**Variable Threshold User Instructions**

**Installation**

Requires FIJI

Copy the Variable\_Threshold.jar file into FIJI plugins folder.

**Running the Plugin**

Start up FIJI

Import Image Sequence

Save this stack as a TIF. Filename must not have space, parentheses, etc

Go to Plugins, slide down and over to Variable Threshold

First step is to create background using a median,

There is a dialog box that allows you to set the range of slices used to calculate a median.

Also there is an item ScaleFactorLightPix that specifies the use of pixels brighter than the background for inclusion into the analyzed images. A value of 1.0 gives equal weight to light and dark pixels. A value of 0 will only include pixels darker than the background in the analyzed images.

After calculating the background, the next dialog box “Variable Threshold Process” sets the image process parameters.

**Beginning Threshold:** Set to the lowest threshold value sufficient to capture all particles. May need to experiment with this parameter initially

**MaxGray2ThreshFactors:** Converts the maximum intensity of particle (absolute value) into a tentative threshold value for that particle.

**Number of Thresholds:** Number of bins for thresholding particle

**Max Value for Reset Threshold**: , Upper limit for threshold;

**Minimum Circularity:** , to be considered for inclusion in results

**Maximum Circularity:** , to be considered for inclusion in results;

**Minsize pixels:** , to be considered for inclusion in results

**Maxsize pixels:** , to be considered for inclusion in results

**InitialDilateErodeSteps:** , prior to variable thresh algorithm

**FinalDilateErodeSteps** if Do Hull not selected, will perform after variable thresh algorithm

**Label interval for collage particles**:,

**Image Frame Width**: ,

**Image Frame Height**: ,

**Save Results** , checkbox;

**Do Hull Analysis** , checkbox performs convex hull analysis after variable threshold , fills in arcs, completes joined fragments,

**Enhance Contrast collage particles** checkbox

**Label collage particles** , checkbox

The final dialog box, “Set Measurements” , only appears if Save Results checkbox was checked. The available parameters are described under 30.7 “Set Measurements” at the following link

<https://imagej.nih.gov/ij/docs/guide/146-30.html>

A log file listing the settings used for the measurement will be saved as a text file. The name consists of a prefix (= name of the TIF image stack being analyzed) +”log”+ a sequential number

The results file is saved as a xls file

The name consists of a prefix (= name of the TIF image stack being analyzed) + a sequential number

These files are stored in the same folder as the saved TIF image stack.

The collage may be optionally saved by using the “File” “Save as” menu. Save as TIF.

If the collage, so saved, is re-opened in FIJI, the overlay with particle boundaries and numbers will be shown. If opened in other applications, the overlay may not be displayed. To join the overlay with the collage, use “Image” “Overlay” “Flatten” from the menu. Then save as TIF. Now the collage and overlay will be shown in other applications (though in FIJI, you will no longer be able to hide the overlay).