

# Leica Histo-Fluo

## USAGE INSTRUCTIONS

### Log in and start

1. Switch on:
  - a. The illuminator (if *Multi-Fluo* or *Single -Fluo* configuration will be used) **1**
  - b. The microscope controller Leica CTR6 LED **2**
  - c. The fluorescence camera (if *Multi-Fluo* or *Single -Fluo* configuration will be used)
2. Log in with your credentials (choose your Group Name from listed Users)
3. To open the software LasX double click on LasX icon and choose the desired configuration (among *Histology*, *Multi-Fluo*, *Single-Fluo*)



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

4. **Important!** In Histology mode the camera is already loaded by default (DMC2900) and the microscope asset has to be with the top right switch **3** completely in, while in fluorescence mode (*Multi-Fluo* and *Single-Fluo*) the camera has to be changed on the LasX software (select *Preferences* -> *Set Camera To* -> *Camera Andor Zyla VSC-04470 sCMOS*) and on the microscope the switch on the top right has to be completely out
5. In order to visualize the specimen on camera, the left switch on the microscope **4** should be completely out (100% of light to the camera)

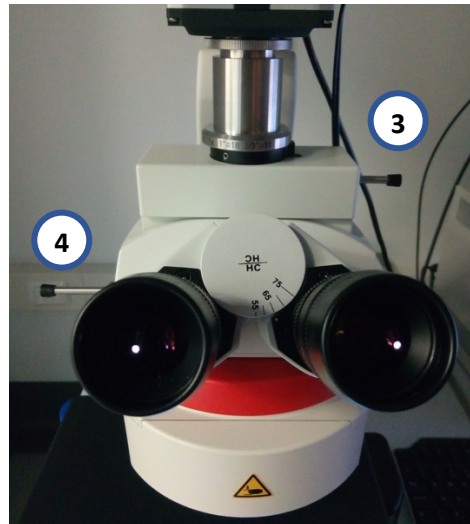
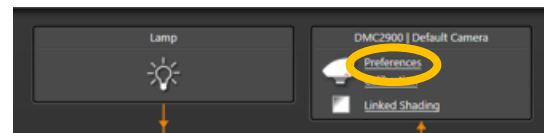


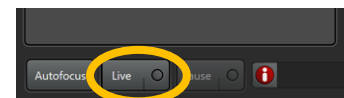
Figure 1 - Microscope top components

### Histology image acquisition

1. Set the image bit depth as 8 bit (select *Preferences -> Set Digitization to -> 8 bit*)

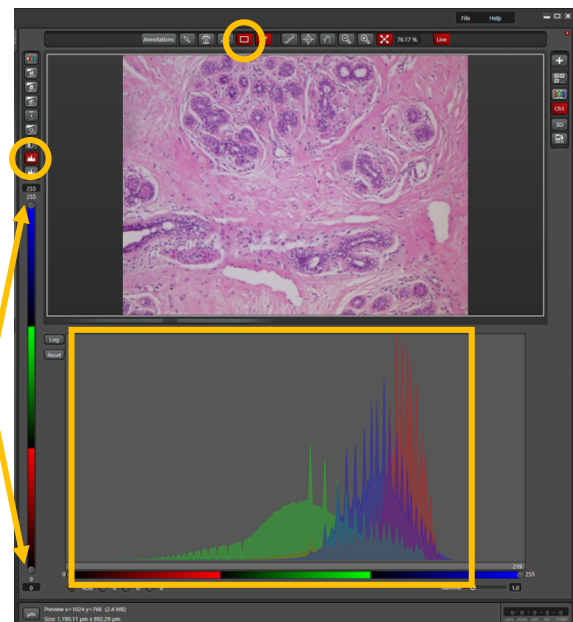


2. Press Live button and focus the sample (with camera on the computer screen or with ocular)



3. From the display visualization bar set the minimum tag at 0 and the maximum tag at 255

4. Press the Histogram button to visualize the image histogram and adjust the exposure time and the light intensity until you reach an adequate histogram range (the minimum should be around 0 and the maximum should reach 230)



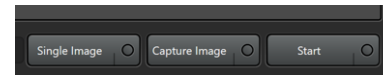
5. White balance correction: click on the rectangle icon, select an empty region eventually by manually moving the stage, and click on *White Balance* from the appeared menu
6. Activate Linked Shading Correction by checking the related setting (Linked shading correction active for acquired images or for live image). When active the button will be green<sup>1</sup>

<sup>1</sup> It is recommended to acquire also the reference images for the Linked Shading correction by following the instructions contained into the Wizard window (Linked Shading pop out)



Figure 2 - Linked Shading pop out

7. Set the image acquisition parameters (usually you should consider just the **a** and **b** parameters):
  - a. **Intensity**
  - b. **exposure time**
  - c. Image Format (no Binning option is set as default)
  - d. Live Format (no Binning option is set as default)
  - e. Gain
  - f. Eventually add z-stack or time dimension (Z and t button on Acquisition mode on the top) and set the related parameters
    - i. for z-stack: z-begin and z-end, z-slice dimension or step number or keep the default option *System Optimized*
    - ii. for time lapse: time interval, duration, cycles etc.
8. Click the Single Image button or Capture Image button (to capture the single displayed image) or click the Start button to perform the entire acquiring procedure.



## Multi-Fluo image acquisition<sup>2</sup>

1. Set the top-right switch of the microscope completely out (see *Log in and start - point 4*)
2. Set the fluorescence camera through *Preferences -> Set Camera To -> Camera Andor Zyla VSC-04470 sCMOS*
3. Keep the desired fluorescence channel discarding the unwanted channels (minus symbol under the Channel panel) and keep/delete the transmission channel
4. 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 or with ocular)
5. Click the Autoscaling button: the image will be displayed with an optimized brightness and contrast for visualization; eventually manually adjust the brightness

<sup>2</sup> For further details please see section *Multi-Fluo image acquisition* in the Multi-Fluo microscope instructions

and contrast by moving the minimum and maximum tag (bar on the left-side of the image)

6. Set the image acquisition parameters (usually you should consider just the **a** and **b** parameters):
  - a. **light intensity (FIM)<sup>3</sup>**
  - b. **exposure time**
  - c. Image Format (if you want to apply any Binning size)
  - d. Eventually add z-stack or time dimension (Z and t button on Acquisition mode on the top) and set the related parameters
    - for z-stack: z-begin and z-end, z-slice dimension or step number
    - for time lapse: time interval, duration, cycles etc.
7. Click the Single Image button (to capture the single displayed image), or Capture Image button (to capture image of fluorescence channel in the selected x,y plane) or click the Start button to perform the entire acquiring procedure.

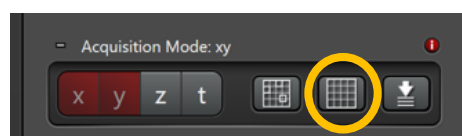
### Single Fluo image acquisition

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 5 6 7 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
3. Click the Spiral button to display a preview of the specimen region formed by stitched fields of view



4. Use the mouse wheel to change the screen zoom. Keep pressed the left click to move the displayed field of view with the mouse

<sup>3</sup> For instance 30% value for FIM and 50 ms for Exposure Time

- 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



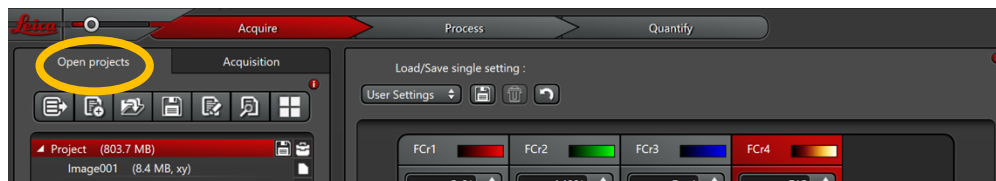
- To visualize and save the stitched fields of view, select the Mosaic Merge sheet 4 and press Merge on the right bottom corner of the LasX window



- Close the LasX Navigator 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:
  - .../DATA(E:)/Archivio/Users/[PI folder]/[Your Folder]/[Experiment Folder]/Project name.lif
- Start a new experiment by clicking the New Project button on Open Project window and Rename it according to the corresponding specimen

## Data transfer

- The files will be automatically copied on the HPC server every night. The files are accessible here:

From PC: <\\hpcifs.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](https://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