

## Olympus FV1000 MPE Mosaic Protocol

### 1) *Initialize the software.*

- ☞ Open the Olympus FluoView software.
- ☞ Set the laser to the appropriate wavelength. Turn on the laser and allow it to reach **Mode Locked** status.
- ☞ Ensure the correct lens is selected on the FluoView software.

### 2) *Set the laser power and gain settings for each desired channel.*

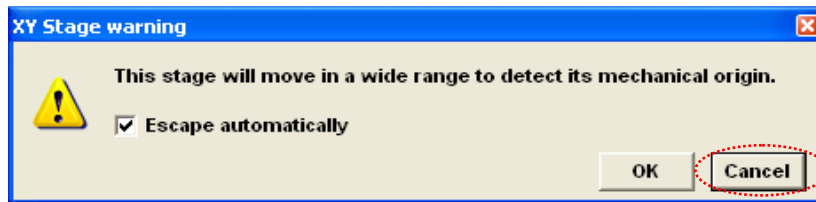
### 3) *Set the Z-stack or Time Lapse parameters, if applicable.*



### 4) *Setup the Multi Area Time Lapse.*

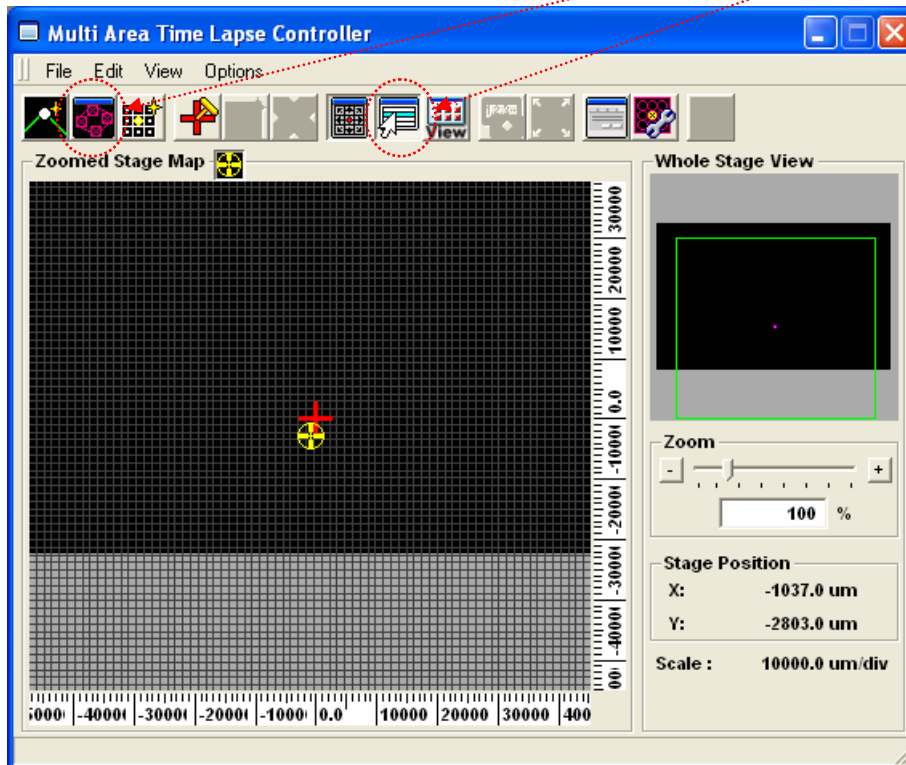
- ☞ Open **Device > Multi Area Time Lapse...** menu.
  - The **XY Stage warning** will appear. Click **Cancel**.



**WARNING: Failure to follow this step could result in damage to the lens or specimen!!!**




- ☞ Ensure the **Mosaic Outline** and **Registered Point List** menus are open by selecting  and  from the **Multi Area Time Lapse Controller** window.



☞ Set the boundaries of your tile scan.

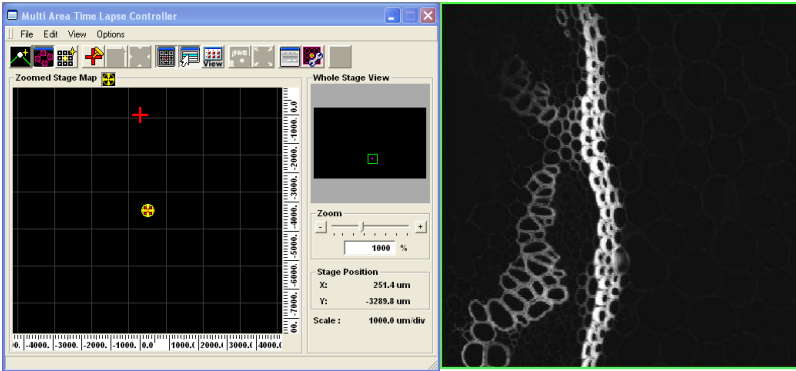
- Navigate to the boundaries of your sample and add these positions



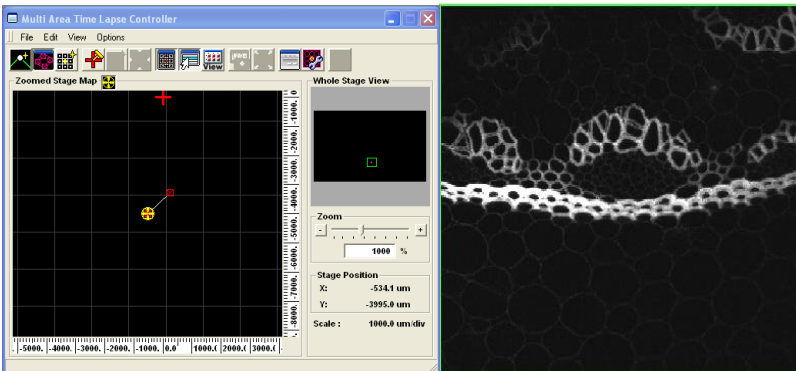
by using the  button in the **Mosaic Outline** menu.

- The montage can be any shape. For example, see following boundaries for a circular sample.

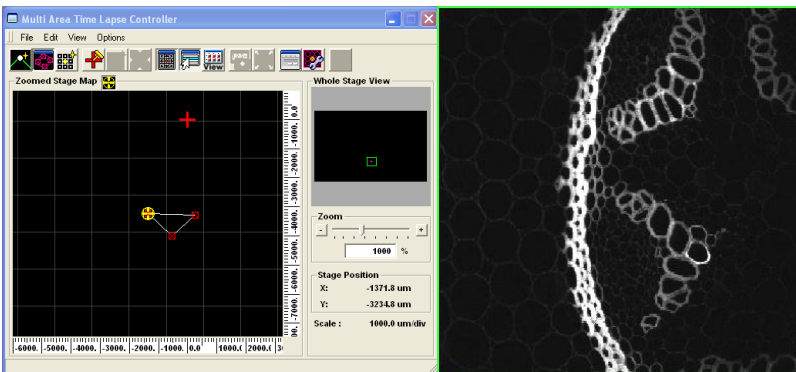
Position 1



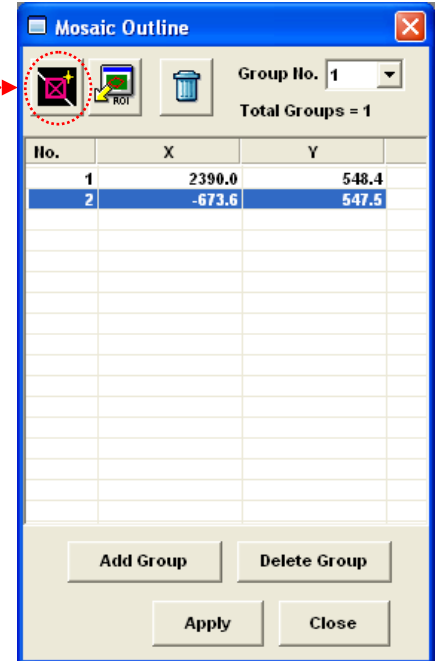
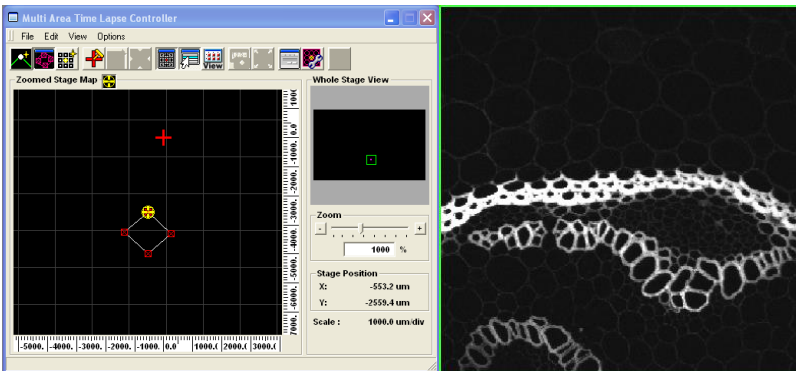
Position 2



Position 3



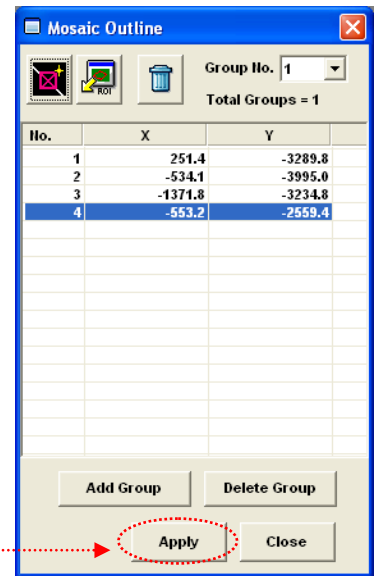
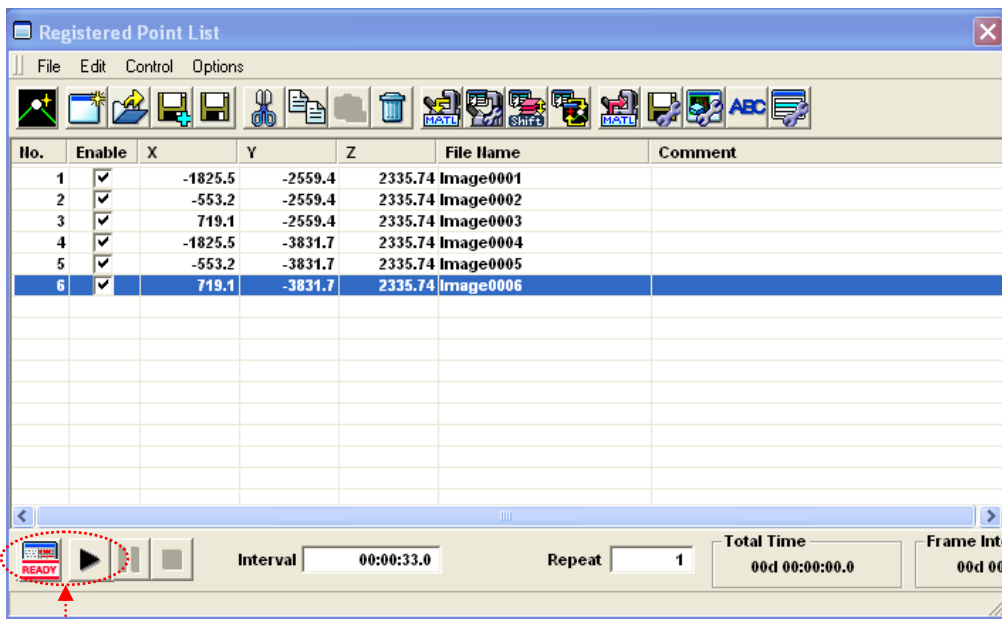
Position 4





*Convallaria rhizome test slide,  
Leica Microsystems*

Click **Apply** in the **Mosaic Outline** menu once boundaries have been set.

- The **Registered Point List** window will now display a series of positions that make up the montage.

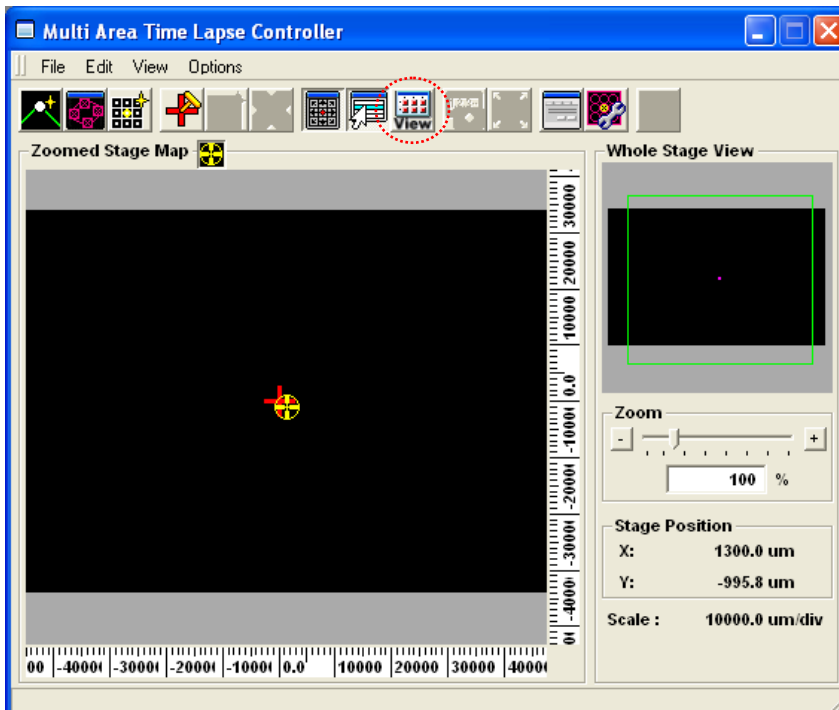



5) *Acquire the image.*

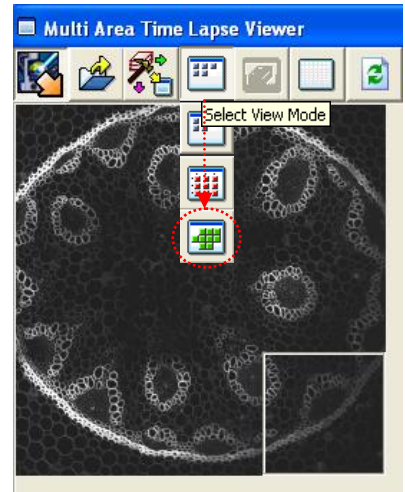
In the bottom of the **Registered Point List** window, click  then  to begin the tile scan.


Once the images are collected, click the  button in the **Multi Area Time Lapse Controller** window.

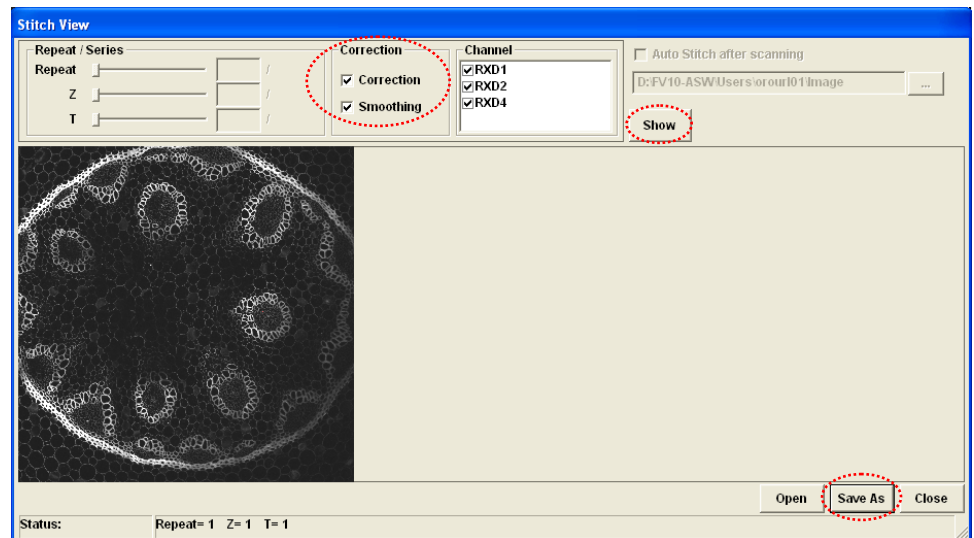
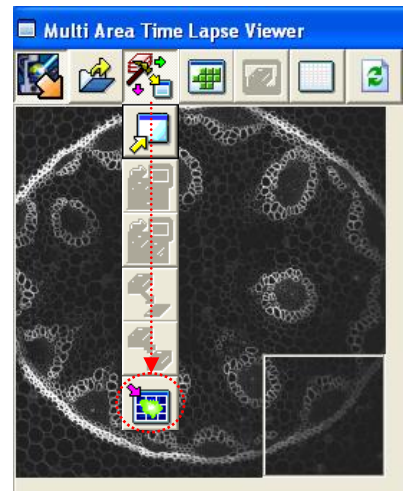
- This will open the **Multi Area Time Lapse Viewer** window.



- ☞ In the **Multi Area Time Lapse Viewer**, ensure the  button is selected from the **Select View Mode** dropdown menu (4<sup>th</sup> icon along top menu bar).



- ☞ Stitch the montage by selecting  from the 3<sup>rd</sup> dropdown.
- The **Stitch View** window will appear.
  - Enable **Correction** and **Smoothing**.
  - Click **Show**.



6) **Saving the image.**

- ☞ Click **Save As** in the **Stitch View** menu to save the tiled image.
- Save as type: OIF File.
  - **OPTIONAL:** When Z-stacks are acquired, enable the “Series” option in the **Save As** menu, save, and reopen the OIF file to select the intensity projection.