BIOMEDICAL APPLICATIONS OF OPTO-THERMAL HEATING AND ULTRASOUND

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Abstract

This thesis describes two projects in which electrical engineering is applied towards biomedical applications.

The first project involves the development of an opto-thermocapillary microrobot system. These gas-bubble microrobots are capable of manipulating cells or micro-objects, and are controlled using computer-generated holograms from a spatial light modulator (SLM). A program was developed in LabVIEW allowing for the automatic actuation of these microrobots in parallel without the need of a human operator. Two major functions have been implemented in this program. The first is a system that prevents microrobots from colliding with one another. The second function is a sequence generator that allows a user to create paths for the actuation of multiple microrobots over a programmable amount of time. The sequence generator creates the necessary holograms and sends them to the SLM, thus controlling the microrobots. With this program, the microrobots were successfully able to automatically manipulate and assemble micro-objects.

The second project explores the feasibility of an ultrasonic bone-marrow harvester. This device uses a piezoelectric actuator enclosed in a concave casing to generate focused ultrasound that can disrupt bone marrow. Pressure maps of the measured output of the device were created, with a maximum positive peak pressure of 0.190 MPa and a maximum negative peak pressure of 0.176 MPa at the center of the device. Experimental data indicates that 100-ms of ultrasound exposure is able to disrupt the cellular matrix of red bone marrow, resulting in up to 28.1% less force needed to penetrate marrow. At the same time, viability of peripheral cells remains high, between 92 to 95% for yellow marrow. This shows the potential of using an ultrasonic device to disrupt bone marrow for eventual harvesting.
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Chapter 1. Introduction

According to the Centers for Disease Control and Prevention, the leading causes of death among those living in the United States in 2013 were heart disease, cancer, and chronic lower respiratory disease (COPD) [1]. In the most serious cases, heart disease and chronic lower respiratory disease may require the need of an organ transplant, while cancer may require chemotherapy. However, chronic rejection of transplanted organs can occur, while chemotherapy can cause the abnormal production of blood cells. As a result, both medical professionals and scientists continue to look for superior and more efficient methods to treat disease.

This is currently being done in the field of biomedical engineering, which involves scientists and engineers from various backgrounds. Biomedical engineering involves the use of engineering techniques in medicine and biology for healthcare purposes. The collaboration of engineers from different backgrounds has led to significant advances in healthcare. Through the development of artificial limbs and organs [2, 3], imaging techniques [4, 5], and methods for the administration of drugs [6, 7], biomedical engineering has enhanced healthcare, thus improving the quality of life. These developments have helped advance a number of fields that biomedical engineers cover, including biomedical electronics, medical imaging, tissue engineering, and bionanotechnology. With biomedical engineering, it will be possible to improve the current methods used to treat disease, including heart disease, cancer, and COPD.

For this thesis, work has been done on two distinct biomedical projects. First, the work done for an opto-thermocapillary flow-addressed (OFB) microrobot system that is able to manipulate both particles and cells will be presented in Chapter 2. Much of the work done in this section uses LabVIEW (National Instruments), a visual programming platform. Chapter 3 discusses the development of an ultrasonic bone-marrow harvester that could potentially make
the process of donating bone marrow less painful and risky compared to current procedures.

Chapter 4 concludes the thesis.
Chapter 2. Cell and Particle Manipulation

Scientists have longed for ways to grow synthetic organs; recently, scientists have managed to successfully grow organs from a patient’s own cells, such as blood vessels [8] and bladders [9]. By using a patient’s own cells to grow the organs, immune systems will be less likely to reject synthetic organs, resulting in a more prolonged life and preventing the need to take debilitating drugs. Scientists have been attempting to make further progress on other types of organs, particularly with major organs like the heart, liver, kidney, and lungs. Some progress has been made; Lu et al. (2013) has managed to grow human heart tissue using induced pluripotent stem cells [10], while Song et al. managed to create bioengineered kidneys using a transplantable graft that holds the potential to be built with a patient’s own cells [11]. Newer methods have found ways to repair tissue, such as Zhang et al., which has developed a “Tissue-Velcro” bio-scaffold which could be used to grow, assemble, and disassemble cells to repair dysfunctional or damaged organs [12].

Although the growth of synthetic organs has seen a rise over the past few years, the grafts and scaffolds developed only serve as building blocks to an artificial organ. Further assembly may be required to place cells, scaffolding, and grafts to develop a fully-functioning organ, particularly at the microscale level [13]. Therefore, it is necessary to develop systems that are capable of manipulating micro-objects in the nano- or micro-scale range. Throughout this chapter, some methods that can be used to manipulate micro-objects will be explored, in particular, through the use of optical methods. A novel optical manipulation system will be explained near the middle of the chapter, with work done on a program used to run the system being detailed in Sections 2.3 and 2.4.
2.1 Optical Manipulation: Survey of Current Methods

One method that can be used to manipulate nano- and micro-scale particles would be to use optical forces, which has become a major field in biomedical research. Optical forces, including light, have been used to trap and manipulate both non-biological and biological objects in aqueous media. Through the use of optical forces, various tasks can be accomplished, including the sorting, assembly, and analysis of cells [14].

The manipulation of particles can be accomplished in a number of different ways. One such way to manipulate particles would be to use direct optical forces, which convert photon energy to a mechanical force such as a gradient force. One example of direct optical manipulation would be optical tweezers, which were first discovered by Arthur Ashkin in 1986. Optical tweezers are optical traps that hold cells using a force ranging from a tenth to hundreds of piconewtons which can be used to manipulate cells and particles in three dimensions [15]. In order to trap particles, optical tweezers form a gradient force that results from a single, tightly focused laser beam using high numerical aperture optics. Particles with indexes of refraction higher than its surrounding media become attracted to the focal point, the area with the highest light intensity. If the laser beam is not focused, the particle will only be trapped laterally, causing the particle to move across the laser beam due to radiation pressure. Typically, the gradient force generated by the tightly focused laser beam will be greater than radiation pressure, allowing the particle to be stably trapped. This concept is shown in Figure 2.1.
Figure 2.1: Visual explanation of optical tweezers. (i) 3D drawing of optical tweezers. Particles experience both lateral and vertical displacements as a result the gradient force, causing it to be attracted to the focal point of the laser. (ii) Ray diagram of a lateral displacement case. The particle will move to the left, towards the focal point of the laser. (iii) Ray diagram of a vertical displacement case. The particle will move down towards the focal point of the laser. (iv) and (v) show free-body diagrams of the cases seen in (ii) and (iii), respectively.

A single laser beam can also be used to form multiple traps. This can be accomplished using a spatial light modulator (SLM), which uses diffraction patterns to split a single beam of light into multiple beams of light. This method, otherwise known as holographic optical tweezers (HOT), allows for the manipulation of multiple particles on a single sample [16]. The holograms generated by the SLM dynamically change in real time depending on the location of each trap, which are often simply controlled using a computer program [17]. HOT systems have been shown to be capable of patterning cells in different ways; this has been shown through the patterning of 5-μm embryonic stem cells in lines and curves [18]. HOT systems are also useful for the optical sorting and manipulation of cells in a microfluidic channel; this sorting can be done by size, shape, or even fluorescence [19].

While optical tweezers have various applications in manipulating biological materials, one major concern is the potential photodamage that may occur to the cell. This can occur due to
the high optical intensities in an optical trap, which can be as high as megawatts per cm$^2$ [20]. This depends on a number of factors, including the type of cell under manipulation and the laser wavelength used, which in turn affects how much energy a cell can absorb. As an example, lasers with wavelengths at 870 and 930 nm can cause maximum photodamage to \textit{E. coli}, while minimum photodamage was seen at wavelengths at 830 nm and 970 nm [21]. Photodamage can also be exhibited through the examination of the cloning efficiency of a cell. For example, a 90 to 100 percent cloning efficiency was seen for a Chinese ovary cell with a power density of 9*10$^9$ J/cm$^2$ at wavelengths between 950 to 990 nm. However, at wavelengths between 700 to 760 nm with the same power density, cloning efficiency drops to 0 to 25 percent. Therefore, the wavelength of the laser chosen to manipulate cells has a major effect on the amount of photodamage a cell can experience.

One such method that can reduce photodamage is to use a dual-beam optical trap. Unlike optical tweezers, a dual-beam optical trap uses the optical scattering force, which pushes particles in the direction of light propagation. The optical scattering force cause particles to be stably trapped by two co-axial dual laser beams directed towards each other [14]. If these laser powers are equal, the particle will be trapped at the center of the laser beams. Similar to optical tweezers, dual-beam traps can be used for manipulation in microfluidic channels [22] or for the analysis of a cell [23]. Dual-beam optical traps do not require high numerical aperture optics or a tightly focused beam; therefore, less photodamage on cells or biomolecules will occur [14]. However, if multiple traps are needed, the setups required can become complicated as it would require the use of multiple laser beams. As a result, this system is less commonly seen in particle manipulation systems that require multiple traps to be produced.
2.2 Microrobots

There are other ways to remedy the issue of photodamage, including through the use of indirect methods to manipulate biological cells. One such method for micro-object manipulation would be to use microrobots, which are untethered sub-millimeter microactuators. Microrobots can be used to indirectly manipulate and assemble cells using various methods, which include but are not limited to electromagnetic [24] and electrostatic methods [25]. However, these methods are limited in their ability to actuate multiple microrobots independently due to the reliance on global electric or magnetic fields. This causes microrobot movement to be intrinsically coupled to these fields, making it difficult to individually control each microrobot. Therefore, in order to achieve the parallel manipulation of microrobots, a different method is needed.

2.3 Opto-thermocapillary Flow-Addressed Bubble Microrobots

Instead of moving particles using with electric or magnetic fields, thermal forces can be used to indirectly manipulate particles. Similar to direct optical forces, opto-thermal forces can be used to manipulate particles. However, unlike direct optical forces, thermal forces are formed through the absorption of light to generate heat in a material. The heat generated by opto-thermal forces can be used to drive a temperature gradient to form a surface-tension gradient in order to cause a mass-transfer along a liquid-gas interface. This phenomenon is known as either the thermocapillary or thermal Maragoni effect [26].

The opto-thermocapillary force generated by the thermocapillary effect is able to form microbubbles [26, 27], which can be referred as opto-thermocapillary flow-addressed (OFB) microrobots. These microrobots can be individually addressed through the use of a control
system similar to those used with optical tweezers. Multiple localized hot spots can be formed through the use of optical patterns, allowing for the actuation of multiple bubble microrobots. Devices used to form multiple optical tweezers, such as micromirrors [28], scanning mirrors [29], and spatial light modulators (SLMs) [16], can also be used to form multiple microrobots. These devices can allow for the parallel manipulation of microbubbles, allowing for multiple microrobots to be addressed individually at once. Compared to optical tweezers, OFB microrobots are not as dependent on the optical, electrical, and magnetic properties of the surrounding media or the object under manipulation. These microrobots are capable of manipulating various types of cells in two dimensions, including yeast cells [30] and NIH/3T3 cells [31]. Figure 2.2 shows an example of multiple OFB microrobots being manipulated to form different patterns on the workspace.

![Figure 2.2 Example of microrobots being manipulated (time format minutes:seconds) to form different patterns. Reproduced with permission from Hu et al. (2014) [32].](image)

### 2.4 Experimental Procedure and Setup

In order to form an OFB microrobot, a light source such as a laser is focused on an absorbing substrate such as amorphous silicon [32]. The absorbing layer converts the light energy into thermal energy, creating a localized hot spot. The fluid near the hot spot will then
vaporize, resulting in the nucleation of the microbubble. The microbubble can then be actuated on the sample using a surface-tension gradient [27]. The temperature gradient creates a surface-tension gradient that causes a fluidic flow from the hot region (the hot spot) to a cooler region, causing the microrobot to move to the hot spot. A diagram of the OFB microrobot is shown in Figure 2.3.

Figure 2.3: Side view of the mechanism used to generate an OFB microrobot. A laser is focused on an absorbing layer (amorphous silicon). This creates a localized hot spot which vaporizes the fluid near it, nucleating the microbubble. Actuation of the microrobot is then done by moving the laser beam to a different location on the absorbing surface. The temperature gradient can be used to create a surface-tension gradient, resulting in fluidic flow from the hot spot to the cooler region, causing the microbubble to move to the hot spot. Reproduced with permission from Hu et al. (2014) [32].

The creation of a program that is capable of generating and actuating OFB microrobots will be detailed in this thesis. A program was previously developed to control OFB microrobots using a scanning mirror [32]; this thesis work focuses on using a SLM to create the optical patterns for OFB microrobot actuation and control. Scanning mirrors use time-sharing, where a single laser is rapidly redirected to multiple locations [33, 34], enabling control of multiple OFB microrobots using a single input laser. In contrast, SLMs are able split a laser beam into several different components at once, without the need for time sharing. This is a distinct advantage over the functionalities of a scanning mirror, since each trap controlled by the SLM will always be active on the workspace, whereas with the scanning mirror, each trap is only active for a small period of time; the more time the scanning mirror needs to cycle through all traps, the likelier it is that it will lose the microbubble, limiting the amount of microrobots that can be controlled at
once. By using a SLM, there is the potential to control many microrobots at once, an important step needed in order for the assembly and cultivation of artificial organs. However, automated processes will be needed to control many microrobots, since a single human operator would be unable to control large numbers of microrobots at once. Therefore, the control software developed in this thesis aims to simplify the process needed to actuate multiple microrobots, while also introducing additional capabilities that allow for the automated control of each microrobot.

The experimental setup is shown in Figure 2.4. First, a computer sends a hologram to the SLM. This will be controlled by a customized program that is able to control the holograms being sent onto the SLM. Once this is done, a laser is focused onto the SLM using a beam shaper. The beam shaper helps to focus the laser light onto the SLM, which then splits the single beam of light into several different beams. These beams are then directed to a mirror, which reflects the beams of light into a microscope objective. The microscope objective focuses each beam of light into the fluidic chamber containing the microrobots, as seen in Figure 2.3. The resulting change is recorded by the camera, which is connected to the computer being used to control the holograms on the SLM. The videos and images taken by the camera can then be used for further processing on the computer, which will be explained in Section 2.5.
The computer will generate holograms for the SLM to use, which can then control the amount of microrobots along with their positions on the substrate. Modified from Rahman et al. (2015) [35].

2.4.1 The OFB Microrobots Program

The program that has been developed for OFB microrobot manipulation is based off the open-source program Red Tweezers, developed by Dr. Richard Bowman at the University of Cambridge [36]. This program, which was created in LabVIEW, was originally designed to control multiple optical tweezers at once using an SLM. The program allows for the manipulation of optical tweezers in a three-dimensional environment by continuously sending holograms to the SLM. An OpenGL shader engine interprets the position of each optical spot of the workspace, creating the hologram sent to the SLM. The program also allows for various parameters of the program to be controlled, including the intensity of each optical trap. The position of a microrobot can be controlled in two ways: the user can either drag the microrobot using a mouse, or they can enter the position of each microrobot as a coordinate. All control is
done on the x- and y-coordinates; the z-coordinate is assumed to be at zero for all cases as the system is only currently capable of 2D trapping. The interface is shown in Figure 2.5.

![Figure 2.5: The OFB microrobots program interface which was designed in LabVIEW. Users can either move the microrobots using either a mouse directly on the interface or entering coordinates into the position array, as highlighted with a box.](image)

All data in Red Tweezers is stored in arrays. This data includes the position for each microrobot (at its center) along with the intensity of the optical spot, which is used for the nucleation and actuation of microrobots. A numbered identification system is used to access the data of each microrobot. The first microrobot inserted into the workspace would be given the ID number 0. If an additional microrobot is inserted into the workspace, it would be given the ID number 1. The ID number will increase by 1 every time a new microrobot is inserted into the workspace.
Since *Red Tweezers* was originally designed as to operate a holographic optical tweezers system, a number of modifications along with new functions were needed so it could serve as the control program for the actuation of OFB microrobots. Many of these functions were developed in LabVIEW, a design platform developed by National Instruments which uses a visual programming language. LabVIEW is capable of data acquisition and instrument control, two important components required for an OFB microrobots system. The functions and modifications developed in LabVIEW are detailed in this section.

### 2.4.2 Bubble Collision Avoidance System

One issue with OFB microrobots is potential collisions with one another since these are gas bubbles in a liquid media. This can occur due to the intensity of the optical spots holding the microrobots; if the intensities of the microrobots are not equal to one another, a collision is more likely to occur. A collision could also occur if the distance between two or more microrobots is too small. If a collision occurs, the microrobots will combine and increase in size, forming one large microrobot, causing instability within the system. To prevent this, the control system could be set such that the distance between two microrobots is equal to the diameter of the largest microrobot in all cases; however, this could not be done in the original *Red Tweezers* program. This could also be theoretically prevented by having enough laser power to distribute to all microrobots; however, this problem will scale up with the number of microrobots and would be also limited by the power of the laser itself. Therefore, this issue must be prevented through the development of a collision avoidance system which limits the distance between two microrobots.

In the bubble collision avoidance system, each microrobot is given a concentric buffer zone. The buffer zone can be controlled by the user and can be adapted for microrobots of different sizes. The program will calculate the distance between the controlled microrobot and
other microrobots. If the microrobot were to cross the buffer zone of another microrobot, the
program will prevent any further movement. This applies for cases where the microrobot is
either moved using a mouse or if coordinates are entered. The function will apply whether if one
or multiple microrobots are being controlled. Therefore, this function will help prevent
microrobot collisions from occurring. The concept of this function is shown in Figure 2.6.

![Diagram of collision avoidance](image)

**Figure 2.6**: Visual concept of the collision avoidance function. If the buffer zones, represented by the
dashed lines for each microrobot, cross or intersect when the microrobot is moved by the user, the
program will automatically prevent the microrobot from moving near the other microrobot. Reproduced
from Rahman et al. (2015) [37].

The development of the collision avoidance system was done through LabVIEW. Figure
2.7 shows the block diagram of this function as it was originally designed; it only worked for
inputs from the mouse. If one microrobot is selected by the user, then moved using a mouse, the
system will check whether there are any other microrobots in the workspace through the use of a
while loop. If there is another microrobot in the workspace, the distance between the selected
microrobot and the other microrobot is calculated, according to

\[ d = \sqrt{(x_1 - x_2)^2 + (y_1 - y_2)^2} \]  

(Eq. 1),

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where $x_1$ and $y_1$ are the $x$ and $y$-coordinates of the selected spot, and $x_2$ and $y_2$ are the $x$ and $y$-coordinates of another spot. If this distance is less than two times the buffer zone width, the microrobot will be unable to move, as the buffer zones are about to cross each other. The only way for the microrobot to move again would be to increase the distance $d$, which can be done if the selected microrobot is moved away from the microrobot it is about to collide into. If the buffer zone of the microrobot does not cross into the buffer zone of the other microrobot, the system will check if there are other microrobots in the workspace. Once the distances between the selected microrobot and all other microrobots are checked, the system will allow the movement to occur.

On the other hand, if there were multiple microrobots selected, the system will check the microrobot that is first according to the ID system. For example, if there are four microrobots in the system with IDs 0, 1, 2, and 3, with 0 and 2 being selected, the system will check the distances between 0 and the other microrobots first, followed by the distances between 2 and the other microrobots. If at any instance the buffer zone of a microrobot crosses the buffer zone of another microrobot, the microrobot will not move until the distance $d$ is increased. After the distance between the selected microrobots and the other microrobots are checked, the system will allow the microrobots to be moved.
Figure 2.7: Block diagram outlining the bubble collision function using a mouse.

A similar block diagram applies if the coordinates for a microrobot are entered. The distance between each selected microrobot and the other microrobots are checked. If the microrobot is given coordinates such that it will intersect another microrobot, the system will display an error string. This string indicates whether the movement is acceptable or not. If the proposed coordinates will cause a potential collision, the movement is automatically stopped by the collision avoidance system.
Through the development of the collision avoidance system, microrobots will be prevented from merging with one another. Using simple formulas, logical statements, and a combination of for and while loops, the collision avoidance system prevents potential issues that could be encountered by the user by stopping any potential movement that could destabilize a workspace.
2.4.2.1 Results and Discussion

Figure 2.9 shows the implemented function in action in Red Tweezers, where one microrobot is controlled using a mouse and moved towards the microrobots. Each microrobot is checked using a combination of for and while loops to alternate between the microrobots that need to be checked, based off the selected microrobot. If a collision is about to occur, as calculated using Equation 1, the bubble collision system will stop the movement and alert the user through the use of a logical Boolean variable labeled “Hit.” If the buffer zones intersect, this variable will obtain a value of 1, which prevents the microrobots being controlled from moving in the workspace and in the Red Tweezers interface. The variable can only be changed back to 0 if the user has the microrobot move away from the microrobot it is about to collide into. On the other hand, if the variable has a value of 0, the microrobots being controlled will be able to move freely until it experiences a potential collision. Once the distance between the selected microrobot and the other microrobots are checked, the function will switch to the next microrobot in numerical order. Once the distances between all selected microrobots and the other microrobots are checked, the function will process any other movement done until a different microrobot is selected or the program is stopped.
Figure 2.9: The bubble collision system in action using a mouse. (left) The selected microrobot (in red) is at a position where it is not crossing a buffer zone. This microrobot will be able to move throughout the designated workspace. (center) The buffer zone of the selected microrobot is crossing with the buffer zone of the right microrobot, resulting in the “Hit” function turning on. (right) The buffer zone of the selected microrobot is crossing with the buffer zone of the bottom microrobot, resulting in the “Hit” function turning on. In the latter cases, the selected microrobot must be moved away from the buffer zone it is crossing in order to move the selected microrobot again.

The function works similarly when coordinates are entered. Using Equation 1, the distances between the selected microrobots and the other microrobots are checked. If any one of the buffer zones of a selected microrobot crosses with another buffer zone, then the movement will be automatically prevented with an error message indicating that the movement is not acceptable. Figure 2.10 shows the function in action.
Figure 2.10: The bubble collision system in action when entering coordinates. The other microrobot has coordinates (0,0). The buffer zone is set to 40 pixels, where 1 pixel equals to 1 microunit, meaning that the microrobots can move no closer than 80 pixels to each other. (i) Original position of selected microrobot. (ii) An acceptable movement to (0, -80 pixels), with the Position Indicator stating that the movement is acceptable since the distance is equal to two times the buffer zone width. (iii) If the movement proposed causes the buffer zones to cross, such as (0, -50 pixels), the Position Indicator will indicate an error, since the microbubbles will be separated by a distance of less than 80 pixels. The selected microrobot will return to its original position.

Despite the successful implementation of this function, there are some limitations which can hamper Red Tweezers, particularly if the microrobots are being controlled with a mouse. First, the processing time to which to check the distances between each microrobot may cause slowdowns on the Red Tweezers system as the number of microrobots increase. With larger numbers of microrobots, the framerate of the program will drop, leading to massive slowdowns in data processing. Since the selected microrobot is checked against all other microrobots in the system, LabVIEW has to process each distance one by one, leading to the slower framerate. Selecting multiple microrobots would increase the processing time needed to check all distances.
A potential way to solve this issue would be to reduce the amount of data load would be to check the microrobots within a specific area instead of the whole workspace. Figure 2.11 shows the concept of the modified function. First, if a microrobot is selected, the coordinates of all microrobots would immediately be checked. A new function would identify the microrobots that are within the specified area declared and store the IDs in an array. The width and length of the scanning area would be equal to a declared distance $2d$ (area in green) plus two times the buffer zone width (area in yellow) and the radius (area in orange) to avoid any potential collisions with microrobots outside of the area. The scan zone is intentionally larger since it needs to factor in the size of the microrobots and the buffer zone width into a potential collision, given the fact that tracking takes place at the center of each microrobot. Then, when the selected microrobot is moved, only the distances between the microrobots within that area are calculated. A new scanning area is only calculated if any part of the microrobot exits the area formed by the declared distance $d$, as indicated by the area in green in Figure 2.11. The function would work similarly for multiple microrobots, with the only addition being that each microrobot would get their own “scan zone.” Once another microrobot is selected, the function will reset. With this additional function, the data load that Red Tweezers would take would be reduced compared to checking all the distances of each microrobot when the selected microrobot is moved. Therefore, the bubble collision system would improve the user experience by having fewer slowdowns.
Figure 2.11: Concept of improved bubble collision system. The distance between the selected microrobot (in red) and all other microrobots (in blue) are checked first. Then, a predetermined square area, which has both dimensions equal to the sum of $2d$ (in green), two times the buffer zone width (in yellow), and two times the radius (in orange), is formed such that the microrobots that fall within this area would be identified. Once the selected microrobot is moved, only the microrobots within that area are checked for distance. If any part of the microrobot moves out this area, the distances are recalculated and a new designated area would be created. In this case, all microrobots are checked, except for the two microrobots on the left, both of which do not fall in either the user-determined area or the buffer zone.

Figure 2.12 shows the algorithm of an improved bubble collision system that would be implemented.
Figure 2.12: Revised bubble collision system block diagram. Adding in an additional step to check the distances between the selected microrobots and the other microrobots beforehand allows the system to determine the microrobots within a specified area. Once the microrobots are moved, the system has to check the distances of the microrobots in the area only.

Another issue which may cause problems for the Red Tweezers system is the possibility that microrobots can cross the buffer zone when controlling a microrobot using a mouse. This can occur if the optical spot holding the microrobot is moved too quickly on the Red Tweezers system. There is a small period of time during which the optical spot can be moved before it is checked by the bubble collision system. If the microrobot is moved too quickly, the system may
not be able to check the microrobot in time before it crosses the buffer zone. This can be a problem if the microrobots are large in size, since that increases the possibility of a potential bubble collision.

This problem can be avoided by introducing a self-correcting mechanism that immediately corrects the position of the microrobot before the hologram is sent to the SLM, such that the distance between the two microrobots is equal to two times the buffer zone width, with the buffer zones intersecting at exactly one point. When using the mouse, a change in position is effectively a linear movement with slope $m$, which is equal to $\frac{y_{\text{final}} - y_{\text{original}}}{x_{\text{final}} - x_{\text{original}}}$. Therefore, the microrobot is moving on a line $y = mx + b$, with $b$ equal to $y_{\text{original}}$. There is a point on this line such that the distance between the buffer zones of each microrobot would match the minimum distance the buffer zones can be apart from each other before intersecting. This point, located at $(x_{\text{corrected}}, y_{\text{corrected}})$, will be exactly at the buffer zone distance specified. Therefore, given $x_2$ and $y_2$, the coordinates of the microrobot that is being checked, a modified version of Equation 1 can be used:

$$d = \sqrt{(x - x_2)^2 + (y_{\text{corrected}} - y_2)^2}$$

where:

$$y_{\text{corrected}} = \frac{y_{\text{final}} - y_{\text{original}}}{x_{\text{final}} - x_{\text{original}}} x + y_{\text{original}}$$

By solving for $x$ given $d$, $x_{\text{corrected}}$ can be found. This value is therefore equal to:

$$x_{\text{corrected}} = x = \sqrt{d^2 - (y_{\text{corrected}} - y_2)^2} + x_2 \quad (\text{Eq. 2})$$

Therefore, the corrected $x$ coordinate can be found using Equation 2. If the microrobot is accidentally moved into the buffer zone, the coordinates $(x_{\text{corrected}}, y_{\text{corrected}})$ would then be sent to the microrobot, thus correcting the microrobot position before the hologram is sent to the
SLM. The “Hit” Boolean variable described earlier in 2.4.2.1 will then activate, preventing any movements that would cause a collision. This mechanism is shown below in Figure 2.13.

Figure 2.13: Concept of self-correcting mechanism for bubble collision system. Since the microrobot travels on a line \( y \), there will be a point (marked in dark red) such that the distance between the two microrobots will be equal to two times the buffer zone width. The self-correcting mechanism will move the selected microrobot (in blue) to the position found with Equation 2.

### 2.4.3 Sequence Generator

Automatic nucleation and control of microrobots is an important goal of this project. Both automatic nucleation and automatic control of the microrobots would be done without operator influence through the detection of micro-objects. Depending on the size and shape of the micro-object, a varying number of microrobots would be created in order to move the object through the use of caging, which will be described in the system applications section in Section 2.5.

In order to allow for automatic nucleation and automatic control, a path-planning function is needed. This path-planning function would allow for microrobots to be actuated to a specific location at certain times. This function would also need to be able to control all microrobots within the workspace and could allow for multiple microrobots to be moved in different paths all at once.

Therefore, a new function, termed the sequence generator, needed to be developed. Since Red Tweezers stores all data, including the positions of every microrobot, in an array, path
planning could be accomplished by using a two-dimensional array to store position data over time. One dimension would contain a sequence number which determines the order by which the frames will appear in, while the other dimension would contain the ID numbers of each microrobot as given by Red Tweezers.

The algorithm for this function is displayed in Figure 2.14. First, in addition to determining which microrobots will be moving in the workspace, the operator must declare the number of frames of the sequence. Also, the framework needed to store the data must be created. This will depend on the number of microrobots that are in the workspace and the number of frames that the sequence will contain. Following this, the operator must select a specific microrobot to “lock onto,” meaning that the program will track the movement of that microrobot specifically. The original position must be marked in order to determine the movement rate. Once the movement is done, the microrobots can be moved to the planned position. These movements are inserted into the framework created earlier, allowing for the storage of the planned movements of all microrobots in the interface. This can be repeated for different sets of microrobots, without the framework needing to be created again.
To complement the sequence generator, a function that is able to play the sequence was also developed. This function, the sequence player, is able to deliver the data stored from the sequence generator into the SLM, allowing for the actuation of the microrobots. The function is able to play the frames at a specific frame rate, meaning that the user can control the speed by which the microrobots are able to move at. The sequence player can also be used to set which frame the *Red Tweezers* interface is displaying. This allows the user to see the resulting movements that would occur as a result of using the sequence generator. With the development of the sequence player, an additional level of control to the sequence generator was added with its ability to play the sequences that are developed while also controlling the data sent to the SLM.
### 2.4.3.1 Results and Discussion

The framework generator, sequence generator, the sequence player are shown in Figure 2.15. In order to allow the user to plan the movements, a button either disabling or enabling *Red Tweezers* to send holograms to the SLM was introduced, which is also shown in Figure 2.15. This prevents unnecessary movements from occurring while keeping the same hologram on the SLM the same until the button is reactivated. If necessary, more frames can be added to the sequence if a user wants to implement additional actions by the microrobots. In addition, changes can be made to the sequence if necessary, for example, if a potential collision occurs with two or more microrobots.

![Diagram of SLM output function, framework generator, sequence generator, and sequence player.](image)

**Figure 2.15:** Images of SLM output function, framework generator, sequence generator, and sequence player, shown in order of which they are applied. (i) The output to SLM button activates a case statement which can prevent holograms to be delivered to the SLM. If false, the most previous hologram will be kept by the SLM. The SLM will receive a new hologram if the case statement is true. (ii) The framework generator allows the framework of the sequence to be created on the maximum number of bubbles and the number of planned sequence frames. (iii) The paths of each microrobot are declared into the framework.
with the sequence generator. (iv) The sequence player allows what was created in (iii) to be played, provided that the SLM output is being allowed.

An application of this function is shown in Figure 2.16. Figure 2.16 shows two microrobots; one microrobot is moved up for 10 frames and then to the right for 10 frames, while the other microrobot is moved down for 10 frames and to the left for 10 frames. Both movements were planned using the sequence generator, reflected by the change in positions in the workspace.

![Figure 2.16: The sequence player playing a 20-frame sequence created by the sequence generator, with frames 0, 9, and 19 displayed. The interface reads from the sequence created and displays the position of each microrobot. The positions are translated into a hologram by the OpenGL hologram engine. The hologram is received by the SLM, which then causes the movement of the microrobot on the workspace. In this case, the red microrobot is moved up from frames 0 to 9, and then moved to the right from frames 10 to 19. The blue microrobot is moved down from frames 0 to 9, and then moved to the left from frames 10 to 19. Scale bar: 50 μm.](image)

An error detector was also added to the sequence generator. In some cases, a user may declare a path for a microrobot that could collide with another microrobot. A user may not be able to catch this error in time, resulting in a bubble collision. Since the sequence generator is

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used as a planning function, the error detector was added to indicate to the user whether a potential collision may occur when the sequence is played. Instead of preventing the user from making the movement, the function will tell the user which frames a collision could potentially occur. Therefore, a user would be able to make corrections to the sequence to prevent the collision from happening.

Additional functions to the sequence generator will be added in the future, including a proper save system. Originally, Red Tweezers did not have a save system that could store the sequence data; it could only save the workspace that existed for only one frame. To solve this issue, a workaround method which sends the data to a separate LabVIEW file was developed. This would allow the sequence to be saved and loaded from this file. This took advantage of the ability of LabVIEW to default data onto a specific array. Therefore, by using this method, the data could be saved and eventually loaded back onto the Red Tweezers interface. However, the method itself is not user-friendly; the file cannot be directly saved in the Red Tweezers interface. Eventually, a more robust save system will need to be created using LabVIEW’s I/O functions.

Potential users may want to repeat a specific action multiple times, such as a specific procedure involving the Red Tweezers system. Since the array used to store data in Red Tweezers is not a traditional two-dimension array, a special function will need to be developed in order to save the data properly in a file. Once then, specific sequences could then be saved and loaded seamlessly on the interface without taking additional steps.

In addition to this, the development of visual aids will be done in conjunction with supervised x96 students using LabVIEW. Visual aids, including microrobot path tracking, are being developed to make it easier for a user of the program to follow the path of a microrobot while also being able to easily see potential collisions between microrobots when using the
sequence generator. The visual aids that are being developed will allow the program to be more accessible and easier to use for those operating this program.

2.5 System Applications

The modified Red Tweezers system has served as a backbone to several conference papers. The first discusses the ability to generate up to 12 OFB microrobots using this program and an SLM [37], an increase of 20% compared to the number generated using a scanning mirror [32]. This paper also demonstrates the idea and implementation of the bubble collision system along with the sequence generator. As shown in Figure 2.17, the sequence generator can help plan the movement of a large number of microrobots at once. Each pattern, as shown in parts (a), (d), and (g), corresponds to a rendered hologram that is sent to the SLM. As shown in (b), (d), and (e). In turn, an optical pattern on the workspace is created as shown in (c), (f), and (i). In this case, the optical pattern was arranged to form the letters “UH” using 50 optical spots which would be used to actuate the microrobots. The average velocity of each optical spot was approximately 80 μm/s. The total power on the substrate was 330 mW, with an average of 6.6 mW per spot. This demonstrates the potential of actuating a large number of microrobots at once, with limited power being the only obstacle in actually being able to actuate this number of microrobots.
Figure 2.17: The development of a sequence arranging 50 optical spots in a square pattern to form the letters “UH.” (a), (d), and (g) contain the patterns created in the Red Tweezers interface, which form the holograms in (b), (e), and (h) respectively. These holograms are sent to an SLM, which forms the optical patterns on the substrate as seen in (c), (f), and (i). Scale bar: 100 μm. Reproduced from Rahman et al. (2015) [37].

Another paper describes the ability of the microrobots system to perform the parallel micromanipulation and microassembly of glass beads using the sequence generator [35]. This is shown in Figure 2.18, with the sequence player running a 230-frame sequence in order for 9 microrobots with an average size of 50 μm to manipulate two 70 μm microbeads within a 1.1 mm to 0.82 mm workspace. The sequence was created using the sequence generator, taking a time of 68 seconds in order to go through all frames, with an average velocity of 100 to 150 μm/s for each microbubble. Thus, automatic assembly was fully demonstrated using the sequence generator system and the sequence player.
Additional work is currently being done on the control program that will help to automate the assembly of larger cells and other particles, allowing for more precise and accurate actuation. A closed-loop caging system is being developed in collaboration with the Chiba Institute of Technology that will allow for OFB microrobots to cage objects of various sizes, allowing for more accurate and precise movements. This will require a number of new functionalities with the program, including particle tracking and path planning. The particle tracking and path planning functionality, which will be written in collaboration with the Chiba Institute of Technology, will eventually be integrated into the OFB microrobots program for further robustness. By accomplishing this task, the assembly of larger cells and molecules can be done using OFB microrobots. This conceptual idea is shown in Figure 2.19.
Figure 2.19: Visual concept of caging for the assembly of particles. (left) The microrobots, represented in blue, move towards the oval-shaped particle, represented in red. The particle tracking system would be able to determine the location of the particle along with the location of the microrobots. (right) The microrobots surround the particle, thus caging it. By moving the microrobots together, the caged particle can then be moved in different directions.

So far, an open-loop feedback system to cage particles has been developed with automatic nucleation and actuation [38]. This system is able to cage a microbead using four microrobots. In order for the system to do this, a particle tracking program would be given the position of a microbead. This allows the program to generate a path for each microrobot to traverse to at a position where each microrobot can assist in caging the particle. This path is then manually inserted into LabVIEW using the sequence generator, with consideration given to the nucleation of the OFB microrobots. Once these paths are inserted into the sequence generator, the microrobots are then generated throughout time. The microrobots would then surround the bead, allowing it to be caged and manipulated throughout the workspace. An outline for this procedure is shown in in Figure 2.20, while the resulting action in Figure 2.21. Eventually, these steps will be automated such that no manual insertion of data is required; all steps would be done by Red Tweezers and an image processing algorithm without any human input.
Figure 2.20: Block diagram of open-loop caging mechanism. Path generation pictured reproduced with permission from Takahashi et al (2016) [38].

Figure 2.21: Caging mechanism demonstrated with OFB microrobots (time format minutes:seconds). The caged microbead (in blue) was caged with four microrobots nucleated at the bottom left corner of the workspace. Each microrobot is moved to a caging position from (a) to (d) such that the four microrobots surround the microbead. In (e), the microrobots are moved closely together and transported from position A to position B. Reproduced with permission from Takahashi et al. (2016) [38].

2.6 Conclusion

The bubble collision system and the sequence generator have increased the functionality of the Red Tweezers program so that it can be applied for OFB microrobots. With the bubble collision system, the potential collision of microrobots can be avoided. The sequence generator helps to
produce paths that a microrobot can travel on and allows the Red Tweezers program to do automated movements given a sequence. These functions will continue to be further developed with the enhancements described in the previous sections, some of which improve the functionality and others which improve the accessibility of the program, including more visual aids.

Through the development of the OFB microrobots program, it is expected that the system will be able to automatically manipulate micro-objects and cells within the microscale range with microscale resolution. This could therefore be used to assemble artificial organs, which have cells organized with microscale resolution [13]. The OFB microrobots system can be used in conjunction with many of the current technologies used in tissue engineering, such as the scaffoldings and platforms developed, which have features in the high nanoscale to the microscale range [39]. OFB microrobots therefore can be used to help in the assembly of cells by manipulating cells or scaffolding to certain positions, allowing for the cells to grow in a suitable environment. This has been shown in previous research done using OFB microrobots. The holographic system has not been used to manipulate cells, a single OFB microrobot has shown to be capable of manipulating NIH/3T3 cells with sizes ranging from 10 to 20 μm in a 450 μm by 350 μm workspace, with the cells remaining viable after assembly [31]. 20-μm thick PEGDA agarose-laden hydrogels with approximate dimensions of 200 by 225 μm were also manipulated using OFB microrobots. Additionally, with the holographic OFB microrobots system, multiple microrobots can be actuated in larger workspaces, as demonstrated the ability to manipulate microrobots with sizes of approximately 70 μm in a 1.1 mm by 0.82 mm workspace [35]. Given the effective area of the SLM of 16 x 12.8 mm [40], it would be possible to work in even larger workspaces. With the holographic OFB microrobots system, cells, scaffoldings, and micro-
objects can be manipulated in sub-millimeter workspaces with higher throughput, greater precision, and lower assembly times. In the future, with further development, the program may be able to assemble objects in three dimensions, an important step in assembling cells into functional organs.
Chapter 3. Bone Marrow

The second project that will be described in this thesis is the validation and feasibility of a novel bone marrow aspiration device, an ultrasonic bone-marrow harvester. The bone marrow is a critical component of the human anatomy. Stem cells come from the bone marrow, a sponge-like tissue that can be found at the center of various bones [41], as seen in Figure 3.1. Stem cells lead to the creation of cells that are vital for living organisms, including white blood cells, red blood cells, and platelets. In turn, these cells help to perform typical bodily functions, including oxygen transportation, blood clotting, and maintenance of the immune system [41].

Figure 3.1: Structure of two common types of bones: the cortical bone and the cancellous bone. Both bones have marrow cavities that contain red marrow. Both also have yellow marrow, as indicated by the sections containing mesenchymal stem cells. Reproduced with permission from Gurevitch et al. [42].
There are two types of bone marrow in a bone: yellow marrow and red marrow [42]. At birth, all mammalians start off with red marrow, which contains hematopoietic stem cells (HSCs). These stem cells serve as the precursor to various cells in the body, including red blood cells, white blood cells, and platelets [41]. As the mammalian ages, some red bone marrow will convert into yellow bone marrow. This type of bone marrow contains mesenchymal stem cells (MSCs) and accumulates fat over time. Although further research is needed, this type of stem cell may have uses in tissue repair and cell therapy [43, 44]. Yellow marrow is unable to produce HSCs, except in emergencies such as extreme blood loss. If this happens, the yellow marrow will lose all the accumulated fat revert back into red marrow, retaining its former functionality.

Blood cells have a short lifespan, with red blood cells having a lifetime of approximately 115 days [45], platelets having a lifespan about 10 days [46], and most white blood cells lasting between hours to days [47]; therefore, it is critical that the bone marrow is functioning in order to replace missing blood cells. However, blood-related disorders, including cancerous diseases such as leukemia and lymphoma can cause irregular and abnormal production of blood cells, with one person being diagnosed with a blood disease every three minutes [41, 48]. Medical treatments that are used to treat cancerous diseases such as chemotherapy or radiation therapy can also destroy both stem and blood cells. If it is determined that irregular or abnormal production of blood cells is occurring, a bone marrow transplant (BMT) would be needed.

3.1 Bone Marrow Transplant Background

The purpose of a BMT is to introduce normal stem cells, which in turn replenish the ability of the body to reproduce blood cells such that bodily functions return to a normal state. Over 21,000 BMTs were performed in 2014 in the United States alone, which was an increase of 20% from the number of transplants in 2010, a number that has been steadily increasing year-to-
year [49]. There are two methods to transport bone marrow to a patient. The first method, the autologous transplant, involves harvesting the bone marrow of the patient before medical treatment and transplanting at a later time [41]. In these cases, the bone marrow of the patient is healthy but is set to be destroyed due to a medical treatment such as chemotherapy. This can also be done if the disease affecting the bone marrow is in remission. About 59.3% this type of BMT involved the use of the autologous method. If the bone marrow of a patient cannot be used for an autologous transplant, an allogeneic transplant will be used in which the donor is another person [41]. Allogenic transplants are less common, with about 41.7% of this type of BMT being done in the United States in 2014 [49].

3.2 Bone Marrow Harvesting: Aspiration Method

In order to perform a BMT, bone marrow harvesting must be done. The most common method, the aspiration method, involves taking a sample of bone marrow tissue from the body, generally at the hip or at the sternum [50]. Approximately 500 to 1200 mL of bone marrow is required for a BMT, which often totals up to about 50 to 300 punctures with an average of 10 mL collected per puncture. The high amount of punctures is needed since the harvested graft becomes smaller during the procedure; thus, more punctures are needed. This procedure, on average, lasts about 60 to 90 minutes, but can vary depending on the physician skill.

It has been shown that the current aspiration method can be dangerous and life-threatening. This is likely due to a number of factors, including the puncturing from aspiration needles and need for general anesthesia [50]. Based on the records of a single hospital, Buckner et al. (1984) [51] found that 6 out of 1,160 donors (0.05%) in an 11-year period (1969 to 1983) had life-threatening complications as a result of being a donor: two had cardiopulmonary issues, two had bacterial infections, and one had a cerebrovascular accident. More recent records between
December 1987 and December 1999 under the National Marrow Donor Program indicated that 125 out of 9282 donors (1.35%) experienced medical complications following the procedures, with 55% of the affected donors experiencing a complication as a result of a mechanical injury and 36% of the affected donors experiencing a reaction to the anesthesia used [52]. For all the cases documented, the donors did recover, although some had prolonged or extended hospital stays. Therefore, these two data sets demonstrate the potential dangers of the bone marrow harvesting procedure.

3.3 Bone Marrow Harvesting: PBSC Method

Another method that can be used to collect stem cells for BMTs would be to use the peripheral blood stem cell (PBSC) method [41]. This method involves collecting stem cells from circulating blood rather than from the bone marrow. Before a PBSC transplant, a donor would need to receive four injections daily of the drug filgrastim that releases stem cells into the blood. After a set period of time, the blood is collected throughout several sessions through a procedure called apheresis, where blood travels from one arm to a blood cell separator, which separates the stem cells from the blood cells. The blood is then returned back to the donor in another arm and the blood is returned back to the patient.

This method is less painful compared to the typical bone marrow harvesting method and is less risky for a donor. From 2004 to 2009, only 0.31% of PBSC donors experienced an adverse reaction from the procedure, compared to 0.99% of BMT donors [53]. Also, it has been shown that PBSC can put a patient at risk for chronic graft-versus-host disease (GVHD), where immune (white blood) cells recognize tissue graft as “foreign” tissue, causing the cells to attack it and thus destroying it. Flowers et al. (2002) [54] has found that GVHD occurred more commonly in patients who received stem cells from PBSC transplants (PBSCT) compared to those who
received a BMT; out of 126 recipients, half of which obtained stem cells from PBSCT, and half which obtained a BMT, 39 of 63 of the recipients (61.9%) developed GVHD after a PBSCT, compared to just 32 of 63 recipients (50.7%) developing GVHD after a BMT. While these numbers are similar, the patients who received GVHD from a PBSCT had longer treatment times compared to those who obtained GVHD after a BMT, with 10 of the 39 patients (25%) needing more than 2 treatment cycles after a PBSCT compared to 3 of the 32 patients (9.3%) needing more than 2 treatment cycles after a BMT. Therefore, this indicates that GVHD is more difficult to treat for a patient receiving a PBSCT compared to those receiving a BMT.

### 3.4 Ultrasound Bone Marrow Harvesting

As a result, a new method which can reduce the potential pain and danger coming from the aspiration method and can reduce the chances of GVHD from a PBSCT would be highly beneficial. This could be accomplished by introducing a novel, more effective way to extract bone marrow which would be less painful than a BMT and less risky compared to a PBSCT. One under evaluated approach would be through the use of an ultrasound-based bone marrow harvesting system [55].

Ultrasound waves are sound waves within the upper limit of audible hearing for a typical person. These waves are typically used for a wide variety of medical purposes, including imaging and physical therapy [55]. Another use for ultrasound in the medical field is its uses for sonoporation, where microbubbles are used to permeate a cell membrane through cavitation. Cavitation involves the generation immediate compression of microbubbles to release a large magnitude of energy (a shockwave) capable of porating tissues and cells, with higher pressures resulting in stronger shockwaves [56, 57, 58]. Thus, ultrasound has the potential to disrupt the cellular matrix of bone marrow, which can then be harvested.
The idea for bone marrow harvesting can be seen in Figure 3.2. First, a technician would drill through a bone. The drill would be larger than the piezoelectric actuator used to generate the ultrasound, which is described further in Section 3.5.1. Then, the drill would be taken out of the drill casing, creating a small hole for the piezoelectric actuator to be placed at. The drill casing remains at the targeted area to provide a stable frame for the piezoelectric actuator in order to maintain contact at the bone marrow cavity. The piezoelectric actuator can then be put into the drill casing, with its face put into contact with the bone marrow cavity. The bone marrow can then be extracted using suction after exposure to ultrasound for an appropriate amount of time. By using the ultrasound to disrupt bone marrow, there would be no need to puncture the hip or the sternum multiple times. Using this method, the potential pain that a donor may experience would be reduced and will help reduce the potential complications that a donor may face.

Ultrasound has been shown to be capable of treating blood clots and destroying soft tissue. Maxwell et al. (2009) [59] demonstrated that blood clots can be eliminated through the use of histotripsy, a method that fractionates soft tissue using high-intensity ultrasound pulses. This has also has been shown on in vitro pig models [60]. This idea could be applied to disrupt the cellular matrix, which will allow for bone marrow extraction by the ultrasound device through the use of suction. However, there is the potential for ultrasound to damage other parts of the body, such as blood vessel walls through ultrasound-induced heating and damage to vessel walls. Therefore, optimization is needed so only the targeted areas are being hit with ultrasound.
Figure 3.2: Bone marrow extraction process. (i) A drill is inserted into the bone in order to penetrate the bone marrow cavity. (ii) The drill is taken out of the drill casing and a hole remains in the bone marrow cavity. (iii) The actuator is inserted into the drill casing into the hole created by the drill. The device can then be used to extract bone marrow.

3.4.1 Focusing the Ultrasound Wave

One way to reduce damage to vessel walls would be to focus the ultrasound wave. This is commonly seen in high-intensity-focused ultrasound (HIFU) devices. In a HIFU device, ultrasound waves from a piezoelectric transducer are focused using a concave transducer face. Thus, when an ultrasound wave travels through the concave transducer face, the wave will be focused at a certain point, reducing the effects caused by ultrasound waves at other areas. Figure 3.3 shows this concept. HIFU has been shown to be capable of liquefying tumor tissue into small fragments while limiting damage to other tissues and other parts of the human body [57].
Figure 3.3: Concept of HIFU using a concave transducer face. The concave transducer face allows for the ultrasound wave to be focused at a certain point. A small membrane layer provides the contact needed for the ultrasound wave to travel through the skin. Based off Shaw et al. 2014 [61].

3.5 Experimental Procedures

The same conceptual idea used to liquefying tumor tissue can be applied to disrupt the cellular matrix of bone marrow, which is essentially flexible tissue. For this thesis, proof of concept will be shown such that it would be possible to disrupt bone marrow for harvesting. This would be done by using focused ultrasound to disrupt the cellular matrix of the bone marrow. With more ultrasound exposure, it is expected that the cellular matrix will become further disrupted, with less force being needed to penetrate the bone marrow. Therefore, with more pressure, bone marrow can be disrupted for eventual extraction. The following section describes the steps needed to develop and test the device.

3.5.1 Device Design and Characterization

In order to develop and characterize the ultrasound-bone-marrow harvester, a piezoelectric actuator needs to be selected. Piezoelectric actuators are the main basis for many transducers and require an AC voltage to operate, which can generate an ultrasound wave.
Depending on the device, a DC voltage may also be required. The generation of an ultrasound wave is primarily based on the converse piezoelectric effect. The piezoelectric effect explains how the actuator generates the ultrasound wave [62]. If the applied voltage on the actuator has the same polarity as the poling voltage, then the actuator will expand vertically and to a lesser extent contract horizontally, as seen in Figure 3.4. On the other hand, if the applied voltage does not have the same polarity as the poling voltage, then the piezoelectric material will contract vertically and to a lesser extent expand horizontally. The continuous expansion and contraction of the piezoelectric material will generate the ultrasound wave needed for bone marrow harvesting. This concept is explained in Figure 3.4.

![Figure 3.4: Visual representation of the piezoelectric effect. (a) The piezoelectric material, unstretched. (b) The piezoelectric material compressed as a result of the applied voltage having the opposite polarity as the poling voltage. (c) The piezoelectric material stretched as a result of the applied voltage having the same polarity as the poling voltage.](image-url)
This concept can be analyzed by examining the two fundamental equations of the piezoelectric effect. First, the strain of the material will be described:

\[
[S] = [s^E][T] + [d][E] \quad \text{(Eq. 3) [63]}
\]

Assuming a constant electric field, the matrix \([s^E]\) represents the elastic compliance of the material when at a constant electrical field, while \([T]\) represents the mechanical stress. The matrix \([d]\) represents the piezoelectric charge coefficient equal to the product of \([s^E]\) and the piezoelectric stress constant \(e\), while \([E]\) represents the electric field. This is essentially a form of Hooke’s Law for elastic materials and what will be used to actuate the piezoelectric material.

The second equation describes the electrical displacement \([D]\):

\[
[D] = [d][T] + [\varepsilon^T][E] \quad \text{(Eq. 4) [63]}
\]

Most of the terms describing the strain can be applied to this equation. The only other different term is the matrix \([\varepsilon^T]\), which represents the permittivity of the material. This equation applies for the direct piezoelectric effect, where an electrical displacement is created as a result of mechanical strain.

Since focus is being placed on the converse piezoelectric effect, emphasis should be placed on the first equation, since a deformation of the piezoelectric material is needed in order to create the ultrasound wave. Assuming no mechanical stress is applied, by applying a certain electrical field \([E]\), a deformation of the piezoelectric material will occur as a result of increased strain, as shown in Figure 3.4. The greater the electric field, the more strain that will be obtained. The continuous straining of the material will result in an ultrasound wave with higher magnitude.

For this case, a four-ring actuator stack (Noliac, NAC2121) electrically connected in series is used to generate the ultrasound wave [64]. The actuator, with a diameter of 6 mm, is pictured in Figure 3.5. The piezoelectric actuator has a capacitance of approximately 240 nF.
It is recommended that the piezoelectric actuators are used below 1/3 of its resonance frequency, which is 159 kHz, in order to experience a flat, steady mechanical displacement from the device [65] (as per personal correspondence with John Klausen, managing director of Noliac operations in North America). To meet this requirement, the piezoelectric material is operated at 50 kHz, which is slightly more than 1/3 of the resonance frequency. To focus the ultrasound wave that will be generated, the piezoelectric material is put into a casing with a 1-cm diameter concave transducer face. The casing was made using SolidWorks and was 3D printed.

In order to drive the piezoelectric actuator, a function generator (National Instruments, PXI-5402) is needed to generate an AC voltage. This function generator is interfaced in a LabVIEW program; however, it is limited to a maximum voltage of 5 V_{pp}. To amplify the voltage, an analog amplifier (Krohn-Hite, 7500) is used. This amplifier has a variable gain

![Figure 3.5](image-url)

Figure 3.5: (top) Example of a 4-stack NAC2121 ring actuator. Each ring is electrically connected in series with each other. (bottom) NAC2121 actuator enclosed in a concave casing. Scale bar: 1 cm.
between 0-40 dB and is also capable of DC offsets of up to 200 V, which is necessary since the NAC2121 operates at voltages from 0 to 200 V.

Before testing the device, a DC offset of 70 V was applied to the actuator for approximately 30 minutes. This constitutes as a “warm-up” time for the piezoelectric actuator due to a property known as the creep effect. When a DC voltage is applied, the piezoelectric actuator slowly expands over time until it reaches a near steady-state condition, thereby affecting the displacement of the actuator [66]. This can therefore contribute to an increase in pressure from testing the device immediately after the DC voltage is applied compared to a later time. The creep effect can be quantified using the following equation:

$$\Delta L(t) = \Delta L(t)_{(t=0)} (1 + \gamma \times \log_{10} \left( \frac{t}{0.1} \right)) \quad \text{(Eq. 5) [66]}$$

This is where $\Delta L(t)$ is the displacement of the actuator at a certain time, $\gamma$ is the creep constant, and $t$ is the time in seconds. For the 4-ring stack NAC2121 actuator, the original, maximum displacement of the actuator is equal to 9.9 $\mu$m, while the creep constant is 4% [65]. Therefore, the creep effect can be plotted, as seen in Figure 3.6.
Figure 3.6: The creep effect changing the displacement of a 4-stack NAC2121 actuator.

The creep effect shows that the maximum displacement of the piezoelectric actuator experiences about a 17% change in its displacement, increasing from 9.9 μm to about 11.6 μm after 35 minutes. Testing done on the piezoelectric actuator indicated that the DC voltage should be left on approximately 30 minutes in order for the actuator to reach a near steady-state condition. At this point, the pressures acquired should be near constant at the same location based on its position to the hydrophone.

Testing of the piezoelectric device was done with a 50 kHz, 800 mV\textsubscript{pp} sinusoidal wave with a cycle interval of 100 ms and a duty cycle of 1% (50 cycles). The small duty cycle is used to prevent potential overheating of the actuator, as recommended by the manufacturer. The amplifier was set with a 40 dB gain to amplify the voltage to 80 V\textsubscript{pp} with a DC offset of 70 V. To evaluate the performance of the device, characterization was done using a 1.0 mm hydrophone probe (Precision Acoustics), which can collect mechanical waves with frequencies between 5
kHz to 15 MHz. The piezoelectric device was placed 2 mm above the hydrophone needle submerged in water. The probe converted the mechanical energy from the ultrasound wave into electrical energy. The signal was then amplified by a submersible amplifier (Precision Acoustics), which buffers the signal and provides a 50 Ω source. The preamplifier was connected to a DC Coupler (Precision Acoustics) which provides power to the submersible amplifier while also acting as a coupler between the preamplifier and the output system. The output of the coupler went into a digitizer (National Instruments, PXI-5122) also interfaced in LabVIEW. The pressure $P$ can then found using the following equation:

$$P = \frac{V(t)}{M(f)} \text{ (Eq. 6) [67]}$$

The pressure $P$ can be calculated by dividing the measured hydrophone voltage $V(t)$ by the hydrophone sensitivity $M(f)$ (V per MPa). At 50 kHz, the sensitivity is 426 V per MPa [68]. This allowed the positive- and negative-peak pressures generated by the piezoelectric actuator to be collected. To characterize the performance of the actuator, pressure readings were taken in 1-mm increments across the 6-mm diameter of the actuator for a total of 49 data points. Each data point resulted from an average of three trials from one cycle interval lasting 100 ms. These data points can be used to create a positive-peak pressure and negative-peak pressure map that can be used to determine the output from driving the piezoelectric actuator. A block diagram showing this system is shown in Figure 3.7.

Figure 3.7: Block diagram describing procedure to obtain pressure measurements using piezoelectric actuator.
3.5.2 Experimental Testing: Force Measurements and Cell Viability

Once characterization is completed, the piezoelectric device can then be tested on yellow bone marrow, followed by red marrow. It is feasible to test with yellow marrow first since it is less dense than red marrow and can provide a look into whether ultrasound can disrupt the cellular matrix of bone marrow. For cases involving yellow marrow, a bovine bone marrow originally at 4°C is obtained from a local supermarket, as shown in Figure 3.8. The bone marrow is warmed up to 37°C to simulate conditions in the human body. Then, a region of yellow marrow is then selected for force measurements. A force meter (Force Mark, M5-5) with a cone gauge tip is used to measure the force needed to penetrate the bone marrow at a control point. A cone gauge tip is used since it resembles the shape that the ultrasound waves travel in as a result of the concave casing used to focus the waveform. Following this, the actuator is placed onto the region selected such that the face is making contact with the bone marrow. About 1 N of force is applied from the actuator to the bone marrow in order to keep the device and the sample in contact with each other. Once this is done, a 80 V_{pp} (after amplification) poling voltage with a frequency of 50 kHz is sent to the actuator with a DC offset of 70 V for 100 ms with a duty cycle of 1% (50 cycles) for 10 seconds. As a result, the bone marrow becomes exposed to 10 ms of ultrasound. The same poling voltage is then sent after a 10 second interval of rest in order to test different exposure times of the ultrasound to the bone marrow. The exposure time is varied from 30 ms to 100 ms in order to gauge how much ultrasonic exposure is needed to disrupt the bone marrow. Figure 3.9 shows the experimental setup, while Figure 3.10 shows the testing of the device on bone marrow. Following this, a sample of the bone marrow is collected and placed in a 0.5%-1% solution of phosphate-buffered saline for cell viability testing.
Figure 3.8: (left) Bovine bone marrow at 4°C. (right) Bone marrow tested at 37°C.

Testing with red marrow involves a similar procedure, although some additional steps are required. At each measurement point, acoustic gel was applied in order to provide contact between the face of the actuator and the bone marrow. Also, more force was required to penetrate the red marrow, so a different force meter (Force Mark, M5-100) was used, which is
capable of measuring forces up to 100 lbf (444.8 N). Exposure times between 30 ms to 200 ms were tested with the same parameters mentioned in the previous paragraph.

Figure 3.10: (left) Spongy bone containing red bone marrow. Testing of piezoelectric device on red bone marrow.

In order to establish that there was a significant disruption to the cellular matrix, two-tailed $p$-values were determined using the paired Student’s $t$-test. A $p$-value is used to test a null hypothesis and indicates the probability that a set of data (the measured force after ultrasound exposure) is significantly different or not compared to a control set of data [69, 70]. If the $p$-value is 0.05 or less, the null hypothesis is rejected, meaning that there is a statistical difference between the control measurements and the measured data points.

To find the $p$-value, assume that are two sets of data, labeled $x_i$, the control value, and $y_i$, the treatment value. For each pair of data, the difference $z$ is calculated. Then, calculate the standard deviation of the differences, $s$. Then, find the $t$-value (with n-1 degrees of freedom), which is equal to

$$t = \frac{z}{\sqrt{s^2}}$$

(Eq. 7) [69],

where $n$ is the sample size. Once the $t$-value is found, $t$-distribution tables can be used to find the $p$-value, which is dependent on the degrees of freedom. This can also be calculated using
programs such as MATLAB or resources such as GraphPad (which is used here). Through the use of the paired Student’s *t*-test, the results obtained from the force measurements can be further evaluated.

To check for cell viability, Trypan Blue is used. Trypan Blue stains dead or non-viable cells, differentiating these cells from viable cells, which remain unchanged. A 0.4% Trypan Blue solution is prepared in order to provide a 1:1 dilution of the cell sample in suspension. The cells are put into a hemocytometer chamber for counting. After 1 to 2 minutes of incubation, the cells are counted under a microscope. An estimate of the number of cells in the sample can then be made by taking the average number of cells in a square. By counting at least four of the squares, taking the average of the number of cells counted for one square, and multiplying this number by the total number of squares, the number of total cells can be counted. The number of dead cells is also counted using the same procedure. By taking the number of dead cells divided by the number of total cells, the cell death percentage can be found. The block diagram for the entire experimental procedure is shown in Figure 3.11.
Figure 3.11: Block diagram describing process to test bone marrow with ultrasound.
3.6 Results and Discussion

The pressure maps that were obtained for the piezoelectric actuator are shown in Figure 3.12 using the procedure described in the previous section:

![Pressure maps](image)

Figure 3.12: Positive (left) and negative (right) peak pressure outputs collected from a NAC2121 actuator 2 mm away from the tip. The maximum positive peak pressure was measured at 0.190 MPa while the maximum negative peak pressure was measured at 0.176 MPa.

The results indicate that the piezoelectric actuator was focused by the concave casing at the center of the device as expected. The maximum positive peak pressure was measured at 0.190 MPa, while the maximum negative peak pressure was measured at 0.176 MPa. Therefore, the pressure maps obtained represent the expected pressures exerted on bone marrow by the piezoelectric actuator.

Table 1 below represents the force measurements obtained from varying the exposure time of yellow bone marrow to ultrasound with a positive peak pressure of 0.190 MPa and a negative peak pressure of 0.176 MPa. Both the change in force between the control measurement and the samples exposed to ultrasound and the calculated \( p \)-value hold greater weight rather than the absolute change in force, since the bone marrow density will vary based on the region of bone marrow selected for testing.
Table 1: Force Measurements on Yellow Marrow

<table>
<thead>
<tr>
<th>Experimental Condition</th>
<th>Force Measurements (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ultrasound exposure</td>
<td>Control?</td>
</tr>
<tr>
<td>time (ms)</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>No</td>
</tr>
<tr>
<td>Change in force</td>
<td>-21.2%</td>
</tr>
<tr>
<td>Calculated p-value</td>
<td>0.78</td>
</tr>
<tr>
<td>70</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>No</td>
</tr>
<tr>
<td>Change in force</td>
<td>25.6%</td>
</tr>
<tr>
<td>Calculated p-value</td>
<td>0.11</td>
</tr>
<tr>
<td>100</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>No</td>
</tr>
<tr>
<td>Change in force</td>
<td>49.4%</td>
</tr>
<tr>
<td>Calculated p-value</td>
<td><strong>0.04</strong></td>
</tr>
</tbody>
</table>

These tests indicate that greater exposure times will cause further disruption to bone marrow. This is particularly seen at exposure times greater than 70 ms, with 46.1% less force needed to penetrate the bone marrow, while a minimal change was seen at 10 ms of exposure. The p-values obtained indicate that there is no significant difference between control points and the measured data points with exposure times at 30 ms and 70 ms. However, with an exposure time of 100 ms, a significant difference was found between the control and the ultrasound-treated sample force measurements with a p-value of 0.04. This indicates that there is a threshold exposure level that will result in yellow bone marrow disruption.

With the knowledge gained from testing yellow bone marrow, red bone marrow was then tested. Table 2 describes the results obtained from red bone marrow.
Table 2: Force Measurements on Red Marrow

<table>
<thead>
<tr>
<th>Experimental Condition</th>
<th>Force Measurements (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ultrasound exposure time (ms)</td>
<td>Control</td>
</tr>
<tr>
<td>30 Yes</td>
<td>62.27</td>
</tr>
<tr>
<td>No</td>
<td>63.17</td>
</tr>
<tr>
<td>Change in force</td>
<td>-1.4%</td>
</tr>
<tr>
<td>Calculated p-value</td>
<td>0.44 (no significant difference)</td>
</tr>
<tr>
<td>100 Yes</td>
<td>62.36</td>
</tr>
<tr>
<td>No</td>
<td>46.23</td>
</tr>
<tr>
<td>Change in force</td>
<td>25.9%</td>
</tr>
<tr>
<td>Calculated p-value</td>
<td>0.0037 (significant difference)</td>
</tr>
<tr>
<td>150 Yes</td>
<td>61.55</td>
</tr>
<tr>
<td>No</td>
<td>41.04</td>
</tr>
<tr>
<td>Change in force</td>
<td>33.3%</td>
</tr>
<tr>
<td>Calculated p-value</td>
<td>0.028 (significant difference)</td>
</tr>
<tr>
<td>200 Yes</td>
<td>58.60</td>
</tr>
<tr>
<td>No</td>
<td>43.37</td>
</tr>
<tr>
<td>Change in force</td>
<td>26.0%</td>
</tr>
<tr>
<td>Calculated p-value</td>
<td>0.005 (significant difference)</td>
</tr>
</tbody>
</table>

Table 2 indicates that the cellular matrix of red marrow is also disrupted using similar parameters. No change is seen with 30 ms of ultrasound exposure, but with greater than 100 ms of ultrasound exposure, 28.1% to 30.9% less force is needed to penetrate the red bone marrow. For cases involving greater than 100 ms of ultrasound, the $p$-values are no greater than 0.0281, indicating significant changes between the control measurements and the ultrasound-treated measurements. The results suggest that reduction in force saturates between 150 to 200 ms, as the change in force is about the same for these cases.

### 3.7 Future Work

Proof of concept for an ultrasound-bone-marrow harvester has been shown by disrupting bone marrow using ultrasound, with less force being needed to penetrate the bone marrow with greater ultrasound exposure times. However, additional work will need to be done on this project.
First, consideration must be given to extracting bone marrow for harvesting. Ring actuators were specifically selected for the ultrasonic bone marrow harvester due to the opening in the middle of the actuator. This opening could be used as the extraction point for the bone marrow, with a separate hollow stick placed in this position in order to provide a pathway for the bone marrow to be extracted from. The bone marrow could therefore be extracted using suction. Aspirating bone marrow will require some type of pulling force which can be accomplished by suction. Extracting bone marrow would require a large amount of negative pressure in order to induce bone marrow to flow within the channel. Current aspiration methods using a syringe plunger can only extract bone marrow at a rate of 3 to 5 mL/s with a 60 N pulling force applied [71]. Therefore, to induce a high bone marrow flow rate, more than 60 N of force from the suction force would be required.

Additionally, to increase the amount of negative pressure to extract bone marrow, a smaller channel could be used. While a smaller channel may not necessarily extract a large amount of bone marrow per second, more pressure can be produced with a decrease in area, resulting in more bone marrow being extracted per milliliter. This has been shown with the extraction of MSCs using syringes of various volumes; the smaller the syringe volume, the more MSCs extracted per milliliter of aspirate [72]. Further study would be required to find the ideal size of the channel, which could be done with further experimentation.

Figure 3.13 shows the original design of the casing with the extraction point. However, an opening at this position would introduce a potential point of leakage into the device, which could cause damage to the actuator. This issue was experienced during the testing of the device, with water leaking into the casing during characterization. Therefore, the casing structure had to be made in a way such it would be impossible for water to leak into the device at this point.
One way that this issue could be solved is by making the hollow stick a part of the casing instead of having it as a separate component. SolidWorks was used to make the modification on the casing. Figure 3.14 shows the modified design made in SolidWorks.

Multiple attempts were made to design this particular casing through the use of 3D printing. However, a successful iteration of this casing could not be implemented due to the resolution of the printers used to-date; these printers were unable to print the hollow stick, which is approximately a millimeter in diameter. Since all components are printed together, the 3D printers used could not precisely make the hollow stick without errors. Therefore, characterization and testing was done without the opening in the casing in order to achieve proof.
of concept. As of the time of this writing, a manufacturer has been lined up to make this type of casing. Therefore, future testing will involve piezoelectric actuators with this modified design. Ultimately, the final design would be able to harvest bone marrow from the cavity, as shown in Figure 3.2.

Additionally, further testing of the piezoelectric material being used is required. Since the ultrasound wave has to travel through an air-material interface, the pressure that is generated by the actuator may not be at its maximum due to an acoustic impedance mismatch. It likely that some of the ultrasound wave is being reflected back to the piezoelectric actuator itself; as a result, there is wasted acoustic energy. By testing different types of casings or introducing a matching layer [63, 73] to reduce the impedance mismatch, more pressure from the device could be potentially obtained. Further research on the acoustic impedance of the piezoelectric actuator material, the NCE51F, will be needed to work on this issue, and further collaboration with those having knowledge on acoustic impedance matching may be necessary to solve this issue. This may also require a change in the piezoelectric device, since acoustic impedance matching is frequency dependent; since the piezoelectric device cannot be operated at more than 1/3 of its resonance frequency, additional changes to the device may be needed. Furthermore, with a change in the device, it may be necessary to reduce the size of the piezoelectric actuator, as it could help lower the pain threshold that a donor may experience as a result of needing to drill through the bone to reach the bone marrow cavity.
Chapter 4. Conclusion

This thesis proposal has demonstrated work on two biomedical projects that aim to improve the current procedures that are needed in order to treat diseases such as heart disease, cancer, and COPD. The first project demonstrated the development of an OFB microrobot actuation program. Two major functions were developed for the program: a bubble collision system which prevents microrobots from colliding with one another, and a sequence generator and player capable allowing for the individual actuation and manipulation of many microrobots at once. The bubble collision system and the sequence generator have seen applications in the manipulation of microparticles, either by pushing and pulling micro-objects or through caging. Further work will be needed to develop a closed-loop caging system, which is being done in collaboration with the Chiba Institute of Technology. Additional work will be also needed to actuate 50 microrobots in order to achieve mass assembly and manipulation of micro-objects. With this system, cells could be eventually assembled into functional tissue for the formation of synthetic organs with a patient’s own cells.

On the other hand, the ultrasonic bone-marrow harvester has been successfully used to disrupt the cellular matrix of bone marrow with high cell viability rates. With further pressures, the device is able to disrupt both yellow and red bone marrow for eventual extraction. Additional work on the project is needed, including the development of an extraction system for bone marrow, testing on different case types for acoustic impedance matching, and further testing of piezoelectric materials to obtained increased pressures from the device. With further development of this project, the bone marrow transplant process will be streamlined, reducing the potential issues that both a donor and a patient would face.
References


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