Documentation for Cell Segmentation

The cell segmentation is based on these steps:

- Adaptive thresholding
- Gaussian filtering of thresholded image
- Global thresholding of gaussian filtered image
- Classification of cells and clusters by segment shape

There are two control panels: one for the segmentation of the whole image („localize“), and one for the segmentation of cell clusters („cluster“). The cluster panel includes the image of the cluster, while the blue image for the cell segmentation (file name in window) and the („red“) red and the green („green“) channel are shown in seperate windows.

Input files have to be stored in a directory, which name is passed to the application. Three versions of images have to be provided which have to contain those strings in the filename:

“DAPI” main channel, basis for cell segmentation
“DsRed” or “_CY5” for red channel
“Alexa” for green channel

1 Whole image segmentation

1.1 Select image

With the „image“ slider the displayed image can be selected. Images are loaded from the directory given as a command line parameter, if this parameter does not exist „images“ is used. In the whole segmented image, cells are marked red, clusters green and junk magenta.

1.2 Switch between original image and overlayed image

The „switch_orig“ slider changes between overlayed (0) image and original image (1).

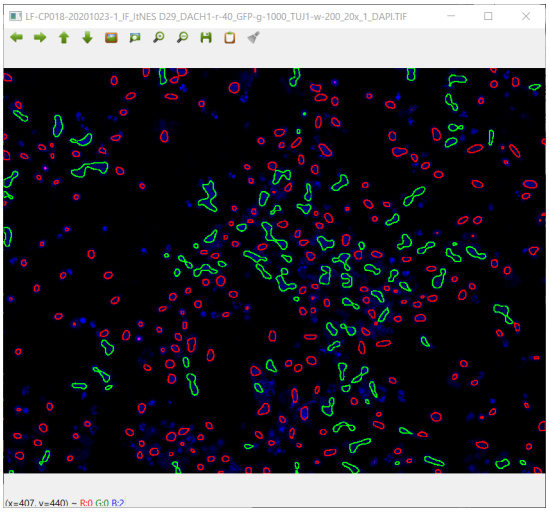
1.3 Adaptive Thresholding

Adaptive thresholding does a sliding window based thresholding to seperate foreground from background. The threshold is determined in the sliding window, every pixel lower than the threshold is background, every pixel higher is foreground. There are two parameters: window size and a constant C.

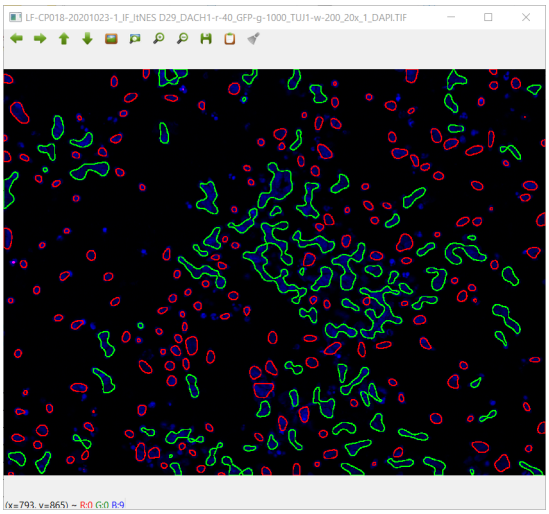
1.4 Window size

The window size of adaptive thresholding defines the size of the sliding window and is called „adapt_thres_win“. Smaller windows reduce the influence to the threshold value of other objects in the neighborhood, while larger windows increase the influence. The window size of the slider is multiplied by 2 and 1 is added to get an odd window size.

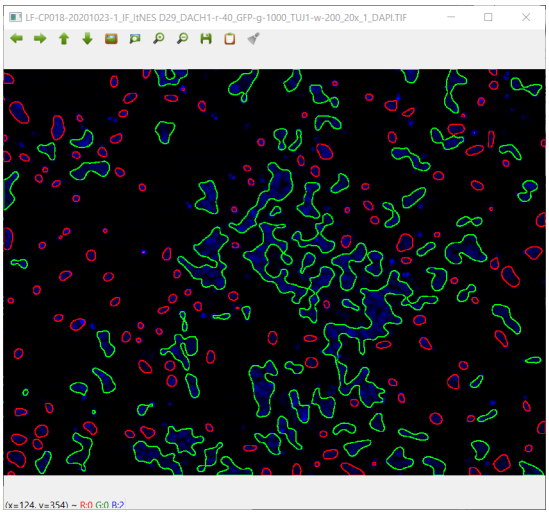
See: https://docs.opencv.org/master/d7/d4d/tutorial_py_thresholding.html



Adaptive Threshold with Window Size 20



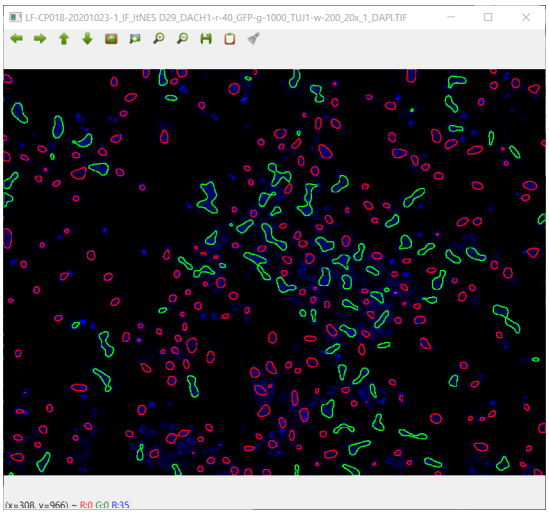
Adaptive Threshold with Window Size 45



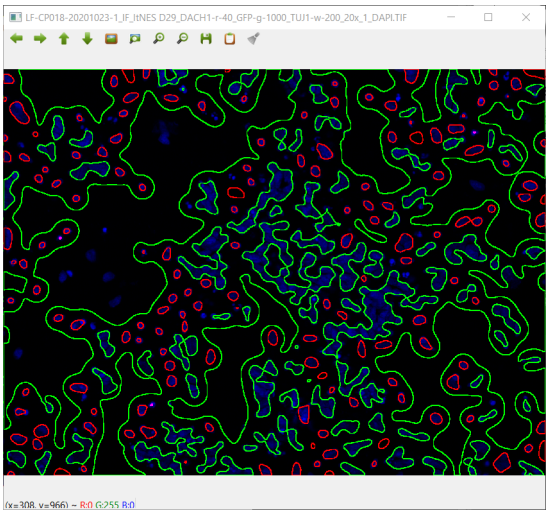
Adaptive Threshold with Window Size 160

1.5 Constant C

A constant C is subtracted from the adaptive threshold and defined in „adapt_thres_c“. 10 is subtracted from the slider value. Larger values reduces the number of foreground pixels since the threshold is lower, lower values increase the number of foreground pixels.



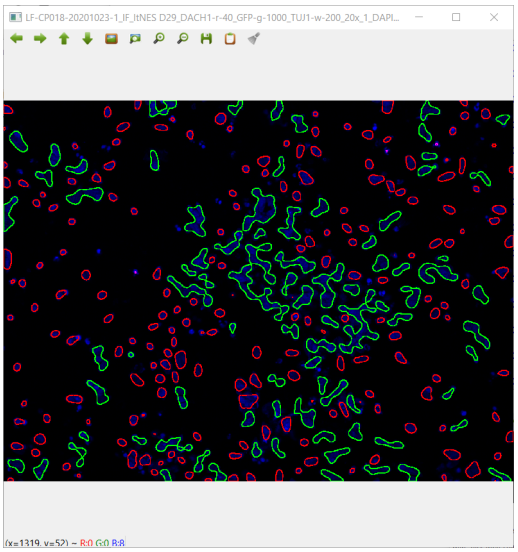
Adaptive Threshold C=9



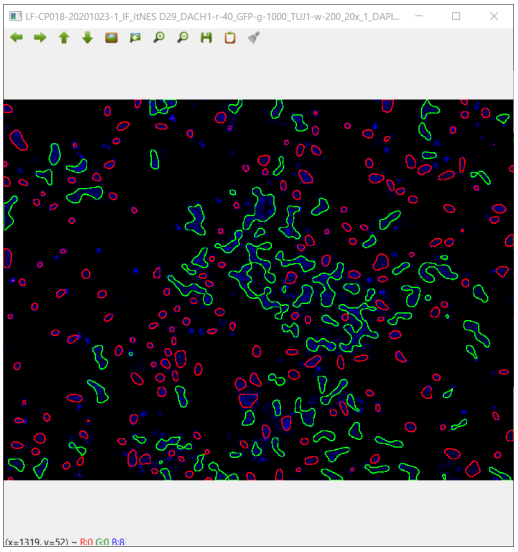
Adaptive Threshold C=11

1.6 Gaussian Filtering

After adaptive thresholding, the binary image is blurred by a gaussian filter. This makes foreground areas „smoother“. For gaussian filtering, a window size is defined by „gaussian_win“. The slider value is multiplied with 2 and 1 is added. Lower windows can detect smaller structures but become more susceptible to noise.



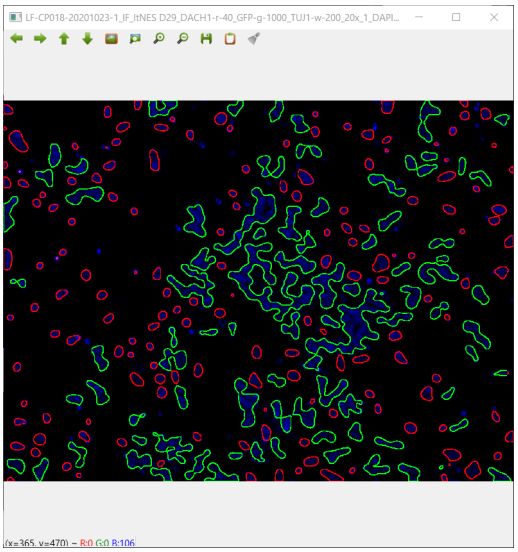
Gaussian Window Size 15



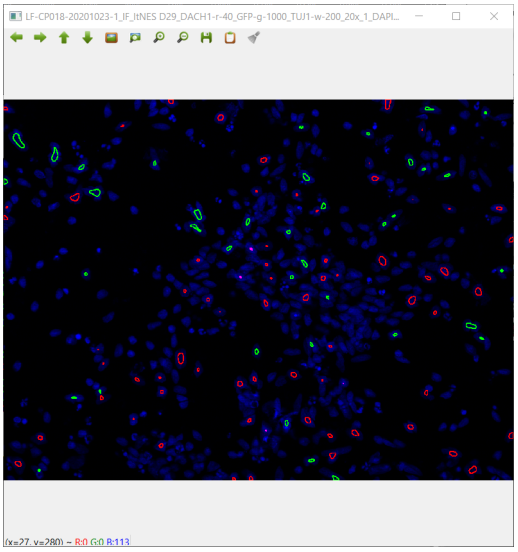
Gaussian Window Size 35

1.7 Thresholding of Gaussian Filtered Image

To get the final foreground seperation, the gaussian filtered image is thresholded by a global threshold, defined in „binary_thres“. The maximal value in the gaussian filtered image is 255, therefore thresholds can be between 0 and 255. Higher thresholds result in smaller segments.



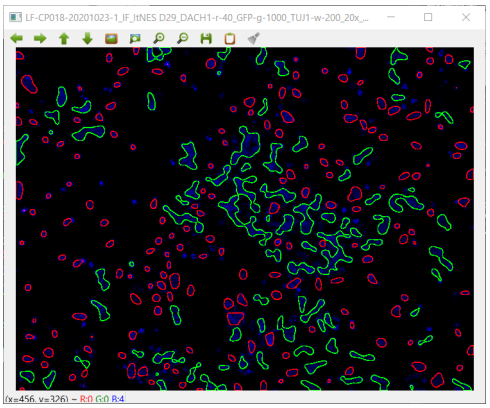
Global Threshold 115



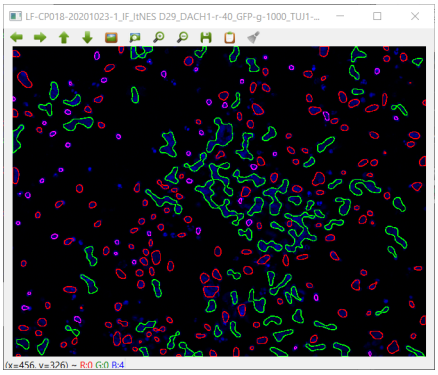
Global Threshold 200

1.8 Sort out small Objects

The „min_cell_area“ slider defines the minimal size of objects in the image in pixels, each smaller object is omitted and marked magenta.



Minimal Cell Area 10



Minimal Cell Area 400

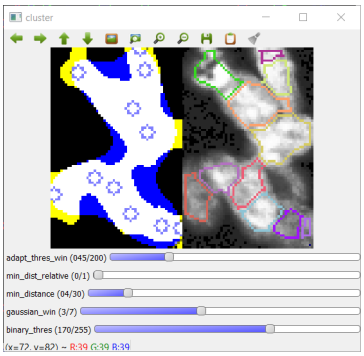
1.9 Quantile of Intensity to sort out bright Objects

To sort out bright object, the 90% quantile of a segment is compared to the quantile set by the slider, which value is in percent. If the 90% quantile of the segment is larger, the segment is omitted.

This feature is currently switched off.

2 Segmentation of Cell Clusters

By clicking in a green segment, the cluster segmentation is activated. A result can look like this:



Example of a clustered segment

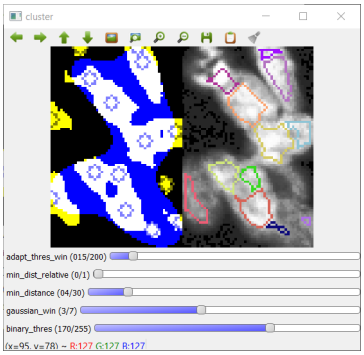
The left image shows the mask of the segment which is currently computed in yellow, the original segmentation from the localization in blue, and the intersection in white. Circles show cell centers which are used for segmentation.

In the right image, the cell cluster is shown with overlayed cell segments. The „switch_orig“ slider from the localization can switch off overlays.

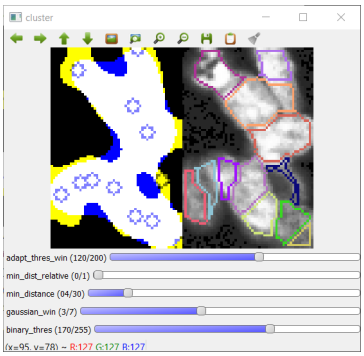
Cell segments with an intersection (white area) with the original segmentation is less than 50% will be omitted.

2.1 Adaptive threshold window size in cluster window

The adaptive threshold window size works the same way as in the localization, now just applied on the cell cluster.



Adaptive Threshold Window Size 15



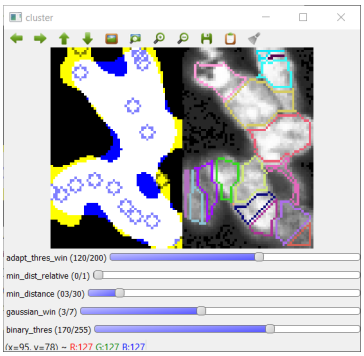
Adaptive Threshold Window Size 120

2.2 Minimal distance between cell centers

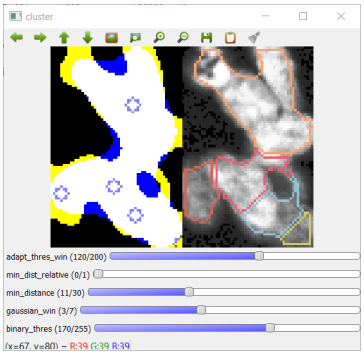
Cell centers are placed by determining local maxima in a distance map. Centers which are too close to other centers are omitted. This minimal distance can be relative to the cluster size or absolute in pixels, which is controlled by the „min_dist_relative“ slider, 0 is absolute, 1 relative.

2.2.1 Absolute distance between cell centers

The absolute distance is set in the „min_distance“ slider, value in pixels.



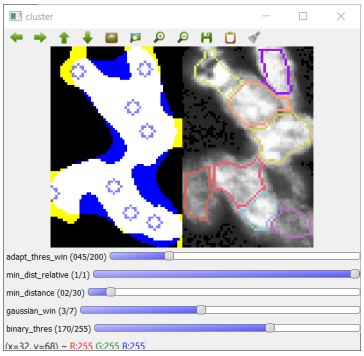
Absolute minimal Distance 3



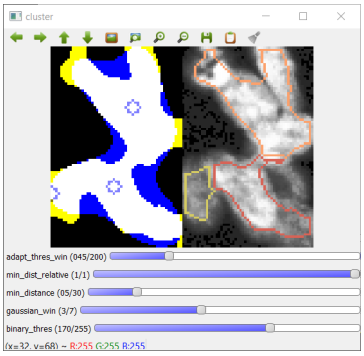
Absolute minimal Distance 11

2.2.2 Relative minimal Distance

If the minimal distance is set to relative, its value is divided by 30 and multiplied with the larger value of height and width of the segment.



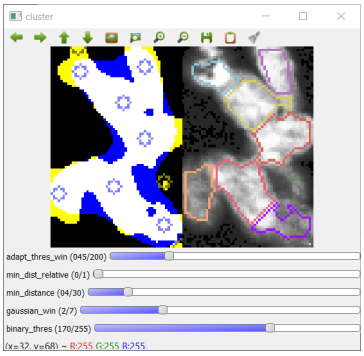
Relative minimal Distance 2



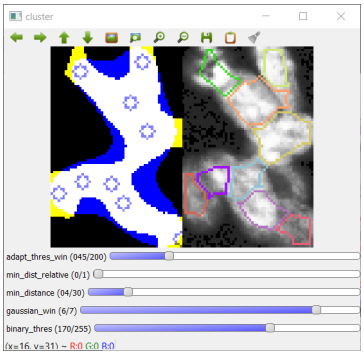
Relative minimal Distance 5

2.3 Gaussian Window Size

The gaussian window is applied to the adaptive thresholded image the same way as in the localization.



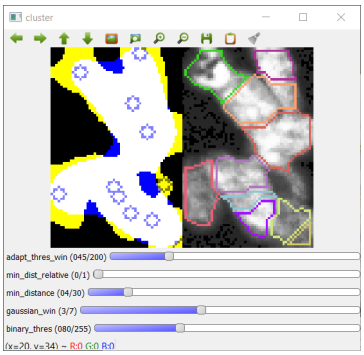
Gaussian Window Size 2



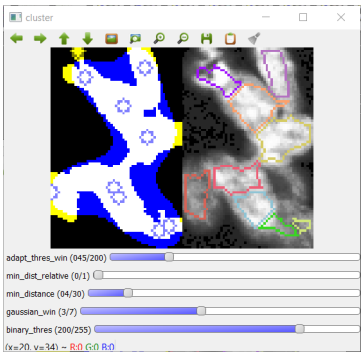
Gaussian Window Size 6

2.4 Thresholding of filtered image

The global thresholding works the same way as in the localization.



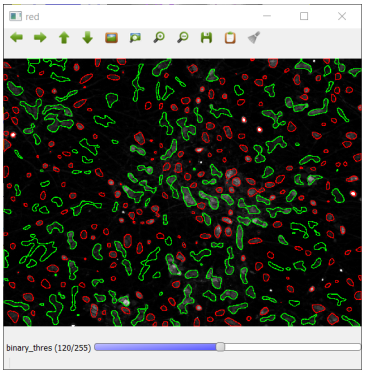
Global Threshold 80



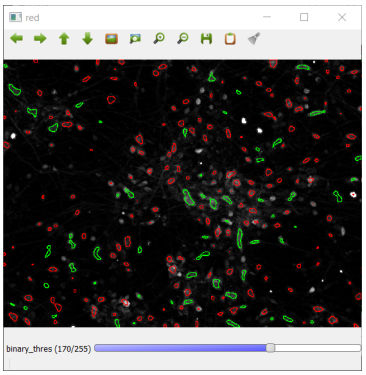
Global Threshold 200

3 Segmentation of red and green channel

For analysis, a foreground/background segmentation of the red and green channel has to be performed. These channels are shown in the „red“ and „green“ window. The same parameters are applied as in the localization, however the „binary_thres“, which is the global threshold, can be modified as shown in the example of the red image:



Global Threshold 120



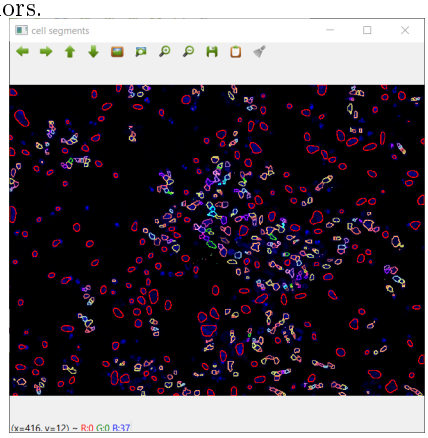
Global Threshold 170

4 Saving Configurations

By pressing keys shift plus 0-9, the current parameters are saved and can be loaded by pressing 0-9.

5 Showing complete segmentation

Pressing „x“ key shows the complete segmentation. The window is closed by pressing „x“ again. Cells are marked red, while cluster segments are in different colors.



Complete Segmentation, Clustering in different (random) Colors

6 Saving Datasets

Pressing „s“ saves the segmentation and mask of all images and the parameter set. A folder with the name „YMDHMS_result“ is created, where Y is the current year, M month, D day, H hour, M minute and S second. The folder contains masks named corresponding to the input files, with pixel values which corresopond to the cell ID.