RESEARCH AIMS TO USE BACTERIA TO POWER NANO-SCALE MACHINERY

Self Powered Microstructures in Low Reynolds Number Fluidic Environments
By Jigar Patel

Synopsis
The Bacteria Actuation, Sensing and Transport team advised by Dr. MinJun Kim is working on harnessing the power of bacterial motors for engineering applications. Members of Dr. Kim’s team include a Ph.D. candidate, Edward Steager, and four mechanical engineering seniors: Jigarkumar Patel (BS/MS candidate), Socheth Bith (BS/MS candidate), Chandan Naik (BS candidate), and Lindsay Reber (BS candidate). For more information on Dr. Kim’s team, see the Team/References.

When fabricating nanoscale motors and in developing micron-scale power sources, the actuating of fluids in microfluidic systems is usually achieved through the use of large external actuators. Dr. Kim’s team is exploring the possibility of using microorganisms as a method for fabricating nanoscale elements.

The team used existing culturing techniques and developed effective, simpler and cheaper techniques to manipulate, control, and track micron scale structures. In the future, this multidisciplinary project will provide an outstanding educational opportunity for both graduate and undergraduate students from various engineering disciplines.

Although the project is in its embryonic state, in future, this technology will power micro-factory that will assemble and disassemble parts of micro-machinery.

Research
Humans have utilized bacteria for centuries to ferment, digest and immunize; as well as to perform tasks within the human body that are necessary for survival. Normal floras (harmless bacteria) are found on all body surfaces exposed to the environment, but the vast majority of bacteria live in and around the human body.

*Serratia marcescens* is a species of bacteria that is commonly found growing in dark damp places such as sewage and soil. These bacteria can cause infection within the human body if not handled properly; but they are also efficient “workhorses” that can be utilized to move objects, diffuse flows, or pump flows in a microfluidic environment. The unique characteristics of *Serratia marcescens*, make it a prime candidate for use in actuating microstructures without an external force.

As the field of engineered micro/nanoscale structures matures, a need has emerged for robust, controllable methods of actuation for miniaturized systems. Various methods have been proposed to actuate microstructures by creating a physical force, a chemical reaction, or a pressure gradient. With current technologies, a microdevice must be actuated through an external force such as ultrasound, electro-osmosis, and electromagnetic field. To make a microdevice move without an
external energy source, a self-propelling power source is required. Mobile microrobots enable microstructures to move from point A to Point B at the expense of extensive computer processing and equipment; but, in microfactory conditions, where a number of robots are required to work in unison, additional use of sensors, actuators, and vision systems will make the situation cumbersome when size is limited.

This research focuses on bacteria and biomotors in an attempt to overcome both physical and computing problems. The goal is to control the movement of the bacteria to a degree that will allow them to perform tasks that result in the assembly, or disassembly, of structures in a biofactory. (Figure 1) The objective is to design microstructures for the microfluidic environment, to attach a monolayer of harmless genetically modified bacteria, Serratia marcescens, to these microstructures using blotting technique, and to analyze the direction and motion of the structures. The flagellated bacterial monolayer acts as the power source, and consumes nutrients in the working fluid as fuel resulting in microdevices that are not only autonomous but also self-contained.

The bacterial transportation systems using PDMS and SU-8 microstructures were studied in an unrestricted, open channel environment. Structures of different geometries (such as the square, circle, and triangle) with aspect ratios of 1:1, 1:2, and 1:3 on the order of 50µm in length, 50µm in width, and 10 µm in depth were designed and tested. The bacteria Serratia marcescens were cultured using a swarm plate technique. A series of steps were developed to release microstructures into the working fluid without damaging the structure or the attached bacteria.

The translational and rotational motion of the microstructures was observed under a phase contrast microscope, and controlled using phototaxis. A localized region within the bacterial swarm became inactive when exposed to UV light, and resumed motion when the UV light source was shuttered. This phototactic control enabled On/Off motion of the microstructures and will be used to control its direction of motion in microchannels. A microstructure tracking algorithm was developed to study the magnitude and direction of motion and could be used as a negative feedback component to adjust the stimulus to keep the microstructure on a predetermined track.
Further Information

Figure 2: Velocity vectors show centroid movement. (a) 50 × 100 µm rectangular barge translates at an average speed of 1.7 µm/s during 25 seconds and rotates 1.48 radians. (b) 50 µm equilateral triangle rotates 3.1 radians during 15 seconds with a stationary centroid then translates 10 µm over the following 7 seconds while rotating 1.5 radians.

Figure 3: A 50 µm × 100 µm rectangular microstructure rotating in open channel motility buffer controlled using phototaxis; (a) rotational motion, (b) still due to UV exposure, (c) resumed motion after UV exposure

Serratia marcescens

*Serratia marcescens* is a rod-shaped, peritrichously-flagellated, gram-negative bacterium about 1 µm in diameter by 2 µm long. It swims at a speed of about 30 µm/s, propelled by the rotation of about 5 µm long, thin, helical filaments, each driven at its base by a flagellar motor. What makes these bacteria so unique and powerful are their flagella. The flagellum is a propulsive organelle that includes a reversible rotary motor embedded in the cell wall, and a filament that extends into the external medium (Berg, 2003). The filament is a long (~10 µm), thin (~20 nm) helix (2.5 mm pitch, 0.5 mm diameter) that turns at speeds of ~100 Hz. Flagella’s rotate at around 20,000 rpm, at energy consumption of only around 10-16 W and with energy conversion efficiency close to 100%. The flagella are randomly distributed over the cell surface and have individual motors that
rotate independent of one another. These flagella form bundles by coordinated clockwise and counterclockwise movements to propel themselves through the low Reynolds number fluid environment. *Serratia marcescens* draw their propulsion energy from the nutrients in the working fluid and hence they can be deemed self-contained.

The flagellum is made by self-assembly of about 20 different proteins about 45nm thick. The rotor ring is made of protein that assembles in the cytoplasmic membrane. This inner cytoplasmic ring of protein controls the direction of rotation. A double-ring structure embedded in the inner membrane comprises the core of the rotor (the MS-ring). A drive shaft (the rod) passes through a bushing (the P- and L-rings) that penetrates the peptidoglycan and outer membrane (Figure 4). An extracellular flexible coupling (the hook) links the driving shaft to a propeller (flagella) (Figure 5). Clustered around the C- and MS-rings are 8 force-generating units comprising proteins called MotA and MotB. Then, other protein molecules attach to the ring, from the base to the tip, to create the motor structure. After the motor has been formed, the flagellar filament, which functions as a helical propeller, is assembled. The flagellar filament is made of 20,000 to 30,000 copies of flagellin polymerized into a helical tube structure.

**Phototaxis**

Phototaxis is the movement of an organism or a cell toward or away from a source of light.

**Swarm plate technique**

Swarming bacteria are especially useful as actuators due to their rigor and size. Swarming bacteria are hyperflagellated, elongated and migrate cooperatively. The surface distribution of the swarm on the agar plate presents a flat, broad surface for the microbarge blotting technique presented below. The swarm plate is inoculated on one edge with 2 µl of Serratia marcescens saturated culture. Agar plates are incubated at 30-34 degrees Celsius, and swarming begins within 8-16 hours (Figure 6). The inoculation site will generally turn pink shortly after the swarming motion develops. The

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swarm itself progresses across the plate in waves that appear as concentric rings with the most active bacteria along the outermost edge of the swarm.

![Swarm plate technique](image)

**Figure 6**: Swarm plate technique. (a) Appearance of 14 cm swarm plate 16 hours after inoculation. Innoculation site near edge appears as opaque, red dot. Swarm edge has propagated roughly 7 cm. (b) Appearance of elongated Serratia marcescens along swarm edge.

**Micromanipulation**

Micromanipulation is a procedure by which bacterial blotted microstructures are extracted from the substrate and released into the working fluid with the aid of the microscope.

**Blotting technique and micromanipulation**

To blot, the chips with microstructures was washed with motility buffer (0.01 M potassium phosphate, 0.067 M sodium chloride, 10-4 M ethylenediaminetetraacetic acid, 0.01 M glucose, and 0.002% Tween-20, pH 7.0) then inverted onto the edge of the swarm plate for 10-15 minutes.

After blotting, the wafer chips were removed from the swarm plate, transferred to a dish with a motility buffer, and lightly agitated to remove unattached bacteria and excess agar. The washed chip was then moved to a fresh Petri dish and submerged under a thin layer of motility buffer for imaging. The microstructures were released while submerged using the second extracting approach (Figure 7 and Figure 8).
Figure 7: Blotting and Releasing Microstructures. (a) Wafer chip washed with motility buffer. (b) Blotting in the swarm plate for 10-15 minutes. (c) Blotted chip rinsed with motility buffer to remove excess agar and unattached bacteria layer. (d) Releasing microstructures.

Figure 8: Freed microstructures

The manipulation was done by using a stereo microscope for imaging, a tweezers to hold the wafer chip in place, and a scalpel blade to extract the barges (Figure 9).
It was later discovered that the bacteria also attached to SU-8, and were able to move SU-8 structures. SU-8 microstructures were fabricated and experiments using the same blotting technique and similar extraction/releasing process were successful. The SU-8 microstructures were fabricated in a simplified single step without having to use molds. They had stronger integrity than PDMS microstructures, and could be easily extracted without damage (Figure 10). Microstructures were released fully intact from the substrate by applying minimal force along one edge with the scalpel blade (Figure 11).
**Microstructure tracking algorithm**

To track the bacterial transport, videos of freed microstructures with live bacteria attached were captured using an inverted phase-contrast microscope. A tracking algorithm was developed in Matlab to calculate the displacement and velocity fields of the microstructure. The analysis of a 100 µm × 100 µm PDMS square microstructure in translation motion and an arbitrary shape (13 µm × 9 µm) in rotational motion which was recorded in an open channel is discussed below to demonstrate the use of tracking an algorithm. A set of consecutive 100 frames with 2048 × 2048 pixels were captured at 4 frames per second, digitized and imported into Matlab for further analysis.

Before getting to the tracking algorithm it is important to know what a digital image is, what pixels are, and what the ‘intensity level’ in a black or a white image are. A digital image is perfectly aligned rows and columns of tiny squares (pixel) that remembers the color value. For an 8 bit black and white picture, the pixel remembers the color and brightness values ranging from 0 to 255, where 0 being the black-most pixel and 255 being white-most pixel. When referred to a 2048 × 2048 pixel image, the image has 2048 columns 2048 rows of pixels. Another important parameter is pixel to pixel distance, which is the distance between centroids of two adjacent pixels; this distance is calculated during the calibration process of the magnification lens and is used to calculate the velocity.

The tracking analysis involved four main steps, namely pre-processing, image enhancement, structure recognition, and velocity calculation. In the pre-processing stage, the brightness levels at the microstructure boundary and time interval between the frames were found using image processing software, ImageJ (NIH). The first step in the image enhancement process involved reading the AVI file and creating the resulting text file. A main ‘For-loop’ in the code scanned one frame at a time that enhanced the image, located the centroid, and calculated the velocity between frames. The matrix of 2048 columns and 2048 rows was created to hold the brightness value for each pixel of 2048 × 2048 frames.
A second sub-For loop scanned each and every pixel, by both length and width, to make the pixels at the boundary completely black (Figure 12 Step 3). This step made the boundary of the structure more distinct and enhanced the image.

The next step was to remove the unwanted gray background surrounding the structure. This was done by detecting the gray pixels and replacing them with white pixels (Figure 12 step 4). The process of making the pixels fall on the extreme intensity level of black and white made it easier to distinguish the structure from bacteria and other unwanted noise in the image.

At this point (Figure 12 Step 4), the structure has black pixels within and surrounding the boundary. The presence of these black pixels generated false x-y boundary coordinates. To overcome this problem, the black pixels were replaced with white pixels (Figure 13 Step 5).
Gray pixels were also replaced with black pixels to make the structure more distinct (Figure 13 Step 6). There were stranded black pixels around the microstructure that were not part of the structure. Every black pixel or structure below a 5 \(\mu m\) size was detected and removed by a horizontal scan of 2048 rows (Figure 13 step 7). At this point, the image was ready for structure recognition and velocity calculation.

Structure was recognized using a vertical scan that detected and recorded the upper and lower boundary of the structure in each of 2048 columns. The vertical scan from the top of the column detected the upper boundary when the threshold limit moved from white (threshold = 255) to black pixels (threshold=0) and a second vertical scan from the bottom of the same column detected the lower boundary.

After obtaining the coordinates of each and every point on the boundary, the centroid was found using the following formula by dividing the microstructure structure into a combination of lines made up of pixels whose areas were known:
The accuracy of the tracking algorithm was tested on structures with predetermined shapes using the time span between frames and the velocity recorded in a video made in Macromedia Flash version 8 (Figure 15). A 20 × 20 mm square with different velocities set between frames was used to validate the accuracy of the code. The computed result showed good agreement with the predetermined values.

Knowing the time span between the two consecutive frames and the distance between the two centroids of the consecutive frames based on pixel-to-pixel distance of 0.124 µm, the velocity of the structure was calculated and plotted (Figure 16). The same procedure was applied to the rotational structure (Figure 17), and other microstructures in the results section.
Figure 16: Step 9, Velocity field of PDMS square microstructure

Figure 17: Rotational movement of a triangular microstructure. Velocity field as calculated by the tracking code