Protocol: Consensus Localization.

Summary: This protocol describes the generation of Consensus Localization image towers.

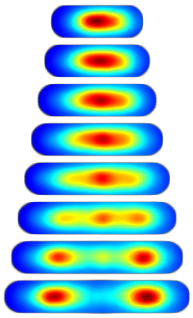

Figure 2: Image tower showing the consensus localization of SeqA-gfp in false color. A consensus image shows the protein concentration as a function of relative cellular position and time relative to the cell cycle.

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. For each cell Interpolate the image tower onto a reference image tower:
    a. Dilate the fluorescence channel and cell mask by 4 times using linear interpolation.
    b. For each frame of the cell stack, generate a reference configuration
        i. Reference Configuration: A rectangle with y width 36 pixels with circular caps with the same length (x width) as the observed cell region
    c. Apply a dilation and shift transformation to each column of the fluorescence image to match the reference configuration.
        i. The dilation and shift are those required to map the column of the region mask to the column of reference configuration.
    d. Interpolate between closest frames to generate an eight frame life cycle of the cell where the cell length interpolates smoothly between 104 pixels and 208 pixels
    e. Scale intensity values in frames 2-7 to leave the areal mean of intensity constant throughout the cell cycle to compensate for the bleaching or the loss of fluorescent protein through proteolysis.
    f. Stack fluorescence images of cells with frame number increasing downwards.
5. Compute mean and standard deviation of stacked images.
6. Normalize false color fluorescence to the brightest pixel in the stack.