

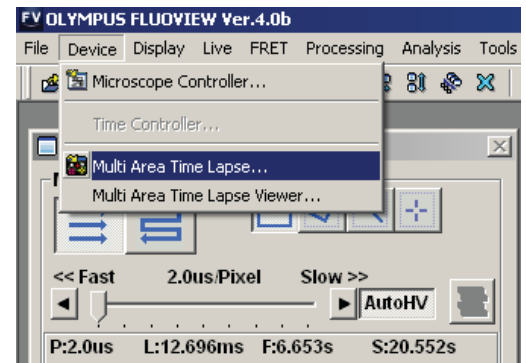
Stitching (I) - Image Acquisition

Set up 'Multi-Area Timelapse' mode

BEFORE you place your slide/dish on the microscope, start the FV1000 software, and select "Multi Area Time Lapse" (MATL) from the "Device" tab on the top of the screen.

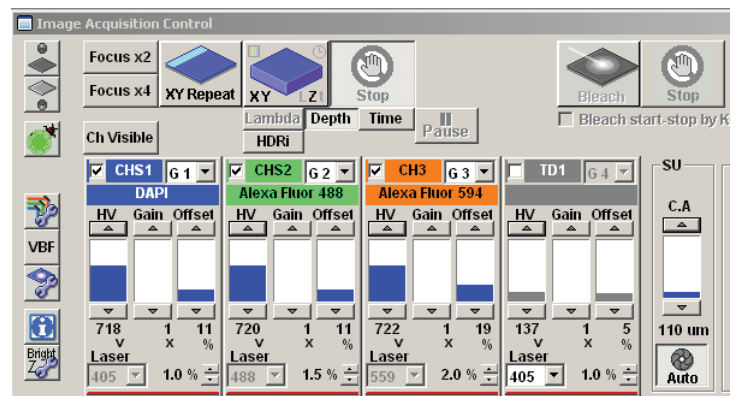
The "XY stage warning" window will pop up with the statement: "This stage will move in a wide range to detect its mechanical origin". Click "OK". This will cause the stage to move around. When it is finished moving, you can place your slide/dish on the stage.

You should see 3 new windows: Stage Control, MATL Control, and Registered Point List (more detail on those later).



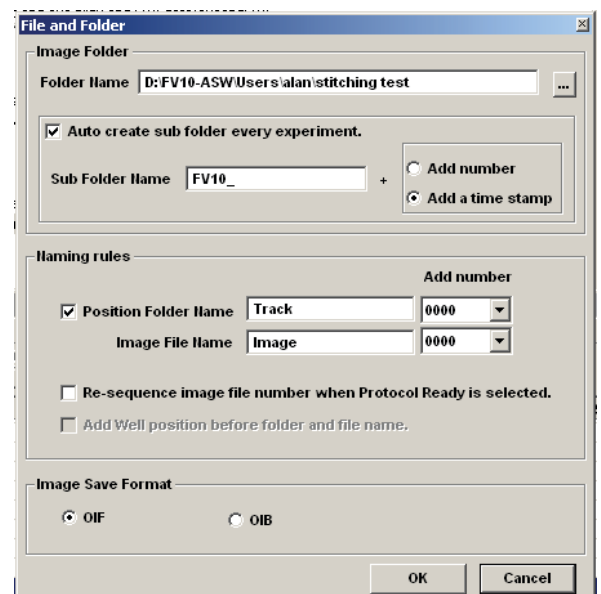
Set up your scan parameters

Load your dyes (virtual phases cannot be done in combination with MATL), find a region of interest on your slide, set exposure levels, etc. If you wish to merge Z-stacks, set your start and end points, then click the "Depth" button in the "Image Acquisition Control" window. It is essential to click "Depth" BEFORE setting up your matrix, otherwise the program will function only in 2 dimensions.



Set Destination for saved files

Click the 4th button from the right (disc & wrench) on the Registered Point List window, which will allow you to set the folder destination (Folder Name window). You also have the option to name your image files (this will show up later on the Registered Point List spreadsheet).



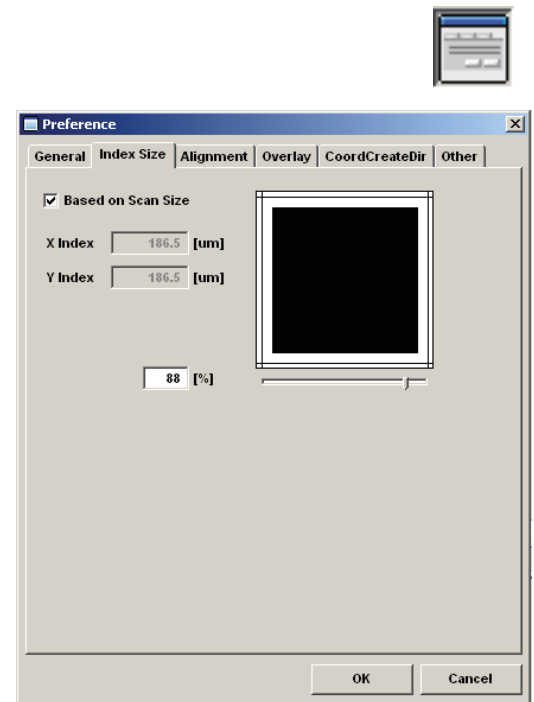
Stitching (II) - Image Acquisition

Set overlap

The 3rd button from the right on the MATL Control window opens the "Preferences" window. Click the "Index Size" tab. The overlap setting is in the window next to the % sign. 100% = 0 overlap. Some overlap is required for optimal image stitching to produce the most seamless combined image. The optimal amount of overlap is determined by the total magnification:

$$((\text{Obj. mag.} \times \text{X zoom\#}) / 8) + 3 = \text{ideal overlap\%}$$

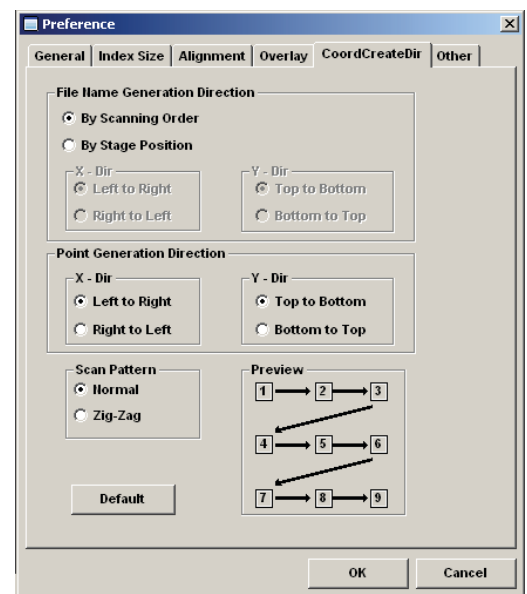
For example, if you are using a 60X objective with a zoom of 1.5, the ideal overlap is around 14%, and you would set the overlap # to 86%.



Set scan order and pattern

Click the "CoordCreateDir" tab:

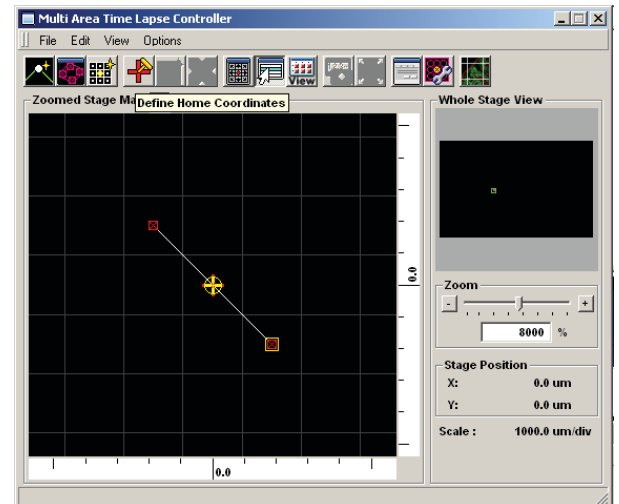
The "Normal" scan pattern is recommended.



Stitching (III) - Image Acquisition

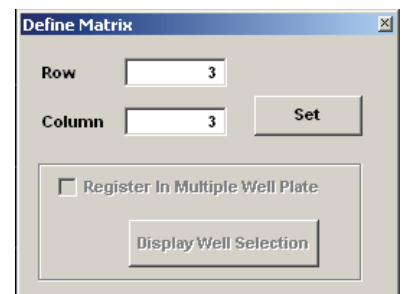
Define your matrix

The MATL Controller window displays a grid. The yellow crosshair shows the position of the sample in the view field on the grid ("you are here"). The red cross marks the center of the grid. The x y coordinates of the yellow crosshair (relative to the center) are shown to the right of the grid (and in the windows of the stage controller). You can zoom in or out with the slider bar to the right.

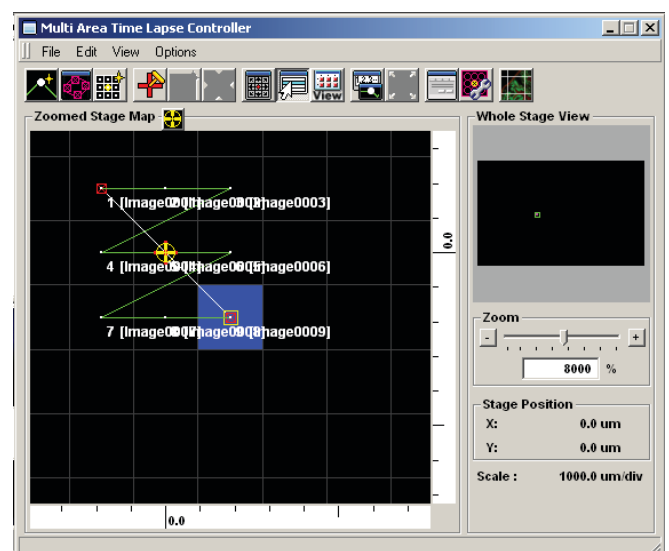


The "Define Matrix" button (3rd from the left in the MATL Controller window) will allow you to specify a set number of squares across and down.

Click "Set" and the yellow crosshairs will be centered in the defined matrix. The coordinates for each square will be loaded into the Registered Point List.



Your matrix should look something like this...



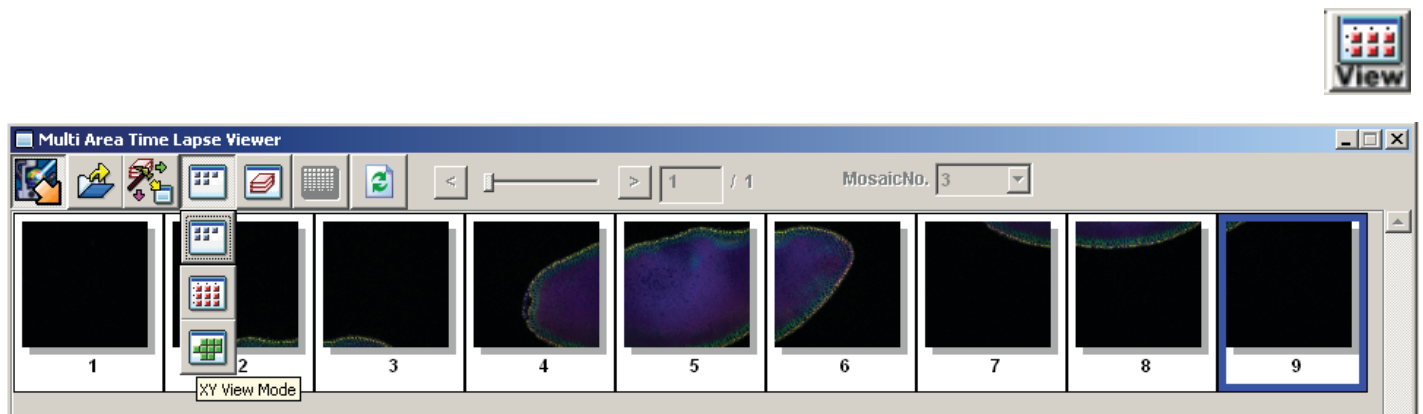
Stitching (IV) - Image Acquisition

Take images

You will use the start controls in the "Registered Point List" window instead of the "Image Acquisition Control" window. First click the "Ready" button (bottom left). When the machine is ready to start, the next button, "Play", will change from a grey arrow to a black arrow. Hit "Play". You should see windows appear for all your channels in the "Live View" window. If you are Z-stacking you should see a z-stack # indicator below the "Ready" and "Play" buttons in the bottom left corner of the Registered Point List window. "Total Time" (bottom right) gives you an estimate of how long the scan will take. The system will scan the entire Z-stack for square 1 (if applicable), then move to the next square.

Registered Point List														
File Edit Control Options														
No.	Enable	X	Y	Z	Z Start	Z End	Scan	Laser(405)	Laser(488)	Laser(559)	(CHS1)-HV	(CHS2)-HV	(CH3)-HV	(TD)-HV
1	<input checked="" type="checkbox"/>	-186.3	186.3	1048.83	1048.83	1046.83	XYZ	1.0	1.5	2.0	718	720	722	137
2	<input checked="" type="checkbox"/>	0.0	186.3	1048.83	1048.83	1046.83	XYZ	1.0	1.5	2.0	718	720	722	137
3	<input checked="" type="checkbox"/>	186.3	186.3	1048.83	1048.83	1046.83	XYZ	1.0	1.5	2.0	718	720	722	137
4	<input checked="" type="checkbox"/>	-186.3	0.0	1048.83	1048.83	1046.83	XYZ	1.0	1.5	2.0	718	720	722	137
5	<input checked="" type="checkbox"/>	0.0	0.0	1048.83	1048.83	1046.83	XYZ	1.0	1.5	2.0	718	720	722	137
6	<input checked="" type="checkbox"/>	186.3	0.0	1048.83	1048.83	1046.83	XYZ	1.0	1.5	2.0	718	720	722	137
7	<input checked="" type="checkbox"/>	-186.3	-186.3	1048.83	1048.83	1046.83	XYZ	1.0	1.5	2.0	718	720	722	137
8	<input checked="" type="checkbox"/>	0.0	-186.3	1048.83	1048.83	1046.83	XYZ	1.0	1.5	2.0	718	720	722	137
9	<input checked="" type="checkbox"/>	186.3	-186.3	1048.83	1048.83	1046.83	XYZ	1.0	1.5	2.0	718	720	722	137

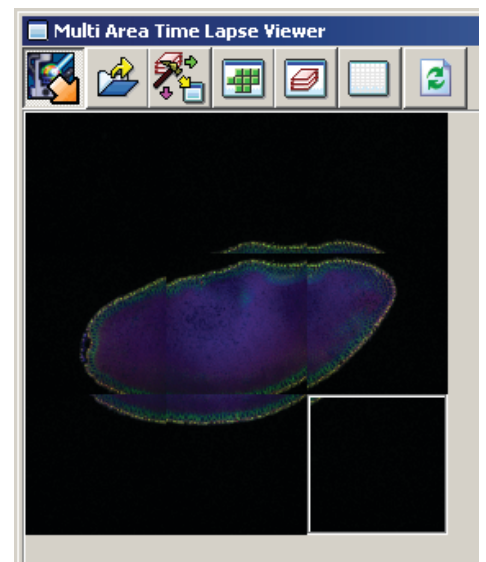
Interval: 00:03:49.5 Repeat: 1 Total Time: 00d 00:00:00.0 Frame Interval Time: 00d 00:00:00.0



Finding the right stitch

When your scan is finished, go to the "Device" tab and select "MATL Viewer". This opens a new window with a picture of imaged squares. Click the 4th button ("Select view mode") and select the bottom button (green tiles) on the drop down menu.

This should arrange all the images in their proper order.

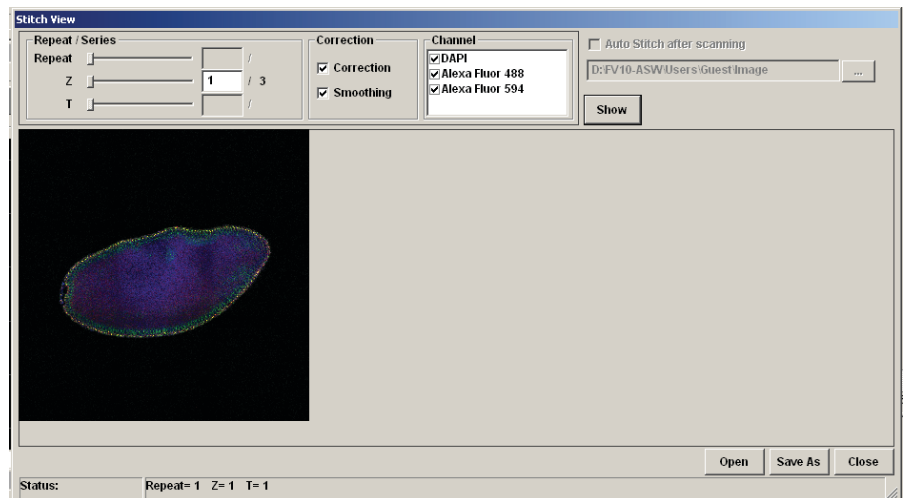
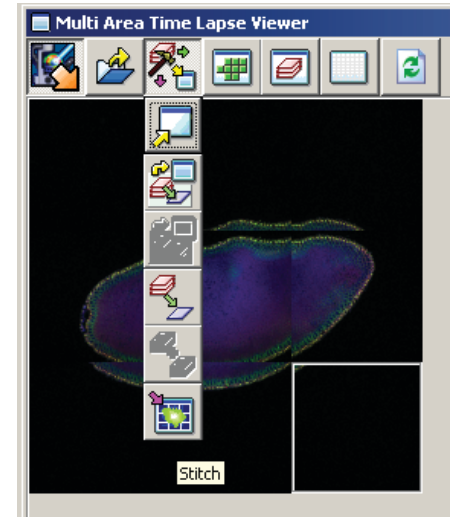


Stitching (V) - Image Stitching

Stitching:

To generate a stitched image go to the 3rd button on the MATL Viewer ("select action") and pick the 6th button ("stitch") in the drop down menu.

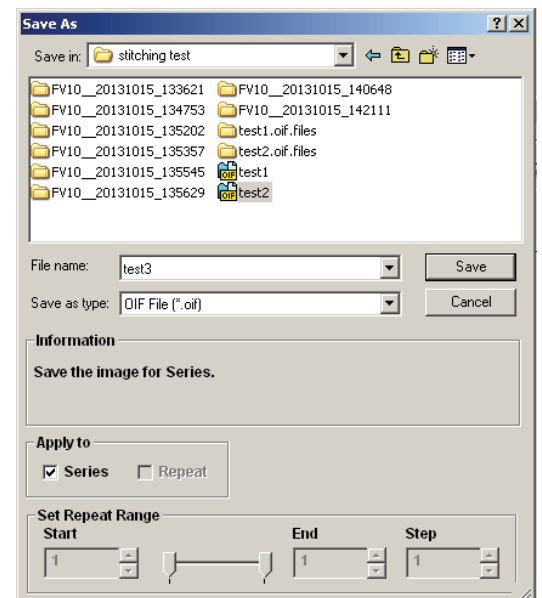
This will open the "Stitch View" window. Click the "Show" button to generate an initial stitched image in the window. Finding the ideal stitch requires a bit of trial and error. If you are stitching Z-stacks, using a plane in the center will usually give you the best results. Use the Z slider to the left to pick a Z plane, and then hit the "Show" button to re-stitch. The channels used are indicated above the stitched image and will be initially all checked. If the image is not optimal you can uncheck some of the channels and re-stitch. The channel with the most contrast works best for guiding stitching. Try different Z-planes and channel combinations to find the best possible image.



Saving the data:

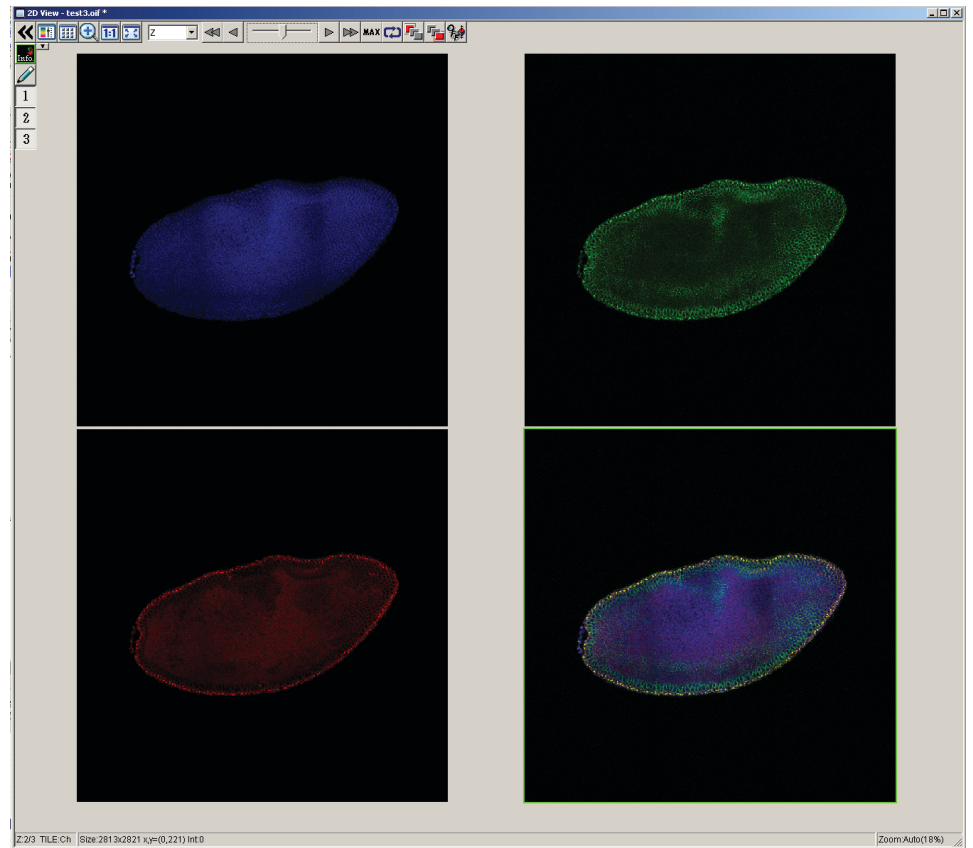
Once you generate a stitch you wish to keep, click the "Save As" button on the bottom right of the Stitch View window.

If you are saving a Z-stack stitch, you must check "Apply to series" to add all of your stacks to the image. Files 1 GB or less can be saved in the "oib" format. Files larger than 1 GB should be saved as an "oif". If you have Z-stacks and/or 20+ tiles you will likely choose "oif".



Stitching (VI) - Image Display

The .Oif file can then be opened in the Olympus FV1000 software, where the z-stack can be scrowled through



The .oif file can also be opened in Imaris software for further advanced image analysis and generating displays for presentations etc.

