Refer to the TIAM\_forOperatta\_userManual.docx for requirements and feel for the orientation and organization of the tool. The some basic details of the computations methods used in tiam\_HT are in tiam\_HT\_compMethods.docx.

It is important to make sure that the tiff files are in consistent order and also are named as the per convention. Example: A - 1(fld 2 wv UV - DAPI). The naming format is used by tiam\_HT to extract well name and field.

The GUI version won’t be very useful as the images are 1024-by-1024 and the GUI only handles 512-by-512. Outlining results are the only way to know. I have now worked out what works for InCell bright field. Detection and outlining parameters are in detection and outlining of DIC are in 040716\_incellHT\_outlineParams.docx.

Use the batch submission file for running tiam\_HT. Two examples in the src folder: (batchHTsubmit\_example/2). Parameters whose values need to set are essentially self-explanatory.

It is best to test out DIC outlining and fluorescence segmentation on a small subset (say from a well) of files. For this one needs to make sure that the lines corresponding to imwrite function (writes tif files) are not commented out in functions tiamHTbatchScript.m and getOutline.m. For flurescence images the outlines need to be overlaid (divide the outline by 255 and then multiply the image and the outline images for overlay).

The segmentation parameters for fluorescence channels may have to modified.

The output is all in the ws/ directory. All the result files and folders have the ‘expName’ in their name. The \*\_outline.tif has the bright field outline in the original image scale. The folder with expName has cropped images of all cells deemed to have good bright field outlines. Both flur and outline (named flurBnd) of the segmented part are included.

The calculated results are in \*.mat files. The main one has \_results.mat and mean values from each of the wells in \_report.mat. There is also \_perWell folder wherein calculated results are stored for each well (file named after the well). These also contain the image and mask arrays stored.

Use the createHistos.m function (in the ws/ folder) to get histogram distributions of many calculated parameters on a per well basis.

All the parameters in the cellData structure are mentioned in the file cellDataFieldDescription.txt