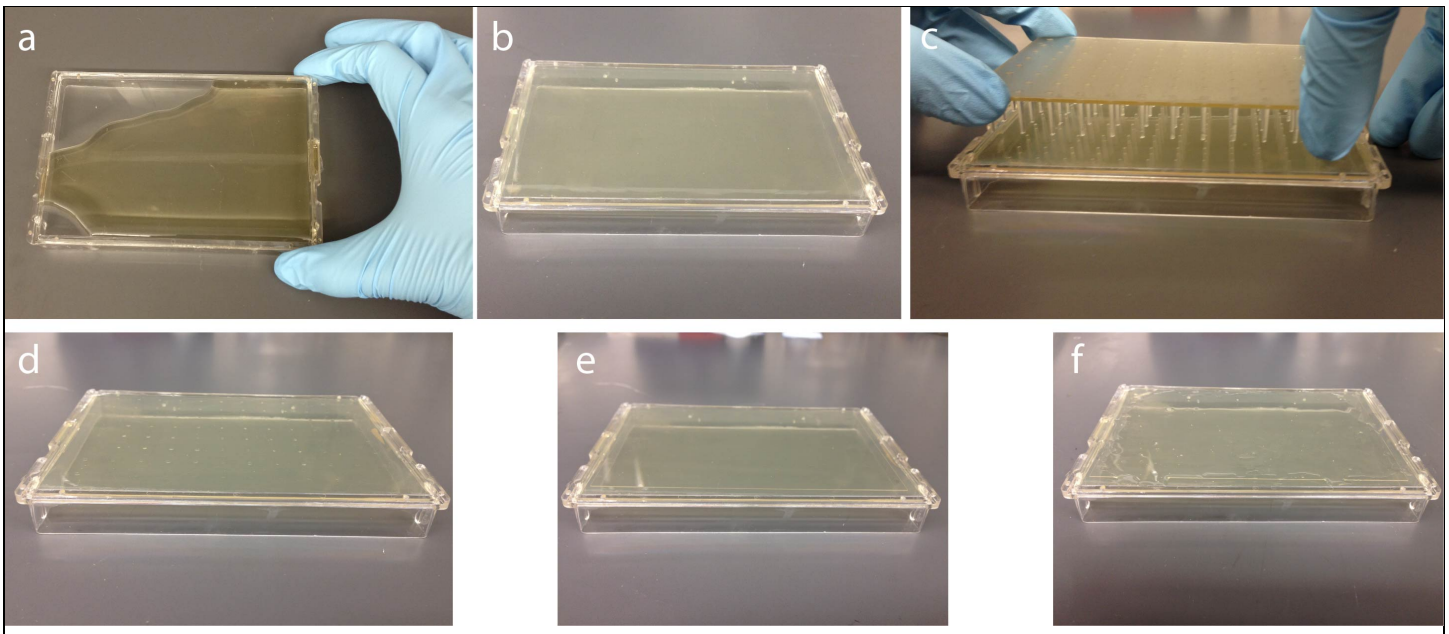
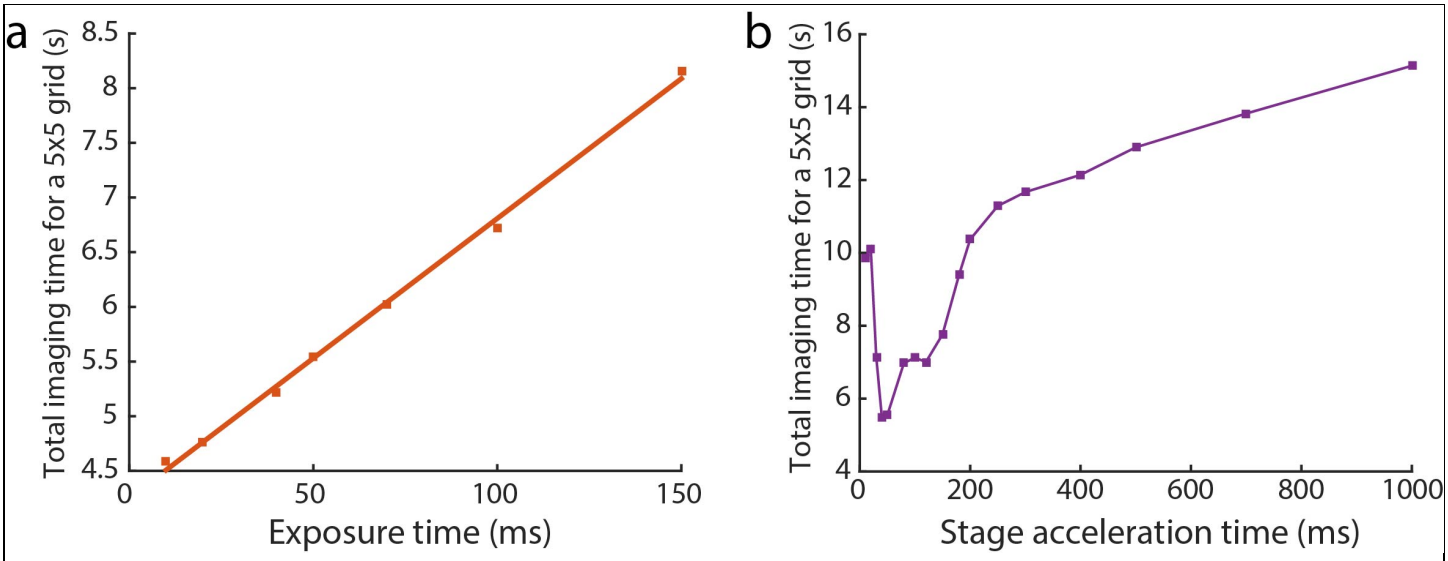


Supplementary Figure 1

Photographs of agar pad and sample preparation.

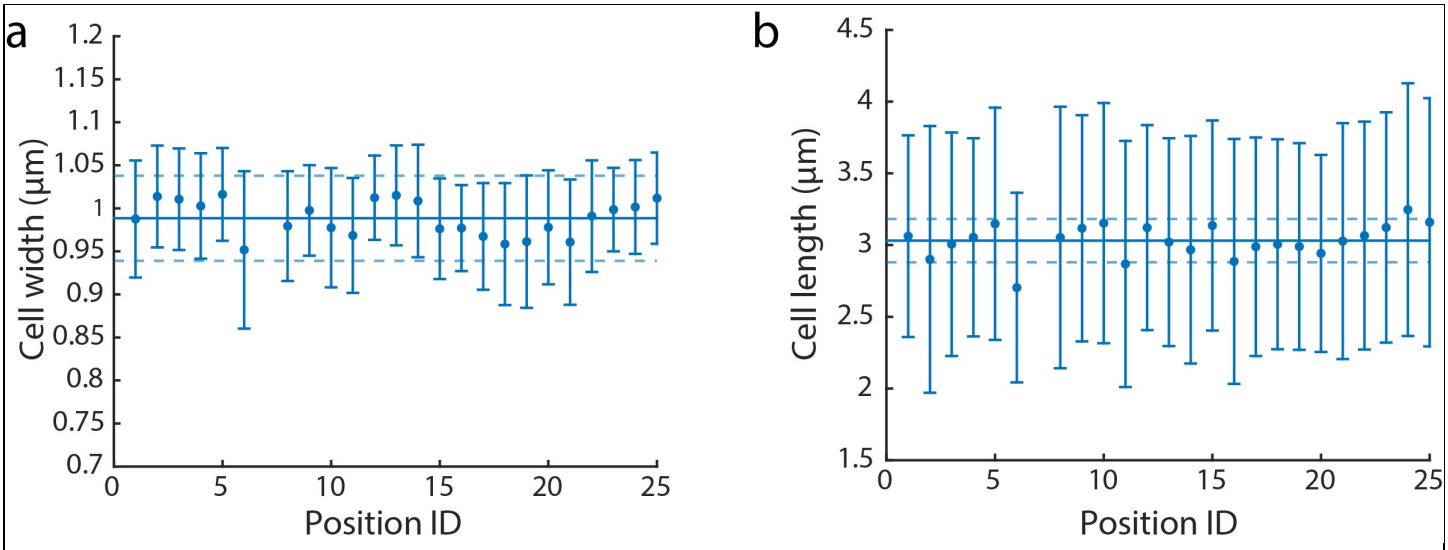
(a) Melted agar is poured onto the bottom surface of a Singer PlusPlate and spread evenly by gently tilting and shaking the plate. (b) A flat agar surface is generally achieved when the plate is left on the benchtop to solidify without disturbance. The agar layer is ~2 mm in thickness. (c, d) Bacterial cultures are transferred onto an agar pad using a 96-well replicator pin. (e) After the agar pad absorbs all the liquid from the cultures, a large glass cover slip is used to cover the agar surface. (f) Immersion oil is applied evenly onto the cover slip for oil-immersion objectives.



Supplementary Figure 2

Optimization of TTL setup.

(a) Imaging time for a 5x5 grid of images for one strain scales linearly with camera exposure time. Reducing exposure time can expedite image acquisition. (b) Total imaging time for a 5x5 grid is dependent on stage acceleration time. In our configuration, an acceleration time of 40-50 ms is optimal.



Supplementary Figure 3

Variation in mean cellular dimensions calculated from individual fields of view is small.

The coefficient of variation of mean cell width (a) and length (b) across the fields of view from one of the wells in the experiment shown in Fig. 3a was ~3%. Data points are mean ± standard deviation, horizontal solid lines are the mean value for cells across all fields of view, and dashed lines are ±5% of the mean. Position #7 happened to have no cells and thus is not included in the plot.