### **Zeiss LSM 710 User Guide**

## Startup

- Turn on **Exfo** lamp to the left of the microscope
- Turn on [Main], [System] and [Component] switches
- If the **Argon** laser is needed (458, 488 or 514nm):
  - Check that the toggle switch is set to [idle]
  - Turn the **key** on the front of the power supply clockwise
  - After 5 minutes switch laser to **[run]**. After another few minutes adjust the **potentiometer** knob to the point just below where the red LED becomes illuminated. (While waiting, continue the startup sequence)
- -Turn on **computer** and log on using your Hawk ID and Hawk password and wait until MacAfee loads.
- -Start [ZEN 2010] software and do not use the microscope while the software loads.

## **Visual Operation**

- -Load slide
  - Select a X10 objective lens. Make sure the stage is sufficiently lowered [Loading position] using the key pad by the microscope or the lower button at the base of the instrument
  - -Load sample onto stage and return using the top left icon on the pad or the upper button.

Note: Do not depend on the preset load and work settings. Samples and slides of different thicknesses may have been used by the previous investigator.

- In Zen in the [Occular] tab, select [Online]
- Select the desired fluorophore or DIC filter
- Select the proper objective lens
- Locate region of interest
- Close the reflected or transmitted light shutter used

## **Confocal Operation**

- Select [Acquisition]
- Select [preset configuration] or use [Smart Setup]
- If you have selected at dye that requires the 561nm line (i.e. AF 568, mCherry etc.) then turn the laser on via the **[Laser]** menu

#### ATTENTION

If you are using a method from the "Smart setup" requiring **more than ONE track** (as seen in the **[Imaging setup]** window, you must follow these guidelines:

- In the [Imaging setup] window select Switch track every [line] instead of [frame] using the pulldown window.
- Check that each track display the proper highlighted emission curve. If not, adjust using the cursor under the emission spectra.

All the tracks selected must have the same setup in their MBS (seen in the [Light Path] window).

- Select [Select all] under the [Channel] window. For each track loaded you will see the laser line necessary for each dye excitation.
- Under the [Light Path] window, select the MBS matching the excitation lines required for your imaging by clicking on the MBS icons (1<sup>st</sup> one and 2<sup>nd</sup> one under the window).



- In the Acquisition Mode menu select the desired parameters for final image
- Select [Auto Exposure] to get initial gain and offset settings
- Select [Live] to find your plan of focus using the focus knobs
- In the [Channels] menu adjust the [laser power], [pinhole], [gain] and [offset] as needed
- Select [Snap] to collect the final image
- Save image to desired location (Do not store on drive C:\ instead use the D:\ drive)

#### **ATTENTION**

## All images left on the D-drive are periodically purged without warning

## **Shutdown**

# Check the online scheduler to determine if someone is scheduled after you (within 1 hour).

## IF YES:

- Turn the Argon laser switch toggle to [idle] and log out of software

### IF NO:

- Turn the Argon laser switch toggle to [idle] and turn off key
- Wait for Argon laser fan to switch off (about 5 min from turning the key off)
- Move stage to load position
- Remove slide
- Gently remove oil from oil lens using lens paper or cotton swabs. Use a cotton swab with a little isopropyl alcohol to remove final traces of oil
- Exit the Zen 2010 software and shut down the computer
- Turn off the [System], [Components] and [Main] switches in this order
- Turn off the Exfo lamp
- Log your usage in the log book by the microscope.

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