Electronic Supplementary Material (ESI) for Analyst.

Supplemental Information: Polydisperse emulsion digital assay to enhance time to detection and extend dynamic range in bacterial cultures enabled by a statistical framework

Image analysis with MATLAB Image Processing Toolbox

Example code and resulting image for in-chip droplet counting:

```
chip = imread('chip_full.png');  % reading image into MATLAB
imshow(chip)  % display image
[centers, radii] = imfindcircles(chip, [5 11], 'ObjectPolarity', 'bright', 'Sensitivity', 0.92, 'EdgeThreshold', 0.09); % parameters for finding circles
viscircles(centers, radii, 'EdgeColor', 'r') % drawing a red outline around each identified circle
```

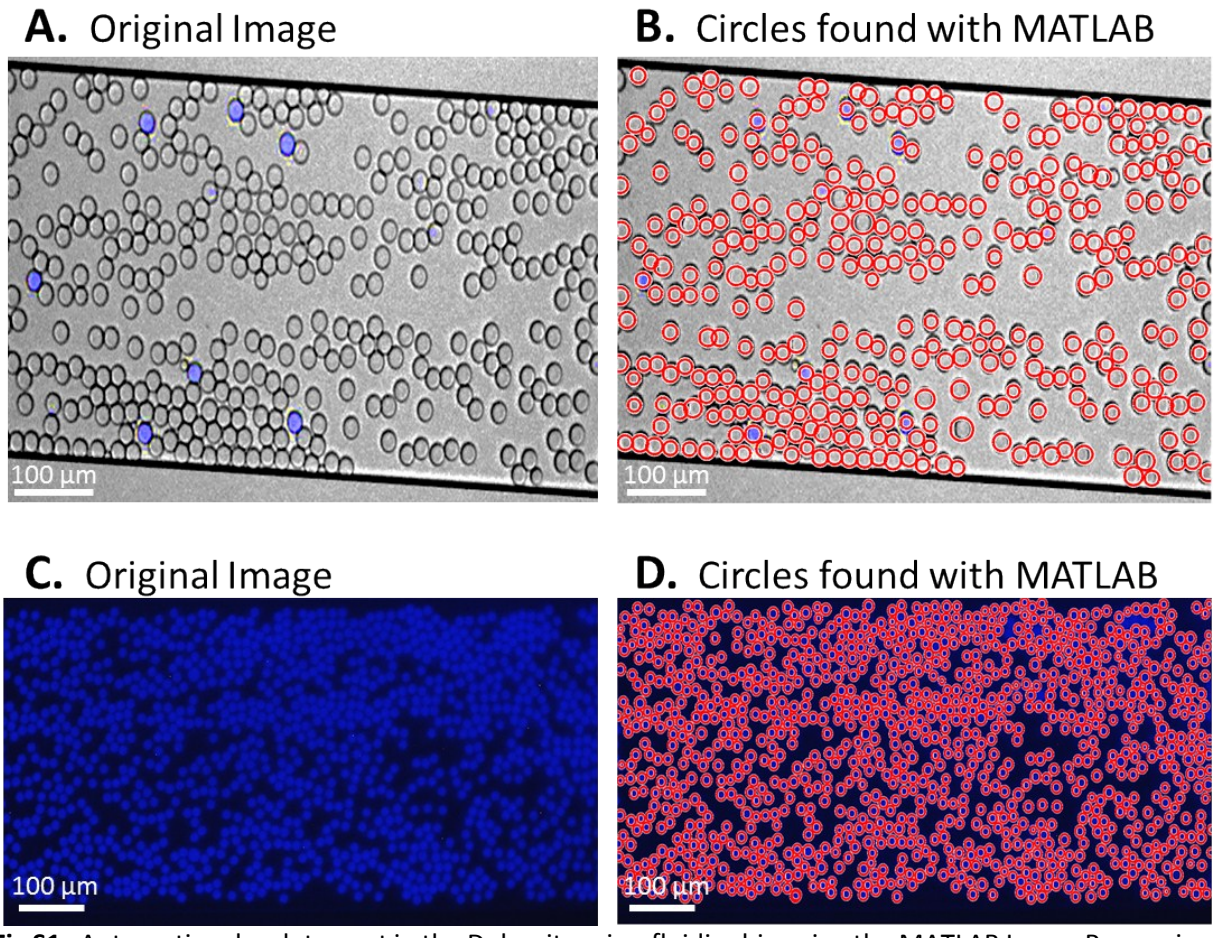


Fig S1. Automating droplet count in the Dolomite microfluidic chip using the MATLAB Image Processing Toolbox. **A.** The original image: brightfield merged with fluorescent image to identify positive (blue) droplets. **B.** The output from MATLAB with red outlines drawn around each identified circle. **C.** Original fluorescent image, and **D.** output from MATLAB with red outlines draw around identified circles.

Example code and resulting image for our method of emulsion formation and droplet counting:

droplet = imread('droplet.png');
imshow(droplet)
[centers, radii] = imfindcircles(droplet, [5,14], 'ObjectPolaraity', 'bright', 'Sensitivity', 0.96, 'EdgeThreshold', 0.15);
viscircles(centers, radii, 'EdgeColor', 'r')

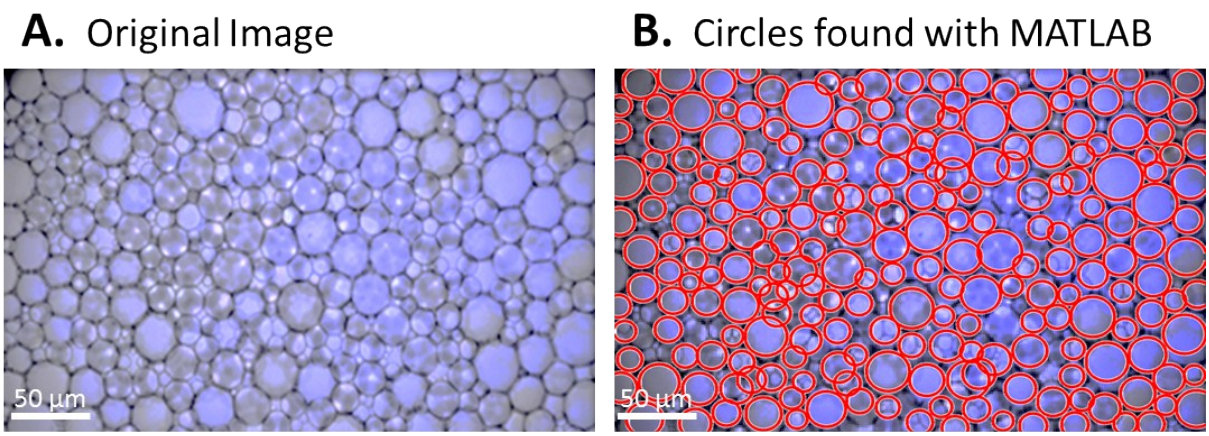


Fig S2. Automating droplet count in for our presented method of droplet preparation using the MATLAB Image Processing Toolbox. **A.** The original image: brightfield merged with fluorescent image to identify positive (blue) droplets with a high input bacterial count, as indicated by many positive droplets. **B.** The output from MATLAB with red outlines drawn around each identified circle. Although the algorithm does not identify every droplet in the polydisperse distribution, it does capture the vast majority (+90%) and does not bias either larger or small droplets, but droplets that are overlapping or obscured.

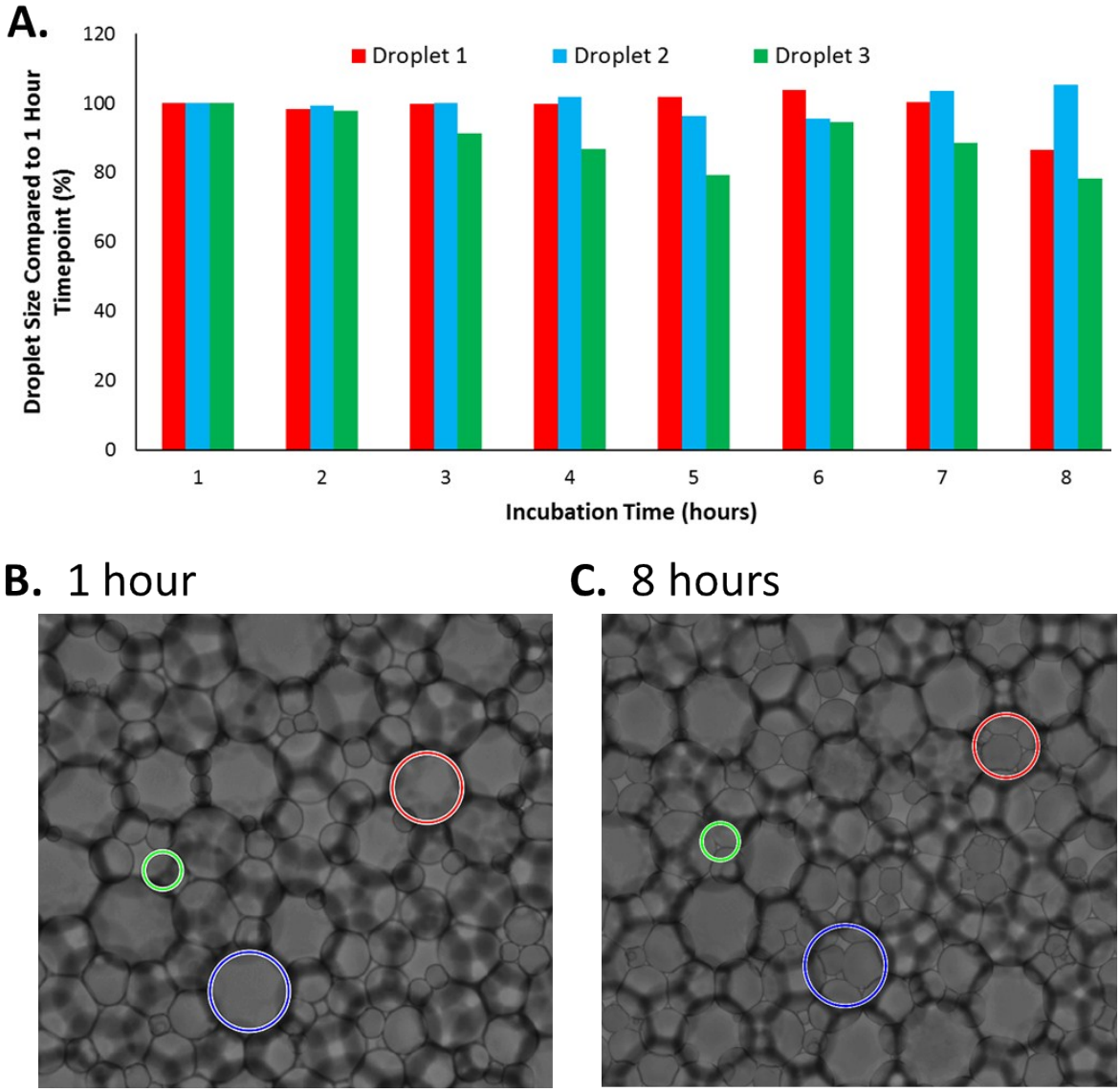


Fig S3. Droplets created with our method remain stable over time. **A.** Measured droplet volumes during an 8 hour incubation at 37°C. **B.** Image of droplets at 1 hour and **C.** 8 hours. There is slight movement of the droplets during the incubation, potentially due to evaporation from imperfect chamber sealing during the 8-hour experiment, but the relative positions and sizes remain stable.

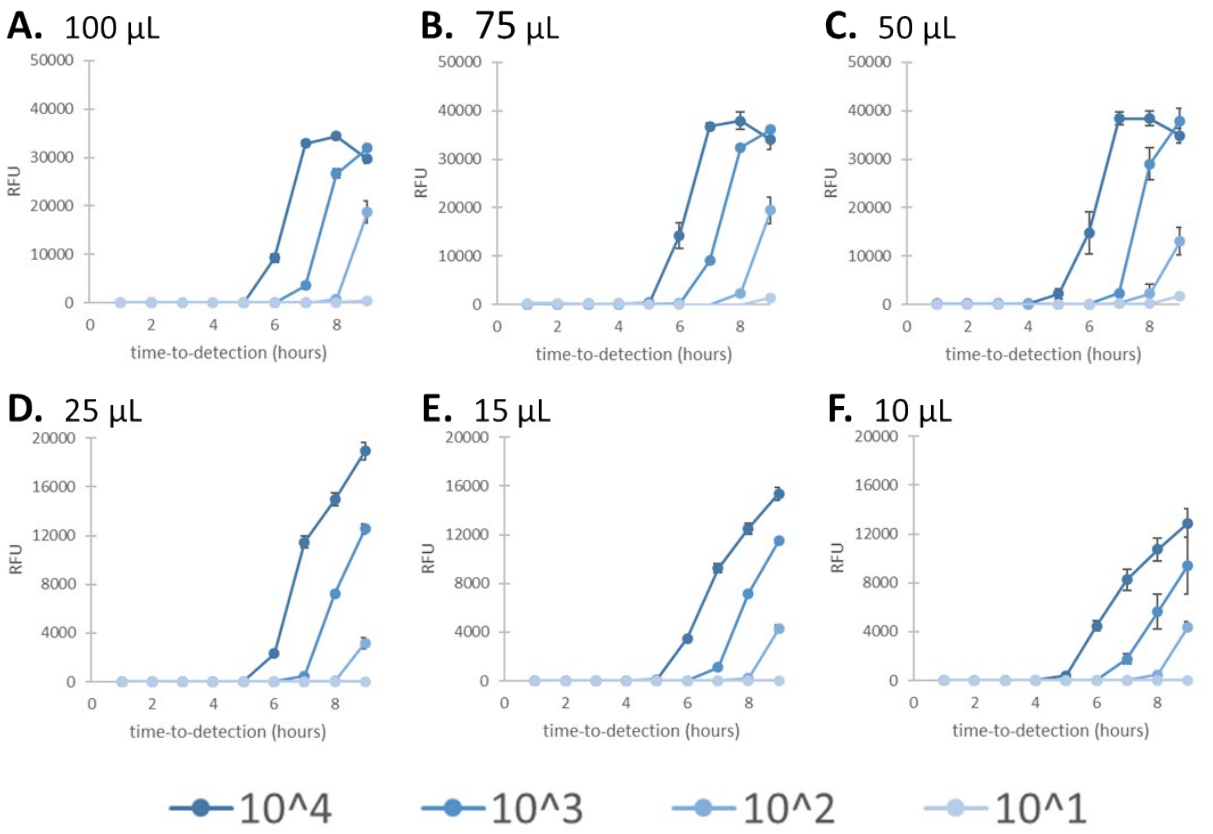
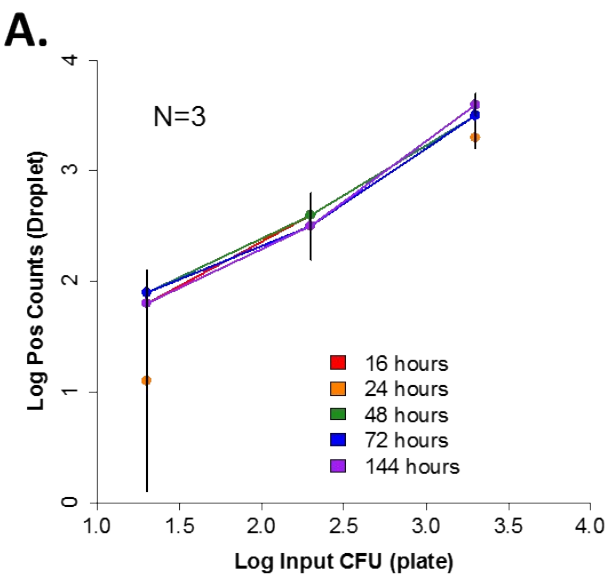


Fig S4. Comparing volumes for bulk culture measured in a well plate using a plate reader. Input amounts of bacteria remained constant across volumes. There was observable difference for time-to-detection across any of the volumes or concentrations of bacteria tested. **A-C** were run in 96-well plates while **D-F** were run in 384-well plates.



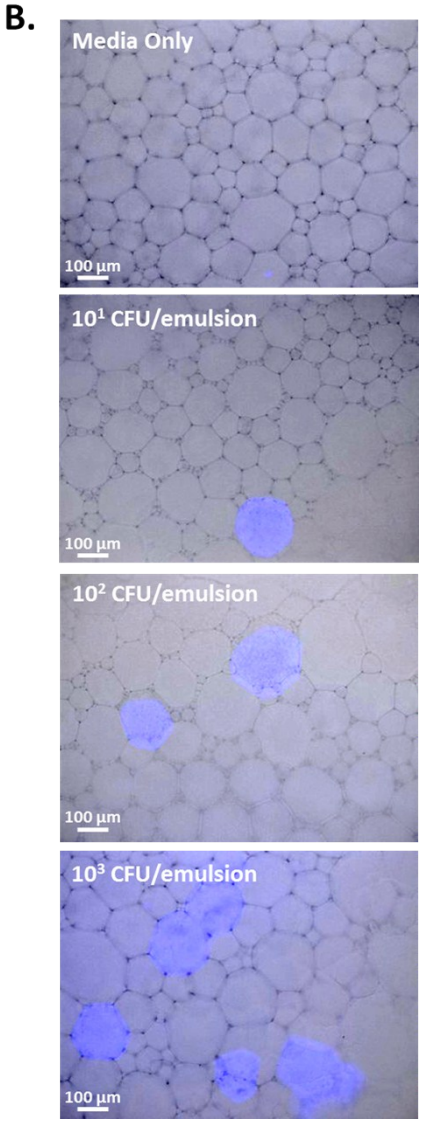


Fig S5. Monitoring droplet stability and environmental isolation over time. **A.** Comparing measured *E. coli* concentration at multiple time points and concentrations to plated culture (plates counted at 24 hours). Averages of N=3 biological replicates are reported with error bars representing ± one standard deviation. *Note, one of the 24 hour samples with 10^1 input had no visible positive droplets making it a count of zero. Averaging this zero resulted in the large error for that point. **B.** Representative images of culture in hand-made droplets at varying concentrations (48 hour time point). Images are a merge of bright field for droplet identification and fluorescence (Ex: 360 nm, Em: 450 nm, 1 sec exposure) for *E. coli* growth.

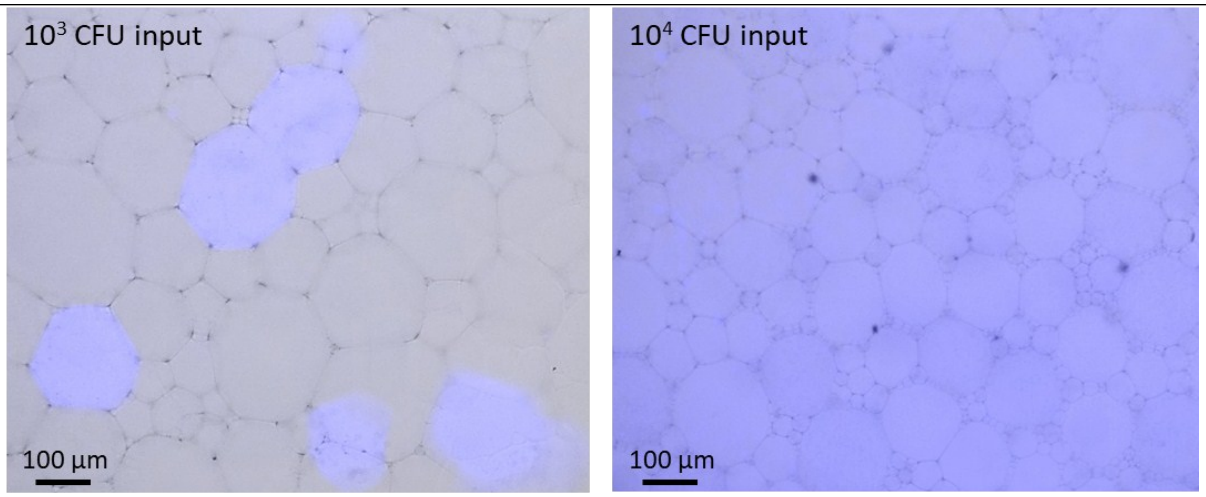


Fig S6. When 10^4 or higher CFU are used as the input to our emulsions, the ability to accurately count positive droplets is saturated, droplets will contain more than one pathogen. Samples with 10^4 or higher CFU/µL exceed the dynamic range of our system.