*Supplementary information:*

# Measuring bacterial adaptation dynamics at the single-cell level using a microfluidic chemostat and time-lapse fluorescence microscopy

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## *Supplementary Movie Captions:*

**Movie S1:** Downshift movie of P5 strain carrying a constitutive P5 promoter GFP reporter. The cells first grew in LB media for ~11 hours, then in M9 minimal media with 0.2% CAA for ~5 hours. Both media contained 50 μg/mL kanamycin. The growth temperature was maintained at 30°C.

**Movie S2:** Downshift movie of P1 strain carrying a ribosomal RNA promoter (rrnB P1) reporter. The cells first grew in LB media for ~6 hours, then in M9 media with 0.2% CAA for ~8 hours. Both media contained 50 μg/mL kanamycin. The growth temperature was maintained at 30°C.

*Supplementary Figures:*

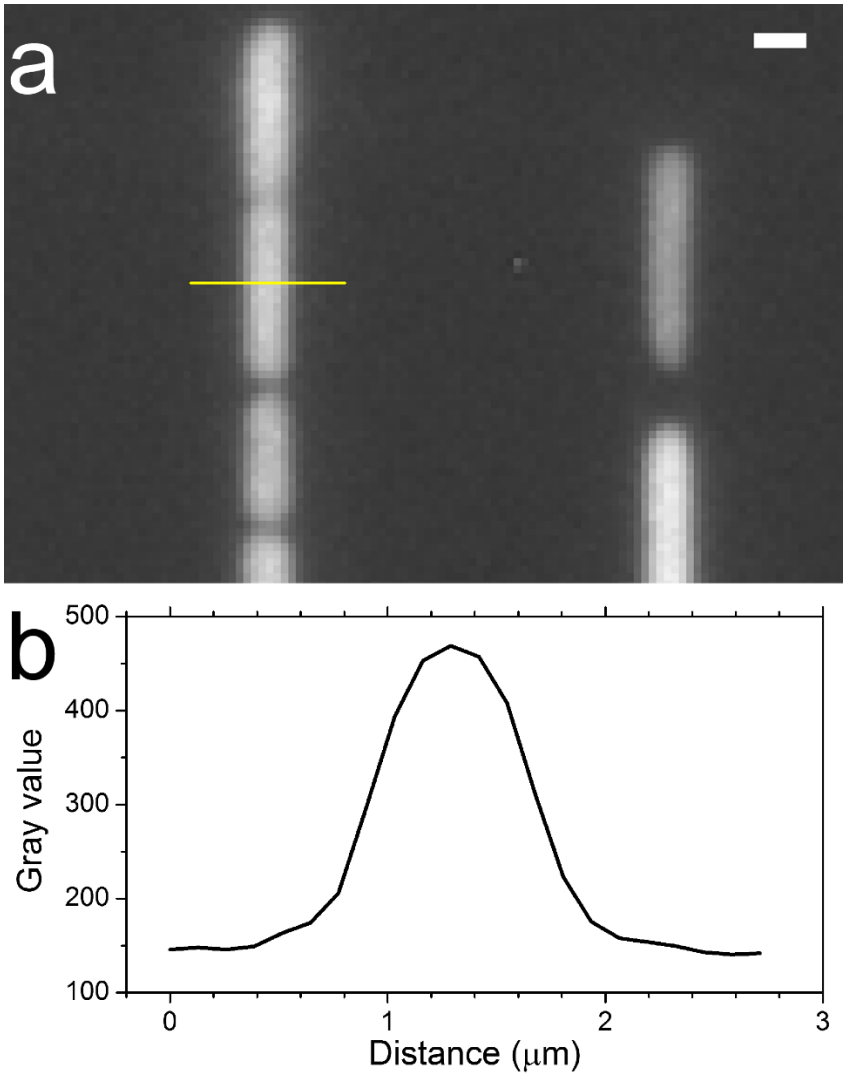
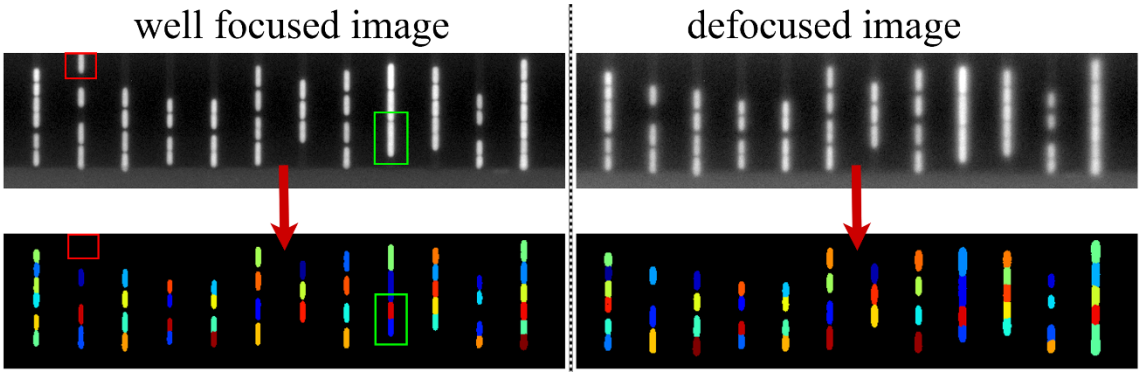



**Figure S1** (a) Enlarged view of the gray-scale fluorescent *E. coli* cells. Scale bar = 1 μm. (b) Intensity profile taken along the yellow line in panel a.




| | | |
|---|---|---|
| Mean cell area (μm²) | 2.29 | 3.07 |
| Mean cell length (μm) | 3.02 | 3.19 |
| Mean cell width (μm) | 0.99 | 1.24 |
| Mean pixel intensity (a.u.) | 0.114 | 0.086 |

**Figure S2** Image segmentation of two consecutive frames with well-focused cells or defocused cells. The identified cells are highlighted with arbitrary coloring. The cell in the red rectangle is excluded because it touches the border. The green rectangle shows an example of mis-segmentation. The cells in both frames were growing in LB medium.

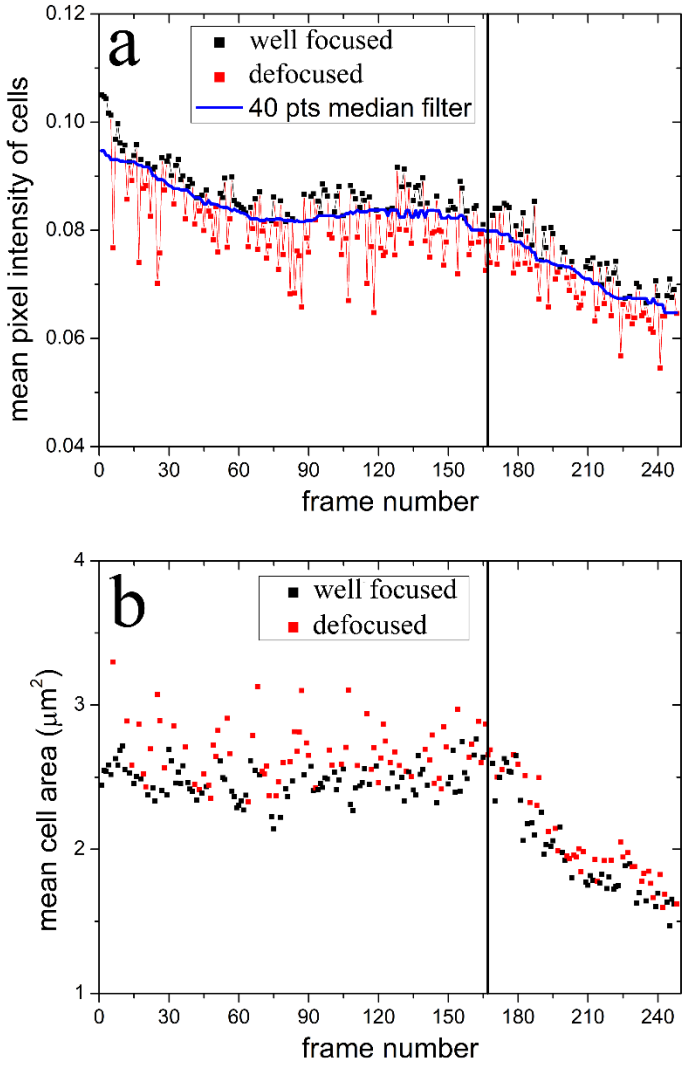



**Figure S3** Protocol for removing the defocused data points. (a) A 40-points median filter is applied to the time-series plot of the mean pixel intensity. The data points below the smooth curve are marked as defocused image frames. (b) All other measurements such as mean cell area from those frames are also masked in subsequent analysis. The vertical solid line indicates the time point of media change.

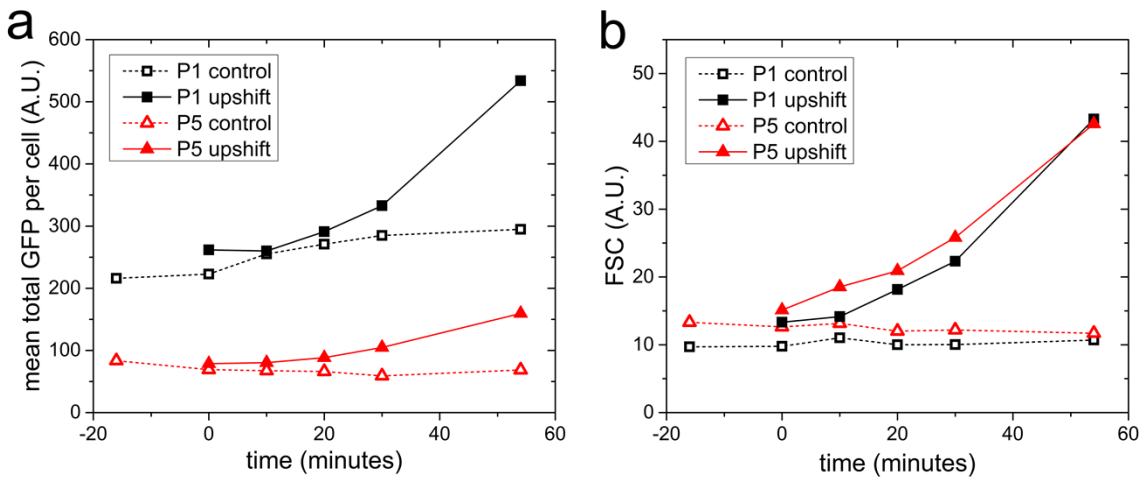



**Figure S4** Flow cytometer upshift experiment. Time-series plots of mean total fluorescence per cell (a) and mean forward scatter (b), measured from typical bulk upshift experiment for cells carrying the rrnBP1 promoter reporter or the T5 phage P5 promoter reporter. At time point 0, the culture growing in minimal medium (M9 with 0.2% casamino acids) was split in two, centrifuged and then re-suspended either in LB (filled symbols) or in the same minimal medium as before (empty symbols). Samples were harvested as described in Experimental, and FSC, SSC and GFP were measured for ~20,000 cells.

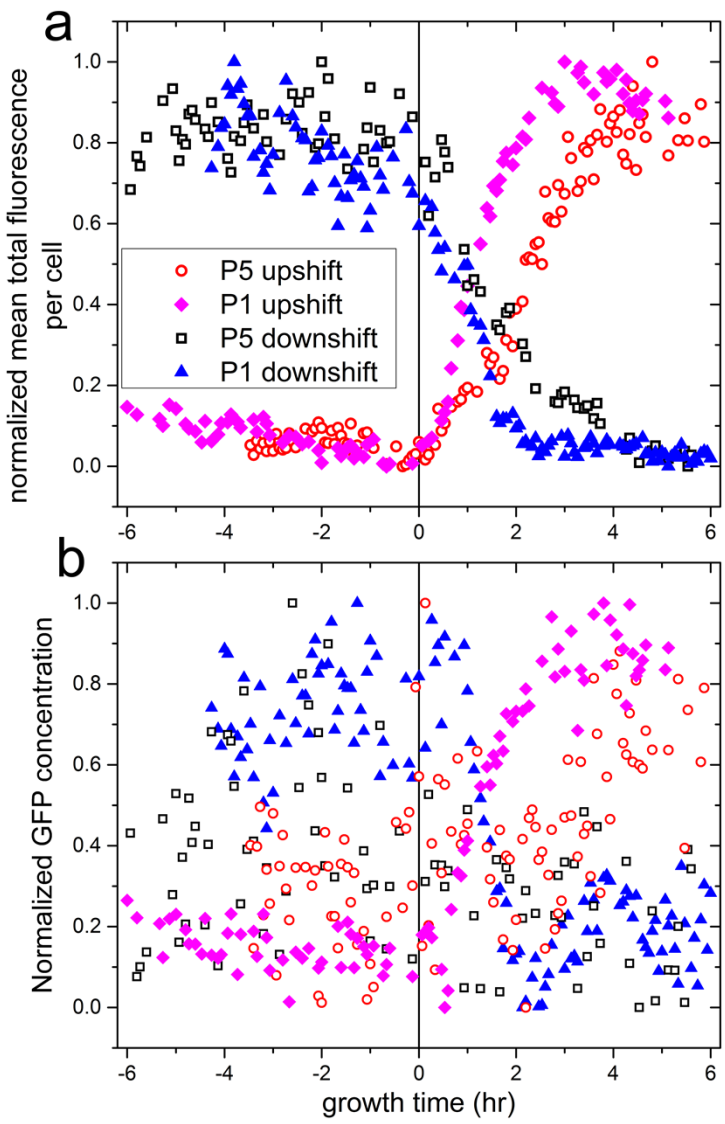



**Figure S5** Normalization of figure 4b (a) and figure 4d (b) such that the values at the minimum and maximum times are 0 and 1, respectively.