**ADJUST THRESHOLDS – 11/01/2021**

**Inputs:** Fused microscopy image and fused thresholded images, one for each channel. You can make these with the Fiji macro 2\_stitch.ijm.

**Outputs**:

* One RGB image called <well>\_fused\_RGB.tif. This is the fused image converted to RGB. It is used by Matlab to draw the network on.
* One thresholded image for each thresholded channe;. The name of each thresholded image is <well>\_th\_<ch\_name>.tif.

**Code**: the Fiji macro 3\_adjustThresholds.ijm inside the folder

CellContactNetwork > Fiji macros > HeLa processing.

**How to run it**

* Open the macro in Fiji and run.
* Fill in the parameters:

**Root**: the output directory where the well folders that contain the processed images are stored. Example:  
M:/tnw/bn/dm/Shared/Lukas/BEP/Experiments/WKS024/20x

**Well:** Name of the well. Only one well at the time can be stitched.

**Names of the thresholded channels**: must be the same as the names you gave the channels when you ran 2\_stitch.ijm.

Graphical user interface, application

Description automatically generated

* A message box will ask you to adjust the brightness/contrast.
* A message box will ask you to fit a circular selection to the boundary of the well. Hold shift and drag your mouse s.t. the selection fits the well:

A picture containing text, red, colorful

Description automatically generated

* Next, you will be asked to paste measurements into the file ‘Well locations.xlsx’. If it doesn’t exist yet, make it and store it inside the **Root**. Manually fill in the titles:

Graphical user interface, application, table, Excel

Description automatically generated

* Then paste the measurement results in the row below:

Table

Description automatically generated

* Next, you will be asked to make adjustments to the thresholds. You can make two adjustments:

1. Remove noise. Do this using the ‘Polygon selection’ tool, and make a selection around the noise. Then press “t” to add the selection to the ROI manager, and continue with the next selection. There is no need to clear it yourself, the code will automatically clear everything inside the selection **on all thresholded channels**.
2. Add foreground pixels. Do this if the stitching did not work properly. First select the ‘color picker’, and click on a white region on the image. Then double-click on the paintbrush tool to change the width of the brush. You can now add foreground pixels to the thresholded images.