Supplementary

Noise removal procedure for Ilastik segmentation

Since Ilastik relies on manual labeling, we manually labeled the same cells and similar background regions to train the pixel classifier. However, the segmentation results contained visually significant noise in the background, reducing similarity. Thererfore, we performed a new expanded manual labeling to remove as much noise as possible.

Initially, both the original (Figure S1a) and RBT-processed (Figure S1f) images were imported into the same Ilastik project and labeled in similar regions (Figure S1b and g). However, since Ilastik learns from all images within a single project, we suspected that the output masks (Figure S1c and h) might interfere with each other. To minimize such interference, the images were subsequently imported into separate projects and labeled with finer detail to reduce background noise (Figure S1d and i).

Before noise removal, the Jaccard index between the Ilastik masks identified from the original and RBT images was 0.7238, with noise being the primary factor lowering the Jaccard index. After noise removal, the segmentation results (Figure S1e and j) achieved a Jaccard index of 0.8159, corresponding to a relative increase of approximately 12.4%. Despite this increase, no major visual differences in the cell regions were observed, suggesting that background noise still largely explains differences.
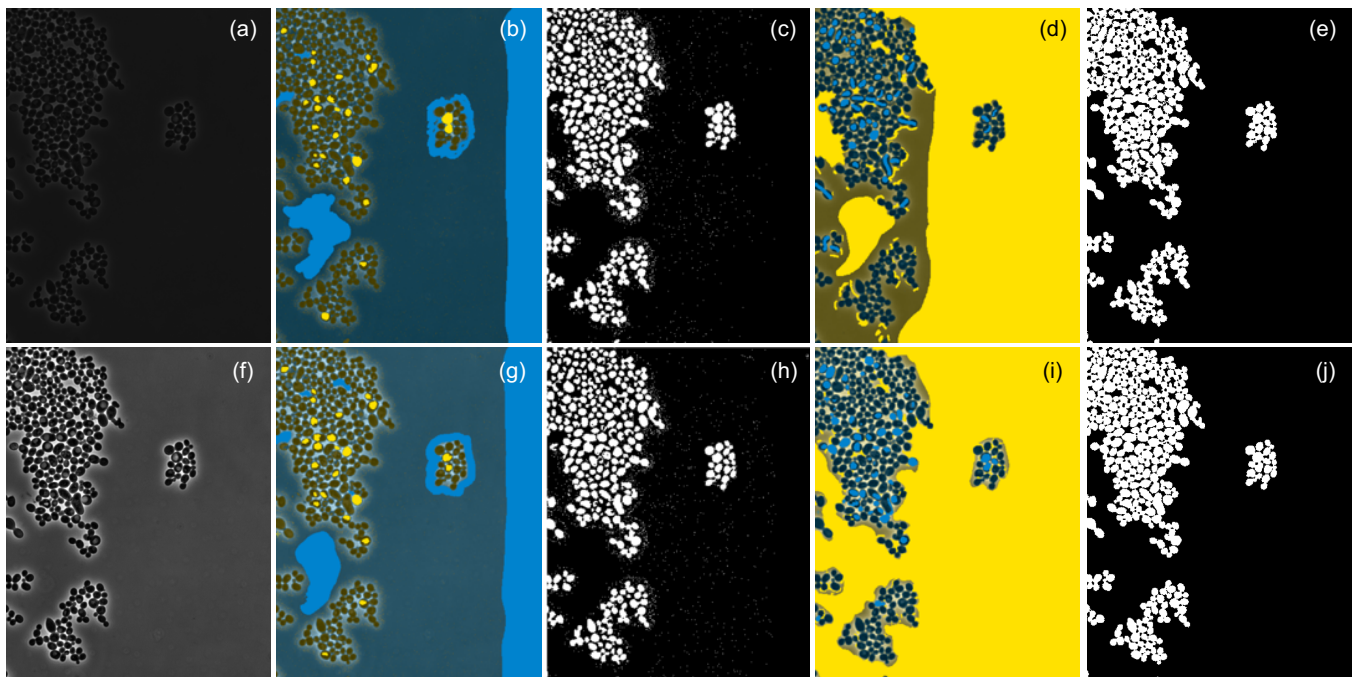

Figure S1: Segmented images, labels, and output masks from Ilastik. The original image (a) and the RBT-processed image (f) were manually labeled. Both images were imported into the same project and labeled in similar regions–background in blue and cells in yellow (b and g)–resulting in the output masks (c) and (h). To minimize interference, the images were also imported into separate projects and labeled with finer detail to reduce background noise; in this setting, the background was labeled in yellow and cells in blue to better highlight regions of noise removal (d and i), resulting in the output masks (e) and (j).