UConn Advanced Light Microscopy Facility Technical Note: Grid Capture and Stitching on the Andor System

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Note: Before beginning, make sure Micro-Manger has proper spatial calibration settings for the objective and camera you are using. If these are not set correctly, stitching will fail.

- In the main Micro-Manager window, select Tools → Options and check "Create metadata.txt file with Image Stack Files. This will create the metadata file that contains the stage coordinates for stitching.
- Unchecking "Save XY positions in separate Image Stack Files" will result in creation of a single metadata file.



• Open the Multi-D Acq. Window and check "Multiple positions (XY)" and "Edit position list..."



• Click on "Create Grid" in the Stage Position List window. This will open the Tile Creator.



• While running live, you can mark boundaries of the grid collection by moving to each and clicking "Set." Or, you can move to the center of the area and define a set grid around your position.



• Here, we center on the area of interest and expand the grid by clicking the "+" button to expand the tile area until it is 4x4 images. Then click "Center Here" to define the grid around the current position.



- Typically, some overlap is set to aid in the stitching and blending of adjacent images. The amount will depend on your magnification and pixel size. Here, the pixels are large with a 10X lens so 50 um is set. For higher mag (like 60 or 100x), only a few microns should be specified. Or, you can set overlap to be something like 20%.
- Click "OK" to send the coordinates to the Stage Position List window.

abel	Position [um]
urrent	PFS Offset(92.925);XYStage(3
Pos_000_000	XYStage(31,498.84,12,101.9)
Pos_001_000	XYStage(32,339.72,12,101.9)
Pos_002_000	XYStage(33,180.6,12,101.9);
Pos_003_000	XYStage(34,021.48,12,101.9)
Pos_004_000	XYStage(34,862.36,12,101.9)
Pos_004_001	XYStage(34,862.36,12,717.5)
os_003_001	XYStage(34,021.48,12,717.5)
Pos_002_001	XYStage(33,180.6,12,717.5); Set Origin
os 001 001	XYStage(32,339.72,12,717.5)
os 000 001	XYStage(31,498.84,12,717.5) Add Offset
tos 000 002	XYStage(31 498 84 13 333 1) G Clear All
ns 001 002	XYStape(32 339 72 13 333 1)
os 002 002	XYStage(33,180,6,13,333,1)
os 003 002	XYStage(34.021.48.13.333.1)
ps 004 002	XYStene/34 882 38 13 333 1)
os_004_002	VVGteen/24 082 38 12 949 7)
os_002_003	VVGteen(24.001.49.12.049.7)
- 002 003	XYOL==(22,100,6,12,040,7)
	X104gg(05,100.0,13,040.7),
- 000 000	×1048ge(32,355.12,13,546.7)
6s_000_003	XYStage(31,498,84,13,948.7)
6s_000_004	XYStage(31,498,84,14,004.3)
'os_001_004	XYStage(32,339.72,14,564.3)
°os_002_004	XYStage(33,180.6,14,564.3);
Pos_003_004	XYStage(34,021.48,14,564.3)
	Load
e	Stage name Save As
e V	Stage name Save As TIZDrive PFS Offset Create Grid



- Start the acquisition of the grid collection by clicking "Acquire" in the Multi-D Acquisition Window.
- The resulting Image Stack File should be saved as a single file by selecting the save option as "Image File Stack." This will give one file with all the images and a single corresponding metadata file. You can also specify individual files.
- To stitch the images, use Fiji and go to Plugins → Stitching → Grid Collection/Stitching.
- Set Type to "Positions from file" and Order as "Defined by image metadata" and click "OK".
- ♥ Grid/Collection stitching

 Type
 Positions from file

 Order
 Defined by image metadata

 ♥
 Define the image coordinates

 img 1.tif; ; (0.0, 0.0, 0.0)
 img2.tif; ; (462.2, 0.2, 10.0)

 img3.tif; ; (1.6, 454.6, 60.1)
 img4.tif; ; (463.1, 456.0, -21.0)

 img5.tif; ; (1.3, 912.9, 0.0)
 Please note that the Stitching is

 based on a publication. If you use it for you research please be so kind to cite us:
 Preibisch et al., Bioinformatics (2009)

 DK
 Cancel
- Set the path to your Image Stack File and the options as shown below. If you saved individual files for each image, browse to one of them and the plugin will detect all the files and stitch them.
- Click "OK" to start the stitching. Depending on which camera you use, you may need to check "invert x x coordinates" or "invert y coordinates" to account for orientation of the sensor.

Grid stitching: Positions from file,	Defined by image metadata	
Multi series file	F:\Grid\Kidney\\Kidney_MMStack.ome.tif	Browse]
Fusion method	Linear Blending	•
Regression threshold	0.30	
Max/avo displacement threshold	2.50	
Absolute displacement threshold	3.50	
T Add tiles as ROIs		
Compute overlap (otherwise	trust coordinates in the file)	
Ignore Calibration		
Increase overlap [%]	• 0	
Invert X coordinates		
T Invert Y coordinates		
Ignore Z stage position		
Subpixel accuracy		
Downsample tiles		
Display fusion		
🗌 Use virtual input images (Sl	w! Even slower when combined with subpixel accura	icy during fusion!)
Computation parameters	Save memory (but be slower)	
Image output	Fuse and display 💌	
This Plugin is developed by St	ephan Preibisch	
http://fly.mpi-cbg.de/preibisch		
		OK Cancel



• Here is the result of 4x4 grid collection with the 10X lens covering 3.5 X 2.5 mm!



