SUPPORTING INFORMATION

Oufti: An integrated software package for high-accuracy, high-throughput quantitative microscopy analysis

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Includes supplementary figures and video legends.

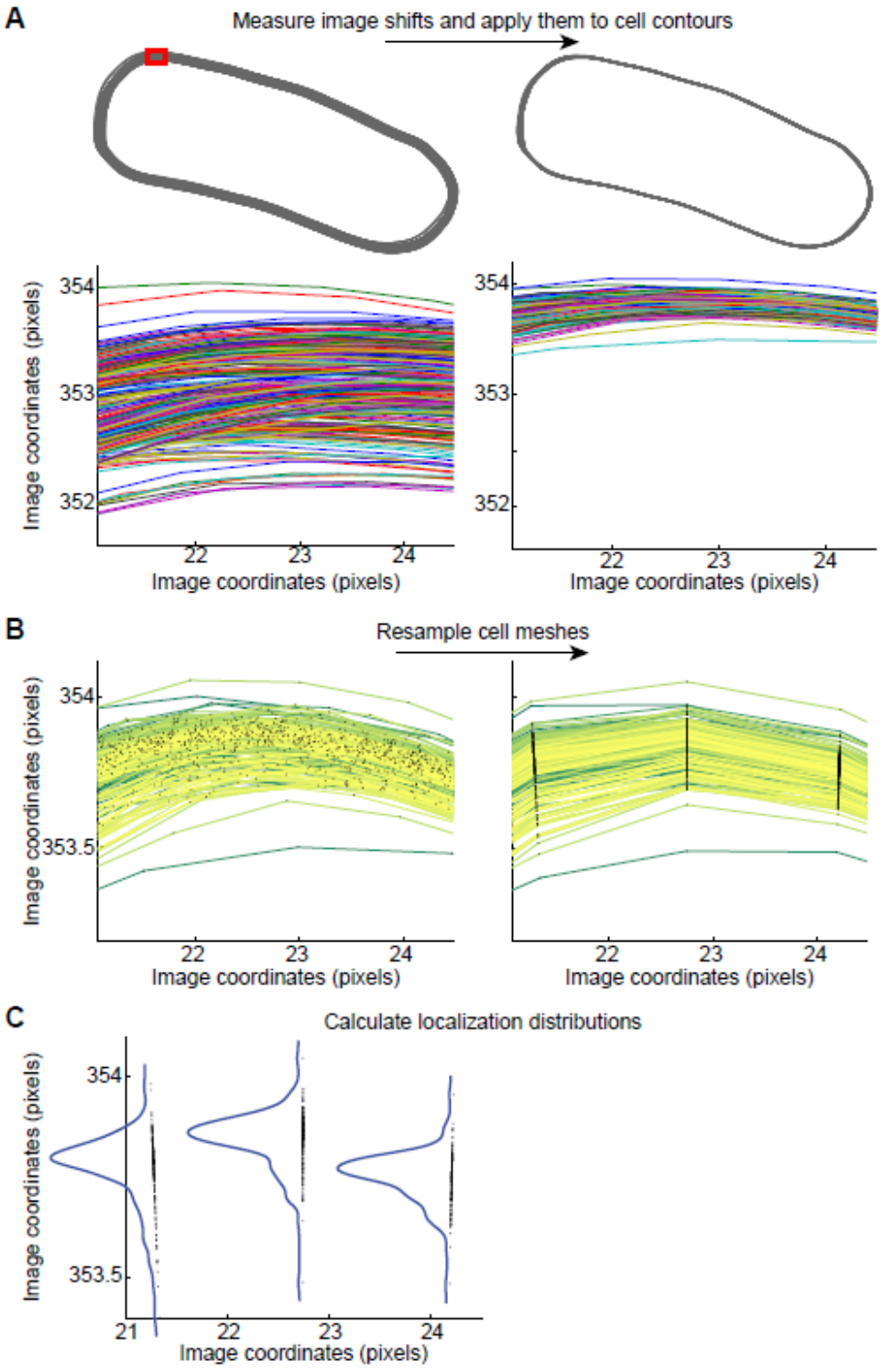

Fig. S1. Method to calculate cell contour precision. Non-growing cells (116) were imaged 301 times in a time-lapse experiment. Cell contours were obtained with the pixel or subpixel algorithms. Here, we show how precision measurements were made, using the 301 cell contours of a single cell (obtained with the subpixel algorithm) as an example. (A) The cell

contours were aligned by applying measured image shifts. The bottom row shows zoomed plots that were generated from the red box. (B) The next step was to create new cell contour vertices (vertices are represented as black dots). To do this, lines orthogonal to cell edges were constructed and used to resample cell contours from all frames. New vertices were identified as the intersection of the orthogonal lines and the cell edges. (C) Localization distributions were then calculated for each new cell contour vertex. Variances of each distribution were measured.

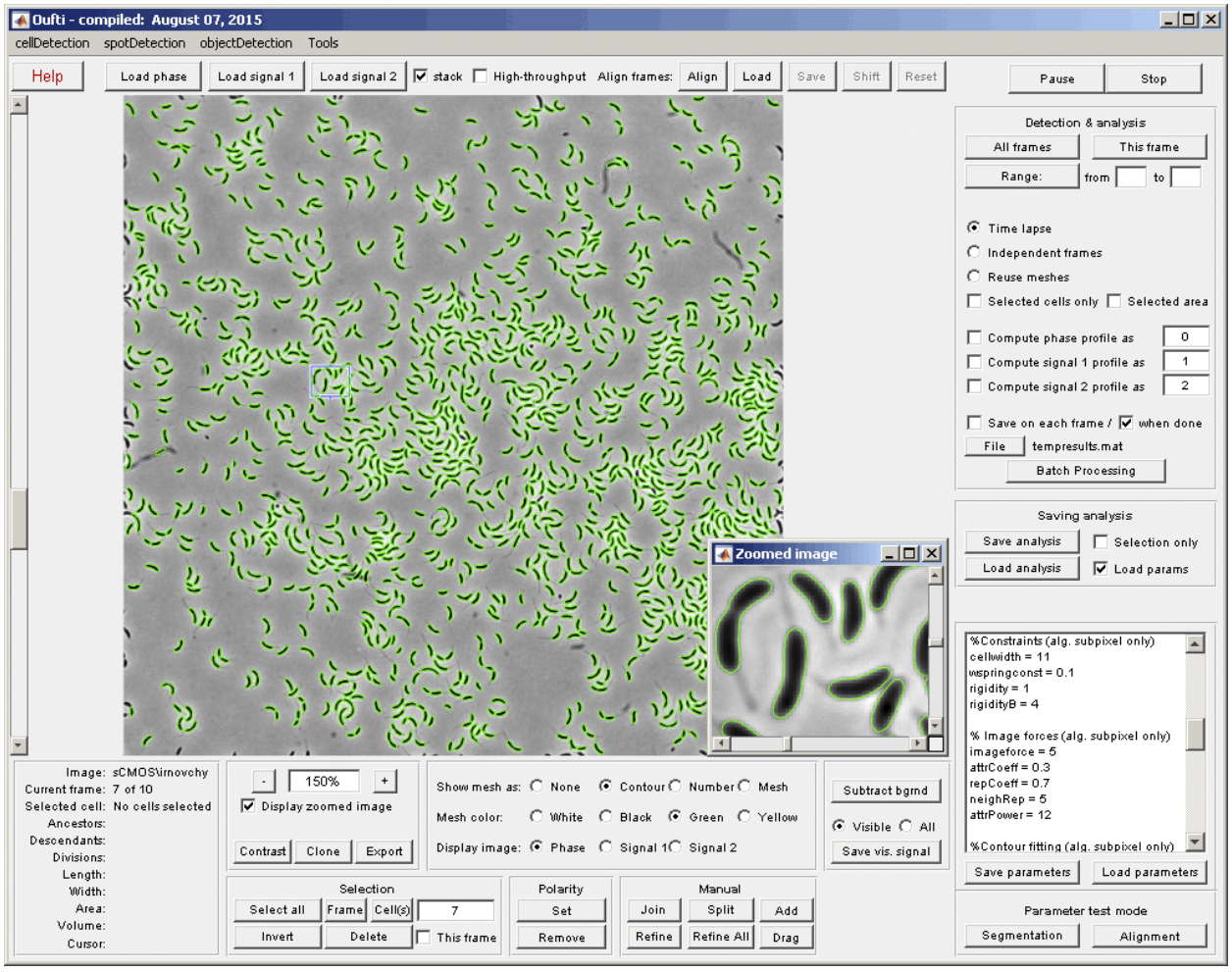

Fig. S2. Detection of *C. crescentus* cells from a 2048x2048-pixel sCMOS image. Cells automatically detected by Oufti's subpixel algorithm using optimized parameters are shown with the green contours.

Video legends

Video 1. **Example of a microfluidic experiment showing *E. coli* cells detected and their growth plotted by Oufti.** Each detected cell (red contour) is associated with a number (in blue). During

the experiment, the growth condition changed around the 4-h time-point, which is apparent by the change in growth rates (i.e., change in growth curve steepness). The codec used for the video is H. 264.

Video 2. **Microfluidic experiment showing *E. coli* cells and their nucleoids detected by objectDetection.** Cell contours are in red and the contour of nucleoids labeled with DNA-binding HU-mCherry are in yellow. This video is related to Fig. 6. The codec used for the video is H. 264.

Video 3. **Image alignment and movie construction in Oufti.** Phase contrast images of *E. coli* expressing FtsZ-YFP (cyan overlay). The 'before' (unaligned) and 'after' (aligned) movies are shown on the left and right, respectively. Scale bar is 1 μm. The codec used for the video is H. 264.