**Zeiss LSM 710 User Guide**

**STARTUP**

 - Turn on **Exfo** lamp to the left of the microscope **1**

- Turn on **[Main] 2**, **[System/PC] 3** and **[Component] 4** switches

 - Turn on **Argon** laser (458, 488 or 514nm):

 - Check that the **toggle switch** **6** is set to **[idle]**

 - Turn the **key** on the front of the power supply clockwise **5**

- After 5 minutes move laser **toggle switch 6** to **[run]**. After another few minutes adjust the **potentiometer** knob **7** to the point just below where the red LED becomes illuminated. (While waiting, continue the startup sequence)

- Turn on **computer 8** and wait for log on window

- On **Exfo** lamp **1** push on the **Open button** **9** once hour display stopped blinking

**FOR THE FIRST USER OF THE DAY**

 log on using: **your HawkID**

 and password: **HawkID password**

 - Start **[ZEN]** software and **do not use the microscope while the software loads.**

**VISUAL OPERATION**

 - Load slide

- Select a X5 or X10 objective lens. Make sure the stage is sufficiently lowered **[Loading position]** using the screen pad on the right of the microscope or use the lower button at the base of the instrument.Select **[Set work position]** on the screen pad.

-Load sample onto stage and focus on your sample then select **[Set work position]** icon on the screen pad.

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 the **[Locate]** tab select the desired fluorophore or DIC filter. Automatic shutter opening/closure upon selection of an imaging mode and TL shutter closes when reflected mode selected.

 - Select the proper objective lens

 - Locate region of interest

- Close the reflected or transmitted light shutter used. To turn off the TL lamp, click on the TL icon and then click on **[ON]** (ON in blue = lamp ON, TL in gray = TL lamp OFF).

**CONFOCAL OPERATION**

1. Select **[Acquisition]**
2. If you have a previously acquired image saved in the format of acquisition (.czi), open that image in the program and click on the **[reuse]** button at the bottom of the image window, or

C- Select **[Smart Setup]** and **[Current]** to keep full control of the confocal acquisition setup and select **[Smartest Line]**, or **[reuse]** parameters from a previous .czi image file

D- The lasers, with the exception of the argon laser, will automatically turn ON if needed from the configuration setup or modify the excitation line under 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, and not using the **[Smartest Line]** 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 displays 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 **[Expand 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 (1st one and 2nd one under the window).

 D- In the Acquisition Mode menu select the desired parameters for final image

 E- Select **[Auto Exposure]** to get initial gain and offset settings

 **OR**

 F- Select **[Live]** to find your plan of focus using the focus knobs

 G- In the **[Channels]** menu adjust the **[laser power], [pinhole], [gain]** and **[offset]** as needed

 H- Select **[Snap]** to collect the final image

 I- Save image to desired location **(Do not store on drive C:\** instead use the **D:\ drive)**

J- **Select .czi format.**

**ATTENTION**

**All images left on the D-drive are periodically purged (every 3 months) without warning**

**SHUTDOWN afterhours:**

**Check the online scheduler to determine if someone is scheduled after you (within 0.5 hour).**

**IF YES:**

- Turn the Argon laser toggle switch to **[idle], close your images BUT let program opened**

**IF NO:**

- Turn the Argon laser toggle switch **6** to **[idle]** and turn off key **5**

 - **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 software then** **shut down the computer 8**

 - Turn off the **[System] 3**, **[Components] 4** and **[Main] 2** switches in this order

- Turn off the **Exfo lamp** **1**

- **Log your usage in the log book** by the microscope.

**The NIH grant number for this instrument should be included in all publications. Grant Number Zeiss LSM710 1 S10 RR025439-01**