Automated cell segmentation was performed through a custom Python based pipeline. mCherry intensity and lifetime images, thresholded to a predefined range to reduce non-specific signal activity, were passed in as initial inputs to the pipeline. Initial thresholding of intensity images through a series of methods methods was used to differentiate foreground vs. background. Next, a left-merge intersection was performed to combine the thresholded intensity image and the range-limited lifetime image. A canny edge detector was used to identify and label individual regions of interest (ROIs) in the resulting image; ROIs were expanded through binary dilation or closing to ensure proper coverage of cell bodies. Following ROI generation, a small-item filter was used based on ROI area to limit noise and non-specific elements in the mask. Ensemble voting was performed via combinatorial analysis of intensity image thresholding and ROI region expansion methods. A maximum intensity projection was generated for each outcome, and a single ‘best’ binary mask was established by maximizing Dice coefficients compared against hand-segmented ground truth images for every image in the dataset. Final segmentation mask quality was assessed through calculations of the Dice coefficient for each image.

\* for different thresholding methods and unique region expansion steps with different methods possible combination outcomes.