

# Leica Multi-Fluo

## USAGE INSTRUCTIONS

### Log in and start

1. Switch on:
  - a. The illuminator 1
  - b. The microscope controller Leica CTR6 LED 2
  - c. The camera
  - d. The computer
2. Log in with your credentials (choose your Group Name from listed Users)
3. Double click on **LasX** icon and choose the desired configuration (*Multi-Fluo* or *Single-Fluo*)

**From now on manage the microscope only through the Software LasX, not through the microscope touch screen**

4. In order to visualize the specimen on camera, the left switch on the microscope should be in the outer position (100% of light to the camera)



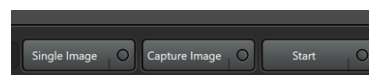
Figure 1 - Microscope controller and illuminator

### Multi-Fluo image acquisition

1. Keep the desired fluorescence channels discarding the unwanted ones (minus symbol under the Channel panel) and keep/delete the transmission channel (channel FCr8)
2. Select manually the objective lens you will need to use, select one of the fluorescence channels and click the Live button to focus the sample (with camera on the computer screen<sup>1</sup> or with the oculars)
3. Click the Autoscaling button: the image will be displayed with an optimized brightness and contrast for visualization; eventually manually adjust the brightness and contrast by moving the minimum and maximum tag (bar on the left-side of the image)

<sup>1</sup> In this case you may have to click the Autoscaling button (see point 3) in order to adjust the visualization on screen

4. Set the image acquisition parameters<sup>2</sup>, considering:
  - a. **light intensity (FIM)**
  - b. **exposure time**
  - c. Image Format (if you want to apply any Binning size, otherwise the default option is *no binning*)
  - d. Eventually add z-stack or time dimension (Z and t button on Acquisition mode on the top<sup>3</sup>) and set the related parameters
    - for z-stack: z-begin and z-end, z-slice dimension or step number or keep the default option *System Optimized*
    - for time lapse: time interval, duration, cycles etc.
5. Click the Single Image button (to capture the single displayed image), or Capture Image button (to capture an image of the selected fluorescence channel in the selected x,y plane) or click the Start button to perform the entire acquiring procedure



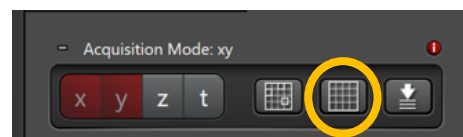
### Single Fluo Image Acquisition

1. Select the desired fluorescence filter
2. Select manually the objective lens you will need to use and click the Live button to focus the sample (with camera on the computer screen or with ocular)
3. See points 3 2 2 of **Multi-Fluo Image Acquisition** section

### LasX Navigator

LasX Navigator plugin allows you to acquire an overview of a specimen area and to use this overview image as (x, y) navigator, allowing you to control the stage (x,y) movement through the software

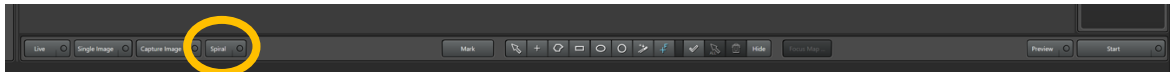
1. Click the LasX Navigator button
2. Click Live button to display the specimen on the computer screen



<sup>2</sup> For instance 30% value for FIM and 50 ms for Exposure Time

<sup>3</sup> To activate the selection of Z and t dimensions you should click the Stop button if you're in Live mode

- Click the Spiral button to display a preview of the specimen formed by stitched fields of view



- Use the mouse wheel to change the screen zoom. Keep pressed the left click to move the displayed field of view with the mouse
- When you've covered the interesting part of your specimen click on Stop button to stop the preview
- To acquire one or more extended fields of view (tile scans), focus on the region of interest and select it with the appropriate shape (rectangle, polygon etc.)



- To acquire a single multichannel image, double click on the region of interest. The stage will move to this position
- Click on the Start button to acquire the single field of view or the selected regions of interest.



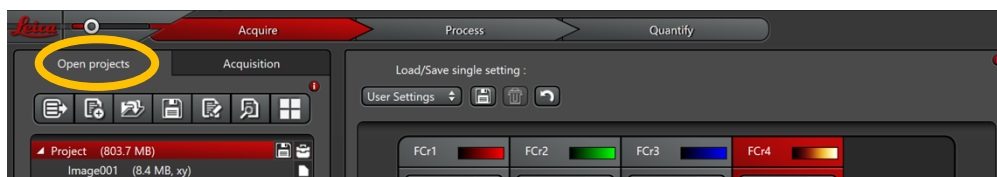
- To visualize and save the stitched fields of view, select the Mosaic Merge sheet and press Merge on the right bottom corner of the LasX window



- Close the LasX Navigator icon to return to LasX Acquisition standard mode

## Saving Data

- In the Open Project window *delete all unnecessary files*



- Rename the active project with the desired name and save the experiment in the following file path:
  - .../DATI(D:)/Users/[PI folder]/[Your Folder]/[Experiment Folder]/Project name.lif

3. Start a new experiment by clicking the New Project button on Open Project window and Rename it according to the corresponding specimen

### Data transfer

1. The files will be automatically copied on the HPC server every night. The files are safely stored there and accessible here:

From PC: <\\hpccifs.ieo.it\techunits\imaging\PublicData>,  
user name= IEODOM\IEO1234 (with the right ieo id)

From Mac: cifs://hpccifs.ieo.it/techunits/imaging/PublicData,  
user name = ieo1234

2. For further information about the data storage please have a look here: <https://ieo.sharepoint.com/sites/ImagingUnit2/> under Documenti
3. To immediately have access to the acquired data, open 'Computer' from the Windows start up icon and type the command row: <\\files.ieo.it>
4. Log in with your IEO credentials
5. Transfer data in Temporary folder. The files are stored under Temporary for a limited period of time (one month)

**The users shouldn't make copies of the raw data on the *files.ieo.it* server. The data storage is on the HPC server**

### Log out

1. Close the LasX software, the illuminator, the microscope controller and the camera
2. Sign out of your user account
3. Keep the computer on