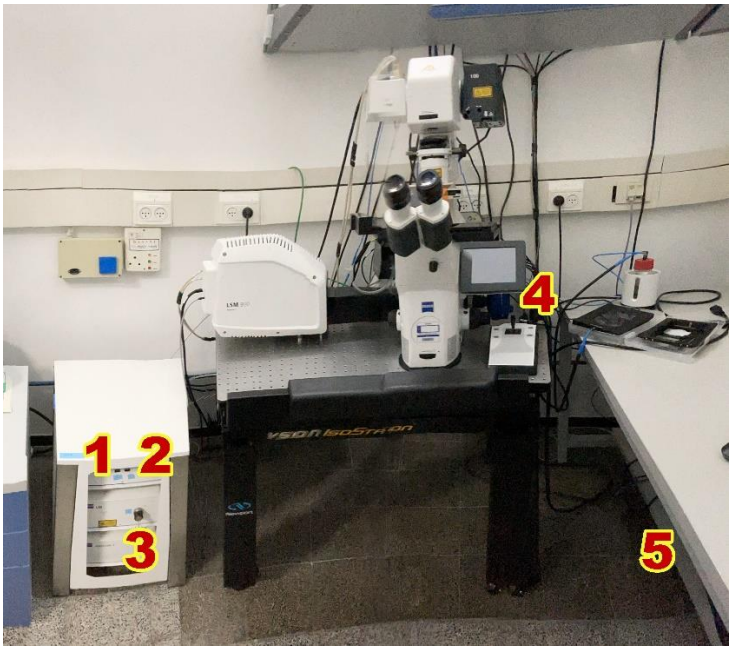
Instruction LSM900 inverted 2026-05-10.docx 2026-05-10

LSM 900 inverted instruction



Switch on

- In the main Electronic rack switch on the following switches **1 → 2**
- Turn ¼ turn clockwise the lasers module key **(3)**
- Make sure the little white light goes on and then off before you turn on the software
- Switch on main cable **4**
- Turn on the computer and log in
  User name: **multilabs**
  Password: **123456**

Position	Objective lens	Magnification	NA	coverslip	Working distance	Immersion
1	Plan Apochromat	x10	0.45	0.17 mm	2 mm	Air
2	Plan Apochromat	x20	0.8	0.17 mm	0.55 mm	Air
3	LD LCI Plan Apochromat	x25	0.8	0-0.17 mm	0.57 mm	Oil Glycerin Water
4	Plan Apochromat	x63	1.4	0.17	0.19 mm	Oil

Software and acquisition

- Log into **BookitLab** and activate your reservation to start **Zen blue 3.5**
- Make sure the white light on the laser module goes on again.
- To acquire images click **ZEN System**

Sample mounting and viewing

- Set the filter turret position to DIC and the condenser to II or III \BF according to the objective lens (see objective lens info on touchpad).
  In the software choose **Switch to > Transmission**
  Set up Kohler illumination if you are going to acquire transmitted light.

Reflected light (fluorescence)

Open fluorescence shutter
Choose the appropriate LED through the touchpad or in **Locate** tab > **Colibri 5/7**

Set the filter turret to the corresponding reflector position

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Image Acquisition - LSM

In **Acquisition** tab:

Load a saved configuration from the **Experiment Manager** or open an image and click **Reuse** or use the **smart setup** wizard.

Choose "best signal" unless your experiment is long and multidimensional. Consult with BCF staff.

If using a configuration for the first time, in **Light Path** tab check the suggested channels one by one:

The emission should start at a wavelength higher than the laser.

If you are using green and red emission fluorophores, the green emission should be up to 530nm.

If you are using red and NIR emission fluorophores, the red emission should be up to 620nm.

DAPI emission should be up to 470nm.

Use emission filters at your discretion; they are not always needed.

In **Channels** tool

With one track selected (lighter color than the others), click **Live** to focus. Optimize each channel separately by checking **Range Indicator** and setting pinhole (same optical section in all channels), detector gain, laser power, offset, digital gain should be left at 1 unless absolutely necessary.

In **Acquisition** tool:

Set acquisition parameters: zoom, averaging; set bits to 8 if acquiring LSM Plus or 16 if regular confocal.

In **Channels** tab make sure all channels are selected.

Press "**Optimal**" or "**LSM Plus**" so that the system will calculate number of pixels according to the optimal Nyquist number for the given objective, channels and zoom.

Strive for pixel dwell time greater than 1µsec.

If scanning time is too high you can consider saving time by:

- setting scanning direction to meander instead of comb if not doing colocalization
- using frame instead of line mode in averaging
- lowering pixel dwell time.
- decreasing number of averaging iterations

Press **Snap** to acquire image

- **Saving images**

Save images as soon as they are acquired

Save image as CZI filetype in **D:\ USERS DATA\PI name\user name**

- If you have acquired an LSM Plus image, go to the **LSM Plus** tab below the image and press "**create LSM Plus**". Save. **Always keep the raw data file as well.**

Multidimensional Aquisition

Check the desired dimensions (Z-stack, Tiles, Time Series)

In the **Z-stack** tool:

Choose scanning mode:

a. All tracks per slice: images one slice for each channel before next slice. Good for co-localization.

b. Full Z-stack per Track: images one channel for all slices before moving to next channel. Fast imaging.

Use the optimal interval, especially if you are using the Airyscan or LSM Plus deconvolution or are interested in colocalization by 3D reconstruction. If you are sampling using a wider interval you cannot use the "3D" option in reconstruction (in deconvolution you can choose the 3rd option: "all current images" which considers each slice as a separate image.

In **Tiles**, you will have to have calibrated the stage. Make sure your overlap is at least 10%. Stitch your images after creating the final LSM Plus or Airyscan image (do not use "online stitching").

If you do **Bleaching**, mark Experimental Regions and **Timed Bleaching**, in **Regions** mark ROI and select (v) bleaching and analysis.
If you are performing multi dimensional acquisition you have to press "**start experiment**".

Image Acquisition - Airyscan

In Zen, **Acquisition** tab:
Open an image and below the image click **Reuse or** use the **smart setup** wizard.
Airyscan is appropriate for x63 oil objective; zoom should be set to 1.3 or higher.
Open the following tools:
Channels, Acquisition Mode
With one track selected, click **Live** to focus. Optimize each channel separately by checking **Range Indicator** and detector gain, laser power, digital gain.
Adjust the master gain or laser power to set the signal range spanning 1/3 to 2/3 of the histogram range.
It is important that no saturated pixels exist.
Set acquisition parameters: zoom, averaging, bits to 8.
Press "**SR**" for super resolution or "**MPLX**" for multiplexing.
Strive for pixel dwell time greater than 1µsec.
If scanning time is too high you can consider saving time by:

- using frame instead of line mode in averaging
- setting scanning direction to meander instead of comb
- lowering pixel dwell time.
- decreasing number of averaging iterations

Press **Snap** to acquire image
For multidimensional images check the desired dimension (Z, time, tile) and after setting the relevant parameters press **Start Experiment**

- **Saving images**
  Save images as soon as they are acquired
  Save image as CZI filetype in **D:\ USERS DATA\PI name\user name**
- After saving the raw data file go to the Airyscan tab below the image, choose "2D" or "3D" or "all images" and press "create Airyscan". Save. **Always keep also the raw data file.**

Data backup

At the end of your session copy your data to a folder under your PI's name on the server (shortcut on the desktop) where it is accessible from any computer in the building following Masha's approval.
PLEASE do not use any type of USB flash drive to retrieve your data from the system computer.
There are computers with active scanning anti-virus software at the Computerized Analysis Room, connected to the servers.

Live Experiment

The system is equipped with an insert for 35mm plates or chamber slides, gas mixer and humidifier.

Ending session

Before you finish, please make sure whether the next user is coming.
If yes:

- Close the ZEN software
- **Log off your reservation at BookItLab**
- Clean oil objectives (if used) with lens paper and benzine.

- Leave nosepiece at 5x or 10x objective lens
- Center the stage
- Leave surfaces clean

If no user is coming in the next two hours, also, **in this order**:

- Close the ZEN software
- Log off your reservation in BookItLab
- Switch off (**4)** (power strip next to the microscope)
- Turn off the laser module key (**3)**
- Switch off **(2)** and then **(1)** in the switch box
- Cover microscope

  Do not turn off computer unless you are the last user before the weekend.