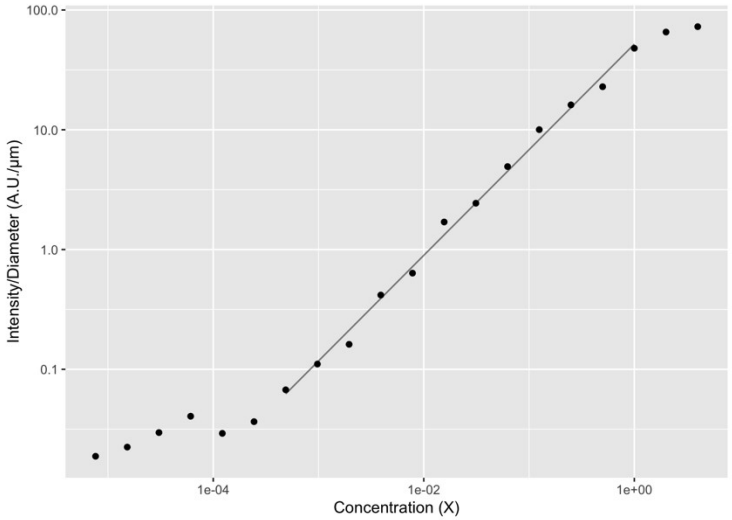
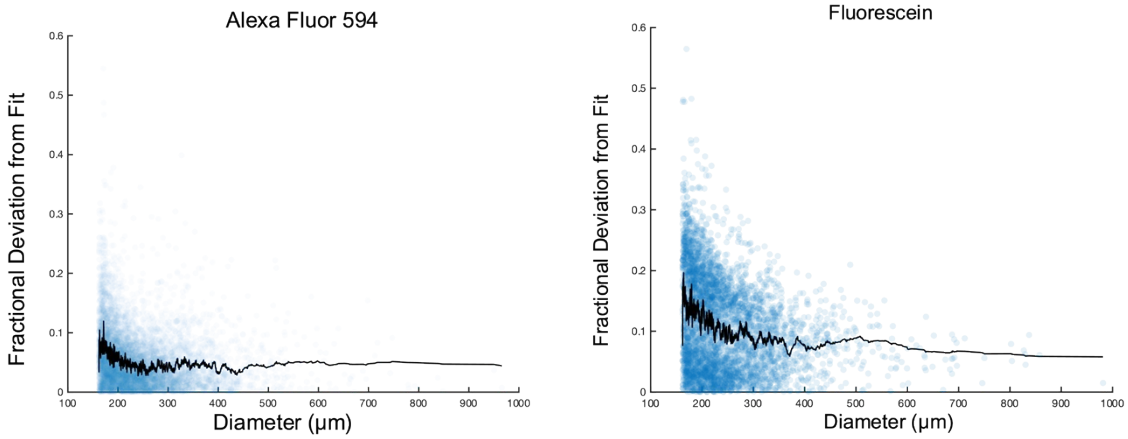
Supplementary Information (SI) for Lab on a Chip.

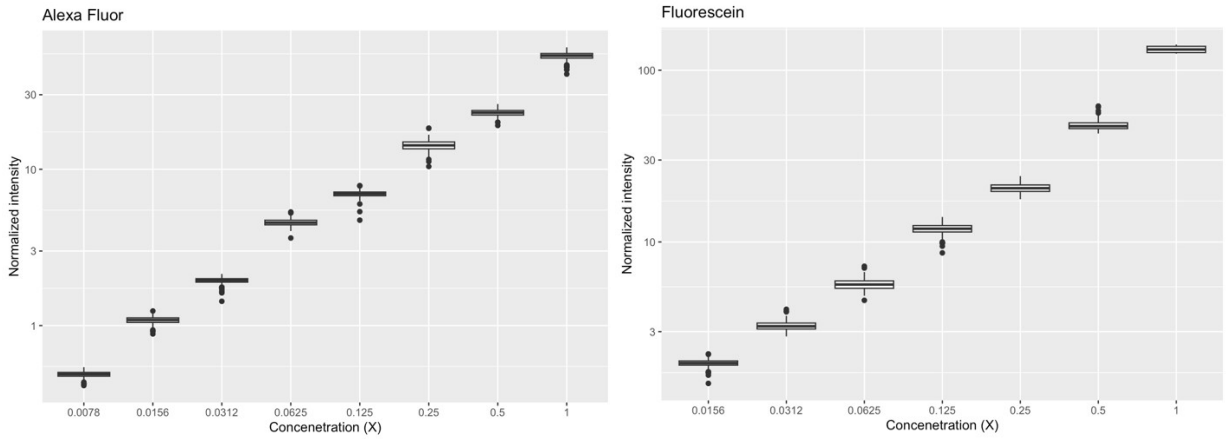
Supplementary Figures and Tables



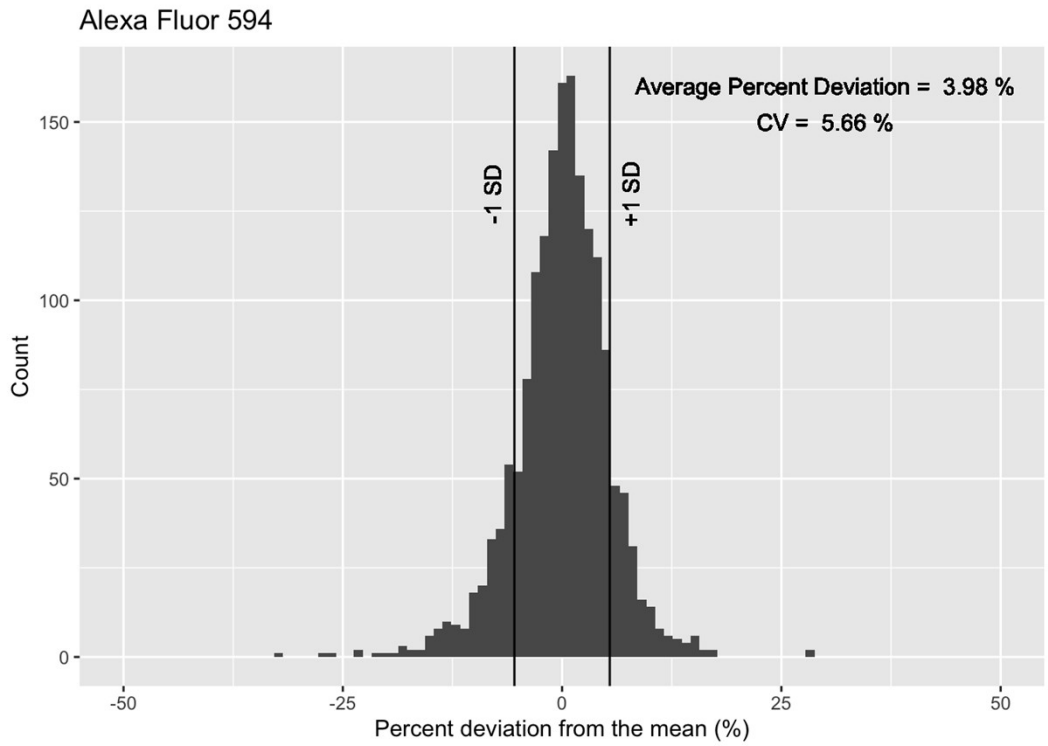
Supplementary Figure 1. Normalized intensity against concentration for two-fold dilutions of fluorescein in droplets. The linear region (0.0003X to 1X, or 0.003 mg/ml to 10 mg/ml) exists between saturation at higher concentrations and a noise-dominated region at lower concentrations.



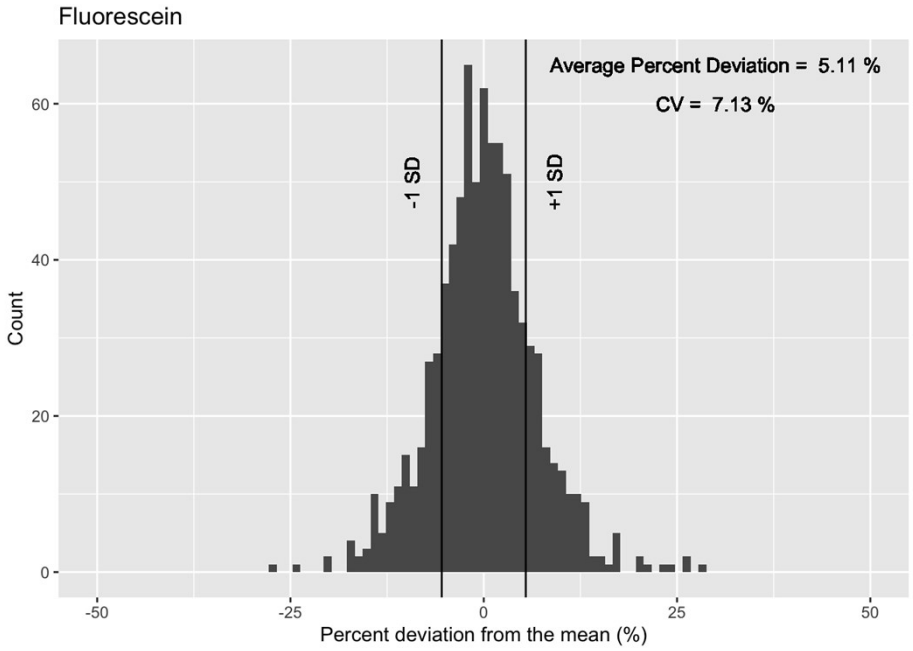
Supplementary Figure 2. Fractional deviation from the line of best fit from Fig. 2c for Alexa Fluor 594 (left)and fluorescein (right), only showing droplets above the diameter cut-off of 150 µm (linear scale). Moving averages have window size of 50 samples. Fractional deviation from fit is calculated as the absolute difference between each droplet's measured intensity and its fitted intensity, normalized by the fitted intensity.



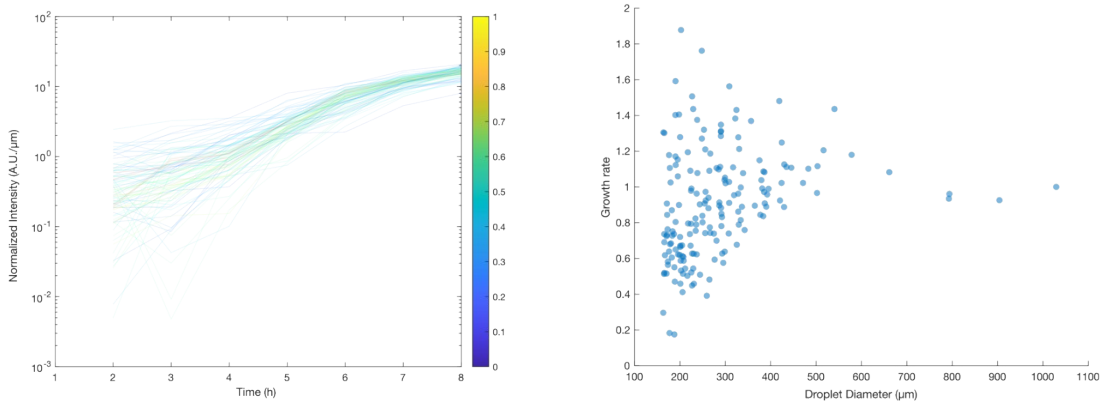
Supplementary Figure 3. Violin plots of normalized intensities for Alexa Fluor 594- (left) and fluorescein- (right), encoded droplets whose diameters range from 200 to 230 µm.



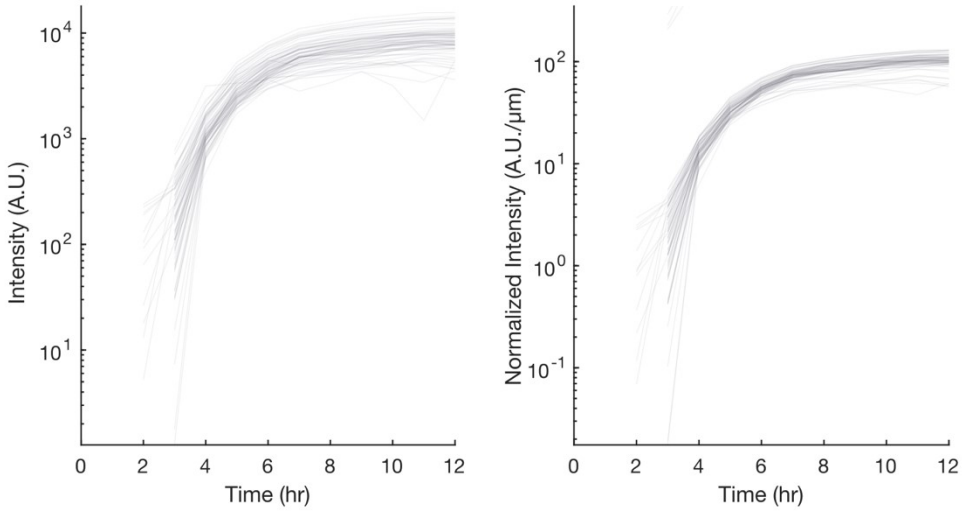
Supplementary Figure 4. Histogram of percent deviation from the mean for Alexa Fluor 594-encoded droplets, diameters 200 to 230 µm, all concentrations aggregated. Lines represent one standard deviation from the mean.



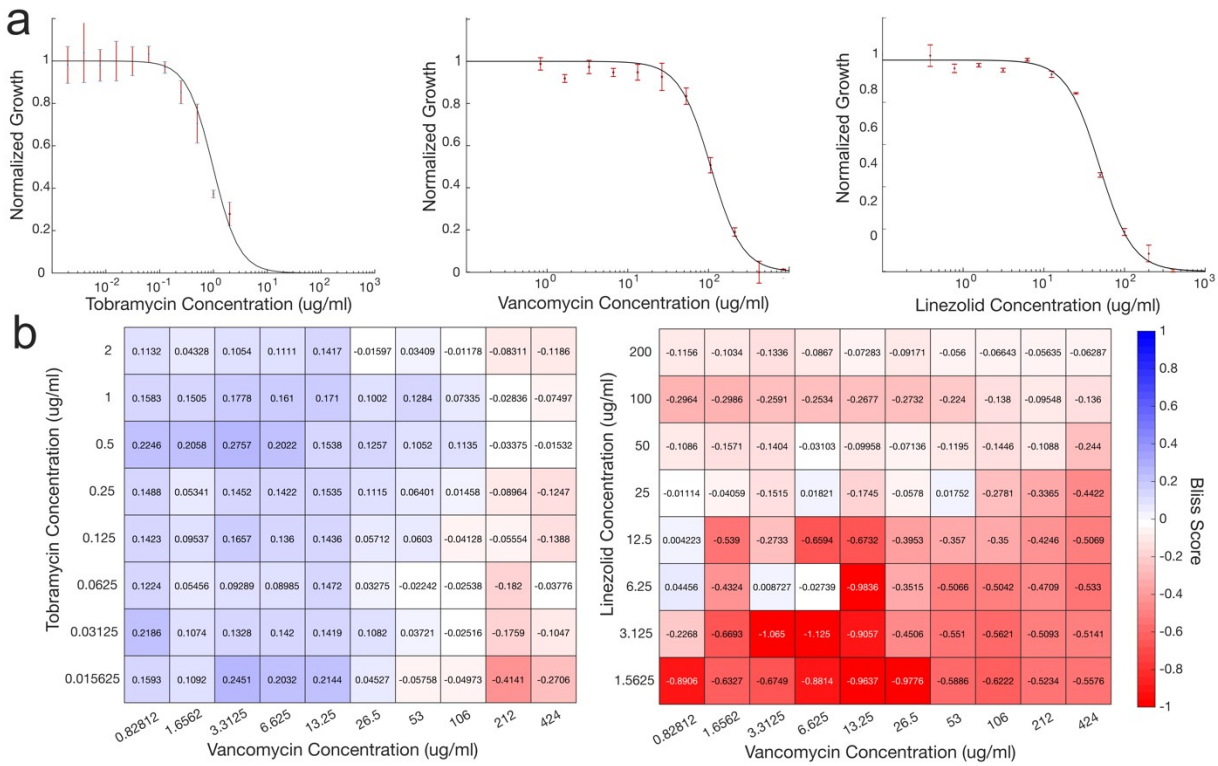
Supplementary Figure 5. Histogram of percent deviation from the mean for fluorescein-encoded droplets, diameters 200 to 230 µm, all concentrations aggregated. Lines represent one standard deviation from the mean.



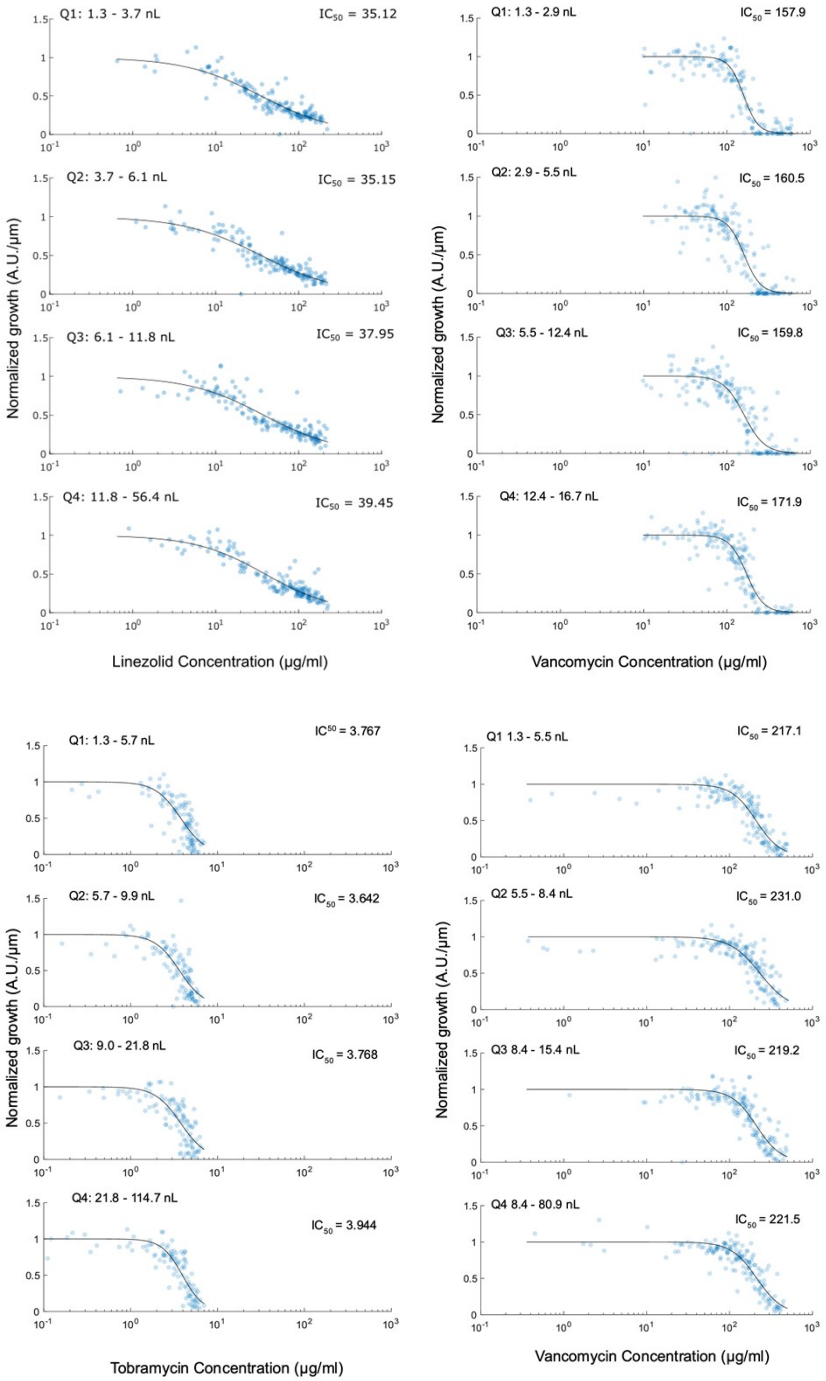
Supplementary Figure 6. Growth curves, colorized by droplet diameter (left) were used to calculate the growth rate for t = 1 hour to t = 6 hours. Growth rates are plotted against respective droplet diameter (right).



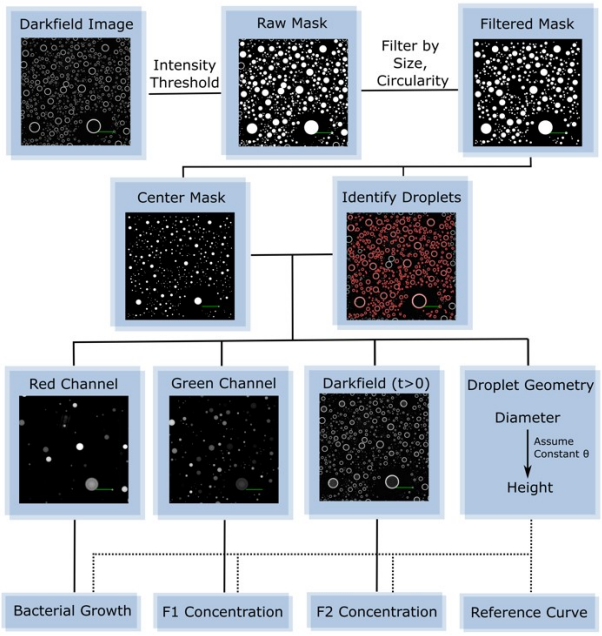
Supplementary Figure 7. Change in darkfield intensity over 12 hours (left) and change in darkfield intensity, normalized by droplet size (right). As with eight hours, the normalization by diameter decreases the growth spread among droplets. The plateau begins around eight hours, levelling off by hour 12.



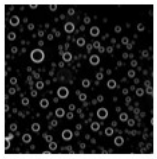
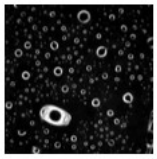
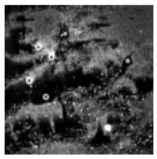
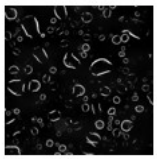
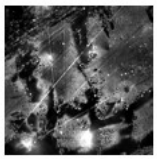
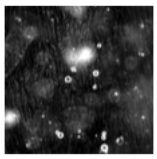
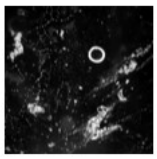
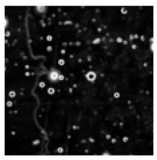
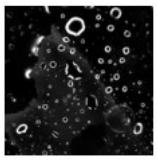
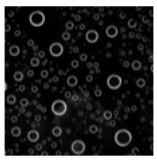
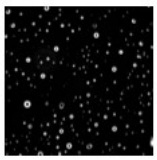
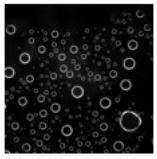
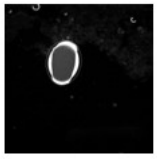
Supplementary Figure 8. (a) Dose-response curves for tobramycin (left), vancomycin (center), and linezolid (right) obtained with well plates. (b) Well plate checkerboard analysis of chosen antibiotics for pairwise synergism (left) and antagonism (right) assays, colored by Bliss Scores. Axes represent antibiotic concentrations at two-fold dilutions. Blue represents synergism, red represents antagonism.



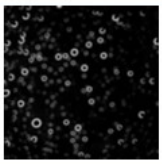
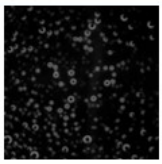
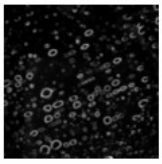
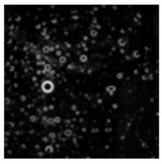
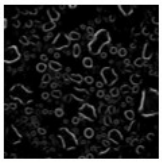
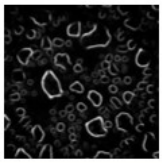
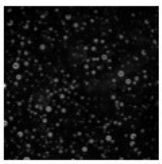
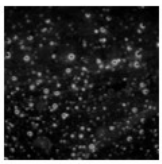
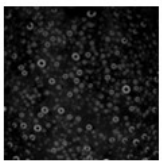
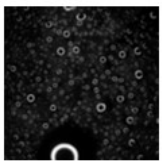
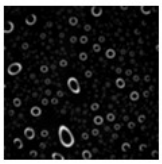
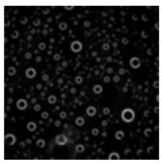
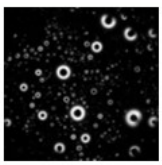
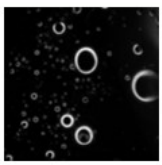
Supplementary Figure 9. Dose-response curves for synergism assay (top, linezolid and vancomycin) and antagonism assay (bottom, tobramycin and vancomycin), sectioned by volume quantile. IC_{50} is the half-max concentration of the antibiotic, obtained from the fitted Hill functions.



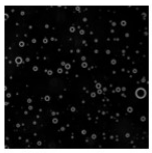
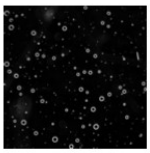
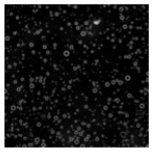
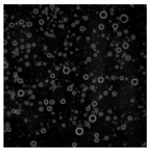
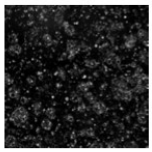
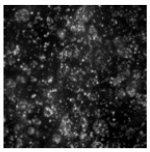
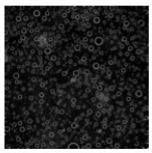
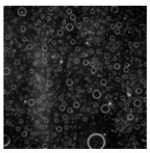
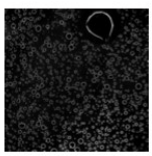
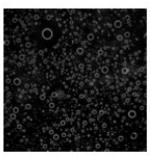
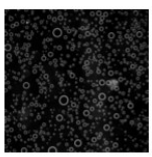
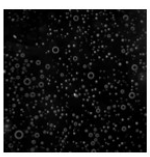
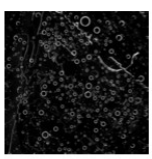
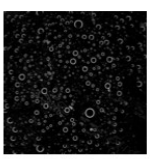
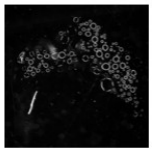
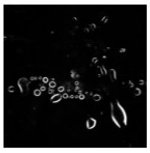
Supplementary Figure 10. Overview of the analysis pipeline. Darkfield images are used to generate masks, which are filtered and used to identify droplets. A center mask is applied to the fluorescence and darkfield images, which, paired with droplet diameter, is used to normalize intensities and calculate concentrations and microbial growth.

Surface	Oil	Image (t=0h)	Image (t=24h)	Comment
FOTS	Silicone Oil			Droplet smearing
SOFT99 Glaco Mirror Coat	Silicone Oil			Droplet evaporation
Minwax Helmsman Spar Urethane	Silicone Oil			Non-circular, Droplet evaporation
Hydrobead Standard Super Water Repellant Coating	Silicone Oil			High background signal
Nasiol Marine Tex	Silicone Oil			Inconsistent contact angle
Krylon UV-Resitant Clear Acrylic Coating	Silicone Oil			Droplet shrinkage
Teflon Tape	Silicone Oil			Inconsistent contact angle, droplet evaporation

Supplementary Table 1. Surface-oil combinations screening.

Surface	Oil	Image (t=0h)	Image (t=24h)	Comment
FOTS	Mineral Oil			Droplet smearing
SOFT99 Glaco Mirror Coat	Mineral Oil			Droplet smearing
Minwax Helmsman Spar Urethane	Mineral Oil			Non-circular
Hydrobead Standard Super Water Repellant Coating	Mineral Oil			High background signal
Nasiol Marine Tex	Mineral Oil			Inconsistent contact angle
Krylon UV-Resitant Clear Acrylic Coating	Mineral Oil			Droplet smearing
Teflon Tape	Mineral Oil			Droplet Smearing

Supplementary Table 1 (continued). Surface-oil combinations screening.

Surface	Oil	Image (t=0h)	Image (t=24h)	Comment
FOTS	FC-40			Used in Main Text
SOFT99 Glaco Mirror Coat	FC-40			Higher droplet inconsistency than FOTS and FC-40
Minwax Helmsman Spar Urethane	FC-40			Non-circular
Hydrobead Standard Super Water Repellant Coating	FC-40			High background signal
Nasiol Marine Tex	FC-40			Inconsistent contact angle
Krylon UV-Resitant Clear Acrylic Coating	FC-40			Droplet shrinking
Teflon Tape	FC-40			High background signal
PGLA	FC-40			Non-circular

Supplementary Table 1 (continued). Surface-oil combinations screening.

SUPPLEMENTARY VIDEOS

Supplementary Video 1. Protocol of droplet spraying and immersing sample in oil. The OmniTray is prepared with double-sided tape and 30 mL of FC-40. Food coloring in water is sprayed, generating combinatorial droplets. The slide is picked up and gently lowered into the oil, then adhered to the double-sided tape by applying pressure. Note: The set-up has been amended for imaging purposes. When working with bacteria, spraying occurs within a plastic container, open side facing the investigator, within a biosafety cabinet. The glass slide is propped up on the far side of the container within a petri dish, and the solutions are sprayed into the container towards the slide.

Supplementary Video 2. Droplets are stable for 12 hours in FC-40 with FOTS surface treatment. Droplets were imaged using 2X objective to display more droplets, while all other videos and images were captured at 4X.

Supplementary Video 3. Silicone oil with surface treated with FOTS allows for droplet movement and shrinkage.

Supplementary Video 4. Insufficient oil volume leads to shrinkage of droplets.

Supplementary Video 5. Droplets sprayed at 19^{o}C and 64% relative humidity. Latter portion is sped up by 20X, as there are only two droplets remaining.

Supplementary Video 6. Droplets sprayed at 19^{o}C and 64% relative humidity, with sample resting upon a chilled glass plate, cooled to 4^{o}C before the experiment and immediately placed under the sample container. Speed is 3X real time.