# SHORT INSTRUCTIONS FOR OPERATING LSM1/2 (Zeiss LSM510) AT CIAN

Version 1.4, September 2014

**Before starting**

To work with LSM1 or LSM2, you need to get trained. Contact the CIAN microscopy team (cian.microscopy@mcgill.ca) to organize a training session.

**General workflow**

1. Turn on microscope, components as needed (see sections 1, 2)
2. Visually inspect objectives; clean as needed (section 2.2)
3. Use test slide to adjust Köhler illumination in bright field (section 2.1)
4. [Set up and adjust DIC illumination]
5. Focus and position experimental sample in transmitted light (BF or DIC)
6. Set up wide-field fluorescence, confocal fluorescence
7. Use acquisition software to acquire images, stacks, time lapses
8. Move data off computer (see section 4)
9. Clean objectives, microscope workstation as needed (section 2.2)
10. Turn off microscope (see section 5), log time in logbook

**General reminders for LSM1 and LSM2**

- Obey 2h/2h rule, i.e. lasers have to be on for at least 2h and off for at least 2h.
- Optical cables are fragile, don't touch, or put things on them
- Avoid bumping into equipment, leaning on air table
- Move data off microscope computer after aquisition
- Limit or omit use of non-confocal (wide field) fluorescence; kills sample and signal

**Useful abbreviations and terms**

LSM = laser scanning microscope

BF = bright field

TL = transmitted light

FA = field aperture, also A or AP = aperture diaphragm

FD = field diaphragm

WFF = wide field fluorescence

DIC = Differential Interference Contrast, a.k.a. Nomarski Interference Contrast

AIM = 'Advanced Imaging Microscopy', acquisition software from Zeiss

Light sources: halogen lamp (for TL), *FluoArc* mercury lamp (for WFF), lasers (for LSM confocal, or TL in channel D)

**More information**

The *Zeiss* LSM510 and Axiovert 2000M manual is found on the LSM computers, and in hard copy in the LSM room.

More extensive, user-friendly operating instructions for the *Zeiss* LSM510 (i.e. the same model as ours) can be found on the internet, e.g. here: http://microscopy.duke.edu/510invmanual.html; keep in mind that the specific setup in our facility is somewhat different.

## 1 - Equipment Setup

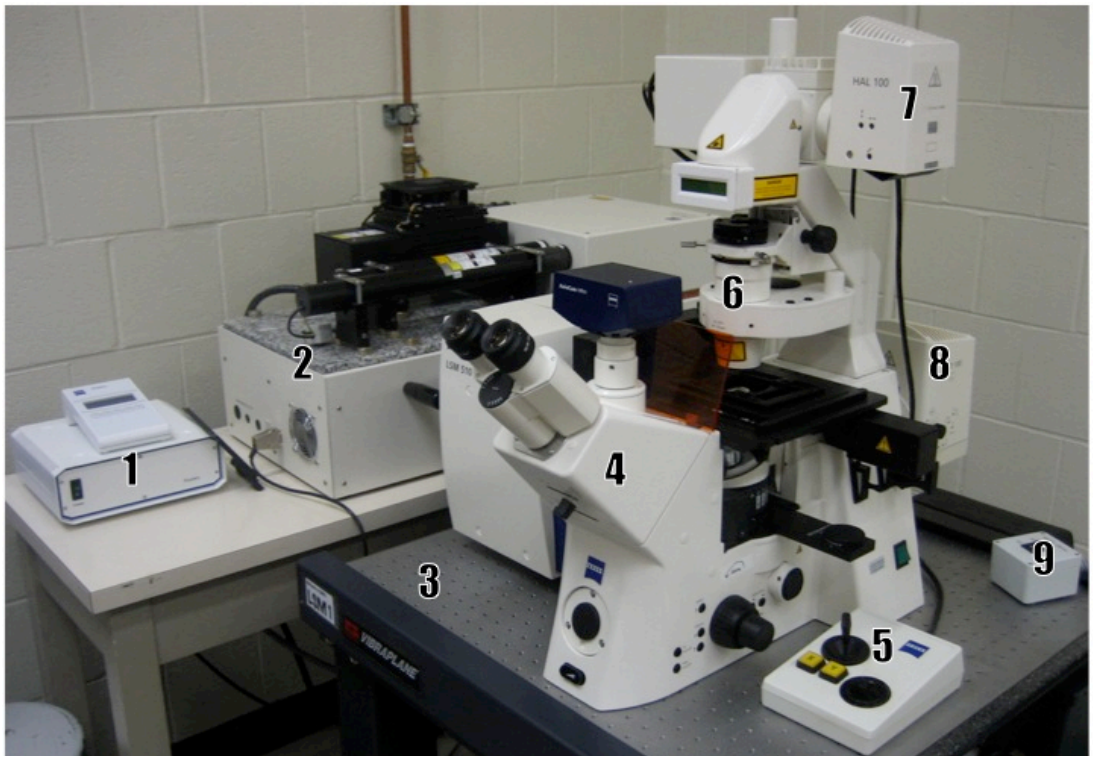



**Fig. 1: LSM1, LSM2 setup**

1 – LED source for WFF
2 – lasers for fluorescence excitation
3 – vibration table
4 – *Zeiss Axiovert 2000M* inverted microscope
5 – joystick for xy stage control (LSM1 only, manual control on LSM2)
6 – condenser
7 – halogen lamp
8 – *FluoArc* mercury lamp
9 – remote control, on/off button

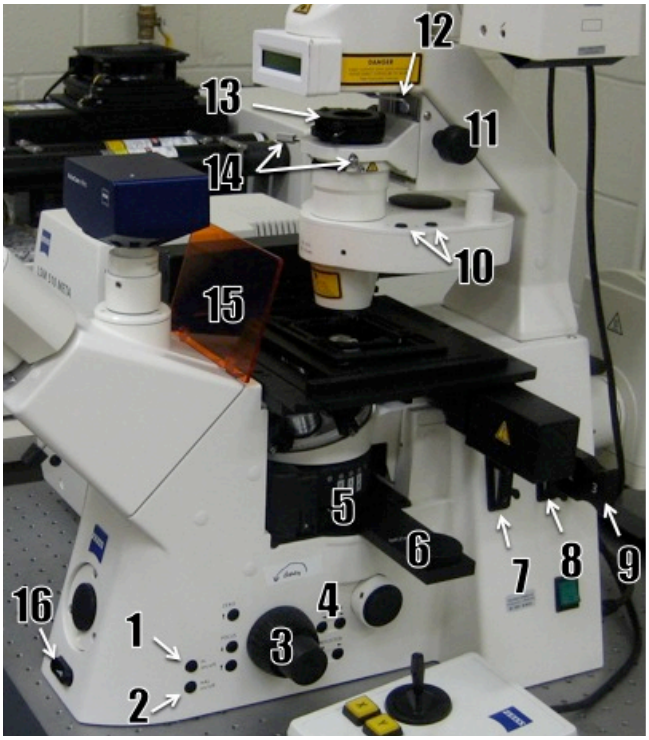


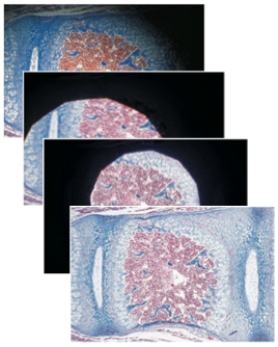

**Fig. 2: LSM control elements for TL, WFF, DIC**

1 – fluorescence shutter, “FL ON/OFF”
2 – halogen lamp shutter, “HAL ON/OFF”
3 – manual focus drive
4 – fluorescence filter cube selection
5 – reflector turret with fluorescence filter cubes
6 – DIC analyzer
7 – Field Diaphragm for WFF
8 – Field Aperture for WFF
9 – neutral density (ND) filters
10 – DIC controls
11 – condenser height (focus) control
12 – Field Diaphragm for TL
13 – DIC polarizer
14 – condenser centering screws
15 – eye protection plate
16 – halogen light intensity switch

## 2 - Starting up LSM1, LSM2

1. Take the **dust cover** off the microscope, put on hook, not the floor
2. Turn on **remote control box**, Fig1-9 (confocal head, laser power control), check that microscope is on, if not, use green button
3. Turn on **computer**
4. **Log in** to your account, launch program
5. Visually inspect **objectives**, clean as needed (see section 2.2)
6. Optional, as needed: turn on **lasers** using software, take special caution with Argon laser (see section 3.1), turn on LED lamp for WFF by using foot pedal only when needed

## 2.1 - Adjusting Köhler Illumination



1. Insert your test slide, adjust light intensity, **focus on specimen** in bright field using 10x objective
2. Close Field Diaphragm (FD) to see edge, **focus** it by adjusting the condenser height, center if needed, **open FD** just enough to illuminate field of view (see images on the left)
3. To adjust Field Aperture (FA), **remove one eyepiece**, use button “A” on condenser base to adjust aperture to 80% of back of objective
4. In case of poor images, clean objective (see section 2.2), repeat procedure; if unsuccessful, notify facility personell and make note in log book
5. Repeat for every objective to be used (or at least do it for objectives used in image acquisition)

## 2.2 - Cleaning objectives

Clean objectives before and after use as follows:

- Take a Kimwipe and fold it in three into a long rectangle.
- Wipe the lens gently by holding either end of the Kimwipe, dragging it gently across the objective lens three times. Use a fresh area of the tissue each time.
- Repeat as needed to remove excess of immersion oil.

Use a test slide to evaluate the optic of the objective, and if needed, clean the objective more thoroughly:

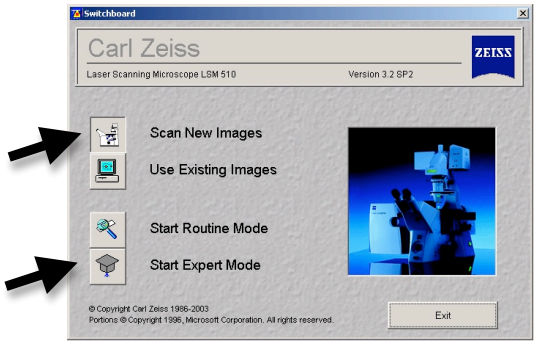
- Fold as above, put a drop or two of lens cleaner (the blue fluid) on the Kimwipe.
- Wipe the lens gently by holding either end of the wet lens tissue dragging it gently across the objective lens three times. Use a fresh area of the tissue each time.
- Repeat with water, then with ethanol.
- Give the objective lens a final wipe or two with a dry Kimwipe to remove any excess fluid.

NEVER wipe the lens in a circular pattern.

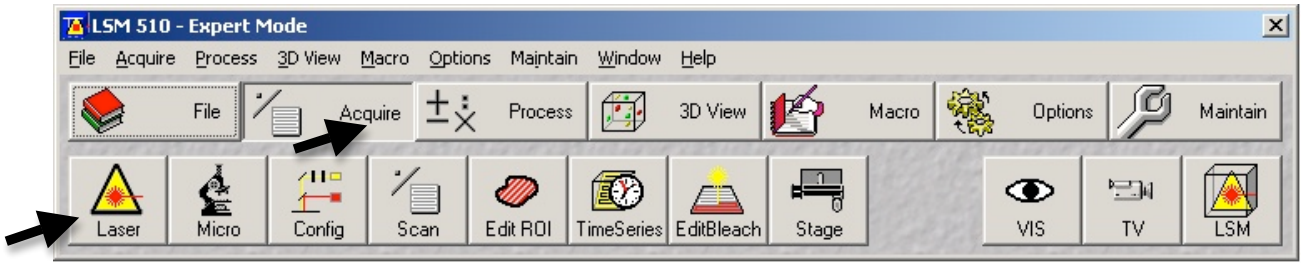
NEVER apply any pressure directly to the lens.

## 3 – Starting acquisition software and lasers

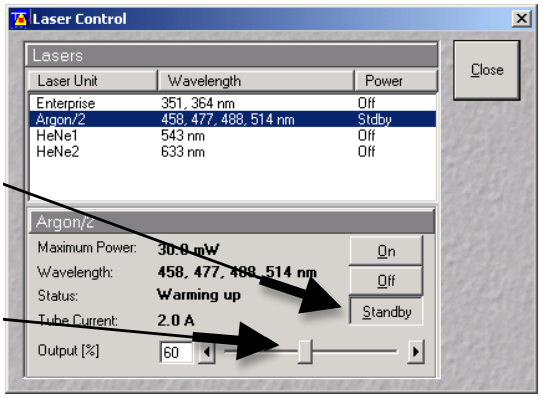

- double-click LSM510 icon on desktop
- for acquisition, select "Scan New Images" and "Start Expert Mode"




### 3.1 Laser ignition




- in Expert Mode menu, select "Acquire", "Laser"
- turn on lasers you will need
- Argon laser must first be turned to "standby", until "on" becomes available, then raise output to 6 Amp.




### 3.2 Note about laser shut-down

Please leave lasers on if someone is scheduled within 2 h after your session.
If the microscope has been left on for you, and you need to cancel your session, it is now your responsibility to turn off the system.

## 4 - Saving data on the Server

Acquisition should always be to your directory in your lab's folder on the D: drive. Afterwards, move data off the microscope computer onto an optical disk (CD-R only, no –RW disks or USB drives allowed), or to the CIAN server as described. Do not delete data that are not copied somewhere else.

1. Connect to the server (start menu, run: \\10.1.0.3), open window for your folder on the server
2. Locate your library on the PC and drag it into your server folder
3. Delete the library from its original location on the hard disk as soon as possible; very large projects will have to be removed immediately after backing up. NO LONG TERM STORAGE WILL BE ALLOWED ON THE COMPUTER
4. You can access your data on the server (Perola, IP address 132.206.213.90) from your networked computer. Note that you have to be connecting from a static IP address within the McGill network, not using a wireless connection (unless you are on VPN).
   A protocol for this is available from the 'Data Storage and Analysis' section of the CIAN website.
5. To avoid data corruption, DO NOT open files on the server. Always make a copy on your local computer to view and analyze.

## 5 - Shutting down LSM1 or LSM2

1. Exit software, computer account; leave computer on if someone is coming to use the system within the next two hours, otherwise shut down the computer
2. Turn off **lasers** (using AIM software) if you are the last user of the day.
   a. For the argon laser: bring output from 6 amp all the way to the left, click on "standby", then "off".
   b. For the 405nm, 543nm and 633nm lasers, click on "off".
3. Exit the software, log off or shut down.
4. Wait for the argon laser fan to stop.
5. Turn off **power to LSM1 or LSM2** with the "remote control" button.
6. **Clean** objectives according to general instructions
7. Carefully replace the **dust cover** on the microscope
8. Make sure that the **LED lamp** is off (no light shining on table)

## Appendix A: Technical data for LSM1 and LSM2

**Microscope Base:**

Zeiss LSM510 on Axiovert 200M, fully motorized (LSM2: motorized focus drive, manual x and y)

**Objectives:**

| Position | Objective LSM1 | Objective LSM2 |
|---|---|---|
| 1 | **10x**/0.3 **dry** | **10x**/0.5 **dry** |
| 2 | **20x**/0.4 **LD* dry** | - empty - programmed 10x/0.3 for maintenance only |
| 3 | **20x**/0.75 **dry** | **25x**/0.8 **oil**-Glyc-water** |
| 4 | - empty - programmed for 40x water (see below) | **40x**/1.3 **oil** |
| 5 | **40x**/1.3 **oil** | **63x**/1.4 **oil** |
| 6 | **63x**/1.4 **oil** | **100x**/1.4 **oil** |
| available | **40x**/1.2 **water** | |
| available | **40x**/0.6 **LD* dry** | |

* long working distance; positioned for 0.17mm cover slips, can be adjusted upon request

** set for oil; can be adjusted for glycerol or water immersion upon request

**Confocal mode: Lasers, fluorescence emission filters (in addition to spectral 'meta' detector)**

| Laser lines | Emission filters LSM1 | | Emission filters LSM2 | | Typical fluorophore |
|---|---|---|---|---|---|
| | Ch2 | Ch3 | Ch2 | Ch3 | |
| 405nm, Diode, Blue (LSM1) | blue and green emitters: LP420, LP475, LP505, BP420-480, BP470-500, BP475-525, BP505-530 | orange, red, far-red emitters: LP505, LP560, LP650, BP500/20IR, BP505-550, BP530-600, BP560-615 | blue and green emitters: LP475, LP505, BP475-525, BP500/20IR, BP505-530, BP530-600, BP505-550 | orange, red, far-red emitters: LP505, LP530, LP560, LP585, BP560-615, BP585-615, LP650 | DAPI |
| 458nm, Argon, 30mW | | | | | CFP |
| 477nm, Argon, 30mW | | | | | |
| 488nm, Argon, 30mW | | | | | FITC, GFP |
| 514nm, Argon, 30mW | | | | | YFP |
| 543nm, HeNe Green,1mW | | | | | Rhod., Cy3 |
| 633nm, HeNe Red, 5mW | | | | | Cy5 |

**Wide-field fluorescence:**

Light source: *FluoArc* mercury arc lamp, 100W

| Filter set/cube | Excitation filter | Beam splitter | Emission filter | Typical fluorophore |
|---|---|---|---|---|
| Filter Set 00 | BP 530-585 | FT 600 | LP 615 | mCherry, AlexaFluor 594 |
| Filter Set 13 | BP 470/20 | FT 493 | BP 505-530 | GFP |
| Filter Set 49 | G 365 | FT 395 | BP 445/50 | DAPI |
| Filter Set 46 (LSM1 only) | BP 500/20 | FT 515 | BP 535/30 | YFP |