

# **INSTRUCTION MANUAL**

## **ZEISS LSM710**

### **LASER-SCANNING CONFOCAL**

### **MICROSCOPE**

**The Microscope Can Only Be Used After Proper Training And Examination**

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## Order of switching:

1. HBO lamp (only if you are looking for a fluorescence light)
2. Main switch
3. System
4. Key (turn to on position)
5. Components



6. Laser : turn key to on (-) position
7. Laser: turn tumbler switch to laser run position
8. Computer



## IMPORTANT

- ! HBO lamp has to be switched only if fluorescent light is required (there is no possibility to switch it on later, as can cause permanent damage in the device)
- ! check the laser power at switching (next to tumbler switch: light control knob: should be at 9 o'clock /middle position at the left side/)
- ! if the LED display in the laser box is red (high power mode): turn down the light control knob
- ! ZEN software should be launched after the laser is warmed up (cca 5 minutes), and the LED display in the laser box lights green.



## Sample insertion

1. open the top of the microscope head
2. turn the microscope slide upside down (cover slip points down)
3. tighten the slide with the two side plates of the specimen holder



- ! 63x and 100x objectives should be used with immersion oil!

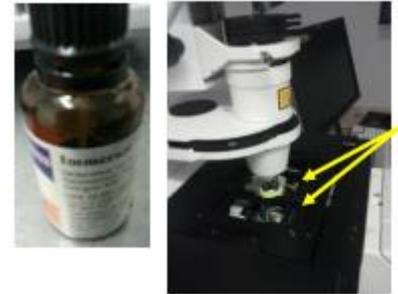
**Usage of immersion oil**

necessary only when using 63x or 100x objectives (marked with rubber ring)

the 1st drop of the dropper should be dropped back to the vial, the 2nd drop should be placed onto the top of the objective without touching the surface of the objective with the dropper

! avoid the flow of oil to the side of the objective lens

WARNING: immersion oil is irritating, avoid direct contact with skin/wash your hands



**Software**

1. enter the **LSM USER**
2. launch the software (double left click with the mouse)



! ONLY AFTER THE LASER HAS ALREADY WARMED UP

3. click the **start system** icon
  - a. the software starts checking the operation of the microscope
  - b. after the checking the following error message is normal:



Do not deal with it!



**Searching the sample in the eyepiece**

- click the **Locate** icon: click **oculars online**: switch on the lamp: adjusting the light intensity of the lamp:



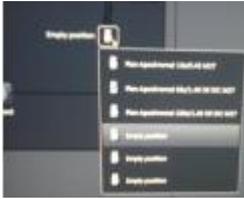
(2 possibilities)



mode a. with the slider in the software

mode b. knob on the body of the microscope

-set the appropriate ocular (2 possibilities):

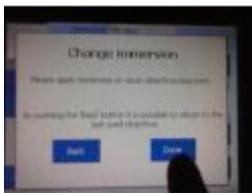


mode a.  
in the software



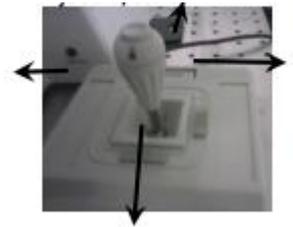
mode b.  
use the touch pad by pressing the required magnification icon

- usage of oil immersion objective requires a confirmation about applying/removing of the oil:



if the request is completed please press DONE in the touch pad

- stage can be moved (forward, backward, leftward, rightward) with the joystick (in the desktop next to the microscope)



x, y moving of the stage can be slowed down with pressing the button on the top of the joystick (jingling sound), (press again to set back the default speed)

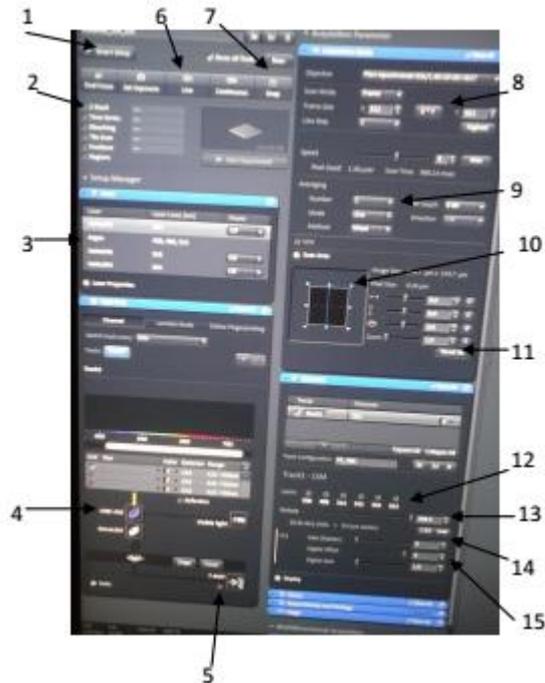
- focusing: micro/macrometer knob located on both sides of the microscope
- after found the sample in the ocular: close the shutter(s)



- upper shutter for transmission light
- lower shutter for fluorescent light (if choose, please set the appropriate filter)

## How to make a snapshot from the sample?

Click to **Acquisition** icon, the following panel can be seen on the screen:

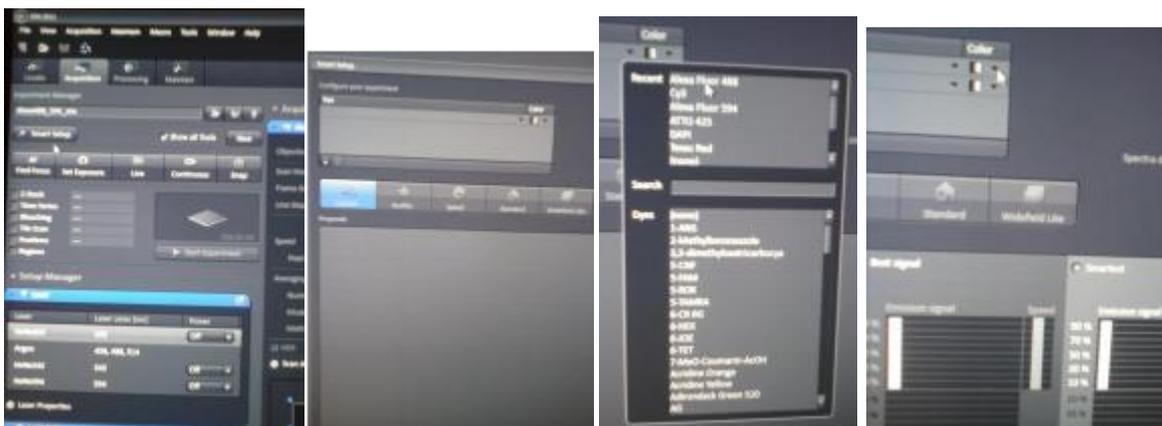


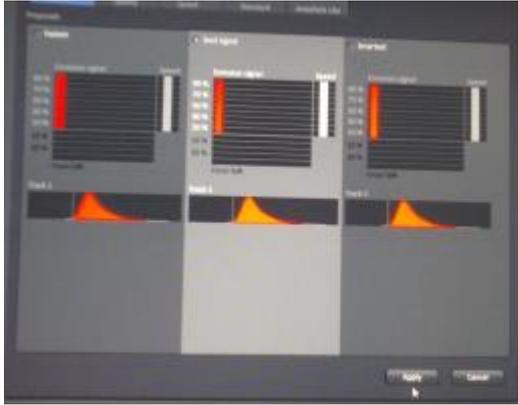
1. Smart setup: select the applied dye and its color
2. Z-stack: optical slicing (check if it is used)
3. lasers (on/off state can be seen)
4. mean beam splitters
5. T-PMT: check if quasi-transmission image is required
6. Live: display image, start image acquisition
7. Snap: make snapshot
8. Frame size (set min. 1024x1024)
9. Averaging: set min. 2 (run 2 times the laser)
10. Scan area: the size of the scanned area
11. Reset all: set back the default size of scanned area
12. Lasers: actually used laser
13. Pinhole: set 1 AU
14. Gain: set the PMT detector

15. Digital offset: to remove background (cca 2)

## Steps of image acquisition:

1. Smart setup: select the applied dye (fluorophore) and its color





After set the dye choose the **Best signal** option then Apply

! When use more than one dyes: 1st choose the Fastest then find the focus (in Best signal: the instrument responds slowly). After set Best signal. Best signal option can remove spectral overlapping. In multiple labeling always use Best signal in image acquisition.



After selecting the given fluorophore the instrument displays its characteristic spectrum, switches the appropriate laser on, select the appropriate filter (check the settings!)

The set spectrum can be manually adjusted further by moving the colorful bar below the spectrum (eg. in spectral overlapping).

2. Settings:

Frame size: min.1024x1024

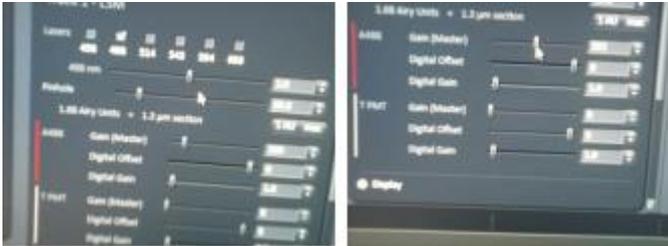
Averaging: 2



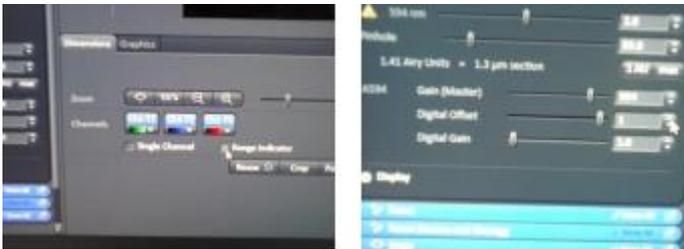
3. Experiment can be launched with the Live button, but before making a snapshot the quality of the image should be verified with the Continuous button



- Set the intensity of the laser and the gain of the PMT (laser intensity is not allowed to be greater than 10, gain: around 900 extremely increases the background)  
Pinhole: set 1 AU



- Range indicator (below the image): shows overexposed regions (red)- in red pattern: decrease laser and/or gain. With **Digital offset** (increase) remove blue from the background.



- Snap: taking photo about the sample. (First press STOP, then press Snap)



- Corrections: below the image with Display button image can be corrected (cut bright and dark intensities off, improve contrast).



- Save image: after taking a photo save it (otherwise the next image overwrites it)

**Order of switching off:**

1. cleaning the objectives: use 96% Ethanol to remove the rest of the oil.  
opposite of switching on:
2. switch off lasers in the software
3. close the software
4. switch of laser box.
  - a. tumbler switch
  - b. turn key to off position

IMPORTANT: after at least 2 minutes the laser stops (banging sound stops)

5. after the laser stops: components
6. turn the key into off position
7. system
8. main switch
9. HBO lamp
10. cover the device with the blue perianth

**How to register?**

At least 48 h before usage one has to book the instrument (Google calendar).

For further information, please contact:

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