The Effects of Hypergravity on Aliivibrio *fischeri*

New Mexico Supercomputing Challenge

Final Report

April 1, 2014

Team members:

Maria Troyer

Victoria Troyer

Kaleb Brown

Project mentor:

Creighton Edington

Contents

Executive Summary

Colonizing exoplanets similar to Earth is becoming more of a feasible idea as technology advances. However, little is known about the effects that increased gravity has on organisms. There has been less research on hypergravity, because it is harder to simulate than microgravity. Commercial centrifuges spin too fast (200-6000 rpm), therefore, to simulate increased gravitational force of about 1.5x that of Earth, a low powered centrifuge had to be engineered. The bacteria Aliivibrio *fischeri* was spun on a homemade centrifuge, for up to one days at a time, in order to determine the effects of hypergravity on a single-celled organism, with the long-term goal of establishing the effects on multicellular organisms. These bacteria luminesce in correlation to the amount of stress it is undergoing, which made it a great test subject because the light emission can be tracked throughout the duration of experimentation. Based on this experiment a program model was written to determine the way the bacteria would be affected within a population exposed to different levels of gravitational force. This research is laying the foundation for future research and the effects of increased gravity will become more apparent and it the possibility of inhabiting exoplanets will be determinable.

Introduction

Exoplanets orbiting other stars are being discovered on a weekly basis (Jones, 2008). As the technology for detection improves, we are finding more and more such bodies in the so-called “Super Earth” category. This means that they are bigger than Earth in mass, anywhere from 1.5 to about 8 Earth masses and are thought to be rocky, terrestrial planets Although we still lack the resolution to fully understand these distant bodies, it is not too soon to begin to think about them as characterizable and potentially habitable environments.

The effects of very different planetary conditions from those we experience on Earth will have shaped the nature of any life forms that may exist on Super Earths. Primarily, the consequence of a much larger mass than Earth is the greater gravitational attraction that these bodies will exert. On our planet, many organisms respond in various ways to the gravitational field. Some organisms orient by means of the up/down vector, some organisms have physical structures that operate using gravity, and some organisms that are very tiny and living in a fluid medium may be relatively unaffected by differences in gravity. These responses have only been investigated to a very limited extent (e.g., Deguchi et al., 2011).

The purpose of this experiment is to test the effects increased gravity has on the organisms Aliivibrio *fischer* (A. *Ficsheri*). The bacterium Aliivibrio *fischeri* is being used to test the effects of increased gravity, simulated in a centrifuge that overcomes Earth’s gravity. This organism is a naturally luminescent bacteria typically found in Hawaiian waters, primarily, in symbiosis with aquatic animals. There is a lot of background information on A. *fischeri* because it has been used for research involving bioluminescence, quorum sensing, and bacterial-animal symbiosis, which is helpful because the outcome of this project is unknown and rather

unpredictable. It is a great candidate for this experiment because it luminesces in response to environmental stress (e.g., Bose, 2008). By measuring the amount of light produced, the amount of stress the organism is undergoing can be inferred.

To determine the effects of increased gravity, a program has been developed in net logo. This program demonstrates the effects of hypergravity on a population of bacteria. All aspects of the organism have been taken into account including its luminosity patters and quorum sensing. With all the factors considered the program gives an approximated stress level depending on the gravitational force based on the amount of light being emitted from the bacteria. The data received to this point is usable information that can be applied to future research projects.

Procedures and Description

Before the Netlogo model could be written the actual experimentation had to take place.

Commercial centrifuges spun too fast, due to the fact that the revolutions per minute (RPM) value needed to simulate a 1.5 g-force (around 100 rpm). Therefore, one had to be built to meet the specifications of this experiment. The centrifuge was constructed using a bike rim and motor and electrical components from various competitions including a Texas Instruments Jaguar speed controller, FRC power distributer, 12 volt battery and breaker and Botball CBC as the brain. For more information on the centrifuge construction see appendix A.

A protocol was developed to track the light emitted by the bacteria throughout the duration of the experiment. This was done by taking pictures of a florescent substance at varied concentrations and comparing the light density on Photoshop. For more information see appendix B.

The bacteria was received in freeze dried form and revived. When the bacteria were stable, they were transferred to test tubes. Two samples were transferred in medium and two samples were transferred without medium into a phosphate buffered saline (PBS) solution. The phosphate solution was clear which made it easier to see the organisms. By creating a cradle in between the spokes, the test tubes were secured to the centrifuge with string. The centrifuge ran for a total of 82 hours. It was stopped at 24, 48, 72, and 82 hours to take pictures and document changes. For more information see appendix C.

Based on this research a program was written to model the experiment. Due to scheduling conflicts, man power, and time dilemmas the experimental project is not yet completed. The current program was written as an intended baseline for this project. The turtles represent the A,

fischeri population and the dark grey represents the medium. As the bacteria absorb the nutrients the medium turns to a light grey. The user has the ability to decide whether or not as the nutrients decreases so does the bacteria population; the turtles can be dependent or independent of the medium. The user is also given the ability to change the size of the bacteria population, and the level of gravity the organisms are being subjected to.

Verification and Validation

To obtain the data we would need for the simulation, we first built a physical model of our project. The physical model however gave off inconclusive results due to unseen errors. Therefore, the data that our Netlogo model is using is unsupported at this time.

Although the experiment is incomplete there has been significant progress made. The protocol to determine light density indicated the path to follow when writing our program. Hence, the results to be expected are given based off of the turtles color change. Once the experiment is completed the data will be added to the program so that accurate results can be taken from the program model.

Results

The results that the Netlogo model display is currently inconclusive as we used data obtained from the physical model that we also built. However, during the construction and testing of the physical model some errors were made rendering our data inaccurate.

Conclusions

The results of my experiment proved to be inconclusive. However, the experiment proves that the centrifuge I built is a reliable way to simulate increased gravitational forces and gather results that can be input to the computer model.

Significant Achievement

The most significant achievement of this project was laying a foundation for future research. This is a novel project with little to no background data to lay the foundation of the experiment on. There has been a protocol developed ensuring successful results for future experimentation that will be used in further development of the program.

Acknowledgments

We would like to thank our mentor Creighton Edingtion for helping us with our code and creating our program and our mentor Talysa Ogas for helping us with the experimental part of the project. We greatly appreciate all the time and effort they have put into helping us.

References

Barjaktarovic´ et al 2007. Time-course of changes in amounts of specific proteins upon exposure to hyper-g, 2-D clinorotation, and 3-D random positioning of Arabidopsis cell cultures. J. Exp. Bot. 58(15):4357-4363.

Bauer, Peter. "Measuring, Counting, and Analyzing Pixels in Photoshop CC." - *For Dummies*. For Dummies, n.d. Web. 05 Mar. 2014.

Bradamante et al 2006. From Hypergravity to Microgravity: Choosing the Suitable Simulator. Bremen Microgravity sci. technol.XVIII-3/4.

Bose, Jeffrey L., Charles S. Rosenberg, and Eric V. Stabb. "Effects of luxCDABEG induction in Vibrio fischeri: enhancement of symbiotic colonization and conditional attenuation of growth in culture." *Archives of microbiology* 190.2 (2008): 169-183.

Deguchi et al 2011. Microbial growth at hyperaccelerations up to 403,627 X g. PNAS 108 (19):7997-8002.

Erdmann et al. 1997. Effect of Gravity Changes on the Cyanobacterium Synechocystis sp. PCC 6803. Curr. Microbio. 35:348–355.

Hoson et al 2005. Signal perception, transduction, and response in gravity resistance. Another graviresponse in plants. Adv. Space Res. 36:1196-1202

Isaac Newton: "In [experimental] philosophy particular propositions are inferred from the phenomena and afterwards rendered general by induction": "Principia", Book 3, General Scholium, at p.392 in Volume 2 of Andrew Motte's English translation published 1729.

Johnson, Elaine. "Development of proper microbiological technique for experimentation with Vibrio fischeri."

Jones, B.W. 2008. “Exoplanets – search methods, discoveries and prospects for astrobiology. *International Journal of Astrobiology*. 7: 279-292.

Kováts, Nora, et al. "COMPARISON OF CONVENTIONAL AND Vibrio fischeri BIOASSAYS FOR THE ASSESSMENT OF MUNICIPAL WASTEWATER \ TOXICITY." *Environmental Engineering & Management Journal (EEMJ)* 11.11 (2012).

Mortimer, M., et al. "High throughput kinetic< i> Vibrio fischeri</i> bioluminescence inhibition assay for study of toxic effects of nanoparticles."*Toxicology in vitro* 22.5 (2008): 1412-1417.

Appendix A: Building the Centrifuge

Materials	Quantity
Base: 12x8x1/4 Plywood	1
Top: 12x8x1/4 Plywood with 4” hole	1
6” strip 2x4 Treated Lumber	4
3” wood screws	16
4x4 metal plate with predrilled wholes	1
CIMple Box, Single Stage Gearbox (am-0734)	1
2.5" CIM Motor (am-0255)	1
20 inch bike rim with nuts	1
½” washers	3
PVC Spacers	3
¼” bolts and nuts	10
Eyebolt and nut	1
Spring	1
Metal plate	
C-Channel	3
Metal bracket	1
Door hinge	2
¼” all thread	3
Power Distribution Board	1
40 Amp Snap Action Breakers	1
Analog Sensor	1
10 inch wheel and hub	1
MK ES17-12, 12 Volt, 17 Amp-Hour Sealed Lead Acid Battery	1
¼ PVC	14 ft
Battery charger	1

Table 1. Materials needed for Part A: Building the centrifuge.

Commercial centrifuges spun too fast, due to the fact that the revolutions per minute (RPM) value needed to simulate a 1.5 g-force (around 100 rpm). Therefore, one had to be built to meet the specifications of this experiment. After much brainstorming and prototyping, a sound design was achieved. The basic idea behind this apparatus design was a gear system. This was

accomplished by using a CIM motor to power a small wheel resting against the rim of a bike tire. The bike rim spokes were an easy place to attach the test tubes holding the bacteria.

The first step was to assemble a wooden table to hold the wheel. The table was made using four 2x4 lumber pieces and two pieces of plywood (one for the base and one for the top). Since the top is four inches shorter than the base, the legs were first attached the top and then aligned with the base to be attached. A hole was drilled into the middle of the top to be used later for placement of the bike rim, as seen in Figure 1.

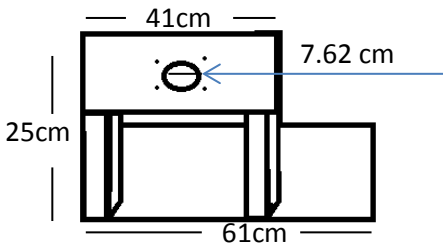


Figure 1. Diagram of the table apparatus.

A square metal plate was specially made to hold the tire rim. There was a 7.62 cm hole drilled into the middle of the plate, so that the rim axel fit snugly and was able to be secured from both sides with nuts (spacers were required to achieve the correct height of the rim), as seen in Figure 2A. The plate was then set over hole in the top and bolted to the table (Figure 2B). Note: make sure each both nuts on either side of rim axel are securely fastened. Over tightening will hinder rims mobility.

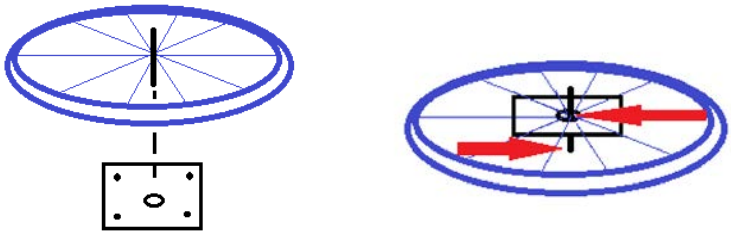


Figure 2 A and B: Diagram displaying the metal plate and the bike rim.

The next step was assembling the AndyMark gearbox, according to package directions.

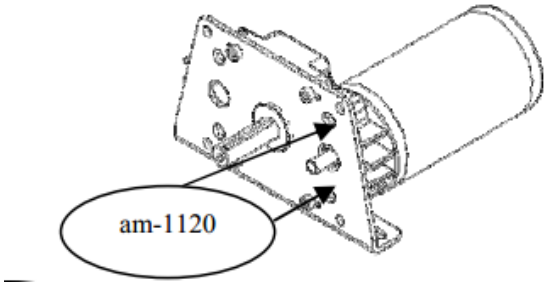


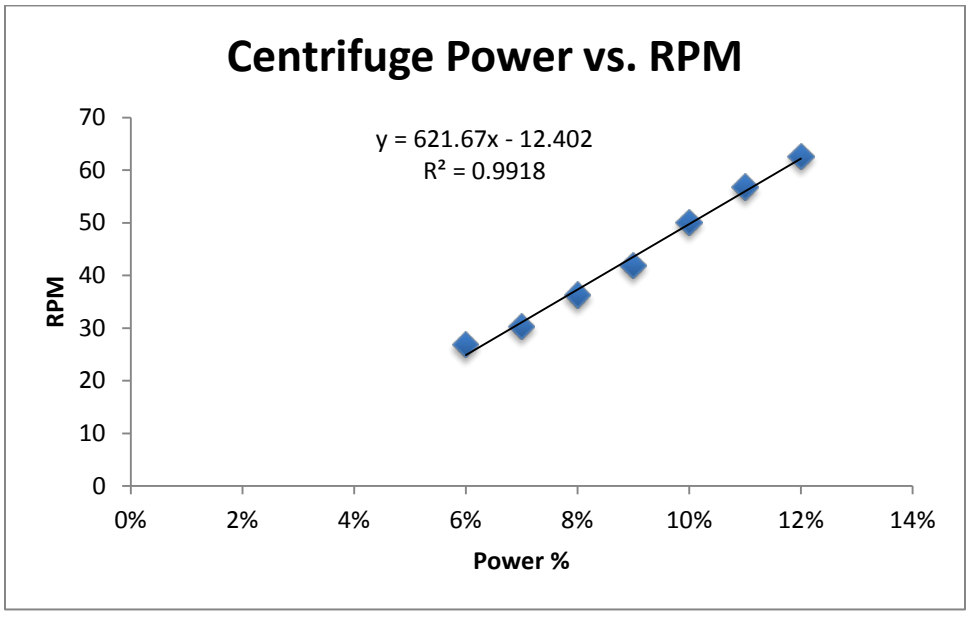
Figure 3. Picture taken from AndyMark.com

When the gearbox was assembled with the motor, two PVC spacers were placed on the hub so the wheel did not rub against the gearbox. The wheel was fastened with a machine key and bolted onto the hub.

The most challenging part of the design was figuring out how to hold the motor at the correct height so that the wheel was able to turn the rim. After much deliberation, it was concluded that a spring loaded arm would work the best. This was made by connecting three pieces of c-channel with all thread. The c-channel was attached to the base of the table using two door hinges. On the upper end of the c-channel, the gearbox was attached to the inner side of the c-channel with the motor pointed into the table. A strip of plumbers tape was run from each of

the top corners of the gear box to the outer c-channel. This increased stability of the motor. On the leg opposite the c-channel arm, a metal bracket was attached. This bracket had one hole drilled through the middle big enough for an eye bolt. A spring was stretched from the top left corner of the gearbox to the eyebolt. This made the contact between the wheel and rim easily adjustable by only having to loosen or tighten the eyebolt.

To determine the rpm an adhesive strip was stuck to the side of the rim. An analog sensor was set up just so that when the rim turned the tape slid through the sensor. A program was written to track the amount of times the strip passed through the sensor. The program was able to determine the rpm level to about 60 rpms which was about 15% power, but after 15% power, the rim was moving so fast that the sensor did not detect the tape on every rotation. To solve this problem, the power values that were giving out correct rpm readings were used to make a linear graph and correctly calculate the power needed to have an accurate rpm, as seen in Graph 1.



Graph 1: Centrifuge Power vs. rpm.

The last step was to build a tent around the centrifuge. The bacteria need 10 – 12 hours of light a day, so the tent allowed for the monitoring of the light without disturbing other people in the lab. The tent is basically a cube-like shape around the upper level of the centrifuge. The first step was to run one bar underneath the short side closest to the motor and another on the other side. On the side closest to the motor, t-connectors were attached to both ends. One more piece of pvc was attached to each connector in the direction of the motor. This is to ensure that the tent extended around the motor. By using a corner bracket at each end, a box was constructed with the remaining pvc pieces. All pieces were securely fastened with machine screws.

After the centrifuge was built, the electronics were secured to the bottom level of the table. A CBC Botball Controller (CBC, see Appendix A) was used as the brain. Connected to it is a Botball motor (when turned starts the rim spinning), two limit switches (one that triggers a display of the rpm value and one that stops the power), and the analog sensor (used to determine rpm). The CBC was connected to the Texas Instruments Black Jaguar speed controller (MDL-BDC24) which was connected to the power distributor. The power distributor was connected to a power breaker and battery.

Rpm Calculations:

a = the acceleration needed to achieve desired gravitational force (m/s^2)

- Earth = $9.8 m/s^2$

r = the radius of the centrifuge (m)

v = the velocity of the centrifuge (m/s)

T = the time it takes to complete one rotation (s)

f = the frequency of the centrifuge (s^{-1})

1) $a = \frac{v^2}{r}$ The acceleration of something in circular orbit

2) $v = \frac{2\pi r}{T}$ The velocity of something in circular orbit.

3) $f = \frac{1}{T}$ frequency (1/s)

4) $v = 2\pi rf$ Substitute 3) into 2)

5) $a = \frac{(2\pi rf)^2}{r}$ substitute 4) into 1)

6) $f = \frac{1}{2\pi * \sqrt{\frac{a}{r}}}$ rearrange

Input Values:

$f = \frac{1}{2\pi} * \sqrt{\frac{\frac{14.7m}{s^2}}{0.21m}}$ a = 1.5x Earth

$f = 1.33\ rps$ = 14.7 m/s^2

$f = 79.97\ rpm$ r = 0.21

Design Evolution:

The biggest change made throughout the entire process was the exchanging of motors. The first time the rpm levels were calculated they came out to a value of about 50. Because the speed was so slow, a high power motor was not necessary and a window motor was used. The motor was connected to a small wheel which was mounted underneath the rim creating a gear system.

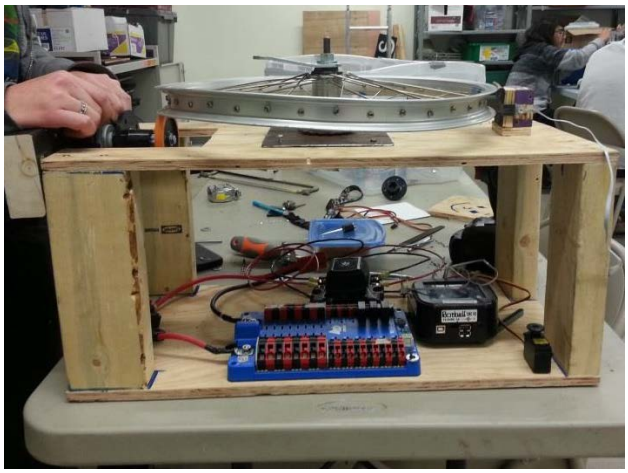


Figure 4. Prototype centrifuge with bad rim.

There were several problems with this design. First, the bike rim being used was not straight the device shook when it was being run. This had a concerning effect on the organism. The actual foundation of the apparatus was not stable either. The entire device shook and the components vibrated, sometimes to the point of shifting positions. Furthermore, the calculations were double-checked and a new value was found to be 110 rpm. The current set up with the window motor was not going to be powerful enough so a bigger motor needed to be used.

The first idea was to have the motor hub connected directly to the rim axel. It was quickly realized that the motor did not have enough power to turn the axel at the speeds needed. The new motor was much bigger and heavier. After trying to adapt the current table to fit the motor, I decided to build a new table and use a spring loaded arm to hold the motor. This design was much more effective allowing leeway between the wheel and tire rim.

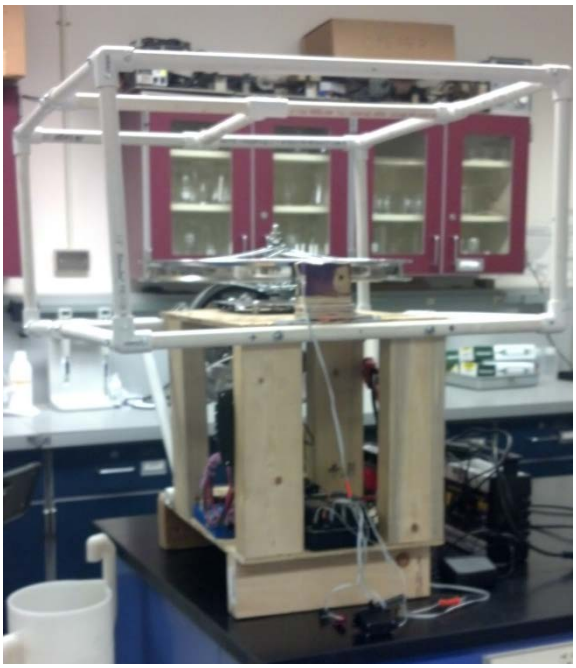


Figure 5. Centrifuge complete with pvc casing and new rim.

Appendix B: Protocol

1. Building the second Centrifuge

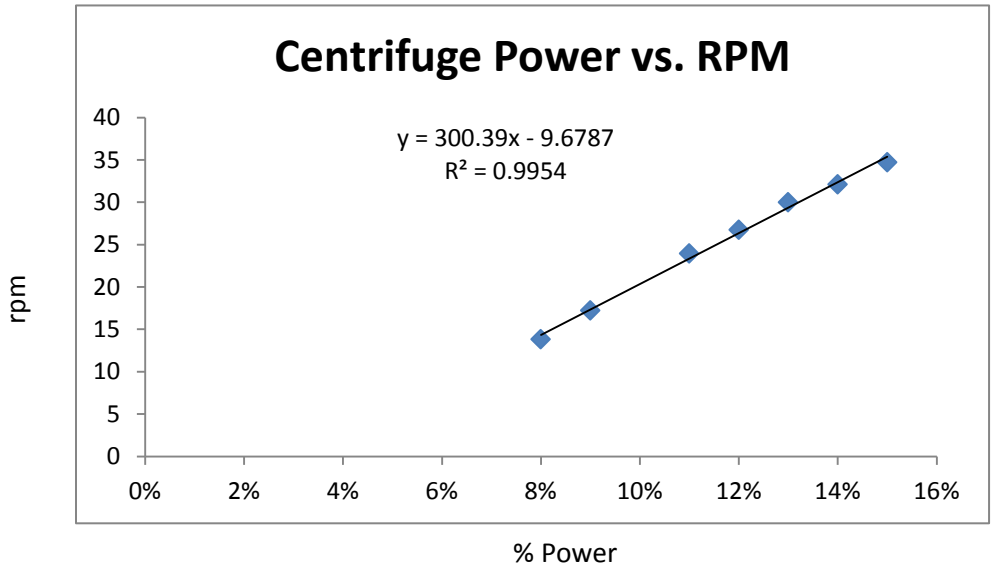
Materials	Quantity
Base: 12x8x1/4 Plywood	1
Top: 12x8x1/4 Plywood with 4” hole	1
6” strip 2x4 Treated Lumber	4
3” wood screws	16
4x4 metal plate with predrilled wholes	1
CIMple Box, Single Stage Gearbox	1
2.5" CIM Motor	1
20 inch bike rim with nuts	1
½” washers	3
PVC Spacers	3
¼” bolts and nuts	10
Eyebolt and nut	1
Spring	1
C-Channel	3
Metal bracket	1
Door hinge	2
¼” all thread	3
Power Distribution Board	1
40 Amp Snap Action Breakers	1
Analog Sensor	1
10 inch wheel and hub	1
MK ES17-12, 12 Volt, 17 Amp-Hour Sealed Lead Acid Battery	1
Battery charger	

Table 2. Materials needed for second centrifuge.

A second centrifuge was built to keep at the School of Dreams Academy for preliminary research on protocols to be done concurrently with actual experimentation at New Mexico Tech. This centrifuge was very similar to the first one; however, because of limited materials the motor

on this centrifuge was smaller. The size allowed for the motor to be positioned facing out. All other components remained the same.

Another rpm linear graph had to be calculated because of the change in motors.



Graph 2. Centrifuge Power vs. RPM for second centrifuge.

2. Comparing the amount of luminescence emitted:

Materials	Quantity
Centrifuge Apparatus	1
Black Light	1
Electrical tape	1
Zip ties	6
10 mL Test Tubes	6
Jig (scrap wood)	1
Camera	1
Chemicals	
Glow stick	1
WD-40	1

Table 3. Materials needed for determining the protocol fluorescence.

The purpose of this part of the experiment is to develop a protocol data collection to be used in actual experimentation. To do this glow stick substance was used. The contents of one glow stick (2mL) were divided into two test tubes. One was set aside as the positive control. A 2x dilution in WD-40 was used for the three remaining test tubes. A test tube with only WD-40 was used as the negative control. To compare results Photoshop was used to count the pixel value and relate between dilutions

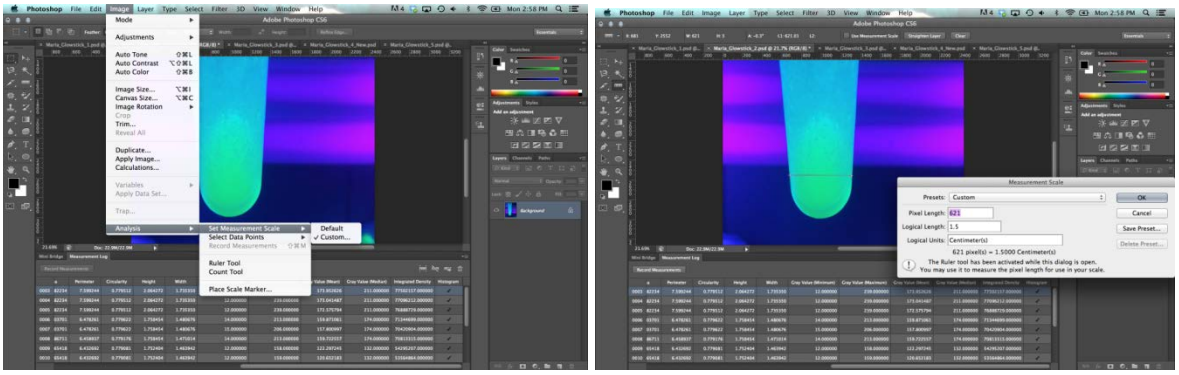


Figure 6 A and B: Screen shots of measuring pixels using Photoshop.

Appendix C: Spinning Aliivibrio *fischeri*

Materials	Quantity
50mL Test Tubes & Stoppers	4
Test Tube Markers	1
String	~ 50 in
Foam Grip	~ 45 in
Identification Tag	4
Centrifuge	1
Photobacterium Broth	
NaCl	30.0g
Sodium glycerol phosphate	23.5g
Pancreatic digest of casein	5.0g
KH_2PO_4	3.0g
Yeast extract	2.5g
$CaCO_3$	1.0g
$MgSO_4$-$7H_2O$	0.3g
$FeCl_3$	0.01g
Phosphate Buffer Saline	
NaCl	
KCl	
Na_2HPO_4	
KH_2PO_4	

Table 4. Materials needed for spinning A. *fischeri*.

The organisms were ordered from the American Type Culture Collection (ATCC) in freeze-dried form. This first step to reviving them was to make the media. Add components (Table 4, Broth) to distilled water and bring volume to 1L, mix thoroughly and autoclave the media for 20 minutes at 121^{o}C. Once the broth is cooled, the freeze-dried pellet of A. *fischeri* could be revived in a small about of media. After a couple weeks, the bacteria are transferred to a larger flask so that the population can grow at a steady rate.

When the bacteria were stable and growth was in the exponential phase, they were transferred to test tubes. Two samples were transferred in medium and two samples were transferred without

medium into a phosphate buffered saline (PBS) solution. The phosphate solution was clear which made it easier to see the organisms. By creating a cradle in between the spokes, the test tubes were secured to the centrifuge with string. The centrifuge ran for a total of 82 hours. It was stopped at 24, 48, 72, and 82 hours to take pictures and document changes. In this run, the tent was not used. This was just a trial run to gain a better understanding of the possible outcomes. Because the bacteria did not luminesce, the results are based from observations. In later trials, the same protocol developed in Part B will be followed.