Optical 2-D Scanning System for Laser - Generated Shockwave Treatment of Wound Infections

A thesis submitted in partial satisfaction of the requirements for the degree Master of Science in Biomedical Engineering

by

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ABSTRACT OF THE THESIS

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Bacterial infections are a burden on the healthcare industry. Biofilms play a major role in allowing bacteria to thrive on surfaces and allow infections to persist. Biofilm formation in wounds is a potential cause of chronic infections. Biofilm disruption therefore, is essential for successful bacterial infection management.

This work focuses on the safety and efficacy of using laser-generated shockwaves on Staphylococcus epidermidis (RP62A) biofilms in vitro and presents the design and implementation of a compact scanning system as a portable and practical solution for future clinical applications of this technique in wound care.

The system uses a Q-switched, ND:YAG pulsed laser with an output wavelength of 1.064 μm that ablates thin Ti film between constraining layer and substrate to generate an
acoustic shockwave. The compressive wave propagates through the layered structures and upon reflection at the interfaces generates a tensile wave which is ultimately responsible for biofilm delamination and dislodging bacteria, thereby making them more susceptible to antibiotic treatment, suggesting a novel treatment option for treating biofilm infected wound infections. Preliminary results find a minimum threshold laser beam of 325mJ energy with a pulse width of 2-6 nanoseconds onto Ti-coated glass substrate focused over 3 mm is required for delamination of biofilm grown on polystyrene petri dishes.

A survey of current methods for treating infected wounds is made to provide context for using this approach. A description of the laser generated shockwave technology and methods used to characterize the shockwave are also presented. Finally, a low-cost portable 2-D scanning platform is designed, constructed and tested successfully.
The thesis of Shahzad Neville Patel is approved.

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I. INTRODUCTION

1.1 Wound Infections

Infected wounds due to trauma or surgery impose a major burden on the US healthcare system. Treatment of infected wounds, both surgical and traumatic, can cause prolonged hospitalization, lead to sepsis, and dramatically increase the cost of patient care\textsuperscript{[1,2]}. One of the main mechanisms that enable bacteria to persist \textit{in vivo} is through the generation of biofilms. Biofilms consist of a three-dimensional matrix, rich with polymeric substances such as polysaccharides, nucleic acids, and proteins that provide a protective and nurturing environment for bacteria to proliferate and reside in\textsuperscript{[3,4]}. They allow the bacteria to mechanically adhere to the wound surface, thereby preventing ingress of white blood cells and antibodies, and also providing a chemical barrier to antibiotics \textsuperscript{[5]}. Bacteria present in mature biofilms are resistant to 50-5000 times the concentrations of antimicrobial agents that are necessary to kill planktonic cells of the same organism\textsuperscript{[4,6]}. Several species of bacteria including genera \textit{Staphylococcus} and \textit{Acinetobacter}, persist in traumatic wounds despite treatment with topical antibiotics, wound irrigation, and surgical debridement, becoming increasingly difficult to manage due to the emergence of bacterial strains that exhibit antibiotic resistance\textsuperscript{[7-13]}.\textsuperscript{\textdagger}
Normal wound healing is a complex process characterized by 3 overlapping phases: inflammatory, proliferative and remodeling[^14]. If the body’s vascular and cellular responses during the inflammatory phase are inadequate to overcome surface microorganisms, the wound becomes predisposed to infection, delaying angiogenesis, tissue granulation, and reepithelialization in subsequent stages, thereby making them chronic[^15]. Chronic wounds are characterized by the formation of a coagulum, accumulation of necrotic debris and leaked protein-containing fluids that serve as a rich medium for bacterial growth. Several advances in wound care have resulted in the current synergistic approach to treat wounds with wound debridement coupled to negative-pressure wound dressings, high pressure irrigation and topical antibiotics[^16^-^20]. However, debridement is often traumatic, time-consuming and must be repeated[^21], since bacterial proliferation can resume after the procedure is finished. Non-healing wounds are biologically characterized by prolonged inflammation, defective re-epithelialization and impaired matrix remodeling, although different abnormalities may well be present in individual patients[^22]. In addition, recent evidence suggests biofilms are a potential cause and mostly present in chronic infections[^23]. From this, one can conclude that successful infectious-wound management requires dislodging, or destroying biofilm to make the bacteria more vulnerable and reduce bioburden. There is widespread acceptance that bioburden under $10^5$ CFU/g of viable wound tissue is necessary for normal healing process to continue[^24^-^26]. Therefore, studying more effective biofilm disruption techniques holds a promising value in reducing the bacterial bio-burden, thereby limiting one of the major contributing factors in persistent wound infections.
In order to survive in the wound environment, micro-organisms need to adapt quickly and resist challenge by the host immune system. This is particularly difficult in clean surgical wounds where both tissue perfusion and the inflammatory response (PMN activity) are normal; microbial contaminants are rapidly cleared from the wound, enabling the wound to heal quickly\cite{21}.

### 1.2 Biofilms

Biofilms are defined as matrix-enclosed (single/multi) bacterial populations’ adherent to each other and to surfaces or interfaces\cite{3}. The microenvironment of each biofilm bacterium is conditioned by the exuberant exopolysaccharide matrix production within the developing biofilm and the juxtaposition of cells of the same species and different species forming microcolonies. Different biofilm bacteria respond to their specific microenvironmental conditions with different growth patterns, and a structurally complex mature biofilm gradually develops. At a higher level of organization, bacteria within biofilms benefit from similar stable juxtaposition and physiological cooperativity and therefore constitute a coordinated functional community that is much more efficient than mixed populations of floating planktonic organisms. Mature biofilms are resistant to 50-5000 times the concentrations of antimicrobial agents that are necessary to kill planktonic cells of the same organism\cite{3}.

### 1.3 Current Treatment Options

#### 1.3.1 Chemical Treatment

Bacteria in biofilms are in a quiescent state, thus diminishing antimicrobial efficacy against biofilms\cite{9,27}. Khoury et al. (1992) demonstrated that bacteria within mature
biofilms resisted up to 5000 times the concentrations of antimicrobial agents necessary to kill planktonic (in suspension) cells of the same organism\[6\]. Surface preparation of medical equipment and implants with antimicrobials has minimized only short-term microbial colonization\[28,29\], while other approaches, like high doses of topical antibiotics\[8-11\], pressurized irrigation, and use of citric acid/zwitterionic surfactant\[30\], have not resulted in eradication of bacteria in biofilms. Desrosiers et al. (2007), reported topical antibiotics in 1000-fold concentrations produced a several log reduction of viable bacteria in \textit{S. aureus} biofilms \textit{in vitro}\[31\]. However, such concentrations are not feasible in a clinical setting. Therefore, chemical treatments alone are not an effective option against fighting biofilm infections.

1.3.2 Low Intensity Ultrasound therapy

LIUS (100KHz to 3MHz) studies, while ineffective on their own\[32-35\], have seen some success when combined with antibiotic therapies\[33,34,36\]. Carmen et al. (2004) showed an enhanced effect of Vancomycin in rabbits when subjected to 48 hours of pulsed LIUS for treating \textit{S. epidermidis} infected implants\[36\]. Similarly, Qian et al. (1999) showed a decrease in enhancing the antibiotic effect of gentamicin as ultrasound frequency increased, suggesting LIUS causes more enhancement of the antibiotic action. Nonetheless, application of ultrasound at similar low-intensities used in acoustic enhanced antibiotic killing does not change the shape of the biofilm nor the arrangement of the bacterial cells in the biofilm\[37\]. In addition, ultrasound waves create standing waves giving rise to thermal generation in tissue\[38,39\]. Moreover, Pitt et al. (2003) showed \textit{E. coli}, \textit{S. epidermidis}, and \textit{P. aeruginosa} growth in biofilm is enhanced by low
frequency, low intensity ultrasound\textsuperscript{[35]}. These studies indicate bactericidal effects when coupled with antibiotics however, on their own LISU treatments are ineffective and could possibly increase bacterial growth.

1.3.3 Negative pressure wound therapy (NPWT)

NPWT promotes chronic wound healing by creating a vacuum through a special sealed dressing to draw out interstitial fluid from the wound, increasing blood inflow to the wound area, and improving capillary circulation at the edges of the wound\textsuperscript{[21]}. The first study investigating the effects of NPWT on the bacterial load showed a decrease in bacterial load around 4 to 5 days compared to control\textsuperscript{[40]}. Three studies thereafter reported opposite results\textsuperscript{[41-43]}. A retrospective clinical study investigating 26 wounds of varying etiology, reported that bacterial colonization increased significantly with NPWT\textsuperscript{[41]}. In a randomized trial, Braakenberg et al. (2006) reported swab cultures showing increased bacteriologic colonization in patients treated with NPWT\textsuperscript{[42]}. Finally, Moues et al. (2011) compared NPWT with conventional moist gauze therapy in 54 patients reporting no quantitative reduction in the number of bacteria between the two treatments\textsuperscript{[43]}. Nonetheless, NPWT has been successful in treating wounds when used in conjunction with debridement, high pressure irrigation, and adjuvant antibiotics in pressure ulcers\textsuperscript{[20]}, infected median sternotomy wounds\textsuperscript{[16,44]} and high energy soft tissue injuries\textsuperscript{[19]}. However, none of the studies reported any bacteriologic sampling data. To summarize, a
majority of these previous studies indicate that NPWT does not reduce bacterial load [43].

1.3.4 Extracorporeal shockwave treatment
ESWT has become the standard non-invasive technique for stone fragmentation[45]. Lithotripters generate a focused, high-intensity acoustic shockwave resulting in mechanical forces and cavitation bubbles in the focused area. This technology has been implemented in osteogenesis and healing of overlying soft tissues in cases involving nonunion and delayed bone healing[46]. ESWT was used in the management of wound care due to its ability to stimulate the regeneration of tissue[47]. Antibacterial effects using ESWT shockwaves have been reported to reduce growth of planktonic S. aureus colonies subjected to 4000 treatments at 2 Hz[48] in vitro. Other studies have also revealed antibacterial effects of high energy shockwaves on planktonic microorganisms[49-52]. A recent in vitro study to treat periodontitis disease showed ESWT (0.4mJ/mm², 3Hz) removed biofilm but did not show any bactericidal effects[53]. These results show an increase in targeted cell permeability under thousands of pulses, however, cavitation-induced phenomenon can further damage mammalian cells[54] due to the tensile component of the mechanical stress waves as it propagates through the media.

1.4 Laser generated Shockwave treatment (LGST)
Lasers have been used in medicine for multiple medical applications including cataract, LASIK, dental applications, ear surgery and extra-luminal laser angioplasty (ELAN). Similar to ESWT, LGST utilizes the same principles to generate mechanical shockwaves, which propagate and interact with biological cells/tissue. The differences
between the two modalities arises in the shockwave generating sources leading to
different waveform characteristics such as pulse rise time, pulse duration and peak stress.
Furthermore, ESWT has a tensile transition that causes cavitation, as compared to LGST
shockwaves which only possess a compressive component. This difference is significant
when analyzing their interaction with biological matter.

LGST uses a laser source to impinge upon a substrate generating mechanical
shockwaves due to the rapid thermal expansion of the medium. In contrast to
ESWT, this produces faster rise times, shorter pulse durations and no cavitation
effects due to the lack of a tensile component. LGST has resulted in enhanced cell
permeability [32-33], thereby facilitating delivery of macromolecules and genes
through cell membranes and skin[57-60]. In addition, LGST shockwaves result in
reduced bacterial viability when coupled with antibiotics [61,62]. The mechanism
for this is thought to be related to the stress wave gradient which has a greater
effect on cell viability than the peak stress of the shockwave[63]. This suggests that
a rapid stress rise-time (2-6ns) should maximize cell wall permeability to allow
maximum drug delivery. Thus, LGST appears to produce a possible bactericidal
improvement with follow-up antibiotic treatment due to enhanced permeability.

Regarding biofilm disruption, Krespi et al. (2008) were able to disrupt
Pseudomonas biofilms on culture plates in vitro[64], while Nigri et al. (2001) showed
no effect of LGST alone on microbial cell viability in vitro[65]. It is possible that the
low peak stress (60 MPa) generated using a polymer instead of a thin metallic
film resulted in this negative result, nonetheless, they showed a log reduction in microbial cell count when they combined LGST with antibiotics[65].

Gupta et al. optimized laser-generated stress wave profiles for measuring the tensile strength of a non-biological thin film interfaces [U.S. Patent 5,438,402] and biological thin films[66-68]. This technique allows us to directly determine the tensile strength of planar interfaces. Ongoing research is involved in studying the adhesion strength of bacterial biofilms grown in static conditions on polystyrene surfaces using this patented technique.
II. LASER GENERATED SHOCKWAVE TREATMENT (LGST) SYSTEM

The laser generated shockwave treatment system was developed using a patented laser-generated shockwave technology [U.S. Patent 5,438,402] to develop a novel minimally invasive modality to disrupt and delaminate bacterial biofilms growing on biological surfaces. This approach will synergistically enhance the function of antimicrobials against bacterial growth and assist in disinfecting wounds.

The technology behind LGST consists of an infra-red laser pulse with sub-nanosecond rise time and pulse width to impinge upon a thin metallic surface (absorber) coated on a non-metallic substrate (like glass). The laser pulse delivers a large amount of energy in a short duration (80ns) onto a constrained metallic surface which causes a rapid thermal expansion and subsequent contraction of the absorbing metallic film. This mechanism translates the optical energy into mechanical energy and generates a travelling compressive shockwave moving away from the laser source. Controlling the variable parameters of this system such as the laser fluence, pulse duration, metallic film, metallic film constraining layer and underlying substrate can optimize the energy
transferred via this mechanism and allow for better fine tuning and refinement.

The model described in this chapter optimizes all the variables listed above and delivers the compressive wave onto the biofilm. Due to the interfaces present between the multiple layers of this model, the peak amplitude of the compressive stress wave attenuates before reaching the biofilm. Some of this lost mechanical energy dissipates while some of it becomes a tensile wave reflecting back from the interface. The interfaces create acoustic impedance mismatches causing reflected tensile waves to be generated. To better understand this acoustic behavior, an analytical modeling approach has been used further in this chapter.

This section discusses the different experimental setups, methods and materials used for this project. The experiments performed included stress wave interferometry, in vitro biofilm delamination studies, mathematical modeling of the biofilm adhesion strength and an ex vivo porcine skin model for shockwave damage studies.

### 2.1 Biofilm delamination experimental setup

*Staphylococcus epidermidis* is a Gram-positive cocci ("spherical-shaped"), coagulase-negative, organism that is part of the *Staphylococcus* genus. It has previously been thought of as a harmless microorganism as it was commonly found on human skin. Currently, *S. epidermidis* is regarded as an opportunistic pathogen that cause nosocomial ("hospital-acquired") infections. This bacterium was chosen as it is commonly the cause of infection in non-healing wounds and is also known for its ability to produce the
polysaccharide adhesion to produce uniform biofilms.

To generate the mechanical shockwave, we used a 1.064μm ND:YAG laser (2-6ns pulse duration) to ablate thin polyethylene film which was RF sputtered with 0.5μm thick Titanium (Ti) coating. The flexible film was coupled to the biofilm layer with deionized (DI) water, shown in Fig 2. Ti was chosen as the ablating metallic film due to its biocompatibility and high absorption coefficient at 1.064μm wavelength. The melting-induced expansion of Ti under confinement generates a compressive stress wave (with a 6-7ns rise time) that propagates towards the biofilm.

A thin 0.5μm titanium film was uniformly deposited on the backside of plain microscopic (soda-line glass) slides (3x1in and 1mm thick, Corning#2947) by a RF sputtering system (Denton Discovery II 550). After metallization, the samples were then coated with waterglass (Sodium Silicate) with uniform thicknesses of 15-20 μm via a spin coater. The waterglass layer acts as the constraining layer and is transparent to the Nd:YAG 1064nm wavelength. The thicknesses of the metallic film, the constraining layer have been previously optimized and are beyond the subject of this discussion.

After 24 hour growth, the petridishes with bacterial solution were washed 5x with phosphate buffer saline (PBS). Any remaining bacteria were considered biofilm as shown in Figure 3.1a. Samples were then stained with Alcian Blue. Alcian blue stains the acidic polysaccharides which are produced by the bacteria in the biofilm. The stain allows for immediate visualization of the biofilm with light microscopy. Alcian blue was prepared using a standard protocol shown here.
The petridishes were then placed atop a water reservoir Figure 3.2. The Ti sputtered
glass slides were introduced by two rigid tapes at the ends that allowed for a \( \sim 1.5 \) mm
coupling thickness. PBS was then applied to couple the shockwave toward the biofilm.
Enough fluid was used until no air pockets or bubbles were seen. Air interfaces can lead
to unwanted reflections of the compressive waves due to the large impedance with air.
The samples were then loaded onto the platform as shown in Figure 1 and were ready for
the shockwave application.

The 2-6 ns Nd:YAG laser pulse was made to focus onto the Ti-coated glass slide
surface using a condenser lens \( (f = 4\text{in}) \) to impinge a \( \sim 3\text{mm} \) area. The absorbed laser
energy by the Ti layer causes a volumetric rapid thermal expansion due to plasma
generation leading to the generation of a compressive shock wave. The waterglass layer
constrains this Ti ablation and directs the compressive shockwave toward the biofilm.
The samples were applied with increasing energies until and later viewed under a visible
microscope.

![Figure 1: Bench-top Experimental setup of the current LGST system used to apply shockwaves to S. epidermidis. (A) Schematic and (B) actual experimental setup](image-url)
2.2 Shockwave Characterization

Interferometry

The displacement interferometer is based on a Michelson Interferometer\cite{69} as shown in Figure 3. The free surface is coated with 500nm reflective Ti coating and acts as the sample arm of the displacement interferometer. Utilizing the constructive and destructive interferences of a fringe pattern from a frequency stabilized 632.8 nm Helium Neon (HeNe) goes through a 50/50 beam splitter, splitting the beam into a reference beam and a sample beam. The reference beam reflects back from the mirror, and the sample beam reflects back from the free titanium free surface. The beams recombine and are focused by a lens onto an ultra-high speed photodetector (Hamamatsu MSM-64178). When the laser generated shockwave arrives at the free surface, the surface displacement offsets the sample beam, causing a phase shift in the signal measured by the photodetector. After amplification, the signal is recorded by a high speed waveform digitizer (Tektronix SCD1000) that can capture at 0.2ns temporal resolution with 5ps rise times.

![Interferometry Setup](image)

Figure 2: Interferometry Schematic to characterize the incoming stress wave.
The resulting waveform is displayed by the digitizer as a sinusoidal waveform of the output voltage from the photodetector as a function of time, with the peaks corresponding to constructive and the troughs to destructive interference.

The output voltage $A_0(t)$ of the photodetector can be expressed as a function of the free surface displacement $u_0(t)$

$$A_0(t) = \frac{(A_{\text{max}} + A_{\text{min}})}{2} + \frac{(A_{\text{max}} - A_{\text{min}})}{2} \sin\left[\frac{4\pi}{\lambda_0} u_0(t) + \delta\right]$$  \hspace{1cm} (2.1)

where $A_{\text{max}}$ and $A_{\text{min}}$ are the global maximum and minimum fringe amplitudes, $\lambda$ is the wavelength of HeNe (632.8nm) and $\delta$ is the phase angle in radians.

The recorded interferograms are analyzed using MATLAB and OriginPro 8 to derive a numerical fit to the above equation and derive the free surface displacement $u_0(t)$.

2.3 Analytical Modeling

The particle displacement function and velocity of any point in a sample is

\[\text{Figure 3: a) Interferometry System. b) Actual interferometer}\]
represented as:

\[ u(x, t) = u_s \left( t + \frac{x}{c} \right) + u_s \left( t - \frac{x}{c} \right) \]  \hspace{1cm} (2.2) \\
\[ v(x, t) = v_s \left( t + \frac{x}{c} \right) + v_s \left( t - \frac{x}{c} \right) \]  \hspace{1cm} (2.3)

where \( s \) represents the substrate, \( c \) is the longitudinal wave velocity in the substrate, assumed to be a constant. Under plane strain and one dimensional wave propagation assumption, the stress and strain along \( x \)-axis can be presented as:

\[ \epsilon(x, t) = \frac{\delta u}{\delta x} = \frac{1}{c} [v_s \left( t + \frac{x}{c} \right) + v_s \left( t - \frac{x}{c} \right)] \]  \hspace{1cm} (2.4) \\
\[ \sigma(x, t) = (\lambda + 2\mu) \frac{\delta u}{\delta x} = \rho c [v_s \left( t + \frac{x}{c} \right) + v_s \left( t - \frac{x}{c} \right)] \]  \hspace{1cm} (2.5)

where under lateral constraints, the stress pulse propagates under uniaxial strain conditions can be related to the Lame constants \( \lambda \) and \( \mu \), the wave velocity \( c \) and the density \( \rho \) of the substrate. Using the boundary condition at the free surface:

\[ \sigma(0, t) = 0 \]  \hspace{1cm} (2.6) \\
\[ v_0(t) = v(0, t) = 2v_s(t) \]  \hspace{1cm} (2.7)

where \( v_0(t) \) is the transient velocity of the free surface obtained by interferometry. Finally, the compressive stress generated can be derived and expressed as:

\[ \sigma_l = \sigma(h, t - \Delta t) = -\frac{1}{2} \rho cv_0(t) \]  \hspace{1cm} (2.8)

The stress wave is generated and propagates as a one-dimensional planar wave over a circular cylindrical region.

The free surface displacement function \( u_0 \) from Eq. 2.1 and the corresponding free
surface velocity can be expressed as:

\[ u_0(t) = \gamma \left\{ \alpha [e^{(-t/\alpha)} - 1] + \beta [e^{(-t/\beta)} - 1] \right\} \]

(2.9)

\[ v_0(t) = \gamma \left[ e^{(-t/\alpha)} - e^{(-t/\beta)} \right] \]

(2.10)

From earlier, \[ A_0(t) = \frac{(A_{\text{max}} + A_{\text{min}})}{2} + \frac{(A_{\text{max}} + A_{\text{min}})}{2} \sin \left[ \frac{4\pi}{\lambda_0} u_0(t) + \delta \right] \]

(2.11)

Combining eq. 2.9 & eq. 2.11 we get,

\[ A_{\text{curve}}(t) = \frac{(u+\nu)}{2} + \frac{(u-\nu)}{2} \sin \left[ \frac{4\pi}{\lambda_0} \gamma \left\{ \alpha [e^{(-t/\alpha)} - 1] + \beta [e^{(-t/\beta)} - 1] \right\} + \delta \right] \]

(2.12)

The function \( A_{\text{curve}} \) is the complete function in order to fit the raw waveform from the photodetector. Six constants \( A_{\text{max}}, A_{\text{min}}, \alpha, \beta, \gamma, \delta \) must be determined and fitted to the data in order to have a unique solution. However, this is very difficult and a different strategy is implemented to determine the surface displacement. From the raw data the time points of the peaks and troughs of the fringes could be obtained. Each peak to trough is separated by a distance of \( \lambda/4 \), where \( \lambda \) is equal to the wavelength of the HeNe frequency stabilized laser of 632.8 \( \text{nm} \). After obtaining the displacement vs. time values, OriginPro 8 was used to fit the displacement function 2.10 vs. time plot to obtain the constants \( A_{\text{min}}, A_{\text{max}} \). The stress generated in the substrate can be directly calculated by combining equations 2.24 and equations 2.11 and the fitted constants as:

\[ \sigma_i = \sigma(h, t - \Delta t) = -\frac{1}{2} \rho c \gamma \left\{ e^{-[(t-\Delta t)/\alpha]} - e^{-[(t-\Delta t)/\beta]} \right\} \]

(2.13)
2.3.1 Analytical 1-D Wave Propagation Model: Matrix Method Modeling

A 1-D, multi-layered wave propagation model developed by Mal et al. to determine the formal solution of the stress field within multilayered anisotropic media subjected to time harmonic disturbances\textsuperscript{[70]}. Using linear elastodynamic theory, the matrix method formulates an elastodynamic boundary value problem wherein stress vectors are prescribed at the top (input stress wave) and bottom (free stress) faces of the plate, while the displacement and stress vectors are continuous at the interfaces. The model created using Matlab\textsuperscript{®} software provides the interfacial stress histories between layers from which the peak tensile stress wave at the desired interface can be estimated. The input stress is determined by interferometry, this is fed into the analytical model as the input stress wave.

Figure 4: Flow chart describing the process to calculate adhesion strength.
2.3.2 Stress propagation problem formulation

Mal et al.\cite{70} formulated a 1-D plane wave propagation problem in a multilayered plate (with total thickness L) is considered (Figure 5) and consists of N layers of homogeneous isotropic and elastic material. The plate is defined from $x_0 < x < x_N$ where the thickness of the nth layer defined between $x_{n-1} < x < x_n$ is $h_n$. The input stress is applied at the top surface where $x = x_0 = 0$, while at $x = x_N = L = \sum_{i=1}^{N} i \cdot h_i$. The longitudinal wave travels (& particle motion) along the x-axis. It is assumed that the layers in the place are perfectly bonded such that the displacement and stresses between the layers are continuous maintaining continuity.

The material for the nth layer can be characterized by its physical density $\rho_n$, and by its longitudinal speed of sound $\alpha_n$. This speed of sound can be solved for by knowing the elastic constants; Young’s Modulus E and Poisson’s ratio $\nu$ by
\[ \alpha_n = \sqrt{\frac{E_n(1-v)}{\rho_n(1+v_n)(1-2v_n)}} \]  
(2.14)

or they be related to the Lame constants \( \lambda_n \) and \( \mu_n \) by

\[ c = \sqrt{\frac{\lambda+2\mu}{\rho}} \]

2.3.3 Governing Equations

Assuming wave propagation in 1-D multilayered medium, the displacement and stress fields can be expressed as \( u_n(x,t) \) and \( \sigma_n(x,t) \) for each sub-layer and must satisfy Navier’s Equations:

\[
\frac{\partial^2 u_n(x,t)}{\partial x^2} = \frac{1}{\alpha_n^2} \frac{\partial^2 u_n(x,t)}{\partial t^2}, \quad x_{n-1} \leq x \leq x_n
\]  
(2.15a)

The next step is to convert the governing equations into the frequency domain by finding the Fourier transform.

\[
\sigma_n(x, t) = \frac{1}{\alpha_n^2} \frac{\partial^2 u_n(x,t)}{\partial t^2}
\]  
(2.15b)
\[
\frac{\partial^2 u_n(x, \omega)}{\partial x^2} + k^2 u_n(x, \omega) = 0
\] (2.16)

where, \(k\) is the wavenumber \(k_n = \frac{\omega}{\alpha} \) and \(\omega\) is the circular frequency.

The solution to this can be shown to be,
\[
\hat{u}_n(x, \omega) = A_n e^{i k_n(x - x_{n-1})} + B_n e^{-i k_n(x - x_{n-1})}
\] (2.17)
such that \(k\) is known as the wavenumber and \(\omega\) is the circular frequency.

where \(A_n\) and \(B_n\) are constants that need to be determined by interface continuity and also the boundary conditions in order to have a unique solution.

The stress field can be obtained by substituting the solution given in equation (2.17) into equation (2.15b) and obtaining
\[
\tilde{\sigma}_n(x, \omega) = i \omega z_n [A_n e^{i k_n(x - x_{n-1})} - B_n e^{-i k_n(x - x_{n-1})}]
\] (2.18)

where, \(z_n = \rho_n \alpha_n\) is the impedance of the material of the \(n\)th layer.

Note that the first component in \(u_n\) and \(\sigma_n\) are the waves propagating along the positive \(x\)-direction while the second term accounts for the waves traveling in the negative \(x\)-direction.

A two-dimensional stress-displacement vector \((S_n)\) in the frequency domain as
\[
\{\tilde{\hat{S}}_n(x, \omega)\} = \{\hat{u}_n(x, \omega)\}
\] (2.19)

All \(w\) arguments are omitted for further discussion.
\[
\{\tilde{\hat{\sigma}}_n\} = \{Q_n\}[E_n(x - x_{n-1})]\{C_n\}
\] (2.20)

where
\[
\begin{bmatrix}
1 & 1 \\
i\omega z_n & -i\omega z_n
\end{bmatrix}
\]  \hspace{1cm} (2.21)

\[
\begin{bmatrix}
everse{E_n(x - x_{n-1})} & 0 \\
e^{-ik_n(x_n - x_{n-1})} & e^{ik_n(x_n - x_{n-1})}
\end{bmatrix}
\]  \hspace{1cm} (2.22)

\[
\{C_n\} = \begin{bmatrix} A_n \\ B_n \end{bmatrix}
\]  \hspace{1cm} (2.23)

### 2.3.4 Continuity Conditions

When applying the interface continuity conditions at \(x = x_{n-1}\), between the \(n\)-th layer and the \(n-1\)-th layers, and knowing that the stress state for the \(n\)-th layer is

\[
\{\tilde{\boldsymbol{S}}_n\} = [Q_n][E_n(x - x_{n-1})]\{C_n\}
\]  \hspace{1cm} (2.24)

then the interface continuity conditions are expressed by

\[
\{\tilde{\boldsymbol{S}}_{n-1}(x_{n-1})\} = \{\tilde{\boldsymbol{S}}_n(x_{n-1})\} = [Q_n]\{C_n\}
\]  \hspace{1cm} (2.25)

From equation (2.24),

\[
\{\tilde{\boldsymbol{S}}_n(x_n)\} = [Q_n][E_n(h_n)]\{C_n\}
\]  \hspace{1cm} (2.26)

Thus,

\[
\{C_n\} = [Q_n]^{-1}[E_n(h_n)]^{-1}\{\tilde{\boldsymbol{S}}_n(x_n)\}
\]  \hspace{1cm} (2.27)

And from (2.20),

\[
\{\tilde{\boldsymbol{S}}_{n-1}(x_{n-1})\} = [P_n]\{\tilde{\boldsymbol{S}}_n(x_n)\}
\]  \hspace{1cm} (2.28)

where

\[
[P_n] = [Q_n][E_n(h_n)]^{-1}[Q_n]^{-1}
\]  \hspace{1cm} (2.29)

\(P_n\) is the ‘propagator matrix’ of the \(n\)-th layer that relates the displacements and
stresses between the nth and n-1 layers.

### 2.3.5 Boundary Conditions

By utilizing the recursive relationship in equation (2.28), we can find that the stresses and displacements at the boundary conditions by

\[
\{ \hat{S}_0(x_0) \} = [P]\{ \hat{S}_N(x_N) \} \tag{2.30}
\]

where, \([P] = [P_1][P_1] \cdots [P_N]\)

Applying the traction boundary conditions at the front \((x=x_0)\) and the back \((x=x_n)\) surfaces of the plate,

\[
\sigma_1(x_0, \omega) = -\hat{f}(\omega) \tag{2.31}
\]

\[
\sigma_N(x_N, \omega) = 0 \tag{2.32}
\]

can be rewritten in the form,

\[
\begin{bmatrix}
\hat{u}_1(x_0, \omega) \\
-\hat{f}(\omega)
\end{bmatrix} =
\begin{bmatrix}
P_{11} & P_{12} \\
P_{21} & P_{22}
\end{bmatrix}
\begin{bmatrix}
\hat{u}_N(x_N, \omega) \\
0
\end{bmatrix} \tag{2.33}
\]

where \(\hat{f}(\omega)\) is the Fourier transform of the applied load \(f(t)\). Thus we obtain the surface displacements given by,

\[
\hat{u}_1(x_0, \omega) = -\frac{P_{11}}{P_{21}} \hat{f}(\omega) \tag{2.34}
\]

\[
\hat{u}_N(x_N, \omega) = -\frac{1}{P_{21}} \hat{f}(\omega) \tag{2.35}
\]

Once the surface displacements are known, the interfacial stresses and displacements can be solved by relating the stress and displacements at the interface, \(x = x_n\) to those at the front surface, \(x = x_N\), through
\[ \{ \hat{S}_n(x_n) \} = [A]\{ \hat{S}_N(x_N) \} \]  
(2.36)

where, \([A] = [P_{n+1}] [P_{n+2}] \cdots [P_N]\)

Rewriting above equation with the appropriate boundary conditions takes the form

\[ \begin{bmatrix} \hat{u}_n(x_n, \omega) \\ \hat{\sigma}_n(x_n, \omega) \end{bmatrix} = \begin{bmatrix} A_{11} & A_{12} \\ A_{21} & A_{22} \end{bmatrix} \begin{bmatrix} \hat{u}_N(x_N, \omega) \\ 0 \end{bmatrix} \]  
(2.37)

which can be solved for the interfacial stresses and displacements as

\[ \hat{u}_n(x_n, \omega) = -\frac{A_{11}}{P_{21}} \hat{f}(\omega) \]  
(2.38)

\[ \hat{\sigma}_n(x_n, \omega) = -\frac{A_{21}}{P_{21}} \hat{f}(\omega) \]  
(2.39)

To obtain the time domain response of the material under an applied load, the inverse Fourier transform can be performed. In the two-layered problem, the interfacial stress can be written explicitly as

\[ \hat{\sigma}_n(x_n, \omega) = -zT \hat{f}(\omega) e^{ik_1h_1(1-e^{2ik_2h_2})} \]  
(2.40)

where, \(T = 1 + R, \ R = \frac{1-z}{1+z}, \ z = \frac{z_2}{z_1} \)

It should be noted that \(T\) and \(R\) are the transmission and reflection coefficients at the interface between the substrate and the coating. The expression (2.40) cannot, in general, be inverted in closed form. Direct inversion using the FFT algorithm is not possible due to the fact that the denominator of equation (2.40) vanishes at frequencies associated with the resonance of the plate. In the present problem, it is convenient to expand the expression in the form

\[ \hat{\sigma}(h_1, \omega) = \hat{\delta}(h_1, \omega) [1 + X + X^2 + \cdots] \]  
(2.41)
where,
\[
\hat{s}(h_1, \omega) = \frac{-2Tf(\omega)e^{ik_1h_1(1-e^{2ik_2h_2})}}{1+Re^{2ik_2h_2}}
\]  
(2.42)

and
\[
X = \frac{e^{2ik_1h_1(e^{2ik_2h_2}+R)}}{1+Re^{2ik_2h_2}}
\]  
(2.43)

It can be shown that \(\hat{s}(h_1, \omega)\) given above is the interfacial stress caused by the interaction of only the forward travelling incident waves with the coating i.e., the interaction of the waves reflected from the back surface of the substrate are not included in this expression. This part of the stress can be inverted using the FFT code in MATLAB.

Since no closed form solution for the place with more than 2 layers can be obtained, a MATLAB script is used to solve the set of equations containing the boundary conditions simultaneously to obtain the stresses at the interfaces. Details of the script can be found in Appendix A.

2.3.6 Mechanical Properties of Layers
The mechanical material properties used in the 5-layer analytical model are presented in Table 1. These include the Young's modulus, Poisson's ratio and density which are used to calculate the longitudinal wave speed in the solid. These parameters are only valid for the solid layers: polystyrene and glass slide. The thicknesses of each layer are measured by a micrometer, aside from biofilm layer which was measured using confocal imaging.

Table 2 lists the longitudinal wave speeds calculated using Lame constants and
density of the material shown in equation (2.15) for all materials except water.

<table>
<thead>
<tr>
<th>Material</th>
<th>Thickness [μm]</th>
<th>Density [kg/cm³]</th>
<th>Young’s Modulus [GPa]</th>
<th>Poisson’s ratio</th>
<th>Longitudinal Wave Speed [m/s]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>1,700</td>
<td>1.00</td>
<td>-</td>
<td>-</td>
<td>1481</td>
</tr>
<tr>
<td>Biofilm</td>
<td>28.49</td>
<td>1.00/1.14</td>
<td>-</td>
<td>-</td>
<td>1,481/1540</td>
</tr>
<tr>
<td>Glass Slide</td>
<td>1,000</td>
<td>2.53</td>
<td>77.4</td>
<td>0.22</td>
<td>5,910</td>
</tr>
<tr>
<td>Polystyrene</td>
<td>830</td>
<td>1.004</td>
<td>3</td>
<td>0.40</td>
<td>2,530</td>
</tr>
</tbody>
</table>

Table 1: Material Properties

<table>
<thead>
<tr>
<th>Material</th>
<th>Height [mm]</th>
<th>λ</th>
<th>μ</th>
<th>Density [g/cc]</th>
<th>Wavespeed [mm/μs]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glass</td>
<td>1.00</td>
<td>2.49E+10</td>
<td>3.17E+10</td>
<td>2.530</td>
<td>5.90</td>
</tr>
<tr>
<td>Water</td>
<td>2.00</td>
<td>-</td>
<td>-</td>
<td>1.050</td>
<td>1.50</td>
</tr>
<tr>
<td>Biofilm</td>
<td>0.10</td>
<td>-</td>
<td>-</td>
<td>1.000</td>
<td>1.52</td>
</tr>
<tr>
<td>Polystyrene</td>
<td>0.70</td>
<td>4.29E+09</td>
<td>1.07E+09</td>
<td>1.004</td>
<td>2.53</td>
</tr>
<tr>
<td>Water</td>
<td>100.00</td>
<td>-</td>
<td>-</td>
<td>1.050</td>
<td>1.50</td>
</tr>
</tbody>
</table>

Table 2: Setup parameters

2.4 *Ex vivo* Porcine Tissue

To complete our preliminary studies, we investigated the mechanical effect of LGST on the connective tissue structure of freshly harvested (<4hr post-mortem) porcine skin tissue. Porcine skin has been shown to have similar histological, physiological and immunological properties to human skin and has been suggested as a good analogue for medical and forensic research.
The tissue samples were subjected to LGST, fixed in 10% formalin, embedded in paraffin, sectioned sagittal (4μm thickness), and stained using H&E and Masson’s Trichrome stains. The results are discussed in Section 2.5.

2.5 Previous Results

In this section, the results obtained using the previous LGST system are discussed.

2.5.1 Biofilm Delamination

Glass shockwave generating substrate

Using the experimental setup discussed in Section 2.1 with glass as the shockwave generating substrate, we obtained the following delamination results for biofilm grown on polystyrene surfaces.

In Figure 6, photomicrographs of biofilm samples shocked at different laser fluences are shown. Biofilm in figure 6(a) is the non-shocked control sample. With the exception of the lowest energy sample shown in figure 6(b), all shocked samples show signs of delamination. The delamination seen in figure 6(c) at 200mJ fluence could be due to delamination or another phenomenon called cavitation. Cavitation is the process by which air-bubbles expand and compress due to the pressure field caused by the acoustic waves.

Biofilm delamination was not evident until a threshold laser energy of 300 mJ was used (Figure 6(d)). This corresponds to peak stress amplitude of ~532 MPa that is generated through the glass slide. However, the peak amplitude propagated through the petri dish was only ~45 MPa. This is the stress wave that entered from the biofilm layer
to the petri dish layer where the biofilm was growing. Also, cavitation-induced bubbles are observed which caused localized delamination as observed in figure 6(c).

Figure 6: Photomicrographs of *S. epidermidis* biofilm shocked using glass substrate with various laser fluence (A) control (B) 100 mJ (C) 200 mJ (D) 300 mJ (E) 400 mJ and (F) 500 mJ. Scale bars = 1mm.

Mylar shockwave generating substrate

In the same setup as above but instead of using a rigid glass substrate, single side Al-coated, bi-directionally oriented, polyethylene terephthalate (boPET), more well known
by its trade name, Mylar was used to generate as the shockwave generating material to provide flexible substrate for clinical applications since glass substrates are an impractical material in a clinical setting. *In vitro* biofilm delamination results using Mylar are shown in Figure 7. Given the vast differences in material properties between Mylar and glass, it is reasonable to presume that the shockwaves generated also vary vastly in magnitude.

Another significant consideration that affects the minimum shockwave magnitude for delamination is the surface upon which biofilm is grown. The stiffness of polystyrene surface does poorly to simulate the physical properties of tissue. Therefore, PDMS was used to better emulate the tissue properties. However, the surface phobicity for PDMS was higher than polystyrene and not conducive for biofilm growth. Therefore, PDMS
surfaces were pretreated with O$_2$ plasma treated to temporally increase surface hydrophilicity. More study needs to be done to further understand and better control the surface chemistry.

**Ex vivo Pig Skin**

Upon analysis of the sections by a blinded pathologist, no significant damage to the stratum cornium, epidermis, dermis, or the epidermal-dermal junction was observed (Figure 8). The collagen structure and orientation remains intact with no signs of thermal damage, and no differences appear when compared to the control (Figure 8). It is important to note that the tissue sections show dead cells due to the fact that samples were harvested post-mortem. Additionally, the physiological and inflammatory responses could not be studied. These preliminary histologic studies suggest no evidentiary findings of tissue damage, however, further studies are necessary.

![Figure 8: Micrographs of ex vivo skin samples subjected to LASER-generated shockwaves. (A) Control (B) 264mJ (C) 498mJ. 40x magnification, Scale bar=50 µm.](image)
2.8.3 Interferometry

The interferograms and stress profile plots from seven difference laser fluence levels are constructed here. Figure 9 shows curve-fitted interferogram plots of two energy levels 11 mJ/mm$^2$ & 30 mJ/mm$^2$. The peaks and troughs of the fringes are aligned as closely as possible.

![Waveforms (11 mJ/mm$^2$)](Waveforms_11mJ.png)

![Waveforms (30 mJ/mm$^2$)](Waveforms_30mJ.png)

Figure 9: Glass raw and fitted interferograms at different fluence levels. (a) 11 mJ/mm$^2$ (77 mJ) (b) 30 mJ/mm$^2$ (210 mJ)

![Input Stress Profile for Glass/Ti](Stress_Profile.png)

Figure 10: Glass input stress profiles from interferometry data at seven laser fluences.
possible to ensure goodness of fit.

The peak stresses generated for different fluences range from 120 MPa at 11 mJ/mm$^2$ to 1.4 GPa at 93mJ/mm$^2$.

<table>
<thead>
<tr>
<th>Constants</th>
<th>Energy Fluences [mJ/mm$^2$]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>77mJ</td>
</tr>
<tr>
<td>$\alpha$</td>
<td>8.6</td>
</tr>
<tr>
<td>$\beta$</td>
<td>8.3</td>
</tr>
<tr>
<td>$\gamma$</td>
<td>1150</td>
</tr>
</tbody>
</table>

Table 3: Input stress constants $\alpha$, $\beta$, $\gamma$ from eq. xx derived empirically after curve fitting the raw data.

2.6 Analytical Model

The analytical model generates 1-D stress profiles as a function of time at each interface. As shown in Figure 5, the compressive peak arrives immediately at the glass surface within 8ns. Graphically, we can verify that the rise time of the input stress matches the 6-7ns pulse window of the Nd:YAG laser. The theoretical propagation time was obtained from the calculated wave speeds and known heights of the layers (Table 4).

At the glass/water interface, the first compressive peak arrives at 92ns compared to ~118ns expected theoretically from Table 4. Due to glass/water impedance mismatch a large portion of the wave is reflected back and travels backward in the glass slide. The peak of this waveform is roughly seen after 340ns again at the glass/water interface with
more attenuation.

At the biofilm/polystyrene interface in figure 11, the first compressive peak arrives at 1595ns compared to ~1656ns expected theoretically from Table 4. The interval between the first compressive wave peak and the first tensile wave is 791ns. This value precisely correlates with the amount of propagation time taken for the first compressive wave to propagate forward and then back through the polystyrene layer after being reflected of the polystyrene/water reservoir interface.

Empirically we determined an ideal sampling interval range from 0.2ns to 0.6ns. The model was run for a period of 5μs at a sampling interval or resolution of 0.3ns. The input given to the model for generating the input stress wave were the curve fitting parameters \( \gamma = 5450; \ a = 8.6ns; \ \beta = 8.3ns \) for a 325mJ pulse and the material properties given in Table 1. The stress profile at 325 mJ is used because it is the minimum laser energy that qualitatively caused a delamination.

<table>
<thead>
<tr>
<th>Material</th>
<th>Height mm</th>
<th>Wavespeed mm/μs</th>
<th>Theoretical Prop. Time ns</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glass</td>
<td>1.00</td>
<td>5.90</td>
<td>118</td>
</tr>
<tr>
<td>Water</td>
<td>2.00</td>
<td>1.50</td>
<td>671</td>
</tr>
<tr>
<td>Biofilm</td>
<td>0.025</td>
<td>1.52</td>
<td>67</td>
</tr>
<tr>
<td>Polystyrene</td>
<td>0.70</td>
<td>2.53</td>
<td>395</td>
</tr>
<tr>
<td>Water</td>
<td>100.00</td>
<td>1.50</td>
<td>67069</td>
</tr>
</tbody>
</table>

*Table 4: Theoretical propagation times in different materials*
In the stress profiles generated by our model, multiple compressive and tensile peaks are observed at varying magnitudes. As briefly discussed earlier, we postulate that these peaks arise due to high impedance mismatch at the glass interfaces causing the initial input compressive waveform to become an attenuating pulse train of compressive waves. Their equally spaced time interval of ~340ns is suggestive of this observation.

![Stress history generated at biofilm/polystyrene interface from 1-D analytical model for 11mJ/mm².](image)

**Figure 11: Stress history generated at biofilm/polystyrene interface from 1-D analytical model for 11mJ/mm².**

Also, in figure 11 we observe some artifact initially in the biofilm/polystyrene interfacial stress profile which can be ignored since the first compressive peak does not arrive at the interface until about 1.5μs. Also, worth noting is the fact that these artifacts are not seen in all stress profiles and do not occur under all scenarios.

Varied mechanical properties will lead to varying impedances of the different layers
and will ultimately lead to varied interfacial stress calculations. Most reported mechanical properties, however, have differing loading types and forces are applied at lower strain rates, thereby allowing viscoelastic effects of the biofilm. However, our strain rates are a lot higher (>10^{-6}) and therefore viscoelastic effects can be ignored. Further work needs to be done in determining the material properties of biofilms under high strain rates. Also, our model assumes uniform material properties where biofilms in fact vary not only in composition but also in adhesive and cohesive forces.

2.7 System Limitations

There are several limitations to the current LGST system, primary of which is the inability to effectively cover target areas larger than the beam spot size. Manual target positioning causes inconsistencies like overlapping regions or spacing between adjacent shocked regions, thereby severely limiting treatment efficacy.

Another limitation of the present system is scalability. With the laser beam covering approximately a ~3mm area, real world applications will require hundreds of shots per target. Furthermore, with manual firing in the current system firing rates < 1 shock/sec, make real world applications infeasible.

In addition to the above limitations, the current system employs a Continuum Nd:YAG laser having a power supply with 4ft x 2ft x 2ft dimensions and the laser head itself 3ft x 1ft x 1ft. This creates a large requirement for space and limits the portability of our system. Over the past few decades due to significant technological advances, compact
Nd:YAG lasers such as the Minilite II Oscillator pulsed laser (Continuum lasers, Santa Clara, CA) are capable of providing the portability along with the required laser fluence necessary for generating the shockwaves. This is discussed further in Chapter V.
III. PORTABLE SCANNING SYSTEM: DESIGN AND CONSTRUCTION

The portable scanning system can be sub-divided into subsystems consisting of the laser source, electro-mechanical and optical subsystems. Excluding the laser source, each subsystem design and their individual components will be discussed in depth before finally proceeding to the construction at the end of this chapter.

Figure 12: Venn diagram overview of LGST subsystems and interfaces
3.1 Scanning System Technologies

The term ‘laser scanning’ is referred to as a controlled deflection of laser beams, visible or invisible. Such a broad definition therefore allows for laser scanning applications to be as diverse as the applications of lasers themselves. Scanned laser beams are used in stereo-lithography, rapid prototyping, object scanning, laser engraving, ophthalmological laser systems for surgery, cytometry, in confocal microscopy, laser printers, laser shows, video displays, LIDAR remote sensing, surveying for topological surveys, military applications and barcode scanners for commercial applications.

The technology used for laser scanning can be broken into two categories, one requiring the mechanical motion of mirrored surfaces and the other which involves a spontaneous change in the optical properties of a transparent medium. The latter is
beyond the scope of discussion here.

3.2 Proposed System
Our present system is not portable primarily due to the laser source and cumbersome requiring specialized training to operate. In addition to the open air laser beam propagation, safety during operation becomes paramount and makes it difficult to operate in real world settings. This section discusses the design criteria for a portable, automated system employing a compact ND:YAG 1064nm pulsed laser coupled to a 2d stepper motor-mirror scanner assembly. The pulse from the laser system hits the scanning assembly which directs the beam onto focusing lenses and ultimately the target. This laser beam impinges a thin metallic film coated substrate on top of the target generating a compressive mechanical shockwave travelling away from the system into the target.
The 2-axis mirror assembly will be sequentially triggered by a microcontroller allowing for high precision and a fast response in a stepwise scanning mode rotating the mirrors. The major components of the system include the laser source, unipolar stepper motors for rotation, controller and drivers, mirrors and a condenser (focusing) lens.

A 532nm green laser will be used for alignment and during assembly & testing to provide visual location feedback to user. A block diagram of the system is shown in Figure 13. The full range of rotation for the mirror will be ±20° based on angular geometry. To synchronize the laser and optical scanner, a pulse generator will be required to trigger the microcontroller. The goal set to scan a 30 mm x 30 mm surface area of the wound with 3 mm diameter spot sizes will be < 10 seconds implying a rate of 10 pulses a second. On completion, the system will be calibrated using thermal paper with 1mm² grids.

3.3 Design Requirements

Below are the major functional (technical) requirements of the laser scanning system:

1) The system shall deliver an Nd:YAG (1064nm) laser pulse to a target surface.
   1.1) The beam diameter shall be not less than 1mm and not greater than 3mm.
   1.2) The laser fluence shall not exceed critical threshold energy value of (50mJ/mm²).

2) The system shall scan a maximum rectangular area (50mm x 50mm) across the target surface.

3) The system shall align the spots such that overlapping region or the spacing between
two spots on the target is less than 1mm.

4) The system shall scan the laser beam within a predefined rectangular area on the target.

5) The system shall deliver only a single laser pulse for each incremental motor step

6) The internal repetition rate on the laser source shall match the repetition rate on the scanning system.

7) The scanning system shall operate at a repetition rate between 10 Hz – 20 Hz.

8) The scanning platform shall not exceed a weight of 10kgs or dimensions of 50cm x 50cm x 50cm.

### 3.4 Design

System design can be broken up into two subsystem assemblies: the electro-mechanical and the optical subsystems. We consider each subsystem independently while developing the design criteria obtained from the requirements discussed in the previous section.

#### 3.4.1 Electro-Mechanical Subsystem

The Electrical subsystem assembly consists of a PC, 2 stepper motors, 1 controller and 2 driver circuits. The PC is used to program the controller via DB9-pin RS232 port using MATLAB® software. The RS232 settings used to communicate with the controller are; Baud rate 9600 kbps, 1 Start Bit, 1 Stop Bit, No Parity, Flow Control none, Terminator ‘CR’. The controller converts commands received via RS232 serial cable
from the PC to two TTL (5V) signals; step and direction. These signals feed into the
driver circuit as inputs. Each terminal from the windings in the motor are connected to
the driver circuit. The controller and driver circuit boards use 12 VDC supply whereas
each motor requires a 5VDC power supply with at least a 1A current rating.

**Stepper Motors**

Stepper motors usually have all windings in the motor are part of the stator, and the
rotor is a permanent magnet. They can be viewed as electric motors without commutators
which reverse current flow periodically in individual armature coils in order to maintain
unidirectional torque as the armature coils move under alternate field poles. Therefore, all
of the electric commutation must be handled externally by the motor controller. This
allows the motor rotor to be held in any fixed position as well as reverse the direction of
rotation. The four coils of a unipolar stepper motor are individually activated and
deactivated sequentially and each time this is done the motor advances a step. Due to
their design, steppers can start and stop spin quickly at controlled orientations.

Between using servomotors and stepping motors. Both types of motors offer similar
opportunities for precise positioning, but differ in a number of ways. Servomotors require
analog feedback control systems of some type. Typically, this involves a potentiometer to
provide feedback about the rotor position, and circuitry to drive a current through the
motor inversely proportional to the difference between the desired position and the
current position. Stepping motors can be used in simple open-loop control systems, most
suited for systems that operate at low accelerations with static loads, but closed loop
control may be essential for high accelerations, particularly if they involve variable loads.
If a stepper in an open-loop control system is over torqued, all knowledge of rotor position is lost and the system must be reinitialized.

Finally, position repeatability for a stepper motor depends on the geometry of the motor rotor, whereas for a servomotor, it generally depends on the stability of the potentiometer and other analog components in the feedback circuit. Given these considerations and their factor on cost, a unipolar stepper motor in open loop was used for this application.

**Motor Mounts**
The initial motor mount required a permanent adhesive to hold the lens. This flaw became a problem when more expensive Nd:YAG mirrors were required for multiple applications.

Therefore the motor mount was redesigned to allow for mirrors to be removable after use. Figure 17, shows a CAD model of the portable scanning system.

Controller Circuit

The controller circuit accepts commands from PC via serial communication and converts them into step and direction TTL signals for the driver circuit. The microcontroller was preprogrammed with commands some of which are shown in Table 5. The dimension of the controller are 78Wx92Lx25H mm.
Figure 17: Preliminary CAD assembly of scanning platform

Figure 18: Controller circuit

Driver Circuit
The Driver circuit (figure 19) accepts Transistor-transistor logic (TTL) inputs (0 - 5V) as step, direction signals from the controller (Figure 18) to drive the stepper motor in PC-controlled mode. A TTL input signal is defined as "low" when between 0 V and 0.8 V with respect to the ground terminal, and "high" when between 2.2 V and 5 V. In free-standing mode, a square-wave oscillator timing pulse generated by IC 4093 is supplied to the OSC output. The frequency of the pulse is controlled via trim pot VR1 and the maximum frequency is set by the 1K ohm resistor in series. IC 4030 inverts the outputs available at Q and Q' which in turn switches the MOSFETs (IRFZ44) on and off in sequence. IRFZ44’s have a low on-resistance and can deliver up to 6A each without a heat sink. Power to the stepper motor is connected to V+ and GND terminals as shown on the overlay (Figure). The ICs are powered at 12V connected to KITV. The free-standing and PC-controlled modes can be set by toggling the SPDT switch from INTERNAL to EXTERNAL. The dimension of the driver are 50Wx75Lx25H mm.

3.4.2 OPTICAL SUBSYSTEM
In this section, we will look at the design considerations for optical alignment and setup. We can classify the optical elements functionally into 3 sets: fixed mirrors for steering, motor-mounted mirrors for scanning, and condenser lenses to focus the beam onto the target.

**Fixed mirrors**

From the laser aperture, the beam is steered onto the scanning platform via fixed table mounted 1 inch coated YAG mirrors. These mirrors are not considered part of the scanning platform. However, they are necessary to deliver the beam from the laser source to the scanning mirror 1 (shown in Figure). These mirrors are routinely checked for alignment before using the laser to ensure the mirrors are aligned and the beam fluence is unaffected.

**Motor-mounted mirrors**

Also referred to as scanning mirrors, these mirrors M1 and M2 are mounted on orthogonal stepper motor rotors discussed previously and are the main components of the scanning platform. Henceforth, we classify M1 rotation providing linear translation in X direction and M2 in Y direction. Each mirror rotation allows linear beam translation in the above orthogonal directions X and Y.

An important criterion while designing the optical setup is to ensure that the laser beam does not skid off due to mirror rotation. To meet this design requirement, basic trigonometric calculations were carried out to calculate the maximum rotation possible before the image skids off the mirror. This constraint limits the total length scanned in the X direction and creates the rectangular shape of the target region rather than a square.
Skidding occurs when the source is rotating. Since the laser source remains stationary, the incident beam on M1 is fixed. Therefore beam skidding does not apply to M1. To determine the beam translation on M2, consider Figure x. An assumption is the centers of the two mirrors are in a straight line. Looking top-down, a right angle triangle is formed between the distance in between the two mirrors and the width of M2. Controlling the distance between the two mirrors and given the diameter of M2, it is possible to determine the half angle (i.e. half the angular rotation of M1).

Figure x illustrates that the tilt of M2 does not impact the distance translated orthogonally across M2.

Beam translation on M2 is dependent only on the rotation of M1 and the distance between the two mirrors ($L_1$). It does not depend on the angular rotation of not M2. Distance $L_1$ is fixed due to design of the motor mount and is fixed at 50mm.
Compound condenser lens system

After steering the beam using the scanning mirrors, the beam is focused onto the target by a compound condenser lens arrangement. Two BK7 plano-convex condenser lenses with the same specifications (FL=200mm, DIA=100mm, R=xx) are coupled together to reduce the equivalent focal length of the assembly. This is shown by using the thin lens approximation below,

$$\frac{1}{f_1} + \frac{1}{f_2} = \frac{1}{f_{eq}} = \frac{1}{8} + \frac{1}{8} = \frac{1}{4}$$

(3.1)

Therefore, the new effective focal length is $f_{eq} = 4$ inches.

These lenses with a 100mm diameter were selected due to a system design consideration to maximize the target size. In the next chapter, it will be shown that this is indeed the case.

It is useful to note that the reason to focus the beam after, and not prior to the scanning mirror assembly is twofold. First, the light rays after steering the laser beam are diverging and focusing them before they reach the target will cause them to diverge again. Second, by focusing the beam onto the scanning mirrors, the energy fluence on the mirrors is drastically increased and may go beyond the 2J/mm$^2$ fluence threshold of the mirrors.

Ray Optics

It is necessary to consider ray optics while designing the optical system to understand the effect of placing the mirrors within the focal length of the compound lens vs outside it on the image formed at the target.
There are two scales on which ray optics will assist in designing the system.

Rays within a single laser beam

Assuming the rays within the laser beam are collimated (planar) i.e. the source is infinity, upon refracting on each lens surface, the rays are focused onto the equivalent focal plane of the compound lens (at FL=100mm).

Multi-beam path while motor is scanning

To utilize the principle of ray optics to understand the beam pathway as the scanning motors are moving, the following assumption is made. Each beam is considered as a ray from a point source. The point source is assumed to be the reflected beam image on mirror M2 which is positioned such that it lies at the (focal point) on the principle axis of the compound lens. This allows us to predict the following

When the reflected beam is orthogonal along the principle axis, the beam path is unaltered. In all other cases, when the incident beam is at an angle, after passing through the lenses the beam travels parallel to the principle axis. Therefore, we can conclude that for every angular rotation of the motor, the resulting beam hitting the target surface at the focal plane is orthogonal to the target and parallel to the principle axis.

3.5 Construction

3.5.1 Assembling Electrical Components

Once the circuit assembly was complete, each board was tested independently to look for connection errors. The motors were connected to each driver via terminal ports M1A, M1B, V+, M2A, M2B, V+. The motors were connected to 5 VDC power supply with a maximum current rating of 6A.
The controller STEP, DIRECTION and GROUND inputs (S1, S2, D1, D2) were connected to each driver input respectively. All circuits (Controller and driver) were powered at 12 VDC.

The PC was connected to the controller via 9 pin female RS232 connector. The RS232 connection was configured to 9600 Baudrate, no parity, 1 start and 1 stop byte and Carrier Return (CR) delimiter.

<table>
<thead>
<tr>
<th>Command</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>POSN</td>
<td>Set the position that motor AA is currently at to be XXXX where XXXX is between -99,999,999 and 99,999,999</td>
</tr>
<tr>
<td>PSTT</td>
<td>Returns the position of motor AA</td>
</tr>
<tr>
<td>AMOV</td>
<td>Move motor AA to the absolute position XXXX where XXXX is between -99,999,999 and 99,999,999</td>
</tr>
<tr>
<td>RMOV</td>
<td>Move motor AA relatively from the current position by XXXX where XXXX is between -99,999,999 and 99,999,999</td>
</tr>
<tr>
<td>STOP</td>
<td>Stop motor AA immediately</td>
</tr>
<tr>
<td>DRON</td>
<td>Turn Direction output (D1-D4) associated with AA on for XXXX * 0.131mS. If XXXX is -1 output will be on until a ‘DROF’ command is received</td>
</tr>
<tr>
<td>DROF</td>
<td>Turn Direction output (D1-D4) associated with AA off immediately from ‘DRON’Command</td>
</tr>
<tr>
<td>SAVE</td>
<td>Saves RATE, ACCI, ACCN, ACCF and OPTN parameters to EEPROM, which are then automatically loaded on the next power up. Valid addresses are 01,05,09 and 13.</td>
</tr>
</tbody>
</table>

Table 5: List of preprogrammed commands for controller

3.5.2 Machining Parts

Mirror Mounts

For machining the 2nd generation mirror mounts, 1 1/2 inch diameter aluminum rods were used as raw stock material. In a lathe, the rods were drilled to create a quarter inch deep blind center hole as shown in Figure. After drilling the pilot hole, material was
extruded using a 1/4, 1/2, 3/4, 7/8 inch drills sequentially. Finally, using a boring tool the hole was sized to an inch in diameter. During the entire process, a caliper was frequently used to measure the width and the depth of the hole. An Nd:YAG reflective mirror was used to verify proper fit.

Without removing the part from the lathe, the exterior surface of the part was faced off to 1 ½ inch outer diameter. This was followed by a rough cut to size the rod into 1 inch piece using a band saw. The 1 inch piece was reloaded onto the lathe and faced off to precisely an inch in thickness. A 5mm (0.197 in) shaft was drilled using a drill press radially. A 6-32 x ½ inch screw was used to secure the part onto the motor. Also, a hole was drilled radially to secure the mirror into the cavity using a 4-40 x ¼ inch set screw.

Motor Mounts

A ¼ inch plate was cut into rectangular section 2in x 3in. Next, M3 size through holes were drilled precisely (1.22 inches) apart in a square pattern using an edge finder on an end mill. The 1.22 inches separation distance is based on the motor specifications drawing in Figure 14.
Figure 21: The portable scanning system after construction and assembly.
3.6 Optical Alignment

Laser alignment is a critical component for optimal functioning of any optical system. The system was aligned using visible and infrared lasers. During construction and assembly, a visible laser was used for system alignment to facilitate construction, testing and ensure safety. Due to the open air design, optical elements can be sensitive to slight disturbances altering optical alignment and causing sub-optimal performance. Therefore, the optical alignment process was performed before each experiment to ensure proper system functionality.

Visible Laser

A visible green (532nm) diode laser was used initially to align the optical elements which include table-mounted fixed mirrors, beam steering motor-mounted mirrors and
the fixed focusing lenses. Alignment was broken down into several ‘segments’ beginning with aligning the laser source and next subsequent optical element.

![Figure 23: Aligning Nd:YAG infrared laser beam using thermal paper.](image)

The degrees of freedom for each optical element were considered during alignment to optimize the process. The optical elements were adjusted to ensure that laser beam was in the center. Alignment was verified visually using a flat piece of paper. Using a visible laser, operator safety was maximized to ensure the infrared (invisible) beam path of Nd:YAG laser is well controlled.

Nd:YAG Alignment

Because the Nd:YAG beam is not visible to the naked eye, aligning infrared lasers possess inherent complexity in comparison to aligning visible lasers. In addition, due to
safety concerns aligning mirrors while Nd:YAG is firing in continuous pulse mode is not possible. Therefore, the only safe approach is to align the Nd:YAG in single pulse mode using thermal paper which ablates upon impingement of laser beam, visually providing location of the beam shown in Figure 23.

**Scanning Logic**

Matlab software was used to communicate with the serial controller to issue motor commands. Once system construction was completed, the visible laser was used to provide location feedback while developing the scanning logic. Pattern shown in Figure 24 are analyzed for efficiency. The algorithm written to scan this pattern was written in Matlab (shown in Appendix).

![Figure 24: Scanning pattern used by the scanning platform to cover a rectangular target region](image)
IV. PORTABLE SCANNING SYSTEM: EXPERIMENTS

In this chapter, test procedures and results for the scanning system constructed previously are discussed. These tests verify and validate the system’s functionality and also characterize the system using novel performance criteria defined here. The tests can be broken into two categories based on the type of laser used in the test case. Two types of lasers are used, 532nm green HeNe visible diode laser and 1064nm infrared Nd:YAG laser.

While the high powered YAG laser is required for generating shockwaves during functional testing, the visible HeNe laser is used as for alignment during design, construction and early testing phase.

The primary advantage of HeNe alignment laser is safety. Especially, when system functionality is not tested and malfunctions are more likely. Other advantages of using a HeNe is that you get immediate feedback which is valuable during design and construction while working on fine tuning motor control. Finally, a disadvantage with using the HeNe is lack of precision and accuracy since marking a spot manually introduces human errors.
On the other hand, using thermal paper to detect the YAG laser pulse is more precise and accurate.

### 4.1 Initial electronics testing

The first set of experiments performed after system construction and assembly are to ensure proper functioning of the electronic circuits. To achieve this, each motor circuit was tested individually by sending commands via the controller from the laptop and an internal clock command on the driver circuits. First, the SPDT switch was set to ‘int’ mode to use the internal clock on A##5 IC in driver circuit. The motor began stepping in one direction, 0.9° per step and completed a single revolution in 400 steps at (frequency Hz). Successfully completing this test establishes that the motors are receiving 4-5V from the power supply and also that the driver circuits are functioning. Using a multimeter, the voltages fed into the motor terminals were recorded between V+ and V_gnd. Following this, the next step was to connect a laptop via serial RS-232 interface to motor controller circuit. Using Matlab, the following code was used to communicate serially to the motors.

```matlab
s=serial('COM6'); #define serial port object
set(s, 'terminator', 'CR'); #set carriage return (CR) terminator
(default Line feed)
set(s, 'baudrate', 9600); # set Baudrate 9600bps
```
Using the listed in manufacturer’s controller manual (Refer Table 5), commands were issued to the microcontroller via RS 232 serial interface. These commands are,

\[
\begin{align*}
\text{fprintf(s, '@01 POSN 0CR');} & \quad \text{# Set current position of motor 1 to 0} \\
\text{fprintf(s, '@01 DRON -1CR');} & \quad \text{# Set direction of motor 1 clockwise} \\
\text{fprintf(s, '@01 DROFF');} & \quad \text{# Reset direction of motor 1} \\
\text{fprintf(s, '@01 RMOV 1 nCR');} & \quad \text{# Move motor 1 by 1 step relative to its current position. Also, n implies no command for motor 2.} \\
\text{fprintf(s, '@01 RMOV n 10CR');} & \quad \text{# Move motor 1 by 0 steps and increment motor 2 by 10 steps relative to its current position.} \\
\text{fprintf(s, '@01 AMOV 0 CR');} & \quad \text{# Absolute move motor 1 to position 0}
\end{align*}
\]

Completing this test ensures, the serial connection is established between the computer and the microcontroller.

Executing the following m file which consist of a basic for loop makes the motors move linearly from position A to point B and return back to position A. Issues commands to only one of the motors.

The following m file consists of two for loops one nested in the other and executing it moves the both motors linearly. This covers a rectangular area across the target.

4.2 Visible laser experiments
4.2.1 Interspacing between two spots

Initially using the visible laser in an incremental fashion the visible spot on the target was traced by hand. The primary goal here was to demonstrate the maximum area that can be covered across the target. This method introduces human error and is not precise but it was a necessary intermediary step required to verify/validate the performance before moving to the next level.

Figure 25: Visible laser pattern traced by hand
Using ImageJ, the average distance between horizontal spots is 3.6 mm and vertical spots is 2.9 mm. The total length scanned across the X and Y directions was not the same because in the vertical direction the width of mirror M2 limits the beam translation. As discussed earlier, this was due to design limitations in selecting Mirror M2.

### 4.2.2 Interspacing as a function of object distance
Establish that the beam reflection on the second mirror is on the principle axis of the condenser lenses. Assume both condenser lenses are in parallel and share a common principle axis. Interspacing does not vary as a function of object distance since it is a function of the angular step of the motors which is constant.

### 4.3 Nd:YAG Experiments

#### 4.3.1 Check for Accuracy & precision
**Accuracy**

To check for line accuracy, a series of shots fired on thermal paper were analyzed in
IMAGEJ by using the distance away from a reference line and comparing this difference across all the shots. It was observed that there was a minor deviation away from the reference line towards the edges.

![Image](image.png)

**Figure 26: Accuracy of shots fired in a straight line**

**Precision**

The scanning system precision is tested under two scenarios. First, to test precision while power to motors is maintained and second, after recycling the power. To test if the motors moved when the coils are initially charged. In the first scenario, the system performed as expected. However, after recycling power, the precision decreased.

**4.3.2 Alignment calibration to optimize beam pattern**

Aligning an infrared laser is more challenging than a visible laser primarily because a visible laser can be left on while alignment thereby providing immediate feedback to align the optics. For infrared lasers, thermal paper provides the necessary feedback to align optics. Nonetheless, applying this technique is tedious, difficult and requires experience to be efficient. In addition, infrared beams cannot be turned on during aligning for obvious safety reasons. Laser alignment requires to be checked each time before using...
the system even if left untouched.

4.3.3 Spot size vs. target distance from focusing lens

For this experiment, the goal was to understand the relationship between the distance of target plane from lens and the laser beam spot size variation on thermal paper. The target plate mounted on a rail was varied in distance away from the focusing assembly starting from 1 inch. The corresponding distance from the ground was recorded using a caliper. Data provided in table xx. Due to physical restrictions the furthest distance the target plane could be varied was 93 mm. This maximum distance corresponded to the thickness of the target mount. From Figure 27, spots labeled 1 through 8 were recorded with spot 1 corresponding to the laser spot generated when target plane was 24 mm (smallest).

Figure 27: Beam spot size decreases as a function of height from 25 mm to 99 mm (Focal plane at 101.6 mm)
From the graph in Figure 28, an inverse trend is observed between increase in the distance and a decrease in laser beam spot size suggesting the target is within the focal length of the compound converging lens assembly. At the largest distance of 93mm apart, the spot size was just about 1mm². Within this distance, the laser beam can be considered converging onto the target.
4.3.4 Asynchronous between scanning platform and laser

The portable scanning system has been used with three lasers. First, the visible HeNe 632nm laser during assembly. Second the infrared Nd:YAG laser 1064nm (Continuum). Third, a portable compact Nd:YAG laser 1064nm (Quantel).

A considerable amount of time was spent to attempt to connect the Continnum laser serially via laptop. After ruling out several options with Continnum technical support, the serial host object on the laser was deemed inoperable and synchronizing the laser with the portable scanning system was abandoned. This created the issue of asynchronicity between the scanning platform and the laser and introduced a new variable frequency. There are three cases possible: First, both equipment (laser and scanning platform) run at 10 Hz.

Out of four cases possible, only three were pursued. Second, the laser is set at a higher
frequency than the scanner. Third, the laser is set at a lower frequency than the scanner. In the second case, multiple laser shots are fired at the same location while in the third case, less than one shot can be fired at a location. Ultimately, we found that firing the laser at a slightly slower frequency than the scanner gave the best result. The scanner ran at 10 Hertz while the laser was run at 7 Hertz.

Figure 29: Illustration shows the diameter of the beam on target as a function of the distance
4.3.5 Generate and analyze patterns generated on thermal paper
Before we can use the system on biological media, the final set of experiments include analyzing system performance on thermal paper. The scanning pattern generated is shown in Figure 30. At the fringes, the spots are not aligned due to the asynchronously between the laser and the scanning system. Also, since the motors are not in a feedback loop, the motor may be in motion as the laser beam is fired causing location discrepancies.
Figure 30: Asynchronous scanned pattern at 7Hz with target plane within focal length
V. FUTURE IMPROVEMENTS & CONCLUDING REMARKS

A proof of concept for a portable scanning system to replace the current laboratory bench top laser-generated shockwave system has been designed, assembled and tested. In this chapter, system improvements and future research directions are discussed.

Several features of this system can be improved and more experiments performed in future studies.

A primary improvement for the scanning system is enabling communication between the platform and laser source to allow synchronous operations via PC. This would improve system performance at the edges of the target region. Additional functionality could include scanning irregular target regions. This functionality would improve the efficacy of the scanning system for real world applications. There are potentially several different approaches to implement this functionality, one of which could be by improving the scanning control logic such that a user can input an image of the irregular target region upon which MATLAB can be used to determine a number of grids and

The next set of studies should include in vitro & in vivo biofilm delamination
experiments. *In vitro* studies on *Staphylococcus epidermidis* biofilms grown under static conditions in petri dishes should be a precursor to *in vivo* studies in animals.

Other improvements could include increasing system precision with geared motors for higher accuracy and speed. This would be beneficial in increasing the scanning system’s efficacy by decreasing the spacing between two positions.
Appendix A: Bacterial Culture Preparation Protocol

Sample Preparation: Bacterial Stock

S. epidermidis (ATCC #35984, Designation: RP62A) is used as the bacterial sample for its ability to produce the polysaccharides adhesive. A bacterial raw sample is delivered by the American Type Culture Collection ("ATTC") in freeze-dried form. It is necessary to develop vials of bacterial stock that can be stored for years in a deep freezer (-80°C) in a glycerol solution. If the cells were directly stored in water, the crystallization of the water can pierce and damage the bacterial cell wall and effectively killing them. The procedure to develop a stock of bacteria is as follows:

1. Prepare food medium by placing 30 g Tryptic Soy Broth (TSB; BD Bacto # 211825) into 1000 mL of deionized (DI) water and autoclave at 121°C
2. Place 40 mL of TSB into a 50 mL conical centrifuge tube (BD Falcon #352070)
3. Use a pipette tip to scrape off frozen bacteria from the sample delivered by ATTC into the 50 mL test tube with TSB
4. Vortex to evenly mix then place into an incubator (37°C & 5% CO2) for 24 hrs.
5. After overnight growth, the 50 mL test tube is centrifuged at 3000 rpm for 15 minutes at room temperature to pellet the bacteria
6. Discard all supernatant and re-suspend the pelleted cells with 10 mL sterile DI water
7. Aliquot 500 uL of sterile 30% glycerol solution in water to 1:7 mL microcentrifuge tubes (Eppendorf #022431081)

8. Aliquot 500 ul of overnight stock into micro centrifuge tubes

9. Vortex and store in cryogenic freezer (-80 C)

Sample Preparation Protocol

Biofilm growth on polystyrene petridish from the stored bacterial stock, a vial is taken and a pipette tip is used to scrape off some of the ice into a 50 mL test tube containing 40 mL of TSB. The vial must not be thawed as a freeze-thaw cycle could potentially kill the cells. The test tube is then placed in an incubator at standard conditions for 24 hours in order to increase cell population. It is then important to know the concentration of bacterial after an overall growth. A spectrometer is used to measure the optical density of a sampled suspension at a wavelength of 600 nm (Biocompare Ultrospec 10 Cell Density Meter). The overnight suspension is then diluted if necessary to an OD600 nm = 0.2 corresponding to a cell density of 4.77x 10^7 CFU/mL. Then, 5 mL of the stock solution was aspirated from the stock into 100 mm x 15 mm polystyrene petri dishes and allowed to grow in an incubator chamber (37 C & 5% CO2) for 24 hours under static conditions.
Appendix B: Wave Propagation Analytical Solution

clc; close all; clear all;
N=5; % Number of layers
c1=5909.947; c2=1481; c3=1540; c4=2530.47; c5=1481; %Wavespeed from EXCEL SPREADSHEET
rho1=2530; rho2=1000; rho3=1140; rho4=1004; rho5=1000;
h1=1e-3; h2=2e-3; h3=0.1e-3; h4=1e-3; h5=100e-3;
Q=1000; % for damping, 100-1000, to remove singularity
for m=1:N
    if (mod(m,5)==0)
        rho(m)=rho5;
        h(m)=h5;
        c(m)=c5;
    elseif (mod(m,5)==4)
        rho(m)=rho4;
        h(m)=h4;
        c(m)=c4;
    elseif (mod(m,5)==3)
        rho(m)=rho3;
        h(m)=h3;
        c(m)=c3;
    elseif (mod(m,5)==2)
        rho(m)=rho2;
        h(m)=h2;
        c(m)=c2;
    elseif (mod(m,5)==1)
        rho(m)=rho1;
        h(m)=h1;
        c(m)=c1;
    end
    z(m)=rho(m)*c(m);
end
T=10e-6;
NP=2^14;
dt=T/NP;
t=0:dt:T;
p0=5450; %for 325mJ 5450 %for 210mJ 4000
b0=8.6;
c0=8.3;
p=0.5*rho1*c1*p0*(exp(-t*10^9/b0)-exp(-t*10^9/c0));
W=2*pi/(T)*(0:NP/2); %Frequency
FP=fft(p,NP); %Fourier transform of source

Pn=inline('[cosh(i*kn*hn) -sinh(i*kn*hn)/(i*w*zn); -i*w*zn*sinh(i*kn*hn) cosh(i*kn*hn)]');
for m=1:NP/2+1
    P=eye(2);
    w=W(m);
    if (w~=0)
        for n=1:N
            Pn=
        end
    end
end
kn=w/c(n)*(1+0.5*i/Q);
hn=h(n);
zn=z(n);
P=P*Pn(kn,hn,w,zn); %P=P(1)P(2)...P(N)
end
if (w==0)
  u(1,m)=0;
u(N+1,m)=0;
else
  u(1,m)=-P(1,1)/P(2,1);
u(N+1,m)=-1/P(2,1);
end
v(1,m)=-i*w*u(1,m);
s(1,m)=-1.0;
v(N+1,m)=-i*w*u(N+1,m);
s(N+1,m)=0.0;
if (w~==0)
  for n=2:N
    D=eye(2);
    for k=n:N
      kn=w/c(k)*(1+0.5*i/Q);
      hn=h(k);
      zn=z(k);
      D=D*Pn(kn,hn,w,zn); %D=P(n+1)P(n+2)...P(N)
    end
    u(n,m)=-D(1,1)/P(2,1);
v(n,m)=-i*w*u(n,m);
s(n,m)=-D(2,1)/P(2,1);
  end
else
  for n=2:N
    u(n,m)=0.0;
v(n,m)=0.0;
s(n,m)=0.0;
  end
end
for k=1:N+1
  for m=NP:-1:NP/2+2
    u(k,m)=conj(u(k,NP-m+2));
v(k,m)=conj(v(k,NP-m+2));
s(k,m)=conj(s(k,NP-m+2));
  end
end
u=conj(u);
v=conj(v);
s=conj(s);
for k=1:N+1
for m=1:NP
    uu(k,m)=u(k,m)*FP(m);
    vv(k,m)=v(k,m)*FP(m);
    ss(k,m)=s(k,m)*FP(m);
end
end
tt=dt*(0:NP/2);
for k=1:N+1
    ut(k,:)=ifft(uu(k,:),NP);
    vt(k,:)=ifft(vv(k,:),NP);
    st(k,:)=ifft(ss(k,:),NP);
    figure(3*k+1);
    time=tt(1:NP/2+1);
    stress=st(k,1:NP/2+1);
    plot(time*10^9,stress*10^-6,:k','LineWidth',2)
    grid on;
    xlim([0 10^9*T/2])
    xlabel('Time(\mus)');
    if k==1
        ylabel('Stress at glass surface (MPa)');
    elseif k==2
        ylabel('Stress glass/water interface (MPa)');
    elseif k==3
        ylabel('Stress water/biofilm interface (MPa)');
    elseif k==4
        ylabel('Stress at Biofilm/Polystyrene Interface (MPa)');
    elseif k==5
        ylabel('Stress at Polystyrene/Water Reservoir Interface (MPa)');
    elseif k==6
        ylabel('Stress at bottom of Water Reservoir surface (MPa)');
    end
end
Appendix C: Scanning Logic

Main Function

```matlab
s=serial('COM7');
fopen(s);
fscanf(s);
set(s,'Terminator','CR');
pause(0.5);
fprintf(s, '@01 POSN 0CR');
fprintf(s, '@02 POSN 0CR');

fprintf(s, '@01 DRON -1CR');
fprintf(s, '@02 DRON -1CR');

for j = 1:20
    if j~=1
        fprintf(s, '@01 RMOV 1 nCR');
    end
    Motor2_forward(s,8);
    beep;
    fprintf(s, '@01 RMOV 1 nCR');
    Motor2_backward(s,8);
    beep;
    j=j+1;
end
Motor1_backward(s,20);
beep;
beep;
```

Subfunction 1: Motor1_forward

```matlab
function [i] = Motor1_forward(s,n)

fprintf(s,'@01 DRON -1CR');
for i = 1:n
    fprintf(s, '@01 RMOV 1 nCR');
    pause(0.125);
    i=i+1;
end
```

Subfunction 2: Motor1_backward

```matlab
function [i] = Motor1_backward(s,n)

fprintf(s,'@01 DROFF');
for i = 1:n
    fprintf(s, '@01 RMOV 1 nCR');
```
pause(0.125);
i=i+1;
end

Subfunction 3: Motor2_forward

function [i] = Motor2_forward(s,n)

fprintf(s,'@02 DRON -1CR');
for i = 1:n
    fprintf(s,'@01 RMOV n 1CR');
    pause(0.125);
i=i+1;
end

Subfunction 4: Motor2_backward

function [i] = Motor2_backward(s,n)

fprintf(s,'@02 DROFF');
for i = 1:n
    fprintf(s,'@01 RMOV n 1CR');
    pause(0.125);
i=i+1;
end
**Appendix D: Histology**

<table>
<thead>
<tr>
<th>Control Sample</th>
<th>Shocked Sample (415 mJ)</th>
</tr>
</thead>
</table>

[Images of histology samples]
VII. REFERENCES


