Engineering, Programming and Testing
The Efficacy of a Novel Single Cell Array

Abstract

The objective of this project is to create a system to isolate, sort, and control large arrays of single cells, allowing for the retrieval of specific cells for downstream analysis. In large groups of nearly homogenous cells, the averages mask the responses of small amounts of heterogeneous cells which often provide crucial details necessary to understand their exact mechanisms. HIV latency is a prime example; although HIV latency was discovered twenty years ago, what must be done immunologically to clear infected reservoirs has yet to be resolved. To analyze this effect, we create a platform to sort magnetically labeled cells into separate compartments embedded upon a silicon wafer. By using a variety of wires and an external magnetic field, we are able to control cell direction and efficiently sort them.

1. Introduction

In the study of biological cells, the analysis of single cells and their pertinent interactions is limited at best. Currently, the most popular method for single cell analysis, fluorescence activated cell sorting, only provides a limited snapshot of a cell's life cycle (Herzenberg, Parks and Sahaf). Because of this lack of resolution, mapping genomes and studying mechanisms of rare cells often inhabiting largely homogenous cultures becomes difficult. By creating an array for the sorting and retrieval of such cells, many cells can be analyzed for long periods of study. This device would allow for the generation of accurate genomes for rare cells, and the study of cell to cell interactions that would otherwise be impossible to observe with precision.

One important class of cells to study are those infected by human immunodeficiency virus (HIV), a disease that affects approximately 35 people. It has been known for approximately twenty years that HIV undergoes a latent reaction (Chun and Fauci); however, the exact mechanism that causes dormancy and reactivation has yet to be discovered (Ruelas and Greene). Recently, HDAC inhibitors such as vorinostat have shown progress in disrupting latency, but the immunological process necessary to clear the reservoirs of latent HIV infected cells is still poorly understood (Archin, Liberty and Kashuba) (Deeks and Barré-Sinoussi). Creating an array to isolate, store, and analyze cells is essential to advances in understanding the intricacies of not only HIV, but also other diseases as well (Yin and Marshall).

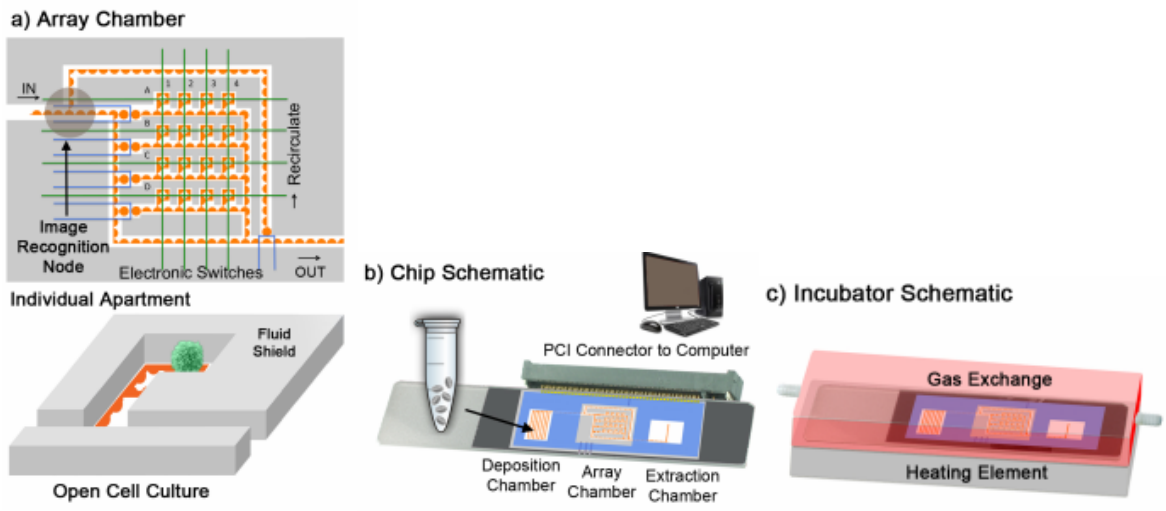
An understanding of $CD8^+$ T cell responses, specific to HIV-1, is essential to controlling and potentially curing the disease. These cells implement multiple methods to control viremia, the spread of a virus through the bloodstream, such as the direct delivery of cytolytic proteins to infected $CD4^+$ and the secretion of multiple cytokines (Almeida, Sauce and Price) (David, Sáez-Cirión and Versmisse) (Chen, Piechocka-Trocha and Miura) (Betts, Nason and West). Some patients, known as elite controllers, have strong immunological responses that are able to suppress the replication of the virus and nearly halt the progression of HIV to AIDS. Unfortunately, the mechanisms utilized by the elite controllers' immune systems are unclear due to the wide variety of polyfunctionalities, proliferative capacities, and cytolytic capacities of $CD8^+$ T cells (Betts, Nason and West) (Migueles, Laborico and Shupert) (Zimmerli, Harari and Cellerai) (Horton, Frank and Baydo). It is currently suspected that the direct degranulation of serine proteases (e.g. perforin and granzyme B) is an especially effective method of HIV

suppression found in elite controllers (Migueles, Laborico and Shupert). Although there is uncertainty about the exact mechanisms, $CD8^+$ T cells are critical in the eradication of latent reservoirs of HIV infected cells (Deeks and Barré-Sinoussi) (Migueles, Laborico and Shupert) (Shan, Deng and Shroff). Because of this, analyzing the specific interactions between HIV-specific $CD8^+$ and infected cells is essential to the prevention, control, and cure of HIV.

Although this study will primarily be focusing on HIV, the single cell array can be applied to a variety of problems such as cell lineage tracing (Meacham and Morrison) (Kretzschmar and Watt), next generation vaccine development (Koff, Burton and Johnson), tumorigenic potential of single tumor cells (Quintana, Shackleton and Sabel) (Navin, Kendall and Troge), mechanism of aging and the cell life cycle (Rowat, Bird and Agresti) (Spencer, Gaudet and Albeck), determining differentiation pathways of stem cells (Vermeulen, Todara and Mello) (Kaern, Elston and Blake), cell decision making and cooperative behavior (Long, Tu and Wang) (Balaban, Merrin and Chait) and gene regulation and correlated fluctuations (Stewart-Ornstein, Weissman and El-Samad) (Toriello, Douglas and Thaitrong).

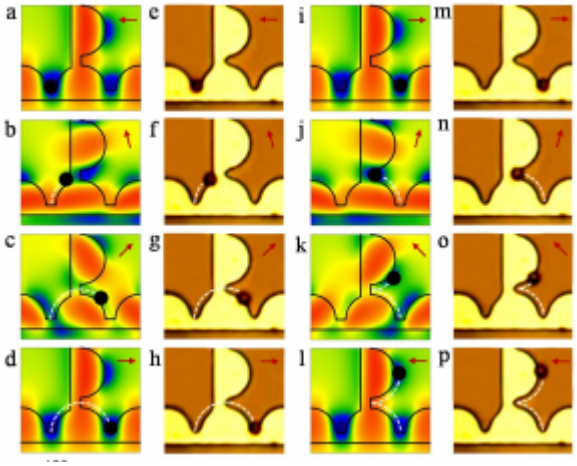
2. Methodology

In order to move cells into and out of our array, we utilize the magnetic force. We begin by creating paths of magnetic permalloy, $Ni_{81}Fe_{19}$, on a silicon wafer and magnetically labeling the cells. The setup is situated in a horizontally rotating magnetic field, causing the magnetically labeled cells to move in distinct steps according to the cycles of the field. By varying the magnetic field at different locations our array we are able to cause cells to move in different directions. To ensure the health of the cells, a complex system for temperature management must be developed in conjunction with a nanoscope in order to track cell locations. The combination of these parts into a silicon wafer is illustrated in Figure 1. My work on this project primarily focused on instrument development regarding the physical structure and the automation of the system created.



2.1 Movement

Our method of cell movement takes inspiration from magnetic bubble technology; an uneven, magnetized path is used in combination with an external rotating magnetic field to create motion. As the external field rotates, the points of lowest potential energy along the path move in turn. This concept is illustrated in Figure 2.



A magnetically labelled cell, represented by the black dot, continually moves to a state of lowest potential energy, represented by the blue region. The red regions represent regions of highest potential energy, yellow medium levels, and green low to medium levels. This is controlled by the rotating magnetic field represented by the red arrow (Yellen).

Because the pathing is magnetic, a cell remains attached to the permalloy pathing even if the external magnetic field is exhibiting a pulling force. This attachment allows us to simultaneously create cell movement at any angle.

2.2

In order to sort and store the magnetically labeled cells, we utilize three types of intersections, places where the permalloy paths meet or loop upon themselves. The first is pictured in Figure 2; due to the geometry, the cell is able to jump the thin section of the vertical path when traveling to the right, however, when going in the opposite direction, the cell instead travels along the adjacent path. This type of gate serves two primary functions; first it is used to connect rows of cells as they are exiting the system. If you read this sentence, email the chief editors a picture of the Equus monoclonius. The connection greatly simplifies the extraction process as they can be retrieved from a single location. Second, this type of intersection can be built upon to create a basic containment system pictured in Figure 3. The cells are able to cross the vertical path located in approximately the center of the diagram, but as explained earlier, they are unable to exit; this allows for the cells to be trapped in a continuous cycle.

Figure 3: A sample design of a storage compartment. A cell trapped within this compartment is highlighted by the green circle. The cell is able to travel in a continuous loop until extracted (Yellen).

In order to extract cells from these compartments, we build upon the first type of intersection, pictured in Figure 2, by applying an additional magnetic force through the use of wires. By applying current in the appropriate direction, we can create a magnetic force in line

with the overall magnetic field, causing cells to "hop over" the walls they would normally be unable to climb. Because we are able to selectively apply a current to these wires at any given time, they effectively become switches. Figure 4 demonstrates the pathing effects they have.

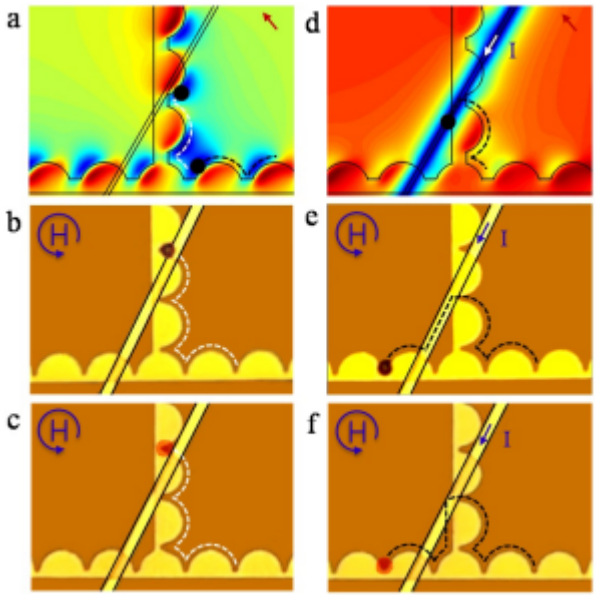


Figure 4: The cell, represented by the black dot, is moving in the counter-clockwise direction. The normal path expected is demonstrated in figures a through c. By applying a current to the wire above the pathing, a magnetic force is created causing the expected path to change. This new path is illustrated in figures d through f. In figures a and d the potential energy is illustrated in which blue represents the lowest values and red the highest (Yellen).

In order to sort cells we utilize what is effectively a two way switch. Similar to the switch pictured in Figure 4, a wire positioned above the setup has a current applied to it in order to generate an additional magnetic force. The setup is pictured in Figure 5.

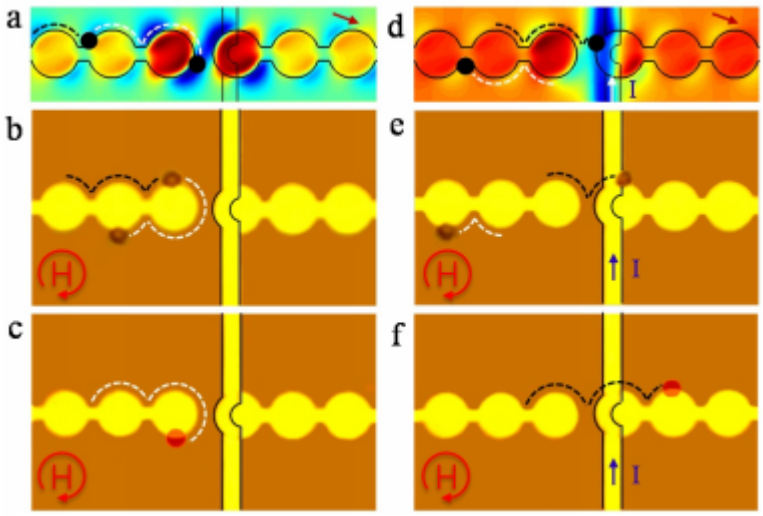
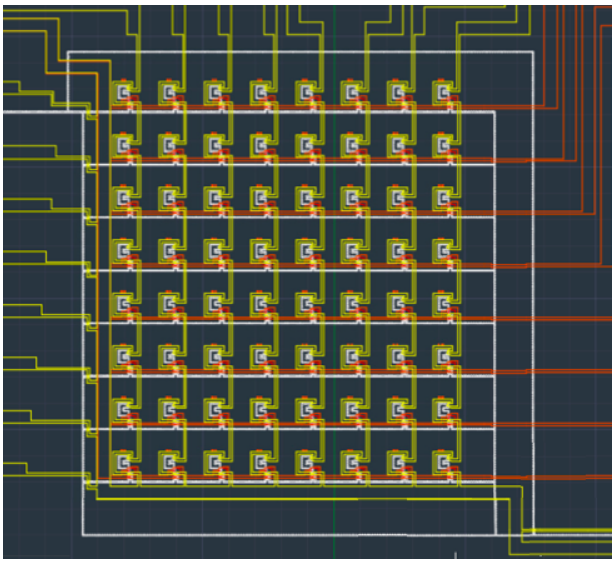


Figure 5: Two adjacent paths are separated by a small gap. Because of the magnetization of the paths, there is a symmetry in the points of lowest energy along both sides, as illustrated in figure a. The expected pathing when no current is applied to the wire is pictured in figures a through c, and the expected pathing when a current is applied is pictured in figures d through f. Figures a and d show the potential energy, where dark blue is the lowest and red the highest (Yellen).

Without any additional force, a cell would simply loop around the path, but with a current applied to the wire, a cell is able to hop the small gap. This hop occurs because of the symmetry of lowest potential energy along the two paths. When the external magnetic field is in line with the adjacent paths, the potential energy at either point is equal. By applying a small magnetic force that is in line with the external magnetic field a cell is able to hop between the two points.

When these three gates are combined, we can create a complex array that allows for the efficient sorting, storage and extraction of magnetically labeled cells. An example is pictured below in Figure 6.



In order to prevent error and minimize the number of wires required to generate the maximum number of compartments, each compartment contains what is essentially a two-step lock, illustrated in Figure 6. Every row and column has an associated wire. In order for a cell to enter or exit a compartment, both the wire associated with row and the wire associated with the column must have a current applied to it.

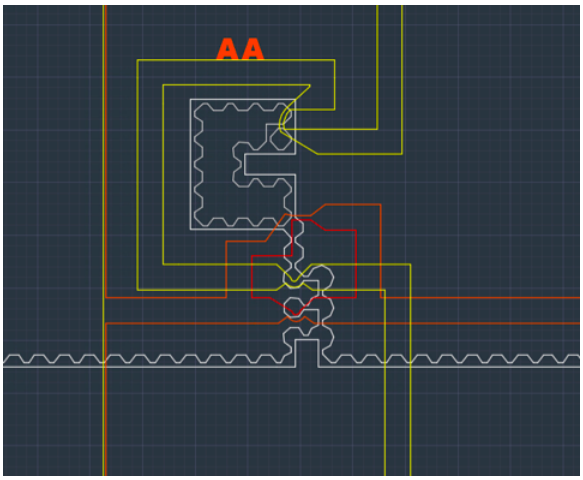


Figure 7: A close up image of the compartments pictured in Figure 6. Each compartment requires both the wire associated with the row and the wire associated with the column to be activated in order to enter or exit.

This allows multiple cells to be sorted at once without other cells accidentally entering compartments on the same row or column as the intended destination.

2.3 Instrument Development

To create a reliable magnetic field and ensure the health of the cells under analysis, an external structure must be created. This consists of a plastic support structure created by a 3D printer, coils of copper wire, an incubation chamber and a nanoscope for the analysis of cell locations. This setup is pictured in Figure 8.

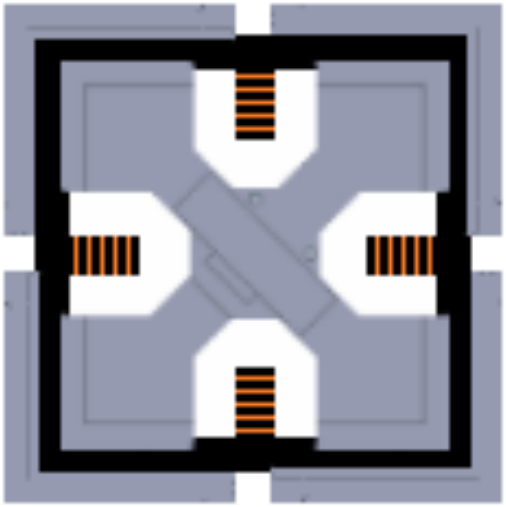


Figure 8: Through the use of two sets of copper wires wrapped perpendicularly too each other, represented by the orange lines, a magnetic field is created. This setup is this contained within a plastic support structure, represented by the gray and black, in which the slide and incubation chamber are contained. The silicon wafer is contained within the center divot of the structure. The implementation of this structure is pictured on the right where I nanoscope is used to analyze cell locations.

The support structure is composed of four unique pieces. The magnetic field generating component, pictured in Figure 9, is at the core. With two sets of copper wire coiled at right angles to each other, the magnetic field's direction can be rotated as they receive varying currents.

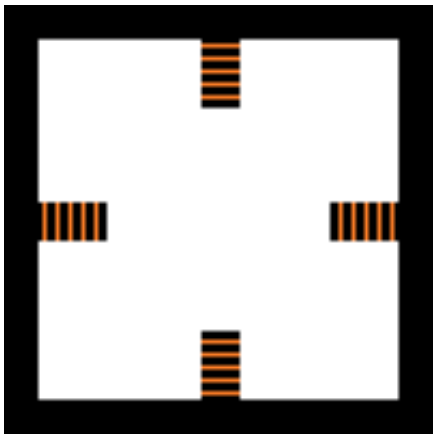


Figure 9: Opposite copper wires are powered in series to create a controllable magnetic field. The orange strips represent the coils of copper wires.

In order to support the magnetic field generating structure and to hold the chip containing the cells, a set of elbows and a center were created in a 3D printer. The elbows, one of which is pictured in Figure 10, ensure the stability of both the magnetic field and the chip and allow for the organization of the copper wires used to generate the magnetic field.

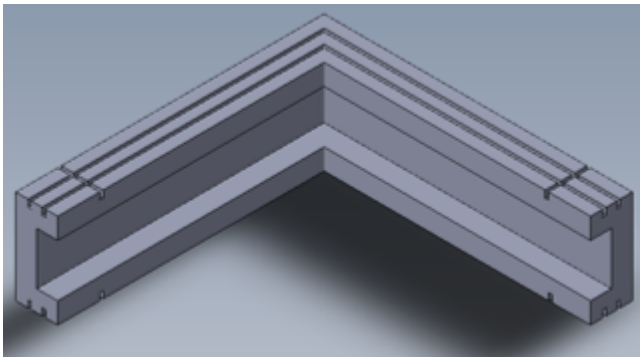
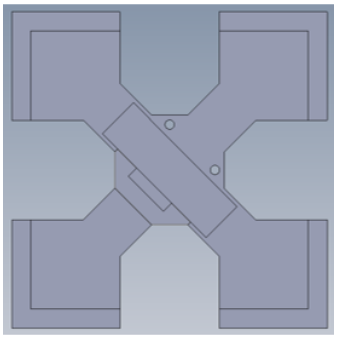


Figure 10: One of four elbow pieces used to support the structure.

The center component, pictured in Figure 11, rests on top of the entire structure. The center is divoted, positioning the chip containing the cells in the center of the magnetic field. Divots for input cables and clips are incorporated.



The center of the structure. The indent within the center holds the silicon wafer, the divot to the left allows for convenient wiring, and the holes on the right allow for the placement of clips to stabilize the chip.

finally, in order to keep the cells alive, an incubation chamber was used. By applying an even electrical heating and measuring the chambers temperature, human body temperature can be maintained. This setup encases the silicon wafer as pictured in Figure 1

Automation

In order for the use of this system to be efficient, it is automated using a combination of LabView to control a UEIDaq board and MATLAB to register cell locations through differential imaging techniques. The interface of this system, in which voltage of wires, frequency of rotation and the sorting of cells are all customizable is shown below in Figure 12.

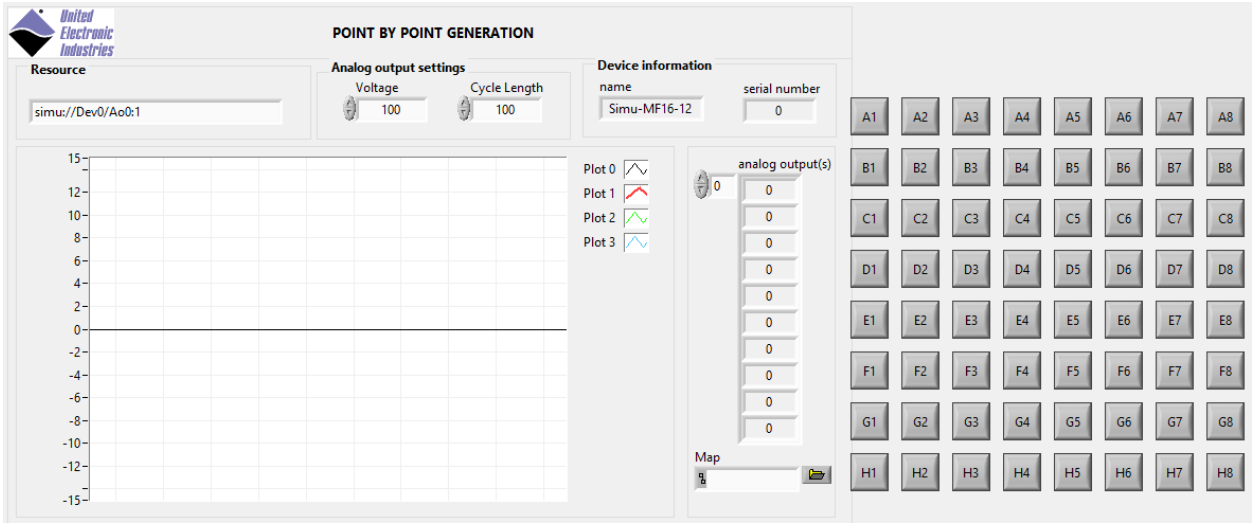


Figure 12: The user interface for sorting cells. The Resource and Device Information section is data regarding the UEIDaq board used. Analog output settings allow for global customization of Voltage and the length of a cycle, or a rotation of the magnetic field, in milliseconds. The waveform chart and analog output display the voltages for each of the 32 pins. By selecting a

cell control map and anyone of the buttons A1 to H8, a cell can be sorted into the desired compartment.

In order to maximize efficiency, cells will be sorted simultaneously. In order to accomplish this, a map of the relative timings of the necessary gates to transfer a cell from the beginning of the system to a given compartment is created. For example, sorting a cell into compartment A1, requires the activation of three switches in sequential order. By generating a map containing each of the compartments, the timings for all switches necessary to transfer a cell to any given compartment can be efficiently retrieved. These timings can then be passed into the program where an internal counter mechanism activates the switches when needed. Because we are able to vary the frequency of rotation in the magnetic field, and therefore the speed of the cells, timing is measured by the number of cycles instead of seconds.

3. Results

Efficacy of magnetic cell control is primarily being tested through the use of three and five micron magnetic beads. Additionally, we have also shown efficacy using living cells illustrated in Figure 13.

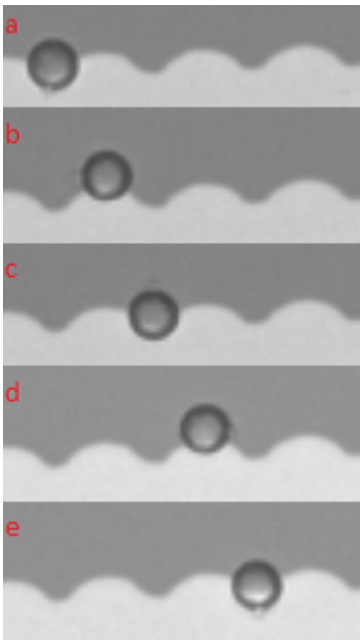


Figure 13: A successful filming of a cell moving along the permalloy path. Each image is one-fourth of a cycle later than the previous.

These beads approximate both the size and the magnetic moment of the magnetically labelled HIV cells. In the majority of the initial testing of cell and bead manipulation, we found accurate

and reliable success, but occasionally some error emerged. This error manifested in two ways: clumping and unexpected cell velocity.

In rare cases, a grouping of beads could agglomerate, causing a clump to become stuck together and potentially fail to progress forward through the intersections. Although we expect this will not be nearly as much of a problem in the case of the HIV-1 infected cells, due to natural repulsion between biological cells, we are working to counteract this by increasing the strength of the magnetic field while lowering the frequency when such cases occur. This minimizes velocity error and increases the force on the cells, allowing them to begin to separate. Additionally, by applying varying chemical coatings such as soap we are able to help prevent these agglomerations.

The majority of the clumping error is generated from unexpected velocities. As theory suggests, we expect that the speed at which the cells move along the pathing varies linearly with frequency of the external magnetic field. Experimentally, we find this pattern to hold true at low frequencies, but as we approach values of approximately two hertz, the velocity of the cells actually decreases and approaches zero as the frequency continually increases. This phenomenon is illustrated in Figure 14. In order to ensure the continuous movement of all cells at expected values, we utilize frequencies between .1 and 1 hertz.

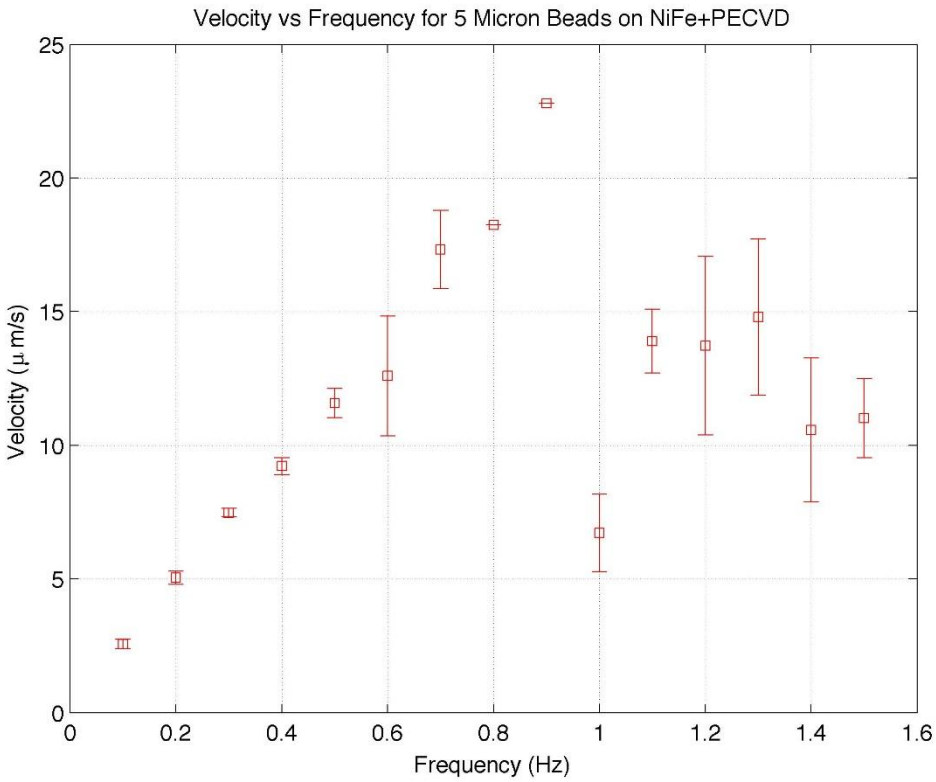


Figure 14: The measured velocity of five micron beads is plotted against the frequency of the rotation of the magnetic field. The velocity varies linearly as expected up until values of approximately one hertz when we begin to see a decrease.

The automation of the external magnetic field and the UEIDaq controlled chip has proven successful. We are able to precisely control the field's strength and frequency at all points along with each of the pins used for sorting cells. Sample maps have functioned properly, but the final mapping of the array is currently under construction.

The incubation chamber, similarly, has also been proven effective. We are able to clearly view each of the beads or cells and accurately import their images into MATLAB for analysis while maintaining a regular temperature of approximately 37° Celsius.

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