**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

The first dialog box is a choice whether to do a Variable Threshold analysis on the stack (default), or to perform filter measurements on a previously processed stack. If the latter option is selected, a new dialog box appears where some options are presented (Save Results, Label collage particles,Label interval for collage particles). Following this panel, the user selects the .zip file containing the set of analyzed regions of interest (ROIs) from a previous analysis). Then the program skips to the filter section (below.)

**Variable Threshold analysis.**

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**:,

**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.

A file containing the ROIs (regions of interest) is stored as a .zip file.

A dialog box is presented providing the option to continue with filtering the results or ending the VT plugin.

If the VT plugin is ended, 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).

**Filtering Results**

Based on the column headings that are in the results table, a set of options for filtering data will be presented in a dialog box. Check the parameters on which filtering will be performed.

A new dialog box appears in which the ranges of each of the parameters to be filtered on may be entered.

The result is a new image stack with a collage of the filtered particles. Behind this image will be a new table with the filtered results. If SaveResults was checked earlier, these files will be saved in the same directory as the original image stack.

A dialog box is presented providing the option to continue with performing another filter study or exiting the plugin. The filtering for the next cycle is based on the original set of ROIs (all particles) not the previous filtered results.