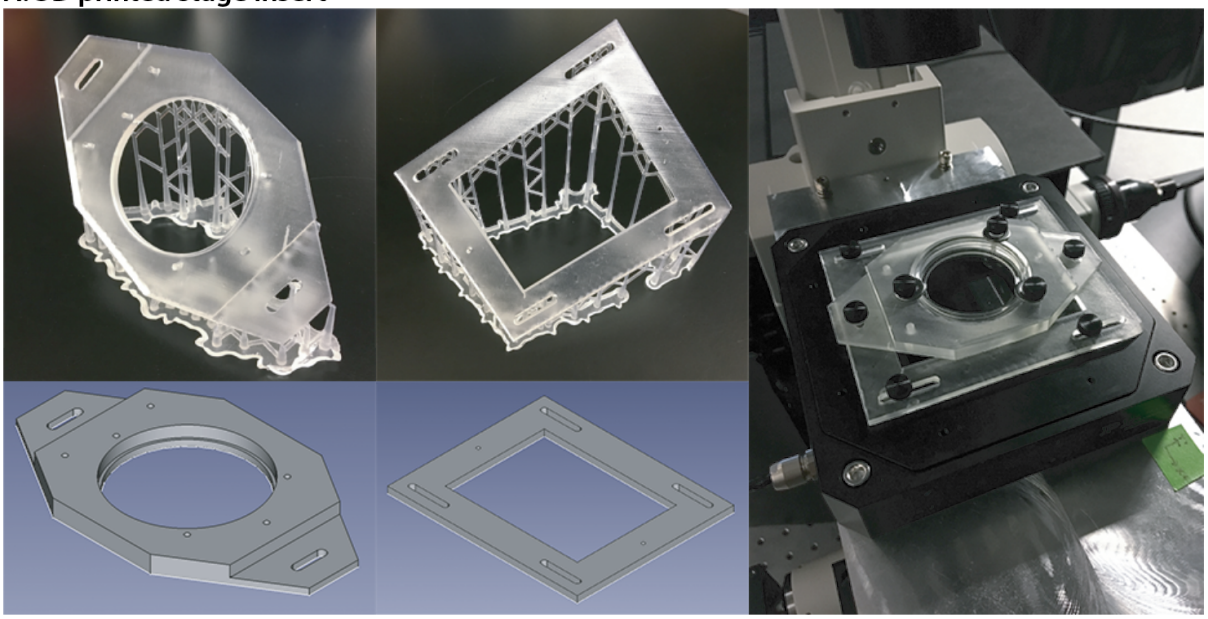
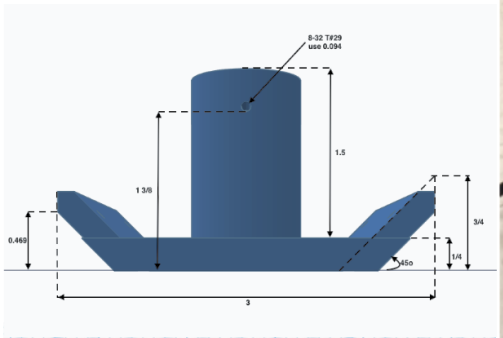
Figure S1. Inexpensive fabrication of stage insert and cell loader with 3D printing.

A. 3D printed stage insert



B. 3D printed cell loader



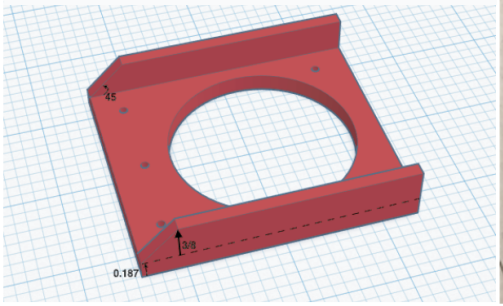


Figure S2: Segmentation accuracy of napari-MM3 Otsu and U-Net methods

To quantify the accuracy of the segmentation masks generated by MM3's Otsu and U-Net segmentation methods, we computed the Jaccard Index [44,59] as a function of the intersection-over-union (IoU) threshold.

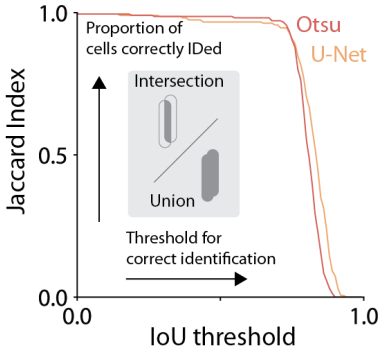


Figure S3: Old-pole aging phenotype is strain specific. Cells imaged with fluorescence often show signs of aging in the old-pole "mother" cell. For instance, in the dataset analyzed in Figure 4 (*E. coli* MG1655 with the fluorescent protein YPet fused to DnaN), we observed systematic differences in cell elongation rate and size between the old-pole cell at the end of the growth channel and its sisters, which inherit the new pole (top center). However, this asymmetry is not universal. Using napari-MM3's Otsu segmentation method, we re-analyzed previously published data obtained without fluorescence illumination [30], and found that the old-pole and new-pole cell elongation rates varied only on the order of 1% (lower center), while in the dataset obtained under fluorescence imaging, the old-pole mother cells grow 7-10% slower than the new pole cells.