Electronic Supplementary Material (ESI) for Analytical Methods.

Microfluidic Biochip and Integrated Diffractive Optics for Bacteria Growth Control and Monitoring

M. Cynthia Goh and Vitali Borisenko

Electronic Supplementary Information

Experimental methods

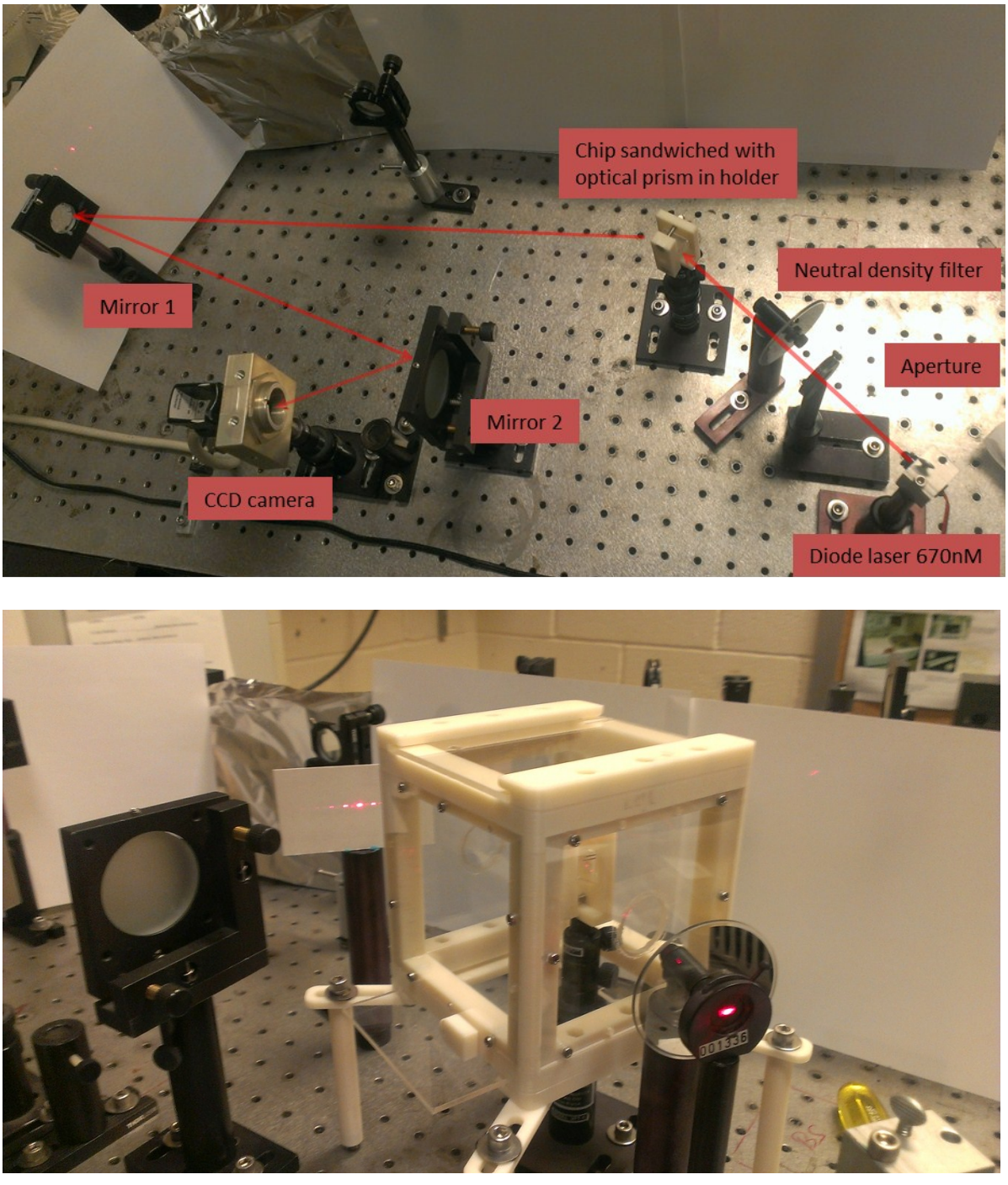
Our microfluidic chips were manufactured using soft lithography technique from PDMS (polydimethylsiloxane) which was moulded and baked on a silicon wafer mould with micron-scale features formed by cross-linked photoresist. The photoresist mould was built using photolithography method.[1] Individual chip for each experiment was cut out from the array of baked PDMS chips and holes were punctured in the chip to allow for the connection of fluid lines. The PDMS chip was then bonded onto coverslip (No. 2 – 0.19 to 0.23 mm thick) using oxygen plasma. The back of the coverslip was coupled to an optical prism using refractive index matching oil. The assembled chip was then placed onto a chip holder inside the miniature home-made environmental chamber for temperature controlling. The custom-made environmental chamber was small enough to accommodate the chip holder and heating coils. It has two double-glass windows for light coming in and out and several tiny openings for tubing and a thermometer. Fluid lines were connected to the chip from various syringes supplying media, cells, or acting as waste reservoir. The flow rate and direction in the chip were controlled by changing the relative heights between the relevant syringes resulting in hydrostatic pressure driven flow. The laser beam was made incident to the prism at an angle such that it would totally internally reflect from the glass-channel interface following a published procedure.[2] A World Star Technologies 670nM 5mW diode laser was used in the setup. A variable neutral density filter was used to attenuate the signal when necessary. A spherical mirror was positioned to focus the light from a single spot in the resulting diffraction pattern onto the digital camera detector surface. We monitored the second-order diffractive spot (the most left in the horizontal pattern in Fig. 2 of the main text). A Silicon Video 2112 CMOS digital camera controlled by a PIXCI D2X capture board and XCAP-STD software (all from EPIX) were used to capture and process images. The images were processed using XCAP-STD by selecting identically sized regions within each image for monitoring diffractive spot. An average intensity value was obtained by averaging all the pixel intensities within the region and it was repeated for each time point. The sampling time for image capturing was set to 5 min. For cell imaging on the chip an Olympus X71 microscope was used. The turbidometry measurements were performed on the Tecan Infinite M1000 reader by monitoring the absorbance at 600 nm.

We used BL21 *E. coli* cells. The bacteria were cultured in LB broth media for a few hours to an optical density of approx. 0.1 @ 600nm and then diluted 1000 time in the same media to make a cell loading solution. For the chip loading we used the approach similar to the published practice.[1] The initial syringe positions were as follows: the media syringe (port A in Fig. 1b of main text) was sitting above the cell loading (port B in Fig. 1b of the main text) and the waste port syringes (port C in Fig. 1b of the main text), with the cell loading syringe to be above the waste syringe and not connected to the port yet. The chip was pre-wetted with media from the media port prior cell loading. After the media reached both the waste and the cell loading ports and making sure that air bubbles were all gone we connected the syringe tubing with cells to the port. Then, we lowered the position of the media syringe by placing it at a level slightly below the cell loading syringe to let the cells to go through the trapping chamber into the waste port. Higher hydrostatic pressure in the media line did not let the cells to flow through the media port. After a while, when we observed the liquid level drops in the cell loading line suggesting the cells were loaded in the traps, the flow direction through the trap chamber was reversed allowing media to flow into all ports, thus supplying the trapped cells with a continuous stream of nutrients. We tried to keep the flow rate as small as possible to prevent the cells to be swept away from the traps due to the shearing forces. We kept the environmental chamber at 36^{o}C.

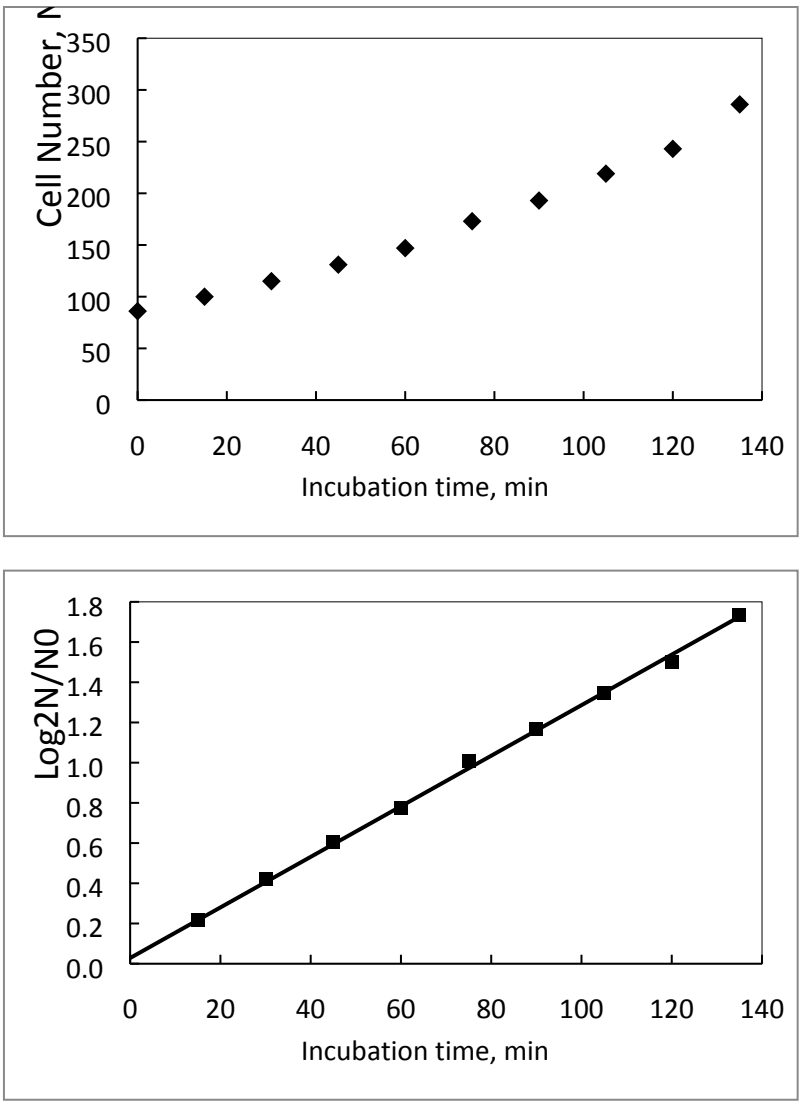
Notes and references for ESI

1 M. S. Ferry, I. A. Razinkov and J. Hasty, *Methods Enzymol.*, 2011, **497**, 295-372.

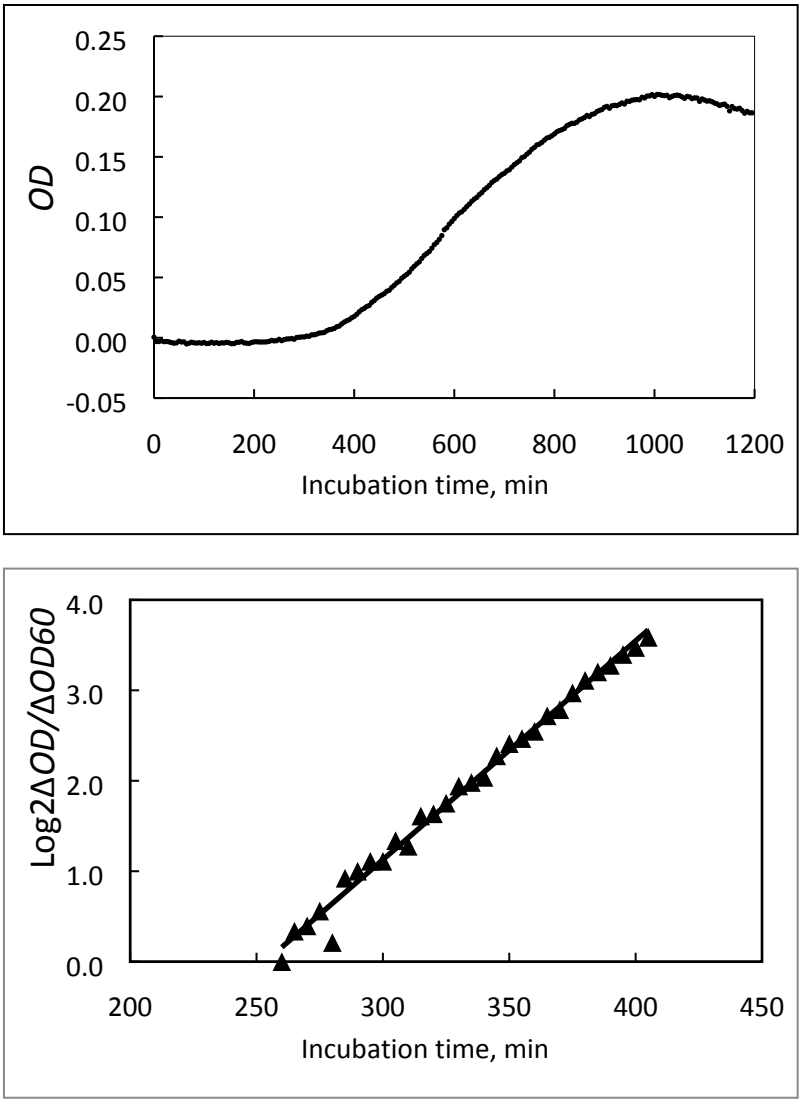
2 J. B. Goh, R. W. Loo, M. C. Goh, *Sensors Actuators B: Chem.* 2005, **106(1)**, 243-248.



Supplementary Figure 1. (Top) Optics layout for diffraction monitoring. (Bottom) Home-made environmental chamber for temperature controlling.



Supplementary Figure 2. (Top) Growth kinetic of *E. coli* cells observed by a bright field microscopy at 60X magnification at 36°C. After the bacteria cells were trapped on the chip, the snapshots of the surfaces of three traps were taken every 15 min under a continuous LB broth flow and then the bacteria cells were counted for every image. Figure 4 of the article and the Supplementary Movie demonstrates the bacteria expansion in the traps that were used for this experiment. (Bottom) Logarithm of growth fitted with the linear function regression. The reciprocal to the slope yields 79.4 min for cell's doubling time. N_0 is the cell number in three traps in the first image of the sequence.



Supplementary Figure 3. (Top) Growth profile of *E. coli* seeded in LB broth and measured at 36°C by turbidometry on the Tecan Infinite M1000 reader (optical density at 600 nm). The LB broth level was subtracted from *E. coli* absorbance. (Bottom) Logarithm of growth fitted with the linear function regression. The reciprocal to the slope yields 41.3 min doubling time of the signal intensity. The signal increase, Δ*OD*, was not observed within the first 3 hours of incubation. ΔOD_{60} is the signal elevation during 200-260 min interval; it was used as a normalization parameter.

Supplementary Movie. *E. coli* expansion in the traps observed by a bright field microscopy at 60X magnification at 36°C. The snapshots of the surfaces of three traps were taken every 15 min under a continuous LB broth flow. The flow goes from right to left. The rectangular traps are ~100μm x 20μm.