**Imaging Center**
**Biomedical Core Facility**
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**BCF**
Biomedical Core Facility

מרכז דימות
מרכז תשתיות ביורפואי
הפקולטה לרפואה ע"ש רות וברוך רפפורט
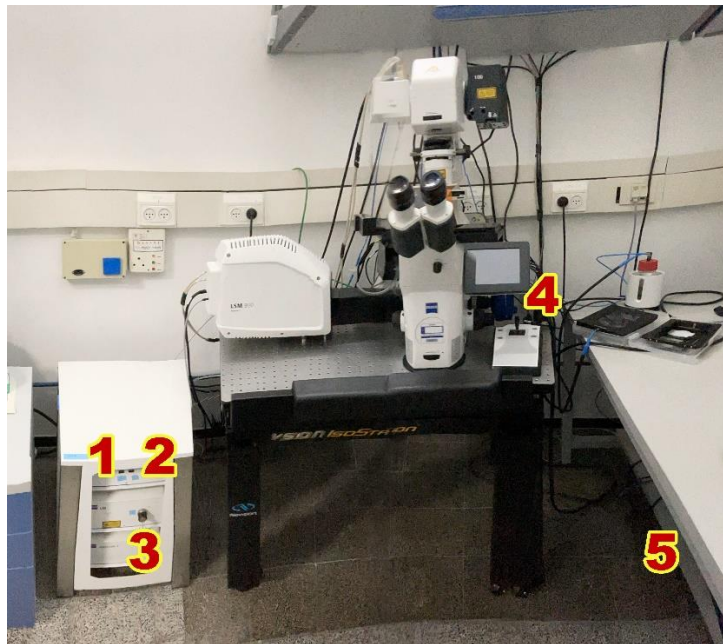הטכניון - מכון טכנולוגי לישראל

**Instruction LSM900 inverted 2024-01-09.docx    2024-03-14**

# 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)**
- 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**
- 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
  Set the filter turret to one of the reflector positions
  Choose the corresponding LED through the touchpad or in **Locate** tab > **Colibri 5/7**

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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** 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), 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).

In **Acquisition** tool:

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

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 dimension (Z-stack, Tiles, Time Series)

In the **Z-stack** tool:

Choose preference 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.

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 dimension 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, offset, 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 pixel 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:

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

Press **Snap** to acquire image

For multidimensional images check the desired dimension (Z, time, tile) and after setting the relevant parameters click **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 save 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 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
- Turn off the laser model key **3**
- Switch off **2→ 1** in the switch box
- Cover microscope
  Do not turn off computer unless you are the last user before the weekend.

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