

NanocontactsTIRF_V5

Inputs: IRM images were used to define the region of the cell interface. Active contour segmentation of the QD605 image was then used to define close contact regions. These regions were then converted to Spots objects in Imaris. The region of the cell interface (contour), close contact regions, and TIRF receptor image stacks were used as inputs for analysis of persistence times.

Purpose of NanocontactsTIRF_V5: CAR intensity was masked to regions of close contacts, and the average intensity for each contact area was then plotted in a histogram. A Gaussian distribution curve centered at the background fluorescence median was overlayed. Contacts that fell within 3 sigma of the Gaussian distribution were considered CAR-, while the higher intensity contacts were considered CAR+. These contacts were then separated into separate image stacks and persistence time for individual contacts were calculated. Contact persistence time was determined by summing the number of frames each binary connected component object existed for and multiplying by the time per frame. Contacts were assumed to not travel more than their diameter per time point.