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Measuring Biological Cell Damage Due to Ionizing Radiation

Kathryn Jacobson
College of Saint Benedict/Saint John's University

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MEASURING BIOLOGICAL CELL DAMAGE DUE TO IONIZING RADIATION

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Kathryn Jacobson

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PROJECT TITLE: MEASURING BIOLOGICAL CELL DAMAGE DUE TO IONIZING

RADIATION

Approved by:

_____

Adam Whitten
Adjunct Assisting Professor of Physics

_____

Dean Langley
Professor of Physics

_____

David Mitchell
Associate Professor of Biology

_____

Dean Langley
Chair, Department of Physics

_____

Emily Esch
Director, Honors Thesis Program

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ABSTRACT

Radiation therapy is used to treat many different types of cancer. Ionizing radiation occurs at very high frequencies and can cause reparable or permanent damage to biological cells. Cancerous tumors are typically located behind layers of healthy tissue and when radiated, the healthy and cancerous cells are damaged. With new technology, radiation machines deliver radiation such that the tissue damage is uniform as a function of tissue depth by filtering out the low-energy photons. The purpose of this research was to determine the validity of using the change in dielectric constant to gauge tissue damage due to radiation. The change in dielectric constant of beef was calculated and analyzed after each dose of two Grays of radiation, totaling four doses. One Gray of radiation is equivalent to the absorption of one joule of radiation energy by one kilogram of matter. Twelve slices of beef were stacked up with aluminum foil between each to simulate the electrodes of a capacitor so that the capacitance could be measured in between radiation doses. Differences in capacitance were converted into changes in dielectric constant. Initial analysis showed problems with the mechanics of the apparatus, particularly, slices 5, 9, 11 and 12 were deemed unusable. Using the valid slices, a weighted average change in dielectric constant per one Gray of radiation was calculated to be 0.49 ± 0.03. Future work includes fixing problems with the apparatus, adapting this technique for living instead of dead tissue as well as determining the mathematical relationship between change in dielectric constant and cellular damage. This would also give insight to reparable damage versus permanent damage.

INTRODUCTION

One of the largest, most collaborated research areas of today's scientific world is the search for a cure for cancer. Within this area, there is a portion of research dedicated to enhancing the current cancer treatments. There are five main cancer treatments: chemotherapy, radiation therapy, immunotherapy, targeted therapies, and transplantation ("Types of Treatment"). Chemotherapy is the use of medication to kill cancer cells (Nordqvist). Radiation therapy is the use of radiation to attack cancer cells. Immunotherapy is treatment that utilizes certain parts of a person's immune system to fight the cancer by either stimulating part of the immune system to work harder and smarter or by giving immune system components to a patient ("What Is Cancer Immunotherapy?"). Targeted cancer therapies are drugs that stop growth of cancer cells by interfering with critical molecules that are involved with cancer growth ("Targeted Cancer Therapies"). Transplantation is a treatment option where the cancer patient receives a new organ or tissue to replace a failing one, or one that has been infested with cancerous cells ("Transplants for Cancer Treatment").

Radiation therapy is most commonly used as a tool to cure or shrink early stage cancer, to prevent cancer relapse, and to treat symptoms subsequent of advanced cancer. A common misconception about radiation therapy is that external beam radiation is the only way to deliver radiation. There are different ways to deliver radiation to cancerous cells. In addition to external beam radiation, techniques such as brachytherapy, internal radiation, and radiopharmaceuticals are used. ("The Science Behind Radiation Therapy")

The goal of radiation therapy is to deliver radiation to the DNA at a critical point in the life cycle such that damage is maximized and cell growth is stopped. This research is derived from the fact that if the radiation dosage delivered to the cancerous tissue is doubled, the cure

rate goes from 5% to 95%. However, radiation does not differentiate between healthy cells and cancerous cells. Whenever radiation is delivered to cancerous tissue, damage to the surrounding healthy tissue comes as a consequence. There has been a lot of research regarding this problem, but there have been few techniques developed that minimize damage to the healthy cells and maximize damage to cancerous cells. One technique is to deliver radiation from multiple angles with the greatest intensity of radiation focused on the cancerous tissue. Another technique is to inject gold particles into the cancerous tissue, which intensifies the radiation absorption in the cancerous tissue. (Broadhurst)

If the low energy photons are not filtered out of the photon radiation beam, there should be a damage gradient present because of the low energy photons would be absorbed in the top layers of tissue. This would result in more damage to the top layers and less damage as the radiation penetrates deeper into the tissue. Unless the tumor is near the surface, this presents a problem. However, technology improvements have created filters to remove the low energy photons so that the radiation damage is consistent throughout the tissue. This is important in radiation therapy because if the cancerous cells are located in deep tissue, damage to the health tissue layers has to be minimized.

However the radiation is delivered, it is important to be able to see how much damage has been imposed on the tissue. Determining the cellular damage directly can be a difficult task. New research is dedicated to finding indirect ways to measure damage.

The purpose of this research was to justify the validity of using changes in dielectric constants as a means of assessing biological cell damage due to ionizing radiation, and to analyze the performance of the apparatus created to measure this change in dielectric constant. The dielectric constant of a material is a measureable value that quantifies the material's ability to

store electrical energy in an electrical field. It is a known fact that the dielectric constant changes with the amount of radiation given to a material. (Barnett et al.) Measuring the capacitance of the tissue and then converting the differences in capacitance, from before and after radiation, to a change in dielectric constant can determine this constant. In order to examine the apparatus's performance, the data collected was initially analyzed to locate problems with the apparatus. If this technique is deemed valid, then it can be used in future research to analyze reparable cell damage versus permanent cell damage. Moreover, this technique can also be used in a clinical setting as a simple and effective method of checking tissue damage after radiation therapy.

THEORY

A biological understanding

Cells are the most basic building blocks of any living subject. Animal cells fall under the category of eukaryotic cells, which means that there are many different membrane organelles within the cell membrane. Figure 1 shows a diagram of a basic biological cell and table 1 gives the functions of the major organelles. The main organelle of interest is the nucleus, because it
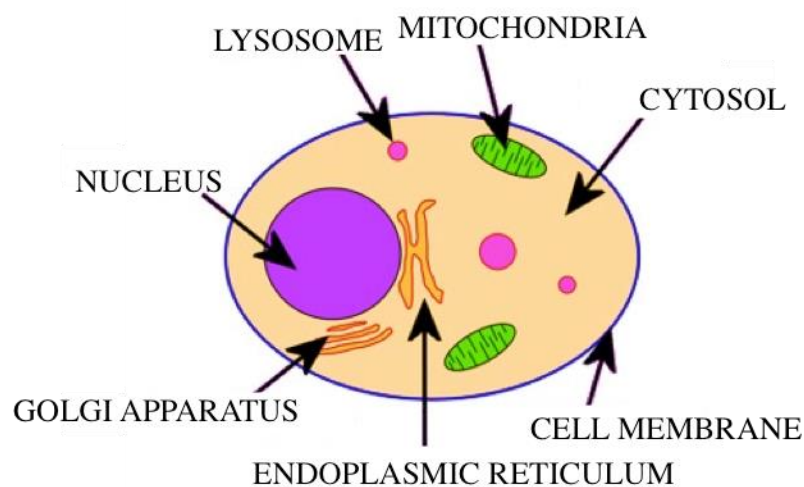

Figure 1. Basic layout of eukaryotic cell. ("Cell Biology")

Table 1. Eukaryotic cell organelles and functions.

Organelle	Function
Cell Membrane	The lipid bilayer that forms the outer boundary of a cell
Cytosol	The gelatinlike aqueous fluid that bathes the organelles on the inside of the cell membrane
Endoplasmic Reticulum	A system of membranous tubules and sacs in the eukaryotic cells that functions as a path along which molecules move from one part of the cell to the other
Golgi Apparatus	A system of membranes in eukaryotic cells that modifies proteins for export by the cell
Lysosome	An organelle containing digestive enzymes
Mitochondria	The organelle that is the site of aerobic respiration in eukaryotic cells
Nucleus	The organelle that contains most of the DNA and directs most of the cell's activities

houses the deoxyribonucleic acid (DNA) of the cell. Analogous to a manual for a machine, the DNA carries the cell's genetic instructions and is one of the most important parts of the cell. The central dogma for biological cells is to use DNA to synthesize messenger ribonucleic acid (mRNA), which is then used to synthesize proteins.

DNA is a negatively charged polymer of nucleotides that encodes the necessary directions to build a cell and information pertaining to storage, replication and execution of cellular information. The information in DNA is stored as a code consisting of four chemical bases – adenine (A), guanine (G), cytosine (C), and thymine (T) – the chemical structures of the bases are shown in figure 2. The information available from the DNA is determined by the order of bases; akin to how letters of the alphabet appear in a certain order to form words and sentences. Attached to each base are a sugar and a phosphate molecule. Combined, these form a nucleotide. Nucleotides are arranged in two long strands that form a double helix; these strands are connected by chemical attractions between base pairs. DNA bases are complementary to each other – A with T, C with G – forming a base pair. Compared to a ladder, the base pairs

form the rungs of the ladder and the sugar and phosphate molecules form the vertical pieces of

the ladder. (Campbell and Reece) The sugar and phosphate molecule make up what is commonly

known as the DNA backbone. DNA has over three billion base pairs in a single strand. Most

cells in the human body are incredibly small, and if the DNA within one cell was arranged in a

single straight piece, the DNA would be almost two meters long. (natureEducation) To package

the DNA such that it fits within the nucleus of a cell, it is wrapped around proteins called

histones to form nucleosomes. The nucleosomes are then coiled into a structure known as

chromatin. Chromatin is then organized into chromosomes, which is the final DNA packaging

molecule. Human cells contain twenty-three pairs of chromosomes in every cell. Figure 3

shows how DNA is compacted such that it can fit inside the nucleus of a cell. How DNA is

manipulated is very important to the functions of the cell. However, before any of these

functions can be discussed, it is helpful to review the cell cycle.
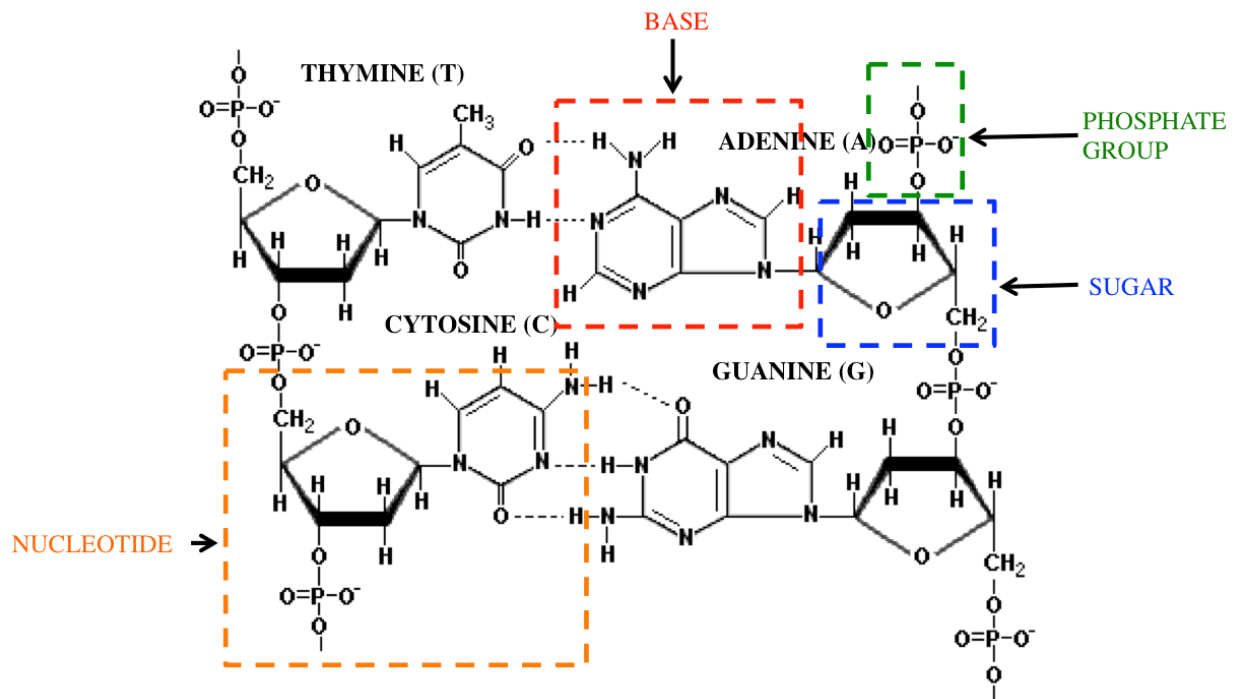
Figure 2. Chemical structures of DNA base pairs. Note
dashed black line show chemical attraction.
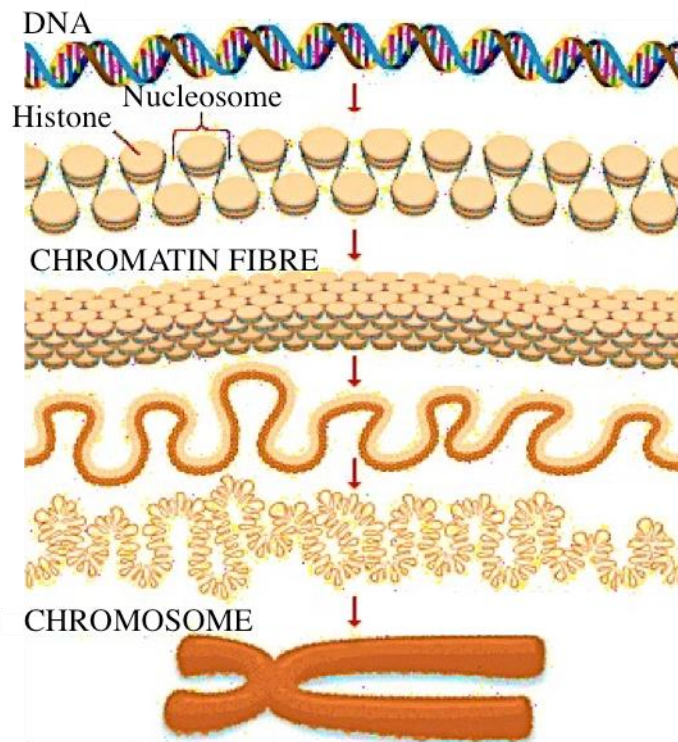
Figure 3. DNA packaging. ("DNA: DNA Packaging into Chromatin and Chromosome")

The cell cycle is the process in which eukaryotic cells divide. There are two main phases in the cell cycle – interphase and mitotic phase – depicted in figure 4. Interphase can be broken down into three separate phases; the first is the G_1 phase. During G_1, the bulk of cell growth happens and the DNA chromosomes are prepared for replication. DNA replication happens in the S phase of interphase. After the DNA is replicated, the cell moves into the G_2 phase in which the cell is prepared for mitosis. (Kimball) During the mitotic phase the original cell, also known as the parent cell, splits into two identical daughter cells. While cells are not actively dividing, they rest in a G_0 phase separate of the cell cycle, but included in the interphase. However, once a cell receives the signal to divide, it moves into the G_1 phase and begins the cell cycle. ("The Science Behind Radiation Therapy") The signals to enter the cell cycle and to complete phases are regulated by proteins, which are produced in a process known as gene expression.
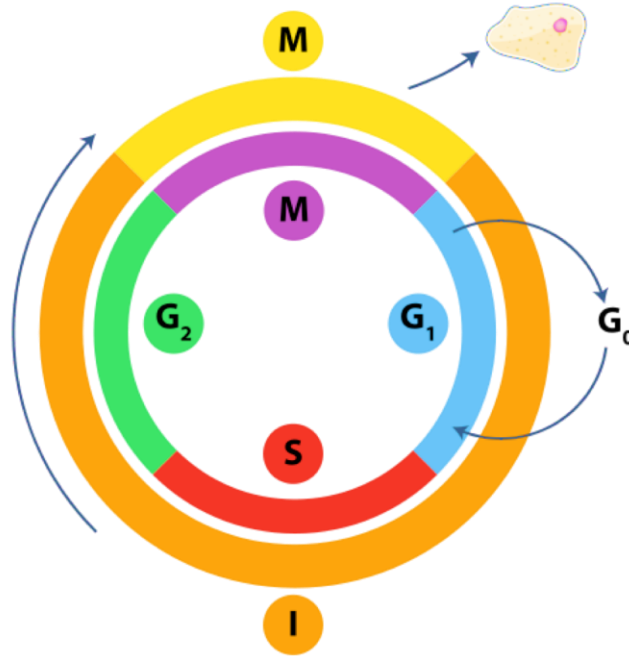
Figure 4. Cell cycle. (Zephyris)

One of the most important roles of DNA is its role in gene expression. Gene expression is the process of decoding the information from DNA and producing proteins. The sequence of DNA bases has a code to make certain proteins needed for cellular functions. In order to decode the DNA, a process known as transcription creates messenger RNAs (mRNAs), from DNA. DNA polymerase, a protein in the cell, then uses the code from the mRNA to produce proteins. This happens during the G_1 phase of the cell cycle when the cell is preparing for DNA replication. (Campbell and Reece) Proteins have many critical functions in the cell. They make most of the structures located within cells, they are used as enzymes to break down food and turn it into fuel for the cell, they are responsible for building new parts, and they act as eyes and ears to alert the cell of any changes in the environment. Proteins carry out most cell functions, so it is important that the proteins are built correctly and are fully functional. (Wiley) As mentioned

before, DNA has over three billion base pairs, but not all of the base pairs are translated during protein synthesis. Gene regulation is the process that regulates gene expression.

Gene expression is one of the most tightly controlled processes in the body; by regulating gene expression, only essential proteins are made for particular cells. Cell properties vary depending on the overall function of the cell. For example, a neuron cell looks different than a skin cell and also has a very different function. However, the DNA in the two cells is identical. Gene expression is regulated based on the environment and needs of the cells. Regulation happens at different stages, namely, transcriptional regulation, post-transcriptional regulation, and translational regulation. (Mandal) Gene expression and regulation are very important to the overall function of the cell, and if genes are misread or damaged, cells can become mutated and cause diseases such as cancer. However, when DNA is damaged, the cell is programmed to attempt to repair the damage.

Genetic stability is vital for any living organism; this not only includes extremely accurate DNA replication, but also mechanisms for repairing damages done to the DNA. Most spontaneous changes in DNA are temporary because of a built in set of processes that correct the change. Causes of these temporary changes include heat, metabolic accidents, natural radiation, and exposure to substances in the environment. Although this type of damage happens daily, only few mutations accumulate in the DNA sequence. The DNA instructs the cell to invest a large amount of energy in making the DNA repair enzymes such that it can tackle the day-to-day disturbance in base pairs. (Alberts et al.) However, sometimes too much damage happens to parts of the DNA sequence, and this can result in a permanent mutation or cell death.

A physical understanding

Just as cells are the basic building blocks of any living subject, atoms constitute all matter. Atoms are composed of three basic particles – protons, neutrons and electrons. Protons and neutrons construct the nucleus of the atom, and possess a positive and neutral electrical charge, respectively. The atomic nucleus is held together by an attractive, strong nuclear force between the protons and neutrons. Strong nuclear forces are extremely powerful but extend only a very short distance. (Young and Freedman) There are also electromagnetic forces between the protons that make them repulse each other. Electrons orbit the nucleus carrying a negative charge. Opposite electrical charges do the work to hold the electrons in orbit around the nucleus, and the farther away the electron is from the atomic nucleus, the less of a pull. Figure 5 illustrates the structure of an atom. Atoms can differ in their number of protons, neutrons and/ or electrons. The number of protons, Z, in an atom determines what element it is. However, the number of neutrons and electrons may differ, which will constitute a change in physical and/ or chemical properties. (US EPA, "What Is an Atom?")
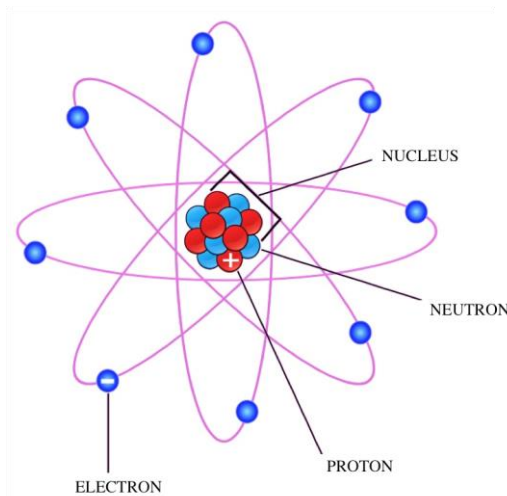

Figure 5. Basic atomic structure. (US EPA, "What Is an Atom?")

Atoms default to the most stable configuration, and when an atom becomes unbalanced it seeks to become more stable. When the ratio of neutrons to protons is unbalanced, the atom can become more stable in several ways: converting neutrons to protons, converting protons to neutrons, or ejecting an alpha particle (two neutrons and two protons) from the nucleus. As a result of changing the number of nucleons (protons and neutrons) the atomic nucleus gives off energy in the form of ionizing radiation. If a proton is gained or lost in this process, the atom becomes a different element with different chemical properties. If there is a gain or loss of a neutron, it becomes an isotope of the element depending on the total number of neutrons; this changes the radiological properties of the atom. When protons and electrons of an atom are unbalanced, the atom is electrically charged and is referred to as an ion of the element. This change affects the chemical reactivity of the atom, but does not affect the stability of the nucleus. (US EPA, OAR) The energy needed to remove an electron from a neutral atom is called the ionization energy. This energy differs for every element because electron's of the distance away from the nucleus. Any source of imbalance in an atom can cause energy to be given off in the form of radiation.

In general, radiation is the process in which electromagnetic waves propagate through a vacuum or matter-containing medium. Not all radiation is the same; different types are characterized by the energy and frequency of the radiation.   Radiation has two major subgroups: non-ionizing and ionizing. Table 2 summarizes the numerical boundaries of the two types of radiation. Two things can happen when matter absorbs radiation: excitation or ionization. Excitation occurs when the radiation increases the kinetic energy of the atoms or molecules, or excites an electron from an occupied orbital into an empty, higher-energy orbital. Ionization occurs when the radiation possesses enough energy to exceed the ionization energy and remove

Table 2. Numerical boundaries for energy (kilojoule per mole of particles) and frequency (TetraHertz) of non-ionizing and ionizing radiation. (Bodner; US EPA, "Ionizing & Non-Ionizing Radiation")

	Non-Ionizing Radiation	Ionizing Radiation
Energy (kJ/mol)	< 298	≥ 298
Frequency (THz)	< 750	≥ 750

an electron from an atom or molecule. (Bodner) Non-ionizing radiation causes excitation and ionizing radiation causes ionization. Figure 6 gives the types of radiation in the electromagnetic spectrum and respective examples of sources of the radiation.

There are two main types of ionizing radiation: photon radiation and particle radiation. Photon radiation is defined as energy propagated by photon waves that have no mass. Photon radiation includes x-rays and gamma rays. Particle radiation is defined as energy propagated by traveling particles that have a definite rest mass, momentum and position at any instant. (Kahn) Particle radiation includes beams of electrons, protons, neutrons, carbon ions, alpha particles, and beta particles. High-energy photon beams are the most common form of radiation used in
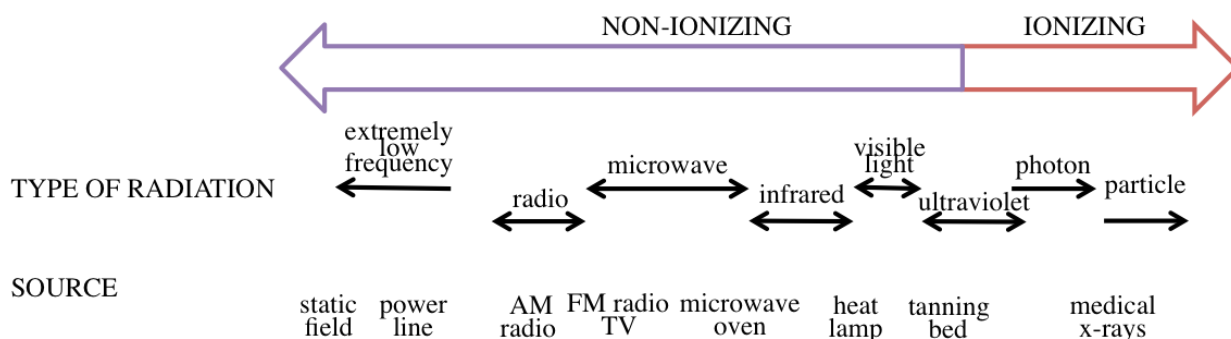
Figure 6. Electromagnetic spectrum with the different types of radiation and respective source examples. Adopted from: (US EPA, "Ionizing & Non-Ionizing Radiation")

cancer treatment. ("The Science Behind Radiation Therapy") Linear accelerators use high

frequency electromagnetic waves to accelerate charged particles, such as electrons, to high

energies through a linear tube. The accelerated electrons are then collided with a target of high

proton material, such as tungsten. The electron energies are converted into a spectrum of

photons as a consequence of the conservation of momentum. (Kahn) The energy of a photon is

characterized by the equation,

$$E = h v$$

where h is Plank's constant,

$$h = 6.62 \times 10^{-34} \text{J} \cdot \text{s}$$

(Joule seconds) and v is the frequency of the photon. Frequency is the speed of light divided by

the wavelength. (Schreiber) Thus, high-energy radiations have a short wavelength and a high

frequency.

When a photon interacts with particles in the material, all or some of the photon's energy

can be lost. If the photon has left over energy, it can continue to affect other particles. This

causes attenuation, or decrease in intensity, of the photon beam. There are four major types of

interactions between photons and material – coherent scattering, photoelectric effect, Compton

scattering, and pair production. (Schreiber) Coherent scattering happens when a photon passes

near an electron setting it into oscillation, which radiates energy at the same frequency as the

incident photon beam. In coherent scattering, there is no energy transfer and is only probable in

high proton number materials with photons of low energy. In the case where the low energy

photons are filtered out, attenuation does not depend on coherent scattering. The photoelectric

effect is the process where the energy from the photon is absorbed by an atom and then

transferred to an electron. If the electron receives an amount of energy larger than the ionization

energy, the electron is ejected from the atom, producing a free radical. The Compton effect is seen when the photon interacts with an electron as though it were a "free" electron. The electron's binding energy is much less than the energy of the photon, making it easier to pull the electron away from the atom. Pair production is when the photon interacts strongly with the electromagnetic field of an atomic nucleus and produces an electron/ positron pair. The attenuation of the beam due to pair production is a function of the number of protons, squared, per atom. (Kahn) In radiation therapy, the Compton effect is the predominant interaction; however, all forms of interactions contribute to the beam attenuation. (Schreiber)

When these photons are incident on a subject, it is important to be able to measure the damage done to the material by the radiation absorbed. In the clinical setting, radiation dose and absorption is measure in Grays (Gy). One Gray is equal to the absorption of one joule of radiation energy by one kilogram of matter. Although this is the most useful quantity, it is the hardest to obtain. Instead of measuring the radiation damage directly, it can be useful to look at the change in dielectric constant of the test subject by measuring the change in capacitance.

A capacitor is a device used to store electric charge. For sake of practicality, a parallel-plate capacitor will be used to discuss capacitors. Note that capacitors can vary in shape and size, but the basic structure includes two adjacent conductors, also known as electrodes, carrying equal but opposite charges. The conductors may be in a vacuum, or separated by an insulating material, which is known as a dielectric. Consider the parallel-plate capacitor shown in figure 7(a), with a distance d between the two conductors, plate area A, and equal magnitude but opposite charges Q on each plate. When in a vacuum, the amount of charge Q stored in a capacitor is directly proportional to the electric potential difference between the plates ΔV.
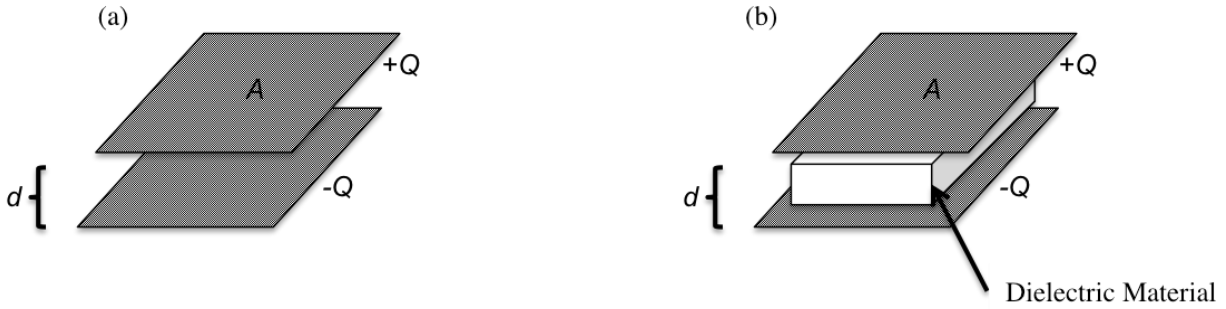
Figure 7. (a) Parallel-plate capacitor. (b) Parallel-plate capacitor with a dielectric.

Charge has units of Coulombs (C) and electric potential difference has units of volts (V). The relationship between Q and ΔV can be written as,

$$Q = C|\Delta V|$$

where C is a positive proportionality constant called capacitance. Physically, capacitance is the measure of the capacity to store electric charge for a given potential difference. (Jeffery) The System International (SI) unit of capacitance is the farad (F). For a parallel-plate capacitor in a vacuum, the capacitance can also be determined by,

$$C = \frac{\varepsilon_0 A}{d}$$

where A is the area of the plates, d is the distance between the plates, and ε_0 is the physical known as the permittivity of free space, which has a value of,

$$\varepsilon_0 = 8.854 \times 10^{-12} \text{ F/m}$$

(farads per meter). There is an electric field produced between the plates of the parallel-plate capacitor as a result of opposite charges on the conductors, shown in figure 8(a). In many capacitors, the conductors are not in a vacuum, but rather have a dielectric material between the plates, as shown in figure 7(b).
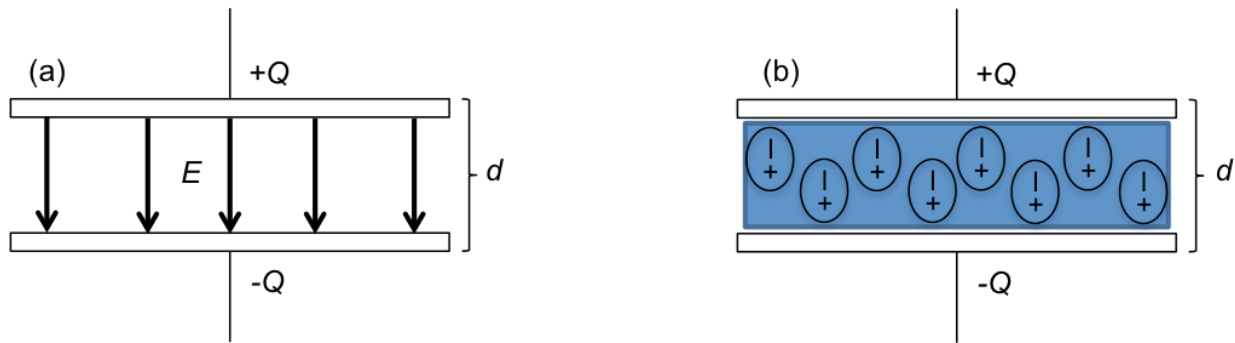
Figure 8. (a) Electric field produced by the oppositely charged plates of the parallel-plate capacitor. (b) Alignment of dielectric material dipoles with electric field produced by parallel-plate capacitor.

When a dielectric material is placed between the two electrodes of a parallel-plate capacitor, the capacitance changes. The capacitance is increased by a dimensionless factor, κ_e, known as the dielectric constant of the dielectric material. The dielectric constant of a material is a physical quantity measuring the ability of the material to store electrical energy in an electrical field. Accounting for the presence of the dielectric, capacitance can be found by,

$$C = \frac{\kappa_e \varepsilon_0 A}{d}$$

if the dielectric constant of the material is known. However, if the dielectric constant is unknown, the equation can be manipulated to solve for κ_e when the capacitance is measured using a measuring device, such as a digital multimeter.

As the physical and chemical properties of the dielectric change, the dielectric constant will change, affecting the capacitance as well. The molecules that make up the dielectric are polar if the center of positive charge within the molecule is not located in the same place as the central negative charge. If the two coincide, the molecule is nonpolar. Figure 9 depicts the difference between a polar and nonpolar molecule. The polarity of a molecule is quantitatively described by its dipole moment. A polar substance is made up of many dipoles, and when placed
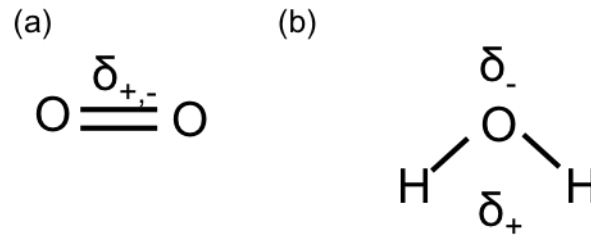
Figure 9. (a) Nonpolar O_2 molecule – positive and negative charge coincide. (b) Polar H_2O molecule – positive and negative charge do not coincide.

in an electric field, the positive charge experiences a force in the direction of the field, while the negative charge experiences a force in the opposite direction. (Semat and Katz) The resulting torque causes the dipoles to align as shown in figure 8(b). Free radicals in the dielectric are also attracted to the oppositely charged plate. This alignment and separation of charge increases the potential difference across the material, thus increasing the dielectric constant.

It is an accepted fact that when a material is radiated, there is a change in dielectric constant. This change is proportional to the damage. Conclusions about tissue damage can be inferred from experimental changes in dielectric constant. (Barnett et al.)

Effects of radiation on a biological cell

If the radiation introduced to the tissue does not have enough energy, no substantial damage will be done to the cell. Therefore, from this point forward it is assumed that the radiation energy is high enough to cause detrimental damage to a cell. When ionizing radiation is delivered to a biological cell, there is a timeline of events that cascade from the interaction between the photons and the cell. First, there are physical interactions between the photon and molecules within the cell. The deposition of energy from the photon excites electrons from different molecules and if the energy is great enough, the molecules can become ionized,

producing free radicals within the nucleus. Next, there are chemical interactions when an electron has been ionized. The material surrounding the ionization site is forced to absorb approximately 33 electronvolts (eV) of energy per electron, or 3,182 kilojoules per mole of electrons. (US EPA, "Ionizing & Non-Ionizing Radiation") This exceeds the binding energy, or the energy required to break neighboring chemical bonds. This can cause major damage to all parts of the cell. The chemical reactions cause the initial damage, but the last part of the timeline is the biological response, which includes mending of the damage and the final condition of the cell. There are three possible outcomes for the cell once it has been damaged: (1) the cell dies as a result of irreparable damage; (2) repairs are done, but the cell retains some permanent damage; (3) or the repair mechanisms return the cell to a healthy functional state ("The Science Behind Radiation Therapy").

All organelles inside the cell are affected by radiation in different ways, ultimately affecting cell functions in different ways. Recall from figure 1 and table 1, the important organelles of a biological cells and its respective function. Table 3 details the consequential effects of radiation on each of the important cell organelles.

The organelle of most interest is the nucleus since it houses the DNA of the cell. The final condition of the cell mostly stems from the damage imposed on the DNA. The DNA can be damaged in two ways: (1) directly with the photon; (2) or indirectly with free radicals produced by the ionization of neighboring molecules. (Perez and Ovid Technologies) DNA damage can happen in different ways, including: base damage, DNA-protein cross-links, single-strand breaks, double-strand breaks, and complex combinations of all of these (Lawrence TS and Ten Haken RK). The explicit mechanism of cell death due to radiation has yet to be established, however, strong evidence supports the notion that double-stranded breaks of DNA are the most

Table 3. Irradiation and biological effects of major cell organelles (Somosy).

Organelle	Irradiation effect	Biological effect
Cell Membrane	changes in cell shape or texture, breaks in lipid bilayer	altered cell communication, cell deformation
Cytosol	unstable free radicals produced	attributes to indirect damage of DNA
Endoplasmic Reticulum	changes in size and shape, changes in formation of blood vessels, fragmentization	molecules no longer can move from one part of the cell to another
Golgi Apparatus	fragmentation and disorganization of strucutre	halts secretion of proteins
Lysosome	number and volume fraction increase in the cell	cell fuel is not broken down correctly and inefficiently
Mitochondria	reactive oxygen species produced in high amounts, alterations in structure, increase in size	decrease in aerobic respiration
Nucleus	changes structure of chromatin, free radicals produced	changes in biological gene expression and cell death mechanism

detrimental cellular effect of radiation. (Alberts et al.) A chemical break like this can lead to an irreversible loss of reproductive integrity of the cell and ultimately cell death.

If the cell retains permanent damage after the cell repairs are finished, the cell might not be fully functional anymore. This can have a great effect on the processes of the cell necessary for cell growth, cell function and programmed cell death. When DNA is damaged, it can cause genes to mutate, which in turn changes the proteins that are made when that gene is expressed. This can cause large problems for the cell because it is not making the correct proteins. Problems arise in the macroscopic scale as well, specifically the development of acute diseases and organ failure.

MATERIALS AND METHODS

Overview

In order to effectively measure the damage caused by ionizing radiation, capacitance of a test subject was measured and then converted into a change in dielectric constant. A change in dielectric constant suggests an alteration in physical structure of the test subject. In this experiment, the test subject (beef) was partitioned into twelve individual slices. An apparatus was constructed to hold the beef in proper alignment with a photon radiation beam produced by a linear accelerator. The capacitance was measured using a switch mechanism to select particular slices of beef. The overall experimental setup is pictured in figure 10.
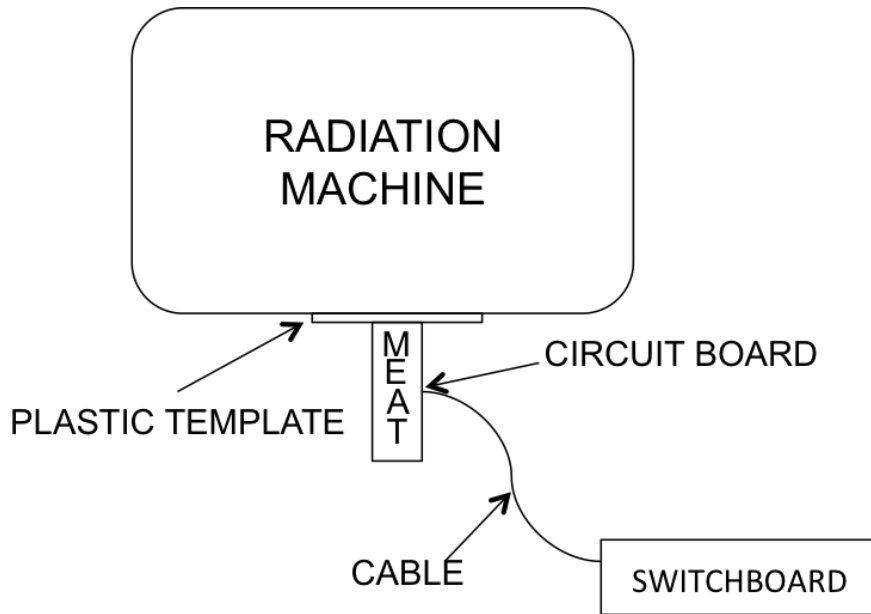

Figure 10. Basic overall experimental set up

Test subject

Twelve slices of organic beef constituted the test subject in this experiment. Each slice was circular in shape with a three-inch diameter and half-inch thick. Twelve slices was chosen because in a clinical setting, tumors are typically located within six inches of the radiation incident site. Each slice was in direct contact with part of the neighboring slices to resemble realistic test subjects, like live animals, as much as possible.

Apparatus

The apparatus used in the experiment is shown with more detail in figure 11. The apparatus was used to constrict the movement of the slices of beef during the radiation process. Four plastic rods (½-inch diameter, 10 ½-inch length) were attached to a plastic template (11 ¾-inch square face, ¼-inch thick) specific to the radiation machine used. Figure 12 shows a top view of the plastic template where the rods were attached. The rods were attached using metal screws and washers; the top 1/8-inch of the rod was shaved, such that the diameter was 3/8-inch. This technique was used because the constant upward force presented by the screws on the rods forced the rods to be held rigid against the bottom of the plastic template. The base that served as a means to compress the meat against the plastic template was constructed using a plastic block (5-inch square face, 1 ½-inch thick) with four holes (½-inch diameter) arranged such that the block can smoothly move to accommodate different size test subjects. Four springs, with a spring constant approximately 400 N·m^{-1}, were individually placed on each pole to support the block. At the bottom of each rod, another washer was used as a base for the spring and was secured to the rod using metal screws.
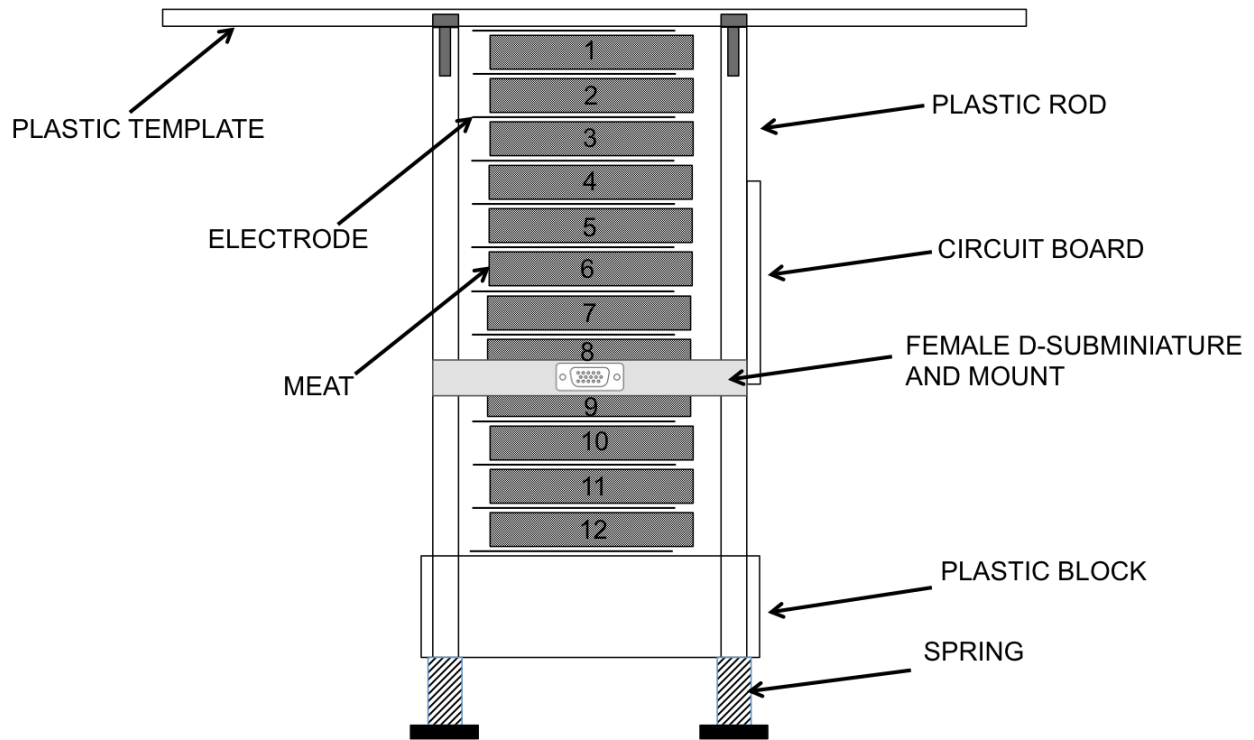
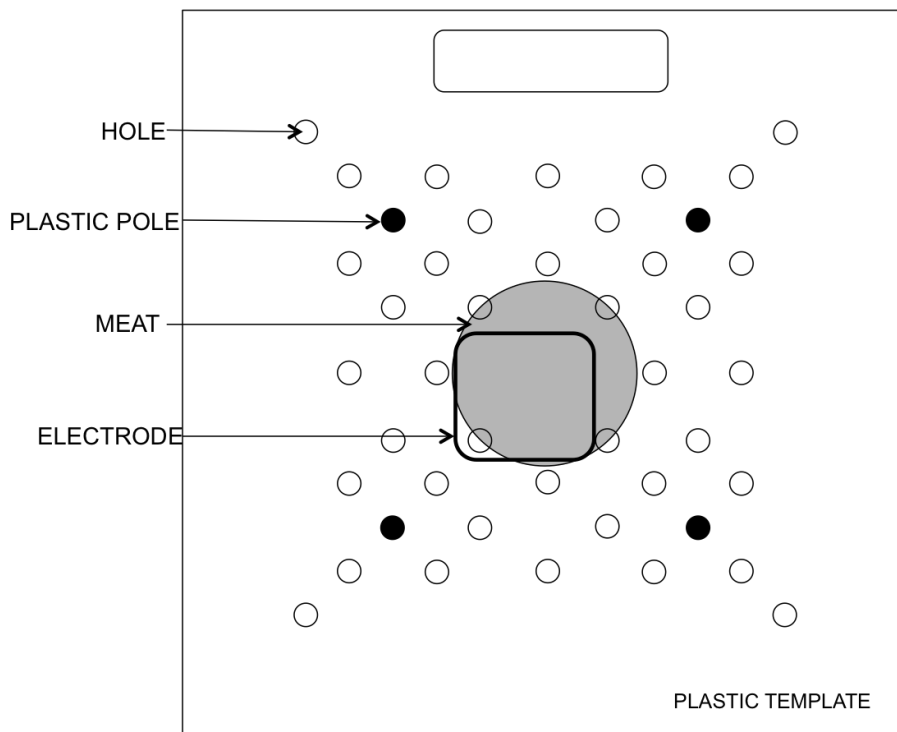Figure 11. Detailed diagram of apparatus. Circuit board and beef are included in this diagram.

Figure 12. Top view of plastic template. Darkened holes represent location of the four rods.
Position of beef and aluminum foil are also shown in this diagram.

The circuit board – used to control which slice's capacitance was measured – was attached to two of the four rods. On two adjacent rods, a female D-subminiature connector was mounted to allow the wires from the circuit board to be lengthened to twenty-five feet so that the capacitance could be safely measured outside of the radiation room.

Circuit board

The circuit board that was used to measure the capacitance of specific slices of meat is schematically shown in figure 13. Thirteen reed relays, specified with a number one through thirteen, were soldered onto the circuit board. Reed relays are constructed of a coil wrapped around a reed switch. The two reeds are ferromagnetic material sealed inside an inert gas tube. When a current runs through the coil, a magnetic field is generated, pulling the reeds together, forming a connection. A schematic diagram of a reed relay is pictured in figure 14. Reed relays were used to minimize the number of wires connected to the circuit board and to allow control over which slice of meat's capacitance was measured.

Connected to the circuit board, through a long cable, was a switchboard consisting of twelve double throw single pole (DTSP) switches. The switches, when "on," determined which slice of beef was measured. Switch one corresponded to the top slice of beef. Similarly, the twelfth switch corresponded to the bottom slice of beef. The DTSP switches were connected to one side of the coil within the reed relay, with the other side of the coil connected to ground. When a DTSP switch was flipped "on," current from the power supply then ran through two coils in adjacent reed relays, closing the reed relay switch. Only one switch was used at a time.
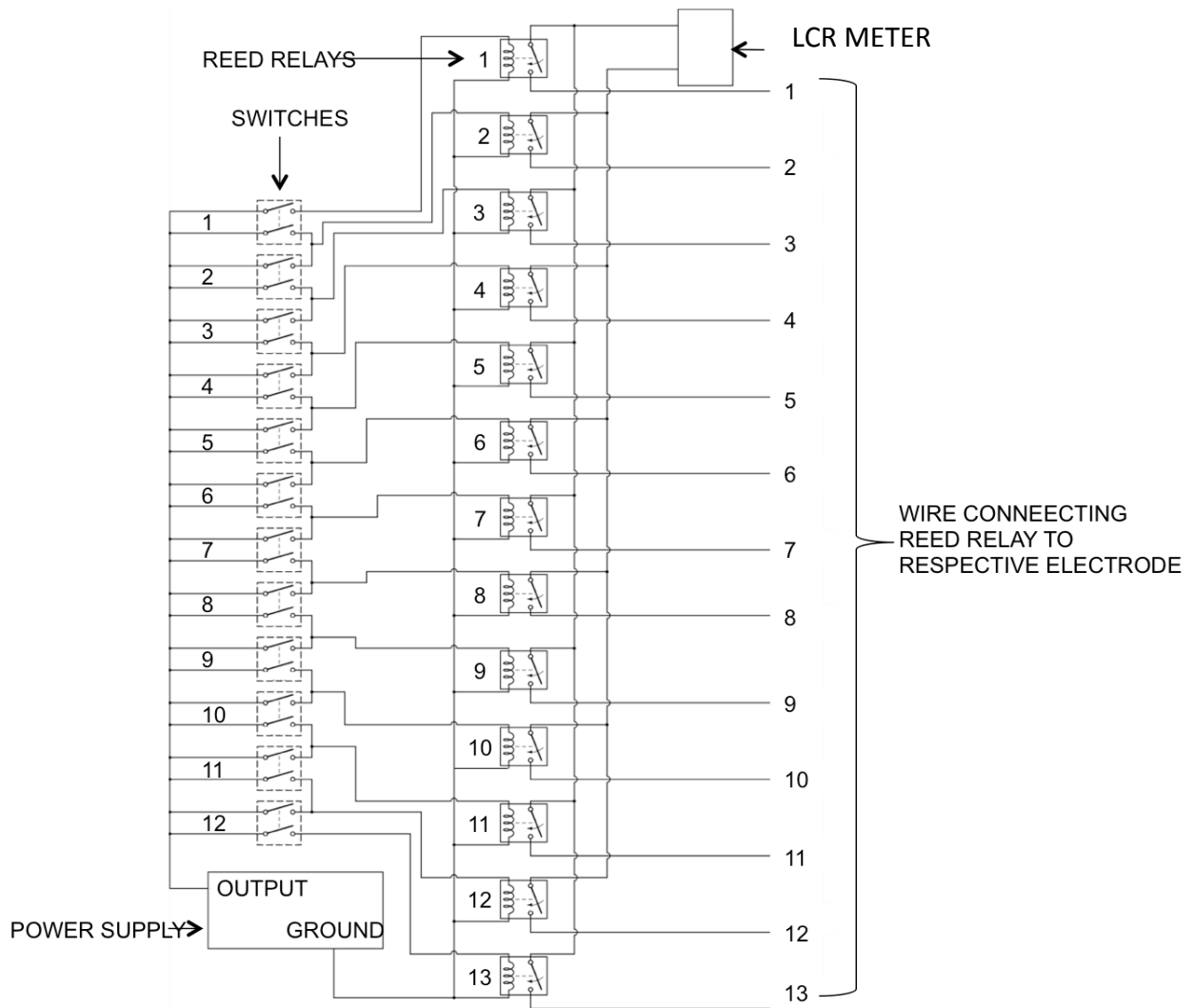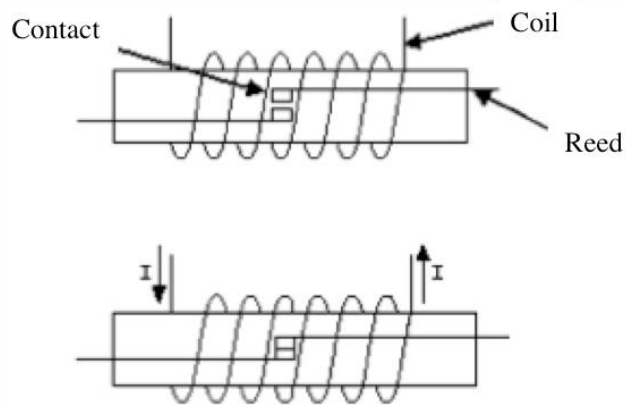
Figure 13. Schematic diagram of circuit board.

Figure 14. Reed relay schematic diagram. ("Reed Relay Protection")

Each reed relay switch was connected to a corresponding electrode. The first reed relay was connected to the top electrode and the thirteenth reed relay was connected to the bottom electrode. Every other reed relay was connected to one of two wires, labeled "even" and "odd," analogous to the number assigned to the reed relay. The two wires were connected to a BK Precision Model 875B LCR Meter.

Radiation source

Radiation was delivered to the meat using a linear accelerator radiation machine. The specific model used was the Varian TrueBeam Radiotherapy System machine at Fairview Hospital in Minneapolis, Minnesota. Alternating potentials accelerated electrons from a hot filament. Once the electrons acquired enough energy, they collided with a block of tungsten, releasing new electrons into a chamber of gas. Collisions between the new electrons and gas particles inside the chamber caused a change in momentum of the new electrons. As a consequence of momentum conservation, photons were then emitted and directed towards the subject.

Data collection

Before the meat was radiated, initial capacitances of each slice were measured and recorded. Then two Grays (Gy) of radiation were delivered to the meat and after waiting 15 minutes to allow stabilization, the capacitance of each slice were measured using a DMM. After the first measurement, another two Gy of radiation were delivered for a total of four Gy. In similar fashion, the capacitance was measured and recorded 15 minutes following the radiation. These steps were repeated until a total of eight Gy of radiation were delivered to the meat.

RESULTS & DISCUSSION

The purpose of this research was to assess one method of measuring biological damage due to ionizing radiation. Figure 15 displays raw capacitance measurements with uncertainty. The legend depicts the amount of total radiation imposed on all twelve slices of beef previous to the measurement. The red oval highlights capacitor reading < 0.1 nF. These measurements are interpreted as a capacitance reading equivalent to zero and are deemed unusable. The reed relays used in this apparatus can develop a residue on the reeds as a result of the inert gas tube not being completely sealed; oxygen reacts with the ferromagnetic material that constitutes the reeds
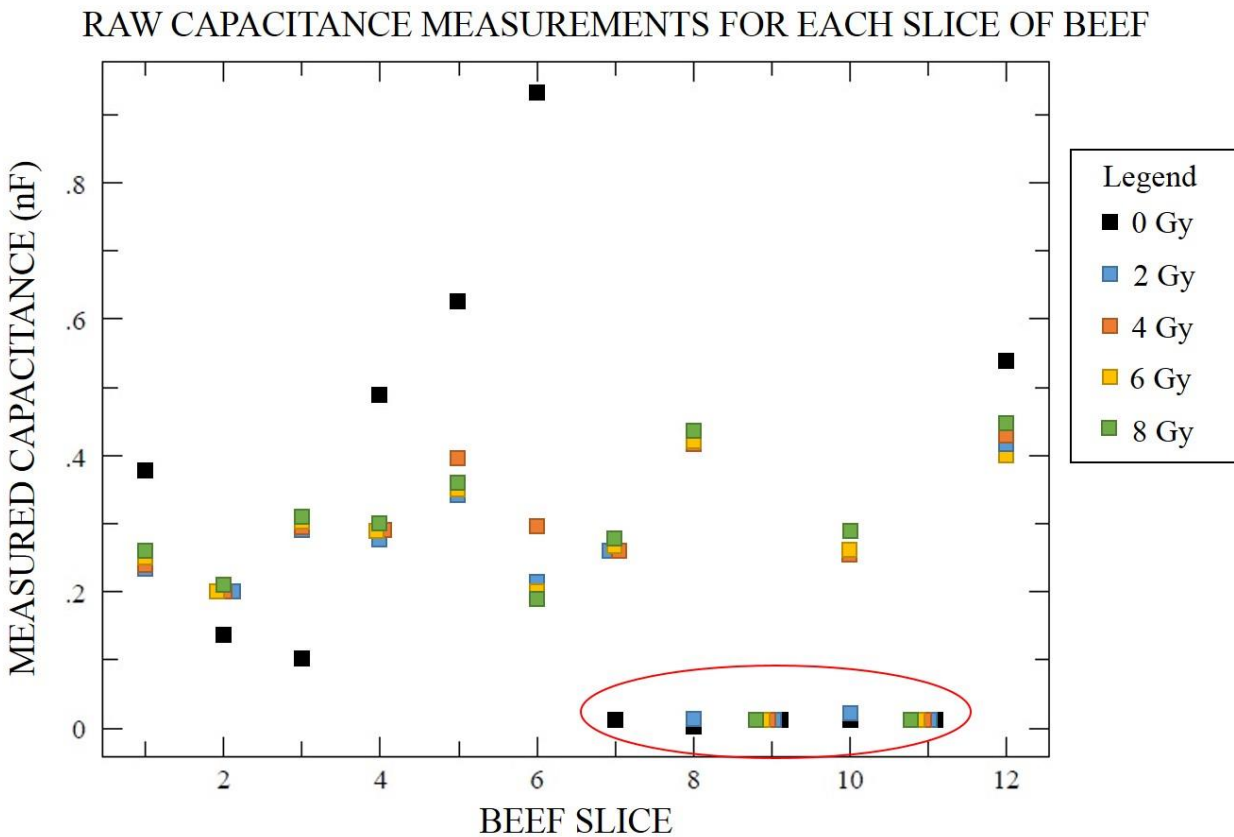

Figure 15. Raw capacitance measurements, uncertainties are included
but are too small to be visibly seen. (n = 12)

and creates the residue. Slices 7 – 11 encompassed such faulty measurements. However, slices 7, 8, and 10 yield usable data after 2 or 4 Grays of radiation were given. This suggests that by closing the read relays and opening several times, the residue was removed, and the reeds could make a connection. These unusable data points are omitted in any further figures or calculations.

In order to evaluate the performance of the apparatus used, the raw data was converted from measured capacitance to dielectric constant using,

$$\kappa = \frac{C \cdot d}{\varepsilon_0 \cdot A}$$

where C is the measured capacitance in farads (F), d is the distance between electrodes (or thickness of slice) in meters (m), A is the area of the electrode in squared-meters (m^2), and ε_o is the permittivity of free space in farads per meter ($F \cdot m^{-1}$). Table 4 reports the values for d, A, and ε_o for the calculations. Uncertainties in dielectric constant were calculated using,

$$\delta\kappa = \kappa \cdot \left(\frac{\delta C}{C} + \frac{\delta d}{d} + \frac{\delta A}{A} \right)$$

Table 3 reports the uncertainties in measured capacitance, slice thickness, and electrode area. Slice thickness and electrode area uncertainties were fractional uncertainties of 12.5% and 10%, respectively. Note that δd is a random error determined by how the butcher slices the beef and that δA is a systematic error since the aluminum electrodes were cut from a single stack.

Table 4. Values and respective uncertainties used in dielectric constant calculations.

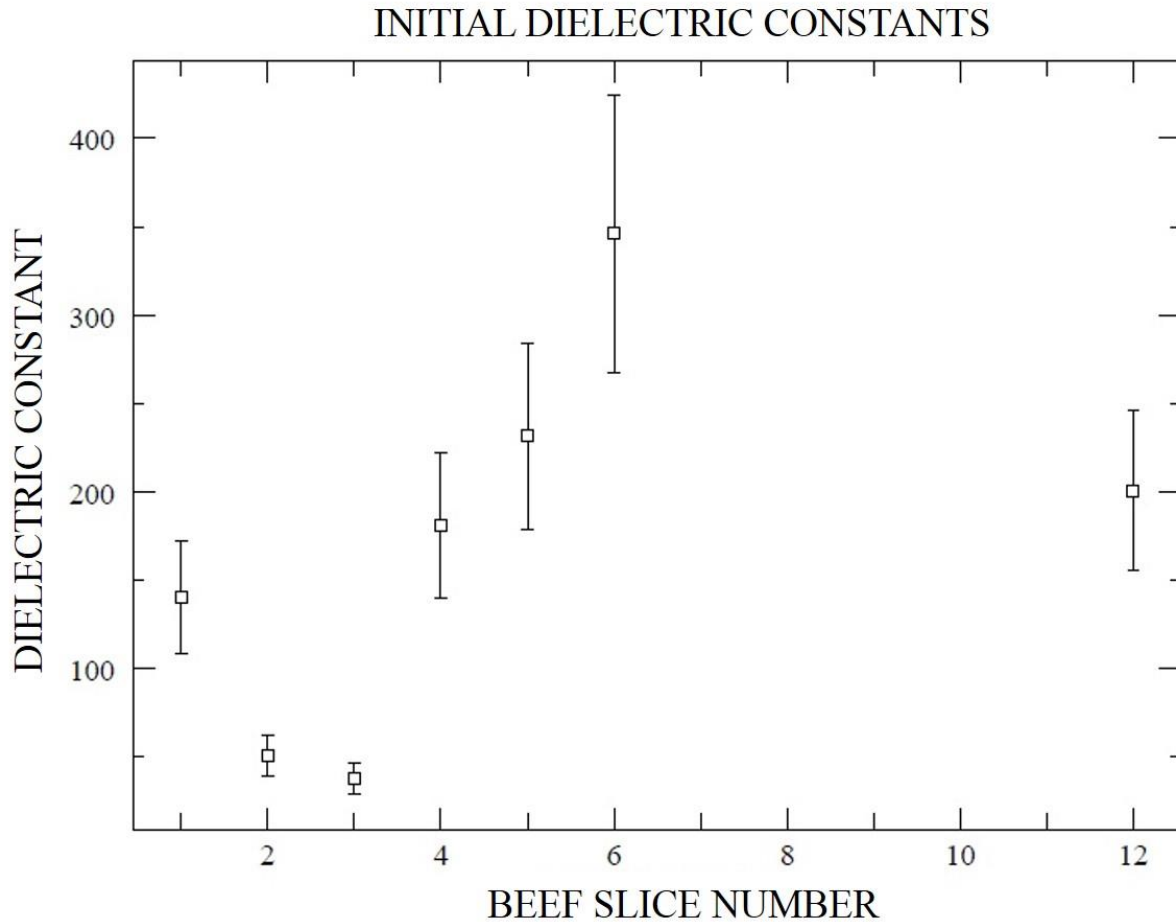| Variable	Value	Uncertainty
Capacitance (nF)	Distinct for every slice	0.001
Thickness of slice (m)	0.0127	0.0016
Area of electrode (m^2)	0.0040	0.0004
Permativity of space ($F \cdot m^{-1}$)	8.854×10^{-12}	

Figure 16. Initial dielectric constants of various beef slices.
Slices 7 − 11 omitted in this figure. (n = 7)

Figure 16 presents the initial dielectric constants of all twelve slices of meat with uncertainties. An important characteristic of the test subject would be to have all twelve slices of beef were comparable in initial dielectric constant. This would indicate the beef slices were of comparable composition and eliminates a source of error in the apparatus. However, as figure 16 shows, not all of the beef slices are comparable within uncertainty. Beef slices 2 and 3 agree within uncertainty of each other, but not with the other slices included in the figure. Furthermore, slices 1, 4 − 6, and 12 agree nicely with each other within uncertainty.

Differences in the dielectric constant of the beef are most likely due to variances in composition of each slice. Slices with a high fat concentration are expected to have lower dielectric constant, about 40 in a 50:50 mix at 20°C. Slices with a low fat concentration are expected to have a larger dielectric constant, about 150-250 in at least a 75:25 mix at 20°C. (Farag et al.) Since the initial dielectric constants shown in figure 16 agree with the expected dielectric constants, the corresponding slices are valid to use in further analysis.

Aside from a few unusable slices, the change in dielectric constant of the beef is still essential to the overall functionality of the apparatus. Uniformity between the slices was important in this analysis and since the initial dielectric constants did not completely agree and the dielectric constants after two Grays did, seen in figure 17, changes in dielectric constants were used to identify other bad readings affected by reed switch failure.
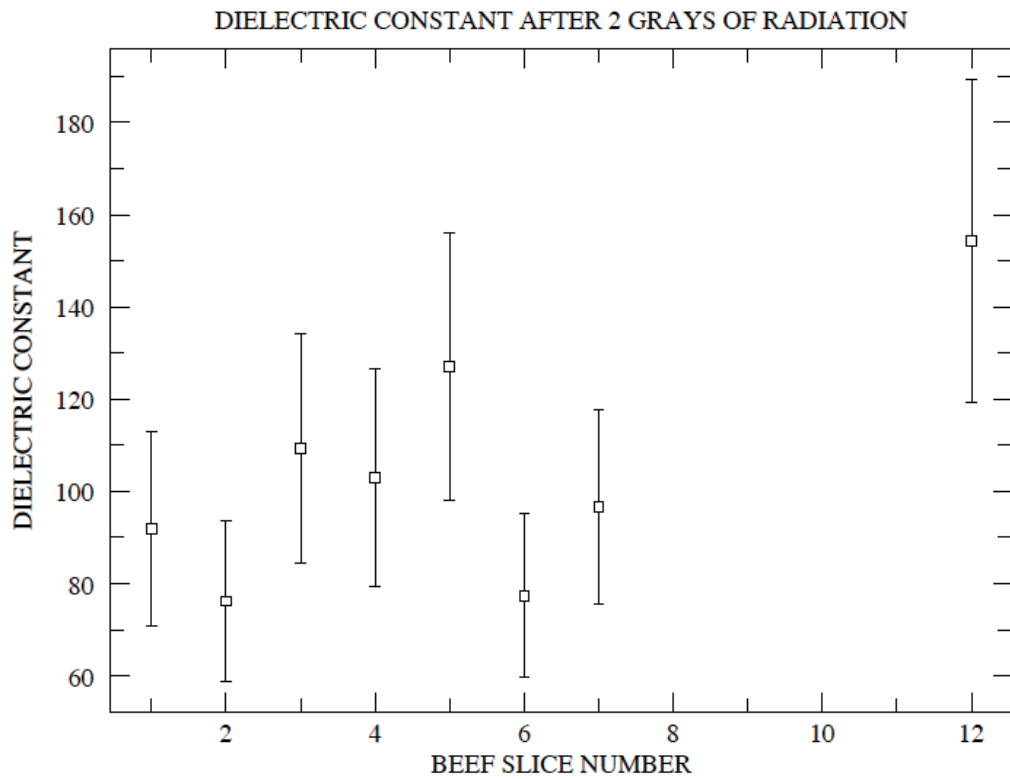

Figure 17. Dielectric constant of various beef slices after two Grays of radiation.
Slices 8-11 omitted. (n = 8)

Changes in dielectric constant were calculated for the valid slices, including slices 1-6, and 12.

The changes in dielectric constant were calculated using,

$$\Delta \kappa = \kappa_n - \kappa_{n-2}$$

where $n = 4, 6, 8$ is the amount of total radiation that has been given. Changes in dielectric

constant for valid slices are presented in table 4. Slices 8 and 10 did not have valid changes in

dielectric constant for $n = 4$ as a result of the aforementioned reed relay temporary malfunction.

This data was used to rule out invalid slices.

Theoretically, when radiation is delivered to tissue the change in dielectric constant

should be a positive change. From table 5, slices 5, 6, and 12 have negative changes in dielectric

constant for one or more intervals of radiation. This might be interpreted as problems with the

apparatus construction for many more slices than initially anticipated. However, using

functional slices, the dielectric constants were fitted to a linear function to obtain $\frac{\Delta \kappa}{\Delta n}$, or the slope

of the fit. Slopes, uncertainties and the reduced chi-squared values for each usable slice are

presented in table 6.

Table 5. Changes in dielectric constant with uncertainty of certain slices.
Recall, n is the total amount of radiation given to the beef.

Beef Slice Number	$\Delta \kappa$ (n = 4)	$\Delta \kappa$ (n = 6)	$\Delta \kappa$ (n = 8)
1	0.17	0.25	0.17
2	0	0	0.08
3	0.34	0.17	0.25
4	0.50	0	0.42
5	4.4	−3.9	0.41
6	−0.08	0.08	−0.17
7	0	0.33	0.67
8	N/A	0.42	0.8
10	N/A	0.08	2.0
12	0.9	−1.3	2.8

Table 6. Slope of linearly fitted function to dielectric constants for valid slices
with uncertainties and corresponding reduced chi-squared.

Beef Slice Number	Slope $= \Delta\kappa/\Delta n$	$\bar{\chi}^2$
1	$0.44 \pm .03$	0.00003
2	$0.06 \pm .03$	0.00007
3	$0.54 \pm .05$	0.00008
4	$0.6 \pm .2$	0.0008
7	$0.7 \pm .2$	0.002
8	$1.3 \pm .2$	0.0003
10	2.3 ± 1.3	0.02

The results displayed in table 5 show the average change in dielectric constant per one

Gray of radiation attainted from the fitted linear function. Note, slice 2 presents a much smaller

slope than the others and is near zero, which is interpreted as slice 2 is nonfunctional and not

used in calculations. The reduced chi-squared values are small as a result of the huge error bars

on the individual dielectric constant quantities used in analysis, which suggest an overestimate in

the uncertainties in the thickness and areas. The weighted average for the change in dielectric

constant per one Gray of radiation was calculated using,

$$\overline{\left(\Delta\kappa/\Delta n\right)} = \frac{\sum \left(\Delta\kappa/\Delta n\right)_i \cdot w_i}{\sum w_i}$$

where the w_i is the weight for a specific slice given by,

$$w_i = \frac{1}{\delta\left(\Delta\kappa/\Delta n\right)_i^2}$$

The uncertainty for the weighted average of change in dielectric constant per one Gray of

radiation is,

$$\delta\overline{\left(\Delta\kappa/\Delta n\right)} = \frac{1}{\sqrt{\sum w_i}}$$

The experimental weighted average of the change in dielectric constant per one Gray of radiation is 0.49 ± 0.03. This value can be used estimate the outcome of research using a functional apparatus to measure the change in dielectric constant as radiation doses are delivered to beef slices.

CONCLUSION

After presenting the experimental results, it can be concluded that the apparatus used in this research is not sufficiently functional. However, this does not indicate that this method is an invalid approach to measure damage due to ionizing radiation. The results presented in table 5, along with a $\frac{\Delta \kappa}{\Delta n} \sim 0.5 \text{ Gy}^{-1}$, would primarily be used in future work as a basis for the range of values to expect if the apparatus is properly functioning. Alterations to the apparatus need to be completed before more testing can be done to check the validity of this method. Alterations include replacing the reed relays, troubleshooting the circuit board and switches for wiring problems and replacing the aluminum foil with carbon foil. The reed relays initially used were inexpensive and resulted in a residue forming as a consequence of the reeds being exposed to air. The circuit board and switch wiring in the apparatus certainly need to be fixed, indicated by the unusable beef slices, namely, slices 9 and 11. These two alterations will potentially fix the hardware problems and give twelve usable beef slices. The purpose of replacing the aluminum with carbon foils is because carbon is abundant in beef, where as aluminum is not. Eliminating the foreign material may also improve results.

Future work would be to improve the equipment in order to obtain larger quantities of data, to adapt the technique to living tissue to gain insight on reparable versus permanent cell

damage, and to potentially derive a mathematical model for the relationship between change in dielectric constant and cellular damage.

ACKNOWLEDGEMENTS

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