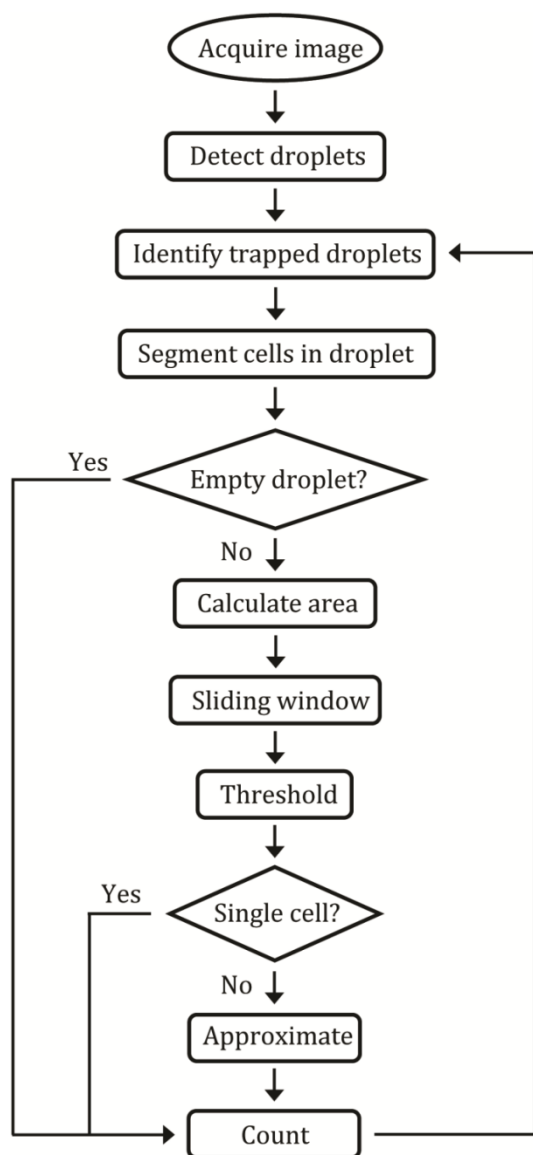# Supporting Information

Automated analysis of dynamic behavior of single cells in picoliter droplets

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Figure S1. Different steps for automatic quantification of cells in individual droplets are presented as a flowchart. Droplets are automatically detected in bright field images by the circular Hough transform. The identified droplets are labeled with a number for further analysis. Cells in each droplet are segmented from background of the fluorescent images. The area of the cells is calculated and a sliding window is applied to normalize the area of segmented cells over time. A threshold is defined by averaging the area of a single cell. The number of cells in individual droplets was approximated based on the area of a single cell.
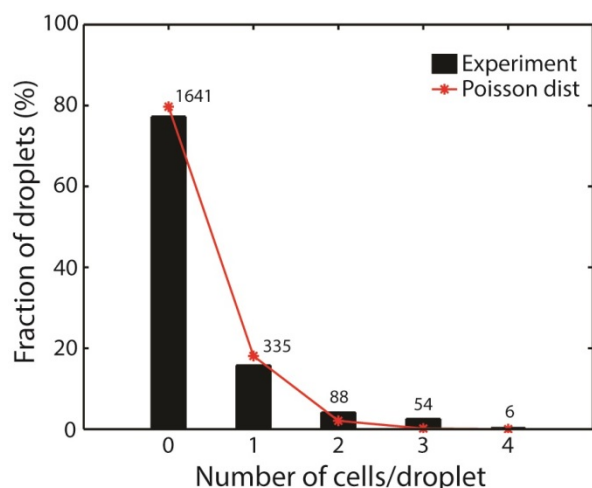
Figure S2. Distribution of cells per droplet. The fractions of droplets with different cell densities is in good agreement with the Poisson distribution (the red line). About 73% of droplets are found to be empty and the average number of cells in each droplet to be 0.22.

Image analysis time

A PC with a Windows 7 64-bit operating system and a CPU of 3.4 GHz and 16 GB RAM was used to carry out the image analysis. The total computation time for detecting the trapped droplets and to count number of cells in each droplet at each time point was less than 4 minutes.
A formula for calculating the total analysis time is:

$$Total\ Analysis\ time = number\ of\ areas \times (A+nB)$$

where A is the typical computational time for detecting and identifying the trapped droplets in the first frame, n is number of time points analyzed and B is the typical time for counting number of cells at each frame.
The total analysis time for all areas was 3.93 minutes.
The time series analysis for each area of the chip which contained on average 40 circular wells was less than 4 seconds.

Movie S1.

This movie shows a time series of florescent images of two cells encapsulated in a picoliter droplet over the incubation time of 11 hours. The imaging interval was 17 minutes.