Closed-Loop Control of Magnetotactic Bacteria

by

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Magnetotactic bacteria have the potential to controllably reach deep-seated regions of the body by vessels and achieve targeted drug delivery. In this application, motion of the magnetotactic bacteria was influenced by the strength of the external magnetic field. Here, the swimming characteristics of mangetotactic bacteria was investigated (Magnetospirillum gryphiswaldense strain MSR-1) under the influence of uniform and adaptive magnetic fields inside microfluidic chips with depth of 5 µm and width 600 µm. It was found that under the influence of magnetic field reversal with approximately twice the field strength, the diameter of the u-turn trajectories taken by the magnetotactic bacteria was decreased by 63%. In addition, the adaptive magnetic field decreased the size of region-of-convergence of the controlled bacteria within the vicinity of the reference position by 65.5%, compared to control using uniform magnetic field. The comparisons between control systems were done on the same culture of magnetotactic bacteria and using the same cell in each control trial.
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<td>Magneto Tactic Bacteria</td>
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<td>PWM</td>
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<td>Transmission Electron Microscope</td>
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<td>PCB</td>
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Chapter 1

Introduction

1.1 Motivation

Chemotherapy is used in the treatment of cancer tumors. The chemotherapeutic agent circulates the human body killing the tumorous cells. However, this treatment can damage other fast growing healthy cells in the process such as the hair and blood cells. There are too many side-effects associated with this treatment such as the lose of hair. Patients also suffer from blood disorder due to the damage of the production of new blood cells in the bone marrow, which leads to low blood counts. Other side-effects such as fatigue, sore in the mouth due to the damage of cells was noticed [4]. In order to mitigate the damage caused to the healthy cells, it has been suggested by many researchers to inject the cancerous cells with the drug to limit its spread [5, 6], however the injection pressure can cause the tumor cells to explode and spread even further [7]. It has been devised to use targeted drug delivery of biodegradable micro or nano materials which can control the drug release around tumour cells to reduce their side-effects. However the challenge remained to be on how can they be positioned inside the body to target the cancerous area only [8]. Recently, certain robots are used to carry the drugs and release it at target areas. In order to make drug delivery to different body parts the robots should be able to move inside very small diameter arteries, veins and capillaries, for that reason micro and nano robots were introduced. There are two types of micro/nano robots, the artificial and the biological ones [7].

The artificial microrobots as the name suggests are man made and they need external power to actuate them. There are several types of propulsion mechanisms suggested to move these types of robots. The first type of powering a microrobot was done through the use of a chemical powering of the robot, an electrophoresis propulsion due to a chemical reaction will produce ions from head to tail and cause it to be propelled (moved),
Figure 1.1: A simulation of the motion of an artificial helically-shaped microrobot moving by flagellar propulsion inside a blood vessel. The microrobot can be used in clearing blood clots (yellow substance) and other non-trivial biomedical applications. Image courtesy of Abdelrahman Oseya.

however this can be toxic if implemented in vivo. Another chemically powered technique was the bubble propulsion which was done by oxygen bubble thrust produced by the reaction between the surface of the microrobot and a hydrogen peroxide solution which act as a fuel, the speed of the oxygen propelled microrobot can reach 1000 body-length-per-second. However, The use of hydrogen peroxide in vivo will be challenging as it might put the body at risk. The second type of powering was by the use of ultrasonic waves, where experiments showed that the ultrasonic acoustic waves can be used to propel a metallic nano robot at a speed of 200 µm/s. The third way of powering artificial micro/nano robots was by magnetic or electric powering, as for the magnetic driven micro/nano robot, it will be pulled by an external magnetic field gradient which is highly recommended due to its high maneuverability [9]. The magnetically driven microrobot can be propelled using different techniques such as helical propulsion, wave propulsion and pulling with magnetic field gradient. As for the helical propulsion, the microrobot was in the shape of a cork and a screw attached to it, this design was inspired from the bacteria flagella. When a rotating magnetic field was applied to the microrobot its tail rotates causing the robot to move forward (figure 1.1). The wave propulsion design was inspired from the eukaryotic flagella where the wave motion of its tail cause it to move forward using either piezoelectric bilayer actuators or current carrying coils [10]. Since the powering of artificial robots on the micro/nano scale to be able to move them is hard and still under research, the idea of using ready motile biological microorganisms was introduced which are considered biological microrobots too.
Biological microrobots discussed in this part have something in common which is that both do not need an external source of power and that they move by means of flagella. Two types of biological microscale robots were investigated within the scope of this thesis.

The first type is the magnetotactic bacteria, this bacteria synthesize magnetic grains inside their cell called magnetosomes. The bacteria helical flagellum rotates producing thrust force in order to overcome the fluid drag force. Each cell contains magnetosomes making the single bacterium has a magnetic dipole moment (M). Figure 1.2 shows that when a bacterium cell was subjected to a magnetic field (B) indicated by parallel arrow lines, and there is an angle (θ) between the axis of the magnetic field and the magnetic dipole moment the cell , this angle will derive a torque indicated by (T) which will act on the cell and rotate it such that it aligns itself with the magnetic field direction (B). As the bacteria are aligned no further force will be acting on it (notice that the dead cell will also align itself to the magnetic field, because of its magnetosomes which are still there). The Magnetic Dipole moment of the bacteria is an important parameter when trying to make motion control over it [10].

A second biological microrobot is the sperm, but because its not magnetic, ways to make it magnetic were introduced, one approach was by genetically modifying it, but it has resulted in affecting the vital function of the organism, another introduced approach was by making the sperm head covered by a magnetic microtube. This way the sperm that is naturally moving can be controlled by similar mechanism to the magnetotactic
bacteria. Refer to figure 1.3 which shows a sperm held inside a magnetic beam and its tail which allow it to move was protruding from the back. This configuration allow magnetic field to affect the direction of motion of the sperm since it is now connected to a magnetic beam trying to align itself to the field and the sperm moving it with its flagella.

Another important aspect of dealing with microrobots either biological or artificial is how to localize the position of this robots. The localization of the microrobot is very important in the application of a feedback controller on the microrobot itself. There are different ways of finding the location of the robots. One way was through the use of electromagnetic tracking, where a magnet attached to a microrobot will generate a low frequency magnetic field which will induce voltage on a sensor, the voltage induced will be a function of the distance and orientation. However, this technique can be challenging if there are other parts near the sensor emitting magnetic gradient as well, this may require calibration of the sensor to overcome it. Ultrasound was also suggested to localize microrobot, as it has high speed, minimal effect on health, good resolution and low cost, however this technique can be inaccurate if the ultrasonic waves were rejected by bones, for instance. A final way was the use of vision techniques which is done by means of a microscope, camera and image processing for localizing it, this was the approach used in this research as it is less costly and it gives accurate results [10].
1.2 Objective

The purpose of this research was to implement a 2D position control by using a proportional gain and calculating the region at which the bacterium converged. This research also shows the effect of increasing the fields on this region of convergence. In order to be able to carry out the experiments the bacteria was cultured following the supplier indicated protocol. The feedback supplied to the closed-loop controller was image based so the bacteria was placed in microfluidic chips and placed over a microscope stage and viewed through a camera where the images acquired from the camera was then used to position the bacteria along $x$-axis, $y$-axis. This image was provided to an implemented tracking algorithm using the differencing technique in tracking the motion.

1.3 Thesis Structure

The remainder of this thesis is organized as follows:

- Chapter 2 provides a literature review of previous work done in the field of micro-robotics.
- Chapter 3 introduces the biological microrobot used in this work which was the Magnetotactic bacteria. This chapter give a brief description of the MTB, the strain used as well as how they were cultured. It also shows the different technique used to characterize the magnetic dipole moment of this microrobot.
- Chapter 4 provides a description of the mechanical and electrical set up used in conducting control.
- Chapter 5 shows a description of the developed tracking algorithm for the tracking of the magnetotactic bacteria needed as a feedback to the closed-loop controller.
- Chapter 6 provide a description of the magnetic fields as a vector of $b_x$, $b_y$, $b_z$, at different applied currents in the work space of the experiments.
- Chapter 7 presents the experimental work conducted as well as the results reached from this experiments.
- Chapter 8 provides the conclusions and directions for future work.
Chapter 2

Literature Review

Since the discovery of Magnetotactic bacteria [11], these micro-organisms have been the focus of many research groups [12–17]. MTB have attracted much attention because of its motility, size and magnetic properties. One study is about the use of MTB for micro-carrier, which might be beneficial in drug delivery and bio-sensing. The bacteria can be used to push the bead of micro to nano size in aqueous medium as a preparation for them to be used inside the human body. The small size of the bacteria, and the bead can allow them to move inside small vessels and access hard to reach areas. This research focus on studying the effect of the wall on the motion of the bacterium as well as the effect of attaching a bead on the behaviour of the bacterium. The thrust force of the bacterium was studied through measuring its average velocity and the influence of attaching the bead on its velocity. Reynolds number was calculated and was very low (Re ≪ 1) which means that the inertial force of the bacteria was negligible compared to drag force, thus the velocity of the bacterium remains constant. Accordingly the thrust force was equated to the drag force and used to estimate the thrust. The drag force increase as a bead was added to the bacteria due to the increase in the surface area. Results showed a drop in the speed was as a bead was attached and as the bead size gets bigger the velocity drop increases as well [18, 19]. Another study was carried out by the same research group which studied the control of a swarm of bacteria for manipulation of larger bead which can be further implemented for drug delivery or microassembly of larger parts. The idea was showed by building a pyramid of microbeads by the using swarm of robots to move them and this swarm was being controller by an external computer. The bead instead of getting attached to the bead as the previous study showed this time the bead was being moved with the swarm without the need to attach to the bead, this way the same swarm can be reused to move the next bead in order to finally build the pyramid. The swarm was collected by using a special non uniform field forcing them to concentrate at a specific location. The thrust force needed to move the blocks of the pyramid was
the total of each bacterium thrust force which was around 4 pN calculated using Stokes law. The results showed that the swarm was able to move the beads(block) around in a certain trajectory by the use of controlled magnetic field and the pyramid was built which shows the effect of using swarms in micro assembly or delivery. The greater the needed thrust force the more bacteria was needed in the swarm [20]. S. Martel et al. also studied the possibility of using the MTB as an oxygen sensor. It was found that the bacteria formed microaerophilic bands and was using aerotaxis to move to preferred oxygen concentration if not affected by a magnetic field trying to direct it to another region which was usually at 0.5% oxygen concentration [21]. Due to this behaviour the magnetotactic bacteria can reverse the direction of its motion so it can turn from north to south seeking bacterium. This behaviour explained why in the control of the bacteria some was being repelled instead of attracted to a certain magnetic field [22]. Another research was to blindly control the motion of the MTB toward a tumor cell with no image feedback because small narrow vessels that will be navigated in vivo will not be able to be shown using technologies used. Since a patient on 1891 had cancer recovered when infected by a bacteria [23], science tried to focus on ways to treat cancer using bacteria [24–30]. Some bacteria are stimulated by chemical gradient, others are affected by light or oxygen level and can change their motion accordingly however this was hard to control inside the body since its hard to change this factor inside it to make them reach solid tumors, however in the case of the bacteria where its motion can be controlled by a magnetic field this makes it a very good candidate. In this research work of blindly controlling the bacteria to reach tumour cell using a magnetic system where a software having the model of the bacteria would generate the currents at each coil to have certain fields at each one of the magnetic coils. Since the environment was unknown when the bacteria hits an obstacle it will move 90 degrees to the obstacle which made it harder to expect the behaviour of the control over it. However, the results showed that some of the bacteria was able to navigate inside the tumour cell [31].

The magnetotatic bacterial control was the focus of other researchers, one of this studies focused the closed-loop control of MTB in a micro-fabricated maze and compared this results to experiments carried out in a capillary tube which was bigger in size compared to the micro fabricated maze. This would show the effect of the channel wall on the motion and control of the bacteria. The maze had a width of 10µm and the capillary tube had width of 200 µm. The control was done using 4 orthogonal electromagnets and current was applied to them to get the needed magnetic field calculated by the controller, The Controller used was a proportional differential (PD) controller. The closed-loop control was repeated inside and outside the fabricated maze and the velocity as well as the region of convergence was measured in the paper. The results showed that the velocity of the bacterium was reduced when placed in the maze which was of narrow
diameter and this enhanced the region of convergence which was smaller than that reached in the capillary tube [32]. Another research paper was about studying the effect of increasing the frequency of an alternating magnetic field on the velocity of mtb and the region of convergence. Figure 2.1 shows the effect of increasing the frequency on the velocity of the mtb, it was shown that increasing the frequency would reduce the velocity due to the perturbation of the motion of the bacterium by increasing the frequency. In this research a null space control based on the redundancy of the magnetic setup was devised. This controller has two part the first try to change the orientation of the MTB while the other generate alternating field to reduce the velocity of the MTB. The results showed that the using a normal PD controller made the ROC equals to 20 $\mu$m and a velocity of 20 $\mu$m/s while using the new null space controller showed that the ROC was reduced to 13 $\mu$m and the velocity was reduced to 15 $\mu$m/s [33]. Finally a comparative study was done between control of MTB and that of a microjet where both were self propelled. The microjet as mentioned was a self propelled microrobot which benefit from the large projection distance of the magnetic fields compared to microrobots propelled by gradients in the fields, this microjets as well as a magnetotactic bacterium share this
property which allow them to be used in micro-assembly [34, 35], micro-actuation [36], micro-manipulation [37]. The microjet propel itself by conversion of chemical energy to kinetic energy, where it react with a hydrogen peroxide medium and emit oxygen bubbles which propel it. The result showed that the microjet has greater value for linear and rotational drag, as well as average speed and dipole moment. However, it was shown that the ROC of the bacterium was less than that of the microjet by about 94.5% which means that accurate positioning was better reached with the magnetotactic bacteria [2]. It should also be noticed that the main focus of this research work was to control microrobots for drug delivery and the use of microjet was difficult since the propulsion was based on reaction with hydrogen peroxide which was toxic to the body, also the microjet has length and diameter of 50 µm and 5 µm respectively which might be difficult to penetrate narrow vessels inside the human bodies that’s why the MTB was chose for this research work since MTB was small in size, self propelled and non toxic to the human.
Chapter 3

Culturing and Characterization of Magnetotactic Bacteria

Magnetotactic bacteria was discovered in 1970 when it was noticed that one of the samples of bacteria when viewed under the microscope collected swarms toward one edge and when the sample slide was moved around they swarmed toward a new point in a unidirectional manner. It has been confirmed that this motion is due to magnetic stimulus when a magnet was brought near to the sample under the microscope and the bacteria started to respond to the magnet by either moving away from it or toward it depending on whether they were north seeking or south seeking. This bacteria is either polar or axial where an axial bacteria can move to ward either the north or the south and they can flip their direction, while the polar bacteria would only be attracted to one pole either the north and they were found in the northern hemisphere and called north seeking bacteria or attracted to the south pole and was called south seeking bacteria. It was found that this bacteria contains iron grains inside their membrane which made them have a permanent dipole moment. The bacteria having a dipole moment ($M$) at an angle $\theta$ with respect to a magnetic field ($B$) experience a torque which would rotate the bacterium. This idea was the base of the research work presented here which will be further shown in the following sections. This bacteria move by means of a flagella at a speed which vary from 10 $\mu$m to 250 $\mu$m [11].
3.1 The Culturing

![SEM image of Magnetospirillum magnetotacticum](figure3_1.png)

**Figure 3.1:** A figure showing a SEM image of the *Magnetospirillum magnetotacticum* with two flagella bundles in the top of its cell body [3].

*Magnetospirillum gryphiswaldense* strain (MSR-1) was ordered from the German collection of microorganisms and cell cultures DSMZ. The magnetospirillum growth medium (DSMZ 380) preparation was with reference to catalogue number DMS 6361 in which detailed information of the chemical used and the concentration quantities needed to prepare one liter of the medium growth was provided. In order to attain the best sterilizing conditions the medium growth was autoclaved at 121 °C for 15 minutes. Afterward aseptically fill screw-capped containers to full capacity with sterile medium, then inoculate heavily leaving no head space of air, and screw down closures tightly. The strain was inoculated in with an oxygen concentration of 1%. The cultures were then cultivated at 26 °C for two to four days. Samples were harvested and selected for experiments based on responsiveness to external magnetic fields, indicating presence of individual magnetic chains were a rotating magnetic field was applied and if there was swirling this would indicate the presence of magnetic bacteria. In order to separate the north and south seeking bacteria means of two magnets discs were used with north and south orientation to differentiate between them. Refer to figure 3.1 showing an image of the mtb.
3.2 Characterizing the Magnetic dipole moments

The Magnetotactic bacteria when subjected to magnetic field it experience a torque and force over it which is represented in equation 3.1 and 3.2, respectively,

\[ T(p) = M \times B(p), \]  

where \( T(p) \) is the torque, \( M \) is the dipole moment of the bacterium and \( B(p) \) is the magnetic field acting on point \( p \). The force equation is given by 3.2

\[ F(p) = (M \cdot \nabla)B(p), \]

where \( F(p) \) is the force experienced by the bacterium which depend of the dipole moment of the bacteria. As both equations show, both the torque and force experienced by the bacterium depends on it dipole moment, which differ from one bacterium to another and need to be measured. There are several ways to measure the dipole moment of the bacteria. The Transmission electron microscopy (TEM) images is one of the ways to calculate the dipole moment of the bacterium whether motile or non motile. Another way to measure is through the flip time technique which is suitable for non motile bacteria and finally the rotating field and the \textit{u-turn} techniques which are only suitable for motile ones.

The TEM image shows the ferromagnetic nano crystals inside the bacterium membrane that causes them to have a dipole moment. This nano crystals have a cuboctahedral morphology. From the TEM image the number of nano crystals and the volume of each of them is recorded, Then the magnetic dipole moment can be calculated as equation 3.3.

\[ M = \sum_{i=1}^{n} M_s V_i, \]

where the \( M_s \) is the saturation magnetization of magnetite and the total \( M \) will be the summation of \( n \) times nano crystals with volume \( V_i \). Figure 3.2 shows how a TEM image of magnetic bacterium will appear and how the nano crystals can be counted and the volume can be calculated for each in order to determine their dipole moments. The second technique is the flip time technique, it can only work with the non motile bacteria. When the magnetic field over the bacteria is reversed the bacterium will flip with it due to its magnetic dipole moment.
Scanning electron microscopy. The left side showing the *magnetospirillum magnetotacticum*. The right showing the strain *magnetospirillum magneticum*.

\[ \tau = \frac{\alpha}{|M| |\mathbf{B}(p)|} \ln \left( \frac{2 |M| |\mathbf{B}(p)|}{kT} \right). \]  

(3.4)

The flip time \( \tau \) is recorded and is given by equation 3.4, where \( k \) and \( T \) are the Boltzmann constant and the temperature of the medium \[38\], and \( \alpha \) is the rotational drag which is calculated according to equation 3.5

\[ \alpha = \frac{\pi \eta L^3}{3} \left[ \ln \left( \frac{L}{d} \right) + 0.92 \left( \frac{d}{L} \right) - 0.662 \right]^{-1}, \]  

(3.5)

where \( \eta \), \( L \) and \( d \) are the medium viscosity, length and diameter of the bacterium respectively \[39\]. So substituting in this equation will derive the dipole moment of the non motile bacterium.

The third Method is the rotating field method where the rotating field influence the magnetic torque as well as the angular velocity of the MTB (\( \omega \)). The relation between both is shown in equation 3.6

\[ |M| |\mathbf{B}(p)| \sin \beta + \alpha \omega = 0, \]  

(3.6)

where \( \beta \) is the angle between the magnetic field and the magnetic dipole moment \[40\]. In order to be able to calculate the dipole moment the boundary frequency (\( \omega_b \)). This frequency is determined by gradually increasing the rotating fields and observing the frequency at which the MTB no longer follow the field so substitute in 3.6 with \( \omega = \omega_b \) and \( \sin \beta = 1 \) and then calculate the dipole moment.
Finally the u-turn technique, when a magnetic field acting on the bacteria is reversed the bacteria undergo a u-turn trajectory where the diameter of the u-turn is given by

\[ D = \frac{\alpha \pi v}{|M||\mathbf{B}(\mathbf{P})|}, \]

where \( v \) is the linear velocity of the MTB [39]. The u-turn technique was the one used in this thesis to characterize the dipole moment of the MTB this is because unlike the TEM it is not costly, and it is suitable for motile bacteria which is the focus of this thesis since motility is needed to have closed-loop positioning of the bacterium, accordingly the flip time technique was not considered and finally the rotating field technique could have been used but the frequency of the field need to be increased till the bacteria can no longer follow it, which would take more time to implement for each bacterium unlike the u-turn technique where you apply just one magnetic field and reverse it to get the dipole moment (\( M \)). Figure 3.3 shows how the MTB will move in a u-turn by reversing the current [41].
Chapter 4

Mechanical Setup and Electrical Components

4.1 The Mechanical parts

The control was done using four orthogonal electromagnets with 970 turns using copper wire of thickness 0.7 mm coated with non conductive material. The diameter of the electromagnet is equal to 30.5 mm and length of 31 mm and a core of length 46 mm and diameter 8 mm. Refer to Appendix A where figure A.1 which shows a schematic of the electromagnet. The size of the electromagnet were chose to be small in order to fit over the stage table of an inverted microscope used in the experiments as well as that the bacteria can be manipulated with magnetic fields as small as 0.4 mT, so a bigger electromagnet was not necessary.

The four electromagnets were kept in place and prevented from being pulled by each other magnetic fields by means of a 3D printed setup with the dimensions shown in figure A.2 in appendix A. This setup was designed such as to be hollow from the middle bottom in order to allow the lens to pass the objective length to the channel containing the bacteria, and it was hollow from the middle top part to allow light from a ring shaped light source to pass through the chips to the objective lens. The setup had protruding parts in the center to prevent magnets from pulling each other in the center if their polarity is opposite each other and strong enough. The setup together with the electromagnets are placed over the inverted microscope stage and they are illuminated from the top as shown in figure 4.1.
Figure 4.1: Setup with electromagnets placed orthogonally over a microscope stage. One electromagnet is able to produce up to 5.27 mT and a flea 3 camera with an field of view of 93 x 70 µm width and height.

The sample was illuminated by a ring shaped light source to have a dark field illuminate of the sample instead of bright field because when bright light was used the bacteria was not showing this is because the bacteria is transparent and very little contrast is shown between the bacteria and the medium which is also colourless, so a way to solve this was to stain the bacteria, but for the MTB a way to stain it without affecting its motility and magnetic property is not found yet. The other solution was to implement dark field illumination which would not direct light beams directly into the sample to the objective lens but rather illuminate the sample from the sides, hence the ring shaped light source, and the image acquired excludes the unscattered beam from the image. As a result, the field around the bacteria, which is not scattered by the bacteria, is generally dark.

The bacteria is then placed in a chip having channels of depth 5 µm and width 600 µm (figure 4.2). This chip is then placed at the center between the electromagnets over the microscope stage between the 3d setup, under the light source. The depth of the channel helped the bacteria to remain in focus most of the time since the average length of the bacterium is 5 µm and diameter of 0.5 µm, so bacterium couldn’t swim out of focus.
4.2 The Electrical Parts

The four electromagnets were then connected to the output ports of four electric drivers manufactured by Cytron technologies, model md10c. This drivers where picked since they could stand up to 13 A current, work at high frequencies and allow sign-magnitude Pulse Width Modulation (PWM) as its input. This allowed the reversal of the current which helped in reversing the fields directions during the experiments. The drivers received the PWM value and the direction of flow of the current as from a connected micro-controller which is arduino uno. The arduino was powered by a usb connected to the pc while the drivers where powered by an external power supply. The driver, arduino and the power supply were connected via a designed PCB board shown in figure A.3 in the attached appendix A. The V+ and V- is connected to the power supply which will be used to power the drivers indicated by d1, d2,d3,d4 in figure A.3 in the appendix. The arduino is then connected to the board pins from 0 to 13 which will then be used to determine the PWM and direction of applying the currents to electromagnets via the drivers, it will also provide a common ground to the drivers.
Chapter 5

The Tracking Algorithm and the Controller Code

In order to provide the controller with the bacterium position in $x$ and $y$, the channel containing the bacteria mentioned in 4 is placed on an inverted microscope with an objective lens of magnification 50X and dark field was used to illuminate the sample. A point grey flea3 model FL3-U3-13S2M-CS camera was used with a pixel size of 3.63 $\mu$m. The streaming of the bacteria was done at 30 FPS using this camera which is connected to the microscope and it was calculated that 1 pixel is 0.1452 $\mu$m in real life.

secondly, The image acquired from the camera is then processed in order to track a single bacterium. The Image processing part was developed using C++ on visual studio programming platform by the help of the opencv library. Thirdly, after the position of a single bacterium is detected by the tracking algorithm the controller would calculate the control action values and by using the Boost library these values are sent over to the Arduino by serial communication which would produce the needed output PWM to the drivers connected to four orthogonal electromagnets. and would accordingly provide the needed magnetic field.

5.1 Image processing: Tracking algorithm

The tracking is done using the frame differencing technique which would take 2 frames, one at time (t-1), and another at time t. The first frame is considered as a background while the other frame will be considered as the image of the moving object, this two frames are then subtracted and saved in a new image called the difference image, this image will be black except for the moving bacterium which would be white. The Tracking algorithm was as follows:
Algorithm 1: The Tracking Algorithm to Return the Position of the Bacteria Throughout the Experiment

**Input**: Streaming video of the bacteria (\textit{vid}), previous bacteria position (\textit{prev\_bacteria\_pos}), thresholding value (\textit{threshold\_value}), current time (\textit{t}), blurring matrix size (\textit{blur\_size})

**Output**: current bacteria position (\textit{current\_bacteria\_pos})

```plaintext
tracking=true;
while tracking do
    frame1 ← read(vid, t - 1);
    frame2 ← read(vid, t);
    diff\_image ← diff(frame1, frame2);
    threshold\_image ← threshold(diff\_image, threshold\_value);
    blurred\_image ← blur(threshold\_image, blur\_size);
    vector\_of\_bacteria ← findblob(blurred\_image);
    current\_bacteria\_pos ← compare(vector\_of\_bacteria, prev\_bacteria\_pos);
    prev\_bacteria\_pos ← current\_bacteria\_pos;
end
```

- Frame1 is acquired at time \( t-1 \) and then Frame2 is acquired at time \( t \), both are gray scale images since the camera is Monochromatic.

- A diff\_image would hold the absolute difference between both Frame1 and Frame2. Please refer to Figure 5.1

![Figure 5.1: Left Image from frame1 taken at t-1 and the right image is from subtracting frame1 and frame2 from each other and saving it in a new image called diff\_image](image)

- A threshold value is then selected to get a binary image from the difference image so the image would now be only black or white, no intermediate values. This image is called the Threshold\_image.

- The threshold\_image is then blurred to remove any noise in the result.

- The blurred\_image would have white blobs for the moving bacterium and black background, this image is passed to a method responsible for finding different blobs and put them in a vector of blobs called vector\_of\_bacteria. Refer to figure 5.2
Figure 5.2: an image showing 2 sliders one called the threshold, which adjust the threshold value of the frames takes to select the threshold value and a blur slider which adjust the blur up matrix in order to reduce the image noise

- The blob of interest is then selected and saved in prev_bacteria_pos, then the vector_of_bacteria is compared with the selected position prev_bacteria_pos and the closest blob to the selected position is then tagged as the blob of interested called current_bacteria_pos.

- Each time the vector of blobs is reformed it compares the position of each blob in the vector with the tagged blob from the previous time step and the closest blob to the last one would be selected again as the tagged blob of interested and this is repeated as long as the tracking is activated and at each time step the bacterium position in x and y is provided to the controller. Refer to figure 5.3

Figure 5.3: The green circle indicates the bacterium position at each time frame and is selected using the feature tracking algorithm. This bacterium swims at an average speed of 20 µm/s
5.2 The Controller Code

After the tracking algorithm provide the actual position of the MTB in x and y, this position is then compared to the selected reference position and the error in x and in y is calculated. The control law is then implemented and the corrective action for the four electromagnets is then sent to the serial port via the use of the BOOST library which is an open source library. The code also has an option to switch between controller and u-turn mode, where in the u-turn mode a constant field value is sent to the serial port which alternate between the left and right magnet. The number of characters sent over the serial port is chosen to be 12 characters which is the pulse width modulation value of the 4 electromagnets separated by ”/”. The Arduino is then reading the values from the serial port and separating them based on finding the character ”/” . The Strings was then converted to Integers and then the direction of the fields together with the PWM value is supplied to the drivers which will accordingly change the field.
Chapter 6

Magnetic Fields and Gradients at Different Currents

6.1 The Magnetic Fields Produced by the Electromagnetic System

In order to manipulate the movement of a single bacterium the magnetic fields of the four electromagnets shown is changed. At first the idea was to place the chip at the center of the electromagnets core and work at the center of the work space and for that the magnets would work as follows if the bacterium is at the left of a reference point then the right electromagnet is activated and vise versa, and if the bacterium is above the reference point then the bottom electromagnet is activated and vise versa. This idea was to work at the center of the cores so the direction of field vector is only having a component in the direction we want the bacterium to move to and negligible components in the other directions. This was tested by a Comsol model and the value of the fields for currents of 0.1,0.5,1,2,3 ampere was shown when only the left magnet magnet is activated. Refer to table 6.1. The table show that when we want to direct the bacterium to the left the $B_x$ is greater than $B_y$ by 99.42% or more, so it was assumed that $B_y$ is negligible compared to $B_x$, also $B_x$ is greater than $B_z$ by 97.74% or less, and it is assumed to have very little effect as well. So according to this model we can activated either the left or right and either the top or bottom electromagnet to manipulate the direction of a single bacterium to a reference position.
Afterward due to the focal length of the used objective lens the chip containing the bacterium had to brought below the core center by 15 mm so it would be closer to the objective lens. This changed the effect of the magnetic field at the center of the sample and when they were recalculated at this different altitude they were as table 6.2 shows. From the table we can see that still the By component can be neglected. However, the Bz component now is about 70.96% of the Bx value.

In order to cancel the Bz effect and have a more homogeneous magnetic field two magnets instead of one is switched on, for example if the bacterium is at the left of a reference point then not only will the right electromagnet be switched on by certain current for the equivalent magnetic field but also the left electromagnet is switched on by negative this current value (opposite direction) by using this approach the Bx value would be greater than before because it is the effect of both electromagnets but also the Bz is reduced since both electromagnet will have opposing Bz fields. This was shown by Comsol simulations in table 6.3. So by switching on two electromagnets instead of one Bz is back to just 1.69 % of Bx value. The By is slightly increased but it is still 1.3 % of Bx value so we can assume that it is negligible as well.
The results from the model had to be compared with results from actual measurement to see if the deviation is in the acceptable range. This is shown in figure 6.1. The deviation between the comsol and the real measurements is of maximum 0.09 mT, which is considered in the acceptable region.

Table 6.3: Magnetic fields below the cores by 15 mm, 2 electromagnet switched on

<table>
<thead>
<tr>
<th>current (A)</th>
<th>Bx (mT)</th>
<th>By (mT)</th>
<th>Bz (mT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>-0.61</td>
<td>0.0</td>
<td>-0.01</td>
</tr>
<tr>
<td>0.5</td>
<td>-3.04</td>
<td>0.04</td>
<td>-0.05</td>
</tr>
<tr>
<td>1</td>
<td>-6.08</td>
<td>0.08</td>
<td>-0.1</td>
</tr>
<tr>
<td>2</td>
<td>-12.17</td>
<td>0.16</td>
<td>-0.2</td>
</tr>
<tr>
<td>3</td>
<td>-18.26</td>
<td>0.24</td>
<td>-0.31</td>
</tr>
</tbody>
</table>
Chapter 7

Experiments and Results
Chapter 8

Experiments and Results

The objective of the work is to be able to have closed-loop control for accurate positioning of the bacterium in the vicinity of a reference point. The control of the magnetotactic bacterium was done by implementing the fact that the direction of motion of the bacterium can be manipulated by changing the magnetic field acting on it, causing it to rotate due to a torque acting on it as shown in equation 3.1. The closed-loop block diagram explaining the flow of data is shown in figure 8.1, the closed-loop control to position the bacteria used a selected reference position and compared it with the bacterium current position provided by the tracking algorithm discussed in chapter 5 and then the error along $x$-axis ($e_x$) and the error along $y$-axis ($e_y$) was calculated as shown in 8.1 (the $x$-axis and $y$-axis are as shown in figure 8.2).

$$e = p_{\text{ref}} - p_{\text{act}},$$

where $e$ is the error along $x$-axis and $y$-axis, the $p_{\text{ref}}$ is the reference point along $x$-axis and $y$-axis and $p_{\text{act}}$ is the actual position of the bacterium provided by the tracking algorithm. It should be noticed that the error will never converge to zero since the bacteria is always moving with its flagella with a certain velocity which depends on the bacterium characteristics so instead of converging to a zero the bacteria will always converge to a region around the reference point which is called the region of convergence (ROC), the smaller this region the better the controller. The bacterium at the start of the control would move in diagonal lines to reach the reference point and once it reaches the reference point it start to move in curves around the reference point which we called the ROC. The proposed controller for the work presented here was a proportional differential integral (PID) controller. However after further investigation it was decided to work with only a proportional controller for the following presented reasons:
• PD controller: This type of controller was discarded since the differential part of the controller will always add a gain to the change of error with time, as we previously mentioned, the velocity of the bacterium is constant and can’t be changed so the change of the error with time will be almost constant at the start of the control and as the bacteria approached the reference point and assuming it moves in a perfect circular behaviour around it (ROC) this change of error with time would become zero. So adding this controller would mean that the corrective action will start with big values and this value will drop with time. It will be shown in this chapter that it is advised to start with small magnetic fields and once the bacterium reach the reference point this corrective action should increase in order to get a smaller ROC. So accordingly The PD controller was not implemented.

• PI controller: This type of controller was discarded although it might reach satisfactory ROC, however since the error is not going to zero but a region around the reference, this controller might reach saturation very fast even before being close to the reference point which would consume a lot of energy. So, it was decided that a proportional controller is better than the PI control system.

There are two types of proportional controller presented in this work, first a proportional controller with a constant Kp value, and another controller with an adaptive kp value. A comparison of the performance of the two controller regarding there effect on the ROC will be shown in the end of the chapter.
8.1 Closed-Loop Control using a Constant Proportional Controller

Under the influence of external magnetic fields, an MTB is subjected to the following torque mentioned in equation 3.1. The $e_x$ and the $e_y$ are used to get the corrective action in $x$ and in $y$ using a proportional gain $K_p$. The $K_p$ value was selected such that the magnetic fields will not reach saturation at big errors in $x$ and in $y$ while still attaining satisfactory behaviour. The correction ($B$) which is the magnetic field in $x$ and in $y$ was calculated as in equation 8.2 provided to the electromagnets in the form of currents to produce the needed magnetic field.

$$B = K_p e.$$  \hspace{1cm} (8.2)

Depending on the sign of the $e_x$, the direction of the magnetic field lines of both the right and left electromagnet is determined while keeping their magnitude the same. The direction is determined such that if the $e_x$ is positive it will make current flow in the positive direction in the left electromagnet and in the negative direction in the right electromagnet this will allow the $B_x$ to be doubled and the $B_z$ to cancel each other. This is explained in details in chapter 6. The same way the $e_y$ will affect the direction of current flowing in the top and bottom electromagnet (the right, left, top and bottom electromagnets are indicated in figure 4.1. The magnetic field ($B$) will always be a vector pointing towards the reference point which will cause a torque on the bacterium due to the angle ($\theta$) between the orientation of the bacterium and the magnetic field lines.

An example of an experiment using closed-loop control with constant $k_p$ to position the bacterium at a reference position is given in figure 8.2, in the figure the blue dot indicate the reference positions selected and the green circle indicate the position of the bacterium. This experiments was done using 3 reference points and Figure 8.3 show the trajectory of the bacterium in blue around 3 reference positions indicated by black lines. The results showed that the bacterium followed the fields toward a reference point but will circulate around it in a circular behaviour called the region of convergence (ROC). The maximum ROC measured for this experiment was 51 $\mu$m, 38.9 $\mu$m and 38.2 $\mu$m for a bacterium with 17.4 $\mu$m/s velocity. Using the same controller while using different bacteria yield different value of the average ROC, so in order to have an idea of the average ROC relation with different bacteria, this experiment was repeated using different bacteria with different properties. The data gathered from this experiments is shown in table 8.1. The velocity of each bacterium, the dipole moment and the region of convergence (ROC) is illustrated in the figure. The results showed that the ROC is directly proportional to the the velocity divided by the dipole moment. Although the
Figure 8.2: A time frame representative constant proportional closed-loop control of a magnetotactic bacterium (MTB), i.e., Magnetospirillum gryphiswaldense strain MSR-1, under the influence of the controlled magnetic fields. The closed-loop control system localizes the MTB within the vicinity of the reference position (small blue circle).

results is satisfactory for some bacterium of certain properties which gave small ROC other showed large ROC.

Table 8.1: The data gathered from different bacteria using same constant proportional controller

<table>
<thead>
<tr>
<th>Experiment</th>
<th>( v(\text{m/s}) )</th>
<th>( m(\text{a.m}^2) )</th>
<th>( \frac{v}{m} )</th>
<th>ROC (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.40E-05</td>
<td>2.40E-20</td>
<td>5.83E+14</td>
<td>2.84E-06</td>
</tr>
<tr>
<td>2</td>
<td>1.53E-05</td>
<td>1.64E-20</td>
<td>9.30E+14</td>
<td>3.70E-06</td>
</tr>
<tr>
<td>3</td>
<td>1.65E-05</td>
<td>1.65E-20</td>
<td>1.00E+15</td>
<td>4.85E-06</td>
</tr>
<tr>
<td>4</td>
<td>1.44E-05</td>
<td>1.27E-20</td>
<td>1.13E+15</td>
<td>6.18E-06</td>
</tr>
<tr>
<td>5</td>
<td>1.59E-05</td>
<td>6.00E-21</td>
<td>2.64E+15</td>
<td>6.67E-06</td>
</tr>
<tr>
<td>6</td>
<td>2.75E-05</td>
<td>3.92E-21</td>
<td>7.03E+15</td>
<td>1.19E-05</td>
</tr>
</tbody>
</table>

8.2 Effect of increasing the magnetic field (B) value on the ROC of the bacterium

The ROC is usually a circular region which depends on the torque acting of the bacterium at all times. The torque doesn’t only depend on the dipole moment of the bacterium but also on the magnetic field strength. Increasing the field acting on a bacterium should increase the torque and make the rotation of the bacterium stronger. Also if the field is rotating as in the case when the bacterium reach the ROC, this increase in the field strength should have an effect in reducing the radius of curvature the bacterium undergoes and hence reduce the ROC. This can be noticed in the \textit{u-turns} experiments discussed in Chapter 3, where increasing the reversed fields can make the \textit{u-turns} narrower and vice versa. This was shown in an experiment carried out at two different fields, see figure 8.4.
Figure 8.3: Three reference positions (vertical black lines) are given to the control system and the MTB is localized within their vicinity. The diameter of the regions of convergence of the three reference positions are 51 µm, 38.9 µm, and 38.2 µm. The speed of the MTB is calculated to be 17.4 µm/s, in this trial. The green circle indicates the position of the MTB and is assigned using the feature tracking algorithm.

The radius of u-turn was reduced from 19 µm at magnetic field \( B \) of 4.1 mT (indicated in the figure by the blue trajectory) to 7 µm at magnetic fields \( B \) of 8.3 mT (indicated in the figure by the red trajectory). This observation of the u-turn having smaller radius of curvature was the reason for studying the effect of increasing the magnetic field on the ROC. Experiments to show the effect of increasing the \( k_p \) , which reflects in an increase in the applied magnetic fields for the same error, on the ROC was implemented.

An experiment was done on a bacterium such that the average magnetic field \( B \) started from 1 mT and then it was increased to 1.4 mT and the average ROC was recorded. Figure 8.5 shows the effect of the increasing the magnetic field \( B \) from 1 to 1.4 mT.
Figure 8.5: The influence of the magnetic field on the size of the region-of-convergence is analyzed on the same magnetotactic bacterium (MTB), i.e., Magnetospirillum gryphiswaldense strain MSR-1, by increasing the magnitude of the magnetic field. The MTB is localized within the vicinity of the reference position (vertical black line) and the magnetic field is increased from 1.0 mT to 1.4 mT at time. The size of the region of convergence is decreased from 18.4 µm to 11.3 µm. The red line indicates the path of the MTB under the influence of the proportional control system. Left: The path taken by the controlled MTB towards the reference position (vertical black line). Middle: Position of the MTB along x-axis. Right: Position of the MTB along y-axis.

on the region of conversion. The time at which the increase in the magnetic field (B) occurred is indicated by the change in the background gray color. As indicated in the figure, the increase in the magnetic fields (B) resulted in a decrease in the ROC of the bacterium from 18.4 µm to 11.3 µm. The data gathered from 6 different bacteria using this experiment is shown in figure 8.6. It is observed that regardless of using different MTB for each experiment the behaviour remains the same which is a noted decrease in the ROC by increasing the magnetic fields (note: the experiments were tested using different count of magnetic fields, this is due to the loss of tracking). According to the mentioned relation between increasing the magnetic fields and the decrease in the ROC a new controller is introduced which is the adaptive proportional controller mentioned in the next section.
Figure 8.6: Closed-loop control of magnetotactic bacteria (MTBs) at different magnetic fields is achieved. Increasing the magnitude of the magnetic fields results in a decrease in the region-of-convergence of the controlled magnetotactic bacterium (MTB) and does not have an influence on its swimming speed. The 6 closed-loop control trials are done using MTBs from the same culture (Magnetospirillum gryphiswaldense strain MSR-1). Each trial is done using the same MTB.

8.3 Closed-Loop Control using adaptive Proportional controller

According to the results shown in the previous parts of this chapter, which is the bacteria moving with its average velocity will reach a reference point with a large region of convergence and increasing the magnetic fields when the bacterium is close to the reference point will reduce it radius of curvature and accordingly reduce its region of convergence. It was decided to design a proportional controller which will have an adaptive Kp value depending on the error such that the Kp will be small as long as the bacterium is still far from the reference point and increase gradually when it gets smaller to reduce it radius of curvature and accordingly the ROC. The Kp function is shown in equation 8.3 and the kp relation with the error is shown in figure 8.7.

\[ K_p = k_1 \exp\left(\frac{e}{k_2}\right) + k_3 \prod, \]

where \( k_1 \), \( k_2 \) and \( k_3 \) are positive control gain, and \( \prod \) is the identity matrix. This controller allow us to increase the magnetic field strength for error that are relatively small. An experiment is conducted using this new controller and the result is shown in figure 8.8 the blue dot indicate the reference positions selected and the green circle indicate the position of the bacterium. This experiments was done using 4 reference points and Figure 8.9 show the trajectory of the bacterium in blue around 4 reference positions indicated by black lines. The results showed that the bacterium followed the
Figure 8.7: A plot representation of the Kp equation mentioned 8.3, which shows the Kp value of the controller in relation with the error in pixels measured, where the Kp value is small for large errors and increase gradually as the error becomes smaller.

fields toward a reference point. The maximum ROC measured for this experiment was 18.1 µm, 13.9 µm, 12.3 µm and 14.7 µm for a bacterium with 17.4 µm/s velocity. This experiment was conducted using the same bacterium as the one used previously using the constant proportional controller in figure 8.3, it was shown that the ROC became significantly smaller when using the new adaptive proportional controller, where there ROC was reduced by 65.5.

Figure 8.8: A time frame representative adaptive proportional closed-loop control of a magnetotactic bacterium (MTB), i.e., Magnetospirillum gryphiswaldense strain MSR-1, under the influence of the controlled magnetic fields. The closed-loop control system localizes the MTB within the vicinity of the reference position (small blue circle).
Figure 8.9: four reference positions (vertical black lines) are given to the control system and the MTB is localized within their vicinity. The diameter of the regions of convergence of the three reference positions are 18.1 µm, 13.9 µm, 12.3 µm and 14.7 µm. The speed of the MTB is calculated to be 17.4 µm/s, in this trial. The green circle indicates the position of the MTB and is assigned using the feature tracking algorithm.

Table 8.2: Comparison between the two different proportional controllers

<table>
<thead>
<tr>
<th>Experiment</th>
<th>v/m</th>
<th>ROC(m)</th>
<th>total reduction in %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>old controller</td>
<td>new controller</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>7.59E+15</td>
<td>3.56E-06</td>
<td>0.92E-6</td>
</tr>
<tr>
<td>2</td>
<td>1E+16</td>
<td>1.35E-06</td>
<td>0.84E-6</td>
</tr>
<tr>
<td>3</td>
<td>2.37E+15</td>
<td>3.96E-06</td>
<td>1.76E-6</td>
</tr>
<tr>
<td>4</td>
<td>3.08E+15</td>
<td>9.30E-07</td>
<td>0.65E-6</td>
</tr>
<tr>
<td>5</td>
<td>4.86E+15</td>
<td>2.20E-06</td>
<td>0.24E-6</td>
</tr>
</tbody>
</table>

More experiments were conducted to study the difference between using the two controllers (constant proportional and adaptive proportional controller) and the average ROC for each experiment was recorded using the same bacterium for each comparative experiment. The data gathered from 5 different experiments are shown in table 8.2. We can notice that the ROC is reduced with the new controller (adaptive kp) compared to the old one (constant proportional). This results showed that increasing the Kp gradually with the decrease in the error shows decrease in the average ROC. The decrease in ROC changes from one bacterium to another and the average decrease was of 52.8%.
Chapter 9

Conclusions and Future Work

9.1 Conclusions

In this study, we control the motion of magnetotactic bacteria, *Magnetospirillum Gryphiswaldense* strain MSR1, using constant proportional controller and adaptive proportional controller. The constant proportional controller achieved localization of the bacterium but with relatively large ROC. It was shown that the ROC of the MTB can be reduced by increasing the strength of the magnetic fields and accordingly the adaptive proportional controller was implemented. This controller had a Kp value which increased gradually as the error becomes smaller. In order to have a fair comparison between the 2 controllers, the same bacterium was used to implement a position control experiment. The adaptive controller reduced ROC by 65.5%.

9.2 Future Work

As part of future studies, motion control of magnetotactic bacteria will be achieved inside microfluidic channels with controlled time-varying flow rate. This study is necessary to translate these microorganisms into *in vivo* applications. The motion control characteristics will be studied along and against the flowing streams of the growth medium to analyze the ability of the flagella propulsive force to overcome the force due to time-varying flow and drag. In addition, magnetotactic bacteria will be controlled towards cancer cells and the cell uptake will be experimentally investigated (in a stationary fluid) under the influence of controlled magnetic fields. Focus on the motion control of magnetotactic bacteria using electromagnetic systems with open configuration will be inducted. Therefore, the electromagnetic system will be redesigned to allow for the
incorporation of a robotic arm to move the electromagnetic coils and the microscopic system.
Appendix A

An Appendix

Figure A.1: Drawing of an electromagnet used in the control of the MTB via magnetic fields, it is a coils of 970 turns, 31 mm in length with an outer diameter of 30.5 mm. The coil has an iron core of 46 mm length and 8 mm diameter. Four electromagnets are used in the setup with an orthogonal configuration.
Figure A.2: top view of the setup which accommodates the 4 electromagnets in an orthogonal configuration

Figure A.3: PCB board showing V+ and V- where the power supply is connected, it also shows d1, d2, d3 and d4 where the drivers are connected and pin 1 to 13 where the arduino is connected
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