BEGINNERS' SAFETY MANUAL FOR
LEICA M205 FCA STEREOSCOPE SYSTEM

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1. Safety Guidelines

Please adhere to the safety guidelines for your own safety and health. When in doubt, always approach the bioimaging facility for assistance.

2. Training

Use of confocal microscopy and the online booking system is authorized by facility staff only. All users must attend the confocal training session conducted by the Bioimaging facility staff before they are allowed to access the confocal system.

2.1 To arrange a training session:

- Please email the Bioimaging team (bioimaging@tll.org.sg) for training session.
- Go to http://microscopy.tll.org.sg/pages/conf_training_form.html to fill in the necessary particulars.
  - *(E.g. Lab, PI, sample, and a brief description of your project)*
- We will help you identify the most appropriate microscope for your imaging needs.
- We will then arrange training session according to your availability within a week.
- Training sessions usually last 0.5 - 1 hours depending on users' previous experience, the types of samples they plan to use.

Once you have completed the training session, we will grant you access to both the confocal PC and the online booking system. Please keep in mind that only bioimaging facility members are allowed to conduct the training. None of your lab members are allowed to conduct the training for you.

If you need a refresher or some specific advice on anything microscopy related, please approach any of the Bioimaging staff for assistance/help.

We also offer training on the various types of image analysis and image processing software available here at TLL, including ImageJ/Fiji, Huygens and Imaris.

3. Acknowledgements

If you use the TLL Microscopy and Imaging facility and/or have been trained or assisted any of the bioimaging facility members in your research, then this should be acknowledged appropriately in your publications and presentations.

4. Operation Procedures

Every confocal system in TLL Bioimaging Facility has its specific instructional manual which are found in every confocal rooms. Strictly adhere to the correct order of operation for all system. Failure to do so will result in disciplinary action from the facility. Any issues encountered during the operation of the system are advised to seek help from the Bioimaging facility.

Modification, exchange, or removal of components beyond this operational manual is strictly prohibited and is only carried out by the manufacturer, Bioimaging facility or by experienced users approved by bioimaging facility.

4.1 Switch ON Protocol

1. SIGN IN the Logbook and record your START-TIMING.
2. Switch on Main power switch **(1)** and PC **(2)**.

     ※ The fluorescence lamp uses LED illumination. It requires no warm-up/cool-down time and can be used any time you like.
3. Wait for PC to start up and log in with your TLL username.
4. Load LASX software.

4.2 Available fluorescence settings

| Fliter	Excitation (nm)	Emission (nm)	Used for
RFP LP	541-551	590LP	RFP, mCherry, chlorophyll
CFP ET	426-446	460-500	CFP, DAPI
ET GFP	450-490	500-550	YFP, TRITC

4.3 Software initiation

Turn on the machine following the instructions pasted on the wall and inserted in the log book. Wait for complete initialization of microscope and Windows after logging in with your TLL username and password.

Double click on:

The LASX configuration screen will appear:
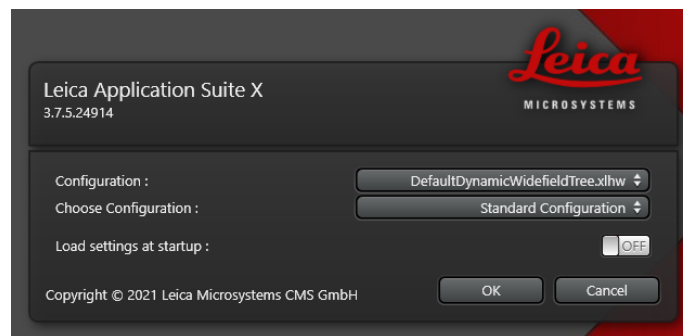
Configuration: `DefaultDynamicWidefieldTree.xlhw` must be selected.

Microscope: Check that **`Standard Configuration`** is selected.

Click on **OK** and wait for complete initialization. Do not move anything on the microscope during the initialization process.

4.4 Using the Acquisition tab

Select the **Acquisition** tab, in which you can set the parameters for your imaging:

- Acquisition Mode (right): Shows the dimensions where you can acquire your image.
    - ※ *x* and *y* are on by default: these are the image plane dimensions.
- Image (right): Sets the image exposure time and binning.
    - ※ Exposure [ms]: Sets exposure time in milliseconds. Longer exposure time means a brighter image, but is less appropriate for samples that move a lot.
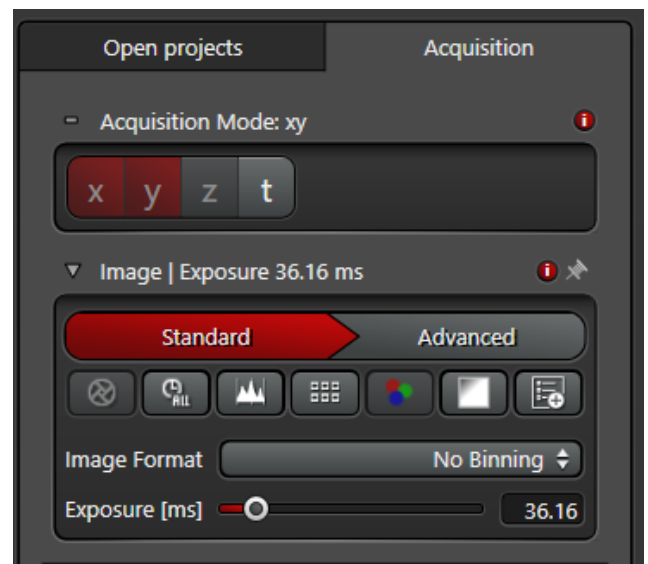
※ Binning: Increases signal-to-noise ratio by grouping adjacent pixels together into one single pixel at the cost of reducing image size by $\frac{1}{(bin\ number)^2}$. This system has a base resolution of 2048×2048, and it can bin down to 512×512 resolution.

- Illumination settings (right):
  - ※ TL Light: white light, bottom illumination
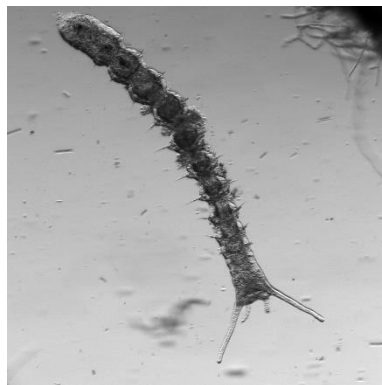   - Intensity: Sets brightness of the light
    - Aperture (BF/RC only): Sets size of light opening
    - Balance (RC only): Shifts aperture position up/down
    - Scene:
      - BF: Bright Field
      - RC: Rottermann Contrast – a Leica-specific contrast method that shows the changes of the refractive index as differences in brightness
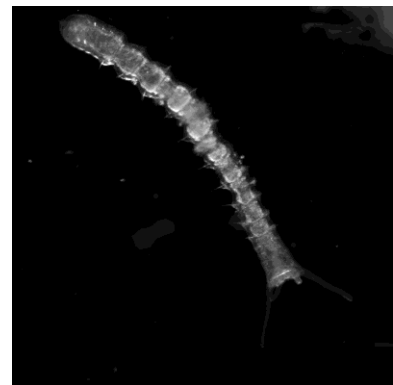   - DF: Dark Field
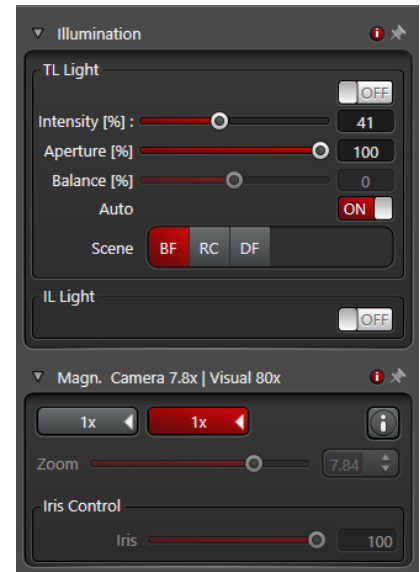
Comparison of contrast types
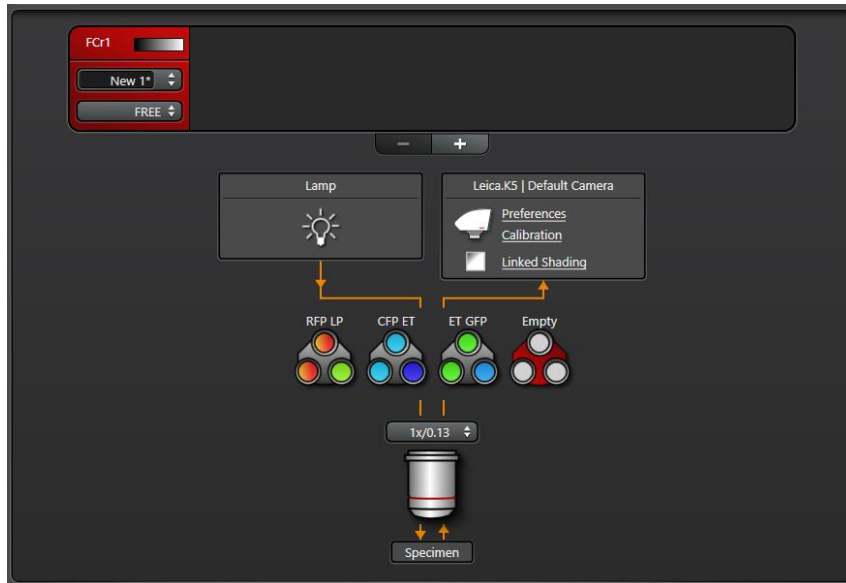
Bright field   Rottermann Contrast   Dark Field

- Magnification (right): selects the objective lens. Use the second 1× setting for the current lens fitted on the stereoscope

4.5 Selecting illumination & fluorescence settings

1. Click on the channel you want to change settings for (FCr). You can add or remove channels by clicking on the (+) or (-) buttons below the channel overview.
2. To change the colour filter for the particular channel, click the channel and press a filter set. Press Empty for normal transmitted light.
3. Click on the gradient on the channel to change the colour.
4. The objective lens can be changed on this window as well. Click on the drop-down menu just above the objective lens icon.

4.6 Image view

There are controls that you can toggle to change the view on the system around the LAS X image view.

Left side (dynamic range and pseudocolour, top to bottom)

- Look-Up Table (LUT): Toggles between black/white monochrome, pseudocolour (if set, otherwise it remains black/white) and over/underexposure highlighting.
    - ※ Normal exposure is in white/orange/black
    - ※ Underexposure is in green
    - ※ Overexposure is in blue

- Manual/Auto scale: Auto scale automatically adjusts the dynamic range for best contrast. Manual scale will require manual adjustment of the dynamic range.

Top bar (annotations)

- Add scale bar: Click this button, then click and drag on the item to be measured. This is auto calculated from the metadata provided by the image based on the settings of the image.

- Select annotation: Click this button, then click on an annotation to select it. Right click selected annotations and press Properties to change features such as the length, angle, colour, and font of the annotation.

  ※ To select or drag a scale bar, click on the line for the scale bar, not the distance measurement.

- Select all annotations: Click this button to select all annotations.

- Delete selected annotation(s): Click this button to delete annotations.

Right bar

- Frame overlays: Click this button to cycle between viewing overlay of all the channels, viewing individual channels, and viewing individual channels together with the overlay.

- Channel select: Click to show or hide channels.

- Overlay select: Click to show or hide overlay of channels selected in the previous option.

4.7 Time lapse

- Press the **t** button on Acquisition mode
- Click on the numbers in Time Interval to specify the amount of time between acqusitions

  ※ This will be grayed out if you press Minimize

- Choose one of the following:
  - Acquire Until Stopped: Once you press Start, the camera will keep acquiring pictures until you press Stop.
  - Duration: Specify the total length of the acquisition. Number of pictures taken will depend on the length of the Time Interval
  - Cycle: Specify the number of cycles you want to take (similar to a camera intervalometer)
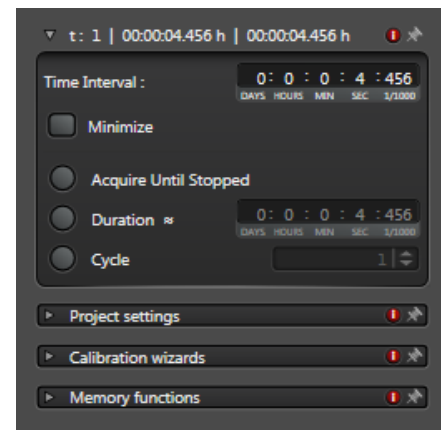
## 4.8 How to save your images and reuse settings

Taking snapshots:
- Right click on the image you would like to take a snapshot of.
- Click on **Snapshot** to get an RGB image of the desired image.
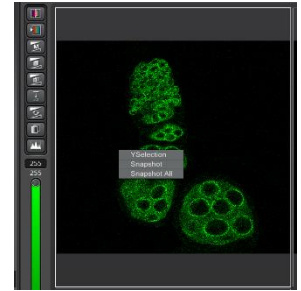
To store an image:
- Select the **Projects** tab and it will list the images you have acquired (either with the **Capture Image** or **Start buttons**. **Live** images are not held in the memory.
- Click on the **Save** button.
- Your images will be saved in a `*.lif` proprietary format.
- Please save in your folder. For example:
  - ➢ Option 1: In TLL `\research_cmn\` drive folder in your respective lab folders.
  - ➢ Option 2: In your own lab drive.
  - ➢ Option 3: In your personal hardisk or thumbdrive. Keep in mind that the data transfer will be slow if you are not using a USB 3.0 drive. Connect it to the blue port at the back of the terminal for maximum speed.
- You can also change the name of each image or delete selected images to your liking by right-clicking on the name and selecting **Rename**.
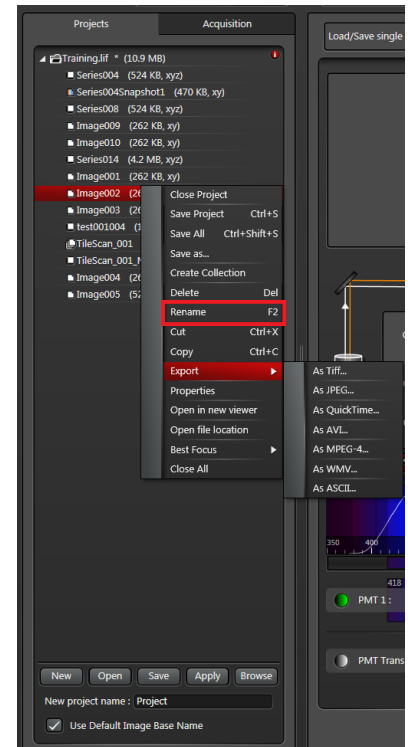
To export an image:

1. Right click on an image you want to export and press Export Image.
2. In the Export as… window, you are given a choice to export as an image or a movie.
3. You can export your image in TIFF, BMP, JPG

   ※ Export LUT exports your image in the colour that you have set for the selected channel(s)

   ※ RAW image exports the full dynamic range (0-65535)

   ※ Scaled Viewer Image exports the narrowed dynamic range as you have set in Image View
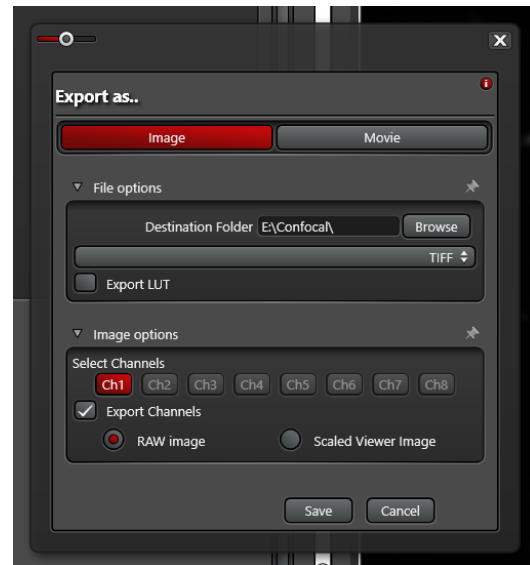
<mark>Important: Remember to back up your data to the network!</mark>

To reuse previous settings:

- Open previous images.
- Right-click on the image you would like to repeat and select **Properties**. Here you will see a detailed record of the settings used to acquire that image, and all its properties.
- Click on **Apply Settings** to re-establish the microscope to the same setting.

4.9 Switching OFF Protocol

Check through TLL microscopy resource booking if there is a user immediately after you. If there is a user immediately after you, leave the system on and log off the PC. Otherwise switch off the system.

(a) Save your images to the TLL common drive folder.

(b) Lower down the objective lens and carefully clean the objective lenses with Whatman lens paper.

(c) Make sure to switch your lens back to the 10x/0.40 objective lens.

(d) Clean the sample holder with Kimwipes and 100% ethanol to remove any oil residues.

(e) If no one is using the microscope within 30 minutes of you finishing your session, switch off the MHL **(4)**, exit LAS X software and switch off the PC terminal **(5)**

　※ If you are only logging off to let someone else use it later, you can simply leave the MHL on, exit the LAS X software, and log off.

(f) Switch off the Camera **(3)**, Super Z control unit **(2)**, and Microscope body **(1)**.

(g) Fill in the logbook with the objective lens used and mention if the MHL is still ON/OFF.