ULTRASOUND SPECTRAL PARAMETERS OF MICRO- AND NANO- PARTICLES:
MEASUREMENT SOFTWARE AND MODELING

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ABSTRACT

Ultrasound tissue characterization offers information of potential diagnostic value which is unavailable to human viewers of B-mode images. Ultrasonic spectral parameters are obtained from frequency analysis of radiofrequency (RF) signals. Spectral slope (m), midband fit (M), and intercept (I) (measured in dB/Hz, dB and dB respectively) are used to characterize tissues.

The objectives of this study are: to create a Graphic User Interface (GUI) for measuring ultrasonic spectral parameters, interface the software with a high frequency ultrasound (HFUS) imaging system, and compare analysis of scattering samples gathered with the software to predictions from an established model. Software was created in LabVIEW to measure spectral parameters, which also interfaced with the Vevo660 HFUS imaging system (Visualsonics, Toronto). Additional software was created to predict spectral parameters for test samples. Values for m, M and I were predicted theoretically and measured experimentally, and compared. Goodness of fit (R$^2$) was used as a metric to judge the reliability of the experimental measurement of the three spectral parameters.

Test samples were made by suspending scatterers in 1.5% agarose gel in plastic capsules. Two types of particles were used as scatterers: 1) PLA (poly lactic acid) microparticles (2.0 µm) at concentrations between 1.91E+7 and 9.57E+8 particles / ml; and 2) PS (polystyrene) particles (500 nm) at concentrations between 1.8E+10 and 1.08E+11 particles / ml.

Theoretically predicted midband fits showed the best agreements with measured values when adjusted by a clumping factor (3.5 for PLA, 2.9 for PS). This factor adjusted the effective scatterer radius to account for the possible aggregation of particles during gel preparation. Predicted results for spectral slope and intercepts were also improved when the clumping factor was applied.

In this study, software that enables ultrasonic data acquisition and analysis based on either bench
top setups or HFUS imaging systems was successfully created. This software provides convenient interface for further studies of developing nanoparticles or microparticles as targeted ultrasound contrast agents, or in vivo tissue characterization.
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CHAPTER 1

INTRODUCTION

1.1 Background

Ultrasound contrast agents have been in clinical use since the first commercial agent, Albunex. These agents enhance image contrast such that anatomical structures can be more easily detected in ultrasound scans. For example, microbubble ultrasound contrast agent may be administered before echocardiography to enhance blood-tissue contrast. The effectiveness of contrast agents is typically measured by image enhancement, as perceived by human viewers.

Spectral parameters derived from ultrasonic radiofrequency data are used for tissue characterization with potential to diagnose cancer or other diseases based on the physical properties of tissue. These parameters are obtained from analysis done by computer algorithms, and may offer additional diagnostic information not available through human observation of images. Compared with healthy tissue, cellular microstructures in cancerous tissue have different physical characteristics. These changes are reflected in their ultrasound backscatter, which can be quantified by spectral parameters. Targeted ultrasound contrast agents may also change tissue spectral parameters in addition to the appearance of B-mode images.

The goal of this study is to create and evaluate a spectral parameter-based method which can be applied to detecting the influence of ultrasound scattering substances. To accomplish these goals, a graphic user interface (GUI) was created and interfaced with a high frequency ultrasound imaging system. The software was used for RF data acquisition and spectral parameter analysis of micro- and nano- particle suspensions in agarose gel in this study to demonstrate its application.
1.2 Research Objectives

This study contains three objectives:

Objective 1: create a GUI allowing the user to acquire ultrasonic radiofrequency data, and to measure spectral parameters.

Objective 2: interface a high frequency ultrasound (HFUS) system with the GUI.

Objective 3: analyze data acquired from micro- and nanoparticle suspensions using the GUI, and compare the measured spectral parameters to model prediction.

This GUI developed in this study allows the measurement and recording of spectral parameters from any ultrasound RF source. This software also allows interfacing with a high frequency ultrasound system (Vevo 660, VisualSonics, Inc.), and accepts input from any A-mode source. The software developed to accomplish these goals was used to test the predictions based on a well accepted model that estimates the spectral parameters from subresolution scatterers (Lizzi et al 1996).

For the second objective, a commercially available high frequency ultrasound system was interfaced with the GUI. The Visual Sonics Vevo660 system operates with interchangeable scanheads, enabling the use of transducers operating at different frequencies. This system also made it possible to record images of test samples.

To accomplish the third goal, changes in spectral parameters were predicted using a Matlab simulation based on the Lizzi model. Samples containing varying concentrations of PLA particles were prepared in agarose gel and scanned with the system.

1.3 Significance and outline

Significance:

We hypothesized that adding particles to gel capsules would change the overall spectral parameters in such a way that could be predicted with an existing model. This would simulate the effect of adding contrast agent to biological tissues. Software developed in this study has potential application to targeted contrast agent studies, since spectral parameters might provide additional objective measures of
tissue properties. For example, while a targeted contrast agent may not accumulate in sufficient quantities to produce a detectable change in contrast, their presence may be detected through spectral parameters.

Outline:

The background and history of contrast agents are described in Chapter 2, to provide the literature information of current methods. Tissue characterization methods and spectral parameter analysis are presented, along with their respective merits and limitations. Chapter 3 describes in detail the development and structure of the scanning software, including recommend operation procedures. The theoretical model developed by Lizzi et al is adopted for theoretical predications of the spectral parameters of micro- and nanoparticle gel suspensions. The restrictions and applicability of this model are described. The experimental results and their analysis are discussed in Chapter 4. The theoretical predications based on Lizzi model were compared to those obtained experimentally from samples using a high frequency ultrasound imaging system. The confounding factors in this study are discussed in Chapter 4.
CHAPTER 2

LITERATURE REVIEW

2.1 Ultrasound tissue characterization

The aim of ultrasound tissue characterization is to quantify the nature of scattering from tissues and provide additional diagnostic information. The goal is to detect features that may not be immediately visible in images by applying characterizing methods. Two main forms are intensity based and frequency based (spectral) methods. The following methods are generally applied to data extracted from images unless stated otherwise. In most cases where entire images are used as the source data, a region of interest (ROI) must be extracted from the main image and rearranged before the characterizing algorithm can be applied. Both spectral and non-spectral methods may require calibration steps to remove effects of the scanning system and transducer before analysis.

Tissue characterization allows the study and quantification of ensembles of cells that are not individually resolvable but produce variation in signals as a group. In ultrasound, scatterers such as cells, microbubbles and nanoparticles are not resolvable due to large differences between scanning wavelength and scatterer diameter. Higher resolution imaging methods may allow imaging of larger individual scatterers, but not their general distribution. To address these problems, tissue characterization methods are used to quantify the nature of scatterer groups.

2.1.1 Intensity-based methods

Intensity-based methods of ultrasound characterization are a function of reflected or transmitted pulse amplitudes from tissues. Two main non-spectral methods of analysis are backscatter and attenuation
coefficient. Digitized images expand the non-spectral options to texture and grayscale analysis. While intensity-based methods offer speed and simplicity, they may be sensitive to error from misalignment.

**Analog signal methods**

Analog signal methods do not require an ultrasound signal to be digitized before analysis. The two methods are backscatter coefficient and attenuation coefficient. Backscatter coefficient measures the ratio of energy sent to a sample that is returned as backscatter. Typically, one transducer acts as both transmitter and receiver. The sample is placed in an immersion bath at the focal range of the transducer, and oriented for maximum echo. The original standard substitution method for planar transducers was developed by (Sigelmann and Reid 1973), and later a modified standard substitution method was developed by (Wang and Shung 1997) for use with focused transducer.

Measuring attenuation coefficient requires two transducers to measure the amount of sound energy transmitted through a sample. The sample is placed between two transducers in a water bath during measurement. Here, one transducer acts as the transmitter while another acts as the receiver.

**Pixel - based methods**

Digital imaging allows pixel value comparison, since intensity values are stored as numbers in an array to represent images. Averaging these grayscale values and comparing their mean value between images is one way of measuring intensity differences between images. This can provide a quantitative measure of the average image brightness, and has been used in measuring the effects of contrast agents (Liu et al 2006). This method is especially useful in B-mode ultrasound images, since each pixel can be represented by a single number.

Texture measurement methods also take advantage of the quantified grayscale levels in images. Creating a histogram of grayscale levels or computing the skewness, kurtosis, dispersion or variance are statistical methods possible with digitized data (Zhang et al 1998).

**2.1.2 Spectral methods**

Spectral methods rely on information obtained by Fourier transforming signals reflected or transmitted through tissues. Spectral characterization methods always involve transforming time-domain
signals into frequency components with an FFT. These methods may be more robust to noise, in addition to providing information not visible to human viewers.

Integrated backscatter is a function of the overall energy contained in a signal over a selected frequency range. Measurement involves first measuring the backscatter signal from a sample, performing an FFT, and then integrating over a selected frequency range. It has been used in liver preservation damage (Vlad et al 2005) and heart tissue (Wu et al 2000) studies.

Spectral parameters are the slope, midband value and intercept (m, M and I respectively) of a calibrated backscatter spectrum. Figure 2.1 demonstrates calculating spectral parameters from an arbitrary FFT. They are intended to provide an index or quantitative measurements of FFT data which can be used to quickly compare sets of raw data. R² is also returned by spectral analysis, although it is not used in characterizing tissues. Instead, it provides a reliability and precision metric for the other variables.

Having high R² values is important to ensure precision, since a data set returning low R² has uncertain spectral parameters. Allowing a reasonable number of samples for linear analysis and choosing relatively low-noise regions of spectrum for analysis tends to raise R². Values for R² in the context of spectral analysis will not be at the same level as for other forms of analysis. When analyzing groups of stochastic scatterers, low R² values are expected. Other studies have not reported R² values.

![Diagram of spectral parameters](image)

**Figure 2.1.** Explanation of spectral parameters. Based on (Lizzi et al 1976)
First, ultrasound backscatter is first obtained using a similar setup as for recording backscatter coefficient. Another source is m-mode data, or individual scan lines extracted from b-mode images. This data is then transformed to the frequency domain through FFT and plotted on a log scale. Next, a best-fit line is calculated for the data within a selected frequency range ($f_1$ and $f_2$), inside the -6 dB bandwidth of the transducer. The spectral slope ($m$) is the increase or decrease in amplitude divided by the frequency range. The midband value, or midband fit ($M$) is the value of the best-fit line evaluated at the frequency between $f_1$ and $f_2$. Spectral intercept ($I$) is the value of the best-fit line extrapolated to 0 Hz.

Initially, spectral parameters were designed as a tissue characterization method for use on eye and orbit tissue (Lizzi et al 1976). In that study, spectral fit and absolute reflectance were returned. This required computer post-analysis of strip chart records. With analysis bandwidth centered around $f_c$, absolute reflectance would be equivalent to midband fit as previously defined.

Previously, spectral parameters have been investigated as a method of diagnosing cancers of the breast (Tateishi et al 1998) and prostate (Feleppa et al 1995, Feleppa et al 1996, Feleppa et al 1997, Scheipers et al 2004). Other studies tracked the effects of apoptosis (Czarnota et al 1999, Kolios et al 2001, Baddour et al 2002, Kolios et al 2002, Kolios et al 2003, Tunis et al 2005) or liver tissue damage during preservation (Vlad et al 2005) on one or more of these spectral parameters. In all of those cases, changes in cell size or structure were hypothesized to change spectral parameters. In cancer diagnosis, differences in spectral parameters were hypothesized to reflect cell morphology differences.

The Lizzi model has also been used inversely to predict scatterer size based on spectral parameters (Kolios et al 2002). When used inversely, the spectral parameters of cells treated with chemotherapeutic drugs were measured and compared to controls. By comparing the measured parameters to those predicted by the Lizzi model, effective scatterer radii were inferred. In this way, the model was used inversely to predict a physical measure instead of being used as a comparison metric.
2.2 Microbubbles and nanoparticles

Microbubbles are the only FDA approved ultrasound contrast agents. New imaging agents that are effective and capable of disease targeting are highly desirable. This section reviews the materials used to build, coat or target particles, along with their applications. Albunex, the first commercially available contrast agent was microbubble-based, and constructed of sonicated human serum albumin (Lindner et al 2004). Sonicating shell-forming materials in the presence of inert gases has remained a widespread synthesis method. It has also been successfully used as a method of forming nanoparticles. The main two applications of microbubbles and nanoparticles, ultrasound contrast enhancement and substance delivery, are also discussed.

2.2.1 Structural and coating components

Resorbable materials are a common choice for microparticles and nanoparticles. These include the polymers poly caprolactone, PLA (poly lactic acid), PGA (poly glycolic acid) and a copolymer of lactic and glycolic acids, PLGA. In these last three polymers, acid monomers are covalently bonded together in chains. The copolymer PLGA is approved by the FDA as an implantable biomaterial. Proteins are also used as shell-forming agents, as in the example of Albunex. Biodegradable polymers and proteins avoid accumulation or toxicity problems by slowly degrading into benign substances.

Another advantage of PLA, PGA and its copolymers is the option of conjugating amines to its carboxyl end group with the EDC-NHS (dimethylaminopropyl carbodiimide / N-hydroxsucinimide) reaction. This reaction has been used to conjugate targeting ligands (Farokhzad et al 2004, Lathia et al 2004, Liang et al 2006) to the surfaces of particles.

Polystyrene (PS), a non-resorbable polymer, has also been investigated as a structural material for solid nanoparticles but has two major disadvantages. First, PS particles are rapidly removed from the bloodstream by the liver due to their hydrophobicity. Although coating PS particles with hydrophilic polymers has been attempted (Dunn et al 1994), the particles continued to be removed from the bloodstream and accumulated in the liver.
In an optoacoustic imaging study, gold was conjugated with Herceptin to produce a targeted contrast agent for HER2/neu positive cells (Copland et al 2004). Like PS particles, all inert particles lack the ability to be resorbed, which may lead to undesired effects. It also eliminates the possibility of creating time-release delivery agents that rely on carrier degradation.

When non-resorbable materials are used, surface modification is the only applicable technique for either drug delivery or contrast enhancement. Due to the differences between total volume and surface area, emulsifying or blending the active agent with a targeted nanoparticle also allows a larger dose per particle to be delivered than if the agent was only conjugated to the surface. Biodegradable polymers allow time-release of active agents, since a polymer blended with active agent will release the active agent while degrading. For these reasons, inert particles are generally limited to in-vitro experiments.

**Emulsifiers and other components**

The solvent-evaporation method is the most common method for forming nanoparticles. This process requires emulsifiers and a solvent in addition to the structural polymer. Emulsifiers are used in the formulation of nanoparticles in order to ensure binding of structural components and distribution of active ingredients. They also make smaller nanoparticle size possible (Dong and Feng 2005), which is important for reducing their vulnerability to the immune system. Longer circulation time makes smaller particle size advantageous. Examples of emulsifiers are poly vinyl alcohol (PVA) (Dong and Feng 2005, Kumar et al 2004) and montmorillonite clay (MMT) (Dong and Feng 2005, Yuancui and Feng 2005). Acetone is widely used as a solvent in the solvent-evaporation method (Venkatraman et al 2005).

When used as a bubble component, the blood protein albumin provides the stability of microbubbles (Lathia et al 2004). Albunex is the first commercially available microbubble contrast agent made from sonicated albumin (Lathia et al 2004). Phospholipids are also used as encapsulating agents for microbubbles. They have been produced by agitating dissolved coating material by vigorous shaking or sonicating (Moran et al 2006) or with a homogenizer (Kumar et al 2004).

**Coating agents**

Smaller particles are less likely to be removed, and particles smaller than 250 nm in diameter avoid pulmonary entrapment (Dayton and Ferrara 2002). Aggregation is a common problem in nanoparticle
synthesis, producing clumped instead of distinct particles which are removed more readily from the bloodstream by the immune system. Creating contrast agents from particles smaller than this threshold increases their circulation time in the bloodstream. Coating the particles with PEG (polyethylene glycol) helps to make them more hydrophilic, and less likely to be attacked by the immune system (Gref et al 1995).

2.2.2 Targeting

The goal of all targeted contrast agents is to increase the concentration of a contrast-enhancing substance near specific cells or tissues. Two approaches have been used in making targeted microbubbles for contrast imaging. The first is to select lipids or membrane forming materials that preferentially adhere to diseased cells, such as phospholipids that selectively adhere to disease, and the second is to bind ligands such as monoclonal antibodies or peptides to the surface of the microbubble which will bind to matching ligands on disease cells (Lindner “Molecular” 2004).

Targeted contrast agents are designed to accumulate near a specific organ or tissue type, and change the echogenicity of these tissues. The goal is to have a tissue with many of the contrast bubbles surrounding it, so that the echogenicity of the targeted tissues will be much different than non-targeted tissues. While non-targeted ultrasound contrast agents are used to enhance imaging of blood vessels or highly vascular tissues, targeted agents are designed to selectively accumulate near specific tissues. This is accomplished through attaching ligands to the surfaces of microbubbles. In this strategy, the ligands attached to the microbubbles bind with other ligands present on the surface of the targeted tissues. For example, an antibody to a specific membrane protein may be bonded to the microbubble surface so that it will selectively adhere to cells expressing the corresponding antigen.

Doxil (doxorubicin) is an anticancer drug, which is delivered as an active agent encapsulated within liposomes. While not conjugated with a specific antigen that targets it towards cancer cells, passive targeting through size allows Doxil to become more concentrated near tumor cells (Freakel et al 2006).

Targeted agents have also been applied therapeutically. in this approach, the hybrid particles are used for both targeting and drug delivery, which are released during sonication (Bloch et al 2004). Since microbubbles can be destroyed by subjecting them to high amounts of acoustic energy, this opens the
possibility of drug delivery. First, microbubbles containing therapeutic agent are targeted to tissues through either passive or active targeting. After they have been administered and given time to concentrate around the selected tissues, they are exposed to ultrasound energy which ruptures them, releasing the therapeutic agent in specific areas. In one study, pulsed high intensity focused ultrasound exposure did not appear to enhance delivery of Doxil-containing liposomes (Frenkel et al 2006).

Approaches to targeting include both passive and active targeting (Kim and Nie 2005). EPR (enhanced permeation and retention) effect, tumor environment and direct local delivery are three examples of passive targeting mechanisms (Kim and Nie 2005). Lectin-carbohydrate, ligand-receptor and antibody-antigen interactions are three forms of active targeting mechanisms (Kim and Nie 2005). The nanoparticles in this study rely on the last form, antibody-antigen interaction between Herceptin and the HER-2 receptor.

The effectiveness of contrast agents can be evaluated with in vitro and in vivo tests. In vivo scans directly test how agents affect images and signals from test subjects. Here, contrast agent is administered to a living organism, and scanned to detect changes in contrast. Mouse anatomy is convenient for the study of nanoparticle effects on liver US contrast due to their organs being located within millimeters of the surface. Their low mass and body volume also make delivery of high doses possible.

In vitro tests require exposing a cell culture to contrast agent and then scanning for changes. Cells are first cultured, and then prepared as small samples through either centrifuge pelleting or mixing with ultrasonically transparent material. Higher numbers of cells are needed when scanning cells in pellet form, since centrifuging packs the cells into a small volume. This leads to a smaller possible gate length for analysis, and less data for analysis.

Another option for exposing cells to agent is the parallel plate perfusion chamber, where culture medium is flowed through a small area between parallel glass plates by a syringe pump (Villanueva et al 1998). Conditions as found in blood vessels are more closely simulated by flowing culture medium than static fluids. While this setup exposes cells to conditions more similar to those found in vivo, it does not provide a large enough sample of exposed cells for ultrasound scanning.

The drawbacks of in-vitro tests are that the cells are being scanned in a configuration different from their natural arrangement. The density of cells per volume may be much higher or lower than their
natural state as a tissue. When scanning cells in gel, spaces between the cells are filled with ultrasonically transparent material, and so will be at a lower concentration than they would be in tissue. Pelleted cells may be at an artificially higher volume concentration. Any dissociation agents such as trypsin may have induced structural changes in the cells. Another drawback is that tissues may include a combination of cells, some of which are missing when the cells are scanned as a monoculture.

Alternative methods exist for characterizing nanoparticles or microbubbles experimentally after synthesis. These are sometimes used as a substitute or in conjunction with ultrasonography. Microscopy provides size and texture information on particles. Light microscopy is only appropriate for particles larger than 1 μm since the wavelength of light cannot resolve structures smaller than this size. The advantage of light microscopy is that living cells can be imaged. The relative abundance of observed particles can also be an indicator of their targeting ability. Electron microscopy allows structures much smaller than 1 μm to be resolved. However, its usefulness is limited in cases where living cells are to be imaged. Fixing methods for electron microscopy are harsh and limit samples to fixed cells or nonliving structures.

2.2.3 Applications

Nanoparticles and microbubbles have been applied as both contrast and delivery agents. Contrast agents are used during clinical or experimental imaging to enhance the visibility of structures in imaging. This allows selected structures to be separated from surrounding tissues more effectively when enhanced diagnostic ability is required. Delivery agents allow substances that would ordinarily be excluded from cells through natural mechanisms. It also allows toxic substances which can easily enter tissues to be more precisely delivered to specific tissues and in smaller doses, rather than administering a large dose which reaches all tissues evenly. Acoustically reflective liposomes have also been tested as an atherosclerosis targeting agent (Bloch et al 2004).

Due to their echogenicity, both microbubbles and nanoparticles can be used as ultrasound contrast agents. As untargeted contrast agents, microbubbles have already been in clinical use since Albunex (Lathia et al 2004). Untargeted contrast agents are currently used in clinical diagnostic tests. Doppler flow ultrasound and echocardiography determine the degree of blockage affecting heart vessels. Contrast agents in these scans enhance the visibility of blood flowing in these vessels, which helps to reveal blockage.
Stress tests involve either exercising the patient or administering an inotropic agent to increase the cardiac output of the heart. During a stress test with echocardiography, the contrast agent is administered intravenously shortly before the scan.

While clinical contrast agents are either microbubbles or liposomes, nanoparticles have also shown the ability to enhance contrast (Liu et al 2006). Compared to microbubbles, nanoparticles are more resilient, since they are homogenous and lack the gas-filled space of microbubbles. Without a hollow cavity, collapse is not a risk. Larger agents are more echogenic, but an upper size limit exists since agents must be small enough to pass through capillaries without impeding flow (Lindner “Microbubbles” 2004).

Other studies have used targeted nanoparticles as carriers for chemotherapy drugs, most commonly Taxol (paclitaxel) (Huang and Feng 2000, Venkatraman et al 2005, Dong and Feng 2005, Zhang and Feng 2006, Kang et al 2004) and 5-FU (5-fluorouracil) (Venkatraman et al 2005). To form delivery agents, drugs are combined within the material of particles, or conjugated to their surfaces.
CHAPTER 3

MATERIALS AND METHODS

3.1 Data acquisition software development

Custom software was written to collect and analyze data for this study. This software allows spectral analysis and recording of data from an arbitrary source with a triggering pulse. For this study, all data was recorded with the Visual Sonics 660 high frequency ultrasound (HFUS) system. The following explains user operation along with software development and structure of the program. An alternative set of acquisition hardware is also discussed.

This study required a method of ultrasonically characterizing and detecting the effects of scatterers dispersed in agarose gel samples. The tissue characterization method selected for scatterer samples in this study needed several characteristics. First, it had to be resistant to small variations in transducer alignment. Secondly, it needed to be computationally efficient enough to quickly calculate results from data after acquisition. A third desired quality was for the method to use available hardware. Spectral analysis offered to fulfill these three requirements.

3.1.1 Interface and operation

"US data acquisition with calibration.vi" is a graphical user interface (GUI) providing signal acquisition, spectral analysis, and data recording. It automates analysis of ultrasound spectral analysis according to a previously published protocol (Lizzi et al 1983). A screenshot of the user interface is shown in figure A.1. The program interface shows four windows on the screen: one on the left and three aligned vertically on the right. The left window is used in both oscilloscope and spectral averaging modes to display the signal as it is acquired live. Above the left window are acquisition parameters (sampling
interval, triggering settings and delay time). Directly below the left window is a panel showing the two
cursor positions, which are used to gate time domain data. A numeric indicator near the lower right corner
of this panel shows the ROI length. Further below the left window is a control for the number of averages,
mode control buttons and spectral parameter indicators grouped within a perimeter.

On the right, beginning from the top of the screen are three windows showing the ROI / FFT of
ROI, calibration spectrum, and calibrated spectra. The top right window serves two purposes: displaying
gated ROI as it is acquired in time during oscilloscope mode, and showing the current uncalibrated
spectrum in spectral averaging mode. The middle right window shows the calibration spectrum, which is
subtracted from the current FFT ROI. The lower right window shows the calibrated spectra, which is the
result of subtracting the calibration spectrum from the current uncalibrated spectrum. Between the windows
for calibration spectra and calibrated spectra are two control buttons that allow the user to either reset the
calibration spectrum to zero, or set the current spectrum as calibration spectrum.

3.1.2 Program steps

There are two operating modes for the program, oscilloscope and spectral averaging mode. In
oscilloscope mode, the program operates as a software-based oscilloscope, showing data acquired in real
time. Here, acquisition parameters such as number of points acquired, sampling interval and triggering
information can be set. These acquisition parameters are shared by both modes. Data can only be viewed as
it is acquired in oscilloscope mode, it cannot be analyzed or saved. Oscilloscope mode is used mainly for
setting the acquisition parameters and setting the gating bounds on the ROI. Spectral averaging mode
provides the abilities to collect data, perform spectral analysis, and save the results to file.

In spectral averaging mode, the user is given the opportunity to set or reset the calibration
spectrum, change linear regression bounds and record data to file. Setting the calibration spectrum
temporarily saves the current spectrum displayed in the ROI FFT window as the calibration spectrum.
Resetting the calibration spectrum sets all values in the calibration spectrum to zero dB. To change the
linear regression bounds, the user can move cursors on the calibrated spectrum graph, which changes the
results of the regression analysis. The calibration spectrum displayed in the left middle graph persists
through subsequent acquisitions until it is reset or substituted with a different spectrum. The GUI allows
arbitrary spectra to be designated as the calibration spectrum, which is subtracted from the current spectra. Since all spectra are in log dB units, subtraction allows the influence of the calibration spectrum to be removed from test spectra. Had the spectra remained in linear scale, the calibration spectrum would have needed to have been divided.

Spectral averaging mode also allows the user to save analysis results. Information from all three graphs along with the results of linear regression is saved to file when recording data. Each recorded file has five columns of data: the frequency scale, selected ROI's from current FFT, calibration FFT, calibrated FFT, and a combined data column. This last column contains the upper and lower bounds of the analyzed frequency range, slope, intercept, MBF and R². Analysis is not limited to a single frequency range, since the user can reselect frequency ranges and record additional data files during spectral analysis mode. Additionally, the user is not limited to saving data used in analysis. The entire three spectra along with a frequency scale can be recorded by setting the frequency range to its maximum, and recording a file. In this way, data can be saved for analysis by other means outside of the program.

The first step in running an experiment is to place samples in the focal zone of the transducer within the water immersion bath. Next, the program is run and the acquisition parameters are set. External triggering is always set to coordinate data acquisition with pulses sent from either the pulser/receiver or Vevo imaging system. Pulser/receiver settings and specifications are listed in table 3.1.

The Acqiris digitizer cards are capable of sampling at several rates, ranging from their maximum down to 100Hz, multiples of 1, 2, 2.5, 4, and 5 (Rothenberg 2004). While other sampling intervals are available, the shortest sampling interval was set to provide the highest sampling frequency in all experiments. Table 3.2 shows the specifications for both Acqiris cards tested in this study. Data was collected only with the DP310 card.
Table 3.1. Panametrics 5900 PR pulser / receiver specifications

<table>
<thead>
<tr>
<th>Pulser / Receiver Settings (Panametrics 5900 PR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRF pulse repetition frequency</td>
</tr>
<tr>
<td>No-load pulse voltage</td>
</tr>
<tr>
<td>Energy (μJ)</td>
</tr>
<tr>
<td>Damping (Ω)</td>
</tr>
<tr>
<td>HP filter</td>
</tr>
<tr>
<td>LP filter</td>
</tr>
<tr>
<td>Attenuators</td>
</tr>
<tr>
<td>Gain</td>
</tr>
</tbody>
</table>

Table 3.2. Digitizer specifications

<table>
<thead>
<tr>
<th>Digitizers</th>
<th>Acqiris DP105</th>
<th>Acqiris DP310</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximum sampling frequency (MHz)</td>
<td>500</td>
<td>420</td>
</tr>
<tr>
<td>Minimum sampling interval (sec)</td>
<td>2.00E-9</td>
<td>2.38E-9</td>
</tr>
<tr>
<td>Bits</td>
<td>8</td>
<td>12</td>
</tr>
<tr>
<td>Maximum input range</td>
<td>± 5V</td>
<td>± 10V</td>
</tr>
<tr>
<td>Minimum input range</td>
<td>± 50 mV</td>
<td>± 250 mV</td>
</tr>
</tbody>
</table>

Next, a delay can be set to exclude signals occurring before the targeted echoes. To finely select the region of interest (ROI), the two cursors on the leftmost window can be set to exclude segments of the signal occurring slightly before and after the desired range. Next, the desired number of averages is set. Clicking on ‘Averaging mode’ enters the program into spectral averaging mode, and collects the requested number of signals. The average spectrum is now displayed on the top rightmost window as the current spectrum. Since the first spectrum represents the calibration spectrum, the button ‘Set acquired as calibration spectrum’ is clicked to set this spectrum as the calibration spectrum.

After collecting and temporarily storing the calibration spectrum, the flat test reflector is removed and replaced with a test capsule. After realigning the transducer, similar steps are followed. First, the signal is acquired in oscilloscope mode to bring the signals within view as before. Then, the cursors are placed around the desired segment to define the ROI. Now, as before, spectral averaging mode collects the requested number of signals and displays the averaged spectrum in the top right window. The calibrated
spectrum appears on the bottom right graph. To select the desired frequency range, cursors are moved to set the upper and lower frequency bounds. As these cursors are moved to change the frequency range, the updated regression results are shown on the screen in a group of indicators. After setting the desired frequency range, clicking on 'save data' opens a dialog box to save the information to a specified file name. Here, the current, calibration and calibrated spectra are recorded, along with the results of regression analysis. To collect data for more test samples, the same steps are followed. Since the calibration spectrum is shared among all test samples, resetting or changing the calibration spectrum is not necessary. The user must be careful not to reset or change the calibration spectrum during a study, since it needs to be consistent among all test samples. While it is not absolutely necessary to perform these steps in the order shown, this is the general method used in this study.

All development was performed in LabVIEW 8.0 on a 3 GHz desktop PC running Windows XP. LabVIEW is a graphical programming language representing steps and components as interconnected blocks instead of textual commands. Virtual instruments (VI's) are the program equivalent of executable programs. In place of subroutines or include files, VI's can include other VI's as sub-VI's. Appendix A provides the full source code of the GUI, along with source code of sub-VI's. All frames in appendix A were generated automatically by the documentation feature in LabVIEW, and are referred to by their frame numbers. All signal processing, averaging of FFT's, linear regression and data recording takes place on the main program frame, shown in figure A.2.

Many of the later frames belong to "AqDx Example Scope.vi" and are included for completeness. They were not modified during development, but are still necessary for operation. Due to redundancy, some frames have been duplicated.

The purpose of the program is to interface with a data source and perform spectral analysis while simplifying user operation. This is accomplished through a signal processing algorithm, documented graphically in the source code, and procedurally in the list below. These signal processing steps extract spectral parameters from raw ultrasound RF data.
The signal processing steps can be summarized as follows, based on (Lizzi et al 1983):

1. Acquire data as set of lines (signals).
2. Multiply each line by a Hamming window.
3. Perform an FFT on each line.
4. Calculate squared magnitude of each FFT.
5. Average together FFT's to calculate spectrum.
6. Convert to logarithmic scale.
7. Record one set of data from a reflective surface, and set as the calibration spectrum. Subtract this from subsequent test spectra.
8. Perform linear regression to extract spectral parameters.

The first step in spectral averaging mode is to acquire data. First, this is gated according to the position of the two cursors in the left graph. The gated ROI is then buffered to $2^X$ size. This is accomplished by taking the base-two logarithm of the ROI size, and then rounding up to the next whole number. The number two is raised to this rounded number, which provides the end size of the buffer. The remaining elements are added to the ROI to produce a $2^X$ sized buffered array. In effect, the size of the array is being rounded up to the next number in the $2^X$ series, which minimizes any effects of changes in vector length due to ROI sizes between test samples. After buffering, the array is multiplied by a Hamming window, fast Fourier transformed, and the square magnitude is taken. Then, the result is multiplied by a scaling factor of the number of total points divided by the number of valid, original points. Beginning from acquisition, these steps are repeated for each spectrum averaged in the result. These steps are performed to collect both calibration and test spectral data. The result of this averaging represents the average spectrum of the data. From here, the average spectrum is converted to log dB scale by taking the base-ten logarithm.

To calculate the calibrated spectra, the calibration spectrum is subtracted from the current spectrum. This was possible since both spectra were in log units instead of linear scale. Subtracting out the calibration spectrum reveals any differences between test samples when the common components of the test spectrum are removed. The option of resetting the calibration spectrum to zero was included to allow
the option of performing linear regression on uncalibrated spectra. It also provided a method of clearing
confusion in case the calibration spectrum was not needed. While this study did not make use of this
option, it was made available for future studies.

This approach to averaging frequency domain data instead of time domain data has two
advantages. First, it prevents mis-triggered data from influencing the end results. For example, a mis-
triggered echo is slightly offset in time from more accurately triggered echoes. When considering this in the
average of the pulses, it appears as if an additional, smaller echo was present in the results. This noise may
have propagated into the frequency analysis, appearing as changes in frequency. Secondly, it prevents
smoothing effects from time-based averaging from distorting the frequencies of collected data. During
acquisition, high frequency noise in the time domain data may also appear in the FFT. Had time domain
averaging taken place, these components may have been filtered out. By averaging only the frequency-
domain data, all frequencies are available for analysis.

The last step in analysis is linear regression. This is carried out by first selecting a frequency range
on the calibrated spectra to exclude regions of the spectrum not valid for the transducer, or to select linear
regions of the calibrated spectra. This ROI is extracted from the main spectrum, and an x-array containing
the same number of points and ranging from the lower frequency bound to the higher bound is constructed
by a sub-VI. Linear regression is performed on these two arrays, which returns the slope and intercept of
the best fit line passing through their points. Goodness-of-fit analysis is also performed, providing the $R^2$
parameter. To calculate the midband fit (MBF), another parameter provided by spectral analysis, the results
of spectral analysis are used. The midband fit is the value on the regression line in the middle of the
analyzed frequency range. Based on the results of linear regression, equations for MBF are:

\[
\text{MBF} = m \times (f_1 + f_2) / 2 + b, \text{ or}
\]
\[
\text{MBF} = m \times f_c + b
\]

where $m =$ spectral slope, $f_1, f_2 =$ lower and upper frequency bounds, $b =$ spectral intercept, $f_c =$ transducer
center frequency.
After being transformed into log-scale frequency data, spectral parameters were extracted. This involved setting a frequency range corresponding to the sensitive range of the transducer, and performing linear regression on the data there. This returned three parameters: midband fit, slope, and intercept. The midband fit is the value in dB of the linear regression line at the center of the frequency range. The spectral slope is returned in dB / MHz, and represents the change in intensity with respect to frequency. The intercept, in dB, is the value of the regression line extrapolated to zero MHz. The $R^2$ goodness of fit parameter was also considered in the analysis.

Since $R^2$ is a commonly used measure, its application in this study needs clarification. With regard to the calibrated FFT, $R^2$ describes the degree of linearity measured among the points in a frequency range used to return spectral parameters. Here, it reflects the validity of a specific set of spectral parameters and not any predictive ability. In another context, $R^2$ may be used to measure the degree of linearity that spectral parameters have with respect to another variable. In these cases, it is a measure of the predictive ability of a best-fit line.

Collecting data with immersion transducers required only four major electronic components: the PC, digitizer card, pulser/receiver, and transducers. This setup is illustrated in figure 3.2. Analog signals were sampled at 420MHz and 12 bits by an Acqiris DP310 PCI card. This card also received the triggering signals from the pulser/receiver. Data was collected for initial experiments with two transducers. Initially, Panametrics XMS-310 and V316-SU transducers with center frequencies of 11.89 MHz and 20MHz were used. Specifications for these transducers are listed in table 3.4. These were driven by a Panametrics 5900 PR pulser/receiver. However, this setup was substituted with the Visual Sonics Vevo660 imaging system, as illustrated in figure 3.1. The RMV-30B and RMV-608 scanheads were used to collect data in this setup. Table 3.5 shows specifications for both scanheads.
<table>
<thead>
<tr>
<th>Water – gel interface (mm)</th>
<th>30 MHz (RMV-30B)</th>
<th>55 MHz (RMV-608)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transducer focal length (mm)</td>
<td>12.7</td>
<td>4.5</td>
</tr>
<tr>
<td>Gated volume top (mm)</td>
<td>12.2</td>
<td>4.0</td>
</tr>
<tr>
<td>Gated volume bottom (mm)</td>
<td>13.2</td>
<td>5.0</td>
</tr>
</tbody>
</table>

Table 3.3. Gel capsule placement and gating volume dimensions

<table>
<thead>
<tr>
<th>Panametrics immersion transducer specifications</th>
<th>XMS-310</th>
<th>V316-SU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Center frequency (MHz)</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>Aperture diameter (mm)</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Focal length (in.)</td>
<td>(none)</td>
<td>1.00</td>
</tr>
<tr>
<td>Focus shape</td>
<td>Planar (unfocused)</td>
<td>Spherical</td>
</tr>
</tbody>
</table>

Table 3.4. Panametrics immersion transducer specifications

<table>
<thead>
<tr>
<th>Visual Sonics scanhead specifications</th>
<th>RMV-608</th>
<th>RMV-30B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Center frequency</td>
<td>55 MHz</td>
<td>30 MHz</td>
</tr>
<tr>
<td>Aperture radius (a)</td>
<td>0.978 mm (calculated)</td>
<td>3.024 mm (calculated)</td>
</tr>
<tr>
<td>f - number</td>
<td>2.300</td>
<td>2.100</td>
</tr>
<tr>
<td>Focal length</td>
<td>4.5 mm</td>
<td>12.7 mm</td>
</tr>
<tr>
<td>Depth of field</td>
<td>1.5 mm</td>
<td>1.7 mm (30C scanhead)</td>
</tr>
<tr>
<td>-6dB bandwidth</td>
<td>100% (55 MHz)</td>
<td>100% (30 MHz)</td>
</tr>
<tr>
<td></td>
<td>27.5 to 82.5 MHz</td>
<td>15 to 45 MHz</td>
</tr>
</tbody>
</table>

Table 3.5. Visual Sonics scanhead specifications
Figure 3.1. Acquisition with Visual Sonics VeVo660 imaging system

Figure 3.2. Acquisition with immersion transducer

Specific regions of backscatter were analyzed to obtain spectral parameters, as shown in figures 3.6 and 3.7. In order to locate an appropriate segment of backscatter for spectral analysis, its location in
time relative to the main echo needs to be known. The speed of sound through water is approximately 1450 m/s, or 1.450 mm / μs. Considering that an echo returning to the transducer takes twice the amount of time to travel any distance, the effective propagation speed is halved. This gives an approximate propagation velocity of 0.725 mm / μs. With respect to time, the two focal distances of the transducers are:

RMV-608 (55MHz): $4.5 \text{ mm} / (0.725 \text{ mm} / \mu\text{s}) = 6.21 \mu\text{s}$

RMV-30B (30MHz): $12.7 \text{ mm} / (0.725 \text{ mm} / \mu\text{s}) = 17.52 \mu\text{s}$

Capsules were placed at a consistent distance from the transducer when scanning to prevent variations due to in scanning intensity. The gated volume of each sample was spaced a constant distance from the transducer aperture, as shown in table 3.3. The water-gel interface was placed slightly before the transducer focal length, and the gated volume was centered about the focal length. After buffering, each FFT had 1024 samples.

![Image of ultrasound transducer](image_url)

**Figure 3.3.** Ultrasound transparency of gel-only capsule at 30 MHz
Figure 3.4. Visual Sonics b-mode image of PLA scatterer capsule, 30 MHz

Figure 3.5. Visual Sonics m-mode image of PLA scatterer capsule, 30 MHz
Figure 3.6. Example analysis of PLA backscatter, expanded view

Figure 3.7. Example analysis of PLA backscatter with ROI selection
Figure 3.8. Example analysis of PLA scatterer spectra, expanded view

Figure 3.9. Example analysis of PLA scatterer spectra, analysis range
After being transformed to frequency domain data, the spectra were analyzed over the entire transducer bandwidth. Both RMV scanheads had 100% bandwidth about their center frequencies. These are listed in table 3.5. For the RMV-30B (30MHz) transducer, this range was 15-45 MHz. The -6 dB bandwidth of the RMV-608 transducer was 27.5-82.5MHz. Figure 3.9 shows an example of linear regression performed on data collected with the 30 MHz probe over its -6 dB bandwidth. This figure also demonstrates the spectral noise present in many of the calibrated spectra, which is suspected to be the main cause of error in this study. Chapter 4.3 discusses this in further detail.

3.1.3 Subprograms and borrowed components

Four subprograms (sub-VI's) were written and used in the main program. These are shown in appendix A, in figures A.46 through A.49. All four of these sub-VI's were written to avoid clutter on the main program frame. "Hamming, fft, mag, scaling.vi" performs signal processing steps on each set of time domain data, preparing them to be averaged. Two similar sub-VI's, "subarray 1D.vi" and "subarray 2D.vi", extract subarrays from larger 1D and 2D arrays, respectively. They are implemented on the main program frame in figure A.2. "X_array_maker.vi" creates an array of arithmetically progressing numbers used as frequency values in linear regression. Placing the blocks for these subroutines helps to organize the program.

"Subarray 1D.vi" accepts the arguments of array, point one, point two and sampling interval. This sub-VI first sorts point one and point two according to higher and lower values, in case the cursor positions have been reversed. After being sorted, these were divided by the sampling interval to find the numerical indices of the range. Then, the subarray between these indices is selected and extracted from the main array and returned. The upper and lower limits are also returned as output. "Subarray 2D.vi" works in a similar manner, returning a 1D subarray along with upper and lower limits. "Subarray 2D.vi" selects a 2D subarray from an initial 2D array. The operation is similar since there is useful information in only one dimension of the initial 2D array. "Subarray 2D.vi" also accepts an offset value, in case there has been an index offset added to the graph.

"Hamming, fft, mag, scaling.vi" accepts the main array to be processed, along with the original number of valid points before they have been added to a $2^x$-sized buffer. The first step is to multiply the
incoming array by a Hamming window. Next, an FFT is performed on the array, and take the squared magnitude. Components for the Hamming window, FFT and complex number manipulation are included in the LabVIEW development environment. Last, the array is multiplied by a scaling factor. This factor is the number of total points divided by the number of valid, original points. For example, if the accepted array has 1024 points \(2^{10}\) points, and the original ROI had 962 points, the scaling factor would be \(1024 / 962\), or 1.064. The array is returned after performing these signal processing steps.

"X_array-maker.vi" creates a 1D array of arithmetically progressing numbers. This provides the X-array required for the linear regression step which occurs in spectral averaging mode. Since the user can define the range of analysis, a new array must be defined for each analysis. This sub-VI accepts three parameters: number of values, lower value, and upper value. An array with the appropriate number of values ranging from the lower to upper values is returned. For example, if the lower and upper values are 26 and 42, and the number of values is 5, the result is 26 30 34 38 24.

All interfaces between the PC and Acqiris card are originally written by Acqiris, and are borrowed from "AqDx.lib." The main program interface and controls for acquisition parameters were borrowed from "AqDx Example Scope.vi." Sub-VI's providing array manipulation, fast Fourier transforms (FFT), Hamming windowing, linear regression, and goodness of fit calculations are originally included in the LabVIEW development environment. A complete list of all program frames are shown in figures A.2 through A.44, and sub-VI's are shown in figures A.46 through A.48. Figure A.45 shows a hierarchy describing the organization of all included sub-VI's.

LabVIEW was a good choice for several reasons. First, it allowed the precision calculations necessary for calculating spectral parameters. Secondly, an example interface between digitizer and computer was already available, which was incorporated into the overall design. The interface between digitizer and computer would have required additional coding had another environment been chosen. Lastly, LabVIEW already had data recording capability.

3.2 Simulation model

Software based on the Lizzi model was created in Matlab to simulate experimental conditions. Modeling software was based on the model equations shown in equations 1 – 8. A theoretical model
predicting the parameters spectral slope, midband value and intercept (m, M and I) from physical characteristics of scatterers is described in (Lizzi et al 1996). The Lizzi model is valid for data collected with a focused transducer, when the scanned volume is positioned within the focal plane of the transducer as shown in figures 3.3, 3.4, and 3.5.

Where:
- \( R \) = distance from transducer aperture to gated volume (mm)
- \( L \) = gated length of scatterer volume (mm)
- \( a \) = transducer aperture radius (mm)

![Diagram](image)

Figure 3.10. Simulation parameters and scanning geometry

Acoustic impedance values for PLA were unavailable at the time of this study, so the value was calculated based on \( E \) (Young’s modulus or tensile modulus) and \( \rho \) (density). The connection between \( K \) and \( E \) was established by the following equation:

\[
K = \frac{E}{3(1-2\nu)}
\]

Where:
- \( K \) = bulk modulus (MPa)
- \( \nu \) = Poisson’s ratio (dimensionless)
- \( E \) = Young’s modulus or tensile modulus (MPa)
When $v = 0.333$ is assumed, $K = E$. This is a reasonable value, as many materials range between 0.3 - 0.4. Similarly, the acoustic impedance value for PS was calculated from sound speed and density. This should be considered an estimate, since the actual value of $Z$ may vary with frequency. The goodness of fit of the linear regression lines were not predicted by this model, and have not been reported in previous studies.

3.2.1 Applicability and restrictions

Restrictions apply to experimental conditions in order for the Lizzi model to predict accurate results (Lizzi et al 1996). First, the scatterers must be smaller than the transducer -3dB beamwidth. This requirement was satisfied since the scatterers were between 0.5 and 3.0 $\mu$m in diameter. They were assumed to be smaller than the -3dB beamwidth since they were not resolvable as individual scatterers in images. Only homogenous scatterer ensembles can be simulated in this model. This was satisfied since only the added scatterers were known to scatter sound energy. This was confirmed by imaging the gel-only control samples. Heterogeneous mixtures of scatterers were not considered.

These predictions are also limited to frequencies within the -15dB bandwidth of the transducer. To consider this restriction, the more conservative -6dB transducer bandwidth was used. This set of equations applies to data collected by the transducer from a gated sample volume immersed in liquid. Three kinds of scatterer morphologies are allowed in the Lizzi model: isotropic, cylindrical, and planar. Both nanoparticles and microparticles are nearly spherical, and were modeled as isotropic scatterers.

3.2.2 Equations

A goal of this study was to compare simulated spectral parameters based on physical qualities of the samples with experimentally measured spectral parameters. Simulated spectral parameters were obtained with software in Matlab, while the experimentally measured parameters were calculated with a program in LabVIEW. Expected values of spectral parameters were calculated in Matlab for cell-only and nanoparticle-only gel pellets.

The simulation model software calculates and graphs spectral parameters based on the variation in input values. All equations from (Lizzi et al 1996) used to create the model are shown on the following page. Figure 3.10 shows the arrangement of parameters a, R, and L with respect to the transducer and gated
volume. The following table 3.6 shows general relationships between predictive variables and spectral parameters:

<table>
<thead>
<tr>
<th>Q</th>
<th>r</th>
<th>C</th>
<th>a</th>
<th>R</th>
<th>L</th>
<th>fc</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>-</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>-</td>
<td>m</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+ , then -</td>
<td>+ , then - M</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>I</td>
</tr>
</tbody>
</table>

Key: N = no effect, + = increases parameter, 1 = decreases parameter, - = varies

Table 3.6. Relationship between variables and predicted spectral parameters

**Model equations based on (Lizzi et al 1996)**

\[ M = 4.34 \ln T + 4.34 \ln (r^{-n-1} C Q^2) + g_1(f, b) n + g_2(f) r^2 \]  (eq. 1)  (Lizzi et al 1996)

\[ m = g_3(f, b) n - g_4(f) r^2 \]  (eq. 2)  (Lizzi et al 1996)

\[ I = M - m f \]  (eq. 3)  (Lizzi et al 1996)

\[ g_1(f, b) = 4.34 \left[ \ln \frac{f}{2} - \frac{1}{2} \ln \left( 1 - \frac{b}{2} \right) - \frac{1}{b} \ln \left( \frac{2 + b}{2 - b} \right) \right] \]  (eq. 4)  (Lizzi et al 1996)

\[ g_2(f) = -17.6f^2 \left( 3 + \frac{b^2}{4} \right) \]  (eq. 5)  (Lizzi et al 1996)

\[ g_3(f, b) = 26.058 \left( \frac{b - \left( 1 - \frac{b^2}{4} \right) \ln \left( \frac{2 + b}{2 - b} \right)}{b^2 f} \right) \]  (eq. 6)  (Lizzi et al 1996)

\[ g_4(f) = 105.5 f \]  (eq. 7)  (Lizzi et al 1996)

**Isotopic scatterers:**

\[ T = 0.64 \left( \frac{2 \pi}{c} \right)^{\frac{1}{3}} \frac{a^2}{3 R^2 L} : n = 4 \]  (eq. 8)  (Lizzi et al 1996)

\[ Z = p \times c \quad c_{obsd} = \sqrt{E \over p} \]
First, values for all parameters were stored in their respective variables. Next, the values of \( g_1 \) through \( g_4 \) were calculated based on these values, using equations 4-7. The next step was to calculate the \( T \) value using equation 8. Unless changing transducers, \( T \) remains constant across different groups of scatterers since it is based entirely on transducer properties. For this study, only the equation relevant to isotropic scatterers was used. No planar or cylindrical scatterers were simulated. Equations 9 and 10 are shown only for completeness. Lastly, the midband value, slope and intercept were calculated using equations 1, 2 and 3 respectively. The Lizzi model does not predict \( R^2 \), which is used exclusively as a thresholding measure. Table 3.7 shows physical property values used in the simulation.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value and units</th>
<th>Name and meaning</th>
</tr>
</thead>
<tbody>
<tr>
<td>L</td>
<td>1.0 mm</td>
<td>Gate length, how much distance is being analyzed</td>
</tr>
<tr>
<td>R</td>
<td>4.0 mm (RMV-608)</td>
<td>Transducer range</td>
</tr>
<tr>
<td></td>
<td>12.2 mm (RMV-30B)</td>
<td>Transducer range</td>
</tr>
<tr>
<td>a</td>
<td>0.978 mm (RMV-608)</td>
<td>Transducer aperture radius</td>
</tr>
<tr>
<td></td>
<td>3.024 mm (RMV-30B)</td>
<td>Transducer aperture radius</td>
</tr>
<tr>
<td>b</td>
<td>100%</td>
<td>Fractional transducer bandwidth</td>
</tr>
<tr>
<td>fc</td>
<td>55 MHz (RMV-608)</td>
<td>Transducer center frequency</td>
</tr>
<tr>
<td></td>
<td>30 MHz (RMV-30B)</td>
<td>Transducer center frequency</td>
</tr>
<tr>
<td>Zo</td>
<td>1.48E6 Pa-s/m³ (Rayls)</td>
<td>( Z_{\text{water}} ) (Phillips et al 1998). Mean acoustic impedance of surrounding material</td>
</tr>
<tr>
<td>Z</td>
<td>2.556E6 Pa-s/m³ (PS)</td>
<td>Mean acoustic impedance of scatterers (PS or PLA particles)</td>
</tr>
<tr>
<td></td>
<td>1.749E6 Pa-s/m³ (PLA)</td>
<td>Mean acoustic impedance of scatterers (PS or PLA particles)</td>
</tr>
<tr>
<td>C</td>
<td>Variable (scatterers/ml)</td>
<td>Effective scatterer concentration</td>
</tr>
<tr>
<td>n</td>
<td>4</td>
<td>Number set by type of scatterer, from eq. 8</td>
</tr>
<tr>
<td>E</td>
<td>3000 MPa (PLA)</td>
<td>Young’s modulus (Lu and Mikos 1999)</td>
</tr>
<tr>
<td>c</td>
<td>1450 m*s⁻¹</td>
<td>Speed of sound through water</td>
</tr>
<tr>
<td>r</td>
<td>Variable (mm)</td>
<td>Effective scatterer radius</td>
</tr>
<tr>
<td>( \rho )</td>
<td>1.05 g/cm³ (PS) (Pu 1999)</td>
<td>Density (g/cm³)</td>
</tr>
<tr>
<td></td>
<td>1.02 g/cm³ (PLA)</td>
<td>Density (g/cm³)</td>
</tr>
<tr>
<td>I</td>
<td>returned by simulation (dB)</td>
<td>Spectral intercept</td>
</tr>
<tr>
<td>M</td>
<td>returned by simulation (dB)</td>
<td>Midband fit</td>
</tr>
<tr>
<td>m</td>
<td>returned by simulation (dB / Hz)</td>
<td>Spectral slope over specified range</td>
</tr>
</tbody>
</table>

Table 3.7. Simulation parameters and values
When scanning materials with ultrasound, it is important to create an acoustically conductive interface between the transducer and sample. For clinical imaging using contact transducer, acoustically transparent gels are commonly used to increase acoustic conductance between the transducer and patient. An immersion bath filled with distilled water acoustically coupled the immersion transducer and sample capsules (part #00731, lot #2304 material: polyethylene, volume: 2.5 ml, height: 31.5 mm, wall thickness: 0.83 mm, mass: 1.55 g. Kartell Labware Division. Noviglio, Italy) similarly to (Straub et al 2005, El-Sherif and Wheatley 2001, Lathia et al 2004).

### 3.3 Sample preparation

Samples were prepared for scanning to test both the accuracy of the Lizzi model and abilities of the software. These samples required both a scanning medium and scatters. Supporting scatterers in 1.5% agarose gel (Cambrex, Baltimore, MD) provides a means of increasing the backscatter gate length. All gel was prepared by heating a measured mass of dry, powdered agarose in water while stirring. The volume of the dry powder was ignored in the calculations.

Previous studies have used agarose gel as a support to hold the cells for scanning (Sakamoto et al 2005, Liu et al 2006). Agarose gel was tested for ultrasound transparency before using as a suspension media. Gel samples at 1.0, 1.5 and 2.0% were prepared in capsules and scanned with both 10 and 20 MHz transducers in A-mode. Here, the boundaries between water and gel were either undetectable or insignificant. 1.5% gel-only samples only produced echoes only from the water-gel interface, without producing backscatter in all images acquired with the Vevo system. Figure 3.3 shows the ultrasound transparency of a gel-only capsule.

Three groups of scatterers were scanned at varying concentrations as test samples set in low-melting point agarose gel: two for PLA particles scanned at both 30 and 55 MHz, and a third for polystyrene particles scanned at 30MHz. All PLA particles were 2.0 μm in diameter, and were scanned at both 30 and 55 MHz. PLA particles were scanned at concentrations of 0%, 50%, 40%, 30%, 20%, 10% and 1% of the initial solution (2.340E9 scatterers/ ml), for values of 0, 1.17E9, 9.36E8, 7.02E8, 4.68E8, 2.34E8 and 2.34E7 scatterers/ ml. Each sample contained 1000 μl of gel. The initial PLA scatterer concentration is derived on the following page.
Scatterers / ml in original PLA solution:
Mass concentration = 10 mg / ml
Diameter = 2.0 μm
Volume = \(4 \pi / 3 \times r^3 = 4 \pi / 3 \times (2.0 / 2)^3 = 4.189 \, \mu m^3\)
Density = 1.02 g / cm³

\[
\frac{10 \, mg}{ml} = 4.189 \, \mu m^3 \times x \times 1.02 \times \frac{g}{cm^3}
\]

\[
x = \frac{10 mg}{ml} \times \frac{1}{4.189 \, \mu m^3} \times \frac{1E6 \, \mu m}{1E2 \, cm} \times \frac{1cm^3}{102 g} \times \frac{1g}{1E3 \, mg}
\]

\[
x = 2.340E9 \frac{scatterers}{cm^3}
\]

All PS particles were 200 nm in diameter, and scanned at 30 MHz. These particles were 500nm in diameter and were scanned in concentrations of 0, 1.8E+10, 3.6E+10, 7.2E+10, 9E+10 and 1.08E+11 scatterers / ml. These samples were also used in a previous study (Liu et al 2006).

3.4 System configuration

The scatterer samples were scanned after being allowed to gel. First, the capsules were affixed to the scanning tank with a tape loop. Then, distilled water was slowly added as the coupling medium. The samples were scanned with a Vevo 660 imaging system (Visual Sonics, Toronto, Ontario) interfaced to a PC with digitizer running the acquisition program. A b-mode image of the sample was recorded which placed the gel-water interface near the transducer focal zone. After selecting an artifact-free scan line within the b-mode image, the scanner was switched to m-mode to allow acquisition of a single line. Data from this single scan line and a triggering signal were sent to the digitizer. This acquisition setup is shown in figure 3.1. During each acquisition session, the gel-only capsule was scanned and recorded first to set the calibration spectrum. This process was repeated for each test capsule, in order of capsule number.
CHAPTER 4

RESULTS AND DISCUSSION

4.1 Simulation results

All simulation results are listed in section 4.2 in figures 4.1 through 4.12 and tables 4.1 through 4.3. None of the relationships between source variables and spectral parameters are necessarily linear, so predictions are shown at each concentration value. Concentration is varied for both PLA and PS scatterers, while frequency is only varied for PLA scatterers. Both simulated and experimental data are shown on each graph for comparison.

According to the Lizzi model, spectral slope ($m$) varies only with scatterer type, scatterer size ($r$), bandwidth, and center frequency ($f_c$). Therefore, change in $m$ is not expected with respect to concentration ($C$). This is reflected by the constant value of predicted $m$ values with respect to $C$ in figures 4.1, 4.4 and 4.10. Increasing $r$ or $f_c$ is expected to decrease $m$. The effect of increased $f_c$ from 30 to 55MHz is reflected by the decrease in predicted $m$ in figure 4.7.

The Lizzi model predicts that spectral intercept ($I$) will be dependent on $C$, $r$, $f_c$, transducer dimensions ($a$, $R$ and $L$) and impedance mismatch ($Q$). The relationships with $C$ and $f_c$ are of main interest. $I$ is predicted to increase with both of these variables. The increase of $I$ with respect to $C$ is shown as positive trends in figures 4.3, 4.6 and 4.10. The increase in $I$ with respect to $f_c$ is shown in figure 4.9.

Midband fit ($M$) is defined in terms of $I$, $m$ and $f_c$. When the vertical scales of graphs of $M$ and $I$ versus $C$ are normalized, their profiles will match. This is due to $m$ being invariant with respect to $C$. Here, the predicted $M$ will be explained in terms of relationships directly to source variables, although it is a function of $I$ and $m$. This way, confusion from complex relationships or intermediate values can be avoided.
M is dependent on C, r, fc, a, R, L and Q. M increases with both C and fc, and decreases with r. Figures 4.2, 4.5 and 4.11 show the predicted increase in M with respect to C as positive trends. The two sets of data in figure 4.8 show the predicted increase in M with respect to fc.

While the Lizzi model returns results for very low concentrations of scatterers, gel-only calibration capsules were not compared with predictions. To generate valid spectral parameters, a calibration needs to be subtracted from the test spectra before performing linear regression. The calibration spectra were used to calibrate other spectra, and so were not calibrated themselves. For this reason, they were not included in the comparison.

4.2 Experimental results

All experimental results are presented graphically in figures 4.1 through 4.12, and in tabular form in tables 4.1 through 4.3. The data are displayed next to theoretical results in tables for comparison. In each graph, error bands for experimental data show ± SD (standard deviation). In tables 4.1 and 4.2, the data are arranged with respect to capsule order, beginning with capsule 0 (gel only) and ending with capsule 6 (10% original concentration). In table 4.3, the data are arranged beginning with the gel-only capsule data and ending with 30% original concentration capsule data.

A clumping factor was added to the model to account for changes in effective scatterer radius from particle aggregation. This factor adjusted the effective scatterer radius to provide the best match for midband fit data. The effect of aggregation was only considered on the scatterer radius. Clumping factor improved the agreement of I and m, though to a lesser degree. Clumping factors for PLA and PS data sets were 3.5 and 2.9, respectively. The predicted values for spectral parameters when clumping is ignored or considered in tables 4.1, 4.2 and 4.3 are indicated by red or blue fields, respectively.

The same clumping factor of 3.5 improved both 30 and 55MHz PLA data sets, which supports the possibility of clumping more than if only one data set were improved. An increase of 3.5 in the effective scatterer radius is reasonable, since this does not require a large number of scatterers. This does not strictly imply that 3.5 scatterers aggregated on average, but rather that the effective scatterer radius increased by a factor of 3.5 on average. Effective decrease in scatterer concentration was ignored.
Before aggregation effects are considered, the Lizzi model and experimental results agree best for spectral slope values. Figures 4.1, 4.4 and 4.10 show predicted and measured m values. According to the model, m is predicted to stay constant with respect to C, and decrease with fc. In figure 4.1, the trend in m values with respect to concentration is not visible. Similarly, figures 4.4 and 4.10 show the absence of clear positive or negative trends with respect to C.

Figures 4.7, 4.8 and 4.9 compare data between 30 and 55 MHz scans of 2.0 μm PLA particles for m, M and I, respectively. The Lizzi model predicts that m decreases with fc, and that M and I increases with fc. The decrease in m with respect to fc is visible in figure 4.7. The general level of m decreases when fc is increased from 30 to 55 MHz. Figures 4.8 and 4.9 show the increase of M and I with respect to fc, respectively. For all three comparisons, only the clumping-adjusted models are shown.

Figures 4.3, 4.6 and 4.12 show predicted and measured I values with respect to C. The model predicts that C increases I, which is shown by limited positive trends in the data. Measured values of I did not appear to correlate as well with the model compared to M.

When the clumping factor is included in the model, predicted values of M show good agreement with experimental data. Figures 4.2, 4.5 and 4.11 show predicted and measured M values with respect to C. The Lizzi model predicts that M increases with respect to C. This positive trend is most visible in figures 4.2 and 4.11.
Figure 4.1. Spectral slope for 2.0 μm PLA scatterers scanned at 30 MHz

Figure 4.2. Spectral midband fit for 2.0 μm PLA scatterers scanned at 30 MHz
Figure 4.3. Spectral intercept for 2.0 \( \mu m \) PLA scatterers scanned at 30 MHz.
<table>
<thead>
<tr>
<th></th>
<th>C (#/ml)</th>
<th>0</th>
<th>1.17E+9</th>
<th>9.36E+8</th>
<th>7.02E+8</th>
<th>4.68E+8</th>
<th>2.34E+8</th>
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<td>PLA1 m (dB/Hz)</td>
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<td>3.37E-7</td>
<td>5.82E-7</td>
<td>5.00E-7</td>
<td>5.81E-7</td>
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</tr>
<tr>
<td>PLA2 m (dB/Hz)</td>
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<td>2.93E-7</td>
<td>-2.75E-7</td>
<td>2.65E-7</td>
<td>3.30E-7</td>
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</tr>
<tr>
<td>PLA3 m (dB/Hz)</td>
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<td>7.35E-7</td>
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<td>7.06</td>
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<td>6.73</td>
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<td>PLA2 I (dB)</td>
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<td>6.08E-7</td>
<td>6.08E-7</td>
<td>6.08E-7</td>
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</tr>
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<td>-24.89</td>
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</tr>
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<td>Simulated (clumping = 3.5) m (dB/Hz)</td>
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<td>5.73E-7</td>
<td>5.73E-7</td>
<td>5.73E-7</td>
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Table 4.1. Experimental and theoretical spectral parameters for 2.0 µm PLA particles, 30 MHz
**Figure 4.4.** Spectral slope for 2.0 μm PLA scatterers scanned at 55 MHz

**Figure 4.5.** Spectral midband fit for 2.0 μm PLA scatterers scanned at 55 MHz
Figure 4.6. Spectral intercept for 2.0 μm PLA scatterers scanned at 55 MHz
<table>
<thead>
<tr>
<th></th>
<th>C (#/ml)</th>
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<th>9.38E+8</th>
<th>7.02E+8</th>
<th>4.68E+8</th>
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<tr>
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<td>3.06E-7</td>
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<td>I (dB)</td>
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<td>2.37</td>
<td>1.25</td>
<td>4.06</td>
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<td>6.12</td>
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Table 4.2. Experimental and theoretical spectral parameters for 2.0 µm PLA particles, 55 MHz
Figure 4.7. Comparison of spectral slope between 30, 55MHz for 2.0 μm PLA scatterers

Figure 4.8. Comparison of spectral midband fit between 30, 55MHz for 2.0 μm PLA scatterers
**Figure 4.9.** Comparison of spectral intercept between 30, 55MHz for 2.0 μm PLA scatterers

**Figure 4.10.** Spectral slope for 500 nm PS scatterers scanned at 30 MHz
Figure 4.11 Spectral midband fit for 500 nm PS scatterers scanned at 30 MHz

Figure 4.12. Spectral intercept for 500 nm PS scatterers scanned at 30 MHz
<table>
<thead>
<tr>
<th></th>
<th>C(#/ml)</th>
<th>0</th>
<th>1.8E+10</th>
<th>3.6E+10</th>
<th>7.2E+10</th>
<th>9E+10</th>
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<td>PS1</td>
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<td>PS2</td>
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<td>1.73E-7</td>
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<td>PS3</td>
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<td><strong>Average</strong></td>
<td>M (dB)</td>
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<td>1.31E-7</td>
<td>2.24E-7</td>
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<td><strong>Average</strong></td>
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<td>L (dB)</td>
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<td>6.1E-7</td>
</tr>
<tr>
<td>Simulated (no clumping)</td>
<td>M (dB)</td>
<td>-8.37</td>
<td>-5.37</td>
<td>-2.96</td>
<td>-1.39</td>
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<td>Simulated (clumping = 2.9)</td>
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<td>-23.66</td>
<td>-20.66</td>
<td>-19.69</td>
<td>-18.90</td>
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</table>

Table 4.3. Experimental and theoretical spectral parameters for 500 nm PS particles, 30 MHz
4.3 Confounding factors and limitations

Several confounding factors existed in this study. Most influential was the lack of linearity in much of the FFT data subjected to spectral analysis. Secondly, values for parameters used in the Lizzi model were drawn from literature rather than measurements. Preparation protocol, image artifact and system noise are among the less influential factors.

Low $R^2$ values were recorded for many of the experimental groups, which are the major source of error between predicted and measured spectral parameters. These low $R^2$ values were caused by lack of clearly linear regions in many calibrated spectra. As stated before in chapter 3, calibrated spectra are a subtracted difference between the test spectrum and calibration spectrum. If only a small difference exists between the calibration and test spectra, the resulting calibrated spectrum will have low amplitude. If these differences are small and random instead of systematic and significant, the resulting calibrated spectrum will likewise be noisy, lacking in shape and structure. When data lacks linearity, the precision of extracted spectral parameters are put into question. This lack in linearity is suspected to be the main cause of divergence from the Lizzi model.

$Z$ (acoustic impedance) values for PLA, saline and water which were used to predict spectral parameters may have also contributed error. The $Z$ value for PLA was based on literature values of $E$ (Young’s modulus) and $\rho$ (density) for bulk material. Therefore, the actual $Z$ values of PLA in this study may have been much different than used for simulation. Using measured values of $Z$ for the scattering material may have provided more accurate predictions.

The acoustic impedance mismatch of agarose gel may be another source of error. While initial scans confirmed the absence of scattering from within the agarose gel, echo between water and gel revealed impedance mismatch between the two media. Echoes from the water-gel interface were visible in images taken at both scanning frequencies. The Lizzi model accounts for two materials (water and PLA), but in reality three were present (water, agarose and PLA). Spectral slope would have remained unaffected from this influence. Including this effect in the model or using a different model altogether may have returned more accurate predictions.
A confound related to the scanning protocol is the ability of gel samples to absorb water. Since the gel is not at osmotic equilibrium with distilled water, it tends to absorb water and swell. This increases the distance between scatterers, and decreases their effective concentration. During any scanning session, several hours would have elapsed between the first and last scan, providing time for capsules to absorb water.

Testing over wider ranges of physical variables may have shown the trends more clearly. Choosing logarithmically spaced values for concentration and radius may have made trends in spectral parameters more apparent. Using figure 4.2 as an example, the largest change in $M$ occurs between the first and second concentration points, and is followed by less significant changes.
CHAPTER 5

CONCLUSION

Ultrasound imaging provides rapid, inexpensive and noninvasive diagnostic images. The need for diagnosing small tumors exists, and may be enhanced through targeted contrast or delivery agents. Combining proven ultrasound technology with tissue characterization algorithms and nanoparticle or microparticle based contrast agents offers a new possibility of diagnosis. An advantage to combining spectral analysis with ultrasound imaging is the lack of hardware changes. The changes in analysis method require only software addition.

The three objectives of this study were to create a graphical user interface (GUI) capable of measuring spectral parameters, interface the GUI with an HFUS system, and compare the measurements with an existing model. This software provides a user-friendly means of measuring spectral parameters from any triggered ultrasound source. During acquisition, the program provides means of locating a signal in time, and recording spectral data with redundancy for later analysis. When compared with an existing model, the data from this program showed agreement of spectral slope data.

While this software is operational in its current state, future versions of this software might include means of recording time data and all acquisition parameters in addition to spectral data. Interfacing with other digitizers to enhance portability or integrating the algorithms with imaging system software are other possibilities. Adding other forms of tissue characterization is another possibility related to nanoparticle and microparticle studies.

Future studies made possible by this software include toxicity-related studies of nanoparticles or microparticles. Since the spectral parameters of cells change during apoptosis, subtle reactions of cells to
these materials might be detected when they are added to cell cultures. Another possible in-vitro study might involve testing for a difference in spectral parameters between cells which have internalized nanoparticles or microparticles compared to cells which have merely adhered to them.

A possible in-vivo study could test for a difference in spectral parameters in tissues before and after they have absorbed nanoparticles or microparticles. This would apply to targeted contrast agents especially, since they have the ability to become more concentrated in regions of cells expressing targeted molecules. Measuring spectral parameters may detect a difference between samples which goes unnoticed when compared with other methods.
LIST OF REFERENCES


GLOSSARY

**Backscatter**: ultrasound signals reflected back from samples to the direction they come from.

**Calibrated backscatter spectrum**: a backscatter frequency spectrum which has been adjusted for system effects. When using logarithmic units, the system spectrum will have been subtracted.

**Clumping factor**: coefficient increasing effective scatterer radius, used to account for particle aggregation.

**FFT**: fast Fourier transform, a method of transforming time-domain data to frequency domain.

**Goodness of fit (R²)**: measure ranging from 0 to 1 describing the linearity of a data set.

**HFUS**: high frequency ultrasound.

**PLA**: poly(lactic acid), a biodegradable polymer.

**PS**: polystyrene, a non-resorbable polymer.

**ROI**: region of interest. A selection from a time-domain or frequency-domain signal.

**Spectral intercept (I)**: value of regression line at 0 Hz.

**Spectral midband fit (M)**: value of regression line in center of analysis region.

**Spectral analysis**: a tissue characterization method returning the slope (m), midband fit (M), intercept (I), and goodness of fit (R²) from a linear regression line applied to a calibrated backscatter spectrum. These values are known collectively as spectral parameters.

**Spectral slope (m)**: change in spectral amplitude divided by change in frequency.

**Tissue characterization**: algorithms designed to quantify the nature of signals from biological tissues. Often used in combination with imaging.

**VI**: virtual instrument. Any program created in LabVIEW.

**Sub-VI**: a VI called within another VI, similar to a subroutine.
APPENDIX A

LABVIEW SOFTWARE DOCUMENTATION
Figure A.3. Frame 1 of “US acquisition with calibration VI”

Figure A.4. Frame 2 of “US acquisition with calibration VI”
Figure A.5. Frame 3 of “US acquisition with calibration.VI”

Figure A.6. Frame 4 of “US acquisition with calibration.VI”
Figure A.7. Frame 5 of “US acquisition with calibration.VI”

Figure A.8. Frame 6 of “US acquisition with calibration.VI”
Figure A.9. Frame 7 of "US acquisition with calibration.VI"

Figure A.10. Frame 8 of "US acquisition with calibration.VI"

Figure A.11. Frame 9 of "US acquisition with calibration.VI"
Figure A.12. Frame 10 of “US acquisition with calibration.VI”

These frames check for changes to the parameters and reconfigure the digitizer as needed.

Figure A.13. Frame 11 of “US acquisition with calibration.VI”

Configure Vertical if settings change, new channel, or new digitizer.

Figure A.14. Frame 12 of “US acquisition with calibration.VI”
These frames check for changes to the parameters and reconfigure the digitizer as needed.

Figure A.15. Frame 13 of “US acquisition with calibration.VI”

Figure A.16. Frame 14 of “US acquisition with calibration.VI”

Stop acquisition if Stop or Single is pressed or parameters change.

Figure A.17. Frame 15 of “US acquisition with calibration.VI”
Figure A.18. Frame 16 of “US acquisition with calibration.VI”

These frames check for changes to the parameters and reconfigure the digitizer as needed.

Figure A.19. Frame 17 of “US acquisition with calibration.VI”

Figure A.20. Frame 18 of “US acquisition with calibration.VI”

Figure A.21. Frame 19 of “US acquisition with calibration.VI”
Figure A.22. Frame 20 of “US acquisition with calibration.VI”

Figure A.23. Frame 21 of “US acquisition with calibration.VI”

Figure A.24. Frame 22 of “US acquisition with calibration.VI”
Figure A.25. Frame 23 of “US acquisition with calibration. VI”

Figure A.26. Frame 24 of “US acquisition with calibration. VI”

Figure A.27. Frame 25 of “US acquisition with calibration. VI”
Figure A.28. Frame 26 of “US acquisition with calibration.VI”

Figure A.29. Frames 27 and 28 of “US acquisition with calibration.VI”

Figure A.30. Frames 29 and 30 of “US acquisition with calibration.VI”
Figure A.31. Frame 31 of “US acquisition with calibration.VI”

Figure A.32. Frame 32 of “US acquisition with calibration.VI”
Figure A.33. Frame 33 of “US acquisition with calibration.VI”

Figure A.34. Frame 34 of “US acquisition with calibration.VI”
Figure A.35. Frame 35 of “US acquisition with calibration.VI”

Figure A.36. Frame 36 of “US acquisition with calibration.VI”

Figure A.37. Frame 37 of “US acquisition with calibration.VI”

Figure A.38. Frame 38 of “US acquisition with calibration.VI”
Figure A.39. Frame 39 of “US acquisition with calibration.VI”

Figure A.40. Frame 40 of “US acquisition with calibration.VI”

Figure A.41. Frame 41 of “US acquisition with calibration.VI”
Figure A.42 Frame 42 of “US acquisition with calibration.VI”

Figure A.43 Frame 43 of “US acquisition with calibration.VI”

Figure A.44 Frame 44 of “US acquisition with calibration.VI”
Figure A.45: Hierarchy of sub-VI’s used in program
valid points
\[ x \rightarrow y \]
scaling factor = \# total points / \# valid points

steps according to protocol:
1. hamming
2. FFT
3. squared magnitude of complex data
4. scaling factor

Figure A.46. Sub-VI: “hamming, fft, mag, scaling.vi”

Figure A.47. Sub-VI: “subarray_selector1D.vi”
Figure A.48. Sub-VI: “subarray_selector2D.vi”
create an array with values from [lower bound] to [upper bound] in \([N]\) steps
first value = [lower bound], last value = [upper bound]

Formula for cells:
\([\text{array}] = (\text{[upper bound]} - \text{[lower bound]})/(\text{[N]} - 1) \times [\text{cell}] + \text{[lower bound]}\)

initialize shift register with value \([\text{lower bound} - (\text{[upper bound]} - \text{[lower bound]})/(\text{[N]} - 1)]\)
so that it will be equal to \([\text{lower bound}]\) for the first number.
after that, add the value \((\text{[upper bound]} - \text{[lower bound]})/(\text{[N]} - 1)\) each iteration

Figure A.49. Sub-VI: "X_array_maker.vi"