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PREPARATION OF EMBOLIC PROTECTION DEVICES FOR EMBOLIC
CHARACTERIZATION

A Thesis

Presented to

The Faculty of the Program of General Engineering

San Jose State University

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

by

Arundhati Deshpande

December 2007

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ABSTRACT

PREPARATION OF EMBOLIC PROTECTION DEVICES FOR EMBOLIC CHARACTERIZATION

by Arundhati Deshpande

Techniques to prepare embolic protection filters for micro computed tomography (micro CT) and subsequent histopathology, and which can eventually be used to bring about tissue characterization in terms of Hounsfield Units (HU), were compared. The techniques evaluated in this study included embedding a specimen each in a) a paraffin cassette, b) a polymethyl methacrylate (PMMA) block, c) paraffin followed by sectioning and mounting on glass slides, and d) PMMA followed by sectioning into 800 μ m wafers. Micro CT was performed on these specimens. The images were used to reconstruct and detect the HU of the tissue. Embedding the specimen in paraffin was found to be best for preparing specimens suitable for micro CT and subsequent histopathology. Using this technique, CT images with higher resolution were obtained, with adjacent regions having different pixel values. HUs could be clearly differentiated.

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CHAPTER ONE

INTRODUCTION

1.1 Importance of Embolic Protection

An embolus is defined as a tissue fragment that detaches from the inside of a blood vessel, travels through the circulatory system, and can obstruct blood flow [1]. Emboli are commonly found to be detached portions of lesions^{*} [2]. Arterial embolism is the disruption of blood supply to a particular part of the body caused due to blockage or ‘occlusion’ of an artery by such detached emboli. Such an occlusion can prevent the tissue downstream from obtaining the necessary amount of blood flow and oxygen, resulting in damage and death of the tissue. The phenomenon of embolization is depicted in Figure 1. An occluded blood vessel with thrombus^{**} formation is seen in Figure 1(a). The lesion grows in size due to accumulation of cells and causes vessel blockages. If an embolus dislodges from the plaque or thrombus as shown in Figure 1(b) and flows downstream it can occlude a smaller artery thus causing tissue death as shown in Figure 1(c). Strokes and heart attacks are commonly caused by such emboli. Atherosclerotic Heart Disease^{***} is considered to be one of the primary causes of death in the United States. It has been reported that the deposition of low-density lipoprotein and fibrin cause the development of plaque^{****} over time [3]. Plaque causes the narrowing of the

^{*} A lesion is defined as “any abnormal tissue change caused by disease or injury.” [2].

^{**} A thrombus is a blood clot.

^{***} Atherosclerotic heart disease (atherosclerosis) is the result of the build-up of plaque on the inner walls of a blood vessel which causes it to narrow down and obstruct blood flow.

^{****} Plaque is the build up of fat cells in the walls of an artery. It could also be caused by the build-up of cholesterol.

inner diameter of arteries. Obstruction of blood flow and consequent tissue infarction* are the penalties of atherosclerosis [3].

Figure 1. Mechanism of embolization (adapted from [4]).

The atheroma** so developed is viewed as an active, inflammatory, and unstable environment[3]. The activation of certain atheroma cells is reported to play a crucial role in causing plaque instability. Unstable atheroma causes parts of the plaque (emboli) to become disjointed and travel downstream leading to infarcts. Therefore, the prevention of embolization is considered to be a preliminary step towards avoiding the ill effects of atherosclerosis.

* Infarction can be defined as the death of tissues due to lack of oxygen supply.

** Atheroma is the accumulation of cells or cell products within the blood vessel.

Surgical or interventional removal of plaque is one of the most important causes of embolization, [5] resulting in several peri- or post-operative complications.

Angioplasty* can be one of the sources of emboli. In order to prevent the emboli from traveling in the blood stream during angioplasty, embolic protection devices have been designed, developed, and marketed. It has been reported that embolic protection leads to an 8-10 fold decrease in death rate [6]. A stroke is one fatal effect of embolization. It is the third-leading cause of death in the United States [7]. 282,000 fatalities from stroke have been forecasted for the year 2007 [7]. Therefore, creation of peri-operative embolic protection devices has been a focus of the medical device industry. The total number of carotid artery stenting procedures done from 1997 to 2003 has been reported to be equal to 12,392 [7]. A progressive increase in the use of embolic protection devices over the years is seen in Figure 2. It can be seen that in the year 1997, the use of embolic protection devices was only 0.013 times of that in the year 2003. By the year 2002, the use of embolic protection devices rose to 0.865 times of that in the year 2003.

1.2 Types of Embolic Protection Devices

Embolic protection devices may be classified into distal protection devices and proximal protection devices. Distal embolic protection devices were introduced by Jacques Theron [5] for cerebral protection during angioplasty and stenting in the carotid arteries. Currently, distal embolic protection devices have applications in carotid stenting, coronary artery stenting, and as interventions on degenerated saphenous vein

*Angioplasty is a surgical procedure in which a balloon catheter is inserted into a narrowed or blocked blood vessel. The balloon is inflated, causing the vessel to open and restoring blood flow. It is also called Percutaneous Transluminal Coronary Angioplasty (PTCA).

grafts. Distal protection devices range from balloon systems that cause temporary occlusion of the vessel distal to the lesion to filter systems that preserve blood flow through their pores but capture emboli. An alternative to distal protection devices is proximal protection devices. Here, a balloon is placed proximal to the lesion causing occlusion of the artery, thereby initiating reverse flow from the artery [5].

Figure 2. Use of embolic protection devices [8].
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1.3 Need to Characterize Emboli

Characterization is the process of bringing about a structural classification of a diverse group of material. Emboli traveling in the blood stream could be made up of several constituents or could be of a single substance. Some of the common constituents of emboli are blood clots (thromboembolism), fat globules (fat embolism), calcifications, cholesterol embolism (atheroembolism), plaque, and even emboli-carrying bacteria. These constituents have unique physical and chemical properties, each having different

effects on embolic protection devices. For example, hard and sharp embolic calcifications could cause a thin polymeric embolic protection filter bag to rupture, thereby negating its purpose. Characterizing the constituents of emboli can help in the optimization of embolic protection device design and material selection.

Characterization can also help build a library of information which relates a constituent to its particular characteristic. One of the most important reasons for characterizing embolic material is to serve as predictors for treating patients. Physicians may be able to predict the embolic constituents with the help of characterization and the history of the patient. With further research, it might be possible to relate the presence or absence of the particular constituent to a unique diseased state. These data can then be used as predictors for treating patients. Another potential application of embolic characterization is to understand the type and cause of embolization. Information about the parent lesion can also be collected. Data obtained from characterization could also pilot a histological procedure to the appropriate region of interest (ROI). Detection, visualization, and quantification of structural parameters such as volume estimation of constituents within an emboli or lesion would be possible. Characterization of emboli is also important to the selection of an appropriate embolic protection device before a surgical procedure. If the constituents of a lesion and their volumes can be diagnosed before a procedure, then physicians can use this information to plan and perform clinical procedures accordingly. Such characterization would enable the medical device community to take a step closer to the ultimate goal of helping the physician reduce risk during minimally invasive surgery and interventional procedures, especially in cardiology and radiology.

1.4 Current Method Used to Characterize Embolic Constituents

One of the common methods used to characterize emboli is via histopathological testing with the help of electron or light microscopy. Histology^{*} is an inexpensive technique and large areas can be studied with good magnification. Histological and pathological^{**} methods can be used only after the embolus is removed from the body [9,10]. They are time-consuming, destructive methods that need the embolus specimen to be sectioned, sliced and stained according to the requirements of histopathological analysis. The original structure of the collected embolus cannot be retained after the histopathological analysis, thus preventing any further analysis on the embolic material. Therefore, there is need for a non-destructive method of embolic characterization.

^{*} Histology is defined as “The study of cells and tissue on the microscopic level” [9].

^{**} Pathology is defined as “the study and diagnosis of the structural and functional changes in cells, tissues and organs” [10].

CHAPTER TWO

LITERATURE REVIEW

Risk reduction has been one of the main foci of diagnostic medicine. Several investigations have been conducted over the years to understand vascular diseases and improve methods to diagnose them. A review of the literature on lesions, emboli, diagnostic imaging techniques, and characterization is presented in the following sections.

2.1 Classification of Lesions and Constituents of Emboli

Lesions developed within vessels change their composition and structure with time as described by H.C. Stary *et.al* [3]. Various attempts have been made to classify lesions in order to have a standard nomenclature instead of a variety of vague terms. The American Heart Association (AHA) developed a classification system based on histological and pathological composition and structure. The types of lesions are shown in Figure 3, where it can be seen that each type of lesion has unique cells and tissues. For example, in a Type II lesion macrophage foam cells are visible within the adaptive thickening of the cell wall; however, in a Type V lesion fibrous thickening of the cell wall is also apparent. The stages in evolution of atherosclerotic lesions can be seen in Figure 4. The histological types are defined in the left column. The arrows in the second column indicate the sequence of progression of lesions. Thrombotic deposits are found to form repeatedly in stages V and VI. Researchers identify this as the principle mechanism of occlusion. Emboli evolving from the lesions may contain any or all of the tissue types

mentioned. Some of the constituents of emboli are platelets, collagen, fibrin, elastin, calcifications, plaque, foam cells, and cholesterol [11].

Figure 3. Cross sectional views of six left anterior descending coronary arteries [3].
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Nomenclature and main histology	Sequences in progression	Main growth mechanism	Earliest onset	Clinical correlation
Type I (initial) lesion isolated macrophage foam cells	<pre> graph TD I((I)) --> II((II)) II --> III((III)) III --> IV((IV)) IV --> V((V)) V --> VI((VI)) VI --> V </pre>	growth mainly by lipid accumulation	from first decade	clinically silent
Type II (fatty streak) lesion mainly intracellular lipid accumulation				
Type III (intermediate) lesion Type II changes & small extracellular lipid pools			from third decade	
Type IV (atheroma) lesion Type II changes & core of extracellular lipid		accelerated smooth muscle and collagen increase	from fourth decade	clinically silent or overt
Type V (fibroatheroma) lesion lipid core & fibrotic layer, or multiple lipid cores & fibrotic layers, or mainly calcific, or mainly fibrotic				
Type VI (complicated) lesion surface defect, hematoma-hemorrhage, thrombus		thrombosis, hematoma		

Figure 4. Sequence of progression of atherosclerosis in human arteries [3].
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2.2 Diagnostic Medical Imaging and Imaging Techniques

The term diagnostic medical imaging represents methods by which an area of the body not visible externally is examined and assessed by creating and analyzing its corresponding images [12]. Medical imaging may be used by radiologists to diagnose specific diseases in human tissues (pathology) or to understand tissue development. It

could also include the development of novel methods for creating these images by radiologists, scientists, and engineers. The technological features of medical imaging are known as radiography. The basic principle of imaging techniques is based on inverse mathematics which infers the properties of tissues from the corresponding signals recorded during the imaging process. For example, in magnetic resonance imaging (MRI) radiofrequency signals and magnets are used to obtain images. These images are used to infer the properties of the examined tissue.

Some of the available imaging techniques currently used in the medical industry are summarized in the following sections.

2.2.1 Projection Radiography

Projection radiography is commonly used in the diagnosis of bone fractures, lung pathology, visualization of the structure of intestines, and sometimes in the diagnosis of colon cancer [12]. In projection radiography, X-rays (electromagnetic radiation) are passed through the body under observation. The projection of the body is recorded on film. The clarity of the image depends on the density of the tissue in the path of the X-ray beam. This method is commonly known as “X-rays”. Contrast media may also be used if internal organs need to be studied. A projection radiograph of a human chest is shown in Figure 5. Variations in grayscale corresponding to different tissue radio densities can be seen. However, the difference between radio densities of soft tissues is less apparent.

Figure 5. Projection radiographic image of rib cage [13].
(Reprinted with permission.)

2.2.2 Fluoroscopy

Fluoroscopy is commonly used to observe the digestive tract and in image-guided procedures such as balloon angioplasty, and urological surgery [14]. It has applications in orthopedic surgery [15]. Fluoroscopy is a real-time imaging technique in which x-rays and contrast media are used on a live patient. A beam of X-rays is transmitted through the body. The body transmits the rays onto a fluorescent plate at intensities that depend on the density of the tissue. The plate is coupled to an image intensifier which in turn is coupled to a camera. It is possible to watch the images live on a screen. A fluoroscopic image of the intestine is shown in Figure 6. Variations in grayscale corresponding to different tissue radio densities can be observed even for soft tissues.

2.2.3 Magnetic Resonance Imaging (MRI)

Magnetic resonance imaging (MRI), also known as nuclear magnetic resonance imaging (NMRI), is used in scanning images of the spine, soft tissues, and the brain [16].

It may also be used to differentiate between normal and diseased tissue and to examine joints [12].

Figure 6. Fluoroscopic image of the intestine [14].
(Reprinted with permission.)

Since MRI does not use ionizing radiation, it finds several applications in examining tissues in pregnant women [12]. MRI is an imaging technique in which magnetic properties, radio waves, and computers are used to generate images of internal structures of the body [16]. Signals are produced by the excitation of hydrogen nuclei present in water molecules within tissues with the help of magnets [12]. MRI produces two-dimensional images in the form of slices. MRI image is not useful when scanning tissues that do not primarily contain water molecules, such as bones. This gives MRI an advantage in imaging soft tissues without the interference of bone images [12]. An MRI image of the human head is shown in Figure 7. Soft tissue can be differentiated on the MRI image. Variations in the gray scale of the lobes of the brain can be seen as compared to the medulla.

Figure 7. MRI of the head [17].
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2.2.4 Ultrasonography (Ultrasound)

Ultrasonography is used to obtain information about a fetus, and to examine abdominal organs and the heart. Tissues reflect high-frequency sound waves in the range of 2-10 MHz to various degrees. These reflections are used to obtain two-dimensional images on a television monitor [12]. Real-time data capturing can be done to understand the function of organs. A comparison of the image of an actual fetal specimen with its MRI and ultrasound images is shown in Figure 8. The morphology of the organs is more clearly visible on the MRI image as compared to the ultrasonic image; however, ultrasonography has relatively shorter imaging procedures, is cheaper, and causes less patient discomfort.

2.2.5 Tomography

“Tomography refers to the cross-sectional imaging of an object from either transmission or reflection data collected by illuminating the object from many different directions” [18], using X-rays. Basically, tomography is aimed at reconstructing an image from its projections. The focal spot and image receptor are moved to obtain detailed radiographic images of only one plane which is in sharp focus [19].

Tomography is also known as “body section radiography, planigraphy, laminography and stratigraphy”[19].

Figure 8. Comparison of an MRI image versus an ultrasonograph of a fetus [20].
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Several different types of tomography are available. In linear tomography, as the source of X-rays and the image receptor move from one position to another, every point on the focal plane is projected to the same location on the image receptor [19]. In Figure 9, the projections of points a, b, c are a”, b”, and c” in Position 1, and a’, b’, and c’ in Position 2. Since the points above and below the focal plane are out of focus, their

data are not recorded on the film. This type of tomography is no longer in use and has been replaced by computed tomography [19] which is explained in Section 2.2.6. In polytomography, a complex set of geometrical movements is programmed. Some of them include circular, hypocycloidal, and elliptical motions of the source and signal receptor. The 'Polytome', manufactured by Philips is an example of this type of device.

Figure 9. Principle of linear tomography (adapted from [19]).

Zonography is similar in principle to linear tomography [12]. A restricted arc of travel is used as in the case of intravenous urograms (IVU). Computed tomography (CT) or computed axial tomography (CAT) is the most common form of tomography used today. It is helical tomography that is used to generate two-dimensional images in the form of slices of the structure under examination.

2.2.6 Principles of Rotational Computed Tomography

In rotational computed tomography, a source of X-rays and X-ray sensors rotate around the body and capture data in the form of image scans or snapshots [21]. The source and the sensors are positioned on the opposite side of a circular trajectory. Many data snapshots are taken successively as the body is steadily passed through the gantry. The data scans are mathematically combined together by a process called *Tomographic Reconstruction* to form a single slice [21]. Various software tools are then used to assemble these slices and generate a three dimensional virtual model of the whole structure under study. The basic principle behind computed tomography (CT) is that materials have the ability to attenuate X-rays exponentially, depending upon the length of travel within the tissue and its attenuation coefficient, as shown in Figure 10 [22]. Consider an X-ray beam of intensity I_0 passing through a material with an attenuation coefficient of μ^* and of length d [23]. The intensity of the beam exiting the material would then be equal to

$$I = I_0 * e^{-d\mu} \quad \text{Equation 1}$$

This equation is also represented as

$$I = I_0 * e^{[-(\mu/\rho) / \rho * t]} \quad \text{Equation 2}$$

where ρ is the specimen density, t is the specimen thickness, and (μ/ρ) is the mass absorption coefficient. Data collected by CT are saved on a computer digital form as an image [24]. The image can be viewed on a television monitor or printed on film. This image consists of a matrix of pixels. Conventionally, these pixels number up to

* A factor expressing optical power loss per unit of length, expressed in dB/Km [23].

65,000 [24]. A CT number, measured in Hounsfield units,* is given to each of these pixels. This number indicates the amount of I_0 absorbed by the tissue at each point in the specimen under study [24]. This number increases with an increase in the density of the tissue and ranges between -1,000 HU and +1,000 HU corresponding to air and bone respectively [24]. The range of Hounsfield Units commonly seen among tissues is shown in Figure 11. It can be seen that tissue of the lungs and fat lie on the negative side of the HU scale whereas soft tissues and bone lie on the positive side of the HU scale.

Figure 10. Principle of tomography (adapted from [22]).

A large amount of data are produced during CT. These data can be manipulated with the help of a process called windowing, “the process of using the calculated Hounsfield Units to make an image” [21], to exhibit the ability of structures to block an X-ray beam. Radiolucent materials have greater transparency to X-ray photons whereas

* The CT number in Hounsfield Units is a quantitative unit used to describe *radio density* which is “the property of relative transparency to the passage of X-rays through a material” [25].

radiodense materials have greater opacity to them [25]. As a point of reference the Hounsfield Unit of distilled water at Standard Temperature Pressure (STP) is zero [25]. The HU of some common substances is given in Table 1.

Figure 11. Hounsfield Units of various tissues (adapted from [24]).

Table 1. HUs of Some Common Substances [25].

Substance	HU
Air	-1000
Fat	-120
Water	0
Muscle	+40
Bone	+1000

The CT image obtained is displayed with a unique shade of gray for each unique CT number in HUs; since the human eye cannot differentiate among 1,500 shades of gray, the *windowing* system is used. Radio density amplitudes are mapped to 256 shades of gray that can be spread over a broad range of HU values [25]. These ranges help construct an overview of the material shape and structure that attenuate the beam of X-

rays to considerable extents. The shades of gray can be spread over a narrow range of HU values around the average HU value of the material, helping to distinguish subtle variations in the structure.

2.3 Present Research in the Field Of Diagnostic Imaging

The importance and use of diagnostic imaging has increased over the years because it offers a technique of gathering information about organs and tissues that are not normally visible. Several techniques of diagnostic imaging, as described in Section 2.2, have been developed and evaluated for specific applications. Techniques have been compared to evaluate their properties in specific applications. For example, research has been conducted to compare computer tomography with fluoroscopy. The sensitivity* of computed tomography (CT) is considered to be higher than that of fluoroscopy in the detection of coronary artery calcification [26-28]. Spiral CT is considered to have a higher negative prediction value** when compared to fluoroscopy [26]. One study has shown no difference between sensitivity and the negative predictive value for high-speed CT and double-helical CT [26]. Spiral or helical computed tomography is considered as an appropriate non-invasive diagnostic technique for coronary heart disease (CHD) [26]. Fluoroscopic images help to better identify large to moderate calcifications as compared to small deposits of calcified plaque [28]. Researchers have shown that fluoroscopy images detect only 52% of calcifications seen on a high resolution electron beam CT [29]. However, the mean calcium density detected by electron beam CT during this study

* "Sensitivity of a test with respect to some class is the probability that the test correctly classifies cases of that class" [26].

** The negative predictive value is defined as "the proportion of patients with negative test results who are correctly diagnosed" [26]. Negative predictive value = $\text{Number of True negative} / (\text{Number of True negative} + \text{Number of False negative})$.

was +99 HU as compared to +546 HU in fluoroscopy indicating that only larger and highly-calcified lesions are evident with fluoroscopy [29]. This explains why CT is less specific* [30] but more sensitive in the detection of calcified plaques. R. Rienmuller and M. J. Lipton have shown that the calcified vessels detected with the help of CT were 50% greater in number than those detected with fluoroscopy [31]. Fluoroscopy has some other disadvantages. Besides having a medium to low sensitivity, the proficiency and experience of the operator, variability of equipment, and the number of views examined also play an important role in the detection of calcified plaque [26]. Quantification of calcification is not possible with fluoroscopy [28]. Radiography, which is another imaging technique using X-rays, does not aid in easy detection of calcifications in coronary arteries [26]. The accuracy of detecting calcifications with radiographs is only 42% as compared to fluoroscopy [26] which is not a very sensitive technique either [32]. Spiral or helical CT has advantages over conventional CT. The scan times are shorter, being one second on average for spiral CT, as compared to 30-90 seconds for conventional CT; the imaging time could be as short as 0.6 seconds as compared to a minimum 20-30 seconds for conventional CT [28]. It has been shown that helical CT has high sensitivity and specificity [33]. Other researchers have also shown that cardiac motion causes the blurring of calcifications at these accelerated scan times and small calcified deposits are not noticed at these speeds [34]. However, spiral CT is still considered better than conventional CT and fluoroscopy in detecting calcification [26].

* "The specificity of a binary classification test with respect to a given class is the probability that the test correctly classifies cases not belonging to that class" [30].

Intra vascular ultrasound (IVUS) is one of the recent methods used to diagnose atherosclerosis [35]. Catheters with transducers are used during cardiac catheterization to obtain cross-sectional images of the arteries. The sonograms obtained provide details about both the lumen of the artery and the thickness of the arterial wall [26]. Studies have compared angiography to IVUS [36, 37]. They found that the IVUS was more sensitive in diagnosing calcified deposits at the lesion. However, IVUS is invasive. Therefore, it has limited application in the diagnosis and screening of lesions, but it may be helpful in the case of patients who have already been diagnosed with atherosclerosis and in defining the structure of lesions before angioplasty.

Magnetic resonance imaging (MRI) has limited application in detecting calcifications [28] because it is generally characterized by lower signal intensity primarily due to the low density of protons in calcified lesions [37]. Also, MRI shows calcified lesions as discrete areas with lower signal intensity [38]. Small calcified areas are difficult to detect [28] because of its sensitivity to heterogeneous magnetic susceptibility occurring in calcifications [38].

2.4 Present Developments of Computed Tomography (CT) in Diagnostic Research

The applications of CT in the field of diagnostic research have increased over the past few years. Studies have shown that the composition of microscopic plaque varies significantly, being over a millimeter in thickness at times, and is as important as the extent of stenosis [39-41]. Histology is the standard method for such analysis [42] but it has some drawbacks. Histological analysis of tissues requires time-consuming

destructive specimen preparation. This method could affect the integrity and shape of the plaque, and subsequent studies on the specimens may not be possible [3, 39-41, 43, 44].

Micro CT has been newly introduced as a method for ex-vivo imaging with high resolution [45]. Micro CT has the advantage of higher resolution as compared to conventional CT. Mohr et al. studied the potential of micro CT for estimation of atherosclerosis in carotid arteries [42]. Their study was conducted on human cadaver carotid artery specimens stored in buffered formalin. Micro CT was carried out perpendicular to the axis of the carotid artery. The CT parameters used were 0.125 mA, 40 keV, 60 μm isotropic resolution, with a slice thickness of 60 μm . The scanning time for CT was 232 to 283 minutes for 176 to 215 slices. Another 60 minutes were needed for each specimen for data transfer and image analysis. CT imaging was performed on these specimens that had been stored in formalin. These specimens were not subjected to histopathological analysis after CT and, therefore, this research did not study specimen preparation for subsequent histopathological analysis. According to this study, the plaque consisted of both calcifications and soft plaque that illustrated a high contrast with respect to each other and the intra-luminal air. The densities varied even within the calcified regions on the tomography, indicating the ability of micro CT to expose the level of calcification. However, the density differences between the soft tissues were less apparent [42]. Volume rendering provided information on the amount of calcified and soft plaque within the entire specimen, as shown in Figure 12. The entire specimen is shown in Part A of Figure 12, whereas soft tissue and calcified tissue are shown in Part B, and Part C respectively.

A. Entire specimen. B. Soft tissue. C. Calcified tissue.

Figure 12. A–C Three-dimensional renderings of specimen [42].

(Reprinted with permission.)

Becker *et al.* analyzed calcified and non-calcified coronary artery atherosclerotic lesions with the help of computed tomographic angiography (CTA) and compared their findings with histopathological results [46]. Eleven human hearts from patients whose cause of death was not heart disease were examined. Methylcellulose and contrast enhancement agents were injected into the ostium of the coronary artery. A four-detector-row CT scanner was used with the following parameters: 80 mA, 120 kV, 0.5 seconds rotation time, and 3 mm per rotation table feed. The total scan time was 80 seconds for the entire heart. The reconstruction was done with slice thicknesses of 0.6 mm and in increments of 0.6 mm, generating approximately 350 individual slices of the heart for each study. The lesions seen on the angiograph, as analyzed by radiologists, were classified into the following categories: “completely calcified, mixed calcified, and non-calcified plaques” as shown in Figure 13. After scanning, macroscopic inspection (MI) was carried out on the lesions. The hearts were pathologically sectioned and then

put into formaldehyde. The lesions were sectioned by tissue blocks of 5 mm and embedded in paraffin. They were then prepared into histological sections. Each section was 4 μ m thick. Next, the blocks were stained with hematoxylin and elastica-van-Gieson. The atherosclerotic lesions were subsequently classified according to the American Heart Association (AHA) criteria. A comparison of the pathological images with the CT images is shown in Figure 13. It can be seen from Part A of Figure 13 that the soft tissue region is lipid-rich and has a mean HU of 40. Part B shows that fibrous plaque without calcium has a mean HU of 90, which is higher than the lipid-rich plaque. Part C shows that the specimen contained calcified regions with a mean HU of 110. The study showed that out of 40 to 50 lesions, only 33 lesions were recognized on computed tomographic angiograph (CTA) and macroscopic inspection (MI). Type-I and II lesions were missed on both CTA and MI, while other types of lesions were detected. MI detected ten type-III lesions whereas CTA detected only one among them, corresponding to the one containing calcium with a sensitivity of 10%. The sensitivity of CTA for type-IV to V_c lesions, compared to MI, ranged between 70 to 100%. Histopathological testing detected only one AHA type-VI lesion as compared to CTA which detected eleven. Spotty and calcified lesions appeared to be missed by MI but were detected by CTA. The CT attenuation for all non-calcified plaques, type-IV, V_a (lipid rich), V_b, and V_c (fibrous rich), was around 76 ± 35 HU, 47 ± 9 HU, and 104 ± 28 HU, respectively. Becker *et al.*'s research discusses some of the possible reasons for the variability in the type of results obtained. Their paper mentions that CTA was not successful in determining type-I and II lesions, probably because of the presently available spatial resolution [46]. Also,

the Hounsfield Units could be used in non-invasively assessing the predominant tissue types that contribute to the composition of the advanced type of coronary artery lesions. However, since the specimens were fixed and embedded after performing CT scanning on them, some amount of tissue shrinkage and degeneration during the CT imaging procedure is possible. Since the intensity of the X-rays that are transmitted depends upon the attenuation coefficient and the thickness of the tissue, this shrinkage can change the HU of the tissue.

A. Type-VI atherosclerotic lesion (40HU). B. Type-V_c (90 HU).
C. Contains calcified lesions (110 HU)

Tissue type/Material/region	Intra-plaque hemorrhage	Lipid-rich plaque	Intima	Media	Fibrous Plaque	Calcium
Abbreviation	Hb	Lc	I	M	F	Ca

Figure 13. Comparison of pathological images with CT images [46].
(Reprinted with permission.)

2.5 Literature Review Summary

Lesions and emboli are made up of different types of tissues, each having different physical and chemical properties. Embolic characterization will help in understanding the constituents of emboli and parent lesions. There is a need to characterize embolic constituents but suitable techniques for specimen preparation and subsequent characterization have not been developed as yet. There are several imaging techniques that are used in the medical industry. These imaging techniques can be used as non-destructive methods of analysis for tissue characterization. Micro CT has high sensitivity and specificity and is a method suitable for tissue characterization, especially for calcifications. Studies have shown that micro CT has the potential to differentiate between the extent of calcification and show differences between calcified and non-calcified lesions in the carotid artery. Studies have also shown that micro CT is able to detect more type VI lesions as compared to even histology and therefore, micro CT can be used for embolic characterization. However, all studies involved specimen that were larger in size than embolic debris. According to these studies, the specimens were either embedded in paraffin or just removed from formalin before CT scanning. Little research has been done on preparation of embolic specimens for tissue characterization using micro CT. This research will help the medical industry take a small but important step towards risk reduction by providing a technique of embolic specimen preparation suitable for non-destructive tissue characterization using micro CT and subsequent histopathology.

CHAPTER THREE

RESEARCH OBJECTIVES

Morphological typing is an attempt to assemble functionally-related materials based on similarity of structure, bringing about structural classification to a diverse group of embolic material types. Morphological typing attempts to recognize a set of similar constituents by simple visual examination requiring no physiological measurements on cells. If an unambiguous typing system can be generated, then it could be used to study the properties of emboli and bring about tissue characterization. The technique may be able to predict the effects of embolic materials on the body as well as on embolic capture devices. Conversely, if the system is ambiguous, such that the characteristics cannot be easily interpreted, its worth as a predictive tool for the study of embolic constituents and properties is lost.

One of the ways in which characterization may be possible is by subjecting the same specimen to micro CT and subsequent pathological analysis and reading. For example, consider the image of a single slice of a specimen which is subjected to micro CT imaging as shown in Figure 14. The Hounsfield Units (HU) equal to x and y of constituents A and B, respectively, are obtained from the CT image. Subsequent histopathological analysis reveals that constituent A is calcified plaque and constituent B is cholesterol on the slide. By comparing the micro CT image and the histopathological image for geometric identity, it can be concluded that the CT number in HU of calcified plaque is x and that of cholesterol is y .

Figure 14. Schematic diagram of morphological typing.

For this technique to be successful, the specimen needs to be compatible with both micro CT equipment and mounting techniques, and histopathological procedures. Therefore, an optimal specimen preparation technique needs to be developed for such an application. The technique developed should be such that the Hounsfield Unit (HU) of the specimen can be determined successfully without hindering the ability to analyze the specimen by histopathology.

The hypothesis for this research was that specimens prepared by following one or more of the following techniques will be suitable for micro CT and subsequent pathological analysis in order to enable tissue characterization.

The specimen preparation techniques are:

- 1) Specimen embedded in a paraffin cassette.
- 2) Specimen embedded in a polymethyl methacrylate (PMMA) block.
- 3) Specimen embedded in a paraffin cassette, sectioned, and mounted on glass slides.
- 4) Specimen embedded in PMMA and sliced into wafers.

The objective of this research was to evaluate the efficacy of these techniques of specimen preparation for three-dimensional micro CT and subsequent histopathological analysis for tissue characterization.

CHAPTER FOUR

METHODOLOGY

The purpose of this research was to determine and compare different techniques of specimen preparation for three-dimensional micro CT and subsequent histopathological analysis to enable embolic debris characterization. The efficacy of these techniques is based upon the ability of the specimen to be scanned using micro CT and then analyzed histopathologically. A flow chart of the experimental procedure is shown in Figure 15. Five filter bags filled with embolic specimens, collected during clinical trials and subsequently fixed in formaldehyde, were provided by Boston Scientific Corporation. The nickel-titanium loop was removed. One specimen was embedded in paraffin and another one in polymethyl methacrylate (PMMA). A third specimen was embedded in paraffin, sectioned, and mounted on glass slides. The last two specimens were embedded in PMMA and sliced into wafers that were 800 μm thick. Three-dimensional computer tomography was performed on each specimen to obtain images in the form of slices. These images were used to detect the Hounsfield Units (HU) of the constituents of these emboli using 3D DoctorTM, a software tool. Based upon the success of micro CT, specimens were sent for histopathological staining and analysis. These images were used to compare the different methods of specimen preparation required for micro CT and subsequent pathological analysis.

Figure 15. Research methodology.

4.1 Specimens and Materials Used For the Study

An unused FilterWire EZ™ is shown in Figure 16 [47]. It consists of a polyurethane filter bag with pores that are 110 μm in diameter. The bag is bonded onto a radiopaque nickel-titanium loop. This subassembly is then mounted onto a polytetrafluoroethylene (PTFE) coated guide wire. The other components help improve the efficiency and performance of the FilterWire EZ™.

Figure 16. Illustration of the FilterWire EZ™ [48].
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Filter bags with emboli collected during clinical trials and subsequently fixed in formaldehyde were provided by Boston Scientific Corporation. These specimens were selected based on a Boston Scientific Corporation grading system, such that all specimens had grossly visible, large human tissue particles. Other materials used during the research are listed in Table 2. Paraffin wax and polymethyl methacrylate were used as embedding media. All specimens were mounted on glass slides before histopathological staining with hematoxylin and eosin (H&E) stain and trichrome stain. 3D Doctor™ was used to evaluate the Hounsfield Unit of the tissues.

4.2 Experimental Equipment

A SkyScan-1172 micro CT scanner, manufactured by Micro Photonics. Inc. that was used is shown in Figure 17 [47]. It is a desk top micro CT scanner. The stage on which the specimens were placed for micro CT scanning is shown in Figure 18. The

specimen holders used are shown in Figure 19. These specimen holders, made out of brass, are 2.5 inches and 0.5 inch in diameter and can be mounted onto the stage with the help of a screw. They have flat surfaces on which a specimen can be placed.

Table 2. Materials Used for Specimen Preparation, Pathological Analysis, and micro CT Image Analysis.

Material	Purpose
Paraffin wax	Used as an embedding medium that is firm enough to provide support and rigidity to the tissue so that it could be sectioned, without damage.
Polymethyl methacrylate (PMMA)	Used as an embedding medium that is firm enough to provide support and rigidity to the tissue so that it could be sectioned, without damage.
Glass slides	To mount the specimen sections for staining and subsequent pathological analysis.
Hematoxylin and Eosin stain (H&E)	To aid pathological analysis of slides.
Trichrome Stain	To aid pathological analysis of slides.
3D Doctor TM (Software)	To evaluate the Hounsfield Unit of the tissue.

Figure 17. SkyScan 1172 micro CT scanner.

Figure 18. Stage on which the specimen is placed.

Figure 19. Two specimen holders that may be used to scan specimen of this size.

4.3 Experimental Procedures

Five specimens were processed and scanned using micro CT. Based upon the quality of the images and efficacy of each technique, the specimens were sent for subsequent histopathological analysis. The filter bag containing embolic debris was selected based on a Boston Scientific Corporation grading system, such that the specimen had grossly visible, large human tissue particles. The stainless steel wire and nickel-

titanium loop were carefully removed. The specimens were embedded and scanned using micro CT. The DICOM images of the specimen were analyzed using 3D DoctorTM software. Histopathological sectioning, staining, and analysis were subsequently done. Further details on the rejection or acceptance of a technique are described in Chapter 5 – Results. All experimental procedures are described in Section 4.3 while the corresponding results and discussion are presented in Chapter 5.

4.3.1 Specimen 1: Specimen Embedded in a Paraffin Cassette

This specimen is shown in Figure 20. The original length of the filter bag from loop to tip, after removing the loop, was 14 mm in its longest dimension, which was longer than the height of the available tissue cassette. Therefore, the filter bag with emboli was bisected. The specimen was cut at a distance of 7 mm from the tip of the filter bag. This subsequently allowed sectioning from the tip, moving progressively through the device towards the loop before mounting the tissue on slides for pathological reading. The bisected filter bag was then embedded in paraffin, as shown in Figure 21. The resulting paraffin cassette with the filter bag embedded in it was scanned using micro CT.

Figure 20. Specimen 1 removed from the loop.

Figure 21. Specimen 1 embedded in a cassette.

During micro CT one cassette was scanned at a 10 μm pixel size; this did not give promising results. The micro CT image appeared gray without showing any detail of the tissue within the paraffin cassette. Changing the pixel size to 4-5 μm would have considerably increased the resolution since a smaller volume of tissue is imaged using the same X-ray intensity and scanning parameters [48]. However, the size of the cassette with paraffin was too large to use a smaller pixel size for the scan because the entire specimen would not fit into the field of vision of the camera. Therefore, a small paraffin block containing the tissue and minimal paraffin was cut from the paraffin cassette, as shown in Figure 22.

Figure 22. Schematic diagram of Specimen 1 cut out from larger block.

This small block was then subjected to micro CT imaging using the parameters listed in Table 3. The smaller block allowed more X-rays to pass through the specimen providing better resolution and less noise. The micro CT imaging results are presented in Chapter 5.

Table 3. Imaging Parameters for Specimen 1.

Scanning parameters	Value
Source Voltage (kV)	40
Source Current (uA)	250
Number of Rows	1048
Number of Columns	1500
Image Pixel Size (um) – slice thickness (voxel)	4.99
Filter	No filter
Rotation Step (deg)	0.400
Frame Averaging	ON (5)
Smoothing	0
Ring Artifact Correction (%)	10
Beam Hardening Correction (%)	40

The DICOM* images of the specimen were analyzed using 3D Doctor™ software [49]. The Hounsfield Unit was calculated for each slice. The following is an example of such an evaluation. The image of the paraffin block as seen in 3D Doctor™ is shown in Figure 23. The gray region at the periphery corresponds to the paraffin. The central lighter gray area is the tissue within the filter bag. Bright white spots can be seen within the inner gray region. These spots correspond to tissue with high radio density, which absorb most of the incident X-rays and allow very little transmission.

* “Digital Imaging and Communications in Medicine (DICOM) is a comprehensive set of standards for handling, storing and transmitting information in medical imaging. It includes a file format definition and a network communication protocol.”[49].

Figure 23. Specimen 1 as seen on 3D Doctor™.

The parameters specified in Table 4 were maintained during image reconstruction to evaluate the Hounsfield Unit of the tissue.

Table 4. Reconstruction Parameters for Slice #556 of Specimen 1.

Parameter	Setting
Contrast Gray Scale Range (Gray scale)	0 to 102
Image Threshold (Pixel values)	76 to 117

The regions corresponding to pixel values ranging from 76 to 117 were selected. All of the regions on slice #556 that had a pixel value ranging from 76 to 117 were highlighted in red as shown in Figure 24. A report of the pixel values and HU for the selected segments of the specimen under study was generated. The micro CT image results are presented in Chapter 5.

Figure 24. Reconstruction parameters for Specimen 1.

In general during histopathological analysis, a paraffin-embedded tissue cassette is subjected to sectioning and staining. The sectioning is commonly done with a microtome to obtain sections of 5-10 μm in thickness. These sections are mounted on glass slides and stained for pathological analysis. During this research, the paraffin block was dissected into a smaller block which had its disadvantages. To obtain paraffin

sections for further histological staining, the dissected block with the specimen needed to be re-embedded in a paraffin cassette. This required the melting of some of the paraffin surrounding the device to ensure that it would sufficiently blend into the new surrounding paraffin and not move during sectioning. This melting often affects the tissue properties such as density, and thus histopathological analysis was tried but was not successful on this specimen.

4.3.2 Specimen 2: Specimen Embedded In Poly Methyl Methacrylate (PMMA)

The stainless steel wire and nickel-titanium loop were carefully removed. The specimen so obtained is shown in Figure 25. The specimen was embedded in poly methyl methacrylate (PMMA), an acrylic resin. The size of the resin block thus obtained was limited to an approximate trapezoidal block of 20 mm x 18 mm x 10 mm on the longest edges.

The resin block containing the specimen was scanned using micro CT at a 10 μm pixel size which did not give promising results, as was the case with Specimen 1. Changing the pixel size to 4-5 μm would have considerably increased the resolution; however the resin block was too large for a smaller pixel size to be used.

Performing a histopathological analysis on this specimen was not beneficial to this research because of the absence of the micro CT images required for characterization. Therefore, histopathological analysis was not performed.

Figure 25. Specimen 2 with metal loop removed.

4.3.3 Specimen 3: Specimen Embedded in Paraffin and Mounted on Glass Slides

The original length of this filter bag, from loop to tip after removing the metal loop, was 14 mm at its longest dimension, which was longer than the height of the available tissue cassette. Therefore, the filter bag with emboli was bisected. The cut was made at 7 mm from the tip of the filter bag. The specimen, now in two parts, was processed and embedded in two individual paraffin cassettes. However, it can be seen from the results of Specimen 1 that a smaller paraffin block volume to tissue volume was required to get a better resolution. Therefore, the paraffin block was sectioned on a microtome to a slice thickness of 5 μm and mounted on standard laboratory glass slides before they were sent for micro CT imaging. The glass slide was CT scanned using the parameters specified in Table 5. The DICOM images of the specimen could not be analyzed using 3D DoctorTM software to obtain the Hounsfield Units. Micro CT was unsuccessful on the specimen mounted on glass slides. Performing a histopathological

analysis on this specimen was not helpful for this research because of the absence of CT images required for characterization and was therefore not performed.

Table 5. Imaging Parameters for Specimen 3.

Scanning parameters	Value
Source Voltage (kV)	40
Source Current (uA)	250
Number of Rows	1048
Number of Columns	1500
Image Pixel Size (um) – slice thickness (voxel)	8.99
Filter	No filter
Rotation Step (deg)	0.400
Frame Averaging	ON (5)
Smoothing	0
Ring Artifact Correction (%)	10
Beam Hardening Correction (%)	40

4.3.4 Specimen 4: Specimen Embedded in Poly Methyl Methacrylate (PMMA) and Sectioned into Wafers

A specimen consisting of the filter bag with the metal loop was encased in clear plastic (PMMA). The metal loop was removed after encasing so that the bag remains open and does not collapse while encasing in plastic. The encased specimen was then cut into ‘wafers’, each 800 μm in thickness, using a diamond wire saw. The size of a single wafer was approximately 25 mm x 18 mm. Six such wafers were obtained from one specimen, as described in Table 6. It can be seen that Wafers 1 and 2 did not contain any tissue. Wafers 3 to 6 contained grossly visible tissue along with pieces of metal wire. The wafers were CT scanned using the micro CT parameters specified in Table 7. The Hounsfield Units were evaluated following the same methodology as presented in Section 4.3.1.

Table 6. Wafers of Specimen 4.

Wafer #	Picture	Specimen Description
1		Specimen appeared as if it did not contain any tissue with it. However, parts of the stainless steel wire were present on the wafer.
2		Specimen appeared as if it did not contain any tissue with it. However, parts of the stainless steel wire were present on the wafer.
3		Tissue was observed on Wafer 3. A section of the stainless steel wire was also present.

Table 6. Wafers of Specimen 4 (Continued).

Wafer #	Picture	Specimen Description
4	<p>The micrograph shows a cross-section of a wafer. At the top, the handwritten number '706105' is visible. Below it, a dark, irregular shape is labeled 'Metal wire' with an arrow pointing to it. A circular region is outlined, containing a small, dark, irregular shape. The bottom of the image shows a series of small, dark, rectangular features.</p>	<p>Tissue was observed on Wafer 4. A section of the stainless steel wire was also present.</p>
5	<p>The micrograph shows a cross-section of a wafer. A dark, irregular shape is labeled 'Metal wire' with an arrow pointing to it. A circular region is outlined, containing a small, dark, irregular shape. The bottom of the image shows a series of small, dark, rectangular features.</p>	<p>Tissue was observed on Wafer 5. A section of the stainless steel wire was also present.</p>

Table 6. Wafers of Specimen 4 (Continued).

Wafer #	Picture	Specimen Description
6		Tissue was observed on Wafer 6. A section of the stainless steel wire was also present.

Table 7. Scanning parameters for Specimen 4.

Scanning parameters	Value
Source Voltage (kV)	40
Source Current (uA)	250
Number of Rows	1048
Number of Columns	1000
Image Pixel Size (um) – slice thickness(voxel)	4.99
Filter	No filter
Rotation Step (deg)	0.400
Frame Averaging	ON (5)
Smoothing	0
Ring Artifact Correction (%)	4
Beam Hardening Correction (%)	60

Micro CT was successful on all of the six wafers. After micro CT, the wafers were mounted by gluing them onto a plastic slide, ground using an ExaktTM grinder, and

polished to an optical finish. H&E,^{*} a basic stain used for assessing the biological response of the tissues surrounding a biomaterial, and trichrome,^{**} a stain used traditionally in paraffin and poly methyl methacrylate (PMMA) microtome sections as well, were used to stain the slides. The stained slides were sent to the pathologist for analysis.

4.3.5 Specimen 5: Specimen Embedded in Poly Methyl Methacrylate (PMMA) without Metal and Sectioned into Wafers

The specimen was prepared in a manner similar to Specimen 4 with the difference that all metallic elements were removed carefully from Specimen 5. The specimen was encased in PMMA. The encased specimen was then cut into wafers, each 800 μm in thickness, using a diamond wire saw. The size of a single wafer was approximately 25 mm x 18 mm. Six such wafers were obtained from one specimen, as described in Table 8. It can be seen that tissue was observed on Wafers 1 to 6. Wafer 7 had very small traces of tissue on it. The wafers were CT scanned using the parameters specified in Table 9. The DICOM images of the specimen were analyzed using 3D DoctorTM software. The Hounsfield Units were evaluated following the same methodology as described in Section 4.3.1. Micro CT was successful on the seven wafers. Based on the

^{*} Hematoxylin and eosin stain (H&E) is commonly used in histopathology. The Hematoxylin stains basophilic structures blue or purple and eosin stains eosinophilic structures bright pink. The terms 'basophilic' and 'eosinophilic' is derived from their affinity to Hematoxylin and Eosin and is irrespective of its acidic/basic nature.

^{**} The Trichrome stain is a tricolor stain commonly used in histopathology to distinguish cells from their surrounding connective tissue. Keratin and muscle fibers appear red, and collagen and bone appear green. The cytoplasm of the cell appears pink where as cell nuclei appear dark brown.

micro CT results, these wafers were not mounted on glass slides, ground, polished or stained using H&E and trichrome stains.

Table 8. Wafers of Specimen 5.

Wafer #	Picture	Specimen Description
1 and 2		Tissue was observed on Wafers 1 and 2.
3 and 4		Tissue was observed on Wafers 3 and 4.
5 and 6		Tissue was observed on Wafers 5 and 6.

Table 8. Wafers of Specimen 5 (Continued).

Wafer #	Picture	Specimen Description
7		Very small traces of tissue were observed on Wafer 7.

Table 9. Scanning Parameters for Specimen 5.

Scanning parameters	Value
Source Voltage (kV)	40
Source Current (uA)	250
Number of Rows	1048
Number of Columns	1000
Image Pixel Size (um) – slice thickness(voxel)	4.99
Filter	No filter
Rotation Step (deg)	0.400
Frame Averaging	ON (5)
Smoothing	0
Ring Artifact Correction (%)	4
Beam Hardening Correction (%)	60

CHAPTER FIVE

EXPERIMENTAL RESULTS AND DISCUSSION

Five techniques to prepare embolic protection devices for micro CT and subsequent pathological analysis, in order to enable tissue characterization, were evaluated. The paraffin embedding technique produced high resolution micro CT images. The micro CT images from the glass slide technique and the PMMA block technique did not provide any information about the constituent tissue within the specimens. The PMMA wafer technique produced low resolution micro CT images with artifacts and noise. A detailed description of the results and discussion is presented in this chapter. A comparison of the techniques is presented in Section 5.6.

5.1 Specimen 1: Specimen Embedded in a Paraffin Cassette

The micro CT images for a slice from each wafer of the specimen were analyzed using 3D Doctor TM to derive the Hounsfield Unit (HU) for the tissue. The HUs were analyzed from the images and are listed in Table 10. The images shown represent the HUs derived for single slices. Three examples of such an analysis are given in Table 10. Similarly, the HUs can be determined for all of the 900 to 1,000 slices that were generated during micro CT. For example, slice 556 was analyzed with an image threshold of 76 to 117 in terms of pixel values. The mean HU for the selected tissue was -376.89. The mean HU for slice 582 was 231.12, where as for slice 247 it was -406.6 when analyzed for an image threshold of 116 to 227 and 69 to 104, respectively. A variation in grey scale over the specimens can be seen. Regions of high and low radio density can be differentiated. Most of the regions within the tissue from a single slice

appear to be more radio dense than the surrounding paraffin, which shows that the paraffin absorbed less radiation than the tissue itself, therefore representing a Hounsfield value that was closer to that of the original tissue than to that of the combination.

Micro CT imaging was successful on the dissected paraffin block as compared to histopathological analysis. To obtain paraffin sections for further histological staining, the dissected block containing the specimen needed to be re-embedded in a paraffin cassette. This required the melting of some of the paraffin surrounding the device to ensure that it would sufficiently blend into the new paraffin and not become dislodged during sectioning. This melting can affect the tissue properties such as density, and thus histopathological analysis was tried but was not successful on this specimen.

The technique of using a paraffin cassette had several advantages. Micro CT images of high resolution were obtained and adjacent regions having different pixel values and HUs could be clearly differentiated. Since the density of the paraffin was less than the density of the tissue, only a very small amount of radiation was absorbed by the paraffin and most of it was transmitted to the tissue. The Hounsfield Units (HUs) obtained represented a value that was closer to that of the original tissue than to that of the combination. Also, since the whole filter bag was scanned at one time, image reconstruction that gave a virtual image of the filter with debris was possible as shown in Figure 26. This property can be used for performing a volumetric analysis on the tissue types. Finally, the cost of scanning was lower than for the other methods such as the PMMA wafer technique, since the whole specimen was scanned at once.

Table 10. Results for Specimen 1.

Specimen 1: Specimen embedded in a paraffin cassette				
Slice #	Image Thresholds (Pixel Values)	Image	Mean Hounsfield Unit (HU)	Discussion
556	76-117		-367.89	The micro CT image of a single slice of the paraffin block is shown. The pixel value was set to a range of 76-117. The tissue corresponding to a pixel value of 76-117 can be seen highlighted in red. The average HU for the selected tissue was -367.89.

Table 10. Results for Specimen 1(Continued).

Specimen 1: Specimen embedded in a paraffin cassette				
Slice #	Image Thresholds (Pixel Values)	Image	Mean Hounsfield Unit (HU)	Discussion
582	116-227		231.12	The micro CT image of a single slice of the paraffin block is shown. The pixel value was set to a range of 116-227. The tissue corresponding to a pixel value of 116-227 can be seen highlighted in red. The average HU for the selected tissue was 231.12.

Table 10. Results for Specimen 1(Continued).

Specimen 1: Specimen embedded in a paraffin cassette				
Slice #	Image Thresholds (Pixel Values)	Image	Mean Hounsfield Unit (HU)	Discussion
247	69-104		-406.6	The CT image of a single slice of the paraffin block is shown. The pixel value was set to a range of 69-104. The tissue corresponding to a pixel value of 69-104 can be seen highlighted in red. The average HU for the selected tissue was -406.6.

This technique also had its limitations. The ratio of the size of the paraffin cassette to that of the tissue was too large for micro CT. Once the tissue with minimal paraffin was dissected from the larger block, pathology was not successful because the integrity of the tissue was lost during the re-melting procedure. Also, since the metallic loop was removed before embedding the tissue, the filter bag folded in on itself at several locations before the paraffin was set. However, this limitation can be overcome by first embedding the tissue in paraffin and letting it set. The loop would give the bag enough opposition to keep it open. Once set, the section containing the loop can be carefully removed. This phenomenon can be seen in the virtual model shown in Figure 26.

Figure 26. Virtual model of specimen embedded in a paraffin block.

5.2 Specimen 2: Specimen Embedded in Poly Methyl Methacrylate (PMMA)

The micro CT images of the specimen could not be analyzed using 3D DoctorTM because the images did not show any variation in density between the tissue and the poly methyl methacrylate (PMMA) block. The raw images as obtained from the micro CT scanning are shown in Table 11.

Table 11. Results for Specimen 2.

Specimen 2: Specimen embedded in polymethyl methacrylate (PMMA)			
Block Orientation	Transmittance	Image	Discussion
Block lying flat	10.2%		The block was scanned by lying it flat on the stage. A minimum transmittance of 10.2% was observed within the block. The tissue within the block cannot be differentiated from the PMMA block.

Table 11. Results for Specimen 2 (Continued).

Specimen 2: Specimen embedded in polymethyl methacrylate (PMMA)			
Block orientation	Transmittance	Image	Discussion
Block in upright position (1)	8.2%		The block was scanned in an upright position on the stage. A minimum transmittance of 8.2% was observed within the block. The tissue within the block cannot be differentiated from the PMMA block.
Block in upright position (2)	15.7%		The block was scanned in a second upright position on the stage. A minimum transmittance of 15.7% was observed within the block. The tissue within the block cannot be differentiated from the PMMA block.

In general, for successful imaging, the minimum transmittance of a micro CT scan should be above 8% [48]. When the block was scanned by laying it flat on the stage, a minimum transmittance of 10.2% was obtained within the block. When the block was scanned in two different upright positions on the stage, minimum transmittances of 8.2% and 15.7% were obtained within the block. These transmittance values show that transmittance was not an issue with this technique of sample preparation. However, since the tissue within the block could not be differentiated from the PMMA block, the difference in the absorbed energy between the PMMA and the tissue was small and this difference made it difficult to distinguish between the PMMA and the tissue when this specimen was scanned. Ideally, two distinct materials should have large differences in their absorbed X-ray energies to be scanned successfully [48]. Also, it has been shown in Section 2.2.6 that the thickness of the material plays a role in the amount of energy absorbed. Therefore the effect of the thickness of the PMMA block on the images needs to be further investigated.

Micro CT imaging was not successful for this specimen. Further analysis on this specimen was discontinued because of the absence of micro CT images to use for characterization after histopathological analysis. Therefore the histopathological analysis was not performed on this specimen.

This technique had a few advantages over the paraffin cassette technique described in Section 5.1. A PMMA block smaller than the paraffin cassette was obtained which helped obtain a smaller pixel size. A cassette was not needed to hold the PMMA material unlike in the case of paraffin. Also, the PMMA block is transparent to visible

light, and therefore the morphology of the specimen within the block can be visually observed.

This technique also had its limitations. Micro CT was not successful when the whole filter bag was embedded into the PMMA block. Even though the PMMA block was smaller than the paraffin cassette, the ratio of the size of the block to the size of the tissue was still too high for successful micro CT imaging and without micro CT, this technique had no advantages with respect to characterization in the present application.

5.3 Specimen 3: Specimen Embedded in Paraffin and Mounted on Glass Slides

Since the ratio of the embedding material to that of the tissue within it was too large for micro CT, the next option was to embed the tissue in media thin enough to reduce the ratio between the embedding media and the tissue. The results of an image of a single slide are summarized in Table 12. The minimum transmittance for the glass scan was 0.4%. The highest value for the minimum transmittance that was obtained was 2.7%, which is still much lower than 8%. Therefore, it was not possible to scan a specimen that consisted of a paraffin mold on a glass slide because the glass slide absorbed much of the X-rays, permitting minimal transmission. As a result, micro CT imaging was not successful for this specimen. This specimen was not analyzed further, and therefore histopathological analysis was also not performed on this specimen.

One advantage of this technique was that the ratio of the paraffin with the glass slide to the tissue is small. If the transmittance of glass would have been suitable for micro CT imaging, a higher resolution image may have been possible. Since the specimen was already mounted on a glass slide, subsequent histological staining and

pathological analysis were possible. In addition, the same section of tissue can be subjected to micro CT scanning and pathological testing. Therefore, direct comparison of pathological slides with the micro CT image would have been possible, thereby aiding in tissue characterization. The transmittance of the glass slide was too low, and therefore most of the X-rays were absorbed within it. Therefore a glass slide cannot be used during micro CT imaging.

Table 12. Results for Specimen 3.

Specimen 3: Specimen embedded in paraffin, and mounted on glass slides		
Raw CT image	Transmittance	Image
Glass Slide	0.4%

5.4 Specimen 4: Specimen Embedded in Poly Methyl Methacrylate (PMMA) and Sectioned into Wafers

The next option was to use an embedding medium that did not require a glass slide to maintain the integrity of the section. A specimen embedded in PMMA and sliced into wafers of 800 μm thickness was evaluated. The micro CT images for a slice for each wafer of the specimen were analyzed using 3D DoctorTM to derive the Hounsfield Unit (HU) for the tissue. The HUs were analyzed from the images and are listed in Table 13. The images shown represent the HUs derived for single slices and such HUs can be determined for all the 900 to 1,000 slices that were generated during micro CT scanning. The CT image of a single slice of Wafer 1 is shown in Table 13. The pixel value was set to a range of 20 to 247. The average HU for the selected material was 1,174.38. From the image of Wafer 1 shown in Table 6 in comparison with that shown in Table 13, it can be seen that this material corresponds to the nickel-titanium loop that remained within the wafer. The CT image of a single slice of Wafer 2 is also shown in Table 13. The pixel value was set to a range of 152-254. The material corresponding to a pixel value of 152-254 can be seen highlighted in red. The average HU for the selected material was 286.11. The white region corresponds to the nitinol wire that remained in the wafer. The streaking is an artifact caused by the presence of high density regions beside low density regions. The average HU for the selected material was 606.73. Similarly, the HUs for all other wafers were calculated and are presented in Table 13. All the images show a variation in gray scale; however, it is not as clear as that seen on the paraffin specimen although regions of high and low radio density can be easily differentiated. The PMMA

displayed more noise as compared to the paraffin specimen shown in Table 10 of Section 5.1. There were several regions in the tissue which seem to have the same radio density as the PMMA, thus making it difficult to differentiate between the two even though the wafer thickness was only 800 μm .

Table 13. Results for Specimen 4.

Specimen 4: Specimen embedded in polymethyl methacrylate (PMMA) and sectioned into wafers					
Wafer #	Slice #	Image Thresholds (Pixel Values)	Image	Mean Hounsfield Unit (HU)	Discussion
1	178	20-247		1174. 38	The CT image of a single slice of Wafer 1 is shown. The pixel value was set to a range of 20-247. The material corresponding to a pixel value of 20-247 can be seen highlighted in red. The average HU for the selected material was 1174.38. From the image shown in Table 6, it can be seen that this material corresponds to the nitinol wire that remained within the wafer.

Table 13. Results for Specimen 4 (Continued).

Specimen 4: Specimen embedded in polymethyl methacrylate (PMMA) and sectioned into wafers				
Wafer #	Slice #	Image Thresholds (Pixel Values)	Image	Mean Hounsfield Unit (HU)
2	485	152-254		286.11
The CT image of a single slice of Wafer 2 is shown. The pixel value was set to a range of 152-254. The material corresponding to a pixel value of 152-254 can be seen highlighted in red. The average HU for the selected material was 286.11. The white region corresponds to the nitinol wire that remained in the wafer. The streaking is an artifact caused by the presence of high density regions beside low density regions.				

Table 13. Results for Specimen 4 (Continued).

Specimen 4: Specimen embedded in polymethyl methacrylate (PMMA) and sectioned into wafers				
Wafer #	Slice #	Image Thresholds (Pixel Values)	Image	Mean Hounsfield Unit (HU)
3	375	118-128		146.14
Discussion				The micro CT image of a single slice of Wafer 3 is shown. The pixel value was set to a range of 118-128. The material corresponding to a pixel value of 118-128 can be seen highlighted in red. The average HU for the selected material was 146.14. The white region corresponds to the nitinol wire that remained in the wafer. The streaking is an artifact caused by the presence of high density regions beside low density regions.

Table 13. Results for Specimen 4 (Continued).

Specimen 4: Specimen embedded in polymethyl methacrylate wafers (PMMA) and sectioned into wafers				
Wafer #	Slice #	Image Thresholds (Pixel Values)	Image	Mean Hounsfield Unit (HU)
4	425	133-171		166.22
				The micro CT image of a single slice of Wafer 4 is shown. The pixel value was set to a range of 133-171. The material corresponding to a pixel value of 133-171 can be seen highlighted in red. The average HU for the selected material was 166.22. The white region corresponds to the nitinol wire that remained in the wafer. The streaking is an artifact caused by the presence of high density regions beside low density regions.

Table 13. Results for Specimen 4 (Continued).

Specimen 4: Specimen embedded in polymethyl methacrylate (PMMA) and sectioned into wafers				
Wafer #	Slice #	Image Thresholds (Pixel Values)	Image	Mean Hounsfield Unit (HU)
5	385	127-140		40.01
				<p>The micro CT image of a single slice of Wafer 5 is shown. The pixel value was set to a range of 127-140. The material corresponding to a pixel value of 127-140 can be seen highlighted in red. The average HU for the selected material was 40.01. The white region corresponds to the nitinol wire that remained in the wafer. The streaking is an artifact caused by the presence of high density regions besides low density regions. The majority of the tissue has a low HU (= 40.01) which shows the presence of low radio density tissue.</p>

Table 13. Results for Specimen 4 (Continued).

Specimen 4: Specimen embedded in polymethyl methacrylate (PMMA) and sectioned into wafers				
Wafer #	Slice #	Image Thresholds (Pixel Values)	Image	Mean Hounsfield Unit (HU)
6	377	194-255		606.73
				<p>The micro CT image of a single slice of Wafer 6 is shown. The pixel value was set to a range of 194-255. The material corresponding to a pixel value of 194-255 can be seen highlighted in red. The average HU for the selected material was 606.73. The white region corresponds to the nitinol wire that remained in the wafer. The streaking is an artifact caused by the presence of high density regions beside low density regions.</p>

Histopathological analysis was successfully done on these wafers. The results at 4X, 10X, or 20X magnification are listed in Table 14. Wafer 1 did not contain any tissue. Wafers 2 through 6 had some amount of plaque. Cholesterol crystals and granular amorphous debris were identified by the histopathological analysis on them.

One of the advantages of the PMMA wafer technique was that the ratio of the PMMA material to the tissue was small, which permitted successful micro CT imaging. The same section of tissue can be subjected to micro CT and pathological reading. Direct comparison of pathological slides with the micro CT image was possible, thereby helping in tissue characterization. Also, specimen handling was simple. However, it had a few limitations. High density material caused streaking and artifacts over low density material [48] thus changing the pixel value of the image. Due to the presence of metallic material beside the low density tissue on the wafer, artifacts were observed. These artifacts alter the pixel value of the CT image, thereby causing an inaccurate derivation of the Hounsfield Unit (HU). This limitation can be resolved by removing all metallic structures from the specimen before wafer preparation. Also, the resolution of the image was not as high as that on the image obtained from the paraffin embedded tissue. Image resolution in pathology is often determined by the physical method of creating the slides [50]. Microtomy gives a better resolution than the Exakt method [50]. However, microtomy is commonly done on paraffin embedded tissue and produces thinner sections 4 to 5 μm thick as compared to 800 μm obtained from the Exakt method. Since these wafers were processed using the Exakt method, a much lower resolution on the pathological image was obtained.

Table 14. Pathological images for Specimen 4.

Wafer #	Image	Discussion
1	Wafer 1 did not contain any tissue on it.	N/A
2	<p>The image shows a microscopic view of a specimen at a scale of 1 inch. The membrane of the filter bag is visible as the 'EP Sieve Membrane'. A minimal amount of tissue is seen on the bag. Some amount of plaque can be seen near the walls of the filter bag. A magnified image of the tissue is also shown outlined in red. The tissue was identified by the histopathological analysis as plaque.</p>	<p>The picture represents the microscopic image of Wafer 2 of the specimen at a scale of 1": 250um. The membrane of the filter bag as represented as the 'EP Sieve Membrane' is shown. A minimal amount of tissue is seen on the bag. Some amount of plaque can be seen near the walls of the filter bag.</p> <p>The magnified image of the tissue is also shown outlined in red. The tissue was identified by the histopathological analysis as plaque.</p>

Table 14. Pathological images for Specimen 4 (Continued).

Wafer #	Image	Discussion
3	<p>Ep_angr298 bag1 wafer3 HE4x</p> <p>plaque</p> <p>inside EP capture device</p> <p>250um</p> <p>Ep_angr299 bag1 wafer3 HE10x</p> <p>cholesterol crystals</p> <p>granular amorphous debris</p> <p>EP membrane</p> <p>EP membrane pore</p> <p>100um</p>	<p>The picture represents the microscopic image of Wafer 3 of the specimen at a scale of 1”: 250um. The membrane of the filter bag is shown. Most of the tissue was identified during the pathological analysis as plaque. The metal wire can be seen at the bottom of the image.</p> <p>The magnified image of the tissue is also shown outlined in red. The picture represents the microscopic image of Wafer 3 of the specimen at a scale of 1”: 100um. Within the tissue, cholesterol crystals and granular amorphous debris were identified by the histopathological analysis.</p>

Table 14. Pathological images for Specimen 4 (Continued).

Wafer #	Image	Discussion
4	<p>The top image is a low-magnification micrograph (4x) showing a dark, irregular mass labeled 'Plaque' and a lighter area labeled 'Membrane'. The bottom image is a magnified view (10x) of the plaque, showing dark, crystalline structures labeled 'Cholesterol crystals' and lighter, granular areas labeled 'Granular amorphous debris'. A scale bar is visible in the bottom right corner of the magnified image.</p>	<p>The picture represents the microscopic image of Wafer 4 of the specimen at a scale of 1": 250um. The membrane of the filter bag as represented is shown. Tissue is seen on the filter bag. Some amount of plaque can be seen near the walls of the filter bag.</p> <p>The magnified image of the tissue is also shown outlined in red. Within the tissue, cholesterol crystals and granular amorphous debris were identified by the histopathological analysis.</p>

Table 14. Pathological images for Specimen 4 (Continued).

Wafer #	Image	Discussion
5	<p>The top image is a low-magnification histological view (HE4x) showing a large, dark, irregular mass labeled 'Plaque' with an arrow pointing to a higher magnification view (HE10x) below it. The bottom image shows a magnified view of the tissue, with a red outline highlighting a specific area. A scale bar is visible in the bottom right corner of the magnified image.</p>	<p>The picture represents the microscopic image of Wafer 5 of the specimen at a scale of 1": 250um. The membrane of the filter bag is shown. Tissue is seen in the filter bag. Histopathological analysis has identified the tissue as plaque.</p> <p>The magnified image of the tissue is outlined in red. Within the tissue, cholesterol crystals and granular amorphous debris were identified by the histopathological analysis.</p>

Table 14. Pathological images for Specimen 4 (Continued).

Wafer #	Image	Discussion
6	<p>The top image is a low-magnification histological view (HE4x) showing a large, dark, irregular mass labeled 'Plaque' with a line pointing to it. The bottom image is a high-magnification histological view (HE10x) of the same area, showing detailed cellular structure and a scale bar in the top right corner.</p>	<p>The picture represents the microscopic image of Wafer 6 of the specimen at a scale of 1": 250um. The membrane of the filter bag is shown. Tissue is seen in the filter bag. Histopathological analysis has identified the tissue as plaque.</p> <p>The magnified image of the tissue is outlined in red. Within the tissue, cholesterol crystals, fibrin and granular amorphous debris were identified by the histopathological analysis.</p>

5.5 Specimen 5: Specimen Embedded in Poly Methyl Methacrylate (PMMA) and Sectioned into Wafers

One of the limitations is the presence of artifacts in the images of specimens which have high density metallic material beside the low density tissue on the wafer. In order to evaluate the possibility of improving image resolution by the removal of metals, Specimen 5 was processed in the same way as Specimen 4, except that all metal was removed from the specimen.

The CT images for each wafer of the specimen were analyzed using 3D Doctor™ to derive the Hounsfield unit (HU) for the tissue. The HU were analyzed from the images and are listed in Table 15. The images shown represent the HUs derived for single slices. Wafer 1 did not have any tissue on it. The slides appeared very hazy with little or no variation in pixel values. The CT image of a single slice of Wafer 2 is also shown in Table 15. The pixel value was set to a range of 173-255. The material corresponding to a pixel value of 173-255 can be seen highlighted in red. The average HU for the selected material was 707.76. Noise originating from the PMMA was observed. The HUs for all six wafers were analyzed and are presented in Table 15. The image resolutions on all slices are lower than Specimen 1 which was embedded in paraffin as shown in Table 10 of Section 5.1. All these images corresponding to Specimen 5 did not show as much of a variation in grey scale as Specimen 4. Regions of high and low radio density could hardly be differentiated. Similar to Specimen 4, the PMMA displayed more noise as compared to the paraffin specimen. There were several regions on the tissue which had the same radio density as the PMMA, which made it

difficult to differentiate between the two. The HUs can be determined for all the 900 to 1,000 slices that were generated during CT.

Table 15. Results for Specimen 5.

Specimen 5: Specimen embedded in polymethyl methacrylate (PMMA) and sectioned into wafers					
Wafer #	Slice #	Image Thresholds (Pixel Values)	Image	Mean Hounsfield Unit (HU)	Discussion
1	No tissue was seen on the micro CT image. Slices appeared very hazy, with little or no variation in pixel value.				
2	564	173-255		707.76	The CT image of a single slice of Wafer 2 is shown. The pixel value was set to a range of 173-255. The material corresponding to a pixel value of 173-255 can be seen highlighted in red. The average HU for the selected material was 707.76. Noise originating from the PMMA is observed.

Table 15. Results for Specimen 5 (Continued).

Specimen 5: Specimen embedded in polymethyl methacrylate (PMMA) and sectioned into wafers					
Wafer #	Slice #	Image Thresholds (Pixel Values)	Image	Mean Hounsfield Unit (HU)	Discussion
3	534	123-197		100.5	The CT image of a single slice of Wafer 3 is shown. The pixel value was set to a range of 123-197. The material corresponding to a pixel value of 123-197 can be seen highlighted in red. The average HU for the selected material was 100.5. Noise originating from the PMMA is observed. The image resolution is lower than Specimen 1 which was embedded in paraffin.

Table 15. Results for Specimen 5(Continued).

Specimen 5: Specimen embedded in polymethyl methacrylate (PMMA) and sectioned into wafers					
Wafer #	Slice #	Image Thresholds (Pixel Values)	Image	Mean Hounsfield Unit (HU)	Discussion
4	623	136-235		254.13	The CT image of a single slice of Wafer 4 is shown. The pixel value was set to a range of 136-235. The material corresponding to a pixel value of 136-235 can be seen highlighted in red. The average HU for the selected material was 254.13. Noise originating from the PMMA is observed. The image resolution is lower to Specimen 1 which was embedded in paraffin.

Table 15. Results for Specimen 5 (Continued).

Specimen 5: Specimen embedded in polymethyl methacrylate (PMMA) and sectioned into wafers					
Wafer #	Slice #	Image Thresholds (Pixel Values)	Image	Mean Hounsfield Unit (HU)	Discussion
5	520	120-237		46.83	The CT image of a single slice of Wafer 5 is shown. The pixel value was set to a range of 120-237. The material corresponding to a pixel value of 120-237 can be seen highlighted in red. The average HU for the selected material was 46.83. Noise originating from the PMMA is observed. The image resolution is lower than Specimen 1 which was embedded in paraffin.

Table 15. Results for Specimen 5 (Continued).

Specimen 5: Specimen embedded in polymethyl methacrylate (PMMA) and sectioned into wafers					
Wafer #	Slice #	Image Thresholds (Pixel Values)	Image	Mean Hounsfield Unit (HU)	Discussion
6	435	121-222		136.22	The CT image of a single slice of Wafer 6 is shown. The pixel value was set to a range of 121-222. The material corresponding to a pixel value of 121-222 can be seen highlighted in red. The average HU for the selected material was found to be equal to 136.22. Noise originating from the PMMA is observed. The image resolution is lower than Specimen 1 which was embedded in paraffin.

Table 15. Results for Specimen 5 (Continued).

Specimen 5: Specimen embedded in polymethyl methacrylate (PMMA) and sectioned into wafers					
Wafer #	Slice #	Image Thresholds (Pixel Values)	Image	Mean Hounsfield Unit (HU)	Discussion
7	591	127-254		172. 12	The CT image of a single slice of Wafer 7 is shown. The pixel value was set to a range of 127-254. The material corresponding to a pixel value of 127-254 can be seen highlighted in red. The average HU for the selected material was found to be equal to 172.12. Noise originating from the PMMA is observed. The image resolution is lower than Specimen 1 which was embedded in paraffin.

The only difference between the specimen preparation for Specimens 4 and 5 was the removal of all metallic structures from Specimen 5 to reduce artifacts from the CT image. From Section 5.4, Specimen 4, it was seen that pathological analysis can be successfully done on such a specimen processed onto a PMMA wafer. Pathological analysis would have been successful for Specimen 5. However, the wafers from Specimen 5 were not sent for pathology because of the following limitations. The clarity of the images was not good enough to differentiate between adjacent tissues of varying radiopacities. Therefore, the HU of different tissues was not easily identifiable. During the image analysis of Specimen 4, it was hypothesized that this differentiation was not evident because of the presence of artifacts caused by metallic structures. However, Specimen 5 showed similar limitations. One possible cause is the nature of PMMA itself. The radio density of PMMA is equal to or higher than most of the tissue, as observed on the wafer images shown in Figure 27. Therefore, differentiation between the PMMA, and tissues with different radio densities was not evident.

Figure 27. Comparison of radio density of PMMA versus tissue.

Also, image resolution in pathology is often determined by the physical method of creating the slides [50]. Microtomy gives a better resolution than the Exakt method [50]. However, microtomy is commonly done on paraffin embedded tissue and produces thinner sections 4 to 5 μm thick. Since these wafers were processed using the Exakt method, a much lower resolution on the pathological image was obtained.

5.6 Comparison of Different Methods of Specimen Preparation

The objective of this research was to evaluate techniques to prepare embolic protection devices for micro CT and subsequent pathological analysis in order to enable tissue characterization. A comparison of the five techniques of specimen preparation is presented in Table 16. The advantages and limitations of each technique along with their suitability for characterization is presented. The paraffin embedding technique was found to be the best technique for tissue characterization. The glass slide technique and the PMMA block technique were not suitable for tissue characterization. The PMMA wafer techniques can be used for characterization but because of the presence of noise and artifacts, the derived HUs is not representative of the actual HU of the tissue.

Table 16. Advantages and Limitations of Different Techniques of Specimen Preparation.

Technique	Advantages	Limitations	Performance
Paraffin Embedding technique	<ol style="list-style-type: none"> 1. High resolution micro CT images were seen. 2. Adjacent regions having different CT Numbers could be clearly differentiated. 3. Paraffin density appeared lower than the tissue density, therefore less radiation was absorbed and more was transmitted. 4. Specimen virtual reconstruction was possible. 5. Volumetric analysis was possible. 6. Cost of scanning was lower than PMMA wafer methods. 7. It is a non-destructive method of sample preparation. 	<ol style="list-style-type: none"> 1. Ratio of size of the paraffin cassette to that of the tissue needs to be kept low. 2. If a smaller block is dissected from the cassette, then pathological sectioning is difficult. 3. There can be a loss of tissue integrity during paraffin remelting. 	Best option for characterization

Table 16. Advantages and Limitations of Different Techniques of Specimen Preparation (Continued).

Technique	Advantages	Limitations	Performance
Glass slide technique	<ol style="list-style-type: none"> 1. Histopathological analysis was possible. 2. Ratio of the paraffin with the glass slide to the tissue was small thus facilitating a high resolution image. 3. The same section of tissue can be subjected to micro CT scanning and pathological testing. Therefore, direct comparison of pathological slides with the micro CT image would have been possible thereby helping in tissue characterization. 	<ol style="list-style-type: none"> 1. The transmittance of X-rays through glass was too low. Therefore micro CT images provide no information about the constituents. 	Not suitable for characterization
PMMA Block technique	<ol style="list-style-type: none"> 1. PMMA block smaller than the paraffin cassette was obtained. 2. A smaller pixel size was possible. 3. A cassette was not needed to hold the PMMA. 4. The PMMA block was transparent to visible light, and therefore the morphology of the specimen within the block could be visually observed. 5. Histopathological processing and analysis was possible. 	<ol style="list-style-type: none"> 1. Micro CT images provided no information about the constituents. 2. The ratio of the size of the block to the size of the tissue was too high for successful micro CT imaging. 	Not suitable for characterization

Table 16. Advantages and Limitations of Different Techniques of Specimen Preparation (Continued).

Technique	Advantages	Limitations	Performance
PMMA wafer technique with metal	<ol style="list-style-type: none"> 1. The ratio of the PMMA material to the tissue was small, which permitted successful micro CT imaging. 2. The same section of tissue can be subjected to micro CT and pathological reading. Direct comparison of pathological slides with the micro CT image was possible, thereby helping in tissue characterization. 3. Histopathological analysis was successful 	<ol style="list-style-type: none"> 1. Low resolution micro CT images were obtained because of the noise originating from PMMA. 2. Artifacts caused by the presence of high density metal were observed. 3. Image resolution was not as high as that on the image obtained from the paraffin embedded tissue. 4. Since these wafers were processed using the Exakt™ method, a much lower resolution on the pathological image was obtained 	Characterization may not be as accurate as paraffin method
PMMA wafer technique without metal	<ol style="list-style-type: none"> 1. The ratio of the PMMA material to the tissue was small, which permitted successful micro CT imaging. 2. The same section of tissue can be subjected to micro CT and pathological reading. Direct comparison of pathological slides with the micro CT image was possible, thereby helping in tissue characterization. 3. Histopathological analysis was successful. 	<ol style="list-style-type: none"> 1. Low resolution micro CT images were obtained because of the noise originating from PMMA. 2. Image resolution was not as high as that on the image obtained from the paraffin embedded tissue. 	Characterization may not be as accurate as paraffin method

CHAPTER SIX

CONCLUSION

Based on the results of micro CT, embedding the specimen in paraffin was found to be the best technique for preparing specimens for micro CT imaging and subsequent histopathology among the techniques evaluated in this research. The paraffin embedding technique produced micro CT images with a resolution higher than all the other techniques evaluated. Adjacent regions having different pixel values and Hounsfield Units could be clearly differentiated. Virtual image reconstruction was also possible. However, the ratio of the size of a standard paraffin cassette to that of the captured embolic tissue was too large for micro CT imaging. Care needs to be taken while re-melting the paraffin in order to prevent the loss of integrity of the tissue. Another option is to limit the size of the paraffin block such that it surrounds the tissue minimally instead of dissecting it out from a standard cassette. The other techniques that were evaluated did not produce promising results. The polymethyl methacrylate (PMMA) block was not suitable for micro CT imaging due to its size. Even though the PMMA block was smaller than the paraffin cassette, the ratio of the size of the block to the size of the tissue was still too high for successful micro CT imaging. The paraffin sections and glass slide technique did not work well with micro CT imaging due to the transmittance of X-rays through glass.

The PMMA wafer technique was found to be the second best technique. The ratio of the PMMA material to the tissue is small, which permitted successful micro CT imaging. Histopathology was successfully done with this technique. The same section of

tissue can be subjected to micro CT imaging and pathological reading. Therefore, direct comparison of pathological slides with the micro CT image is possible, thereby helping in tissue characterization. However, this technique produced images with noise and lower resolution than the paraffin embedding technique and therefore the HU of different tissues may not be easily identifiable.

CHAPTER SEVEN

RECOMMENDATIONS FOR FUTURE WORK

Additional work is needed in the area of specimen preparation for characterization of embolic tissue. A method of re-embedding paraffin within a new cassette without damaging the tissue needs to be developed. The paraffin-embedded specimen can then be subjected to micro CT and subsequent pathological analysis. The micro CT images can be correlated to the pathological images to evaluate the Hounsfield Units (HU) for different tissue types. A library of such Hounsfield Units corresponding to different tissue/cell types can be generated. This library can help physicians diagnose the constituents of emboli and parent lesions. With further research, it might be possible to relate the presence or absence of the particular constituent to a unique diseased state and to pilot a histopathological, surgical, or interventional procedure. These data can then be used as predictors for treating patients.

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