Zeiss LSM800-03 Inverted confocal microscope LBE 22/11/ 2019

System description:

Inverted confocal microscope, ZEN Blue 2.6 Hotfix-8

Motorized stage for multiple position imaging

Objectives:

- Position1: 5x (reserved for Calibration lens)
- Position2: Objective Plan-Apochromat 10x / NA 0.45 Air (WD=2.1mm)
- Position3: Objective Plan-Apochromat 20x / NA 0.8 Air
- Position4: Objective Plan-Apochromat 40x / NA 1.3 Water
- Position5: Empty
- Position6: Objective Plan-Apochromat 63x / NA 1.4 Oil

- Laser lines: 405, 488, 555, 639 nm

2 PMT detectors with free choice of the spectral range. (Uses a tunable short pass and band pass filter in front of the PMTs)T-PMT detector for transmitted light.

Simultaneous detection of only two dyes possible +ESID (TPMT), more than two have to be acquired sequentially

I. Starting up:

1. Switch on the multiple socket (1). Provides power to microscope controllers
1. Turn on (by pressing Big blue on/off button) Cool LED, if you need it for visual examination and don't forget to close the shutter when you don't need it or at the end of your session.

2. Switch on the system(3) and Components (4)
3. Switch on the computer (5)
4. Login: LSM User (Useme!11)
5. Open ZEN Blue Software and press "Start System" -> Restart the ZEN if it was used by the previous user **(to prevent bugs and objective crash)**
6. "Image Processing" is for offline analysis of your images

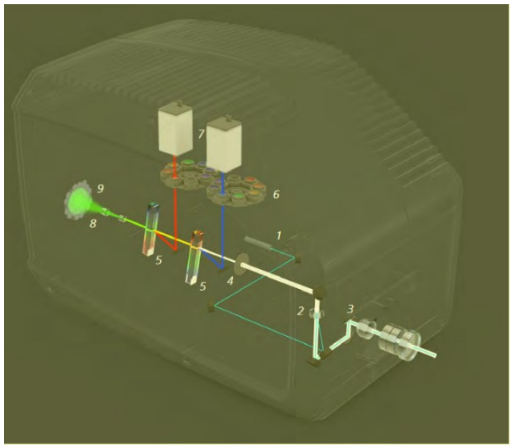
II. Visual examination of the sample

1. Press “Locate” in the main microscope menu and “Oculars Online”
2. You can change objectives automatically on the small touch screen attached on the right side of the microscope by selecting “microscope” menu and “objectives”
3. Please make sure the chosen objective corresponds to the one shown in the software. If this is not the case please restart the system. (Otherwise your pinhole and pixel sizes as well as the sectioning thickness are all incorrect.)
4. If you wish to view your sample in Brightfield press BF button in the “Locate” menu
5. For visual examination of fluorescence press the buttons DAPI, GFP or RFP (open the RL shutter from the microscope touchscreen and the RL button on the right side of the microscope stand)

III.

IV. Acquiring confocal images

1. Select “Acquisition” in the main menu of Zen 2011
2. Go to “Smart Setup” choose your dyes (set the LUT) and configuration, you can choose between simultaneous (fastest) and sequential (best signal) scan, press ”Apply”.
3. **Check the settings and make sure that Red and FarRed dyes are set to 2nd detector (Due to the Variable dichroic arrangement)**



4. If you are using the detector for transmitted light: activate T-PMT (ESID) represented in the light path scheme. Make sure Köhler illumination is set properly if you are getting bad bright field images!
5. Press the “AF” (Autofocus) button to find your focus, alternatively go to “Live” acquisition modus and find it manually
6. Press the “Auto exposure” button and the software will adjust the gain of the PMTs not the laser power, according to the brightness of your dyes.

5.1 What Auto exposure does is to increase the PMT voltages until about 1% of all pixels are overexposed. If the resulting gain is below 400 V decrease the laser power. If the gain is above 900 V increase the laser power.

7. Set pinhole to Airy 1 in the channel dialog to achieve confocality.
8. In the channel dialog you can adjust the pinhole, gain and laser power manually.

9. To zoom in your image go to “dimensions” below the acquisition window. With “Crop” you can select the desired area of your image, then go to “Live” or “Snap” to apply, “Reset all” will remove the zoom again. Depending on the scan speed, the lowest zoom is 0.5 and not 1.0.
10. Before taking an image press “optimal” in the “acquisition mode” to adjust optimal number of pixels and the pixel size according to Nyquist sampling criteria.
    Note: More pixel than ”optimal” almost never make sense but feel free to decrease the pixel number to increase acquisition speed at the cost of resolution.
11. To capture single images, click on ‘Snap’

12. **Setting up Z-stack:**

- Enable Z-Stack and the Z-Stack dialog will appear
- Press “Live” and set your first and last slice
- The software will suggest optimal slice number and interval which you can change manually
- Press “Start experiment”

13. **Setting up time series:**

- Enable “time series”
- Time series dialog will open
- Set cycle number and interval time
- Press “start experiment”

14. **Tile Scan (in this mode you have: Tiles, Multipositions):**
15. Enable “tile scan”
16. Tile scan dialog will open
17. Set desired number of horizontal and vertical tiles
18. You can choose different regions to scan. Choose regions and press button “+” to add it.
19. Press “start experiment”

Advanced Set up allows you to do preview scan of region of interest.

20. **Data Saving:**

Save your data to D: data folder. Note that this computer is not backed up. Please move your data as soon as possible using portable storage devices. Since this is a Demo instrument it’s not being connected to our network. You can also use “Auto save”, your experiment will go directly to your folder.

V. Switching off:

1. Close the ZEN Software
2. Shut down the computer (3)
3. Turn off the Cool LED (2)

4. Switch of the components (4) and system(3)
5. Switch off multiple socket (1)

Bleaching Experiments:

In order to perform bleaching experiments you need to:
1) Activate the experimental regions
2) Activate the Bleaching
3) Draw the ROI of interest (the height “Y-axis” is important -> the narrower ROI in Y, the lower time required for bleaching)
4) If you want to bleach time dependent, choose when the bleaching starts and the number of iterations.
5) If you want interactive bleaching, choose the graphics menu, draw an ROI. Now when you start the acquiring of images the mouse is converted to the defined ROI and where you click the bleaching performed.

Laser Safety Instructions

- During operation of class 3B and class 4 lasers red warning lights have to be switched on manually
- Red warning light at the door of the room, containing laser-based equipment, prohibits the entrance
- Optical path of the laser beam at all setups has to stay intact and should never be disassembled by a user. Users are never permitted to disconnect optical connections (pipes, fibers etc), remove protective coverings or disassemble any parts of the setups, especially those parts that are labeled with laser-warning signs.
- User has to make sure, that objectives mounts are blocked by objectives or light- blocking plugs, before switching the system on or starting the work
- Any cleaning activities (objectives, stage cleanings) as well as changing of objectives or filters have to be performed only after blocking of the laser light is ensured. This can be ensured by closing the scanhead shutter or switching off the laser.
- Laser class-specific warnings at each setup have to be observed and considered
- Eye contact with direct beam of Class 3B laser, or eye contact with mirror reflection from class 3B laser, should be avoided at all times
- Eye or skin contact with direct or diffuse light of Class 4 laser, should be avoided at all times
- Laser safety goggles are situated at all workspaces and should be used in any situation where potential contact of eyes with Laser light of the classes 3B or 4 is possible, according to the previous two points
- Laser safety goggles have to be worn at all times of operation of Laser Class 4
- Laser safety goggles have to be worn at all times of operation laser Class 3 at the optogenetic setups
- Laser safety goggles are assigned to each setup and matched to the corresponding laser wavelengths. Matching laser safety goggles should be used at all times, and should not be carried over between the setups
- Only one person is allowed to be in the corresponding compartment during Laser Class 4 operation and optogenetic setup operation
- Users are not allowed to wear any reflective objects (rings, watches etc) during laser operation
- Using of the equipment is only allowed after the introduction from a laser safety officer of IST Austria. Introduction has to be done individually for each setup.
- Changing experimental conditions, that involves changes in the laser application, have to be reported to the laser safety officer prior to the start of experiment

Users have to understand that any violations against the instructed rules and also withholding information leading to safety hazards will ultimately result in denial of admission to all laser equipped instruments at the IST Austria.