Cell detection by canny Edge detection and CHT of transmitted images.

Detected cells outlined based on the edges. Only those detected events with outlines that resemble cells (based on size and shape descriptors) are considered further.

Segmentation of fluorescence is done by Otsu thresholding on Gaussian blurred images. The separation parameter is used to decide whether segmentation is of acceptable quality. For reporter channel (currently PKC theta staining) triangle thresholding on the raw images gives better outlines. This is done only if the otsu separation parameter is above the threshold. pSMAC/ICAM1 has lot of speckles in background, which presents a challenge for segmentation. Otsu segmentation is done on raw images. Small binary components are removed, which represent speckles. Further when the major component is chosen smaller speckles are disregarded. Largest segment that satisfies a set of criteria is chosen. Option of choosing multiple components for pSMAC exists. I could include watershed as well, but this option is not used at present.

Flur intensity is background subtracted for reporting. A ring outside the dilated DIC outline is considered for local background subtraction. Filling the pSMAC hole and circularity measurement allow for determining the pSMAC status (hole, broken, no hole).

Synapse symmetry is the ratio of weighted cSMAC over the radius of the cell (taken based on the effective radius of the average of the DIC area and pSMAC convex hull area).

Reporter enrichment: mean intensity of reporter within the mask of the costim segment over the mean intensity outside the costim mask.