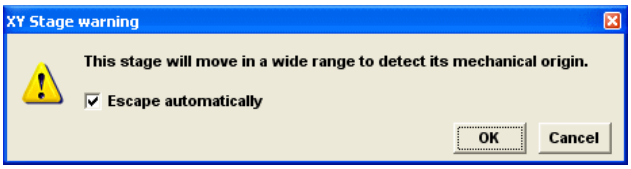
# Stitch Image Acquisition by MATL

MATL; Multi Area TimeLapse allows to acquire adjacent images with automated XY stage, and newly released “MATL Viewer” enables to stitch all images automatically to get high recolution, wide area 3D image.

<Procedure>

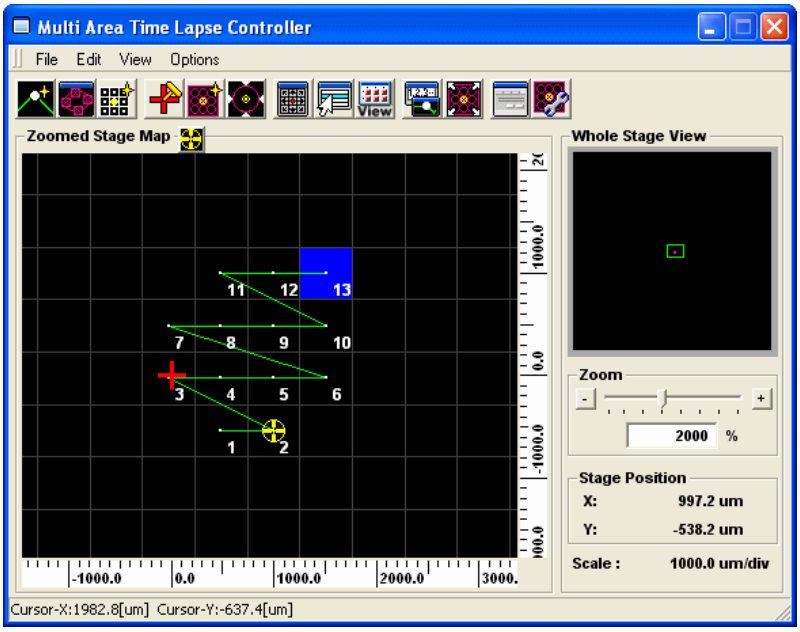
Image acquisition

1. Select [Multi Area Time Lapse] from [Device] menu on [OLYMPUS FLUOVIEW] window.




2. Click on <OK> button so that the stage is initialized to mechanical origin.
   In case no initialization is required, click on <Cancel> button.

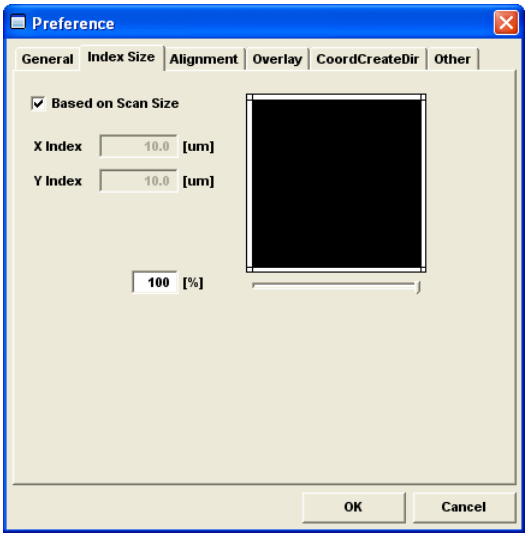
3. MATL will start and [Multi Area Time Lapse Controller] window will appear.




Set up [Preference].

1. [Preference] dialog box is displayed by selecting [Preference] from [Options] menu on [Multi Area Time Lapse Controller] window.

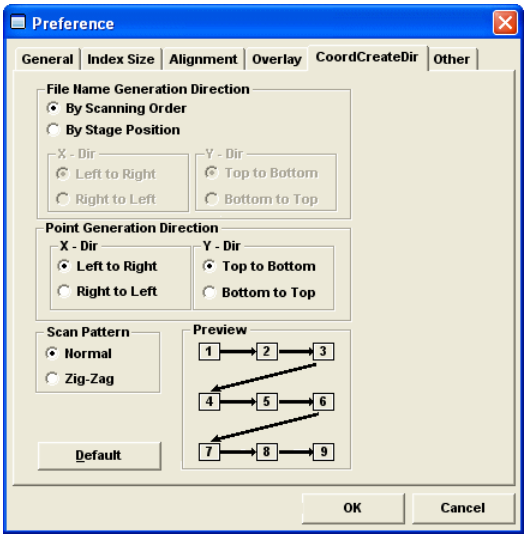
2. Select [Index Size] Tab and set margin overlapped with adjacent images.
   Check on [Based on Scan Size] button and define the ratio of index size on the textbox.. If you set 100%, there is no margin. If you set 98%, margin is 1% on each side.




**TIP**

Ratio 96%–98% is appropriate. If you set big margin, the photobleach at adjacent area will be distinct especially in 3D images.

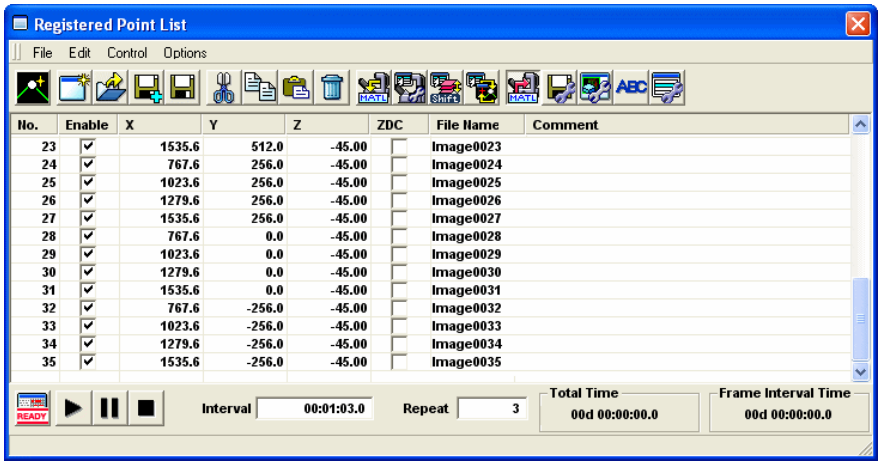
3. Select [Coord Create Dir] Tab and set movement of automated XY stage.

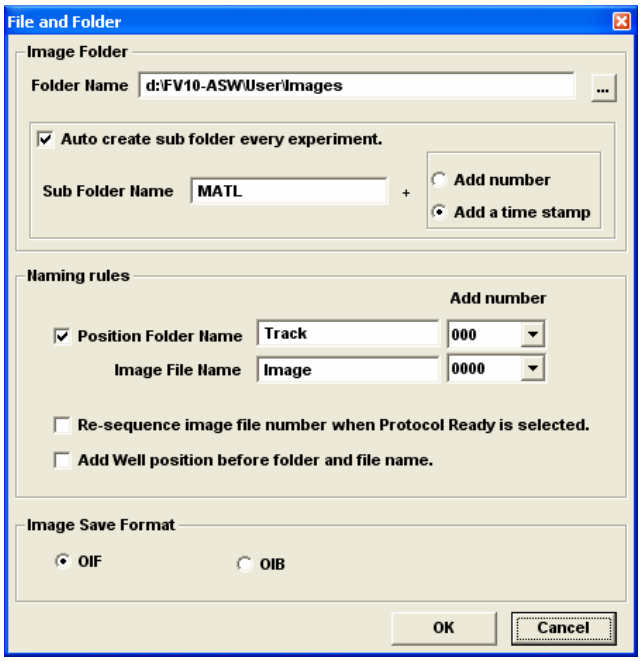


## Set up File Folder.

To open [ File and Folder] window, click [File and Folder]button on [Registered Point List] window.

Define the File name, Image Folder and Image Format to save acquired images.

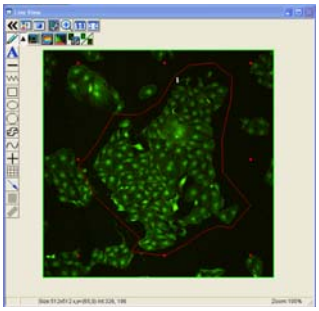


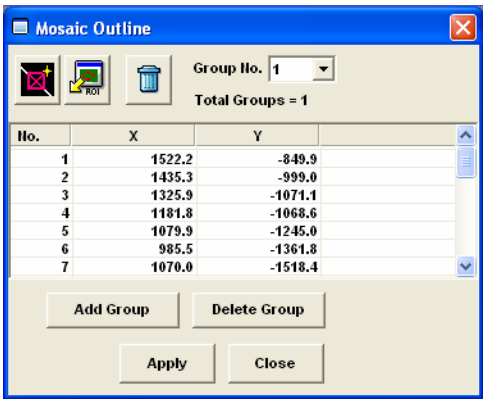
## Coordinate Registration using [Extract ROI] function

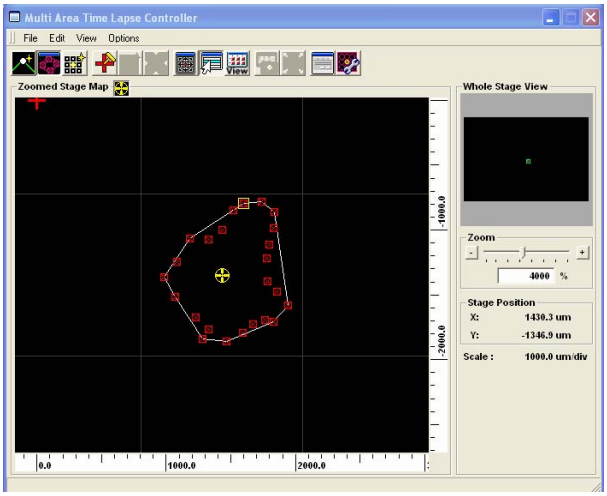
[Extract ROI] function is the new function of ASW1.7. This function allows you to define imaging region with lower magnification objective lens and image acquisition will be executed with higher magnification to get high resolution image.

1. Use lower magnification objective lens i.e. 10X, and show image on [Live View] window. Draw a ROI on the area that should be observed.
   **TIP:**The ROI that can be loaded is only (Rectangle) or (Polygon)

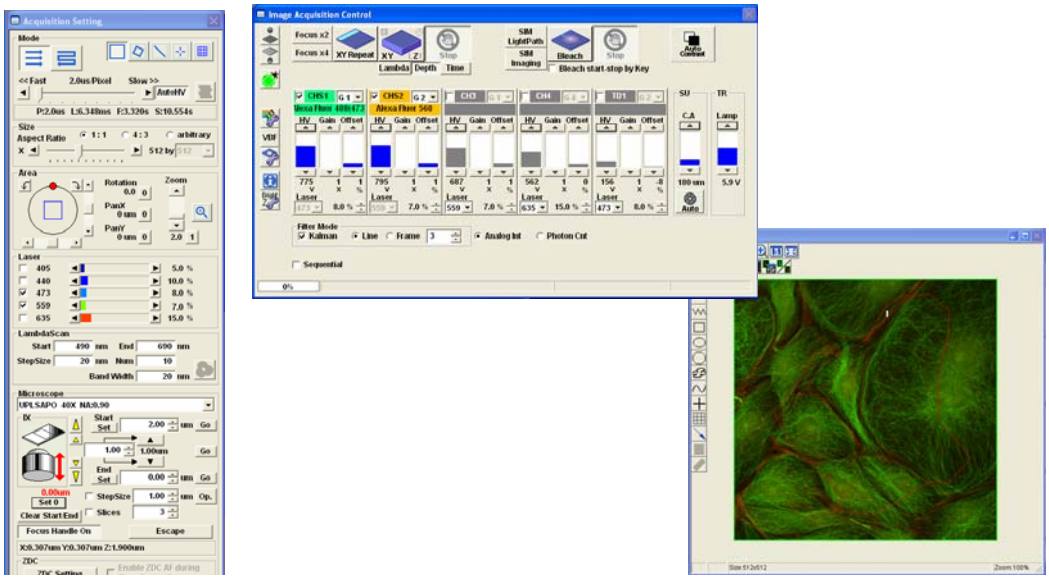


2. Click on <Extract ROI> button on [Mosaic Outline] window
   ROI information will be loaded as contour coordinates to MATL and displayed on the stage map on [Multi Area Time Lapse Controller] window and [Mosaic Outline] window.



3. Change Objective lens to higher magnification i.e. 40X. and adjust imaging condition on [Acuisition Setting] and [Image Acquisition Control] window.



**TIP:**

CA; confocal aperture is recommended to be opened instead of using Auto.

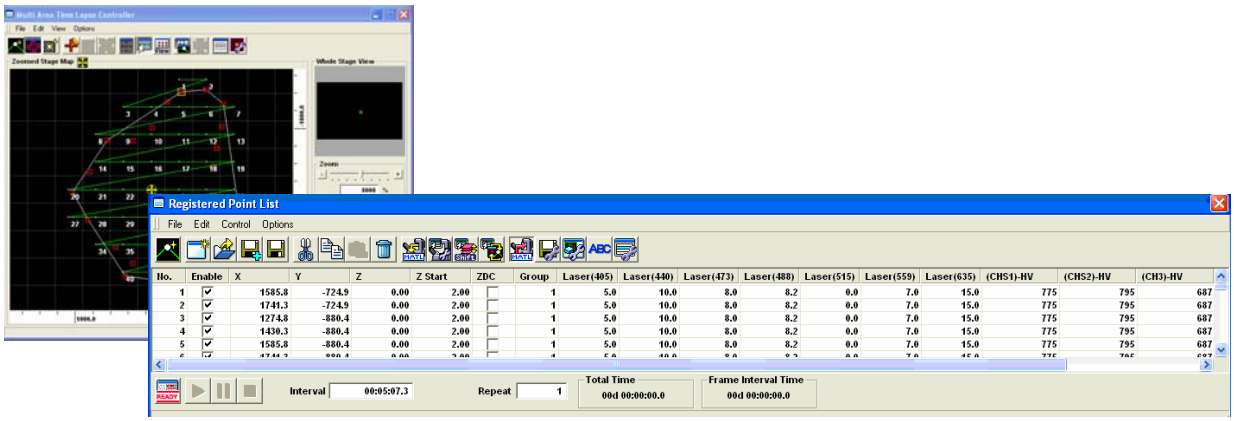
Zoom maginification is recommended to around 2X.

These setting will improve unevenness at peripheral edge of image.

4. Click ON 

(Apply) button on [Mosaic Outline] window.

Contour coordinates will be converted to the coordinates of an image to be acquired and registered on [Coordinate] list on [Registered Point List] window.



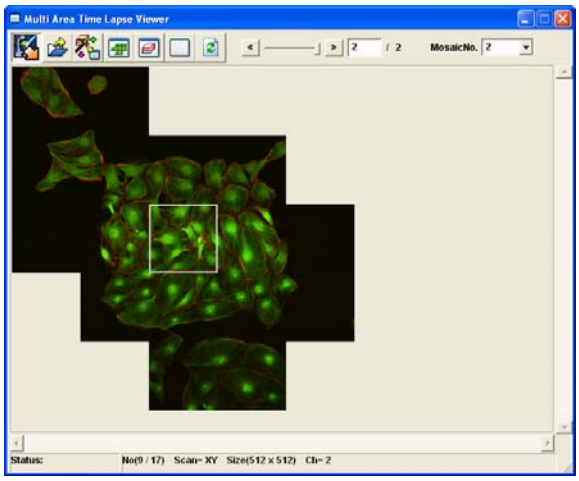
## Image Acuisition

Click 

<Ready>button and then click 
<start>button to start image acquisition.

## MATL Viewer

MATL Viewer enables to stitch all imaged to one image.
MATL Viwer window will appear if [Multi Area Time Lapse Viewer] is selected on [View] menu on [Multi Area Time Lapse Controller] window.
It will also appear by selecting [Multi Area Time Lapse Viewer] on [Device] menu on the main window.



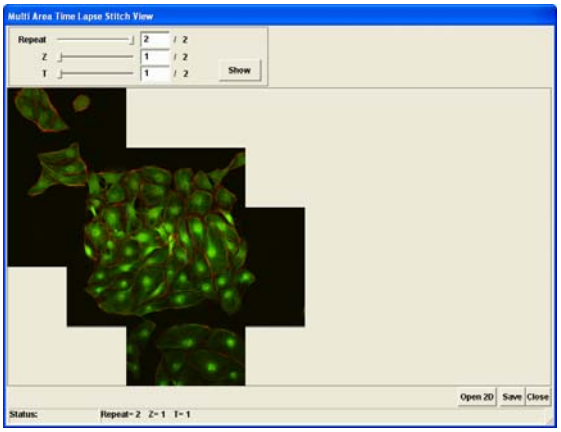

1. Specify image data to be stitched using <Open>button on MATL Viewer.

   On the [Open] dialogue box, specify the file "MATL_ Mosaic.log" file of experiment.

2. Select View Mode button to <XY View Mode> button. Images will be aligned in every XY coordinate axis.

3. Click <Select Action>button and select <Stitch>button.

   [Multi Area Time Lapse Stitch View] will appear.



Click Show <Show>button, the stitched image will display on [Stitch Image Display] region with the conditions set.

4. Click Open 2D <Open 2D>button, a stitch image will be displayed on new [2D View] window and closes this window. When the stitch image is not saved, [Save As] dialog box will appear. In accordance with the dialog box, save the image.

**TIP: Restrictions**

- On this window, it is possible to display an image that was acquired by specifying coordinates on [Mosaic Outline] window or [Define Matrix] dialog box.
- Image size that can be output is of 1 x 1 through 15000 x 15000 pixels.
- The uppermost limit of the number of coordinate points that can be stitched is of 30000 points.
- The uppermost limit of memory for images that can be stitched is approximately 800MB.
  Depending upon image size or number of data, it may exceed the uppermost limit of memory and, in such a case, the stitch cannot be performed.