# BEGINNERS SAFETY MANUAL FOR
# ZEISS Axio Observer WF 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.

## 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 objective lens. Let the oil drip onto the lens surface without touching the objective lens with the applicator.
- 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 kim wipes 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 Mercury Lamps (HBO)

HBO lamps are fluorescence lamps that emit a visible light range of 300 – 650nm, enabling us to observe fluorescence signals such as GFP, DAPI and RFP. HBO lamps use high pressure mercury lamps which contains mercury vapour that is extremely toxic to the human body. 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 350hrs.
- Do not look directly at the HBO lamp when it is switched on as it can damage your eyes.
- If there are no users in the next 30mins, HBO lamp must be turned off.
- Never attempt to change a bulb yourself.  Please contact facility staff when you notice that a bulb has reached between 300 - 350hrs 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 team (*bioimaging@tll.org.sg*) for training session.
- Fill in this training form: *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.

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

Each microscopy system in TLL Bioimaging Facility has its specific instructional manual which is found in every confocal room. 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. During operation of laser microscopy systems, do not look into the laser beam directly as they are all Class 3b and Class 4 lasers.

## 4.1 Switching ON Protocol

1. SIGN IN the Logbook and record your START-TIMING.
2. Switch on Power supply box SMC2009 (1).
3. Press on the Focus Controller (2).
4. Switch on the Microscope power supply (3).
5. Press on the Microscope button, rear left side (4) and wait for initialization.
6. Switch on the camera (5).
7. Switch on the HBO/Mercury lamp (6), if needed.
8. Switch on the LEDs (7), if needed.

   ※ If you want to use the LEDs, there is a lever behind the microscope that you will need to turn before you can switch to the LEDs. You cannot use the LEDs together with HBO illumination.

9. Switch on the PC (8) and log in with your Wonderland.
10. Load the MetaMorph program from the desktop.
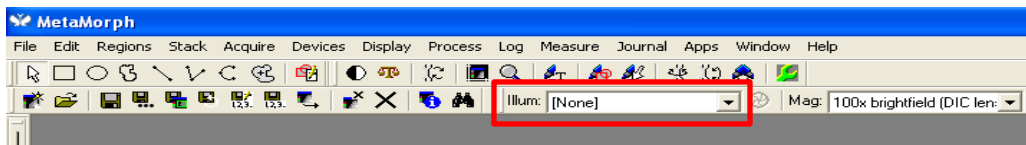11. Fill in the log book.

**4.2 Objective Lens**

| Objective lens | Immersion media |
|---|---|
| 10x/0.3 Plan Neofluar | Air |
| 20x/0.5 Plan Neofluar | Air |
| 40x/1.1 C Apochromat | Water |
| 40x/1.3 Plan Neofluar | Oil |
| 63x/1.4 Plan Apochromat | Oil |
| 100x/1.4 Plan Apochromat DIC I Ph3 | Oil |

**4.3 Software initiation**

Turn on the machine following the instructions pasted on the wall or inserted in the log book, (switches are numbered to make things easier). Wait for complete initialization of microscope and Windows after logging in with your TLL username and password.

Double click on MetaMorph icon 

You will observe the main menu bar as below. You will need to select the Illumination option to the emission you wish to view and select current shutter.



This software is an open-loop control software. **Settings changed on the microscope body will not be reflected in the program,** so set your illumination settings and objective lens using MetaMorph only.

**4.4 Definite Focus 2**

This microscope has a feature to stabilise focus to guard against drift in long sessions. It functions by detecting the distance from the end of the objective lens to the bottom surface of the sample you are using (well plate, dish, slide)

This feature works best with the following objective lens:

| Objective lens | Immersion media |
|---|---|
| 10x/0.3 Plan Neofluar | Air |
| 20x/0.5 Plan Neofluar | Air |
| 40x/1.1 C Apochromat | Water |

As a general rule, Definite Focus 2 does not work well with oil immersion lens.

Once you have confirmed that you are using the objective lens as listed above, follow the instructions below to have stabilised focus.



1. Mount and focus your sample.
2. Press the On button on the Definite Focus tab in the touchscreen. The Definite Focus tab is available in the Home and Microscope page.
3. If Definite Focus 2 is able to lock focus, the controller box will show a **Monitoring** status with a percentage number.
   - ※ The percentage number does not matter as long as there is a number. You don't have to wait for it to reach 100%.
   - ※ You can use the focus knobs or the focus controls on MetaMorph to change the focus level without affecting the operation of Definite Focus 2. Definite Focus 2 will stabilise to the new focus distance you have selected after you have finished adjusting focus.
4. If the sample is disturbed through any means other than operation of the focus knob, the controller box will show a **Correcting** status and the focus will adjust until it reaches the focus distance you have defined.
5. If Definite Focus 2 is unable to lock focus or the sample is moved in such a way that Definite Focus 2 is unable to adjust for it, the controller box will show an **Error** status and automatically turn off Definite Focus. Press the button on the touch screen to turn it back on again after checking your sample.
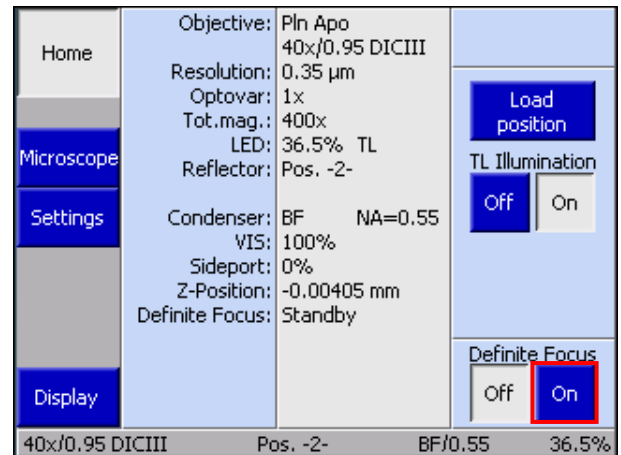
**4.4 Acquisition**

To acquire image, select "Acquire" on both the menu bar and in the drop-down menu.

In the Acquire window,

- **Acquire** lets you capture an image of your sample.
- **Exposure Time** option lets you decide how long your sample should be exposed to an excitation source for imaging. You can set the duration from milliseconds, to seconds, and minutes.
- **Auto Expose** The software uses a range of exposure times. This usually takes 1-4 minutes and might result in bleaching.
- **Show Live** lets you view your sample in real time.
- **Binning** will combine pixels to give you a brighter image. This is done by selecting how many pixels you want to combine into one pixel. Keep in mind that binning shrinks your image by $\frac{1}{(binning\ size)^2}$ and would generally result in excessively small images after binning size 3.

In the "Camera Area",

- **Full Chip**: Uses the whole CCD Chip to image your sample
- **Center Quad**: Uses the center area of the CCD chip to image your sample.
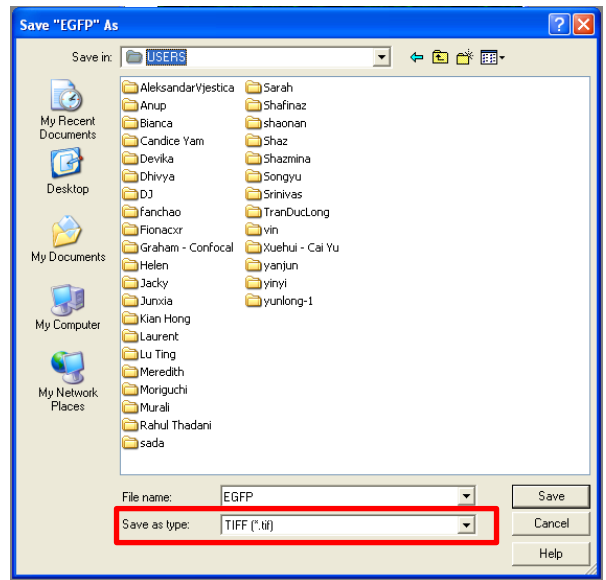- **Use Active Region:** Select a region of interest to image.
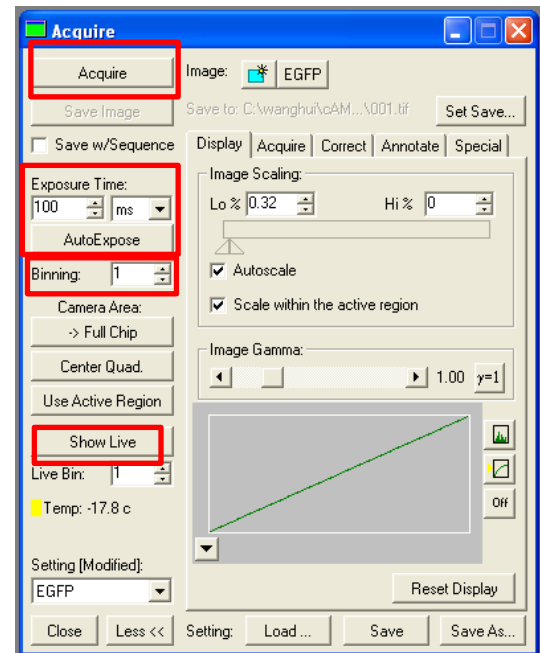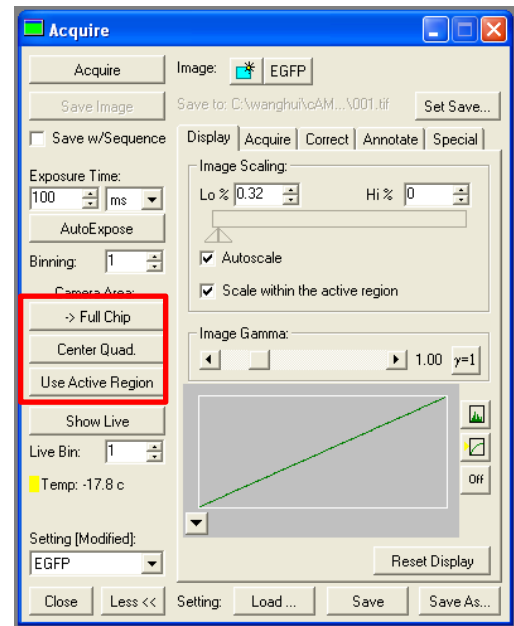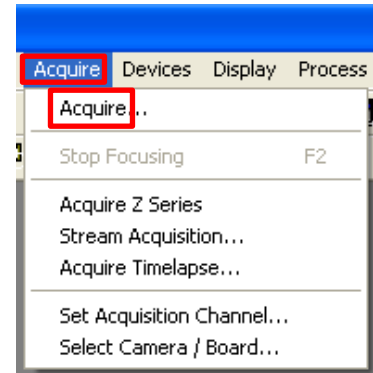
To save an image select **"File"**

Next select **"Save As"**

This will open a window, here select D/E drives. Next select the folder "Users" and create the folder with your name.

You must specify what file type to save your image in. It is recommended to save your image in TIFF format, as it is a lossless format.



**4.5 Multi Dimensional Acquistion**

The Multi Dimensional Acquistion App lets you do more than one type of acquisition simultaneously or consecutively.

Select "Apps" then click on "Multi-Dimensional Acquisition"

A dialogue box opens, that allows you to set all the imaging parameters. Tick your desired acquisition mode. Under the main tab: select the type of imaging, i.e. timelapse, multiple stage positions, multiple wavelengths, z-series.
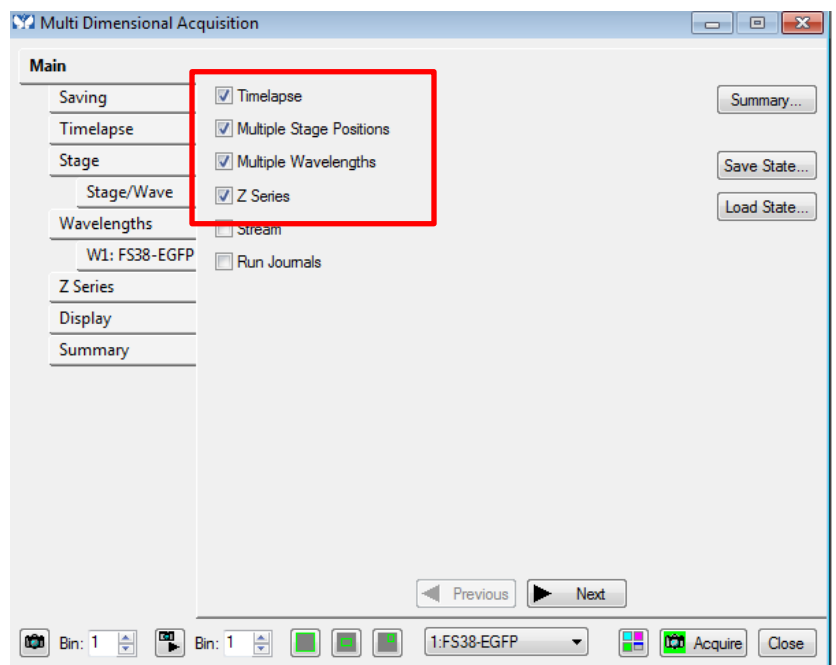
### 4.6 Saving data

You need to set where your data will be saved before starting your experiment and setting your desired acquisitions.

Click Select Directory and select the D:/ E: drive. Create a folder with your name to automatically save your data in. (Do not save in C drive)

Save "Base Name" as test 001 and tick "increment base name if file exists". This will ensure that the program will save your data files with the name with a running number sequence especially when you are doing a Z stack and or time series experiment.
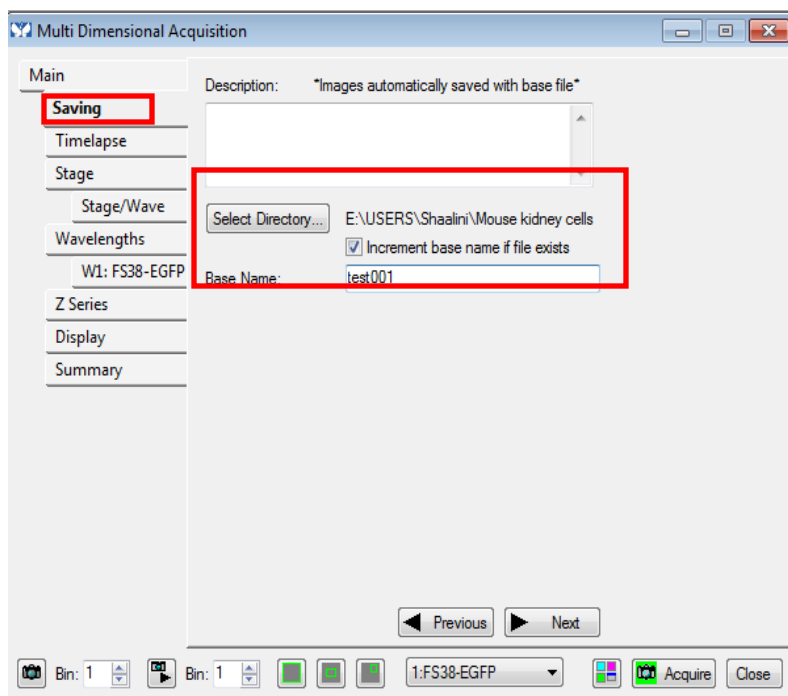
### 4.7 Timelapse

Under the "Timelapse" tab, you can 2 different settings.

*IMPORTANT:* Always choose your timelapse settings as last as the software will indicate the minimum time interval between 2 time points after configuring the wavelengths and Z-series.

1. **Number of time points:** Is the number of images you wish to acquire during your time frame.
2. **Duration:** Time taken for your experiment.
- **Time Interval:** How many seconds/mins in between each time point. It cannot be less than "estimated time interval'
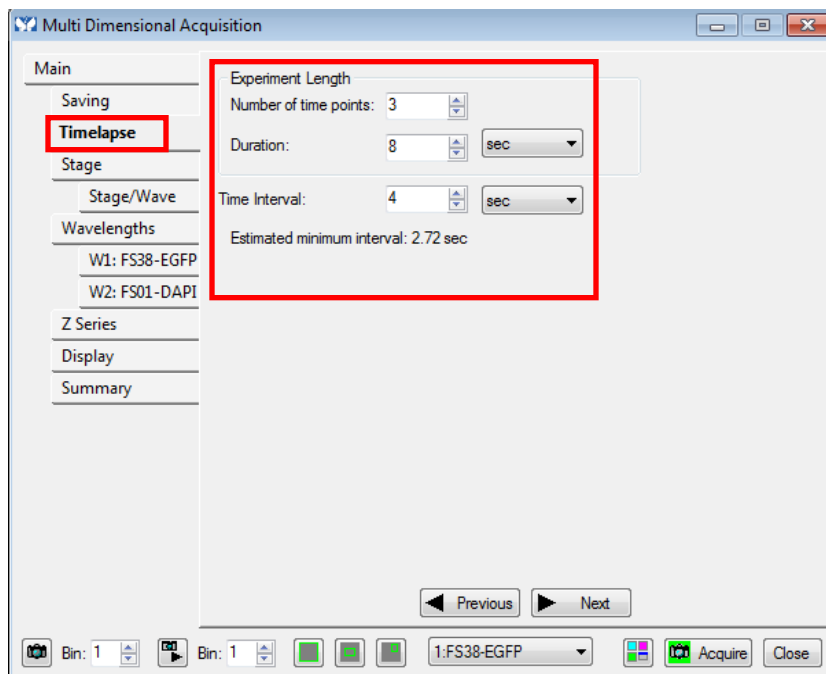
## 4.8 Multiple Stage Position

Under the Multiple Stage position, you can view in live mode to:

**+** **Add** multiple positions of your sample where you wish to acquire your image.

**X** **Remove** positions that you do not want to image

If you desire to go back to your previous positions, you could click "move to positions".
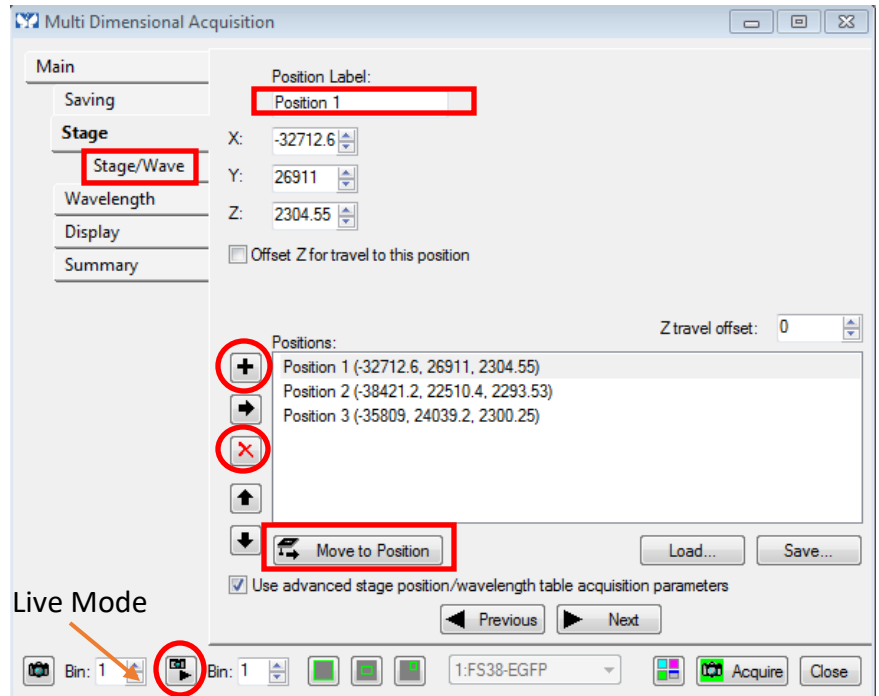
## 4.9 Multiple Wavelengths

Under **Wavelengths tab**, you can select the number of wavelengths or fluorophores you choose to acquire.

Set your **illumination** according to your fluorophores and adjust the **exposure time** under live mode. Increasing Digitizer increases acquisition speed but also increases noise. NOTE: use the same digitizer for all wavelengths.

"**Acquire**" option lets you decide if you want to acquire at every time point or alternate time point for a particular wavelength.
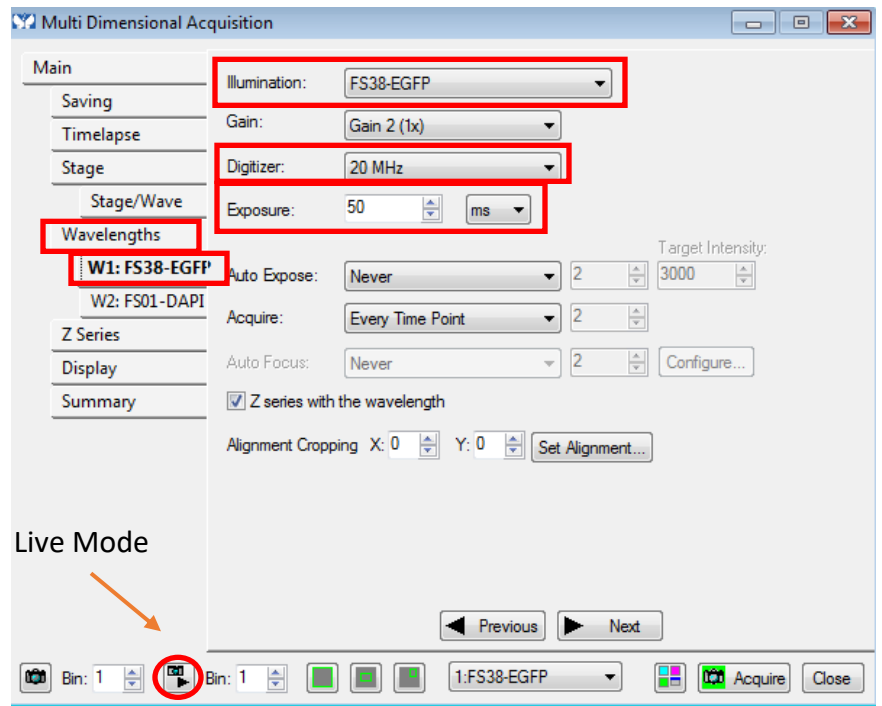
**4.10 Z-Stack**

*This option is not as useful on wide field systems as on confocal systems*

Shows images at different depths of the cell, allowing you to gain more information of your sample.

Under the Z series tab, choose "acquire Z-series for one wavelength at a time" for fast acquisition.

There are 2 ways to set Z



1. **"Range around current":** In case you want to image few microns up and down, from current focal point. Select step size and range to define the Z-series.

2. Setting the Top and the bottom. Uncheck range around current.
   - Click on "Live" to see your image on screen.
   - Use the fine focusing knob of the microscope to focus on the upper position of your specimen volume and click **"Set Top to Current"** button to set the upper position of the Z-stack.
   - Then focus on the lower position of your specimen volume, where you want your z-stack to end and Click **"Set Bottom to Current"** button to set the lower position.
   - **Step Size** tells you at which depth it acquires the image. For example, step size of 6um, acquires an image at every 6um for the depth you have selected.
   - **Number of steps:** indicates the number of images you are going to acquire for your selected depth and Step size. In this case, 7 images.
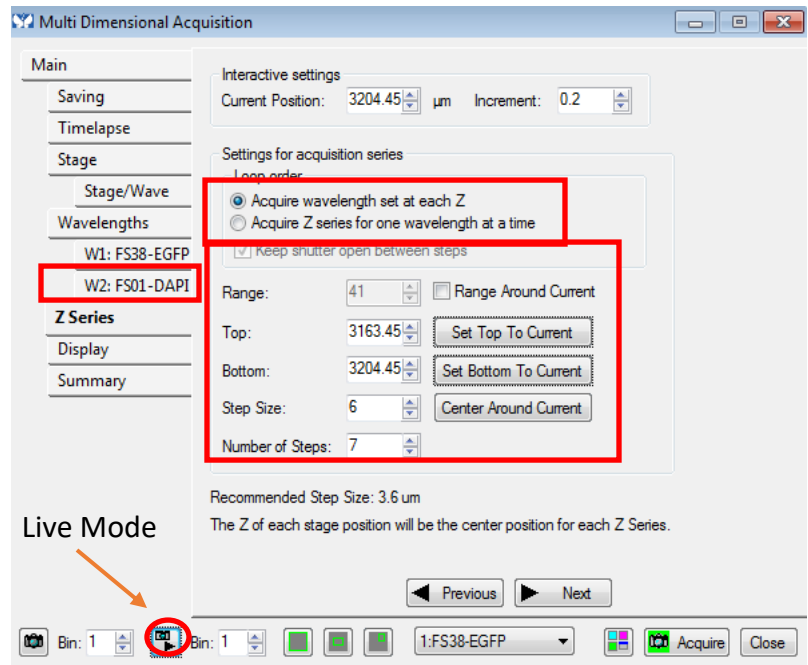
### 4.11 Summary

Under summary, it gives you all the parameters that you have chosen for all the different acquisitions mode including Image Saving requirements.

Once, you've confirmed all parameters, click "**Acquire**' and a progress report will pop up to inform you of the current acquisitions it is capturing.
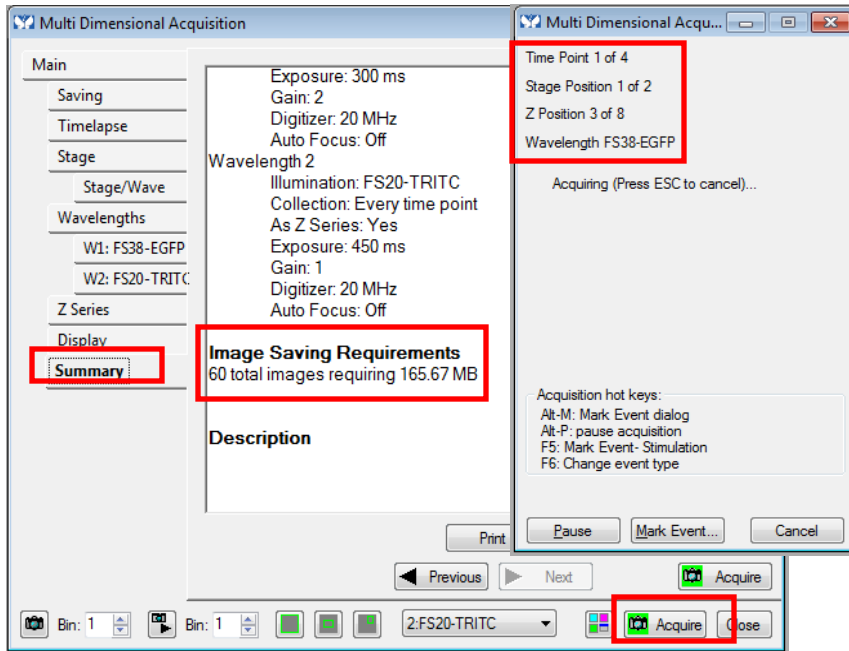


### 4.12 Reviewing Multi-Dimensional App

Reviewing multi-dimensional App allows you to view, filter, organize, and analyze your saved images in a Multi-Dimensional Data Set (*.nd) and generate new images based on your viewing criteria.

Select "Apps" then click on "Review Multi-Dimensional Acquisition"

Select the "**Base Files**" and then "**select directory**" where you have saved your files. All your acquired images will be saved under the Data sets. You could select the individual file (*.nd) or you could select all and click **"View"**.
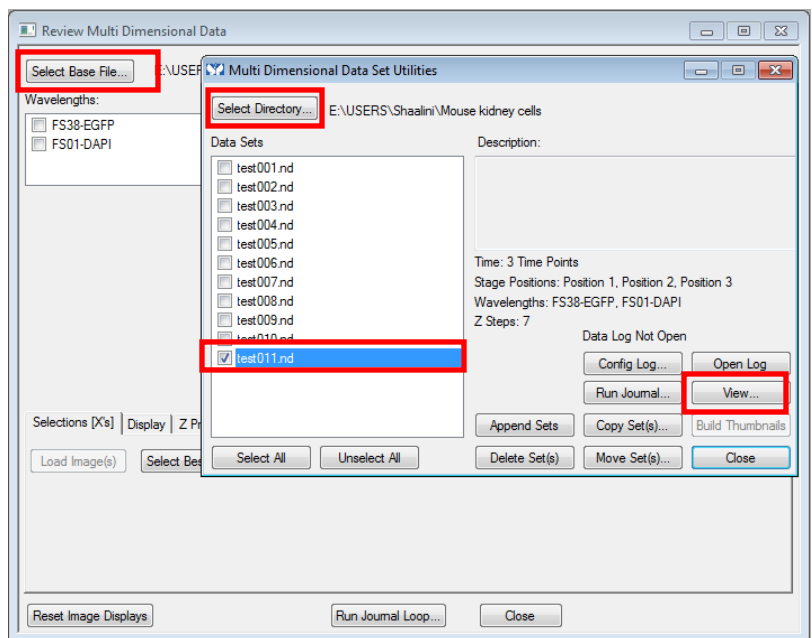
Check the wavelengths to view your merged images. To view, click on each of the cubes to view the image according to your settings.

- **Vertical column** highlights the different images acquired in z-stack.
- **Horizontal row** highlights the images at different timepoints in timelapse.
- **Stage position** allows you to view the images at different positions

Under the **display tab**, set the colors you would like to view your wavelength/fluorophores in. It would automatically give you the image according to your displayed settings and parameters. Save the merged images as tiff format.
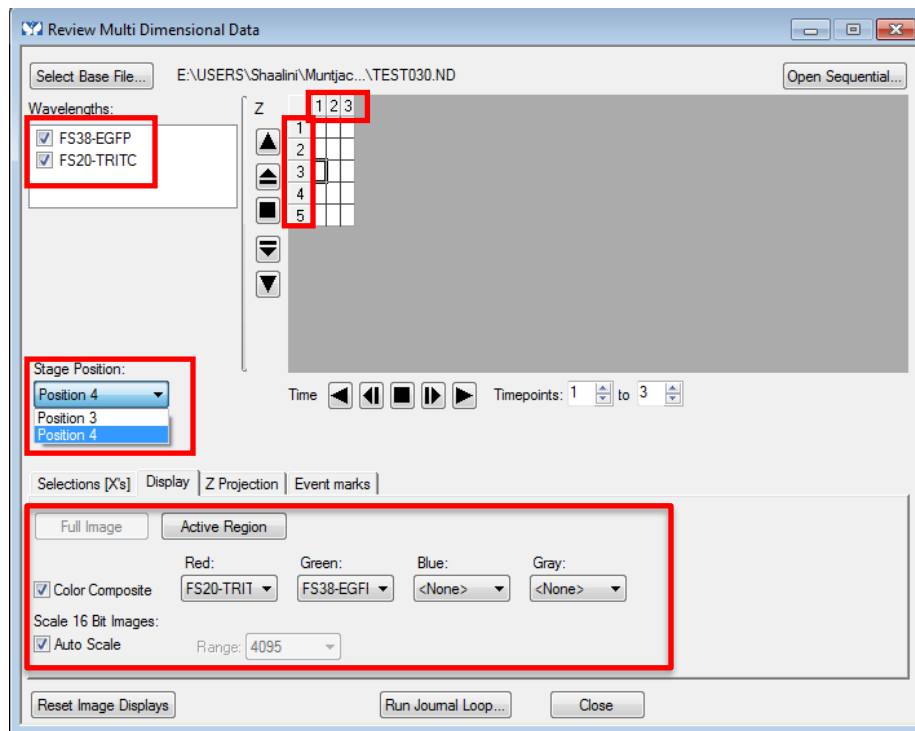
**4.13 Switching OFF Protocol**

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 computer. Otherwise switch off the system.

(a) Save your images through TLL common drive folder. Images saved in computer D/E drives will be ***deleted within a month without prior warning***.
(b) Carefully clean the objective lens with Whatman lens tissue (use 100% ethanol for oil).
(c) Exit MetaMorph, backup your data, and shut down the PC (8).
(d) Switch off the LEDs (7), if they have been on.
(e) Switch off the HBO/Mercury lamp (6), if it has been on.
(f) Switch off the camera (5).
(g) Press off the Microscope button, rear left side (4).
(h) Switch off the Microscope power supply box (3).
(i) Press off the Focus Controller (2).
(j) Switch off Power supply box SMC2009 (1).
(k) Fill in the logbook.