Microfluidic reveals growth rate of E. coli in LB

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Using a microfluidic device we examined the growth rate of GFP expressing E. coli. We discuss the experimental procedure and discuss possible improvements to our setup. Using a LB nutrient bath, our E. coli grew exponentially with a doubling time between forty to sixty minutes butnever reached a linear growth rate regime. Future directions of this setup could include rapid switching of media or exposure to transcription factors.

Microfluidics │ E. coli │ growth rate

Introduction

Designed to force fluids into tiny spaces, microfluidic devices are simple, cheap tools which have a variety of applications. Some examples that the Groisman Lab at UCSD is working on include: chemotaxis and gradient response; perfusion chambers to study rolling, adhesion, migration of blood cells; substrate rigidity sensing and TIRF microscopy of mammalian cells; Imaging of the development and behavior of C. elegans worms; oxygen tension and hypoxia; kinetics and thermodynamics of protein folding; biolistic delivery with a gene gun; and microbial cultures in microchambers.

Here we use a microfluidic device to examine growth rates of Escherichia coli (E. coli), a gram-negative, facultative anaerobic, rod-shaped bacterium that is commonly found in the lower intestine of warm-blooded organisms, that has been genetically modified to express green fluorescent protein (GFP). Our microfluidic device allows us to image the growth of an E. coli colony in real time with single cell resolution. It also provided a way to rapidly switch media using a small pressure differential between the media flow lines, although this capability was not utilized in our experimental design.

After trapping cells in our growth chambers, we recorded images of the cell colonies as they grew using GFP to indicate the area covered by the cell populations. Tracking the area across time allowed us to estimate the doubling time of the cells under the conditions provided.

Experimental procedure

Materials. This experiment requires working air and vacuum pumps, a pressure regulator, a computer nearby, the microfluidic device, and a microscope, preferably one with a camera already attached. The air and vacuum pumps need to be readily accessible to adjust air/vacuum pressure and intensity. Other small equipment include a sturdy hose for the air/vacuum, numerous containers for water, bacteria, media, and BSA, various sizes of tubing, stiff and orange, straw-like tubes, tweezers, and tape.

Small but thick tubes made up most of the necessary components. The elastic material of these thicker tubing allowed for flow-stopping clips to be clipped on without resulting in the collapse of the tube. We then fit a more slender and fragile tube into the thicker tubes to be fitted into fluid holding containers. The orange straw pieces are then placed into the thick tubing. These thin, orange, straw-like pieces were obtained from the Hwa Lab and were the parts that were plugged into the device. The straw was cut to a shorter length to reduce unnecessary clutter it might have caused. We followed the advice of Severin, a member of the Hwa Lab, and cut an end at an angle to form a sharp tip. This sharp tip reduced the hassle to fit the straw into the device's openings, which, before, had been a challenge. We bought and attached a special pressure hose for the air pump.

We used an upright microscope which was already set up for use. Already attached above the microscope was the EMCCD camera. The EMCCD camera required, and will most likely continue to require, fine tuning with the Andor computer program prior to experimental use, otherwise it simply displays useless gray blobs on the computer screen. The Andor computer program, once fiddling around with the different settings and with the help of Google, became a very useful image capture tool. The program allowed for clear videos and pictures of our device, many of which came out to be very good on the first run.
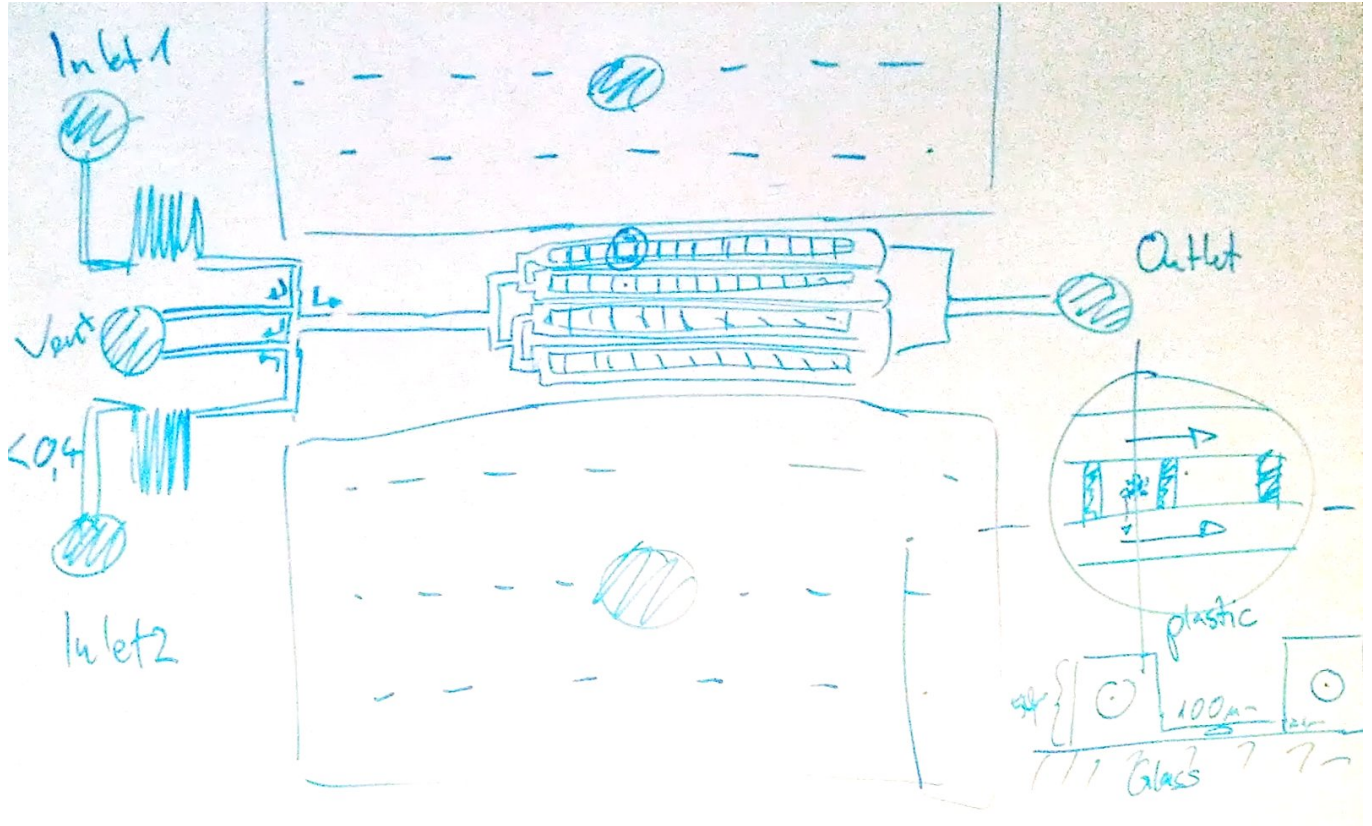
Fig. 1. A schematic of the microfluidic device we used. Note the two inlets on the left flank the vent and are across from the outlet. This configuration is important to remember as you insert tubes.

Preparation. Place the Microfluidic device in front. By now the tubing should be set up and ready to insert. Keep the exit ends of the tubes above the level of the unpressurized containers to reduce wasted liquids.

$$\frac{1}{2}\rho v^2 + \rho g z + p = \text{constant} \qquad [\mathbf{1}]$$

Bernoulli's principle, described by equation [1], tells us that the pressure of tubes apply on the device are directly related to the height of the tube inlet so keep all containers at or above the level of the microfluidic device to prevent backflow. BSA must be inserted into the device first to coat the inner walls to prevent bacteria from adhering to the walls and clogging the device. To do this, clear the BSA flowing tubes of large bubbles and let a droplet form at the tip. This will reduce air bubbles from flowing into the device as much as possible. Clip the tubing, leaving the droplet formed on the tip. Use the pointed end to slide it into the vent of the de-

vice. Turn air pressure regulator to about 3.0 PSI, release the clip and wait. Soon, the BSA will flow all through the system and the device will slowly begin excreting BSA bubbles from the other openings. Plug these openings, starting with the other outlet, and ending with the two inlets and wait for up to ten minutes.

At this point, checking the device through the microscope is recommended. Large air bubbles inside the device will start to be squeezed out through the air-permeable walls of the device. Once the large bubbles are gone, continue waiting for the air in the growth chambers to be removed. Meanwhile, ready the clipped E. coli tube in the same way as the BSA. Once the air bubbles inside the device are gone, insert into the outlet of the device. Remove the other plugs and insert clipped media tubes. Begin flowing E. coli through the device. Once several cells have been captured in the growth chanmbers, apply vacuum to the vacuum lines to hold the cells in place. It may help to apply and release vacuum to help flush cells into the growth chambers.
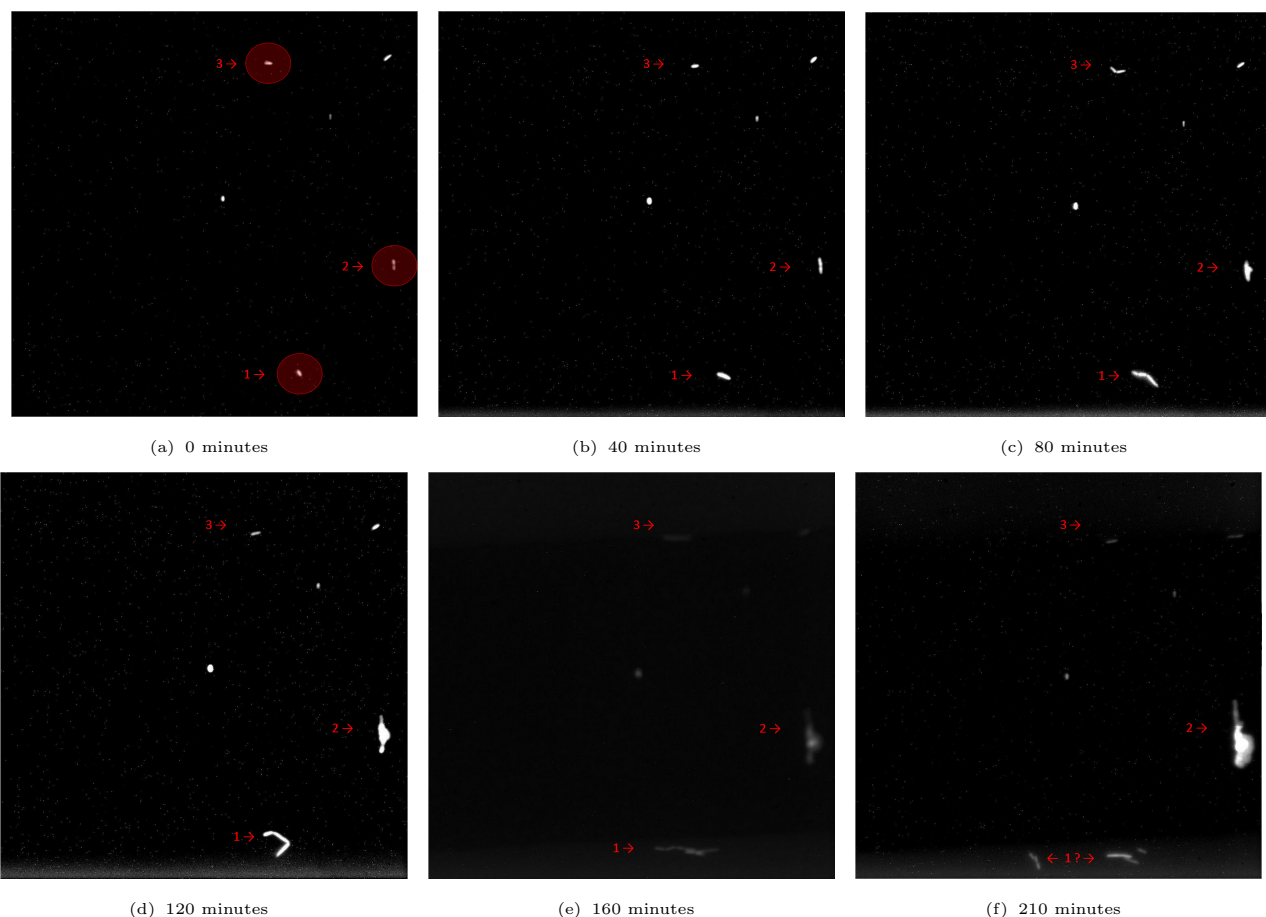
Fig. 2. Images captured of the colonies as they grew. ImageJ allowed us to track area without having to worry about intensity and contrast.

Once, several cells are in the growth chambers, the experiment can begin.

Experiment. We grew E. coli using lysogeny broth, LB, and took a set of 10 photos of the fluorescent bacteria in 1 chamber, approximately, every 40 minutes. Each photo set was taken within 10 seconds to reduce the effects of photobleaching as much as possible. We attempted to maintain an experimental temperature of about 35° C using an air heater with a temperature feedback sensor attached to our microscope.

Results

With the assistance of Phil Tsai, we used adjusted the image threshold to contast the appoximate area of the E. coli and measured these areas for three separate populations in ImageJ. The numbers were plotted in MS Excel to determine and visualize the growth rates of the colonies. These images are shown in figure 2 and the area measurements are presented in table 1 and graphically in figure 3.
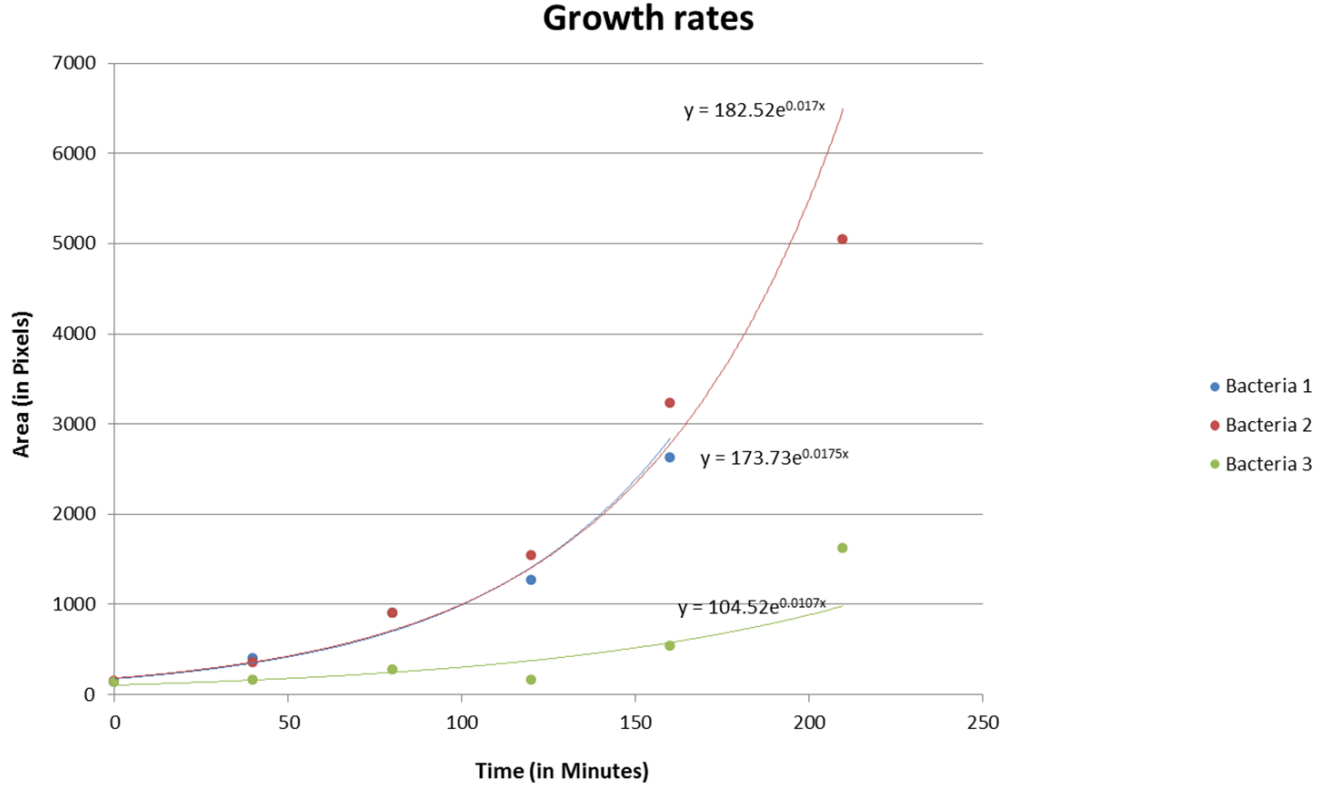
Growth rates

Fig. 3. Measured population growth rates as well as fitted models. It should be noted that the measurement for bacteria colony 1 for 210 minutes was omitted as an outlier. We suspect that some of the cells may have been washed away.

Analysis

We assume that bacterial growth is described by

$$\frac{dM}{dt} = M\lambda \qquad [2]$$

where M is the mass of bacteria and λ is the growth rate. Solving equation [2] simply gives us

$$M(t) = M_0 e^{\lambda t} \qquad [3]$$

where M_0 is the initial mass of the bacteria.

In the case of our microfluidic device, the growth chamber has a very small constant height (dt) due to the vacuum pressure we apply, and assuming constant bacterial density (ρ) we can consider only area (A)

$$A(t) = \frac{M(t)}{\rho dt} = \frac{M_0}{\rho dt} e^{\lambda t} = A_0 e^{\lambda t} \qquad [4]$$

with A_0, our initial area of the colony.

Minimizing the sum of squared residuals, we fit our model to estimate the parameters for each different population. The parameters we obtained are available in table 2. We would like to note that we omitted the measurement of colony 1 at 210 minutes as an outlier due to experimental error.

We can define another useful quantity, the doubling time (T) such that

$$\frac{A(t + T)}{A(t)} = 2 \qquad [5]$$

which simply means that the mass or in this case area doubles after a doubling time. Using equation [4], we have

$$\frac{e^{\lambda(t+T)}}{e^{\lambda t}} = 2 \qquad [6]$$

which trivally yields the relationship between the doubling time and the growth rate

$$T = \frac{\ln(2)}{\lambda} \qquad [7]$$

thus, using the fitted parameter λ, we can estimate the doubling time, T, for each of our populations which are also included in table 2.

Conclusion

Although our results are not necessarily profound, we outline a reliable method to set up and image E. coli in a microfluidic device.

There were many possible improvements that would make the procedure easier. Firstly, using an inverted microscope instead of an upright microscope would be helpful to allow the tubing to be easily handled, instead of propping it with lab scraps. Also, trapping would be much easier if we had a vacuum pressure regulator as well. Our pressure regulator didn't work at negative PSI and the vacuum from the wall forced all our cells to the edges of the chambers, possibly biasing our results. Also, while we did use temperature control, a better system which enclosed the experimental area would likely improve the reliability of the results. Finally, setting up an electronic shutter to take pictures at pre-set intervals would allow more experiments to be run withough having to stay there for hours.

If any of these improvements can be made, more interesting experiments could be performed such as seeing how growth rate and doubling time depends on media, temperature, or transcription factors.

Appendix: Experiment Lab Manual

To view a slideshow of our setup, including videos we recorded of the media switching go to `http://goo.gl/8GPYw`.

Table 1. Population growth measurements

Time (minutes)	0	40	80	120	160	210
Colony 1	143	397	902	1273	2627	(75)
Colony 2	149	352	902	1540	3229	5040
Colony 3	143	160	276	159	537	1620

Table 2. Model parameters

	λ (minutes^{-1})	A_0 (pixels)	T (minutes)
Colony 1	0.0175	173.73	39.6
Colony 2	0.017	182.52	40.8
Colony 3	0.0107	104.52	64.8