**spot\_distance\_cutoff**: max distance between for 2 spots to be considered 1

NUCLEI DETECTION PARAMETERS:

**nucl\_id\_contrast\_enh\_type**: method to use for contrast enhancement of DAPI image - 2 options: **none** (no contrast-enhance) or **rescale\_percentile**

**nucl\_id\_ce\_percentile**: the top/bottom percentile of pixels to set to the image maximum/minimum when rescaling pixel values. Similar to % “saturated pixels” in Imagej’s Contrast Enhance. \*NOTE\* if this is set to 0, then it is the same as setting **none** for **nucl\_id\_contrast\_enh\_type** (no contrast-enhance)

**nucl\_id\_med\_filter\_size**: size of median filter to apply prior to nucleus detection

**nucl\_id\_watershed** 0/1: use watershed to separate touching nuclei

**nucl\_id\_ws\_gauss\_sigma**: stdev for gaussian kernel, gauss filter is applied to distance transform prior to watershed

**nucli\_id\_ws\_min\_dist**: peaks in the distance map must be at least this distance away (if closer, only larger peak will be kept)

**nucl\_id\_th:** thresholding algorithm to define nucleus objects (e.g. “otsu” or “li”)

**nucl\_id\_min\_solidity**: minimum solidity to be included as a nuclei (otherwise object is discarded for the analysis)

**nucl\_id\_min\_area**: minimum area of nuclei, objects smaller will be discarded

**nucl\_id\_max\_area**: maximum area of nuclei, objects larger will be discarded

BLOB DETECTION PARAMETERS:

**blob\_min\_sigma**: min. stdev for gaussian kernel. keep low to detect smaller blobs

**blob\_max\_sigma**: max. "

**blob\_num\_sigma**: number of intermediate values of stdev to consider

**blob\_th\_GFP**: intensity threshold for GFP image, local maxima smaller than thresh are ignored. Reduce this to detect blobs with less intensities.

**blob\_th\_RFP**: intensity threshold for RFP image, local maxima smaller than thresh are ignored. Reduce this to detect blobs with less intensities.

**blob\_overlap** (0-1): if area of 2 blobs overlaps by a fraction greater than this threshold, the smaller blob is eliminated

**white\_tophat** 0/1: apply white tophat - returns the bright spots of an image that are smaller than the structuring element

**tophat\_disk\_size**: structuring element is a disk, specify size here

GFP\_ce\_percentile\_ll: percentile of pixels saturated from the bottom (low intensity)

GFP\_ce\_percentile\_ul: percentile of pixels saturated from the top (high intensity)

RFP\_ce\_percentile\_ll

GFP\_ce\_percentile\_ul