



BEGINNERS SAFETY MANUAL FOR ZEISS AXIOPLAN 2 COLOUR WF SYSTEM

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1. Safety Guidelines	2
1.1 Immersion Oil.....	2
1.2 Metal Halide Lamps (MHL)	2
2. Training	3
2.1 To arrange a training session:.....	3
3. Online Booking and System Access.....	3
3.1 Acknowledgements.....	4
4. Operation Procedures.....	5
4.1 Switching ON Protocol	5
4.2 Objective lens.....	5
4.3 Microscope operation.....	6
4.3.1 Right side controls	6
4.3.2 Left side controls.....	8
4.3.3 Condenser	9
4.4 Software initiation	11
4.5 Adjusting for acquisition	11
4.6 Measuring distances (scale bar tool)	14
To create a scale bar	14
To measure distances	16
4.7 To publish your image (to view in other programs)	17
4.8 Acquiring video	17
4.9 Switching OFF Protocol	19
5. Troubleshooting.....	20

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.

1.1 Immersion Oil

Modern immersion oil has no known hazards to human beings so far, yet they can cause discomfort to a person if the immersion oil has been left on skin for too long or inhaled. Therefore, please take note of the following safety guidelines for using immersion oil:

- Use the applicator to apply the immersion oil onto the slides for upright system.
- Do not touch the applicator oil directly on the objective lens as this will scratch or break the lens. Instead, allow the drop of oil to contact the lens surface.
- If the bottle containing immersion oil has oil on the sides, wipe down with kimwipes and ethanol. Wash hands immediately after with soap and water.
- Wipe immersion oil off objective lenses after use using lens paper and wash hands immediately after with soap and water.
- Always clean up any spilled oil or residue from oily slides immediately after your session.

1.2 Metal Halide Lamps (MHL)

Metal halide lamps are arc fluorescence lamps that emit a high-power visible light which has a range of 300 – 650nm, enabling us to observe fluorescence signals such as GFP, DAPI and RFP with the appropriate filter sets. MHL bulbs contain mercury vapour which is extremely toxic to the human body if allowed to escape through breakage or other damage. Therefore, please take note of the following safety guidelines when working with the mercury lamp:

- Ensure that the mercury lamp has been off for at least 30 mins before switching it on. Once the lamp has been turned on, it must remain on for a minimum of 30 minutes to allow sufficient time for it to warm up and run properly.
- Check the timer on the power box to ensure the mercury lamp does not run past 2,000 hours.
- Do not look directly at the MHL when it is switched on as it can damage your eyes.
- If there are no users in the next 30 mins, MHL must be turned off.
- Never attempt to change a bulb yourself. Please contact facility staff when you notice that a bulb has crossed 2,000 hours or is not functioning properly.

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 Bioimaging Facility (bioimaging@tll.org.sg) for a training session.
- Fill in the necessary particulars in the form here:
http://microscopy.tll.org.sg/pages/conf_training_form.html
(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 1 - 3 hours depending on users' previous experience.
- Users receiving training are highly encouraged to bring their own samples so that staff can adjust trainings to your imaging needs, but if not possible, staff have their own samples that they can work with.

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 Yang Fan 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. Online Booking and System Access

- Booking of all Light microscopes prior to use is **COMPULSORY** through microscopy resource booking via TLL Intranet
(https://intranet.tll.org.sg/App/tll_intranet/booking_searches)
- Users are only allowed to book the confocal system that they have received training on. If they wish to book other confocal system, they have to receive a separate training.
- Users are entitled to advance bookings of up to 2 weeks. They are advised to plan their experiments accordingly to avoid any disappointments.
- During Office Hours (Weekdays from 8.30am to 6pm)
 - Each user is entitled to a **MAXIMUM of 2 bookings** per system per week.
 - Users whom are trained on multiple systems are entitled to a maximum of 3 bookings per week but it has to be shared across the systems that they have received training on.

- Each booking must not exceed 3 hours.
- If users have utilized all their entitled bookings for the week, “**24Hr Rule**” can be applied where they can book the system in less than 24 hours in advance according to its availability. If extra slots are booked within the 24-hour period, a note of “**24Hr Rule**” should be made in the booking description.
- During Non - Office Hours (Weekdays after 6pm, Sat, Sun & Public holidays)
 - If users require more slots in a particular week, they can book on weekdays during non-peak hours (*after 6pm*), on weekends (*Sat and Sun*) and on public holidays.
 - If extra time is required, bookings can be extended out of peak hours (*E.g. 3 – 7pm*).
- Bookings exceeding these limits are subjected to cancellation without prior warning.
- Simultaneous bookings of two or more different systems are not allowed. Multiple bookings for the same system on the same day during office hours is strictly prohibited.
- If users cannot attend a booked session for any reason, it is **COMPULSORY** that they cancel their booking through TLL intranet and email (confocal@tll.org.sg) to announce the availability. If they are the last users for the day, they need to check if the system has been switched off completely.
- If users are swapping a session with another user, they must change the booking details accordingly.
- Under any circumstances, users are not allowed to make bookings on behalf of other people. Users who have received training but are yet to gain access to microscopy resource booking may approach TLL Bioimaging department for booking assistance if they need to use it urgently.
- If a user fails to show up within the first 30 minutes of their booking, the slot is forfeited and is free for any user to use it.
- If any users violate any of these Booking rules, users will be subjected to the 3 strikes policy.

1st offence	A warning will be issued along with the reminder of the rules.
2nd offence	A second warning will be issued and your respective PI will be notified.
3rd offence	Banned from using any of the facility’s microscope for 2 weeks.

3.1 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.

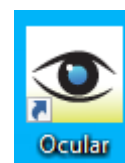
4. Operation Procedures

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 Switching ON Protocol

- (a) SIGN IN the Logbook and record your START-TIMING.
- (b) Press on the Microscope button, right side **(1)**.
- (c) Switch on the MHL if required **(2)**.
(Warning: If you switch on/off the MHL while the camera is still on, the camera will crash and you will need to restart it.)
- (d) Switch on the camera in-line power **(3)**.
- (e) Switch on the PC **(4)** and log in with your Wonderland account.
- (f) Load the Ocular program from the desktop.
- (g) Fill in the log book.



4.2 Objective lens

Objective lens	Immersion media	Available illuminations
5x/0.15 Plan-NEOFLUAR	Air	BF, DF
10x/0.30 Plan-NEOFLUAR	Air	BF, DF, Ph1
20x/0.75 Plan-APOCHROMAT	Air	BF, DF, DIC II
40x/0.75 Plan-NEOFLUAR	Air	BF
63x/1.4 Plan-APOCHROMAT	Oil	BF, DIC III
100x/1.4 Plan-APOCHROMAT	Oil	BF

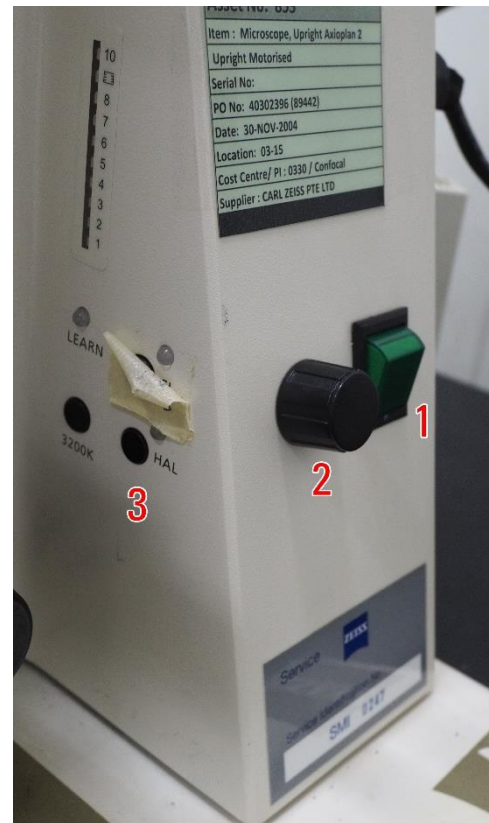
BF: Bright Field; DF: Dark Field; Ph#: Phase contrast; DIC: Differential Interference Contrast

See **Section 4.3.3 Condenser** for a guide on how to set illumination.

4.3 Microscope operation

The Zeiss Axioplan 2 is a semi-automatic system that is not connected to the computer. All operations will need to be done manually on the microscope body itself.

4.3.1 Right side controls



The right side of the microscope contains the following functions:

- (1) Power switch: flip this to turn the microscope on
- (2) Brightness knob: Turn this to increase the halogen lamp brightness
- (3) Halogen lamp switch: Press this to turn the halogen lamp on/off. You will need to switch it off when you are using fluorescence imaging.
 - Press the 3200K button to fix the lamp at a constant 3200K colour temperature. This is very bright so look for the switch in (7) to reduce the amount of light that passes into the sample.
- (4) Fluorescence aperture lever: Push down to reduce the **amount** of fluorescence light that reaches your sample. Do not confuse with the field limiter.
- (5) Fluorescence shutter bar: Push this in to shut off fluorescence light if you are not actively viewing your sample
- (6) Fluorescence field limiter lever: Push down to reduce the **size** of the circle that you are going to excite your sample with fluorescence. Do not confuse with the aperture lever.

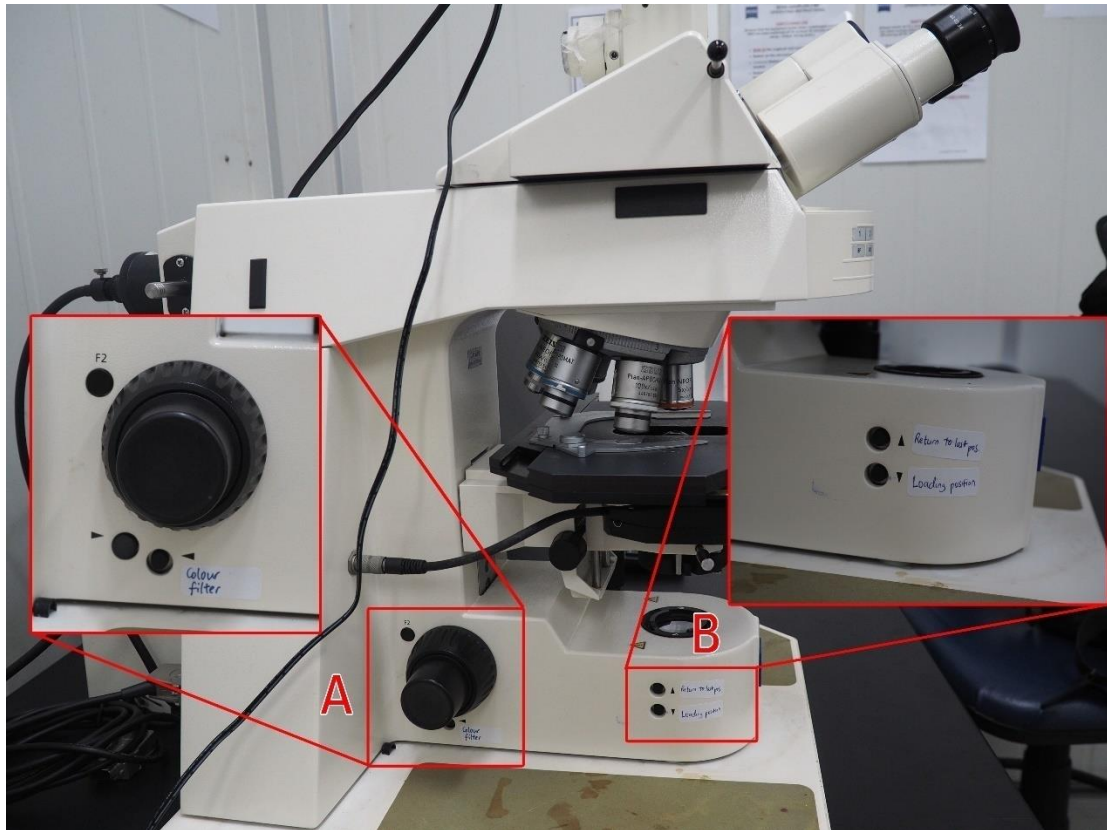
(7) ND filter: This is a filter that reduces the amount of light that passes through from the lamp to the sample *without* changing the colour temperature of the lamp like dimming it does. Press the triangle buttons to switch between filter densities to halve or double the amount of light passing through to your sample.

- The wheels will indicate how much light is passing from the lamp to the sample. To calculate the amount of light passing through, multiply the two numbers you see on the wheel shown, then divide by 100 to get the actual percentage of light passing through. In the picture shown, the percentage of light passing through is **0.4%.**

There is a piece of tape on one of the buttons. It is the fluorescence shutter button that only works if the Zeiss HBO lamp is fitted to it. Since this system uses an MHL from a different company it does nothing.



4.3.2 Left side controls



The left side of the microscope contains the following functions:

(A) Colour filter changer: Changes the colour filter. View the current state of the colour filter from the top window as shown below.



(B) Quick stage height mover: Use it to more efficiently load your sample

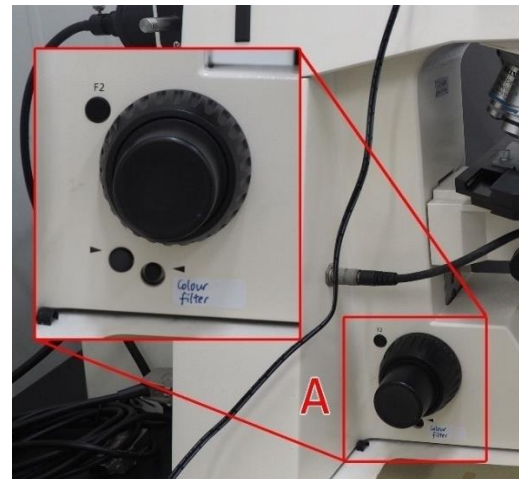
- Press the lower button marked “Loading position” to lower the stage for changing your sample.
- Once your sample is loaded, press the upper button to move the stage back to the previous position.

4.3.3 Condenser

This system is a semi-automatic system, meaning that while operations are motorised, they are not connected to the computer and settings do not come in one package. Condenser settings will need to be changed manually.

Default: Bright Field

By default you should be using bright field for your samples.



1. Look up at the colour filter changer and check that the number is 1, for bright field. A list of available colour filter settings is also shown on the microscope.
2. If the colour filter setting is wrong, press the buttons near the left focus knob to change the colour filter settings until the number is 1.

3. Then for the condenser, press the \leftrightarrow buttons to cycle through the condenser filter options until I/H is shown.



For Phase Contrast:

1. Check if your current objective can use phase contrast. A marking showing **Ph#** should appear on the objective lens.
2. Go to the condenser and press the \leftrightarrow buttons to cycle through the condenser filter options until the correct setting is shown. For phase contrast you should use the Arabic numeral that matches the marking shown on the objective lens. Also make sure the condenser lens is swung in.

For DIC:

1. Look for a black insert in the lens turret on top of the objective that you are using. This is a DIC prism, and if it is present you can use DIC on this objective:
2. Remove the DIC prism from where it is positioned and take note of the Roman numeral in the prism. For the example given at the right, the appropriate DIC setting is II.
3. Carefully insert the DIC prism back into the objective lens turret.
4. As with the Phase Contrast setting, press the \leftrightarrow buttons to cycle through the condenser settings until the correct setting is shown.



For dark field:

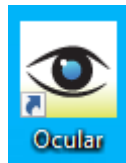
Set the system to bright field first, then press the \leftrightarrow buttons to cycle through the condenser filter until D is shown. You may need to increase the amount of light so that it is visible in the eyepiece.

For fluorescence:

1. We recommend using the 2F microscopes instead. They are more sensitive, the operation is computer control enabled, and multiple fluorescence stacking is available for these systems.
2. If you still choose to continue using this microscope for fluorescence, we strongly urge you to reconsider anyway, but you can select the desired colour filter by pressing the colour filter setting buttons until the correct fluorescence is set by looking at the colour filter display number.

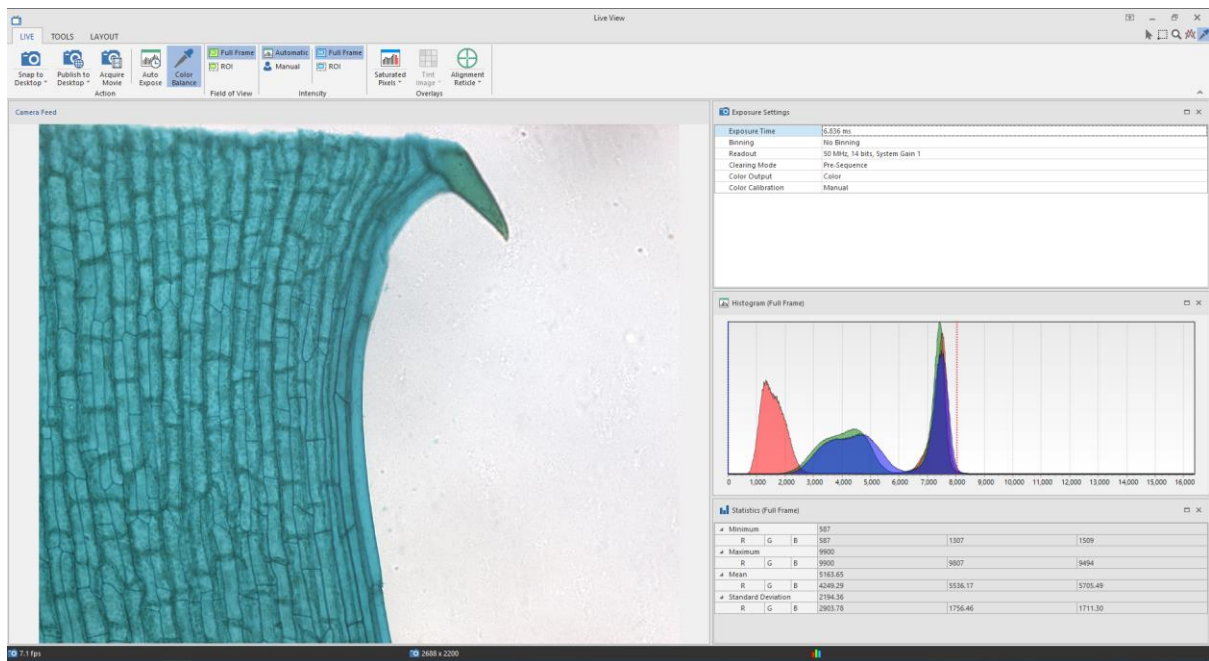
4.4 Software initiation

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:

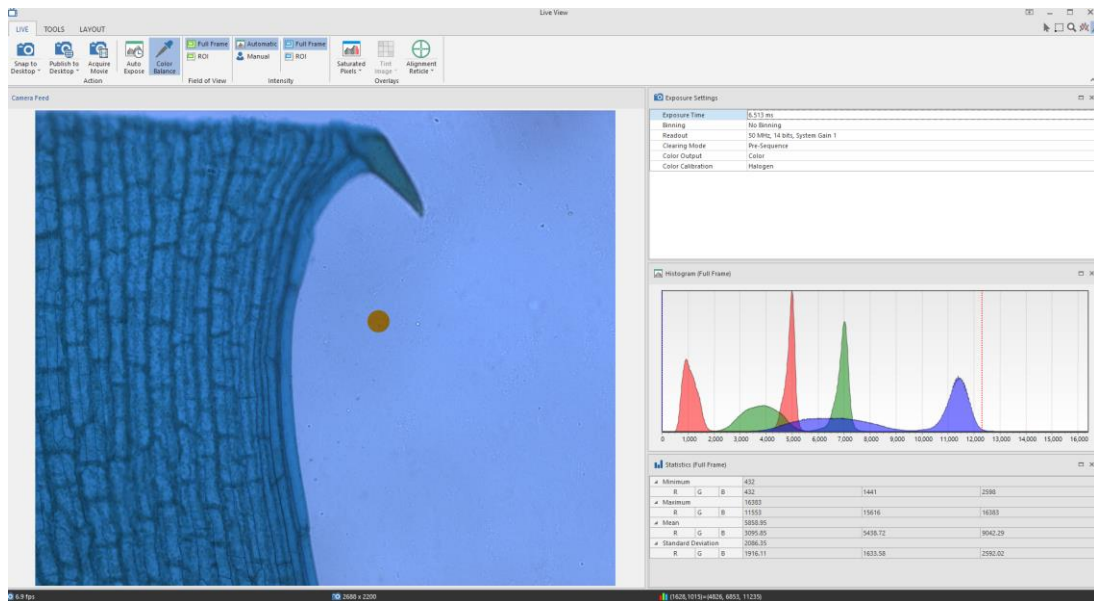
The program starts up immediately and Live View starts up.



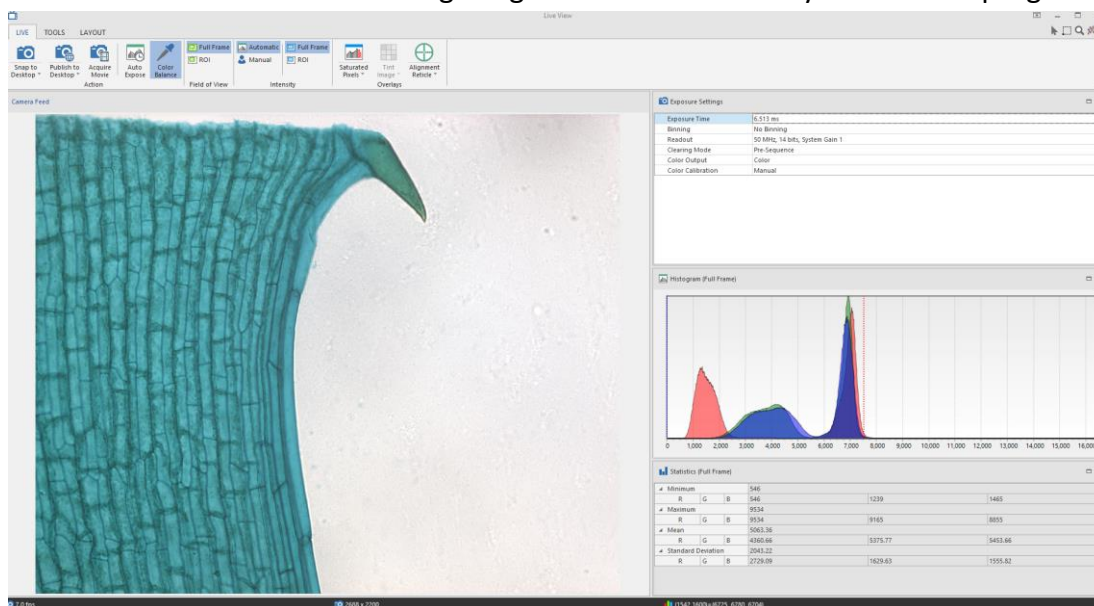
4.5 Adjusting for acquisition

1. Press the 3200K button on the microscope body for the most consistent colour temperature for the halogen lamp.
2. Cycle through the ND filter buttons so that the sample does not blind you through the eyepiece.
3. Find and focus your sample.
4. Move your sample so that a blank area can be seen through the camera live view.
5. Press the **Auto Expose** button to automatically set exposure.

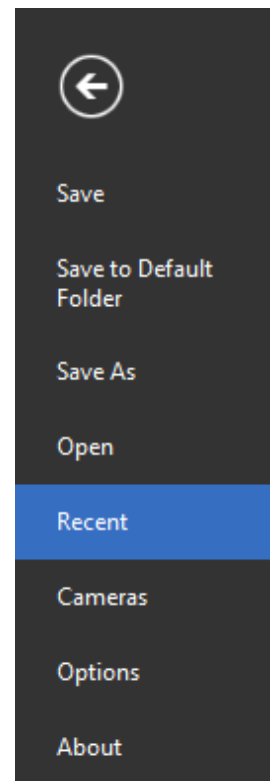
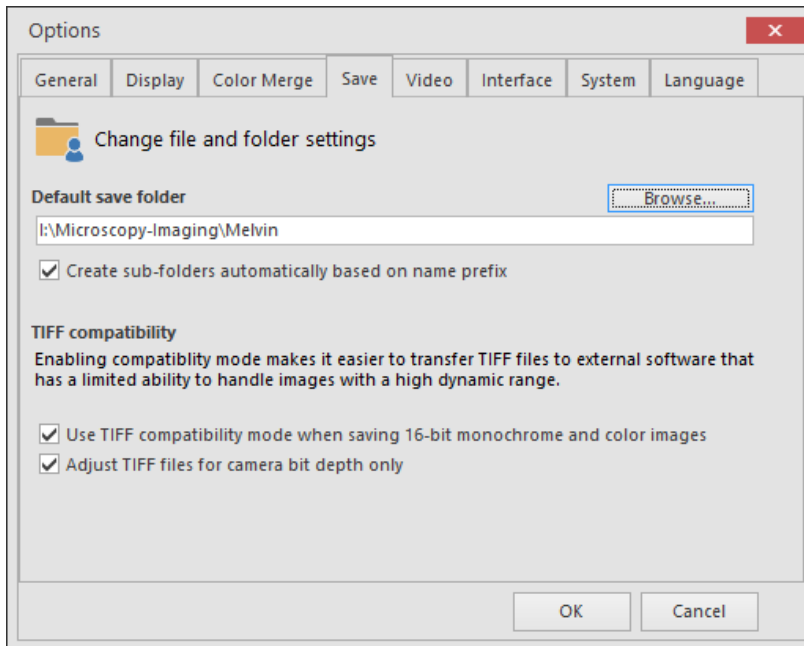
- Press the **Color Balance** button on the ribbon, then click and drag to define a region to set as the white point. It will show up as a negative-colored space.



- Once you are happy with your settings, press **Snap to Desktop**. (**Warning:** "Desktop" means the Ocular program desktop, not the Windows desktop. You will need to save the resulting image somewhere before you close the program.)



- If you want to save somewhere directly when you press Snap (like in a network drive), press **File** and go to **Options** (right):
- Go to the **Save** tab, then define a Default save folder.



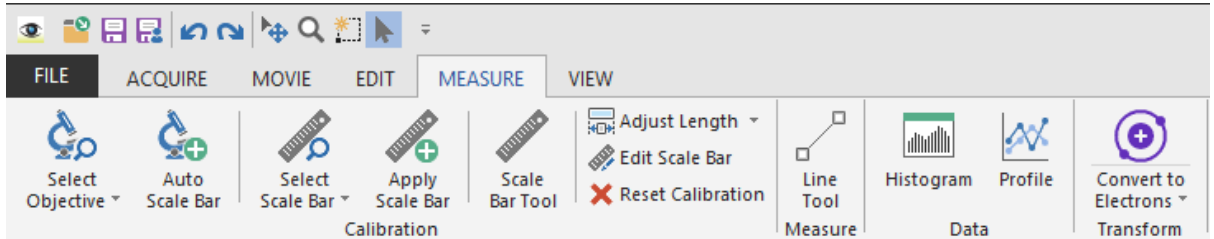
- Then in Live View you can press the text instead and press **Snap to Default**.
The snapped picture will not show up in the Ocular desktop. Go to **Recent files** and find your snapped picture.
- Once the image is snapped, you can set up your picture for measurements and other functions. **Make sure you have written down your microscope settings somewhere because the program will not save them for you.**

4.6 Measuring distances (scale bar tool)

Note: Scale bar creation and distance measuring is only available for raw snapped images, not Published images.

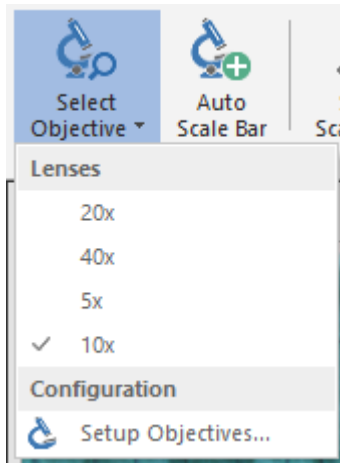
To create a scale bar

1. Open your snapped image into the Ocular desktop, then navigate to the **Measure** tab.

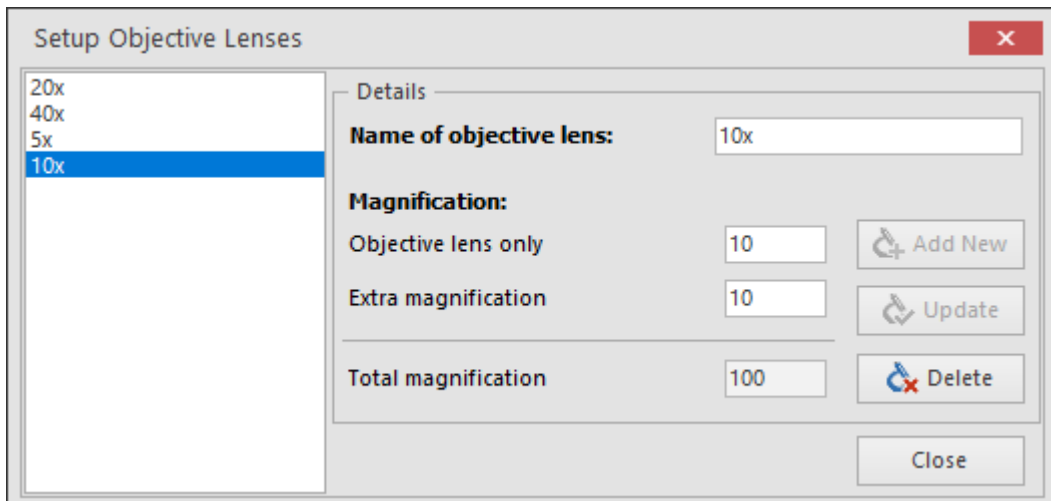


2. Press **Select Objective**, then select the objective lens that you have used if it is available.

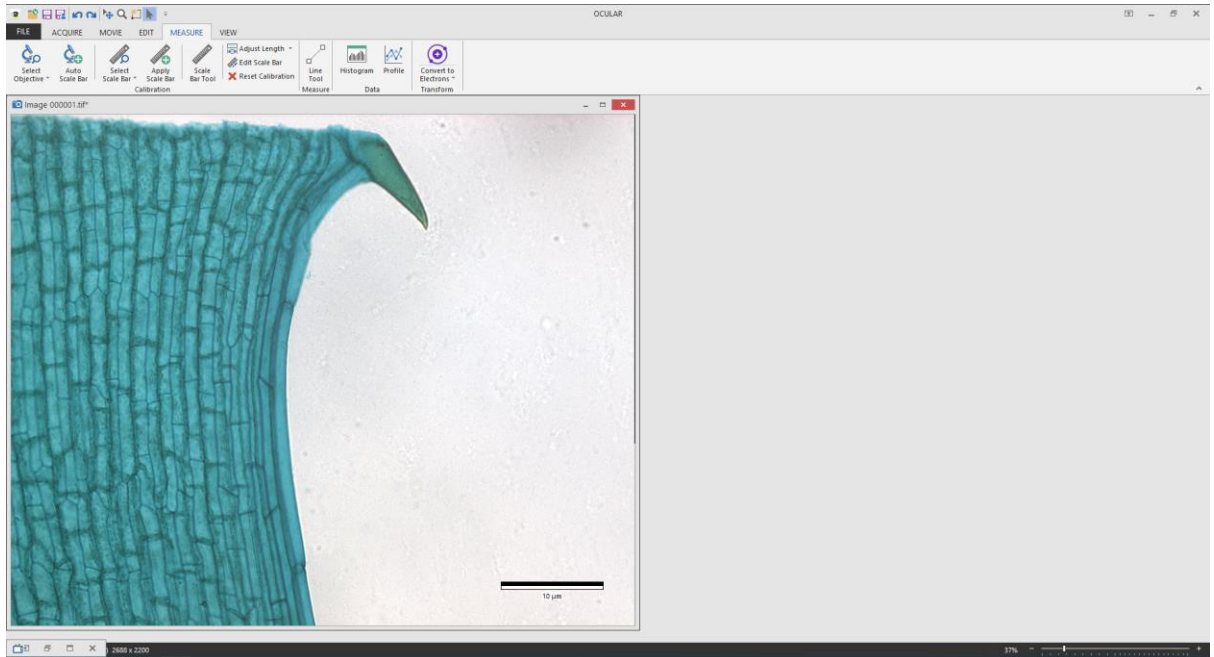
If your objective lens is not available in the dropdown, press **Setup Objectives...**

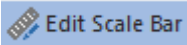


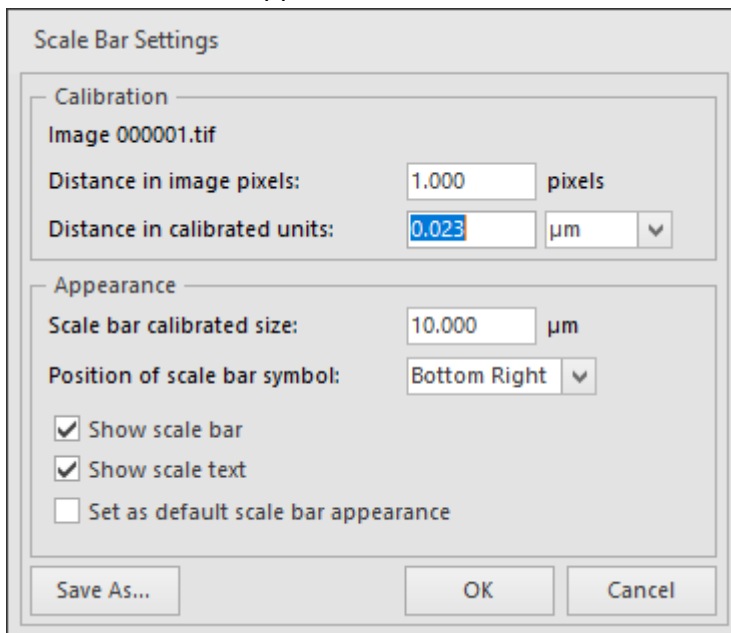
3. In this window you will specify your magnification. Set the name of the objective lens (we recommend just putting the magnification number of the objective lens), then in Magnification, set the following values:
 - a. **Objective lens only:** the value seen on the objective.
 - b. **Extra magnification:** 10.



4. Press **Add New** to create the objective lens setting, then confirm that your picture is using it by opening the dropdown and selecting the objective lens you have created.
5. Press **Auto Scale Bar** to create the scale bar with the calibrations you have set.



6. If you want to change the length or position of the scale bar, press  and a window will appear:



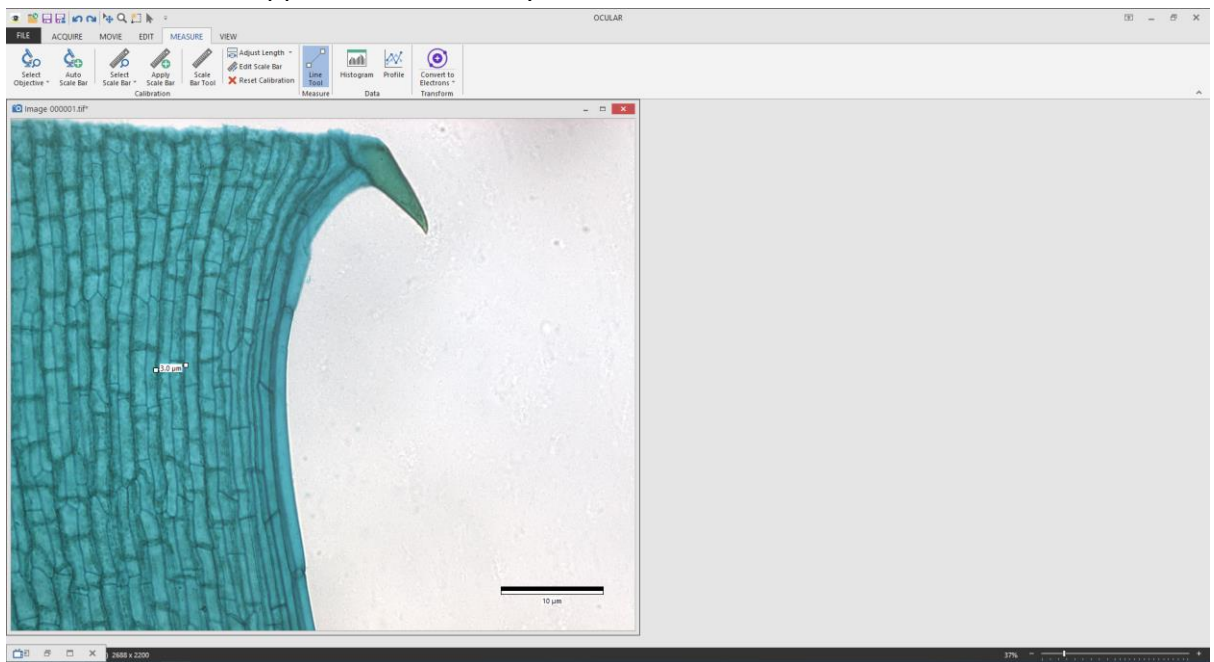
- a. **Do not touch Calibration**
- b. Set **Scale bar calibrated size** to the length that you want.
- c. Choose from the dropdown the **Position of scale bar symbol**. It is not possible to freely change the position other than the predefined positions available.

To measure distances

1. Follow steps 1 to 4 of the previous section where applicable.



2. Press Line Tool **Measure** and draw a line in your sample showing the distance you want to measure.
3. The measurement appears automatically.

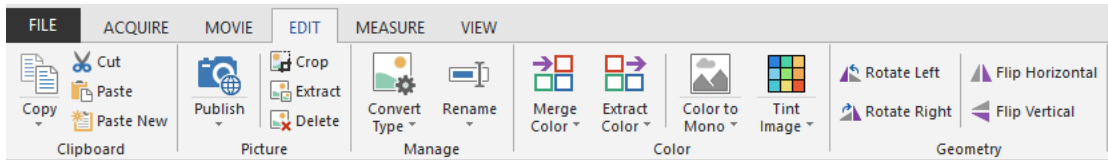


4. You can move or resize it to your preference. Click and drag the centre of the line to move it, or you can click and drag one of the end points to resize it.

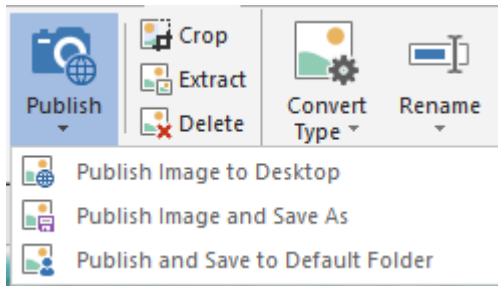
4.7 To publish your image (to view in other programs)

Publishing your image burns all the scale bars and measurements currently present in the last step of the previous section into your image and makes the image viewable in other programs that are not 16-bit compatible or are unable to view the contrast settings made by Ocular.

1. Once your image is ready, go to the **Edit** ribbon and press **Publish**.



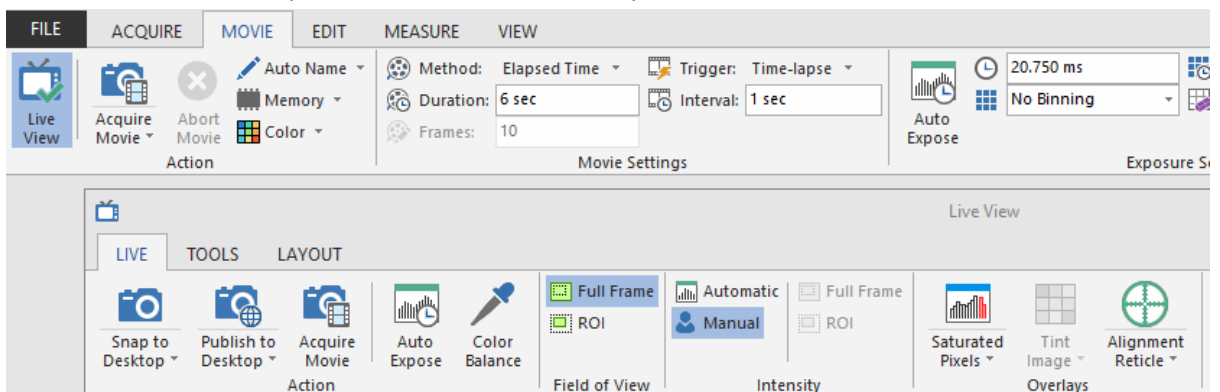
2. You will be presented with the following options:



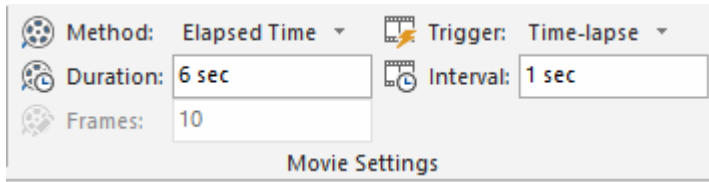
- a. **Publish Image to Desktop** sends the published image to the Ocular desktop **but does not save it yet**. You will need to manually save the published image.
- b. **Publish Image and Save As** publishes the image to the Ocular desktop and presents a window for you to define a place to save the image to.
- c. **Publish and Save to Default Folder** publishes the image to the Ocular desktop and sends it to the folder which you have defined in Section 4.5.

4.8 Acquiring video

1. On the main window (not the Live View window), switch to the Movie tab.



2. In the subtab Movie Settings, there are a few options you can select:



a. **Method**

- i. **Elapsed Time:** Takes a movie for a defined amount of time as specified in **Duration**.
- ii. **Frame Count:** Takes a movie for a defined number of frames as specified in **Frames**.
- iii. **Unlimited:** Takes a movie until the **Stop Movie** button is pressed. The entire movie is saved starting from the time **Acquire Movie** is pressed.
- iv. **Circular:** Takes a movie until the **Stop Movie** button is pressed. Only the last frames as specified in **Frames** will be saved.

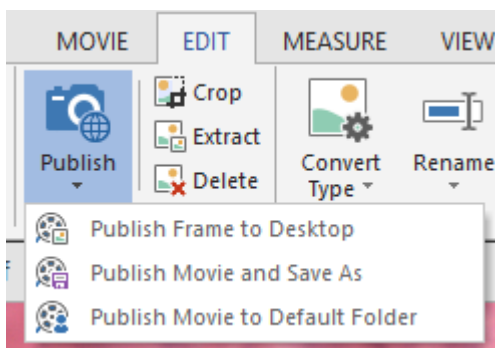
b. **Trigger**

- i. **Time-Lapse:** Each frame is taken in intervals defined by the value specified in **Interval**.
- ii. **No Delay:** The camera takes images in the fastest interval that it can manage. See the lower left corner of the Live View window for the framerate.
- iii. **External trigger** options are not useful here because there are no external triggers installed in this microscope camera.

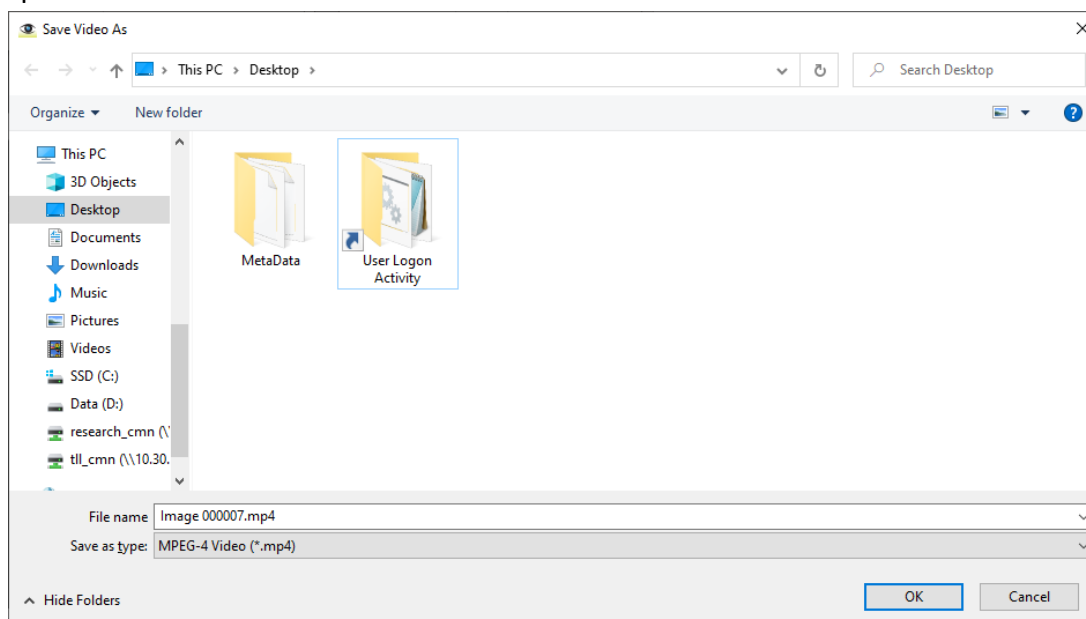
3. Once you have set your options, press **Acquire Movie** on either the main window or the Live View window. Either of them will work and will use the same settings as defined previously.

4. After finishing your movie acquisition, make sure to save the raw file. It will be saved as .tiff.

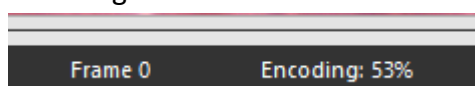
5. To export your video, you may go to the **Edit** tab, press **Publish**, then click **Publish Movie and Save As**. Publish Frame to Desktop will only publish the frame you have shown on the window.



6. A window will pop up asking you where to save your video. Specify it, then press OK. You may encode your video in MPEG-4 (mp4), AVI or QuickTime Movie (mov). MPEG-4 is recommended because it gives higher quality for the same amount of space used.



7. Encoding will take some time. To see its progress, look at the bottom toolbar.



8. Your video is now encoded.

4.9 Switching OFF Protocol

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

1. Transfer your images into the TLL network, your own USB, or a movable hard disk. Images saved in computer desktop or D/E drives will be **deleted within a month without prior warning**.
2. Carefully clean the objective lens with Whatman lens tissue (use 100% ethanol for oil).
3. Exit Ocular software and shut down the PC **(4)**.
4. Turn off the camera in-line switch **(3)**.
5. Switch off the Metal Halide lamp **(2)**, if it has been turned on.
6. Switch off the Microscope.
7. Fill in the logbook.

5. Troubleshooting

Issue	Explanation & Remedial steps
Ocular software does not turn on Live View/Ocular software hangs on startup/Live View is frozen.	Camera may have crashed. This may have been caused by a power spike caused by switching on the MHL while the camera is on. <ol style="list-style-type: none">1. Turn off the camera in-line switch (3).2. Close Ocular software if it is still open.3. Turn on the camera in-line switch (3).4. Wait for 5 seconds.5. Open Ocular software and confirm that the Live View is working again.
Unable to make a scale bar/measure distances	Select an objective, then press Auto Scale Bar. Scale bar creation and distance measuring is only available for raw snapped images, not Published images. If you are using a Published image, look for the raw image that was used to publish it and then follow the steps in Section 4.6.