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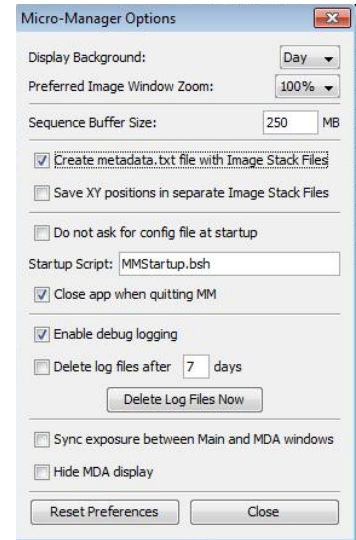
- Open the Multi-D Acq. Window and check "Multiple positions (XY)" and "Edit position list…"



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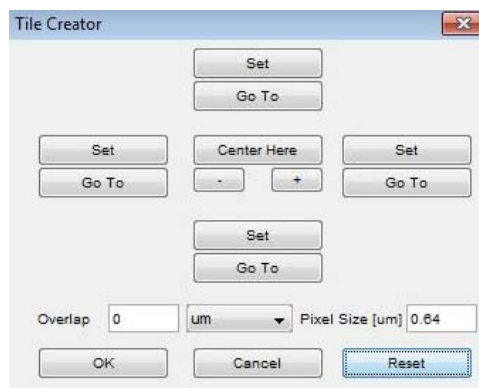


- While running live, you can mark boundaries of the grid collection by moving to each and clicking "Set." Or, you can move to the center of the area and define a set grid around your position.

- 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.
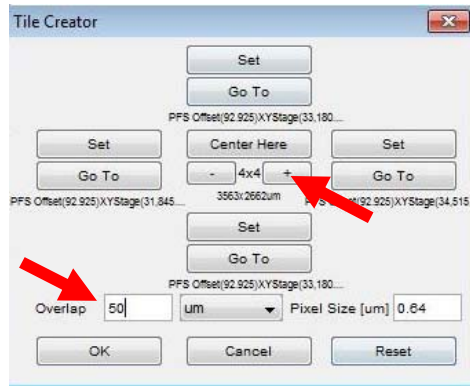


- Typically, some overlap is set to aid in the stitching and blending of adjacent images. The amount will depend on your magnification and pixel size. Here, the pixels are large with a 10X lens so 50 um is set. For higher mag (like 60 or 100x), only a few microns should be specified. Or, you can set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

- Click "OK" to send the coordinates to the Stage Position List window.

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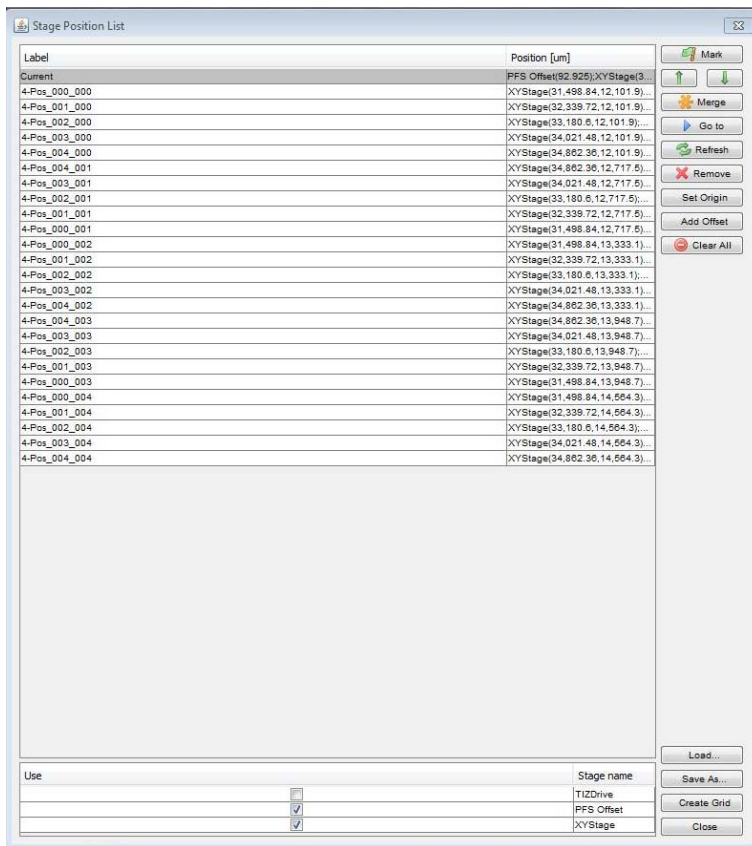
- Start the acquisition of the grid collection by clicking "Acquire" in the Multi-D Acquisition Window.

- 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".
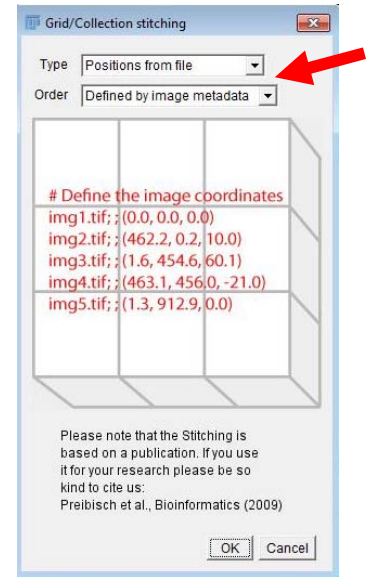
- Set the path to your Image Stack File and the options as shown below. If you saved individual files for each image, browse to one of them and the plugin will detect all the files and stitch them.

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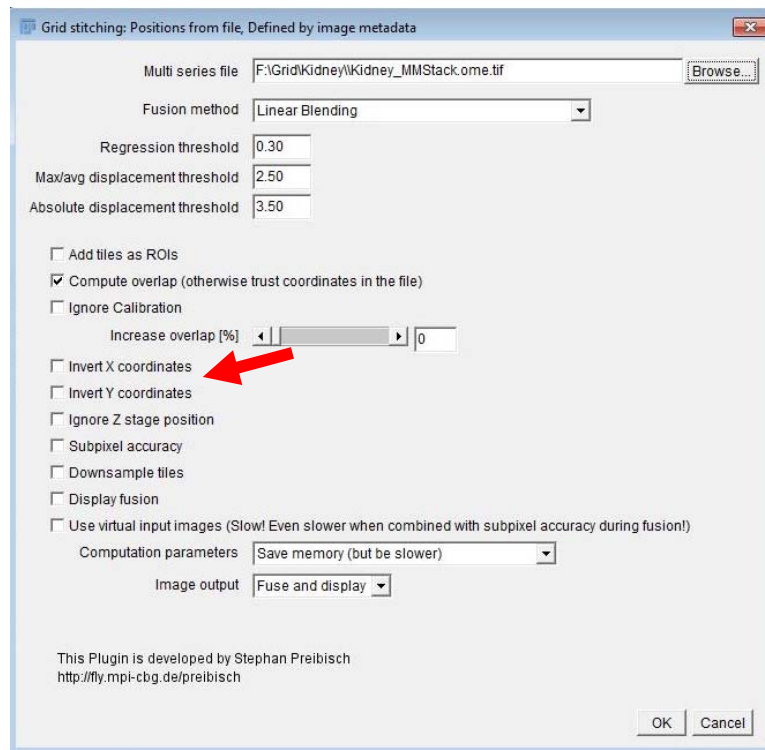
- Here is the result of 4x4 grid collection with the 10X lens covering 3.5 X 2.5 mm!