Electronic Supplementary Material (ESI) for Lab on a Chip.
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High-Throughput Screening of Antibiotic-Resistant Bacteria in Picodroplets

Supporting information

1. MIC measurement procedure

Organism streak-out preparation:

Streak out for single colonies from frozen stocks of HS151 onto TSA+SB. Incubate at 37˚ C overnight.

Plates were sealed with parafilm and stored at 4° C for up to 4 weeks.

Inoculum overnight preparation:

For HS151 inoculate a single colony into 3 mL of TSB, incubate shaking at 225 rpm overnight at 37° C.

Prepare drug dilution and assay plates:

Prepare assay plates, prepare 2x the top drug concentrations in column 1 in 100 µL of 4% DMSO.

An example of how to set up the controls is shown in the table below. Controls are dissolved in 100% DMSO.

compound	start µg/mL	2x µg/mL	stock mg/mL	stock µL	DMSO µL	dH20
Fusidic Acid	64	128	5	1.28	0.72	48

Add 50 µL of 4% DMSO to columns 2-12.

Samples will be serially diluted 1:2 (50 µL into 50 µL) across the plate to column 11, leave column 12 as a no drug growth control. Remove extra 50 µL from column 11 and discard.

Inoculate assay plates:

Dilute HS151 TSB overnight culture 10^{-4}. First, dilute overnight culture 30 µl into 3 mL TSB for 10^{-2}, and then further dilute 10^{-2}, 30 µL into 3 mL 2x TSB for 10^{-4}.

Add 50 µL to assay plate, giving a final 100 µL assay volume in 1x TSB.

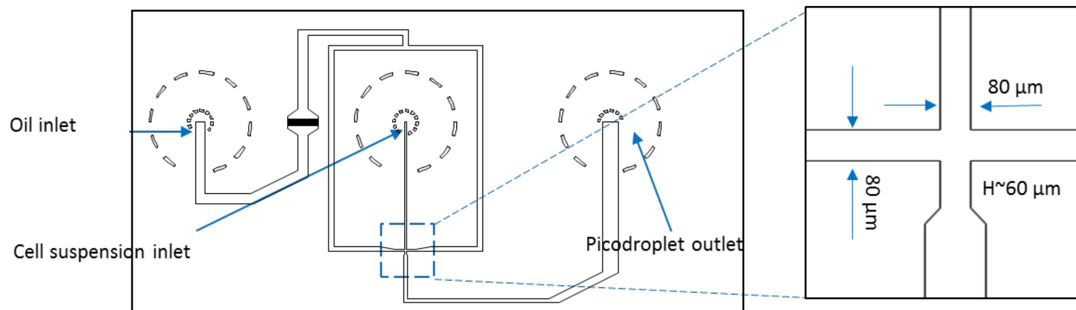
Incubate overnight at 37˚ C for 20-22 hours.

Read plates:

Use the microtitre plate viewer to score wells for the minimal inhibitory concentration (MIC) for each compound.

2. Picodroplet generation

<u>Biochip Design:</u>



Flow Conditions:

Oil Phase	3 % Pico-Surf™ 1 in Novec™ 7500
Aqueous Phase	Cell library sample
Flow Rates	Oil Phase: 3500 µL/h Aqueous Phase: 2000 µL/h
Picodroplet Generation Rate	**1714 Hz**
Picodroplet Size	324 pL/85 micrometer

3. Procedures for breaking the emulsion

Pico-Break™ 1 is used to induce phase separation of an aqueous emulsion stabilised with a fluorous surfactant. Pico-Break™ 1 contains a proprietary, orange-coloured, fluorocarbon dye that is soluble in fluorophilic solvents. The dye is used as a phase contrast reagent in order to more easily visualise the boundary layer between the two liquid phases. The procedure includes:

1. Briefly spinning the emulsion in a (micro) centrifuge to ensure the emulsion is floating on top of the fluorous oil.

2. Using a pipette to carefully remove the Pico-Surf™/oil (bottom layer).

3: Adding an equal volume of Pico-Break™-1

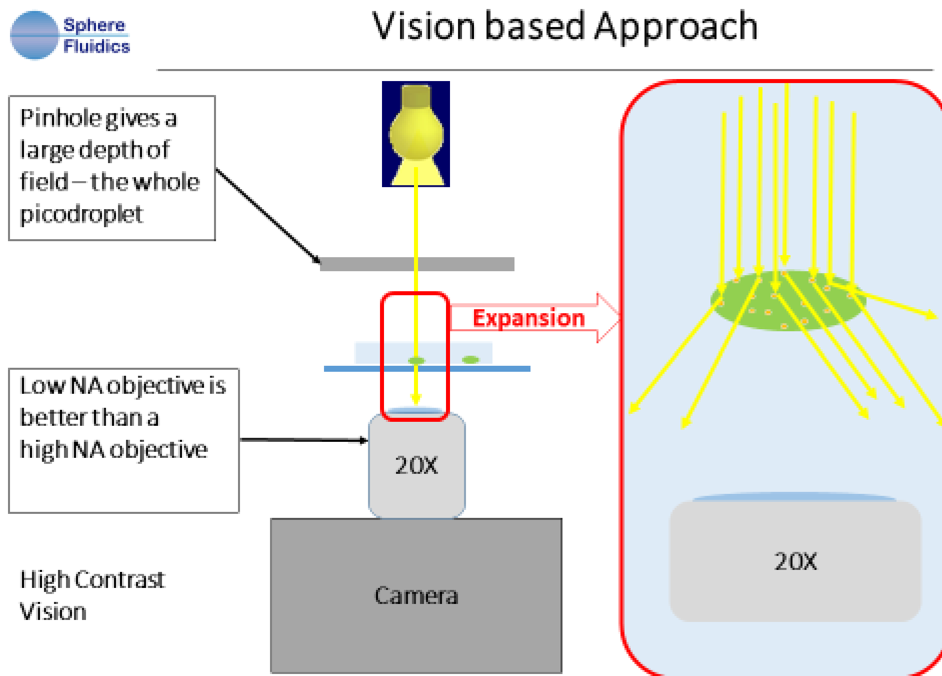
4. Gently rocking the tube by hand for a few seconds.

5. Briefly spinning the tube.

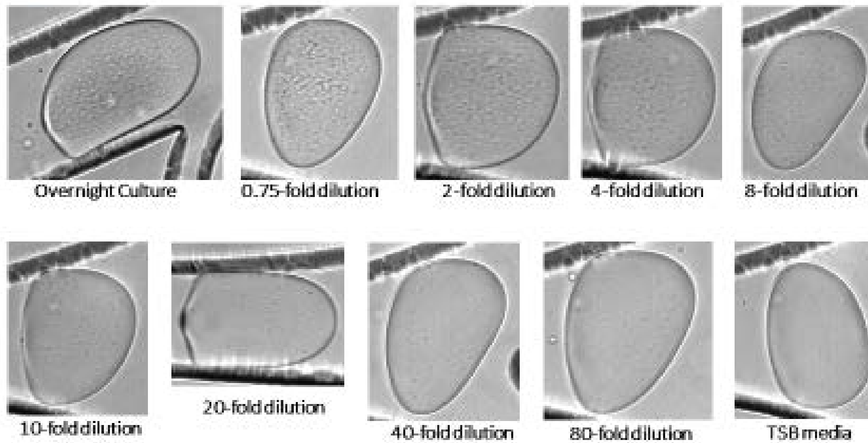
6. Pipetting out the top layer.

4. Vision Based Approach for determining the bacterial occupancy of picodroplets

Optical Arrangement: The microfluidic biochip is illuminated using a pinhole aperture to give a large depth of field while using a Zeiss LD-Plan Neofluar, 0.4 NA, 20X objective. Movies were collected using a Vision Research Phantom Miro eX4 4GB black and white fast camera. See slide below.

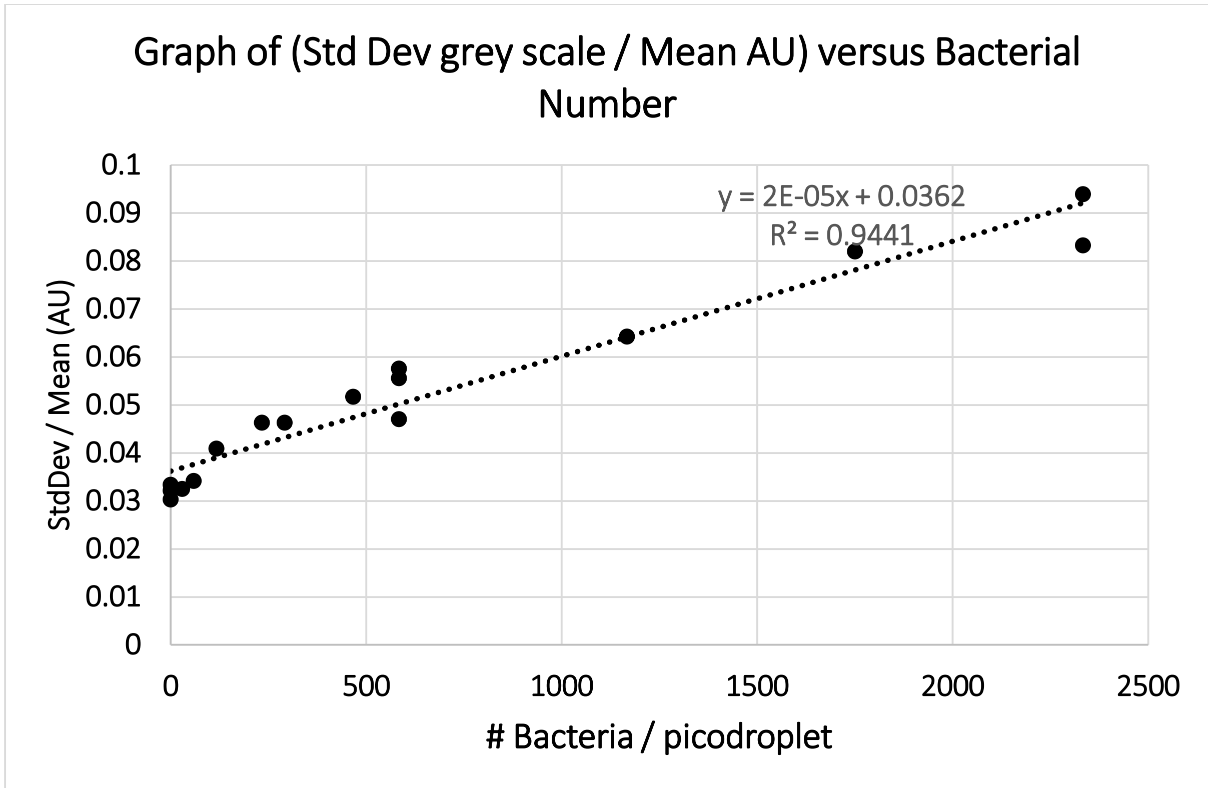


An overnight culture of *Escherichia coli* (HS151) cells in TSB media were stored on ice and picodroplets were generated on a picodroplet generator sorting biochip using 3% Pico-Surf^{TM} 1 in Novec™ 7500 (300 µL/h) and an aqueous flow rate of 200 µL/h, to give picodroplets with a volume of 778 pL. These large picodroplets were made in this generation sorting device (height 50 µm) so that these picodroplets would be flattened and become "pancake-like", so that the videos collected could be used to make grey-scale measurements on the interior of the picodroplet rather than being partially masked by the picodroplet interface. A few micrographs are shown in the slide below. The overnight culture medium was then diluted and again picodroplets were generated using the same oil and aqueous flow rates. A fast camera movie of the picodroplets generated at each bacterial concentration was taken and analysed in a custom LabView programme.



Picodroplet Generator sorting chip with 105 µm optical fibre,
3% Pico-Surf™ 1 in Novec™ 7500, flow rate=300 µL/h,
Parental strain bacteria flow rate=200 µL/h,
Picodroplet volume c.a.778 pL

Assuming that the bacterial overnight culture contained $3.0X10^9$ CFU/mL, then the picodroplets (778 pL) made using the overnight culture contained approximately 2,333 CFU/picodroplet. Subsequent dilution with TSB media allowed the bacterial CFU of the picodroplet to be estimated. Following image analysis, an essentially linear plot was obtained from the standard deviation of the grey scale/Mean AU versus the bacterial CFU/picodroplet, see graph below. This grey scale analysis indicated that the amount of scattered light was proportional to the number of bacteria in the picodroplets, within the range of occupancy tested. Therefore, this vision based approach that discrimination between picodroplets containing parental type bacteria and those containing mutants could be achieved in a label free manner. This vision based approach would allow sorting decisions to be made, but may not give the highest throughput. Therefore, in order to allow a higher picodroplet screening throughput, again based on an optical discriminatory method we decided to implement a light scattering based assay rather than a vision analysis based assay.



5. Bacteria grow curve in picodroplets

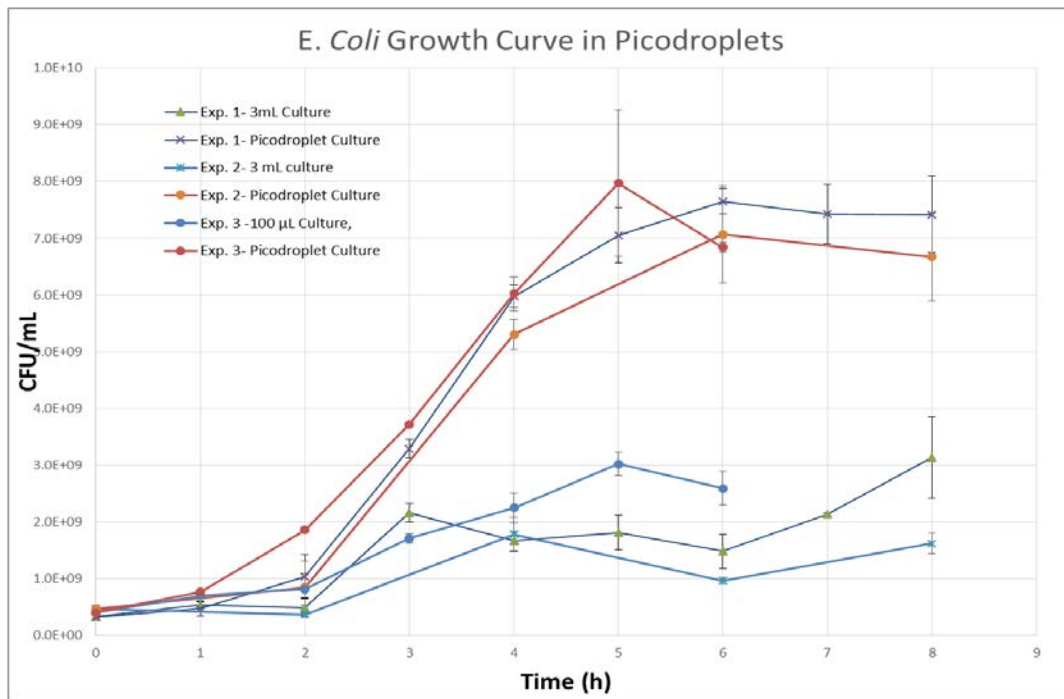


Chart shows growth curve of three separate experiments using 3E8 cells/mL as starting concentration.

6. Flow chart of Sorting of spontaneous antibiotic resistant mutants