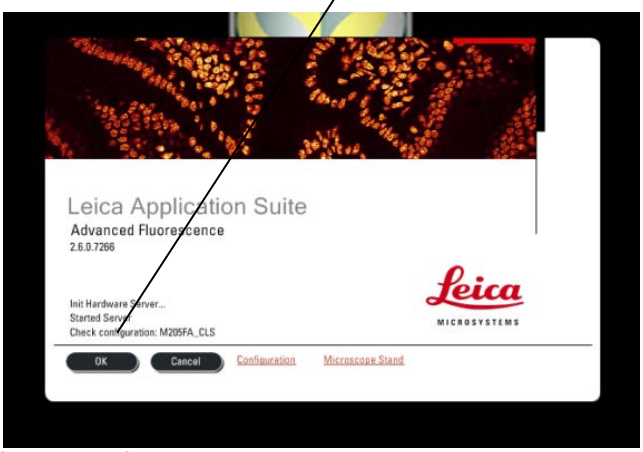
To start:

- Turn on the microscope and required light sources.
    - The microscope has two switches, one at the back of the base and one at the back of the column, both where the power cables are.
    - There are two light sources, a bright field light box to the left hand side of the microscope and UV light also on the left hand side.
    - If you don't need to use the UV for fluorescence leave it off.
    - The microscope also has a transmitted light base. If you wish to use this replace the black/white plate with the glass plate.

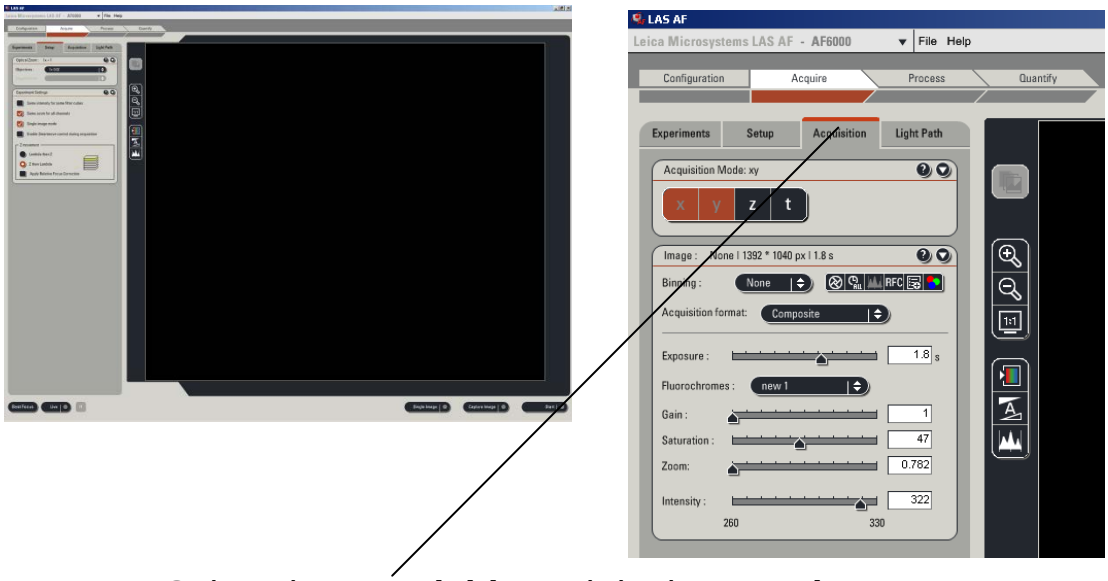
- Turn on the PC and monitor and log on (see log in page below).

- Open the Leica LAS-AF software from the desktop. 

    - Allow the microscope and software to run the start-up checks.
    - When prompted to confirm configuration check the setting:
        - M205FA_CLS allows the software to control the ring light.
        - M205FA_TLRCi allows the software to control the transmitted light base.
        - To change, click **configuration** and select the set up you require and confirm 'OK'.



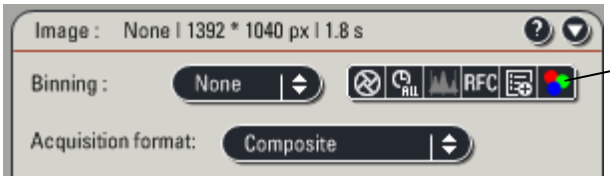
- When asked if the clamp settings have been changed select ‘No’.
- The window below will open.



- Select the **Acquisition** tab in the **Acquire** menu to access the exposure time, white balance etc.

To view your sample with Bright field settings:

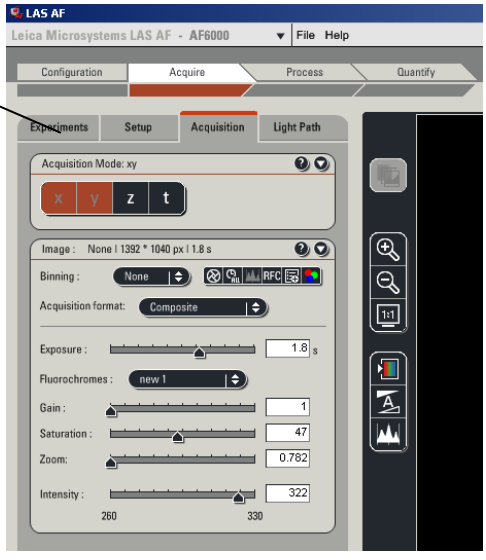
- Push the silver camera port all the way in to view your sample through the eye pieces.
- Push the **Shutter** button on the bright field light box to turn on the ring light.
- Use the dial on the touch screen to zoom (bottom dial) and focus (top dial) your sample.
- Pull the camera port out and click the **Live** button (bottom left hand corner of the window) to see your sample on the screen.
- Select the **white balance** to correct the camera colours.
  - Click on the icon below, select **auto white balance** and click **Measure and apply**.



- Adjust your focus and use the **Acquisition** settings (exposure etc.) to improve your image.

To take an image:

- Click **Single Image**. The file will be listed in the left hand side of the screen in the **Experiments** tab.

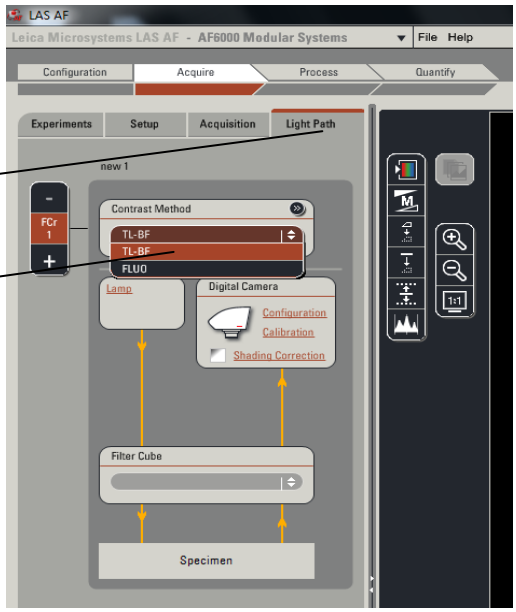


To view your sample under Fluorescence:

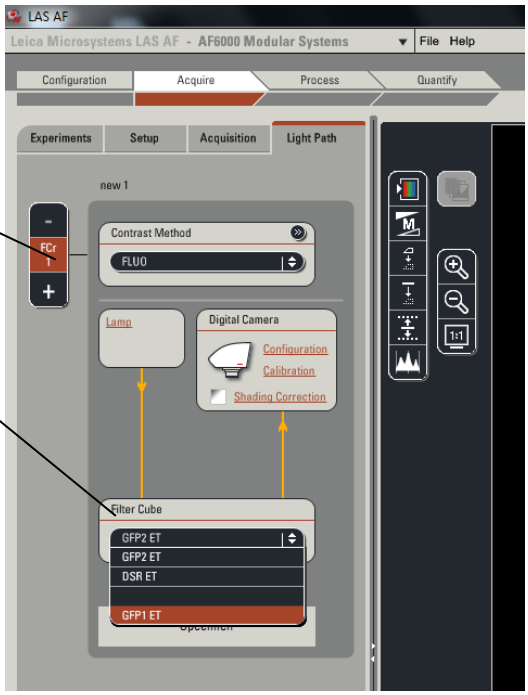
- Select which filters you require and position them in the filter carrier (please ask if you need help with this).
- Each filter has a small transponder which should be recognised by the filter carrier and show in the touch pad screen under the **Light** menu.
- If the filter carrier does not recognise the filter, remove it and refit, this usually solves the problem.
- The filter carrier will hold four filters but you will need to use a blank if you require a bright field image with your fluorescent image.
- Using the touch screen pad select the filter you wish to view your sample with. The filter carrier will automatically position it.
- Make sure the UV protection screen is positioned down and click **Live** on the main screen. The UV light should come on automatically.
- You can view your sample on the screen by pulling the silver camera port out or through the eye pieces by pushing it in.
- Adjust your focus and use the Acquisition settings (exposure etc.) to improve your image.
- If your fluorescence has a weak signal dim or turn off the room lights as this can help eliminate background interference.

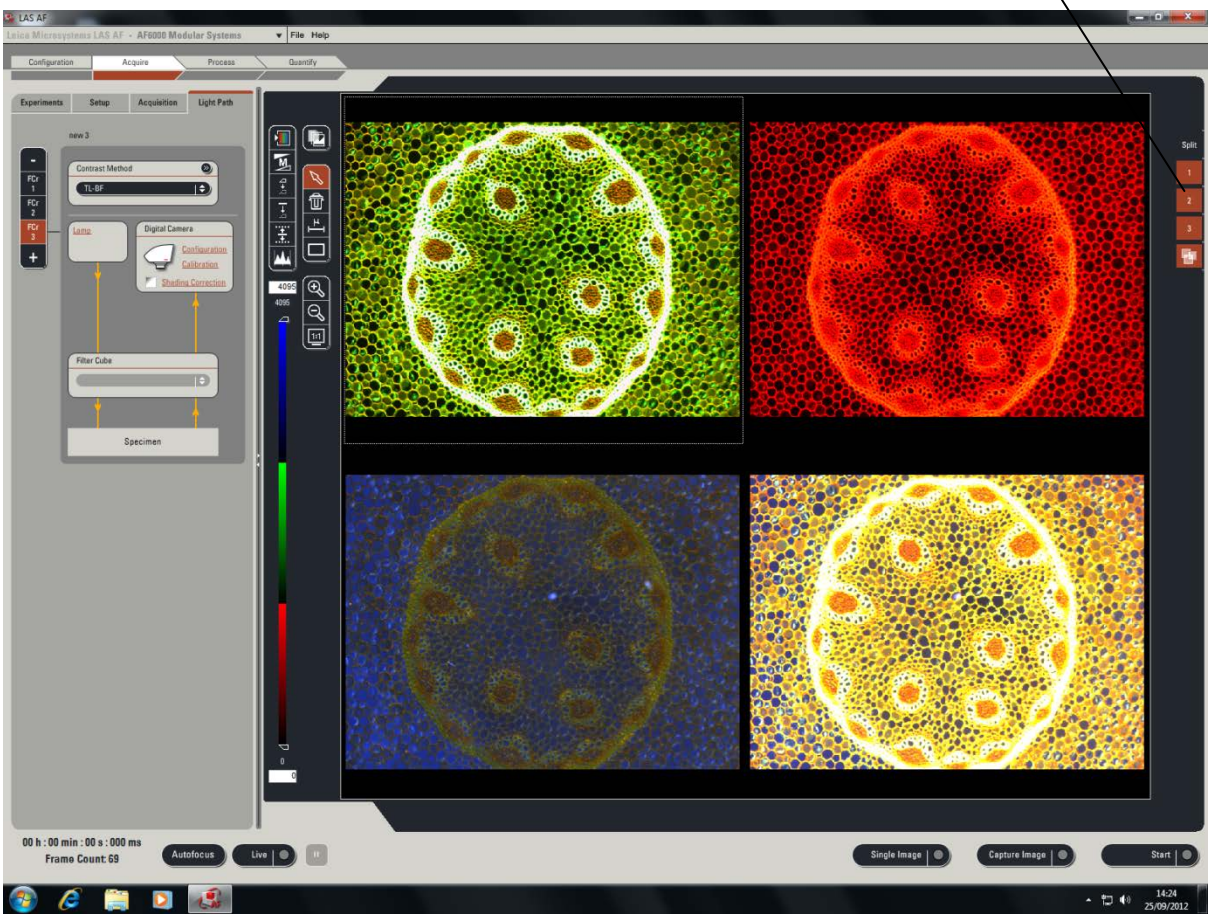
To take an image:

- Select **Light Path** and change the contrast method from **TL-BF** to **FLUO**.



- Select **FCr 1** and assign a filter cube using the drop down menu. The filters listed will be the ones in the filter carrier.
- Click the **+** to add a second filter (FCr 2) or leave blank for a bright field image. You can add up to four filters.
- Click **Capture Image.** The software will take an image with each filter selected (see below) with a final image of all channels overlaid.
- You can view each channel as a single image using the numbered tabs to the right hand side.





Leica M205 FA User notes

To save your images:

- Go to the **Experiment** tab.
- Right click on your experiment or image file.
- Click **Save**. The **Save as** window will open showing the file path of the last user. Click **Browse** to find your destination folder.
- Save in your folder on D:\userdata\ or bioimage_tmp on salt.
- The files will be saved as *.lif* files with all the meta-data so you can return to them later to add scale bar, change settings etc. These can only be viewed with LAS.
- Right click on your experiment export as a tif to your network folder (I: drive). You can select to add a scale bar, time stamp, date etc. when the save window opens. The tif files can be viewed in Photoshop etc.
- When exporting multi fluorescent image
- You can export the image files singly, however the options to add a scale bar, time stamp, date etc. are unavailable for single image files and only active for exporting whole experiments.

To close down:

- Shut down the software and turn off the PC first.
- Turn off the microscope and light boxes.
- Replace dust cover.

LAS-AF has numerous options such as time lapse, multi focus and z-stack images. If you wish to use any of these applications please speak to Kirstie who will be able to help you further.

PLEASE NOTE: This software is quite buggy!

- If you have any problems in the first instance check the camera is not live as this will lock out a number of functions.
- If you cannot see a live image check the camera port is open on the left hand side of the eye pieces.
- If either of the above do not solve your problem restart the software.
- If that doesn't work turn off the PC and microscope and restart from the beginning. This seems to solve most problems.
- If in doubt ask Kirstie or Jean – we are here to help!