
Summary: This protocol describes the generation of single-cell image towers.

Figure 1: Image tower showing the single-cell localization of SeqA-gfp in false color. The localization in a newborn cell is shown in the first frame. The final frame shows the cell just prior to division.

1. Segment and link data to generate cell stacks. (Protocol)
2. Background subtract fluorescence images by subtracting the background level defined as the mean fluorescence, throughout the frame, in regions outside of cells.
3. Rotate cell image and mask in each frame to align the major axis of the mask with the x-axis and placing the old pole (the new pole is created in the last division) on the left hand side.
4. Center each image on the y axis
5. Stack masked fluorescence images of cells with frame number increasing downwards.
6. Normalize false color fluorescence to the brightest pixel in the stack.