**12. Stitching Data Images in Preparation of Image Analysis**

Image stitching is the process of taking individual image tiles and combining the tiles in XY coordinate space to produce a single image. This process is necessary for nearly all analyses of MITOMI experiments. Stitching is typically more reliable if the images have been flat-field corrected since the vignette in uncorrected images often obscures features and confuses the stitching algorithm.

If features in the images are positioned identically (see Page 15), a single stitched image is sufficient for generating the stitching coordinates for all subsequent images in the dataset. If features are not positioned identically, it may be necessary to stitch images individually, if possible.

**Stitching an Image**

For stitching MITOMI images, it is often best to start by stitching the protein image first due to the ubiquitous presence of protein in the chambers. These high signal features are easily distinguished and make the stitching process reliable. Once the coordinate file has been generated, subsequent images can be stitched identically. Stitching the bound DNA image alone can be difficult due to the fact that not all oligos bind to the surface, which leads to fewer distinguishable features to reliably stich with.

1. Using FIJI, Plug-ins > Stitching > Grid/Collection Stitching
2. Type > Grid: column-by-column
3. Order > {Setup1: Down & Left , Setup2: Up & Left}

* Grid size x: maximum xxx+1 in > source\_imagefile\_xxx\_yyy.ome.tif
* Grid size y: maximum yyy+1 in > source\_imagefile\_xxx\_yyy.ome.tif
* Tile overlap [%]: 20
* First file index i: 1
* File names for tiles: (if FFCorrection was used) {i}.tif
* Output textfile name: TileConfiguration.txt
* Fusion method: Linear Blending
* Regression threshold: 0.30
* Max/avg displacement threshold: 2.50
* Absolute displacement threshold: 3.50
* Compute overlap (Check)
* Invert X coordinates: (Setup1 Only)
* Invert Y coordinates: (Setup2 Only)
* Computation parameters: Save computation time
* Image output: Fuse and display

1. Save image as TIFF if correct

**Identically Stitched Images**

If all images from a given experiment are expected to have features in identical locations, the coordinates of the first stitched image can be used to stitch the remainder with the following steps.

1. Stitch one image with FIJI using desired parameters (normal Grid/Collection stitching)
2. Copy the TileConfiguration.registered.txt file from the stitched folder to the directories that are to be stitched with the same coordinates
3. Prepare the settings for the next image

a. Type - Positions from file

b. Order - Defined by TileConfiguration

c. Layout file - TileConfiguration.registered.txt

d. Uncheck all boxes

1. Save image as TIFF

**Automated Stitching with MIJI**

Using Matlab, it's possible to automate the FIJI stitching with call to MIJI. Refer to the official documentation for the MIJI module in FIJI. Since each optical setup requires different settings for stitching, settings should be varied as needed. A sample of the automated script can be found in the FFCorrection.m script in the MIJI section. Revise as needed in a standalone script if desired.