Inverted Olympus Fluoview 1000S Automated Stage and Multi-Area Time Lapse (MATL)

For best results

- Restart the windows computer prior to starting the FLV1000S Software
- Scan the stage (described below)

Multi Area Time Lapse (MATL) allows the user to accomplish the following:

- Control the X, Y Stage
- Define multiple regions to image
- Image adjacent images
- Outline an area and image all the locations within the defined region
- Image the defined areas over time
- Image well plates
- Align images, side-by-side using the MATL viewer
- Stitch images into a single view

How to use MATL (Details Follow)

1. Start MATL from the Device Menu
2. Program well plate if the case well plate is used
3. Move the stage
4. Register coordinates.
   Repeat as needed for additional locations
5. Adjust image acquisition settings for each coordinate (as needed).
   Re-register point
6. Post view setting.(As needed basis)
7. Execute protocol
8. Stitch Setting.(As needed basis)
9. Close MATL.
Start MATL

- Warning dialog box as shown below will appear.

![XY Stage warning](image)

- Scan the stage (OK)
- MATL will start and [Multi Area Time Lapse Controller] window will appear

Well Plate Setting (if used)


![Multi Area Time Lapse Controller](image)

[Preference] dialog box will appear

Select [Overlay] tab.

Turn [Well plate] check box on [Overlay Options] group box to checked state and program well plate to appear on stage map.

Click on <OK> button and close [Preference] dialog box.

Note: The color of the well plate map can be changed
2) Select the well plate to be used


In [Well Name] drop down list, select name of well plate to be used. If a well plate is not used, select "None". When a unique setting is desired, select "User Custom".

3) Click on (Apply) button.

4) The well is displayed on stage map.
5) Fine adjust the mapping
[Register the center of well] dialog box will appear.
6) Select [Start registration] radio button
7) Click on <Next> button.
Dialog box as shown below will appear.

**Note:** Leave the [Auto Escape] check box checked. The objective lens is moved down during the movement of the stage.

When stage move is completed, the stage will return to original Z position.

8) Click on <Go to A1> button.
Stage will start moving to center of well A1 previously registered.

9) Wait until the dialog box appears.
10) Locate the center of the well using the confocal image mode or viewing mode.
11) Click on register

12) Exit

June 13, 2014
Ways to Move the Stage

(1) Double click the mouse button on a position in the stage map shown on the [Multi Area Time Lapse Controller] window and the stage will be driven to that location.

(2) Move the stage using move button on [Stage Controller] Window

- Select Fine or Course
- Depending upon speed, the <Move> button will change appearance.

- Click on <Move> arrow in the desired direction.
Note: When <Fine> or <Coarse> is selected, the stage keeps moving while <Move> button is pressed and held.

(3) Move the stage by specifying the coordinates directly

- Enter coordinates of the position where the stage should be moved in [X] and [Y] text box respectively on [Stage Controller] window.
- Click on (GO) button.

(4) Move to the center of well (If the well plate setting is used.)

- If the stage is currently positioned in well, it will move to center of well.
- If the stage is currently positioned outside well, it does not move.

June 13, 2014
**Coordinate Registration (Max 3000 points)**

**Note:**
The current imaging conditions will be registered with the point when it is registered. To change conditions, see 5, How to Change for Image Acquisition Conditions for Registered Points.

If images are going to be stitched, the image must be acquired as 1:1 (the field must be square). Select [1:1] in [Size] on [Acquisition Setting] window and then, register the coordinate.

Coordinates cannot be registered while the MATL is running a scan.

**Methods to select points**

1) Separate points
   a. Move the stage to the desired location
   b. Select
      i. Add current location form the Edit menu in the MATL window
      ii. Or, click on the MATL or [Registered Point List] window

2) Adjacent coordinates
   Or select
   b. The [Define Matrix] box will appear
c. Enter number of coordinate points in lateral direction with reference of current stage position as center in [Column] text box.

d. Enter number of coordinate points in longitudinal direction with reference of current stage position as center in [Row] text box.

e. Click on (Set) button.

f. Coordinates will be registered to stage map and [Coordinate] list on [Registered Point List] Window.

In case coordinate point is registered with "Column3xRow3"

![Coordinate registration example]

3) Register all the points in a contour (MOSAIC)


c. Move the stage to the edge of the observation area.

d. Click on (Regist) button on [Mosaic Outline] window (or use F7)

e. The XY coordinates of current stage position will be registered to [Contour Coordinate] list.

f. Repeat to outline the area to be imaged.

g. To delete coordinates, select the row applicable in [Contour Coordinate] list and click on (Delete) button.

h. When contour designation is done, click on (Apply) button.

i. The [Coordinate] list on [Registered Point List] window will be updated with the, XY coordinates and the current image acquisition information.

Note: Contours are created by connecting points located at the outside edge of the selected region. For this reason, coordinate points inside the contour will be ignored even if they are registered as contour coordinates. (See Following Examples)
If the imaging conditions for individual points need to be adjusted, update the settings.

Edit Menu in the [Registered Point List]

1. Select [Edit Acquisition Parameters].
2. [TC [Imaging Setting]] dialog box will appear.
3. Change acquisition parameters.
4. When complete, click on <OK> button on [TC [Imaging Setting]] dialog box to close the dialog box.

To change the XY coordinates, ZDC control, folder of file, file name or comment, use [Coordinate] list on [Registered Point List] window and edit data directly.

Note:
If a coordinate position is registered when a focus offset position has not yet been set, the [ZDC] check box will appear in yellow.

**To enable ZDC control for a position indicated in yellow, there are 2 methods available as follows.**

1) Re-registration by setting a focus offset position
   - In [Coordinate] list on [Registered Point List] window, set [ZDC] check box in [Coordinate] list of which ZDC control should be enabled to checked state.
   - Set (Set Track info to FV with position movement) button on [Registered Point List] window to "pressed" state.
   - Double-click [No.] of the row of which ZDC control should be enabled.
   - At the said registration point, click on <Set Offset> button on [ZDC Control] window.
   - Select [Only Selected point] in the dialog box displayed by clicking (Re-Register) button, and click <OK> button.
Since the acquisition conditions including a focus offset position are re-registered, the [ZDC] check box in [Coordinates] list will turn to white.

2) Running a protocol without specifying a focus offset position
   o In [Coordinate] list on [Registered Point List] window, set [ZDC] check box in [Coordinate] list of which ZDC control should be enabled to checked state.
   o When a protocol is executed, this software will set a focus offset position automatically at the first image acquisition time.

Re-registering image acquisition to update imaging conditions
1) On [Coordinate] list of [Registered Point List] window, select the row of condition for image acquisition that should be re-registered.
2) Select [Re-Register] from [Edit] menu on [Registered Point List] window.
3) Select [Only Selected point] in [Re-registration Options] dialog box displayed, and click <OK> button.
4) The row selected in [Coordinate] list will be re-registered with current stage position and current condition for image acquisition.

Re-registration Options

- Only Selected point
- All points in the same group
- All Registered Points

Or click the (Re-Register) button

To change the Z stage position for a point in MATL
   OR
   Click (Shift Z axis) button on tool bar.
To change Image Processing


OR

Click (Image Process Setting) button on tool bar.

To re-edit contour coordinates

1) If contour coordinates are separated by group, select them in [Group No.] drop down list on [Mosaic Outline] window.
2) Select the stage map on [Multi Area Time Lapse Controller] window will move to the coordinates selected.
3) To add a contour coordinate, double-click on a place to add on coordinates of the stage move and move the stage and then, click on (Regist) button on [Mosaic Outline] window.
4) To delete contour coordinates, select the row of the coordinates that should be deleted in [Contour Coordinate] list on [Mosaic Outline] window and then, click on (Delete) button.
5) To edit X or Y position of the contour coordinates, edit it directly in [Contour Coordinate] list on [Mosaic Outline] window.
6) When contour coordinates are added, deleted or edited, the coordinates for image acquisition will return to contour coordinates on Stage map.
7) When edits for contour coordinates are completed, click on (Apply) button.
8) The contour coordinates are converted to the coordinates for image acquisition.
Execute Image Acquisition (Protocol)

- The image acquisition (protocol) is started based on the [Coordinate] registered coordinate list.
- Enter number of repeat cycles for image acquisition (protocol) in (Repeat) text box on [Registered Point List] window.
- If the number of repeat cycles is set at more than 2 times, the interval time between start of a series of image acquisition ([Coordinate] list No.1) and start of next series of image acquisition ([Coordinate] list No.1) should be set with (Interval).
- Click on (Ready) button to enter preparation for image acquisition (protocol).
- Click on (Start) button.
- Image acquisition (protocol) will be started.
- Image will be saved immediately after acquisition.

Note:

While a protocol is executing, the following edits can be performed.

[Shift Z axis] dialog box that appears when (Shift Z axis) button is clicked

If either one of the following events is edited while a protocol is running, the post view function on [Multi Area Time Lapse Viewer] window cannot be used.

- In case a file name that should be saved with [File Name] on [Registered Point List] window is changed
- In case [Enable] check box on [Registered Point List] window is unchecked and an image acquisition at that coordinates is cancelled (Even if [Enable] check box is ticked off at next repetitive acquisition time, the post view function cannot be used.)

If a protocol is executed by setting (Track) button on [Multi Area Time Lapse Viewer] window to "pressed" state, a thumbnail of the image acquired will immediately be displayed on the same window (i.e., post view of the image acquired can be displayed).

Even if a protocol is executing, the display method of a thumbnail image can partly be changed.
Viewing Files Collected with MATL

It is possible to verify acquired images while the MATL is running by using the aligning (post view function). Also, it is possible to align and view images already acquired.

To display images acquired while a protocol is executing

- [Multi Area Time Lapse Viewer] window will appear.
- Set (Track) button on [Multi Area Time Lapse Viewer] window to "pressed" state.
- The thumbnails of images acquired will immediately be displayed on [Post view display] region.

To Display an image acquired upon completion of protocol

- [Multi Area Time Lapse Viewer] window will appear.
- Click on (Open) button on [Multi Area Time Lapse Viewer] window.
- [Open] dialog box will appear.
- Select [MATL_Mosaic.log] file in a folder where the image acquired is saved and then, click on <Open> button.
- The thumbnails of images acquired will be displayed on [Post view display] region on [Multi Area Time Lapse Viewer] window.
- [MATL_Mosaic.log] file is automatically created in a folder of the image file that was acquired with use of MATL.
Stitch Setting

Multiple images acquired by using MATL are stitched in accordance with the XY coordinates to create a single frame image.

Notes:
- Image can be output from 1 x 1 up to 10000 x 10000 pixels.
- Up to 3000 coordinate points can be stitched.
- If the image size or number of data points exceeds the limit stitching cannot be performed.
- If the (Projection) button is selected from the (Select Image Type) button on the [Multi Area Time Lapse Viewer] window, stitching for projection images can be performed.

Select the (Stitch) button from the (Select Action) button on the [Multi Area Time Lapse Viewer] window.

The [Stitch View] window appears.

TIP:
In the [Condition Setting] region, set to display stitch images.

Click the (Show) button.
The entire stitched image will appear on the [Stitch Image Display] region.

To save the stitch image, click the (Save As) button.
The settings to save can be set in the displayed [Save As] dialog box.
Save the image according to the instructions in the [Save As] dialog box.

To display a stitch image on the [2D View] window, click the (Open) button.
Finishing the MATL


All the windows related to MATL will be closed.

TIP:

If the protocol is not saved, a message to confirm save will appear.

To save the protocol, click on <Yes> button and save the protocol in accordance with the dialog box appeared.

Otherwise click on the <No> button.
**Limits and Restrictions**

There are a number of restrictions on [Multi Area Time Lapse Viewer] window and [Stitch View] window that are used for Post view and or Stitch.

Restrictions by Observation Conditions

Projection image or Append image can be created with the following images.

- Projection image: XYZ, XYZT
- Append image: XY, XYZ, XYT, XYZT

Executable/non-executable (OK/No) of Post view or Stitch function, depending on observation condition is described.

<table>
<thead>
<tr>
<th>Observation condition</th>
<th>Display on [Multi Area Time Lapse Viewer] window (Post view)</th>
<th>Display on [Stitch View] window (Stitch)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Condition set to Lambda series, Line Scan or Point Scan</td>
<td>OK(*)</td>
<td>No</td>
</tr>
<tr>
<td>Ratio of index size is set to a value other than 100% on [Index Size] tab on [Preference] dialog box.</td>
<td>OK(*)</td>
<td>OK</td>
</tr>
<tr>
<td>Image size is set to a value other than [1:1] on [Size] group box on [Acquisition Setting] window.</td>
<td>OK(*)</td>
<td>OK</td>
</tr>
</tbody>
</table>

* On [Post view display] region on [Multi Area Time Lapse Viewer] window, a thumbnail image acquired will appear. The thumbnail image will be edited to 86 x 86 pixels.
Restrictions by mixing of observation condition

Using MATL for image acquisition, it is possible to create image files with observation mixed conditions.

However, there are restrictions and; executable or non-executable for creation of Post view, Stitch or Append image is described.

<table>
<thead>
<tr>
<th>Pattern of File Type</th>
<th>Differentia</th>
<th>Example</th>
<th>Display on [Multi Area Time Lapse Viewer] window (Post view)</th>
<th>Display on [Stitch View] window (Stitch)</th>
<th>Creation and display of Append image on [Multi Area Time Lapse Viewer] window</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observation condition differs, depending on the repeat number on the same coordinate.</td>
<td>Observation mode</td>
<td>XY mode at the 1st repeat time and XYT mode at the 2nd repeat time.</td>
<td>OK</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Number of series (T, Z)</td>
<td>2 slices of T series at the 1st repeat time and 4 slices of T series at the 2nd repeat time.</td>
<td>OK</td>
<td>No</td>
<td>OK (T series)</td>
<td>No (Z series)</td>
</tr>
<tr>
<td>Acquisition size</td>
<td>512 x 512 pixels at the 1st repeat time and 4096 x 4096 pixels at the 2nd repeat time.</td>
<td>OK</td>
<td>No</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Number of channels</td>
<td>2 channels at the 1st repeat time and 3 channels at the 2nd repeat</td>
<td>OK</td>
<td>No</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>File name</td>
<td>&quot;Image&quot; at the 1st repeat time and &quot;Observation&quot; at the 2nd repeat time.</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>-----------</td>
<td>---------------------------------------------------------------------</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td></td>
</tr>
<tr>
<td>X or Y coordinate</td>
<td>[X:0, Y:0] at the 1st repeat time and [X:10, Y:10] at the 2nd repeat time.</td>
<td>OK</td>
<td>OK</td>
<td>OK</td>
<td></td>
</tr>
<tr>
<td>Observation condition differs in an image file that is to be displayed as one XY image.</td>
<td>Mode of images for coordinate order (1, 1): XY Mode of images for coordinate order (1, 2): XYZ</td>
<td>OK</td>
<td>No</td>
<td>OK</td>
<td></td>
</tr>
<tr>
<td>In case &lt;Select View Mode&gt; button on [Multi Area Time Lapse Viewer] window is set to (XY View Mode) button or image stitch is done on [Stitch View] window</td>
<td>Coordinate order for images (1, 1) to be aligned: T series 2 slices Coordinate order for images (1, 2) to be aligned: T series 4 slices</td>
<td>OK</td>
<td>No</td>
<td>OK</td>
<td></td>
</tr>
<tr>
<td>Number of series (T, Z)</td>
<td>Coordinate order for images (1, 1) to be aligned: T series 2 slices Coordinate order for images (1, 2) to be aligned: T series 4 slices</td>
<td>OK</td>
<td>No</td>
<td>OK</td>
<td></td>
</tr>
<tr>
<td>Acquisition size</td>
<td>Size of images for coordinate order (1, 1): 512 x 512 pixels</td>
<td>OK</td>
<td>No</td>
<td>OK</td>
<td></td>
</tr>
<tr>
<td>Number of channels</td>
<td>Number of channels for coordinate order of images (1,1): 2 channels</td>
<td>OK</td>
<td>No</td>
<td>OK</td>
<td></td>
</tr>
<tr>
<td>--------------------</td>
<td>------------------------------------------------------------------</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Number of channels for coordinate order of images (1,2): 3 channels</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total number for repeat per coordinate is different.</td>
<td>Coordinate No.1 is set with total number of 10 and coordinate No.2 is set with total number of 9.</td>
<td>OK</td>
<td>(Blank data appears at missing place)</td>
<td>OK</td>
<td>(Blank data appears at missing place)</td>
</tr>
</tbody>
</table>