LSM 710 Confocal Microscope Updated Operation Protocol

Turning ON the system:

1. On the power remote switch, push "Main Switch" ON (Fig 1A).

2. Switch ON "Systems/PC" (Fig 1B).

3. Turn ON the computer and wait for the welcome page to show.

4. Switch ON the "Components" button (Fig 1C).

5. Start the ZEN software and press "Start System".

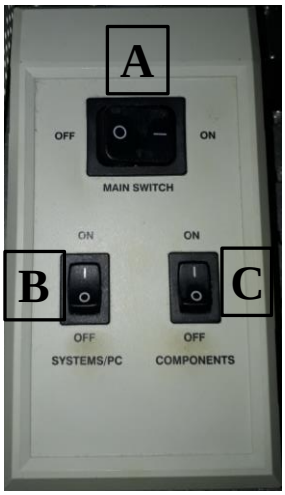

Figure 1

6. If the Ar-ML laser is required, turn the "key" on the power supply to 1 (Fig. 2A). <u>Warm up period is 15 min!</u>

N.B: Always keep the "toggle switch" ON (Fig. 2B).

Figure 2

7. Push the "idle-run-switch" (Fig. 3A) upwards to switch from standby to run mode, after the laser warm up time of 15 min is already completed.

8. After switching the laser into "Run mode" (Fig. 3A), wait for <u>1 minute</u> (Fig. 3C). Adjust the required power with the "Control Knob" (Fig. 3B) to the right (only red light is ON then to the left until only green light is ON) (Fig. 3C).

Figure 3

Turning OFF the system:

(Switch to 10X objective before proceeding)

1. Click the Exit button in the File tab to leave the ZEN software.

2. Shut down the computer.

3. Put the Ar-ML laser to Stand by position 1st,with the "Control Knob" (Fig. 3B) turned all the way to the left and 2nd, with the "idle-run-switch" (Fig. 3A) downwards.

4. Switch OFF the Ar-ML laser by the "key" switch (from 1 to 0) on the power supply (Fig. 2A). Wait until the fan of the Argon laser has switched OFF.

N.B: DO NOT turn OFF the "toggle switch" (Fig. 2B).

5. On the power remote switch, turn OFF "Components" and then "System/PC" switch (Fig. 1).

6. Finally, switch OFF the "Main Switch" (Fig. 1A).

- Not adhering to this procedure has and will result in no start and will increase down time.

- DO NOT use excess oil on your slides when engaging the 63X and 100X oil lenses.

- Make sure to clean BOTH, the slides and the oil lenses (with appropriate tissues), before turning back into dry lenses.

- Do not leave your slides, consumables and debris in the facility.

Z-stacks:

1. **Choose your "First slice" and "Last Slice" by using the one dye that corresponds to your protein of interest.**
2. **Go to advanced settings**
3. **Select all dyes**
4. **Click optimize sectioning to get a common area between all dyes (You can decrease / increase the number of slices as long as you still have a common area between all your dyes)**
5. **Start experiment**

Tile scan:

1. **Go to advanced settings and choose 15 % overlap, online stitching and 0.7% threshold.**
2. **If using a confocal dish center it to the middle of the objective to get the whole area.**