Appendix A. Supplementary material

Materials and methods

Materials

A reference strain of *E. coli* (ATCC 25922) as well as a multi-drug resistant strain of *E. coli* (ATCC BAA 2471) were purchased from ATCC (Manassas, VA, USA). Of note, the multi-drug resistant strain is reported to be resistant to a variety of drug classes including carbepenems, β-lactams, and aminogycosidases (eg: gentamicin). During experiments, bacteria were suspended in Mueller-Hinton II Cation-adjusted broth purchased from Sigma-Aldrich (St. Loius, MO, USA). Resazurin sodium salt was used as the bacterial viability indicator and was purchased from Sigma-Aldrich along with the antibiotic gentamicin at a stock concentration of 50 mg/mL. Microfluidic devices were treated with Aquapel (Pittsburgh Glass Works, LLC) to render their surface hydrophobic. During droplet generation, the surfactant used for the continuous phase was poly(ethylene glycol) di-(krytox-FSH amide) and was purchased from RAN Biotechnologies (Beverly, MA, USA).

Bacterial culture conditions

Reference *E. coli* (ATCC 25922) and multi-drug resistant *E. coli* (ATCC BAA-2471) were separately plated, and an isolated colony from each was grown unto log phase in tryptic soy broth (TSB). The bacteria were then counted via plating in tryptic soy agar (TSA), and stocks were aliquoted and frozen with 20% glycerol (v/v) at -80 °C. Prior to each experimental run, a fresh aliquot of bacteria was thawed and washed twice with Mueller-Hinton broth.

Single-bacteria confinement in droplets

Confinement of bacteria in droplets can be described as a Poisson process (Collins et al., 2015; Guan et al., 2015; Köster et al., 2008). Therefore, the probability of finding *x* bacteria in a droplet is given by

$$P(x) = \frac{\lambda^x}{x!} e^{-\lambda} \quad (1)$$

where λ is the average number of bacteria per droplet, as determined by product of the input concentration of bacteria and the droplet volume. For experiments in this work, a bacterial concentration of 10^7 CFU/mL and a droplet volume of 20 pL is utilized, which equates to 1 bacterium in every 10 droplets. $\lambda = 0.1$ is chosen here to ensure that single-cell encapsulated droplets are easily observed with microscopy, while minimizing the encapsulation of 2 or more cells in a droplet. Indeed, the probability of digital encapsulation, *P(0) + P(1)*, in this case is 99.53%, ensuring that the vast majority of droplets contain only 0 or 1 cell.

Data analysis

A custom-built MATLAB program was developed for analyzing the raw fluorescence intensity data acquired from the APD. From the fluorescence intensity time trace of each experimental run, the program looks for individual droplets by surveying and quantifying peak widths and average peak heights. Once droplet position and average fluorescence intensity are identified for all droplets in a sample, the program plots the intensities as a histogram with a bin size of approximately 10 photon counts. Resulting histograms typically follow a bimodal distribution, and in order to classify the respective subpopulations without applying an arbitrary threshold, a Gaussian mixture model (MATLAB) is used. This method uses statistical mixture modeling (expectation maximization) to fit Gaussian curves to each population in the histogram. Following the curve fit, we can find the relative weights (or probability density) of each fitted curve in order

to separate and quantify the "positive" histogram population (droplets with growing bacteria) from the "negative" population (droplets with no growing bacteria). The reported p-value used to differentiate the positive droplet populations of the no-antibiotic control experiments from the gentamicin experiments for the reference strain of *E. coli* was obtained from a Student's t-test performed in GraphPad Prism.

Supplementary Figures:

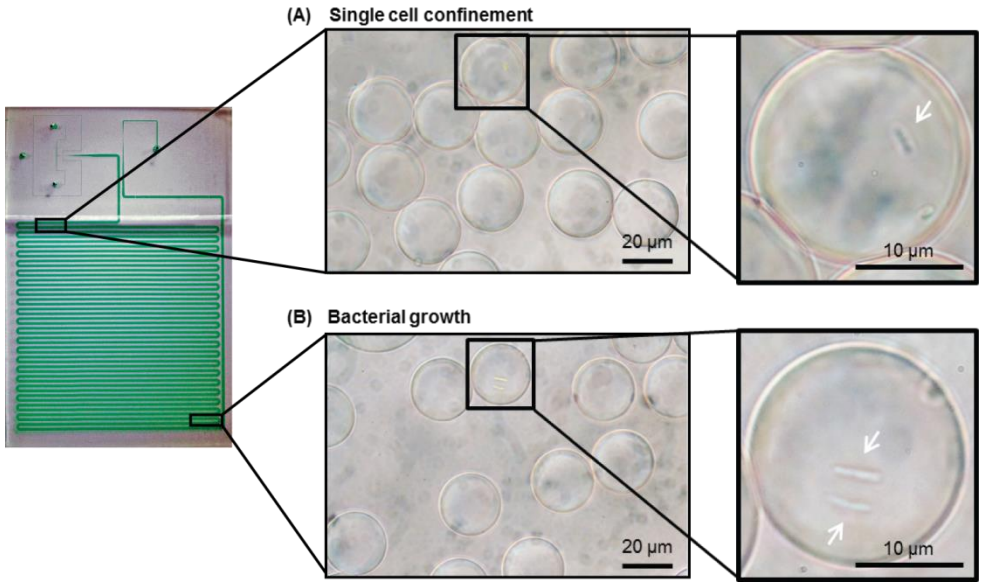

Figure S1: Micrographs of generated droplets confirm (A) digital single-cell confinement after droplet generation and (B) bacterial growth in droplets after 60 minutes of on-chip incubation at 37 ^{0}C. Arrows indicate presence of single *E. coli* cell.

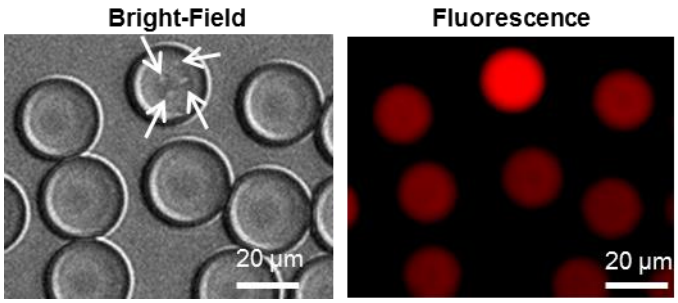

Figure S2: (A) Bright field micrograph of cells growing droplets after 1 hour of incubation. (B) Fluorescence micrograph, with red pseudocolor for clarity, corresponding to the droplets shown in (A) shows that droplets either strongly fluoresce (indicative of bacterial growth) or weakly fluoresce (empty droplets).

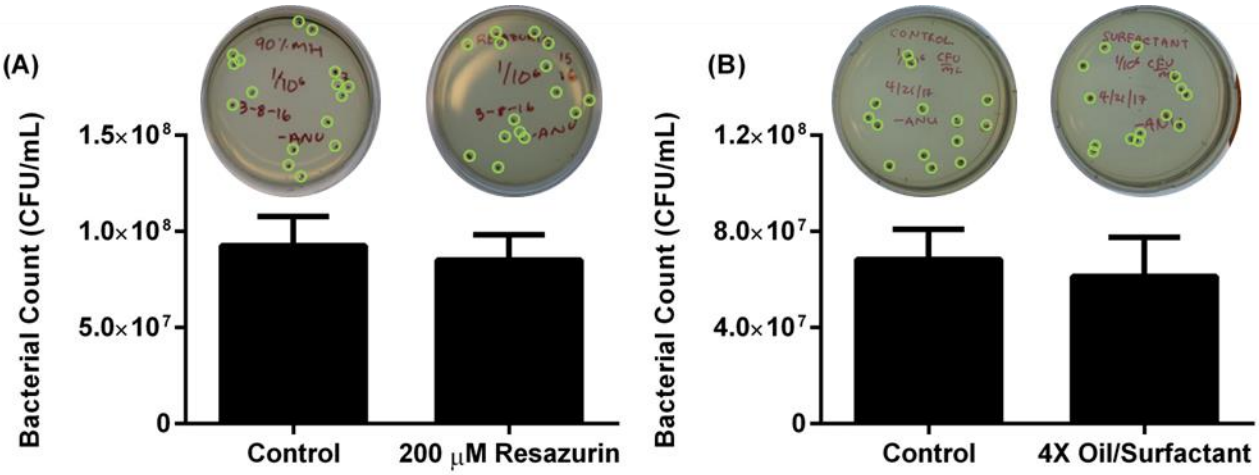

Figure S3: No significant effect of resazurin and surfactant on bacterial growth. (A) After overnight plating of *E. coli* with and without the addition of 200 µM resazurin, the grown colonies (circled in green) were quantified. The difference between the colony concentration without resazurin (9.2×10^7 CFU/mL) and with resazurin (8.6×10^7 CFU/mL) is not significant.

Thus the addition of resazurin has negligible effect on *E. coli* growth and is therefore a suitable assay for the drop FAST platform. (B) In a similar manner, *E. coli* growth with and without the presence of 5% (poly(ethylene glycol) di-(krytox-FSH amide)) in FC-40 oil was plated and quantified. Colony concentration with the surfactant present (6.1×10^7 CFU/mL) is not significantly different from the control (6.8×10^7 CFU/mL).

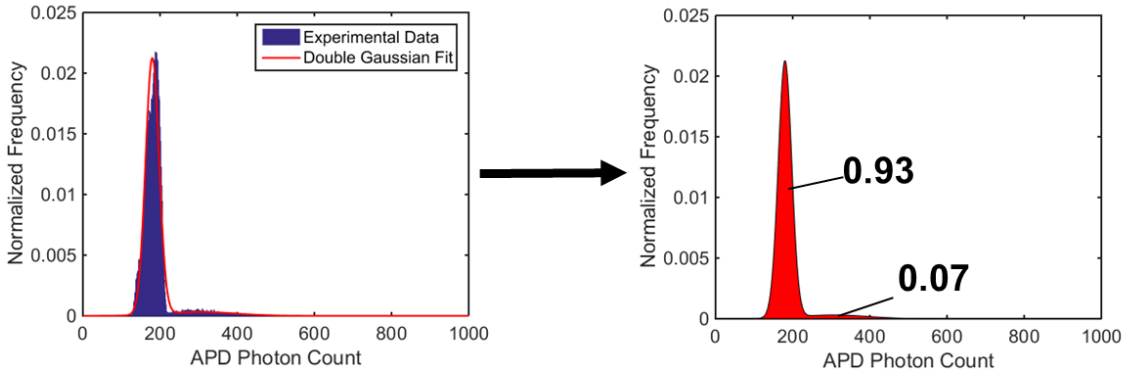

Figure S4: A Gaussian mixture model (GMM) is used to fit the histograms generated from acquired data. The GMM allows us to represent the data as the sum of two component probability density functions. We are then able to extract the total probability of each full population in the mixture, and this allows us to quantify the frequency of a "positive" droplet.

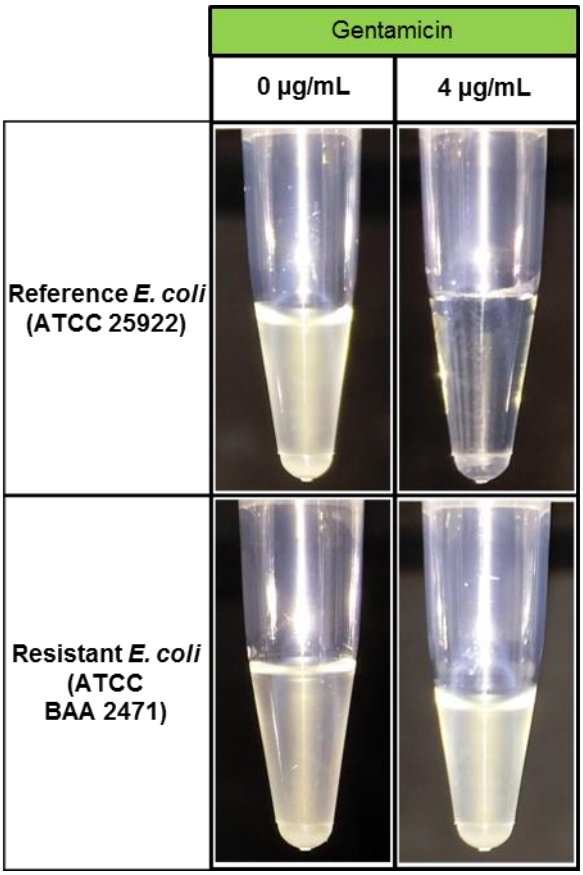

Figure S5: Bulk broth dilution test of reference and resistant strains of *E. coli* against gentamicin. Benchtop (16 hour) incubation of *E. coli* along with 4µg/mL gentamicin shows no growth for the reference strain, whose tube is optically transparent compared to the no-antibiotic condition, and growth (via turbidity) for the resistant strain.