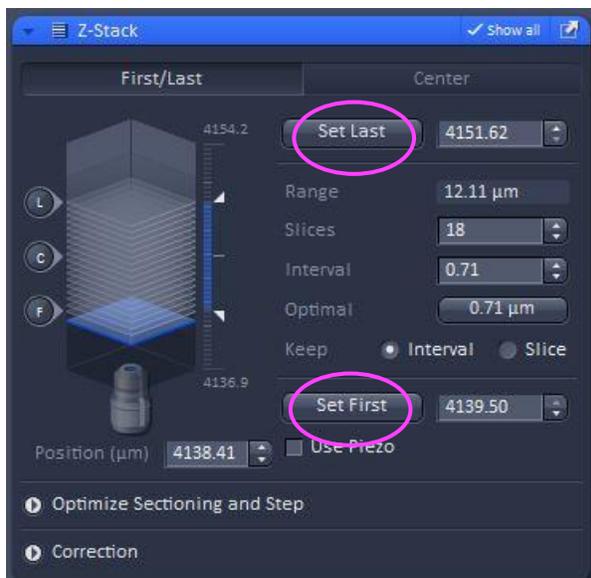
Experiments are for a protocol more advanced than a single z slice.

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

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

Execute experiments through **Start Experiment** button

6.4 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.

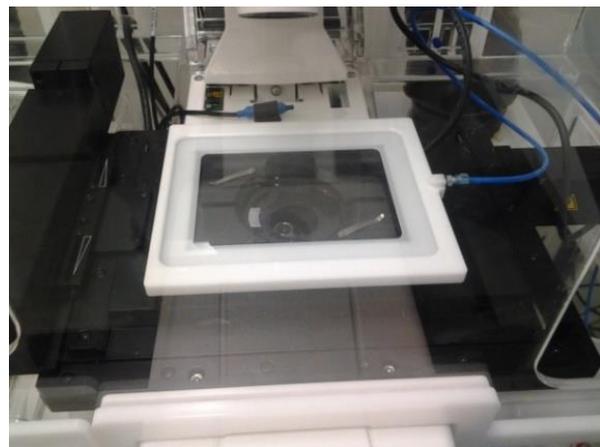
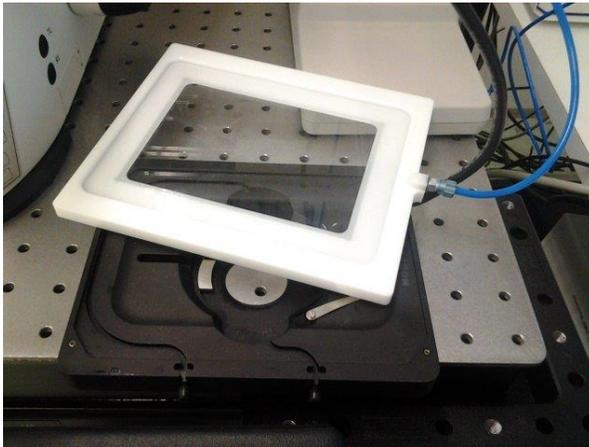
Execute the procedure with the **Start Experiment** button

7. Live Cell experiments

7.1 Live imaging equipment set up



Check that there is distilled water in the humidifier bottle.



Place the heating insert onto the stage. Place your sample into the insert.

Place the CO₂ incubator lid onto the heating insert. Make sure the air holes on the lid are facing the sample.



Switch on/off the incubator and CO₂ through the software. Typically, 37°C and 5% CO₂ are used for normal cell imaging.

7.2 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 11)

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 runs.

8. Troubleshooting

8.1 *Do not see fluorescence in the Locate mode*

Check that that the HBO 100 lamp unit is on and manually open the **FL shutter** in the touch-screen control unit.

8.2 *Weak signal with the Argon laser*

Check that the switch is in **Run mode, not Idle**.

8.3 *Argon does not start*

The LASOS cooling unit is perhaps switched off.