SIMULATIONS FOR INVESTIGATING THE CONTRAST MECHANISM OF BIOLOGICAL CELLS WITH HIGH FREQUENCY SCANNING ACOUSTIC MICROSCOPY

A Dissertation in
Acoustics
by
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ABSTRACT

Scanning Acoustic Microscopy (SAM) is one of the most powerful techniques for nondestructive evaluation and it is a promising tool for characterizing the elastic properties of biological tissues/cells. Exploring a single cell is important since there is a connection between single cell biomechanics and human cancer. Scanning acoustic microscopy (SAM) has been accepted and extensively utilized for acoustical cellular and tissue imaging including measurements of the mechanical and elastic properties of biological specimens. SAM provides superb advantages in that it is non-invasive, can measure mechanical properties of biological cells or tissues, and fixation/chemical staining is not necessary. The first objective of this research is to develop a program for simulating the images and contrast mechanism obtained by high-frequency SAM. Computer simulation algorithms based on Matlab® were built for simulating the images and contrast mechanisms. The mechanical properties of HeLa and MCF-7 cells were computed from the measurement data of the output signal amplitude as a function of distance from the focal planes of the acoustics lens which is known as V(z). Algorithms for simulating V(z) responses involved the calculation of the reflectance function and were created based on ray theory and wave theory. The second objective is to design transducer arrays for SAM. Theoretical simulations based on Field II© programs of the high frequency ultrasound array designs were performed to enhance image resolution and volumetric imaging capabilities. Phased array beam forming and dynamic apodization and focusing were employed in the simulations. The new transducer array design will be state-of-the-art in improving the performance of SAM by electronic scanning and potentially providing a 4-D image of the specimen.
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Chapter 1

Introduction

1.1 Background and motivation

According to previous research, time-resolved scanning acoustic microscopy has been used to characterize biological tissue, which mainly consists of a collection of single cells in a cellular matrix, and to investigate the mechanical properties of cells in a sub-cellular level. Furthermore, recent research has revealed that several cell mechanisms are controlled by mechanical properties of cytoplasm such as cell division, cell adhesion, cell differentiation, cell volume regulation, apoptosis and proliferation [3]. In addition, there is a direct connection between single-cell biomechanics and human cancer [5] and there is a subtle interaction among cell shape, invasiveness of tumor cells, cytoskeleton organization and gene expression [5]. Therefore it is important to explore a single cell base and assess its mechanical properties.

Unlike other techniques that have been used for investigation of the mechanical properties of biological cells, such as optical tweezers, laser tweezers, and atomic force microscopy, it has been shown that acoustic microscopy provides information on the mechanical properties of a cell’s interior structures including cytoskeleton, cytoplasm, nucleus and not just those of the cell membrane [5]. Moreover, acoustic microscopy is considered a minimal invasive technique since its operating frequencies are in the GHz range and also yields excellent spatial resolution [3]. Time-resolved acoustic microscopy is an ideal technique that allows all necessary parameters of a cell to be measured including cell thickness, cell density, sound velocity, and sound attenuation [3] making it a powerful tool in biomedical research and applications for detecting abnormalities of cancer cells or other diseased cells [2]. Previous studies have shown that among most cancers,
cervical cancer is the third most common cancer in women worldwide and also is the leading cause of cancer mortality in women in developing countries. Early detection of cervical cancer is still a critical step in treatment.

The purpose of this research is to develop a computer simulation program for high-frequency time-resolved acoustic microscopy that will be able to determine the mechanical properties of a single cell with sub-micrometer resolution. This program has enormous potential for biomedical research in the early detection of cervical cancer and future study on its dynamic processes. In the simulations, a single cell model will be constructed based on the physiology of the HeLa cell, a cell line derived from human cervix carcinoma [74]. It has been shown that HeLa cells are among the best studied, characterized cancer cell line. In order to optimize and enhance the time-resolved scanning acoustic microscopy analysis, different specifications will be tested, such as the number of array elements, and the focal properties and resolutions of linear array and phased array. The simulation program is based on the FIELD II Ultrasound Simulation Program.

1.2 Specific aims and methodology used

The objectives of this research are as follows:

1. To develop a program for simulating the images and contrast mechanisms obtained by high-frequency SAM. We will focus on the mechanical properties of HeLa cells, which can be analyzed from the V (z) response which depicts the output signal amplitude from the specimen being tested as a function of distance from the focal planes of the acoustics lens. Two new algorithms for simulating V (z) responses are constructed based on Ray theory and Wave theory (angular spectrum) and will involve calculations of the reflectance function for coupling the medium/cell/substrate system.
In this study, the RF data obtained by time-resolved scanning acoustic microscopy measurements were used to estimate material properties, calculate the reflectance function of the system, then simulate the V(z) curve, and compare to published V(z) measurements. The V(z) curve also allows the calculation of the Rayleigh wave speed and its attenuation. The V(z) simulation done in this study proved the hypothesis that the Rayleigh wave speed can be calculated from the V(z) curve of each material being tested. The V(z) curve pattern with its period and the reflectance function are also distinctive for each type of material being tested.

2. To design transducer arrays for SAM. 1-D and 2-D linear array and phased arrays will be simulated using Field II® to analyze image resolution as well as volumetric imaging capabilities.

We used the RF data obtained by time-resolved scanning acoustic microscope measurements were used to estimate material properties and simulate the acoustical images from the RF data set. Then generate the scatterer map from the acoustical image and use the scatterer map as the phantom target in Field II® for transducer simulations to obtain B-mode image in order to analyze image resolution.

### 1.3 Dissertation outline

Chapter 1 is the introduction providing the background and motivation of this work, the specific aims of the research, and an overview of this thesis. Chapter 2 focuses on the background in understanding the principles of scanning acoustic microscopy (SAM). Details are provided on acoustic lenses, coupling media, acoustic microscope resolution, and imaging procedures. Olympus UH3 SAM and SAarland Scanning Acoustic Microscopy (SASAM) are provided as examples of commercial SAM, and the applications of SAM in pulse mode and tone burst mode.
are explored. The use of SAM in exploring the properties of biological cells is also provided. Chapter 3 focuses on the background in understanding the $V(z)$ curve. Information is presented on $V(z)$ theory including Ray models and Fourier Angular Spectrum Theory or the Wave Theory Model. Chapter 4 discusses the background in understanding the properties of biological cells. The contrast factor, velocity measurement, and layer model and reflectance function of biological cells are discussed. Specific attention is given to HeLa cells. Chapter 5 provides the background in understanding ultrasound transducer arrays including the types of arrays, focusing mechanisms and ultrasound imaging. Specifically discussed are single element, linear and phased arrays; axial and lateral resolution and focusing; and B-scan, P-scan and C-scan imaging. Chapter 6 details the methods and simulations employed in this research. Information is provided on the calculation of the reflectance function for biological cells and substrates, $V(z)$ curve simulation, FFT analysis, mechanical properties of cells from time-resolved SAM, and specific transducer simulations. Chapter 7 details the simulation results. Specific attention is given to the reflectance function for biological cells and substrates, $V(z)$ curves, the mechanical properties of cells, transducer simulations. Chapter 8 provides analysis and interpretation of the results presented in Chapter 7. Finally, Chapter 9 provides summary and conclusion as well as suggestions for future work.
Chapter 2

Background in Understanding Scanning Acoustic Microscopy

2.1 Principles of Scanning Acoustic Microscopy

There are two main categories of scanning acoustic microscope (SAM); a transmission SAM and a reflection SAM. Figure 2-1 illustrates the lens geometry of the transmission SAM [2], [3]. The transmission SAM consists of a symmetrical pair of lens elements, which is built by polishing a small concave spherical surface at the end of a sapphire rod, connected by a small volume of coupling liquid. These two acoustic lenses are identical and arranged confocally. The illumination and the detection process can be performed by focusing these lenses. Each lens is composed of a single spherical interface between the liquid and a lens rod. On the top of the opposite end of the sapphire rod, there is a thin film piezoelectric transducer mounted at the center on the axis of the lens surface.
Figure 2-1. The lens geometry of the transmission SAM (Lemons and Quate, 1979. Yu and Boseck, 1995).

For the reflection SAM, the same lens is used for both transmitting and receiving the acoustic signal as shown in Figure 2-2. Reflection SAM will be discussed further throughout this work.
The first scanning acoustic microscope operated in transmission mode and there is difficulty in adjusting and setting up the lenses confocally, especially with high frequencies and shorter wavelengths. As a result, most of the recent development and application of SAM has been done with the reflection type microscope. In this research we focus on the reflection SAM.

### 2.1.1 Acoustic lens

The lens system, which is used for generating and receiving ultrasound waves, is considered as the crucial part of a SAM. Figure 2-3 depicts the schematic of an acoustics lens system. The lens is constructed of single crystal sapphire rod cut along the crystallographic $c$ axis.
of the sapphire. Sapphire is the most widely used for the lens material for the reason that it has low acoustic attenuation, high acoustic impedance, and high velocity. With the low acoustic attenuation property of sapphire, the energy loss of high frequency acoustic waves traveling inside the lens decreases. Sapphire also has a comparatively high acoustic velocity (11000 m/s) compared to water (1500 m/s), yielding high refractive index of 7.4. With a high refractive index, it is possible for the acoustic waves to be focused sharply with negligible spherical aberration in high numerical aperture lenses since spherical aberration is inversely proportional to the square of the refractive index [4]. By comparison, an optical microscope has a refractive index usually less than 1.5 [4].

![Figure 2-3: The schematic of an acoustic lens system.](image)

As seen in Figure 2-3, a concave spherical or cylindrical surface is scraped in the center of the other end of the rod surface, providing the focusing action. Generally, the radius and aperture angle of the cavity are determined by the frequency and applications. To optimize transmission of the acoustic waves, it is also coated with a quarter-wavelength-thick matching layer [2]. The large difference of acoustic velocity between sapphire and water allows for negligible spherical
aberration; however, the high impedance mismatch between them is a disadvantage since it allows only a very small amount of acoustic energy to pass through the cavity interface. Therefore, a matching layer is needed and usually deposited onto the cavity surface in order to solve this problem. This matching layer typically has a thickness of a quarter-wave length and impedance much smaller than that of the sapphire.

On the other end of the rod a piezoelectric transducer, normally a thin film of Radio Frequency (RF)-sputtered ZnO, is mounted. (RF sputtering is a technique used to fabricate the thin films.) It is necessary to use coupling fluid, typically water, between the lens and the specimen. Plane acoustic waves are generated once the transducer is excited with a short RF pulse, approximately 30 ns in duration. The waves propagate through the rod and become a focus beam on the axis of the lens by refraction at the concave spherical interface between the lens and the liquid. The focused beam travels to the object to be imaged which is located at the focus of this lens. The acoustic waves are then partially reflected at the interface. The echoes created traverse the system conversely and are converted back into an electrical pulse by the transducer acting as a light-sensitive receptor and coherent detector. The strength of this pulse is in proportion to the acoustic reflectivity of the object at the point being investigated. An acoustic image can be constructed by mechanically scanning the object in a raster pattern and display it on a TV monitor afterward.

### 2.1.2 Coupling medium

As mentioned in section 2.1.1, it is essential to provide proper coupling medium that acoustic waves can travel through. Air is not a viable choice for high frequency acoustic wave propagation due to its high attenuation. Neither will a solid medium since it cannot support the easy mechanical scanning of the acoustic beam [4]. Therefore, liquid is the better choice of coupling
medium for scanning acoustic microscopy at present. Water is typically the best and the most extensively used coupling medium because it has good wetability, comparatively low acoustic attenuation, and chemical inertness [4]. Nevertheless the acoustic wave attenuation in water is proportional to the square of the frequency making it difficult to use water in high frequency operation. To alleviate this problem, the attenuation in water can be improved by elevating the water temperature. For example, in general for frequencies below 400 MHz, water at room temperature (25°C) is acceptable, but for frequencies above 400 MHz, the temperature of the water needs to be 60°C in order to diminish attenuation. In the case of high frequencies above 1.5 GHz, significant attenuation in water will still occur even at elevated temperatures. Cryogenic couplants possess a much lower attenuation and lower sound velocity. Here, liquid argon and liquid helium have often been used [4], [5]. Not only is the attenuation of acoustic waves in the liquid phase of the isotope helium 4 negligible, but its acoustic velocity is also only one-eighth of that of water [4],[6]. Other non-cryogenic, low-attenuation couplants, such as gallium and mercury, have also been used at room temperature. However, specific equipment for handling these couplants is necessary and the applications of non-cryogenic couplants are quite restricted.

2.1.3 Resolution of the acoustic microscope

Since the aberrations in the acoustic microscope are insignificant, the resolution of an acoustic lens can be determined almost entirely by diffraction limitations.

The resolution, \( R \), of SAM can be expressed as:

\[
R = 0.51 \frac{\lambda_w}{N.A}
\]

where \( \lambda_w \) is the wavelength of sound in liquid, and N.A is the numerical aperture of the acoustic lens, which is the ratio of focal distance to an aperture dimension [7], [8]. Usually N.A is
approximately 1 for a high-frequency lens providing a resolution of $0.5\lambda_w$. For a well-designed lens, the diameter of the focal spot approaches the acoustic wavelength, which is about 0.4 µm at 2 GHz in water. The resolution of an acoustic microscope may be tested by imaging a specimen with a fine grating ruled on it. Figure 2-4 shows an acoustic image of an optical grating with a period of 0.4 µm at 2 GHz. In this case, the acoustic microscope can attain a resolution comparable to that of an optical microscope.

An alternative way to improve the resolution of a SAM is to make the wavelength smaller since resolution is proportional to the wavelength in the liquid $\lambda_w$. The wavelength depends on the velocity of sound in the liquid, $c_w$, and the frequency, $f$, such that $\lambda_w=\frac{c_w}{f}$. Assuming that the frequency can be indefinitely increased, virtually unlimited resolution could be accomplished.
However, the use of very high frequency is limited due to the attenuation of the coupling medium and the available radius of curvature of the lens. As stated previously regarding the coupling medium, it is necessary to have a liquid medium between the acoustic lens and the specimen. The attenuation of acoustic waves is in proportion to the frequency squared in most liquids at or near room temperature according to their linear viscosity. To increase the frequency, it is crucial to reduce the liquid path length between the lens and the specimen. Therefore, the focal length of the lens and, in turn, its radius of curvature must be small. However, it is quite difficult to fabricate lenses with a very small radius. In addition, even if it is possible to grind lens radii as small as required (approximately 15 µm), for a lens operating in a pulsed mode with higher frequencies, there are still significant issues with high-speed switches to achieve sufficiently narrow pulses to prevent the specimen from being swamped by the lens echo. The highest frequency for a microscope with water coupling at 60°C is typically about 2 GHz.

As mentioned in section 2.1.2, the attenuation of acoustic waves in water is reduced by increasing its temperature. The nonlinear properties of the coupling liquid have also been used to enhance resolution. It is possible to improve the resolution of the microscope by at least a factor of 1.4 by the generation of harmonics [9]. It is preferred to utilize a liquid with either a lower sound velocity or a lower absorption coefficient. Cryogenic liquids such as super fluid helium are potentially good candidates [4].

2.1.4 Imaging procedure

By mechanically scanning the acoustic lens and the specimen in a raster pattern, a two dimensional image can be built by SAM. The acoustic lens scans in one direction with high frequency, at high speed and the stage moves the other direction perpendicularly. The signals received by the lens are amplified and displayed on a TV monitor via a frame storage memory [4].
The grey value of each pixel on the image corresponds to the received signal amplitude. A high frequency SAM has an exceptionally accurate small field mechanical scanner to attain high resolution yielding a magnification range from 100 to 2000. It usually takes approximately 10-20 seconds to acquire a typical 512-line image. The actual scanning and imaging procedures may vary from microscope to microscope.

2.2 Examples of commercially available SAMs

There are several manufacturers building and designing scanning acoustic microscopes. One such example is the Olympus UH-3 Scanning Acoustic microscope system, which is manufactured by the Olympus Optical Company, Japan. The Olympus UH-3 is a reflection type SAM that can be operated both in qualitative imaging (scanning) mode and quantitative (non-scanning) mode. There are three main features of the Olympus UH-3 SAM: surface imaging, subsurface and internal imaging, and quantitative evaluation of surface acoustic wave velocity. There are two modes of operation: tone burst mode and pulse mode. The tone burst mode uses tone burst signal as the excitation signal whereas the pulse mode employs a very short pulse signal as the excitation signal. Figure 2-5 depicts tone burst mode with a long duration (narrow band frequency) excitation signal and pulse mode with a short spike pulse (broad band frequency) excitation signal.
Figure 2-5. (a) tone burst mode uses a long duration (narrow band frequency) excitation signal and (b) pulse mode uses a short spike pulse (broad band frequency) excitation signal.

A second example of a commercial SAM is the combined optical and acoustic microscope, which is used to assess the measurement possibilities of time-resolved acoustic microscopy on living cells [10]. A high frequency time-resolved acoustic microscope, SAaerland Scanning Acoustic Microscopy (SASAM), has been developed by Weiss et al (2007). The conventional scanning acoustic microscope is an imaging system in which a radio frequency (RF) electrical pulse is used to excite acoustical signal waves in a lens. The lens has a spherical cavity that focuses sound onto a spot whose size is comparable to the acoustic wavelength in the coupling fluid. The acoustic echoes reflected by the object are collected by the same lens and are detected by the piezoelectric
transducer. The lens is scanned over the sample mechanically line by line in a raster pattern. During the scan, the RF-signal is recorded for each position. The integral of the envelope of the RF-signals can be used to reconstruct a gray scale image. The new combined optical and acoustic microscope can be characterized by a combination of operating principles and design features distinguishing it from other high-frequency acoustic microscopes. These principles and design features are: it operates in time-resolved mode; it is designed as an attachment to an inverse optical microscope; it is fully automated, so it can remember the positions of several cells and acquire acoustical images of these cells for several hours or even days; and measurement can be done at 37°C. This new system is also composed of four main modules: an acoustical lens, an optical module, a scanning unit, and high-frequency electronics [10].

For the acoustical lens module, a high-frequency lens is used with a semi-aperture angle of 45° manufactured by Kramer Scientific Instrument. The spectrum of the lens has a center frequency of 860 MHz and a -6 dB bandwidth of 30%. At this frequency, the lens provides a lateral resolution of 1.5µm [10].

For the optical module, it is composed of an inverted optical microscope (Olympus IX81) with a scanning unit attached to a rotating column that allows the user to switch between the condenser and the acoustical lens. The inverted optical microscope has two operating modes, and thus two light paths: reflection mode when the light is coming from the lens located below the sample, and transmission mode, when the light from a condenser, located above the sample, passes through a sample and then is collected by a lens, located below the sample. The transmission mode provides a good contrast for cell interfaces and therefore the optical images of the cells are obtained in the transmission mode before starting acoustical imaging. Imaging in the reflection mode is used for adjusting the focus of the acoustical microscope and for aligning the optical axis of the optical microscope and the vertical axis of the acoustical microscope. This setup makes it possible to obtain optical trans-illumination, epi-fluorescence, confocal optical, and acoustical images, in conjunction
with synchronous acoustic data acquisition from the same cell [33]. Part of the optical microscope with the microscope stage is covered by a special chamber (Solent Scientific Limited, Segensworth, UK). This configuration provides temperature control for the cells placed on the optical microscope stage. Inside the chamber the temperature can be kept constant at room temperature or 37 ± 0.5°C. Optical images are acquired using a digital camera with on-chip image amplification from Hamamatsu Photonics (Hamamatsu, Japan).

For the scanning module, the scanner used to perform the raster movement of the lens over the sample consists of a manual mechanical stage for aligning the acoustical lens and the optical path (OWIS, Staufen, Germany), a scanning stage for lateral (x, y) scanning, and one mechanical stage with a direct current (DC) motor to move the lens in the vertical (z) direction with a resolution of 0.1 µm (Physik Instrumente, Karlsruhe, Germany). The acoustic scanner is approximately the size of an optical condenser and, therefore, can be attached to almost any commercially available inverted optical microscope. The piezo scanning stage has a scanning range of 100 µm x 100 µm and a resonant frequency of several hundred hertz. The lateral position of the lens is detected with a capacitive position sensor with a resolution of several nanometers and is controlled by a power supply developed for atomic force microscopes.

The high-frequency electronics module is used for generating and receiving short acoustical pulses. It consists of a short-pulse generator with 1 ns pulse duration, 1 GHz center frequency, 10 V_{pp} amplitude, and a pulse repetition frequency of 500 or 800 kHz, acoustical lens, a high-frequency switch, a broadband low noise amplifier with amplification of 40 dB, and a fast analogue-to-digital (A/D) converter DC211 (Acqiris, Geneva, Switzerland). After amplification, the echo signal is digitized. The digitization rate for the amplified RF signal is 4 G samples/second and the digitization resolution is 8 bits. The trigger signals and the signals for driving the piezo stage are generated by a microcontroller. The step size of the raster pattern can be arbitrarily set between 0.05 and 5 µm.
2.3 Applications of SAM in pulse mode (time-resolved scanning acoustic microscopy)

Both pulse mode and tone burst mode of operations can be employed for imaging and the quantitative measurement of material acoustic properties. When operating in pulse mode, SAM is referred to as time-resolved acoustic microscopy [4], [11]. Either longitudinal waves or shear waves are utilized in time-resolved SAM for both imaging and gathering acoustic property measurements. An extremely short excitation pulse is applied in time-resolved SAM in order to resolve echoes from different interfaces. As depicted in Figure 2-6, an example of time-resolved SAM for a specimen structure, acoustic signal 1, reflected from the specimen surface, can be separated from the acoustic signal 2, reflected from the specimen/substrate interface. Acoustic signal 2 can be used to image the specimen/substrate interface. In addition, the disparity between acoustic signal 1 and acoustic signal 2 can be employed to investigate and evaluate specimen material properties.

Figure 2-6. The schematic diagram of SAM operating in pulse mode.
For example, in 2008 Jikai Du utilized the time-resolved SAM technique for the measurement of longitudinal velocities of various waterborne epoxy coatings with 30-100µm on steel substrate [4]. His results revealed that waterborne epoxy coating have lower longitudinal velocity and Young's modulus compared to traditional epoxy coatings. Reinforcing additives can increase the longitudinal velocities of the waterborne epoxy coatings [4].

Christopher et al. in 1989 used time-resolved SAM to characterize tissue sections with a thickness between 10 and 15 µm. A resolution of about 8 µm was achieved. Additionally, acoustic wave velocity, acoustic impedance, section thickness, and frequency dependent acoustic attenuation in the range of 100-500 MHz were measured with the application of FFT and inverse FFT techniques [12]. Additional details about time-resolved SAM will be discussed in Chapter 4.

### 2.4 Applications of SAM in tone burst mode (V(z) technique)

When SAM is operating in tone burst mode, it is able to image specimen surface and subsurface features with high resolution. The most important application of SAM in tone burst mode is the V(z) technique. Weglein and Wilson [4],[13], first studied the V(z) phenomena by operating SAM on a variety of planar samples at 375 MHZ and discovered that the highest peak of the received signal always occurred as the acoustic lens was focused at the sample surface (focal distance of the acoustic lens). In addition, they also found that when the distance between the lens and the sample surface diminished, the signal amplitude would exhibit a series of oscillations on some samples; nevertheless there were no such oscillations on other samples. They described the phenomena of these oscillations were caused by the generation of leaky Rayleigh waves. They explained that signal oscillations would occur only when the critical angle for the Rayleigh wave generation was smaller than half of the aperture angle of the acoustic lens. This behavior of signal oscillations was uniquely material dependent leading Weglein and Wilson to define the term
Acoustic Material Signature (A.M.S.). Weglein [14] used a model based on acoustic ray theory to describe this acoustic material signature phenomenon and established the relationship between the acoustic material signature and material properties.

The A.M.S. is also known as V(z) response. V represents the amplitude of the received signal as a function of z which represents the distance between the specimen and the acoustic lens. Theory of V(z) will be explained in Chapter 3.

2.5 Explore biological cells and tissue with SAM

It has been shown that SAM is able to explore the cellular and subcellular mechanical properties of biological cells as well as tissue level properties. Currently, there are two types of microscope available to investigate these properties: the scanning laser acoustic microscope (SLAM) operated at approximately 100 MHz and the scanning acoustic microscope (SAM) operated in reflection mode at operating frequencies up to 2 GHz. This yields lateral resolution in the submicrometer range and axial resolution in the range of approximately 30-50 nm [15]. However, the SAM images of cells and tissues are complicated, making them difficult to interpret and require careful attention to detail. Numerous researchers have developed a variety of techniques to extract the information from the images obtained by SAM using mathematical solutions. Examples of the applications of SAM for exploring biological specimen are presented next.

2.5.1 Investigate cells in culture with SAM

It has been revealed that the acoustic properties of the biological specimen can be determined from the measurement data obtained by SAM. For example, longitudinal sound
velocity, attenuation, cell thickness, and reflectivity of sound can be measured by SAM. The estimated cell shape or topography can also be seen from the pattern of interference fringes in acoustic images. An example of interference fringes in acoustic image is shown in Figure 2-7.

Figure 2-7. Interference fringes in acoustic image of Xenopus tadpole endothelial heart cell (XTH-2) compared to sound velocity distribution. (a) The left ordinate denotes thickness (continuous line) and the right ordinate denotes longitudinal sound velocity (bars) along the scanning line through the XTH-2 cell are marked by arrow heads in (b). A gap appears in (a) since no measurements are attainable in the center of the cell area. (b) The SAM image of the two living XTH-2 cells scanned at 1GHz (ELSAM®, Leica, Wetzlar, F.R.G.). Scanning line and intensity distribution along this line are presented. The arrowhead marks the range displayed in (a). (J. Bereiter-Hahn, 1995, Andrew Briggs, 1998)
The zones of equal acoustical path lengths between reflecting boundaries of the cell/culture medium and cell/substratum are defined by these interference fringes in SAM images [15]. This pattern is just a first approximation of surface topography since local variations in longitudinal velocity change the position of an interference fringe. It also appears that viscosity of the cytoplasm causes the attenuation of the sound waves. When interpreting the SAM images of cells, it is basically assumed that the cytoplasm is homogeneous. However, this is not valid in reality due to the distribution of cytoskeletal elements, granules, vacuoles, and the endomembrane system. Vacuoles and granules, which are easily noticeable, can be discarded from the calculation when they exceed the limits of the lateral resolution of SAM. Also, the endomembrane system possibly contributes sound-reflecting boundaries depending on the physiological state of the cells [15]. In the case of soft tissue, calculations of impedance differences from the acoustic signal are not complicated since the contribution of surfaces waves and Rayleigh waves can be ignored. It has also been shown that intracellular sound velocity is associated with the reflectivity of a cell boundary. Additionally, by knowing impedance differences at the boundary, the reflectivity coefficient can be calculated. Due to distinct areas filled with cytoskeletal elements of various functional states, the longitudinal velocity will likely change along the acoustical path inside of a cell. In general, the physical state of the cytoplasm could be treated as either a viscoelastic fluid (the commonly accepted approach) or a quas-solid, fibrous matrix filled with fluid. These basic assumptions affect the interpretation that the longitudinal sound velocity can be determined by bulk modulus or Young’s modulus as it can be expressed as:

\[ v_c^2 = \frac{E}{\rho} \]  \hspace{1cm} 2.2

Where \( v_c \) is the longitudinal sound velocity in cytoplasm, \( E \) is the appropriate modulus of elasticity, and \( \rho \) is the density.
2.5.2 Investigating biological specimens with time-resolved SAM

It has been known that time-resolved SAM is capable of characterizing soft and hard biological tissues. The short impulse excitation allows separate echo pulses from the top and bottom of a thin specimen to be identified in the received signal allowing the vertical structure in specimens to be resolved. With the invention of the ultrasound microscope by Kannigiesser, it extends these earlier developments and opens new applications in biology and medicine [15]. The variation of the acoustic pulse length over a wide range, high scanning speed, and the ready adaptation to an inverted light microscope are important features of the development of the SAM. Lemons and Quate first invented the time-resolved SAM at Oxford with a confocal system. Such a system is capable of providing acoustic sectioning similar to the way a confocal laser-scanning microscope can be used for optical sectioning. This kind of imaging of soft biological samples is often obstructed due to attenuation of samples and high reflectivity of the supporting surface combined with low reflectivity of specimens [3]. The time-resolved SAM technique has a major advantage in that it requires no prior assumptions about the acoustic properties of the specimen. Further details about time-resolved measurement technique will be described in Chapter 4.

Chapter summary

In this chapter, the principles of scanning acoustic microscopy were described. The two main categories of scanning acoustic microscope (SAM); a transmission SAM and a reflection SAM were explained. Furthermore, examples of commercially available SAMs, and applications of SAM operating in pulse mode and tone burst mode were also discussed. In the next chapter, the theory of V(z) response will be presented.
Chapter 3

Background in Understanding V(z) curve

3.1 The V(z) curve

The scanning image of a specimen at the surface or subsurface of an object can be acquired by mechanically scanning the object plane. The object is fixed at an (x, y) position whereas the acoustic lens moves towards the object in the z direction. One then observes a series of oscillations in the transducer video output as a function of distance z. This effect is also known as the acoustic material signature (A.M.S.) as mentioned in the previous chapter. Weglein and his colleagues first pioneered this effect experimentally in 1979. This effect was also theoretically studied by a number of authors such as Atalar and Wickramasinghe, both, in 1979 [16], [17]. Figure 3-1 illustrates some of the main features of V(z) for a quartz specimen, obtained by a commercial instrument known as the Ernst Leitz scanning acoustic microscope (ELSAM) [2].
In Figure 3-1, there is the strong central maximum centered on the focal plane at $z=0$, a characteristic of the sample-coupling liquid interface due to the primary reflection’s existence. This region is independent of the material properties of the sample being scanned. The curve for positive $z$ attenuates rapidly as distance $z$ is increased since this region the sample surface is farther away than the focal plane. Most of the acoustic energy is reflected away outside the acoustic lens and only a less convergent beam is detected and received by the transducer. Conversely, there are strong oscillations on the negative $z$ side where a series of periodic maxima and minima occur and are characterized by a period $\Delta z$, which allows for the calculation of the acoustic properties of the sample. These periodic depths of the minima and the relative magnitudes of the maxima vary with the material, indicating different acoustic properties for each material. $\Delta z$ may be multivalued or may vary with distance $z$ for a layered solid. The material-dependent information is exhibited in
this region and is defined as the acoustic material signature which is an important technique for the nondestructive evaluation of material properties.

3.2 Theory of V(z)

There have been two main theoretical models developed for the explanation of V(z) phenomena: ray models based on an effect of ray interference principles and wave theory or Fourier angular spectrum models.

3.2.1 Ray models

An approximate expression of the null spacing in terms of Rayleigh wavelength and Rayleigh critical angle was first introduced by Weglein [4] [13][14]. This first model is simple and related acoustic materials signatures to the leaky surface wave velocities, however it is not quite accurate. Later, Parmon and Bertoni developed a more accurate expression for the V(z) curve based on Weglein’s model by including interference between the specularly reflected ray and leaky surface wave ray [4] [18]. It has been shown that Parmon and Bertoni’s model yielded a better fit to the experimental data as well as agreed better with the ray properties of the acoustic lens than that of Weglein’s models. In addition, Parmon and Bertoni’s model could also be used to calculate the null spacing and predict the shape of V(z) curves.

3.2.1.1 Weglein’s model

In this model, it is believed that the interference of two acoustic rays near the Rayleigh critical angle causes the V(z) phenomena. Weglein explained that the null spacing of the V(z)
curve can be empirically described in terms of the Rayleigh wave velocity and the Rayleigh critical angle. Figure 3-2 illustrates the mechanism of V(z) phenomena.

![Diagram of V(z) phenomena](image)

Figure 3-2. The mechanism of V(z) phenomena based on Weglein model.

The two acoustic rays propagate into the liquid near the Rayleigh critical angle ($\theta_R$) as the acoustic lens translates toward or defocuses with a distance $\delta z$. Ray A is specularly reflected from the sample surface with an incident angle very close to the critical Rayleigh angle. Ray B is a leaky Rayleigh wave whose incident angle equals the Rayleigh critical angle and also displaces with a lateral shift ($r$) compared to the specularly reflected acoustic ray. This leaky Rayleigh wave reradiates energy into the coupling medium at the Rayleigh angle in the form of longitudinal waves, while it is propagating along the sample surface [4]. The two acoustic rays are assumed to have the same propagation delays, excluding this lateral shift. The phase difference between the specularly reflected ray (A) and the laterally shifted (B) ray can be calculated as:
\[ \phi = 2\pi \left( \frac{\delta z \tan \theta_R}{\lambda_R} \right) \] 3.1

Where \( \lambda_R \) is the Rayleigh wavelength. The variation of the transducer output affected by this phase difference can be determined by the following expression:

\[ V_{out} = V_0 \sin \left( \frac{m \delta z \tan \theta_R}{\lambda_R} \right) \] 3.2

The periodic null spacing \( \Delta Z_N \) can be calculated as:

\[ \Delta Z_N = \frac{\lambda_R}{\tan \theta_R} \] 3.3

3.2.1.2 Parmon and Bertoni's model

Parmon and Bertoni later improved a more accurate expression for calculating the null spacing in the \( V(z) \) curve based on the interference effect between the specularly reflected axial ray and the leaky wave ray [4],[18]. Their model yields the expression for calculating the surface wave velocity as follows:

\[ V_R = \frac{c_w}{\left( 1 - \left( 1 - \frac{c_w}{2f\Delta Z} \right)^2 \right)^{1/2}} \] 3.4

where \( V_R \) is the surface wave velocity, \( c_w \) is the velocity in water, \( f \) is frequency, and \( \Delta Z \) is the periodic null spacing.
3.2.1.3 Bertoni’s model

Bertoni proposed a more thorough model that can determine the absolute phase and amplitude of the received signal of a point focus lens and also enable the prediction of the shape of the V(z) curve [4],[18]. Therefore, it is a better model compared to both Weglein’s model and Parmon and Bertoni’s model since those two models yield no information about the shape of the V(z) curve influenced by various attenuation factors. Bertoni’s model involves the reflectance function at the interface between the coupling liquid and the sample. The reflected acoustic field was divided into two parts: a geometrically reflected acoustic field at the sample surface and an acoustic field produced from the excitation and radiation of the leaky Rayleigh waves. The formulas used in the calculation of the transducer outputs were based on ray optical theory. Bertoni’s model yields the advantages of the separation of acoustic fields and the consideration of attenuation factors. The separation of the acoustic fields indicated that when the two acoustic fields were out of phase, the minima on the V(z) curve occurred. Regarding attenuation factors, attenuation occurs in water, by the radiation of leaky Rayleigh waves, and by the damping of the leaky Rayleigh waves. Bertoni included these factors in his model and described how the attenuation of each factor related to or affected the shape of the V(z) curve. He also developed the same expression as Weglein’s model and Parmon and Bertoni’s model for the relationship between the null spacing and the Rayleigh wave velocity. It has been shown that this model was in a good agreement with experiments for predicting the shape of V(z) responses [19]. Additionally, Bertoni’s model proved that the geometry and acoustic parameters of the acoustic lens and sample could have an effect on the shape of V(z) curves [4].
3.2.2 Fourier Angular Spectrum Theory or Wave Theory model

Atalar first introduced and developed the wave theory model based on the Fourier angular spectrum theory [4], [20]. In this model, the incident acoustic field distributions were decomposed at various planes in the acoustic microscope system into angular spectrum of plane waves in order to achieve an integral expression for \( V(z) \) curves. Figure 3-3 illustrates the schematic diagram of an acoustic field for the wave theory model by Atalar.

![Diagram](image)

Figure 3-3. The coordinates and geometry for the lens and transducer as used in the analysis (Atalar et al., 1979)

From Figure 3-3, the acoustic field distributions are divided into 4 planes; plane 0 is the transducer plane, plane 1 is the back focal plane, plane 2 is the front focal plane, and plane 3 is the substrate-sample plane. When a plane acoustic wave for which an angular frequency \( \omega \) is incident on the transducer plane, the acoustic field at the transducer plane is expressed as \( u_0(x, y)e^{j\omega t} \). The
acoustic fields of the planes, which are denoted as $i$, where $i=1, 2$ and 3, are expressed by a spatial distribution $u_i^\pm(x, y)$ or a frequency distribution $U_i^\pm(k_x, k_y)$. Here, superscript $(\pm)$ and $(\mp)$ indicate the direction of field travel in $+z$ or $-z$ respectively. The angular spectrum at the transducer plane ($z = z_0$) can be expressed as [20]:

$$U_0^+ = F[u_0^+(x, y)] = \int \int_{-\infty}^{\infty} u_0^+(x, y) \exp[-j(k_x x + k_y y + k_z (z - z_0))] \, dx \, dy$$

where $F$ denotes the two dimensional Fourier transform. At the transducer plane, $z$ equals $z_0$; thus, the term $k_z(z - z_0)\big|_{z = z_0}$ in the right-hand side in Equation 3.5 becomes zero. As a result, we obtain:

$$U_0^+ = \int_{-\infty}^{\infty} u_0^+(x, y) \exp[-j(k_x x + k_y y)] \, dx \, dy$$

The relationships between the spatial and frequency distributions are generalized by the following equations [20]:

$$U_i^\pm = F[u_i^\pm(x, y)]$$
$$u_i^\pm = F^{-1}[U_i^\pm(x, y)]$$

where $F$ denotes the two dimensional Fourier transform and $F^{-1}$ denotes the two dimensional inverse Fourier transform.

It should be noted that only the phase related to the distance traveled ($|z_i - z_0|$) is changed as the waves propagate from the transducer plane ($z_0$) to the back focal plane ($z_1$). Thus, we obtain [20]:

$$U_1^+(k_x, k_y) = U_0^+ \exp[jk_z(z_1 - z_0)]$$
Also, the pupil function \( P(x, y) \) indicating the shape of the lens is expressed as:

\[
P(x, y) = \text{circ}\left[ \frac{(x^2 + y^2)^{1/2}}{R} \right]
\]  

3.9

where \( R \) is the radius of the pupil and \( \text{circ} \) is expressed as:

\[
\text{circ}(r) = \begin{cases} 
1, & r < 1 \\
0, & r > 1 
\end{cases}
\]  

3.10

Applying the Fresnel approximation and the thin lens model, \( u_2^+(x, y) \) is expressed as [20]:

\[
u_2^+(x, y) = \frac{\exp[jk_0f(1 + \bar{e}^2)]}{j\lambda_0f} \times \int \int u_1^+(x_1, y_1)P_1(x_1 + x_2, y_1 + y_2)\exp[-j\frac{2\pi}{\lambda_0f}(x_1x_2 + y_1y_2)]dx_1dy_1
\]  

3.11

where \( k_0 \) is the wave number in the coupling medium, \( f \) is the front focal distance, \( \lambda_0 \) is the wavelength in the coupling medium, \( P_1 \) is the pupil function from the lens to the specimen, and \( \bar{e} \) is defined as:

\[
\bar{e} = \frac{C_2}{C_1}
\]  

3.12

where \( C_1 \) is the longitudinal wave velocity of the buffer rod and \( C_2 \) is the longitudinal wave velocity of the coupling medium.
Suppose that \( x_2 \ll x_1 \) and \( y_2 \ll y_1 \) at the front focal plane, the pupil function from the lens to the specimen is expressed as [20]:

\[
P_1(x_1 + x_2, y_1 + y_2) \approx P_1(x_1, y_1)
\]  

Equation 3.11 can then be rewritten as:

\[
u_2^+(x, y) = \frac{\exp[jk_0 f (1 + \bar{c}^2)]}{j\lambda_0 f} F[u_1^+(x, y)]
\]  

\[
U_2^+ = F[u_2^+(x, y)]
\]

Combining Equations 3.13 and 3.14 yields,

\[
U_2^+ = \left\{ \frac{\exp[jk_0 f (1 + \bar{c}^2)]}{j\lambda_0 f} F[u_1^+(x, y)] \right|_{k_x = \frac{k_0 x}{f}, \quad k_y = \frac{k_0 y}{f}} 
\]

\[
= j\lambda_0 f \exp[jk_0 f (1 + \bar{c}^2)]u_1^+ \left[ -\left( \frac{f}{k_0} \right) k_x, -\left( \frac{f}{k_0} \right) k_y \right] \times P_1 \left[ -\left( \frac{f}{k_0} \right) k_x, -\left( \frac{f}{k_0} \right) k_y \right] 
\]

Suppose that \( k_x^2 + k_y^2 \ll k_0 \), the following approximation is obtained:
3.17

\[k_z = (k_0^2 - k_x^2 - k_y^2) \approx k_0 - \frac{1}{2} \left( \frac{k_x^2 + k_y^2}{k_0} \right)\]

Then \(U_3^+(k_x, k_y)\) can be written as:

\[U_3^+(k_x, k_y) = U_2^+(k_x, k_y) \exp[jk_0 z] \exp \left[-j \left( \frac{k_x^2 + k_y^2}{2k_0} \right) z \right] \quad 3.18\]

\[z = |z_3 - z_2| \quad 3.19\]

where \(z_2\) and \(z_3\) are values along the Z axis at the front focal and the specimen planes respectively.

\(U_3^-\) is the angular spectrum of the reflection wave at the interface between the specimen and the coupling medium. It can be written as:

\[U_3^-(k_x, k_y) = U_3^+(k_x, k_y) R \left[-j \left( \frac{k_x}{k_0}, \frac{k_y}{k_0} \right) z \right] \quad 3.20\]

where \(R\) is the reflectance function.

The angular spectrum of the reflection wave at the front focal plane is expressed as [20]:

\[U_2^-(k_x, k_y) = U_3^-(k_x, k_y) \exp[jk_0 z] \exp \left[-j \left( \frac{k_x^2 + k_y^2}{2k_0} \right) z \right] \quad 3.21\]
\[ U_2^{-}(k_x, k_y) = -j \lambda_0 f \exp[j k_0 f (1 + \bar{e}^2)] u_1^{+} \left[ -\left( \frac{f}{k_0} \right) k_x, -\left( \frac{f}{k_0} \right) k_y \right] \]
\[
\times P_1 \left[ -\left( \frac{f}{k_0} \right) k_x, -\left( \frac{f}{k_0} \right) k_y \right] \exp(j 2k_0 z) \exp \left[ -j \left( \frac{k_x^2 + k_y^2}{2k_0} \right) \right] \mathcal{R} \left( \frac{k_x}{k_0}, \frac{k_y}{k_0} \right) \]
\[ 3.22 \]

\[ u_2^{-}(x, y) = F^{-1}[U_2^{-}(k_x, k_y)] \]
\[ 3.23 \]

Similarly \( u_1^{-}(x, y) \) is achieved,

\[
\begin{align*}
 u_1^{-}(x, y) &= \frac{\exp[j k_0 f (1 + \bar{e}^2)]}{j \lambda_0 f} \times \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} u_2^{-}(x_2, y_2) P_2(x_1 + x_2, y_1 + y_2) \exp[-j \frac{2\pi}{\lambda_0 f} (x_1 x_2 + y_1 y_2)] dx_2 dy_2 
\end{align*}
\[ 3.24 \]

where \( P_2 \) is the pupil function from the coupling medium to the lens. The same approximation is applied to Equation 3.13 to yield:

\[
P_2(x_1 + x_2, y_1 + y_2) \equiv P_2(x_1, y_1) \]
\[ 3.25 \]

Thus,

\[
\begin{align*}
 u_1^{-}(x, y) &= \frac{\exp[j k_0 f (1 + \bar{e}^2)]}{j \lambda_0 f} \left. F[u_2^{-} P_2(x, y)] \right|_{k_x = k_0 f, k_y = k_0 f} 
\end{align*}
\[ 3.26 \]

Combining equations 3.23 and 3.26 yields:

\[
\begin{align*}
 u_1^{-}(x, y) &= \frac{\exp[j k_0 f (1 + \bar{e}^2)]}{j \lambda_0 f} P_2(x, y) U_2^{-}\left( \frac{k_0}{f}, x, \frac{k_0}{f}, y \right) 
\end{align*}
\[ 3.27 \]
Finally, combining equation 3.22 and 3.27 yields:

\[
\begin{align*}
    u_i^- (x, y) &= -\exp\{j2k_0[z + f (1 + c^2)]\} \times u_i^+ (-x, -y) P_1(-x, -y) P_2(x, y) \\
    &\times \exp\left[ - j \left( \frac{k_0 z}{f^2} \right)(x^2 + y^2) \right] R\left( \frac{x}{f}, \frac{y}{f} \right)
\end{align*}
\]

3.28

Equation 3.28 illustrates the reflected field at the back focal plane in terms of the incident field at the same plane, the pupil functions of the lens, the reflectance function of the reflector object, and the position of the object.

Since the position of the focal point varies, the variation of transducer voltage is governed by the movement of the acoustic lens along the z axis and is expressed as [20]:

\[
V(z) = \int \int_{-\infty}^{\infty} u_i^- (x_1, y_1) u_i^+ (x_1, y_1) dx_1 dy_1
\]

3.29

The wavenumbers can be expressed as:

\[
k_z' = (k_0^2 - k_x^2 - k_y^2)^{1/2}
\]

3.30

where \( k_0 \) is the wavenumber of the coupling medium.

The acoustic field at the back focal plane (plane 1) can be written as:

\[
u_i^+ (x_1, y_1) = u_i^+ (x_1, y_1) * F^{-1}[\exp(jk_z'd)]
\]

3.31

The reflected acoustic field at the transducer plane (plane 0) can be expressed as [20]:

\[
\text{...}
\]
\[ u_0^-(x_1, y_1) = u_1^-(x_1, y_1) * F^{-1}[\exp(jk'_z d)] \]  
\[ d = |z_1 - z_0| \]  

By substituting Equation 3.32 into Equation 3.29, the variation of transducer voltage expressed in Equation 3.29, can also be rewritten as:

\[ V(z) = \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} u_0^+(x_1, y_1) \left( \int_{-\infty}^{\infty} u_1^-(\xi, \eta) \times F^{-1}[\exp(jk'_z d)] \right) d\xi d\eta \, dx_1 dy_1 \]  

By applying the definition of convolution to the term inside the curly bracket in Equation 3.34 above, we have

\[ V(z) = \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} u_0^+(x_1, y_1) \left( \int_{-\infty}^{\infty} u_1^-(\xi, \eta) \times F^{-1}[\exp(jk'_z d)] \right) \bigg|_{\substack{y = y_1 - \eta \\ y = \eta - \xi}} \, d\xi d\eta \, dx_1 dy_1 \]  

By changing the order of integration of the above equation, we obtain [20]:

\[ V(z) = \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} u_1^-(\xi, \eta) \left( \int_{-\infty}^{\infty} u_0^+(x_1, y_1) \times F^{-1}[\exp(jk'_z d)] \right) \bigg|_{\substack{x = x_1 - \xi \\ y = y_1 - \eta}} \, d\xi d\eta \]  

Because the wavenumber \( k'_z \) in Equation 3.30 is an even function of \( k_x \) and \( k_y \). Therefore, we have
\[ F^{-1}[\exp(jk'z)] \bigg|_{x=x_1-z}^{y=y_1-\eta} = F^{-1}[\exp(jk’z)] \bigg|_{x=x_1-y}^{y=y_1-\eta} \] \hspace{1cm} 3.37

Equation 3.34 can then be rewritten as:

\[ V(z) = \int \int_{-\infty}^{\infty} u_1^-(\xi, \eta) \left\{ u^+_0(\xi, \eta) \ast F^{-1}[\exp(jk'z)] \right\}_{x=\xi}^{y=\eta} d\xi d\eta \] \hspace{1cm} 3.38

The term in the curly bracket in Equation 3.38, \( \left\{ u^+_0(\xi, \eta) \ast F^{-1}[\exp(jk'z)] \right\}_{x=\xi}^{y=\eta} \), is defined as \( u_1^+(x_1, y_1) \), as shown in Equation 3.31. Thus, Equation 3.38 can be rewritten as [20]:

\[ V(z) = \int \int_{-\infty}^{\infty} u_1^-(\xi, \eta) u_1^+(\xi, \eta) d\xi d\eta \] \hspace{1cm} 3.39

\[ V(z) = -\exp\left\{ j2k_0[z + f(1+\varepsilon^2)] \right\} \int_{-\infty}^{\infty} u_1^+(-x, -y) u_1^+(x, y) \times P_1(-x, -y) P_2(x, y) \]
\[ \times R\left( \frac{x}{f}, \frac{y}{f} \right) \exp\left[ -j \left( \frac{k_0}{f^2} \right) z(x^2 + y^2) \right] dx dy \] \hspace{1cm} 3.40

Omitting the constant factor, \(-\exp\{ j2k_0[z + f(1+\varepsilon^2)] \}\), equation 3.40 can be expressed as [20]:

\[ V(z) = \int \int_{-\infty}^{\infty} u_1^+(-x, -y) u_1^+(x, y) P_1(-x, -y) P_2(x, y) \times R\left( \frac{x}{f}, \frac{y}{f} \right) \exp\left[ -j \left( \frac{k_0}{f^2} \right) z(x^2 + y^2) \right] dx dy \] \hspace{1cm} 3.41
We can also write these the mathematical expressions in cylindrical coordinates since the acoustic field distributions are also symmetric about the z axis in the scanning acoustic microscope [20].

For a circularly symmetry system, we have

\[
R\left(\frac{x}{f}, \frac{y}{f}\right) = R\left(\frac{r}{f}\right); \quad u_1^+(x, y) = u_1^+(r); \quad \text{and} \quad P(x, y) = P(r)
\]

Therefore, Equation 3.41 can be expressed as [20]:

\[
V(z) = 2\pi \int_0^\infty [u_1^+(r)]^2 P_1(r) P_2(r) R\left(\frac{r}{f}\right) \times \exp\left[-j \frac{k_0}{f^2} z r^2\right] r dr
\]

Equation 3.42 shows that the acoustic field of the back focal plane, the lens, the reflectance function, and the defocal distance are the main factors in the output voltage.

Although the shape of the amplitude distribution of the acoustic field at the back focal plane is determined by the size of transducer and the frequency of the acoustic wave, in general, its shape forms that of a Gaussian distribution. The pupil function is typically constant. Therefore, the reflectance function affects the V(z), which depends on the elastic properties of the material. Additionally, the V(z) changes in accordance with the defocusing distance(z).

Later Sheppard and Wilson (1981) derived Atalar’s model in a simpler form [2] [21]. Figure 3-4 illustrates the geometry and coordinate system used for analyzing field distributions.
Figure 3-4. Geometry and coordinate system used for analyzing field distributions (Yu and Boseck, 1995).

The transducer generated a plane wave of unit amplitude which travels through a distance $h$ until it reached the lens at the lens-coupling medium interface. The acoustic field $u_1$ represents the acoustic field on the back side of the lens as shown in Figure 3-4 at the plane I. The acoustic field $u_1$ may be calculated by using the diffraction theory of the piston transducer [2].

Once the plane acoustic wave propagates through the acoustic lens, the illumination function at the converging spherical wave-front surface is:

$$U_1(\theta) = u_1(\theta) P(\theta) \cos^{1/2} \theta$$  \hspace{1cm} 3.43
where $\theta$ is the incident angle of the acoustic wave. $P(\theta)$ denotes the generalized pupil function of the acoustic lens for waves propagating in this direction. The pupil function explains the complex amplitude of the acoustic wave as it traverses through the lens. The $\cos^{1/2}\theta$ term describes the acoustic imaging system being considered as an aplanatic system for completeness. This $\cos^{1/2}\theta$ term is canceled out in the calculation afterward [2][21]. The mathematical expression in Equation 3.43 was derived excluding the paraxial approximation or the assumption of the thin-lens model, therefore the result is applicable for any large aperture of the acoustic lens.

After that the acoustic wave propagates and reaches an object at the focus. The acoustic wave is then reflected by the object with a reflectance function $R(\theta)$. The amplitude of the reflected wave can be calculated by:

$$ U_2(\theta) = u_1(\theta)P(\theta)R(\theta)\cos^{1/2}\theta $$

This reflected wave is subsequently refracted by the acoustic lens again before it finally reaches the transducer. In addition, the pupil function of the lens is again $P(\theta)$. Here, the assumption is that the pupil function $P$ is the same in both directions. The acoustic field at the transducer can be expressed as:

$$ U_3(\theta) = u_1^2(\theta)P^2(\theta)R(\theta) $$

Therefore, the unnormalized signal at the transducer is achieved by:

$$ V(0) = \int_0^{\infty} P^2(\theta)u_1^2(\theta)R(\theta)2\pi rdr $$
By substituting \( r = f \cdot \sin\theta \), \( dr = f \cdot \cos\theta \cdot d\theta \), where \( f \) is the focal length, into Equation 3.46, we obtain

\[
V(0) = 2\pi f \int_0^{\theta_m} P^2(\theta)u_1^2(\theta)R(\theta) \sin\theta \cos\theta \, d\theta
\]

where \( \theta_m \) is the half aperture angle of the acoustic lens. Beyond this given limit, \( P^2(\theta)u_1^2(\theta)R(\theta) \) becomes zero and is disappeared. When the lens moves towards the reflecting surface of the object by a distance \(-z\) from the focus, the phase of the wave incident at a given point on the surface will progress by \( k_w \cdot z \). The waves returning to the lens will advance their phase by twice this. The normalized signal at the transducer becomes [23], [24]:

\[
V(z) = \int_0^{\theta_m} P^2(\theta)u_1^2(\theta)R(\theta) \times \exp[-ik_0z \cos\theta] \sin\theta \cos\theta \, d\theta
\]

where \( k_0 \) is the wave number of sound in the liquid and \( z \) is the defocus.

Typically, the function \( P(\theta) \) and \( u_1(\theta) \) are known for a given lens. Therefore, the \( V(z) \) is entirely determined by the reflectance function \( R(\theta) \). The \( V(z) \) function is associated with the elastic properties of the specimen since \( R(\theta) \) is a complex function of the elastic properties of the specimen being tested. The reflectance function \( R(\theta) \) will change as a consequence of any changes of materials, and therefore affect the \( V(z) \) response.
Chapter summary

In this chapter, the theory of the V(z) curve was described. The details of the two main theoretical models: Ray model and Fourier Angular Spectrum Theory (Wave Theory Model) were provided. The following chapter will discuss about the properties of biological cells, the contrast mechanism, velocity measurement, and layer model and reflectance function of biological cells, respectively.
Chapter 4

Background in Understanding Biological Cell

It has been revealed that the mechanical properties of cells, tissues, and the underlying structures respond to the ultrasound [25]. The mechanical properties also greatly affect cell regulation such as segmentation, division, motility, etc. In soft tissues, the underlying structure is made up of a matrix containing a group of single cells. Ultrasound scattering effects observed in the frequency range from 10 to 100 MHz possibly take place at a subcellular level in structures of sizes less than and up to the order of magnitude of the ultrasound wavelength[15], [25]. Therefore, it is useful to examine the mechanical properties of a single cell level as it potentially presents insight into the mechanism of scattering from an aggregate of cells, such as tumors [25], [26], [27].

The examination of the cells in vitro on a microscopic level as well as the mechanical properties can be accomplished by scanning acoustic microscopy (SAM). Resolutions of 1 µm can be reached by utilizing frequencies up to 2 GHz [28]. Acoustical properties that can be measured by SAM are sound velocity, sound attenuation, cell thickness, and the elasticity and stiffness of intracellular components [25], [29], [30], [31]. There are two different techniques used in SAM as described in chapter 2: time resolved SAM [32], [33], [34], [35] and V(z) [30], [36], [37]. Time resolved SAM, which employs a short broad band pulse, appears to be the most promising technique for quantitative measurements. The acoustic pulses need to be separated for the time resolved analysis [15]. Another well known approach is reflection confocal acoustic microscopy, which applies a monochromatic tone burst, to enable the acquisition of V(z) or V(f) signatures.

However, the disadvantage of these techniques is that data collection at multiple defocus positions is required. As the transducer approaches the specimen at multiple depth positions, it
induces shear stress due to the scanning motion. In addition, this considerably increases the data acquisition time [15].

In SAM imaging, the contrast is produced from the acoustic property variation of the cellular substructure including possibly other physical effects such as surface wave modes on the substrate, interference fringes due to modulation of the substrate echo by the cell surface echo, and others. As a result, the need for using specialized and toxic chemical dyes that are used in optical microscopy is not required [25].

4.1 Contrast mechanism for acoustic imaging of biological cells and tissues

Contrast in the acoustic images of biological cells and tissues is not generally determined by the difference in reflection coefficients because their acoustic impedances are close to those of either the coupling medium or culturing liquid. Instead, contrast in the acoustic images of biological cells and tissue can be created by the difference in attenuation. It has been shown that the difference in attenuation of the biological specimens can be increased and maximized by using a background substrate composed of highly reflective materials such as sapphire which has an acoustic impedance about 44.3x10^6 kg·m^(-2) [38], or silica glass which is used as a substrate when operating SAM with frequencies in a range of 200 – 600 MHz for imaging biological specimens. The corresponding contrast mechanism varies with the V(z) curve. In addition, some information about the adhesive condition between the cells and the substrate can be revealed by the scanning reflection acoustic microscope. The V(z) curve can be explained by a mathematical expression as follows [38]:

\[
V(z) = C^{-1} \int_{0}^{\infty} u^2(r) P^2(r) R \left( \frac{r}{f} \right) \exp \left\{ ikz \sqrt{1 - \left( \frac{r}{f} \right)^2} \right\} rdr
\]  

4.1
where \( u \) is the acoustic field, \( P \) is the pupil function of the lens geometry, \( R \) is the reflectance function, \( k \) is the wave number in the coupling medium, and \( f \) is the focal length.

By substituting \( r = f \sin \theta \) into equations 4.1 and 4.2, we obtain the following equations [38]:

\[
V(z) = C^{-1} \int_0^\infty u^2(\theta)P^2(\theta)R(\theta) \exp(ikz \cos \theta) \sin \theta \cos \theta \, d\theta 
\]

\[ V(z) = C^{-1} \int_0^\infty u^2(\theta)P^2(\theta) \sin \theta \cos \theta \, d\theta \]

where \( \theta \) is half the aperture angle of the lens.

By replacing \( k_z = k \cos \theta \) in equations 4.3 and 4.4, we obtain the following expressions [38]:

\[
V(z) = C^{-1} \int_{k_0}^{k \cos \theta} Q^2(k_z)R(k_z) \exp(ikz \cos \theta) \, dk_z 
\]

\[
C = \int_{k_0}^{k \cos \theta} Q^2(k_z) \, dk_z 
\]

\[ Q^2(k_z) = u^2(k_z)P^2(k_z)k_z \]

We also obtain the following expression from equation 4.5 [38]:

\[
F^{-1}\{V(z)\} = C^{-1} Q^2(k_z)R(k_z) 
\]

where \( F^{-1}\{\} \) is the inverse Fourier transformation.
The contrast in this case is initiated by the reflectance function, which varies with the cell and its peripheral conditions [38]. For example, in the case that the acoustic beam is emitted from the acoustic lens onto the healthy cell grown on the substrate in the coupling medium, the reflectance function can be computed from the layered media consisting of the culturing medium, the cell, and the substrate as depicted in Figure 4-1 (a). In the case of an injured cell, the reflectance function can be calculated from the layered media consisting of the coupling medium, the cell, the fluid, and the substrate as illustrated in Figure 4-1 (b).

![Figure 4-1](image)

(a) healthy cell (b) injured cell (Maev, 2008).

### 4.2 Layer model and reflectance function of biological cells

Biological cells or tissues can be treated as thin films with near zero shear modulus [4]. Figure 4-2 illustrates the schematic of a cell and substrate structure. Here, the biological cell is assumed to have zero shear velocity so the structure can be treated as a liquid-liquid-solid system.
By employing the velocity potentials of the acoustic waves, the acoustic waves in a coupling medium, the biological cell, and the substrate can be expressed as follows [4].

Coupling medium (layer I)

\[ \Phi^{I+} = A^{I+} \exp\{i(\omega t - k_x^I x - k_z^I z)\} \]  
\[ \Phi^{I-} = A^{I-} \exp\{i(\omega t - k_x^I x + k_z^I z)\} \]

Biological cell (layer II)

\[ \Phi^{II+} = A^{II+} \exp\{i(\omega t - k_x^{II} x - k_z^{II} (z - d))\} \]  
\[ \Phi^{II-} = A^{II-} \exp\{i(\omega t - k_x^{II} x + k_z^{II} (z - d))\} \]

Substrate (layer III)

\[ \Phi^{III+} = A^{III+} \exp\{i(\omega t - k_x^{III} x - k_z^{III} (z - d))\} \]  
\[ \Psi^{III+} = B^{III+} \exp\{i(\omega t - k_x^{III_B} x - k_z^{III_B} (z - d))\} \]

where \( \Phi \) is the potential of the longitudinal wave, \( \Psi \) is the potential of the shear wave; \( A \) is the amplitude of the potential of the longitudinal wave; \( B \) is the amplitude of the potential of the shear wave; the superscript + indicates waves propagating in the positive direction of z axis; the superscript − indicates waves propagating in the negative direction of z axis; \( k \) is the wave number;
\( \omega \) is the angular frequency and Roman numerals I, II, and III indicate media layer of wave propagation; \( d \) is the thickness of biological cell or tissue.

According to Snell’s law, which explains the relationship between the angles and the velocities of the sound waves, the ratio of longitudinal sound velocities in each media layer equals to the ratio of the sine’s of the incident and the refracted angles. Snell’s law can also be expressed in terms of a ratio of wavelengths:

\[
\frac{v_{l_1}}{v_{l_2}} = \frac{\lambda_1}{\lambda_2} = \frac{\sin \theta_1}{\sin \theta_2}.
\]

By using the relationship between wavenumber and wavelength; \( k = \frac{2\pi}{\lambda} \). Hence, Snell’s law can be written as:

\[
\frac{k_2}{k_1} = \frac{\sin \theta_1}{\sin \theta_2} \text{ or, } k_2 \sin \theta_2 = k_1 \sin \theta_1
\]

We have, \( k_x = k_L \sin \theta_L \) and \( k_x = k_L \cos \theta_L \), where \( k_L \) indicates the wave number for a longitudinal wave; \( \theta \) is an incident angle or a reflected angle; a subscript \( L \) indicates a longitudinal wave. In the substrate layer (layer III), we also have \( k_x^{III_B} = k_S^{III} \sin \theta_S^{III} \) in addition to the longitudinal wave component, where \( k_S \) indicates the wave number for a shear wave; \( \theta \) is an incident angle or a reflected angle; a subscript \( S \) indicates a shear wave.

We can write the wavenumbers in each medium layer as \( k_x^I, k_x^II, k_x^{III}, k_x^{III_B} \) for layer I, II, and III respectively:

\[
\begin{align*}
    k_x^I &= k_L^I \sin \theta_L^I \\
    k_x^{II} &= k_L^{II} \sin \theta_L^{II} \\
    k_x^{III} &= k_L^{III} \sin \theta_L^{III} \\
    k_x^{III_B} &= k_S^{III} \sin \theta_S^{III}
\end{align*}
\]
By applying Snell’s law, the wavenumbers in x-axis in each medium layer \( k_x^I, k_x^{II}, k_x^{III}, k_x^{III_B} \) are equal:

\[
k_x^I = k_x^{II} = k_x^{III} = k_x^{III_B} = q
\]

where \( q \) is a constant.

And let, \( \alpha = k_x = k_L \cos \theta_L ; \quad \beta = k_z^{III_B} = k_s^{III} \cos \theta_s^{III} \)

By substituting 4.13, 4.14 into equations 4.7-4.12, we obtain

**Coupling medium (Layer I)**

\[
\Phi^{I+} = A^{I+} \exp\{i(\omega t - qx - \alpha^I z)\}
\]

\[
\Phi^{I-} = A^{I-} \exp\{i(\omega t - qx + \alpha^I z)\}
\]

**Biological cell (Layer II)**

\[
\Phi^{II+} = A^{II+} \exp\{i(\omega t - qx - \alpha^{II} (z - d))\}
\]

\[
\Phi^{II-} = A^{II-} \exp\{i(\omega t - qx + \alpha^{II} (z - d))\}
\]

**Substrate (Layer III)**

\[
\Phi^{III+} = A^{III+} \exp\{i(\omega t - qx - \alpha^{III} (z - d))\}
\]

\[
\Psi^{III+} = B^{III+} \exp\{i(\omega t - qx - \beta (z - d))\}
\]

In a case of a linear, homogeneous, and isotropic medium, the potential of the longitudinal wave (\( \Phi \)) and the potential of the shear wave (\( \Psi \)) are associated to the tangential components and normal components of displacements and stress vectors as follow [80].

1. Tangential components of displacement (\( u \)), [80]:

\[
u = \frac{\partial \Phi}{\partial x} - \frac{\partial \Psi}{\partial z}
\]
2. Normal components of displacement ($\xi$), [80]:

$$\xi = \frac{\partial \Phi}{\partial z} + \frac{\partial \Psi}{\partial x} \quad 4.22$$

Normal components of particle velocity ($v$) can be determined by taking time derivative of the normal component of displacement:

$$v = \frac{\partial \xi}{\partial t} = \frac{\partial}{\partial t} \left( \frac{\partial \Phi}{\partial z} + \frac{\partial \Psi}{\partial x} \right) \quad 4.23$$

3. Tangential stress vector ($\sigma$), [80]:

$$\sigma = \lambda \nabla^2 \Phi + 2\mu \frac{\partial^2 \Phi}{\partial z^2} + 2\mu \frac{\partial^2 \Psi}{\partial x \partial z} \quad 4.24$$

where,

$$\nabla^2 = \frac{\partial^2}{\partial x^2} + \frac{\partial^2}{\partial z^2}$$

thus,

$$\sigma = \lambda \left( \frac{\partial^2 \Phi}{\partial x^2} + \frac{\partial^2 \Phi}{\partial z^2} \right) + 2\mu \frac{\partial^2 \Phi}{\partial z^2} + 2\mu \frac{\partial^2 \Psi}{\partial x \partial z} \quad 4.25$$

4. Normal stress vector ($\tau$), [80]:

$$\tau = 2\mu \frac{\partial^2 \Phi}{\partial x \partial z} + \mu \left( \frac{\partial^2 \Psi}{\partial x^2} - \frac{\partial^2 \Psi}{\partial z^2} \right) \quad 4.26$$

The boundary conditions for the coupling medium-cell-substrate layer are the particle velocity in the direction of the $z$-axis and the continuity of stress [4].

At $z=0$;

$$\sigma_z^I = \sigma_z^{II} \quad \text{and} \quad v_z^I = v_z^{II} \quad 4.27$$

At $z=d$;

$$\sigma_z^{II} = \sigma_z^{III}, \tau_{xz}^{III} = 0 \quad \text{and} \quad v_z^{II} = v_z^{III} \quad 4.28$$
where $\sigma_z$ is the normal stress along z direction; $\tau_{xz}$ is the horizontal shear stress; and $v_z$ is the particle velocity component along the z direction.

Particle velocity and stress in each medium layer can be calculated from Equation 4.15-4.20 and substituted into Equation 4.27 and 4.28 to satisfy the boundary conditions [4].

By applying Equations 4.23 and 4.25 to Equations 4.15 and 4.16, particle velocity and stress in layer I (coupling medium) in the direction of the z-axis can be obtained as following:

Particle velocity in layer I,

$$v_z^l = \frac{\partial}{\partial t} \left( \frac{\partial \Phi^1}{\partial z} + \frac{\partial \Phi^I}{\partial z} \right)$$

$$v_z^l = [\omega(-\alpha') \cdot A'^+] + [\omega(\alpha') \cdot A'^-] = (-\omega \alpha') \cdot [A'^+ + (-A'^-)]$$

$$-\frac{v_z^l}{\omega} = \alpha' \cdot [A'^+ + (-A'^-)]$$  \hspace{1cm}  4.29

Stress in layer I,

$$\sigma_z^l = \lambda^l \left( \frac{\partial^2 (\Phi^1 + \Phi^I)}{\partial z^2} \right) + 2\mu^l \frac{\partial^2 (\Phi^1 + \Phi^I)}{\partial z^2}$$

$$\sigma_z^l = (\lambda^l + 2\mu^l) \cdot \left( \frac{\partial^2 (\Phi^1 + \Phi^I)}{\partial z^2} \right)$$

$$\sigma_z^l = (\lambda^l + 2\mu^l) \cdot [\alpha'^2 \cdot A'^+ + \alpha'^2 \cdot A'^-]$$

$$\sigma_z^l = (\lambda^l + 2\mu^l) \alpha'^2 \cdot [A'^+ + A'^-]$$  \hspace{1cm}  4.30

We rearrange Equations 4.29 and 4.30 in terms of matrices,

$$\left[ \begin{array}{c} \sigma_z^l \\ v_z^l \\ \frac{v_z^l}{\omega} \end{array} \right] |_{z=0} = \left[ \begin{array}{ccc} (\lambda^l + 2\mu^l) \alpha'^2 & 0 & [A'^+ + A'^-] \\ 0 & \alpha' & [A'^+ + A'^-] \end{array} \right]$$  \hspace{1cm}  4.31

Likewise, particle velocity and stress in layer II (biological cell) in the direction of the z-axis can be obtained by applying Equations 4.23 and 4.25 to Equations 4.17 and 4.18:
Particle velocity in Layer II,

\[ u_{z}^{II} = \frac{\partial}{\partial t} \left( \frac{\partial \Phi^{II+}}{\partial (z-d)} + \frac{\partial \Phi^{II-}}{\partial (z-d)} \right) \]

\[ \frac{\partial}{\partial t} \left( \frac{\partial \Phi^{II+}}{\partial (z-d)} \right) \bigg|_{z=0} = A^{II+} \omega (-\alpha^{II}) e^{j(\alpha^{II})(z-d)} \bigg|_{z=0} \]

\[ = \omega A^{II+} (-\alpha^{II}) e^{j(\alpha^{II})(-d)} \]

By using Euler’s formula for complex analysis:

\[ e^{j \alpha^{II} d} = \cos(\alpha^{II} d) + j \sin(\alpha^{II} d) \]

\[ \frac{\partial}{\partial t} \left( \frac{\partial \Phi^{II+}}{\partial (z-d)} \right) \bigg|_{z=0} = -\omega A^{II+} \alpha^{II} [\cos(\alpha^{II} d) + j \sin(\alpha^{II} d)] \]

Also,

\[ \frac{\partial}{\partial t} \left( \frac{\partial \Phi^{II-}}{\partial (z-d)} \right) \bigg|_{z=0} = A^{II-} \omega (\alpha^{II}) e^{j(\alpha^{II})(z-d)} \bigg|_{z=0} \]

\[ = \omega A^{II-} (-\alpha^{II}) e^{j(\alpha^{II})(-d)} \]

\[ \frac{\partial}{\partial t} \left( \frac{\partial \Phi^{II-}}{\partial (z-d)} \right) \bigg|_{z=0} = \omega A^{II-} \alpha^{II} [\cos(\alpha^{II} d) - j \sin(\alpha^{II} d)] \]

Therefore we have particle velocity in Layer II,

\[ u_{z}^{II} = -\omega A^{II+} \alpha^{II} [\cos(\alpha^{II} d) + j \sin(\alpha^{II} d)] + \omega A^{II-} \alpha^{II} [\cos(\alpha^{II} d) - j \sin(\alpha^{II} d)] \]

\[ -\frac{v_{z}^{II}}{\omega} = A^{II+} \alpha^{II} [\cos(\alpha^{II} d) + j \sin(\alpha^{II} d)] - A^{II-} \alpha^{II} [\cos(\alpha^{II} d) - j \sin(\alpha^{II} d)] \]

By rearranging the equation 4.42 in terms of matrices,

\[ -\frac{v_{z}^{II}}{\omega} = \begin{bmatrix} j \alpha^{II} \sin \alpha^{II} d & \alpha^{II} \cos \alpha^{II} d \end{bmatrix} \begin{bmatrix} A^{II+} & A^{II-} \end{bmatrix} \]

Stress in layer II,

\[ \sigma_{z}^{II} = \lambda^{II} \left( \frac{\partial^{2} (\Phi^{II+} + \Phi^{II-})}{\partial (z-d)^{2}} \right) + 2 \mu^{II} \left( \frac{\partial^{2} (\Phi^{II+} + \Phi^{II-})}{\partial (z-d)^{2}} \right) \]
\[
\sigma_z^{II} = (\lambda^{II} + 2\mu^{II}) \cdot \left( \frac{\partial^2 (\Phi^{II+} + \Phi^{II-})}{\partial (z-d)^2} \right)
\]
\[
= (\lambda^{II} + 2\mu^{II}) \cdot \left( \frac{\partial^2 (A^{II+} e^{i(\omega t-qx-\alpha^{II}(z-d))} + A^{II-} e^{i(\omega t-qx+\alpha^{II}(z-d))})}{\partial (z-d)^2} \right)
\]
\[
\sigma_z^{II} = (\lambda^{II} + 2\mu^{II}) \cdot [\alpha^{II2} \cdot A^{II+} + \alpha^{II2} \cdot A^{II-}]
\]

By solving the boundary equations, the set of first order simultaneous equations relating to the potentials can be obtained as follows:

\[
\begin{bmatrix}
-\sigma_z^n \\
\frac{v_z}{\omega}
\end{bmatrix}
|_{z=0} = \begin{bmatrix}
\lambda^L k_L^2 & 0 \\
0 & \alpha^L
\end{bmatrix}
\begin{bmatrix}
A^{II+} \\
A^{II-}
\end{bmatrix} + A^{II+}
\]
\[
\begin{bmatrix}
-\sigma_z^n \\
\frac{v_z}{\omega}
\end{bmatrix}
|_{z=d} = \begin{bmatrix}
\lambda^{II} k_L^{II2} & 0 \\
0 & \alpha^{II}
\end{bmatrix}
\begin{bmatrix}
A^{II+} \\
A^{II-}
\end{bmatrix} + A^{II+}
\]

where \(\lambda\) and \(\mu\) are Lame’s constants for each media; biological specimen, coupling medium, and substrate. Then, by solving the equations 4.23 and 4.24, the reflectance function can be obtained as follows [4]:

\[
2q\alpha^{III} A^{III+} + (q^2 - \beta^2) B^{III+} = 0
\]
\[
\frac{A^I}{A^{I^+}} = \frac{(C_1 - \gamma C_2) \cos \alpha_{II} d - i(\gamma C_1 - C_2) \sin \alpha_{II} d}{(C_1 + \gamma C_2) \cos \alpha_{II} d + i(\gamma C_1 + C_2) \sin \alpha_{II} d}
\]

where

\[
\gamma = \frac{\rho_I \alpha_{II}}{\rho^{III} \alpha_I}
\]

\[
C_1 = (k_s^{III}^2 - 2q^2)^2 + 4q^2 \alpha_{III}^2 \beta
\]

\[
C_2 = \frac{\rho^{II} \alpha_{III}}{\rho^{III} \alpha_{II}} k_s^{III}^4
\]

Once the reflectance function is determined by employing the above equations, the \(V(z)\) curve can then be simulated using either ray theory model or angular spectrum theory (wave theory) model, and the longitudinal velocity of the biological cells can be calculated [4].

### 4.3 Mechanical properties measurements for single cells and biological tissues

Scanning acoustic microscopy is capable of measuring the mechanical properties of a single cell’s interior such as the cytoskeleton, cytoplasm, and nucleus [39], [34]. All necessary parameters of a cell can be measured by employing the time-resolved acoustic microscope [28], [32], [40]. In time-resolved acoustic microscopy, a short pulse is emitted to the layered system consisting of coupling medium, cell, and substrate, and then the echo signal from the layered structure is recorded. This technique enables the measurement of cell thickness, variation of sound velocity, attenuation, and density inside the cell [28], [32], [40]. Time-resolved SAM with a center frequency of 500 MHz has been used to measure the elastic properties of thin fibroblast cells whose thickness did not exceed 5 \(\mu\)m [32]. Nevertheless, it was inadequate to distinguish the internal
structure of the cell with the resolution at 500 MHz. In order to achieve a better, possibly near optical resolution, and to measure the acoustic properties of different cell structures, a shorter pulse with a central frequency of about 1 GHz is required. There are, however, the high attenuation of sound in water in the gigahertz frequency range and the small differences in the acoustical impedance between cell and coupling medium. As a result, the echo reflected from the surface of the cell is weak and its amplitude becomes similar to the level of noise in the acoustical system [41], [42]. Therefore, the main challenge in constructing a time-resolved SAM operating at 1 GHz is to increase the signal-to-noise ratio. In 2007, Weiss et al. developed a new version of the high frequency, time-resolved SAM with an operating frequency in the gigahertz range to study dynamic processes in biological cells. The signal-to-noise ratio in the acoustical images was increased by averaging the detected radio frequency signal over 10 measurements at each scanning point. However, to conduct quantitative measurements of the acoustic parameters of cells, the signal had to be averaged over 2000 measurements. This technique allows for the acoustical properties of a single cell to be measured \textit{in vivo} and the elastic parameters of subcellular structures to be measured. [35]

### 4.3.1 Mechanical properties measurement by time-resolved SAM

Several SAM approaches have been developed to measure the acoustical parameters of cells. For example, Bereiter-Hahn et al. in 1990 revealed that sound velocity can be deduced from the sum and differences of the amplitudes in adjacent constructive and destructive interference fringes in high-frequency SAM images [43]. Kundu et al. in (2000) reported that longitudinal wave speed, attenuation, and the thickness profile of a biological cell can be obtained from the voltage versus frequency curve at a fixed depth or V(f, Z) curves [36]. Nevertheless, these techniques have some disadvantages. For example, the technique that measures the interference fringes has
limitations as it can only be used for the peripheral area of a single cell since the central part lacks the fringes, and it can only be applied for characterizing large cells such as XT2 fibroblasts (approximately 100 µm in length) [44]. The V(f) curve measurement technique has disadvantage as it requires the high accuracy of the position of the focus which is difficult to attain in practice [45].

Time-resolved acoustic microscopy used in quantitative measurement utilizes pulses that are adequately short to enable the reflections from the top and the bottom of the cell to be separated in the time domain [28], [32], [40]. Figure 4-3 represents notations for different echoes measured by the time-resolved SAM.

![Time-resolved SAM for quantitative measurements.](image)

As seen in Figure 4-3, \( t_0 \) is the arrival time of the echo reflected from the substrate outside the cell known as “reference echo”, \( t_1 \) is the arrival time of the echo reflected from the top surface of the cell known as “top echo”, and \( t_2 \) is the arrival time of the echo reflected from the cell-
substrate interface known as “bottom echo”. The time delays of different echoes can then be defined as follow. Let $\tau_1$ be the time delay between top and referenced echoes, $\tau_1 = t_0 - t_1$, $\tau_2$ be the time delay between bottom and reference echoes, $\tau_2 = t_0 - t_1$, and $\tau_{12}$ be the time delay between bottom and top echoes, $\tau_{12} = t_1 - t_2$. The thickness of the cell ($d$) at any scanning position of the microscope (x, y) can be computed using a simple geometrical optic formula once the time delay ($\tau_1$) is measured and the sound velocity in the coupling medium ($c_w$) is known:

$$d(x, y) = \frac{c_w \tau_1(x, y)}{2}$$ \hfill (4.32)

In addition, the time delay $\tau_1$ is a function of the scanning position (x, y) as $\tau_1(x, y)$. After the thickness of the cell is computed, the longitudinal sound velocity of the specimen can be determined by measuring either the time delay $\tau_{12}$:

$$c(x, y) = \frac{2d(x, y)}{\tau_{12}} = \frac{c_w \tau_1(x, y)}{\tau_{12}(x, y)}$$ \hfill (4.33)

or the time delay $\tau_2$:

$$\tau_2(x, y) = 2d \left( \frac{1}{c} - \frac{1}{c_w} \right) = \tau_1(x, y) \left( \frac{c_w}{c} - 1 \right)$$ \hfill (4.34)

$$c = c_w \left( \frac{\tau_2(x, y)}{\tau_1(x, y)} + 1 \right)$$ \hfill (4.35)

In the case that the velocity inside the cell is higher than that outside of the cell, $\tau_2$ will be negative. The cellular fluid often has the density and compressibility close to that of the coupling medium. The velocity of ultrasonic waves in the cellular fluid can be computed by taking account of...
contributions of various kinds of intra- and inter- molecular interactions [46]. Additionally, the longitudinal sound speed is also related to the elastic bulk modulus $K$ of the cell,

$$K = \rho c^2$$

where $\rho$ is the density of the cell and $c$ is the longitudinal sound velocity of the cell.

This bulk modulus is valid under the assumption that longitudinal wave velocities are much higher than shear wave velocities. This also applies for several cell types of which interior cellular fluid or material possess small shear modulus, such as myocytes which have a shear wave velocity about 15 times lower than that of longitudinal waves [28], [34], [39].

### 4.3.2 Time-resolved techniques and elastic microanalysis

Information about the elastic properties of biological cells and tissue sections can be obtained by using the time-resolved technique. Time-resolved measurements employ pulses that are sufficiently short to allow the reflections from the top and the bottom surfaces of a layer system [38]. The elastic properties of the layer then can be determined from the amplitude and arrival times of the two echoes. Figure 4-4 illustrates the echoes from such a situation; (a) is the reference echo from the glass substrate at defocus $z_0$, (b) is the reflected signal from an MCF-7 cell attached to a glass substrate.
Figure 4-4. RF- signals from time-resolved measurement. (a) Reference signal, $S_0(t)$, reflected from a glass substrate at focus without specimen. (b) Reflected signal, $S(t)$, from an MCF-7 cell attached to a glass substrate. (Experimental data by Eric Strohm and Michael Kolios, 2007)
The reference signal as shown in Figure 4-4 (a) can be expressed as [28]:

\[ S_0(t) \equiv A_0 r(t - t_0) \ast g(t, z_0) \]  

From Equation 4.37, the left-hand side of the equation, \( S_0(t) \) represents the measured value in the experiment, and the right-hand side of the equation describes an interpretation in terms of the quantities of interest. \( A_0 \) is the reflection coefficient at the water-substrate interface, \( r(t - t_0) \) is the two-way lens response disregarding any effects of focusing or attenuation in the coupling medium, \( g(t, z) \) represents the distortion to the wave-shape due to defocus and attenuation, the symbol \( \ast \) indicates the convolution operation which allows the primary waveform to be expressed by \( r(t) \), with adjustments as a result of the function \( g(t, z) \). Both of these functions can be obtained experimentally. For the waveform distortion function \( g(t, z) \), it may be arbitrarily set to unity at the defocus distance \( z = 0 \). Subsequently, it is possible to obtain the lens response function by measuring with the substrate at the focus of the lens. The measurements of the reflected signal can then be carried out for a range of values of \( z \). The function \( g(t, z) \) can be determined for each of the values of \( z \) by deconvolution due to \( r(t) \) is independent of \( z \). By applying the convolution theorem, deconvolution can be performed: first, the functions are Fourier transformed, then deconvolution is replaced by a division to yield \( \tilde{g}(f, z) \), and lastly \( g(t, z) \) can be obtained by taking inverse Fourier transform of \( \tilde{g}(f, z) \). It should be noted that when the defocus becomes positive (\( z > 0 \)), the shape of the waveform is almost independent of defocus over the related range of \( z \), as a result \( g \) will be a function of \( z \) only. Thus, the convolution in Equation 4.31 can be expressed by way of a simple multiplication by a constant determined by the value of \( z \) due to \( g \) is now independent of \( t \). The functions in Equation 4.37 can be complex functions. The function
\( r(t - t_0) \) can be restrained to be real variable because the measured function \( S_0(t) \) is real, which is consistent with setting \( g(t, 0) = 1 \). In the other cases of values of \( z \), the function \( g(t, z) \) would need to be allowed to be complex. Nevertheless, it is typically sufficient to set \( g \) as a real variable when the defocus is positive \((z > 0)\) [28].

There will be two echoes appeared when the lens moves toward a region of the substrate that is attached to a biological specimen: one from the top surface of the specimen, indicated by subscript 1, and one from the interface between specimen and the substrate, indicated by subscript 2. This signal is illustrated in Figure 4.5 (b) and can be expressed as:

\[
S(t) = A_1 r(t - t_1) \times g(t, z_1) + A_2 r(t - t_2) \times g(t, z_2)
\]  

It is sufficient, when the defocus becomes positive, to restrain the function \( g \) to be a real function of \( z \) only and be independent of \( t \), provided that the lens and substrate being used having been measured earlier just as in the case of the reference signal. It should be noted that this approximation works best when refraction in the layer is small, however, corrections for refraction in the thicker layer can be made as well. By ways of scanning through and measuring the minimum positive value at which the shape of the waveform is approximately constant as a function of \( z \), the optimum value of \( z \) can be achieved. Additionally, the amplitude is possibly varied but that is inconsequential because it is accounted for in the dependence of \( g \) upon \( z \). In addition, the large value of the defocus is undesirable due to the attenuation in the coupling fluid would then reduce the signal [28]. Equation 4.37 and 4.38 can be expressed, with the approximation of the independence of the waveform shape on \( z \), as:

\[
S_0(t) = A_0 r(t - t_0) \times g(z_0)
\]
\[ S(t) = A_1 r(t - t_1) \times g(z_1) + A_2 r(t - t_2) \times g(z_2) \]  \hspace{1cm} 4.40

In the case of the length of the pulse is less than the difference between \( t_1 \) and \( t_2 \); the two echoes from the top and the bottom surfaces, can be measured by calculating the normalized correlation of \( S_0(t) \) and \( S(t) \)

\[ C(t) = \frac{\int_{-\infty}^{\infty} S(t') S_0(t' + t) dt'}{\int_{-\infty}^{\infty} S_0(t')^2 dt'} \]  \hspace{1cm} 4.41

Generally, the limits of the integrals in Equation 4.41 are the range of available data in practice. The two peaks should be appeared in the correlation, corresponding to the optimum match between the reference signal \( S_0(t) \) and the two echoes contained in the signal \( S(t) \). In the case that the two echoes are sufficiently separated in time as in the case in Figure 4-4 (b), it is usually best to isolate them and determine their correlations separately, in particular if their magnitudes are greatly different. By knowing the height and position of each maximum, the four essential parameters; \( t_0 - t_1, t_0 - t_2, \frac{A_1}{A_0}, \frac{A_2}{A_0} \), can be measured. When the sound velocity in coupling medium \( c_w \), the acoustic impedance of the coupling medium \( Z_0 \), and attenuation (average over the bandwidth) \( \alpha_0 \) of the coupling medium and the impedance \( Z_s \) of the substrate are known, all of the acoustic properties of the layer can be calculated [28].

1. The thickness \( d \) of the biological cell can be calculated once the difference in time between the reference signal \( t_0 \) and the reflection from the top of the cell \( t_1 \) and the sound velocity in coupling medium \( c_w \) are known.

\[ d = \frac{1}{2} (t_0 - t_1) c_w \]  \hspace{1cm} 4.42
2. The longitudinal sound velocity in the cell \((c)\) can be determined from the time \(t_2\) of the echo from the interface between the cell and the substrate, and the times of the other two echoes.

\[
c = c_w \frac{t_0 - t_1}{t_2 - t_1}
\]

3. The impedance of the cell \((Z)\) can be calculated from the ratio of the magnitude of the reflection \(A_1\) from the top of the cell to the magnitude of the reference signal \(A_0\), and the impedance \(Z_0\) of the coupling medium.

\[
Z = Z_0 \frac{1 + A_1}{1 - A_1}
\]

4. The density of the cell \((\rho)\) can be determined once we know velocity and impedance of the cells calculated from Equation 4.43 and 4.44, respectively.

\[
\rho = \frac{Z}{c}
\]

5. The attenuation in the cell \((\alpha)\) can be calculated from the attenuation (average over the bandwidth) \(\alpha_0\) of the coupling medium, the amplitude \(A_2\) of the echo from the interface between the layer and the substrate and the amplitudes of the other two echoes, as well as the impedance of the substrate \(Z_s\), the impedance of the cell \(Z\), the impedance of the coupling medium \(Z_0\).

\[
\alpha = \alpha_0 + \frac{1}{2\pi} \log_e \left\{ \frac{A_0 Z_s - Z}{A_2 Z_s + Z (Z + Z_0)^2} \frac{Z_s Z_0}{Z - Z_s} \right\}
\]
The attenuation in the cell is in units of nepers per unit length, with attenuation taken as positive [28].

\( S_0(t) \) should be measured close to where \( S(t) \) is measured to yield the best results, and also on the same substrate material. In the case of two closely spaced echoes, the best accuracy in separating them can be achieved when the two signals are of comparable magnitude. This can be acquired if the ratio of the cell impedance to the coupling fluid impedance is slightly less than the ratio of the substrate impedance to the cell impedance, in order that the two terms in Equation 4.40 are of comparable magnitude, providing a positive defocus \( g(z_2) \) to be less than \( g(z_1) \). It is recommended that a polymer substrate should be used for biological cell and tissue, which would potentially support lateral longitudinal waves but not Rayleigh waves. However, lateral waves and surface waves are not typically a problem due to measurements of this kind are made at \( z \geq 0 \) [28].

In the case that two signals are so close together that they are not well separated in the correlation in Equation 4.42, it is better to describe the signals in the frequency domain and apply Fourier transformations, denoted by a bar. If the pulse shapes are independent of the defocus and that the frequency dependence of \( \bar{g}(z) \) can be neglected and let the left-hand term correspond to the measurement value and the right-hand term describe the quantities of interest, then the Fourier transformation of \( S_0 \) and \( S \) can be expressed as [28]:

\[
\bar{S}_0(f) = A_0 \bar{r}(f) g(z_0) \exp(i2\pi ft_0) \tag{4.47}
\]

and

\[
\bar{S}(f) = A_1 \bar{r}(f) g(z_1) \exp(i2\pi ft_1) + A_2 \bar{r}(f) g(z_2) \exp(i2\pi ft_2) \tag{4.48}
\]

One can enhance such data in the frequency domain by applying a Wiener filter. Each contribution, in the time domain, can be represented as a Dirac delta function \( \delta(t - \tau_m) \) with amplitude
$A_m$ convolved with the lens time-response $s(t)$. (The Dirac delta function $\delta(x)$ equals zero except when $x = 0$, and the area under the function is 1.) The values of $t_m$ and $A_m$ are required, which could be determined by deconvolving the lens time-response. Ideally, the Fourier transformation of the measured signal $\bar{s}(f)$ can be divided by the reference signal $\bar{s}_0(f)$ by the convolution theorem. However, there is noise in the system, which occasionally causes division by zero and contributes in wild distortions at frequencies where the reference signal is very small. As a result, it is necessary to multiply both signals with the complex conjugate of the reference signal to make the denominator become a real number and then the real number will be added to the denominator to assure good behavior when it reaches or goes below the noise level. When one applies a true Wiener filter, the noise power spectrum is added in the denominator whereas in a simpler pseudo-inverse filter, a constant $M$ is employed which is selected to be bigger than the maximum value of the noise power spectrum over the frequency range of interest. Therefore, the filtered signal with complex conjugate denoted by * is expressed as:

$$
\bar{S}_2(f) = \frac{\bar{s}(f)\bar{s}_0^*(f)}{\bar{s}_0(f)\bar{s}_0^*(f) + M}
$$  \hspace{1cm} 4.49

A shorter pulse in the time domain can be obtained by taking inverse transformed of the filtered signal and it can then be examined by the correlation from Equation 4.41. As a result of the constant $M$, the reference signal must first pass through the same Wiener filter as well.

In addition, Oppenheim and Schafer (1975) proposed another powerful approach for analyzing the Fourier transformed signals starting with cepstral filtering [28]. By taking the logarithm of the modulus of each of the two equations, and then the first is subtracted from the second, we obtain:
\[
\ln |\tilde{S}(f)| - \ln |\tilde{S}_0(f)| = \ln |A_1 \tilde{r}(f)g(z_1) \exp(i2\pi ft_1) + A_2 \tilde{r}(f)g(z_2) \exp(i2\pi ft_2) | \\
- \ln |A_0 \tilde{r}(f)g(z_0) \exp(i2\pi ft_0) | \quad 4.50
\]

In cepstral analysis, data are treated in the frequency domain as if it were in the time domain and vice versa [28]. By means of cepstral analysis, the value comes from the observation that the logarithm of the power spectrum of a signal including two echoes with an additive periodic component because of the presence of the two echoes. As a result, a peak at the time arrival between the two echoes appears in the Fourier transformation of the logarithm of the power spectrum. The additive component in the logarithm of the power spectrum obtains from a multiplicative component in the power spectrum itself, while the subtraction of the logarithms in Equation 4.50 corresponds to the division in Equation 4.49. The spectra \( \tilde{S}_0(f) \) and \( \tilde{S}(f) \) are normalized so that the two logarithms on the left-hand side of Equation 4.43 are of comparable magnitude in order to achieve the most accuracy in the following analysis. By applying logarithms, similar benefits to the factor \( M \) in Equation 4.49 can be obtained. The goal, as in any filtering procedure, is to improve the information that is of interest though the information has been discarded. By cancelling \( \tilde{r}(f) \) throughout the right-hand side of Equation 4.50, getting rid of the phase factor \( \exp(i2\pi ft_0) \) and letting

\[
A_1' \equiv \frac{A_1 g(z_1)}{A_0 g(z_0)}, \quad A_2' \equiv \frac{A_2 g(z_2)}{A_0 g(z_0)}
\]

by substituting \( A_1' \), \( A_2' \) into Equation 4.43, we obtain:

\[
\ln |\tilde{S}(f)| - \ln |\tilde{S}_0(f)| = \ln |A'_1 \exp(i2\pi ft_1) + A'_2 \exp(i2\pi ft_2) | \\
= \ln |A'_1 \{ \cos(2\pi ft_1) + i \sin(2\pi ft_1) \} + A'_2 \{ \cos(2\pi ft_2) + i \sin(2\pi ft_2) \} | \quad 4.51
\]
Then we multiply the right-hand side by the complex conjugate to acquire the modulus, thus we obtain:

\[
\ln|\tilde{S}(f)| - \ln|\tilde{S}_0(f)| = \frac{1}{2} \ln \left[ 2 \left( A_1'{}^2 + A_2'{}^2 \right) + A_1' A_2' \cos(2\pi ft_1) \cos(2\pi ft_2) + \sin(2\pi ft_1) \sin(2\pi ft_2) \right]
\]

4.52

Equation 4.5 above can also be expressed as

\[
\ln|\tilde{S}(f)| - \ln|\tilde{S}_0(f)| = \frac{1}{2} \ln \left[ B + C \cos(2\pi f(t_2 - t_1)) \right]
\]

4.53

where,

\[
B = A_1'{}^2 + A_2'{}^2 ; \quad C = 2 A_1' A_2'
\]

4.54

4.4 HeLa cells

A HeLa cell is an immortal cervix epithelial cell line that has been widely used as a standard sample in cancer research. The cell line was derived from cervical cancer cells taken from Henrietta Lacks, who died from her cancer on October 4, 1951. These cells proliferate rapidly, even when compared to other cancer cells. It has been revealed that HeLa cells have an active version of the enzyme telomerase during cell division, which inhibits the incremental shortening of telomeres that is involved in aging and eventual cell death. Therefore, HeLa cells avoid experiencing the Hayflick limit, which is the restricted number of cell divisions that most normal cells can undergo before dying out [73] [74].

The three phenotypic differences between normal and cancerous cell are cell size and shape distribution, nuclear-to-cytoplasmic volume ratio, and volume of extracellular space (D C Walker et al., 2003). These differences between normal and cancerous cells affect the mechanical
properties of those cells; the mechanical properties change with each phenotype change. Velocity of the longitudinal wave, density, thickness, and attenuation of the longitudinal waves inside of the cells can be determined from measurement data obtained by SAM. B-scan and C-scan images of the cells attained by SAM exhibit distinct patterns inside the cell at different stages of the cell cycle. For example, high frequency SAM imaging of dividing HeLa cells has revealed that acoustical images of the HeLa cells become darker during cell division as shown in Figure 4-5.

Figure 4-5. C-scan of HeLa cells obtained at defocus -10 µm. Cell A has almost a spherical shape indicating that it is most likely in the early stages of mitosis. Cells B, C have passed mitosis stage as their contrast is noticeably dark, and cell D is possibly in the interphase stage. (P.V. Zinin et al, 2007).

Figure 4-5 depicts the c-scan acoustical image of HeLa cells at different stages of the cell cycle. The dark contrast of the dividing HeLa cells was attributed to an increase of sound attenuation in the cytoskeleton. It was also found that attenuation increased after the cell division by 50%. The increase of the attenuation can be attributed to the polymerization of the f-actin [48].
**Morphological diagram of cervical squamous epithelium**

**Normal tissue**

Cervical squamous epithelium is composed of several layers of cells with various morphological characteristics. In addition, the layers of epithelium can be further divided into a number of different classes, based on stages of increasing maturation: basal (*stratum cylindricum*), parabasal (*stratum spinosum profundum*), intermediate (*stratum spinosum superficiae*) and superficial (*stratum corneum*).

When cells experience changes in morphology or function during their life span, this process is known as maturation. A cell will shift progressively from the basal layer towards the epithelial surface while maturation occurs; it also becomes gradually more flattened as well as nucleus reduces in size, but an overall increase in cell volume.

Basal cells are detached from the underlying stroma by a fine carpet of collagen fibers. The shape of basal cells is almost cuboidal. Additionally, basal cells possess the largest a nuclear-to-cytoplasmic ratio of normal squamous cells.

In addition, the cell junctions are varied throughout the squamous epithelium along with a non-uniform distribution of extracellular space. The tight junctions remove extracellular space and create a semi-permeable barrier between the lumen, where the membranes of adjacent cells are partly fused, and underlying tissue. Tight junctions are prevalent in the superficial layers close to the lumen [47], [51]. Different types of specialized contact areas of the cell membranes are noticed in the less mature layers of the tissue. The cell membranes in these layers are composed of large protein molecules or networks of microfilaments efficiently fused together the membranes of adjacent cells. Furthermore, the extra-cellular volume in these layers is considerably larger than that of in the superficial layer [47], [52], [53], [54].
Cervical intraepithelial neoplasia (CIN) is defined as a continuous spectrum of change, where the epithelium possibly become gradually more abnormal in appearance known as dysplastic. Histologists and gynecologists employ CIN to explain the changes that occurs in the squamous epithelium of the cervix as a precursor to an invasive carcinoma [47]. Transformation in the squamous epithelium usually involves with levels of abnormality as displayed in Figure 4-6.

Figure 4-6 illustrates an interpretation of the development from normal cervical epithelium phase through the precancerous phase to cervical carcinoma phase. This is known as cervical intraepithelial neoplasia (CIN) or squamous intraepithelial lesions (SIL). The conditions of CIN changes result in an increased nuclear-cytoplasmic ratio, an increased volume of extra-cellular space, and a decreased layer of flattened cells close to the surface [47], [49], [50].
There are three stages of CIN: CIN I, CIN II, and CIN III, which are based on the severity of the dysplasia, and the level of the epithelium affected. Nevertheless, there are no explicit quantitative descriptors for these stages. Hence it is very highly subjective for diagnosis. Table 4.1 provides a general guideline for diagnosis.

Table 4.1. General guideline for diagnosis three stages of cervical intraepithelial neoplasia (CIN) (D C Walker et al, 2003).

<table>
<thead>
<tr>
<th>Stage</th>
<th>Histological description</th>
</tr>
</thead>
<tbody>
<tr>
<td>CIN I: mild dysplasia</td>
<td>Dysplastic cells, which possess enlarged nuclei and poor maturation, show in bottom of 1/3 epithelium.</td>
</tr>
<tr>
<td>CIN II: moderate dysplasia</td>
<td>Abnormalities display in 2/3 of epithelium.</td>
</tr>
<tr>
<td>CIN III: severe dysplasia or carcinoma <em>in situ</em></td>
<td>Abnormalities exhibit throughout entire epithelial thickness.</td>
</tr>
</tbody>
</table>

The stages of CIN approximately associate with the proportion of the dysplastic cells to the total epithelial thickness. For example, for a typical CIN I, tissue will be normal throughout the upper two-thirds of its thickness, but enlarged nuclei possibly present in the lower third. In the case of CIN II, the lower two-thirds of the epithelium most likely appear abnormal. In the case of CIN III, abnormal nuclei and cell structures present throughout entire epithelial thickness including the surface of the tissue [47].

It seems that the most obvious tissue structural change due to the development of CIN is the loss of differentiation and stratification. Typically, the rate of cell production and proliferation in normal tissue is well controlled. On the other hand, the control mechanisms are disrupted and
cells may possibly grow at an abnormally fast rate, or experience unusual differentiation processes in neoplastic tissue. In addition, with the progress of CIN, the tendency to grow in volume of small and cuboidal basal cells becomes slowly deteriorate and the basal cells become less flattened towards the surface. This will ultimately result in tissue that is homogenous throughout its thickness in relation to distribution of cell sizes and shapes [47].

Furthermore, there is an indication of an increase in the nuclear-cytoplasmic ratio with increasing level of dysplasia. In general, this increase in nuclear size is also a common characteristic of cancer and precancerous development.

The increase in volume of extra-cellular space is also another major feature associated with the progress of CIN, even though it is imperceptible on the scale of Figure 4-6. Also, when severely dysplastic or CIN III stage takes place in the epithelium, micro invasion into the underlying stroma is most likely to occur as well as metastasis spreading via the lymphatic system. By making a diagnosis in the early CIN stages prior to such a critical stage is reached, it provides the better chance for successful treatment [47].

**Phenotypic characteristics of cancer cells.**

There has been a lot of research focused on identifying the phenotypic characteristics of *in vitro*-transformed cells that associate with the growth of a cancer *in vivo* [55].

The general characteristics of transformed malignant cells are shown in Table 4.2. It should be noted that a number of these changes as listed in Table 4.2 may be characteristic of certain rapidly proliferating cell populations and not be specific for the malignant state. Also, it should be emphasized that only a single criterion is not adequate to identify cancerous cells. That is to say some non-transformed cells have one or more of these characteristics whereas some transformed cells lack one or more of them. Taken together, however, these criteria appear to define a cell that
will become malignant. A number of these properties only associate with malignant cells growing in cell culture. Nevertheless, a number of the properties of transformed cells in culture are also characteristic of cancer growing in an animal or patient [55].

Although, the characteristics in Table 4.2 are not used in this research for SAM simulations, they are presented here so that the readers can see the general characteristics of transformed malignant cells in addition to the mechanical properties.

Table 4.2. Properties of transformed malignant cells growing in cell culture and/or in Vivo (R.W. Ruddon, 1995).

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cytologic alterations similar to those of cancer cells in vivo, as well as increased cytoplasmic basophilia, increased number and size of nuclei, increased nuclear/cytoplasmic ratio, including formation of clusters and cords of cells.</td>
</tr>
<tr>
<td>2</td>
<td>Changes in growth characteristics:</td>
</tr>
<tr>
<td></td>
<td>a. Immortality of transformed cells in culture. Transformed malignant cells become immortal as they can be passaged in culture indefinitely.</td>
</tr>
<tr>
<td></td>
<td>b. Reduced density-dependent inhibition of growth or loss of contact inhibition. Transformed cells often grow to a higher density than their normal counterparts. They possibly will pile up in culture rather than stop growing when they make contact.</td>
</tr>
<tr>
<td></td>
<td>c. Reduction of serum requirement. Typically, transformed cells need less concentration of serum or growth factors to replicate in culture than non-transformed cells do.</td>
</tr>
<tr>
<td></td>
<td>d. Loss of anchorage-dependence as well as acquisition of ability to grow in soft agar.</td>
</tr>
<tr>
<td></td>
<td>e. Loss of cell cycle control. Transformed cells are not able to stop in G₁ or at the G₁/S boundary in the cell cycle as they are subject to metabolic restriction of growth.</td>
</tr>
<tr>
<td></td>
<td>f. Resistance to apoptosis, or programmed cell death.</td>
</tr>
<tr>
<td>3</td>
<td>Alteration in cell membrane structure and function as well as increased agglutinability by plant lectins, changes in composition of cell surface glycoproteins, proteoglycans, glycolipids, and mucins; appearance of tumor-associated antigens; and increase uptake of amino acids, hexoses, and nucleosides.</td>
</tr>
<tr>
<td>4</td>
<td>Loss of cell-cell and cell-extracellular matrix interactions that promote cell differentiation.</td>
</tr>
<tr>
<td>5</td>
<td>Loss of response to differentiation-inducing agents including changed cellular receptors for these agents.</td>
</tr>
<tr>
<td>6</td>
<td>Altered signal transduction mechanisms, as well as constitutive rather than regulated function of growth factor receptors, phosphorylation cascades, and dephosphorylation mechanisms.</td>
</tr>
<tr>
<td>7</td>
<td>Increased expression of oncogene proteins as a result of chromosomal translocation, amplification.</td>
</tr>
<tr>
<td>8</td>
<td>Loss of tumor suppressor gene protein products due to deletion or mutation.</td>
</tr>
<tr>
<td>9</td>
<td>Genomic imprinting error that causes the overproduction of growth promoting substances.</td>
</tr>
</tbody>
</table>
10. Increased or unregulated production of growth factor.

11. Genetic instability leading to progressive loss of regulated cell proliferation, increased invasiveness, and increased metastatic potential. “Mutator” genes may be involved in this effect.

12. Alteration in enzyme patterns. Transformed cells have increased levels of enzymes involved in nucleic acid synthesis and produce higher levels of lytic enzymes, e.g., proteases, collagenases.

Chapter summary

In this chapter, the properties of biological cells and the measurements of these properties by means of using scanning acoustic microscopy techniques were explained. In addition, the contrast mechanism, velocity measurement, layer model and reflectance function of biological cells, the features of HeLa cells, including the characteristic of tissue structure associated with the progression of cervical intraepithelial neoplasia were discussed. In the next chapter, the principles of ultrasound transducer array will be presented.
Chapter 5

Background in Understanding Ultrasound Transducer Arrays

The ultrasound imaging technique was first applied to oceanography, military, and nondestructive evaluation purposes prior to medical imaging applications. An ultrasound imaging technique has been developed from the echo ranging method, which is similar to that employed by some animals such as dolphins and bats to determine distance from objects. The behavior of ultrasound waves propagating in tissue when reflected off of internal structures is similar to when sound is reflected off of the walls of a large room. It is quite simple for the image construction procedure theoretically, yet it is still complicated in practice [1] [56]. Nevertheless, it is not complicated by using modern technology to measure the time delay for the reflected echo traveling back to the source. Then the distance to the target can be calculated by using the measured time delay assuming the speed of sound is known. To construct an image, some variations of the time delay measurement techniques are utilized in ultrasound imaging methods.

The purpose of this chapter is to provide a review of ultrasound transducer array technology. Understanding this material is essential for grasping important concepts introduced in Chapter 7. The material here is provided for the convenience of the reader and no new innovations are claimed. Some readers may wish to skip directly to Chapter 6.

The most commonly used mode in ultrasound imaging is the B-scan. The B-scan imaging mode depicts a two-dimensional image of a slice from a volume of tissue; the two axes of the B-scan image are the scan depth from the transducer and the length of the transducer array. A modern B-scan system utilizes the use of a transducer array instead of a mechanically scanned single-element transducer. For example, one dimensional arrays consist of multiple small transducers that
are electronically switched, focused, and steered in order to develop the transmit and the receive beams [56]. This scheme of the focusing and steering procedure is referred to as beamforming which yields different results depending on the linear or phased array being used. The scanning and image formation procedure for an array is depicted in Figure 5-1.

Although the B-scan is the most widely used modality and can be employed in a variety of ways; ultrasound imaging also offers other imaging modes and various applications. For example, most imaging systems have electronics designed for detecting Doppler shifted frequencies, which can be used for distinguishing the blood flow or the tissue movement.

Figure 5-1. Scanning and image formation procedure for an array (Ritter, 2000).
As seen in Figure 5-1, the image can be acquired line-by-line as the following procedure. First, the transmit beamformer, together with the computer controller and the transmit demultiplexer, chooses the number of array elements for transmitting an ultrasound beam and consecutively determines the size of the active transmit aperture as well as the shape of the emitted acoustic field. A focused beam can then be acquired by exciting each element of the array with a time-delayed signal, which is similar to a relative time-delay from the center to the edge of the aperture provided by a lens in a mechanically focused transducer [56]. In order to diminish the side-lobe levels, increase the focal depth, or enhance the shape of the transmitted beam; apodization technique can be applied by the transmit beamformer. The most widely used approach of apodization is to apply amplitudes that vary across the active aperture such as a Hanning window function. It is also common to apply these three techniques of selecting the active aperture, applying time delays for focusing/steering, and applying apodization to the receive beamformer. Then the received signals are summed into a single RF line by the receive beamformer. After the signal leaves the receive beamformer, it will be amplified by time-gain compensation (TGC), which is utilized to compensate for signal losses caused by attenuation in tissue and the diffraction of the ultrasound beam. After that the amplified signal will be decreased its dynamic range by logarithmic compression so that both low-intensity echoes and echoes from strong reflectors are within a range of 40 dB [56]. Subsequently the envelope detection demodulates the signal and filters the carrier. Finally, the demodulated signal is adjusted to be displayed in the scan converter unit. This entire image formation procedure produces one scan line of information. In general, a typical image entails between 48 and 196 lines, and a real-time display requires approximately 30 per second for a frame rate [56]. There have been more developments nowadays in ultrasound imaging technology such as three-dimensional imaging, harmonic imaging, the used of contrasts agents, and elastograpy.
5.1 Transducers and Arrays

Transducers usually consist of one or more piezoelectric elements. The transducers convert electrical energy into acoustical energy and vice versa as a result of the piezoelectric effect. The basic properties of transducers can be explained in terms of single-element transducers [56], [57].

The piezoelectric effect was discovered by Pierre and Jacques Curie in 1880. It is the phenomenon that a crystal or a material such as quartz and tourmaline transforms it physical dimensions with regard to the application of an electric field, and conversely [58]. Typically, naturally occurring crystals have weak piezoelectric properties. The most popular piezoelectric material used in transducer is a polycrystalline ferroelectric ceramic, lead zirconate titanate (Pb(Zr,Ti)O$_3$ or PZT). PZT has very strong piezoelectric properties following polarization. The polarization process of a ferroelectric material is accomplished by heating the material to a temperature just above a certain level, depending on its Curie temperature, and letting it cool gradually where a strong electric field is applied by means of two electrodes in the direction in which the piezoelectric effect is desired. As a result, the dipoles along the direction of polarization are aligned [58]. There has been variety of ferroelectric materials developed. For example barium titanate (BaTiO$_3$) was among the first developed; lead metaniobate (PbNb$_2$O$_5$) and lithium niobate (LiNbO$_3$) have also been utilized. Furthermore, by depositing selected impurities or dopants, certain piezoelectric properties of PZT can also be improved [58].

When an electric field is applied to a piezoelectric material without strain or it is clamped, it generates the so-called the piezoelectric stress constant, $e$, in units of newtons/m$^2$ or coulombs/m$^2$. In addition, when an electric field is applied to a piezoelectric material without external stress, the transmitting constant or piezoelectric strain constant, $d$, in coulombs/newtons is produced. The relationship between these two properties is $e = c^E \cdot d$, where $c^E$ is the elastic constant of the material without an electric field. The open circuit electric-field per unit applied
stress is defined as the receiving constant, \( g \), in units of \( \text{v-m/newton} \). The piezoelectric material’s dielectric constant, \( \varepsilon \), relies upon the degree of freedom of the material [58]. In the case that the material can move without restraint, the dielectric constant measured is defined as \( \varepsilon^T \). The relationship between the transmitting constant and the receiving constant is expressed as \( d = g \varepsilon^T \). On the other hand, if material is clamped so that it cannot move when a field is applied; the dielectric constant measured is called the clamped dielectric constant, \( \varepsilon^s \).

The electromechanical coupling coefficient (ECC) is used to exhibit the ability of a material to convert energy [58]. The ECC is determined by [58]:

\[
ECC = \frac{\text{stored mechanical energy}}{\text{total stored energy}}
\]

The ECC indicates the performance of a material as a transducer because only the stored mechanical energy is useful. The ECC, however, is different from the efficiency of the transducer. For example, it is considered that when the transducer is lossless, its efficiency is 100%. However, it’s not required for the ECC to be 100% since part of the energy is possibly stored dielectrically, a form of potential energy other than that which is being stored as mechanical energy [58]. The relationship between the ECC and the piezoelectric stress constant, \( e \), is expressed as [58]:

\[
ECC = \frac{e^2}{cR} \varepsilon^s = \frac{\varepsilon^T}{\varepsilon^s} - 1
\]

Other type of materials that have also been discovered and utilized in various applications is piezoelectric polymers such as polyvinylidene difluoride (PVDF), which is semicrystalline. PVDF has some advantages as a transducer, for example it is economical, flexible, and has a relatively high receiving constant. Most of the transducers with a wide operating bandwidth can be
simply fabricated from PVDF. There are, however, some drawbacks for this material such as it possesses a very low transmitting constant, substantial dielectric loss, and low dielectric constant. In addition, piezoelectric composite materials have been developed and become the most potential cutting edge materials in transducer technology. For example, the development in fabrication technology for PZT polymer 1-3 composites, which is composed of small PZT rods embedded in a low-density polymer, for applications in the range of 1 to 7.5 MHz. Nevertheless, the fabrication cost for the composite materials is expensive. Furthermore, it has also been found that fine-grain PZT and single-crystal ferroelectric materials are promising materials for high frequency applications such as Pb(Zn$_{1/3}$Nb$_{2/3}$)O$_3$-PbTiO$_3$ (PZN-PT) and Pb(Mg$_{1/3}$Nb$_{2/3}$)O$_3$-PbTiO$_3$ (PMN-PT), which exhibits a higher ECC and a similar dielectric constant compared to conventional PZT [58].

5.1.1. Single element transducer

A single element piezoelectric transducer usually consists of five parts; the electrical impedance matching network, the backing material, the piezoelectric material, the matching layers, and a lens for focusing the beam. The detail of each part will be explained next. Figure 5-2 depicts a cross section of a typical single element transducer [56].
From Figure 5-2, the transducer has the electrical impedance matching circuit for providing the matching electrical impedance of the transducer to the impedance of the drive/receive electronics [56]. A 50 ohm source is usually used to generate an electrical impulse excitation in most devices. On the other hand, when the devices are switched to serve as a receiver, subsequently they drive a 50 ohm load. The electrical tuning should be effective for the entire passband of the device since broad bandwidth is required for these transducers. Additionally, to acquire nominal 50 ohm real impedance at the center frequency, the dielectric permittivity and area of the piezoelectric must be chosen. After that a series or shunt tuning inductor can be applied to eliminate capacitive reactance. The impedance match can be calculated by employing equivalent circuit models [56] [59].

Next, the backing material located on the top of the piezoelectric provides three crucial functions to the transducer: reinforcing structural support for piezoelectric crystal, decreasing the mechanical Q of the device, and attenuating back-reflections [56] [59]. The backing material gives the fragile piezoelectric material reinforcement. Furthermore, backing material that has an acoustic
impedance close to that of the piezoelectric material can be used to damp vibrations and decrease the overall Q for the device. The small value of Q can enhance image resolution because of a shorter time-domain response [56] [59]. Nevertheless, the low Q property reduces the transmit sensitivity by way of absorbing energy that could otherwise be transmitted into tissue. In addition, the attenuation of any mechanical excitation coupled to the backing material so that any spurious signals would reflect within the backing material itself. Therefore, most backing materials are made of soft polymers filled with particles or gas-filled bubbles to yield maximum attenuation. There are some other minor functions of the backing materials such as contributing an electrical interconnection to the element as well as heat dissipation [56] [59].

The next component, the piezoelectric material serves as a resonant device, whereby its thickness regulates the frequency of vibration. There are thin electrode layers deposited on both faces of the piezoelectric material where an electric field will be applied. Moreover, these layers are wired to the impedance matching circuit. The doped lead zirconate titanate compositions (Pb(Zr,Ti)O$_3$), or PZT are the most commonly used piezoelectric materials because they possess a range of dielectric constants and high piezoelectric coefficients. Furthermore, a number of relaxor ferroelectric materials with outstanding piezoelectric properties have also been explored and utilized, such as single crystals of the solid solution of Pb(Zn$_{1/3}$Nb$_{2/3}$)O$_3$ –PbTiO$_3$ and Pb(Mg$_{1/3}$Nb$_{2/3}$)O$_3$ –PbTiO$_3$ [56] [59].

Next, the matching layers, which can be composed of one or more layers, located in between the piezoelectric material and the tissue. They perform as transformers, which is similar to the transmission line transformers used in communication theory [56] [59]. The matching layers with a quarter of a wavelength thickness can transform the low acoustic impedance of tissue (1.5 MRayls) to match the high acoustic impedance of the piezoelectric (> 25 MRayls). It should be noted that in order to acquire the maximum sensitivity and shortest ringdown, it is critical to properly choose the thickness and acoustic impedance of the matching layers. Matching layers are
usually the composites made from polymers and small particles of graphite, aluminum oxide, silver, or tungsten [56] [59].

In the case of transducer arrays, the same basic structure as the single-element device is also applied. However, each element of the arrays is designed to perform as a single, isolated, transceiver. One-dimensional linear and phased arrays, multi-dimensional arrays (such as 1.5-D, and fully sampled 2-D arrays), and annular arrays are the most widely used arrays types [59]. The advantages of the multi-dimensional arrays are that they can enhance image resolution and improve image quality. Annular arrays also provide excellent image quality, but they require mechanical scanning in order to construct a two-dimensional image [59].

The last component of the transducer, a lens is utilized for focusing the beam. It should be noted that the lens material must be carefully chosen so that the acoustic impedance of the lens matches the acoustic impedance of the human tissue. The shape of the emitted field can be calculated by using diffraction theory. For example, in the case of a planar source, the beamwidth at the transition from the Fresnel to the Fraunhofer zone will be too wide to achieve good lateral resolution in the image. A narrower shape beamwidth at the focal point can be acquired by using either a focused element or a focusing lens. The depth-of-field will be, however, limited as the image resolution is improved [56] [59].

5.1.2 Arrays

Arrays are transducers composed of a number of piezoelectric material elements. For example, a linear or 1-D array consists of rectangular piezoelectric elements aligning to each other; a 2-D array typically has a square-shaped and the elements are assembled in rows and columns; an annular array has a ring-shaped and the elements are arranged concentrically, as shown in Figure 5-3.
Figure 5-3. Geometry of (a) a linear array, (b) 2-D array, and (c) an annular array (Shung and Zipparo, 1996).

To operate a linear array, voltage pulses are applied to groups of elements in sequence as shown in Figure 5-4. By electronically moving an ultrasound beam across the face of the transducer, an ultrasound image can be generated similar to that achieved by manually scanning a single element transducer [58]. A real-time image can be produced in this manner if the electronic sequencing or scanning is recurring fast enough, approximately 30 frames per second. Typically, linear arrays are composed of 128 to 512 elements and are approximately 1 cm wide and 10 to 15 cm long [58]. The linear array composed of a backing material, a piezoelectric material inserted between two electrodes, and two matching layers as illustrated in Figure 5-5.
Figure 5-4. A group of linear array elements are excited simultaneously generating a sharper ultrasonic beam (Shung and Zipparo, 1996).

Figure 5-5. Schematic diagram of the acoustic stack of an array (Shung and Zipparo, 1996).
The gap between the centers of two elements is called the pitch and usually ranges from $\lambda/2$ to $3\lambda/2$ or greater, where $\lambda$ is the wavelength in the medium into which the ultrasound is fired. It should be noted that the pitch for a linear array is not as crucial as in a phased array [58]. The space between two elements is defined as the kerf, which is often etched into layers as well as backing material and can be filled with air or an acoustic isolating material in order to diminish acoustic crosstalk between adjoining elements [58].

The structure of the phased array is analogous to the linear array; however, it operates in a different way. Generally, a phased array is composed of about 64 to 256 piezoelectric elements, and its size is smaller than the size of the linear array. A phased array produces the ultrasonic beam that can be both focused and steered by appropriately delaying either the transmission signals or the receiving signals. Figure 5-6 shows the dynamic focusing and steering of an ultrasonic beam by a phased array [56] [58].

![Dynamic focusing and steering scheme of a phased array](image_url)

Figure 5-6. Dynamic focusing and steering scheme of a phased array (Shung and Zipparo, 1996).
From Figure 5-6, let the path length difference between the center element and element number \( n \) be \( \Delta r_n \), at a point \( P(r, \phi) \), the time difference is expressed by [58]:

\[
\Delta t_n = \frac{\Delta r_n}{c} = \frac{x_n \sin \phi}{c} + \frac{x_n^2}{2cr}
\]  

5.3

The \( \frac{x_n \sin \phi}{c} \) and \( \frac{x_n^2}{2cr} \) terms on the right side of the equation represent the time differences due to focusing and steering, respectively. In order to get the pulses to arrive at point \( P \) concurrently, the excitation pulse at the center element must be delayed by a time, \( \Delta t_n \), relative to the excitation pulse at the element \( n \). The far field radiation pattern of an array consisting of \( N \) elements can be expressed as [58]:

\[
p(u) = \text{sinc} \left( \frac{bu}{\lambda} \right) \cdot \sum_{m=1}^{N} \delta \left( u - m \frac{\lambda}{g} \right) \ast \text{sinc} \left( \frac{Lu}{\lambda} \right)
\]  

5.4

where \( u = \sin \phi \), \( p(u) \) is the pressure at an angle of \( \phi \), \( b \) is the width of the element, \( g \) is the pitch, \( L \) is the length of the array, the symbol * represents convolution, \( \delta \) is the delta function, and sinc indicates the sinc function = \( \frac{\sin x}{x} \). Grating lobes, high side-lobes for arrays with consistently spaced elements, often occur at certain angles due to constructive interference. These angles can be expressed in terms of the wavelength and the pitch as [58]:

\[
\phi_g = \sin^{-1} \left( \frac{n \lambda}{g} \right)
\]  

5.5

where \( n \) is an integer = \( \pm 1, \pm 2, \cdots \). In the case that the pitch \( g \) is smaller than \( \lambda/2 \), the grating lobes occur at angles greater than 90°; therefore, the array is considered as being fully sampled. The width of the element, \( b \) can be used to determine the magnitude of a grating lobe with respect to the main lobe. The smaller the width of the element, the larger the relative magnitude of the grating lobe.
There are several techniques to restrain the grating lobe such as randomizing the spaces between elements, which distributes the energy of the grating lobe in all directions yielding a “pedestal” sidelobe [58].

Typically, linear arrays can be focused and steered only in the azimuthal plane. A lens is usually employed to focus in the elevation plane perpendicular to the imaging plane, and thus the slice thickness of the imaging plane can be determined [56] [58]. The contrast resolution normally degrades in the near field and beyond the focal zone due to the broadened beam width in these two regions. To resolve this problem, multidimensional arrays such as 1.5-D or 2-D arrays are employed. An example of a 1.5-D design is illustrated in Figure 5-7.

![Piezoelectric element](image)

**Figure 5-7.** An example of 1.5 D array (Shung and Zipparo, 1996).

The additional elements of 1.5-D arrays in the elevation are utilized to offer limited dynamic focusing. However, these additional elements raise the number of electronic channels and complexity in array fabrication. Additionally, there are apprehensions associated with 1.5-D arrays
that do not occur in 1-D arrays: an augmented aperture size/footprint, and grating lobes in the
elevation plane as a result of the small number of elements [58].

Smith et al. at Duke University studied the two-dimensional arrays to operate high-speed
3-D ultrasonic imaging [60], [61]. According to their study, their 2-D array design, which consists
of 42x42 (1764 elements) at 2.5 MHz with 484 elements wired, has suffered from difficulty with
electrical interconnection because of the large number of elements and channels. Additionally, a
low signal-to-noise ratio on account of electrical impedance mismatching, as well as small element
size has caused problems. To resolve the interconnection problem and array stack design, the use
of fiber optics and multilayer architecture are promising options [61].

Biplane focusing can also be acquired by using an annular array. Focus through the field
of view can be achieved by applying proper externally controllable delay lines or dynamic focusing.
In addition, the width of each annulus can possibly be adjusted to sustain the beam intensity over
the dynamic focal zones. Nevertheless, annular arrays have a major drawback in that is mechanical
steering must be operated in order to produce two dimensional images [58].

5.2 Focusing

5.2.1 Axial and Lateral Resolution

Axial resolution is defined as the resolution along the axis of the ultrasound beam. It is a
measurement of the ability of an ultrasound system to distinguish echoes from distinct structures
located at different depths along the axis of the beam and display them separately in the ultrasound
images. There are some other factors that affect axial resolution such as gain settings, the selected
size of the field of view, and the technique of echoes processing. Additionally, the axial resolution
is mostly governed by the physical length of the pulses being used to form the beam which is known
as the spatial pulse length (SPL) [62]. The SPL depends on several factors such as transducer frequency, transducer design, damping material, and the amplitude of the excitation impulses. The SPL is also determined by the depth of the field of view in some cases; as an example some systems employ longer pulses with larger energy content to obtain deeper penetration and thus switching to a lower frequency can be avoided. The best theoretical resolution achievable is half of the SPL [62]. If the interfaces are closer together than half of the SPL along the axis of the beam, the leading edge of the echo from the second interface will overlap the tail of the echo from the first interface. As a result, the receiving circuits will perceive one long echo rather than two separate echoes and the interfaces will not be resolved. In order to get the two interfaces to be resolved, the interfaces must be at least half the SPL apart so that the two echoes can be separated in time and thus the receiving circuits distinguish the two separate signals [62]. The relationship between axial resolution and SPL can be expressed as [62]:

\[
Axial\ Resolution\ (AR) = \frac{SPL}{2}
\]

The relationship above implies that the axial resolution limit can be made extremely small by making the pulse extremely short. Nevertheless, it is not practical to improve the axial resolution in this manner because when the level of damping is increased; the energy content of the pulse is diminished and as a result the depth of penetration is decreased. Therefore, there is a trade-off between enhancing axial resolution and maintaining sufficient penetration depth [62]. The frequency of the transducer determines the wavelength of the sound, thus affecting the axial resolution. Higher frequencies are associated with shorter pulses. As the frequency increases, the wavelength decreases, and the SPL also decreases. A rule of thumb rule is the higher the frequency, the better the axial resolution capability. The axial resolution can be calculated for various
transducer frequencies if the number of wavelengths in each pulse is known by using the relationship, \( c = \frac{\lambda}{f} \), where \( c \) is the speed of sound in a medium, \( \lambda \) is the wavelength, and \( f \) is the frequency. For example, the wavelength for 5 MHz sound in soft tissue is 0.31 mm; if a pulse consists of four wavelengths, then the SPL is approximately 1.2 mm, and thus the axial resolution limit is approximately 0.6 mm. In the case that a pulse consists of only two wavelengths, then the SPL is approximately 0.6 mm and the axial resolution limit is about 0.3 mm. Two different 5 MHz transducers can provide different axial resolution limits depending on the length or a different number of wavelengths of the pulses the transducers produce. It should be emphasized that the governing factor for the axial resolution is the spatial pulse length, not the frequency.

Because the pulse length does not change considerably with depth, the axial resolution does not change with increasing distance from the transducer face. However, it is affected by the size of the field of view (FOV) selected [62]. At a particular FOV setting, the echoes from two close interfaces may be exhibited in adjacent pixels and consequently not resolved. If the FOV is decreased by using more pixels per unit area of patient, echoes are then spread out more in the display and spatial resolution is improved. This relates to the selection of the field of view of any given image [62]. The axial resolution is limited by the SPL for a small field of view. For a large FOV, it is limited by the distance represented by each pixel which changes with FOV. The two echoes must be separated by two or more pixels in order to be distinguished in an image display. As the FOV increases, the distance represented by two pixels also increases [62].

In addition, the output power of the transducer slightly affects the axial resolution. The larger the amplitude of the voltage impulse, the longer the time of crystal vibration. The longer the pulse leads to slightly decreasing the axial resolution.

Furthermore, gain settings also impinge on the axial resolution indirectly because they have an effect on the length of the voltage signals produced by echoes striking the crystal of the
transducer, even though they have no effect on the transmitted pulse length. As a result, the higher the gain, the poorer the axial resolution.

The other factor that can affect the axial resolution is the beamwidth. As depicted in Figure 5-8, when the specular interface is angled to the beam, the finite beamwidth results in echoes from the interfaces being displayed over a certain distance along the central axis where echoes supposedly occur and each interface remains within the beam. This happens due to the angle of the beam to the interfaces. For a narrow beam, the echoes from each interface are separated, hence they can be resolved. For a wide beam, there is an overlap of the echoes from each interface, thus only one long echo would be distinguished instead of two separate echoes [62].

![Figure 5-8. Beamwidth and approaching angle affect on the axial resolution (Gent, 1997).](image)

Consequently, the most excellent axial resolution can be acquired by using the highest possible transducer frequency which is the major factor affecting the axial resolution; applying proper power and gain settings; and choosing appropriate selection of the field of view.
The axial resolution ability of a transducer can be determined in practice by scanning the closely spaced wires in an ultrasound test phantom [62]. There is a depth for which no axial resolution exists known as the dead space with some transducers, especially mechanical sector types. The dead space is caused by a combination of reverberation effects and crystal recovery time. The dead space can also be measured using a phantom by scanning a series of superficial wires and determining the distance that the interface must be located from the transducer surface before it can be resolved [62].

Lateral resolution is the resolution perpendicular to the beam axis. It is a measurement of the ability of the system to separately display echoes occurring from different interfaces located at the same depth from the transducer, but at different positions across the beam perpendicular to beam axis. Compared to the axial resolution, the lateral resolution capability of a diagnostic ultrasound system is normally much inferior [62]. The comparatively poor resolution in the lateral dimension associates with the finite beamwidth of an ultrasound. The finite width of an ultrasound beam has an effect on linearity of the echoes in a sonogram to some extent. For example, in the case of the echo from a point reflector is displayed as a line in the sonogram instead of a dot. The echo is recorded for as long as the interface stays within the beam as it sweeps throughout the region [62]. The echo is written in memory as the line is also moving to response to the instantaneous position of the central axis of the beam. Consequently, the point reflector will reflect an echo over a finite time, which is the time of passage of the beamwidth through the reflector. As a result, the echo will be recorded as a line on the display; the larger the beamwidth, the longer is the line as illustrated in Figure 5-9 [62].

It should be noted that the axis of each linear echo is always perpendicular to the axis of the beam as the echo being recorded. Thus, when scanning with a sector transducer, there will be oblique lines due to the radial arrangement of the lines of sight [62].
Figure 5-9. (a) Linear echoes from point reflectors (b) Point reflectors and their separation relative to the beamwidth resulting in the conditions of resolved or not resolved lateral resolution (Gent, 1997).

For any given position of the beam, all received echoes take place from its central axis responding to the single line along which echoes are recorded into memory. As a result, separate interfaces located at the same depth and within the beam at the same time as it sweeps through the
region will each produce echoes assumed to have originated from the same point at the central axis. Thus, only one echo will be recorded [62].

In the case that the interfaces are placed further apart than the width of the beam while it sweeps through the region including the reflectors, there will be a period during which neither reflector is within the beam and no echo will be recorded. This creates a space between the recordings of an echo from each interface and two separate echoes will exhibit on the display. That is to say, in order to achieve the resolved lateral resolution, the interfaces must be further apart than the width of the beam [62]. The lateral resolution can be calculated from:

$$Lateral\ Resolution = (f\#)\lambda$$

where $f\# = \frac{focus\ distance}{aperture\ length}$ and $\lambda$ is the wavelength of the ultrasound.

The beamwidth is a major factor affecting the lateral resolution. The beamwidth can be determined by several factors as well as the diameter of the aperture, transducer frequency, focus, and dynamic range [62]. As the diameter of aperture increases, the beamwidth also enhances close to the transducer face. In terms of the frequency, the higher the frequency beams, the more directional the beams are along with longer near field and less divergence. Focusing scheme also affects the beamwidth either by using single or multiple zones; the beamwidth is not uniform with depth particularly with a single focal zone. In addition, a higher dynamic range improves beamwidth as well. Furthermore, the output power setting has an effect on the beamwidth; higher power slightly enhances the width of the beam. Additionally, the gain setting affects the beamwidth; the beamwidth can be increased efficiently by adjusting high gain setting [62].

The beamwidth also changes with the depth of ultrasound beams and thus the lateral resolution varies with depth as well. Therefore, it should always be asserted for the depth at which
the measurement of lateral resolution capability is made. The best lateral resolution can be achieved where the beam is narrowest, which is in the near field for a non-focused transducer or in the region of the focal zones for a focus transducer [62].

In conclusion, when the echoes reflected from two targets either in the axial or in the lateral direction can be distinguished by the use of the emitted pulse duration and the beamwidth of either -3 dB or -6 dB beamwidth, the axial and lateral resolutions of a transducer can then be evaluated, respectively. The axial and the lateral resolutions of a transducer can be enhanced by means of increasing the bandwidth, utilizing backing and/ or matching and focusing, correspondingly. Additionally, the spectrum of an ultrasonic pulse is frequency dependent and also varies as it propagating through the tissues. As the ultrasound beam propagates deeper through the tissue, its center frequency and bandwidth will diminish and thus deteriorate the axial resolution. In commercial scanners, time-gain setting controls pulse shape and duration including some signal processing [58] [59] [62].

5.2.2 Focusing

Focusing is the most widely used technique to enhance imaging quality for diagnostic ultrasound transducers or to focus acoustic energy at a target for the therapeutic ultrasound transducer. For diagnostic ultrasound, the image resolution can be improved by ways of focusing in the plane along the line of the aperture [63]. In addition, for the therapeutic ultrasound, the acoustic pressures and intensities involved can be enhanced within the focal zone. Generally, there are two main focusing techniques; by applying an electronic delay, and by applying an acoustic lens at the face of the transducer. Figure 5-10 illustrates the two focusing schemes.
As depicted in Figure 5.10 (a), an acoustic lens can provide focusing in the elevation direction by controlling the contributions of the pressure field from all elements of the aperture to reach at the field point simultaneously. Nevertheless, there is a drawback of using an acoustic lens for that the focal point cannot be adjusted. The continuous phase delay, $\phi_n$ in the unit of radian, along the elevation direction of each element varies with the thickness of the lens and can be written as [72] [75]:

$$\phi_n = \frac{2\pi d_{lens}(n)}{\lambda_n}$$

where $d_{lens}$ is the thickness of the lens in the elevation direction divided by the number of arrays [m], and $\lambda_n$ is the wavelength of the lens material [m]. By summing the Green’s function of each ultrasound field from a simple virtual source of the elevation position responding to a phase delay, we can determine the total ultrasound field, $p$, in the unit of Pa as follow [72] [75]:

$$p = \sum_{n=1}^{N} \frac{e^{i(kR_n+\phi_n)}}{R_n}$$

where $k$ is the wave number [m$^{-1}$], and $R_n$ is the distance between the field point and the position of a simple virtual source [m].
For electronic focusing as seen in Figure 5-10 (b), a time-delay is applied to each element to produce individual ultrasound beams. Focusing during transmission can be performed by adding delays to the excitation pulse of each element of the aperture. The aperture then transmits a concave shaped ultrasound beam which is focused at the focal point and determined by the time-delay. The focal depth for electronic focusing can be adjusted by changing the time-delay applied to the individual element. The time-delay \( t_i \) for the physical element \( i \), can be calculated from [72] [75]:

\[
t_i = \frac{1}{c} \sqrt{(x_i - x_f)^2 + (y_i - y_f)^2 + (z_i - z_f)^2}
\]

where \( c \) is the speed of sound [m/s], \( (x_i, y_i, z_i) \), is the position of the center for the physical element \( i \) [m], and \( (x_f, y_f, z_f) \), is the position of the focal point [m] [75].

### 5.3 Ultrasound Imaging

The most widely used ultrasonic imaging modes are B-scan and C-scan. Depending on a scan technique and a scan modality, the B-scan and the C-scan can be further classified. For example phased array scanning technique or mechanical scanning technique as well as a linear scan mode, or a sector scan mode and so on. Additionally, ultrasound imaging can also be categorized into real time or non-real time modes as well.

In this section 5.3, the two main categories of ultrasound imaging modes; B-scan and C-scan will be explained respectively.
5.3.1 B-scan

B-scan, also known as a “2D mode”, is a brightness-modulated image, providing a two dimensional image in which the depth along the \( z \) axis and the azimuth along the \( x \) axis, as well as a cross sectional reflection image of the target being scanned. Here the excellent reference of Havlice and Taenzer [64] is cited. The interested reader may wish to look at this reference directly for additional details.

The transducer usually performs both as a transmitter for transmitting the acoustic beam which is swept through a region, and as a receiver for positioning the received echoes to construct a B-scan image and display on a monitor such that the display scan line associate with the direction of the acoustic propagation in the tissue can be observed [64].

One of the dimensions of the B-scan image can be deduced from the arrival time of echoes of a short acoustic signal being reflected from the targets along a straight line path. The echoes from the targets near the transducer arrive earlier than the ones from the targets further away from the transducer [64]. Another dimension of the B-scan image in a transverse direction can be achieved by either mechanically or electronically moving the transducer to transmit another short acoustic signal and receive the echo from the target, producing a different straight line path. This B-mode image scanning procedure is carried on until the transducer completes scanning the entire region of interest. It is necessary to utilize some techniques for tracing the propagation path through the target to efficiently delineate the image [64].

A gray-scale display is considered as one of the most significant developments in acoustic imaging and typically encompasses 10 or more distinct brightness levels. In general, the brightness levels are assigned by the imaging system; starting from a small range of echo intensities and then dispensing into the brightness levels. For instance, the brighter brightness levels indicate the strong echo intensities whereas the lower brightness levels imply the weaker echo intensities [64].
In addition, there is another development of ultrasound imaging display; a color display. The color display has also been employed with different echo intensities levels being exhibited as different colors. However, compared to a gray-scale display, a color display provides images with quite dramatic appearances, but there is no more information in such a color display than in a gray-scale display with the same number of distinct brightness levels [64].

A projection of B-scan slices to yield a 3D volumetric image is defined as a P-scan display mode which can be generated by cascading display of parallel cross section plans across from top to the bottom at which the slices are free selectable [78]. Later in Chapter 7 and Chapter 8, the volumetric images are presented as a P-scan display mode.

5.3.2 C-Scan

A C-scan image displays a two-dimensional orthographic image of an object as the scanning plane is fixed at a constant depth. It should be noted that the arrival time plays no important role in either of the two dimensions of a C-scan image, on the contrary with the B-scan, of which one dimension of the image is deduced from the arrival time of an acoustic pulse. In addition, C-scan images are similar to images acquired by an X-ray fluoroscopy. As a result, the C-scan images are frequently more readily interpretable as compared to B-scan images [64].

An image plane for C-scan imaging can be selected by means of time gating the received echo waveform. The time-gating circuit unit operates as an on-off switch; when there is no trigger pulse, the time-gating circuit is turned off; but it can be turned on when there is an excitation pulse for a time duration controlled by the pulse length. To obtain a C-scan image, a time gate is triggered by the electronic pulser and delayed by a time period equivalent to the depth. In the C-scan image, the X-Y plane represents a plane parallel to the scanning plane of the transducer and perpendicular
to the ultrasonic beam, whereas in B-scan image the X-Y plane being scanned is a plane containing the ultrasonic beam [64].

Chapter Summary

In this chapter the principle of ultrasound transducers including single element transducer and arrays was explained. Additionally, focusing schemes for ultrasound transducers as well as the image resolution, namely the axial resolution and the lateral resolution were described. Furthermore, the types of ultrasound imaging modes were also explained. In the next chapter, methods and simulations used in this research will be discussed in detail.
Chapter 6

Methods and Simulations

6.1 Calculation of reflectance function for biological cell and substrate

The reflectance function for the biological cell and substrate system is calculated by employing the layer model as described in Chapter 4, section 4.2. Here, biological specimens were treated as thin films with near zero shear modulus attached on a substrate. The biological specimens used in the calculations were kidney tissue, HeLa cells, and MCF-7 breast cancer cells. The substrates used in the calculations were fused quartz and glass. Figure 6-1 shows the schematic of a specimen and substrate system, which is treated as a liquid-liquid-solid system since the biological specimen is assumed to have zero shear velocity.

Figure 6-1. Schematic diagram of a layer structure for biological cell-substrate system used in the calculation of reflectance function (Maev, 2008).
6.2 Simulations of V(z) curves

Once the calculations of the reflectance function for the biological specimen and substrate were completed, the V(z) curves for the system were simulated. The V(z) curves were simulated using MATLAB® according to the following procedures.

1. Assign the parameters of the acoustics lens, the biological specimen (e.g. HeLa cells, kidney tissue), and the glass substrate. Examples of the parameters of the acoustic lens are shown in Table 6.1 and the parameters of HeLa cell, glass substrate (Silica) are shown in Table 6.2. The thickness of the HeLa cell is about 12 µm and its longitudinal velocity is 1534 m/s. The attenuation inside of the HeLa cell is about 0.03 Neper/µm. The glass substrate has shear wave velocity about 5845 m/s.

2. Calculate the parameters of acoustic field at the back focal plane, the pupil function of the lens, and the reflectance function.

3. Calculate and plot the V(z) curves as described in Chapter 3. Figure 6-2 illustrates the schematic diagram of V(z) curve calculations by means of the Fourier Angular Spectrum method. In Figure 6-2, the cell is now taken into account as the plane 4. The method of calculation is the same procedure as of those described in section 3.2.2 of Chapter 3, however, the cell is now included. Figure 6-3 shows the schematic diagram of the V(z) curve calculations by means of the Ray theory method.
Table 6-1. Parameters of the acoustic lens (Tittman and Miyasaka, 2002)

<p>| | |</p>
<table>
<thead>
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<th></th>
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<tbody>
<tr>
<td>Transducer material</td>
<td>ZnO</td>
</tr>
<tr>
<td>Radius of Transducer</td>
<td>383.00 µm</td>
</tr>
<tr>
<td>Frequency</td>
<td>1 GHz</td>
</tr>
<tr>
<td>Buffer rod</td>
<td>Al₂O₃</td>
</tr>
<tr>
<td>Aperture Angle of Lens</td>
<td>120°</td>
</tr>
<tr>
<td>Focal distance</td>
<td>577.52 µm</td>
</tr>
</tbody>
</table>

Table 6-2. Acoustic properties of the coupling liquid, HeLa cell, and substrate (Weiss et al., 2007)

<table>
<thead>
<tr>
<th></th>
<th>( \rho ) (kg/m(^3))</th>
<th>( C_L ) (m/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coupling liquid (water)</td>
<td>1000</td>
<td>1501</td>
</tr>
<tr>
<td>HeLa cells</td>
<td>1100</td>
<td>1534</td>
</tr>
<tr>
<td>Glass substrate (Silica)</td>
<td>2250</td>
<td>5968</td>
</tr>
</tbody>
</table>
Figure 6-2. Schematic diagram for $V(z)$ calculation by employing the Wave Theory or Fourier Angular Spectrum approach (Yu and Boseck, 1991).

Figure 6-3. Schematic diagram for $V(z)$ calculations using the Ray theory method (Yu and Boseck, 1991).
6.2.1 FFT analysis of V(z) curves

In chapter 3, it was explained that the V(z) response is caused by the interference between the specularly reflected acoustic waves and the leaky surface acoustic waves. By knowing the null spacing $\Delta z$, we can calculate the leaky surface wave velocities. The experimental procedures for V(z) curve analysis will be provided in this section.

The FFT analysis of V(z) curve was originally proposed by Kushibiki et al. in 1985. There are two components for a V(z) response: $V_L(z)$ and $V_R(z)$. Figure 6-4 shows these two components. $V_L(z)$ is the characteristic lens response where the effects of the leaky surface waves are not included. $V_R(z)$ is varied on the propagation of the surface acoustic waves, which explains the interference effects between specularly reflected acoustic waves and leaky surface acoustic waves. In addition, $V_R$ can be used for extracting the surface acoustic wave velocities.
Figure 6-4. Analysis of a $V(z)$ curve (Kushibiki et al. 1985)
The procedures of FFT analysis for V(z) response is explained as follows and illustrated in the flow charts in Figures 6-5 and 6-6.

1. Load the V(z) curve obtained from the simulation.
2. Convert V(z) data from a logarithmic scale to a linear scale.
3. Compute \( V'_I(z) \) by subtracting the \( V'_L(z) \) from the V(z). (\( V'_L(z) \) is V(z) curve for lead, Pb.)
   (Note: Lead is selected because no waves are excited in its surface.)
4. Apply the digital low pass filtering, e.g. moving average filter to attenuate frequency components higher than those corresponding to the interference periodicity, and synthesize a \( \Delta V_L(z) \) curve.
5. Determine \( V_I(z) \) by subtracting \( \Delta V_L(z) \) from \( V'_I(z) \).
6. Sample the \( V_I(z) \) curves with the distance interval \( \Delta z_s \). Sampling points \( N_S \) are distributed from the waveform in the characterization region. Here, we add additional \( N_d \) dummy sampling points both in front of and behind \( V_I(z) \) curves so that it is a sufficiently high frequency resolution. The total points \( N \) equal \( N_d + N_s \). The frequency interval \( \Delta k \) in the frequency domain is given by \( \Delta k = 2\pi/(N \cdot \Delta z_s) \).
7. Apply the FFT analysis to the waveform in order to obtain spectral distribution.
8. From the spectral distribution obtained from step 7, we find a center frequency \( \eta \) which yields a maximum spectrum corresponding to the characteristic dip interval \( \Delta z \), which can be calculated from \( \Delta z = 2\pi/k_{peak} \). Here, \( k_{peak} \) is the sampling index (frequency) of the peak amplitude. \( k_{peak} = 2\pi n/N \Delta z \), where \( N \) is the number of sampling points, \( n \) is the sampling index of the peak amplitude, and \( \Delta z_s \) is the sampling interval. Additionally, we have the other two specified spectra of \( F(\eta) \) and \( F(\eta + \pi/z_w) \) that will be used in calculating the attenuation of the leaky surface acoustic wave.
9. Calculate the surface wave velocity $V_{lsaw}$ and the attenuation factor $\alpha_{lsaw}$.

$$v_{lsaw} = \frac{v_w}{\sqrt{(1-(1-v_w/2f\Delta z)^2)}}$$ \hfill 6.1

$$\alpha_0(n) = \frac{(\frac{\pi}{2z_w})|F(\xi(n)\pm\frac{\pi}{2z_w})|}{\left\{|F(\xi(n))|^2-|F(\xi(n)\pm\frac{\pi}{2z_w})|^2\right\}^{1/2}}$$ \hfill 6.2

$$\gamma(n) = \frac{\alpha_0 \cos \theta_l(n) + 2\alpha_w}{2 \sin \theta_l(n)}$$ \hfill 6.3

$$\gamma = \frac{2\pi f \alpha}{v_{lsaw}} \quad ; \quad \alpha_{lsaw} \approx \alpha$$ \hfill 6.4
Figure 6-5. Flow chart for FFT analysis of V(z) curves (Kushibiki et al. 1985)
In this research, $V(z)$ of fused quartz is used to demonstrate the FFT analysis of $V(z)$ curve and obtained by simulation. Also, $V_L(z)$ is obtained by simulating the $V(z)$ curve of lead (Pb), which has a relatively low surface wave velocity. Figure 6-7 illustrates waveform FFT analysis of $V(z)$ of fused quartz obtained from the simulation at a frequency of 400 MHz.
Figure 6-7. Waveform FFT analysis of V(z) of fused quartz. (a) V(z) curve of fused quartz. (b) V(z) curve of lead. (c) $V'_1(z)$. (d) FFT of $V'_1(z)$. (This simulated data was processed by Juntarapaso.)
The FFT analysis of $V(z)$ curve of fused quartz yields $\Delta z$ around 19.1 $\mu$m and gives the surface wave velocity $V_{lsaw}$ approximately 3449.6 m/s, which is close to the value in the literature of 3410 m/s.

It should be noted that FFT analysis of $V(z)$ curve is useful for extracting surface acoustic wave velocity $V_{lsaw}$ and the attenuation factor $\alpha_{lsaw}$ for solid materials that usually generate surface acoustic waves. However, it is difficult to implement this technique for soft materials such as biological cells because the Rayleigh wave or surface acoustic wave may not be produced within a specimen due to the critical angle of the Rayleigh wave or surface acoustic wave of soft materials is typically high. In addition, the relative attenuation of soft material is often high; thus, the $V(z)$ curve may not have enough oscillations for the FFT analysis even though the Rayleigh wave is generated.

Nevertheless, the mechanical properties of biological specimen can be measured by time-resolved SAM technique as explained in section 4.3 of Chapter 4. This research focuses on obtaining the mechanical properties of biological specimen by means of time-resolved SAM technique. The simulation methods used with time-resolved SAM for extracting the mechanical properties of biological cells will be presented next, in section 6.3.

6.3 Extracting the mechanical properties of biological cells from time-resolved SAM

6.3.1 Acoustical images and data acquisition

The acoustical images of the cells are usually filled with noise due to the high attenuation of ultrasound in the gigahertz frequency range and the small difference in the acoustical impedance between the cell and coupling liquid. The total loss due to attenuation in the coupling medium at the 1GHz frequency and of conversion of electrical to acoustical energy and vice versa is
approximately -33 dB for a lens with a focal length of 100 µm. If the reflection coefficient of a cell surface is approximately 1%, then the received voltage is only $5 \times 10^{-6}$ of the excitation voltage, which is about 10 V. Usually multifold averaging up to 2000 is applied to the signal recorded at each scanning point to increase the signal-to-noise ratio and this allows for the detection of the echo reflected from the surface of a cell.

Acoustical images are constructed from a fixed number of pixels, small squares assigned specific grey level and a specific coordinate on the image plane $(x_i, y_i)$, and aligned as a grid. The coordinates $(x_i, y_i)$ establish the position of the pixel on the grid. There are two operating modes employed in this time-resolved SAM: C-scan mode and RF-scan mode. By combining multiple RF scans, one can construct P-scan volumetric image.

6.3.1.1 C-scan mode

For the C-scan mode, the acoustic microscope mechanically scans the sample in a plane parallel to the sample surface at a fixed defocus position ($z$). Three dimensional imaging can be constructed from acoustical images of different layers within the sample by varying the defocus position. A tenfold averaging of the signals was applied in order to increase the signal-to-noise ratio on these images. It usually takes approximately 16 seconds to scan an area of 50µm x 50µm with a step increment of 0.2 µm. The C-scan mode has a relatively fast scanning speed and low rate of signal averaging to provide a fast acoustic image of the cell.

6.3.1.2 RF-scan mode

Grey scale images are produced from the RF datasets in the C-scan. Let $Z$ be the defocus distance of the lens, and $s(t, x_i, y_i, Z)$ be the RF signals measured at any pixel positions $(x_i, y_i)$ as a function of time $t$. Let $I(x_i, y_i, Z)$ be the brightness of the acoustical images at the defocus $Z$ and at any pixel position $(x_i, y_i)$. $I(x_i, y_i, Z)$ is an integral over time of a squared RF signal $s_n(t, x_i, y_i, Z)$:
\[ I(x_i, y_i, Z) = \frac{1}{N} \sum_{n=1}^{N} \int_{\Delta T} [s_n(t, x_i, Y_i, Z)]^2 \, dT \]

N is the total number of measurements taken at the same position. \( \Delta T \) is the time gate shown in Figure 6-8. Integrating further over 3-5 images will suppress noise in the image.

Figure 6-8. RF signal of the HeLa cell with resolved echoes from the top and bottom of the cell measured in focus. The first echo is due to the reflection from the top surface of the cell. The second echo is due to the reflection of the cell and substrate interface [10].

The RF-scan mode has a relatively slow scanning speed and a high rate of signal averaging. This mode was designed for quantitative measurements of sound velocity and sound attenuation inside of cells. The defocus Z is fixed, and the RF signal is recorded at each pixel position. Figure
6-8 shows the RF signal measured at one point inside of HeLa cells. The echo signal with a strong top echo in Figure 6-8 can be detected only within small areas of the cell.

A cross section of the cell along one scan line can be generated from the recorded RF signals by taking the average of the positive part of the RF signals:

\[ I_B(t, x_B, y, Z) = \frac{1}{N} \sum_{n=1}^{N} s_n(t, x_B, y, Z) \]  

At each pixel position, the signal is the average of N times.

The positions and values of the maxima of the echo signals reflected from the top of the cell and the cell/substrate interface as shown in Figure 6-8 can be determined by applying the Hilbert transform H(s).

6.3.1.3 P-scan mode

For the volumetric image of the cell, it can be generated by cascading each grey scale image slice produced from the RF datasets as explained above to give a P-scan volumetric image, which is a projection of the grey scale image slices and parallel cross section plans across from top to the bottom at which the slices are free selectable. The position of each image slice started from the top of the cell, then moved toward the cell layer, and finally reached the bottom of the cell. Each P-scan volumetric image consists 30 slices of grey scale simulated image, cutting through the cell at a different depth in the Z-axis direction for each X-Y plane. The volumetric images of the MCF-7 and the HeLa cells are presented as a P-scan display mode in Chapter 7 and 8.
6.3.2 Thickness, sound velocity, and attenuation of cells

MATLAB codes were written to simulate and calculate the local thickness, sound velocity, and attenuation of cells by using the formulas described in sections 4.3.1 and 4.3.2 in Chapter 4. Figure 6-9 shows the echoes of the RF signals used in the calculations.
Figure 6-9. (a) Echo signal from the interface between the coupling medium and the substrate. (b) Echo signal from an MCF-7 cell attached to a glass substrate resolved echoes from the surface and bottom of the cell measured in the defocus $z = -10 \, \mu m$. The first echo is due to the reflection from the surface or the top of the cell. The second echo is due to the cell/substrate interface. (Experimental data by Eric Strohm and Michael Kolios, 2007)
The cell thickness, \( d \), at any scanning position \((x, y)\) can be calculated from:

\[
d(x, y) = \frac{c \tau_1(x, y)}{2}
\]

6.7

After the thickness is calculated, we can calculate the longitudinal sound velocity of the cell using:

\[
c(x, y) = \frac{2d(x, y)}{\tau_{12}(x, y)}
\]

6.8

By measuring the amplitudes of the reflected signal, we can then calculate the impedance of the cell. Let \( A_0 \) be the amplitude of the signal reflected from the coupling medium-substrate interface and \( A_1 \) be the amplitude of the signal reflected from the coupling medium-cell interface.

\[
Z_1 = Z_0 \frac{A_0 + A_1}{A_0 - A_1}
\]

6.9

Where \( Z_0 \) is the acoustic impedance of the coupling medium. After we know the velocity and the impedance, the local density of the cell can be determined from:

\[
\rho_1(x, y) = \frac{Z_1(x, y)}{c(x, y)}
\]

6.10

The local attenuation can be calculated by using the expression proposed in 1995 by Briggs et al.

\[
\alpha_1 = \alpha_0 + \frac{1}{2d} \log_e \left( \frac{A_0 Z_s - Z_1}{A_2 Z_s + Z_1} \right) \left( \frac{4Z_1 Z_0}{Z_s + Z_0} \right)
\]

6.11

Where \( \alpha_0 \) is the attenuation in the coupling medium, \( A_2 \) is the amplitude of the echo from the interface between the cell and the substrate (Figure 6-9 b), and \( Z_s \) is the impedance of the substrate.
6.4 Transducer Simulations

The transducer simulations were performed and developed based on the Field II Ultrasound simulation program. Field II programs employ the concept of spatial impulse response developed by Tupholme and Stepanishen in 1971. The pressure generated by the transducer can be found from the Rayleigh integral:

\[ h(r, t) = \int_S \delta(t - \frac{|\vec{r}|}{c}) \frac{dS}{2\pi|\vec{r}|} \tag{6.12} \]

\(|\vec{r}|\) is the position of the field point in space, \(c\) is the speed of sound, and \(S\) is the transducer surface.

The emitted pressure field can be expressed as:

\[ p(\vec{r}, t) = \rho \frac{\partial v_n(t)}{\partial t} * h(\vec{r}, t) \tag{6.13} \]

\(v_n(t)\) is the surface velocity of the transducer and \(\rho\) is the density of the medium. The spatial impulse response describes how the transducer shape emits sound in space and can be seen as the impulse response for the linear system at a particular point in space. Since linear acoustics is used, the effect of apodization of the transducer surface can readily be included and responses from different transducer elements can be directly added for array transducers.

In addition, the scattered field and the received response can be determined from the spatial impulse response. The received signal from the transducer can be calculated from:

\[ p_r(\vec{r}, t) = v_{pe}(t) *_t f_m(\vec{r}) *_r h_{pe}(\vec{r}, t) \tag{6.14} \]

\(*_r\) indicates spatial convolution and \(*_t\) denotes temporal convolution. \(v_{pe}\) is the pulse-echo impulse which includes the transducer excitation and the electro-mechanical impulse response during emission and reception of the pulse. \(f_m\) accounts for the inhomogeneities in the tissue due to the density and propagation velocity perturbations which give rise to the scattered signal. \(h_{pe}\) is the
pulse-echo spatial impulse response that relates the transducer geometry to the spatial extent of the scattered field. \( v_{pe} \), \( f_m \), and \( h_{pe} \) can be expressed as follows:

\[
v_{pe}(t) = \frac{\rho}{2c^2} E_m(t) \star t \frac{\partial^3 v(t)}{\partial t^3}
\]

6.15

\[
f_m(\vec{r}_1) = \frac{\Delta \rho(\vec{r})}{\rho} - \frac{2 \Delta c(\vec{r})}{c}
\]

6.16

\[
h_{pe}(\vec{r}, t) = h_t(\vec{r}, t) \star h_r(\vec{r}, t)
\]

6.17

The received response can then be calculated by finding the spatial impulse response for the transmitting and the receiving transducer and then convolving with the impulse response of the transducer. A single radio frequency (RF) line in an image can be calculated by summing the response from a collection of scatterers, in which the scattering strength is determined by the density and speed of sound perturbations in the tissue. Therefore, homogeneous tissue can be made from a collection of randomly placed scatterers with a scattering strength of a Gaussian distribution, where the variance of the distribution is determined by the backscattering cross-section of the particular tissue.

### 6.4.1 Point targets

The first synthetic phantom consists of five point targets placed with a distance of 10 mm. apart starting at 30 mm from transducer surface. A linear sweep image is made of the points and the resulting image is compressed to show a 40 dB dynamic range. This phantom is used for showing the spatial variation of the point spread function for a particular transducer, focusing, and apodization scheme.
6.4.2 Cyst phantom

This phantom consists of a cyst region. This can be used for characterizing the contrast-lesion detection capabilities of an imaging system. The scatterers in the phantom are generated by finding their random position within a 60 x 50 x 10 mm cube, and then assigning a Gaussian distributed amplitude to each scatterer.

If the scatterer resides within a cyst region, then the amplitude is set to 0. A linear scan of the phantom was done by a 64, 128, and 192 element transducer with a Hanning apodization in transmission and reception. The height of the element was 5 mm, width was one wavelength, and the kerf was 0.55 mm. A single transmit focus was done at 60 mm and the receiving focus at 20 mm intervals beginning at 30 mm from the transducer surface.

6.4.3 Cell model

A bitmap image is generated from the scattering strength of the acoustical image of the cells. This map then determines the factor multiplied onto the scattering amplitude generated from the Gaussian distribution and models the difference in the density and speed of sound perturbations in the tissue. The scatterer maps used in this research were based on the acoustical e-scan images from the experimental data set of HeLa cells and MCF-7 as explained in previous steps. The phantom of the acoustical image of cells has been made with 100,000 scatterers that were randomly distributed within the phantom. A Gaussian distributed scatter amplitude with a standard deviation was calculated from the scatter map. The phantom was scanned with frequencies of 7, 70, 100, 600, 800 MHz. 64, 128, and 256 element phased array were designed with approximately half wavelength spacing and Hanning apodization. A single focus on transmission was used, and dynamic focusing was used during reception. Each image consists of 50 scan lines.
The phantom was also scanned with a 100 MHz, 20x20 element array 2D fully populated array transducer with approximately half a wavelength spacing and Hanning apodization. A single transmission focus transducer was used with multiple focusing during reception. Each image is composed of 30 lines.

Chapter summary

This chapter provided the methods and simulations used in this research. The details of the calculation of reflectance function for biological cell and substrates, the simulations of $V(z)$ responses, extracting the mechanical properties of biological cells from the SAM data, as well as transducer simulations were described. The following chapter will present the simulation results.
Chapter 7

Simulation Results

In this chapter, the results of the calculation and simulation for; (1) the calculation of the reflectance function of the layer model consisting of the coupling medium, the biological specimen, and the substrate; (2) the simulation results of V(z) responses; (3) the SAM images of HeLa cells and MCF-7 cells as well as extracting the mechanical properties of the cells from the time-resolved SAM measurement data; and (4) the transducer simulations, will be presented in section 7.1, 7.2, 7.3, and 7.4 respectively.

The results of calculation and simulation for the reflectance function of the layer model consisting of the coupling medium, the biological specimen, and the substrate, including the simulation results of the V(z) responses as well as the SAM images simulation of HeLa cells and MCF-7 cells, and the transducer simulation are new. Though there are some experimental data done by some other researchers, no one has done the simulations for the layer model before. The calculation program is invaluable to calculate the reflectance function and the V(z) of any specimen being studied.
7.1 Reflectance function for biological cells and substrate

7.1.1 Fused quartz substrate

A reflectance function $R$ is a complex function. The reflectance function $R$ of fused quartz is shown in Figure 7-1.

![Reflectance function of Fused quartz and coupling medium](image)

Figure 7-1. Reflectance function of fused quartz substrate and water as a coupling medium. (Top) the modulus of the reflectance function $R$; the vertical axis indicates the modulus of $R$, and the horizontal axis indicates the incident angles (degree). (Bottom) The phase of the reflectance function $R$; the vertical axis indicates the phase of $R$ (radian), and the horizontal axis denotes the incident angles (degree).

The modulus of $R$, as seen in Figure 7-1 (top), is characterized by the following features: (1) the modulus of $R$ equals 0.8 at the incident angle $\theta = 0$; (2) the cusp near the incident angle $\theta = 14.5^\circ$ where the modulus of $R$ rises to about 0.9, which corresponds to the longitudinal wave critical angle of fused quartz; (3) the incident angle $\theta = 23.5^\circ$ where the modulus of $R$ rises to one, which corresponds to the shear wave critical angle for fused quartz. It should be noted that beyond the
shear wave critical angle no energy can be propagated into the solid, and thus the modulus of $R$ beyond this point must be unity. This result is similar to those found in Yu and Boseck in 1995 [2].

The phase of $R$, as seen in Figure 7-1 (bottom), experiences very small dip at around the longitudinal wave critical angle $\theta = 14.5^\circ$; it also shows the dramatic behavior changes by almost $2\pi$ at approximately the shear wave critical angle $\theta = 23.5^\circ$. It should be noted that this phase transition entails the existence of nonspecular reflections, which are diffuse reflections at the surface, in the system. In this system consisting of fluid-liquid interface, Rayleigh waves can be generated at around few degrees beyond the shear wave critical angle at which waves in the liquid can couple into Rayleigh waves in the surface of the solid, which can be seen the phase changes by almost $2\pi$.

7.1.2 Kidney tissue 3 µm thickness on fused quartz substrate

A reflectance function of kidney tissue with 3 µm thickness mounted on fused quartz substrate is illustrated in Figure 7-2.
Figure 7-2. Reflectance function of kidney tissue with 3 µm thickness attached to a fused quartz substrate. (Top) The modulus of the reflectance function $R$; the vertical axis indicates the modulus of $R$, and the horizontal axis indicates the incident angles (degrees). (Bottom) The phase of the reflectance function $R$; the vertical axis indicates the phase of $R$ (radians), and the horizontal axis denotes the incident angles (degrees).

From Figure 7-2(top), at the incident angle $\theta = 0$, the modulus of $R$ of kidney tissue with 3 µm thickness attached to a fused quartz substrate system is approximately 0.74, which is less than that of the fused quartz substrate only ($|R| = 0.8$), as shown in Figure 7-1 (top). At the point near the incident angle $\theta = 14.2^\circ$, the modulus of $R$ reaches to about 0.9, which corresponds to the longitudinal wave critical angle of the system. At the incident angle $\theta = 22.5^\circ$, the modulus of $R$ reaches unity, which corresponds to the shear wave critical angle for the system. The phase transition of $R$ begins at this angle.

The phase of $R$, as seen in Figure 7-2 (bottom), displays small fluctuations at around the longitudinal wave critical angle $\theta = 14.2^\circ$. The dramatic behavior of phase transition occurs at approximately the shear wave critical angle $\theta = 22.5^\circ$. 
The reflection function $R$ of the fused quartz substrate only and the reflectance function $R$ of kidney tissue with 3 µm thickness attached to a fused quartz substrate system, as displayed in Figure 7-1 and Figure 7-2 respectively, are different due to the existence of the kidney tissue is taking into account for the latter system, which is treated as the liquid-liquid-solid system as explained in section 4.2 of Chapter 4.

The existence of the soft thin layer biological specimen, which is treated as the liquid-liquid-solid system, contributes in progressively reduction of the Rayleigh waves. Sometimes the Rayleigh waves cannot be generated in the soft thin biological specimen. In the fluid-liquid system, Rayleigh waves can be excited at around few degrees beyond the shear wave critical angle at which waves in the liquid can couple into Rayleigh waves in the surface of the solid, as seen in the change of the phase by almost $2\pi$. As seen in Figure 7-1 (bottom) for the phase change of the fused quartz substrate-water (the solid-fluid system), it was approximately $2\pi$ due to the existence of the Rayleigh waves. But for the phase change in Figure 7-2 (bottom) for the water-kidney tissue-fused quartz substrate (the liquid-liquid-solid system), it was less than $2\pi$ that implies about the progressively reduction of Rayleigh waves as explained previously.

### 7.1.3 HeLa cell 5.27 µm on fused substrate

Figure 7-3 shows a reflectance function of a HeLa cell with a 5.27 µm thickness attached to a fused quartz substrate.
Figure 7-3. Reflectance function of a HeLa cell with 5.27 µm thickness attached to a fused quartz substrate. (Top) The modulus of the reflectance function R; the vertical axis indicates the modulus of R, and the horizontal axis indicates the incident angles (degrees). (Bottom) The phase of the reflectance function R; the vertical axis indicates the phase of R (radians), and the horizontal axis denotes the incident angles (degrees).

From Figure 7-3(top), at the incident angle $\theta = 0$, the modulus of R of a HeLa cell with a 5.27 µm thickness attached to a fused quartz substrate is approximately 0.78. At the incident angle $\theta = 14.6^\circ$, the modulus of R rises to about 0.92 corresponding to the longitudinal wave critical angle of the system. At the incident angle $\theta = 23.5^\circ$, the modulus of R becomes unity, which corresponds to the shear wave critical angle of the system.

The phase of R, as seen in Figure 7-3 (bottom), exhibits the dramatic behavior of phase transition starting at approximately the shear wave critical angle $\theta = 23.5^\circ$.

The reflection function R of kidney tissue with 3 µm thickness attached to a fused quartz substrate system and the reflectance function R of a HeLa cell with 5.27 µm thickness attached to
a fused quartz substrate system, as displayed in Figure 7-2 and Figure 7-3 respectively, are different because their mechanical properties such as thickness, density, and longitudinal velocity are different. The reflectance function changes as a consequence of any changes of materials being tested as described in section 4.2 of Chapter 4.

7.1.4 HeLa cell 11.9 µm on fused substrate

Figure 7-4 illustrates a reflectance function of a HeLa cell with 11.9 µm thickness attached to a fused quartz substrate.

Figure 7-4 Reflectance function of a HeLa cell with 11.9 µm thickness attached to a fused quartz substrate. (Top) The modulus of the reflectance function R; the vertical axis indicates the modulus of R, and the horizontal axis indicates the incident angles (degrees). (Bottom) The phase of the reflectance function R; the vertical axis indicates the phase of R (radians), and the horizontal axis denotes the incident angles (degrees).
From Figure 7-4 (top), at the incident angle $\theta = 0$, the modulus of $R$ of a HeLa cell with a 11.9 $\mu$m thickness attached to a fused quartz substrate is approximately 0.78. The modulus of $R$ increases to about 0.93 corresponding to the longitudinal wave critical angle of the system, at the incident angle $\theta = 14.6^\circ$. At the incident angle $\theta = 23.5^\circ$, the modulus of $R$ rises to one, which corresponds to the shear wave critical angle of the system.

The phase of $R$, as shown in Figure 7-4 (bottom), displays the dramatic behavior of phase transition beginning at approximately the shear wave critical angle $\theta = 23.5^\circ$.

The reflection function $R$ of a HeLa cell with 5.27 $\mu$m thickness attached to a fused quartz substrate system and the reflectance function $R$ of a HeLa cell with 11.9 $\mu$m thickness attached to a fused quartz substrate system, as displayed in Figure 7-3 and Figure 7-4 respectively, are different in terms of their phase changes because the thicknesses are different. As explained in section 4.2 of Chapter 4, the thickness of the cell also plays important role in the calculation of the reflectance function.

7.1.5 HeLa cell 12.6 $\mu$m on fused quartz substrate

A reflectance function of a HeLa cell with 12.6 $\mu$m thickness attached to a fused quartz substrate is shown in Figure 7-5.
Figure 7-5. Reflectance function of a HeLa cell with 12.6 µm thickness attached to a fused quartz substrate. (Top) The modulus of the reflectance function $R$; the vertical axis indicates the modulus of $R$, and the horizontal axis indicates the incident angles (degrees). (Bottom) The phase of the reflectance function $R$; the vertical axis indicates the phase of $R$ (radians), and the horizontal axis denotes the incident angles (degrees).

As shown in Figure 7-5 (top), the modulus of $R$ of a HeLa cell with a 12.6 µm thickness attached to a fused quartz substrate is approximately 0.68, at the incident angle $\theta = 0$. The modulus of $R$ increases to about 0.91 corresponding to the longitudinal wave critical angle of the system, at the incident angle $\theta = 14.6^\circ$. At the incident angle $\theta = 23.5^\circ$, the modulus of $R$ rises to one, which corresponds to the shear wave critical angle of the system.

The phase of $R$, as displayed Figure 7-5 (bottom), exhibits the dramatic behavior of phase transition beginning at approximately the shear wave critical angle $\theta = 23.5^\circ$.

The reflection function $R$ of a HeLa cell with 11.9 µm thickness attached to a fused quartz substrate system and the reflectance function $R$ of a HeLa cell with 12.6 µm thickness attached to
a fused quartz substrate system, as illustrated in Figure 7-4 and Figure 7-5 respectively, are different for their phase changes due to their different thicknesses. The thickness of the cell affects the reflectance function, as described in section 4.2 of Chapter 4.

### 7.1.6 HeLa cell 15.6 µm on fused quartz substrate

A reflectance function of a HeLa cell with 15.6 µm thickness attached to a fused quartz substrate is shown in Figure 7-6.

![Figure 7-6. Reflectance function of a HeLa cell with 15.6 µm thickness attached to a fused quartz substrate. (Top) The modulus of the reflectance function R; the vertical axis indicates the modulus of R, and the horizontal axis indicates the incident angles (degrees). (Bottom) The phase of the reflectance function R; the vertical axis indicates the phase of R (radians), and the horizontal axis denotes the incident angles (degrees).](image-url)
As illustrated in Figure 7-6 (top), the modulus of \( R \) of a HeLa cell with a 15.6 µm thickness attached to a fused quartz substrate is approximately 0.79, at the incident angle \( \theta = 0 \). The modulus of \( R \) rises to about 0.93 corresponding to the longitudinal wave critical angle of the system, at the incident angle \( \theta = 14.6^\circ \). At the incident angle \( \theta = 23.5^\circ \), the modulus of \( R \) becomes unity, which corresponds to the shear wave critical angle of the system.

As shown in Figure 7-6 (bottom), the phase of \( R \) displays the dramatic behavior of phase transition beginning at approximately the shear wave critical angle \( \theta = 23.5^\circ \).

The reflection function \( R \) of a HeLa cell with 12.6 µm thickness attached to a fused quartz substrate system and the reflectance function \( R \) of a HeLa cell with 15.6 µm thickness attached to a fused quartz substrate system, as illustrated in Figure 7-5 and Figure 7-6 respectively, are different for their phase changes because of their different thicknesses. The thickness of the cell has an effect on the reflectance function, as explained in section 4.2 of Chapter 4.

In conclusion, the new Matlab algorithm developed in this study can be used in the calculation of the reflectance function for the biological specimen. The reflectance function calculated from the program leads the calculation of the \( V(z) \) curves which is considered as a material signature. As we seen in these reflectance function calculation results, the liquid-liquid-solid layer structure consisting of the coupling medium-soft thin layer biological specimen-substrate, causes increasingly reduction of the Rayleigh waves, which are finally dominated by the interference between reflections from the top and the bottom surfaces of the cell. Rayleigh waves can be generated in the fluid-liquid layer structure at which waves in the liquid can couple into Rayleigh waves in the surface of the solid, as appeared in the change of the phase by -
approximately almost $2\pi$, for example the phase change of the fused quartz substrate-water shown in Figure 7-1 (bottom). However, in the case of the coupling medium-soft thin biological specimen-substrate or the liquid-liquid-solid system, the phase change was less than $2\pi$ due to the greatly reduction of Rayleigh waves. It should be noted that often the Rayleigh waves cannot be generated in the soft thin biological specimen.

7.2 $V(z)$ curves

7.2.1 $V(z)$ curve of fused quartz

Figure 7-7 shows the results of the $V(z)$ curve simulation of the fused quartz substrate.
Figure 7-7. (top) $V(z)$ curve simulation results of the fused quartz substrate using ray theory at a frequency of 400 MHz. (bottom) $V(z)$ curve simulation using wave theory or angular spectrum technique at a frequency of 400 MHz.
The features of the $V(z)$ curves illustrated in Figure 7-7 and Figure 7-8, the features of $V(z)$ curve are explained in the following paragraph. At the center of the focal plane ($z = 0$), there is the strong central maximum which is a characteristic of the substrate and the coupling medium interface and because of the existence of the primary reflection. It should be noted that this region is independent of the material properties of the substrate being tested. For the region of positive distance $z$, the curve attenuates rapidly as distance $z$ increases because the substrate surface is far away from the focal plane, therefore most of the acoustic energy is reflected outside the lens and only small amount of the acoustic energy is detected by the transducer. For the region of negative distance $z$, the curve exhibits strong oscillations with a series of periodic maxima and minima distinguished by a period $\Delta z$. This region of periodic oscillations is characteristic of the material acoustic properties. The patterns of oscillations vary with the material being tested, as well as the depths of the minima and the relative magnitudes of the maxima. The phase transition of the reflectance function $R$ as shown in Figure 7-1 is responsible for these oscillations in the $V(z)$ curve.

Figure 7-7 (top) shows the $V(z)$ curve of the fused quartz substrate based on Ray Theory
at center frequency 400 MHz; the period $\Delta z$ is approximately 18.8 $\mu$m. The $V(z)$ curve of the fused quartz substrate based on angular spectrum technique at center frequency 400 MHz is shown in Figure 7-7 (bottom); the period $\Delta z$ is approximately 18.5 $\mu$m. By knowing $\Delta z$, the Rayleigh wave velocity $v_R$ can be calculated from (Weglein, 1985):

$$v_R = \frac{c_w}{\sqrt{1 - \left(\frac{c_w}{2f\Delta z}\right)^2}}$$

7.1

where $c_w$ is the longitudinal sound velocity of coupling medium and $f$ is the frequency.

In this simulation, the coupling medium is water at 20°C with $c_w$ about 1482 m/s, and the center frequency is 400 MHz. By substituting $c_w$, $f$, and $\Delta z$ in Equation 7.1, we obtain Rayleigh wave velocity of fused quartz substrate, $v_R = 3423.8$ m/s for the $\Delta z$ using Ray theory, and $v_R = 3397.8$ m/s for the $\Delta z$ using Angular spectrum approach. The Rayleigh wave velocity of fused quartz, in literature, is typically about 3410 m/s [28]. The values obtained by the simulations are sufficiently close to the value from the literature.

The variation of curves for the two methods can be explained as following. In the angular spectrum method, the explicitly postulation of the existence of leaky Rayleigh waves is not needed since the equations used for determining the reflectance function analytically take into account for their behavior that satisfy the boundary conditions for acoustic waves in a fluid-fluid-solid layers. It should be noted that this reflectance function produces oscillations in $V(z)$ as found experimentally. The change of the phase of nearly $2\pi$ in the reflectance function at around an angle of incidence slightly above the shear wave critical angle causes the oscillations in the $V(z)$ curve. A simpler and more intuitive model that is the ray method model is also justified. In this ray method, the two important rays that are the specularly reflected axial ray and the leaky wave ray as explained in section 3.2.1 of Chapter 3. The ray method predicts approximately the same periodicity of the oscillations in $V(z)$ as the angular spectrum or Fourier method. It also accounts more
straightforwardly for the fact that these oscillations happen only when defocusing towards the lens. As we seen in the simulations of the V(z) curves above, the two methods gave very similar oscillation patterns. In order to make the simulation more intuitive, the ray method was applied in further calculation and simulation in this study.

7.2.2 V(z) curve for biological specimens attached on substrate

7.2.2.1 V(z) curve of the kidney tissue with 3 μm thickness attached on fused quartz substrate

The V(z) curve simulation of the kidney tissue with 3 μm thickness mounted on a fused quartz substrate is shown in Figure 7-9.

![V(z) curve simulations of the kidney tissue with 3 μm thickness attached on fused quartz substrate](image)

Figure 7-9. V(z) curve simulations of the kidney tissue with 3 μm thickness attached on fused quartz substrate at a frequency 400 MHz.
Figure 7-10. $V(z)$ curve simulations for the kidney tissue with 3 µm thickness attached on fused quartz substrate at a frequency of 400 MHz and $V(z)$ curve of fused quartz substrate at a frequency of 400 MHz.

Figure 7-9 shows the $V(z)$ curve of the kidney tissue with 3 µm thickness attached on fused quartz substrate at center frequency 400 MHz; the period $\Delta z$ is approximately 18.3 µm. By substituting this $\Delta z$ in Equation 7.1, we obtain Rayleigh wave velocity of the kidney tissue with 3 µm thickness attached on fused quartz substrate, $v_R = 3380.3$ m/s. Figure 7-10 displays $V(z)$ curve simulations of the kidney tissue with 3µm thickness attached on fused quartz substrate at a frequency of 400 MHz along with $V(z)$ curve of fused quartz substrate only at a frequency of 400 MHz. The surface wave velocity of kidney tissue in the literature is 3351 m/s [38]. The surface wave velocity value obtained from the simulation is close to the value provided in the literature.

As seen in Figure 7-9 and Figure 7-10, the periods $\Delta z$ of $V(z)$ curve of the kidney mounted on the fused quartz are different from those of the fused quartz substrate only because of the different layer model used to determine reflectance functions of these two cases. This agrees with the literature that $V(z)$ curve is unique for each material and enable the calculation of the Rayleigh wave velocity of a thin biological specimen attached on a substrate.
7.2.2.2 \textit{V(z) curve of a HeLa cell attached on a fused quartz substrate}

\(V(z)\) curves of HeLa cells with different thicknesses attached on a fused quartz substrate are shown in Figure 7-11 to Figure 7-14.

![Graph of V(z) curve of a HeLa cell with 5.27 \(\mu\)m thickness mounted on a fused quartz substrate at a frequency of 400 MHz.]

Figure 7-11. \(V(z)\) curve of a HeLa cell with 5.27 \(\mu\)m thickness mounted on a fused quartz substrate at a frequency of 400 MHz.

Figure 7-11 depicts \(V(z)\) curve of a HeLa cell 5.27 \(\mu\)m thickness attached on a fused quartz substrate at a frequency of 400 MHz; the period \(\Delta z\) is approximately 19.2 \(\mu\)m. By substituting this \(\Delta z\) in Equation 7.1, we obtain Rayleigh wave velocity of the HeLa cell with 5.27 \(\mu\)m thickness attached on fused quartz substrate, \(v_R = 3481.9\) m/s.
Figure 7-12. V(z) curve of a HeLa cell with 5.27 µm thickness mounted on a fused quartz substrate at a frequency of 400 MHz along with V(z) curve of the kidney tissue with 3 µm thickness attached on fused quartz substrate at a frequency of 400 MHz.

Figure 7-12 shows V(z) curve of a HeLa cell with 5.27 µm thickness mounted on a fused quartz substrate at a frequency of 400 MHz along with V(z) curve of the kidney tissue with 3 µm thickness attached on fused quartz substrate at a frequency of 400 MHz. The periods of the V(z) curve for the HeLa cell attached on a fused quartz substrate also are different from those of the kidney tissue. It can be seen that the V(z) curves have similar patterns but different periods ∆z for each case.
Figure 7-13. V(z) curves of HeLa cells with different thicknesses attached on fused quartz substrate at a frequency of 400 MHz; HeLa cell with 5.27 µm thickness, HeLa cell with 11.5 µm thickness, a HeLa cell with 15.6 µm thickness, and HeLa cell with 12.6 µm thickness were used in the simulations.

Figure 7-13 shows V(z) curves of HeLa cells with different thicknesses of 5.27, 11.5, 15.6, and 12.6 µm thickness attached on fused quartz substrate at a frequency of 400 MHz; the periods ∆z are 19.2, 19.9, 20.6, 19.6, respectively. By substituting these periods ∆z in Equation 7.1, we obtain Rayleigh wave velocities of the HeLa cell with 5.27, 11.5, 15.6, and 12.6 µm thickness attached on fused quartz substrate, \( v_R = 3458.1, 3517.5, 3575.8, \) and 3492.2 m/s, respectively. Nevertheless, there are no experimental data or literatures available at present for the Rayleigh wave velocity of HeLa cells. The Rayleigh wave velocities obtained from the simulations here can possibly be used as suggested values for any future measurements.
Figure 7-14. V(z) curves of biological specimen with different thicknesses attached on fused quartz substrate at a frequency of 400 MHz; kidney tissue with 3 µm thickness, HeLa cell with 5.27 µm thickness, HeLa cell with 11.5 µm thickness, HeLa cell with 11.5 µm thickness, a HeLa cell with 15.6 µm thickness, and HeLa cell with 12.6 µm thickness were used in the simulations.

Figure 7-14 depicts V(z) curves of HeLa cells with different thicknesses of 5.27, 11.5, 15.6, and 12.6 µm thickness attached on fused quartz substrate at a frequency of 400 MHz along with the V(z) curve of the kidney tissue with 3 µm thickness attached on fused quartz substrate at a frequency of 400 MHz. Each V(z) curve shown in Figure 7-14 gives different values of periods Δz as mentioned previously. The simulation of the V(z) curve agrees with the literature that the V(z) curve is distinctive for each material, which allows the calculation of the Rayleigh wave velocity of a thin biological specimen attached onto a substrate.
7.3 Physical characteristic of cells

7.3.1. Imaging of HeLa cells and MCF-7 cells

MATLAB codes were written to simulate the acoustical images of the cells using the RF data collected from the time-resolved measurements.

The acoustical images of MCF-7 cells and HeLa cells are shown in Figures 7-15 to Figure 7-17.

![Acoustical image of MCF-7 cells at 800 MHz](image)

Figure 7-15. Acoustical image of MCF-7 cells at 800 MHz. (Experimental data provided by Pavlos Anastasiadis).

Figure 7-15 displays an acoustical image of two MCF-7 cells being scanned at a frequency of 800 MHz (experimental data were provided by Pavlos Anastasiadis). The cellular membrane and the nucleus are visible in this acoustical image. The darker area indicates the high attenuation of the sound. The polymerization of the f-actin possibly causes the high attenuation of the sound inside the cells.
In addition to the MCF-7 cells seen earlier, two different HeLa cells were used in this work for the transducer simulations. Figure 7-16 shows an acoustical image of HeLa cells being scanned at a frequency of 400 MHz immediately following cell division (experimental data were provided by Pavlos Anastasiadis). Figure 7-17 also displays an acoustical image of a HeLa cell, but a different cell from the ones shown in Figure 7-16, being scanned at a frequency of 400 MHz (experimental data were provided by Pavlos Anastasiadis). The nucleoli of the cells are visible in all images.

Figure 7-16. Acoustical image of HeLa cells at 400 MHz. (Experimental data provided by Pavlos Anastasiadis).

Figure 7-17. Acoustical image of HeLa cells at 400 MHz. (Experimental data provided by Pavlos Anastasiadis).
7.3.2 Extracting the mechanical properties of biological cells from the time-resolved SAM technique

Thicknss, sound velocity, acoustic impedance, density, attenuation, and bulk modulus of the MCF-7 cells were calculated by analyzing the radio frequency (RF) signals collected from the time-resolved method as described in Chapter 6. The Figure 7-18 depicts the MCF-7 cells and their measured positions. The image and measurement data were provided by Eric Strohm. The cells were measured at a frequency of 375 MHz. The cell #2 and the cell #4 were selected for the calculations due to the measurement data were available for only these two cells and the results for the mechanical properties of MCF-7 cells were presented in Table 7-1.

Figure 7-18. MCF-7 cells and their measured positions. The cells were being measured at a frequency of 375 MHz. (The image and measurement data were provided by Eric Strohm.)
Table 7-1. Calculation results for the mechanical properties of MCF-7 cell # 2 and cell #4 at 375 MHz from Figure 7-18. All experimental data that were used in the calculations were provided Eric Strohm and Michael Kolios.

<table>
<thead>
<tr>
<th>Mechanical Properties</th>
<th>Cell # 2</th>
<th>Cell # 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thickness [µm]</td>
<td>11.8</td>
<td>11.2</td>
</tr>
<tr>
<td>Sound velocity [m/s]</td>
<td>1573</td>
<td>1580</td>
</tr>
<tr>
<td>Acoustic impedance [MRayls]</td>
<td>1.60</td>
<td>1.56</td>
</tr>
<tr>
<td>Density [kg/m³]</td>
<td>1017</td>
<td>987</td>
</tr>
<tr>
<td>Attenuation [Neper/µm]</td>
<td>0.16</td>
<td>0.18</td>
</tr>
<tr>
<td>Bulk modulus [GPa]</td>
<td>2.51</td>
<td>2.46</td>
</tr>
</tbody>
</table>

Thickness, sound velocity, acoustic impedance, density, attenuation, and bulk modulus of the HeLa cells were calculated by analyzing the radio frequency (RF) signals collected from the time-resolved method as described in Chapter 6. The Figure 7-19 shows an acoustical image of the HeLa cells used in the calculation of HeLa cell mechanical properties. The cells were measured at a frequency of 400 MHz and the results of the mechanical property calculations are presented in Table 7-2. (The image and measurement data were provided by Pavlos Anastasiadis).

Figure 7-19. Acoustical image of HeLa cells at 400 MHz. (The image and measurement data were provided by Pavlos Anastasiadis)
Table 7-2. Calculation results for the mechanical properties of the HeLa cell at 400 MHz. (The experimental data used were provided by Pavlos Anastasiadis. Due to the limitation of the measurement data, the calculation here was made for only the one cell.)

<table>
<thead>
<tr>
<th>Mechanical properties</th>
<th>Cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thickness [µm]</td>
<td>5.3</td>
</tr>
<tr>
<td>Sound velocity [m/s]</td>
<td>1500</td>
</tr>
<tr>
<td>Acoustic impedance [MRayls]</td>
<td>1.78</td>
</tr>
<tr>
<td>Density [kg/m³]</td>
<td>1186</td>
</tr>
<tr>
<td>Attenuation [Neper/µm]</td>
<td>0.21</td>
</tr>
<tr>
<td>Bulk modulus [GPa]</td>
<td>2.67</td>
</tr>
</tbody>
</table>

It should be noted that the sound velocity, the acoustic impedance, the density and the bulk modulus of the cells were close to those of the coupling medium. The calculation results agree with the published data in the literature, which measured a sound velocity of 1534.5 ± 33.6 m/s in HeLa cells [10] and 1582.25 ± 11.76 m/s in MCF-7 cells [76]. Because there is a lack of published data on individual cell properties; the other parameters cannot be compared at the present time.

7.4 Transducer simulations

The transducer designed for SAM simulations are presented in this section. The 1D-phased array transducers simulation results are presented in section 7.4.1. The 2D-phased array transducer simulation results are shown in section 7.4.2. The volumetric images are presented in section 7.4.3.

In section 7.4.1, the phantoms, which were made of scatterer maps based on the acoustical images from the experimental data set of HeLa cells and MCF-7 cells, were scanned with frequencies of 100, 400, 600, 800, and 1000 MHz. 64, 128, and 256 element phased array were designed with half wavelength of each frequency for the spacing (7.7 µm, 1.9 µm, 1.3 µm, 0.96 µm, 0.77 µm, for 100, 400, 600, 800, and 1000 MHz respectively) and Hanning apodization. A single focus on transmission was employed along with dynamic focusing during reception. The reason that a single focus on transmission was used is to avoid slowing the frame rate whereas the
multiple transmission makes the frame rate slow by a factor equal to the number of the transmit foci used. If, however, the multiple transmission focal zones were to be employed, the lateral resolution could have been improved. For dynamic focusing during reception allowed focusing throughout the entire scan depth which was divided into multiple zones each of which was assigned focal length during reception. Each image of HeLa cells is composed of 50 scan lines, which the number of scan lines came from the successive simulations to yield the scan lines that could assemble a simulated images within three days of simulation time for each HeLa cells image. Each image of MCF-7 cells consists of 64 lines, which the number of scan lines was a result from the successive simulations to give the scan lines that could build the simulated images within four days of simulation time for each MCF-7 cells image. Figure 7-20 to Figure 7-64 displays the simulated B-scan results of MCF-7 cells and two different sets of HeLa cells, respectively.

In section 7.4.2, the phantom, which was made of scatterer maps based on the acoustical C-scan images from the experimental data set of MCF-7 cells, was scanned with a 100 MHz, 20x20 element array 2D fully populated array transducer with approximately half a wavelength spacing and Hanning apodization. A single transmission was utilized with multiple focusing during reception. The image consists of 30 scan lines, which the number of scan lines obtained from the successive simulations to generate the scan lines that could construct the simulated images within four days of simulation time for each MCF-7 cells image, as shown in Figure 7-65.

It should be noted that FIELD II® program usually performs better using a 100 MHz sampling and approximate calculations rather than using higher frequency sampling, especially in a GHz range, as expressed in FIELD II® user guide manual [75].

In section 7.4.3, the volumetric images of the cells are presented as a P-scan display mode, which is a projection of a B-scan to create a 3D image by cascading B-scan slices, in Figure 7-66 to Figure 7-95. Each volumetric image consists of 30 slices of B-scan simulated images, slicing through the cells at a different depth in the Z-axis direction for each X-Y plane.
7.4.1 1D-Phased array transducers

7.4.1.1) 64 elements with 100 MHz center frequency – MCF7 cells

Figure 7-20.(Left) An acoustical image of MCF-7 cells used as a phantom. (Right) B-scan image of MCF-7 cells with a 64 element phased array transducer at a center frequency of 100 MHz.

Figure 7-20 displays a B-scan simulated image of MCF-7 cells being scanned with a 64 element phased array transducer at a center frequency of 100 MHz. The sampling frequency was 1400 MHz. The phased array was designed with approximately half wavelength (7.7 μm) spacing and Hanning apodization. The element height was 5 mm, the width was 6 μm, and the kerf was one-tenth of a wavelength. A single transmit focus was at 6 mm. The focusing during the reception is at 1 mm to 15 mm in 1 mm increments. The simulated image consists of 64 lines with 1.4 degrees between the lines, which the number of scan lines came from the successive simulations to yield the scan lines that could assemble a simulated images within four days of simulation time for each MCF7 cells image.
7.4.1.2) 128 elements with 100 MHz center frequency - MCF7 cells

Figure 7-21. (Left) An acoustical image of MCF-7 cells used as a phantom. (Right) A B-scan image of MCF-7 cells with a 128 element phased array transducer at a center frequency of 100 MHz.

A B-scan simulated image of MCF-7 cells being scanned with a 128 element phased array transducer at a center frequency of 100 MHz is shown in Figure 7-21. The sampling frequency was 1400 MHz. The dimensions of each element are: the element height was 5 mm, the width was 6 µm, and the kerf was one-tenth of a wavelength. The phased array was designed with approximately half wavelength (7.7 µm) spacing and Hanning apodization. For transmission, a single transmit focus was set at 6 mm. For the reception, the multiple receiving foci during the reception were at 1 mm to 15 mm in 1 mm increments. The simulated image consists of 64 lines with 1.4 degrees between the lines, which the number of scan lines came from the successive simulations to yield the scan lines that could assemble a simulated images within four days of simulation time for each MCF7 cells image.
7.4.1.3) 256 elements with 100 MHz center frequency – MCF7 cells

Figure 7-22. (Left) An acoustical image of MCF-7 cells used as a phantom. (Right) A B-scan image of MCF-7 cells with a 256 element phased array transducer at a center frequency of 100 MHz.

Figure 7-22 displays a B-scan simulated image of MCF-7 cells being scanned with a 256 element phased array transducer at a center frequency of 100 MHz. The sampling frequency was 1400 MHz. The phased array was designed with approximately half wavelength (7.7 μm) spacing and Hanning apodization. The element height was 5 mm, the width was 6 μm, and the kerf was one-tenth of a wavelength. A single transmit focus was at 6 mm. The focusing during the reception is at 1 mm to 15 mm in 1 mm increments. The simulated image consists of 64 lines with 1.4 degrees between the lines, which the number of scan lines came from the successive simulations to produce the scan lines that could construct a simulated images within four days of simulation time for each MCF7 cells image.
7.4.1.4) 64 elements with 400 MHz center frequency – MCF7 cells

Figure 7-23. (Left) An acoustical image of MCF-7 cells used as a phantom. (Right) A B-scan image of MCF-7 cells with a 64 element phased array transducer at a center frequency of 400 MHz.

A B-scan simulated image of MCF-7 cells being scanned with a 64 element phased array transducer at a center frequency of 400 MHz is displayed in Figure 7-23. The sampling frequency was 5600 MHz. The dimensions of each element are; the element height was 1 mm, the width was 1 µm, and the kerf was one-tenth of a wavelength. The phased array was designed with approximately half wavelength (1.9 µm) spacing and Hanning apodization. For transmission, a single transmit focus was set at 1 mm. For the reception, the multiple receiving foci during the reception were at 1 mm to 10 mm in 1 mm increments. The simulated image consists of 64 lines with 1.4 degrees between the lines, which the number of scan lines came from the successive simulations to yield the scan lines that could build a simulated images within four days of simulation time for each MCF7 cells image.
7.4.1.5) 128 elements with 400 MHZ center frequency – MCF7 cells

Figure 7-24. (Left) An acoustical image of MCF-7 cells used as a phantom. (Right) A B-scan image of MCF-7 cells with a 128 element phased array transducer at a center frequency of 400 MHz.

Figure 7-24 shows a B-scan simulated image of MCF-7 cells being scanned with a 128 element phased array transducer at a center frequency of 400 MHz. The sampling frequency was 5600 MHz. The phased array was designed with approximately half wavelength (1.9 μm) spacing and Hanning apodization. The element height was 1 mm, the width was 1 μm, and the kerf was one-tenth of a wavelength. A single transmit focus was at 1 mm. The focusing during the reception was at 1 mm to 10 mm in 1 mm increments. The simulated image consists of 64 lines with 1.4 degrees between the lines, which the number of scan lines came from the successive simulations to yield the scan lines that could assemble a simulated images within four days of simulation time for each MCF7 cells image.
7.4.1.6) 256 elements with 400 MHz center frequency – MCF7 cells

Figure 7-25. (Left) An acoustical image of MCF-7 cells used as a phantom. (Right) A B-scan image of MCF-7 cells with a 256 element phased array transducer at a center frequency of 400 MHz.

A B-scan simulated image of MCF-7 cells being scanned with a 256 element phased array transducer at a center frequency of 400 MHz is displayed in Figure 7-25. The sampling frequency was 5600 MHz. The dimensions of each element are; the element height was 1 mm, the width was 1 µm, and the kerf was one-tenth of a wavelength. The phased array was designed with approximately half wavelength (1.9 µm) spacing and Hanning apodization. For transmission, a single transmit focus was set at 1 mm. For the reception, the multiple receiving foci during the reception were at 1 mm to 10 mm in 1 mm increments. The simulated image consists of 64 lines with 1.4 degrees between the lines, which the number of scan lines came from the successive simulations to generate the scan lines that could assemble a simulated images within four days of simulation time for each MCF7 cells image.
7.4.1.7) 64 elements with 600 MHz center frequency – MCF7 cells

Figure 7-26. (Left) An acoustical image of MCF-7 cells used as a phantom. (Right) A B-scan image of MCF-7 cells with a 64 element phased array transducer at a center frequency of 600 MHz.

Figure 7-26 displays a B-scan simulated image of MCF-7 cells being scanned with a 64 element phased array transducer at a center frequency of 600 MHz. The sampling frequency was 8400 MHz. The phased array was designed with approximately half wavelength (1.3 μm) spacing and Hanning apodization. The element height was 1 mm, the width was 1 μm, and the kerf was one-tenth of a wavelength. A single transmit focus was at 1 mm. The focusing during the reception is at 1 mm to 10 mm in 1 mm increments. The simulated image consists of 64 lines with 1.4 degrees between the lines, which the number of scan lines came from the successive simulations to produce the scan lines that could construct a simulated images within four days of simulation time for each MCF7 cells image.
7.4.1.8) **128 elements with 600 MHz center frequency – MCF7 cells**

Figure 7-27. (Left) An acoustical image of MCF-7 cells used as a phantom. (Right) A B-scan image of MCF-7 cells with a 128 element phased array transducer at a center frequency of 600 MHz.

A B-scan simulated image of MCF-7 cells being scanned with a 256 element phased array transducer at a center frequency of 400 MHz is displayed in Figure 7-27. The sampling frequency was 8400 MHz. The dimensions of each element are; the element height was 1 mm, the width was 1 µm, and the kerf was one-tenth of a wavelength. The phased array was designed with approximately half wavelength (1.3 µm) spacing and Hanning apodization. For transmission, a single transmit focus was set at 1 mm. For the reception, the multiple receiving foci during the reception is at 1 mm to 10 mm in 1 mm increments. The simulated image consists of 64 lines with 1.4 degrees between the lines, which the number of scan lines came from the successive simulations to yield the scan lines that could build a simulated images within four days of simulation time for each MCF7 cells image.
7.4.1.9) 256 elements with 600 MHz center frequency – MCF7 cells

Figure 7-28. (Left) An acoustical image of MCF-7 cells used as a phantom. (Right) A B-scan image of MCF-7 cells with a 256 element phased array transducer at a center frequency of 600 MHz.

Figure 7-28 shows a B-scan simulated image of MCF-7 cells being scanned with a 256 element phased array transducer at a center frequency of 600 MHz. The sampling frequency was 8400 MHz. The phased array was designed with approximately half wavelength (1.3 μm) spacing and Hanning apodization. The element height was 1 mm, the width was 1 μm, and the kerf was one-tenth of a wavelength. A single transmit focus was at 1 mm. The focusing during the reception is at 1 mm to 10 mm in 1 mm increments. The simulated image consists of 64 lines with 1.4 degrees between the lines, which the number of scan lines came from the successive simulations to generate the scan lines that could assemble a simulated images within four days of simulation time for each MCF7 cells image.
7.4.1.10) *64 elements with 800 MHz center frequency – MCF7 cells*

Figure 7-29. (Left) An acoustical image of MCF-7 cells used as a phantom. (Right) A B-scan image of MCF-7 cells with a 64 element phased array transducer at a center frequency of 800 MHz.

A B-scan simulated image of MCF-7 cells being scanned with a 64 element phased array transducer at a center frequency of 800 MHz is displayed in Figure 7-29. The sampling frequency was 11200 MHz. The dimensions of each element are; the element height was 1 mm, the width was 0.75 µm, and the kerf was one-tenth of a wavelength. The phased array was designed with approximately half wavelength (0.96 µm) spacing and Hanning apodization. For transmission, a single transmit focus was set at 1 mm. For the reception, the multiple receiving foci during the reception is at 1 mm to 10 mm in 1 mm increments. The simulated image consists of 64 lines with 1.4 degrees between the lines, which the number of scan lines came from the successive simulations to produce the scan lines that could build a simulated images within four days of simulation time for each MCF7 cells image.
7.4.1.11) 128 elements with 800 MHz center frequency – MCF7 cells

Figure 7-30. (Left) An acoustical image of MCF-7 cells used as a phantom. (Right) A B-scan image of MCF-7 cells with a 128 element phased array transducer at a center frequency of 800 MHz.

Figure 7-30 displays a B-scan simulated image of MCF-7 cells being scanned with a 128 element phased array transducer at a center frequency of 800 MHz. The sampling frequency was 11200 MHz. The phased array was designed with approximately half wavelength (0.96 μm) spacing and Hanning apodization. The element height was 1 mm, the width was 0.75 μm, and the kerf was one-tenth of a wavelength. A single transmit focus was at 1 mm. The focusing during the reception is at 1 mm to 10 mm in 1 mm increments. The simulated image consists of 64 lines with 1.4 degrees between the lines, which the number of scan lines came from the successive simulations to generate the scan lines that could construct a simulated images within four days of simulation time for each MCF7 cells image.
7.4.1.12) 256 elements with 800 MHz center frequency – MCF7 cells

Figure 7-31. (Left) An acoustical image of MCF-7 cells used as a phantom. (Right) A B-scan image of MCF-7 cells with a 256 elements phased array transducer at a center frequency of 800 MHz.

A B-scan simulated image of MCF-7 cells being scanned with a 256 element phased array transducer at a center frequency of 800 MHz is displayed in Figure 7-31. The sampling frequency was 11200 MHz. The dimensions of each element are; the element height was 1 mm, the width was 0.75 μm, and the kerf was one-tenth of a wavelength. The phased array was designed with approximately half wavelength (0.96 μm) spacing and Hanning apodization. For transmission, a single transmit focus was set at 1 mm. For the reception, the multiple receiving foci during the reception is at 1 mm to 10 mm in 1 mm increments. The simulated image consists of 64 lines with 1.4 degrees between the lines, which the number of scan lines came from the successive simulations to yield the scan lines that could assemble a simulated images within four days of simulation time for each MCF7 cells image.
7.4.1.13) 64 elements with 1000 MHz center frequency – MCF7 cells

Figure 7-32. (Left) An acoustical image of MCF-7 cells used as a phantom. (Right) A B-scan image of MCF-7 cells with a 64 element phased array transducer at a center frequency of 1000 MHz.

Figure 7-32 shows a B-scan simulated image of MCF-7 cells being scanned with a 64 element phased array transducer at a center frequency of 1000 MHz. The sampling frequency was 14000 MHz. The phased array was designed with approximately half wavelength (0.77 µm) spacing and Hanning apodization. The element height was 0.8 mm, the width was 0.6 µm, and the kerf was one-tenth of a wavelength. A single transmit focus was at 1 mm. The focusing during the reception is at 1 mm to 10 mm in 1 mm increments. The simulated image consists of 64 lines with 1.4 degrees between the lines, which the number of scan lines came from the successive simulations to generate the scan lines that could build a simulated images within four days of simulation time for each MCF7 cells image.
7.4.1.14) 128 elements with 1000 MHz center frequency – MCF7 cells

Figure 7-33. (Left) An acoustical image of MCF-7 cells used as a phantom. (Right) A B-scan image of MCF-7 cells with a 128 element phased array transducer at a center frequency of 1000 MHz.

A B-scan simulated image of MCF-7 cells being scanned with a 128 element phased array transducer at a center frequency of 1000 MHz is shown in Figure 7-33. The sampling frequency was 14000 MHz. The dimensions of each element are; the element height was 0.8 mm, the width was 0.6 µm, and the kerf was one-tenth of a wavelength. The phased array was designed with approximately half wavelength (0.77 µm) spacing and Hanning apodization. For transmission, a single transmit focus was set at 1 mm. For the reception, the multiple receiving foci during the reception are at 1 mm to 10 mm in 1 mm increments. The simulated image consists of 64 lines with 1.4 degrees between the lines, which the number of scan lines came from the successive simulations to produce the scan lines that could construct a simulated images within four days of simulation time for each MCF7 cells image.
7.4.1.15) 256 elements with 1000 MHz center frequency- MCF7 cells

Figure 7-34. (Left) An acoustical image of MCF-7 cells used as a phantom. (Right) A B-scan image of MCF-7 cells with a 256 element phased array transducer at a center frequency of 1000 MHz.

Figure 7-34 displays a B-scan simulated image of MCF-7 cells being scanned with a 256 element phased array transducer at a center frequency of 1000 MHz. The sampling frequency was 14000 MHz. The phased array was designed with approximately half wavelength (0.77 µm) spacing and Hanning apodization. The element height was 0.8 mm, the width was 0.6 µm, and the kerf was one-tenth of a wavelength. A single transmit focus was at 1 mm. The focusing during the reception is at 1 mm to 10 mm in 1 mm increments. The simulated image consists of 64 lines with 1.4 degrees between the lines, which the number of scan lines came from the successive simulations to produce the scan lines that could construct a simulated images within four days of simulation time for each MCF7 cells image.
7.4.1.16) 64 elements with 100 MHz center frequency- HeLa cells

Figure 7-35. (Left) An acoustical image of HeLa cells used as a phantom. (Right) A B-scan image of HeLa cells with a 64 element phased array transducer at a center frequency of 100 MHz.

A B-scan simulated image of HeLa cells being scanned with a 64 element phased array transducer at a center frequency of 100 MHz is shown in Figure 7-35. The sampling frequency was 1400 MHz. The dimensions of each element are; the element height was 5 mm, the width was 6 µm, and the kerf was one-tenth of a wavelength. The phased array was designed with approximately half wavelength (7.7 µm) spacing and Hanning apodization. For transmission, a single transmit focus was set at 6 mm. For the reception, the multiple receiving foci during the reception is at 1 mm to 15 mm in 1 mm increments. The simulated image consists of 50 lines with 1.8 degrees between the lines, which the number of scan lines came from the successive simulations to yield the scan lines that could assemble a simulated images within three days of simulation time for each HeLa cells image.
Figure 7-36. (Left) An acoustical image of HeLa cells used as a phantom. (Right) A B-scan image of HeLa cells with a 128 element phased array transducer at a center frequency of 100 MHz.

Figure 7-36 shows a B-scan simulated image of HeLa cells being scanned with a 128 element phased array transducer at a center frequency of 100 MHz. The sampling frequency was 1400 MHz. The phased array was designed with approximately half wavelength (7.7 μm) spacing and Hanning apodization. The element height was 5 mm, the width was 6 μm, and the kerf was one-tenth of a wavelength. A single transmit focus was at 6 mm. The focusing during the reception is at 1 mm to 10 mm in 1 mm increments. The simulated image consists of 50 lines with 1.8 degrees between the lines, which the number of scan lines came from the successive simulations to generate the scan lines that could construct a simulated images within three days of simulation time for each HeLa cells image.
7.4.1.18) 256 elements with 100 MHz center frequency – HeLa cells

Figure 7-37. (Left) An acoustical image of HeLa cells used as a phantom. (Right) A B-scan image of HeLa cells with a 256 element phased array transducer at a center frequency of 100 MHz.

A B-scan simulated image of HeLa cells being scanned with a 256 element phased array transducer at a center frequency of 100 MHz is displayed in Figure 7-37. The sampling frequency was 1400 MHz. The dimensions of each element are; the element height was 5 mm, the width was 6 µm, and the kerf was one-tenth of a wavelength. The phased array was designed with approximately half wavelength (7.7 µm) spacing and Hanning apodization. For transmission, a single transmit focus was set at 6 mm. For the reception, the multiple receiving foci during the reception is at 1 mm to 15 mm in 1 mm increments. The simulated image consists of 50 lines with 1.8 degrees between the lines, which the number of scan lines came from the successive simulations to produce the scan lines that could assemble a simulated images within three days of simulation time for each HeLa cells image.
7.4.1.19) 64 elements with 400 MHz center frequency – HeLa cells

Figure 7-38. (Left) An acoustical image of HeLa cells used as a phantom. (Right) A B-scan image of HeLa cells with a 64 element phased array transducer at a center frequency of 400 MHz.

Figure 7-38 displays a B-scan simulated image of HeLa cells being scanned with a 64 element phased array transducer at a center frequency of 400 MHz. The sampling frequency was 5600 MHz. The phased array was designed with approximately half wavelength (1.9 µm) spacing and Hanning apodization. The element height was 1 mm, the width was 1 µm, and the kerf was one-tenth of a wavelength. A single transmit focus was at 1 mm. The focusing during the reception is at 1 mm to 10 mm in 1 mm increments. The simulated image consists of 50 lines with 1.8 degrees between the lines, which the number of scan lines came from the successive simulations to yield the scan lines that could build a simulated images within three days of simulation time for each HeLa cells image.
7.4.1.20) 128 elements with 400 MHz center frequency – HeLa cells I

Figure 7-39. (Left) An acoustical image of HeLa cells used as a phantom. (Right) A B-scan image of HeLa cells with a 128 element phased array transducer at a center frequency of 400 MHz.

A B-scan simulated image of HeLa cells being scanned with a 128 element phased array transducer at a center frequency of 400 MHz is shown in Figure 7-39. The sampling frequency was 5600 MHz. The dimensions of each element are; the element height was 1 mm, the width was 1 µm, and the kerf was one-tenth of a wavelength. The phased array was designed with approximately half wavelength (1.9 µm) spacing and Hanning apodization. For transmission, a single transmit focus was set at 1 mm. For the reception, the multiple receiving foci during the reception is at 1 mm to 10 mm in 1 mm increments. The simulated image consists of 50 lines with 1.8 degrees between the lines, which the number of scan lines came from the successive simulations to generate the scan lines that could assemble a simulated images within three days of simulation time for each HeLa cells image.
7.4.1.21) 256 elements with 400 MHz center frequency – HeLa cells

Figure 7-40. (Left) An acoustical image of HeLa cells used as a phantom. (Right) A B-scan image of HeLa cells with a 256 element phased array transducer at a center frequency of 400 MHz.

Figure 7-40 shows a B-scan simulated image of HeLa cells being scanned with a 256 element phased array transducer at a center frequency of 400 MHz. The sampling frequency was 5600 MHz. The phased array was designed with approximately half wavelength (1.9 μm) spacing and Hanning apodization. The element height was 1 mm, the width was 1 μm, and the kerf was one-tenth of a wavelength. A single transmit focus was at 1 mm. The focusing during the reception is at 1 mm to 10 mm in 1 mm increments. The simulated image consists of 50 lines with 1.8 degrees between the lines, which the number of scan lines came from the successive simulations to produce the scan lines that could construct a simulated images within three days of simulation time for each HeLa cells image.
7.4.1.22) 64 elements with 600 MHz center frequency – HeLa cells I

Figure 7-41. (Left) An acoustical image of HeLa cells used as a phantom. (Right) A B-scan image of HeLa cells with a 64 element phased array transducer at a center frequency of 600 MHz.

A B-scan simulated image of HeLa cells being scanned with a 64 element phased array transducer at a center frequency of 600 MHz is displayed in Figure 7-41. The sampling frequency was 8400 MHz. The dimensions of each element are; the element height was 1 mm, the width was 1 μm, and the kerf was one-tenth of a wavelength. The phased array was designed with approximately half wavelength (1.3 μm) spacing and Hanning apodization. For transmission, a single transmit focus was set at 1 mm. For the reception, the multiple receiving foci during the reception are at 1 mm to 10 mm in 1 mm increments. The simulated image consists of 50 lines with 1.8 degrees between the lines, which the number of scan lines came from the successive simulations to generate the scan lines that could build a simulated images within three days of simulation time for each HeLa cells image.
7.4.1.23) 128 elements with 600 MHz center frequency – HeLa cells I

Figure 7-42. (Left) An acoustical image of HeLa cells used as a phantom. (Right) A B-scan image of HeLa cells with a 128 element phased array transducer at a center frequency of 600 MHz.

Figure 7-42 displays a B-scan simulated image of HeLa cells being scanned with a 128 element phased array transducer at a center frequency of 600 MHz. The sampling frequency was 8400 MHz. The phased array was designed with approximately half wavelength (1.3 μm) spacing and Hanning apodization. The element height was 1 mm, the width was 1 μm, and the kerf was one-tenth of a wavelength. A single transmit focus was at 1 mm. The focusing during the reception is at 1 mm to 10 mm in 1 mm increments. The simulated image consists of 50 lines with 1.8 degrees between the lines, which the number of scan lines came from the successive simulations to yield the scan lines that could construct a simulated images within three days of simulation time for each HeLa cells image.
7.4.1.24) 256 elements with 600 MHz center frequency- HeLa cells I

Figure 7-43. (Left) An acoustical image of HeLa cells used as a phantom. (Right) A B-scan image of HeLa cells with a 256 element phased array transducer at a center frequency of 600 MHz.

A B-scan simulated image of HeLa cells being scanned with a 256 element phased array transducer at a center frequency of 600 MHz is shown in Figure 7-43. The sampling frequency was 8400 MHz. The dimensions of each element are; the element height was 1 mm, the width was 1 µm, and the kerf was one-tenth of a wavelength. The phased array was designed with approximately half wavelength (1.3 µm) spacing and Hanning apodization. For transmission, a single transmit focus was set at 1 mm. For the reception, the multiple receiving foci during the reception are at 1 mm to 10 mm in 1 mm increments. The simulated image consists of 50 lines with 1.8 degrees between the lines, which the number of scan lines came from the successive simulations to generate the scan lines that could assemble a simulated images within three days of simulation time for each HeLa cells image.
7.4.1.25) 64 elements with 800 MHz center frequency – HeLa cells I

Figure 7-44. (Left) An acoustical image of HeLa cells used as a phantom. (Right) A B-scan image of HeLa cells with a 64 element phased array transducer at a center frequency of 800 MHz

Figure 7-44 shows a B-scan simulated image of HeLa cells being scanned with a 64 element phased array transducer at a center frequency of 800 MHz. The sampling frequency was 11200 MHz. The phased array was designed with approximately half wavelength (0.96 μm) spacing and Hanning apodization. The element height was 1 mm, the width was 0.75 μm, and the kerf was one-tenth of a wavelength. A single transmit focus was at 1 mm. The focusing during the reception is at 1 mm to 10 mm in 1 mm increments. The simulated image consists of 50 lines with 1.8 degrees between the lines, which the number of scan lines came from the successive simulations to produce the scan lines that could construct a simulated images within three days of simulation time for each HeLa cells image.
7.4.1.26) 128 elements with 800 MHz center frequency – HeLa cells I

Figure 7-45. (Left) An acoustical image of HeLa cells used as a phantom. (Right) A B-scan image of HeLa cells with a 128 element phased array transducer at a center frequency of 800 MHz.

A B-scan simulated image of HeLa cells being scanned with a 128 element phased array transducer at a center frequency of 800 MHz is displayed in Figure 7-45. The sampling frequency was 11200 MHz. The dimensions of each element are; the element height was 1 mm, the width was 0.75 µm, and the kerf was one-tenth of a wavelength. The phased array was designed with approximately half wavelength (0.96 µm) spacing and Hanning apodization. For transmission, a single transmit focus was set at 1 mm. For the reception, the multiple receiving foci during the reception is at 1 mm to 10 mm in 1 mm increments. The simulated image consists of 50 lines with 1.8 degrees between the lines, which the number of scan lines came from the successive simulations to yield the scan lines that could assemble a simulated images within three days of simulation time for each HeLa cells image.
7.4.1.27) 256 elements with 800 MHz center frequency – HeLa cells I

Figure 7-46. (Left) An acoustical image of HeLa cells used as a phantom. (Right) A B-scan image of HeLa cells with a 256 element phased array transducer at a center frequency of 800 MHz.

Figure 7-46 displays a B-scan simulated image of HeLa cells being scanned with a 256 element phased array transducer at a center frequency of 800 MHz. The sampling frequency was 11200 MHz. The phased array was designed with approximately half wavelength (0.96 μm) spacing and Hanning apodization. The element height was 1 mm, the width was 0.75 μm, and the kerf was one-tenth of a wavelength. A single transmit focus was at 1 mm. The focusing during the reception is at 1 mm to 10 mm in 1 mm increments. The simulated image consists of 50 lines with 1.8 degrees between the lines, which the number of scan lines came from the successive simulations to generate the scan lines that could construct a simulated images within three days of simulation time for each HeLa cells image.
7.4.1.28) 64 elements with 1000 MHz center frequency – HeLa cells I

A B-scan simulated image of HeLa cells being scanned with a 64 element phased array transducer at a center frequency of 1000 MHz is displayed in Figure 7-47. The sampling frequency was 14000 MHz. The dimensions of each element are; the element height was 0.8 mm, the width was 0.6 µm, and the kerf was one-tenth of a wavelength. The phased array was designed with approximately half wavelength (0.77 µm) spacing and Hanning apodization. For transmission, a single transmit focus was set at 1 mm. For the reception, the multiple receiving foci during the reception is at 1 mm to 10 mm in 1 mm increments. The simulated image consists of 50 lines with 1.8 degrees between the lines, which the number of scan lines came from the successive simulations to generate the scan lines that could construct a simulated images within three days of simulation time for each HeLa cells image.
7.4.1.29) 128 elements with 1000 MHz center frequency – HeLa cells I

Figure 7-48. (Left) An acoustical image of HeLa cells used as a phantom. (Right) A B-scan image of HeLa cells with a 128 element phased array transducer at a center frequency of 1000 MHz.

Figure 7-48 shows a B-scan simulated image of HeLa cells being scanned with a 128 element phased array transducer at a center frequency of 1000 MHz. The sampling frequency was 14000 MHz. The phased array was designed with approximately half wavelength (0.77 μm) spacing and Hanning apodization. The element height was 0.8 mm, the width was 0.6 μm, and the kerf was one-tenth of a wavelength. A single transmit focus was at 1 mm. The focusing during the reception is at 1 mm to 10 mm in 1 mm increments. The simulated image consists of 50 lines with 1.8 degrees between the lines, which the number of scan lines came from the successive simulations to produce the scan lines that could assemble a simulated images within three days of simulation time for each HeLa cells image.
7.4.1.30) 256 elements with 1000 MHz center frequency – HeLa cells I

Figure 7-49. (Left) An acoustical image of HeLa cells used as a phantom. (Right) A B-scan image of HeLa cells with a 256 element phased array transducer at a center frequency of 1000 MHz.

A B-scan simulated image of HeLa cells being scanned with a 256 element phased array transducer at a center frequency of 1000 MHz is shown in Figure 7-49. The sampling frequency was 14000 MHz. The dimensions of each element are; the element height was 0.8 mm, the width was 0.6 µm, and the kerf was one-tenth of a wavelength. The phased array was designed with approximately half wavelength (0.77 µm) spacing and Hanning apodization. For transmission, a single transmit focus was set at 1 mm. For the reception, the multiple receiving foci during the reception are at 1 mm to 10 mm in 1 mm increments. The simulated image consists of 50 lines with 1.8 degrees between the lines, which the number of scan lines came from the successive simulations to generate the scan lines that could construct a simulated images within three days of simulation time for each HeLa cells image.
7.4.1.31) 64 elements with 100 MHz center frequency – HeLa cell II

Figure 7-50. (Left) An acoustical image of HeLa cells used as a phantom. (Right) A B-scan image of HeLa cells with a 64 element phased array transducer at a center frequency of 100 MHz.

Figure 7-50 displays a B-scan simulated image of HeLa cells being scanned with a 64 element phased array transducer at a center frequency of 100 MHz. The sampling frequency was 1400 MHz. The phased array was designed with approximately half wavelength (7.7 μm) spacing and Hanning apodization. The element height was 5 mm, the width was 6 μm, and the kerf was one-tenth of a wavelength. A single transmit focus was at 6 mm. The focusing during the reception is at 1 mm to 15 mm in 1 mm increments. The simulated image consists of 50 lines with 1.8 degrees between the lines, which the number of scan lines came from the successive simulations to produce the scan lines that could assemble a simulated images within three days of simulation time for each HeLa cells image.
7.4.1.32) 128 elements with 100 MHz center frequency – HeLa cell II

Figure 7-51. (Left) An acoustical image of HeLa cells used as a phantom. (Right) A B-scan image of HeLa cells with a 128 element phased array transducer at a center frequency of 100 MHz.

A B-scan simulated image of HeLa cells being scanned with a 256 element phased array transducer at a center frequency of 100 MHz is displayed in Figure 7-51. The sampling frequency was 1400 MHz. The dimensions of each element are; the element height was 5 mm, the width was 6 µm, and the kerf was one-tenth of a wavelength. The phased array was designed with approximately half wavelength (7.7 µm) spacing and Hanning apodization. For transmission, a single transmit focus was set at 6 mm. For the reception, the multiple receiving foci during the reception are at 1 mm to 15 mm in 1 mm increments. The simulated image consists of 50 lines with 1.8 degrees between the lines, which the number of scan lines came from the successive simulations to generate the scan lines that could build a simulated images within three days of simulation time for each HeLa cells image.
7.4.1.33) 256 elements with 100 MHz center frequency – HeLa cell II

Figure 7-52. (Left) An acoustical image of HeLa cells used as a phantom. (Right) A B-scan image of HeLa cells with a 256 element phased array transducer at a center frequency of 100 MHz.

Figure 7-52 shows a B-scan simulated image of HeLa cells being scanned with a 256 element phased array transducer at a center frequency of 100 MHz. The sampling frequency was 1400 MHz. The phased array was designed with approximately half wavelength (7.7 μm) spacing and Hanning apodization. The element height was 5 mm, the width was 6 μm, and the kerf was one-tenth of a wavelength. A single transmit focus was at 1 mm. The focusing during the reception is at 6 mm to 15 mm in 1 mm increments. The simulated image consists of 50 lines with 1.8 degrees between the lines, which the number of scan lines came from the successive simulations to yield the scan lines that could construct a simulated images within three days of simulation time for each HeLa cells image.
7.4.1.34) 64 elements with 400 MHz center frequency – HeLa cell II

Figure 7-53. (Left) An acoustical image of HeLa cells used as a phantom. (Right) A B-scan image of HeLa cells with a 64 element phased array transducer at a center frequency of 400 MHz.

A B-scan simulated image of HeLa cells being scanned with a 64 element phased array transducer at a center frequency of 400 MHz is shown in Figure 7-53. The sampling frequency was 5600 MHz. The dimensions of each element are; the element height was 1 mm, the width was 1 μm, and the kerf was one-tenth of a wavelength. The phased array was designed with approximately half wavelength (1.9 μm) spacing and Hanning apodization. For transmission, a single transmit focus was set at 1 mm. For the reception, the multiple receiving foci during the reception is at 1 mm to 10 mm in 1 mm increments. The simulated image consists of 50 lines with 1.8 degrees between the lines, which the number of scan lines came from the successive simulations to generate the scan lines that could assemble a simulated images within three days of simulation time for each HeLa cells image.
7.4.1.35) 128 elements with 400 MHz center frequency – HeLa cell II

Figure 7-54. (Left) An acoustical image of HeLa cells used as a phantom. (Right) A B-scan image of HeLa cells with a 128 element phased array transducer at a center frequency of 400 MHz.

Figure 7-54 displays a B-scan simulated image of HeLa cells being scanned with a 128 element phased array transducer at a center frequency of 400 MHz. The sampling frequency was 5600 MHz. The phased array was designed with approximately half wavelength (1.9 μm) spacing and Hanning apodization. The element height was 1 mm, the width was 1 μm, and the kerf was one-tenth of a wavelength. A single transmit focus was at 1 mm. The focusing during the reception is at 1 mm to 10 mm in 1 mm increments. The simulated image consists of 50 lines with 1.8 degrees between the lines, which the number of scan lines came from the successive simulations to yield the scan lines that could build a simulated images within three days of simulation time for each HeLa cells image.
7.4.1.36) 256 elements with 400 MHz center frequency- HeLa cell II

Figure 7-55. (Left) An acoustical image of HeLa cells used as a phantom. (Right) A B-scan image of HeLa cells with a 256 element phased array transducer at a center frequency of 400 MHz.

A B-scan simulated image of HeLa cells being scanned with a 256 element phased array transducer at a center frequency of 400 MHz is displayed in Figure 7-55. The sampling frequency was 5600 MHz. The dimensions of each element are; the element height was 1 mm, the width was 1 μm, and the kerf was one-tenth of a wavelength. The phased array was designed with approximately half wavelength (1.9 μm) spacing and Hanning apodization. For transmission, a single transmit focus was set at 1 mm. For the reception, the multiple receiving foci during the reception are at 1 mm to 10 mm in 1 mm increments. The simulated image consists of 50 lines with 1.8 degrees between the lines, which the number of scan lines came from the successive simulations to produce the scan lines that could construct a simulated images within three days of simulation time for each HeLa cells image.
7.4.1.37) 64 elements with 600 MHz center frequency- HeLa cell II

Figure 7-56. (Left) An acoustical image of HeLa cells used as a phantom. (Right) A B-scan image of HeLa cells with a 64 element phased array transducer at a center frequency of 600 MHz.

Figure 7-56 shows a B-scan simulated image of HeLa cells being scanned with a 64 element phased array transducer at a center frequency of 600 MHz. The sampling frequency was 8400 MHz. The phased array was designed with approximately half wavelength (1.3 μm) spacing and Hanning apodization. The element height was 1 mm, the width was 1 μm, and the kerf was one-tenth of a wavelength. A single transmit focus was at 1 mm. The focusing during the reception is at 1 mm to 10 mm in 1 mm increments. The simulated image consists of 50 lines with 1.8 degrees between the lines, which the number of scan lines came from the successive simulations to generate the scan lines that could assemble a simulated images within three days of simulation time for each HeLa cells image.
7.4.1.38) 128 elements with 600 MHz center frequency - HeLa cell II

Figure 7-57. (Left) An acoustical image of HeLa cells used as a phantom. (Right) A B-scan image of HeLa cells with a 128 element phased array transducer at a center frequency of 600 MHz.

A B-scan simulated image of HeLa cells being scanned with a 128 element phased array transducer at a center frequency of 600 MHz is displayed in Figure 7-57. The sampling frequency was 8400 MHz. The dimensions of each element are; the element height was 1 mm, the width was 1 μm, and the kerf was one-tenth of a wavelength. The phased array was designed with approximately half wavelength (1.3 μm) spacing and Hanning apodization. For transmission, a single transmit focus was set at 1 mm. For the reception, the multiple receiving foci during the reception are at 1 mm to 10 mm in 1 mm increments. The simulated image consists of 50 lines with 1.8 degrees between the lines, which the number of scan lines came from the successive simulations to yield the scan lines that could build a simulated images within three days of simulation time for each HeLa cells image.
7.4.1.39) 256 elements with 600 MHz center frequency- HeLa cell II

Figure 7-58. (Left) An acoustical image of HeLa cells used as a phantom. (Right) A B-scan image of HeLa cells with a 256 element phased array transducer at a center frequency of 600 MHz.

Figure 7-58 displays a B-scan simulated image of HeLa cells being scanned with a 256 element phased array transducer at a center frequency of 600 MHz. The sampling frequency was 8400 MHz. The phased array was designed with approximately half wavelength (1.3 μm) spacing and Hanning apodization. The element height was 1 mm, the width was 1 μm, and the kerf was one-tenth of a wavelength. A single transmit focus was at 1 mm. The focusing during the reception is at 1 mm to 10 mm in 1 mm increments. The simulated image consists of 50 lines with 1.8 degrees between the lines, which the number of scan lines came from the successive simulations to produce the scan lines that could construct a simulated images within three days of simulation time for each HeLa cells image.
7.4.1.40) 64 elements with 800 MHz center frequency- HeLa cell II

Figure 7-59. (Left) An acoustical image of HeLa cells used as a phantom. (Right) A B-scan image of HeLa cells with a 64 element phased array transducer at a center frequency of 800 MHz.

A B-scan simulated image of HeLa cells being scanned with a 64 element phased array transducer at a center frequency of 800 MHz is shown in Figure 7-59. The sampling frequency was 11200 MHz. The dimensions of each element are; the element height was 1 mm, the width was 0.75 μm, and the kerf was one-tenth of a wavelength. The phased array was designed with approximately half wavelength (0.96 μm) spacing and Hanning apodization. For transmission, a single transmit focus was set at 1 mm. For the reception, the multiple receiving foci during the reception is at 1 mm to 10 mm in 1 mm increments. The simulated image consists of 50 lines with 1.8 degrees between the lines, which the number of scan lines came from the successive simulations to generate the scan lines that could assemble a simulated images within three days of simulation time for each HeLa cells image.
7.4.1.41) 128 elements with 800 MHz center frequency- HeLa cell II

Figure 7-60. (Left) An acoustical image of HeLa cells used as a phantom. (Right) A B-scan image of HeLa cells with a 128 element phased array transducer at a center frequency of 800 MHz.

Figure 7-60 shows a B-scan simulated image of HeLa cells being scanned with a 128 element phased array transducer at a center frequency of 800 MHz. The sampling frequency was 11200 MHz. The phased array was designed with approximately half wavelength (0.96 μm) spacing and Hanning apodization. The element height was 1 mm, the width was 0.75 μm, and the kerf was one-tenth of a wavelength. A single transmit focus was at 1 mm. The focusing during the reception is at 1 mm to 10 mm in 1 mm increments. The simulated image consists of 50 lines with 1.8 degrees between the lines, which the number of scan lines came from the successive simulations to yield the scan lines that could build a simulated images within three days of simulation time for each HeLa cells image.
7.4.1.42) 256 elements with 800 MHz center frequency – HeLa cell II

Figure 7-61. (Left) An acoustical image of HeLa cells used as a phantom. (Right) A B-scan image of HeLa cells with a 256 element phased array transducer at a center frequency of 800 MHz.

A B-scan simulated image of HeLa cells being scanned with a 256 element phased array transducer at a center frequency of 800 MHz is displayed in Figure 7-61. The sampling frequency was 11200 MHz. The dimensions of each element are; the element height was 1 mm, the width was 0.75 μm, and the kerf was one-tenth of a wavelength. The phased array was designed with approximately half wavelength (0.96 μm) spacing and Hanning apodization. For transmission, a single transmit focus was set at 1 mm. For the reception, the multiple receiving foci during the reception are at 1 mm to 10 mm in 1 mm increments. The simulated image consists of 50 lines with 1.8 degrees between the lines, which the number of scan lines came from the successive simulations to produce the scan lines that could construct a simulated images within three days of simulation time for each HeLa cells image.
7.4.1.43) 64 elements with 1000 MHz center frequency - HeLa cell II

Figure 7-62. (Left) An acoustical image of HeLa cells used as a phantom. (Right) A B-scan image of HeLa cells with a 64 element phased array transducer at a center frequency of 1000 MHz.

Figure 7-62 displays a B-scan simulated image of HeLa cells being scanned with a 64 element phased array transducer at a center frequency of 1000 MHz. The sampling frequency was 14000 MHz. The phased array was designed with approximately half wavelength (0.77 μm) spacing and Hanning apodization. The element height was 0.8 mm, the width was 0.6 μm, and the kerf was one-tenth of a wavelength. A single transmit focus was at 1 mm. The focusing during the reception is at 1 mm to 10 mm in 1 mm increments. The simulated image consists of 50 lines with 1.8 degrees between the lines, which the number of scan lines came from the successive simulations to generate the scan lines that could assemble a simulated images within three days of simulation time for each HeLa cells image.
7.4.1.44) 128 elements with 1000 MHz center frequency - HeLa cell II

Figure 7-63. (Left) An acoustical image of HeLa cells used as a phantom. (Right) A B-scan image of HeLa cells with a 128 element phased array transducer at a center frequency of 1000 MHz.

A B-scan simulated image of HeLa cells being scanned with a 128 element phased array transducer at a center frequency of 1000 MHz is shown in Figure 7-63. The sampling frequency was 14000 MHz. The dimensions of each element are; the element height was 0.8 mm, the width was 0.6 μm, and the kerf was one-tenth of a wavelength. The phased array was designed with approximately half wavelength (0.77 μm) spacing and Hanning apodization. For transmission, a single transmit focus was set at 1 mm. For the reception, the multiple receiving foci during the reception is at 1 mm to 10 mm in 1 mm increments. The simulated image consists of 50 lines with 1.8 degrees between the lines, which the number of scan lines came from the successive simulations to yield the scan lines that could construct a simulated images within three days of simulation time for each HeLa cells image.
7.4.1.45) 256 elements with 1000 MHz center frequency - HeLa cell II

Figure 7-64. (Left) An acoustical image of HeLa cells used as a phantom. (Right) A B-scan image of HeLa cells with a 256 element phased array transducer at a center frequency of 1000 MHz.

Figure 7-64 shows a B-scan simulated image of HeLa cells being scanned with a 256 element phased array transducer at a center frequency of 1000 MHz. The sampling frequency was 14000 MHz. The phased array was designed with approximately half wavelength (0.77 μm) spacing and Hanning apodization. The element height was 0.8 mm, the width was 0.6 μm, and the kerf was one-tenth of a wavelength. A single transmit focus was at 1 mm. The focusing during the reception is at 1 mm to 10 mm in 1 mm increments. The simulated image consists of 50 lines with 1.8 degrees between the lines, which the number of scan lines came from the successive simulations to produce the scan lines that could build a simulated images within three days of simulation time for each HeLa cells image.
7.4.2 2D- Phased array transducers

20 x 20 elements fully populated arrays with 100 MHz center frequency- MCF7 cells

Figure 7-65. (Left) An acoustical image of MCF-7 cells used as a phantom. (Right) A B-scan image of MCF-7 cells with 20x20 elements 2D fully populated array transducer at a center frequency of 100 MHz.

A B-scan simulated image of MCF-7 cells, as shown in Figure 7-65, was scanned with a 100 MHz, 20x20 element 2D fully populated array transducer with approximately half a wavelength (7.7 µm) spacing and Hanning apodization. The sampling frequency was 1400 MHz. The dimensions of each element are; the element height was 5 mm, the width was 6 µm, and the kerf was one-tenth of a wavelength. For transmission, a single transmit focus was set at 6 mm. For the reception, the multiple receiving foci during the reception are at 1 mm to 10 mm in 1 mm increments. The simulated image consists of 30 lines with 0.05 degrees between the lines, which the number of scan lines obtained from the successive simulations to generate the scan lines that could construct the simulated images within four days of simulation time for each MCF-7 cells image.
7.4.3 Volumetric Images

In this section, the volumetric images of the MCF-7 and the HeLa cells are presented as a P-scan display mode, which is a projection of a B-scan to yield a 3D image by cascading B-scan slices, in Figure 7-66 to Figure 7-95. Each volumetric image is composed of 30 slices of B-scan simulated images, slicing through the cells at a different depth in the Z-axis direction for each X-Y plane.

Figure 7-66. P-scan display mode for volumetric images.

7.4.3.1) 64 elements with 100 MHz center frequency – MCF7 cells

Figure 7-67. (Left) An acoustical image of MCF-7 cells used as a phantom. (Right) A P-scan mode volumetric image of MCF-7 cells with a 64 element phased array transducer at a center frequency of 100 MHz.
Figure 7-67 displays a P-scan mode volumetric image of MCF-7 cells created from B-scan slices obtained from the simulations of a 64 element phased array transducer at a center frequency of 100 MHz. The phased array was designed with approximately half wavelength spacing and Hanning apodization.

7.4.3.2) 128 elements with 100 MHz center frequency – MCF7 cells

Figure 7-68. (Left) An acoustical image of MCF-7 cells used as a phantom. (Right) A P-scan mode volumetric image of MCF-7 cells with a 128 element phased array transducer at a center frequency of 100 MHz.

A P-scan mode volumetric image of MCF-7 cells generated from B-scan slices obtained from the simulations of a 128 element phased array transducer at a center frequency of 100 MHz is shown in Figure 7-68. The phased array was designed with approximately half wavelength spacing and Hanning apodization.
7.4.3.3) 256 elements with 100 MHz center frequency – MCF7 cells

Figure 7-69. (Left) An acoustical image of MCF-7 cells used as a phantom. (Right) A P-scan mode volumetric image of MCF-7 cells with a 256 element phased array transducer at a center frequency of 100 MHz.

Figure 7-69 shows a P-scan mode volumetric image of MCF-7 cells created from B-scan slices obtained from the simulations of a 256 element phased array transducer at a center frequency of 100 MHz. The phased array was designed with approximately half wavelength spacing and Hanning apodization.
7.4.3.4) 64 elements with 400 MHz center frequency – MCF7 cells

Figure 7-70. (Left) An acoustical image of MCF-7 cells used as a phantom. (Right) A P-scan mode volumetric image of MCF-7 cells with a 64 element phased array transducer at a center frequency of 400 MHz.

A P-scan mode volumetric image of MCF-7 cells generated from B-scan slices obtained from the simulations of a 64 element phased array transducer at a center frequency of 400 MHz is displayed in Figure 7-70. The phased array was designed with approximately half wavelength spacing and Hanning apodization.
7.4.3.5) 128 elements with 400 MHz center frequency – MCF7 cells

Figure 7-71. (Left) An acoustical image of MCF-7 cells used as a phantom. (Right) A P-scan mode volumetric image of MCF-7 cells with a 128 element phased array transducer at a center frequency of 400 MHz.

Figure 7-71 displays a P-scan mode volumetric image of MCF-7 cells created from B-scan slices obtained from the simulations of scanned with a 128 element phased array transducer at a center frequency of 400 MHz. The phased array was designed with approximately half wavelength spacing and Hanning apodization.
7.4.3.6) 256 elements with 400 MHz center frequency – MCF7 cells

Figure 7-72 (Left) An acoustical image of MCF-7 cells used as a phantom. (Right) A P-scan mode volumetric image of MCF-7 cells with a 256 element phased array transducer at a center frequency of 400 MHz.

A P-scan mode volumetric image of MCF-7 cells generated from B-scan slices obtained from the simulations of a 256 element phased array transducer at a center frequency of 400 MHz is shown in Figure 7-72. The phased array was designed with approximately half wavelength spacing and Hanning apodization.
7.4.3.7) 64 elements with 600 MHz center frequency- MCF7 cells

Figure 7.73. (Left) An acoustical image of MCF-7 cells used as a phantom. (Right) A P-scan mode volumetric image of MCF-7 cells with a 64 element phased array transducer at a center frequency of 600 MHz.

Figure 7-73 shows a P-scan mode volumetric image of MCF-7 cells created from B-scan slices obtained from the simulations of a 64 element phased array transducer at a center frequency of 600 MHz. The phased array was designed with approximately half wavelength spacing and Hanning apodization.
**7.4.3.8) 128 elements with 600 MHz center frequency - MCF7 cells**

Figure 7-74. (Left) An acoustical image of MCF-7 cells used as a phantom. (Right) A P-scan mode volumetric image of MCF-7 cells with a 128 element phased array transducer at a center frequency of 600 MHz.

A P-scan mode volumetric image of MCF-7 cells generated from B-scan slices obtained from the simulations of a 128 element phased array transducer at a center frequency of 600 MHz is displayed in Figure 7-74. The phased array was designed with approximately half wavelength spacing and Hanning apodization.
7.4.3.9) 256 elements with 600 MHz center frequency- MCF7 cells

Figure 7-75. (Left) An acoustical image of MCF-7 cells used as a phantom. (Right) A P-scan mode volumetric image of MCF-7 cells with a 256 element phased array transducer at a center frequency of 600 MHz.

Figure 7-75 displays a P-scan mode volumetric image of MCF-7 cells created from B-scan slices obtained from the simulations of a 256 element phased array transducer at a center frequency of 600 MHz. The phased array was designed with approximately half wavelength spacing and Hanning apodization.
7.4.3.10) 64 elements with 800 MHz center frequency - MCF7 cells

A P-scan mode volumetric image of MCF-7 cells generated from B-scan slices obtained from the simulations of a 64 element phased array transducer at a center frequency of 800 MHz is shown in Figure 7-76. The phased array was designed with approximately half wavelength spacing and Hanning apodization.
7.4.3.11) 128 elements with 800 MHz center frequency - MCF7 cells

Figure 7-77 (Left) An acoustical image of MCF-7 cells used as a phantom. (Right) A P-scan mode volumetric image of MCF-7 cells with a 128 element phased array transducer at a center frequency of 800 MHz.

Figure 7-77 shows a P-scan mode volumetric image of MCF-7 cells created from B-scan slices obtained from the simulations of a 128 element phased array transducer at a center frequency of 800 MHz. The phased array was designed with approximately half wavelength spacing and Hanning apodization.
7.4.3.12) 256 elements with 800 MHz center frequency- MCF7 cells

Figure 7-78. (Left) An acoustical image of MCF-7 cells used as a phantom. (Right) A P-scan mode volumetric image of MCF-7 cells with a 256 element phased array transducer at a center frequency of 800 MHz.

A P-scan mode volumetric image of MCF-7 cells generated from B-scan slices obtained from the simulations of a 256 element phased array transducer at a center frequency of 800 MHz is displayed in Figure 7-78. The phased array was designed with approximately half wavelength spacing and Hanning apodization.
7.4.3.13) 64 elements with 1000 MHz center frequency- MCF7 cells

Figure 7-79. (Left) An acoustical image of MCF-7 cells used as a phantom. (Right) A P-scan mode volumetric image of MCF-7 cells with a 64 element phased array transducer at a center frequency of 1000 MHz.

Figure 7-79 displays a P-scan mode volumetric image of MCF-7 cells created from B-scan slices obtained from the simulations of with a 64 element phased array transducer at a center frequency of 1000 MHz. The phased array was designed with approximately half wavelength spacing and Hanning apodization.
7.4.3.14) 128 elements with 1000 MHz center frequency- MCF7 cells

Figure 7-80. (Left) An acoustical image of MCF-7 cells used as a phantom. (Right) A P-scan mode volumetric image of MCF-7 cells with a 128 element phased array transducer at a center frequency of 1000 MHz.

A P-scan mode volumetric image of MCF-7 cells constructed from B-scan slices obtained from the simulations of a 128 element phased array transducer at a center frequency of 1000 MHz is displayed in Figure 7-80. The phased array was designed with approximately half wavelength spacing and Hanning apodization.
7.4.3.15) 256 elements with 1000 MHz center frequency- MCF7 cells

Figure 7-81. (Left) An acoustical image of MCF-7 cells used as a phantom. (Right) A P-scan mode volumetric image of MCF-7 cells with a 256 element phased array transducer at a center frequency of 1000 MHz.

Figure 7-81 shows a P-scan mode volumetric image of MCF-7 cells created from B-scan slices obtained from the simulations of a 256 element phased array transducer at a center frequency of 1000 MHz. The phased array was designed with approximately half wavelength spacing and Hanning apodization.
7.4.3.16) 64 elements with 400 MHz center frequency – HeLa cells I

Figure 7-82. (Left) An acoustical image of HeLa cells used as a phantom. (Right) A P-scan mode volumetric image of HeLa cells with a 64 element phased array transducer at a center frequency of 400 MHz.

A P-scan mode volumetric image of HeLa cells constructed from B-scan slices obtained from the simulations of a 64 element phased array transducer at a center frequency of 400 MHz is displayed in Figure 7-82. The phased array was designed with approximately half wavelength spacing and Hanning apodization.
7.4.3.17) 128 elements with 400 MHz center frequency – HeLa cells I

Figure 7-83. (Left) An acoustical image of HeLa cells used as a phantom. (Right) A P-scan mode volumetric image of HeLa cells with a 128 element phased array transducer at a center frequency of 400 MHz.

Figure 7-83 displays a P-scan mode volumetric image of HeLa cells generated from B-scan slices obtained from the simulations of a 128 element phased array transducer at a center frequency of 400 MHz. The phased array was designed with approximately half wavelength spacing and Hanning apodization.
Figure 7-84. (Left) An acoustical image of HeLa cells used as a phantom. (Right) A P-scan mode volumetric image of HeLa cells with a 128 element phased array transducer at a center frequency of 600 MHz.

A P-scan mode volumetric image of HeLa cells created from B-scan slices obtained from the simulations of a 128 element phased array transducer at a center frequency of 600 MHz is shown in Figure 7-84. The phased array was designed with approximately half wavelength spacing and Hanning apodization.
7.4.3.18) 128 elements with 800 MHz center frequency – HeLa cells I

Figure 7-85. (Left) An acoustical image of HeLa cells used as a phantom. (Right) A P-scan mode volumetric image of HeLa cells with a 128 element phased array transducer at a center frequency of 800 MHz.

Figure 7-85 shows a P-scan mode volumetric image of HeLa cells constructed from B-scan slices obtained from the simulations of a 128 element phased array transducer at a center frequency of 800 MHz. The phased array was designed with approximately half wavelength spacing and Hanning apodization.
7.4.3.19) 128 elements with 100 MHz center frequency – HeLa cells II

volumetric image of HeLa cell 128 elements, 100MHz

Figure 7-86. (Left) An acoustical image of HeLa cells used as a phantom. (Right) A P-scan mode volumetric image of HeLa cells with a 128 element phased array transducer at a center frequency of 100 MHz.

A P-scan mode volumetric image of HeLa cells generated from B-scan slices obtained from the simulations of a 128 element phased array transducer at a center frequency of 100 MHz is shown in Figure 7-86. The phased array was designed with approximately half wavelength spacing and Hanning apodization.
7.4.3.19) 128 elements with 400 MHz center frequency – HeLa cells II

Figure 7-87. (Left) An acoustical image of HeLa cells used as a phantom. (Right) A P-scan mode volumetric image of HeLa cells with a 128 element phased array transducer at a center frequency of 400 MHz.

Figure 7-87 displays a P-scan mode volumetric image of HeLa cells created from B-scan slices obtained from the simulations of a 128 element phased array transducer at a center frequency of 400 MHz. The phased array was designed with approximately half wavelength spacing and Hanning apodization.
7.4.3.20) 128 elements with 600 MHz center frequency – HeLa cells II

Figure 7-88. (Left) An acoustical image of HeLa cells used as a phantom. (Right) A P-scan mode volumetric image of HeLa cells with a 128 element phased array transducer at a center frequency of 600 MHz.

A P-scan mode volumetric image of HeLa cells constructed from B-scan slices obtained from the simulations of a 128 element phased array transducer at a center frequency of 600 MHz is displayed in Figure 7-88. The phased array was designed with approximately half wavelength spacing and Hanning apodization.
7.4.3.21) 128 elements with 800 MHz center frequency – HeLa cells II

Figure 7-89. (Left) An acoustical image of HeLa cells used as a phantom. (Right) A P-scan mode volumetric image of HeLa cells with a 128 element phased array transducer at a center frequency of 800 MHz.

Figure 7-89 shows a P-scan mode volumetric image of HeLa cells generated from B-scan slices obtained from the simulations of a 128 element phased array transducer at a center frequency of 800 MHz. The phased array was designed with approximately half wavelength spacing and Hanning apodization.
7.4.3.22) 128 elements with 1000 MHz center frequency – HeLa cells II

A P-scan mode volumetric image of HeLa cells created from B-scan slices obtained from the simulations of a 128 element phased array transducer at a center frequency of 1000 MHz is displayed in Figure 7-90. The phased array was designed with approximately half wavelength spacing and Hanning apodization.

Figure 7-90. (Left) An acoustical image of HeLa cells used as a phantom. (Right) A P-scan mode volumetric image of HeLa cells with a 128 element phased array transducer at a center frequency of 1000 MHz.
7.4.3.23) 256 elements with 100 MHz center frequency – HeLa cells II

volumetric image of HeLa cell 256 elements, 100 MHz

Figure 7-91. (Left) An acoustical image of HeLa cells used as a phantom. (Right) A P-scan mode volumetric image of HeLa cells with a 256 element phased array transducer at a center frequency of 100 MHz.

Figure 7-91 displays a P-scan mode volumetric image of HeLa cells constructed from B-scan slices obtained from the simulations of a 256 element phased array transducer at a center frequency of 100 MHz. The phased array was designed with approximately half wavelength spacing and Hanning apodization.
7.4.3.23) **256 elements with 400 MHz center frequency – HeLa cells II**

A P-scan mode volumetric image of HeLa cells generated from B-scan slices obtained from the simulations of a 256 element phased array transducer at a center frequency of 400 MHz is displayed in Figure 7-92. The phased array was designed with approximately half wavelength spacing and Hanning apodization.
7.4.3.23) 256 elements with 600 MHz center frequency – HeLa cells II

Figure 7-93. (Left) An acoustical image of HeLa cells used as a phantom. (Right) A P-scan mode volumetric image of HeLa cells with a 256 element phased array transducer at a center frequency of 600 MHz.

Figure 7-93 shows a P-scan mode volumetric image of HeLa cells created from B-scan slices obtained from the simulations of a 256 element phased array transducer at a center frequency of 600 MHz. The phased array was designed with approximately half wavelength spacing and Hanning apodization.
7.4.3.24) 256 elements with 800 MHz center frequency – HeLa cells II

Figure 7-94. (Left) An acoustical image of HeLa cells used as a phantom. (Right) A P-scan mode volumetric image of HeLa cells with a 256 element phased array transducer at a center frequency of 800 MHz.

A P-scan mode volumetric image of HeLa cells constructed from B-scan slices obtained from the simulations of a 256 element phased array transducer at a center frequency of 800 MHz is shown in Figure 7-94. The phased array was designed with approximately half wavelength spacing and Hanning apodization.
7.4.3.25) 256 elements with 1000 MHz center frequency – HeLa cells II

Figure 7-95. (Left) An acoustical image of HeLa cells used as a phantom. (Right) A P-scan mode volumetric image of HeLa cells with a 256 element phased array transducer at a center frequency of 1000 MHz.

Figure 7-95 displays a P-scan mode volumetric image of HeLa cells generated from B-scan slices obtained from the simulations of a 256 element phased array transducer at a center frequency of 1000 MHz. The phased array was designed with approximately half wavelength spacing and Hanning apodization.
7.4.3.26) 20 x 20 elements fully populated arrays with 100 MHz center frequency- MCF7 cells

Figure 7-96. (Left) An acoustical image of MCF-7 cells used as a phantom. (Right) A P-scan mode volumetric image of MCF7 cells with 20x20 elements 2D fully populated array transducer at a center frequency of 100 MHz.

A P-scan mode volumetric image of MCF-7 cells, as shown in Figure 7-96, created from B-scan slices obtained from the simulations of a 100 MHz, 20x20 element 2D fully populated array transducer with approximately half a wavelength spacing and Hanning apodization.

Chapter summary

In this chapter, the simulation results of reflectance function for biological cells and substrate; V(z) curves; transducer simulations were presented. In the following chapter, the interpretation of the simulation results will be presented.
Chapter 8

Analysis

In this chapter, section 8.1 will present the resolution analysis for the phased array used in this study. Then the main results from the simulations, mirror the primary objectives that are (1) the calculation of the reflectance function of the layer model consisting of the coupling medium, the biological specimen, and the substrate; (2) the simulation results of $V(z)$ responses; (3) the SAM images of HeLa cells and MCF-7 cells as well as extracting the mechanical properties of the cells from the time-resolved SAM measurement data; and (4) the transducer simulations will be summarized and discussed in section 8.2, 8.3, 8.4, and 8.5 respectively. Lastly, the simulation code repository will be presented in section 8.6

8.1 Resolution Analysis

In section 5.2.2 of Chapter 5, we explained about electronic focusing giving the terms in Equation 5.10 for transmission from an array; this is also valid for reception of the ultrasound wave because of acoustic reciprocity. This means that it is possible during reception to change the focus as a function of time so that a dynamic tracking focus can be achieved. Table 8.1 shows the resolution analysis for phased arrays.
Table 8.1. The resolution analysis for phased array transducer used in the simulations.

<table>
<thead>
<tr>
<th>Center frequency, $f_0$ [MHz]</th>
<th>Speed of sound, $c$ [m/s]</th>
<th>Wavelength, $\lambda$ [µm]</th>
<th>Number of array elements</th>
<th>F-number</th>
<th>Lateral resolution [µm]</th>
<th>Axial resolution [µm]</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>1540</td>
<td>15.4</td>
<td>64</td>
<td>12.5</td>
<td>192.1</td>
<td>7.7</td>
</tr>
<tr>
<td>100</td>
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<td>15.4</td>
<td>128</td>
<td>6.2</td>
<td>95.8</td>
<td>7.7</td>
</tr>
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<td>400</td>
<td>1540</td>
<td>3.9</td>
<td>64</td>
<td>11.3</td>
<td>43.6</td>
<td>1.9</td>
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<td>3.9</td>
<td>128</td>
<td>5.7</td>
<td>21.8</td>
<td>1.9</td>
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<td>400</td>
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<td>3.9</td>
<td>256</td>
<td>2.8</td>
<td>10.9</td>
<td>1.9</td>
</tr>
<tr>
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<td>1540</td>
<td>2.6</td>
<td>64</td>
<td>12.5</td>
<td>32.1</td>
<td>1.3</td>
</tr>
<tr>
<td>600</td>
<td>1540</td>
<td>2.6</td>
<td>128</td>
<td>6.2</td>
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<td>1.3</td>
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<td>1.9</td>
<td>64</td>
<td>11.6</td>
<td>22.4</td>
<td>0.96</td>
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<tr>
<td>800</td>
<td>1540</td>
<td>1.9</td>
<td>128</td>
<td>5.8</td>
<td>11.2</td>
<td>0.96</td>
</tr>
<tr>
<td>800</td>
<td>1540</td>
<td>1.9</td>
<td>256</td>
<td>2.9</td>
<td>5.6</td>
<td>0.96</td>
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<td>1.5</td>
<td>64</td>
<td>12.5</td>
<td>19.2</td>
<td>0.8</td>
</tr>
<tr>
<td>1000</td>
<td>1540</td>
<td>1.5</td>
<td>128</td>
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</tr>
</tbody>
</table>

As seen in Table 8.1, theoretically, the higher frequency of the phased array, the better the resolutions can be achieved. The number of array elements and their dimension are also important. In the simulations run in this study, the array element has the dimensions that meet the criteria for phased arrays; the ratio of height to width of each element is more than 4:1, and the pitch of the array is about half wavelength. At each center frequency, the dimensions of the individual elements are kept constant; therefore, more number of the elements consists of the array, the length of the array will be increased as:

$$L = nw + (n - 1)k$$  \hspace{1cm} (8.1)

where $L$ is the length of the aperture, $n$ is the number of array elements, $w$ is the width of the individual element, $k$ is the kerf of the individual element.

The length of the aperture ($L$) affects the F-number of the arrays due to:

$$F\# = \frac{\text{focal length}}{L}$$  \hspace{1cm} (8.2)
Here we fixed the focal length to be constant, hence the larger the aperture, the smaller of the F-number. F-number plays an important role in calculating the lateral resolution as:

\[
\text{Lateral resolution} = (F\#) \times \lambda
\]

8.3

At the same operating frequency, the wavelength is constant, thus the lateral resolution depends on the F-number. The smaller number of F-number, the smaller and the better the lateral resolution. As shown in Table 8.1, at center frequency of 100 MHz, the 256 elements gives the smallest F-number and hence the smallest lateral resolution value compared to the 128, and the 64 element respectively.

The axial resolution can be calculated as described in section 5.2.1 of Chapter 5. It should be noted that theoretically the axial resolution limit will be very small if the pulse being transmitted extremely short. However, in practice, it is difficult to improve the axial resolution in this approach due to when the level of damping is increased; the energy content of the pulse is decreased, as a result the depth of penetration is diminished. As a result, it should be kept in mind that there is a trade-off between improving axial resolution and maintaining sufficient penetration depth. Theoretically, at center frequency of 1000 MHz, the 256 elements phased array will give the best lateral resolution as well as the axial resolution as shown in Table 8.1.

8.2 Reflectance function of biological cells and substrate

The reflectance function of each biological specimen attached to a substrate system plays an important role in the V(z) response of the materials because it varies specifically with the elastic properties of specimen/substrate system being tested. From the results shown in section 7.1 of Chapter 7, we can see that different kinds of cells yield different reflectance functions. For example, the kidney tissue gave different reflectance function from that of the HeLa cells. In Figure 7-2, the reflectance function of the kidney tissue showed that at the incident angle \( \theta = 0^\circ \), the
modulus of the reflectance function is approximately 0.74 and also at the longitudinal wave critical angle of the system $\theta = 14.2^\circ$ corresponding to the modulus of the reflectance function reaches to about 0.9; the phase of the reflectance function appears to have a small fluctuation at around the longitudinal wave critical angle of the system at $\theta = 14.2^\circ$ and the dramatic behavior of the phase transition takes place at around the shear wave critical angle $\theta = 22.5^\circ$. In Figure 7-3, the reflectance function of the HeLa cells showed that at the incident angle $\theta = 0^\circ$, the modulus of the reflectance function is about 0.78 and at the longitudinal wave critical angle of the system $\theta = 14.6^\circ$ corresponding to the modulus of the reflectance function rises to about 0.92; the phase of the reflectance function shows a small fluctuation at approximately the longitudinal wave critical angle of the system at $\theta = 14.6^\circ$ and the dramatic behavior of phase transition occurs at around the shear wave critical angle $\theta = 23.5^\circ$.

The importance of the specimen/substrate system cannot be overemphasized. Holding all other factors unchanged, the reflectance function may still change depending upon the substrate used. Researchers and biomedical personnel must carefully match or choose the necessary substrate in any work being done with the reflectance functions of HeLa and MCF-7 cells. For example, Figure 8-1 shows a reflectance function of a HeLa cell with a 5.27 µm thickness attached to different substrate materials.
Figure 8-1. Reflectance function of a HeLa cell with 5.27 µm thickness attached to a sapphire substrate and a fused quartz substrate. (Top) The modulus of the reflectance function $R$; the vertical axis indicates the modulus of $R$, and the horizontal axis indicates the incident angles (degree). (Bottom) The phase of the reflectance function $R$; the vertical axis indicates the phase of $R$ (radian), and the horizontal axis denotes the incident angles (degree).

From Figure 8-1(top), at the incident angle $\theta = 0$, the modulus of $R$ of a HeLa cell with a 5.27 µm thickness attached to a sapphire substrate is approximately 0.93; whereas the modulus of $R$ of a HeLa cell for the case of a fused quartz substrate is about 0.78. For a sapphire substrate case, at the incident angle $\theta = 7.7^\circ$, the modulus of $R$ rises to about 0.94 corresponding to the longitudinal wave critical angle of the system; whereas for a fused quartz case, at the incident angle $\theta = 14.6^\circ$, the modulus of $R$ rises to about 0.92 corresponding to the longitudinal wave critical angle of the system. For a sapphire substrate case, at the incident angle $\theta = 12.5^\circ$, the modulus of $R$ becomes unity, which corresponds to the shear wave critical angle of the system. The phase of $R$, as seen in Figure 8-2 (bottom), exhibits the dramatic behavior of phase transition starting at approximately
the shear wave critical angle $\theta = 12.5^\circ$; whereas for a fused quartz substrate, the dramatic behavior of phase transition starting at approximately the shear wave critical angle $\theta = 23.5^\circ$.

In addition to the type of cell being analyzed, the thickness of the cell has an effect on the modulus and the phase of the reflectance function. As seen in section 7.1 of Chapter 7, HeLa cells of differing thickness yielded very different reflectance functions. For example, Figure 8-2 shows the reflectance function of HeLa cells with different thickness attached to a fused quartz substrate as previously calculated and shown in section 7.1 of Chapter 7.

![Figure 8-2. Reflectance function of a HeLa cell with different thicknesses attached to a fused quartz substrate. (Top) The modulus of the reflectance function $R$; the vertical axis indicates the modulus of $R$, and the horizontal axis indicates the incident angles (degree). (Bottom) The phase of the reflectance function $R$; the vertical axis indicates the phase of $R$ (radian), and the horizontal axis denotes the incident angles (degree).]
The calculation of the reflectance function is required in order to simulate the V(z) curve of the material. As before with the choice of substrate being used, any future research or work must carefully match and use the appropriate cell thickness or they risk inaccurate results and false conclusions.

These results reflect the findings of previous literature such as Maev, 2008 which indicate that the reflectance functions should vary with cell type and thickness. Therefore, the code generated in my work should be applicable to calculate the reflectance function of any biological specimen attach to any substrate system.

8.3 V(z) curves

The V(z) calculations using the two theory; ray theory and angular spectrum approach (wave theory) were presented in section 7.2.1 of Chapter 7. Figure 7-7 (top) shows the V(z) curve of the fused quartz substrate based on Ray Theory at center frequency 400 MHz; the period Δz is approximately 18.8 µm. The V(z) curve of the fused quartz substrate based on angular spectrum technique at center frequency 400 MHz is shown in Figure 7-7 (bottom); the period Δz is approximately 18.5 µm. By knowing Δz, Rayleigh wave velocity \( v_R \) can be calculated as mention in section 7.2 of Chapter 7. Table 8.2 shows the calculations of the Rayleigh wave velocity for fused quartz.

Table 8.2. The calculation results for Rayleigh wave velocity for fused quartz material.

<table>
<thead>
<tr>
<th></th>
<th>( \Delta z ) (µm)</th>
<th>Rayleigh wave velocity, ( v_R ) (m/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ray Theory</td>
<td>18.8</td>
<td>3423.8</td>
</tr>
<tr>
<td>Angular Spectrum Approach</td>
<td>18.5</td>
<td>3397.8</td>
</tr>
<tr>
<td>Literature value [28]</td>
<td>N/A</td>
<td>3410</td>
</tr>
</tbody>
</table>
The $v_R$ of fused quartz value obtained by using ray theory gives an error about 0.40% from the published value in the literature [28]. The $v_R$ of fused quartz value obtained by using angular spectrum approach gives an error approximately 0.36% from the published value in the literature [28].

The ray theory and the angular spectrum (wave theory) both enable the calculation of $V(z)$ response. The wave theory is a complete integral solution of the problem, whereas the ray theory is the discretized solution of the same problem. For the wave theory for the integral analysis, the knowledge of Rayleigh wave modes does not require. Additionally, the effects of all rays incident at all incident angles are automatically included. For the ray theory, the critical angles must be determined first so the $V(z)$ can then be calculated.

Each $V(z)$ curve, as shown in section 7.2 of Chapter 7, gives different values of periods $\Delta z$. In particular, as before with reflectance functions, the $V(z)$ curves differ due to cell type and thickness. For example, in Figures 7-12 and 7-14 respectively, the periods of the $V(z)$ curve for the HeLa cell attached on a fused quartz substrate are different from those of the kidney tissue. In those images, the same substrate was used. Therefore, any change in the $V(z)$ curve is a result of the type of cell being analyzed and cell thickness. In addition, when the type of cell and substrate were held constant but the thickness was different, the periods of these $V(z)$ curves were also different because the thickness played an important role to the reflectance function as seen in Figures 7-13 and 7-14 for the $V(z)$ curves of HeLa cells attached on a fused quartz substrate.

From this work, it can be seen that the $V(z)$ curves have different patterns and different periods $\Delta z$ for each material being tested as well as the thickness of that material. The simulation of $V(z)$ curve agrees with the literature that $V(z)$ curve is distinctive for each material. Therefore, the code generated in my work are applicable to calculate and simulate the $V(z)$ response of any types of biological specimen attached to a substrate system.
The V(z) curve allows for the calculation of the Rayleigh wave velocity of a thin biological specimen attached on a substrate. In this work, the Rayleigh wave velocity of the kidney tissue with 3 µm thickness attached on fused quartz substrate was \( v_R = 3380.3 \text{ m/s} \). The surface wave velocity of kidney tissue in the literature is 3351 m/s [38]. The value obtained from the simulation gives 0.87\% error from the literature value and is considered to be sufficiently close to the value in the literature. This indicates that the code generated in this work can calculate the Rayleigh wave velocity, theoretically, of the biological specimen attached to a substrate system.

Unfortunately, there are no experimental data or literature available currently for the Rayleigh wave velocity of HeLa cells. However, the Rayleigh wave velocities obtained from the simulations completed in this study can be used as suggested values for any future measurements. Future field measurements should be compared to the values here in order to test the validity of this code and/or determine the accuracy and precision of future field measurement techniques.

8.4 Mechanical properties and physical characteristics of cells

An acoustical image of the MCF-7 cells being scanned at a frequency of 800 MHz was shown in section 7.3.1 of Chapter 7. Also, the acoustical images of two different HeLa cells being scanned at a frequency of 400 MHz were also presented in section 7.3.1 of Chapter 7. The nucleoli of the cells are visible in all images. The cellular membrane and the nucleus are visible in the acoustical image of the MCF-7 cells only. The darker area appearing in the acoustical image indicates the high attenuation of the sound.

In section 7.3.2 of Chapter 7, thickness, sound velocity, acoustic impedance, density, attenuation, and bulk modulus of the MCF-7 cells and the HeLa cells were calculated by analyzing the radio frequency (RF) signals collected from the time-resolved method and were presented in Table 7-1 and Table 7-2.
The calculation results in this study agree with the published data in the literature, which measured a sound velocity of $1534.5 \pm 33.6$ m/s in HeLa cells [10] and $1582.25 \pm 11.8$ m/s in MCF-7 cells [76]. Table 8.3 summarizes the calculation results and compare to the published value in the literature.

Table 8.3 The calculation results for the acoustical/mechanical properties of biological cells

<table>
<thead>
<tr>
<th>Properties</th>
<th>MCF-7 cells</th>
<th>HeLa cells</th>
<th>coupling medium (water), Published value [28]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Calculation results</td>
<td>Published value</td>
<td>Calculation results</td>
</tr>
<tr>
<td>Longitudinal sound velocity [m/s]</td>
<td>1573 ± 15</td>
<td>1582.25 ± 11.8 [76]</td>
<td>1500 ± 10</td>
</tr>
<tr>
<td>Thickness [µm]</td>
<td>11.8 ± 1.2</td>
<td>N/A</td>
<td>5.3 ± 0.5</td>
</tr>
<tr>
<td>Acoustic impedance [MRayls]</td>
<td>1.60 ± 0.01</td>
<td>N/A</td>
<td>1.78 ± 0.01</td>
</tr>
<tr>
<td>Density [kg/m³]</td>
<td>1017 ± 16</td>
<td>N/A</td>
<td>1186 ± 14</td>
</tr>
<tr>
<td>Attenuation[Neper/µm]</td>
<td>0.16 ± 0.05</td>
<td>N/A</td>
<td>0.21 ± 0.05</td>
</tr>
<tr>
<td>Bulk modulus [GPa]</td>
<td>2.51 ± 0.09</td>
<td>N/A</td>
<td>2.67 ± 0.07</td>
</tr>
</tbody>
</table>

Nevertheless, because there is a lacking of published data on individual cell properties, unfortunately the other parameters cannot be compared at the moment. It should be noted that the sound velocity, acoustic impedance, density, and bulk modulus of the cells were close to those of the coupling medium as shown in Table 8.3. This indicated that the code generated and methods used in this study can calculate the mechanical properties of the biological cells from the RF data collected by the time-resolved measurement approach. For future work, it is highly recommended for more experiments on measuring either individual cells or the cells undergo different stages of cell cycle so the trend of how the properties change under specific conditions can be established. The sound velocity of the cells calculated above was then used in the transducer simulations. The attenuation inside the cell can be applied to the transducer simulations as well.
8.5 Transducer simulations

In section 7.4.1 of Chapter 7, the phantoms were constructed from scatterer maps based on the acoustical images created from the experimental data set of HeLa cells and MCF-7 cells. The phantoms consist of 100,000 scatterers. They were then scanned with frequencies of 100, 400, 600, 800, and 1000 MHz to simulate 50 RF lines for HeLa cells, 64 RF lines for MCF-7 cells. The 64, 128, and 256 element phased arrays were designed with approximately half wavelength spacing and Hanning apodization.

The simulations of B-scan images showed that the cells can be seen even though they were not sharp in contrast. The MCF-7 cells can be seen in the simulated image and look similar to the acoustical image of MCF-7 cells. Likewise, the HeLa cells are visible in the simulated image and look similar to their acoustical images. However, the simulated images are not well defined. This is probably due to limitations of the FIELD II®, which usually runs better using a 100 MHz sampling and approximate calculations rather than using higher frequency sampling, especially in a GHz range, according to FIELD II® user guide manual. In the simulations of this research, the sampling frequencies used were much higher than 100 MHz. In addition, the bitmap images of the acoustical images provided the low level of details and blur possibly due to improper focusing.

It should be noted that the dark areas within the cells in both the acoustical images and the simulated images indicates the high attenuation of the sound. Nevertheless, the cells are visible in the B-scan images. This indicates that the code generated in this study can be used as a guide to explore the B-scan imaging obtained by transducer arrays and can be extended in further research that would like to focus on transducer array design for SAM.

From the simulation results, it appears that the 64 and 128 element phased arrays operating at frequencies of 100, 400, and 600 MHz yielded better images when compared to the 256 element phased array at the same operating frequencies. The cell membrane of MCF-7 cells are somewhat
visible in the simulated image using the 64 and 128 element phased arrays operating at frequencies of 100, 400, and 600 MHz. However, they are barely visible when using the 256 element phased array at the same operating frequency. The inside of the MCF-7 cells are visible as a very dark area in the B-scan simulated images where the attenuation appears in the acoustical images. For the HeLa cells, the cell membranes are barely visible but not very sharp in contrast in the acoustical images that are used as a phantom for the simulations. The inside of the HeLa cells are also seen in the B-scan simulated images where the attenuation appears in the acoustical images. For the higher operating frequencies of 800 and 1000 MHz, the image results appeared to be blurred.

It was expected that, theoretically, the higher frequencies would give the better image resolutions as explained previously following Table 8.1; however, that was not the case. I believe this is due to the limitations of FIELD II® program related to sampling frequency range as mentioned previously. Therefore, the resulting calculations might not be as accurate as those of lower sampling frequencies.

From the calculations shown in Table 8.1, the higher the frequencies, the better the axial resolutions. However, as the penetration depth of the ultrasound decreased, the frequency increased. Therefore, a careful balance between axial resolution and penetration depth must be sought.

In section 7.4.2 of Chapter 7, the phantom was made of scatterer maps based on the acoustical images from the experimental data set of MCF-7 cells and then scanned with a 100 MHz, 20x20 element array 2D fully populated array transducer with approximately half a wavelength spacing and Hanning apodization. A single transmission was utilized with multiple focusing during reception as a dynamic focusing. The cells can be seen in the simulated B-scan image but the contrast was still obscure. The cell membrane of MCF-7 cells can be vaguely seen in the simulated image. The inside of the MCF-7 cells where the attenuation appears in the acoustical images are also seen as a very dark area in the B-scan simulated images. Again, the limitations of the FIELD
II® program and its sampling frequency issue as mention previously and the low level of details in bitmap acoustical image used as a phantom may have caused the problem to the simulated image. A better design of 2D array will be worth for exploring for the future works since 2D arrays can provide C-scan image mode instantly.

It should be noted that the attenuation inside the cells that causes the dark area in the images of the cells depends on the condition of the cells. This includes normal characteristics of the cell relating to life cycle such as the cell division. Also, the distribution of the f-actin inside the cells may have caused the increase of the attenuation.

In section 7.4.3 of Chapter 7, the volumetric images of the cells are presented as a P-scan display mode, which is a projection of a B-scan to yield a 3D image by cascading B-scan slices. Each P-scan volumetric image consists of 30 slices of B-scan simulated images, slicing through the cells at a different depth along the Z-axis direction for each X-Y plane. The volumetric image results were still not easy to interpret due to the limitation of experimental data. A better volumetric image result could potentially be generated if more experimental data are available.

8.6 The simulation codes repository

(a) Single execution

The algorithm for calculate the reflectance function and the V(z) curves simulations were written in MATLAB®. The codes were run by using the personal computer (PC) using MATLAB® 2008a. For the algorithm for executing the transducer simulations, they were written in MATLAB® based on FIELD II® program. The codes were written to simulate the RF lines from a phantom created from a bitmap acoustical image of the cell and then created a simulated B-scan image of the cell. The numbers of lines were 64 lines for MCF-7 cells simulations; and 50 lines for both of the HeLa
cells simulations. The codes can be run by utilizing the personal computer (PC); however, the time for a single simulation of B-scan images of the cell took up to several days using PC. (The PC runs under Windows 32 and 64 bits using MATLAB® R2008a.) According to FIELD II® guide, the time of simulation can be decreased by using a low sampling frequency. However, we set up the transducer for a center frequency range from 100 to 1000 MHz which means the sampling frequency used in the simulations were much higher (at least twice of the center frequency). Although, spatial impulse responses were calculated in this study employing sampling frequencies in range of GHz, so the sharp discontinuities of the responses occur. FIELD II® manages these sharp discontinuities by accurately keeping track of the time position of the responses including employing the integrated spatial impulse response as an intermediate step in the calculations. However FIELD II® usually performs better employing a 100 MHz sampling and estimated calculations than using the exact analytic expression and a GHz range sampling frequency.

In order to be able to run all the simulations with less time consuming, the codes were then run as parallel execution instead of single execution which will be discussed next.

(b) Parallel execution

To decrease the time for the simulations, all the codes were submitted to run parallel on the Lion-X series cluster of Research Computing and Cyberinfrastructure (RCC) of the Pennsylvania State University, which provides high performance computing systems. (The Lion-X series cluster runs under Linux on 64 bits and MATLAB R2009b) The simulations were split into concurrently run sessions. First we generated the phantom data same as we did for single execution and then store them in a file. After that the phantom data file was employed by a number of workstations to simulate the RF signal for different imaging directions, which were then stored in separate files; one for each RF line. These RF line files were subsequently used to assemble a simulated B-scan
image. The whole procedures were executed for simulating the RF lines for both MCF-7 cells and HeLa cells images.

Chapter Summary

In this chapter, the interpretation of the simulation results of reflectance function for biological cells and substrate; V(z) curves; transducer simulations were presented. In the next chapter, a conclusion including suggested future work will be discussed.
Chapter 9

Conclusion and Future Work

The overall work summary and suggestions future work will be presented in section 9.1 and 9.2 respectively.

9.1 Summary and conclusion

The calculation and the Matlab algorithm developed in this study is a novel and powerful tool for the calculation of the reflectance function and simulating the V(z) of any coupling medium-biological specimen-substrate system being studied and for related future research. The use of V(z) curve simulation is important and yield the individual characteristics of the systems as it’s being considered as a material signature. Although there are some experimental data done by some other researchers, no one has done the simulations for the layer model before. Thus, the calculation and simulation program developed in this study is new and very useful tool for further studying about cells and their physical characteristics including their acoustic images in the future.

High-frequency time-resolved scanning acoustic microscopy is a powerful method for measuring the mechanical properties of biological cells. By employing high frequency time-resolved SAM, the mechanical properties of biological specimens can be determined.

In this research, the original contributions were as follows;

1) The codes developed in this study using Matlab are able to determine the reflectance function of the layer model consisting of the coupling medium, the biological specimen, and the substrate as a liquid-liquid-solid system. Once the calculation of the reflectance function is completed, then V(z) curves of the system can be simulated. These original codes were a powerful tool for simulating the contrast mechanisms of biological specimens (e.g. MCF-7 cells and HeLa cells)
attached to a substrate. This is indicated by the results presented in section 7.1 and section 7.2.

The reflectance function simulation was useful in the analysis of the surface acoustic waves in the layer structure that was composed of the biological specimen, which has a very low (near zero) shear modulus. Theoretically, simulations of V(z) responses based on well-known established Ray theory and Wave theory can be simulated after the reflectance function is determined.

2) The codes developed in this study using Matlab yield simulation results of V(z) responses. V(z) curve is considered a material signature of which its FFT analysis is useful for extracting surface acoustic wave velocity and the attenuation factor for solid materials. However, in the case of soft materials, such as biological cells, the Rayleigh wave or surface acoustic wave may not be produced within a specimen because the critical angle of the Rayleigh wave or surface acoustic wave of soft materials is typically high as is its relative attenuation. Therefore, the V(z) curve may not have enough oscillations for the FFT analysis even though the Rayleigh wave is generated. According to reflectance function calculation results of this study, the liquid-liquid-solid layer structure consisting of the coupling medium-soft thin layer biological specimen substrate causes an increasing reduction of the Rayleigh waves. They are eventually dominated by interference between reflections from the top and the bottom surfaces of the cell. Rayleigh waves can be generated in the fluid-liquid layer structure where the waves in the liquid can couple into Rayleigh waves at the surface of the solid. This appeared in the phase change by approximately $2\pi$, such as see in the phase change of the fused quartz substrate-water. However, in the case of the coupling medium-soft thin biological specimen-substrate or the liquid-liquid-solid system, the phase change was less than $2\pi$ due to the significant reduction of Rayleigh waves.

In the angular spectrum method, the explicit postulation of the existence of leaky Rayleigh waves was not needed since the equations used for determining the reflectance function analytically take into account their behavior that satisfy the boundary conditions for acoustic
waves in fluid-fluid solid layers. It should be noted that this reflectance function produces oscillations in $V(z)$ as found experimentally. The change of the phase of nearly $2\pi$ in the reflectance function at around an angle of incidence slightly above the shear wave critical angle causes the oscillations in the $V(z)$ curve. A simpler and more intuitive model for use, the ray method model, is also justified. In the ray method, two important rays are the specularly reflected, axial ray and the leaky wave ray. The ray method predicts almost the same periodicity of the oscillations in $V(z)$ as the angular spectrum or Fourier method. In addition, it was more direct since these oscillations happen only when defocusing towards the lens. As we saw in the simulations of the $V(z)$ curves in this study, the two methods gave very similar oscillation patterns. In order to make the simulation more intuitive, the ray method was applied in the calculations and simulations in this study.;

3) The codes developed using Matlab for calculating the mechanical properties of the cells and simulating acoustical images for the RF signals were measured by time-resolved SAM. In addition to this being a new application of SAM, these original codes also proved excellent for calculating the mechanical properties of the MCF-7 cells and the HeLa cells as shown in Chapter 7. The calculation results agree with the previously published data.

4) The transducer simulations designed and performed through Matlab codes were written and developed based on the Field II Ultrasound simulation program in order to simulate the acoustical images of the cells using the RF data collected from the time-resolved measurements. Various high frequency 1D and 2D transducer arrays have been designed and simulations have been performed to investigate image resolution and volumetric imaging capabilities. The transducer array designs in this research are novel and have the potential to improve the performance of SAM with electronic scanning capability.

The codes generated in this research for transducer simulations do not give clear simulated B-scan images of the cell as of yet. Also, the limitations of the FIELD II® program
using the sampling frequency of more than 100 MHz range may have contributed an error in the calculations. Any future work developing computer software which can overcome the limitation of FIELD II® program in terms of sampling frequency for transducer might yield a better image and more accurate calculations.

9.2 Suggested future work

The codes and methodologies developed and utilized in this study have a profound implication for future research.

1. For SAM experiments, it is encouraged for future studies to employ a time-resolved measurement technique to measure and extract the acoustical/mechanical properties values of individual cell properties. This will be very valuable for use as reference information. As mentioned in Chapter 8, due to a lack of published data on individual cell properties, some of the other parameters cannot be compared at present. Future work to focusing on the measurement of the properties of cells during the various stages of cell cycles, a comparison between healthy cells and cancerous cells, and using different cell lines are needed. Having this data available as a reference will benefit microbiologists or clinical personnel in diagnosing cellular abnormalities that may lead to cancer or other illnesses of cellular origin.

2. The transducer simulations of B-scan images were successful in that the cells can be seen but the contrast of the simulated images were not as sharp as expected. The MCF-7 and HeLa cells were visible in the simulated images and look similar to their acoustical images. However, the simulated images were not able to provide as much detail as desired at present. There are two possible factors that may contribute to these problems; the limitation of FIELD II®
and the bitmap images with low level details of the acoustical images used as the phantom.

FIELD II® works excellent with sampling frequencies in 100 MHz range. In any future work to overcome these problems, one should develop a new ultrasound simulation software that can handle the high sampling frequency in GHz range. For the bitmap image to be used as a phantom for the simulation, it is highly recommended that images with greater detail be used and/or to enhance the contrast level of the bitmap image prior to creating a phantom. Care should also be taken in choosing the decimation factor when assembling the image from the simulated RF-line to avoid temporally aliasing the RF-lines.

3. For the transducer simulations of the B-scan image in this study, we employed only a single transmission with dynamic focusing for the reception. Future studies should explore more options of focusing in order to achieve better simulated images such as employing multiple transmit focal zones to allow good lateral resolution to be constant throughout the depth of the field of view.

4. The transducer simulations for 2D array in this study provide the foundation for a better transducer design for SAM. Future studies should investigate the use of 2D array designs more fully to create a better volumetric image.
Bibliography


Appendix A
MATLAB Codes

I. Codes for calculating reflectance function and simulating $V(Z)$ based on Ray theory

A. Fused quartz

```matlab
%% SIMULATION OF $V(Z)$ CURVE - FUSED QUARTZ
%% May, 2009
%% Yada Juntarapaso
%% Graduate Program in Acoustics

%% Reference
%% Science, Technology and Education of microscopy: an overview. pp. 325–344

%clear all;
close all; clc;

%% Default figure setting
set(0,'Defaultaxesfontsize',16);
set(0,'Defaulttextfontsize',16);
lw=2.;
scrsz=get(0,'ScreenSize');
scrwidth=scrsz(3)-scrsz(1);
scrheight=scrsz(4)-scrsz(2);
scrw1=scrwidth*0.1;   scrw2=scrwidth*0.8;
scrh1=scrheight*0.1;  scrh2=scrheight*0.7;

!!!!!!!!!!!!!!! Assign Acoustic lens property!!!!!!!!!!!!!!!
```
freq=200e6; % The center frequency of acoustic lens [Hz]
a=0.383e-3; % Radius of transducer [m]
theta_m=60/180*pi; % Half aperture angle of the lens [radian]

% Buffer rod properties (Sapphire)
cL_rod=11175; % Longitudinal wave speed of Sapphire [m/s]
cS_rod=6950; % Shear wave speed of Sapphire [m/s]
rho_rod=3980; % density of Sapphire [kg/m^3]

% Coupling medium property: in this case = Water
cW=1500; % Longitudinal wave speed of water [m/s]
rhoW=1000; % density of water [kg/m^3]
alphaW=1100; % Absorption coefficient of water
focal=577.52e-6; % Focal distance [m]
R=focal*(1-cW/cL_rod); % Radius of the spherical recess [m]
lambda=cL_rod/freq; % Wavelength in the buffer rod [m]
L0=6122.00e-6; % Length of the buffer rod (Focal distance assumption)

% AntireflecS_rodon coating
cL_anti=2250; % Longitudinal wave speed [m/s]
rho_anti=4640; % Density [kg/m^3]
t_anti=2.451e-6; % Thickness [m]

% Specimen solid = Fused quartz
cL_fq=5970; % Longitudinal wave speed of fused quartz [m/s]
cS_fq=3765; % Shear wave speed of fused quartz [m/s]
rhoS=2200; % density of fused quartz [kg/m^3]

% Pupil function
% wavenumbers
kW=freq*2*pi/cW; % wave number in water [1/m]
kL=freq*2*pi/cL_rod; % Acoustic lens (Longitudinal wave number) [1/m]
kT=freq*2*pi/cS_rod; % Acoustic lens (Shear wave number) [1/m]

% Number of calculation points
Npoint=400;
Tpoint=1000;
Zpoint=2^14;
% sampling point in the z-diresction for V(z) response
z_span=linspace(-250e-6,150e-6,Zpoint);

% r-position along the lens surface
r=linspace(0,R*sin(theta_m),Npoint);

% Angular span
theta_span=asin(r./R);
theta_incd=theta_span-asin(cW/cL_rod*sin(theta_span));
theta_trans=asin(cW/cL_rod*sin(theta_span));

%% Transmission coefficient
% from lens to water
Z_Ln=rho_rod*cL_rod./cos(theta_span);
Z_Wn=rhoW*cW./cos(theta_trans);
T_LW=2*Z_Wn./(Z_Ln+Z_Wn);
P1=T_LW;

%% Pupil function
figure('Position',[scrw1,scrh1,scrw2,scrh2])
plot(theta_incd*180/pi,abs(P1),'linewidth',2);
title('Pupil function P1')
xlabel('deg.')
ylabel('Normalized amplitude');

%% Pupil function for P2
figure('Position',[scrw1,scrh1,scrw2,scrh2])
plot(theta_incd*180/pi,phase(P1),'linewidth',2)
xlabel('k_x/k_f')
ylabel('Phase (rad)');

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

%%% calculate reflectance function
%% Define angles

TL_span = asin(cL_fq/cW*sin(theta_incd)); % Transmitted angles for longitudinal waves
theta_span = asin(cS_fq/cW*sin(theta_incd)); % Transmitted angles for shear waves

ZW = rhoW*cW./cos(theta_incd); % Acoustic impedance in the water
Z2L = rhoS*cL_fq./cos(TL_span); % longitudinal Acoustic impedance in the specimen
Z2S = rhoS*cS_fq./cos(theta_span); % shear Acoustic impedance in the specimen

num = Z2L.*(cos(2*theta_span)).^2 + Z2S.*(sin(2*theta_span)).^2 - ZW;
denum = Z2L.*(cos(2*theta_span)).^2 + Z2S.*(sin(2*theta_span)).^2 + ZW;
Refl = num./denum; % Reflectance function

%% Plot the reflectance function
figure('Position',[scrw1,scrh1,scrw2,scrh2]) % Location figure, and set background to white
subplot(211)
plot(theta_incd*180/pi,abs(Refl),'linewidth',2);
title('Reflectance function of Fused quartz and coupling medium')
xlabel('deg.')
ylabel('Normalized amplitude');

subplot(212)
plot(theta_incd*180/pi,phase(Refl),'linewidth',2);
xlabel('deg.')
ylabel('Phase (rad)');

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%  Numerical integral for the lens back-surface field
%%  Integration element
z=L0; % Lens back-plane
n=round(4*a/lambda); % Number of element in the transducer radius
m=round(n*pi); % Number of element in the transducer circumference

%% integration element
Dsigma=1./(n*a/lambda);
Dsi=pi/m;
ul=0; % initialization
Z=z./(a^2/lambda);
X=r./(a^2/lambda);
L=sqrt(R.^2+Z.^2);
%% Numerical integral based on the Rayleigh-Sommerfeld integral
for (p=1:m)
    si_p=Dsi*(p-.5);
for (q=1:n)
    sigma_q=Dsigma*(q-.5);
gamm=atan(X./Z);

    DSq=sigma_q*Dsigma*Dsi;
    L_pq=sqrt(L.^2+sigma_q.^2-2*L.*sigma_q.*sin(gamm).*cos(si_p));
    integ=1./L_pq.*exp(i*2*pi*(a/lambda)^2*L_pq).*DSq;
    ul=ul+integ;
end
end

%% Plot the integration result
figure(['Position',scrw1,scrh1,scrw2,scrh2]) % Location figure
subplot(211)
plot(theta_incd*180/pi,abs(u1),'linewidth',2);
title('u1')
xlabel('deg.')
ylabel('Normalized amplitude');

subplot(212)
plot(theta_incd*180/pi,phase(u1),'linewidth',2);
xlabel('deg.')
ylabel('Phase (rad)');

%% V(z) response
%% calculation of V(z) curves
for n3=1:length(z_span)
    X3=z_span(n3);
    intg=ul.^2.*P1.*P2.*Refl.*exp(j*2*kW*X3.*sqrt(1-r.^2./focal^2)).*r;
    Vz(n3)=trapz(r,intg);
end
dB_Vz=20*log10(abs(Vz)./max(abs(Vz)));
figure('Position',[scrw1,scrh1,scrw2,scrh2]) % Location figure
plot(z_span*1e6,dB_Vz,'linewidth',2)
grid on;
title('V(z) curve of Fused Quartz')
xlabel('z (\mum)')
ylabel('(dB ref. z=0 \mum)');
dz=(46.5e-6)-(11.85e-6);
vSAW=cW/sqrt(1-(1-cW/2/freq/dz)^2);

save Vz_quartz
save z_span
save Vz
save dB_Vz
save freq
save dz
save vSAW

B. HeLa cell attached on substrate

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%Calculate Reflectance function and V(z) curve_HeLa cell normal
%phase with sapphire substrate
%stage: Cell D
% Yada Junarapaso
% Graduate Program in Acoustics
% May 2009 EST
% Layer model: medium I = coupling liquid = water
% medium II = HeLa cell cell A (before division stage, thickness 12
micron, sound velocity 1550 m/s, density 1239 kg/m^3)
% medium III= substrate (sapphire)

clear all; close all; clc;

%% Default figure setting
set(0,'Defaultaxesfontsize',14);
set(0,'Defaulttextfontsize',14);
lw=2.;
scrsz=get(0,'ScreenSize');
scrswidth=scrsz(3)-scrsz(1);
scrheight=scrsz(4)-scrsz(2);
scrw1=scrswidth*0.1; scrw2=scrwidth*0.8;
scrh1=scrheight*0.1; scrh2=scrheight*0.7;

%%% assign acoustics lens property

freq=400e6; % Center frequency of the acoustics lens [Hz]
a=0.383e-3; % Radius of the transducer [m]
Thetam=60/180*pi; % Half aperture angle of the lens [rad]
cL_buffer=11175; % Longitudinal wave speed of the buffer rod, Sapphire [m/s]
cS_buffer=6950; % Shear wave speed of the buffer rod, Sapphire [m/s]
rho_buffer=3980; % density of the buffer rod [kg/m^3]

%%% properties of each medium layer
%%% medium layer I = coupling liquid = water in this case
cL_I=1500;
rho_I=1000;

%%% medium layer II = biological cell = HeLa cell before division (cell A)
cL_II=1550;
rho_II=1239;

%%% medium layer III = substrate = sapphire in this case
cL_III=cL_buffer;
cS_III=cS_buffer;
rho_III=rho_buffer;

% Focal property of lens
focal=557.52e-6; % focal distance [m]
R=focal*(1-cL_I/cL_buffer); % Radius of the spherical recess
lambda=cL_buffer/freq; % wavelength in the buffer rod
L0=6112e-6; % Length of the buffer rod

% Number of calculation
Npoint=400;
Tpoint=1000;
Zpoint=512;
z_span=linspace(-300e-6,150e-6,Zpoint); % sampling point in the z-direction for V(z) response
%z_span=linspace(-150.8e-6,150e-6,Zpoint);
%z_span=linspace(-120.8e-6,50e-6,Zpoint);

r=linspace(0,R*sin(Thetam),Npoint); % r-position along the lens surface
theta_span=asin(r./R);
theta_inc_span=theta_span-asin(cL_I/cL_buffer.*sin(theta_span)); % incident angle
\[
\theta_{trans\ span} = \arcsin\left(\frac{c_L_I}{c_L\_buffer} \cdot \sin(\theta_{span})\right);
\]
% transmitted angle

%%% Calculation of Reflectance function

%%% calculation from layer I
\[
k_{L\_I} = 2\pi f / c_L\_I; \quad \text{wave number in layer I (water)}
\]

\[
\theta_{L\_I} = \theta_{\text{inc\_span}}; \quad \text{incident angle of longitudinal wave}
\]

\[
s = k_{L\_I} \cdot \sin(\theta_{L\_I});
\]

\[
\alpha_{I} = k_{L\_I} \cdot \cos(\theta_{L\_I});
\]

%%% calculation from layer II (cell)
\[
k_{L\_II} = 2\pi f / c_L\_II;
\]

\[
\theta_{L\_II} = \arcsin\left(\frac{s}{k_{L\_II}}\right);
\]

\[
\alpha_{II} = k_{L\_II} \cdot \cos(\theta_{L\_II});
\]

%%% calculation layer III; substrate (sapphire)
\[
k_{S\_III} = 2\pi f / c_S\_III;
\]

\[
\theta_{S\_III} = \arcsin\left(\frac{s}{k_{S\_III}}\right);
\]

\[
\beta_{III} = k_{S\_III} \cdot \cos(\theta_{S\_III});
\]

\[
k_{L\_III} = 2\pi f / c_L\_III;
\]

\[
\theta_{L\_III} = \arcsin\left(\frac{s}{k_{L\_III}}\right);
\]

\[
\alpha_{III} = k_{L\_III} \cdot \cos(\theta_{L\_III});
\]

%%% calculate $B_1$, $B_2$, $\gamma$
\[
B_1 = \left( (k_{S\_III})^2 - 2s^2 \right)^2 + 4s^2 \alpha_{III} \beta_{III};
\]

\[
B_2 = \left( \frac{\rho_{II} \alpha_{III}}{\rho_{III} \alpha_{II}} \right) (k_{S\_III})^4;
\]

\[
\gamma = \frac{\rho_{I} \alpha_{II}}{\rho_{II} \alpha_{I}};
\]

\[
d = -12.6e-6; \quad \text{% thickness of the cell 12.6 micron (cell A: before division)}
\]

\[
\text{numerator} = (B_1 - (\gamma \cdot B_2)) \cdot \cos(\alpha_{II} \cdot d) + (j \cdot ((\gamma \cdot B_1) - B_2) \cdot \sin(\alpha_{II} \cdot d));
\]

\[
\text{denominator} = (B_1 + (\gamma \cdot B_2)) \cdot \cos(\alpha_{II} \cdot d) - (j \cdot ((\gamma \cdot B_1) + B_2) \cdot \sin(\alpha_{II} \cdot d));
\]
Refl=numerator./denominator;

%%% Plot the reflectance function
figure('Position',[scrw1,scrh1,scrw2,scrh2]) % Location figure
subplot(211)
plot(theta_inc_span*180/pi,abs(Refl),'linewidth',2);
title('Reflectance function : layer model : coupling medium + HeLa cell (after division : cell A) + substrate (sapphire)');
xlabel('deg.'),
ylabel('Normalized amplitude');

subplot(212)
plot(theta_inc_span*180/pi,phase(Refl),'linewidth',2);
xlabel('deg.'),
ylabel('Phase (rad)');

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%% Define Pupil functions
%% wavenumbers
kL_I=freq*2*pi/cL_I; % Coupling medium
kL_lens=freq*2*pi/cL_buffer; % Acoustic lens (Longitudinal)
kS_lens=freq*2*pi/cS_buffer; % Acoustic lens (Shear)

%% Transmission coefficient
%% from lens to water
Z_Ln=rho_buffer*cL_buffer./cos(theta_span);
Z_Wn=rho_I*cL_I./cos(theta_trans_span);
T_LW=2*Z_Wn./(Z_Ln+Z_Wn);
T1=T_LW; % Pupil function
T_WL=2*Z_Ln./(Z_Ln+Z_Wn);
T2=T_WL;

% P1=1; P2=1;

%%% Plot Pupil function for P1
figure('Position',[scrw1,scrh1,scrw2,scrh2]) % Location figure
subplot(211)
plot(theta_inc_span*180/pi,abs(P1),'linewidth',2);
title('P1')
xlabel('deg.'),
ylabel('Normalized amplitude');

subplot(212)
plot(theta_inc_span*180/pi,phase(P1),'linewidth',2);
xlabel('deg.'),
ylabel('Phase (rad)');

%%% Plot Pupil function for P2
figure('Position',[scrw1,scrh1,scrw2,scrh2]) % Location figure
subplot(211)
plot(theta_inc_span*180/pi,abs(P2), 'linewidth', 2);
title('P2')
xlabel('deg.')
ylabel('Normalized amplitude');

subplot(212)
plot(theta_inc_span*180/pi,phase(P2), 'linewidth', 2)
xlabel('deg.')
ylabel('Phase (rad)');

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%  Numerical integral for the lens back-surface field
%%   Integration element
z=L0;                           % Lens back-plane
n=round(4*(a/lambda));          % Number of element in the transducer radius
m=round(n*pi);                  % Number of element in the transducer circumference

%% integration element
Dsigma=1./(n*a/lambda);
Dsi=pi/m;

u1=0;                           % initialization
Z=z./(a^2/lambda);
X=r./(a^2/lambda);
L=sqrt(R.^2+Z.^2);

%% Numerical integral based on the Rayleigh-Sommerfeld integral
for (p=1:m)
    si_p=Dsi*(p-.5);
    for (q=1:n)
        sigma_q=Dsigma*(q-.5);
        gamm=atan(X./Z);
        DSq=sigma_q*Dsigma*Dsi;
        L_pq=sqrt(L.^2+sigma_q.^2-2*L.*sigma_q.*sin(gamm).*cos(si_p));
        integ=1./L_pq.*exp(i*2*pi*(a/lambda)^2*L_pq).*DSq;
        u1=u1+integ;
    end
end

%%  Plot the integration result
figure('Position', [scrw1,scrh1,scrw2,scrh2])    % Location figure
subplot(211)
plot(theta_inc_span*180/pi,abs(u1), 'linewidth', 2);
title('u1')
xlabel('deg.')
ylabel('Normalized amplitude');
subplot(212)
plot(theta_inc_span*180/pi,phase(u1), 'linewidth', 2);
xlabel('deg.'
ylabel('Phase (rad)');

figure('Position', [scrw1, scrh1, scrw2, scrh2])  % Location figure, and
set background to white
subplot(211)
plot(theta_inc_span*180/pi,abs(u1.^2.*P1.*P2), 'linewidth', 2);
%xlim([0 1])
title('u1.^2*P1*P2')
xlabel('deg.')
ylabel('Magnitude (normalized value)');

subplot(212)
plot(theta_inc_span*180/pi,phase(u1.^2.*P1.*P2), 'linewidth', 2)
%xlim([0 1])
xlabel('deg.'
ylabel('Phase (rad)');

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%%%%%%%%%%%%
%% V(z) response
%% calculation of V(z) curves

for n3=1:length(z_span)
    X3=z_span(n3);
    intg=u1.^2.*P1.*P2.*Refl.*exp(j*2*kL_I*X3.*sqrt(1-r.^2./focal^2)).*r;
    Vz(n3)=trapz(r,intg);
    %Vz(n3)=trapz(theta_span,intg);
end

dB_Vz=20*log10(abs(Vz)./max(abs(Vz)));

%% Plot the V(z) curves
figure('Position', [scrw1, scrh1, scrw2, scrh2])  % Location figure
plot(z_span*1e6,abs(Vz)/max(abs(Vz)),'linewidth',2)
grid on;
title('V(z) curve:HeLa cell (before division: cell A, thickness
12.6\mum) on sapphire substrate');
xlim([min(z_span)*1e6 max(z_span)*1e6]);
ylim([0 1]);
xlabel('z (\mum)'
ylabel('Normalized value');

figure('Position', [scrw1, scrh1, scrw2, scrh2])  % Location figure
plot(z_span*1e6,dB_Vz, 'linewidth', 2)
grid on;
II. Codes for calculating reflectance function and simulating V(Z) based on Wave theory

A. Fused quartz

%% SIMULATION OF V(Z) CURVE - FUSED QUARTZ  By Angular spectrum method
%% (Atalar method)
%% June, 2009
%% Yada Juntarapaso
%% Graduate Program in Acoustics

%% Reference
%% [1] A. Atalar, An angular spectrum approach to contrast in
pp. 5130-5139
%% [2] Z. Yu, Scanning acoustic microscopy and its applications to
material
%% 863-891.
quisition
%% of biological cells and soft tissues with scanning acoustic
microscopy.
%% 325-344

clear all; close all; clc;

%% Default figure setting
set(0,'Defaultaxesfontsize',16);
set(0,'Defaulttextfontsize',16);
lw=2.;
scrsz=get(0,'ScreenSize');
scrwidth=scrsz(3)-scrsz(1);
scrheight=scrsz(4)-scrsz(2);
scrlw=scrwidth*0.1;      scrw2=scrwidth*0.7;
scrhl=scrheight*0.1;     scrh2=scrheight*0.8;
% Assign Acoustic lens property

freq=400e6; % The center frequency of acoustic lens [Hz]
Ra=0.383e-3; % Radius of transducer [m]
theta_m=60/180*pi; % Half aperture angle of the lens [radian]

% Buffer rod properties (Sapphire)
cL_rod=11175; % Longitudinal wave speed of Sapphire [m/s]
cS_rod=6950; % Shear wave speed of Sapphire [m/s]
rho_rod=3980; % density of Sapphire [kg/m^3]

% Buffer rod properties (Sapphire)
cL_rod=11175; % Longitudinal wave speed of Sapphire [m/s]
cS_rod=6950; % Shear wave speed of Sapphire [m/s]
rho_rod=3980; % density of Sapphire [kg/m^3]

% Coupling medium property: in this case = Water

cW=1500; % Longitudinal wave speed of water [m/s]
rhoW=1000; % density of water [kg/m^3]
alphaW=1100; % Absorption coefficient of water
focal=577.52e-6; % Focal distance [m]

R=focal*(1-cW/cL_rod); % Radius of the spherical recess [m]
lambda=cL_rod/freq; % Wavelength in the buffer rod [m]
L0=6122.00e-6; % Length of the buffer rod (Focal distance assumption)

k=2*pi/lambda; % Wave number in the buffer rod [1/m]

% AntireflecS_rodion coating

cL_anti=2250; % Longitudinal wave speed [m/s]
rho_anti=4640; % Density [kg/m^3]
t_anti=2.451e-6; % Thickness [m]

% Specimen solid = Fused quartz

cL_fq=5970; % Longitudinal wave speed of fused quartz [m/s]
cS_fq=3765; % Shear wave speed of fused quartz [m/s]
rhoS=2200; % density of fused quartz [kg/m^3]

% Pupul function

kW=freq*2*pi/cW; % wave number in water [1/m]
kL=freq*2*pi/cL_rod; % Acoustic lens (Longitudinal wave number) [1/m]
kT=freq*2*pi/cS_rod; % Acoustic lens (Shear wave number) [1/m]

% Number of calculation points

Npoint=501; % should be odd
Tpoint=1000;
Zpoint=512;

z_span=linspace(-50e-6,10e-6,Zpoint);  % sampling point in the z-direction for V(z) response

r=linspace(-R*sin(theta_m),R*sin(theta_m),Npoint);  % r-position along the lens surface

theta_incd=asin(r./R);  % Angular span
theta_trans=theta_incd-asin(cW/cL_rod*sin(theta_incd));  % angular span of transmitted angle

A = 1; x0 = 0; y0 = 0;
sigma_x = 2*Ra;
sigma_y = 2*Ra;

x=linspace(-(Npoint-1)/2,(Npoint-1)/2,Npoint)*(lambda/5);
y=x;

f=freq;  %frequency [Hz]
N=length(x);
w=-pi/2:pi/(N-1):pi/2;
kx=(f.*w)./cL_rod;
ky=(f.*w)./cL_rod;
[X, Y] = meshgrid(x,y);

a=(X-x0).^2/(2*sigma_x^2);
b=(Y-y0).^2/(2*sigma_y^2);
gaus=A*exp(-(a+b));
u0=gaus;
figure;mesh(X,Y,u0)
title('u0')
xlabel('x (\mu m)')
ylabel('y (\mu m)')
zlabel('z (\mu m)')

%%%%%%%%%%%%%%%% U0plus %%%%%%%%%%%%%%%%%

u0plus=abs(u0);
U0plus = fftshift(fft2(u0plus));
figure;
imagesc(kx,ky,20*log10(abs(U0plus)));
title('U0plus')
xlabel('kx')
ylabel('ky');
\texttt{mesh(\textit{kx,ky,20*log10(abs(U0plus))});
\texttt{title('U0plus')
\texttt{xlabel('kx')
\texttt{ylabel('ky')

\texttt{%break

\texttt{%%%%%%%%%%%%%%%%%%% U1plus %%%%%%%%%%%%%%%%%%%%%%%

\texttt{k0=2*pi*f/cW;
\texttt{kzz=sqrt(k0.^2-kx.^2-ky.^2);
\texttt{kz=meshgrid(kzz,kzz);

\texttt{U1plus=U0plus.*exp(j.*kz.*L0);
\texttt{figure;imagesc(kx,ky,20*log10(abs(U1plus)));
\texttt{title('U1plus')
\texttt{xlabel('kx')
\texttt{ylabel('ky')
\texttt{%figure; mesh(kx,ky,20*log10(abs(U1plus)));
\texttt{title('U1plus')
\texttt{xlabel('kx')
\texttt{ylabel('ky')

\texttt{u1plus=ifft2(U1plus);
\texttt{figure;mesh(X,Y,abs(u1plus));
\texttt{title('u1+')
\texttt{xlabel('x \text{ (mum)}')
\texttt{ylabel('y \text{ (mum)}')
\texttt{zlabel('abs(u1plus)')

\texttt{%%%%%%%%%%%%%%%%%%%% U2plus %%%%%%%%%%%%%%%%%%%%%%%%

%%%% pupil function%%%%%%

\texttt{rr=meshgrid(r+1e9,r+1e9);
\texttt{arg=sqrt(X.^2+Y.^2)./rr;
\texttt{figure; mesh(arg)

\texttt{%% Transmission coefficient
\texttt{%% from lens to water
\texttt{Z_Ln=rho_rod*cL_rod./cos(theta_trans);
\texttt{Z_Wn=rhoW*cW./cos(theta_trans);
\texttt{T_LW=Z_Ln/(Z_Ln+Z_Wn);
\texttt{P1=T_LW;
\texttt{P1=meshgrid(P1,P1);
\texttt{P1=1;
\texttt{T_WL=Z_Ln/(Z_Ln+Z_Wn);
\texttt{P2=T_WL;
\texttt{P2=meshgrid(P2,P2);
\texttt{P2=1;}}}
cbar=cW/cL_rod;
kk0=meshgrid(k0,k0);
lambda0=2*pi./kk0;

u2plus=((exp(j.*kk0.*(focal.*(1+cbar^2)))./(j.*lambda0.*focal))).*(ffts
hift(fft2(abs(u1plus.*P1))));

figure; mesh(X,Y,abs(u2plus));title('u2plus');xlabel('x
\mum');ylabel('y \mum');zlabel('abs(u2plus)');

U2plus=fftshift(fft2(abs(u2plus)));
figure;
imagesc(kx,ky,20*log10(abs(U2plus)));title('U2plus');xlabel('kx');ylabel('ky');
figure; mesh(kx,ky,abs(U2plus));
title('U2plus');xlabel('x');ylabel('y');zlabel('abs(U2plus)');

%UP=(fft2(u1plus.*P1));
%figure; imagesc(log10(abs(UP))); title('fft[(u1+)*P1]')
%figure; mesh(abs(UP)); title('fft[(u1+)*P1]')

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%%% U3 plus%%%%%%%%%%%%%%%%%%%%

k3x=meshgrid(kx,kx);
k3y=meshgrid(ky,ky);
Z=linspace(-50e-6,10e-6,Npoint);
Z=meshgrid(Z,Z);
U3plus=U2plus.*(exp(j.*kk0.*Z)).*exp(-j.*(((k3x.^2+k3y.^2)/(2*kk0)).*Z));

figure;
imagesc(kx,ky,20*log10(abs(U3plus)));title('U3plus');xlabel('kx');ylabel('ky');
figure; mesh(kx,ky,abs(U3plus));
title('U3plus');xlabel('kx');ylabel('ky');zlabel('abs(U3plus)')

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

%%%%% calculate reflectance function

%% Define angles

TL_span=asin(cL_fq/cW*sin(theta_trans)); % Transmitted angles for
longitudinal waves
TS_span=asin(cS_fq/cW*sin(theta_trans)); % Transmitted angles for
shear waves
\[ Z_W = \rho_W c_W \cos(\theta_{\text{trans}}); \]  
% Acoustic impedance in the water
\[ Z_{2L} = \rho_S c_{L_\text{f}q} \cos(TL_{\text{span}}); \]  
% longitudinal Acoustic impedance in the specimen
\[ Z_{2S} = \rho_S c_{S_\text{f}q} \cos(TS_{\text{span}}); \]  
% shear Acoustic impedance in the specimen

\[ \text{num} = Z_{2L} \ast (\cos(2TS_{\text{span}})) \ast ^2 + Z_{2S} \ast (\sin(2TS_{\text{span}})) \ast ^2 - Z_W; \]
\[ \text{denum} = Z_{2L} \ast (\cos(2TS_{\text{span}})) \ast ^2 + Z_{2S} \ast (\sin(2TS_{\text{span}})) \ast ^2 + Z_W; \]

\[ \text{Refl} = \frac{\text{num}}{\text{denum}}; \]  
% Reflectance function

\[ \text{Refl2} = \text{Refl}((N\text{point} - 1)/2:end); \]

%% Plot the reflectance function
figure('Position', [scrw1, scrh1, scrw2, scrh2])  
% Location figure, and set background to white
subplot(211)
plot(theta_trans((Npoint - 1)/2:end) \ast 180/pi, abs(Refl2), 'linewidth', 2)
title('Reflectance function of Fused quartz and coupling medium')
xlabel('deg.')
ylabel('Normalized amplitude');

subplot(212)
plot(theta_trans((Npoint - 1)/2:end) \ast 180/pi, phase(Refl2), 'linewidth', 2)
xlabel('deg.')
ylabel('Phase (rad)');

Refl = meshgrid(Refl, Refl);

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%U3minus%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

U3minus = U3plus \ast \text{Refl};
figure;
imagesc(kx, ky, 20 \ast \log10(abs(U3minus)));title('U3minus');xlabel('kx');ylabel('ky')
figure; mesh(kx, ky, abs(U3minus));title('U3minus')
xlabel('kx');ylabel('ky');zlabel('abs(U3minus)')

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%U2minus%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
U2minus=U3minus.*(exp(j.*kk0.*Z)).*exp(-j.*((kkx.^2+kky.^2)/(2*kk0)).*Z);

figure;
imagesc(kx,ky,20*log10(abs(U2minus)));title('U2minus');xlabel('kx');ylabel('ky');zlabel('abs(U2minus)')
figure; mesh(kx,ky,abs(U2minus));title('U2minus');xlabel('kx');ylabel('ky');zlabel('abs(U2minus)')

u2minus=ifft2(U2minus);
figure; mesh(X,Y,abs(u2minus));title('u2minus');xlabel('x (\mum)');ylabel('y (\mum)');zlabel('abs(u2minus)')

u1minus=((exp(j.*kk0.*(focal*(1+cbar^2))))./(j.*lambda0*focal)).*P2.*U2minus;
figure; mesh(X,Y,abs(u1minus));title('u1minus');xlabel('x (\mum)');ylabel('y (\mum)');zlabel('abs(u1minus)')

U1minus=fftshift(fft2(abs(u1minus)));
figure;
imagesc(kx,ky,20*log10(abs(U1minus)));title('U1minus');xlabel('kx');ylabel('ky');zlabel('abs(U1minus)')
figure; mesh(kx,ky,abs(U1minus));title('U1minus');xlabel('kx');ylabel('ky');zlabel('abs(U1minus)')

%%%%%%%%%%%%%%%%%u0minus%%%%%%%%%%%%%%%%%

bb=ifft2(exp(j.*kz.*L0));
%u0minus=conv2(abs(u1minus),bb,'same');

%%%%%%%%%%%%%%%% VZ curves%%%%%%%%%%%%%%%%%

% V(z) response
% calculation of V(z) curve

intg=u1plus((Npoint-1)/2:end,(Npoint-1)/2).^2.*P1((Npoint-1)/2:end,(Npoint-1)/2).*P2((Npoint-1)/2:end,(Npoint-1)/2).*transpose(Refl2);

vz=transpose(intg);
r=r((Npoint-1)/2:end);
ZZspan=linspace(-250e-6,150e-6,2point);
for n3=1:length(ZZspan)
X3=ZZspan(n3); intg=vz.*exp(-j.*(k0.*X3/focal^2).*r.^2).*r;%.*Refl2; Vz(n3)=trapz(r,intg);

end

dB_Vz=20*log10(abs(Vz)./max(abs(Vz)))

%% Plot the V(z) curves
figure('Position',[scrw1,scrh1,scrw2,scrh2]) % Location figure
plot(ZZspan*1e6,abs(Vz)/max(abs(Vz)),'linewidth',2)
grid on;
title('V(z) curve of Fused Quartz (Angular spectrum method)')
xlabel('distance z (\mum)')
ylabel('normalized Vz')

figure('Position',[scrw1,scrh1,scrw2,scrh2]) % Location figure
plot(ZZspan*1e6,dB_Vz,'linewidth',2)
grid on;
title('V(z) curve of Fused Quartz (Angular spectrum method)')
xlabel('distance z (\mum)')
ylabel('dB Vz')

B. HeLa cell attached on substrate

%% SIMULATION OF V(Z) CURVE - HeLa cell and FUSED QUARTZ By Angular spectrum method
%% (Atalar method)
%% June, 2009
%% Yada Juntarapaso
%% Graduate Program in Acoustics

%% Reference
%% Science, Technology and Education of microscopy: an overview. pp. 325-344
clear all; close all; clc;

%% Default figure setting
set(0,'Defaultaxesfontsize',16);
set(0,'Defaulttextfontsize',16);
lw=2.;
scrsz=get(0,'ScreenSize');
scrwidth=scrsz(3)-scrsz(1);
scrheight=scrsz(4)-scrsz(2);
scrw1=scrwidth*0.1;
scrw2=scrwidth*0.7;
scrh1=scrheight*0.1;
scrh2=scrheight*0.8;

%%%%%%%%%%%%%%%% Assign Acoustic lens property%%%%%%%%%%%%%%%%

freq=400e6;  % The center frequency of acoustic lens [Hz]
Ra=0.383e-3;  % Radius of transducer [m]
theta_m=60/180*pi;  % Half aperture angle of the lens [radian]

%Buffer rod properties (Sapphire)
cL_rod=11175;  % Longitudinal wave speed of Sapphire [m/s]
cS_rod=6950;  % Shear wave speed of Sapphire [m/s]
rho_rod=3980;  % density of Sapphire [kg/m^3]

%% Coupling medium property: in this case = Water
cW=1500;  % Longitudinal wave speed of water [m/s]
rhoW=1000;  % density of water [kg/m^3]
alphaW=1100;  % Absorption coefficient of water
focal=577.52e-6;  % Focal distance [m]

R=focal*(1-cW/cL_rod);  % Radius of the spherical recess [m]
lambda=cL_rod/freq;  % Wavelength in the buffer rod [m]
L0=6122.00e-6;  % Length of the buffer rod (Focal distance assumption)

k=2*pi/lambda;  % Wave number in the buffer rod [1/m]

%% AntireflecS_rodion coating
cL_anti=2250;  % Longitudinal wave speed [m/s]
rho_anti=4640;  % Density [kg/m^3]
t_anti=2.451e-6;  % Thickness [m]

%%%% properties of each medium layer
%%% medium layer I = coupling liquid = water in this case
cL_I=1500;
rho_I = 1000;

%%% medium layer II = biological cell = HeLa cell normal phase (cell D)
cL_II = 1501;
rho_II = 1184;
att = 2.12e-4; % attenuation coefficient in cell [dB/m*Hz]

%%% medium layer III = substrate = fused quartz in this case
cL_III = 5970;
cS_III = 3765;
rho_III = 2200;

%%% Pupil function
%%% wavenumbers
kW = freq * 2 * pi / cW; % wave number in water [1/m]
kL = freq * 2 * pi / cL_rod; % Acoustic lens (Longitudinal wave number) [1/m]
kT = freq * 2 * pi / cS_rod; % Acoustic lens (Shear wave number) [1/m]

%%% Number of calculation points
Npoint = 501; % should be odd
Tpoint = 1000;
Zpoint = 512;
z_span = linspace(-50e-6, 10e-6, Zpoint); % sampling point in the z-direction for V(z) response
r = linspace(-R * sin(theta_m), R * sin(theta_m), Npoint); % r-position along the lens surface
theta_incd = asin(r ./ R); % Angular span
theta_trans = theta_incd - asin(cW / cL_rod * sin(theta_incd)); % angular span of incident angle
%theta_trans = asin(cW / cL_rod * sin(theta_incd)); % angular span of transmitted angle
A = 1; x0 = 0; y0 = 0;
sigma_x = 2 * Ra;
sigma_y = 2 * Ra;
x = linspace(-(Npoint - 1) / 2, (Npoint - 1) / 2, Npoint) * (lambda / 5);
y = x;
f = freq; % frequency [Hz]
N = length(x);
w = -pi/2:pi/(N-1):pi/2;
kx = (f.*w)./cL_rod;
ky = (f.*w)./cL_rod;
[X, Y] = meshgrid(x, y);

a = (X-x0).^2/(2*sigma_x^2);
b = (Y-y0).^2/(2*sigma_y^2);
gaus = A*exp(-(a+b));
u0 = gaus;
figure; mesh(X, Y, u0)
title('u0')
xlabel('x (\mum)')
ylabel('y (\mum)')
zlabel('z (\mum)')

%%%%%%%%%%%%%%%% U0plus %%%%%%%%%%%%%%%%%%%%%%%

u0plus = abs(u0);
U0plus = fftshift(fft2(u0plus));
figure;
imagesc(kx, ky, 20*log10(abs(U0plus)));
title('U0plus')
xlabel('kx')
ylabel('ky')

%%%%%%%%%%%%%%%%%%% U1plus %%%%%%%%%%%%%%%%%%%%%%

k0 = 2*pi*f/cW;
kzz = sqrt(k0.^2-kx.^2-ky.^2);
kz = meshgrid(kzz, kzz);

U1plus = U0plus.*exp(j.*kz.*L0);
figure; imagesc(kx, ky, 20*log10(abs(U1plus)));
title('U1plus')
xlabel('kx')
ylabel('ky')
figure; mesh(kx, ky, 20*log10(abs(U1plus)));
title('U1plus')
xlabel('kx')
ylabel('ky')
u1plus = ifft2(U1plus);
figure; mesh(X,Y,abs(u1plus));
title('u1+');
xlabel('x (\mu m)');
ylabel('y (\mu m)');
zlabel('abs(u1plus)');

U2plus = fftshift(fft2(abs(u2plus)));
%%%%%% U3 plus%%%%%%%%%%%%%%%%%%%%

kx=meshgrid(kx,kx);
kky=meshgrid(ky,ky);
Z=linspace(-50e-6,10e-6,Npoint);
Z=meshgrid(Z,Z);
U3plus=U2plus.*(exp(j.*kk0.*Z)).*exp(-j.*(((kx.^2+kky.^2)./(2*kk0)).*Z));

figure;
imagesc(kx,ky,20*log10(abs(U3plus)));title('U3plus');xlabel('kx');ylabel('ky');
figure; mesh(kx,ky,abs(U3plus));title('U3plus');xlabel('kx');ylabel('ky');zlabel('abs(U3plus)'

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

%% Transmission coefficient
%% from water to cell
Z_cell=rho_II*cL_II./cos(theta_trans);
Z_Wn=rhoW*cW./cos(theta_trans);
T_WC=Z*Z_cell./(2*Z_Wn+Z_cell);
T1=T_WC;
T1=meshgrid(T1,T1);
T_CW=2*Z_Wn./(Z_Wn+Z_cell);
T2=T_CW;
T2=meshgrid(T2,T2);

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

% Calculation of Reflectance function
% calculation from layer I
kL_I=2*pi*freq/cL_I; % wave number in layer I (water)
thetaL_I=theta_trans; % incident angle of longitudinal wave
s=kL_I.*sin(thetaL_I);
alpha_I=kL_I.*cos(thetaL_I);

%calculation from layer II (cell)
kL_II=2*pi*freq/cL_II;
rho_II=1184;
att=2.12e-4; %attenuation coefficient [dB/m*Hz]
thetaL_II=asin(s./kL_II);
\[ \alpha_{II} = k_{L_{II}} \cdot \cos(\theta_{L_{II}}); \]

% calculation layer III; substrate (fused quartz)

\[ k_{S_{III}} = 2\pi \cdot \text{freq} / c_{S_{III}}; \]
\[ \theta_{S_{III}} = \arcsin(s./k_{S_{III}}); \]
\[ \beta_{III} = k_{S_{III}} \cdot \cos(\theta_{S_{III}}); \]

\[ k_{L_{III}} = 2\pi \cdot \text{freq} / c_{L_{III}}; \]
\[ \theta_{L_{III}} = \arcsin(s./k_{L_{III}}); \]
\[ \alpha_{III} = k_{L_{III}} \cdot \cos(\theta_{L_{III}}); \]

%%%%% calculate B1, B2, gamma

\[ B1 = ((k_{S_{III}})^{2} - 2\cdot s^{2})^{2} + 4\cdot (s^{2}) \cdot \alpha_{III} \cdot \beta_{III}; \]
\[ B2 = ((\rho_{II} \cdot \alpha_{III}) / (\rho_{III} \cdot \alpha_{II})) \cdot (k_{S_{III}})^{4}; \]
\[ \gamma = (\rho_{I} \cdot \alpha_{II}) / (\rho_{II} \cdot \alpha_{I}); \]

\[ d = \text{linspace}(-5e-6,0,N_{point}); \]
\[ d = -5e-6; \quad \text{% thickness of the cell 5.27 micron (cell D: normal phase or at interphase)} \]

\[ \text{numerator} = ((B1 - (\gamma \cdot B2)) \cdot \cos(\alpha_{II} \cdot d)) + (j \cdot ((\gamma \cdot B1) - B2) \cdot \sin(\alpha_{II} \cdot d)); \]
\[ \text{denominator} = ((B1 + (\gamma \cdot B2)) \cdot \cos(\alpha_{II} \cdot d)) - (j \cdot ((\gamma \cdot B1) + B2) \cdot \sin(\alpha_{II} \cdot d)); \]

\[ \text{Refl} = \text{numerator} \div \text{denominator}; \]
\% break

\[ \text{Refl2} = \text{Refl}((N_{point}-1)/2:end); \]

%% Plot the reflectance function
figure('Position', [scrw1,scrh1,scrw2,scrh2]) \quad \% Location figure, and set background to white
subplot(211)
plot(theta_trans((Npoint-1)/2:end)*180/pi,abs(Refl2),'linewidth',2)
title('Reflectance function of HeLa cell+the coupling medium+fused quartz substrate')
xlabel('deg.')
ylabel('Normalized amplitude');

subplot(212)
plot(theta_trans((Npoint-1)/2:end)*180/pi,phase(Refl2)/(2*pi),'linewidth',2)
xlabel('deg.')
ylabel('Phase (rad)');
Refl = meshgrid(Refl, Refl);

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%% assign attenuation function %%%%%%%%%%%%%%%%%%%%%%

%attx = att*f*d.*x;
%atty = attx;
%ATT = meshgrid(attx, atty);
%ATT2 = ATT((Npoint - 1)/2:end, (Npoint - 1)/2);

dd = meshgrid(d, d);
%U4plus = U3plus.*T1.*exp(j.*kz.*dd).*exp(-j.*ATT);
U4plus = U3plus.*T1;
figure;
imagesc(kx, ky, 20*log10(abs(U4plus))); title('U4plus'); xlabel('kx'); ylabel('ky');
figure; mesh(kx, ky, abs(U4plus)); title('U4plus'); xlabel('kx'); ylabel('ky'); zlabel('abs(U4plus)');
break

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

U4minus = U4plus.*Refl;
figure;
imagesc(kx, ky, 20*log10(abs(U4minus))); title('U4minus'); xlabel('kx'); ylabel('ky');
figure; mesh(kx, ky, abs(U4minus)); title('U4minus'); xlabel('kx'); ylabel('ky'); zlabel('abs(U4minus)');

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

%U3minus = U4plus.*T2.*exp(j.*kz.*dd).*exp(-j.*ATT);
U3minus = U4plus.*T2;
break
figure;
imagesc(kx, ky, 20*log10(abs(U3minus))); title('U3minus'); xlabel('kx'); ylabel('ky');
figure; mesh(kx, ky, abs(U3minus)); title('U3minus'); xlabel('kx'); ylabel('ky'); zlabel('abs(U3minus)');

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

%U2minus = U3minus.*(exp(j.*kk0.*Z)).*exp(-j.*(kx.^2 + kky.^2)/(2*kk0)).*Z;
U2minus = U3minus.*(exp(j.*kk0.*Z));
figure;
imagesc(kx,ky,20*log10(abs(U2minus)));title('U2minus');xlabel('kx');ylabel('ky')
figure; mesh(kx,ky,abs(U2minus));title('U2minus')
xlabel('kx');ylabel('ky');zlabel('abs(U2minus)')

u2minus=ifft2(U2minus);
figure; mesh(X,Y,abs(u2minus));title('u2minus')
xlabel('x (\mu m)'); ylabel('y (\mu m)');zlabel('abs(u2minus)')

%%%%%%%%%%%%%%%%%u1minus%%%%%%%%%%%%%%%%%%%%%%%

t=1;

u1minus=((exp(j.*kk0.*(focal*(1+cbar^2))))./(j.*lambda0*focal)).*P2.*U2

figure; mesh(X,Y,abs(u1minus));title('u1minus')
xlabel('x (\mu m)'); ylabel('y (\mu m)');zlabel('abs(u1minus)')

U1minus=fftshift(fft2(abs(u1minus)));
figure; imagesc(kx,ky,20*log10(abs(U1minus)));title('U1minus');xlabel('kx');ylabel('ky')
figure; mesh(kx,ky,abs(U1minus));title('U1minus')
xlabel('kx');ylabel('ky');zlabel('abs(U1minus)')

%%%%%%%%%%%%%%%%%u0minus%%%%%%%%%%%%%%%%%%%%%

bb=ifft2(exp(j.*kz.*L0));

%u0minus=conv2(abs(u1minus),bb,'same');

%%%%%%%%%%%%%%%% VZ curves%%%%%%%%%%%%%%%%%%%%

%% V(z) response
%% calculation of V(z) curve

%intg=ulplus((Npoint-1)/2:end,(Npoint-1)/2).^2.*P1((Npoint-1)/2:end,(Npoint-1)/2).*P2((Npoint-1)/2:end,(Npoint-1)/2).*transopse(Refl2);
intg=ulplus((Npoint-1)/2:end,(Npoint-1)/2).^2.*P1((Npoint-1)/2:end,(Npoint-1)/2).*P2((Npoint-1)/2:end,(Npoint-1)/2).*T1((Npoint-1)/2:end,(Npoint-1)/2).*T2((Npoint-1)/2:end,(Npoint-1)/2).*transpose(Refl2);
vz=transpose(intg);
r=r((Npoint-1)/2:end);

ZZspan=linspace(-250e-6,150e-6,2point);

for n3=1:length(ZZspan)
    X3=ZZspan(n3);

\[ \text{intg} = v_z^* \exp(-j \cdot (k_0 \cdot x_3 / \text{focal}^2) \cdot r^2) \cdot r; \]
\[ \%\text{intg} = v_z^* \exp(-j \cdot (k_0 \cdot x_3 / \text{focal}^2) \cdot r^2) \cdot r \cdot \text{transpose}(\exp(-j \cdot \text{ATT}2)). \cdot \text{transpose}(\exp(-j \cdot \text{ATT}2)); \]

\[ V_z \text{Hela}(n3) = \text{trapz}(r, \text{intg}); \]
\[ \text{end} \]

\[ \text{dB}_{V_z \text{Hela}} = 20 \cdot \log_{10}(\text{abs}(V_z \text{Hela}) / \text{max}(\text{abs}(V_z \text{Hela}))); \]

% Plot the V(z) curves
figure('Position', [scrw1, scrh1, scrw2, scrh2]) % Location figure
plot(ZZspan*1e6, abs(VzHela)/max(abs(VzHela)), 'linewidth', 2)
grid on;
title('V(z) curve of Hela cell at normal phase with fused quartz substrate (Angular spectrum method)')
xlabel('distance z (\mum)')
ylabel('normalized Vz')

figure('Position', [scrw1, scrh1, scrw2, scrh2]) % Location figure
plot(ZZspan*1e6, dB_VzHela, 'linewidth', 2)
grid on;
title('V(z) curve of Hela cell at normal phase with fused quartz substrate (Angular spectrum method)')
xlabel('distance z (\mum)')
ylabel('dB Vz')

**III. FFT analysis of V(z) curve**

% V(z) curve analyze-- Kushibiki method
% Yada Junantarapaso Acoustics Program at Penn State
% June 2009

% Reference
% JUN-ICHI KUSHIBIKI AND NORIYOSHI CHUBACHI. IEEE Transactions on
% [2] Z. Yu, Scanning acoustic microscopy and its applications to
281

%% 863-891.

close all; clear all; clc;

load Vz; 
load Vz_Pb; 

Vz_quartz=Vz; 
Vz_lead=Vz_Pb;

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%% 
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%% 
%% Default figure setting 
set(0,'Defaultaxesfontsize',16); 
set(0,'DEFAULTtextfontsize',16); 
lw=2.; 

lw=2.;  

figure('Position',[scrw1,scrh1,scrw2,scrh2]); 
plot(z_span*1e6,abs(Vz_quartz),'

figure('Position',[scrw1,scrh1,scrw2,scrh2]); 
plot(z_span*1e6,abs(Vz_lead),'

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%% 
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%% 

% V(z) curve analysis 
% From reference [1], section C. Spectral Analysis of Acoustic Properties. 
% and figure 12 page 198 

% Define VI'
\[ V_{I\text{-dash}} = \text{abs}(V_{z\text{-quartz}}) - \text{abs}(V_{z\text{-lead}}); \]

\[ X_w = V_{I\text{-dash}}; \]

% synthesize delta VL by using moving-average and define VI : procedure

% and figure 12e

\[
\text{for } n = 3:\text{length}(X_w), \\
\quad \text{dVL}(n) = (X_w(n) + (X_w(n-1)) + \ldots + (X_w(n-29)) + (X_w(n-30)) + \ldots + (X_w(n-47)) + (X_w(n-48)) + (X_w(n-49))) / 50; \quad \text{the filtered signal} \\
\quad V_{I}(n) = V_{I\text{-dash}}(n) - \text{dVL}(n); \]

\[
\text{end} \\
\]

% plot VI

\[
\text{figure('Position', [scrw1, scrh1, scrw2, scrh2])} \\
\text{plot(z_span*1e6, (V_I), 'linewidth', 2)} \\
\text{ylabel('amplitude')} \\
\text{xlabel('distance Z \text{\mum}') } \\
\text{title('VI')} \\
\text{grid on} \\
\]

% sampling VI (procedure of figure 12f)

\[
v_{\text{samples}} = V_{I}(1:128:\text{length}(V_{I})); \\
zz_{\text{span}} = z_{\text{span}}(1:128:\text{length}(z_{\text{span}})); \\
\]

% add dummy points

\[
\text{Ns} = \text{length}(v_{\text{samples}}); \quad \% \text{number of sampling point of sampled VI} \\
N = 8192; \quad \% \text{Total number of sampling points} \\
Nd = N - Ns; \quad \% \text{number of dummy point} \\
vv = [\text{zeros}(1, (Nd/2)) \ v_{\text{samples}} \ \text{zeros}(1, (Nd/2))]; \quad \% \text{Sampled VI including dummy points} \\
f = 200e6; \quad \% \text{center frequency of acoustics lens [Hz]} \\
c = 1500; \quad \% \text{sound speed in water [m/s]} \\
w = 0:pi/(N-1):pi; \\
k = (f.*w)/c; \quad \% \text{wave number}
%k=w./c;
ws=0:pi/(Ns-1):pi;
ks=(f.*ws)./c;

% execute FFT of sampled signal
vv_samp=abs(vv);
VV=fftshift(fft(vv_samp,N));

figure('Position', [scrw1,scrh1,scrw2,scrh2])
subplot 211
stem(k*1e-6,abs(VV),'fill')
xlabel('wavenumber k [rad/micron]
ylabel('amplitude')
title('FFT of VI')
grid on

subplot 212
stem(k*1e-6,abs(VV)/max(abs(VV)),'fill')
xlabel('wavenumber k [rad/micron]
ylabel('normalized amplitude')
%xlim([1.5e5 2.8e5])
grid on

% calculate deltaZ
% from the fft plot, we got the wavenumber giving the maximum amplitude in spectra = 2.0947e5 rad/m; k=2.0947e5 [rad/m] = 0.209475 rad/micron

% Using equation (30) pp. 195 ref. [1]
dZ_from_fft=2*pi/2.0947e5

% load value of dz of Vz curve (fused quartz) from previous simulation
% 'Yada_Vz_fused_quartz_May_2009.m'
load dz
dz_sim=dz;

% Calculation dz by using equations (1),(2)on pp.869 and equation (4) pp.870
% in reference [2]
ps=0.16; % poisson ration of fused quartz
beta=3765; % shear wave velocity of fused quartz

vR=beta/(1.14418-(0.25771*ps)+(0.12661*ps*ps)); % using equation (1) ref.[2]pp.869 Rayleigh wave velocity

vw=1500; % sound speed in water [m/s]
\[
\lambda_w = \frac{\text{vw}}{f};
\]

\[
\theta_R = \frac{\text{asin}(\text{vw}/v_R)}{\text{Rayleigh angle, using equation (2) ref. [2] pp. 869}};
\]

\[
d_{Z_{\text{calc}}} = \frac{\lambda_w}{2(1 - \cos(\theta_R))}; \quad \% \text{Calculation } dZ \text{ using equation (4) ref. [2] pp. 870}
\]

\[
\% \text{ percent errors}
\]

\[
\text{percent_error}_{dZ_1} = \frac{\left| \left( d_{Z_{\text{from \text{fft}}} - d_{Z_{\text{sim}}}} / d_{Z_{\text{sim}}} \right) \right| \times 100}{\% \text{ compare value from fft method and the simulation value}}
\]

\[
\text{percent_error}_{dZ_2} = \frac{\left| \left( d_{Z_{\text{from \text{fft}}} - d_{Z_{\text{calc}}}} / d_{Z_{\text{calc}}} \right) \right| \times 100}{\% \text{ compare value from fft method and calculation value}}
\]

\[
\text{percent_error}_{dZ_3} = \frac{\left| \left( d_{Z_{\text{calc}} - d_{Z_{\text{sim}}}} / d_{Z_{\text{sim}}} \right) \right| \times 100}{\% \text{ compare value from the calculation and the simulation values}}
\]

\% Calculate the velocity of leaky SAW. Using equation (15) pp. 194 ref. [1]

\[
\text{vw} = 1500; \quad \% \text{sound speed of water } [\text{m/s}]
\]

\[
\text{aa} = \frac{\text{vw}}{(2 \times f \times d_{Z_{\text{from \text{fft}}}})};
\]

\[
V_{\text{LSAW}} = \frac{\text{vw}}{\sqrt{1 - (1 - \text{aa})^2}}
\]

\% Calculate the normalized attenuation factor

\% using equations (28), (29) pp. 195 and equation (12) pp. 193 ref. [1]

\[
\theta_{\text{LSAW}} = \frac{\text{acos}(1 - (\text{vw}/(2 \times f \times d_{Z_{\text{from \text{fft}}}})))}{\% \text{calculate the normalized attenuation factor}}
\]

\[
\alpha_{\text{W}} = (25.3e15 \times f^2); \quad \% \text{attenuation coefficient in water. ref. [1] pp. 193}
\]

\% Calculate \( \alpha_0 \) using equation (28) pp. 195

\[
\alpha_0 = (0.0181e6 \times 1)/\sqrt{((1)^2 - (0.14046)^2}) \quad \% \text{at } \text{k} = .2276e6
\]

\[
\delta = (0.2276 - .20947)e6; \quad \% \text{normalized amplitude at } 2276 = .14046
\]

\% Calculate \( \gamma \) using equation (29) pp. 195

\[
\gamma = (\alpha_0 \times \text{cos}(\theta_{\text{LSAW}}) + (2 \times \alpha_{\text{W}})) / (2 \times \sin(\theta_{\text{LSAW}}))
\]

\[
\alpha_{\text{LSAW}} = \gamma \times V_{\text{LSAW}} / (2 \times \pi \times f)
\]

\% percent error for \( \alpha_{\text{LSAW}} \) compare with the value from the table III \% from ref. [1] pp. 203 \% the measured value of \( \alpha_{\text{LSAW}} = 3.90 \times 10^{-2} \)
percent_error_alphaLSAW=((abs(alpha_LSAW-3.90e-2))/3.9e-2)*100

IV. MCF phantom

% Creates a phantom for MCF7 cell.
% Developed by Yada J. Graduate Program in Acoustics. PSU.
% Based on Example by Jürgen Arendt Jensen and Peter Munk, Ver. 1, March 26, 1997.

function [positions, amp] = mcf_phantom(N_tot)

% The total number of scatterers is N_tot=N*N_group
% memory problems forces to split the calculation up in parts
% The sub calculation size is 1000

N=1000;
N_group=floor(N_tot/N);
if N_group~=floor(N_tot/N)
    error('Group split is not a multiplum of the total number of scatterers');
end

% Define image coordinates

x_size = 0.8/1000;
z_size = 0.6/1000;
y_size = 0.15/1000;
z_start = 0.2/1000;

% Load input map

[samimage, MAP,out3]=bmpread('powerImage.bmp');

% Find the white structures and generate data for them

index=1;
strong = (samimage > 250);
[n,m]=size(strong);
for i=1:n
    disp([num2str(i/n*100),' % finished'])
    for j=1:m
        if (strong(i,j))
            strong_pos(index,:)=[(j/m-0.5)*x_size 0 i/n*z_size+z_start];
    end
index=index+1;
end
index=index+1;
end
end
end
disp(['num2str(i),' strong pixel values found'])

for mm=1:N_group

% calculate position data
x0 = rand(N,1);
x = (x0-0.5)*x_size;
z0 = rand(N,1);
z = z0*z_size+z_start;
y0 = rand(N,1);
y = (y0-0.5)*y_size;

positions((mm-1)*N+(1:N),:) = [x y z];

% Amplitudes with different variance must be generated according to the
% input map.
% The amplitude of the samimage image is used to scale the variance

var_value(:,mm)=diag(samimage(round([1+z0*(size(samimage,1)-
1)]),round([1+x0*(size(samimage,2)-1)]))));
amp(:,mm)=var_value(:,mm).*randn(size(var_value,1),1);
end

% Make it into column vectors
amp=reshape(amp,N*N_group,1);

% Include the strong scatterers
positions=[positions];
amp=[amp];
end

V. HeLa Phantom

% Creates a phantom for HeLa cell.
% Developed by Yada J. Graduate Program in Acoustics, PSU.
% Based on Example by Jürgen Arendt Jensen and Peter Munk, Ver. 1, March 26, 1997.

function [positions, amp] = hela_phantom(N_tot)

% The total number of scatterers is N_tot=N*N_group
% memory problems forces to split the calculation up in parts
% The sub calculation size is 1000

N=1000;
N_group=floor(N_tot/N);
if N_group~=(N_tot/N)
    error('Group split is not a multiplum of the total number of scatterers');
end

% Define image coordinates
x_size = 0.85/1000;
z_size = 0.6/1000;
y_size = 0.2/1000;
z_start = 0.2/1000;

% Load input map
[samimage, MAP, out3]=bmpread('powerImagethree.bmp');

% Find the white structures and generate data for them
index=1;
strong = (samimage > 250);
[n,m]=size(strong);
for i=1:n
disp([num2str(i/n*100),' % finished'])
    for j=1:m
        if (strong(i,j))
            strong_pos(index,:) = [(j/m-0.5)*x_size 0 i/n*z_size+z_start];
            index=index+1;
        end
    end
end
disp([num2str(i), ' strong pixel values found'])

for mm=1:N_group
    % calculate position data
    x0 = rand(N,1);
x = (x0-0.5)* x_size;
z0 = rand(N,1);
z = z0*z_size+z_start;
y0 = rand(N,1);
y = (y0-0.5)* y_size;

positions((mm-1)*N+(1:N),:) = [x y z];

% Amplitudes with different variance must be generated according to the
% input map. The amplitude of the fetus image is used to scale the variance

var_value(:,mm)=diag(samimage(round([1+z0*(size(samimage,1)-1)]),round([1+x0*(size(samimage,2)-1)])));
amp(:,mm)=100*var_value(:,mm).*randn(size(var_value,1),1);
end

% Make it into column vectors
amp=reshape(amp,N*N_group,1);

% Include the strong scatterers
positions=[positions];
amp=[amp];
end

VI. Transducer simulation

% Phased array scan of MCF7 cells
% This script assumes that the field_init procedure has been called
% Here the field simulation is performed and the data is stored
% in rf-files; one for each rf-line done. The data must then
% subsequently be processed to yield the image. The data for the
% scatterers are read from the file pht_data.mat, so that the procedure
% can be started again or run for a number of workstations.
% Developed by Yada Juntarapaso. PSU Acoustics. January 2011
% Based on example by Joergen Arendt Jensen and Peter Munk,
% Version 1.1, April 1, 1998, JAJ.
% Ver. 1.1: 1/4-98: Procedure xdc_focus_center inserted to use the new focusing scheme for the Field II program

% Generate the transducer apertures for send and receive

field_init

f0=1000e6;                    % Transducer center frequency [Hz]
fs=14000e6;                   % Sampling frequency [Hz]
c=1540;                       % Speed of sound [m/s]
lambda=c/f0;                  % Wavelength [m]
width=0.6/1e6;                % Width of element
element_height=0.8/1000;     % Height of element [m]
kerf=lambda/10;               % Kerf [m]
focus=[0 0 0.6]/1000;         % Fixed focal point [m]
N_elements=256;               % Number of physical elements

% Set the sampling frequency

set_sampling(fs);
set_field ('show_times', 5)

% Generate aperture for emission

xmit_aperture = xdc_linear_array (N_elements, width, element_height, kerf, 1, 5, focus);

% Set the impulse response and excitation of the xmit aperture

impulse_response=sin(2*pi*f0*(0:1/fs:2/f0));
impulse_response=impulse_response.*hanning(max(size(impulse_response)));
xdc_impulse (xmit_aperture, impulse_response);

excitation=sin(2*pi*f0*(0:1/fs:2/f0));
xdc_excitation (xmit_aperture, excitation);

% Generate aperture for reception

receive_aperture = xdc_linear_array (N_elements, width, element_height, kerf, 1, 5, focus);

% Set the impulse response for the receive aperture

xdc_impulse (receive_aperture, impulse_response);

% Load the computer phantom

load mcfpht_data
% Set the different focal zones for reception

focal_zones=[0.5:0.5:10]'/1000;
Nf=max(size(focal_zones));
focus_times=(focal_zones-0.5/1000)/1540;
z_focus=0.6/1000; % Transmit focus

% Set the apodization

apo=hanning(N_elements)';
xdc_apodization (xmit_aperture, 0, apo);
xdc_apodization (receive_aperture, 0, apo);

% Do phased array imaging

no_lines=64; % Number of lines in image
image_width=90/180*pi; % Size of image sector [rad]
dtheta=image_width/64; % Increment for image
%dtheta=0.7/180*pi;

% Do imaging line by line

for i=1:8
    if ~exist(['rf_ln',num2str(i),'.mat'])
        cmd=['save rf_ln',num2str(i),'.mat i']
eval(cmd)
        % Set the focus for this direction
        theta= (i-1-no_lines/2)*dtheta;
        xdc_focus (xmit_aperture, 0, [z_focus*sin(theta) 0
        z_focus*cos(theta)]);
        xdc_focus (receive_aperture, focus_times, [focal_zones*sin(theta)
        zeros(max(size(focal_zones)),1) focal_zones*cos(theta)]);
        % Calculate the received response
        [rf_data, tstart]=calc_scat(xmit_aperture, receive_aperture,
        phantom_positions, phantom_amplitudes);
        % Store the result
        cmd=['save rf_ln',num2str(i),'.mat rf_data tstart']
eval(cmd)
    end
end

% Free space for apertures
xdc_free (xmit_aperture)
dxdc_free (receive_aperture)
Appendix B

FIELD II ultrasound simulation program commands

Field II user’s guide

<table>
<thead>
<tr>
<th>Command</th>
<th>field_debug</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purpose:</td>
<td>Procedure for initialize debugging. This will print out various information about the programs inner working. Initially no debugging is done.</td>
</tr>
<tr>
<td>Calling:</td>
<td>field_debug(state)</td>
</tr>
<tr>
<td>Input:</td>
<td>State - 1: debugging, 0: no debugging.</td>
</tr>
<tr>
<td>Output:</td>
<td>none.</td>
</tr>
</tbody>
</table>

Field II user’s guide

<table>
<thead>
<tr>
<th>Command</th>
<th>field_end</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purpose:</td>
<td>Procedure for terminating the Field II program system and releasing the storage.</td>
</tr>
<tr>
<td>Calling:</td>
<td>field_end ;</td>
</tr>
<tr>
<td>Input:</td>
<td>none.</td>
</tr>
<tr>
<td>Output:</td>
<td>none.</td>
</tr>
</tbody>
</table>

Field II user’s guide

<table>
<thead>
<tr>
<th>Command</th>
<th>field_guide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purpose:</td>
<td>Procedure for displaying the Field II users’ guide (this guide) using the Adobe acrobat reader.</td>
</tr>
<tr>
<td>Calling:</td>
<td>field_guide</td>
</tr>
<tr>
<td>Input:</td>
<td>none.</td>
</tr>
<tr>
<td>Output:</td>
<td>The Field II guide is displayed in a separate window using acrobat reader.</td>
</tr>
</tbody>
</table>

Note that the Adobe pdf reader must be installed on the system, and it must be accessible under Matlab under the name acroread. The users guide should be in the search path of Matlab, preferably in the same directory as the m-files for Field II with the name users_guide.pdf.

Field II user’s guide

<table>
<thead>
<tr>
<th>Command</th>
<th>field_info</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purpose:</td>
<td>Procedure for showing information about the Field II program. The information is printed in the Matlab window.</td>
</tr>
<tr>
<td>Calling:</td>
<td>field_info</td>
</tr>
</tbody>
</table>
Input: None.
Output: Information is printed in the Matlab window.
For boolean variables a value of 1 indicates true and 0 for false.

**Field II user’s guide**

**set_sampling**

**Purpose:** Set the sampling frequency the system uses.
Remember that the pulses used in all apertures must be reset for the new sampling frequency to take effect.
This procedure has been superseded by set_field, and it is for portability reasons better to use set_field.
**Calling:** set_sampling(fs);
**Input:** fs - The new sampling frequency.
**Output:** none.

**Field II user’s guide**

**set_field**

**Purpose:** Set various parameters that determines the function of the program.
**Calling:** set_field(option_name, value);
**Input:**
- use_att: Whether to use attenuation (< 0 for attenuation)
- freq_att: Frequency independent attenuation in dB/m.
- freq_2: Frequency dependent attenuation in dB/m Hz around the center frequency.
- att_f0: Attenuation center frequency in Hz.
- debug: Whether to print debug information (1 = yes)
- c: Set the speed of sound in m/s.
- fs: Set the sampling frequency.
- show_time: Show calculation times during calculation. (yes = any positive number). A number larger than 2 is taken as the time in seconds between the printing of estimates.
- use_rectangles: Use rectangles for the apertures. (1 = yes)
- use_triangles: Use triangles for describing apertures. (1 = yes)
- use_lines: Use lines for describing apertures. (1 = yes)
- fast_integration: Whether to use fast integration (1) of the responses for bound lines and triangles. Fast integration uses a simple trapezoidal time integration of the responses, else a Romberg integration, as described in Numerical Recipes, are used.

**Output:** none.
**Field II user’s guide**

**xdc_apodization**

**Purpose:** Procedure for creating an apodization time line for an aperture

**Calling:** xdc_apodization (Th, times, values);

**Input:**
- Th: Pointer to the transducer aperture.
- times: Time after which the associated apodization is valid.
- values: Apodization values. Matrix with one row for each time value and a number of columns equal to the number of physical elements in the aperture.

**Output:** none.

---

**Field II user’s guide**

**xdc_baffle**

**Purpose:** Procedure for setting the baffle condition for the aperture.

**Calling:** xdc_baffle (Th, soft_baffle);

**Input:**
- Th: Pointer to the transducer aperture.
- soft_baffle: Whether to use the soft-baffle condition:
  - 1 - using soft baffle
  - 0 - using rigid baffle (default for apertures)

**Output:** none.
**Field II user's guide**

**xdc_center_focus**

**Purpose:** Procedure for setting the center point for the focusing. This point is used as a reference for calculating the focusing delay times and as a starting point for dynamic focusing.

**Calling:** `xdc_center_focus (Th, point);`

**Input:**
- `Th` Pointer to the transducer aperture.
- `point` Focus center point.

**Output:** none.

**Field II user's guide**

**xdc_concave**

**Purpose:** Procedure for creating a concave transducer.

**Calling:** `Th = xdc_concave (radius, focal_radius, cde_size);`

**Input:**
- `radius` Radius of physical elements.
- `focal_radius` Focal radius.
- `cde_size` Size of mathematical elements.

**Output:** `Th` A pointer to this transducer aperture.

**Field II user's guide**

**xdc_convert**

**Purpose:** Procedure for converting an aperture from a rectangular description to a triangular description.

**Calling:** `xdc_convert (Th);`

**Input:**
- `Th` Aperture to be converted.

**Output:** None.

**Note:** The number of mathematical elements gets to be twice as large since one rectangle is modeled by two triangles.
**Field II user’s guide**

**xdc_convexFocused_array**

**Purpose:** Procedure for creating a mechanical elevation focused convex array aperture.

**Calling:** Th = xdc_convexFocused_array (no_elements, width, height, kerf, Reconvex, Rfocus, no_sub_x, no_sub_y, focus);

**Input:**
- no_elements: Number of physical elements.
- width: Width in x-direction of elements.
- height: Width in y-direction of elements.
- kerf: Distance in x-direction between elements.
- Reconvex: Convex radius.
- Rfocus: Radius of elevation focus.
- no_sub_x: Number of sub-divisions in x-direction of elements.
- no_sub_y: Number of sub-divisions in y-direction of elements.
- focus: Fixed focus for array (x,y,z). Vector with three elements.

**Output:** Th A pointer to this transducer aperture.

---

**Field II user’s guide**

**xdc_dynamic_focus**

**Purpose:** Procedure for using dynamic focusing for an aperture.

**Calling:** xdc_dynamic_focus (Th, time, dir_zx, dir_zy);

**Input:**
- Th: Pointer to the transducer aperture.
- time: Time after which the dynamic focus is valid.
- dir_zx: Direction (angle) in radians for the dynamic focus. The direction is taken from the center for the focus of the transducer in the z-x plane.
- dir_zy: Direction (angle) in radians for the dynamic focus. The direction is taken from the center for the focus of the transducer in the z-y plane.

**Output:** none.
**Field II user's guide**  

**xdc_convex_focused_array**

**Purpose:** Procedure for creating a mechanical elevation focused convex array aperture.

**Calling:**  
\[ \text{Th} = \text{xdc_convex_focused_array} \text{ (no_elements, width, height, kerf, Reconvex, Rfocus, no_sub_x, no_sub_y, focus);} \]

**Input:**  
- no_elements: Number of physical elements.
- width: Width in x-direction of elements.
- height: Width in y-direction of elements.
- kerf: Distance in x-direction between elements.
- Reconvex: Convex radius.
- Rfocus: Radius of elevation focus.
- no_sub_x: Number of sub-divisions in x-direction of elements.
- no_sub_y: Number of sub-divisions in y-direction of elements.
- focus[3]: Fixed focus for array (x,y,z). Vector with three elements.

**Output:**  
\[ \text{Th} \]  
A pointer to this transducer aperture.

---

**Field II user's guide**  

**xdc_dynamic_focus**

**Purpose:** Procedure for using dynamic focusing for an aperture.

**Calling:**  
\[ \text{xdc_dynamic_focus (Th, time, dir_zx, dir_zy);} \]

**Input:**  
- Th: Pointer to the transducer aperture.
- time: Time after which the dynamic focus is valid.
- dir_zx: Direction (angle) in radians for the dynamic focus. The direction is taken from the center for the focus of the transducer in the z-x plane.
- dir_zy: Direction (angle) in radians for the dynamic focus. The direction is taken from the center for the focus of the transducer in the z-y plane.

**Output:** none.
**Field II user’s guide**

**xdc_excitation**

**Purpose:** Procedure for setting the excitation pulse of an aperture

**Calling:** xdc_excitation (Th, pulse);

**Input:**
- Th Pointer to the transducer aperture.
- pulse Excitation pulse of aperture as row vector

**Output:** none.

**Field II user’s guide**

**xdc_focus**

**Purpose:** Procedure for creating a focus time line for an aperture

**Calling:** xdc_focus (Th, times, points);

**Input:**
- Th Pointer to the transducer aperture.
- times Time after which the associated focus is valid.
- points Focus points. Vector with three columns (x,y,z) and one row for each field point.

**Output:** none.

**Field II user’s guide**

**xdc_focused_array**

**Purpose:** Procedure for creating an elevation focused linear array transducer.

**Calling:** Th = xdc_focused_array (no_elements, width, height, kerf, Rfocus, no_sub_x, no_sub_y, focus);

**Input:**
- no_elements Number of physical elements.
- width Width in x-direction of elements.
- height Width in y-direction of elements.
- kerf Distance in x-direction between elements.
- Rfocus Radius of elevation focus.
- no_sub_x Number of sub-divisions in x-direction of elements.
- no_sub_y Number of sub-divisions in y-direction of elements.
- focus[] Fixed focus for array (x,y,z). Vector with three elements.

**Output:** Th A pointer to this transducer aperture.
Purpose: Procedure for creating a linear, elevation focused array transducer with an number of rows (1.5D array)

Calling: TH = xdc_focused_multirow (no_elem_x, width, no_elem_y, heights, kerf_x, kerf_y, Rfocus, no_sub_x, no_sub_y, focus);

Input:  
- no_elem_x: Number of physical elements in x-direction.
- width: Width in x-direction of elements.
- no_elem_y: Number of physical elements in y-direction.
- heights[]: Heights of the element rows in the y-direction. Vector with no_elem_y values.
- kerf_x: Width in x-direction between elements.
- kerf_y: Gap in y-direction between elements.
- Rfocus: Radius of elevation focus.
- no_sub_x: Number of sub-divisions in x-direction of elements.
- no_sub_y: Number of sub-divisions in y-direction of elements.
- focus[]: Fixed focus for array (x, y, z). Vector with three elements.

Output: TH A pointer to this transducer aperture.
**Field II user’s guide**

### xdc_focus_times and xdc_times_focus

**Purpose:** Procedure for creating a focus time line for an aperture. All the delay values are supplied by the user. The previous time line is replaced by this time line.

Note that the two procedures perform the same operation. xdc_times_focus has been added due to compatibility with the PC version of Field, and should be the procedure generally used.

**Calling:** xdc_focus_times (Th, times, delays); or xdc_times_focus (Th, times, delays);

**Input:**
- Th: Pointer to the transducer aperture.
- times: Time after which the associated focus is valid.
- delays: Delay values. Matrix with one row for each time value and a number of columns equal to the number of physical elements in the aperture.

**Output:** none.

---

**Field II user’s guide**

### xdc_free

**Purpose:** Procedure for freeing the storage occupied by an aperture

**Calling:** xdc_free(Th);

**Input:** Th: Pointer to the transducer aperture.

**Output:** none.

---

**Field II user’s guide**

### xdc_get

**Purpose:** Procedure for getting data for an aperture

**Calling:** data = xdc_get(Th, info_type);

**Input:**
- Th: Pointer to the transducer aperture.
- info_type: Which information to get (text string). The possibilities are:
  - rect: information about rectangular elements
  - tri: information about triangular elements
  - focus: focus time line
  - apo: apodization time line

**Output:** data: data about the aperture
**Field II user’s guide**

**xdc_impulse**

**Purpose:** Procedure for setting the impulse response of an aperture.

**Calling:** `xdc_impulse (Th, pulse);`

**Input:**
- `Th` Pointer to the transducer aperture.
- `pulse` Impulse response of aperture as row vector.

**Output:** `none`.

**xdc_linear_array**

**Purpose:** Procedure for creating a linear array aperture.

**Calling:** `Th = xdc_linear_array (no_elements, width, height, kerf, no_sub_x, no_sub_y, focus);`

**Input:**
- `no_elements` Number of physical elements.
- `width` Width in x-direction of elements.
- `height` Width in y-direction of elements.
- `kerf` Distance in x-direction between elements.
- `no_sub_x` Number of sub-divisions in x-direction of elements.
- `no_sub_y` Number of sub-divisions in y-direction of elements.
- `focus[]` Fixed focus for array (x, y, z). Vector with three elements.

**Output:** `Th` A pointer to this transducer aperture.

**xdc_linear_multirow**

**Purpose:** Procedure for creating a linear multi-row array aperture, where the transducer has been diced to create a two-dimensional matrix of elements. The individual rows can have different heights.

**Calling:** `Th = xdc_linear_multirow (no_elem_x, no_elem_y, heights, kerf_x, kerf_y, no_sub_x, no_sub_y, focus);`

**Input:**
- `no_elem_x` Number of physical elements in x-direction.
- `width` Width in x-direction of elements.
- `no_elem_y` Number of physical elements in y-direction.
- `heights[]` Heights of the element rows in the y-direction. Vector with `no_elem_y` values.
- `kerf_x` Width in x-direction between elements.
- `kerf_y` Gap in y-direction between elements.
- `no_sub_x` Number of sub-divisions in x-direction of physical elements.
- `no_sub_y` Number of sub-divisions in y-direction of physical elements.
- `focus[]` Fixed focus for array (x, y, z). Vector with three elements.

**Output:** `Th` A pointer to this transducer aperture.
**Field II user's guide**

### xdc_lines

**Purpose:** Procedure for creating an aperture bounded by a set of lines.

**Calling:** \( \text{Th} = \text{xdc_lines} (\text{lines}, \text{center}, \text{focus}); \)

**Input:**
- \( \text{lines} \)  Information about the lines. One row for each line. The contents is:

<table>
<thead>
<tr>
<th>Index</th>
<th>Variable</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>no_phys</td>
<td>The number for the physical element starting from one</td>
</tr>
<tr>
<td>2</td>
<td>no_mat</td>
<td>The number for the mathematical element starting from one</td>
</tr>
<tr>
<td>3</td>
<td>slope</td>
<td>Slope of line (NaN is infinity slope)</td>
</tr>
<tr>
<td>4</td>
<td>infinity</td>
<td>True if slope is infinity</td>
</tr>
<tr>
<td>5</td>
<td>intersect</td>
<td>Intersection with y-axis (slope&lt;&gt;NaN) or x-axis if slope is infinity</td>
</tr>
<tr>
<td>6</td>
<td>above</td>
<td>Whether the active aperture is above or to the left (for infinite slope) of the line</td>
</tr>
</tbody>
</table>

- \( \text{center} \)  The center of the physical elements. One line for each element starting from 1.
- \( \text{focus} \)  The fixed focus for this aperture.

All dimensions are in meters.

Notice that this procedure will only work for flat elements positioned in the x-y plane.

**Output:**  A handle \text{Th} as a pointer to this transducer aperture.

---

### xdc_piston

**Purpose:** Procedure for creating a flat, round transducer

**Calling:** \( \text{Th} = \text{xdc_piston} (\text{radius}, \text{ele_size}); \)

**Input:**
- \( \text{radius} \)  Radius of physical elements.
- \( \text{ele_size} \)  Size of mathematical elements.

**Output:**  \text{Th}  A pointer to this transducer aperture.
**Field II user’s guide**

**xdc_rectangles**

**Purpose:** Procedure for creating an aperture consisting of rectangles.

**Calling:** 

```
Th = xdc_rectangles (rect, center, focus);
```

**Input:** 

- `rect` Information about the rectangles. One row for each rectangle. The contents is:

<table>
<thead>
<tr>
<th>Index</th>
<th>Variable</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><code>no</code></td>
<td>The number for the physical aperture starting from one</td>
</tr>
<tr>
<td>2-4</td>
<td><code>x1,y1,z1</code></td>
<td>First corner coordinate</td>
</tr>
<tr>
<td>5-7</td>
<td><code>x2,y2,z2</code></td>
<td>Second corner coordinate</td>
</tr>
<tr>
<td>8-10</td>
<td><code>x3,y3,z3</code></td>
<td>Third corner coordinate</td>
</tr>
<tr>
<td>11-13</td>
<td><code>x4,y4,z4</code></td>
<td>Fourth corner coordinate</td>
</tr>
<tr>
<td>14</td>
<td><code>apo</code></td>
<td>Apodization value for this element</td>
</tr>
<tr>
<td>15</td>
<td><code>width</code></td>
<td>Width of the element (x direction)</td>
</tr>
<tr>
<td>16</td>
<td><code>heigh</code></td>
<td>Height of the element (y direction)</td>
</tr>
<tr>
<td>17-19</td>
<td><code>c1,c2,c2</code></td>
<td>Center point of the rectangle</td>
</tr>
</tbody>
</table>

All dimensions are in meters.

**Output:** A handle `Th` as a pointer to this transducer aperture.

---

**Field II user’s guide**

**xdc_show**

**Purpose:** Procedure for showing information about an aperture.

**Calling:** 

```
xdc_show(Th, info_type);
```

**Input:** 

- `Th` Pointer to the transducer aperture.
- `info_type` Which information to show (text string). The possibilities are:
  - `elements` - information about elements
  - `focus` - focus time line
  - `apo` - apodization time line
  - `all` - all information is shown

The argument is optional, and by default all information is shown.

**Output:** ASCII output on the screen about the aperture.
**Field II user's guide**  

**xdc_focus_times and xdc_times_focus**

**Purpose:** Procedure for creating a focus time line for an aperture. All the delay values are supplied by the user. The previous time line is replaced by this time line.

Note that the two procedures perform the same operation. `xdc_times_focus` has been added due to compatibility with the PC version of Field, and should be the procedure generally used.

**Calling:** `xdc_focus_times (Th, times, delays); or xdc_times_focus (Th, times, delays);`

**Input:**
- `Th` Pointer to the transducer aperture.
- `times` Time after which the associated focus is valid.
- `delays` Delay values. Matrix with one row for each time value and a number of columns equal to the number of physical elements in the aperture.

**Output:** none.

---

**Field II user's guide**  

**xdc_2d_array**

**Purpose:** Procedure for creating a two-dimensional (sparse) array aperture.

**Calling:** `Th = xdc_2d_array (no_ele_x, no_ele_y, width, height, kerf_x, kerf_y, enabled, no_sub_x, no_sub_y, focus);`

**Input:**
- `no_ele_x` Number of physical elements in x-direction.
- `no_ele_y` Number of physical elements in y-direction.
- `width` Width in x-direction of elements.
- `height` Width in y-direction of elements.
- `kerf_x` Distance in x-direction between elements.
- `kerf_y` Distance in y-direction between elements.
- `enabled` Matrix of size `(no_ele_x, no_ele_y)` indicating whether the physical element is used. A 1 indicates an enabled element and zero that it is not. `enable(1,1)` determines the state of the lower left element of the transducer when seen in the x – y plane.
- `no_sub_x` Number of sub-divisions in x-direction of elements.
- `no_sub_y` Number of sub-divisions in y-direction of elements.
- `focus[]` Fixed focus for array `(x, y, z)`. Vector with three elements.

**Output:** `Th` A pointer to this transducer aperture.
**Field II user’s guide**

**ele_apodization**

**Purpose:** Procedure for setting the apodization of individual mathematical elements making up the transducer. This apodization is also multiplied onto the spatial impulse response for the mathematical element regardless of the value of the apodization of the physical element and its dynamic apodization.

**Calling:** `ele_apodization (Th, element_no, apo);`

**Input:**
- `Th` Pointer to the transducer aperture.
- `element_no` Column vector with one integer for each physical element to set apodization for.
- `apo` Apodization values. Matrix with one row for each physical element and a number of columns equal to the number of mathematical elements in the aperture.

**Output:** none.

**Field II user’s guide**

**ele_delay**

**Purpose:** Procedure for setting the delay of individual mathematical elements making up the transducer. This can be used to model a fixed lens in front of the aperture.

**Calling:** `ele_delay (Th, element_no, delays);`

**Input:**
- `Th` Pointer to the transducer aperture.
- `element_no` Column vector with one integer for each physical element to set delay for.
- `delays` Delay values. Matrix with one row for each physical element and a number of columns equal to the number of mathematical elements in the aperture.

**Output:** none.

**Field II user’s guide**

**calc_h**

**Purpose:** Procedure for calculating the spatial impulse response for an aperture.

**Calling:** `[h, start_time] = calc_h(Th, points);`

**Input:**
- `Th` Pointer to the transducer aperture.
- `points` Field points. Vector with three columns `(x, y, z)` and one row for each field point.

**Output:**
- `h` Spatial impulse response in m/s.
- `start_time` The time for the first sample in h.
**Field II user’s guide**

**calc_hhp**

**Purpose:** Procedure for calculating the pulse echo field.

**Calling:** \[ \text{hp, start\_time} = \text{calc\_hhp(Th1, Th2, points)}; \]

**Input:**
- \( \text{Th1} \) Pointer to the transmit aperture.
- \( \text{Th2} \) Pointer to the receive aperture.
- \( \text{points} \) Field points. Vector with three columns \((x,y,z)\) and one row for each field point.

**Output:**
- \( \text{hp} \) Received voltage trace.
- \( \text{start\_time} \) The time for the first sample in \( \text{hp} \).

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**Field II user’s guide**

**calc_hp**

**Purpose:** Procedure for calculating the emitted field.

**Calling:** \[ \text{hp, start\_time} = \text{calc\_hp(Th, points)}; \]

**Input:**
- \( \text{Th} \) Pointer to the transmit aperture.
- \( \text{points} \) Field points. Vector with three columns \((x,y,z)\) and one row for each field point.

**Output:**
- \( \text{hp} \) Emitted pressure field.
- \( \text{start\_time} \) The time for the first sample in field.
**Field II user's guide**

### `calc_scat`

**Purpose:** Procedure for calculating the received signal from a collection of scatterers.

**Calling:** `[scat, start_time] = calc_scat(Th1, Th2, points, amplitudes);`

**Input:**
- `Th1`: Pointer to the transmit aperture.
- `Th2`: Pointer to the receive aperture.
- `points`: Scatters. Vector with three columns (x,y,z) and one row for each scatterer.
- `amplitudes`: Scattering amplitudes. Row vector with one entry for each scatterer.

**Output:**
- `scat`: Received voltage trace.
- `start_time`: The time for the first sample in `scat`.

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### `calc_scat_all`

**Purpose:** Procedure for calculating the received signal from a collection of scatterers and for each combination of transmit and receive elements in the aperture. This corresponds to a full synthetic aperture scan, with each element transmitting and all elements receiving. Note that the raw data is calculated. No focusing or apodization is employed on the data and this has to be done on the data afterwards.

Note that this routine can give a lot of data, when many elements are used. A 32 elements transducer gives 1024 signals. The data can therefore be decimated after calculation of the response. This still gives exactly the same response, but with fewer samples in the result. It just has to be ensured that the decimated sampling frequency (`fs/dec_factor`) is large enough to not give aliasing in the response.

**Calling:** `[scat, start_time] = calc_scat_all (Th1, Th2, points, amplitudes, dec_factor);`

**Input:**
- `Th1`: Pointer to the transmit aperture.
- `Th2`: Pointer to the receive aperture.
- `points`: Scatters. Vector with three columns (x,y,z) and one row for each scatterer.
- `dec_factor`: Decimation factor for the output sampling rate. The sampling frequency is then `fs/dec_factor`, where `fs` is the sampling frequency set in the program. The factor must be an integer.
- `amplitudes`: Scattering amplitudes. Row vector with one entry for each scatterer.

**Output:**
- `scat`: Received voltage trace. The matrix is organized with one received signal for each receiving element and this is repeated for all transmitting element, so the first signal is transmitting with element one and receiving with element one. The transmitting with element one receiving with element two and so forth. The it is repeated with transmitting element 2, etc.
- `start_time`: The time for the first sample in `scat`. 
Field II user’s guide

Purpose: Procedure for calculating the received signal from a collection of scatterers and for each of the elements in the receiving aperture.

Calling: \[ \text{[scat, start\_time]} = \text{calc\_scat\_multi}(\text{Th1, Th2, points, amplitudes}); \]

Input:
- \text{Th1} Pointer to the transmit aperture.
- \text{Th2} Pointer to the receive aperture.
- \text{points} Scatterers. Vector with three columns \((x, y, z)\) and one row for each scatterer.
- \text{amplitudes} Scattering amplitudes. Row vector with one entry for each scatterer.

Output:
- \text{scat} Received voltage trace. One signal for each physical element in the receiving aperture.
- \text{start\_time} The time for the first sample in \text{scat}. 


VITA

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Yada Juntarapaso was born in Chumphon, Thailand. She received her Bachelor of Engineering degree in Electrical Engineering from King Mongkut’s University of Technology Thonburi, Thailand in 2002. Her bachelor’s thesis was “Surge protection in low voltage system.” After college graduation, she obtained an honor scholarship from Ministry of Science and Technology of Thailand to pursue Ph.D. study in the United States. She joined The Graduate Program in Acoustics at The Pennsylvania State University in 2004 to earn her Ph.D. degree. Her research involves with developing computer simulation programs for ultrasonic transducers designs for high frequency scanning acoustic microscopy (SAM) with biomedical applications and imaging. Her research interests are in the area of ultrasound transducers and acoustical imaging and simulations.