IMPEDANCE-BASED DETECTION OF TISSUE USING
A MULTI-ELECTRODE DEVICE

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Abstract

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By

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Melanoma skin cancer is the abnormal growth of the melanocytes – the pigmented cells located in the epidermis. The current gold standard diagnostic technique for determining whether a lesion is cancerous involves subjectively examining suspicious lesions and performing an invasive biopsy to confirm melanoma. This method may neglect some lesions or cause scarring from biopsies that turn out to be benign. Thus, impedance-based detection using a multi-electrode device was investigated as a noninvasive technique to diagnose melanoma skin cancer. The multi-electrode device was designed with 8 equally spaced Ag/AgCl electrodes surrounding one central electrode at a 5 mm radius. The electrodes were held in place by a vice-like mechanism using three circular Delrin sections. The electrodes were interfaced to an 8:1 multiplexer and National Instruments Educational Laboratory Virtual Instrumentation Suite (ELVIS) for measurement control and impedance analysis. The ELVIS system, multiplexer, and electrode device were validated for accuracy with various values of resistors and capacitors. Raw and cooked chicken thigh meat and skin were tested to evaluate the capabilities of the electrode device to discern different tissue types and tissue moisture contents by impedance measurements. EpidermTM and Melanoma tissue-engineered skin analogues, provided by MatTek Corporation, were tested to mimic the in situ disease state. The electrode device was found to produce reliable measurements for known electrical components with resistances between 10 ohms and 100 k-ohms and capacitances between 10 nF and 10 uF. The measurements from the chicken tissues and tissue-engineered skin constructs – excluding cooked chicken skin data – fell within the reliable range of the electrode device and were thus considered reliable as well. All analyses concluded that a statistical difference between the impedances of raw meat and raw skin, cooked meat and raw meat, and EpidermTM and Melanoma existed. Therefore, the hypothesis that a multi-electrode device could differentiate between melanoma and healthy skin tissues based on impedance measurements was satisfied.

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Introduction

Thesis Objective

The objective of this thesis is to manufacture, assemble, and test a device that uses a matrix of electrodes to measure the electrical impedance of skin tissue. This device will be used to measure the differences in electrical impedance between healthy tissue and cancerous tissue, specifically melanoma. Statistical differences in impedance can potentially provide a non-invasive diagnosis for melanoma and save the patient from unnecessary biopsies. The matrix of electrodes allows for the acquisition of impedance in multiple directions, which can help identify the boundaries of the tumor.

To ensure that this electrode device functions properly, the number of electrodes and electrode pattern have been kept small and simple, respectively. Thus, the objective of this thesis is also to review possible alterations to the device for future work, which may increase the resolution of the device and allow for accurate identification of tumor boundaries.

Thesis Purpose

The purpose of this thesis is to: 1) present information on skin cancer and provide current clinical practices in diagnosing, staging, and treating melanoma, 2) introduce a relatively new technique for non-invasive diagnosis of skin cancer, 3) validate this new technique by providing and analyzing experimental data using the constructed electrode matrix device, 4) draw practical and theoretical conclusions on the data, and 5) speculate on alterations for future work, clinical utilization, and possible interplay between radiofrequency tissue ablation treatment devices.

Background

Skin Anatomy and Physiology

To understand the scientific theory behind impedance analysis of skin, it is important to understand the overall structure, cellular composition, and normal functioning of skin. Skin is considered the body's largest organ and serves multiple functions including thermoregulation, sensation, vitamin D synthesis, and barrier functions [1]. Thermoreceptors in the skin transmit signals to the brain, which can induce vasoconstriction or vasodilation when it is cold or hot, respectively [1]. Similar to thermoreceptors, there are an abundance of nerve endings in the skin that allow for the sensation of touch. Vitamin D synthesis begins in the skin from exposure to ultraviolet (UV) B rays and ultimately leads to calcitriol, which serves numerous functions as a hormone in the body [1]. Finally, the skin serves a barrier function to prevent bacteria, fungi, and water from entering the body through the skin. It also functions to retain the water in the body. Due to its large surface area and its location as a barrier to the external world, the skin receives the most physical injuries in the form of cuts, scraps, UV radiation and more [1]. However, with skins high regeneration rate, the physical traumas that occur are quickly repaired [1].

All of the aforementioned functions occur from the cellular makeup of skin. Figure 1 shows the three main layers of skin, which are the hypodermis, dermis, and epidermis, in order from deepest to most superficial [1]. The hypodermis is the deepest layer of skin and is mainly comprised of adipose tissue [1]. It is highly vascularized and is thus an attractive sight for drug injections known as subcutaneous injection [1]. The

adipose tissue connects the skin to underlying structures, insulates the body, and acts as padding in sensitive areas [1].

The dermis is the middle layer of the skin, superficial to the hypodermis, and is mainly connective tissue with collagen and elastin fibers and fibroblast cells [1].

Incorporated into the dermis are blood vessels, sweat glands, and nerve endings, which contribute to the functions of sensation and thermoregulation [1]. At the border between the dermis and the epidermis, there exist wave-like structures called dermal papillae [1]. These dermal papillae mesh with epidermal ridges and oppose shearing forces placed on the skin [1].

Figure 1: Skin Anatomy

A cross section showing the three layers of skin and their components [1]

The outermost (superficial) layer of skin is known as the epidermis. The epidermis is important to this thesis because it contains the cells that grow uncontrollably

during formation of most common skin tumors [1]. The epidermis is broken down into further layers known as the stratum basale, stratum spinosum, stratum granulosum, stratum lucidum, and stratum corneum, from deepest to most superficial [1]. The stratum basale is uniquely comprised of stem cells, melanocytes, keratinocytes, and tactile cells [1]. Epidermal stem cells, found in the stratum basale, produce new keratinocytes and gradually push old keratinocytes to the surface of the skin in approximately 30-40 days [1]. Melanocytes are pigment-producing cells that release a brown to black pigment known as melanin [1]. This melanin is taken up through phagocytosis by keratinocytes and is accumulated on the side of the nucleus that receives the most UV rays [1]. Melanin absorbs these harmful UV rays and inhibits DNA damage [1]. Tactile cells are touch receptors and are coupled to nerve fibers found in the dermis to transmit pressure and texture information to the brain [1]. Keratinocytes are the most abundant cell in the epidermis and are found in all five layers. Keratinocyte secretion of lipids in the stratum granulosum and stratum spinosum is responsible for the hydrophobic barrier that retains water in the body [1]. Keratinocytes also have many desmosomes and tight junctions – types of intercellular connections – that oppose shearing forces and relate to the toughness of skin [1]. The last cell, dendritic cells, are found in the stratum granulosum and stratum spinosum and act as macrophages for any pathogens that enter the epidermis through abrasions, scratches, etc [1]. Together, the intercellular connections, as well as the different layers of skin, influence the electrical properties of healthy skin tissue. Changes in the type and number of cells during tumorigenesis are discussed in the section *Skin Cancer*.

Cancer

In general, a tumor is the term used to describe the uncontrolled growth of abnormal cells in the body [2]. A tumor may be slow growing masses that are encapsulated with fibrous tissues (benign) or fast growing masses that can release cells into the systemic circulation (malignant) [1]. The latter of these is the more dangerous type of tumor and is often referred to as cancer. The five major classes of cancer and the cells in which they originate from are carcinoma from epithelial cells, melanoma from melanocytes, sarcoma from bone or muscle, leukemia from blood forming tissues, and lymphoma from lymph nodes [1].

Causes

In normal, healthy cells, when the life cycle reaches its end, extrinsic (cytokines) and intrinsic (cytochrome-c) factors stimulate the cell to fragment the DNA and begin destroying itself [3]. This is known as apoptosis. Phagocytic cells, such as macrophages or dendritic cells, remove and degrade the fragments of the apoptotic cells [3]. The death of cells is counteracted by the controlled generation of new cells from stem cells. For instance, in the stratum basale layer of the epidermis, stem cells continuously regenerate keratinocytes to renew the skin [1]. However, in tumorigenic cells, key genes that regulate differentiation are damaged in the DNA [1]. All of these genes are not known, but some of them include *sis*, *ras*, and *p53*, which, in their abnormal states, secrete excessive amounts of angiogenic growth factors, cause abnormally sensitive growth factor receptors, and fail to inhibit the enzymes of the cell cycle, respectively [1].

DNA damage occurs through various mutagens and mechanisms that include chemicals, excessive alcohol, environmental toxins, sunlight exposure, genetics,

radiation, and viruses [4]. Some chemicals known for their carcinogenic potential are benzene, asbestos, ethylene oxide, formaldehyde, tobacco, and many more [4].

Effects and Symptoms

Cancer symptoms are highly dependent on the classification and location of the tumor. For these reasons, the effects and symptoms described in this section may not correspond to every cancer. One of the first symptoms of cancer is fatigue [2]. Fatigue stems from the fact that the tumors have a high metabolic rate due to the continuous differentiation of cells [2]. Tumors secrete angiogenic (capillary-forming) factors that increase the blood flow and nutrients to it [2]. The flow of more nutrients to the tumor depletes the resources used for other healthy cells; the result is a general feeling of weakness. Muscle and adipose tissue may even degrade to provide extra nutrients to the tumor, which leads to weight loss [2].

In some cancer cases, especially in leukemia, the immune system is either preoccupied with fighting the cancer or abnormal due to the cancer. This allows for opportunistic infections to overwhelm the weakened immune system and cause serious illness or death. The growth of the tumor itself has further implications than just metabolic need. Growing masses can infiltrate normal tissues and cause dysfunction [1]. If serious enough, the dysfunction can lead to death as in the inability to oxygenate blood in lung cancer [1]. Tumor growth may also place pressure on airways or blood vessels and can inhibit breathing and oxygenation to healthy tissues [1]. Finally, it is important to note that while the primary cancer is detrimental, in most cases, the metastatic tumors that infiltrate other regions of the body cause the more severe symptoms [1].

Treatment

Cancer treatment options are similar to symptoms in that they vary greatly with the classification and location of the mass. However, the three main treatments used in most cancers are chemotherapy, radiation therapy, and surgery [2]. Chemotherapy involves administering drugs that are cell cycle arresters (i.e. inhibit differentiation) [2]. Experimental chemotherapy drugs are tagged with certain proteins to hone directly to and affect only the cancerous cells [2]. Yet, most chemotherapy drugs still inhibit differentiation in all cells in the body and thus cause significant side effects, such as poor appetite, loss of hair, fatigue, bleeding, and mouth sores [2].

Radiation therapy uses multiple X-ray beams that meet at the tumor and produce a high amount of radiation at its location [2]. Just as radiation can damage DNA and cause cancer, high amounts of radiation can further damage DNA and cause cell death [2]. There are few side effects to radiation therapy, but may include irritation or damage to cells surrounding the tumor [2]. Radiation therapy and chemotherapy are often used synergistically to shrink the tumor for surgery [2]. Depending on the location of the cancer, a surgeon will cut into the body and remove all tumor cells in the area.

Figure 2: Basal Cell Carcinoma

A basal cell carcinoma lesion showing the beaded edge and central depression [5]

Skin Cancer

Now with the knowledge of skin anatomy and an overview of cancer, we can specifically investigate the nuances of skin cancer. As of 2007, skin cancer is the most common type of cancer, although there is a high survival rate associated with the disease [1, 6]. There are three common types of skin cancer that are classified by the cell type in which they originate. These classifications are further lumped into melanoma and non-melanoma cancer categories.

Non-Melanoma

Basal cell carcinoma and squamous cell carcinoma are the most common types of non-melanoma skin cancer [7]. Non-melanoma cancers are generally less severe than melanoma cancers [7]. Basal cell carcinoma derives from the cells in the stratum basale – the deepest layer of the epidermis [7]. While basal cell carcinoma is the most prevalent type of skin cancer, it is also the least hazardous to health because of the low risk of metastasis [7]. The lesion of basal cell carcinoma usually grows from a small, shiny bump to a larger one with a beaded edge and central depression as shown in Figure 2 [7]. Squamous cell carcinoma derives from keratinocytes in the stratum spinosum layer of the epidermis [7]. This form of skin cancer is more malicious but still controllable if diagnosed early. These lesions appear red and scaly and usually precede more ulcerative lesions as in Figure 3 [1]. Other forms of non-melanoma skin cancer that are less common include Kaposi's sarcoma, Merkel (tactile) cell carcinoma, and cutaneous lymphoma [7]. Even though the majority of these skin cancers are more prominent than melanoma skin cancer, they are often less dangerous and will not be investigated in this thesis.

Melanoma

Melanoma skin cancer is considered the most deadly form of all skin cancers [8]. It was estimated in 2010 that 68,130 men and women would be diagnosed with melanoma and 8,700 men and women would die from melanoma [8]. Melanoma stems from the abnormal growth of melanocytes in the epidermis and may occasionally be found in the pigmented segments of the eyes and intestines [6].

Figure 3: Squamous Cell Carcinoma

A squamous cell carcinoma lesion with advanced ulcerative feature [9]

Risk Factors

Abnormal growth of melanocytes occurs from damage to the DNA. Most scientists believe that UV rays damage the DNA directly, however, there are many other factors that have a role in the development of melanoma. Skin complexion is highly correlated to melanoma production [8]. Persons with fairer skin pigment cannot protect

against the UV rays as well as persons with dark complexions. This results in an approximately 30 fold greater chance of melanoma in white persons compared to Asian and Black persons [8]. Similarly, increased sun exposures or intense sunburns will cause more UV ray damage and a greater likelihood of skin cancer development [10]. As such, areas of the body that experience the most sun exposure, which are the back, neck and face, have a higher probability of developing cancerous lesions [10]. Genetics also plays a role in melanoma development. Family members with previous history of melanoma are likely to pass the dysfunctional gene to their offspring [10]. Recent studies in melanoma etiology have found that mutations in the BRAF, p53, or Rb genes are often seen repeated among family members that exhibit the disease [11]. These genes are thought to control the suppression of growth, which, when mutated, cause immortalized cancer cells [11]. Age is also responsible for development of the disease considering that the median age of melanoma diagnosis is 60 years [8]. One article believes that the manifestation of melanoma in later years is due to a gradual buildup of oxidative stress caused by melanin-derived reactive oxygen species [11]. Finally, individuals should scrutinize the appearance of dysplastic nevi, or moles. Nevi, which are benign growths, have the capability to begin changing into melanoma or other skin cancer lesions [10]. Tips for identifying malicious lesions are discussed in the *Diagnosis* section next.

Diagnosis

Early diagnosis of melanoma is critical in survival rates. When the cancer is diagnosed early – before the lesion has spread to lymph nodes or other tissues – the relative survival percentage at 5 years after the diagnosis is 98% [8]. However, when the cancer has metastasized, the relative survival percentage at 5 years is 15.9% [8]. Current

diagnosis techniques utilize a combination of visual inspection and histological testing. The visual inspection of the nevi is governed by the ABCDE rules – an updated version of the ABCD rules. A physician uses these rules during an examination, but they recommend annual self-examinations using mirrors [12]. The following rules are as follows [10]:

- A = Asymmetry – Two halves of the nevus do not match
- B = Border – The borders of the nevus are uneven or blurred
- C = Color – Two or more distinct colors are found. These colors may be brown, tan, black, red, or blue.
- D = Diameter – The diameter of the nevus is larger than ¼” or 6 mm
- E = Evolving – Any change in rules A, B, C, or D, or any bleeding, itching, or crusting, is cause for concern.

Images depicting benign and malignant lesions for all aspects of the ABCDE rules are shown in Figure 4. If any of the rules are satisfied and a nevus appears to be malignant, then a biopsy is performed. The biopsies performed depend on the appearance of the lesion and the location. Most commonly, excisional biopsies are performed, which includes cutting away the entire lesion, as well as some surrounding healthy tissue [4]. Incisional biopsies are similar to excisional biopsies, but only incorporate cancerous cells – no healthy tissue is removed [4]. Punch biopsies remove a cylindrical section of the epidermis and dermis similarly to a circular cookie cutter [4]. Punch biopsies are beneficial because they incorporate dermis tissue that is helpful for staging [4].

After the biopsy, a sample of the tissue is sent to the laboratory where histology is performed by staining the sample and viewing cells with a microscope [4]. Trained

laboratory technicians subjectively analyze the cell morphology of the sample and determine if cancer is a proper diagnosis. Often, multiple technicians view the histology slides to reduce error from technician subjectivity [4]. If cancer is determined to be an appropriate diagnosis, trained professionals stage the cancer and determine a prognosis. Many times, further excisional biopsies are needed to remove all cancerous cells.

Figure 4: ABCDE Rules

Example images of the difference between benign nevi and malignant melanomas in reference to the ABCDE rules of diagnosis [10]

Since the ABCDE rules and histological inspections are based on the physician or technician's subjectivity, the consistency of results may vary between persons. Thus it is important to create a diagnostic technique that eliminates subjectivity. Even more important, is creating a diagnostic technique that is non-invasive so that surgery does not have to be performed. For example, scarring will occur from an excisional biopsy, while the results of the biopsy may yield benign results. Besides scarring, with diagnostic techniques being invasive, physicians may decide that a lesion does not appear severe enough to justify surgery. The physician attempts to eliminate unnecessary scarring while balancing the risk of overlooking a malignant tumor. This risk would be nearly non-existent if an accurate non-invasive diagnostic technique could provide objective results.

Attempts at producing new diagnostic techniques include multispectral digital dermoscopy, confocal scanning laser microscopy, and cellular electrical bioimpedance [12]. Multispectral digital dermoscopy uses a camera that has the ability to project electromagnetic waves at different wavelengths and record the reflected signals [12]. Different wavelengths of electromagnetic radiation can penetrate different thicknesses of skin and obtain images of a lesion that physicians cannot see [12]. The color of the lesion is also recorded and a computer algorithm analyzes all of the data to determine if the lesion is likely malignant; this technique yields a sensitivity of 95-100% [12]. Confocal scanning laser microscopy is similar to multispectral digital dermoscopy, except that it uses a pinhole to focus a low-intensity infrared laser beam [12]. This highly focused laser beam is able to penetrate the skin and record structures at different levels [12]. Thus, it provides slices of tissue, like a digital rendition of histology, to allow for diagnosis. The confocal microscopy technique allows for 98% sensitivity [12]. Cellular electrical

bioimpedance is the diagnostic technique that will be investigated further in this thesis.

The theory behind cellular bioimpedance measurements and its application toward melanoma skin cancer diagnosis are discussed further in *Electrical Properties → Cancer* and *Practical Uses*.

Staging

After successful diagnosis and identification of the type of skin cancer, staging of the lesion begins. Staging includes a series of parameters that can be followed objectively. There are two divisions to staging. The first division encompasses the T, N, and M parameters. These parameters are broken down even further into numbers that relate to the number or size of each parameter. The second division of staging groups the T, N, and M values together to assign a comprehensive value to the stage of the cancer. This comprehensive value will determine the likely prognosis of the patient. The parameters are explained as follows [13]:

- **T = Primary Tumor.** This parameter of staging mainly deals with the thickness of the lesion. The thickness of the lesion is defined as the distance from the top layer of the epidermis to the bottom of the lesion. These measurements are usually performed with micrometers after excision or during the histological sections of the tissue. Imaging technologies, such as confocal microscopy or ultrasound, may also accurately gauge the thickness of the tumor. In addition to the thicknesses, appearances of microscopic ulcerations are taken into account, as well as the rate of mitosis. The rate of mitosis only corresponds to staging in T1 lesions and is given an ‘a’ for mitosis rates less than 1event/mm² or a ‘b’ for rates greater than or equal to 1 event/mm².
 - **TX** – Primary tumor cannot be assessed
 - **T0** – No evidence of primary tumor
 - **Tis** – Melanoma in situ (still in original place)
 - **T1** – Melanoma is 1.0 mm or less in thickness
 - **T2** – Melanoma is 1.01 – 2.0 mm
 - **T3** – Melanoma is 2.01 – 4.0 mm
 - **T4** – Melanoma is greater than 4.0 mm
 - **a** – Lesion contains no ulceration
 - **b** – Lesion contains ulceration

- **N = Regional Lymph Nodes.** This parameter identifies the number of lymph nodes affected by metastasis of the lesion. Metastasis occurs when cellular adhesion molecules break down in the lesion. This allows surface cancerous cells to dislodge from the mass and travel either by the lymphatic system to the lymph nodes or by the circulatory system to distant tissues. Lymph nodes are tested by palpating downstream lymph nodes for inflammation. Lymph fluid is also retrieved from the lymph nodes under local anesthesia as shown in Figure 5. The lymph fluid is stained to look for leukocytes or cancer cells. Further differentiation into 'a' and 'b' are used to describe the size of the metastasis.
 - **NX** – Regional lymph nodes cannot be assessed
 - **N0** – No regional metastases detected
 - **N1-3** – Metastases related to the number of nodes affected
 - **a** – Micrometastasis
 - **b** – Macrometastasis
- **M = Distant Metastases.** This parameter identifies the tissues affected by melanoma metastases and are ranked from 'a' to 'c' based on the type of tissue affected most frequently. Since skin and subcutaneous tissue is adjacent to the original location of the lesion, it is common to get metastasis in these locations. The lungs are also a frequent location for metastatic melanoma. Metastases are imaged by using X-rays, PET scans, and MRIs that allow for visualization of the body. This parameter can also be further differentiated by the serum levels of Lactate Dehydrogenase (LDH).
 - **M0** – No detectable evidence of distant metastases
 - **M1a** – Metastases to skin, subcutaneous, or distant lymph nodes with normal LDH
 - **M1b** – Metastases to lung with normal LDH
 - **M1c** – Metastases to all other visceral sites or elevated LDH

Figure 5: Lymph Node Biopsy

An example of a lymph node biopsy to determine the N stage of melanoma [14]

Given the definitions and parameters for the staging of melanoma, a table compiling the comprehensive stages with the parameters is provided in Table 1 [13].

There are two headings for the stages of melanoma: clinical and pathologic staging. The only difference between the two headings is that pathologic staging incorporates surgical findings of melanoma metastases to lymph nodes.

Table 1: Melanoma Skin Cancer Staging [13]

ANATOMIC STAGE/PROGNOSTIC GROUPS							
Clinical Staging ³				Pathologic Staging ⁴			
Stage 0	Tis	N0	M0	0	Tis	N0	M0
Stage IA	T1a	N0	M0	IA	T1a	N0	M0
Stage IB	T1b	N0	M0	IB	T1b	N0	M0
	T2a	N0	M0		T2a	N0	M0
Stage IIA	T2b	N0	M0	IIA	T2b	N0	M0
	T3a	N0	M0		T3a	N0	M0
Stage IIB	T3b	N0	M0	IIB	T3b	N0	M0
	T4a	N0	M0		T4a	N0	M0
Stage IIC	T4b	N0	M0	IIC	T4b	N0	M0
Stage III	Any T	≥ N1	M0	IIIA	T1-4a	N1a	M0
					T1-4a	N2a	M0
				IIIB	T1-4b	N1a	M0
					T1-4b	N2a	M0
					T1-4a	N1b	M0
					T1-4a	N2b	M0
					T1-4a	N2c	M0
				IIIC	T1-4b	N1b	M0
					T1-4b	N2b	M0
					T1-4b	N2c	M0
					Any T	N3	M0
Stage IV	Any T	Any N	M1	IV	Any T	Any N	M1

To summarize the table, Stages I and II include in-situ tumors. These tumors are the ones that are most likely survivable if removal of the cancerous cells begins immediately. The letters 'a', 'b', and 'c' that are associated with stages I and II provide the information on the thickness of the tumor. The thicker the tumor, the more likely it is that the mass will penetrate into the dermis and begin metastasis. Stage III incorporates all melanoma metastases to distant lymph nodes. As the number of lymph nodes affected increases, so does the risk of death or other severe complications. Stage IV takes into account metastases to other tissues, which is considered the worst prognosis, depending on the locations of the metastases.

Treatment

With staging of the tumor known, the physician and the patient discuss treatment options. With stage I and II melanoma, there is no involvement of distant tissues or lymph nodes and thus the tumor itself can specifically be targeted for the treatment. One benefit of having a skin cancer is that the mass is found on the surface of the body. Thus, surgical intervention is often easy and fast. Excisional surgery, related to the technique used in the diagnosis of cancer, is often used to treat patients with skin cancer. In this technique, the physician merely cuts away the tumor along with a specified margin of healthy tissue to ensure the removal of all tumor cells [10]. For in situ melanomas, 0.5 – 1 cm of healthy tissue surrounding the tumor is removed during surgery [10]. Melanomas that are 1 mm or less, 1.01 to 2 mm, or 2.01 mm or greater in thickness will have approximately 1 cm, 1 to 2 cm, or 2 cm of healthy tissue removed during the surgery, respectively [10].

One advanced surgical technique that correlates to reduced margins of healthy tissue removed is called Mohs micrographic surgery [6]. In this technique, a thin layer of the tumor is removed and investigated for the presence of tumor cells at the border [6]. These investigations include staining the tissue sections and viewing slides under a microscope. If tumor cells are found in the middle of the section, then more cuts along the thickness of the tumor are needed [6]. If tumor cells are found at the border of the section, then it is likely that all tumor cells are not resected from the skin. Thus, the surgeon removes more marginal healthy tissue until the presence of tumor cells at the border of the sections is non-existent [6]. This surgery is longer than the simple excisional technique but reduces the amount of healthy tissue lost and softens the presence of a scar afterward [6]. However, looking at Figure 6, a scar is typically still noticeable from the surgery.

Figure 6: Mohs Surgery Scar

A noticeable scar created from Mohs micrographic surgery [15]

Another surgical treatment that causes heavy scarring is called electrodesiccation and curettage [16]. Here, the surgeon uses a spoon-like instrument, known as a curette, and scoops the tumor away from the skin [16]. Due to heavy bleeding, an electrode is placed in the crater of the removed tumor and electric current is passed to the tissue to cauterize blood vessels and kill any remaining tumor cells in the area [16]. This technique is quick and easy, but the removal of all tumor cells is not confirmed and scarring is typically present [16].

Techniques that prevent or markedly reduce the appearance of scars are needed as skin cancer treatments. One such technique is known as cryosurgery and is similar to wart removal. Liquid nitrogen is sprayed or applied to the tumor and the intense cold freezes the cells and stops cellular processes, effectively killing the tumor cells [17]. While this may prevent or reduce the appearance of scars, the technique is not effective in killing tumors that have advanced beyond in situ/thin melanoma [17].

Radiofrequency tissue ablation has been suggested as a possible non-invasive treatment for skin cancer, although there are no current publications testing the capabilities of this technique. It is suggested that the principles behind the use of radiofrequency ablation should also work equally well, if not better, in skin cancer due to its location on the surface of the body [18]. This technique sends radiofrequency waves at 300 to 500 MHz frequencies to the tumor [18]. The waves interact with each other at the tumor location and cause the tissue to heat up. The excessive heat damages the cells and causes cell death. If a shape of the tumor is identified using imaging technologies, the radiofrequency waves can be directed to cancerous tissue only [18]. This technique may reduce the appearance of scars and healthy tissue damaged during treatment [18].

There are also treatment techniques that usually treat metastatic cancers but can be used to shrink the tumor. For example, radiation therapy and chemotherapy may be administered to shrink the tumor, which allows for smaller scar development after surgical excision [4]. These techniques are also often used after surgical excision to ensure that all cancer cells are destroyed and that relapses do not occur [4]. Furthermore, these techniques are used to treat melanomas that have metastasized to lymph nodes and distant tissues [4].

Radiation therapy, similar to radiofrequency ablation, uses directed X-ray beams that are shaped from Computer Tomography (CT) images of the tumor to cause extensive DNA damage in cancerous cells [4]. These directed beams enter the body at different locations and intersect at the tumor to cause maximal damage, while leaving the healthy tissue unharmed [4]. While radiation is a cause of cancer, it can also disturb DNA enough at high doses to kill the tumor cells [6].

Chemotherapy uses chemical agents to attack DNA. Some topical agents, such as Fluorouracil and Imiquimod, can be applied to skin to kill cancer cells in the epidermis [6]. These topical agents, however, are only directed toward basal cell and squamous cell carcinomas [6]. There are also systemic chemotherapy drugs that are cell cycle inhibitors [6]. These arrest cells in their cell cycle and prevent them from differentiating. Cancerous cells often cannot handle the cell cycle inhibitors and they die before healthy tissues do. Chemotherapy is a harsh treatment with many side effects due to the non-specific nature of cell cycle inhibitors [6].

Lastly, immunotherapy and gene therapy have been investigated as possible treatments. Immunotherapy utilizes chemicals and compounds in the body to treat the

cancer [4]. The body has many natural defenses but can often times be shut off by the tumor. By isolating these natural defenses, amplifying their numbers, and reinjecting them into the body, we can effectively enhance our immune systems [4]. Interferon (IFN) alpha-2b is an FDA approved therapy indicated for use in high-risk (Stage II, III) melanomas with a high chance of recurrence [4]. Other immunotherapies in research include tumor necrosis factor (TNF) and high dose IL-2. TNF is not FDA approved but may show promise due to its natural tumor fighting capabilities and anti-angiogenic properties [4]. IL-2 is currently FDA approved for stage IV metastatic melanoma. It is known by the name proleukin and activates the body's innate immune system to fight the cancer [4]. Gene therapies seem to still be premature, but consist of delivering genes to cells to enhance or inhibit certain functions. For example, inhibiting the functions of mitogenic genes may slow cancer cell differentiation or inhibiting angiogenic factors from generating capillaries may prevent the delivery of nutrients to the tumor [10].

With all of this in mind, it is important to protect one's self from harmful UV rays. Wearing sun block, long sleeved shirts, long pants, and sunglasses and clothing that absorb UV radiation can help prevent the occurrence of cancer [6]. With that said, 1 in 52 people of white complexion will get skin cancer in their lifetime [8]. Thus, it is important to consistently perform self-examinations in order to find suspicious nevi in early stages of development. Better, non-invasive, objective diagnostic techniques may increase the ability to diagnose early tumors. For this reason, one diagnostic technique in particular, multi-electrode impedance analysis, will be investigated further in this thesis.

Electrical Properties

When describing the theory behind multi-electrode impedance analysis, it is important to have an understanding of basic electrical properties. This section will begin with a brief overview of electrical theory, followed by a discussion of electrical properties of healthy and cancerous skin tissue, and ending with a summary of previous work accomplished in the field of Electrical Impedance Spectroscopy (EIS) as a diagnostic technique for cancer.

Introduction to Electrical Theory

There are various electrical parameters that describe circuits and circuit components. This thesis will focus exclusively on the impedance of skin cells. Impedance (Z) describes the amount of opposition to the flow of current and is associated with a phase angle (θ); the phase accounts for the difference in cycles of the voltage waveform compared to the current waveform [19]. A phase difference results from the retardation of the current waveform as it travels through the measurement medium. Current (I) is the flow of electric charges through a medium and is traditionally defined as the flow of positive charges. Impedance is related to current through Ohm's law, which states that the voltage (V) divided by the current equals the impedance, where the voltage is the potential energy that drives the current [19].

Equation 1 – Ohm's Law

$$Z = \frac{V}{I}$$

Impedance may be broken down into separate vectors along a real and imaginary (complex) coordinate plane [19]. The real (X-axis) component of impedance is the resistance [19]. Resistance (R) is the opposition to DC current in a circuit and thus does

not depend on frequency. The imaginary (Y-axis) component of impedance is the reactance (X) and is the opposition of capacitance and inductance to current [19]. Capacitance (C) is the measure of a components ability to store charge across separated conductive plates and is measured in Farads (F) [19]. Equation 2 gives the reactance associated with the capacitive component of a circuit [19]. The capacitive reactance at low frequencies (DC) causes the capacitor to behave as an open switch. Conversely, with high frequencies, a capacitor behaves as a short.

Equation 2 – Capacitive Reactance

$$X_C = -\frac{1}{2\pi fC}$$

Where:

f = Frequency (Hz)

C = Capacitance (F)

Inductance (L) is the measure of a components ability to store charge through coil-induced electric fields and is measured in Henrys (H) [19]. Equation 3 gives the reactance associated with the inductance of a component [19]. At low (DC) and high frequencies, an inductor acts as a shorted wire and open switch, respectively.

Equation 3 – Inductive Reactance

$$X_L = 2\pi fL$$

where L is the inductance in Henrys. Impedance, phase, resistance, and reactance are measured in Ohms and related by the following geometric relationships [19]:

Equation 4 – Impedance Components

$$Z = \sqrt{R^2 + X^2}$$

Equation 5 – Phase Angle

$$\theta = \arctan\left(\frac{X}{R}\right)$$

Skin Electrical Properties

As discussed in the section *Skin Anatomy and Physiology*, skin consists of three main layers. Within the dermis and epidermis layers are various distributions of hair follicles, pores, and sweat ducts [20]. Furthermore, moving from deep to most superficial the cellular shape, structure, and composition changes, producing a dense, scaly layer of dead keratinocytes at the stratum corneum [1]. For these reasons, the skin is considered to be anisotropic and heterogeneous [20]. These features of skin can make electrical measurements in various directions particularly troublesome. However, there exists simplified equivalent circuit and mathematical models that aid in predicting electrical properties.

Figure 7: Skin Tissue Equivalent Circuit Model

The skin equivalent circuit model consists of intra and extracellular fluid resistances (R1 and R2) and a capacitance associated with the cell membrane (C1) [21].

A common equivalent circuit model of a single cell that can also be used for skin tissue is shown in Figure 7 [21]. For single cells, the intra and extracellular fluid resistances are denoted R_1 and R_2 , respectively [21]. There is also a capacitive element to the circuit (C_1) due to the cellular membrane [21]. The cellular membrane is composed of a phospholipid bilayer, which is impenetrable by many ions and molecules [22]. The separation of ions on either side of the membrane, established in large part by ATP-dependent sodium-potassium pumps, mimics the function of parallel-plate capacitors [22]. The capacitance of the cellular membrane is usually taken as 1 uF/cm^2 of membrane surface area [22].

When dealing with skin tissue as a whole, Figure 7 is used again, but the values of the three components change to accommodate a lumped sum of all the intracellular and extracellular fluid resistances. The capacitive component value changes to reflect an increased capacitance associated primarily with the stratum corneum. As discussed earlier, an important function of the skin is to retain water and prevent bacteria, fungi, and water from entering the body [1]. This is accomplished by keratinocytes between the stratum granulosum and spinosum [1]. In this region, tight junctions interconnect keratinocytes [1]. Secretion of lipids and the formation of an insoluble protein layer on the inner surface of plasma membranes further enhance the function of the epidermal water barrier [1]. Superficial to this barrier, keratinocytes undergo apoptosis [1]. All of these aspects of the epidermal water barrier create high impedance regions, which may detract from reliable impedance measurements of deep tissues and tumors [23].

A study performed by *Yamamoto* and *Yamamoto* in 1976 derives an equation to represent the resistivity of skin at different layers in the stratum corneum [23]. The

resistivity, also known as the specific resistance, is a way to normalize resistance measurements based on the distance between, and the surface area of, electrodes (Equation 6) [18]. Equation 7 represents an exponential decay of the resistivity from the most superficial layer of the stratum corneum to deeper regions [23]. This is determined empirically by consecutive impedance measurements after cellulose tape stripping of a few layers of stratum corneum [23].

Equation 6 – Specific Resistance

$$R_s = R_M * S_A / D_E$$

Where:

R_s = Specific resistance

R_M = Measured resistance

S_A = Electrode surface area

D_E = Distance between electrodes

Equation 7 – Stratum Corneum Resistivity Profile

$$\rho(x) = \rho_o e^{-\alpha x}$$

Where:

ρ_o = Initial resistivity found as $7.7 \times 10^5 \Omega\text{-m}$

α = Attenuation factor found as $2 \times 10^5 \text{ m}^{-1}$

x = Depth into stratum corneum

The resistivity of a tissue is an important parameter in electrical impedance measurements because it serves as a normalized value to compare to other studies using different electrodes and electrode separations. A critical question in electrical impedance studies is determining the frequency of the source signal. One factor in determining frequency is the desired depth of the measurement. High frequencies allow for greater current depth penetration, while lower frequency currents remain superficial [24]. Thus, in investigated deep melanomas, higher frequencies may be desired. In addition, the

depth of penetration is partially determined by the separation of electrodes – usually taken to be half the distance between electrodes [25]. High frequencies also have another inherent advantage in impedance measurements. Since the equivalent circuit model of skin tissue consists of a capacitive element, it may be short-circuited with a high frequency signal. With the capacitive element shorted, impedance measurements are comprised primarily of the intracellular and extracellular fluid resistances; this provides a method of measuring the hydration of the tissue [26]. The effective shorting of the capacitive element is known as a beta dispersion and can occur anywhere from kHz to MHz [25]. The frequency at which the beta dispersion occurs is dependent on the shape and structure of the cells, as well as the intra and extracellular water content [25].

It is important to note that impedance measurements of skin are dependent on numerous parameters. For instance, the location of the measurement on the body, the age and gender of the subject, and the season (time of year) all influence impedance readings [25, 27]. Also, the length of time of the measurement was found to skew the results; skin resistance decreases over long testing periods [28]. To ensure that as many variations of skin impedance measurements are filtered out, an ipsilateral skin site – one on the same side of the body as the suspicious lesion – is used as a reference point [29].

Cancerous Electrical Properties

All of the previously discussed details of skin electrical properties hold true for cancerous electrical properties with the exception that the cell shape, size, and orientation of cancerous cells differs from that of healthy, benign cells [30]. The permeability of cancer cells to ions increase due to an unknown mechanism in cancer physiology [31]. The increased permeability of the cellular membrane allows increased flow of potassium,

magnesium, and calcium out of the cell, and sodium and water into the cell [31]. The increase in water content due to the change in osmotic pressure causes a spherical shape in cancer cells [31]. A sphere represents the greatest possible volume to surface area ratio and as a result, cancer cells contain increased intracellular fluids. Since the intracellular fluid is a more conductive (less resistive) medium, the impedance of cancerous tissue, especially at the beta dispersion, will decrease [32].

Impedance Device Characteristics

Differences in the physiology and anatomy of cancerous and healthy cells are exploited during diagnosis of melanoma. There are a few variations in the impedance diagnostic techniques that are utilized in the literature; these include degrees of invasiveness, electrode material, and electrode pattern. For their impedance-measuring device, some investigators use a micro-invasive setup [29]. This consists of spiked electrodes that penetrate the thin 10 – 40 um stratum corneum, which provides the advantage of bypassing a high impedance region [29]. *Aberg* et al. discovered that micro-invasive electrodes allowed for accurate impedance measurements of deep tissues, in which malignant melanomas usually reside [29]. Although considered micro-invasive, the electrodes still penetrate the skin and may cause pain, irritation or other effects, which are not specified by the authors. The non-invasive technique, in which this report will focus, suffers from the high stratum corneum impedance; this may skew results for deeper tissues [33]. Mechanisms around the effects of the stratum corneum consist of sourcing high frequency signals to short the capacitor, scrubbing, cleaning, abrading, or moisturizing the skin to remove layers of stratum corneum or increase conductance of the dead keratinocyte layers, or normalizing measurements to an ipsilateral skin site [33].

Electrode material for impedance devices usually consists of either gold or silver-silver chloride (Ag/AgCl) [28, 34]. Gold is a good conductor and used frequently in microelectrode devices. However, gold electrodes tend to be polarizable [35]. Polarizable electrodes are those in which the material is difficult to oxidize [35]. For this reason, ions concentrate around the electrode and create a capacitive area [35]. An overpotential is needed to adjust for the voltage drop across the concentrated ion capacitor [35]. This overpotential may change with time and magnitude of voltage applied creating offsets in the measurements [35]. Ag/AgCl is considered a non-polarizable electrode and allows current to pass from the electrode-electrolyte interface with no capacitive influence [35]. Ag/AgCl electrodes undergo a chemical reaction in which silver oxidizes and binds with Cl^- to form a water-stable AgCl molecule [35]. The chlorine ion is abundant in biological tissues and thus creates a stable reaction. Electrical noise in measurements is also less for Ag/AgCl than with other metallic electrodes [35].

Figure 8: Dinh et al. Electrode Pattern

Depicts a linear electrode pattern with the source and sink electrodes surrounding the reference electrode [28].

Finally, the electrode pattern differs between investigators and depends mostly on the desired measurement depth, resolution, and coverage of the device. *Glickman et al.* and *Har-Shai et al.* utilize rectangular matrices of 8x8 and 8x16 pointed gold electrodes with a footprint of 4x4 and 5x10 mm², respectively [33, 34]. *Dinh et al.* use a four-electrode setup with the source and sink electrodes linearly bounding two reference electrodes; this alignment is shown in Figure 8 [28]. The last electrode pattern variant consists of ring-type electrodes. *Aberg et al.* use four concentric electrode rings where the central electrode performs as the sink and second electrode from the center acts as a guard electrode to direct the current [25]. The outer two rings act in unison as the source electrodes and create a “virtual electrode” between the two rings depending on their voltages [25].

Previously Recorded Data

This section provides previously recorded impedance data on both skin and cancerous tissues from the literature. This information is important because it allows for comparison of data generated during this thesis. The type of data provided depends on the investigators. In some instances, specific resistances and capacitances are given, while others supply only normalized conductance values and sensitivity/specificity measures.

Glickman et al. recorded conductivity and capacitance values at 2 kHz for normal and cancerous skin tissue from nude mice injected with human melanoma cells. The conductance and capacitance of normal skin were 0.44 uS and 0.017 nF, respectively. The conductance and capacitance of a malignant tumor were 2.65 uS and 0.05 nF, respectively. They used a normalized conductance (GN) value of 6.32 to reach 95% sensitivity and 67% specificity [34].

Aberg et al. recorded conductance values for 31 frequencies over the range 1 – 100 kHz. They used a principal components analysis (PCA), which used two statistical principal components to compare values. Their sensitivity and specificity were 92% and 80%, respectively [29].

Har-Shai et al. used normalized conductance at 2 kHz to determine differences in measurements. Control skin conductance measured on the head and neck was 2.23 uS, while the control skin elsewhere was 0.96 uS. A GN of 6.2 was used to obtain a 100% sensitivity [33].

Dinh et al. measured skin resistances at equilibrium and recorded a maximum value of 583,179 Ω . As the salt concentration of the electrolyte increased, the measured resistance decreased [28].

Pethig et al. determined specific capacitance and resistance for the stratum corneum at 1 – 10 kHz as 4.6 nF/cm² and 34.9 k Ω /cm², respectively. At 13.56 MHz, skin conductance was 0.25 S/m [32].

Gabriel et al. compiled conductivity and permittivity values of skin from multiple sources. At 10 kHz, the conductivity, dependent on the hydration of the skin, ranged from 1.0E-4 to 0.5E-1 S/m. At the same frequency, the permittivity ranged from 2.0E3 to 1.0E5 [36].

Hemingway et al. averaged specific resistances of human skin and muscle measured at 500 – 3000 kHz. The specific resistances of skin and muscle were 289 and 110 Ω , respectively. At a lower frequency ($f = 1000$ Hz), the resistance of cadaveric skin immediately after death was 59 Ω [37].

Stante, a previous graduate student at the California Polytechnic State University in San Luis Obispo, initiated campus research on impedance-based measurements to diagnose melanoma skin cancer. Two Ag/AgCl electrodes spaced 1 cm apart

were used as the testing device. The impedance of raw and cooked chicken thigh skin, fat, and meat were measured at 10 kHz. The average impedance values for raw chicken skin, fat, and meat were 3.897, 2.945, and 1.801 k-ohms, respectively. The average impedance values for cooked skin, fat, and meat were 8.455, 27.273, and 2.784 k-ohms, respectively. Tissue engineered skin constructs of EpidermTM and Melanoma, from MatTek Corporation, were also measured at 10 kHz. The average impedance for EpidermTM and Melanoma were 3.088 and 12.856 k-ohms, respectively [18].

The above values differ considerably from each other. This is expected because of the differences in electrode material, measurement site, test preparation techniques, and age and gender of the subject. However, these values, aside from Stante's work, have a similar trend that may serve as a starting point for a comparison with the values found in this thesis. In general, the conductance of melanoma tissue was greater than that of healthy skin tissue. This means that the resistance of melanoma tissue was less than the resistance of healthy skin tissue. While the values of resistance and capacitance for melanoma and healthy skin tissue differ between authors, the normalized conductance values remain consistent at approximately 6.2 to 6.4. Normalizing the measurements is beneficial because it removes the inconsistencies associated with the different experiments and allows for a straightforward comparison to other data. If possible, the normalized conductance values will be compared to the data found in this thesis.

Methods

As indicated in the *Introduction*, this thesis will focus on electrical impedance measurements as a non-invasive, objective technique for diagnosing melanoma skin cancer. To do this, a multi-electrode device was constructed and tested for its ability to differentiate between tissue types and tissue health. This project serves merely as a proof of concept that multi-directional impedance detection has the potential to distinguish between normal and melanoma skin and provide reliable results.

Figure 9: Electrode Device Assembly

The electrode device and fixture system used for impedance measurements.

In this section, a description of the device, shown in Figure 9, and the system used in this thesis for impedance measurements will be presented. The use of certain materials and dimensions will be explained, as well as the software, hardware, and program

running the measurement algorithm. The methods used to validate the hardware, program, and device will be discussed. Finally, the methods used in the acquisition of impedance data for cooked and raw chicken tissue and melanoma and healthy tissue engineered skin analogues will be presented.

Briefly, the measurement system consists of the electrode device that houses and secures the electrodes, the device fixture that secures and actuates the electrode device, the breadboard circuit that routes the measurement signals, National Instruments' Educational Laboratory Virtual Instrumentation Suite (ELVIS) and USB-6251 that generates the measurement signals and acquires data, and National Instruments' LabVIEW that controls the signals and flow of data. During measurement, the electrodes are placed on the surface of tissue and electrical current is passed between two electrodes. The returning voltage is recorded and the impedance of the tissue between the two electrodes is calculated automatically within LabVIEW. Further detail is provided in the following sections.

Electrode Device

The device used in this thesis consisted of four main parts shown in Figure 10: the shaft, the electrode holder, the electrodes, and the device fixture. The composition, design, assembly, and purpose of each main part will be described in individual sections that follow.

Shaft

The shaft housed the electrodes and associated wires to prevent damage from external factors and provided a strain relief point for the wires. The shaft also provided a larger area for gripping if the electrode device was used by hand. It was composed of four

1" diameter, stackable lens tubes from ThorLabs. The lengths (part numbers) of the four stackable tubes in order from tip to tail of the device were 0.5" (SM1L05), 2" (SM1L20), 1" (SM1L10), and 0.3" (SM1L03). These lens tubes all contained external and internal custom threads of 1.035"-40 at the tip and tail of each tube, respectively. This allowed for the simple joining of shaft members that made various assembly tasks, such as soldering electrodes, more accessible. Some alterations were made to the 0.5" and 1" lens tubes to accommodate some features of the entire device.

Figure 10: Electrode Device Exploded View

An exploded view of the electrode device and translational stage with components labeled.

The 0.5" lens tube tip, where the external threads resided, was turned down with a lathe to remove the regions with diameters less than 1". Removing this feature allowed for the insertion of the electrode holder into the tube. Inside the tube, approximately 7 mm from the tip, was a retaining ring with external threads of 1.035"-40. The bottom of

the electrode holder, once inserted into the tube, rested on the retaining ring to prevent further movement into the tube. There were also three holes drilled into the sides of the tube, one of which was tapped with #1-64 threads. The two non-tapped, clearance holes of gauge 50 rested 180-degrees from each other at 1.165 mm from the tip of the tube. The tapped hole was located 90-degrees from the clearance holes and 3.495 mm from the tip of the tube. The clearance holes passed #1-64 nylon screws to interact with the top member of the electrode holder (discussed in the next section); this effectively prevented movement of the electrode holder out of the tube. The tapped hole allowed interaction of a #1-64 screw with the middle member of the electrode holder; this effectively moved the middle member relative to the top and bottom members to act as a vice to the electrodes.

In order for the electrodes to interface with the data acquisition system, wires passed out of the shaft. To accomplish this, a 0.25" by 0.25" square was milled out of the 1"-diameter tube wall at the tail end. The reason the hole was cut at the tail of the tube rather than in the middle was to avoid twisting the wires and electrodes during connection of the 0.3" tube to the 1" tube. Furthermore, the action of screwing the 0.3" tube onto the shaft applied pressure to the wires to prevent moving and tension on the electrode-wire joints.

Electrode Holder

The electrode holder, as alluded to in the previous section, consisted of three components that functioned together to clamp the electrodes in place. The diameter and length of all components were 1" and 2.33 mm, respectively. The three sections were parted with a lathe from a 1" stock Delrin rod. Delrin was used because of its electrical insulation properties, machinability, and availability. Nine 0.0335" diameter holes were

match drilled into the face of all three sections to a total depth of 6.75 mm. The remaining 0.25 mm of material in the bottom section was match drilled with a 0.0325" drill bit. These holes held the 9 electrodes used in this device and were patterned into the section faces such that 8 electrodes circumferentially surrounded 1 central electrode in 45-degree increments at a 5 mm radius (see Figure 11 for clarification). The step in drill bit diameters provided a small ridge for the electrodes to rest. Thus, if the electrodes were manufactured with appropriate tolerances, the extension of the electrodes from the device would be roughly the same length. The pattern of electrodes allowed for impedance measurements in 8 different directions and kept the amount of tissue between an outer electrode and the central electrode constant at 5 mm. A small distance between electrodes also increased the resolution of the device and prevented impedance values from exceeding the measurement capabilities of the hardware. Specifically, a 5 mm radius was used so that measurements could be conducted on the 1 cm diameter tissue engineered skin constructs, which are described in more detail in the *Tissue Engineered Skin Analogues* section.

Figure 11: Electrode Pattern

The eletctrode pattern of 8 outer electrodes surrounding a central one at a 5 mm radius. This is match drilled into the face of all electrode holder sections.

The three separate sections of the electrode holder were needed to act like a vice. The top section's cylindrical face had two #1-64 tapped holes that were 180-degrees from each other and aligned with the clearance holes on the 0.5" lens tube. As mentioned before, the screws held the top section in place and prevented the electrode holder assembly from moving within the shaft. The middle section diameter was slightly turned down on a belt sander to allow for movement within the shaft. The turning of a screw in the threaded hole of the 0.5" lens tube caused translation of the middle section in reference to the top and bottom sections; this effectively pinched each electrode and fixed them in place.

Electrodes

Silver-silver chloride (Ag/AgCl) electrodes were used as the measurement electrodes for this device. They consist of a silver wire coated in silver chloride precipitate. As described in the *Introduction*, Ag/AgCl electrodes are nearly perfectly nonpolarizable and allow current to pass freely from electrode to electrolyte. The chemical reactions responsible for the electrode function are:

The oxidation of bulk silver and the release of an electron and silver ion are followed by the synthesis of silver chloride from the silver ion and a chloride ion found in the electrolyte. Since AgCl is stable and only slightly soluble in water, it precipitates out of solutions and deposits onto the silver wire [35].

The half-cell potential of the Ag/AgCl electrode is determined by the following governing equation:

Equation 8: Silver Half-Cell Potential [35]

$$E = E_{Ag}^0 + \frac{RT}{nF} (\ln K_s - \ln a_{Cl^-})$$

where E is the half-cell potential, E_{Ag}^0 is the standard half-cell potential of silver, R is the gas constant, T is temperature, n is the valence of silver, F is Faraday's constant, K_s is the solubility product, and a_{Cl^-} is the activity of the chloride ion. The standard half-cell potential of silver is found in a standard table. The solubility product is the rate of precipitation out of solution at equilibrium and is constant. All other symbols are constants except for the activity of the chloride ion. However, in biological applications, the chloride ion remains stable [35]. Thus, the half-cell potential of Ag/AgCl electrodes is stable and will not change during experimentation. This reduces transient errors in impedance determination [35].

Furthermore, nonpolarizable electrodes do not develop double charge layers associated primarily with polarizable electrodes [35]. The double charge layer is created by the buildup of ions attracted to the surface of a polarizable electrode [35]. As current passes between electrode and electrolyte, the concentration of ions at the surface of the electrode changes and creates an overpotential [35]. The double charge layer is also responsible for motion artifacts that cause errors in measurements [35]. If an electrode moves, the ions in the double charge layer are disturbed and the half-cell potential of the electrode changes momentarily [35]. Since nonpolarizable electrodes do not have a double charge layer, overpotentials, stability, and electrode noise are avoided.

For this thesis, Ag/AgCl pellet electrodes (E255) were purchased from Warner Instruments. The length and diameter of the active tip of the electrode was 10 mm and 0.8 mm, respectively. This size was chosen primarily for consistency with a previous thesis

entitled “The Electrical Properties of Human Tissue For the Diagnosis and Treatment of Melanoma Skin Cancer” by Glenn Stante. Keeping the electrode dimensions similar allowed for quick cross-referencing to previous results. The small diameter also allowed for more accurate measurements because less tissue was underneath the electrode; the tissue of interest should be between electrodes, rather than underneath, so that the current passes through it. Furthermore, these electrodes were determined to be “suitable for probing tissue” by the manufacturer [38]. Each electrode was soldered to a 20 gauge insulated wire for coupling to the measurement hardware. When needed, electrode tips were placed in 100% ethyl alcohol for three minutes and deionized water for five minutes to clean the surface.

Device Fixture

The electrode device was compact enough for handheld use. However, for experimental purposes, the electrode device was fixed to a micrometer-actuated surface to alleviate motion artifacts and similar user errors. The main device fixture component was a height gauge as shown in Figure 12. This height gauge provided a table for sample placement and a Delrin backing for securing the shaft of the device. A wheel, when turned, allowed for coarse adjustment of the device in the vertical direction. Attached to the Delrin backing was a rotational stage that allowed for rotation of the electrode device. The rotation was necessary for specimens with uneven surfaces, because it evenly distributed the pressure; this was important for reliable measurements. Attached to the rotational stage was a 1” translational stage (PT1) from ThorLabs that allowed for fine adjustment in the vertical direction of the electrode device with a micrometer. The electrode device was affixed to the translational stage with an altered steel bar that was

bent in a way to encircle the device shaft. Screws (0.25"-20) secured the steel bar to the translational stage and applied pressure so that the device did not move during experimentation.

Figure 12: Electrode Device Fixture

The electrode device and fixture system with components labeled.

Device Summary

With an understanding of the anatomy of the electrode device, its function will be discussed briefly to familiarize the reader with its intended use before continuing to the control and data acquisition of the system. The electrodes that protrude from the face of the electrode holder were placed perpendicular to the surface of the tissue. The device fixture was rotated as needed so that each electrode reached the surface of the skin at the same moment. When the program was started, a sinusoidal signal traveled from the central electrode to one of the 8 outer electrodes. The impedance was recorded and the

next outer electrode was selected. This continued, holding the central electrode active and switching between the outer electrodes, until all 8 outer electrodes were selected. The details of the program, hardware, and circuit used to perform these measurements are discussed in the next section.

Device-Computer Interface

Measurements from the electrodes were coupled to a computer through National Instruments ELVIS and USB-6251 data acquisition systems, a breadboard circuit, and National Instruments LabVIEW software. Figure 13 shows the complete device-computer interface, which will be described in more detail in the following sections.

Figure 13: Device-Computer Interface

The device-computer interface showing the device, ELVIS system, multiplexer circuit, USB-6251, and LabVIEW software.

National Instruments ELVIS

ELVIS, shown in Figure 14, stands for Educational Laboratory Virtual Instrumentation Suite and is a National Instruments product that contains 12 different instruments in one integrated suite. It was selected for use in this thesis because of its availability, its economical advantages in having 12 integrated instruments, and its simple integration with National Instruments LabVIEW that allowed for experimentation control, measurement, automation, and most importantly, flexibility [39]. The ELVIS instruments used in this thesis were the Digital Bus Writer, Variable Power Supplies, and Impedance Analyzer. The Digital Bus Writer wrote digital data to the digital output terminal on the ELVIS breadboard and was capable of user-created patterns, ramp, or toggle, and could either produce single or continuous writes. The Variable Power Supplies were capable of supplying -12 to 12 volts DC and supplied a maximum current of approximately 280 mA at 5 V. The Impedance Analyzer provided the resistance, reactance, impedance, and phase at frequencies between 5 and 35,000 Hz. Since most of the literature suggested differences in tissue impedances at 2 and 10 kHz, the ELVIS Impedance Analyzer was sufficient for this project [40].

Figure 14: National Instruments ELVIS

An example NI ELVIS system showing the front panel (dials/switches) and prototyping board [40].

The ELVIS system has a front panel and a prototyping board. The front panel contains dials, switches, BNC connectors, and banana jacks to simulate a traditional analog instrument interface. The prototyping board provides perforations that allow for building temporary circuits that can be easily altered if changes are needed. The board also provides input/output pins for all integrated ELVIS instruments. Using the prototyping board, one could couple an external circuit with the integrated instruments.

National Instruments USB-6251

While the ELVIS system is responsible for the actual control and measurement of the experiment, a data acquisition system is needed to send and receive the data to and from the computer. National Instruments USB-6251 is a 16 bit, M series data acquisition system that includes 16 analog inputs, 2 analog outputs, 24 digital I/O, two 32-bit counters, and is compatible with the NI ELVIS device. The USB-6251 connects to the computer through USB and has transfer speeds of 1.25 and 2.8 mega-samples/second for

the analog input and output, respectively [41]. The transfer speeds, number of available channels, resolution of the signals, and compatibility with the ELVIS system justifies the USB-6251 as a proper data acquisition system for this thesis.

Multiplexer

The ELVIS prototyping board was used to construct a circuit to control the selection of the outer electrodes and to couple the circuit to the integrated instruments. Wire connected the *Current Hi* pin of the Impedance Analyzer to the central electrode of the device. To control which outer electrode was selected for measurement, an 8:1 analog multiplexer (NXP HEF4051BP, 652) was used.

Multiplexers (mux) take one of many inputs, dependent on the digital address, and direct it to a common output [42]. The mux used in this project, the HEF4051B purchased from Avnet, allowed for 8 analog inputs, corresponding to the 8 outer electrodes, and directed the signal to the common output, which was connected to the *Current Lo* pin on the ELVIS prototyping board. This completed the electrical loop from the *Current Hi* pin. With 8 possible inputs, three digital pins determined the active channel. A table of the power states for each digital pin and the corresponding active channel is provided in Table 2. S1, S2, and S3 are the three digital pins, E is an on/off pin that can cause high impedance states in all channels regardless of the states of the digital pins, L is low power (~ 0 V), H is high power (~ 5 V), and X does not matter [43].

Table 2: HEF4051BP, 652 Function Table [43]

Input				Channel ON
$\bar{E}$	S3	S2	S1	
L	L	L	L	Y0 to Z
L	L	L	H	Y1 to Z
L	L	H	L	Y2 to Z
L	L	H	H	Y3 to Z
L	H	L	L	Y4 to Z
L	H	L	H	Y5 to Z
L	H	H	L	Y6 to Z
L	H	H	H	Y7 to Z
H	X	X	X	switches off

The specific pinouts for this device are shown in Figure 15. The device has two negative power supplies, one for the analog inputs (V_{EE}) and the other for the digital inputs (V_{SS}). V_{SS} must be grounded, while V_{EE} is either grounded or set to -5 V dependent on whether the analog inputs are positive or both positive and negative, respectively [42]. Considering that the Impedance Analyzer signal is a sinusoidal waveform, a -5 V supply was used for V_{EE} .

Figure 15: HEF4051BP Pinout Diagram

The diagram shows the specific pins for the multiplexer. Y prefixes indicate analog inputs, S prefixes indicate digital addresses, V prefixes indicate power supplies, and Z is the common output [43].

Breadboard Circuit

The ELVIS system contains some hardware resource conflicts depending on which integrated instruments are used at the same time. The conflicts also depend on the capabilities of the data acquisition system. Fortunately, the Impedance Analyzer, Digital Write Bus, and Variable Power Supplies can be used simultaneously. The prototyping board mainly consists of the multiplexer and the connections between it and the integrated instruments. The central electrode of the device connects to the *Current Hi* pin, the outer electrodes connect to the 8 analog inputs of the multiplexer, and the Z pin (common output) of the multiplexer inserts into the *Current Lo* pin. The V_{SS} and V_{EE} pins of the multiplexer are attached to the common ground of the ELVIS system and the negative supply voltage of the Variable Power Supplies, respectively. The positive variable power supply pin on the ELVIS is wired to the V_{DD} pin on the multiplexer. The negative and positive supply voltage dials on the ELVIS front panel are set to approximately -5 and 5 V, respectively. Four pull-up resistors of 10 k-ohms are used to “pull-up” the current from the digital output bus. The first four channels of the digital output bus connect to the S0, S1, S2, and E pins of the multiplexer. The signals and timing of this circuit are controlled by the NI LabVIEW software and the custom program developed for this thesis.

NI LabVIEW Program

LabVIEW is a programming environment that uses graphical icons and wires to create a flowchart of code execution. LabVIEW comes with large libraries of predefined graphical icons that can be easily altered to create virtual instruments (VI) that have the

proper functionality for the project [44]. Furthermore, many external products have downloadable drivers that can be installed and integrated into the code fairly easy.

The custom program developed for this thesis, found in *Appendix B*, controlled the ELVIS system and required the following user inputs: device name, number of points, high frequency, low frequency, and the time delay between electrode measurements. Overall, the program calculated the variables used in the impedance analyzer, activated the impedance analyzer, and controlled the active channel of the multiplexer. Specifically, the number of points, high frequency, low frequency, and For loop iteration entered a function node with the equation:

Equation 9: Program Iteration Frequency

$$F_i = \frac{F_h - F_l}{N - 1}i + F_l$$

where F_i was the specific iteration frequency, F_h was the high frequency, F_l was the low frequency, N was the number of points, and i was the iteration number. A second For loop commenced to control the selection of the outer electrodes. Inside, the digital write function was initiated and the digital address of the multiplexer channel was written dependent on the second For loop iteration value. The iteration frequency and device name entered the second For loop and the ELVIS Impedance Analyzer subVI, where an algorithm produced the impedance, phase, resistance, and reactance. After storing the data in memory, the digital write function was closed and the second For loop iterated to record all 8 directions. When the second For loop finished execution, the stored recordings were written to a Microsoft Excel file that was named for the first For loop

iteration. This process was repeated in the first For loop until the number of data points requested was reached.

System Validation

The hardware, software, custom program, device, and device fixture worked together to measure the impedance of various tissues. However, it was important to verify the functionality of individual pieces to ensure the system as a whole was working properly. To do this, the ELVIS system, the multiplexer, and the electrode device were tested. The details of each test are described in the sections to follow.

ELVIS Validation

The purpose of this test was to confirm that the ELVIS system could correctly identify the impedance of various resistors and capacitors to within the tolerance thresholds provided by the manufacturer of the electrical components. If the ELVIS system was able to accomplish this task, then we could consider all values recorded on biological tissues to be reliable as well.

The electrical components (Vishay BCcomponents) were inserted directly into the *Current Hi* and *Current Lo* pins of the Impedance Analyzer so that the impedance measured would be from the ELVIS system and the electrical component only. The seven values of resistors used for validation were 1, 10, 100, 1 k, 10 k, 100 k, and 1 M-ohm. Three different resistors for each value were used. Three capacitors for each of five capacitor values were utilized for validation. The capacitor values were 10 u, 1 u, 0.1 u, 10 n, and 1 n-farad. The resistors and capacitors had a threshold accuracy of $\pm 5\%$ and $\pm 10\%$ based on the specifications given by the manufacturer, respectively.

The main custom program was altered for the purposes of this validation. The second For loop was removed because outer electrode selection was not necessary for the ELVIS system alone. Two points were recorded – one each at 2 and 10 kHz frequency. The 2 kHz frequency was selected because of its expected position at the β -dispersion point for skin and its use by Glickman and Har-Shai [33, 34]. The 10 kHz frequency was chosen for its use by Gabriel and Stante [18, 36]. The data recorded by the Impedance Analyzer were compared to the value ranges provided by the manufacturer.

Multiplexer Validation

Multiplexers inherently increase the impedance of the load because of the way they are constructed. For certain applications, like this thesis project, sources of impedance other than the tissue or electrical component being measured can cause errors in the data. Thus, the multiplexer must be validated to ensure that reliable impedance readings are still possible. If not, some post-processing techniques can be applied to eliminate the added impedance from the multiplexer. Since impedances in series act as resistors in series, any component's impedance can be added to arrive at the total system impedance. Therefore, if the impedance of the multiplexer without a load is known, then it can be subtracted from the impedance of the multiplexer with a load to obtain the impedance of the load only.

The multiplexer, pull-up resistors, and all connections were placed onto the prototyping board as described in the *Breadboard* section, except the connections to the electrode device were removed. Instead, the *Current Hi* wire was split into 8 wires leading to the 8 multiplexer inputs as shown in Figure 16. Before each connection to the multiplexer input, a resistor or capacitor was placed. The same amount and value of

resistors and capacitors used in the ELVIS validation were employed again in this validation. An additional test was performed with bare wires connecting the multiplexer inputs to record the intrinsic impedance of the multiplexer; this test was performed three times. All tests were performed at 2 and 10 kHz frequencies. The data recorded for each resistor and capacitor at the two frequencies was compared to the values supplied by the manufacturer to determine the reliability of the system. Then, the average intrinsic impedance was subtracted from the recorded data and compared to the values supplied by the manufacturer.

Figure 16: Multiplexer Validation Setup

Shows the setup for the multiplexer validation tests with a series of resistors in the test strip.

Device Validation

After validating the ELVIS system and the multiplexer, the electrode device was tested to ensure that all electrodes were connected to their respective wires, that the solder joints did not add extraordinary impedance, and that the Ag/AgCl electrodes were capable of measuring the correct resistor and capacitor values. The ELVIS prototyping

board was arranged according to the description in the *Breadboard* section. Since the electrode tips were 0.8 mm in diameter and a solid connection to an electrical element was difficult, an electrical testing hook clip (Figure 17) was utilized. One hook clip was placed on the central electrode and another was placed on an outer electrode. The opposite ends of both hook clip wires attached to either side of an electrical component as shown in Figure 18.

Figure 17: Electrical Testing Hook Clip [45]

An example electrical testing hook clip used for grabbing small components.

The same quantity and value of resistors and capacitors used in the previous validations were tested between the same two electrodes at 2 and 10 kHz frequencies. After testing of the electrical components was complete, the free ends of the hook clips were connected together to measure the system impedance. This was conducted 8 times for each of the 8 outer electrodes. The intrinsic system impedance of the two electrodes tested with electrical components was subtracted from the recorded data and compared to the electrical component values provided by the manufacturer for each frequency. The intrinsic system impedances of all outer electrodes were also checked for homogeneity. If

the system impedances were similar, then we could deduce that the resistor and capacitor values reliably identified by the one tested outer electrode could also be reliably identified by all other outer electrodes.

Figure 18: Electrode Device Validation Setup

Electrical testing hook clips connect two electrodes via an electrical component.

Experimental Validation

With the entire system validated, any measurements conducted on biological tissues that fell within the capabilities of the system could be considered reliable. The experimental hypothesis that the electrode device could differentiate between melanoma and healthy skin tissue based on impedance measurements must now be validated. Raw

and cooked chicken thigh skin and meat were used for preliminary verification.

Following that, melanoma and epidermis tissue engineered skin analogues were tested.

Both experiments are described in more detail in the following sections.

Raw Chicken Skin and Meat

Chicken tissue samples from Foster Farms were purchased from local grocery stores and used for experimentation because of their availability, low cost, and continuity with previous work. While chicken meat and skin did not directly correlate to human melanoma and epidermis skin tissue, the principles behind the detection of melanoma would still exist in the chicken. That is, the anatomy, physiology, and moisture content of chicken skin and meat at a cellular level should allow for differentiation by the electrode device. This validation served as a low cost, preliminary test to determine if the electrode device could differentiate from visually distinct tissues. If it could, then more expensive validation efforts using anatomically and physiologically correct tissues may be employed.

Twenty measurements each were performed on two different chicken thighs. Half of the measurements (ten locations) were performed on the skin, while the other half were tested on the meat. Each measurement consisted of impedance values in all 8 directions at 2 and 10 kHz frequencies. Distinct locations were used for each measurement, while attempting to conserve the orientation of chicken thigh for as many measurements as possible. The orientation could possibly produce different impedance values based on the anisotropic nature of biological tissues. Before beginning experimentation, the chicken thigh was allowed to come to thermal equilibrium with the room. All attempts were made to keep the pressure of each electrode similar to one

another and consistent throughout multiple measurements by rotating the electrode device fixture as needed. Impedance values of chicken thigh meat and skin at each frequency were compared to find statistical differences between the two groups. An example image of the chicken tissue test is shown in Figure 19.

Figure 19: Chicken Tissue Validation Setup

The electrode device is rotated so that all electrodes are flush with the surface of the chicken tissue.

Raw and Cooked Chicken

The previous validation shows that the electrode device can differentiate two visually distinct tissues. However, the differences between normal epidermis and melanoma are much more subtle. As discussed in *Cancerous Electrical Properties*, the changes in membrane permeability cause cell swelling. Thus, the moisture content of

melanoma tissue increases. Using cooked chicken in comparison to raw chicken is an attempt to create a difference in moisture content within the same tissue type. Finding differences in impedance between cooked and raw skin or cooked and raw meat gives a better indication of successful trials with more advanced, better-suited tissue samples.

The same two chicken thighs used in the previous validation were cooked in a convection oven for 35 minutes at 350 degrees Fahrenheit. After reaching thermal equilibrium with the room, the same methods used in the previous validation were employed. Briefly, twenty measurements each were performed on the two chicken thighs – ten on skin, ten on meat. Both 2 and 10 kHz frequencies were used for all test locations. During cooking, the skin became stiffer and lifted away from the meat of the chicken. For experimental purposes, the electrode device was actuated until the skin was in contact with meat again. The impedances of cooked skin and meat were compared to that of raw skin and meat at both frequencies.

Tissue Engineered Skin Analogues

Tissue engineered skin analogues purchased from MatTek Corporation were used for the final experimental validation. EpiDerm™, a human 3-D skin-like tissue structure, and Melanoma, a full thickness human melanoma skin model, were obtained because of their similarities to in-vivo epidermis and melanoma tissues. EpiDerm™ contained normal human epidermal keratinocytes derived from neonatal-foreskin tissue. According to MatTek, EpiDerm™ closely resembled the human skin and consisted of the basal, spinous, granular, and cornified layers. Studies also showed that EpiDerm™ approaches the barrier properties of normal skin [46]; EpiDerm's™ structure is shown at 400x magnification in Figure 20. Melanoma, shown in Figure 21, contained human melanoma

cells, normal human epidermal keratinocytes, and normal human dermal fibroblasts. According to MatTek, the Melanoma analogues closely resembled the progression of melanoma in vivo. Both Epiderm™ and Melanoma analogues were highly reproducible, had highly differentiated cells, were metabolically and mitotically active tissues, and were cultured in serum-free medium on specially prepared cell culture inserts [46].

Figure 20: Epiderm™ Histological Cross-Section

A histological section of Epiderm™ taken at 400x magnification [46].

Epiderm™ was ideal for impedance measurements because of its structural resemblance and barrier function. In vivo, the lipid barrier of the epidermis creates many problems for impedance measurements due to the high capacitance. Thus, it is beneficial that the barrier function was reproduced in the skin analogue to better mimic actual skin. Melanoma was ideal as a melanoma tissue analogue because it was metabolically active and contained different stages of tumor growth. It also was a full thickness sample that consisted of the epidermis and dermis.

Three samples each of EpidermTM and Melanoma were tested. Since the samples were 1 cm in diameter, only one measurement was performed per sample. All samples used had been frozen for approximately 2 years and needed rehydration before testing. Twenty uL of deionized water was placed on a petri dish. The tissue sample being measured was removed from a 10 mL conical tube and deposited atop the water droplet. The electrode device was actuated until all electrodes were contacting the sample and the program was started. All 8 directions were measured at 2 and 10 kHz frequencies. Impedance measurements from EpidermTM were compared to that of Melanoma for a statistical difference. Finding a difference would serve as reasonable evidence for accepting the experimental hypothesis that melanoma could be diagnosed by analyzing the impedance values in multiple directions.

Figure 21: Melanoma Tissue Analogue Histological Cross-Sections

The Melanoma histological cross sections depict melanoma cells at the epidermal/dermal boundary (long arrows) and metastatic melanoma cells (short arrows) [46].

Results

Following the completion of the experiments, all data was organized into tables using Microsoft Excel and transferred to Minitab for statistical analysis. The statistical analysis included Student T-tests, one-way ANOVA analyses, and/or multiple regression models. The data and statistical results will be presented in the following sections. For a discussion of the results and a conclusion about the validity of the device and experimental hypothesis, see the *Discussion* section.

ELVIS Validation Results

The data obtained for the ELVIS validation resistors is presented in Table 3 and Table 4 for the 2 kHz and 10 kHz tests, respectively. The first column labeled “Resistor Value” is the face value of the resistors that is provided by the manufacturer. The “Measured Resistance” column is the resistance component of the measured impedance. The absolute value of the percent error for each measurement is calculated in column three. Since the manufacturer indicates that the tolerance of each resistor is 5%, the measured resistance value may have a calculated percent error of 5% and still be reliable. Thus, column four provides information on whether the measured resistance is within the tolerance allowed by the manufacturer. The value of column four is “Y” for yes if the absolute value of the experimental error is less than 5%. Conversely, a value of “N” for no is shown if the experimental error exceeds 5%.

Table 3: ELVIS Validation Resistor Values at 2 kHz

Resistor Value (ohms)	Measured Resistance (ohms)	Experimental Error (%)	Within 5% Tolerance (Y/N)
1	3.573	257.3	N
1	3.578	257.8	N
1	3.55	255	N
10	12.647	26.47	N
10	12.442	24.42	N
10	12.509	25.09	N
100	101.902	1.902	Y
100	100.747	0.747	Y
100	101.269	1.269	Y
1,000	976.164	2.3836	Y
1,000	991.353	0.8647	Y
1,000	994.461	0.5539	Y
10,000	9939.484	0.60516	Y
10,000	9754.059	2.45941	Y
10,000	9872.324	1.27676	Y
100,000	99273.07	0.72693	Y
100,000	99422.341	0.577659	Y
100,000	100528.066	0.528066	Y
1,000,000	969616.092	3.0383908	Y
1,000,000	983668.296	1.6331704	Y
1,000,000	971611.515	2.8388485	Y

Table 4: ELVIS Validation Resistor Values at 10 kHz

Resistor Value (ohms)	Measured Resistance (ohms)	Experimental Error (%)	Within 5% Tolerance (Y/N)
1	3.48	247.80	N
1	3.48	248.00	N
1	3.48	248.00	N
10	12.57	25.65	N
10	12.35	23.48	N
10	12.45	24.49	N
100	101.84	1.83	Y
100	100.72	0.72	Y
100	101.18	1.18	Y
1,000	976.01	2.40	Y
1,000	996.30	0.37	Y
1,000	995.70	0.43	Y
10,000	9879.43	1.21	Y
10,000	9951.68	0.48	Y
10,000	9766.04	2.34	Y
100,000	98862.71	1.14	Y
100,000	99336.73	0.66	Y
100,000	100879.94	0.88	Y
1,000,000	399099.19	60.09	N
1,000,000	397399.65	60.26	N
1,000,000	397255.33	60.27	N

The capacitor data obtained from the ELVIS validation at 2 kHz and 10 kHz is provided in Table 5 and Table 6, respectively. The face value of the capacitor provided by the manufacturer is located in the column labeled “Capacitor Value” and is given in Farads. The reactance component of the measured impedance in ohms is shown in the second column. The measured reactance is used with Equation 2 to calculate the capacitance in Farads and is presented in column three. Column four shows the calculated percent error from the capacitor face value. The “Within Tolerance” column offers a quick reference to whether the measured capacitor value falls within the 10% tolerance specification provided by the manufacturer.

Table 5: ELVIS Validation Capacitor Values at 2 kHz

Capacitor Value (F)	Measured Reactance (ohms)	Calculated Capacitance (F)	Experimental Error (%)	Within Tolerance (Y/N)
1.00E-09	-78713.11	1.064E-09	6.42	Y
1.00E-09	-75655.15	1.107E-09	10.72	N
1.00E-09	-77777.66	1.077E-09	7.70	Y
1.00E-08	-8231.78	1.018E-08	1.76	Y
1.00E-08	-7783.91	1.076E-08	7.61	Y
1.00E-08	-7926.77	1.057E-08	5.67	Y
1.00E-07	-805.75	1.040E-07	3.96	Y
1.00E-07	-812.38	1.031E-07	3.11	Y
1.00E-07	-791.40	1.058E-07	5.85	Y
1.00E-06	-81.40	1.029E-06	2.90	Y
1.00E-06	-78.33	1.069E-06	6.94	Y
1.00E-06	-81.25	1.031E-06	3.10	Y
1.00E-05	-7.74	1.083E-05	8.29	Y
1.00E-05	-7.55	1.109E-05	10.92	N
1.00E-05	-7.47	1.121E-05	12.12	N

Table 6: ELVIS Validation Capacitor Values at 10 kHz

Capacitor Value (F)	Measured Reactance (ohms)	Calculated Capacitance (F)	Experimental Error (%)	Within Tolerance (Y/N)
1.00E-09	-15039.82	1.058E-09	5.82	Y
1.00E-09	-14530.14	1.095E-09	9.53	Y
1.00E-09	-14910.07	1.067E-09	6.74	Y
1.00E-08	-1591.25	1.000E-08	0.02	Y
1.00E-08	-1528.90	1.041E-08	4.10	Y
1.00E-08	-1499.42	1.061E-08	6.14	Y
1.00E-07	-151.01	1.054E-07	5.40	Y
1.00E-07	-151.84	1.048E-07	4.82	Y
1.00E-07	-148.49	1.072E-07	7.18	Y
1.00E-06	-13.76	1.157E-06	15.66	N
1.00E-06	-13.18	1.208E-06	20.75	N
1.00E-06	-13.72	1.160E-06	16.02	N
1.00E-05	0.17	-9.646E-05	1064.58	N
1.00E-05	0.20	-8.162E-05	916.18	N
1.00E-05	0.22	-7.334E-05	833.43	N

Multiplexer Validation Results

The resistor data obtained from the switch validation experiment at 2 kHz is provided in Table 7. This data is comparable to that obtained from the multiplexer validation experiments with resistors at 10 kHz and capacitors at 2 and 10 kHz in that the majority of measured values fall outside of the allowable tolerance range of 5%.

Therefore, the resistance and reactance values from the three intrinsic multiplexer impedance tests were averaged for each electrode and frequency. Table 8 presents the averaged data for each electrode at 2 and 10 kHz.

Table 7: Multiplexer Validation Resistor Values at 2 kHz

Resistor Value (ohms)	Measured Resistance (ohms)	Experimental Error (%)	Within 5% Tolerance (Y/N)
1	64.50	6350.20	N
1	64.32	6332.10	N
1	64.56	6355.60	N
10	73.59	635.85	N
10	73.37	633.69	N
10	73.57	635.72	N
100	162.46	62.46	N
100	162.83	62.83	N
100	163.24	63.24	N
1,000	1045.77	4.58	Y
1,000	1036.89	3.69	Y
1,000	1037.26	3.73	Y
10,000	10010.84	0.11	Y
10,000	9921.70	0.78	Y
10,000	10142.49	1.42	Y
100,000	98083.38	1.92	Y
100,000	99057.11	0.94	Y
100,000	99169.93	0.83	Y
1,000,000	771537.53	22.85	N
1,000,000	783858.22	21.61	N
1,000,000	779009.45	22.10	N

Table 8: Averaged Intrinsic Multiplexer Impedance Values

Frequency (Hz)	Average Impedance (ohms)	Average Phase	Average Resistance (ohms)	Average Reactance (ohms)	Electrodes
2000	63.597	0.379	63.596	0.421	0
10000	63.877	2.331	63.824	2.598	
2000	63.695	0.409	63.693	0.455	1
10000	63.716	2.338	63.663	2.599	
2000	63.716	0.406	63.714	0.451	2
10000	63.694	2.341	63.640	2.602	
2000	63.653	0.408	63.652	0.453	3
10000	63.601	2.341	63.548	2.598	
2000	63.470	0.411	63.468	0.456	4
10000	63.408	2.344	63.355	2.594	
2000	63.453	0.410	63.451	0.455	5
10000	63.414	2.343	63.361	2.593	
2000	63.337	0.409	63.335	0.452	6
10000	63.304	2.344	63.251	2.590	
2000	63.440	0.409	63.438	0.454	7
10000	63.373	2.347	63.320	2.595	

The average intrinsic resistances from Table 8 were subtracted from the multiplexer validation resistor data based on the frequency and electrode number in which the data was recorded. The new resistor values for the multiplexer validation at 2 and 10 kHz are presented in Table 9 and 10, respectively. Similar to the ELVIS validation results, the tables show the face value of the resistor provided by the manufacturer, the resistance component of the measured impedance, the calculated percent error based on the face value, and whether the percent error falls within the allowable 5% tolerance range.

Table 9: Adjusted Multiplexer Validation Resistor Values at 2 kHz

Resistor Value (ohms)	Measured Resistance (ohms)	Experimental Error (%)	Within 5% Tolerance (Y/N)
1	0.906	9.367	N
1	0.725	27.467	N
1	0.960	3.967	Y
10	9.892	1.083	Y
10	9.676	3.243	Y
10	9.879	1.213	Y
100	98.750	1.250	Y
100	99.111	0.889	Y
100	99.522	0.478	Y
1,000	982.117	1.788	Y
1,000	973.241	2.676	Y
1,000	973.606	2.639	Y
10,000	9947.376	0.526	Y
10,000	9858.233	1.418	Y
10,000	10079.024	0.790	Y
100,000	98019.932	1.980	Y
100,000	98993.662	1.006	Y
100,000	99106.480	0.894	Y
1,000,000	771474.195	22.853	N
1,000,000	783794.783	21.621	N
1,000,000	778946.010	22.105	N

Table 10: Adjusted Multiplexer Validation Resistor Values at 10 kHz

Resistor Value (ohms)	Measured Resistance (ohms)	Experimental Error (%)	Within 5% Tolerance (Y/N)
1	0.902	9.767	N
1	0.989	1.067	Y
1	0.993	0.667	Y
10	9.895	1.053	Y
10	9.793	2.073	Y
10	9.875	1.253	Y
100	98.696	1.304	Y
100	99.219	0.781	Y
100	99.674	0.326	Y
1,000	982.190	1.781	Y
1,000	973.126	2.687	Y
1,000	973.421	2.658	Y
10,000	9945.803	0.542	Y
10,000	9852.997	1.470	Y
10,000	10068.064	0.681	Y
100,000	95902.467	4.098	Y
100,000	95597.675	4.402	Y
100,000	95683.207	4.317	Y
1,000,000	244610.593	75.539	N
1,000,000	263550.992	73.645	N
1,000,000	264127.630	73.587	N

The average intrinsic reactance values from Table 8 were subtracted from the multiplexer validation capacitor reactance data based on the frequency and electrode in which the data was recorded. The adjusted capacitor data recorded at 2 and 10 kHz is shown in Table 11 and Table 12, respectively. The tables provide the face value of the capacitor, the adjusted reactance component of the measured impedance, the calculated capacitance and percent error, and whether the percent error falls within the allowable 10% tolerance range as specified by the manufacturer.

Table 11: Adjusted Multiplexer Validation Capacitor Values at 2 kHz

Capacitor Value (F)	Measured Reactance (ohms)	Calculated Capacitance (F)	Experimental Error (%)	Within Tolerance (Y/N)
1.00E-09	-71385.03	1.115E-09	11.48	N
1.00E-09	-73654.41	1.080E-09	8.04	Y
1.00E-09	-71362.71	1.115E-09	11.51	N
1.00E-08	-7497.88	1.061E-08	6.13	Y
1.00E-08	-7640.46	1.042E-08	4.15	Y
1.00E-08	-7662.36	1.039E-08	3.86	Y
1.00E-07	-761.33	1.045E-07	4.52	Y
1.00E-07	-766.57	1.038E-07	3.81	Y
1.00E-07	-688.00	1.157E-07	15.66	N
1.00E-06	-75.18	1.059E-06	5.85	Y
1.00E-06	-76.33	1.043E-06	4.26	Y
1.00E-06	-75.30	1.057E-06	5.68	Y
1.00E-05	-7.43	1.071E-05	7.10	Y
1.00E-05	-7.41	1.074E-05	7.39	Y
1.00E-05	-7.27	1.094E-05	9.39	Y

Table 12: Adjusted Multiplexer Validation Capacitor Values at 10 kHz

Capacitor Value (F)	Measured Reactance (ohms)	Calculated Capacitance (F)	Experimental Error (%)	Within Tolerance (Y/N)
1.00E-09	-14404.20	1.105E-09	10.49	N
1.00E-09	-14867.63	1.070E-09	7.05	Y
1.00E-09	-14417.19	1.104E-09	10.39	N
1.00E-08	-1518.25	1.048E-08	4.83	Y
1.00E-08	-1545.79	1.030E-08	2.96	Y
1.00E-08	-1553.65	1.024E-08	2.44	Y
1.00E-07	-151.93	1.048E-07	4.75	Y
1.00E-07	-153.76	1.035E-07	3.51	Y
1.00E-07	-137.28	1.159E-07	15.94	N
1.00E-06	-15.01	1.061E-06	6.07	Y
1.00E-06	-15.21	1.046E-06	4.64	Y
1.00E-06	-14.98	1.062E-06	6.23	Y
1.00E-05	-1.49	1.070E-05	6.96	Y
1.00E-05	-1.48	1.072E-05	7.22	Y
1.00E-05	-1.46	1.090E-05	8.96	Y

Device Validation Results

Resistor and capacitor measurements from the device validation experiments suffered from the same high intrinsic impedance as the multiplexer validation experiments. This produced many measurements that were not within the allowable 5% tolerance range. The data, provided in the *Appendix* for completeness, is removed from this section because of its resemblance to Table 7. In post processing, the average intrinsic device impedance values were subtracted from the measured data to yield the actual measured impedance of the electrical component. Table 13 provides the average impedance, phase, resistance, and reactance of the device dependent on the electrode and frequency at which the data was recorded.

Table 13: Averaged Intrinsic Device Impedance Data

Frequency (Hz)	Average Impedance (ohms)	Average Phase	Average Resistance (ohms)	Average Reactance (ohms)	Electrodes
2000	63.913	0.397	63.912	0.443	0
10000	64.207	2.458	64.148	2.754	
2000	64.037	0.434	64.035	0.485	1
10000	64.066	2.428	64.008	2.715	
2000	64.052	0.431	64.050	0.481	2
10000	64.080	2.424	64.023	2.711	
2000	63.843	0.438	63.841	0.488	3
10000	63.936	2.431	63.878	2.712	
2000	63.695	0.435	63.693	0.484	4
10000	63.719	2.438	63.662	2.711	
2000	63.763	0.429	63.761	0.477	5
10000	63.812	2.429	63.754	2.705	
2000	63.624	0.441	63.622	0.489	6
10000	63.707	2.436	63.649	2.707	
2000	62.947	0.398	62.946	0.437	7
10000	63.288	2.443	63.230	2.697	

The average resistance values were subtracted from the measured resistance values based on the electrode and frequency at which the data was recorded. The adjusted resistance values for the device validation at 2 and 10 kHz are shown in Table 14 and Table 15, respectively. Column 1 provides the face value of the resistor in ohms. Column 2 is the resistance component of the measured impedance in ohms. The experimental error is calculated in column 3 based on the resistor face value. The final column shows whether the measured resistance falls within the allowable 5% tolerance as specified by the manufacturer.

Table 14: Adjusted Device Validation Resistor Values at 2 kHz

Resistor Value (ohms)	Measured Resistance (ohms)	Experimental Error (%)	Within 5% Tolerance (Y/N)
1	1.092	9.200	N
1	1.035	3.500	Y
1	1.106	10.600	N
10	10.003	0.030	Y
10	9.961	0.390	Y
10	10.030	0.300	Y
100	99.056	0.944	Y
100	99.949	0.051	Y
100	99.469	0.531	Y
1,000	974.059	2.594	Y
1,000	982.924	1.708	Y
1,000	973.952	2.605	Y
10,000	9954.849	0.452	Y
10,000	10087.475	0.875	Y
10,000	9870.341	1.297	Y
100,000	99112.306	0.888	Y
100,000	99225.581	0.774	Y
100,000	98264.690	1.735	Y
1,000,000	801043.472	19.896	N
1,000,000	791142.239	20.886	N
1,000,000	792843.733	20.716	N

Table 15: Adjusted Device Validation Resistor Values at 10 kHz

Resistor Value (ohms)	Measured Resistance (ohms)	Experimental Error (%)	Within 5% Tolerance (Y/N)
1	1.081	8.067	N
1	1.096	9.567	N
1	1.106	10.567	N
10	10.021	0.207	Y
10	9.927	0.733	Y
10	10.010	0.097	Y
100	99.019	0.981	Y
100	99.880	0.120	Y
100	99.439	0.561	Y
1,000	974.451	2.555	Y
1,000	983.397	1.660	Y
1,000	974.145	2.586	Y
10,000	9962.718	0.373	Y
10,000	10096.420	0.964	Y
10,000	9877.401	1.226	Y
100,000	96582.834	3.417	Y
100,000	96701.997	3.298	Y
100,000	95849.491	4.151	Y
1,000,000	205745.569	79.425	N
1,000,000	198722.483	80.128	N
1,000,000	197578.106	80.242	N

The average intrinsic reactance values were subtracted from the measured capacitance data based on the electrode and frequency in which they were recorded. The adjusted capacitance values for the device validation at 2 and 10 kHz are shown in Table 16 and Table 17, respectively. Column 1 provides the face value of the capacitor in Farads. Column 2 is the reactance component of the measured impedance in ohms. The capacitance in Farads is calculated in column 3 according to Equation 2. Column 4 is the calculated percent error and column 5 shows whether the measured capacitance falls within the allowable 10% tolerance as specified by the manufacturer.

Table 16: Adjusted Device Validation Capacitor Values at 2 kHz

Capacitor Value (F)	Measured Reactance (ohms)	Calculated Capacitance (F)	Experimental Error (%)	Within Tolerance (Y/N)
1.00E-09	-73109.80567	1.08847E-09	8.85	Y
1.00E-09	-71493.74667	1.11307E-09	11.31	N
1.00E-09	-70655.26267	1.12628E-09	12.63	N
1.00E-08	-7492.967667	1.06203E-08	6.20	Y
1.00E-08	-7652.075667	1.03995E-08	3.99	Y
1.00E-08	-7645.348667	1.04086E-08	4.09	Y
1.00E-07	-761.7386667	1.04468E-07	4.47	Y
1.00E-07	-688.1126667	1.15646E-07	15.65	N
1.00E-07	-767.0526667	1.03744E-07	3.74	Y
1.00E-06	-76.44666667	1.04095E-06	4.10	Y
1.00E-06	-75.33566667	1.05631E-06	5.63	Y
1.00E-06	-75.30366667	1.05675E-06	5.68	Y
1.00E-05	-7.384666667	1.0776E-05	7.76	Y
1.00E-05	-7.409666667	1.07397E-05	7.40	Y
1.00E-05	-7.239666667	1.09919E-05	9.92	Y

Table 17: Adjusted Device Validation Capacitor Values at 10 kHz

Capacitor Value (F)	Measured Reactance (ohms)	Calculated Capacitance (F)	Experimental Error (%)	Within Tolerance (Y/N)
1.00E-09	-14847.90833	1.0719E-09	7.19	Y
1.00E-09	-14448.63633	1.10152E-09	10.15	N
1.00E-09	-14356.25333	1.10861E-09	10.86	N
1.00E-08	-1521.620333	1.04596E-08	4.60	Y
1.00E-08	-1556.505333	1.02251E-08	2.25	Y
1.00E-08	-1552.953333	1.02485E-08	2.49	Y
1.00E-07	-152.2203333	1.04556E-07	4.56	Y
1.00E-07	-137.6023333	1.15663E-07	15.66	N
1.00E-07	-154.4563333	1.03042E-07	3.04	Y
1.00E-06	-15.26433333	1.04266E-06	4.27	Y
1.00E-06	-15.02433333	1.05931E-06	5.93	Y
1.00E-06	-15.04733333	1.0577E-06	5.77	Y
1.00E-05	-1.494333333	1.06506E-05	6.51	Y
1.00E-05	-1.489333333	1.06863E-05	6.86	Y
1.00E-05	-1.464333333	1.08688E-05	8.69	Y

Raw Chicken Results

The program used for the chicken experiments output data labeled with the frequency, impedance, phase, resistance, reactance, and electrode number. The data obtained from the experiments on raw chicken meat and skin was transferred to Microsoft Excel where the location number, sample number, and tissue type/condition were added to the spreadsheet. This data was loaded in Minitab and all columns were separated according to frequency. Student's T-tests were performed comparing the impedance of raw chicken meat and raw chicken skin at 2 and 10 kHz. The test hypotheses were established as follows:

$$H_o : \mu_1 = \mu_2$$

$$H_A : \mu_1 \neq \mu_2$$

where μ_1 was the population mean impedance of raw meat and μ_2 was the population mean impedance of raw skin. The p-values of 0.001 and 0.001 for the 2 and 10 kHz t-tests, respectively, were less than the α -value of 0.05 and the null hypothesis (H_o) was rejected. This meant that the impedance values for raw chicken meat and raw chicken skin were statistically different. The Minitab t-test outputs for the 2 and 10 kHz cases are located in *Appendix D*.

Since there were over 600 data points collected for raw chicken meat and skin, the data was depicted with a scatterplot. The scatterplots shown in Figure 22 and 23 graphed the raw chicken meat and skin data based on their resistance (x-axis) and reactance (y-axis) values. Both figures showed two separate linear trends for each group, although the separation between the groups was more prominent in the 10 kHz plot. Also notice that the raw meat had a greater spread of data, which was shown by the standard deviation (StDev) in the t-test outputs in *Appendix D*.

Figure 22: Scatterplot of Raw Chicken Skin and Meat at 2 kHz

Figure 23: Scatterplot of Raw Chicken Skin and Meat at 10 kHz

Cooked Chicken Results

Impedance data obtained during experimentation on cooked chicken skin and meat were compared using a Student's t-test. The statistical hypotheses were as follows:

$$H_o : \mu_1 = \mu_2$$

$$H_A : \mu_1 \neq \mu_2$$

where μ_1 was the population mean impedance of cooked meat and μ_2 was the population mean impedance of cooked skin. The p-values of 0.000 and 0.000 for the 2 and 10 kHz tests, respectively, were less than the α -value of 0.05 and the null hypothesis was rejected. We concluded that the population mean impedance of cooked meat was statistically different than that of cooked skin. The Minitab t-test outputs are provided in *Appendix D*.

Notice that the mean of the cooked skin at 2 kHz was 1.8 Mega-ohms. As seen in the ELVIS, multiplexer, and device validation results, the 1 Mega-ohm resistor could not be correctly identified. The implications of this will be considered in the *Discussion* section. Figures 24 and 25 provide a visual representation of the cooked chicken meat and skin data by graphing their resistances and reactances. Since the cooked skin data was so large and scattered, it made the cooked meat data appear to be a single point. In actuality, all points more negative than a reactance of -100,000 ohms and larger than a resistance of 1 Mega-ohm could probably be considered an outlier because of the measurement limitations of the ELVIS system. However, even with those points removed, there was still a large enough separation between the cooked meat and cooked skin data to produce statistical significance.

Figure 24: Scatterplot of Cooked Chicken Meat and Skin at 2 kHz

Figure 25: Scatterplot of Cooked Chicken Meat and Skin at 10 kHz

Cooked Vs. Raw Chicken Results

Cooked and raw chicken tissue data were compared to each other to see if the device could differentiate between the same tissues under different conditions (i.e. cooked meat vs. raw meat and cooked skin vs. raw skin). With multiple groups, two statistical approaches could be used, either one-way ANOVA or multiple regression models. One-way ANOVA analyses do not adjust the statistics based on the contributions from other parameters. For instance, the impedance data could be separated by frequency, but electrode number, location number, and other similar parameters could not be accounted for in the analysis. For this reason, a multiple regression model was employed.

For the multiple regression model, the frequency, electrode number, and tissue type/condition were used to predict the impedance of the tissue. The tissue type/condition was assigned as an adjusted categorical predictor, meaning that the β -values produced for the categorical predictor represented the estimated average change when moving from one group – the reference group – to another group. A β -value and a p-value were produced in the Minitab multiple regression output. The p-value was the statistical value that determines which of the following hypotheses is true:

$$H_o : \beta_x = 0$$

$$H_A : \beta_x \neq 0$$

where the subscript x denoted a positive integer corresponding to the parameter number in the model. The Minitab multiple regression output for all chicken tissue types is shown in *Appendix D*.

With the reference group automatically assigned to the cooked meat, the p-values tested for a statistical difference of the cooked skin, raw meat, and raw skin groups against the cooked meat group. The corresponding p-values for the cooked skin, raw

meat, and raw skin groups were 0.000, 0.360, and 0.533, respectively. The only p-value less than the α -value of 0.05 was the cooked skin group. This means that the population mean impedance of cooked skin was significantly different from the population mean impedance of cooked meat adjusting for all other variables. However, looking at the “Analysis of Variance” section in the Minitab output above, the p-value for the Lack-of-Fit test was 0.000. The Lack-of-Fit test used a null hypothesis (H_0) that a linear model was the best choice and an alternative hypothesis (H_A) that a non-linear model was the best choice. Since the Lack-of-Fit p-value was less than the α -value of 0.05, the null hypothesis was rejected and a non-linear model was determined to be better.

Figure 26: Boxplot of Chicken Tissue Impedance

The lack of significance of the data and the conclusion that a non-linear model was better could result from the high leverage that the cooked skin data had on the rest of the data. Leverage refers to the characteristic of the cooked skin impedance to obscure

the statistical significance of the other data because of its large values. Figure 26 shows a boxplot of the impedance for each tissue type/condition and frequency. A boxplot shows the minimum value, the end of the bottom line; the lower quartile, the bottom horizontal line; the median, the middle horizontal line; the upper quartile, the upper horizontal line; and the maximum value, the end of the top line, for each group. This figure depicts the leverage of the cooked skin data and the issue with the current multiple regression model. For this reason, the cooked skin data was removed from the analysis and the multiple regression model was conducted again.

The new multiple regression model used frequency, electrode number, and the cooked meat, raw meat, and raw skin to predict the impedance. The Minitab output of this model is located in *Appendix D*. With a cooked meat reference group, the p-values for the raw meat and raw skin groups were 0.000 and 0.000. Both of the p-values were less than the α -value of 0.05. Thus, the null hypothesis was rejected and it was concluded that the population mean impedance of the raw meat and raw skin were significantly different than the population mean impedance of the cooked meat group adjusting for all other variables. The frequency and electrode number p-values were 0.027 and 0.582, respectively. From this data, we concluded that the average impedance from data acquired at 2 kHz was significantly different from that acquired at 10 kHz and that the electrode in which the measurements were recorded had no significant effect on the impedance measured. Furthermore, the Lack-of-Fit p-value was 1.000 indicating that a linear model was a good choice for the data. A boxplot of the impedance values for cooked meat, raw meat, and raw skin and both frequencies is shown in Figure 27.

Differences in the median value and scatter of data for each group was more easily identified when the large cooked chicken skin values were eliminated.

Figure 27: Boxplot of Chicken Tissue Impedances - Excluding Cooked Skin

The mean impedance values for cooked meat, cooked skin, raw meat, and raw skin at 2 kHz were 2788.6, 6571.10, 7130, and 5583, ohms respectively. The mean impedance values at 10 kHz were 2596.5, 3300.41, 6347, and 5278 ohms, respectively. From the mean impedance results, chicken skin increased in impedance when cooked, while chicken meat decreased in impedance. In the raw state, chicken meat had higher impedance than the chicken skin. In addition, testing at 10 kHz yielded smaller impedance values than testing at 2 kHz.

Tissue Engineered Skin Analogue Results

When compiling the data for the tissue engineered skin analogues, all impedance values greater than 1 Mega-ohm were deleted because of the measurement limitations of

ELVIS at such high values. Like the chicken tissue data, the skin analogue data was labeled with frequency, impedance, phase, resistance, reactance, and electrode number by the program. After transferring the data to Minitab, the sample number and sample type parameters were added. A multiple regression model using frequency, electrode number, and tissue type (i.e. EpidermTM or Melanoma) to predict the impedance was employed for the statistical analysis. The Minitab multiple regression model is shown in *Appendix D*.

With an EpidermTM reference group, the p-value for Melanoma was 0.058. While close, the p-value was not less than the α -value of 0.05. Thus, the null hypothesis – that the EpidermTM and Melanoma population mean impedances were equal – was retained. Furthermore, the Lack-of-Fit p-value was 0.998, which was greater than the α -value of 0.05. Therefore, a linear model was the best choice for this data. Two alternatives, namely compiling the data independent of frequency and removing outliers, were conducted.

Reviewing the Minitab multiple regression output above, the p-values for the frequency and electrode number were greater than the α -value of 0.05. This means that the impedance values did not statistically differ from each other based on the frequency and electrode number. Since the frequency did not produce statistical differences, data from both frequencies were combined into one group. A Student's t-test was performed on the integrated frequency data comparing the population mean impedance of EpidermTM and Melanoma. The Minitab t-test output is shown in *Appendix D*.

The t-test p-value was 0.048, which was less than the α -value of 0.05. Thus, the null hypothesis that the population mean impedance of EpidermTM equaled the population mean impedance of Melanoma was rejected; there was a statistical difference between

impedance values from Melanoma and EpidermTM. A boxplot depicting the impedance values of EpidermTM and Melanoma is shown in Figure 28. Notice that the EpidermTM data had a small deviation from the median compared to the wide scatter of the Melanoma data.

Figure 28: Boxplot of Tissue Engineered Skin Analogues

The second alternative that involved removing outliers was valid due to the condition of the tissue. The tissue samples were previously used by other members for other experiments and then frozen for two years until their use in this thesis. Furthermore, the stage of melanoma, as far as growth time, was unknown for the samples because of improper labeling by previous students. The different stages of melanoma, mainly horizontal or vertical growth phases, could have either increased or decreased the number of cancerous cells linearly between two electrodes, respectively. These various environmental unknowns may have lead to variations and errors in the data. For these reasons, 13 outliers, as predicted by Minitab, were removed from the data. A multiple

regression model using frequency, electrode number and tissue type to predict the impedance was employed. The Minitab multiple regression output is provided in *Appendix D*.

With an EpidermTM reference group, the p-value for Melanoma was 0.022, which was less than the α -value of 0.05. The null hypothesis that the population mean impedance of EpidermTM was equal to the population mean impedance of Melanoma was rejected. Therefore, there was a statistical difference between the impedance values of the two groups. Furthermore, the Lack-of-Fit p-value was 0.8989, which was larger than the α -value of 0.05. The null hypothesis that a linear model was sufficient was retained. The average impedance for EpidermTM and Melanoma tissue samples at 2 kHz were 19,469 and 24,276 ohms, respectively. The average impedance for EpidermTM and Melanoma tissue samples at 10 kHz were 16,628 and 23,407 ohms, respectively. The average impedance of the Melanoma tissue samples was larger than that of the EpidermTM samples.

Discussion

A discussion of the results and conclusions made in the previous section will be presented here. Where appropriate, the discussion will reference the overall hypothesis of this thesis – that melanoma skin cancer can be differentiated from normal epidermis tissue through impedance measurements by a multi-electrode device.

Device Design Discussion

The device design was described in the *Methods* section under the *Electrode Device* subtitle. Overall, the design worked well for the 9-electrode arrangement in that the majority of electrodes were fixed in place, breaking of the electrodes rarely occurred, and the assembly was uncomplicated. However, a few issues occurred during the duration of experiments. Firstly, the tolerances of the Ag/AgCl electrodes from Warner Instruments were very loose. All electrodes had approximately the same length, but varying diameters of AgCl due to varying manufacturing and quality control. This created some issues when placing the electrodes through the electrode holder openings. Larger diameter electrodes were difficult to fit through the electrode holder and would stop short of the electrode ridge developed into the design; this created electrodes that protruded further from the device face than other electrodes and could have created differences in impedance measurements due to dissimilar pressures on the electrode tips. To correct the issue, some of the AgCl was gently trimmed by Kelly forceps. Smaller diameter electrodes reached the electrode ridge, but were too small to feel the effects of the vice mechanism. This resulted in free motion into and out of the electrode holder face and could have created varying pressure on the electrode tips. While only occurring to

one electrode, the free movement was corrected by applying a small amount of epoxy to the electrode and electrode holder.

Other issues with the Ag/AgCl electrodes were related to their cost and fragility. Each electrode was approximately \$25.00, which if mass-produced would be an exceptional expense. Furthermore, the fragility of the electrodes created difficulties in placing them within the electrode holder, soldering them to wires without inflicting damage, and testing various tissues and electrical components without bending or breaking the tips. These specific electrodes were chosen for this thesis mainly for continuity with a previous student's thesis. However, if this project was continued, the Ag/AgCl electrodes could be cheaply manufactured by purchasing silver wire and either soaking it in household bleach or applying a current to it while in NaCl or KCl solution [38]. In addition, gold or Ag/AgCl microelectrodes could be produced through wafer fabrication methods. This will be discussed in more detail in the *Future Work* section.

ELVIS Validation Discussion

The data presented in Tables 3 through 6 comprise the ELVIS validation results. The resistor and capacitor values that could be identified by the ELVIS system to within the 5% tolerance range provided by the manufacturer comprised the reliable range of the ELVIS system. Any resistance and reactance value from the chicken tissues or skin analogues that fell within this reliable range could be considered reliable as well. From the data, values of resistors from 100 ohms to 100 k-ohms could be successfully identified to within the 5% tolerance range provided by the manufacturer at both frequencies. The 1 M-ohm resistor could be successfully measured by the ELVIS system at 2 kHz only. With such a high resistor value, it was possible that the injection of

surrounding electrical noise from nearby lights and other electronics disturbed the impedance analyzer algorithm. At 10 kHz the algorithm could have a more difficult time filtering the noise and establishing the amplitude and phase of the signal, resulting in an incorrect resistance reading.

For the ELVIS capacitor results, values from 1 nF to 1 uF could be reliably measured with the ELVIS system at 2 kHz to within the 10% tolerance range provided by the manufacturer. The 1 nF value was included, because the reactance value was reliably recorded 66.7% of the time, with the wrongfully identified value at the cusp of the 10% boundary. At 10 kHz, the ELVIS system reliably identified the capacitance of values from 1 nF to 0.1 uF to within the 10% tolerance range provided by the manufacturer. These capacitor values correlated to reactance measurements from approximately -80,000 to -80 ohms and -15,000 to -150 ohms for the 2 and 10 kHz cases, respectively.

In regards to Glenn Stante's thesis, his recorded resistance values for chicken tissues and tissue engineered skin analogues at 10 kHz ranged from 1 to 50 k-ohms and 1 to 40 k-ohms, respectively. These values all fell within the reliable resistor range of 100 ohms to 100 k-ohms from this thesis project. Thus, it is likely that future chicken tissue and skin analogue resistance measurements will fall within the reliable resistor range as well. Stante's recorded reactance values for chicken tissues and tissue engineered skin analogues at 10 kHz ranged from -12 ohms to -5.5 k-ohms and -100 ohms to -14 k-ohms, respectively [18]. Although all of the values did not fall between the -15,000 to -150 ohm range as determined by this thesis' ELVIS validation, several things could occur that warrant continuation of the experiment. Firstly, the addition of the multiplexer and device may alter the measurement capabilities of the ELVIS system, bringing the predicted

chicken tissue and skin analogue reactances within the correct range. Secondly, the ELVIS system could be inherently different from the ELVIS system used during Glenn Stante's thesis. That is, the DC offset or hardware calibration could differ between ELVIS systems. Thus, the reactances measured from chicken tissues and skin analogues may still be within the tolerable range of the ELVIS system used for this thesis.

This validation established a range of resistance and capacitance values that could be reliably identified by the ELVIS system. While data from Stante's thesis did not completely coincide with this range, additional validations may prove otherwise.

Multiplexer Validation Discussion

As discussed briefly in the *Methods* section under the *Multiplexer Validation* subtitle, multiplexers inherently add impedance to the circuit. The effects of the added impedance in an application in which the accuracy of the impedance is crucial can be seen in Table 7, which shows a reduced ability to identify the resistors and capacitors correctly. Without any post-processing, the measurement capabilities of the ELVIS system are reduced so that only 1 to 100 k-ohm resistors can be reliably measured at 2 kHz. Similar reductions in measurement capabilities are also shown with resistors at 10 kHz and capacitors at 2 and 10 kHz.

Since impedances in series are added together to get the total impedance, and the impedances of the entire measurement system and specimen are in series, the average intrinsic impedance can be subtracted from the raw measurement data based on Equation 10 to produce the impedance of the specimen (i.e. resistor, capacitor, chicken, etc) itself.

Equation 10: Impedance Components in Series

$$Z_{Total} = Z_1 + Z_2 + \dots Z_n$$

where n is a positive integer. Interestingly enough, the measurement capabilities of the ELVIS system improved. Tables 9 through 12 summarize the multiplexer validation data after adjusting for the intrinsic impedance. At 2 and 10 kHz, resistors valued from 10 ohms to 100 k-ohms were identified correctly to within the 5% tolerance range provided by the manufacturer. Even though three 1-ohm resistors were correctly identified between the 2 and 10 kHz tests, the other experimental errors of the 1-ohm resistors were beyond the acceptable range. Thus, the 1-ohm value was not included in the range of resistors that can be reliably measured by the ELVIS system.

After analyzing the adjusted capacitor data, it was concluded that capacitor values from 10 nF to 10 uF could be identified to within the 10% tolerance range for the 2 and 10 kHz tests. Looking at the data in detail, one gross outlier could be seen – the third 0.1 uF capacitor. This value was so much greater than surrounding data, and given the other two correctly identified 0.1 uF capacitors, it was likely that a manufacturing or packaging error occurred. The correctly identified capacitor values correspond to reactance values of -7,500 to -7 ohms and -1,500 to -1.5 ohms for the 2 and 10 kHz cases, respectively. Also take note that the experimental errors of the 1-nF capacitors were on the cusp of the 10% tolerance boundary. Since the reactance values corresponding to the 1 nF and 10 nF capacitors were -15,000 and -1,500 ohms, respectively, it is likely that many reactance values in-between were within the 10% tolerance range.

Adding the multiplexer changes the range of resistance and capacitance values that can be reliably identified by the ELVIS system to within the tolerance provided by the manufacturer. The device validation must be examined to determine the range of resistance and capacitance values for the entire system.

Device Validation Discussion

The addition of the device to the ELVIS and multiplexer system can create further impedance errors with poor solder connections, extra wires, and/or electrode-tissue interfaces. After running the validation experiments with the device, it was discovered that high intrinsic impedances reduced the measurement capabilities of the ELVIS system. However, after filtering out the average intrinsic impedance, the measurement capabilities of the ELVIS system were enhanced. This effect, similar to what occurred in the multiplexer validation, derives from the high accuracy, but narrow range of the ELVIS system. For instance, the 10-ohm resistor could not be properly identified by the ELVIS system alone. However, adding the multiplexer and device raised the impedance to approximately 74 ohms (10 ohms + intrinsic resistance of approximately 64 ohms). The intrinsic resistance raised the value of the 10-ohm resistor to within the measurement range of the ELVIS system. The intrinsic impedance alone was also high enough to be within the measurement range of the ELVIS system. With the high accuracy of the ELVIS system within an acceptable measurement range, it reliably recorded the values of the intrinsic resistance and combined resistance. These values, once subtracted from one another, reliably identified the 10-ohm resistor. The characteristics of the ELVIS system, multiplexer, and device allowed for measurement of values outside of the ELVIS system range.

The data gathered during the device validation is summarized in Tables 13 through 17. At 2 and 10 kHz, resistor values from 10 ohms to 100 k-ohms were reliably identified by the ELVIS system to within the 5% acceptable tolerance range provided by the manufacturer. This range encompassed all of the values recorded by Glenn Stante

during his investigations with chicken tissue and tissue engineered skin analogues. Again, these values were 1 to 50 k-ohms and 1 to 40 k-ohms for the chicken tissues and skin analogues, respectively.

The results from the capacitor data indicated that values from 10 nF to 10 uF could be reliably identified to within the 10% tolerance range provided by the manufacturer. With the same set of resistors and capacitors used in the multiplexer and device validations, the same outlier was present (second 0.1 uF capacitor). Similar to the multiplexer validation, the experimental errors for the 1-nF capacitors were on the cusp of the 10% tolerance boundary. With a wide margin of reactance values between the 1 nF and 10 nF capacitors, it was likely that many values in-between fell within the 10% acceptable tolerance range. The reactance value ranges of correctly identified capacitors were -7,500 to -7 ohms and -1,500 to -1.5 ohms for the 2 and 10 kHz cases, respectively. Considering Glenn Stante's recorded reactance values at 10 kHz that ranged from -12 ohms to -14 k-ohms, there was a region from -1.5 to -14 k-ohms that would be measured incorrectly. However, the values of chicken tissues and skin analogues depended on the specific ELVIS system and measurement electrodes used. Thus, further investigation into the impedances of the chicken tissues and skin analogues must be conducted.

Adding the electrode device did not change the intrinsic impedance greatly. The range of resistance and capacitance values that can be reliably identified by the ELVIS system are identical to that of the multiplexer validation. While Stante's results do not directly coincide with this range, the results from this thesis may.

Raw Chicken Discussion

The results from experimentation on raw chicken tissues are shown in Figures 22 and 23. By looking at the scatterplots, one can see a separation of the black and red dots – more prominently in the 10 kHz plot. The visual separations of data between the raw chicken meat and raw chicken skin serve as a preliminary test for a statistical difference. To ensure that a statistical difference is actually present, the t-test results were analyzed. With p-values for both the 2 and 10 kHz tests less than the α -value of 0.05, the null hypothesis was rejected. Therefore, the population mean impedance of the raw chicken meat and raw chicken skin groups were statistically different.

The average raw chicken meat and raw chicken skin impedances at 2 kHz were 7,130 and 5,583 ohms, respectively. The average raw chicken meat and raw chicken skin impedances at 10 kHz were 6,347 and 5,278 ohms, respectively. At both frequencies, the average raw chicken meat impedance was larger than that of raw chicken skin. Given that skin and meat cells were both structurally and physiologically different, differences in impedance were expected.

The ranges of resistance values for raw chicken meat and raw chicken skin at 2 kHz were 1,962 to 29,396 and 2,793 to 11,464 ohms, respectively. The ranges of resistance values for raw chicken meat and raw chicken skin at 10 kHz were 1,855 to 30,671 and 2,696 to 14,705 ohms, respectively. All ranges of measured resistance values fell between the 10-ohm to 100k-ohm range from the device validation. Thus, all resistances values for raw chicken meat and skin could be considered reliable.

The ranges of reactance values for raw chicken meat and raw chicken skin at 2 kHz were -1,509 to -167 and -989 to -149 ohms, respectively. The ranges of reactance

values for raw chicken meat and raw chicken skin at 10 kHz were -1,338 to 1,786 and -681 to 117 ohms, respectively. All of the 2 kHz reactance values fell between the -7,500 to -7 ohm range determined during the device validation. At 10 kHz, some values were found outside of the device validation range of -1,500 to -1.5 ohms. For raw meat at 10 kHz, there were approximately 15 of 320 measurements that were outside the reactance range. Since only a few measurements were outside this range, the reactance data for raw meat at 10 kHz was determined to be reliable. For raw skin at 10 kHz, approximately one quarter of the reactance values were outside the device validation range. These values tended to be close to zero and mainly recorded on one of the two chicken samples. Since these values, while positive, were close to zero, relatively close to the other reactance data for raw skin, and were not adjusted for the intrinsic impedance of the system, they were taken to be reliable.

With all of the resistance measurements and the majority of the reactance measurements falling in the reliable range of the ELVIS system as determined by the device validation results, the impedance values for raw chicken tissues were considered reliable. Therefore, the electrode device and measurement system are capable of discerning differences in tissues in multiple directions based on impedance.

Cooked Chicken Discussion

The data obtained from the cooked chicken experimentation is depicted in Figures 24 and 25. Visually, one can see a clear separation between the cooked chicken meat and cooked chicken skin data. Statistically, the t-test p-values comparing cooked meat and cooked skin at 2 and 10 kHz were less than the α -value of 0.05. The null hypothesis was rejected and the two groups were determined to be statistically different from each other.

However, a few issues arose during the experimentation on cooked skin. Firstly, after cooking the chicken, the skin became stiff and detached from the chicken meat in many locations. If the skin was not touching the meat during experimentation, the measured impedance would resemble that of an open circuit – approximately 2 M-ohms or greater. To correct the issue, the device was actuated until the electrodes pressed the skin against the meat. Secondly, even with the skin against the meat, the majority of measured impedance values exceeded the allowable range as determined by the device validation. Thus, the impedance values for cooked chicken skin could not be reliably measured with the device and measurement system used in this thesis. However, regardless of the actual impedance values of cooked chicken skin, they were extremely large compared to other groups and could most likely be differentiated from the cooked chicken meat.

The ranges of resistance for the cooked meat and cooked skin at 2 kHz were 985 to 6,959 and 170,921 to 2,494,270 ohms, respectively. The ranges of resistance for the cooked chicken meat and cooked chicken skin at 10 kHz were 791 to 4,325 and 66,735 to 236,744 ohms, respectively. As discussed above, the majority of the cooked skin could not be considered reliable because it exceeded the measurement limitations of the ELVIS system. The cooked meat values all fell within the resistance measurement range of 10 ohms to 100 k-ohms as determined by the device validation.

The ranges of reactance for the cooked meat and cooked skin at 2 kHz were -710 to -126 and -1,978,834 to -22,953 ohms, respectively. The ranges of reactance for the cooked chicken meat and cooked chicken skin at 10 kHz were -176 to 42 and -461,852 to -8,512 ohms, respectively. Again, the reactance values of cooked chicken skin exceeded the measurement capabilities of the ELVIS system and were not considered reliable. At 2

kHz, all of the cooked chicken meat reactance values fell within the acceptable range of -7,500 to -7 ohms. At 10 kHz, the majority of the cooked chicken meat reactance values fell within the acceptable range of -1,500 to -1.5 ohms, with an exception of 15 out of a total 320. Since the majority of the values were acceptable, the values outside of the acceptable range were close to zero, and all values were relatively close to one another, all cooked chicken meat reactance values were considered reliable.

With the resistance and reactance measurements of cooked chicken meat determined to be reliable, the impedance values were reliable as well; impedance consists of both resistance and reactance as per Equation 4. Therefore, the electrode device and measurement system were capable of discerning differences in tissues in multiple directions based on impedance.

Cooked Vs. Raw Chicken Discussion

As was briefly discussed in the *Results* section, and more intimately above, the cooked chicken skin measurements were not considered reliable because they exceeded the measurement capabilities of the ELVIS system as determined by the device validation. However, it was not unreasonable to conclude that cooked chicken skin could be differentiated from all other tissue types by its exceedingly large impedance values. The cooked chicken skin data was eliminated from the multiple regression model, because the data was unreliable. Consequentially, the measurements from the other groups were not overshadowed by the large cooked skin impedance values. Excluding cooked chicken skin, the p-values of raw meat and raw skin when compared to cooked meat were less than the α -value of 0.05. Thus, both raw skin and raw meat impedances were statistically different from that of the cooked meat group. As already established by

a t-test in the *Raw Chicken Discussion* section, raw meat and raw skin impedances were statistically different from each other. Furthermore, as discussed above, the majority of impedance values from the raw meat, raw skin, and cooked meat groups could be considered reliable. Therefore, the electrode device and measurement system could successfully differentiate between different tissue types and tissue moisture contents, the latter of which was expected to be the major difference between melanoma and healthy skin tissue.

The average impedance of cooked meat at 2 and 10 kHz was 2,788 and 2,596 ohms, respectively. These values are smaller than the average impedance of raw meat. While we would expect the raw meat impedance to be lower than the cooked meat impedance because of the reduction in moisture content during cooking, other events on the cellular level, like anatomical and physiological alterations, could have occurred to change the measured impedance. Comparing Glenn Stante's chicken tissue impedances to the ones measured in this thesis, there were a few differences as seen in Table 18. The cooked meat impedances at 10 kHz was nearly identical to that found by Glenn Stante. However, there was a substantial difference between the raw meat and raw skin impedances and their relative order. For example, in this thesis, the raw meat impedance was higher than the raw skin, whereas Stante found the opposite effect. These issues could potentially be explained by the different ELVIS systems, measurement acquisition methods (automatic as compared to Stante's manual approach), number of electrodes used, and/or pressure on electrode tips during experimentation.

Table 18: Comparing Measured Impedances to Previous Work

	Impedance (ohms) at 10 kHz		
Tissue	This Thesis	Glenn's Thesis	%Difference
Cooked Meat	2596	2784	6.75
Raw Meat	6347	1801	252.42
Raw Skin	5278	3897	35.44

Tissue Engineered Skin Analogue Discussion

With the conclusion that the electrode device and measurement system used in this thesis could successfully differentiate between different tissue types and tissue moisture contents based on impedance measurements in multiple directions, it must be shown that the same thing could be accomplished on tissue engineered constructs of melanoma and healthy epidermis. If so, then the objective of this thesis would be satisfied and the ability to differentiate between melanoma and healthy epidermis constructs in multiple directions based on impedance measurements would be successful.

As described in the *Results* section, running a multiple regression model on the EpidermTM and Melanoma data resulted in a p-value higher than the α -value of 0.05. However, the data was found to not depend on frequency based on its p-value. Thus, the frequency data was combined and the two groups were analyzed with a t-test. In addition, outliers were removed from the multiple regression model and tested again. Both alternatives (i.e. t-test or removal of outliers) resulted in p-values less than the α -value of 0.05. Thus, the null hypothesis was rejected and the population mean impedance of EpidermTM and Melanoma tissue constructs were determined to be statistically different.

Now, the data must be checked to ensure that the values were reliable. At 2 kHz, the resistance ranges for EpidermTM and Melanoma constructs were 8,843 to 26,470 and 5,235 to 77,059 ohms, respectively. At 10 kHz, the resistance ranges for EpidermTM and

Melanoma constructs were 8,201 to 22,228 and 4,904 to 77,072 ohms, respectively. All of these values were within the acceptable range of 10 ohms to 100 k-ohms as determined by the device validation. Therefore, the resistance measurements were reliable for both constructs at both frequencies.

Reviewing the capacitor data at 2 kHz, the reactance ranges for Epiderm™ and Melanoma constructs were -3,934 to -765 and -27,041 to -319 ohms, respectively. Only 3 out of 122 reactance values were beyond the acceptable -7,500 to -7 ohm reactance range as determined by the device validation. As discussed in the *Device Validation Discussion* section, since the experimental errors for the 1 nF capacitors were close to 10%, and because there were a wide margin of reactances from 10 nF to 1 nF – namely -7,500 to -75,000 ohms – it was likely that a reactance of -27,041 was still within the reliable range of the ELVIS system. For these reasons, the reactance data at 2 kHz for both constructs were determined to be reliable. At 10 kHz the reactance ranges for Epiderm™ and Melanoma constructs were -4,984 to -239 and -38,659 to -133 ohms, respectively. For this frequency, there were 33 out of 122 reactance values that were beyond the acceptable reactance range of -1,500 to -1.5 as determined by the device validation. Again, with the experimental errors of the 1 nF capacitors so close to 10%, and with the wide range of reactances from 10 nF to 1 nF – namely -1,500 to -15,000 ohms – it was likely that many of the reactances closer to -1,500 ohms were reliable. Overall, there were 6 reactance values that exceeded -15,000 ohms and were ultimately removed during the outlier analysis. Thus, the reactance values included in the statistical analysis for both constructs at 10 kHz were reliable.

With all resistances and the majority of reactances determined to be in an acceptable range for the ELVIS system, the impedance data was concluded to be reliable. Impedance consists of both resistance and reactance as per Equation 4.

The average impedance of EpidermTM constructs was less than that of Melanoma constructs. This relationship was in opposition to the data found in the literature, but consistent with Glenn Stante's findings [18, 33, 34]. Possible reasons for the disagreement with the literature most likely stemmed from the tissue samples themselves. Firstly, other student's in unrelated projects used the tissue samples previously. The handling, care, and storage of the samples were not known during those projects. Secondly, the tissue constructs were frozen for 2 years between thesis projects, possibly causing the samples to dry and increase in impedance. Thirdly, the melanoma impedance measurements should be standardized to healthy skin impedance. Since the samples were not derived from the same person/specimen, a standardization protocol could not be established. Fourthly, the samples were tissue engineered constructs and not actual human specimens. Furthermore, the measurements conducted in this thesis were in vitro, compared to in situ measurements by the authors in previous literature. Conducting the experiments with tissue engineered constructs in a petri dish was drastically different from in situ measurements of melanoma and healthy skin. All of these combined factors made it difficult to compare to previous work. In particular, being unable to normalize the melanoma tissue impedances to the healthy tissue impedances made it impossible to compare the recorded data to the normalized conductance value of 6.2 to 6.4 found in previous work.

Even with the differences from the literature, the objective of the thesis was satisfied and the manufactured electrode device and measurement system were able to differentiate between melanoma and healthy epidermis tissue in multiple directions based on impedance measurements. These experiments increase the possibility that the same device and measurement system could differentiate between in situ melanoma and healthy skin tissues.

Conclusion

Analyzing the data obtained from the validation experiments on the ELVIS system, multiplexer, and electrode device, resistor values from 10 ohms to 100 k-ohms and capacitor values from 10 nF to 10 uF could be reliably measured to within the tolerance range provided by the manufacturer. The measured resistance and reactance values for all chicken tissues, excluding cooked chicken skin, were within the acceptable resistance and reactance ranges as determined by the validation experiments. The measured resistance and reactance values for both tissue engineered skin analogues were within the acceptable resistance and reactance ranges as determined by the validation experiments. Thus, all impedance data, excluding cooked chicken skin was reliable.

Raw chicken skin and raw chicken meat were determined to be statistically different by a t-test. Cooked chicken skin, having extremely large values, was eliminated. However, the large values in themselves, allow for statistical distinction from cooked chicken meat. A multiple regression model showed that the raw chicken and raw meat impedances were statistically different from that of cooked meat. Therefore, the device and measurement system could distinguish between different tissue types and tissue moisture content, the latter of which simulates the melanoma disease state.

EpidermTM and Melanoma skin construct impedances were found to be statistically different after removal of outliers. The electrode device and measurement system could distinguish between tissue engineered skin constructs of EpidermTM and Melanoma in multiple directions based on impedance alone. Accordingly, the objective of this thesis was satisfied and the prospect of differentiating in situ melanoma from healthy skin tissue is substantial.

Future Work

This thesis project serves as a preliminary study into the feasibility of multi-directional impedance analysis to differentiate between melanoma and healthy skin tissue. Currently, work is being performed on the next generation of the electrode device, which is a multi-electrode chip created by clean room wafer fabrication. Other devices in the future may also have coupling abilities to a radiofrequency tissue ablation tool. The following sections will discuss additional tests with the current electrode device and speculate about the possible design, fabrication method, imaging capabilities, and radiofrequency tissue ablation interplay of the next-gen device(s).

Next Steps

Next steps with the current electrode device would include more in vitro tests, animal testing on grey horses, and clinical testing with human subjects. The additional in vitro tests should examine more samples for better statistical results and samples with varying melanoma stages to investigate the sensitivity of the device. Using in situ tissues will allow for the full complexity of the skin to be studied, including the effects from hair, nerve endings, a full dermis, sweat glands, and sweat. Thus, grey horses, an animal with a high occurrence of melanoma, should be examined. After animal testing, human subjects should be tested with the device, followed by a biopsy of the lesion and histological analysis of the tissue for diagnosis. All melanoma measurements should be standardized to an ipsilateral healthy epidermis measurement and a database should be created. With a database of values, statistical analysis can be conducted to establish the specificity and sensitivity of a chosen threshold value. The threshold value is then used as a boundary to differentiate between melanoma and healthy skin tissue.

Next-Gen Device

The goal of the next-gen device is to identify the exact boundaries of the melanoma tumor. Issues with the current electrode device design are that 8 electrodes at 5 mm distances from one another do not have sufficient resolution to discern accurate boundaries. The more electrodes present and the closer the electrodes are to one another, the higher the resolution possible. However, bringing electrodes closer together creates issues with measurement depth. The depth of measurement is dependent on the distance between electrodes and the source signal frequency. The current is estimated to reach depths that are approximately $\frac{1}{2}$ the separation of electrodes [25]. Furthermore, high frequency signals tend to reach deeper locations, while low frequency signals remain superficial [24]. Since melanoma lesions can be approximately 4 mm thick, it is important that measurement signals reach 4 mm into the tissue [13]. For these reasons, electrodes should be placed in a linear fashion so that switching the active electrode to the next one on the line (i.e. switching from adjacent electrodes to one inactive electrode in-between) could alter measurement depth. Thus, a matrix pattern or offset parallel lines may be optimal.

The construction of the microelectrode is completed in a clean room with wafer fabrication techniques. First, a 4" silicon wafer is cleaned with ethanol, acetone, and isopropyl alcohol (IPA) while spinning [47]. The wafer is removed and dried with pressurized air. Then, a positive photoresist, such as SPR 955-2.1, is spun onto the wafer to produce a sacrificial layer [47]. The photoresist is baked on a hotplate, exposed with light to a glass mask patterned with the electrode layout, and ammonia baked to reverse the image of the mask [47]. Another exposure to light is followed by development in MF

701 [47]. After drying, 100 Angstroms of titanium and 1000 Angstroms of gold are deposited onto the wafer through e-beam metal evaporation or sputtering [47]. Lift-off of the unwanted metal (everywhere but the electrodes and interconnects) is performed by breakdown of the sacrificial layer in acetone [47]. After drying, the interconnects can be electrically passivated with SU-8. SU-8 is a negative resist that is spun on the wafer, baked, exposed with light to a glass slide patterned with the passivation layer, baked again, and developed in SU-8 Developer [47].

The above recipe creates a microelectrode pattern, with interconnecting wires, out of gold. The electrodes themselves are exposed, while the interconnects are passivated with SU-8. A few alterations may be made to the recipe: 1) a 2 mil polyimide sticker can be placed on the silicon wafer prior to coating with SPR, 2) silver may be deposited onto the wafer instead of gold and titanium, and/or 3) additional gold can be electrodeposited after the passivation layer. The polyimide sticker may be used when a flexible surface is needed. After fabrication, it can be peeled from the silicon wafer and used more effectively on curved skin surfaces. Silver may be an alternative choice to gold for the same reasons that it is used in this thesis; namely, no overpotential and no motion artifacts [35]. Once deposited, the silver can be electrodeposited with silver chloride to create Ag/AgCl measurement electrodes. If silver is not used, gold can be further electrodeposited to form a beaded surface that protrudes past the passivation layer. The added gold allows for a better contact surface to the skin and increases the electrode surface area, which leads to lower electrode-tissue interface impedances. Other complications that must be fixed include interfacing measurement equipment to the electrodes, having enough input/output ports for controlling the many multiplexers

involved in the circuit, and creating an algorithm to measure all areas and depths of the skin quickly and accurately.

A possible control algorithm should consider the electrode matrix design as an image. Each measurement between electrodes forms a voxel – a 3-dimensional pixel – that identifies the likelihood of melanoma. The microelectrode device would first be placed on an ipsilateral healthy skin location. Each electrode and depth available would be quickly measured and stored in memory. Then, the device would be placed on the suspicious nevus. The algorithm starts recording from the corner and measures all depths possible. The values measured are referenced to the stored values for the ipsilateral location and determined to be cancerous or benign. If benign areas are found, the measurements continue row by row from left to right until a cancerous area is discovered. After measuring all depths possible in the cancerous area, the measurement moves down one row while in the same column (turns right). All depths are checked for cancerous areas. If a cancerous area is discovered, the measurement turns left – in this case, to the right one column while in the same row. If a benign area is discovered, the measurement turns right – to the left one column while in the same row. Continuing this left or right method, the boundaries of a tumor can be found accurately without having to measure the impedance between all electrodes. With this algorithm, a traced 3-dimensional boundary of the tumor is detected and the information can be sent to animation software or therapeutic tools, one of which could be a radiofrequency tissue ablation instrument.

Radiofrequency Tissue Ablation

Radiofrequency tissue ablation is the use of radio waves between frequencies of 375 to 500 kHz to heat tissue [18]. The movement of the electromagnetic waves through

the body creates friction that heats the areas where the electromagnetic waves meet [18]. If multiple waves are directed at the right angle, they can intersect at a tumor and constructively interfere with each other. At 41 degrees Celsius, injury of the tissue occurs and reaches necrosis at 46 degrees Celsius [18]. Since melanoma tumors are found on the surface of the body, radiofrequency tissue ablation is ideal. Ag/AgCl electrodes can be used to source and sink the radio waves and administer the therapy [18]. Thus, the microelectrode device could double as a melanoma therapy when attached to the proper signal generator. Also, the detected boundaries of the tumor could be transferred to a separate radiofrequency ablation device that uses the information to control its own therapeutic regimen.

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Appendix A: Solidworks Drawings

Figure 29: Electrode Device Bill Of Materials

Figure 30: Electrode Holder Top Section Drawing

Figure 31: Electrode Holder Middle Section Drawing

Figure 32: Electrode Holder Bottom Section Drawing

Appendix B: LabVIEW Program

Figure 33: LabVIEW Program Front Panel

The front panel takes the user inputs, in this case number of points, highest frequency, lowest frequency, data acquisition device name, and time delay, and directs them to the program.

Appendix C: Validation Data

The following tables were unused in the main report because of their similarity to other graphs and insignificance to the conclusion of the thesis, but are provided here for completeness.

Table 19: Unadjusted Multiplexer Validation Resistor Values at 10 kHz

Resistor Value (ohms)	Measured Resistance (ohms)	Experimental Error (%)	Within 5% Tolerance (Y/N)
1	64.73	6372.60	N
1	64.81	6381.30	N
1	64.82	6381.70	N
10	73.56	635.58	N
10	73.46	634.56	N
10	73.54	635.38	N
100	162.34	62.34	N
100	162.86	62.86	N
100	163.31	63.31	N
1,000	1045.74	4.57	Y
1,000	1036.67	3.67	Y
1,000	1036.97	3.70	Y
10,000	10009.16	0.09	Y
10,000	9916.35	0.84	Y
10,000	10131.42	1.31	Y
100,000	95965.83	4.03	Y
100,000	95661.04	4.34	Y
100,000	95746.57	4.25	Y
1,000,000	244673.84	75.53	N
1,000,000	263614.31	73.64	N
1,000,000	264190.95	73.58	N

Table 20: Unadjusted Multiplexer Validation Capacitor Values at 2 kHz

Capacitor Value (F)	Measured Reactance (ohms)	Calculated Capacitance (F)	Experimental Error (%)	Within Tolerance (Y/N)
1.00E-09	-71384.575	1.11477E-09	11.48	N
1.00E-09	-73653.959	1.08042E-09	8.04	Y
1.00E-09	-71362.253	1.11512E-09	11.51	N
1.00E-08	-7497.43	1.0614E-08	6.14	Y
1.00E-08	-7640.002	1.04159E-08	4.16	Y
1.00E-08	-7661.907	1.03861E-08	3.86	Y
1.00E-07	-760.875	1.04587E-07	4.59	Y
1.00E-07	-766.121	1.03871E-07	3.87	Y
1.00E-07	-687.58	1.15736E-07	15.74	N
1.00E-06	-74.728	1.06489E-06	6.49	Y
1.00E-06	-75.872	1.04884E-06	4.88	Y
1.00E-06	-74.847	1.0632E-06	6.32	Y
1.00E-05	-6.977	1.14057E-05	14.06	N
1.00E-05	-6.959	1.14352E-05	14.35	N
1.00E-05	-6.82	1.16683E-05	16.68	N

Table 21: Unadjusted Multiplexer Validation Capacitor Values at 10 kHz

Capacitor Value (F)	Measured Reactance (ohms)	Calculated Capacitance (F)	Experimental Error (%)	Within Tolerance (Y/N)
1.00E-09	-14401.608	1.10512E-09	10.51	N
1.00E-09	-14865.036	1.07067E-09	7.07	Y
1.00E-09	-14414.598	1.10412E-09	10.41	N
1.00E-08	-1515.657	1.05007E-08	5.01	Y
1.00E-08	-1543.196	1.03133E-08	3.13	Y
1.00E-08	-1551.053	1.02611E-08	2.61	Y
1.00E-07	-149.334	1.06576E-07	6.58	Y
1.00E-07	-151.168	1.05283E-07	5.28	Y
1.00E-07	-134.677	1.18175E-07	18.18	N
1.00E-06	-12.403	1.2832E-06	28.32	N
1.00E-06	-12.615	1.26163E-06	26.16	N
1.00E-06	-12.384	1.28517E-06	28.52	N
1.00E-05	1.11	-1.43383E-05	243.38	N
1.00E-05	1.118	-1.42357E-05	242.36	N
1.00E-05	1.132	-1.40596E-05	240.60	N

Table 22: Unadjusted Device Validation Resistor Values at 2 kHz

Resistor Value (ohms)	Measured Resistance (ohms)	Experimental Error (%)	Within 5% Tolerance (Y/N)
1	64.038	6303.80	N
1	63.981	6298.10	N
1	64.052	6305.20	N
10	72.949	629.49	N
10	72.907	629.07	N
10	72.976	629.76	N
100	162.002	62.00	N
100	162.895	62.90	N
100	162.415	62.42	N
1,000	1037.005	3.70	Y
1,000	1045.87	4.59	Y
1,000	1036.898	3.69	Y
10,000	10017.795	0.18	Y
10,000	10150.421	1.50	Y
10,000	9933.287	0.67	Y
100,000	99175.252	0.82	Y
100,000	99288.527	0.71	Y
100,000	98327.636	1.67	Y
1,000,000	801106.418	19.89	N
1,000,000	791205.185	20.88	N
1,000,000	792906.679	20.71	N

Table 23: Unadjusted Device Validation Resistor Values at 10 kHz

Resistor Value (ohms)	Measured Resistance (ohms)	Experimental Error (%)	Within 5% Tolerance (Y/N)
1	64.311	6331.10	N
1	64.326	6332.60	N
1	64.336	6333.60	N
10	73.251	632.51	N
10	73.157	631.57	N
10	73.24	632.40	N
100	162.249	62.25	N
100	163.11	63.11	N
100	162.669	62.67	N
1,000	1037.681	3.77	Y
1,000	1046.627	4.66	Y
1,000	1037.375	3.74	Y
10,000	10025.948	0.26	Y
10,000	10159.65	1.60	Y
10,000	9940.631	0.59	Y
100,000	96646.064	3.35	Y
100,000	96765.227	3.23	Y
100,000	95912.721	4.09	Y
1,000,000	205808.799	79.42	N
1,000,000	198785.713	80.12	N
1,000,000	197641.336	80.24	N

Table 24: Unadjusted Device Validation Capacitor Values at 2 kHz

Capacitor Value (F)	Measured Reactance (ohms)	Calculated Capacitance (F)	Experimental Error (%)	Within Tolerance (Y/N)
1.00E-09	-73109.369	1.08847E-09	8.85	Y
1.00E-09	-71493.31	1.11308E-09	11.31	N
1.00E-09	-70654.826	1.12629E-09	12.63	N
1.00E-08	-7492.531	1.06209E-08	6.21	Y
1.00E-08	-7651.639	1.04001E-08	4.00	Y
1.00E-08	-7644.912	1.04092E-08	4.09	Y
1.00E-07	-761.302	1.04528E-07	4.53	Y
1.00E-07	-687.676	1.15719E-07	15.72	N
1.00E-07	-766.616	1.03804E-07	3.80	Y
1.00E-06	-76.01	1.04693E-06	4.69	Y
1.00E-06	-74.899	1.06246E-06	6.25	Y
1.00E-06	-74.867	1.06292E-06	6.29	Y
1.00E-05	-6.948	1.14533E-05	14.53	N
1.00E-05	-6.973	1.14122E-05	14.12	N
1.00E-05	-6.803	1.16974E-05	16.97	N

Table 25: Unadjusted Device Validation Capacitor Values at 10 kHz

Capacitor Value (F)	Measured Reactance (ohms)	Calculated Capacitance (F)	Experimental Error (%)	Within Tolerance (Y/N)
1.00E-09	-14845.211	1.0721E-09	7.21	Y
1.00E-09	-14445.939	1.10173E-09	10.17	N
1.00E-09	-14353.556	1.10882E-09	10.88	N
1.00E-08	-1518.923	1.04781E-08	4.78	Y
1.00E-08	-1553.808	1.02429E-08	2.43	Y
1.00E-08	-1550.256	1.02664E-08	2.66	Y
1.00E-07	-149.523	1.06442E-07	6.44	Y
1.00E-07	-134.905	1.17976E-07	17.98	N
1.00E-07	-151.759	1.04873E-07	4.87	Y
1.00E-06	-12.567	1.26645E-06	26.65	N
1.00E-06	-12.327	1.29111E-06	29.11	N
1.00E-06	-12.35	1.2887E-06	28.87	N
1.00E-05	1.203	-1.32298E-05	232.30	N
1.00E-05	1.208	-1.31751E-05	231.75	N
1.00E-05	1.233	-1.29079E-05	229.08	N

Appendix D: Minitab Outputs

Raw Chicken Results

Two-Sample T-Test and CI: Resistance_2000_2, Condition_2000_2

Two-sample T for Resistance_2000_2

Condition_2000_2	N	Mean	StDev	SE Mean
RawMeat	160	7108	5405	427
RawSkin	160	5569	1880	149

Difference = μ (RawMeat) - μ (RawSkin)

Estimate for difference: 1538

95% CI for difference: (646, 2431)

T-Test of difference = 0 (vs not =): T-Value = 3.40 P-Value = 0.001 DF = 196

Two-Sample T-Test and CI: Impedance_2000_2, Condition_2000_2

Two-sample T for Impedance_2000_2

Condition_2000_2	N	Mean	StDev	SE Mean
RawMeat	160	7130	5415	428
RawSkin	160	5583	1886	149

Difference = μ (RawMeat) - μ (RawSkin)

Estimate for difference: 1548

95% CI for difference: (654, 2442)

T-Test of difference = 0 (vs not =): T-Value = 3.41 P-Value = 0.001 DF = 197

Cooked Chicken Results

Two-Sample T-Test and CI: Impedance_2000_1, Condition_2000_1

Two-sample T for Impedance_2000_1

Condition_2000_1	N	Mean	StDev	SE Mean
CookedMeat	168	2789	817	63
CookedSkin	168	1828459	796568	61457

Difference = μ (CookedMeat) - μ (CookedSkin)

Estimate for difference: -1825670

95% CI for difference: (-1947002, -1704338)

T-Test of difference = 0 (vs not =): T-Value = -29.71 P-Value = 0.000 DF= 167

Two-Sample T-Test and CI: Impedance_10000_1, Condition_10000_1

Two-sample T for Impedance_10000_1

Condition_10000_1	N	Mean	StDev	SE Mean
CookedMeat	168	2496	709	55
CookedSkin	168	330041	101036	7795

Difference = μ (CookedMeat) - μ (CookedSkin)

Estimate for difference: -327544

95% CI for difference: (-342934, -312154)

T-Test of difference = 0 (vs not =): T-Value = -42.02 P-Value = 0.000 DF= 167

Cooked Vs. Raw Chicken Results

General Regression Analysis: Impedance_4_ versus Frequency_4_, Electrode_4_

Regression Equation

Condition_4_1				
CookedMeat	Impedance_4_1	=	19038.9 - 2.60854 Frequency_4_1 - 212.894 Electrode_4_1	
CookedSkin	Impedance_4_1	=	386516 - 2.60854 Frequency_4_1 - 212.894 Electrode_4_1	
RawMeat	Impedance_4_1	=	23135.3 - 2.60854 Frequency_4_1 - 212.894 Electrode_4_1	
RawSkin	Impedance_4_1	=	21826.8 - 2.60854 Frequency_4_1 - 212.894 Electrode_4_1	

Coefficients

Term	Coef	SE Coef	T	P
Constant	19039	4846.81	3.9281	0.000
Frequency_4_1	-3	0.44	-5.8842	0.000
Electrode_4_1	-213	732.20	-0.2908	0.771
Condition_4_1				
CookedSkin	367477	5421.04	67.7872	0.000
RawMeat	4096	4468.85	0.9166	0.360
RawSkin	2788	4468.85	0.6239	0.533

Summary of Model

S = 57212.2 R-Sq = 84.09% R-Sq(adj) = 84.02%
PRESS = 3.843320E+12 R-Sq(pred) = 83.84%

Analysis of Variance

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Regression	5	2.00043E+13	2.00043E+13	4.00086E+12	1222.30	0.000000
Frequency_4_1	1	1.01731E+12	1.13332E+11	1.13332E+11	34.62	0.000000
Electrode_4_1	1	1.08547E+09	2.76723E+08	2.76723E+08	0.08	0.771287
Condition_4_1	3	1.89859E+13	1.89859E+13	6.32864E+12	1933.45	0.000000
Error	1156	3.78386E+12	3.78386E+12	3.27323E+09		
Lack-of-Fit	58	1.73628E+12	1.73628E+12	2.99358E+10	16.05	0.000000
Pure Error	1098	2.04758E+12	2.04758E+12	1.86483E+09		
Total	1161	2.37882E+13				

General Regression Analysis: Impedance_1 versus Frequency_1, Electrode_1, ...

Regression Equation

Condition_1
CookedMeat Impedance_1 = 2898.37 - 0.0571251 Frequency_1 + 24.8358 Electrode_1

RawMeat Impedance_1 = 6994.73 - 0.0571251 Frequency_1 + 24.8358 Electrode_1

RawSkin Impedance_1 = 5686.3 - 0.0571251 Frequency_1 + 24.8358 Electrode_1

Coefficients

Term	Coef	SE Coef	T	P
Constant	2898.37	282.439	10.2619	0.000
Frequency_1	-0.06	0.026	-2.2138	0.027
Electrode_1	24.84	45.046	0.5513	0.582
Condition_1				
RawMeat	4096.36	251.867	16.2640	0.000
RawSkin	2787.93	251.867	11.0690	0.000

Summary of Model

S = 3224.52 R-Sq = 22.54% R-Sq(adj) = 22.23%
PRESS = 10200722583 R-Sq(pred) = 21.74%

Analysis of Variance

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Regression	4	2.93856E+09	2.93856E+09	734640427	70.655	0.00000
Frequency_1	1	5.09594E+07	5.09594E+07	50959352	4.901	0.02707
Electrode_1	1	3.16058E+06	3.16058E+06	3160583	0.304	0.58153
Condition_1	2	2.88444E+09	2.88444E+09	1442220887	138.708	0.00000
Error	971	1.00960E+10	1.00960E+10	10397502		
Lack-of-Fit	43	1.18030E+08	1.18030E+08	2744891	0.255	1.00000
Pure Error	928	9.97794E+09	9.97794E+09	10752095		
Total	975	1.30345E+10				

Tissue-Engineered Skin Analogue Results

General Regression Analysis: Impedance versus Frequency, Electrode, Type

Regression Equation

Type

Epiderm Impedance = 20528.3 - 0.232318 Frequency - 357.751 Electrode

Melanoma Impedance = 26296 - 0.232318 Frequency - 357.751 Electrode

Coefficients

Term	Coef	SE Coef	T	P
Constant	20528.3	3777.69	5.43408	0.000
Frequency	-0.2	0.38	-0.61660	0.539
Electrode	-357.8	667.00	-0.53636	0.593
Type				
Melanoma	5767.8	3016.76	1.91191	0.058

Analysis of Variance

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Regression	3	1.19525E+09	1.19525E+09	398415379	1.44003	0.234651
Frequency	1	8.97994E+07	1.05190E+08	105190448	0.38020	0.538685
Electrode	1	9.40954E+07	7.95941E+07	79594091	0.28768	0.592719
Type	1	1.01135E+09	1.01135E+09	1011351407	3.65541	0.058312
Error	118	3.26474E+10	3.26474E+10	276672469		
Lack-of-Fit	28	3.34342E+09	3.34342E+09	119407959	0.36673	0.998199
Pure Error	90	2.93039E+10	2.93039E+10	325599206		
Total	121	3.38426E+10				

Two-Sample T-Test and CI: Impedance, Type

Two-sample T for Impedance

Type	N	Mean	StDev	SE Mean
Epiderm	58	18049	5141	675
Melanoma	64	23828	22297	2787

Difference = mu (Epiderm) - mu (Melanoma)

Estimate for difference: -5779

95% CI for difference: (-11498, -60)

T-Test of difference = 0 (vs not =): T-Value = -2.02 P-Value = 0.048 DF = 70

**General Regression Analysis: Impedance versus Frequency,
Electrode, Type**
Outliers removed

Regression Equation

Type

Epiderm Impedance = 16989.4 - 0.0855339 Frequency + 518.232 Electrode

Melanoma Impedance = 13236.4 - 0.0855339 Frequency + 518.232 Electrode

109 cases used, 13 cases contain missing values

Coefficients

Term	Coef	SE Coef	T	P
Constant	16989.4	1952.72	8.70039	0.000
Frequency	-0.1	0.20	-0.42538	0.671
Electrode	518.2	350.59	1.47819	0.142
Type				
Melanoma	-3753.0	1611.32	-2.32913	0.022

Analysis of Variance

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Regression	3	550735328	550735328	183578443	2.60780	0.055508
Frequency	1	17649978	12738260	12738260	0.18095	0.671427
Electrode	1	151199386	153817838	153817838	2.18504	0.142351
Type	1	381885963	381885963	381885963	5.42483	0.021767
Error	105	7391573789	7391573789	70395941		
Lack-of-Fit	28	1413430189	1413430189	50479650	0.65019	0.898908
Pure Error	77	5978143601	5978143601	77638229		
Total	108	7942309117				