THE ULTRASONIC PROPERTIES OF COLON CANCER TISSUE

by

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ABSTRACT

THE ULTRASONIC PROPERTIES OF COLON CANCER TISSUE

Cancer is the most threatening disease for human life. Therefore, diagnosis of cancer is very important. For cancer diagnosis, many medical imaging systems have been developed. Ultrasonic imaging is one of them. Colon cancer presents a significant important statistics amongst all cancer types. Colon cancer is the third most common cancer diagnosed in all cancer cases. Both diagnosis of colon cancers and determining tumor limits precisely are quite important for patients' lives and life quality. In this thesis, healthy and tumorous colon tissues from 3 different patients are examined by using Scanning Acoustic Microscopy. Ultrasonic properties of those tissues are presented. The difference of ultrasonic properties between healthy and tumorous colon tissues is observed. For a better determination of the tumor, a new computer program is developed providing detailed images and analysis tool.

ÖZET

KOLON KANSERİ DOKUSUNUN ULTRASONİK ÖZELLİKLERİ

Kanser insan hayatını tehdit eden hastalıkların başında gelmektedir. Bundan dolayıdır ki tanısı oldukça önemlidir. Kanserin tanısı için pek çok görüntüleme sistemi geliştirilmiştir. Ultrasonik görüntüleme de bunlardan biridir. Kanser çeşitleri arasında kolon kanseri oldukça yüksek bir orana sahiptir. Kolon kanseri tüm kanser tanıları içerisinde üçüncü en yaygın kanser çeşididir. Kolon kanserinin hem tanısı hem de tümör sınırlarının düzgünce belirlenmesi hastanın yaşamı ve yaşam kalitesi açısından oldukça mühimdir. Bu tez çalışmasında Taramalı Akustik Misroskop kullanılarak 3 farklı hastadan alınmış sağlıklı ve tümör içeren kolon örnekleri incelenmiştir. Bu dokuların ultrasonik özellikleri ortaya konmuştur. Sağlıklı kolon dokusu ile tümör içeren kolon dokusu arasındaki ultrasonik özelliklerin farklılığı gözlemlenmiştir. Tümörün sınırlarının daha iyi belirlenmesi için daha ayrıntılı bir görüntü veren ve analiz aracı olarak kullanılabilecek yeni bir bilgisayar programı geliştirilmiştir.

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LIST OF SYMBOLS

c	Speed of Sound
m	Meter
mm	Millimeter
MN	Millinewton
s	Second
S	Transmitted Signal
S_{ref}	Reflected Signal from Reference Material
S_{target}	Reflected Signal from Target Material
Z	Acoustic Impedance
Z_{ref}	Acoustic Impedance of Reference Material
Z_{sub}	Acoustic Impedance of Substrate Material
Z_{target}	Acoustic Impedance of Target Material
κ	Elastic Modulus of Material
μm	Micrometer
ρ	Density of Material

LIST OF ACRONYMS/ABBREVIATIONS

2D	Two Dimensional
3D	Three Dimensional
AFM	Atomic Force Microscopy
CT	Computed Tomography
GUI	Graphical User Interface
LSFM	Light Sheet Fluorescence Microscopy
Max	Maximum
MRI	Magnetic Resonance Imaging
PET-CT	Positron Emission Tomography-Computer Tomography
RGB	Red, Green, Blue
SAM	Scanning Acoustic Microscopy
TIRFM	Total Internal Reflection Fluorescence Microscopy

1. INTRODUCTION

Cancer threatens our lives, therefore its diagnosis is vital. Cancer types can be diagnosed by medical imaging techniques such as Computed Tomography (CT), Magnetic Resonance Imaging (MRI), Positron Emission Tomography - Computer Tomography (PET-CT) and Ultrasonography [1]. Those are very well-known and common physical medical techniques in medicine and diagnosis. Apart from those body or body-part scan devices, there are also many microscopy systems for the cellular and molecular imaging such as Light Sheet Fluorescence Microscopy (LSFM), Total Internal Reflection Fluorescence Microscopy (TIRFM), Atomic Force Microscopy (AFM). All of these systems and imaging techniques use different physical phenomena depending on the size of the area of interest. In this thesis, we conduct our research by using an ultrasonography system.

In comparison with CT, MRI, and PET, the ultrasonic imagining system has advantages such as its harmless effect, reasonable cost, portability, and real-time imaging [2]. However, the ultrasonic imagining system generally has a lower resolution than CT and MRI systems. The ultrasonic imaging system is made up of ultrasonic transducers, transmit-receive channels, power suppliers, signal processing devices, and monitors. To receive the ultrasound, the imagining system controls the ultrasonic transducer, and with a set of data from the transducer, it produces an ultrasound image. The images can be two-dimensional (2D) or three-dimensional (3D) according to the type of the transducer and the imaging system. Ultrasound imaging technology has been enhanced with the help of computer technology. Also, system integration has provided a better image quality, data acquisition, analysis, and display [3, 4].

In this thesis, different colon cancer tissues are examined by using Scanning Acoustic Microscopy (SAM) which utilizes ultrasonic properties (acoustic impedance values) to obtain images from cells and tissues [5]. All screenshots of SAM are in Appendix A.

2. SCANNING ACOUSTIC MICROSCOPY

Scanning Acoustic Microscopy (SAM) is a full-field, nondestructive technique capable of detecting changes in elastic properties of solids [6].

Speed of sound changes depending on the type of tissue. The speed of sound is higher in rigid materials [7]. Therefore, observing the speed of sound in various tissues gives us information about elasticity of tissues [7, 8]. SAM is used to calculate the characteristics of the acoustic impedance, elasticity and the speed of sound in tissues.

SAM was developed by Lemons and Quatate at Stanford University at the beginning of 1970's. This primary design is still being used in biomedical researches. Image contrast in SAM depends on biological aspect of tissue, frequency and condition of optical focusing [9].

Generally, tissue consisting of low protein level is similar to distilled water in terms of the speed of sound and acoustic impedance. However, tissue with high collagen content exhibits larger speed of sound and acoustic impedance [10,11]. For this reason, distilled water is used as a reference material for the comparison of a target material. In cancerous cells, due to different energy producing mechanism, the protein level is elevated [12]. It means that the elasticity of tissue including higher protein content has a bigger acoustic impedance values. Therefore, cancerous tissue can be detected by measuring the acoustic impedance of tissues [13].

2.1. System Setup of SAM

SAM has 5 main parts in its setup. Figure 2.2 illustrates the setup of our system.

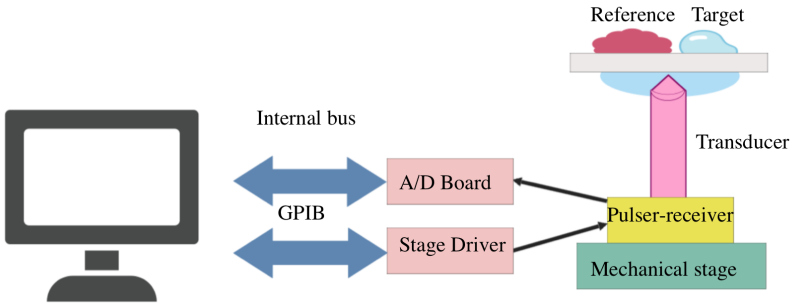

Figure 2.1. System setup of SAM.

Setup of SAM can be listed as follows:

(i) Sample Area

Sample area is where the data are acquired. Target and reference materials are placed on the substrate. The substrate should directly touch the water between transducer and itself in order to receive signals.

(ii) Transducer

Transducer is an ultrasonic prob with an acoustic lens.

(iii) Signal Pulse Generator and Signal Receiver

Signal pulse generator and signal receiver are a computer based signal generator and digital oscilloscope which reads received signals and turns them into meaningful ones, respectively.

(iv) Mechanical Stage

Mechanical Stage is the micro-computer that controls the base.

(v) Computer Screen

Computer screen displays the signals from digital oscilloscope which is one of the parts of the computer based pulse generator - receiver. It also shows the images from samples by turning signal data into pixel by pixel images.

2.2. The Operation Principles of SAM

In order to examine internal structures, interfaces, and surfaces of a substrate harmlessly, ultrasound is used by SAM [12]. The resulting acoustic image is evaluated to point out and define features such as cracks and voids.

SAM performs by using the pulse reflection method. The transducer is the acoustic objective-centerpiece of the microscope [14]. Short sound pulses are generated, transmitted and received by the transducer. High-frequency electromagnetic oscillations are transformed into a mechanical wave by the acoustic lens and these waves work as a plane parallel wave field inside the lens. The sound file on the sample is focused by cavity through the coupling medium such as water. In the sample, the sound is reflected in material interfaces according to the mismatch in acoustic impedance. Sound pulses reflected from the sample are received by the acoustic lens [14]. Sound pulses are converted into electromagnetic pulses by the transducer and these are stored as a function of time (A-scans). Analysis of A-scan results in a pixel with defined gray values. The acoustic objective scans the sample point by point and line by line in order to generate an image [15].

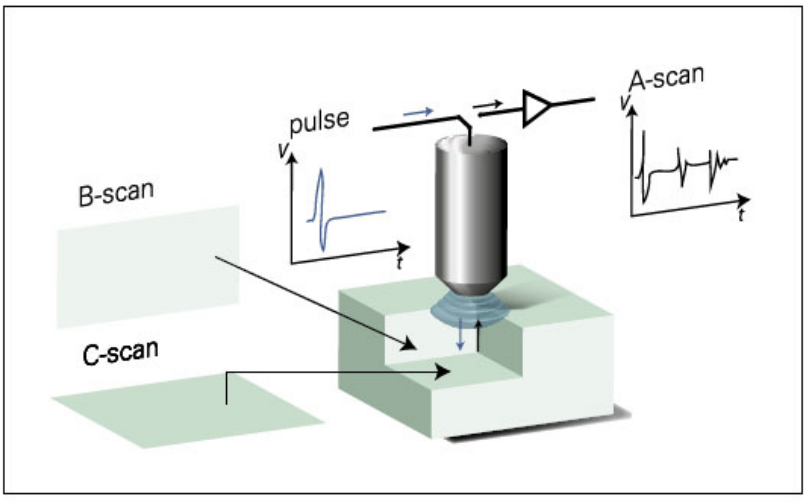

Figure 2.2. Operation principle of SAM.

2.3. Ultrasonic Properties of SAM

The model of the SAM used in this thesis is AMS-50SI. With AMS-50SI, not only sound speed but also acoustic impedance values can be observed. For the acoustic impedance mode, the tissue must be cut approximately 10 μm thickness [16]. Acoustic impedance of tissue can be compared with a reference value such as distilled water [17].

Acoustic impedance of a material is represented by Z.

$$Z = \rho.c \tag{2.1}$$

where ρ is the density of a material, c is the speed of sound in the material which is given by

$$c = \sqrt{\frac{\kappa}{\rho}}. \tag{2.2}$$

Here, κ is the elastic modulus of a material which gives us information about the elasticity of a material [18]. Therefore, Equation (2.1) becomes

$$Z = \sqrt{\rho\kappa}. \tag{2.3}$$

Equation (2.3) shows the dependence of the acoustic impedance on the elasticity κ and density ρ.

Figure 2.3 represents the research data acquisition area and the physical background of the collection of the data set of the acoustic impedance through the transducer of SAM.

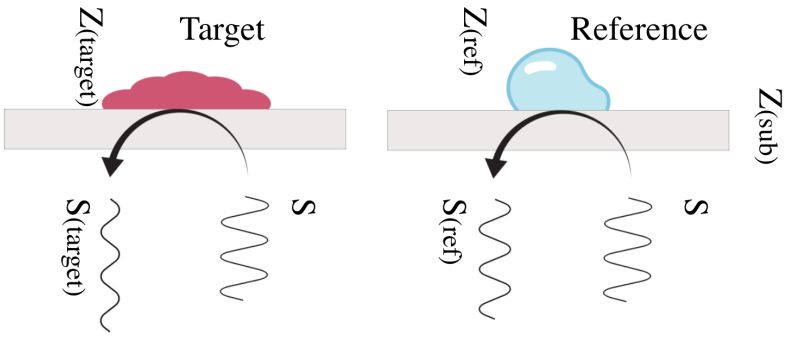

Figure 2.3. Acoustic properties representation of SAM.

In order to derive the acoustic impedance of a target material, let's begin with transmitted signal from a reference material

$$S_{ref} = \frac{Z_{ref} - Z_{sub}}{Z_{ref} + Z_{sub}} S. \tag{2.4}$$

So;

$$S = \frac{Z_{ref} + Z_{sub}}{Z_{ref} - Z_{sub}} S_{ref}, \tag{2.5}$$

$$S_{target} = \frac{Z_{target} - Z_{sub}}{Z_{target} + Z_{sub}} S, \tag{2.6}$$

$$S_{target} Z_{target} + S_{target} Z_{sub} = S Z_{target} - S Z_{sub}, \tag{2.7}$$

$$Z_{target}(S - S_{target}) = Z_{sub}(S + S_{target}), \tag{2.8}$$

$$Z_{target} = \frac{S + S_{target}}{S - S_{target}} Z_{sub}, \tag{2.9}$$

, and

$$Z_{target} = \frac{S(1 + \frac{S_{target}}{S})}{S(1 - \frac{S_{target}}{S})} Z_{sub}. \tag{2.10}$$

Then, by substituting Equation (2.5) into Equation (2.10), we obtain

$$Z_{target} = \frac{1 + \frac{S_{target}}{S_{ref}} . \frac{Z_{sub} - Z_{ref}}{Z_{sub} + Z_{ref}}}{1 - \frac{S_{target}}{S_{ref}} . \frac{Z_{sub} - Z_{ref}}{Z_{sub} + Z_{ref}}} Z_{sub}.$$

where S is the transmitted signal, S_{target} and S_{ref} are reflections from the target and reference materials, respectively. Z_{target}, Z_{ref} and Z_{sub} are the acoustic impedance values for the target, the reference, and the substrate, respectively.

3. EXPERIMENTS AND RESULTS

In this thesis, the data from three different patients diagnosed with colon cancer are examined [17, 19, 20]. From every patient, both healthy and cancerous tissues were taken. The healthy tissues are the control group while the cancerous tissues are the tumor group. The samples were taken from Cerrahpaşa University Medicine Faculty. The samples were delivered in a formaldehyde solution [21].

3.1. Setup Details of Experiments

Figure 3.1. shows the set up used in our work.

Figure 3.1. Experimental setup.

We use Scanning Acoustic Microscopy. In this microscopy, HTD- 1825 (ϕ1.8 F2.5) transducer type was used. Scan size is 4800 μm while scan area is 300 x 300 pixels. Z_{sub} is polystyrene which has the impedance of 2.46 MNs/m^3 and Z_{ref} is distilled water which has the impedance of 1.49 MNs/m^3. The power was 1 Volt [21, 22].

3.2. Results and Data Analysis

Data analysis was done in MATLAB and the value results were taken in ImageJ.

3.2.1. Colon Tissue 1

Images in Figure 3.2 represent healthy tissue from the first colon tissue.

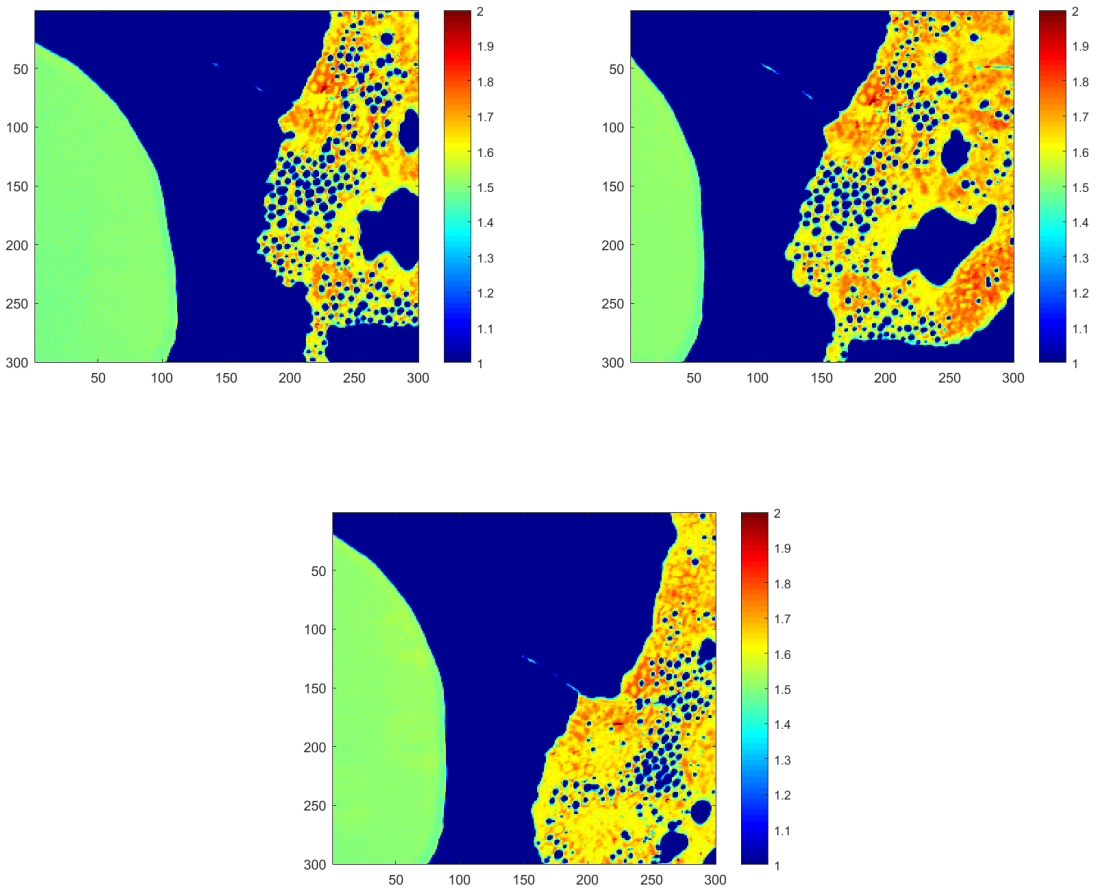

Figure 3.2. First control group from different points.

Distilled water and a control tissue were used for the first patient diagnosed with colon cancer. This control tissue was imaged from different points in order to get the most accurate results. These images are shown in Figure 3.2. Each coordinate represents 300 points corresponding to another 300 points. Total scanned area is 300 x 300 pixels. 300 points correspond to 4.8 mm. The total scanned area is 4.8 x 4.8 mm^2. The color bar represents acoustic impedance values (MNs/m^3) for each pixel.

The results of tissue's analysis in Figure 3.2 are in Table 3.1.

Table 3.1. Acoustic impedance results of the first control group.

Mean	**Standard Deviation**
1.715 MNs/m^3	0.032 MNs/m^3

Images in Figure 3.3 represent tumorous tissue of the first colon tissue.

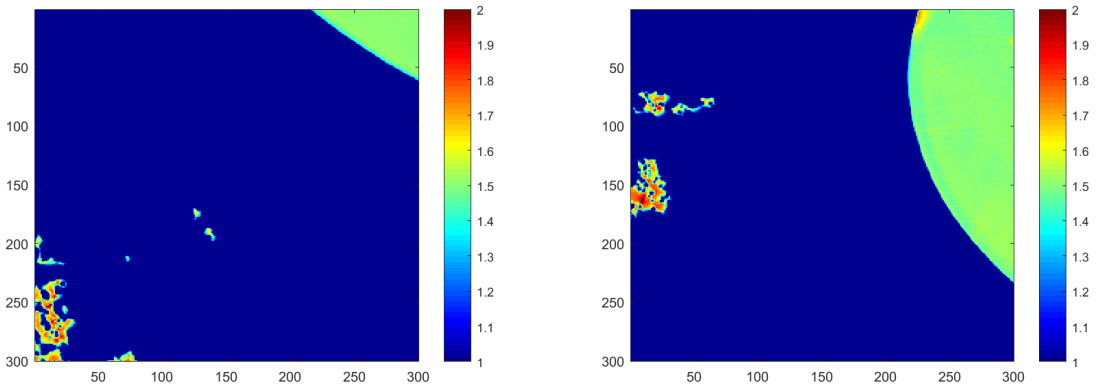

Figure 3.3. First tumor group from different points.

Distilled water and a tumorous tissue were used for the first patient diagnosed with colon cancer. This tumorous tissue was imaged from different points in order to get the most accurate results. These images are shown in Figure 3.3. Each coordinate stands for 300 points corresponding to another 300 points. Total scanned area is 300 x 300 pixels. 300 points correspond to 4.8 mm. The total scanned area is 4.8 x 4.8 mm^2. The color bar represents acoustic impedance values (MNs/m^3) for each pixel.

The results of the tissue's analysis in Figure 3.3 are in Table 3.2.

Table 3.2. Acoustic impedance results of the first tumor group.

Mean	**Standard Deviation**	**Max Value**
1.755 MNs/m^3	0.023 MNs/m^3	2.070 MNs/m^3

3.2.2. Colon Tissue 2

Images in Figure 3.4 represent healthy tissue of the second colon tissue.

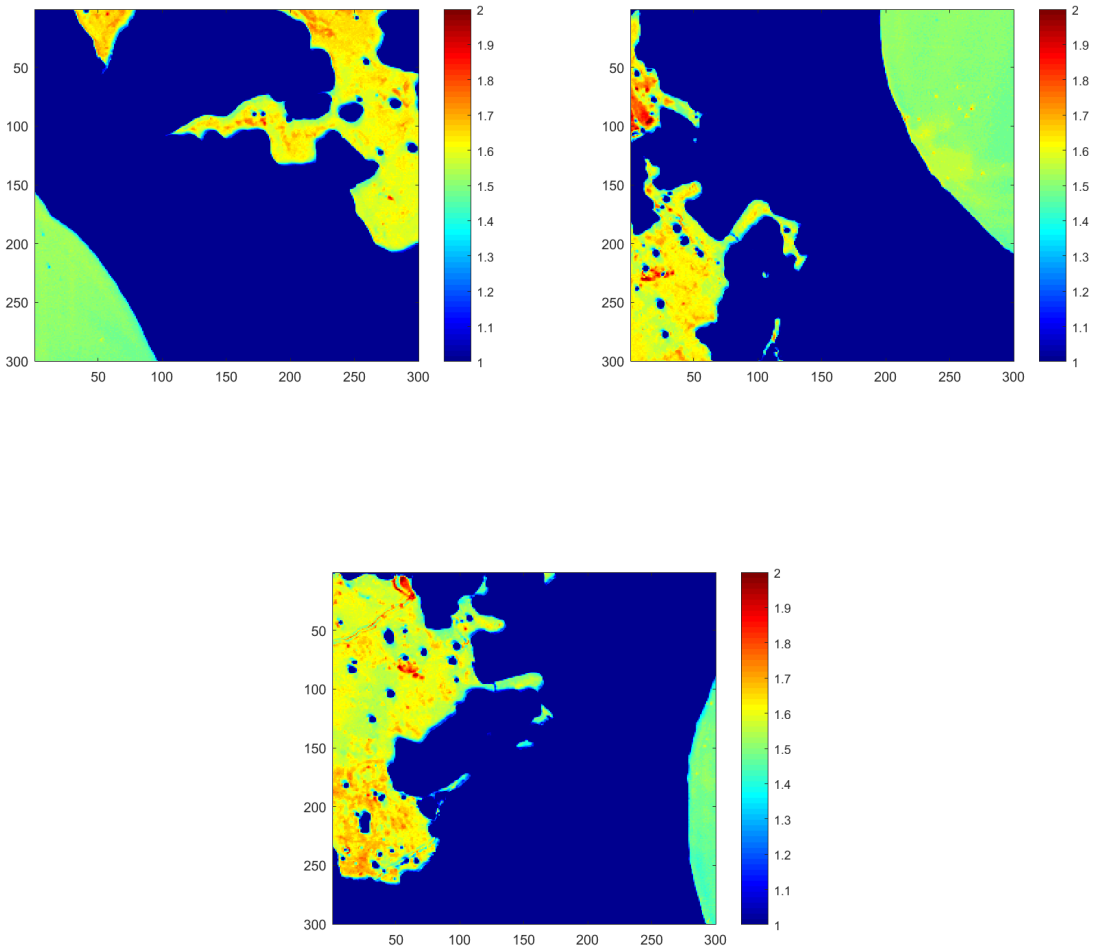

Figure 3.4. Second control group from different points.

Distilled water and a control tissue were used for the second patient diagnosed with colon cancer. This control tissue was imaged from different points in order to get the most accurate results. These images are shown in Figure 3.4. Each coordinate stands for 300 points corresponding to another 300 points. Total scanned area is 300 x 300 pixels. 300 points correspond to 4.8 mm. The total scanned area is 4.8 x 4.8 mm^2. The color bar represents acoustic impedance values (MNs/m^3) for each pixel.

The results of the tissue's analysis in Figure 3.4 are in Table 3.3.

Table 3.3. Acoustic impedance results of the second control group.

Mean	**Standard Deviation**
1.736 MNs/m^3	0.045 MNs/m^3

Images in Figure 3.5 represent tumorous tissue of the second colon tissue.

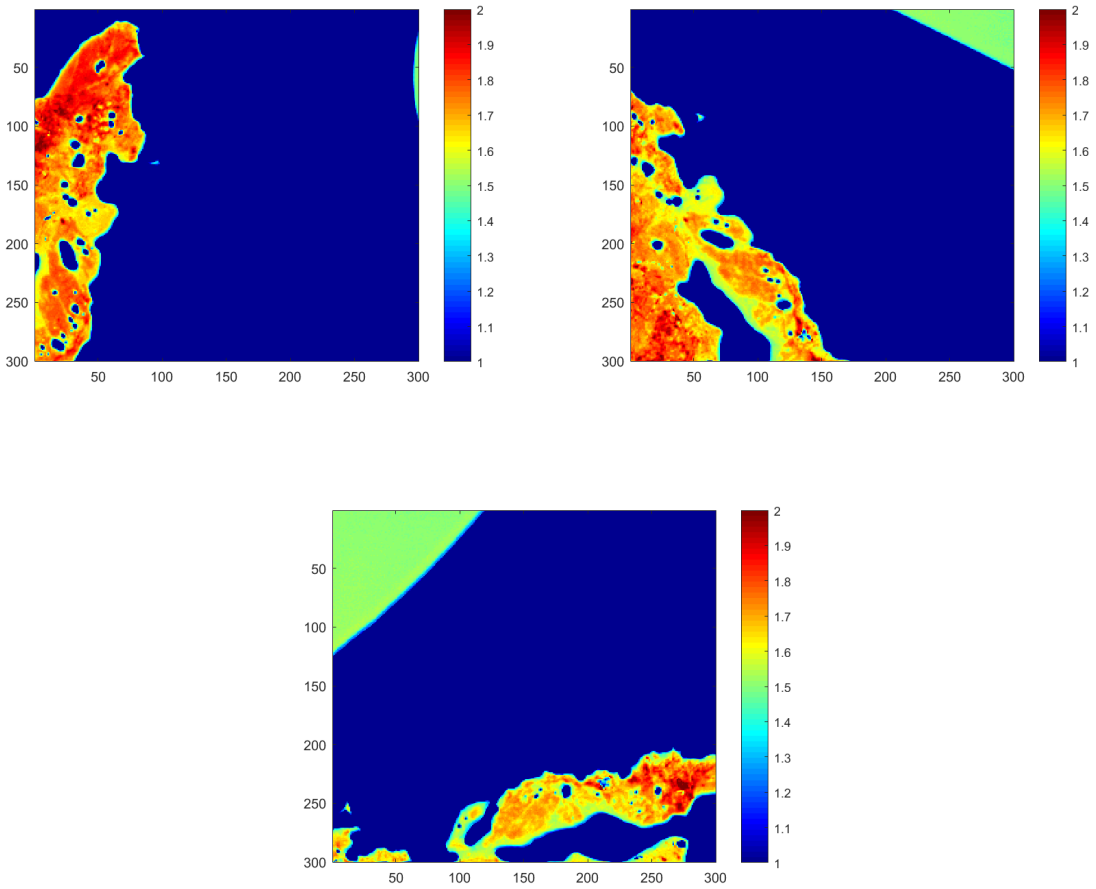

Figure 3.5. Second tumor group from different points.

Distilled water and a tumorous tissue were used for the second patient diagnosed with colon cancer. This tumorous tissue was imaged from different points in order to get the most accurate results. These images are shown in Figure 3.5. Each coordinate stands for 300 points corresponding to another 300 points. Total scanned area is 300 x 300 pixels. 300 points correspond to 4.8 mm. The total scanned area is 4.8 x 4.8 mm^2. The color bar represents acoustic impedance values (MNs/m^3) for each pixel.

The results of the tissue's analysis in Figure 3.5 are in Table 3.4.

Table 3.4. Acoustic impedance results of the second tumor group.

Mean	Standard Deviation	Max Value
2.039 MNs/m^3	0.064 MNs/m^3	2.190 MNs/m^3

3.2.3. Colon Tissue 3

Images in Figure 3.6 represent healthy tissue of the third colon tissue.

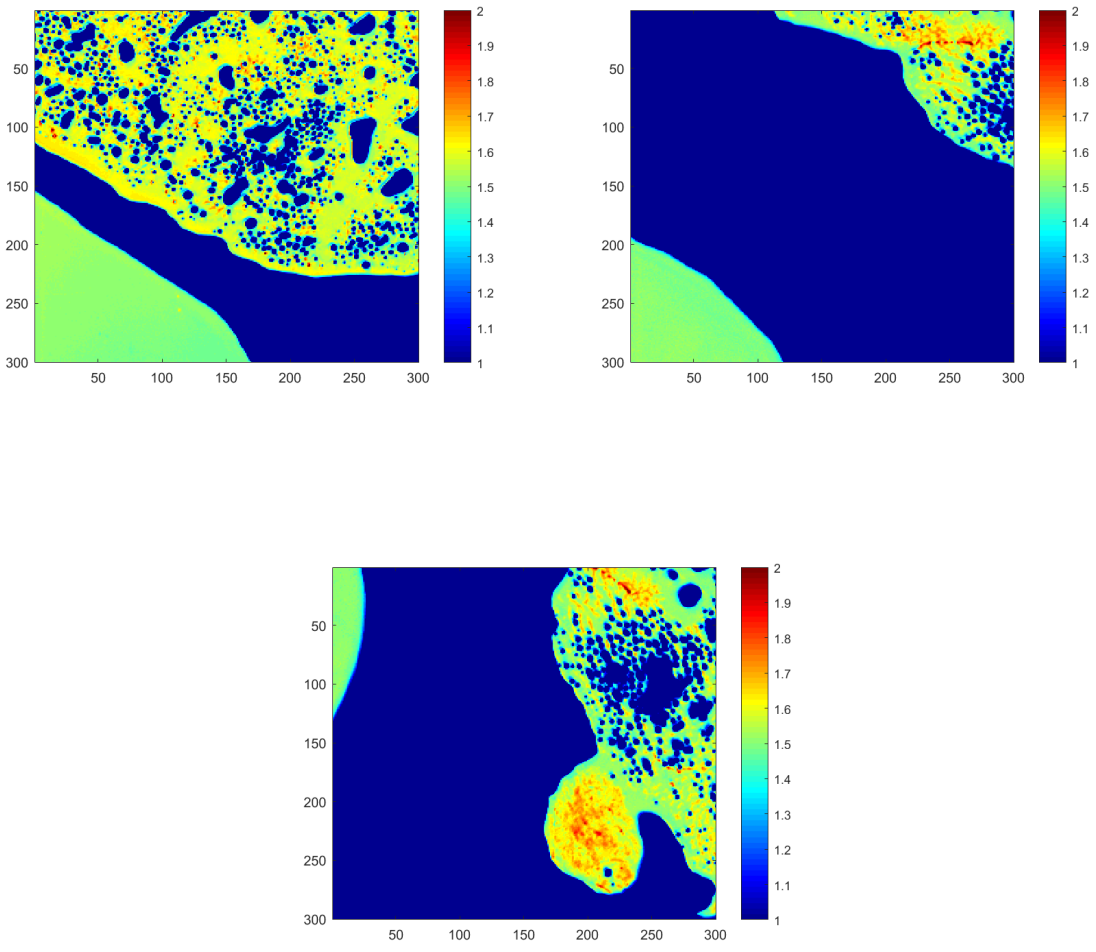

Figure 3.6. Third control group from different points.

Distilled water and a control tissue were used for the third patient diagnosed with colon cancer. This control tissue was imaged from different points in order to get the most accurate results. These images are shown in Figure 3.6. Each coordinate stands

for 300 points corresponding to another 300 points. Total scanned area is 300 x 300 pixels. 300 points correspond to 4.8 mm. The total scanned area is 4.8 x 4.8 mm^2. The color bar represents acoustic impedance values (MNs/m^3) for each pixel.

The results of the tissue's analysis in Figure 3.6 are in Table 3.5.

Table 3.5. Acoustic impedance results of the third control group.

Mean	**Standard Deviation**
1.678 MNs/m^3	0.036 MNs/m^3

Images in Figure 3.7 represent tumorous tissue of the third colon tissue.

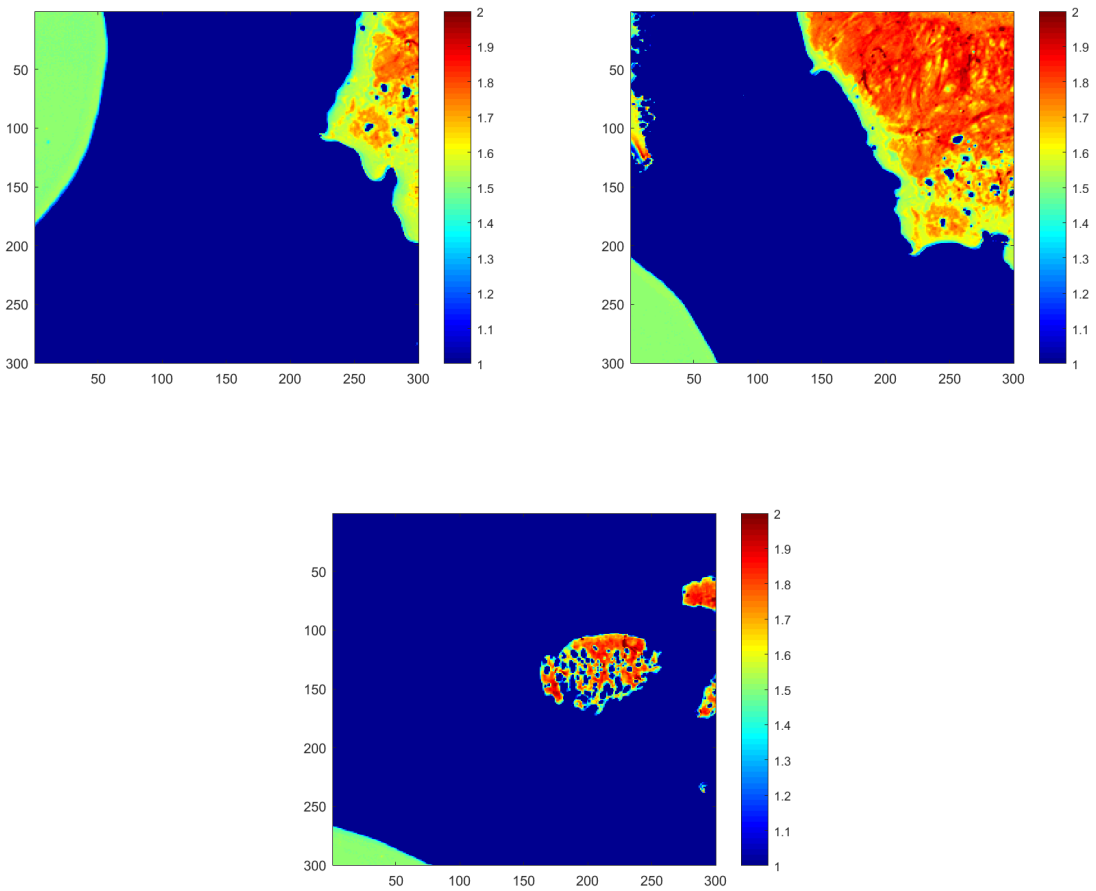

Figure 3.7. Third tumor group from different points.

Distilled water and a tumorous tissue were used for the third patient diagnosed with colon cancer. This tumorous tissue was imaged from different points in order to

get the most accurate results. These images are shown in Figure 3.7. Each coordinate stands for 300 points corresponding to another 300 points. Total scanned area is 300 x 300 pixels. 300 points correspond to 4.8 mm. The total scanned area is 4.8 x 4.8 mm^2. The color bar represents acoustic impedance values (MNs/m^3) for each pixel.

The results of the tissue's analysis in Figure 3.7 are in Table 3.6.

Table 3.6. Acoustic impedance results of the third tumor group.

Mean	**Standard Deviation**	**Max Value**
1.998 MNs/m^3	0.045 MNs/m^3	2.210 MNs/m^3

3.3. Discussion

Healthy and tumorous tissues have different acoustic impedance values due to their different coefficients of elasticity. Cancerous cells contain higher protein level than healthy ones. Higher protein level makes the tissue's coefficient of elasticity larger [12]. It means that the acoustic impedance value in the cancerous tissue is higher than the healthy tissue [13]. In our imaging and data analysis, we use both control and tumorous tissues for 3 different patients. For each patient, control and tumorous tissues are examined by comparing tissues' acoustic impedance values (MNs/m^3) with the acoustic impedance value of distilled water as a reference material. Various parts of all of the tissues are examined to get the most accurate values. All coordinates represent 300 points and all images are formed by 300 x 300 pixels. 300 points correspond to 4.8 mm. The total scanned area is 4.8 x 4.8 mm^2. The color bar is the key for acoustic impedance values for a 1 x 1 pixel area. Every color corresponds to the acoustic impedance value in range 0.00 - 2.00 (MNs/m^3). Images and reference colors are determined in MATLAB.

For control groups, mean and standard deviation values are analysed in ImageJ. The results of maximum value are ignored because, even in control group tissue, there may be tumor cells.

For tumor groups, mean, standard deviation and maximum values are analysed in ImageJ. Maximum value analysis is done in order to determine whether the tissue is tumorous or not.

Comparing mean acoustic impedance values between control groups and tumorous tissue groups gives us information about healthy and tumorous tissues' elasticity [12]. Because there is a correlation between the elasticity of a biological material and protein level, higher protein level can be detected. Therefore, cancerous tissues can be distinguished by checking the acoustic impedance value [13].

4. COMPUTER PROGRAM CONVERTING ACOUSTIC IMPEDANCE VALUES INTO IMAGES AND ANALYSING ACOUSTIC IMPEDANCE DATA

4.1. The Logic of Algorithm

For SAM, the data were taken as .imp format and transformed them to .csv format. ImageJ was used to calculate the mean, the max and the standard deviation values. Data files were obtained from SAM as .csv format then converted to text file (.txt format) to use data in ImageJ to get the mean, the max and the standard deviation values. Even though MATLAB can be used to obtain images by using acoustic impedance values as it was done before in this work, a new algorithm was developed to obtain more detailed images of tissue. In this algorithm, every color for every interval is determined by a typical acoustic values of healthy tissues and characterization of other materials [13, 19, 23].

The algorithm is based on getting every pixel's data from the text file and converting them to colors based on our key colors. 300 x 300 pixels data set is taken from SAM so that it is constructed as 300 x 300 pixels bitmap image. Every line is read from left to right. Whole data set is read line by line from top to bottom. Apart from this code, color key values are set manually in a different text file as stated in the previous paragraph according to characteristic values for the substrate, the reference and the target materials. Red, green, blue (RGB) values are inserted for every interval by hand as text file. Opacity value, alpha, is taken as 255 to get colors as full opaque. This text file is also shown as a color image as bitmap. After constructing and reading two different data sets (data from SAM and data for colors), the two of them are merged in the same program (.exe codes). Every data value is transformed to RGB value as colors. After reading whole data sets in the program, the image is produced in the .bmp (bitmap) format.

For exceptions, if data is out of boundary values, the color is set as full transparent and white. For our data sets, no exception occurs.

The generated images by this program are more detailed, hence, the analysis can be done more precisely and neatly. Some calculation methods for analysing data sets were implemented to the main image generating program by using these more detailed images distinguishing differences more elaborately. The extended program allows to select an area, detecting minimum and maximum values in a selected area and, area size. Because images were formed as pixel by pixel, area size indicates the total number of data in a selected area. Mean, standard deviation and standard error calculations for a selected area were added to the computer program. Therefore, this program can be used as an analysis tool for a specific analysis target. In order to make the computer program more functional and useful, graphical user interface (GUI) was created. The analysis results and a selected area are shown in the our GUI. Hence, this allows us to see which area we are dealing with in our analysis clearly. Figure 4.1. shows the graphical user interface.

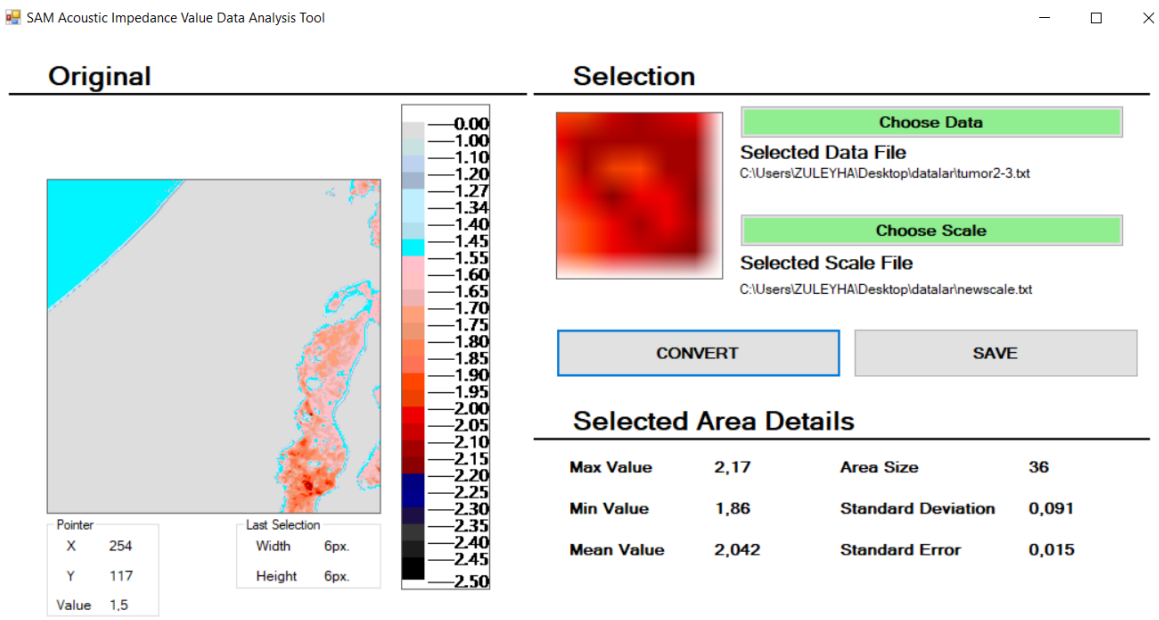

Figure 4.1. GUI for creating images and analysing data sets.

C# programming language is used for the program and .NET framework are chosen. Codes can be found in Appendix B.

4.1.1. Color Key Values

The list shows the key colors for the intervals $[19, 24]$.

Reference Interval Values	RGB Values	Colors
-0.50 - 1.00	221, 221, 221	Light Gray
1.00 - 1.10	202, 225, 225	Light Steel Blue 1
1.10 - 1.20	188, 210, 238	Light Steel Blue 2
1.20 - 1.27	162, 181, 205	Light Steel Blue 3
1.27 - 1.34	191, 239, 255	Light Blue 1
1.34 - 1.40	178, 233, 238	Light Blue 2
1.40 - 1.45	142, 229, 238	Cadet Blue 2
1.45 - 1.55	0, 245, 245	Turquoise 1
1.55 - 1.60	255, 192, 203	Pink
1.60 - 1.65	255, 193, 193	Rosy Brown 1
1.65 - 1.70	238, 180, 180	Rosy Brown 2
1.70 - 1.75	255, 160, 122	Light Salmon 1
1.75 - 1.80	238, 149, 114	Light Salmon 2
1.80 - 1.85	255, 127, 80	Carol
1.85 - 1.90	255, 114, 86	Carol 1
1.90 - 1.95	255, 69, 0	Orange Red 1
1.95 - 2.00	238, 64, 0	Orange Red 2
2.00 - 2.05	238, 0, 0	Red 2
2.05 - 2.10	205, 0, 0	Red 3
2.10 - 2.15	164, 0, 0	Dark Candy Apple Red
2.15 - 2.20	139, 0, 0	Dark Red
2.20 - 2.25	0, 0, 128	Navy Blue
2.25 - 2.30	0, 0, 139	Dark Blue
2.30 - 2.35	28, 15, 69	Dark Strong Blue
2.35 - 2.40	54, 54, 54	Gray 21
2.40 - 2.45	28, 28, 28	Gray 11
2.45 - 2.50	0, 0, 0	Black

4.2. Images and Results of The Computer Program

Images were created and data analysis was done in the computer program.

4.2.1. Colon Tissue 1

Images in Figure 4.2 represent healthy tissue of the first colon tissue.

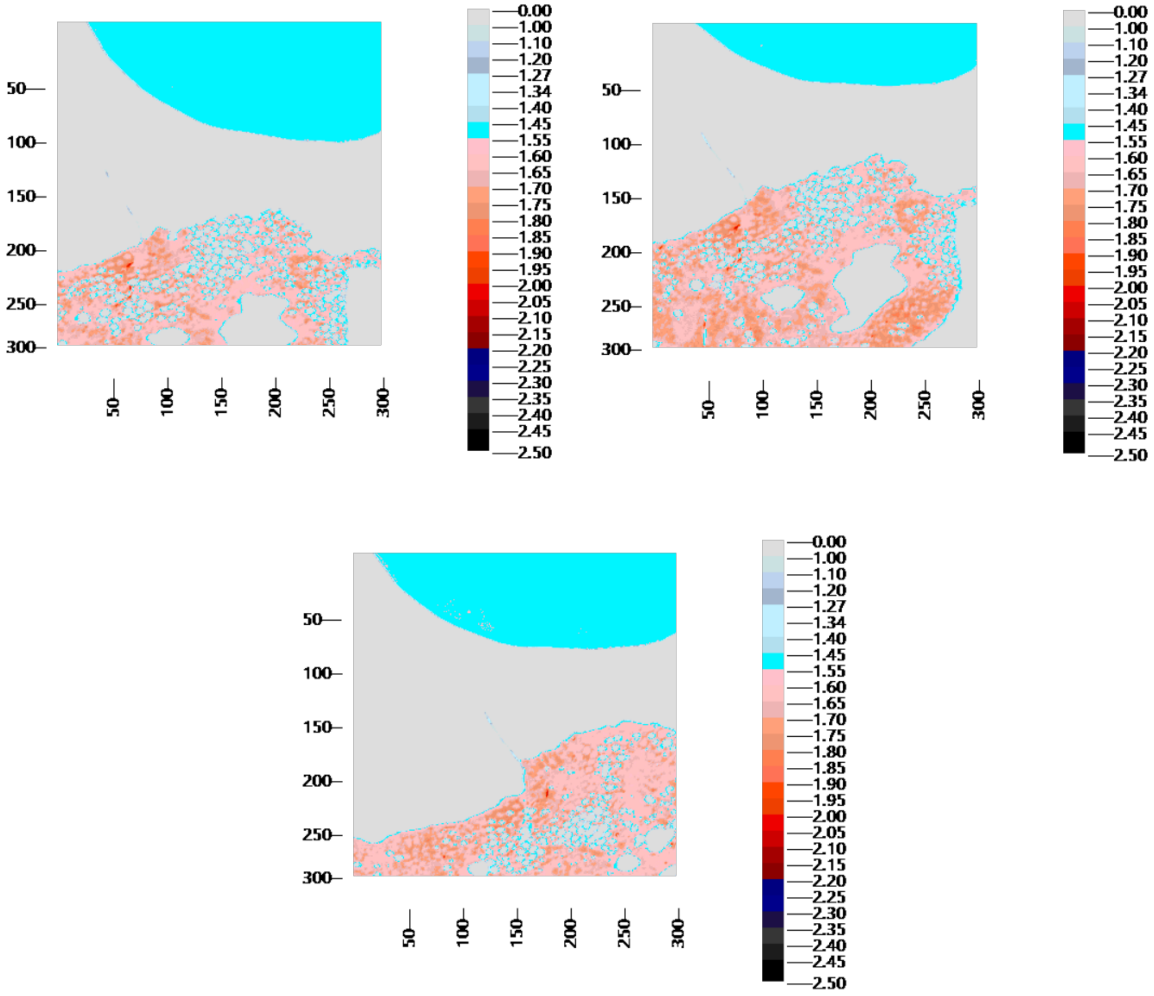

Figure 4.2. Detailed images of the first control group from different points.

Distilled water and a control tissue were used for the first patient diagnosed with colon cancer. This control tissue was imaged from different points in order to get the most accurate results. These images are shown in Figure 4.2. Total scanned area is 300 x 300 pixels. The total scanned area is 4.8 x 4.8 mm^2. The color key represents colors and corresponding acoustic impedance values (MNs/m^3) for each pixel.

The results of the tissue's analysis in Figure 4.2 are in Table 4.1.

Table 4.1. Acoustic impedance results of the first control group analyzed the computer program.

Mean	**Standard Deviation**
1.642 MNs/m^3	0.029 MNs/m^3

Images in Figure 4.3 represent tumorous tissue of the first colon tissue.

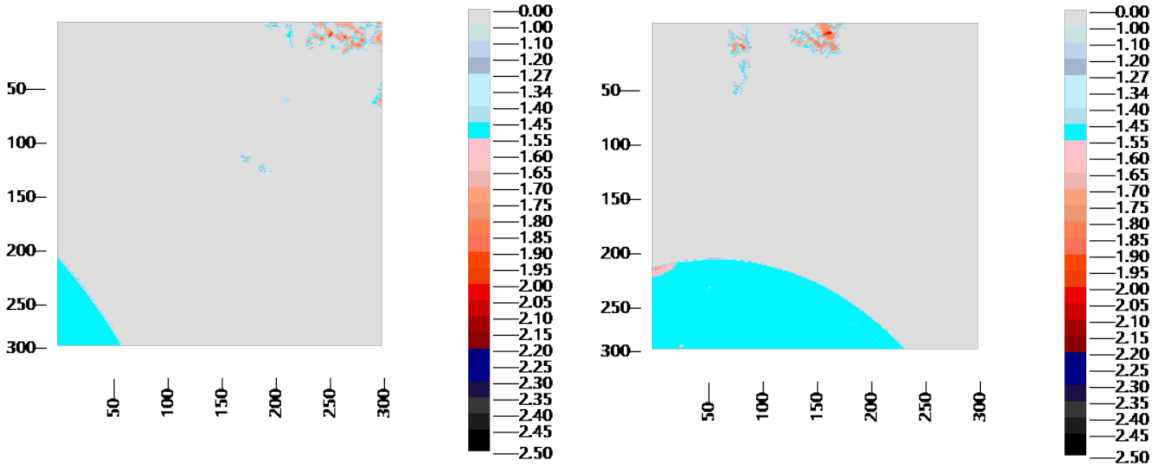

Figure 4.3. Detailed images of the first tumor group from different points.

Distilled water and a tumorous tissue were used for the first patient diagnosed with colon cancer in Figure 4.3. This tumorous tissue was imaged from different points in order to get the most accurate results. Each coordinate stands for 300 points corresponding to another 300 points. Total scanned area is 300 x 300 pixels. 300 points correspond to 4.8 mm. The total scanned area is 4.8 x 4.8 mm^2. The color key represents colors and corresponding acoustic impedance values (MNs/m^3) for each pixel.

The results of the tissue's analysis in Figure 4.3 are in Table 4.2.

Table 4.2. Acoustic impedance results of the first tumor group analyzed in the computer program.

Mean	**Standard Deviation**	**Max Value**
1.941 MNs/m^3	0.034 MNs/m^3	2.070 MNs/m^3

4.2.2. Colon Tissue 2

Images in Figure 4.4 represent healthy tissue of the second colon tissue.

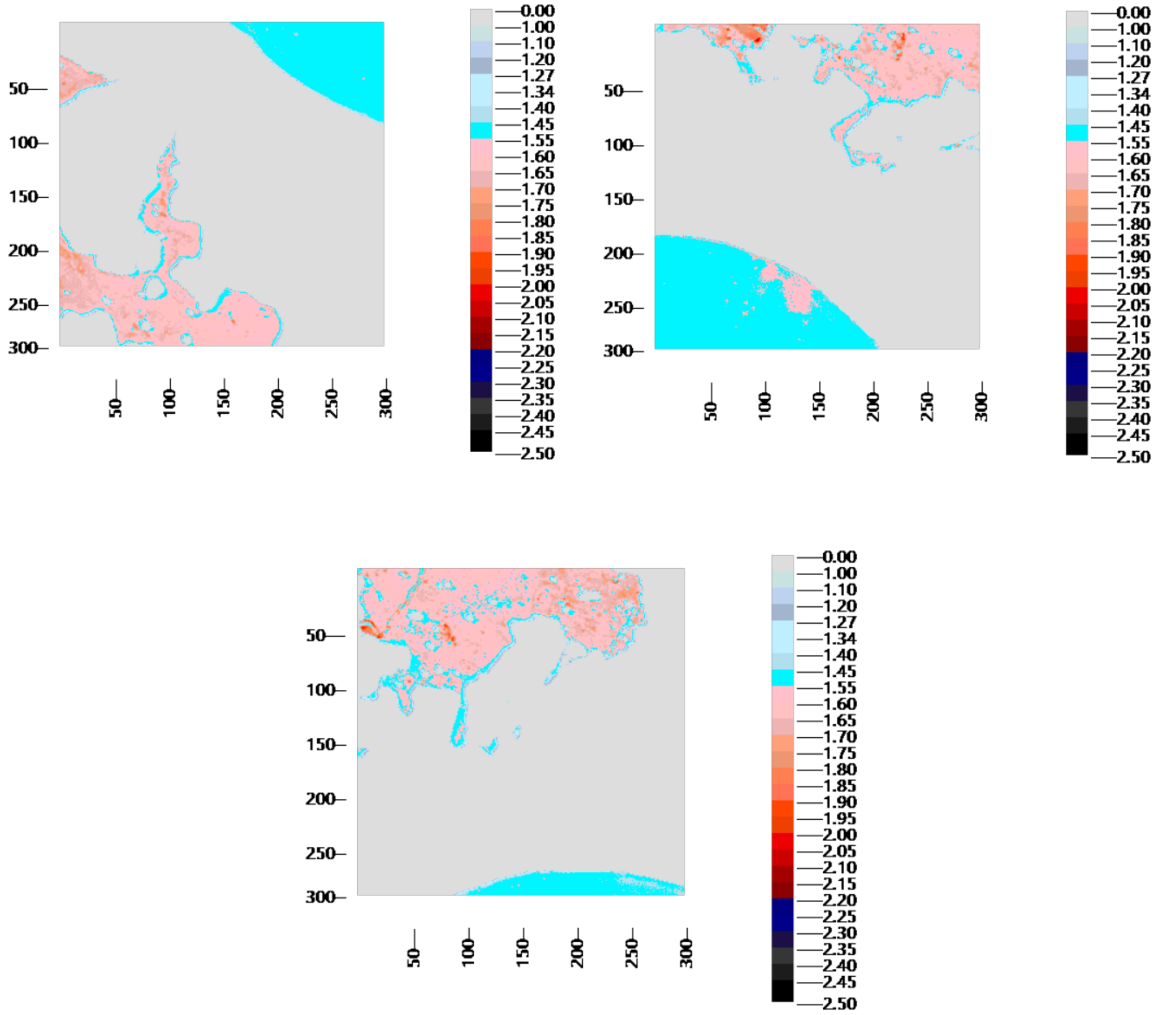

Figure 4.4. Detailed images of the second control group from different points.

Distilled water and a control tissue were used for the second patient diagnosed with colon cancer. This control tissue was imaged from different points in order to get the most accurate results. These images are shown in Figure 4.4. Each coordinate stands for 300 points corresponding to another 300 points. Total scanned area is 300 x 300 pixels. 300 points correspond to 4.8 mm. The total scanned area is 4.8 x 4.8 mm^2. The color key represents colors and corresponding acoustic impedance values (MNs/m^3) for each pixel.

The results of the tissue's analysis in Figure 4.4 are in Table 4.3.

Table 4.3. Acoustic impedance results of the second control group analyzed in the computer program.

Mean	**Standard Deviation**
1.598 MNs/m^3	0.018 MNs/m^3

Images in Figure 4.5 represent tumorous tissue of the second colon tissue.

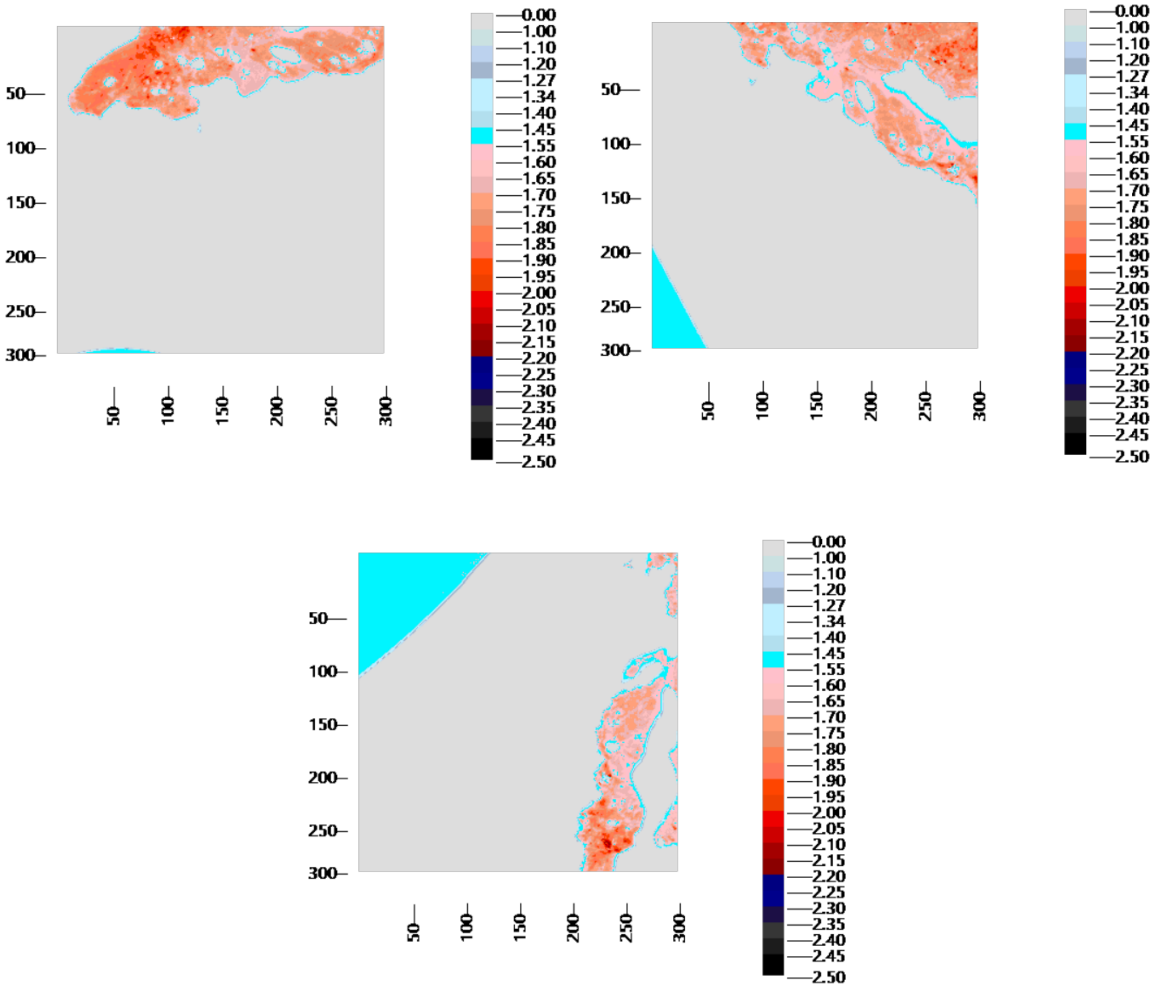

Figure 4.5. Detailed images of the second tumor group from different points.

Distilled water and a tumorous tissue were used for the second patient diagnosed with colon cancer in Figure 4.5. Each coordinate stands for 300 points corresponding to another 300 points. Total scanned area is 300 x 300 pixels. 300 points correspond to 4.8 mm. The total scanned area is 4.8 x 4.8 mm^2. The color key represents colors and corresponding acoustic impedance values (MNs/m^3) for each pixel.

The results of the tissue's analysis in Figure 4.5 are in Table 4.4.

Table 4.4. Acoustic impedance results of the second tumor group analyzed in the computer program.

Mean	Standard Deviation	Max Value
2.017 MNs/m^3	0.060 MNs/m^3	2.190 MNs/m^3

4.2.3. Colon Tissue 3

Images in Figure 4.6 represent healthy tissue of the third colon tissue.

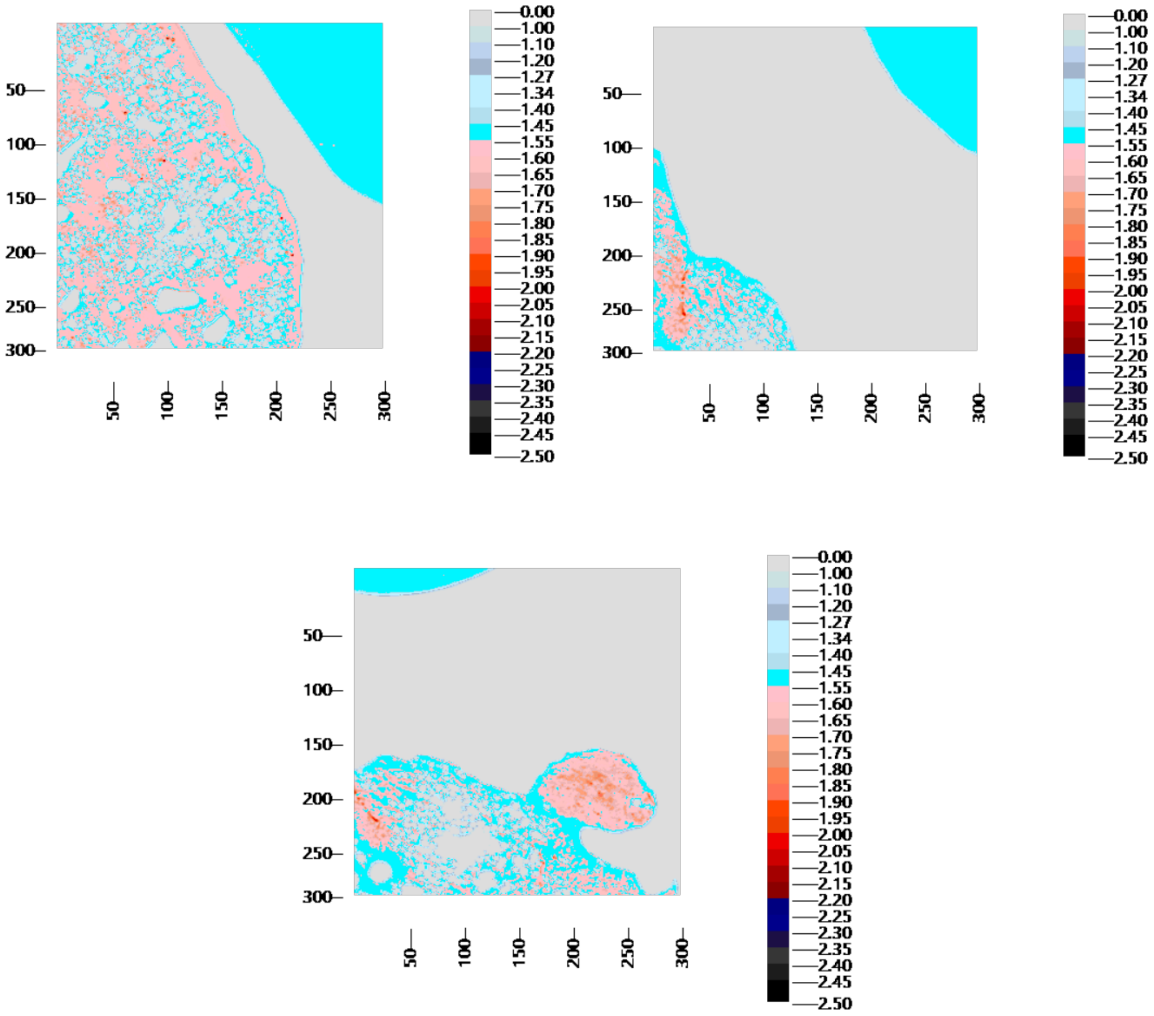

Figure 4.6. Detailed images of the third control group from different points.

Distilled water and a control tissue were used for the third patient diagnosed with colon cancer. Images are shown in Figure 4.6. Each coordinate stands for 300

points corresponding to another 300 points. 300 points correspond to 4.8 mm. The total scanned area is 4.8 x 4.8 mm^2. The color key represents colors and corresponding acoustic impedance values (MNs/m^3) for each pixel.

The results of the tissue's analysis in Figure 4.6 are in Table 4.5.

Table 4.5. Acoustic impedance results of the third control group analyzed in the computer program.

Mean	**Standard Deviation**
1.636 MNs/m^3	0.032 MNs/m^3

Images in Figure 4.7 represent tumorous tissue of the third colon tissue.

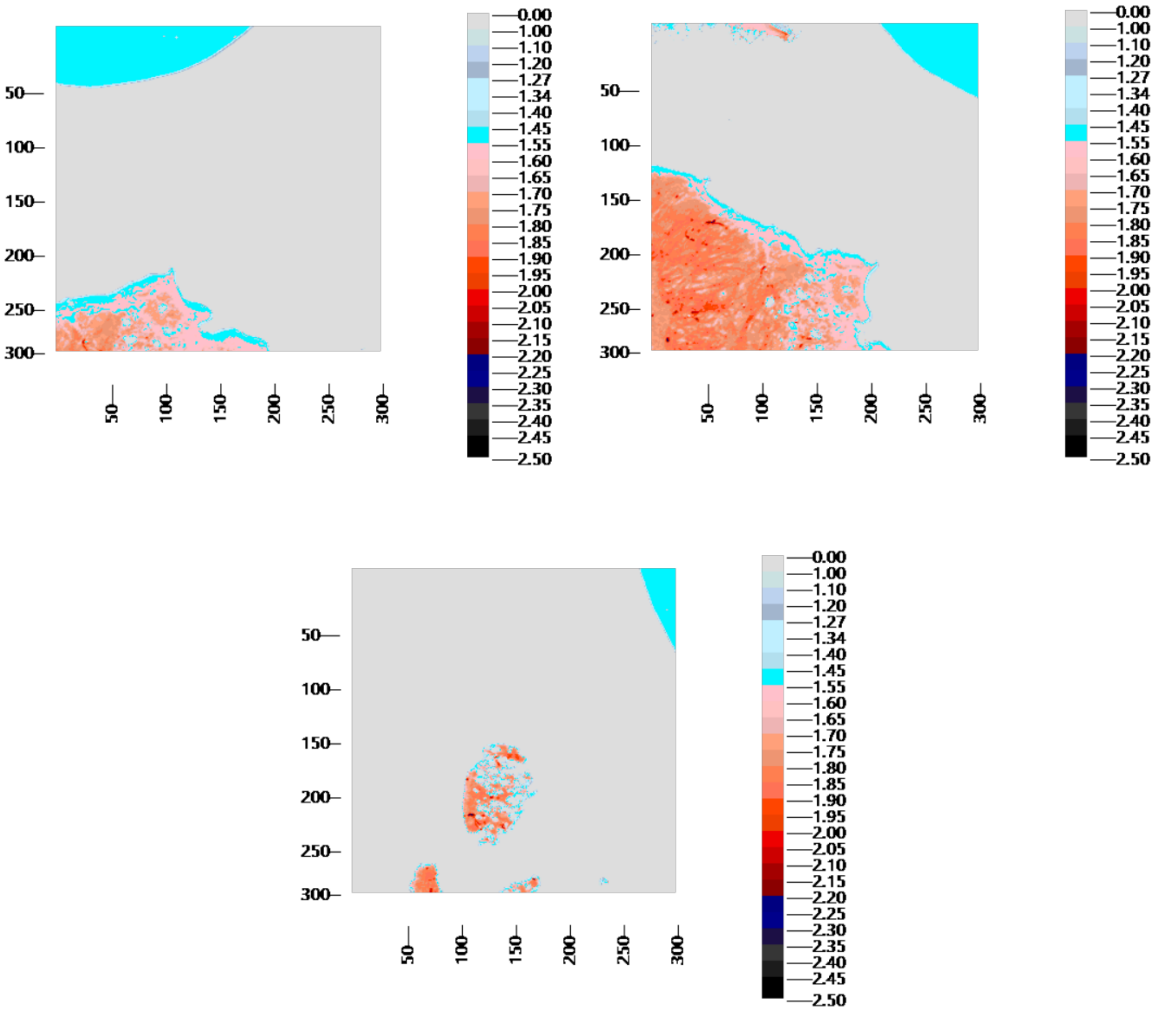

Figure 4.7. Detailed images of the third tumor group from different points.

Distilled water and a tumorous tissue were used for the third patient diagnosed with colon cancer. This tumorous tissue was imaged from different points in order to get the most accurate results. These images are shown Figure 4.7. Each coordinate stands for 300 points corresponding to another 300 points. Total scanned area is 300 x 300 pixels. 300 points correspond to 4.8 mm. The total scanned area is 4.8 x 4.8 mm^2. The color key represents colors and corresponding acoustic impedance values (MNs/m^3) for each pixel.

The results of the tissue's analysis in Figure 4.7 are in Table 4.6.

Table 4.6. Acoustic impedance results of the third tumor group analyzed in the computer program.

Mean	**Standard Deviation**	**Max Value**
2.080 MNs/m^3	0.052 MNs/m^3	2.210 MNs/m^3

4.3. Discussion

As it is discussed in Chapter 3, healthy and tumorous tissues have separate coefficients of elasticity and thus they have different impedance values (MNs/m^3). In comparison with healthy ones, cancerous cells have higher protein levels which make the coefficient of elasticity of the tissues larger [12]. Hence, the acoustic impedance value in the cancerous tissue is higher than the healthy tissue [13]. In Chapter 3, the collected data sets are analysed in MATLAB. However, the images may be more detailed in order to determine the tumorous area more precisely. In Chapter 4, the computer program is written to obtain more detailed images. For figures in Chapter 4, all coordinates represent 300 points and all images are formed by 300 x 300 pixels. 300 points correspond to 4.8 mm. The total scanned area is 4.8 x 4.8 mm^2. The color key represents the acoustic impedance values for a 1 x 1 pixel area. Every color corresponds to an acoustic impedance value in range 0.00 - 2.50 (MNs/m^3).

The results of maximum value are ignored since there may be tumor cells even in control group tissue. Thus, for the tissue of the control groups, mean and standard deviation values of control groups are analysed in the computer program. Mean, standard deviation and maximum value of tumor groups are analysed in the computer program. In order to determine whether the tissue is tumorous or not, maximum value analysis is conducted.

The difference between mean acoustic impedance values between control groups and tumorous tissue groups is informative about the elasticity of healthy and tumorous tissues [12]. Higher protein level can be detected since there is a correlation between the elasticity of a biological material and protein level so that cancerous tissues can be distinguished by checking acoustic impedance value [13]. The computer program works with more elaborated color key reference and because of that, the images indicate the difference between protein levels in detail. This distinct difference can be seen in acoustic impedance results analyzed in the computer program.

5. CONCLUSION

In this study, it is observed that the colon tumor affects the elasticity of tissue directly. The elasticity and acoustic impedance depend on each other. Therefore, different acoustic impedance values are measured by SAM. There is a significant difference between control and tumor groups of the same patients. In ImageJ analysis, for the first patient, while the control tissue's acoustic impedance mean is 1.715 MNs/m^3, the cancerous tissue's acoustic impedance mean is 1.755 MNs/m^3. For the second patient while control tissue's acoustic impedance mean is 1.736 MNs/m^3, the cancerous tissue's acoustic impedance mean is 2.039 MNs/m^3. For the third patient while control tissue's acoustic impedance mean is 1.678 MNs/m^3, the cancerous tissue's acoustic impedance mean is 1.998 MNs/m^3.

The new computer program was developed in order to create more detailed images than MATLAB ones and analyse data on these images more neatly rather than ImageJ. In the computer program analysis, for the first patient, while the control tissue's acoustic impedance mean is 1.642 MNs/m^3, the cancerous tissue's acoustic impedance mean is 1.941 MNs/m^3. For the second patient while control tissue's acoustic impedance mean is 1.598 MNs/m^3, the cancerous tissue's acoustic impedance mean is 2.017 MNs/m^3. For the third patient while control tissue's acoustic impedance mean is 1.636 MNs/m^3, the cancerous tissue's acoustic impedance mean is 2.080 MNs/m^3. It can be understood that defining limits of healthy and tumorous areas more precisely allows us to understand difference in ultrasonic properties between healthy and cancerous tissues more clearly.

Comparing both analysis tools, it can be concluded that acquiring more detailed images helps us to analyze more neatly and determine limits of tumorous areas more precisely. Ultrasonic medical imaging can be developed for detecting tumorous areas and limits of these areas by studying both hardware and software of ultrasonic devices.

From the results of both analysis tools, it can be easily said that tumor makes the tissue's acoustic impedance higher than the healthy ones. Especially for the second and the third cases, the difference between control and tumor tissues is highly significant.

As a future work, various areas and problems can be studied such as physical properties of different types of cancer and compare them with those of healthy tissue. Ultrasonic properties of other types of tissues and materials can be elaborated and examined deeply by using SAM.

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APPENDIX A: SCREENSHOTS OF SAM SOFTWARE

Figures A1-A6 are the screenshots of Honda AMS-50SI Scanning Acoustic Microscope Software.

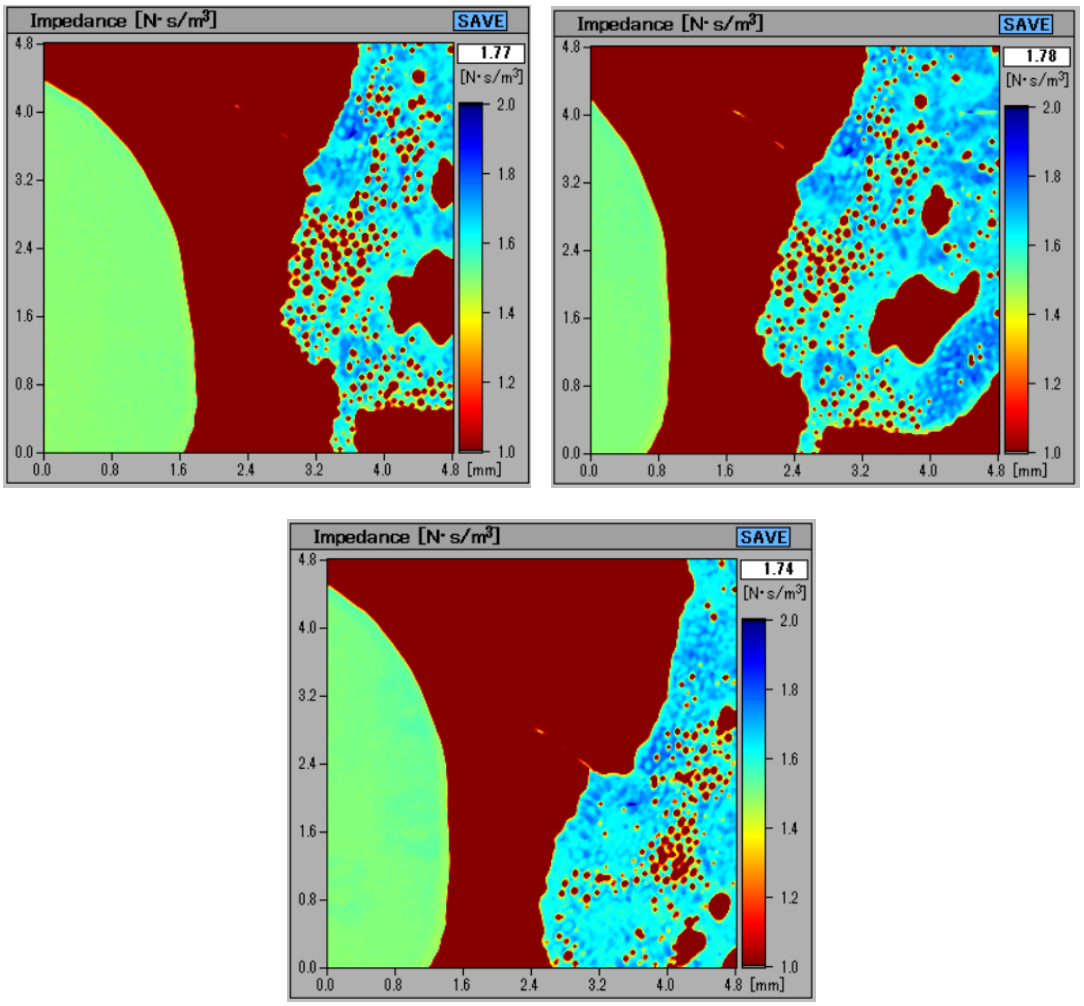

Figure A.1. First Control Group Screenshots of SAM Software.

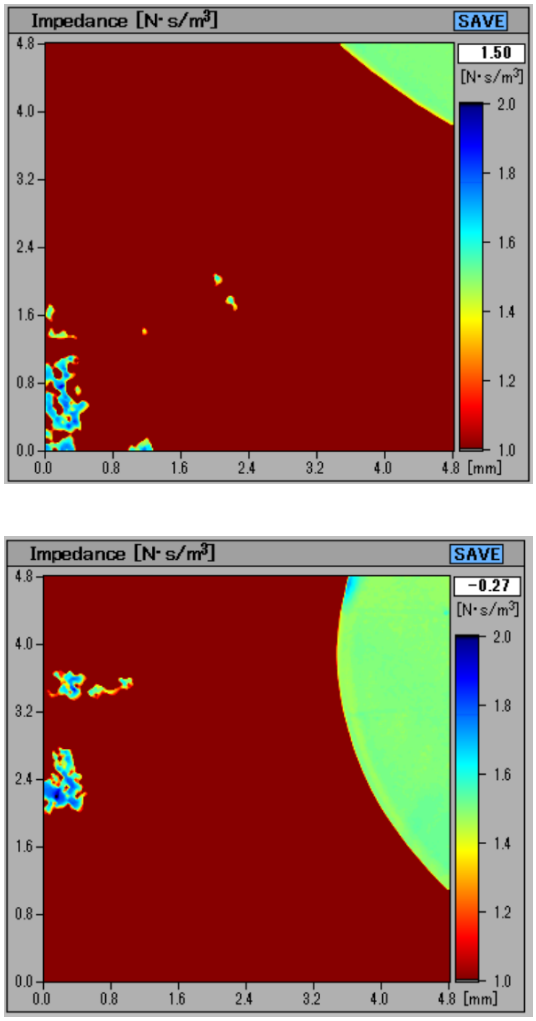

Figure A.2. First Tumor Group Screenshots of SAM Software.

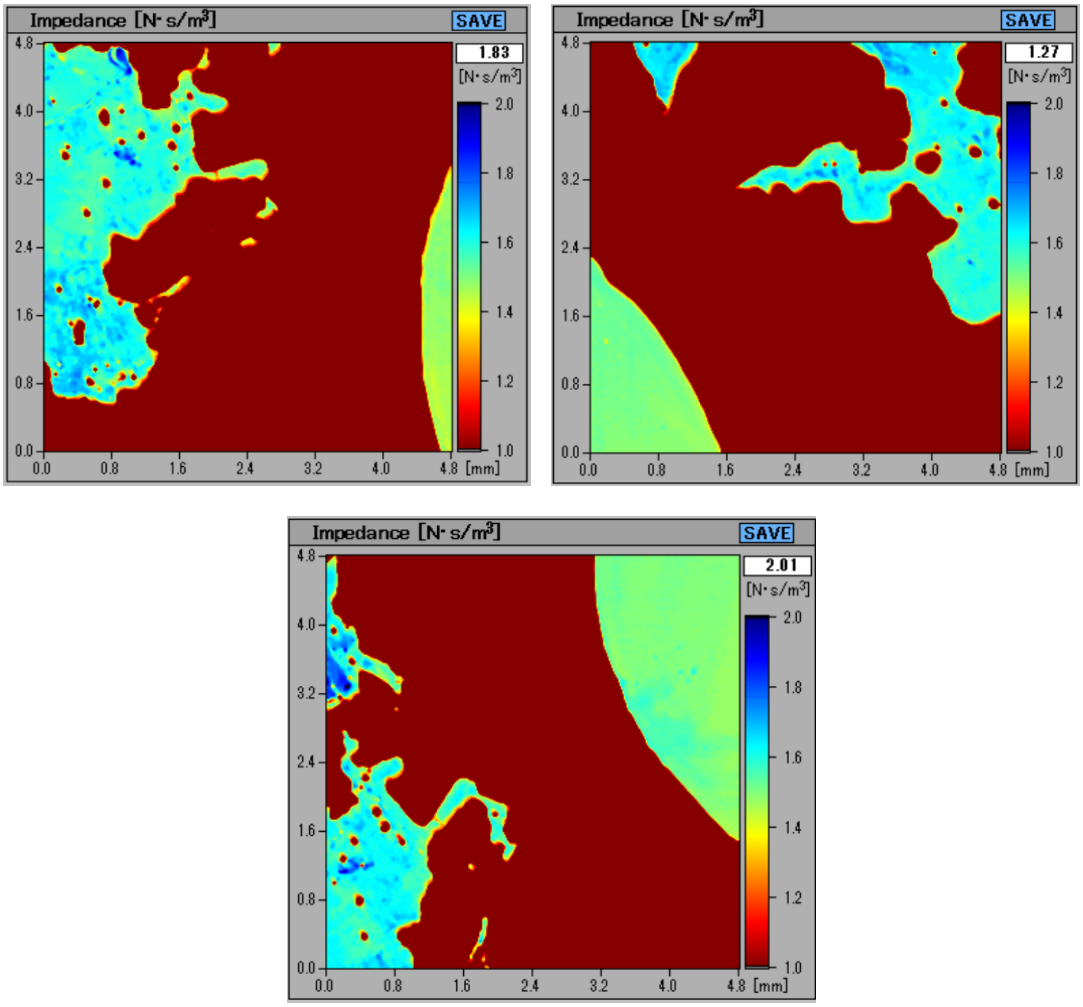

Figure A.3. Second Control Group Screenshots of SAM Software.

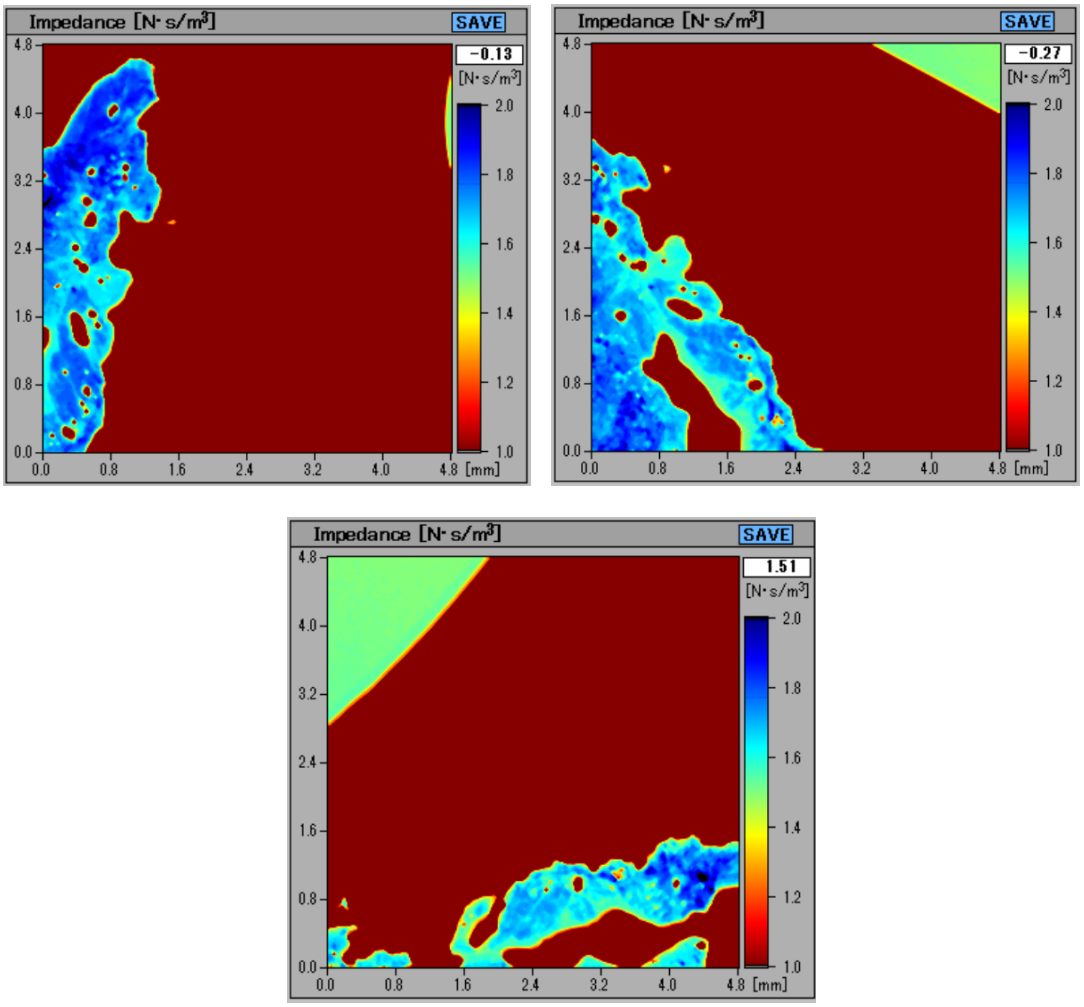

Figure A.4. Second Tumor Group Screenshots of SAM Software.

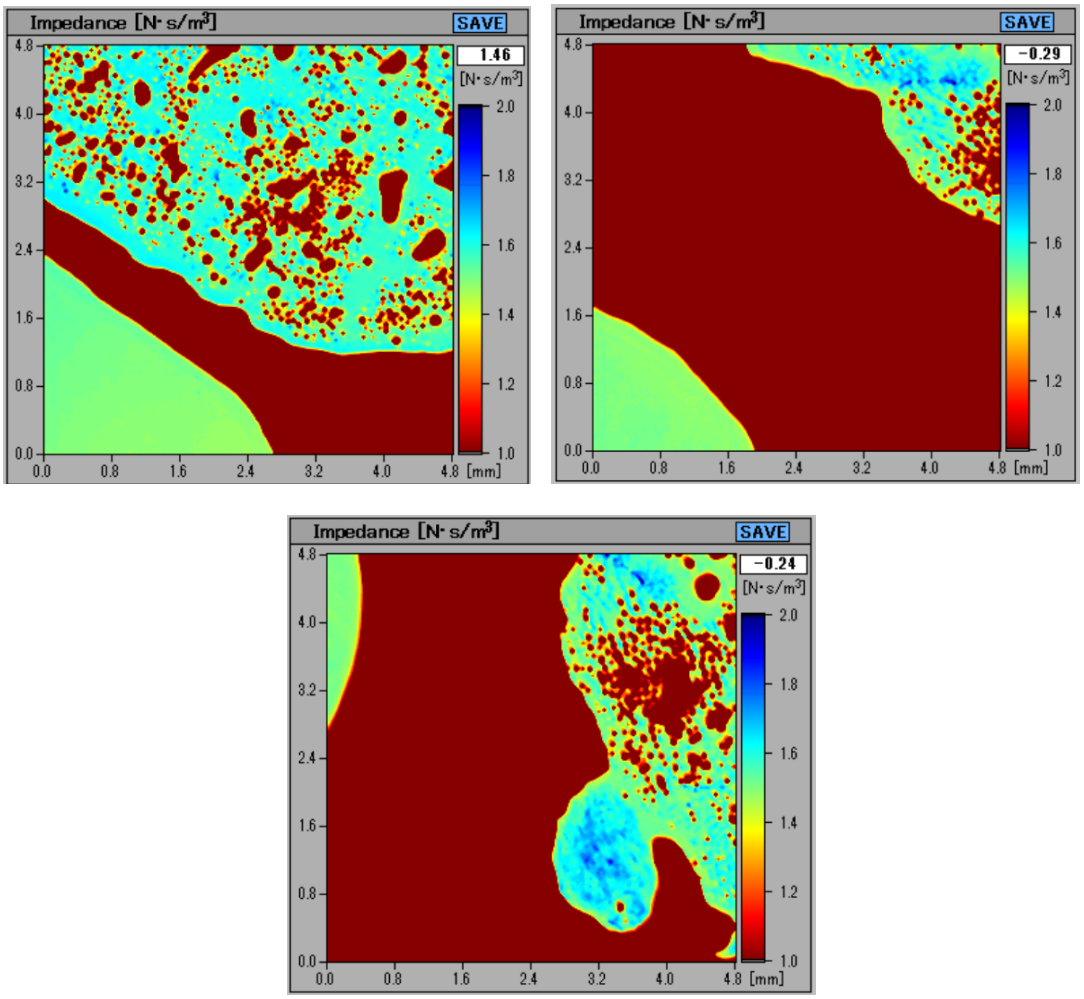

Figure A.5. Third Control Group Screenshots of SAM Software.

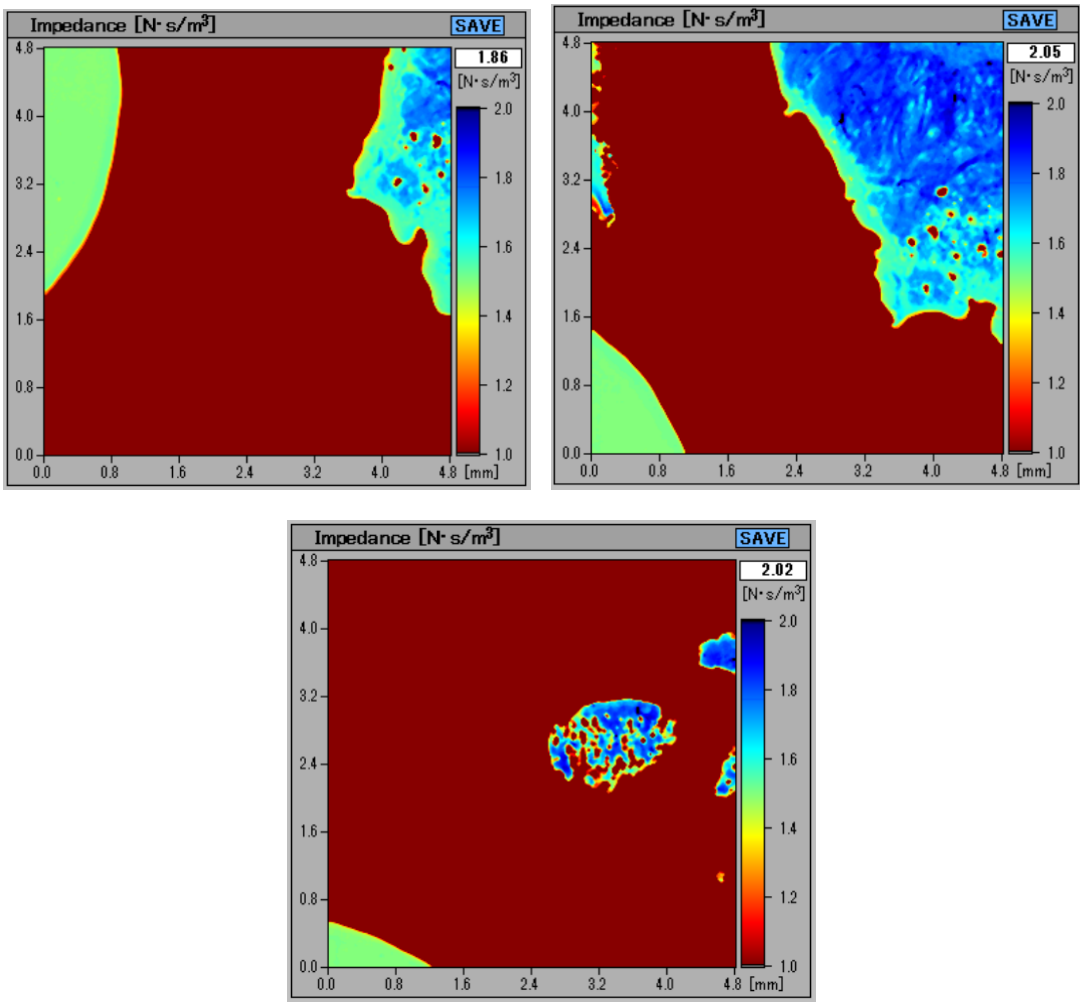

Figure A.6. Third Tumor Group Screenshots of SAM Software.

APPENDIX B: CODES FOR THE COMPUTER PROGRAM

```
using System;
using System.Collections.Generic;
using System.Drawing;
using System.IO;
using System.Linq;
using System.Text;
using System.Threading.Tasks;

namespace SAMAcousticImpedanceValueDataAnalysisTool
{
    internal class RawDataImageConverter
    {
        #region Variable
        private readonly int m_width = 300;
        private readonly int m_height = 300;
        private readonly int m_scale_width = 80;
        private readonly int m_rectangle_w = 20;
        private readonly int m_rectangle_h = 15;
        private Font font = new Font("Segoe UI", 12F);
        #endregion

        #region Ctor
        public RawDataImageConverter(string DataPath,
        string ScalePath)
        {
            this.DataPath = DataPath;
```

```
        this.ScalePath = ScalePath;
        Selection = new List<double>();
    }

    public RawDataImageConverter(string DataPath,
    string ScalePath, string SavedImagePath) :
    this(DataPath, ScalePath)
    {
        this.SavedImagePath = SavedImagePath;
    }

    #endregion

    #region Property
    public string DataPath { get; set; }

    public string ScalePath { get; set; }

    public string SavedImagePath { get; set; }

    public List<List<byte[]>> ProcessedDataList { get;
    private set; }

    public List<List<string>> RawData { get; private set; }

    public List<double> Selection { get; private set; }

    public double SelectionStandardDeviation { get; private set; }

    public double SelectionStandartError { get; private set; }
```

```
#endregion

#region Image Drawing

public Bitmap GetBitmapDrawData()
{
    var x = File.ReadAllLines(DataPath);
    ProcessedDataList = new List<List<byte[]>>();
    RawData = new List<List<string>>();
    foreach (var line in x)
    {
        var new_line = line.Replace('.', ',').Trim();
        var d = new_line.Split('\t').ToList();

        RawData.Add(d);
        ProcessedDataList.Add(ProcessedData(d));
    }
    Bitmap dataDraw = DrawData(ProcessedDataList, m_width,
    m_height);
    return dataDraw;
}

private List<byte[]> ProcessedData(List<string> data)
{
    var temp = new List<byte[]>();

    foreach (var d in data)
    {
        var decimal_data = decimal.Parse(d);

        if (decimal_data >= (decimal)-0.50 &&
```

```
decimal_data < (decimal)1.00)
{
    temp.Add(new byte[] { 221, 221, 221 });
    continue;
}
else if (decimal_data >= (decimal)1.00 &&
decimal_data < (decimal)1.10)
{
    temp.Add(new byte[] { 202, 225, 225 });
    continue;
}
else if (decimal_data >= (decimal)1.10 &&
decimal_data < (decimal)1.20)
{
    temp.Add(new byte[] { 188, 210, 238 });
    continue;
}
else if (decimal_data >= (decimal)1.20 &&
decimal_data < (decimal)1.27)
{
    temp.Add(new byte[] { 162, 181, 205 });
    continue;
}
else if (decimal_data >= (decimal)1.27 &&
decimal_data < (decimal)1.40)
{
    temp.Add(new byte[] { 191, 239, 255 });
    continue;
}
else if (decimal_data >= (decimal)1.40 &&
decimal_data < (decimal)1.45)
```

```
{
    temp.Add(new byte[] { 178, 223, 238 });
    continue;
}
else if (decimal_data >= (decimal)1.45 &&
decimal_data < (decimal)1.55)
{
    temp.Add(new byte[] { 0, 245, 255 });
    continue;
}
else if (decimal_data >= (decimal)1.55 &&
decimal_data < (decimal)1.60)
{
    temp.Add(new byte[] { 255, 192, 203 });
    continue;
}
else if (decimal_data >= (decimal)1.60 &&
decimal_data < (decimal)1.65)
{
    temp.Add(new byte[] { 255, 193, 193 });
    continue;
}
else if (decimal_data >= (decimal)1.65 &&
decimal_data < (decimal)1.70)
{
    temp.Add(new byte[] { 238, 180, 180 });
    continue;
}
else if (decimal_data >= (decimal)1.70 &&
decimal_data < (decimal)1.75)
{
```

```
            temp.Add(new byte[] { 255, 160, 122 });
            continue;
        }
        else if (decimal_data >= (decimal)1.75 &&
        decimal_data < (decimal)1.80)
        {
            temp.Add(new byte[] { 238, 149, 114 });
            continue;
        }
        else if (decimal_data >= (decimal)1.80 &&
        decimal_data < (decimal)1.85)
        {
            temp.Add(new byte[] { 255, 127, 80 });
            continue;
        }
        else if (decimal_data >= (decimal)1.85 &&
        decimal_data < (decimal)1.90)
        {
            temp.Add(new byte[] { 255, 114, 86 });
            continue;
        }
        else if (decimal_data >= (decimal)1.90 &&
        decimal_data < (decimal)1.95)
        {
            temp.Add(new byte[] { 255, 69, 0 });
            continue;
        }
        else if (decimal_data >= (decimal)1.95 &&
        decimal_data < (decimal)2.00)
        {
            temp.Add(new byte[] { 238, 64, 0 });
```

```
            continue;
        }
        else if (decimal_data >= (decimal)2.00 &&
        decimal_data < (decimal)2.05)
        {
            temp.Add(new byte[] { 238, 0, 0 });
            continue;
        }
        else if (decimal_data >= (decimal)2.05 &&
        decimal_data < (decimal)2.10)
        {
            temp.Add(new byte[] { 205, 0, 0 });
            continue;
        }
        else if (decimal_data >= (decimal)2.10 &&
        decimal_data < (decimal)2.15)
        {
            temp.Add(new byte[] { 164, 0, 0 });
            continue;
        }
        else if (decimal_data >= (decimal)2.15 &&
        decimal_data < (decimal)2.20)
        {
            temp.Add(new byte[] { 139, 0, 0 });
            continue;
        }
        else if (decimal_data >= (decimal)2.20 &&
        decimal_data < (decimal)2.25)
        {
            temp.Add(new byte[] { 0, 0, 128 });
            continue;
```

```
}
else if (decimal_data >= (decimal)2.25 &&
decimal_data < (decimal)2.30)
{
    temp.Add(new byte[] { 0, 0, 139 });
    continue;
}
else if (decimal_data >= (decimal)2.30 &&
decimal_data < (decimal)2.35)
{
    temp.Add(new byte[] { 28, 15, 69 });
    continue;
}
else if (decimal_data >= (decimal)2.35 &&
decimal_data < (decimal)2.40)
{
    temp.Add(new byte[] { 54, 54, 54 });
    continue;
}
else if (decimal_data >= (decimal)2.40 &&
decimal_data < (decimal)2.45)
{
    temp.Add(new byte[] { 28, 28, 28 });
    continue;
}
else if (decimal_data >= (decimal)2.45 &&
decimal_data < (decimal)2.50)
{
    temp.Add(new byte[] { 0, 0, 0 });
    continue;
}
```

```
        }
        return temp;

    }

    private Bitmap DrawData(List<List<byte[]>> data, int wi,
    int he)
    {

        Bitmap bmp = new Bitmap(wi, he);
        bmp.MakeTransparent();
        for (int h = 0; h < he; h++)
        {
            var h_data = data[h];
            for (int w = 0; w < wi; w++)
            {
                int r = 0, g = 0, b = 0;
                if (h_data.Count > w)
                {
                    var pixels = h_data[w];

                    r = pixels[0];
                    g = pixels[1];
                    b = pixels[2];

                    bmp.SetPixel(h, w,
                    Color.FromArgb(r, g, b));
                }
                else
                {
```

```
                    bmp.SetPixel(h, w,
                    Color.FromArgb(0, r, g, b));
                }


            }
        }

        return bmp;
    }

    #endregion

    #region Scale Drawing

    public Bitmap GetBitmapDrawDataScale()
    {
        var x_scale = File.ReadAllLines(ScalePath);
        var scale_array = new List<List<byte[]>>();
        foreach (var line in x_scale)
        {
            var new_line = line.Replace('.', ',').Trim();
            var d = new_line.Split('\t').ToList();
            scale_array.Add(ProcessedData(d));
        }
        var scale_height = m_rectangle_h *
        (scale_array.Count + 2);
        Bitmap scale = new Bitmap(m_scale_width, scale_height,
        System.Drawing.Imaging.PixelFormat.Format24bppRgb);
        scale.MakeTransparent();
        for (int i = 0; i < scale_array.Count; i++)
```

```
        {
            var index = GetScaleValue(i);
            var rgb = scale_array[i][0];
            var color = Color.FromArgb(rgb[0], rgb[1], rgb[2]);
            CreateRectangle(scale, 0, i *
            m_rectangle_h + m_rectangle_h,
            m_rectangle_w, m_rectangle_w, color, index);
        }


        using (Graphics graphics = Graphics.FromImage(scale))
        {
            var y = ((scale_array.Count + 1) * m_rectangle_h)
            - m_rectangle_h / 3;
            graphics.DrawString("----2.50", font,
            new SolidBrush(Color.Black), m_rectangle_w, y);
            graphics.Flush();
        }


        return scale;
    }

    private string GetScaleValue(int i)
    {
        var index = "----";
        switch (i)
        {
            case 0:
                index += $"0.00";
                break;
```

```
                case 1:
                    index += $"1.00";
                    break;
                case 2:
                    index += $"1.10";
                    break;
                case 3:
                    index += $"1.20";
                    break;
                case 4:
                    index += $"1.27";
                    break;
                case 5:
                    index += $"1.34";
                    break;
                case 6:
                    index += $"1.40";
                    break;
                case 7:
                    index += $"1.45";
                    break;
                case 8:
                    index += $"1.55";
                    break;
                case 9:
                    index += $"1.60";
                    break;
                case 10:
                    index += $"1.65";
                    break;
                case 11:
```

```
                index += $"1.70";
                break;
            case 12:
                index += $"1.75";
                break;
            case 13:
                index += $"1.80";
                break;
            case 14:
                index += $"1.85";
                break;
            case 15:
                index += $"1.90";
                break;
            case 16:
                index += $"1.95";
                break;
            case 17:
                index += $"2.00";
                break;
            case 18:
                index += $"2.05";
                break;
            case 19:
                index += $"2.10";
                break;
            case 20:
                index += $"2.15";
                break;
            case 21:
                index += $"2.20";
```

```
                break;
            case 22:
                index += $"2.25";
                break;
            case 23:
                index += $"2.30";
                break;
            case 24:
                index += $"2.35";
                break;
            case 25:
                index += $"2.40";
                break;
            case 26:
                index += $"2.45";
                break;
            case 27:
                index += $"2.50";
                break;
            default:
                index += "---";
                break;

        }
        return index;
    }



    #endregion
```

```
#region Unit Drawing

public Bitmap GetBitmapDrawUnit()
{
    var x_scale = new List<string> {"0" ,"50", "100", "150",
    "200", "250", "300" };

    var scale_height = 320;
    var scale_width = 60;

    Bitmap scale = new Bitmap(scale_width, scale_height,
    System.Drawing.Imaging.PixelFormat.Format24bppRgb);
    scale.MakeTransparent();

    //CreateRectangleUnit(scale, 0, 0, 50, m_rectangle_w,
    Color.Transparent, x_scale[0] + "---");//0
    CreateRectangleUnit(scale, 0, 50, 50, m_rectangle_w,
    Color.Transparent, x_scale[1] + "---");//50
    CreateRectangleUnit(scale, 0, 100, 50, m_rectangle_w,
    Color.Transparent, x_scale[2] + "---");//100
    CreateRectangleUnit(scale, 0, 150, 50, m_rectangle_w,
    Color.Transparent, x_scale[3] + "---");//150
    CreateRectangleUnit(scale, 0, 200, 50, m_rectangle_w,
    Color.Transparent, x_scale[4] + "---");//200
    CreateRectangleUnit(scale, 0, 250, 50, m_rectangle_w,
    Color.Transparent, x_scale[5] + "---");//250
    CreateRectangleUnit(scale, 0, 290, 50, m_rectangle_w,
    Color.Transparent, x_scale[6] + "---");//300

    return scale;
}
```

```
public Bitmap GetBitmapDrawUnitBottom()
{
    var x_scale = new List<string> { "0", "50", "100", "150",
    "200", "250", "300" };

    var scale_height = 350;
    var scale_width = 60;

    Bitmap scale = new Bitmap(scale_width, scale_height,
    System.Drawing.Imaging.PixelFormat.Format24bppRgb);
    scale.MakeTransparent();

    //CreateRectangleUnit(scale, 0, 0, 50, m_rectangle_w,
    Color.Transparent, x_scale[0] + "---");//0
    CreateRectangleUnit(scale, 0, 50, 50, m_rectangle_w,
    Color.Transparent, x_scale[1] + "---");//50
    CreateRectangleUnit(scale, 0, 100, 50, m_rectangle_w,
    Color.Transparent, x_scale[2] + "---");//100
    CreateRectangleUnit(scale, 0, 150, 50, m_rectangle_w,
    Color.Transparent, x_scale[3] + "---");//150
    CreateRectangleUnit(scale, 0, 200, 50, m_rectangle_w,
    Color.Transparent, x_scale[4] + "---");//200
    CreateRectangleUnit(scale, 0, 250, 50, m_rectangle_w,
    Color.Transparent, x_scale[5] + "---");//250
    CreateRectangleUnit(scale, 0, 300, 50, m_rectangle_w,
    Color.Transparent, x_scale[6] + "---");//300

    return scale;
```

```
}

#endregion

#region Helper

public void SaveBitmap(Bitmap bitmap)
{
    bitmap.Save(SavedImagePath);
}

public Bitmap MergedBitmaps(Bitmap bmp1,
Bitmap bmp2,int marginwidth = 40)
{

    var h = Math.Max(bmp1.Height, bmp2.Height);
    var margin = new Bitmap(marginwidth, h);
    margin.MakeTransparent();
    var w = bmp1.Width + bmp2.Width + margin.Width;
    Bitmap result = new Bitmap(w, h);
    using (Graphics g = Graphics.FromImage(result))
    {
        var bmp1_h_mean = (h - bmp1.Height) / 2;
        g.DrawImage(bmp1, 0, bmp1_h_mean);
        g.DrawImage(bmp1, 0, bmp1_h_mean);
        g.DrawImage(margin, bmp1.Width, 0);
        g.DrawImage(bmp2, bmp1.Width + margin.Width, 0);
    }
    return result;
}
```

```
public bool CheckedState()
{
    return !(DataPath == string.Empty ||
    ScalePath == string.Empty);
}

public string GetDataFileName()
{
    return $"{Path.GetFileName(DataPath).Split('.')[0]}.bmp";
}

private Bitmap CreateRectangle(Bitmap bitmap, int x, int y,
int height, int width, Color color, string index)
{

    using (Graphics graphics = Graphics.FromImage(bitmap))
    {

        using (SolidBrush myBrush = new SolidBrush(color))
        {
            graphics.FillRectangle(myBrush,
            new Rectangle(x, y, width, height));
            graphics.DrawString(index, font,
            new SolidBrush(Color.Black), x + m_rectangle_w,
            y - (height / 2));
            //graphics.DrawString(index, font,
            new SolidBrush(Color.Black), x + m_rectangle_w,
            y );

        }
        graphics.Flush();
```

```
        }

        return bitmap;
    }

    private Bitmap CreateRectangleUnit(Bitmap bitmap, int x,
    int y, int height, int width, Color color, string index)
    {
        using (Graphics graphics = Graphics.FromImage(bitmap))
        {

            using (SolidBrush myBrush = new SolidBrush(color))
            {
                graphics.FillRectangle(myBrush, new Rectangle(x,
                y, width, height));
                //graphics.DrawString(index, font,
                new SolidBrush(Color.Black), x + m_rectangle_w,
                y - (height / 2));
                graphics.DrawString(index, font,
                new SolidBrush(Color.Black),
                x + m_rectangle_w, y);

            }
            graphics.Flush();
        }

        return bitmap;
    }
    #endregion

    #region Computation
```

```
public void CalculateSelection(int X0,int X1,int Y0,int Y1)
{
    var selectionRawData = new List<double>();
    int minX = Math.Min(X0, X1);
    int minY = Math.Min(Y0, Y1);

    int maxX = Math.Max(X0, X1);
    int maxY = Math.Max(Y0, Y1);

    for (int x = minX; x < maxX; x++)
    {
        for (int y = minY; y < maxY; y++)
        {
            selectionRawData.Add(double.Parse(RawData[x][y]));
        }
    }

    Selection = selectionRawData;
}

public double CalculateStandardDeviation()
{
    double mean = Selection.Sum() / Selection.Count;

    double sum = 0.0;

    Selection.ForEach((d) =>
    {
        sum += Math.Pow((d - mean), 2);
    });
```

```
            SelectionStandardDeviation =
            Math.Sqrt(sum / (Selection.Count - 1));

            return SelectionStandardDeviation;
        }

        public double CalculateStandartError()
        {
            SelectionStandartError =
            CalculateStandardDeviation() / Math.Sqrt(Selection.Count);
            return SelectionStandartError;
        }

        #endregion
    }
}
```