Photo-Actuation: Determining the Effect of Blue Light on the Actuation of Micro-Beads by *Serratia marcescens*

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***Abstract*—MicroBioRobots (MBRs) are robotic systems with both an animate and inanimate component. The animate, or biological, component of MBRs acts as an actuator, providing the source of power for the MBR's movement. Since this biological component is, in fact, a living organism, it is generally very responsive to external stimuli. For this reason, microorganisms, such as bacteria, act as sensors in addition to actuators. In order to utilize the innate sensing of microorganisms effectively, their response to particular stimuli must be understood in a way that is both measurable and predictable.**

Light is a stimulus known to affect the motility of bacteria. I developed a 2D tracking algorithm in MATLAB in order to analyze the motion of MBRs made up of micro-beads actuated by *Serratia marcescens*. I report on the effect of blue light on this motion, finding that a high intensity of blue light leads to an increase in the tumbling of bacteria and an overall decrease in the movement of MBRs, sometimes working strongly against a current.

Through my attempts to obtain accurate and useful data, I also examine the effectiveness of several techniques for creating functional MBRs and acquiring successful video data. This is a complex process of its own and does not yet have a generally agreed upon protocol. I take into account the pros and cons of several techniques, finding that the smaller bead size I observed (3µm) was easier for the bacteria to move and control than the 6.8µm beads. I also found that washing the micro-beads using sonication did appear to promote the natural attachment of the bacteria to the micro-beads. Due to the adhesion of both beads and bacteria to surrounding surfaces, it was best to use Percoll to give the MBRs buoyancy and then observe them on some plane other than the top or bottom to prevent any beads or bacteria that had become stuck in place from affecting the data. Drift is another common problem when observing objects of this size, and the best way I found to control it was through the use of a sealed chamber.

Even with these improvements, future research is necessary to find the optimal way of collecting and analyzing data on MBRs. Future studies should also be conducted with MBRs using lower intensities of blue light to determine if different light intensities will affect the MBRs' motion differently. It would also be useful to conduct further research on how bacteria move beads of varying sizes so that the use of bacteria as an actuation technique is not limited solely to beads with diameters of 3µm.

I. Introduction

Recent advancements have transformed robotic technology, once considered farfetched and futuristic, into an important aspect of many people's daily lives. From communication to medicine, robots are used in all manner of fields. As researchers continue to make enhancements, many robots are being created on a smaller and smaller scale. Microscopic devices have the potential of making an extremely large impact on society. However, objects of this size are not yet capable of their own, controlled motion without the presence of an external force, such as a magnetic field.

By utilizing the natural movement of flagellated bacteria such as *Escherichia coli* and *Serratia marcescens*, an external force is no longer necessary. The combined efforts of many individual bacteria cells can be used to power the movement of a larger device [1]. This process of providing the power behind motion is termed "actuation," and the source of the power, in this

case flagellated bacteria, is an "actuator." In order for this technology to be implemented in any sort of useful way, researchers must be able to control the direction and speed with which the bacteria move these micro-robots. Possible methods for obtaining this control include the use of outside stimuli or the physical alteration of the micro-robots themselves. However, it is necessary to know the effects these methods will have on the bacteria and micro-robots, together making up an MBR, before they can be properly implemented.

II. Background

A. Running and Tumbling of Flagellated Bacteria

The flagella of flagellated bacteria allow them to move and swim in fluid environments. The helical movement of each of the bacteria's flagella is irreversible, and therefore allows the bacteria to move despite their low Reynolds number. These bacteria are constantly either "running" or "tumbling" depending on the direction in which their flagella are rotating. When all of the flagella are rotating counterclockwise, the bacterium is in a running state. In this state, the flagella bundle together and propel the bacterium forward in a single direction. If even one of the bacterium's flagella begins to rotate clockwise, the bacterium will go into a tumbling state. When this occurs, the bacterium rotates in place, allowing it to change direction but resulting in little to no linear movement. By constantly switching back and forth between these two states, a bacterium develops what is sometimes referred to as a "random walk." Without any significant environmental stimuli, a bacterium will generally spend more time in its running mode and tumble approximately once a second [2].

B. Effect of Blue Light

Blue light has been shown to produce a repellant response in *E. coli* cells [3]. Due to their similar structure, it is possible that this same response may also be observed in *S. marcescens*. However, it is not clear that the application of blue light always acts as a repellant to the bacteria. Since the response in *E. coli* cells is caused by several photoreceptors, factors such as the intensity of the light are believed to have slightly different effects on each of the receptors and could therefore change the cells' behavior. It is also important to recognize that blue light could act as a short-term repellant while having a different effect in the long-term or vice versa, as bacteria commonly adapt to their environment [3].

C. pH-Taxis with Bacteria-Driven Micro-Beads

Micro-beads actuated by *S. marcescens* have been previously researched in a microfluidic channel with a stable pH gradient. The cells formed attachments with the micro-beads and moved them to an area with a more desirable pH for the bacteria [4]. Thus, it is reasonable to conclude that the ability of *S. marcescens* to stick to, push, and pull micro-beads is not affected by the desirability of pH. Instead, the creation of a pH gradient primarily affects the direction of the bacteria's motion.

D. Phototaxis with Bacteria-Driven Micro-Beads

The effect of UV light on microstructures actuated by swarming *S. marcescens* has also been observed. Phototaxis may be preferable to chemotaxis because light can quickly and easily be added or removed from an area and is visually detectable. Light also tends to create less of a gradient than its chemical stimuli counterparts, allowing for a more uniform response from the cells. Initial experimentation suggested UV light could be used to halt the motion of microbiorobots [5]. Later research attempted to use UV light to prevent rotational motion while electric fields were used to produce linear motion. In this case, UV light stopped rotational motion completely for only one to two seconds, but the long-term effect was a reduced rotational velocity with each exposure to the light [1].

E. Motility Buffers and Other Techniques for Successful Video Data

Certain chemicals and techniques are commonly used when observing the motion of MBRs, but the key to their successful implementation is balance. While it is necessary that the bacteria and micro-beads attach to one another, any additional attachment they have to surfaces or themselves could impact the data, resulting in inaccurate conclusions with respect to the motion of MBRs. Tween, Bovine Serum Albumin (BSA), Polydimethylsiloxane (PDMS) coating, Percoll, and chambers have all been previously used to prevent unwanted attachments, settling, or drift in a microscopic environment. However, because we are working on a scale where our vision is very limited, it is often difficult to predict the full effect these

techniques will have on the MBRs' motion. It is thus important for different "solutions" to be tested and their effect observed before determining if they should be used in final experimentation.

III. Materials And Methods

A. Bacteria

The flagellated bacteria *S. marcescens* were used for all experiments. Cells were prepared by inoculating a 10ml solution of Luria-Burtani (LB) broth with 10µl of *S. marcescens*. This solution was then incubated for four hours at 34 degrees Celsius. During this incubation period, the solution was moved repeatedly in a circular motion at 180rpm. The *S. marcescens* were used for experiments immediately after their incubation period was complete.

B. Micro-Beads

We used red fluorescent beads with a radius of 3µm. We sonicated and centrifuged the beads five times, alternating between water and IPA, before re-suspending them in motility buffer. This sonication process has been done in previous studies [4], and its purpose was to remove any surfactant on the outside of the micro-beads in order to promote natural attachment between the beads and bacteria.

C. MicroBioRobots

The bacteria and bead solutions were combined on the slide and pipetted back and forth several times to promote attachments between the *S. marcescens* and micro-beads. While BSA and Tween were both tested as methods to prevent the sticking of bacteria and beads to surrounding surfaces, it was eventually decided that using Percoll to suspend bacteria and beads before observing them in a central plane was the best option. Unlike BSA and Tween, Percoll did not appear to have an effect on the attachments of the bacteria and beads to one another.

D. Microscopy and Imaging

I took videos using a RETIGA EXi *FAST* Cooled Mono 12-bit camera by QImaging. I connected the camera to an Axioplan 2 imaging microscope to observe the MBRs using both a 40x water immersion lens and a 10x oil immersion lens. Neither of these lenses was immersed in the fluid sample because the chamber used included a coverslip separating the sample and the lens. Despite this, the lenses still focused properly and were able to provide good images. The videos were taken and put into Audio Video Interleaved (AVI) format with the program Image-Pro Plus 7.0.

E. Tracking

I wrote a MATLAB tracking code to follow and record the paths of objects from frame to frame. The code uses MATLAB's built in function object finder and compares the objects found in each frame as well as their distances from one another to determine how the objects move over time. I then expanded upon this using the Hough transform to track only circular objects. This allowed me to track only micro-beads in videos where both bacteria and micro-beads were present and visible. Using the paths found through this tracking code, I was able to obtain quantitative data from the videos including the mean squared displacements of moving bacteria and/or beads, the direction of their motion, their speeds with relation to each other, and other relevant pieces of information.

IV. Experimental Results

When determining the change in movement of tracked objects, the mean squared displacement (MSD) proved to be a useful statistic. In early experimentation, when only *S. marcescens* were being observed, the average MSD for all bacteria tracked was approximately the same whether or not the blue light was turned on. However, when methods to prevent bacteria from sticking to the slide were implemented, the MSD proved to be significantly smaller when the blue light was turned on. This suggests that the application of blue light increases the rate at which *S. marcescens* tumble. This result was not seen when bacteria were sticking to the slide because such a large portion of *S. marcescens* remained in place regardless of light, leading the average MSD to be so similar that the underlying trend could not be seen. The best solution found for this problem of sticking was to use Percoll to give beads and bacteria additional buoyancy and then observe them on some horizontal plane toward the middle of the sample. When these MBRs were observed, it was clear that they moved significantly less when exposed to blue light. This reaction remained true for all intensities of blue light tested, though stronger intensities did have a stronger impact. It appeared that in this case, where light is shown

on a particular area without creating a gradient to build up to the point of focus, blue light has little impact on the direction of movement but a large impact on the speed and amount of movement.

V. Discussion And Conclusion

After exposing MBRs to blue light, we can conclude that at high intensities, blue light causes an increase in the frequency with which *S. marcescens* tumble and a nearly complete halt in the movement of the MBRs throughout the duration of the blue light exposure. While it did not appear that MBRs outside the scope of the light source were any more likely to move toward the lit area than they had been previously, MBRs that were directly illuminated by the blue light looked almost as if they were becoming "stuck" in place. It seems unlikely that the blue light created this effect solely by increasing the frequency of tumbling in *S. marcescens*. If this were the case, it would be expected that the MBRs would decrease in their movement but still continue to move along with any drift present. Further research is necessary to know how the blue light may have altered the MBRs or the surrounding environment in such a way that would cause the MBRs to halt almost entirely. Once it is better understood why blue light has this effect on MBRs and if it can be reproduced in other environments, blue light may become a useful tool in controlling the motion of MBRs collectively without an outside source of power. Future studies should also look into lower intensities of blue light to determine if they have similar effects.

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