Thesis for the Degree of Master of Engineering

Self-constructed scanning acoustic microscope system for biological tissue characterization

By

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Department of Biomedical Engineering

The Graduate School

Pukyong National University

Feb 2019

Self-constructed scanning acoustic microscope system for biological tissue characterization

(생물 조직 특성 감별을 위한 자가 제작 주사 음향 현미경 시스템)

Advisor: Prof. Junghwan Oh

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Abstract

Scanning acoustic microscope has been developed to investigate acoustic properties of various organs and disease states. As conventional way, in order to assess pathology of a tumor, it is taken out of body through biopsy. Then it would spend on a serial of chemical staining processes before being observed. However, it is difficult to get done during an operation. Normally, it takes several hours to several days to complete the processes. Furthermore, the tissue after being stained, usually loses it biological functions. In previous studies, it has been proved that speed of sound is able to be considered to be a good parameter in order to characterize abnormality of the tissue. The systems which have been used in the studies made by renowned technological companies. They are usually compact and expensive and could be unmodifiable by users. As the result of understanding biological and technical aspects, a lab-based system for such researches are in need. In the scope of the thesis, we focus on technical solutions and in vivo experiments to evaluate the practical aspects of the system.

Keywords: scanning acoustic microscope, speed of sound, tissue characteristic

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Chapter 1. Introduction

1. The overview of single-element ultrasonic transducer

The terms of sound and ultrasound are used to mention about to propagation of mechanical waves in different frequencies range. Ultrasound referred to the range beyond human hearing (typically 20 kHz). The way the acoustic waves propagate to the medium depends on the particular elastic properties of that medium and its mass density.

A crystal terms piezoelectricity develops an electric charge when they are mechanically strained. When a voltage is applied to both sides of the crystal, the thickness of the crystal either stresses or strains, depending on the polarity of the voltage and the distinct properties of the crystals. Single-element transducers are instruments that work in both modes by converting electrical energy into acoustic energy to create acoustic signal and by converting the echo pressure waves into electrical energy.

The center frequency of the transducer is mainly determined by the thickness of the piezoelectric which can be expressed by the equation below

$$f_c = \frac{c_p}{2l_p}$$

Where f_c is referred to center frequency, c_p , l_p is the acoustic velocity and thickness of the piezoelectric, respectively.

When a pulse applies to the piezoelectric which is generated by an instrument called pulser, cause the crystal to vibrate at the resonant frequency (center frequency). While the crystal vibrates at the frequency, it will keep vibrating after the applied pulse finished. The vibration is increasing fading away until all of the energy is dissipated. The effect of the vibration could be controlled by a backing layer which is backed up at the rear side of the crystal. Without the backing layer, the ringing time takes longer to dissipate the energy which results in having longer waveform duration. This probably effect to axial resolution. Another factor also depends on waveform duration is bandwidth of the signal. The longer waveform duration is, the more narrow of its frequency spectrum is.

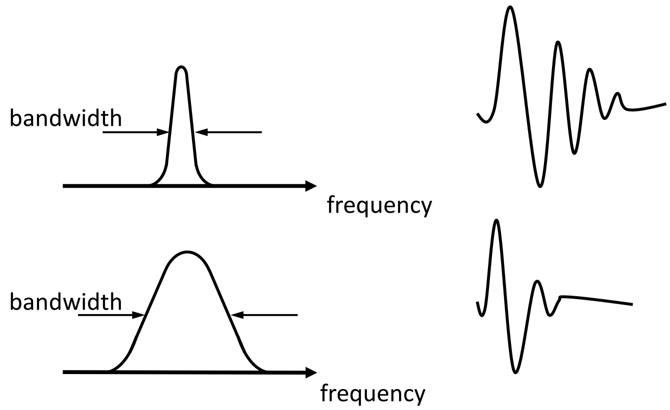


Figure 1 Demonstrate the relationship between waveform duration and bandwidth

Furthermore, as the ultrasonic energy is generated, it propagates two sides of the crystal. The more energy to back to the backing layer, the less energy travel to the target. This affects to the sensitivity of the transducer. So, the overall goal is to have energy in the piezoelectric removed as quickly as it can. Another factor can improve this is matching layers.

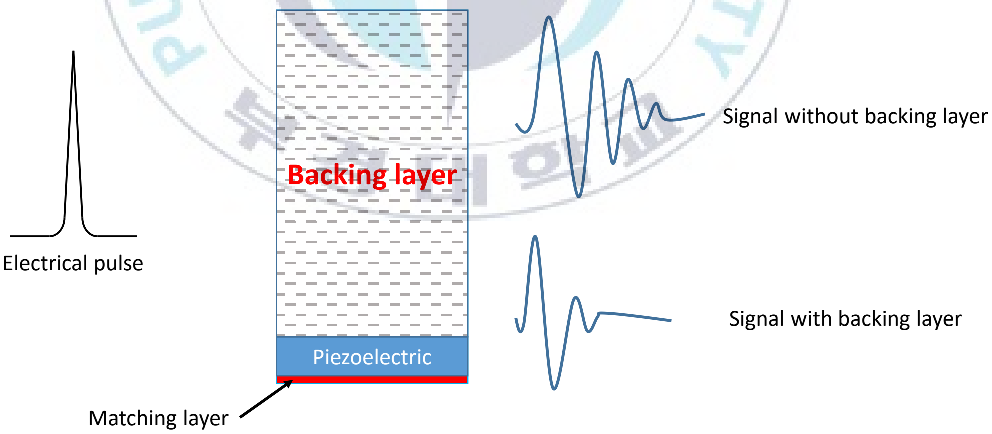


Figure 2 Demonstration of the effect of backing layer

When there is a huge difference in acoustic impedance between two mediums results in large reflection of incident waves. The impedance mismatch here is between crystal and propagating medium. In order decrease the mismatch, a thin layer called a quarter of wavelength is applied on the surface of the transducer. The quarter wavelength matching layer can boost up to 100% of energy transmission but this is only for a specific single frequency. To tolerate the problems, a serial matching layer could be put on the surface of the transducer.

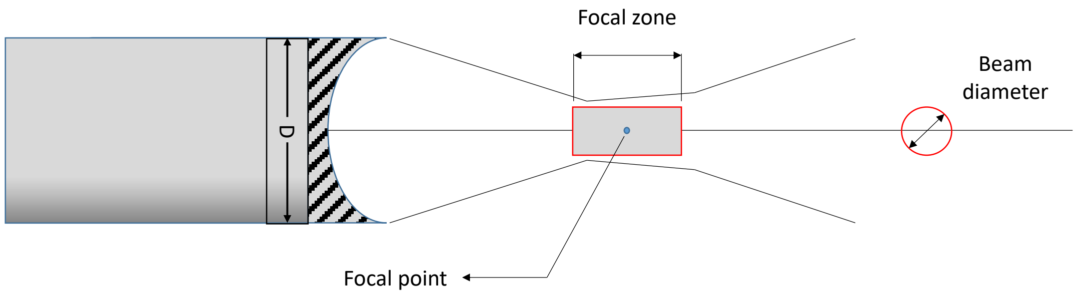


Figure 3 Beam profile of a single-element spherical transducer

In non-destructive testing industry, there are two main types of single-element transducer that is focused and unfocused transducer. For the focused transducer, it is subdivided into cylindrical and spherical transducers. The main different of cylindrical transducers that they focus the energy on a line instead of single point as spherical transducer does. In the scope of the study, we want to construct an 2D image that can shape the whole scanning area. So, we would concentrate to single-element spherical transducer.

In the beam profile of the transducer, the focal point is referred as the center of the spherical geometry where concentrated the highest energy. The focal zone of the transducer is the area along the transducer's axis and expand to both sides of the focal point where the energy dropped by 6dB compared to the maximum value at the focal point. Beam diameter is a circular area centered at focal point and perpendicular with transducer's axis where the energy at the boundary dropped by 6dB. The beam diameter can be approximated by:

$$BD = \frac{Fc}{fD}$$

Where BD is beam diameter and F, c, f, D are focal length, speed of sound of the medium, nominal frequency and diameter of the active element, respectively.

In most of the cases, beam diameter could be used as lateral resolution. Whereas, axis resolution is approximated as haft-wavelength (λ/2).

2. Single-element transducers and application in scanning acoustic microscope

Scanning acoustic microscope or called SAM is term frequently used in non-destructive evaluation and testing methods. The benefits of the methods help evaluate or inspect products that already in completing stages. It is increasingly becoming a crucial standard in quality control. It is particularly sensitive to air gaps when difference of acoustic impedances of material and air is significant.

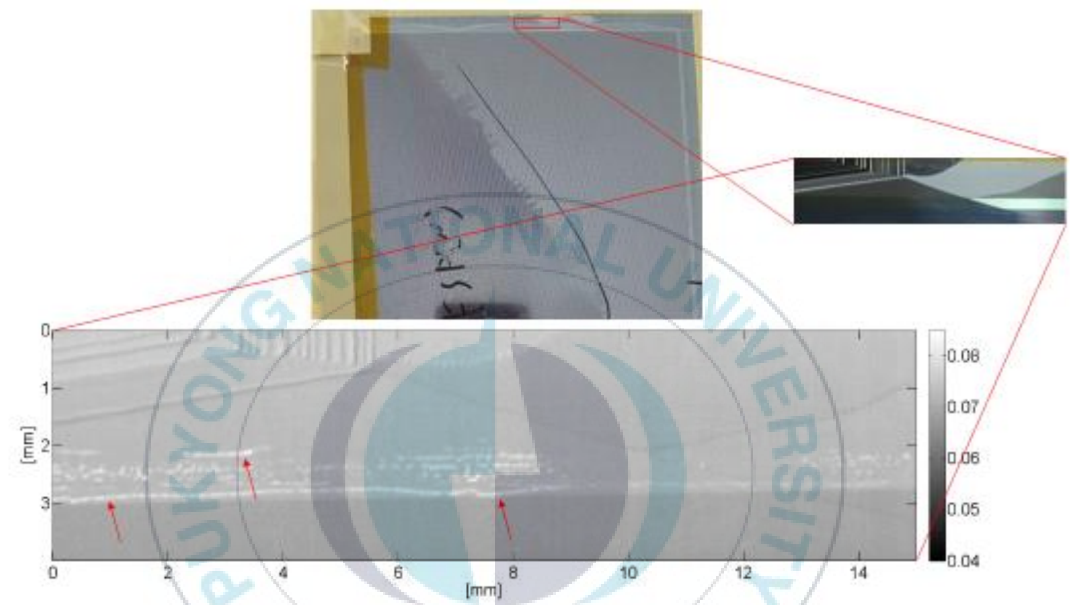


Figure 4 Demonstrates an ultrasonic image of an OLED panel

The figure above shows ultrasonic image of an OLED TV panel. In the manufacturing processes, the attachment of cover glass and panel below is uncompleted. As a result, air gaps called bubbles have been stuck in the middle. Ultrasonic has played a role of a non-destructive evaluation in quality control. As a given grayscale image, a threshold would be set according to practical processes in order to convert to binary image. Since then, an algorithm would be run to evaluate the quantity and size of the bubbles to make a decision that the item passes or fails.

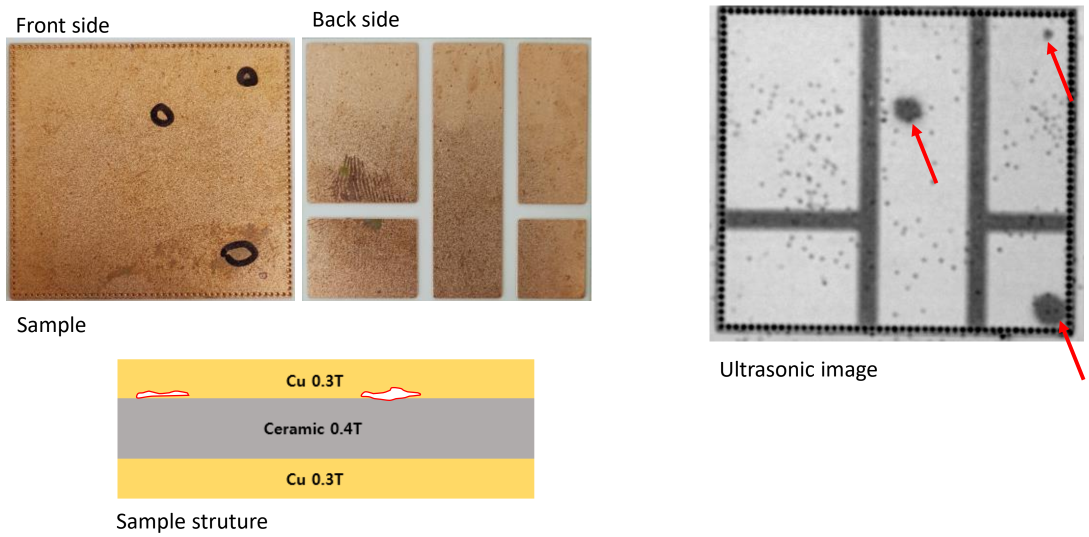


Figure 5 Demonstrates ultrasonic image of a multi-layers structure with air gaps stuck inside

The figure above shows the result of an ultrasonic non-destructive testing. The air gaps remain inside structure. Data processing in the application has a bit difference in comparison with previous one. The reason is that dramatic impedance mismatch between coupling media and copper. As a result, distance between two signal reflections is too close. This make data processing more complicated.

3. RF signal and signal analysis

In fluid and solids, acoustic waves are able to travel in four modes such as longitudinal waves, shear waves, surface waves and plate waves which is based on the way the particles in the medium oscillated when they propagate through. In the scope of the study, the transducer's direction is perpendicular with the sample so we only focus on the effects of longitudinal waves. In longitudinal waves, the oscillations direction and propagating direction is coincidental.

Figure 6 Waveform of a reflective acoustic signal

An electrical pulse applies across the crystal, it vibrates and then generates ultrasonic waves. The waves will propagate through the medium. When it hits a certain obstacle, the incident waves will be divided into two sub-waves: reflective waves and transmissive waves. The percentage of energy according to each part can be calculated by the equation express below:

$$T = \frac{2Z_r}{Z_r + Z_t} \; ; R = \frac{Z_t - Z_r}{Z_r + Z_t}$$

Where T and R is the percentage of energy that transmits and reflects, respectively. Z_r is the acoustic impedance of reflective medium and Z_t is the acoustic impedance of transmissive medium. Acoustic impedance can be obtained by Z=ρc which ρ is material density and c is acoustic velocity of that medium.

The reflective waves are under mechanical energy. After hitting the surface of crystal, it will convert the energy into electrical signal. The properties and the shape of the interfaces the acoustic waves interacts with will be reflected in the magnitude of receiving signal.

In order to work on further analysis, the signal needs to be digitized. As analog signal, after digitizing by an oscilloscope or an digitizer, the information of the signal become discrete data points.

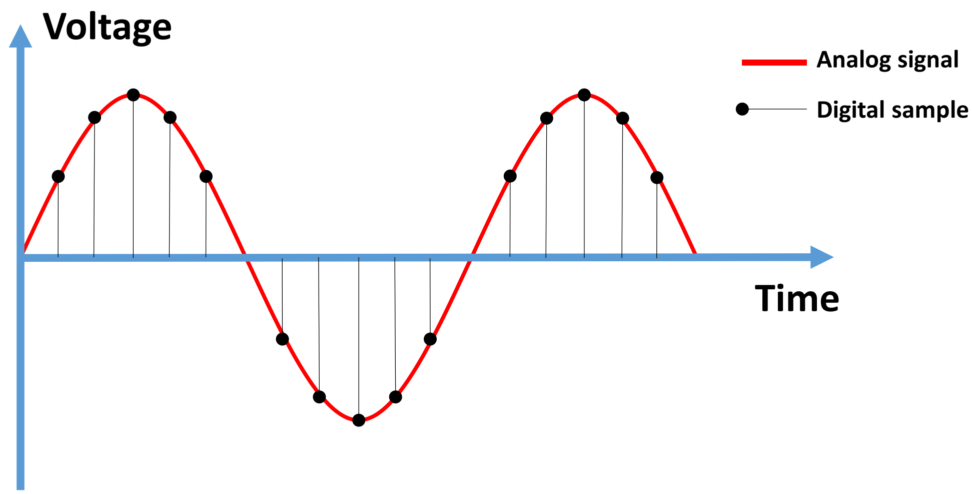


Figure 7 Demonstrate an analog signal after digital converter

The sets of data points after analog-digital converter will reflect how change the signal is in analog form. The faster sampling frequency is, the more real of signal in digital form is. Sampling frequency or sampling rate can be understood as the intervals between two digital data and it is in unit of sample/second. In signal processing, a double sampling frequency of an analog signal can obtained the frequency of analog signal. But in some applications, the maximum values are the key in actuation. So, two times higher sampling frequency compared to the real signal in these cases is not sufficient. A recommendation of the cases is ten times higher frequency than the estimated real frequency.

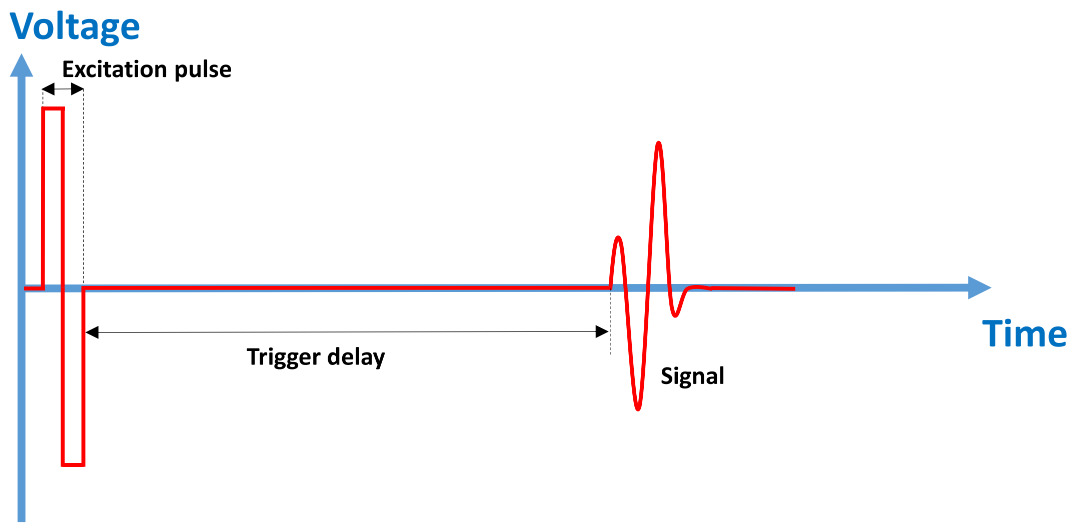


Figure 8 Demonstrate the schematic of an ultrasound signal from the excitation stage to receiving signal stage

There is a period of time between excitation pulse event and receiving signal. The amount of time depends on transducer's characterization such as focal length, delay lines attachment and so on; and distance between transducer's surface and objectives. In digitizing processes, excitation pulse is used as a primary event to trigger the process. In order to get desired signal when working on high sampling rate, it requires digitizer musts acquire a huge of useless data. A resolve for the problem is by apply a factor called trigger delay. The trigger delay basically moves trigger even close to the desire signal. As the result, a reduction of data storage is achieved.

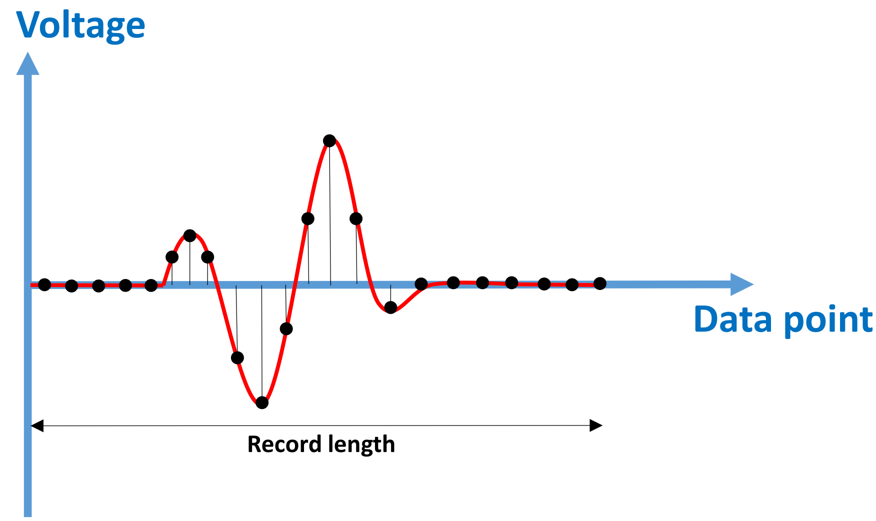


Figure 9 Demonstrate a segment of the signal in digital form

A segment of the signal after acquisition derives 1D array of data. For applications use amplitude based techniques, the maximum value of the absolute 1D array will be achieved and used as a parameter to descript the properties of the interface it interacted with. A further analysis could be conducted in Fourier transform. The product of Fourier transform is a complex function include two primary component: Real and Image. A given real part reveals frequency information of the signal and imagery provides phase information of each frequency component. The equation below is used to conduct discrete Fourier transform:

$$X_k = \sum_{n=0}^{N-1} x_n e^{-j\frac{kn2\pi}{N}}$$

Where N is record length or the total number of data points in the 1D array and n is the order of element in the 1D array. The step between the frequencies is $\text{Resolution} = \frac{SamplingRate}{\mathrm{Re}\,cordLength}$. In theory, the factor k starts from 0 to (N-1). But due to Nyquist limit: (sampling rate/2), the frequency components that over the limit will be the mirror of the component in the front. X_k is complex values in form of A+jB. Intensity values and phases value can be calculated as:

$$Intensity = \sqrt{A^2 + B^2} \quad ; \qquad phase = \tan^{-1}\left(\frac{B}{A}\right)$$

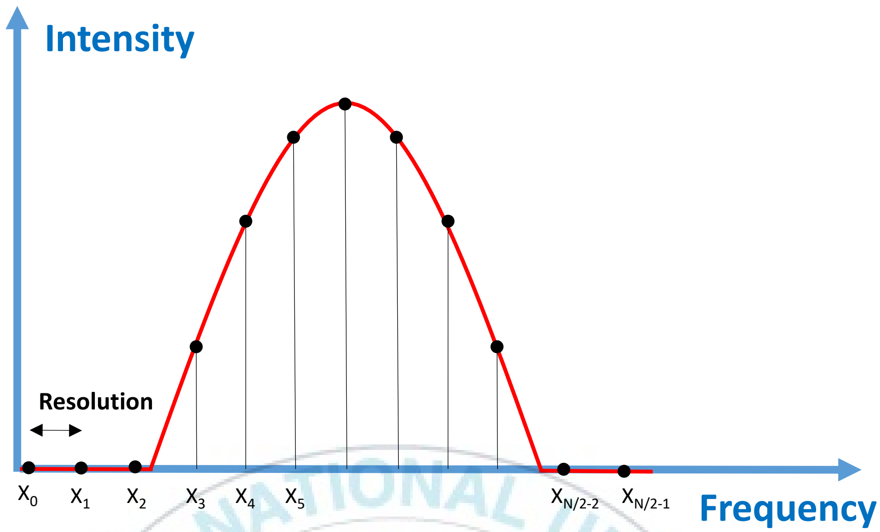


Figure 10 Demonstrate data in frequency domain

Chapter 2. Materials and methods

1. Acoustic waves interaction

As mentioned in the previous chapter, an incident wave hits an interface, it would create two components: transmission and reflection. The transmissive component keeps propagating to next medium. This time, the transmissive component plays a role of an incident wave. The same effect will occur if it keeps hitting another interface and follows the percentage of energy division.

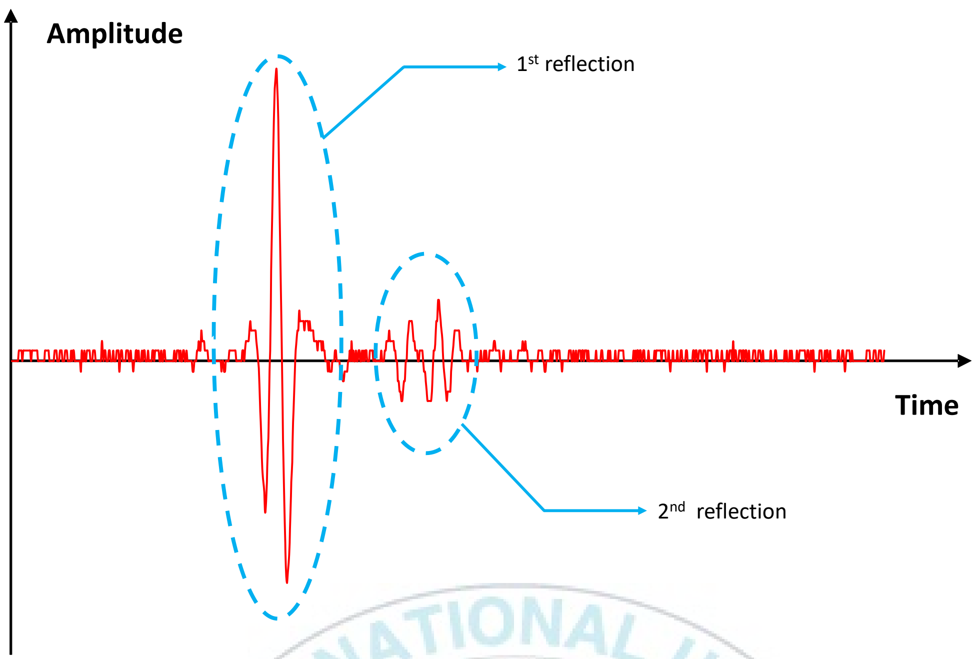


Figure 11 Demonstrate a ultrasonic signal of two reflection

As mentioned in previous parts, the acoustic signal emitted from transducer is in range of frequencies (not single frequency) and imagery production of Fourier transform is phase. When there are more than two reflections, they may have some frequencies being in phase or out phase which result in constructive or destructive interferences. Because each frequency have different wavelength, it means that the second reflection would have different phases at different frequencies. As the result, the frequencies that make their phases reversing would be reduced magnitude in frequency spectrum.

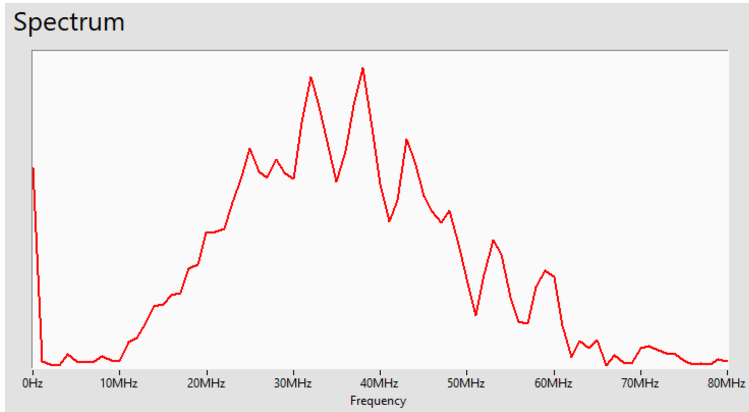


Figure 12 Demonstrate Fourier transform of two reflections which show the reduction of reversed phases

In acoustic propagation, frequencies keep unchanged when it pass through mediums. Depending on speed of sound in that medium, the corresponding wavelengths can be determined. By this way, applications could be used to measure the thickness of an object or the distance of two interfaces when acoustic speed of material is known.

$$f_n = \frac{nc}{2d} \quad (n=0,1,2,\ldots)$$

The equation shows according to a certain speed of sound and a certain thickness, a multiple frequencies could be obtained by analyzing their reduction or disappearance on the spectrum.

2. Calculation model

The Term of SAM which stand for scanning acoustic microscope. In this technique, a single-element transducer or an array transducer is used to scan over the area which results in 3D data of the object and can be transferred to a 2D image to provide information of a certain layer.

In the study, a “d” thickness of a slice of tissue is put on the top of a substrate called sliced glass which commonly used in specimen observation under microscope. A single-element transducer will scan over the area. Signal analysis will be conducted to calculate thickness and speed of sound of every pixel to give a 2D image.

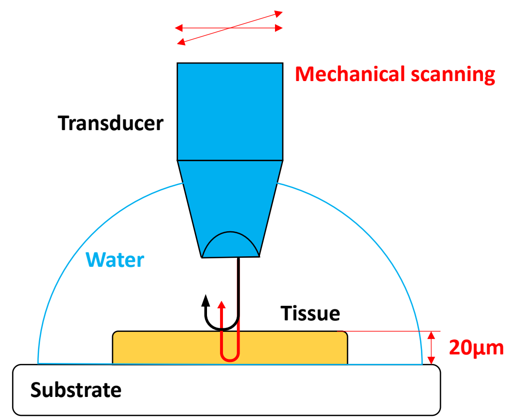


Figure 13 Demonstrate the schematic of tissue characterization by scanning acoustic microscope

The calculation will be perform in frequency domain by Fourier transform. As mentioned above, power spectrum can give some information of two reflections which contains thickness and speed of sound of material. Although the specimens is sliced at a certain parameter of thickness by a dedicated microtome but an error in thickness might be exist. So, the thickness recalculation is needed and nominal thickness parameter set on microtome would play a role of reference values.

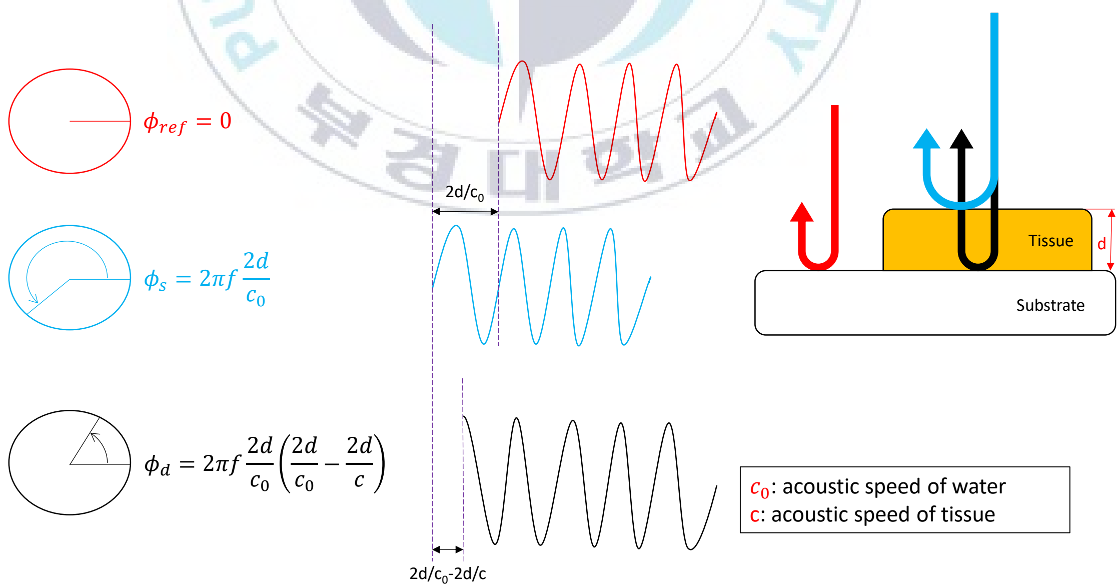


Figure 14 Demonstrate phased distribution in the calculating model

An ideal model is given to make it easy to understand phased analysis processes. The model in the figure above just considers for a single frequency. The case tissue completely attach on substrate is considered. All receiving signal will be perform Fourier transform.

Suppose that phase on the surface of the substrate is used as reference phase. Phase in the front surface is Φ_s and phase in the back surface is Φ_d. Due to the fact that reference phase is zero, the front surface is equal to the time travel round trip according to the thickness d with speed of sound of coupling medium (water is used in this study) and angular velocity according to a frequency f. By choosing positive direction upward, phase of rear surface is calculated from the front phase by adding the similar relation but speed of sound in tissue.

$$\phi_s = \phi_m + (2n-1)\pi = 2\pi f_m \frac{2d}{c_0}; \qquad \phi_d = \phi_m = 2\pi f_m \left(\frac{2d}{c_0} - \frac{2d}{c} \right)$$

In the relation above, f_m and Φ_m is practical variables because the model is just consider for a single frequency. In practice, a transducer have a bunch of frequencies which give corresponding phases. So, f_m and Φ_m need to be obtained. Thickness and speed of sound also derived by the following equation:

$$d = \{\phi_m - \phi_{ref} + (2n-1)\pi\} \frac{c_0}{4\pi f_m}$$

$$c = \left(\frac{1}{c_0} - \frac{\phi_m - \phi_{ref}}{4\pi f_m d} \right)^{-1}$$

The term of adding multiple odd number of Pi have a special meaning to determine f_m and Φ_m. Noting that we need to look for a particular frequency and its phase in order to apply to above equation. To complete a period of oscillation, it means that phase according to that frequency has changed 360 degrees or two Pi. The special frequency will be detectable when two reflections from front and rear faces of tissue will be out of phase and the thickness so that is equal to multiple odd number of wavelength inside it.

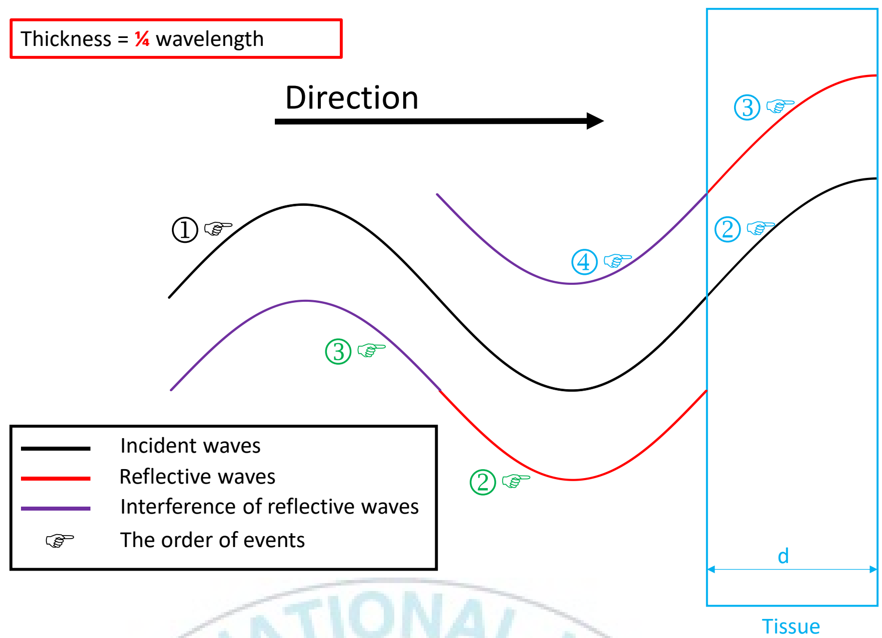


Figure 15 Demonstrate the case of thickness according to a quarter wavelength

In this instance, considering a frequency so that the thickness of tissue correspond to a quarter of its wavelength in that tissue. When an incident wave hit to the surface of tissue, it is divided into two groups those are reflective group and transmissive group. The transmissive group keep propagating inside the tissue until it approach tissue's backside and reflect back, go out tissue and head to transducer. Meanwhile reflective signal also flies back to transducer but take in advance compared to transmissive signal. As it is shown in purple color, the two signals are out of phase which result in destructive interference. The phased difference is 180 degrees of Pi. On the spectrum, the frequencies qualify the conditions that make it two reflection being out of phase will appear as valley points which is also demonstrated in Figure .

Remember that transducer's axial resolution is about half of wavelength. According to a thickness is equal to one fourth of wavelength or n=1 in thickness calculation equation, it is unacceptable. So we will consider the next case, n=2. This is in accordance with thickness being equal to three fourth of the wavelength. The phased difference is 540 degrees of three Pi.

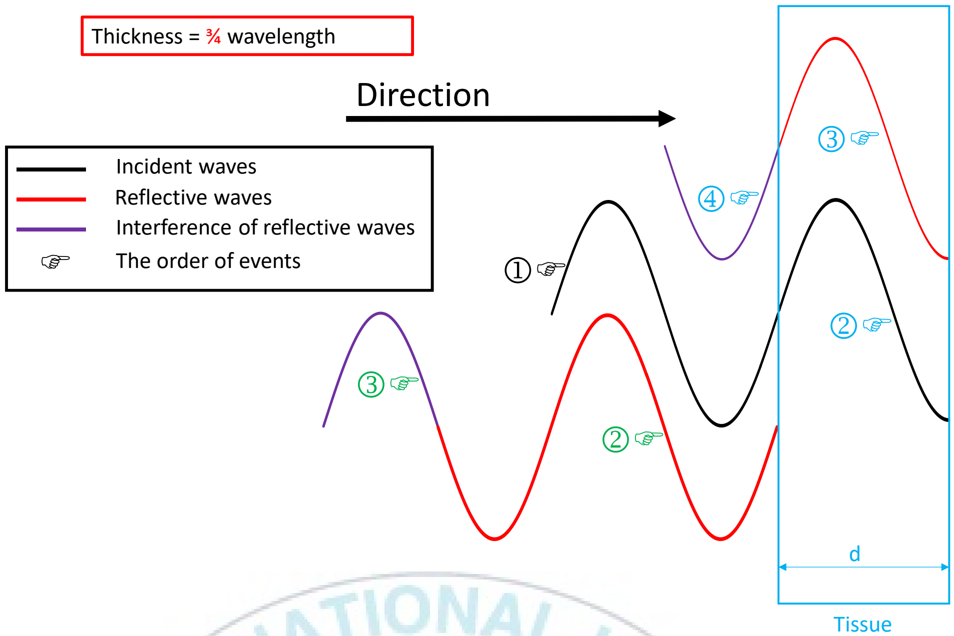


Figure 16 Demonstrate the case of thickness according to three fourth of wavelength

In this case, we also derive the two reversed signals. Generally, if transducer's bandwidth is wide enough, we can encounter multiple valley points on the spectrum with a consistent frequency step. In this study, due to transducer's characterization, n=2 will be sufficient to use in the calculation.

3. Thickness and speed of sound determination.

In the study, a nominal center 60 MHz transducer is used. Its bandwidth spread from 30MHz to 80MHz. Based on previous research, acoustic speed of tissue fluctuates from 1,500m/s to 1,800m/s. It results in the range of measurable thickness from about 11µm to 25µm.

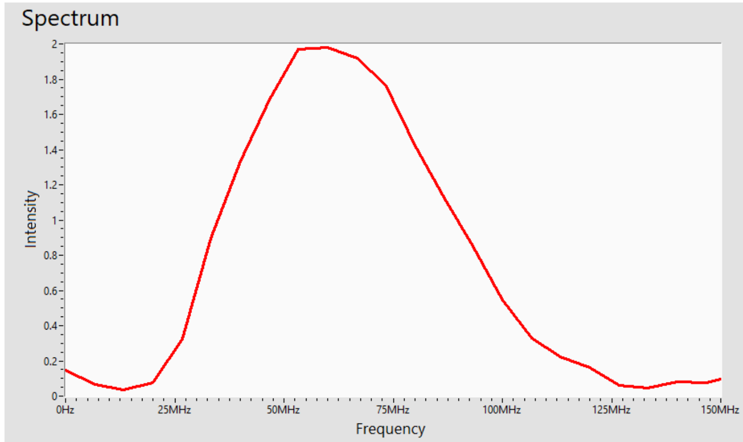


Figure 17 Indicate spectrum of the transducer with its signal reflected from glass surface

For those reasons, a 20μm slice of tissue which is cut by a microtome (Leica brand name) will be used in the experiments.

Initially, the single-element transducer scan over the area. A 3D volumetric data is achieved. Each column of the 3D data represents to data of one pixel (A-scan). A horizontal line of the columns represent to data of one line on scanning (B-scan).

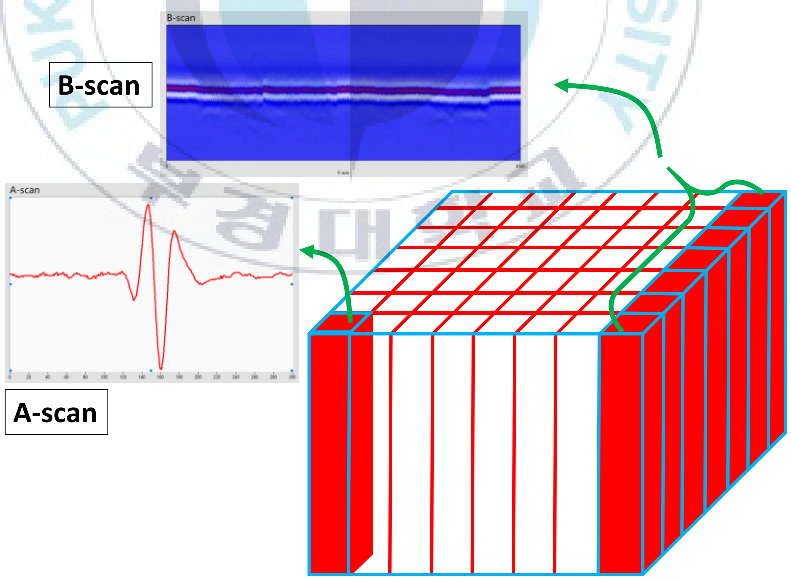


Figure 18 Demonstrate the meanings of A-scan, B-scan on 3D volumetric data

To display an 2D image which its each pixel brings information of thickness and speed of sound, the calculation for every point is conducted.

The experiment is set up so that the first horizontal line and the first vertical line of the scanning is without tissue which is used to determine the tilting of the sample stage. By the way, the tilting of the sample stage is manually adjusted to minimize as much as possible.

The first step in the calculation is to perform Fourier transform the 3D volumetric data into frequency domain which results in achievement of two 3D volumetric data, frequency part which is real part of the complex function and phase part which is imagery part of the complex function. Then, the spectrum of a certain point without tissue is chosen as reference spectrum which is used to normalize other pixel's spectrum (suppose that the spectrum of every point without tissue is the same).

In the next step, the calculation runs over each point. At each point, its spectrum information would be extracted to normalize with the reference frequency by subtracting each other. The necessary of this step due to the fact that although at the specific frequency, the two reflections is out of phase. But magnitude of the first reflection is dominant by the second one. That's why the elimination is not significant as well as it is difficult to detect an expected valley in its spectrum. The dominance of the second reflection over the first one due to their acoustic impedance. Water's acoustic impedance and tissue's acoustic impedance is similar but tissue's and glass's.

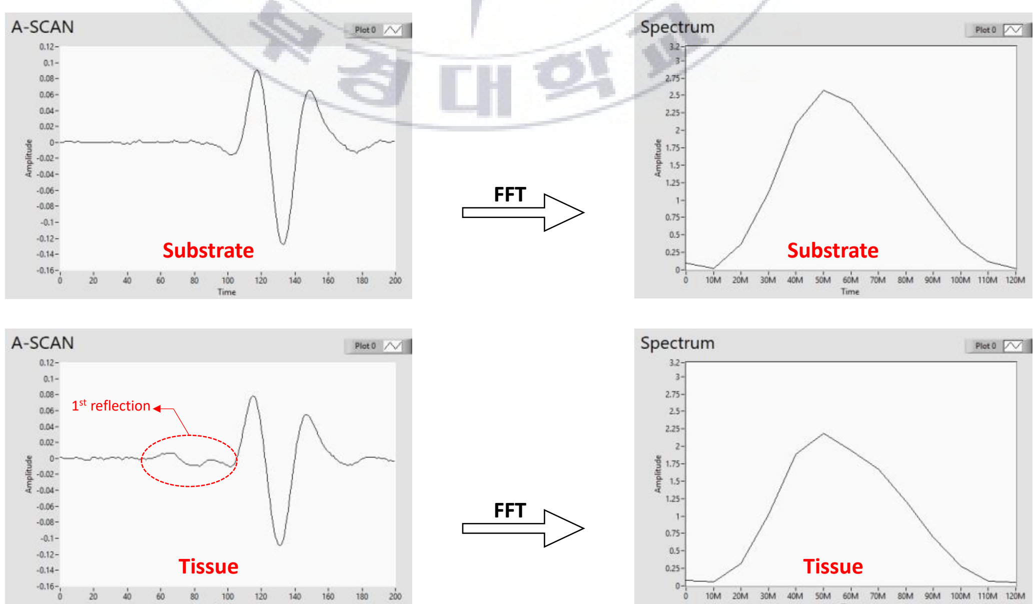


Figure 19 Demonstrate typical signals reflected from tissue and substrate

After normalizing, the nominal spectrum will appear single or several peaks according to single or several valleys in its original spectrum because when subtracting, the biggest reduction is the longest distance in comparison with reference spectrum. In the nominal spectrum, an algorithm will run to detect the highest value. Its frequency value of this highest value in amplitude will be used as value of f_m. By the given frequency, correspondent phase will be achieved and will be used as value of Φ_m.

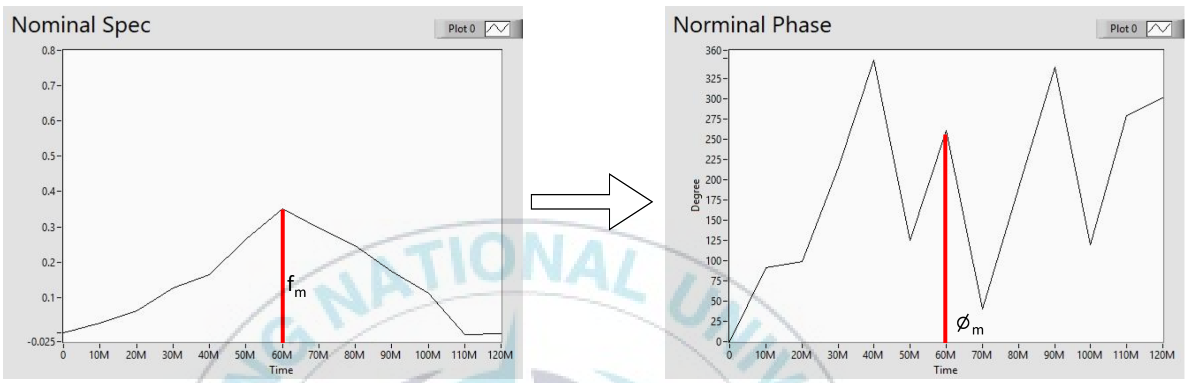


Figure 20 Demonstrate the detection of f_m and Φ_m

Reference phases are the phase of specific frequencies without tissue. Although minimal tilting adjustment has been conducted, phase change due to tilting remainder and mechanical issues is undeniable. So, reference phases at different points are different and its directly measurement is almost impossible. An interpolating algorithm has been used to obtained relatively reference phase of corresponding position. The detail of the algorithm would be descripted in next section.

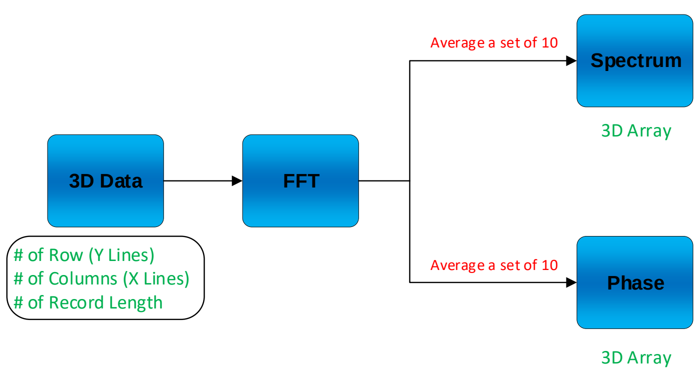


Figure 21 Demonstrate the diagram for data processing-part 1

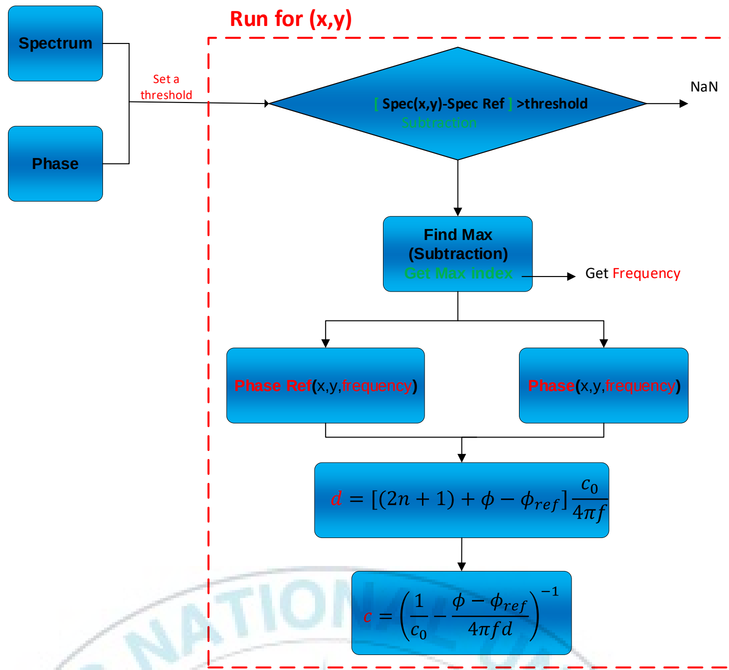


Figure 22 Demonstrate the diagram for data processing-part 2

The speed of sound of water will be measured after each scanning to minimize the errors due to change of surrounding temperature between each scanning.

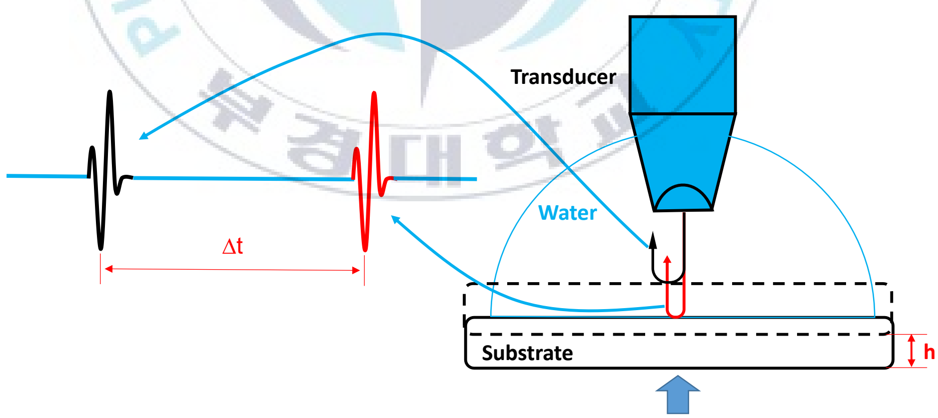


Figure 23 Demonstrate the diagram of acoustic speed measurement

The calculation of speed of sound of water is performed by determining time lag between two reflections at two different positions in Z axis. Initially, after the scanning has finished, the container that includes sample is put on a stage of vertical linear motor. Then, the reflection from the glass (without) tissue is marked. Next, the vertical motor will move the glass up to a height of a known h. The second reflection is also marked. The difference between the reflections in time domain is the time that the speed of sound of water travels round trip of h.

$$c_0 = \frac{2h}{\triangle t}$$

By obtaining the values of Φ_m, Φ_{ref}, f_m, c_0, and n=2, the thickness and then speed of sound of discrete pixels are determined.

Chapter 3: System design

1. Mechanical components

The system is designed to use mainly experimental parts available. The mechanical system is primarily divided into three parts: linearly scanning part, integrated microscope and tilting adjustable stage.

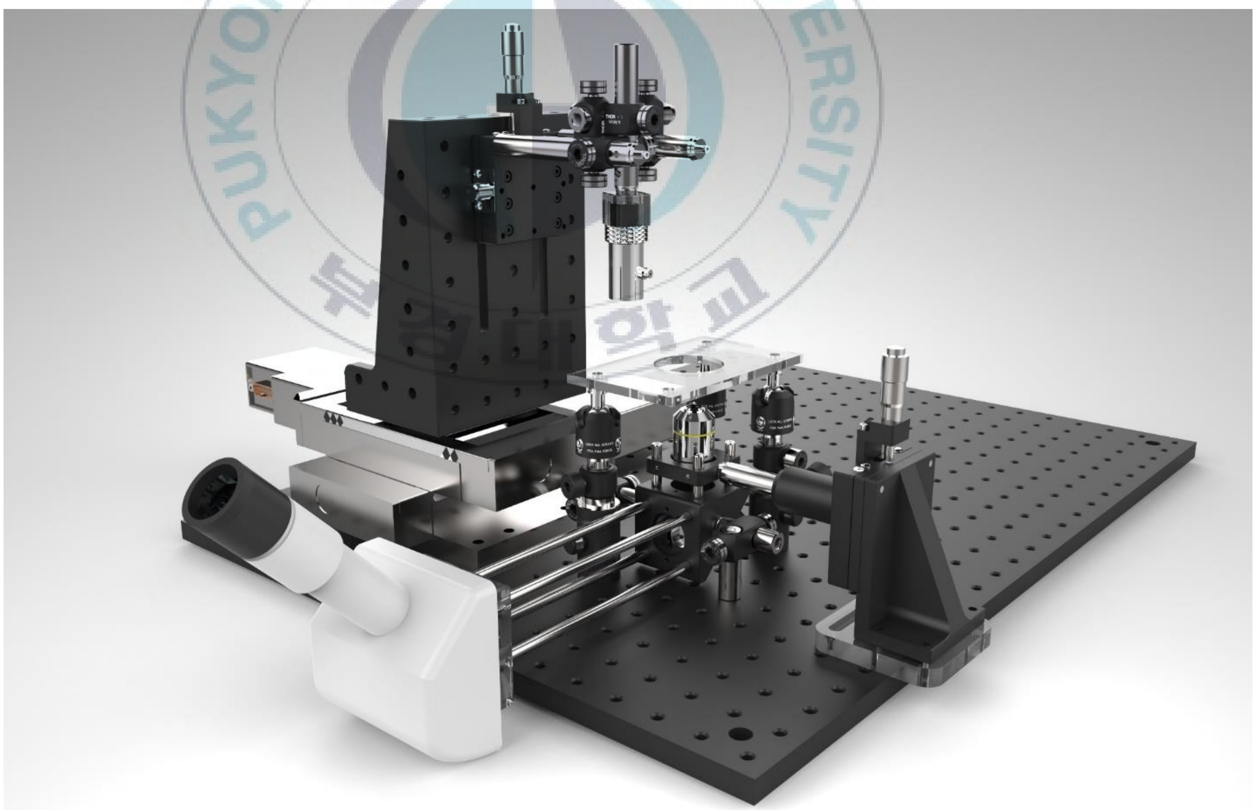


Figure 24 Scanning acoustic microscope for tissue characteristic

The linearly scanning part is made of two Newport linear motor mounted on each other. The top motor is the main scanning motor which defined as X axis while the bottom one is defined as Y axis. A single-element transducer is set up on a self-constructed holding cluster to keep the transducer stable and height-adjustable.

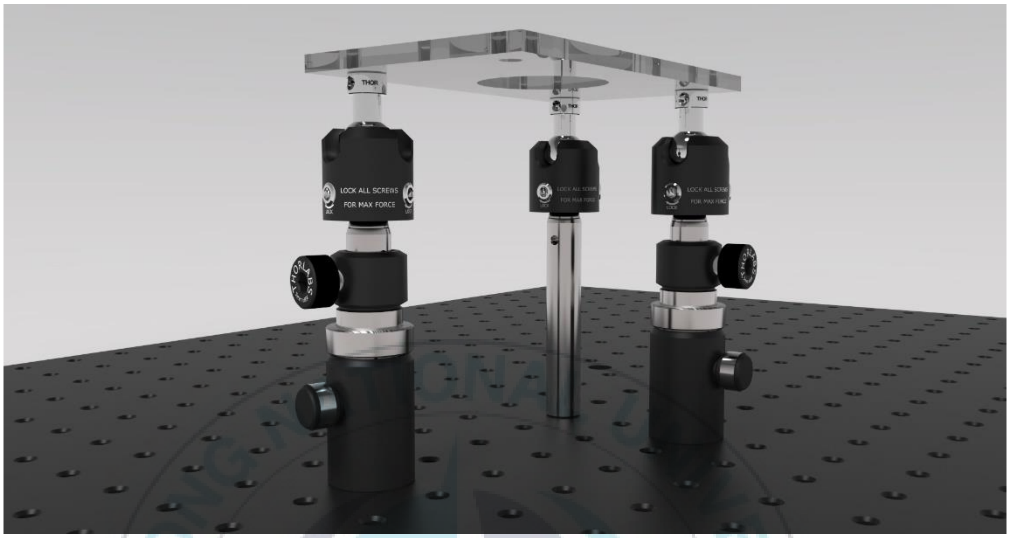


Figure 25 demonstrate tilting adjustable stage

In order to minimize the tilting of the stage, a design of three posts stage was made which is capable of height adjustment at right top corner and left bottom corner but the rest post is fixed at vertical movement. In another word, one post is stay still and the other two posts that one adjusts tilting along X axis. The height of the two post are controlled by a translating post holder (Thorlabs PH2T). When adjusting the height of two vertical adjustable posts, the stage is getting tilting an angle in comparison with the post. For the issue, a locking ball is added between posts and the stage. The function of the locking ball is to keep a connection between posts and the stage. The stage is made of plastic plate which is fabricated by a CNC machine to make shape and a circular hole at the center for microscope checking.

The method to measure the thickness of sample based on phases comparison between top and bottom of the sample as mentioned previously, so the sample have to be completely attach to the sliced glasses. Although the sliced samples were handled with care, but the slices is easy to detach from glasses during chemical processes. As the result, high values of thickness might be obtained.

A special property of tissue is that its naturally negative charge. To minimize the detachment effect, positive charge has been used. By the different charge, tissue would be stronger adhesion to glasses.

To checking the attachment of tissue, a microscope is integrated to the system. The microscope is designed to locate beneath the sample, making it more convenient for checking the attachment while transducer can scan the sample from the other side. The microscope includes three main components: an objective lens, a right angle mirror and an eyepieces. Light from the bottom of sample is collimated by the objective lens with magnification of 10 times. After that the parallel light beams is oriented 90 degrees for comfortable view by the right angle view. Eventually, the eyepieces which is utilized by an old microscope (10x in magnification), focus the collimated light to user's eyes and give a final magnification of 100 times. The three components are assembly on 60mm cases (Thorlabs) to make it compact. To make the sample to be focused, the objective lens's case is mounted on a vertical cluster which is carefully aligned to case's axis.

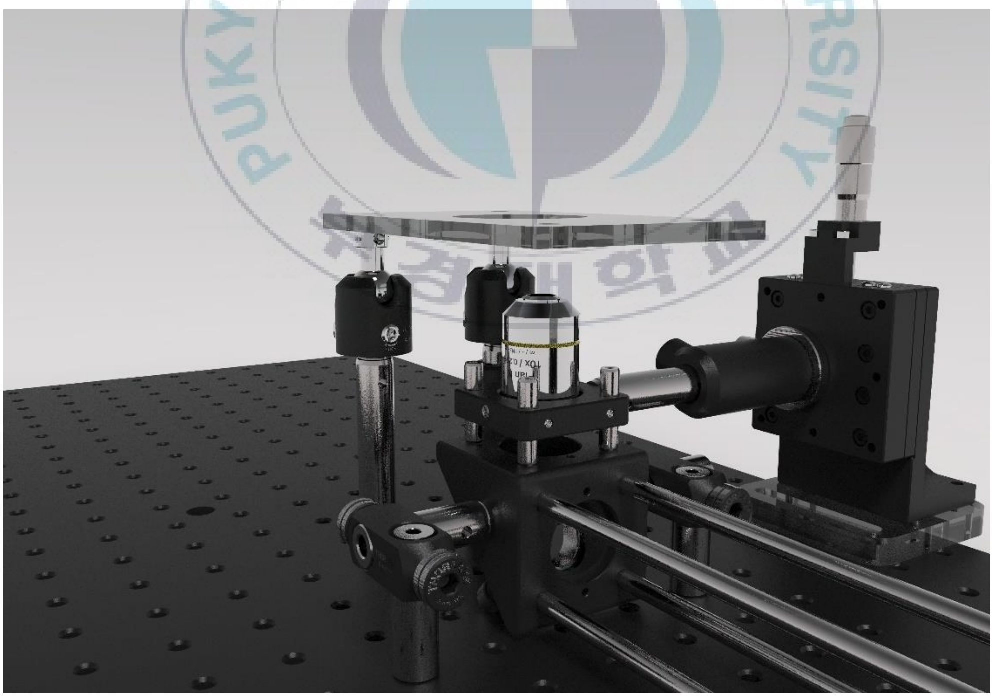


Figure 26 demonstrate a modified view of the microscope in the system

2. Electrical components

The heart to the system is an application which is coded on Labview platform. An PXI has been used as controller. NI-5152 digitizer has been boosted to 2Gs/s(acquire data at rate of two billions sample per second or 0.5 ns for one sample) to improve resolution in phase domain. According to the typical interference frequency around 70 MHz which is equivalent to 16 ns for its period. It means that the step size in phase domain of the digitizer is 10.8 degree.

As digitizer sampling at its internal clock, the clock runs continuously and when it receives a trigger event, its timing could be error within one cycle of the clock. It is called phase noise or jitter and in practice it usually more than one cycle. In other word, the results could be error in multiple of step size or multiple of 10.8 degree, for example.

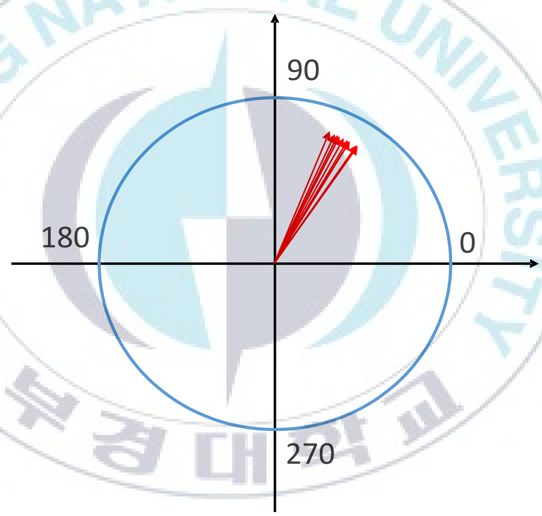


Figure 27 Demonstrate phase noise in phase domain

To reduce the error or improve the accurate of the system, a method called ten times average sampling is applied. By the method, for each data point, it is sampled ten times then the values are average to get it central distribution. In theory, transducer has to acquire ten data for one point before moving to the next. This makes challenge to mechanical system when it musts run discretely. To solve the problem, high trigger frequency is applied to make digitizer acquire ten data of points are supposed to be one. The scanning motor scans sample at speed of 2 mm/s and step size sets up at 20 µm it generates a trigger frequency at 200 Hz. Transducer's pulser runs in internal mode at frequency of 20 kHz. It means the ten points of data would be completed in a distance that is equal to 1/100 of the step size which is acceptable to consider them as a single point.

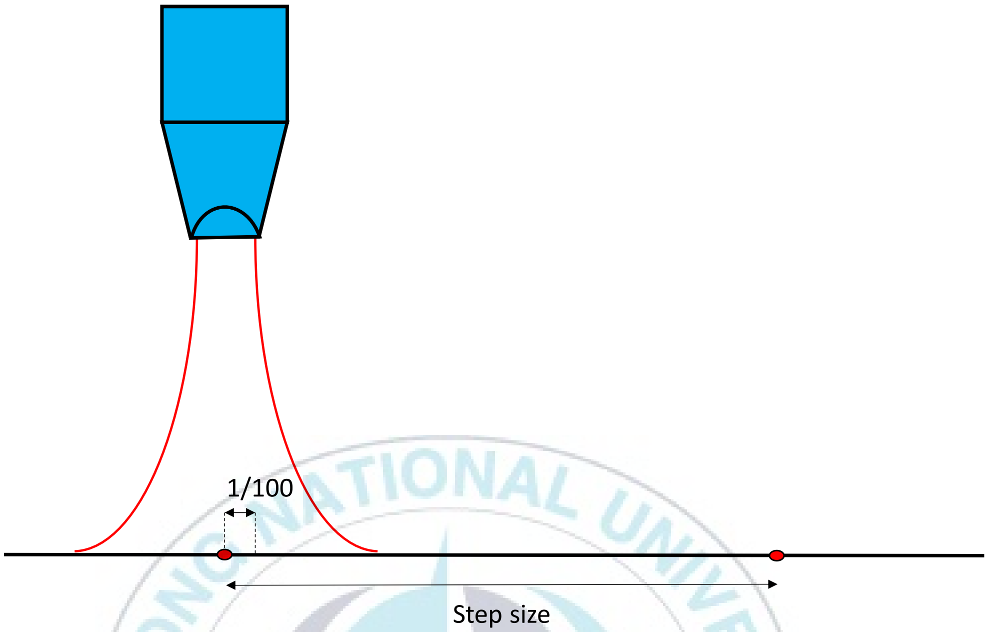


Figure 28 Demonstrate the distance to finish acquiring ten data in comparison with step size

As usual, a pulser excites its transducer in external trigger mode. The motor that the transducer is mounted on would create trigger by an encoder integrated in the motor. This means that trigger frequency would fluctuated around its correspond velocity due to acceleration and deceleration but the same distance between steps size. In this study, 10 times sampling would be performed by synchronizing internal trigger of pulser and external trigger of the motor.

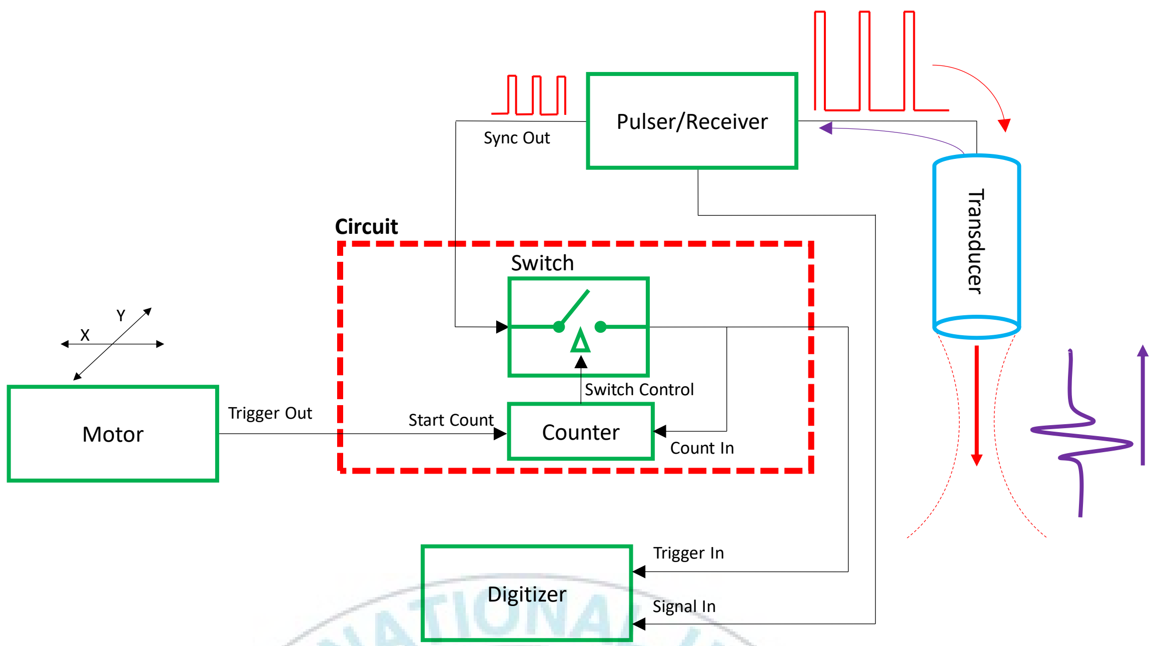


Figure 29 Demonstrate the diagram of synchronization of internal and external trigger

The synchronization is conducted by an external printed circuit board. The main function of the board is to block the internal trigger line activating the digitizer under the control of a switch. The controlled pin of the switch is connected to a counter. The counter has a count in line which links to the output of the board to count the number of pulses go through (ten pulses for this application) and a start count pin connects to trigger output of the motor.

Initially, pulser excites transducer at frequency of 20 kHz. Signal line of the pulser connects to digitizer signal input but the digitizer does nothing without trigger event input. The synchronize out from pulser which basically connects to trigger input of digitizer via the switch is block at the first phase. Counter waits for a rising edge pulse from motor. Since then it open the gate of switch to let trigger from pulser through. The output of switch has a feedback line to count in of the counter. The function of the feedback line is that count enough ten pulses pass through then close the gate. From now on it would wait till a new trigger from motor. The trigger pulses get through the switch would synchronize to signal from pulser.

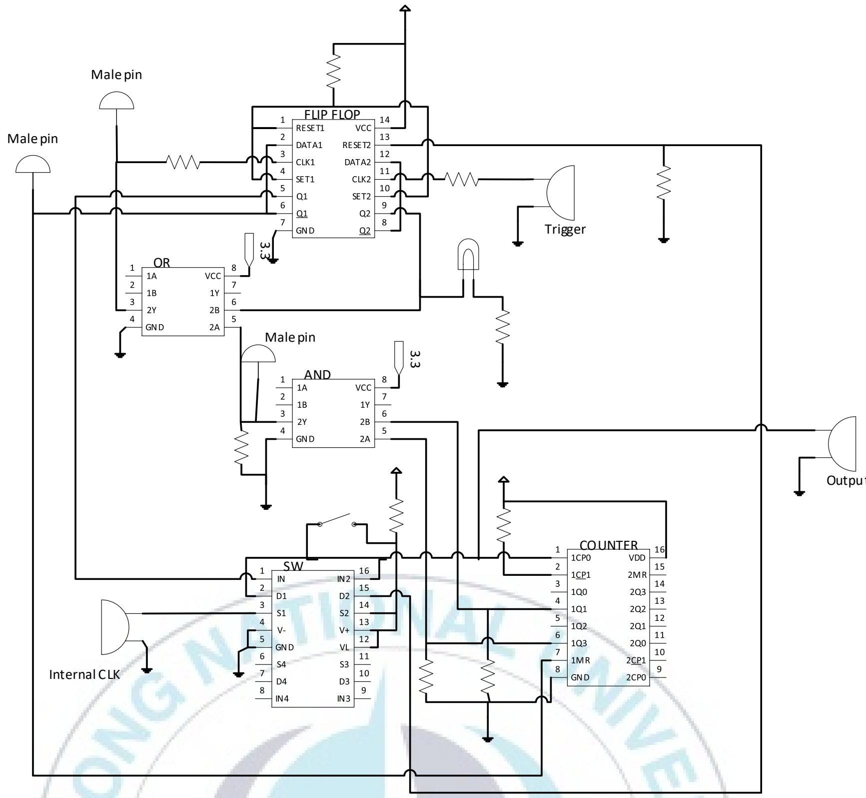


Figure 30 Diagram of ten times sampling external circuit design

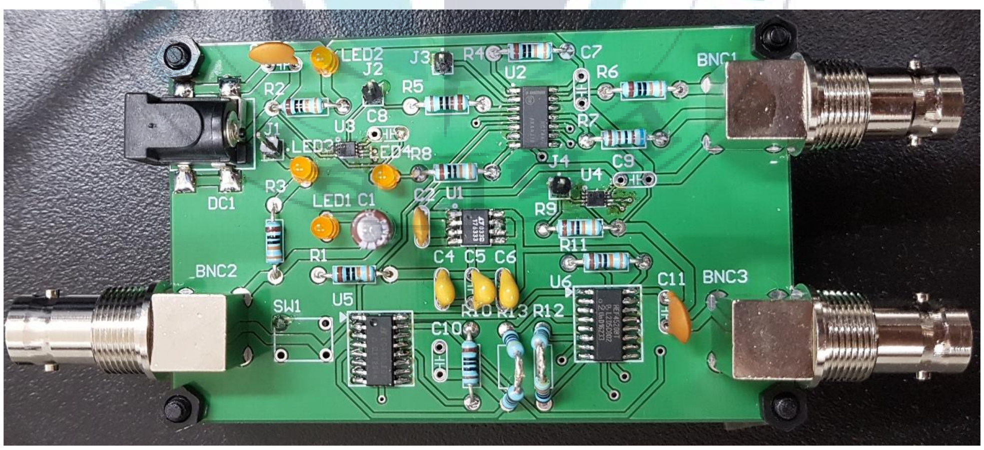


Figure 31 Printed circuit board of ten times sampling design

Counter in the design is and binary counter IC. It has four output pins representing four bits of a binary number. The state of ten according to 1010 means that the first and third pins would be high at the state of ten. The two pins connect to and AND logic gate to output a signal to deactivate the switch. Two flip-flips gate has been used to maintain the state of active for counter and switch. The switch using in the circuit is actually a limiter which mean that it reduces amplitude of pulses from input, not completely block them. So, to avoid the limited pulses trigger digitizer, the trigger level from digitizer was set at level higher than amplitude of the limited pulses.

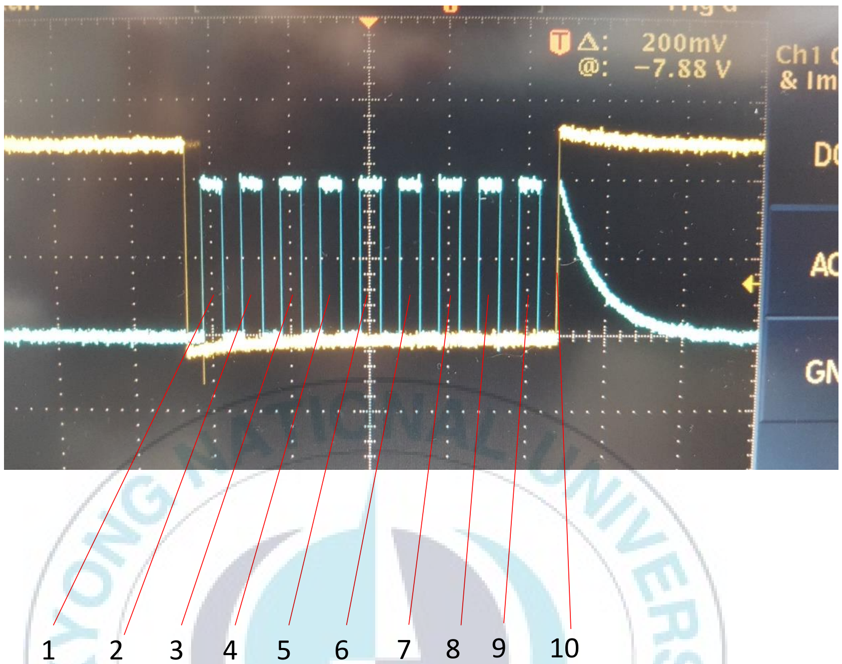


Figure 32 Demonstrate ten pulses has been encounter when an active signal set low

The figure below shows results of discrete ten data in comparison with its average acquired with a static point (when transducer stand still) which descripted on the left versus four average data of ten.

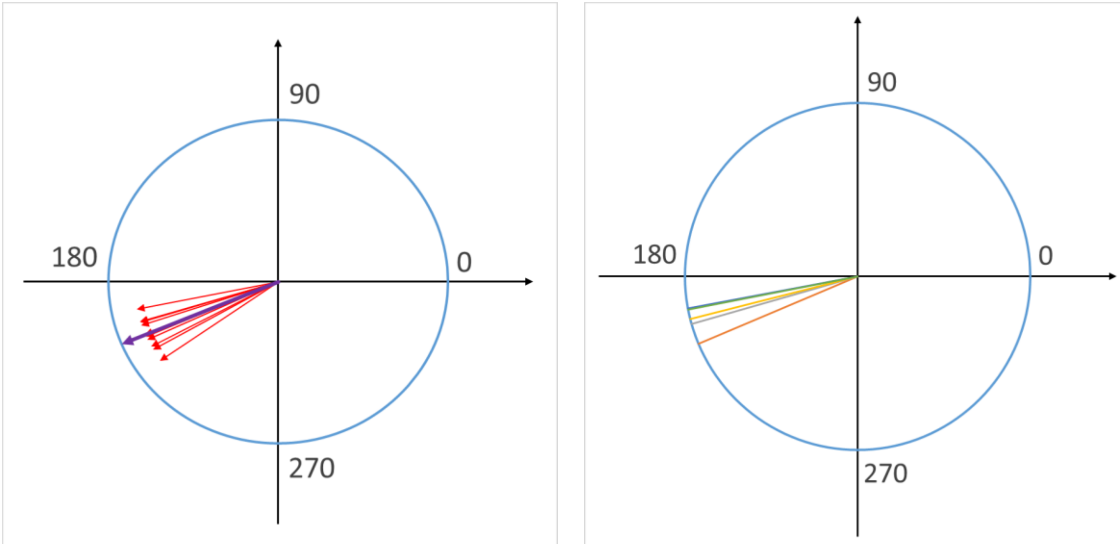


Figure 33 Demonstrate reduction of phase noise effect after average.

3. Technical issues

The ten data of one point after acquiring would be averaged. The result represents for one point of data. With the resolve, phase noise has been improve. Besides, due to phase domain characteristic phase ranges from 0 to 360 degrees. Whenever phase of a signal of a certain frequency distribute around 0 degree, the array of ten data includes values around 0 and around 360 degree which result to an average of significant difference from values of the elements. In this case, an algorithm has been applied to resolve.

The algorithm basically search for a sudden change in the group of ten. If the changes between elements are greater an threshold (we set the threshold here is 180 degrees), the program categorize the group into the case. Then, elements are higher than the threshold or 180 degrees would be supposed to minus values by subtracting 360 degrees. After supposition, elements would be averaged. If the result of average is negative, an implementation is need.

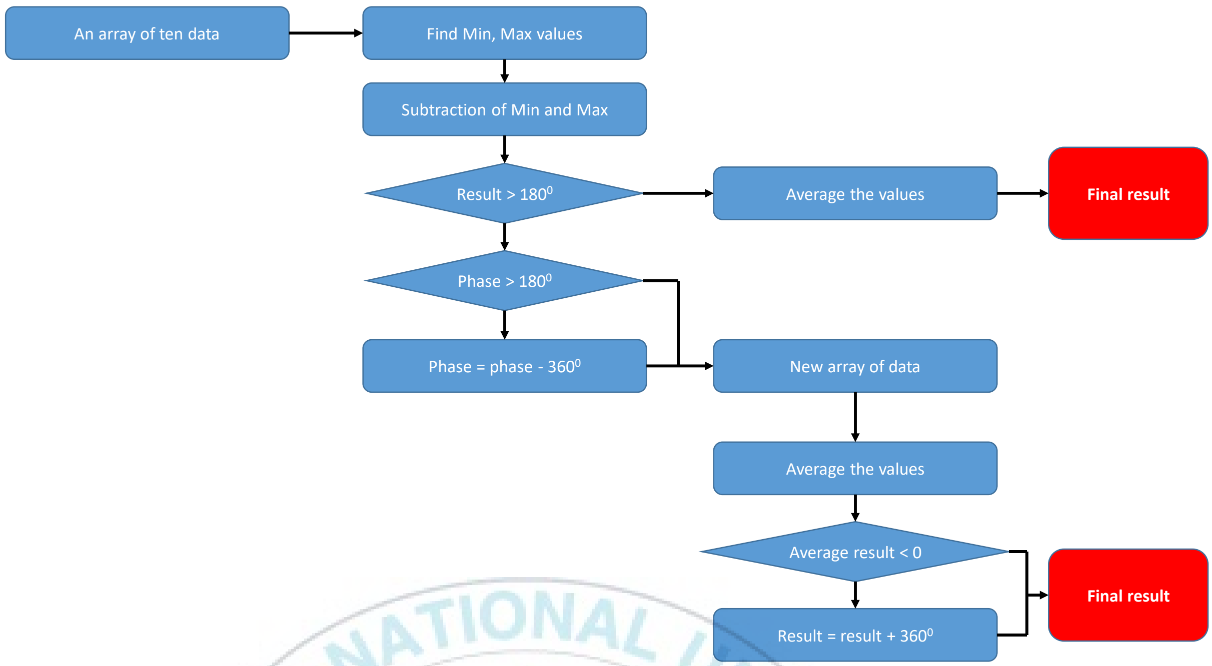


Figure 34 A flow chart for fixing error of average result due to phases distributed around zero

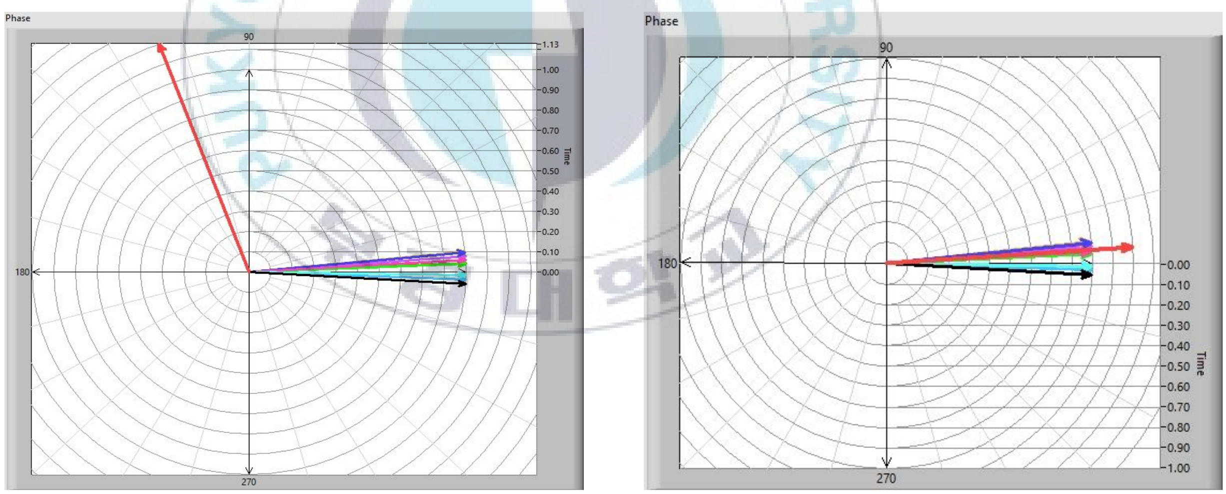


Figure 35 Demonstrate a group of ten phases distributed around zero before and after algorithm applying

Although tilting adjustment has been applied, unavoidable mechanical issues generated by linear scanning motors which make change distance between transducer and sample. As the result, unexpected phase change makes calculation of the program more challenge. When phases along X axis fluctuated around zero or 360 degrees, their values becomes significant difference even though their real phases just slightly changes.

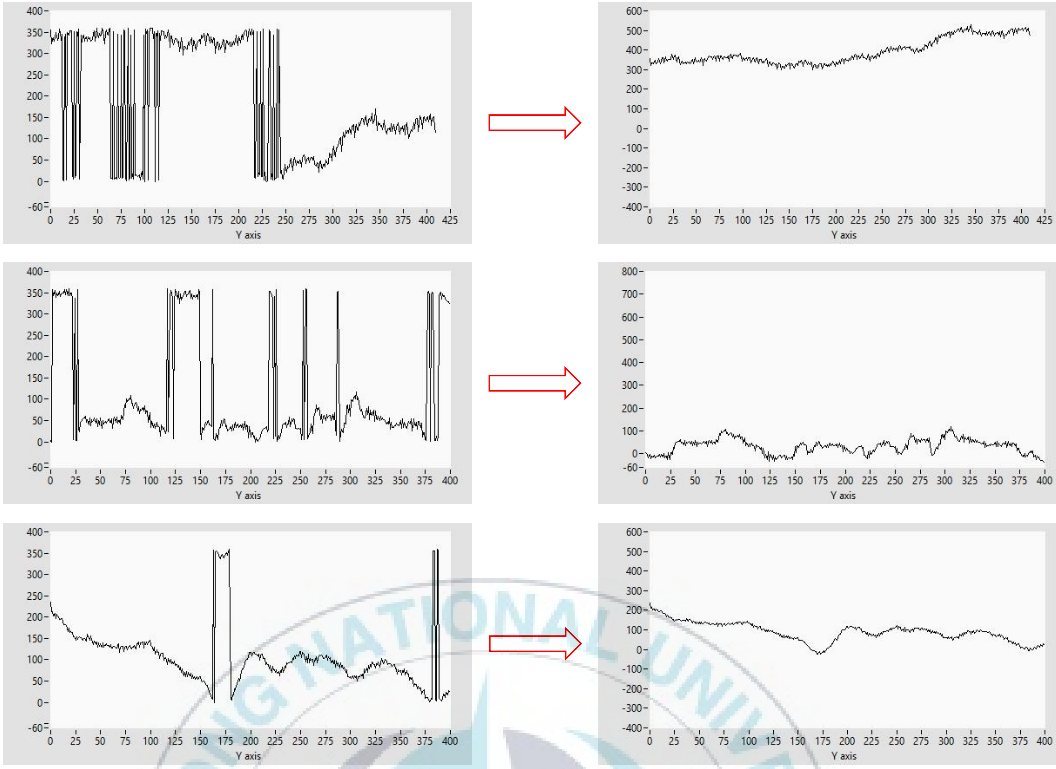


Figure 36 Demonstrate different patterns of unexpected phase changes before and after fixing

The figure above shows three typical pattern of unexpected phase changes. The fixing algorithm is basically average the whole line of data in order to choose the dominated side. After that algorithm scans all data of the line. Whenever subtraction of the current position and its next one over a threshold (200 degrees has been chosen) then one of them would be reversed its value by add or subtract to 360 degree. Although the values after reversing would be out of standard (minus values or over 360 degrees), but their relative values compared with other elements in the line would be acceptable in calculation.

The last issue is to find reference phase for each point. As mentioned in previous section, thickness and speed of sound calculation need to obtain reference phase. Reference phase is a quantity of phase measured in place of calculated point without tissue. A direct measurement of such that thing is a significant challenge. So, a solution to determine phase reference called phase interpolation has been apply. Phase reference is a value which obtained after performing fast Fourier transform. The values reflect relatively the distance between transducer and reflective interfaces. In a perfect plan, this can be done by applying a plan equation. Assume that substrate is a plan. Then, the equation could be made by obtaining phase values of three corner point and its coordinated position. To get know phase of any point in the plan, coordinated positions input are required.

In the study, there is another factor made the sample plan imperfect that is worn shaft of scanning motors. On the current motors, there are curves on scanning route make phases unpredictable. In this case, plan equation is inapplicable. Instead of getting three points at corners to make an equation plan, the first horizontal and vertical lines have been used to predict reference phases.

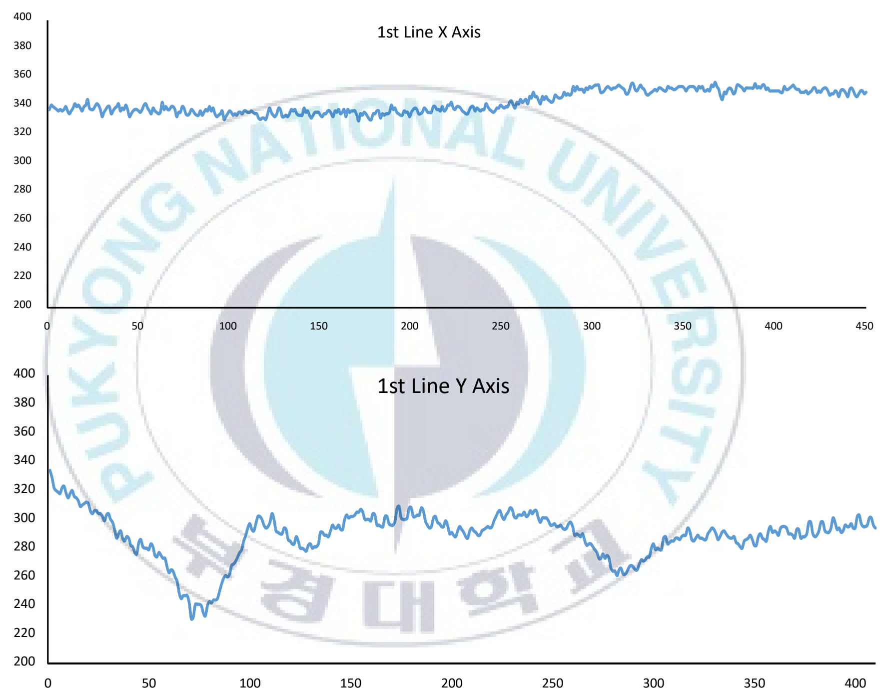


Figure 37 Demonstrate imperfect straight of scanning motors in phase domain at a certain frequency. X axis is known as horizontal line and Y axis is known as vertical line which are extracted from the first lines along X axis and Y axis without tissue

By getting the first line, we can get patterns of scanning lines and we know the patterns repeatable. In this study, roughness of sliced glass is ignored.

$$\boxed{\phi(x,y,f)=\phi_{y_0}(x,f)+\phi_{x_0}(\mathrm{y},\mathrm{f})-\phi_{x_0}(0,f)}$$

The equation above descripts how reference phase at a specific point is calculated. After interferential frequency has been found, it is applied along with the coordinated position to the equation. Φ_{x0} and Φ_{y0} indicate phases on the first horizontal and vertical lines, respectively. The variable x in vertical line means the order element in the vertical line array that we look up its value. The variable y in horizontal line means the order element in the horizontal line array that we look up its value. Keep in mind that the data for first horizontal and vertical lines are a set of 2D array. Each row of the array represents to phase values along that axis at a specific frequency. That is why obtaining interference frequency required.

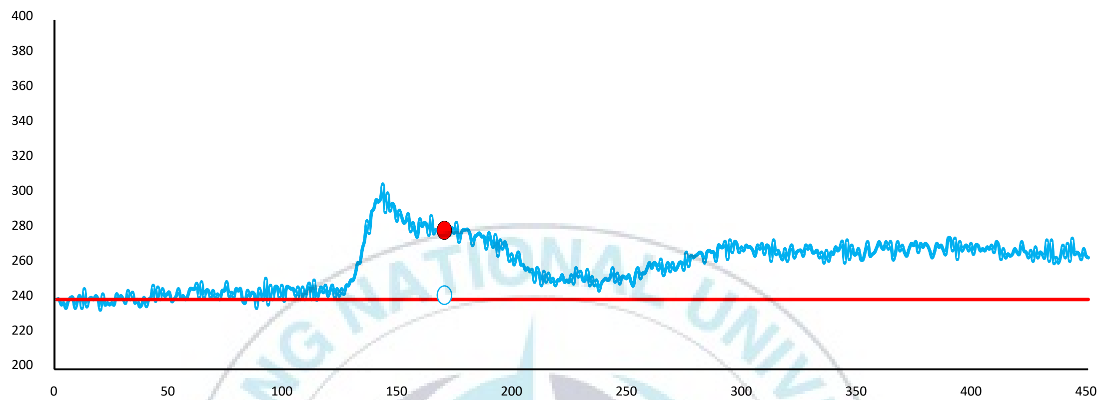


Figure 38 Indicates reference phase calculation at position includes tissue

The figure above shows a calculated values (white point) at a position of tissue (red point). The horizontal red line is used as reference to show an acceptable values of calculation compared to the trend of the base. In the scope of the thesis, a full scale assessment of errors in reference phase calculation has not been conducted. By casually checking, the maximum error of reference phase calculation has ever captured about 30 degrees at 80MHz.

Chapter 4. Results

There are two programs have been made to perform the topic. One is for online acquisition and another one is for offline data processing. The main functions of online acquisition program is primarily configure digitizer and synchronize it with motion control. In the online program, data is stored into TDMS format which have a capability of as high speed storage as binary data and integrated additional information of sample. The program also display A-scan, B-scan, C-scan images which help for alignment and tilting adjustment.

Figure 39 Indicates the interface of online acquisition program

The data after acquiring would be transferred to offline data processing program. In the program, data initially loads to RAM before it is processed. Algorithms and basic information such as speed of sound of water and size of images need to be input. The program is not only a calculating program but is it also for studying and checking phases at every position and showing values of thickness and speed of sound and interference frequency at a desired point and straight pattern of the two first lines. After calculating, color scale for each type of image could be changed to highlight some regions. Finally, images of C-scan, thickness, speed of sound and their raw 2D data could be saved.

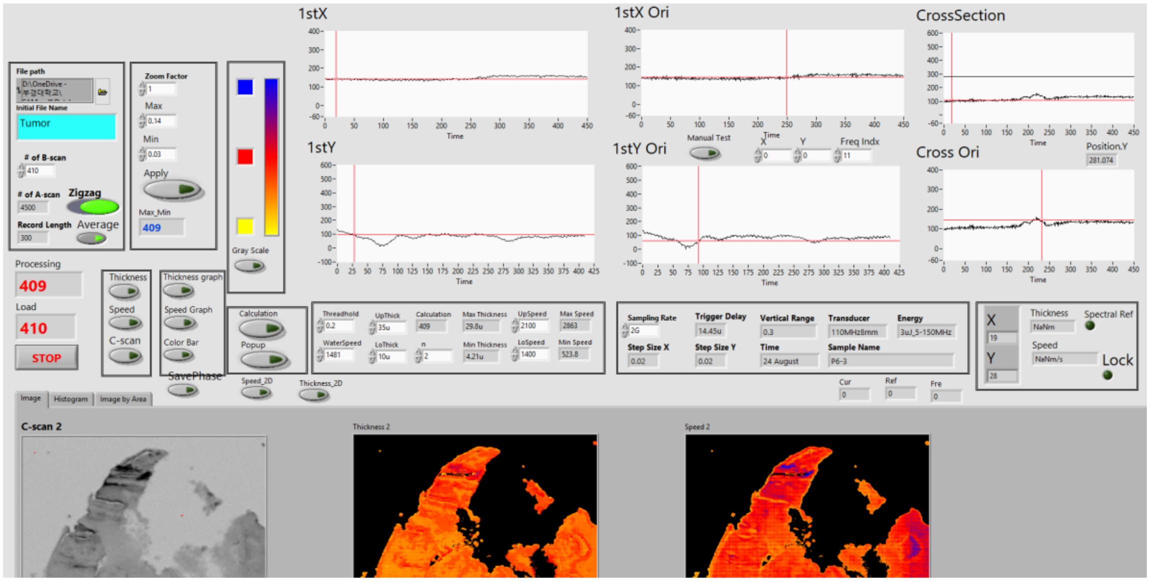
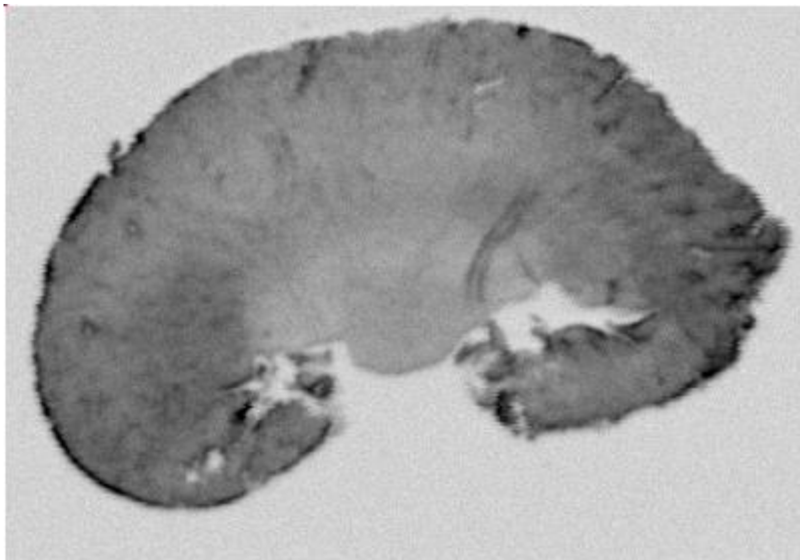
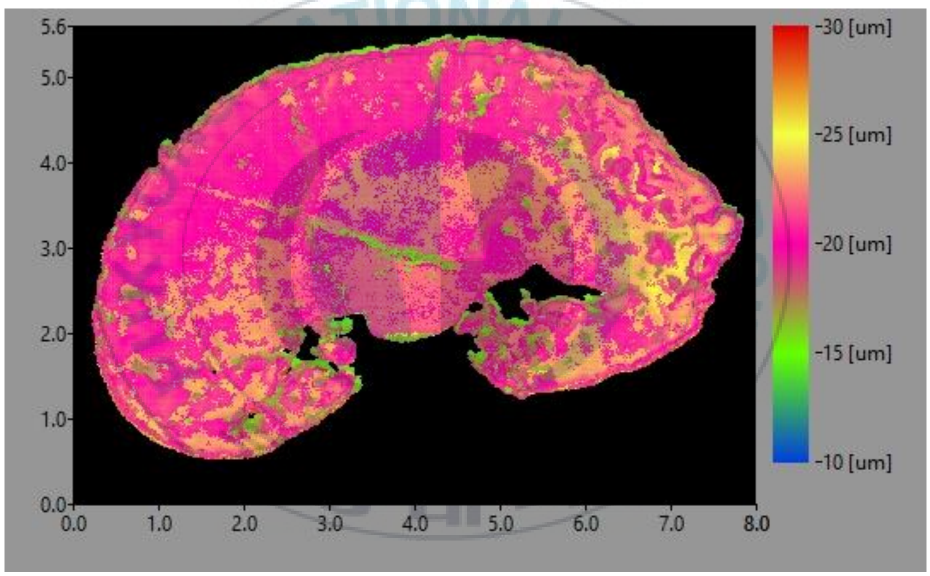


Figure 40 Indicates the interface of offline data processing program

In the offline program, there are six graphs include the first two horizontal and vertical lines which divided into original and fixed groups and cross-section graphs. The terms of original and fixed graphs mean that an algorithm would detect the 1D data if it is the case of unexpected phase changes. The original graphs show phases data before fixing and the other ones indicate phase data after fixing to check suspicious points on the results. The position of points are automatically input by hovering mouse cursor over the point. By doing this, all information such as calculated thickness, speed of sound, interference frequency and phase line that it belongs to would be display on the interface of program.


(a)


5.6
5.0
4.0
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2.0
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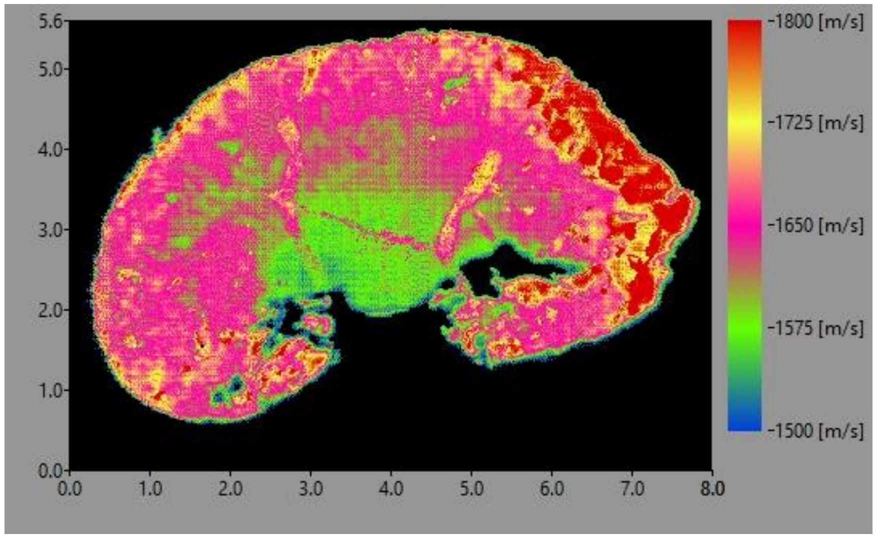
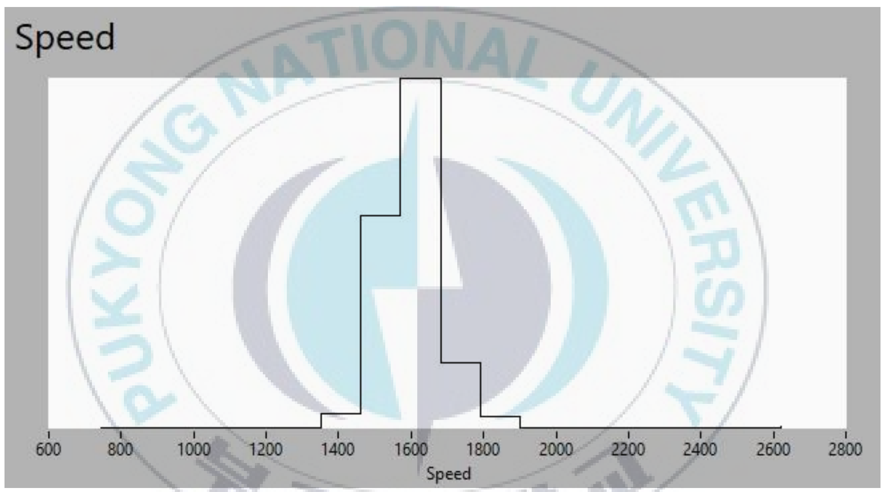
-10 [um]
(b)

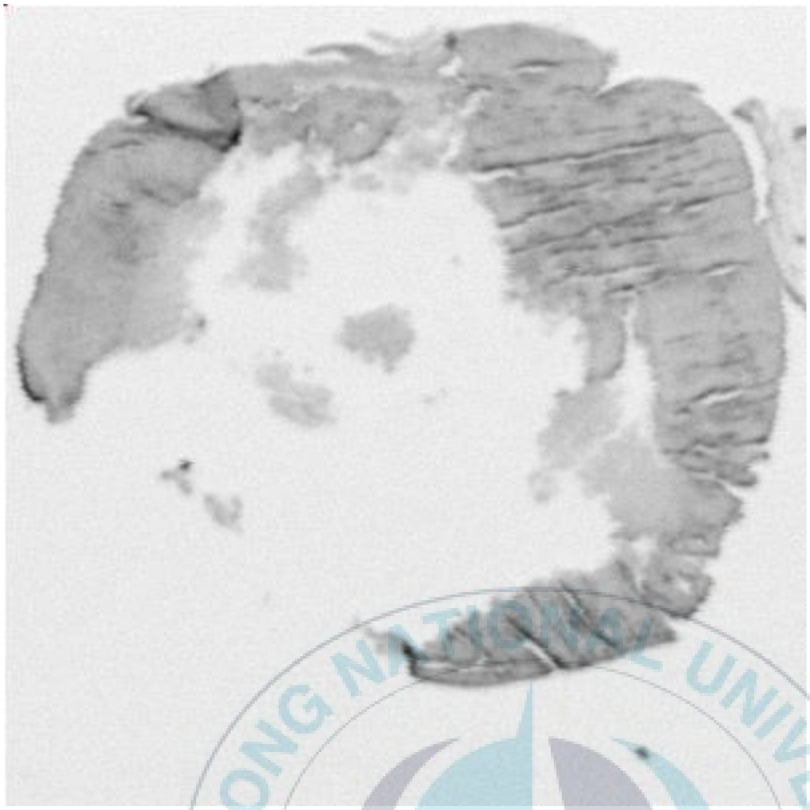


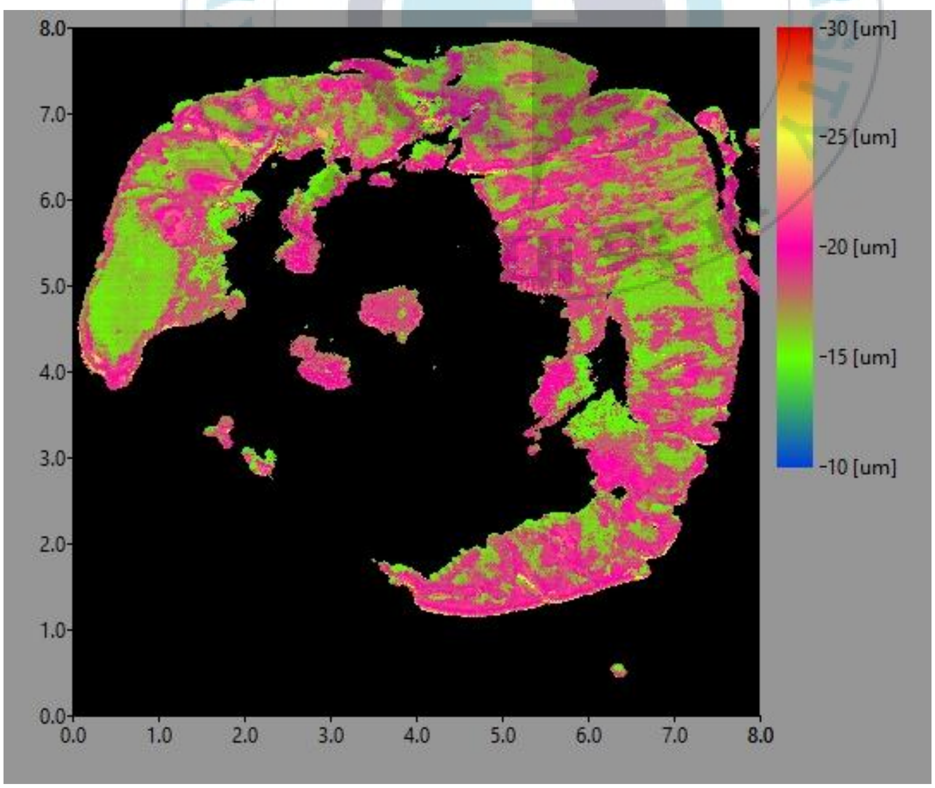
(c)



(d)

Figure 41 Demonstrates results of kidney. Figure (a) displays C-scan image and figure (b) displays thickness image and figure (c) displays speed of show image and figure (d) displays histogram of speed of sound image


(a)



8.0
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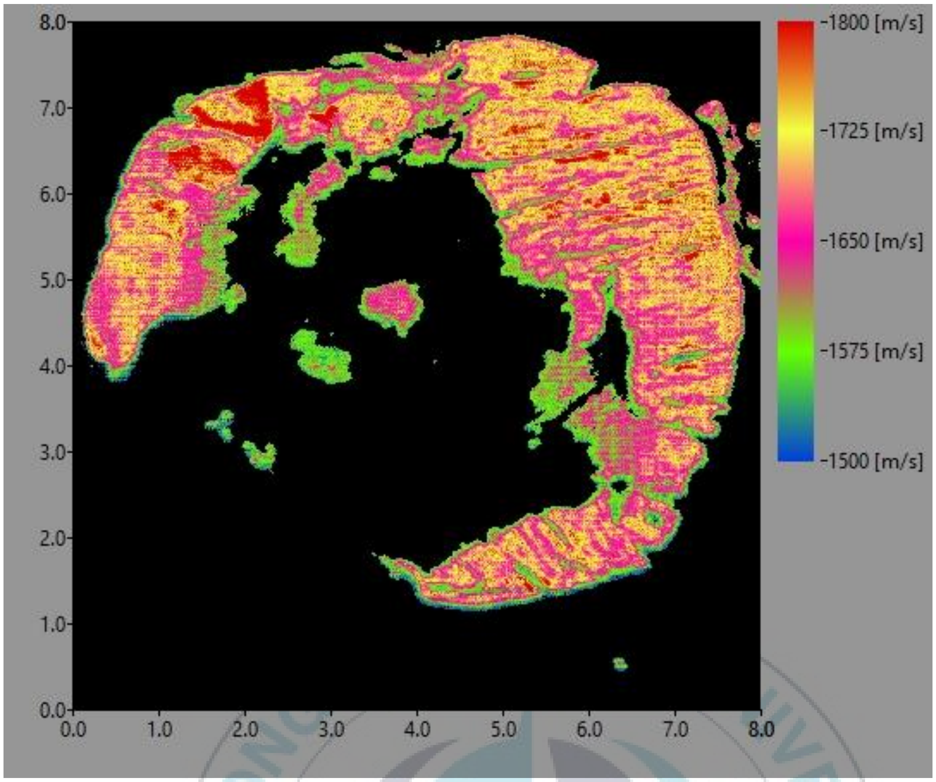
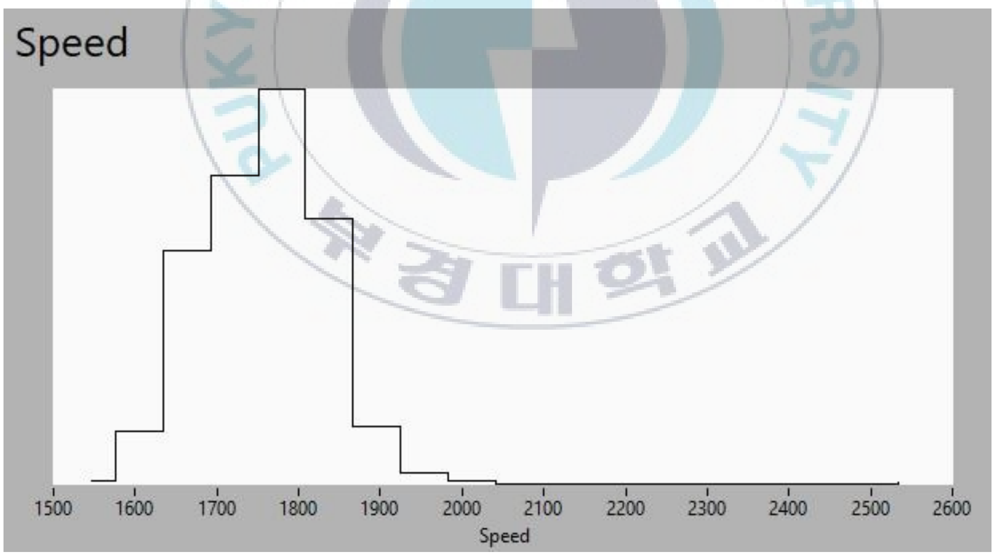
(b)



(c)



(d)

Figure 42 Demonstrates results of tumor. Figure (a) displays C-scan image and figure (b) displays thickness image and figure (c) displays speed of show image and figure (d) displays histogram of speed of sound image

Speed of sound in tissue could reveal elastography of the tissue. As reference papers, tumor has higher rigidity than normal tissue. So, speed of sound is an indirect measurement for assessing elastography of tissue as well as normality and its stage. In the experiment, sample after collecting from mice it is dehydrated and embedded to paraffin before sliced 20µm in thickness by a Microtome. Paraffin initially fixes sample for slicing then be removed by serial chemical processes. Here we used kidney represent for regular tissue. Tumor was grown in other mice by injecting breast cancer cells under skin. The two figures above show results for the two samples. Four type of data show in the figures provide different information. C-scan images or called intensity images provide roughness of tissue's surface. Thickness images show reliability of the experiment set up whether it is in range of nominal thickness (20µm) as well as give information for speed of sound calculation. Speed of sound images provide critical information for the study. It help identify and differentiate normal and abnormal tissue. The histograms show an overview image to determine dominant value of the image.

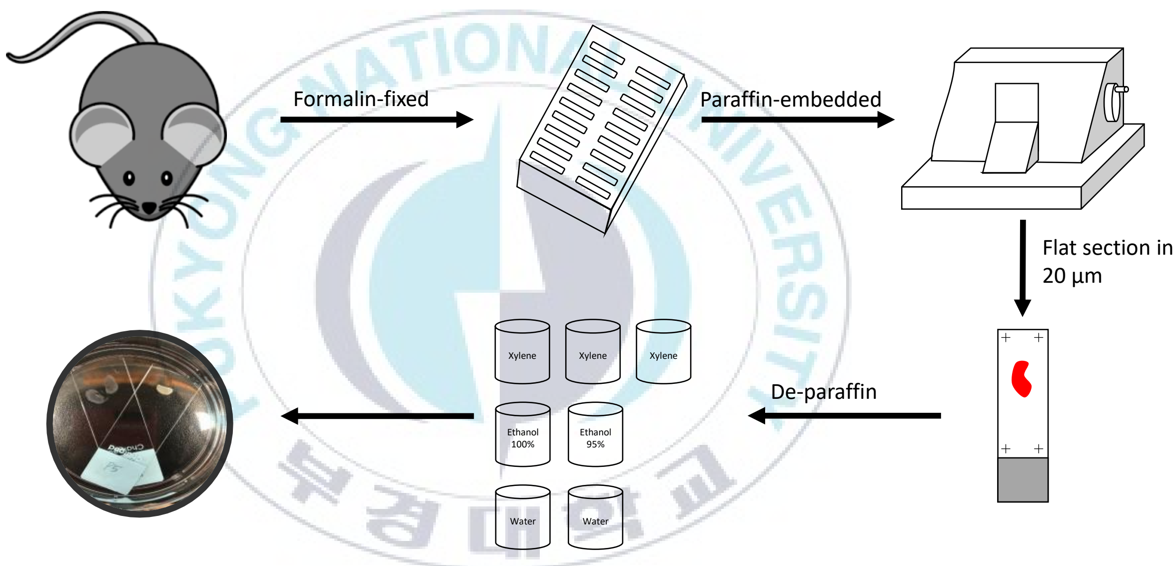


Figure 43 Indicates tissue processing

Chapter 5. Discussion

In the study, there are several steps need to do manually still remain. For reference phase prediction, the first vertical line is still not acquired in the scanning due to no encoder signal. The line data achieve after data reconstruction completes. This is one of the reasons make the current program has not worked online yet.

Tilting adjustment still requires manual works and somehow make the system less automatic. This could be improved by replacing height adjustable posts by two Z axis motors. After putting samples on the stage and performing basic alignment steps, X axis and Y axis motors would scan boundaries to look for first lines. Then, a quick titling profile of substrate is obtained. The program run a calculation to control Z axis motors to minimize the tilting. Since then, the scanning would be performed and real time data could be display.

As previous studies, thickness of tissue should be slices under 10µm. The thickness probably includes one basic layers of cells. This make the measurement more accurate by avoiding an overlap between two different layers. 10µm layer of tissue will also require a higher frequency transducer to qualify interference frequency values. The central frequency is required at least 110MHz. A short focal length is also expected to increase transducer's resolution and improve scanning time.

Chapter 6. Conclusion

In the study, the system could basically measure thickness and speed of sound of 20μm sliced tissues. To assess feasibility as well as reliability of the system, more scanning of different types of tissue such as (tumor and normal tissue) need to be conducted.

The system has been self-constructed mainly based on experimental laboratory instruments. There are few parts need to be fabricated by CNC machine to connect two different parts out-of-shelf. This makes the system becomes low cost so that it could be used in study or be optimized and developed for clinical uses.

As an promising method to characterize tissue that could alternate with conventional staining methods which often take several hours to several days for staining processes before microscope observation. Tissue characterization by scanning acoustic microscope could be modified to reduce prepared steps that adapting to on-site medical operations.

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