THE EFFECT OF SURFACE PLASMON RESONANCE ON BACTERIA MORPHOLOGY

by

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Dedication

I would like to dedicate this thesis to all the little bacterias who continue to live their lives regardless of circumstances.

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ABSTRACT

THE EFFECT OF SURFACE PLASMON RESONANCE ON BACTERIA MORPHOLOGY

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Cells are known to be affected significantly due to persistent exposure to electromagnetic radiation. DNA can be damaged by electromagnetic waves in several ways that may lead to genetic changes. Cellular multiplication plays an important role in the survival of life. This multiplication involves a strand of DNA going through several steps which include proofreading mechanisms to protect DNA from damages. These repair mechanisms range from specific "locate and repair" systems to "damage tolerance" repairs. With many damage events per cell per day the errors that do make it through the repair process are seen as mutations to the genetic code and may be expressed as changes in morphology. Application of the Jaynes-Cummings model

may help to understand these processes in more detail. The interaction of DNA with radiation is studied as an interaction of matter with electromagnetic waves. Through this investigation, the physically expressed response of bacteria exposed to surface plasmons is explored. This approach is used to measure the changes in morphology which may be associated with the interaction of DNA with surface plasmons. After exposure to weak magnetic fields, both the exposed group and the control group expressed similar changes in bacteria size. These results suggest surface plasmons produced in gold may have a larger effect on living organisms than those generated in steel.

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CHAPTER 1:

INTRODUCTION TO THE BIOLOGY OF MUTATIONS

Current advances in the fields of biophysics, biology, and chemistry are constantly pushing for new ways and methods of understanding the increasingly smaller and more complex aspects of biological systems. For this reason new equipment is constantly being developed, such as molecular sensors and biosensors, some of the methods scientists use for observations and making measurements are fluorescence spectroscopy [14] and surface plasmon resonance [46]. These biosensors are and will be used extensively for the foreseeable future and are being entrusted to make precise and accurate measurements and collect vital data. In this thesis I will investigate the effect that surface plasmon resonance will and can have on biomolecules and living organisms. The use of E. coli will be how this investigation will be carried forward. Since surface plasmons produce electromagnetic fields, a study of the effects of these electromagnetic fields will be imperative.

In order to study the effect of electromagnetic fields on the morphology of bacteria, through its interaction with plasmons, we need to understand the bacterial growth mechanism in detail. The bacterial growth is related to the consumption of nutrients by bacteria and its doubling. This process cannot be understood without understanding the chemical composition of cell proteins and its interaction with external fields. Plasmons are a quantum of plasma oscillations and exhibit electromagnetic properties. They interact with the protein molecules and can have an impact on its

structure and morphology. For a deeper understanding of molecular processes, we need to have background knowledge of several approaches used in different fields of science including chemistry and biology, along with physics. In this thesis we mainly apply physical concepts to understand the biological processes. We also use some experimental data and observational results to check our approach. Some of the existing results are used to support our interpretation of these results as well. This thesis is organized as described below.

In the next section we present the cellular composition and the Deoxyribonucleic Acid (DNA) structure in detail and describe the chemistry of DNA and its replication. For this purpose a macroscopic and a small scale (quantum mechanical) approach are taken. The presentation of the following work, from large scale to small scale, is intended to grow in complexity. We will start with the macroscopic structures and develop a more intuitive map of the corresponding relationships of everything as it is decomposed into smaller and more complex and abstract components. The next chapter will then discuss the physical processes themselves relating plasmons and radiation with their effects on biological matter. The final two chapters will describe the experimental process, data, and results along with concluding remarks.

Cellular Structure

All cells, as a basic unit of living organisms, are divided into two categories: prokaryotic cells and eukaryotic cells. Prokaryotic cells can exist as a single unit and make up unicellular forms of life such as bacteria and archaea. Eukaryotic cells make up everything else: plants, animals, protists, and fungi [13]. Both prokaryotic

and eukaryotic cells contain DNA whose code carries all the information about the structure and behavior of the organism. So far no known living organisms have been discovered without DNA [17]. DNA is extremely important to determine how and what living organisms do during their life. The main role of the DNA is to keep information in the form of genetic code.

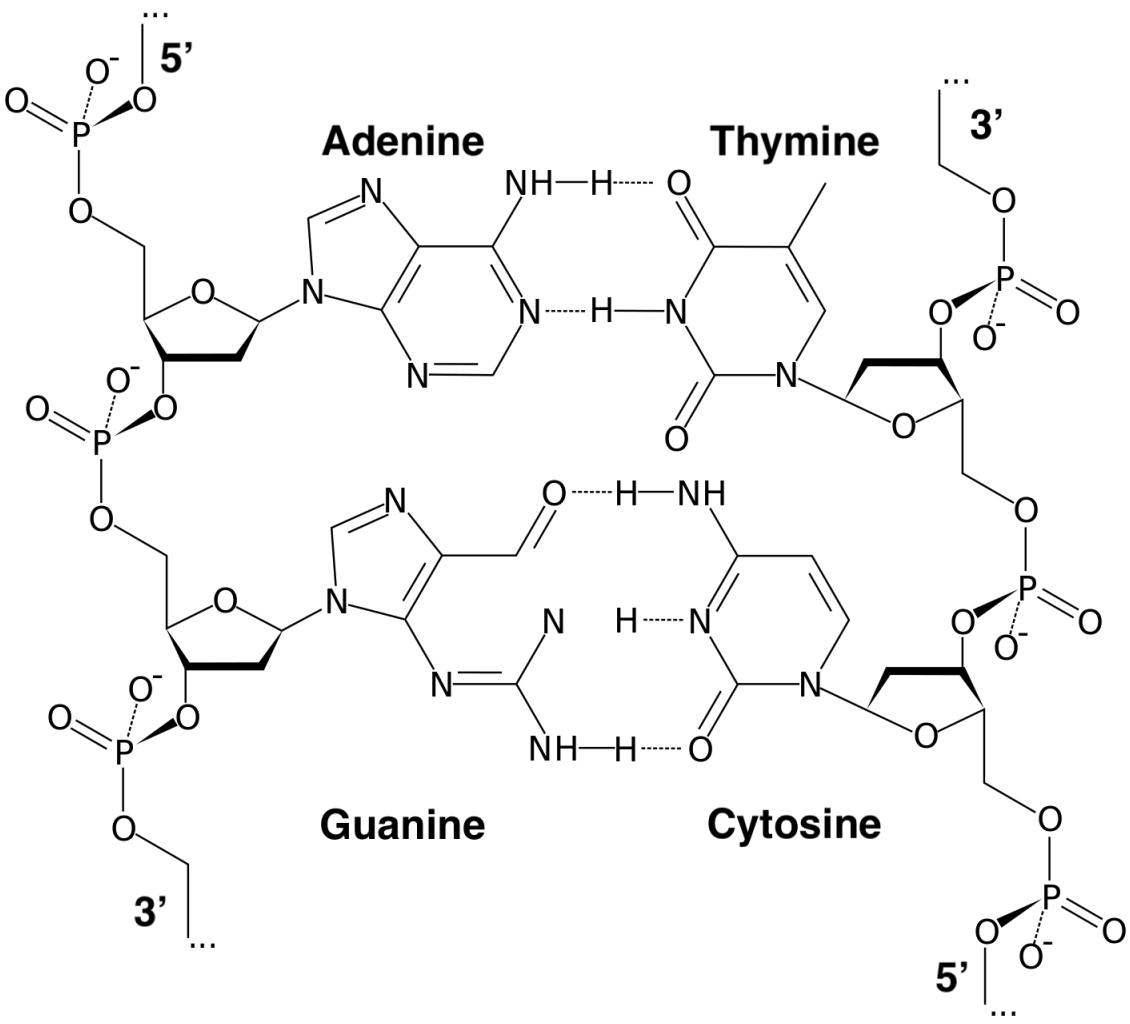
The function of DNA has been under detailed investigation for almost a century. The only known function of DNA is to hold all the information required to keep living organisms alive, such as the protein production processes, gene expression, and allelic variation. Everything that is required for the organism to develop, survive, and reproduce is encoded within the DNA. Central dogma theory is currently the main framework from which the understanding of how the genetic information is contained within the DNA and is accessed and expressed in terms of genes. Restated, central dogma theory states how the flow of information goes from DNA to RNA to proteins.

The significance of DNA has played an important role in navigating the direction of biological research into all the mechanisms, which take place in and around proteins, including DNA. Some of these aspects are the way in which information is encoded, how DNA is produced, how it replicates, how it repairs itself, and how mistakes are expressed. DNA is a unique type of nucleic acid, which is one of the four main organic molecules in all living organisms which is required for life, the other three are proteins, lipids, and carbohydrates. DNA and RNA are both nucleic acids, DNA is the double helix ladder most people are familiar with, more details will be introduced shortly. RNA can come in different shapes dependent on function and is slightly less stable than DNA. Proteins are extremely versatile, having many functions and making up many structures. Currently extensive research is being done in the

field of protein folding [10], the physical process proteins constantly and consistently undergo to acquire their native 3-dimensional structure which is usually biologically functional. Lipids, better known as fats, contain the most energy of all the other organic compounds and play an important roll in cell membranes, hormone signaling, and as steroids. Carbohydrates, also known as sugars, can have their origins traced all the way back to their production during photosynthesis. Used as the main fuel source by the cell, carbohydrates can come in many forms for example sucrose, galactose, and fructose.

The nucleic acid is a polymer made up of monomers. Monomers are a type of molecule that can become chemically bound with other monomers and create long chains. These long chains are called polymers. These individual monomers are called nucleotides, they are each made up of three components. These nucleotides are known as thymine, adenine, guanine, cytosine, and uracil: T, A, G, C, and U respectively. These 5 nucleotides make up DNA and ribonucleic acid (RNA). The three components that bond together to make up these nucleotides are a 5-carbon sugar, a nitrogenous base, and at least one phosphate group. The pairing up of these nucleotides are what give the DNA double helix its structure and encodes all the information for the organism containing it. Shown in Figure 1 are the nucleotides in DNA, they will pair opposite and antiparallel to one another. The strands are said to run antiparallel to one another since they sit side by side and run in opposite directions, the head of one strand is laid against the tail of the other.

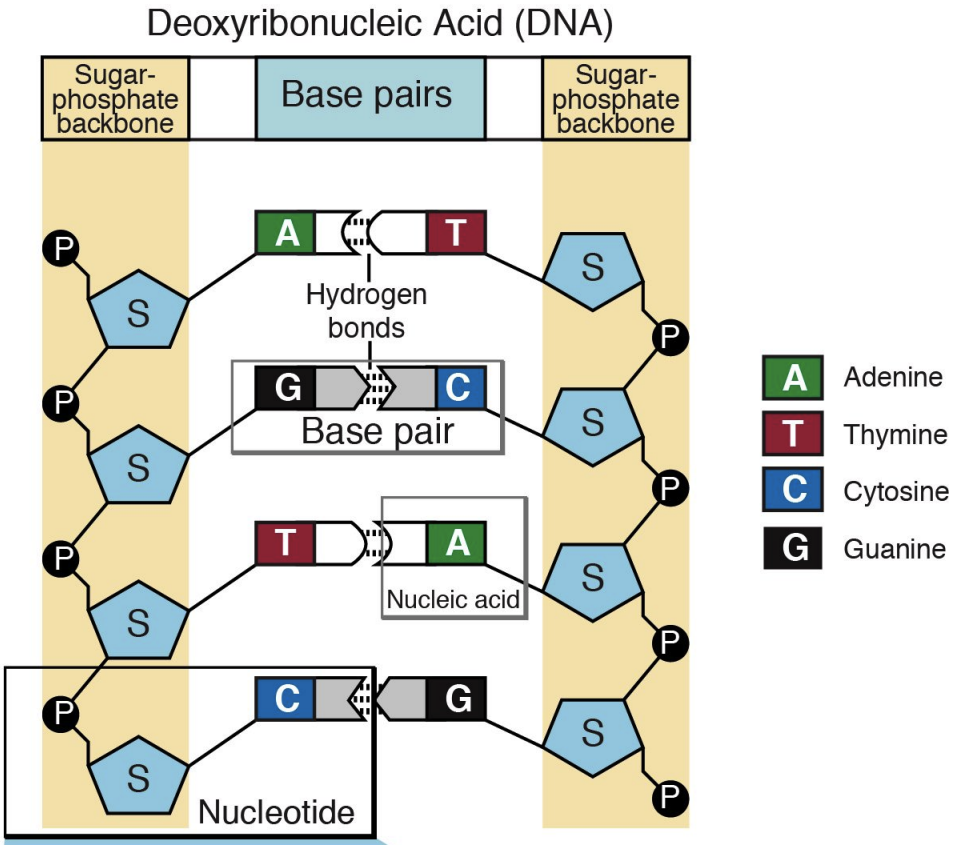
Nucleotides are bonded to one another via chemical interactions known as hydrogen bonds. Although hydrogen bonds contain both covalent and electrostatic contributions, strong evidence implies the primary contributor is covalent [52]. As



https://de.wikipedia.org/wiki/Datei:DNA_structure_formula-de.svg

Figure 1: *A small section of DNA displaying a paired set of nucleotides.*

is seen in Figure 2 the hydrogen bonds reach out from the nitrogenous base of one nucleotide and creates a bond with the other, the correct nucleotide is found due to geometric correspondence between base pairs. Nucleotides can only pair with each other in very specific ways. Thymine pairs with adenine and cytosine pairs with

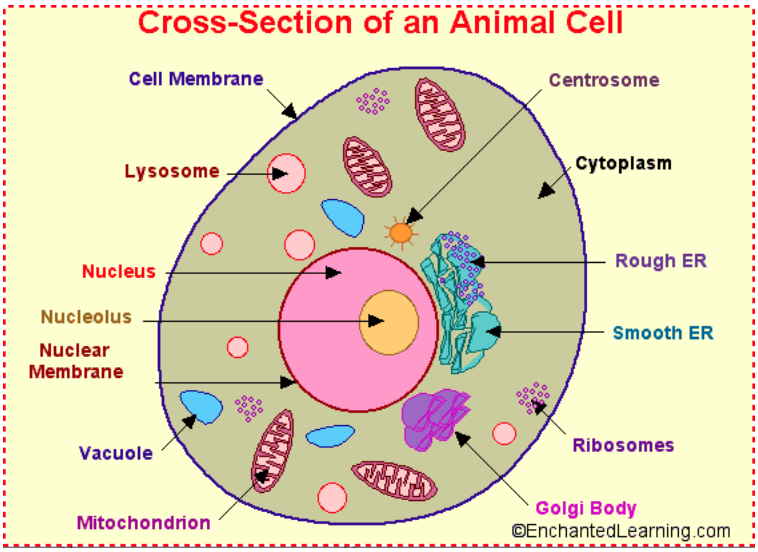


https://knowgenetics.org/nucleotides-and-bases/

Figure 2: *Nucleotides must match in a specific way.*

guanine. In RNA adenine can only pair with uracil and cytosine can only pair with guanine. This pairing is referred to as nucleic acid base pairs or base pairs.

Deconstructing the anatomy of a basic eukaryotic and prokaryotic animal cell will provide a general map of the cells differences and provide insight to its components. The eukaryotic cell is a very dynamic system and contains roughly 12 organelles, Figure 3. All the contents within the cell are collectively called the protoplasm. The cell is enclosed by a **Cell Membrane** which is made of a thin layer of semipermeable protein and fat that allow nutrients to pass into the cell. Within the cell membrane all



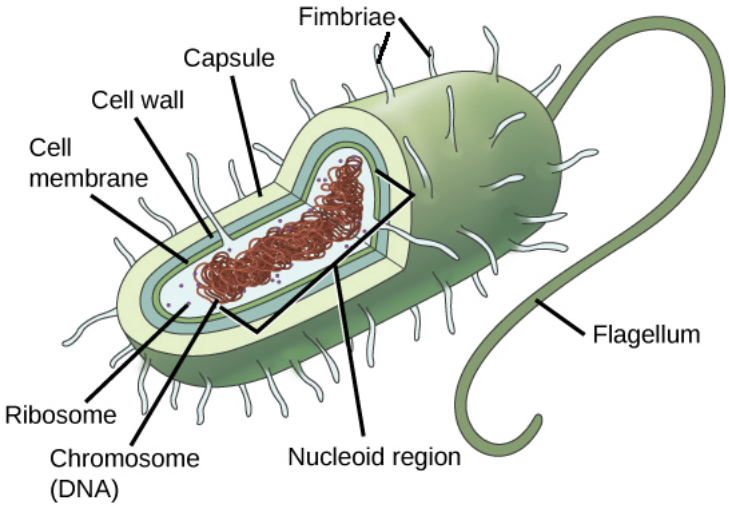
http://sciencewithwindsor.weebly.com/blog/parts-of-cells

Figure 3: *A model of a eukaryotic cell displaying all of its organelles.*

the other organelles reside and participate in the functions necessary to keep the cell alive. The remaining organelles are the **Cytoplasm**, the material outside the nucleus in which the other organelles are located, is where most of the cellular activities take place, the main components of which are cytosol, organelles, and cytoplasmic inclusions. Cytosol is a gel-like substance and makes up about 70% of the cell's volume. The **Golgi Body**, which prepares proteins and carbohydrates for transport out of the cell, also plays an important role in processing proteins for secretions, which contain enzymes that attach sugar monomers to proteins. **Lysosome** contains digestive enzymes (that are imported from the golgi body) and digests nutrients that can break down many kinds of biomolecules acting as the waste disposal system of the cell and can even break-down virus particles. Lysosome is also a part of other cell processes such as plasma membrane repair and cell signaling, any communication process which governs the basic activities of cells. **Mitochondria** (the powerhouse

of the cell!) is responsible for respiration, regulation of cellular metabolism, and converts energy stored in glucose into adenosine triphosphate (ATP). **Centrosome** is the main microtubule organization center and plays a big part as the cell cycle regulator. The **Nuclear Membrane** is made up of two lipid bilayer membranes which surround the nucleus. These two membranes, inner and outer, are punctured by and connected via nuclear pores, large hollow protein complexes. The **Nucleolus** is the site of ribosome biogenesis. The nucleolus also plays a role in the creation of signal recognition particles and partakes in the cell's response to stress. The **Nucleus** controls the functions of the cell by regulating the cell's gene expression and contains all of the cell's genome, better known as DNA. The Nucleus is the control center of the cell. **Ribosome** is the translator where proteins are synthesized. Messenger RNA (mRNA) will provide instructions to the ribosome, via the translation of the genetic code, on how to link amino acids together to form polypeptide chains called proteins. The **Endoplasmic Reticulum** is an organelle that can be broken into two subunits called the rough endoplasmic reticulum and the smooth endoplasmic reticulum. In general the endoplasmic reticulum transports materials through the cell, produces proteins and lipids, and engages in protein folding for transport. And finally the **Vacuole** which is a small enclosed compartment filled with organic and inorganic molecules, including enzymes and water, that play a part in many functions. Some of these functions being a food digester, waste material remover, and isolator of harmful materials.

Prokaryotes, Figure 4, which are simple single-celled organisms that lack a nucleus and membrane-bound organelles, do not fall under the description above. Prokaryotic cells do not have a nucleus but generally have a single chromosome, a circular piece



https://www.khanacademy.org/science/high-school-biology/hs-cells/hs-prokaryotes-and-eukaryotes/a/prokaryotic-cells/

Figure 4: *A generalized model of the prokaryotic cell structure.*

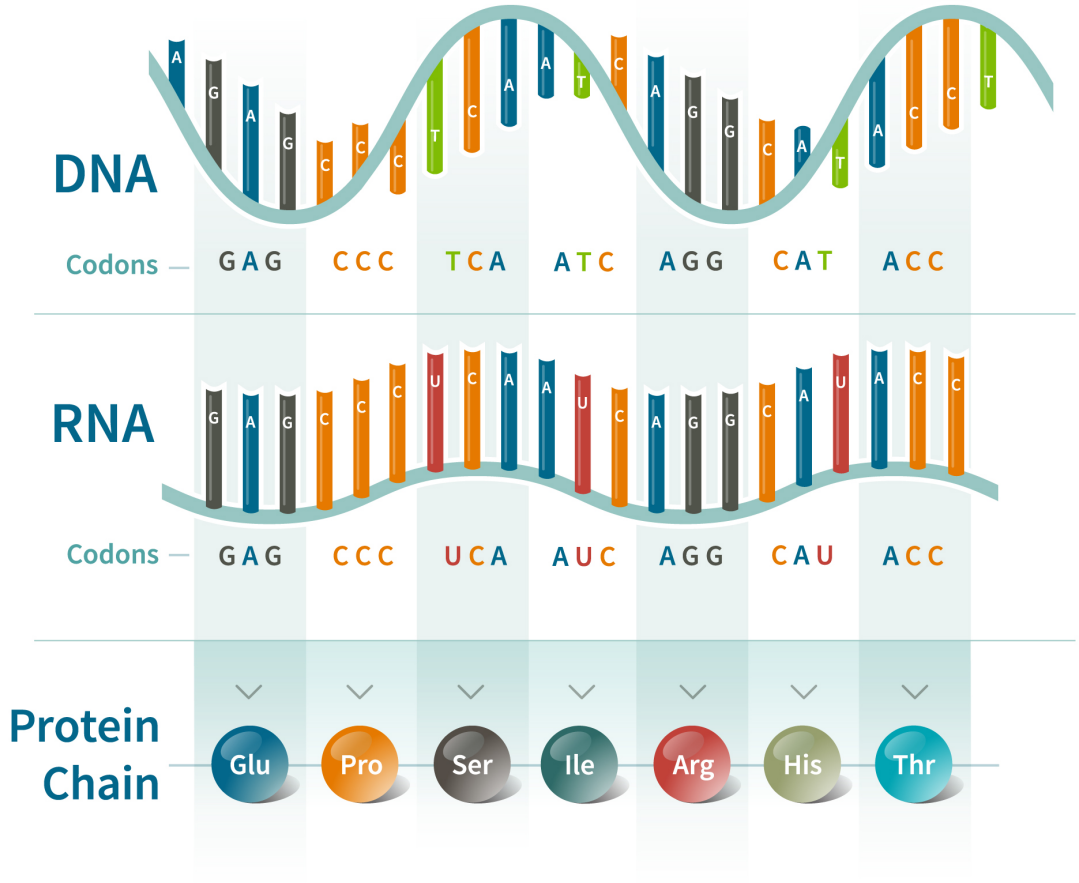
of double-stranded DNA, which resides in an area called the nucleoid. Although prokaryotes can be divided into two domains, Bacteria and Archaea, their basic cell structures are similar. Prokaryotic cells have a **Capsule** which help protect the cell from the outside world. The capsule is covered in **Pili** which are small hairlike structures that allow it to stick to things. Inside of the capsule we have a **Cell Wall**, the composition of which differs between the two domains of prokaryotes and from eukaryotes. The cell wall is there to provide a ridged lay for the cell to hold its shape. Next is the **Plasma Membrane** which regulates what enters and leaves the cell. Within the plasma membrane is the **Cytoplasm** which has the same job as it does inside of the prokaryotic cell. Mentioned earlier the **Chromosomes** are found in the **nucleoid**, an area reserved for the genetic information of the prokaryote. **Plasmids** are also found in the cytoplasm along with the **Ribosome**. Plasmids are circular

gene carrying DNA structures not used in reproduction but are usually exchanged with other prokaryotes in the surrounding environment.

As was mentioned, the DNA of eukaryotic cells resides in the nucleus, that is the command center of the cell. All functions of the cell are controlled by the synthesis of proteins in which the DNA contains all the information. In order for the DNA to function properly there is an entire set of processes in place for replication and repair molecules to constantly check the DNA for any type of mistakes and damage. Damage to DNA is a fairly common occurrence and the cell needs to be ready to catch and repair these damages before those sections of DNA are replicated or are used for synthesizing new proteins. It is important to keep in mind that damage does not mean mutation but can lead to mutation if it cannot be repaired in a timely manner. More details about DNA damages, repairs, and mutations will be given in the following section.

Proteins are molecules made up of chains of amino acids and exhibit the properties of these combined amino acids. The combination of an amino group and acidic carboxyl form the amino acid molecules. The amino group is a compound or functional group which contains a nitrogen atom with two lone pairs, pairs of valence electrons that are not shared with another atom to form a bond. Acidic carboxyl is a part of the carboxyl groups, these are weak acids that release hydrogen ions due to frequent partial dissociation. There are 20 naturally occurring amino acids Figure 6, all of which need to be coded for by the DNA. It was found that the minimum number of nucleotides required to code the information to make one amino acid is three. This group of three nucleotides is called a codon. One or more codons are required to make a protein. A group of codons, whose genetic code contains the information to

produce a protein, is called a gene. In the protein synthesis process a gene provides information which is then transcribed into mRNA by the RNA polymerase and taken to the ribosome for protein synthesis, Figure 5.



https://www.ancestry.com/lp/dna-sequencing/dna-code-codons

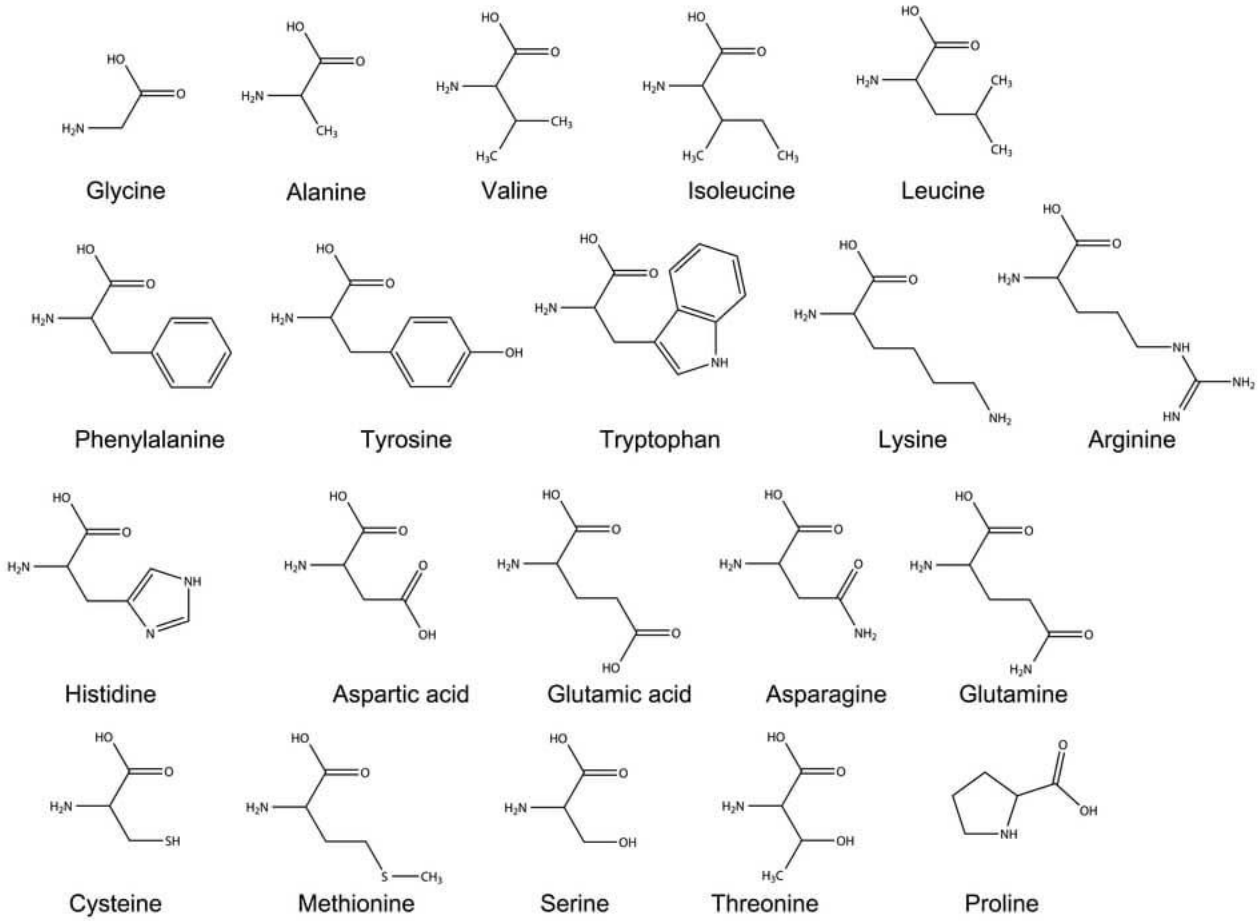
Figure 5: *Depiction of DNA triplets, codons, being transcribed into a protein .*

There are four types of RNA; messenger RNA (mRNA), transfer RNA (tRNA), ribosomal RNA (rRNA), and small nuclear RNA (snRNA). Similar to DNA, RNA is a nucleic acid, but unlike DNA, RNA is usually only found as a single strand. These four different types of RNA each serve a purpose in keeping the organism alive. Messenger

RNA is produced during the transcription process and is the corresponding genetic sequence of a specific gene in the DNA transcribed by the enzyme RNA polymerase. This mRNA is then transported through nuclear pore complexes into the cytoplasm [1] and transcribed and translated by the ribosome into proteins. Once in the cytoplasm the mRNA fits into the ribosome and starts to interact with tRNA. tRNA is a long clover shaped nucleotide chain with an amino acid on one end and an anticodon, a sequence of three nucleotide counterparts, on the other. The anticodon will bind to a specific codon on the mRNA molecule, within the ribosome, then the tRNA will deposit its amino acid and move out of the ribosome. Every tRNA carries a specific amino acid, which when deposited will add to the chain of amino acids left behind by the prior tRNA, building the protein molecule. rRNA is non-coding structural nucleic acid that makes up part of the ribosome and is the most abundant form of RNA in a cell. Finally, snRNA is responsible for converting pre-mRNA into mature mRNA by removing introns from the molecule. RNA molecules partake in many important roles in living organisms as well as viruses, such as coronaviruses, which will encode their genetic information within an RNA genome. The virus then uses the genetic machinery of living organisms to replicate its RNA genome as well as to manufacture the components required to build more viruses, encoded within that same genome.

Deoxyribonucleic Acid (DNA)

DNA is in charge of storing all the information about the structural details and distribution of responsibilities to all components of the living organisms. DNA molecules may undergo several harmful interactions with harmful chemicals and high energy ra-



https://www.purefoodcompany.com/amino-acid-chart/

Figure 6: *A complete chart of the 20 amino acids produced by cells.*

diation. The focus of this thesis is plasmon damage but chemical damage will also be mentioned when needed. When damage occurs, there are steps taken by the cell to assess this damage and attempts are made to repair the damage. Our bodies are constantly repairing DNA damage at an extremely high rate, but all repairs are not successful and not all damage can be repaired. In the case of unrepairable damage the cell undergoes apoptosis, programmed cell death. The consequences of DNA damage

vary in intensity from none at all because repair was successful, to mutation, cell death, or even result in full organism death. Death of a multicellular organism occurs when DNA repair is unable to protect a large amount of cells from dying.

Chemical damage to DNA can occur in a number of ways from multiple sources in one of various times during the DNA replication process or protein synthesis process. Some of the different chemical processes that can damage DNA are caused by biological metabolism, free radicals, heavy metal ions, pesticides, medicine, and oxidative processes. Under certain conditions, during the biological metabolism of the cell, spontaneous molecular isomerization can occur in the DNA base pairs. When this happens the structure of the molecules can change, can shift isomer forms as long as bond energy is roughly equivalent. This means even though the atoms in the molecules remain the same the location of the atoms can change. These new molecules can have roughly the same bond energy and, dependent on the current conditions, can be observed switching between different isomers. This dynamic equilibrium form of isomers are called tautomers. The forming of a different isomer of a given component of the nucleotide can alter the function of the nucleotide or bond strength of the base molecule. This can cause an unwanted break or unexpected change in the nucleotide damaging the DNA at that location.

Free radicals are atoms that have one or more unpaired electrons, become unstable, and react quickly with other substances, stealing their electrons and causing a chain reaction of a potentially destructive nature. Free radicals can occur in different places of the body and affect different aspects of the cells in those locations. Free radicals inside or outside of the cell can steal electrons from the cell membrane weakening and damaging the cell membrane until it destroys the cell. Oxidative damage on the

DNA is caused by free radicals adding an oxygen atom to the DNA bases. Guanine is the most vulnerable base to oxidation, due to free radicals, and is said to have a very low oxidation potential [55]. Cytosine is next in vulnerability to oxidation out of the remaining bases.

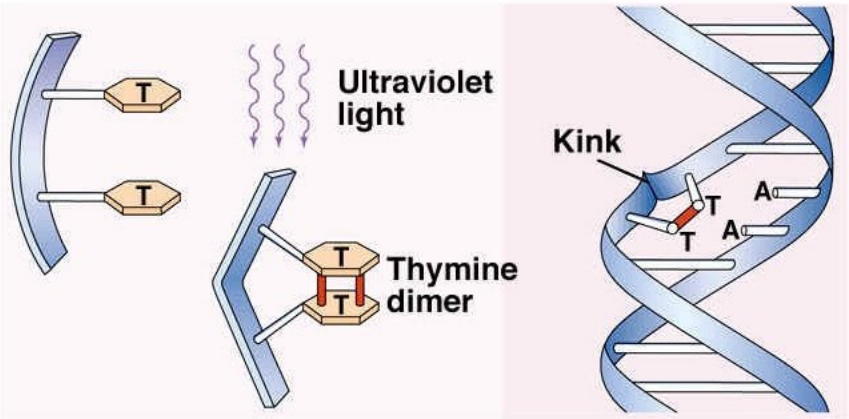
Heavy metal ions, such as arsenic and nickel, are classified as carcinogens since exposure can induce double strand breaks and inhibit certain proteins in the DNA repair pathways [3][29]. Heavy metal exposure can alter how cells repair double strand breaks which will be dependent on the type of metal and the dosage [20]. Heavy metals cause the formation of reactive oxygen species which in turn causes oxidative deterioration of biological macromolecules. Heavy metal ions will also interact with the electron donor groups of the bases which will also alter how they bond to one another. Overall how heavy metals cause double strand breaks and inhibit DNA repair pathways is still unclear [11]. More than 25% of pesticides are classified as oncogenes, where an increase in the number of cancer cases was observed in people in agriculture production using pesticides. Pesticides create free radicals which, similar to heavy metals, produce reactive oxygen species. This can lead to oxidative DNA damage, it can disrupt cellular pathways, cause single and double strand breaks, and inhibit enzymes and receptors [24]. At the base of this process changes in the genetic material are seen from these DNA damage causing pesticides. DNA oxidation is one of the largest contributors to DNA damage with roughly 10,000 events per cell per day. In the cell oxygen requires four electrons in order to be reduced to water (H_2O) and has three different oxidative forms/stages with one, two, and three electrons that can affect the DNA bases. These three oxidative forms, the partial reduction of water, are the superoxide anion ($1e^-$), hydrogen peroxide ($2e^-$), and hydroxyl radical

($3e^-$), one more electron gives H_2O ($4e^-$). Oxidation is a subgroup of the free radical section since these forms of oxygen are all radicals, but it is significant enough to be mentioned separately and will be mentioned again in the radiation section. These are a few of the more obvious ways DNA can be damaged by chemicals, many more exist.

DNA damage caused by radiation will mainly occur from ionizing radiation in which there are two forms, particles and waves. This ionizing radiation can interact with the atoms in the cell in one of two ways; direct (coulomb interaction) and indirect (non-coulomb interaction) [54]. All ionizing radiation can cause damage to DNA. Damage to DNA caused by non-ionizing radiation can also occur, but at a much lower rate and in different ways. Non-ionizing radiation in the higher frequency ranges can cause photochemical reactions and can also produce free radicals that can cause cellular damage. Non-ionizing radiation will mostly produce heat which can also cause damage to occur due to denaturing, easier breaking of bonds, and changes in the characteristics of the molecules. A more in depth view about radiation will be given in the section on radiation.

Ionizing radiation will cause damage to DNA in multiple ways. DNA damage can happen when the ionizing radiation breaks a molecular bond, or produces a free radical [2]. Oxidation can also occur when ionizing radiation enters the cell and initiates the reverse of the reduction of water process, this makes the hydroxyl radical step of the process the most destructive. Oxidation can cause damage to all parts of the DNA strand, the bases, sugars, and phosphodiester backbone. Backbone damage is the most dangerous because it can lead to double strand breaks. Ionizing radiation is a source of chemical damage to the cell, the ionizing radiation comes in causing

chemical changes [34] e.g. breaking chemical bonds and the production of reactive oxygen species [18]. Another way ionizing radiation can cause damage is by causing the production of pyrimidine dimers [5]. These pyrimidine dimers are made up of either thymine or cytosine nucleotides in which consecutive nucleotides form kinks in the DNA strand where hydrogen bonds are broken and form covalent bonds in the carbon-carbon double bonds, Figure 7. Thymine nucleotides have a double bond between the 5th and 6th carbon in the nitrogenous base. When UV light enters the cell it ejects the electron shared in this location breaking this double bond. In order for the carbon to rebalance the valence of this bond consecutive thymine nucleotides will share electrons between the two 5th carbons and between the two 6th electrons and will thus bond with one another. These kinks, which are very stiff, can cause major problems when the cell is trying to replicate.



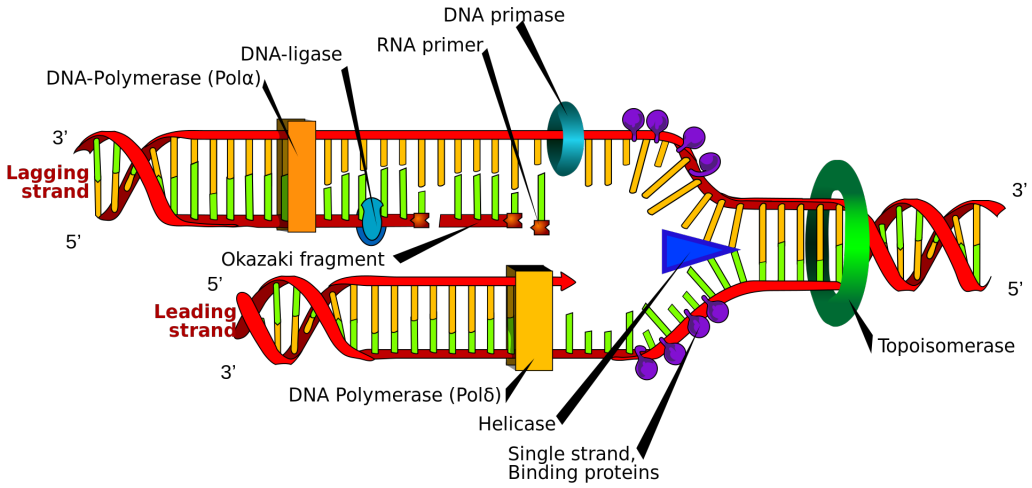
https://www.askiitians.com/forums/Botany/uv-light-causes-1-transition-2-transversion-3_204653.htm

Figure 7: *Incoming UV light causing the formation of pyrimidine dimers in DNA.*

DNA Replication

DNA replication can be broken into three steps; initiation, elongation, and termination. DNA replication happens during the cell division process. This process is necessary for the division of cells in order for each cell to have its own copy of DNA. In cell division the DNA must replicate prior to the division of the cell, this is known as the interphase, which happens right before mitosis/meiosis. When describing DNA replication there are four main enzymes that are key players. These four enzymes are helicase, primase, DNA polymerase, and ligase. Each of these four enzymes carry a different role. In the initiation phase of the replication process specific initiator proteins split the double helix at specific sites and begin pulling apart the DNA to create two parent templates. Replication starts at a location called the origin, eukaryotes have multiple origin points whereas prokaryotes have only one. This origin point is identified by a specific DNA sequence. In order to spit the molecules apart the binding energy of the molecules must be overcome. Helicase, the unzipping enzyme, starts at the origin and travels down the double helix breaking all the hydrogen bonds. This binding energy is overcome by ATP hydrolysis, the breaking of the phosphoanhydride bond lowers the activation energy required to break the DNA nucleotide bonds separating the strands. The process is thought to make use of an active unwinding mechanism [22] which indicates that the helicase applies a force between the separating DNA strands, forcing them apart, while ATP helps lower the energy required to force the two strands apart, thus breaking the bonds. While traveling down the DNA strand another enzyme, topoisomerase, works to prevent supercoiling of the DNA. Supercoiling is the over- or under-winding of the DNA strand and references the

strain on that strand. Single stranded binding (SSB) proteins are then deposited on the open nucleotide binding sites to keep the strands separate. Next primase makes RNA primers on each strand going from 3' - 5' on the template strand. DNA strands run antiparallel to one another and are categorized as the **leading strand** and the **lagging strand**.



https://teachmephysiology.com/basics/cell-growth-death/dna-replication/

Figure 8: *DNA double helix undergoing replication.*

DNA polymerase begins to build the new strand running from $3' - 5'$ on the template strand or building $5' - 3'$ on the daughter strand. Since the leading strand is built continuously from $5' - 3'$, as in Figure 8, the DNA polymerase can continue to move forward unbroken while the DNA is unzipped. The lagging strand continuously requires a new primase to set an RNA primer and a DNA polymerase to then build the next section of DNA, leaving small gaps in the DNA sequence. These partially built DNA fragments are called Okazaki fragments. Finally, on the lagging stand

side, the RNA primers need to be replaced by DNA bases and ligase moves in to close the gaps between Okazaki fragments.

The quantum mechanical description of this is as follows, the electrons in the molecules are in their bound states requiring energy $h\nu = E_n - E_m$ to be bumped into higher energy levels. Electrons in higher energy levels could weaken bonds causing them to break. If the incoming energy is sufficiently high or the activation energy is sufficiently low then the electron will be knocked out of the molecule altogether causing the bond in the molecule to be broken. In this scenario both the hydrogen bonds between the nucleotides and the covalent bonds constructing the phosphodiester backbone can be modeled this way. The electron is bound to very discrete energy levels due to the discrete nature of the molecule energy levels where $V_{min} < E_e < V_{max}$, where E_e is the energy of the electron. Once $E_e > V_{max}$ the electron is ejected from the molecule.

$$\frac{-\hbar}{2m}\nabla\Psi(x) + V(x)\Psi(x) = E\Psi(x) \tag{1}$$

Here $\hbar$ is the reduced Plank constant, $\Psi(x)$ is the wave function of the system dependent on distance x, $V(x)$ is the potential of the system, and E is the total energy. Starting with Schrodinger's equation we can use the finite square well potential to model this interaction. In this interaction

$$V(x) = \begin{cases} V_0, & x < r \\ 0, & -r < x < r \\ V_0, & x > r \end{cases} \tag{2}$$

where r is a given distance dependent on the strength of the potential. Anywhere outside of this distance and the electron continues moving on its own, if inside of this distance it falls into the potential well, in this case creating a bond. In order for the bond to be broken the electron needs to be given an energy $E > V_{max}(x)$ which will kick it out of the potential well. The incoming energy required to remove an electron and break the bond is the binding energy. As DNA helicases unwind the DNA SSB proteins move in and bind with all the open nucleotides which are later removed in the DNA synthesis process. This again can be modeled as a moving particle with $E < V_i$, as the particle moves past a potential it will fall in and form a bond, where V_i is a potential bonding site with specific discrete energy levels. Once the daughter strands begin to take shape, the DNA has entered the elongation phase, elongating the daughter strands as the parent strands are pulled apart.

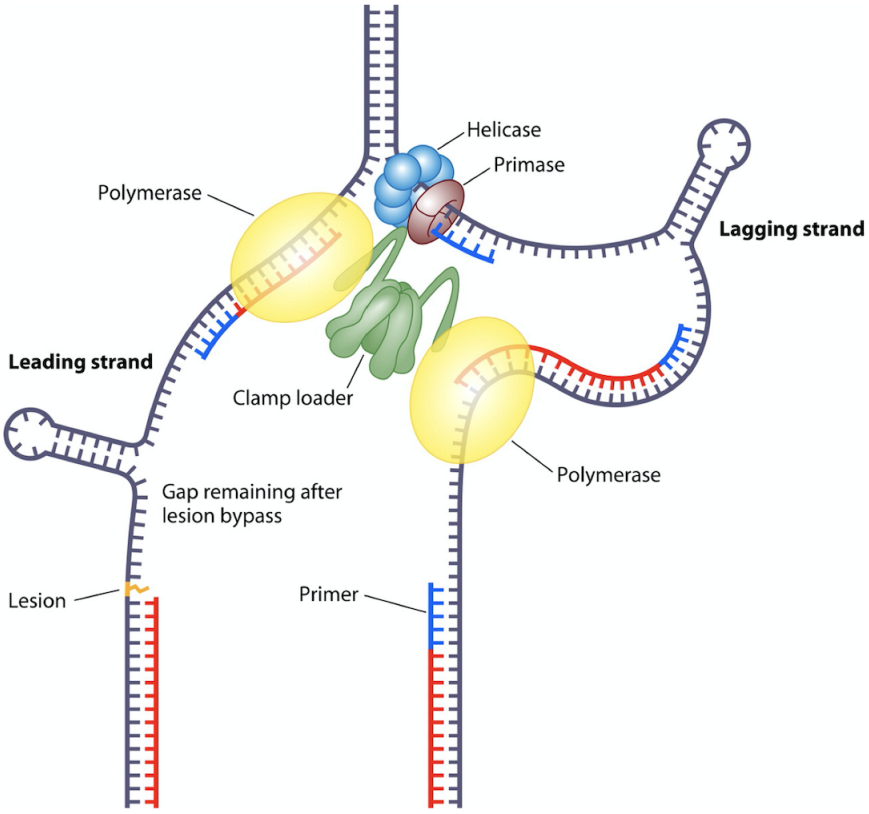
Finally, the termination phase, is the point at which the two daughter strands are complete copies of the original parent strand. By the end of replication there should be two identical strands of DNA molecules.

DNA Repair Mechanisms

With all the different types of DNA damage possible there are DNA repair mechanisms in place to ensure the repair and correction of any damage. For the amount of damaging events, an estimated 1,000,000 per day due to outside sources [25], not including mistakes on behalf of the DNA replication process, that take place only about 1 in 1000 - 10,000 make it through the repair process and lead to permanent change in the DNA sequence which can be propagated as mutations. Many of the DNA repair processes evolved to use the double helix structure to their advantage by using the other half of the DNA strand as a template for the damaged side. This only works if one half of the strand is broken, if a double strand break occurs other pathways are needed. Defects in DNA repair proteins, especially in humans, can lead to the development of diseases which can be characterized by increased occurrences of cancer. If these defects do exist, the frequently produced defected cells in certain parts of the body, then these mutated cells can inhibit the required cellular production needed to continue their growth in a living body, these cells can be identified as cancer cells. An example of this is the two varied copies of the melanocortin 1 receptor (MC1R) gene that people with red hair carry. These varied genes allow for the development of cancer causing genetic mutations to take place at a rate 42% greater than those who do not carry this variation [36]. When MC1R is activated, it produces a pigment called eumelanin. Individuals with darker skin and hair produce a lot of this pigment and are provided UV protection. Individuals carrying this MC1R variant produce a different pigment called pheomelanin, which doesn't allow the skin to tan very well or protect the DNA from damages caused by UV exposure. Usually after UV exposure

the body produces a hormone called α-MSH that binds to MC1R, this complex then binds to the tumor suppressor protein PTEN. People with the MC1R variant produce a mutated complex which cannot bind to PTEN, this allows the cells to grow and divide more quickly [7], which can lead to cancer.

The post-replication mismatch repair system seeks out mismatched nucleotides and replaces the incorrect nucleotide with the correct nucleotide. Mismatch repair in living cells has a mutation rate of about 1 in 10^9 base pairs, this includes pre-replication proofreading and post replication mismatch repair. In all organisms there are two types of proteins which play a role in the mismatch repair mechanism, Mutator S (MutS), mismatch locator, and Mutator L (MutL), mismatch repairer. When these two proteins are inactivated in organisms, ranging from bacteria to humans, high rates of mutation are observed. In order for this process to work well first the mismatched pair must be recognized and then there needs to be a differentiation of the newly synthesized DNA strand from the original. MutS is responsible for the first step in this process, being able to identify almost all mismatched pairs. This is done in three steps (1) MutS attaches to the DNA and bends it in search of a mismatch, (2) it kinks the DNA, when a mismatch is found, by undergoing a conformation, (3) MutS unbends the DNA upon a further conformation to prepare the site for repair [48]. At this point, how the next step occurs differs from organism to organism, essentially MutL, or other versions of the MutL proteins like MutLalpha and MutH, help trap MutS at a DNA mismatch location as well as step in and begin the repair mechanism identifying the newly synthesized DNA strand from the parent strand [33] [15]. In eukaryotes it is still not yet well understood how the different strands are distinguished from one another.



https://mmbr.asm.org/content/74/4/570#sec-2

Figure 9: *Hairpin formation in DNA during replication.*

The mismatch repair system can also catch mistakes made via insertions and deletions. These insertions and deletions occur in almost the exact same way but depend on the strand. A deletion happens when the parent DNA strand is being replicated and there is a hairpin effect in the DNA, Figure 9. This hair-pinning effect occurs when a newly separated single strand of DNA bends and bonds with itself [4]. The DNA polymerase can accidentally skip past this hairpin and continue synthesizing the new DNA strand sans the hair-pinned segment. If the opposite occurs, hair-pinning in the newly formed DNA strand, the DNA polymerase can

repeat a section of the same template region of the parent strand, this is known as insertion. Together these processes are called slipped-strand misreplication and are recognized by MutS. Once recognized the mismatch repair system begins degradation of the newly synthesized DNA strand to replace it with a corrected version. Defects in the mismatch repair system of an organism expresses itself as an increased rate of spontaneous mutation. These mutations in humans have a predisposition to cause various types of cancer.

The mismatch repair system is more of an error repair system whereas damaged regions of DNA are repaired by excision and resynthesis or reversal. Reversal is a literal attempt by specialized enzymes to reverse any unwanted modifications made to the DNA. Alkylating groups, which are potent modifiers of the structure of the DNA bases, can add methyl groups to the DNA bases or DNA backbone. Usually this is a normal process for adenine and cytosine but when this happens with guanine it can be very damaging . This damage is expressed in the form of point mutations, the methyl group attached to the guanine ring will cause the nucleotide to mispair with thymine [31]. In order for this damage to be repaired alkyltransferase, repair protein, removes the methyl group, reversing the damage, and binds it covalently to a cytosine residue in the alkyltransferase active site. Once attached to this site the repair protein is inactivated due to the irreversibility of the methylation. If the gene for the repair protein alkyltransferase is removed or deleted a higher susceptibility of growing tumors arises in organisms, after being exposed to alkylating agents.

The repair mechanism responsible for the most DNA repair is the excision and resynthesis system. Excision repair cuts and removes damaged components of the DNA and replaces them with undamaged components. Excision repair is broken

into two types: base excision repair where a single nucleotide is damaged and needs replacement; and nucleotide excision repair in which a larger chunk of the DNA strand containing multiple nucleotides is damaged and needs to be replaced. In both cases the complementary undamaged strand of DNA is used as a template for inserting the correct sequence of nucleotides. A fairly common and simple way the DNA can be damaged is the breakage of a glycosidic bond which in turn leaves a gap in the DNA, abasic site. Hydrolysis is a simple example of how this bond can be broken, the addition of a water molecule to the glycosidic bond will produce two monosaccharides. When this happens the apyrimidinic endonuclease is the enzyme which initiates the repair process by cleaving the phosphodiester backbone at the abasic site and filling it in by repair DNA polymerase.

Lesions that distort the DNA need to be repaired a different way. Distortion can occur from the formation of pyrimidine dimers, which can usually be reversed by photolyases, except for in placental mammals. In this case and in the other living organisms the main repair pathway used is the nucleotide excision repair pathway to remove an entire section of the DNA about 10 to 30 nucleotides long and replace it with a newly synthesized piece. As can be seen both methods are very similar in that they continuously scan the DNA for any damage, excise the damaged DNA strand on the phosphodiester backbone, and refill the single strand empty site with a new piece of DNA. Nucleotide excision repair can also be broken into two types to help differentiate the timing of the repair process and how they differ in the proteins used to recognize DNA damage. Global genomic repair is happening constantly, scanning the entire genome for any damage to repair. Transcription-coupled repair is only responsible for locating and repairing any damage in the DNA that is stalling the

RNA transcription process. Defects in the different components of the nucleotide excision repair system express themselves as an increased mutation rate and other diseases. This can include increased incidence of cancer and premature aging due to UV exposure from sunlight which is the main source of pyrimidine dimers.

Translesion polymerase is another repair method which allows the DNA to continue replication at an unrepaired lesion without the restoration of the DNA at that point. This repair method is much more error prone with an error rate of about 1 in every 100 - 10,000 lesions. This method is more accurately considered a "damage tolerance" repair mechanism than a complete repair mechanism [38]. This bypassing method although much more prone to errors during replication, is a better alternative than no replication at all. These last few mechanisms are specific repair mechanisms which search for and repair specific types of damage. Next is the DNA damage response which takes care of extensive damage to the DNA.

DNA damage response halts the cells cycle while repair is completed. This damage response is brought about by single or double strand breaks of the DNA. These double and single strand breaks can lead to multiple outcomes such as stalling of replication, collapse of the replication fork, and single stranded DNA to name a few examples. This type of damage in multicellular eukaryotes can lead to apoptosis of the cell, programmed cell death, although in single-celled organisms division will resume even if the damage can not be repaired. The DNA damage response differs in prokaryotes such as bacteria and eukaryotes such as mammals. In bacteria one of the better understood damage responses is the SOS response. Eukaryotes do not have an SOS response, instead their DNA damage response systems are much more complicated involving sensor, regulator, mediator, and effector proteins. The SOS

response in bacteria activates more than 40 other genes which are responsible for the production of different repair proteins. These proteins interact with the single or double strand break and repair them. Different proteins bond together to form larger repair complexes that can bond to and repair the areas where single of double stand breaks occur [32]. This constant activation and inactivation of different genes allows the cell to repair the DNA more effectively. Some of these genes inhibit the cell's division and increase the amount of time the cell has to repair the damaged DNA. The SOS response is also responsible for inducing mutagenesis, this is due to a property of the response as more of a damage tolerance than specific damage repair mechanism. In eukaryotes sensor proteins bind to the damaged site and recruit protein regulator kinases which signal the presence of damage. These regulator proteins phosphorylate which in turn activates the mediator proteins that provide binding sites for the effector proteins, the repair proteins. Similar to the bacteria they also recruit proteins which halt the cell cycle to provide a large repair window. But unlike in bacteria the halting of the cell process is generally irreversible since extreme damage to the DNA in one cell can lead to an increased chance of cancerous changes. Thus multicellular organisms developed a specialized system for ridding the body of these damaged cells, apoptosis.

Nucleotide Tautomers

DNA has four nucleotides: adenine, thymine, guanine, and cytosine. Each of these nucleotides has two tautomers. Tautomers are a form of isomers which are dependent on dynamic equilibrium, they have multiple forms and can easily oscillate between both those forms. The purines have amino and imino forms while the pyrimidines

have a keto and enol form. These two tautomers differ from each other in the same way for all four nucleotides, the hydrogen atom in the nitrogenous base can change location. A change from bonding with a nitrogen atom to bonding with an oxygen atom happens in the keto to enol tautomeric shift, while a change from two hydrogen atoms bonded to one nitrogen atom to one hydrogen bonded with each nitrogen atom happens in the amino to imino tautomeric shift. Other than the movement of the hydrogen atoms the reorganization of the bonding electrons also occurs to maintain the correct number of bonds required to hold the molecule together. Initially in the Watson and Crick model of DNA the nucleotides, thymine and guanine, were thought to spend most of their time in their enol forms which hindered progress in developing the model. Jerry Donohue helped provide the correct interpretation for the tautomeric forms to use which lead to the final correct Watson and Crick model of DNA [23]. In this case the keto-amino forms are the most common whereas the enol-imino forms are more rare.

Once paired the keto form is stabilized by the hydrogen bonds holding the two strands of the double helix together. When replication begins and the strands are pulled apart the nucleotides have a higher chance of changing tautomers now that the hydrogen atoms have one free end. These tautomeric shifts are more likely to occur from the keto form of thymine and guanine to the enol form. As the nucleotide shifts between tautomers it can be incorporated back into the daughter double helix in the enol form which in turn results in a mismatch base pair error [50]. An example of this is a tautomeric shift from keto guanine to enol guanine resulting in the pairing of the guanine nucleotide with a thymine nucleotide rather than the appropriate cytosine nucleotide, Figure 10. Because of the transience of this event once the

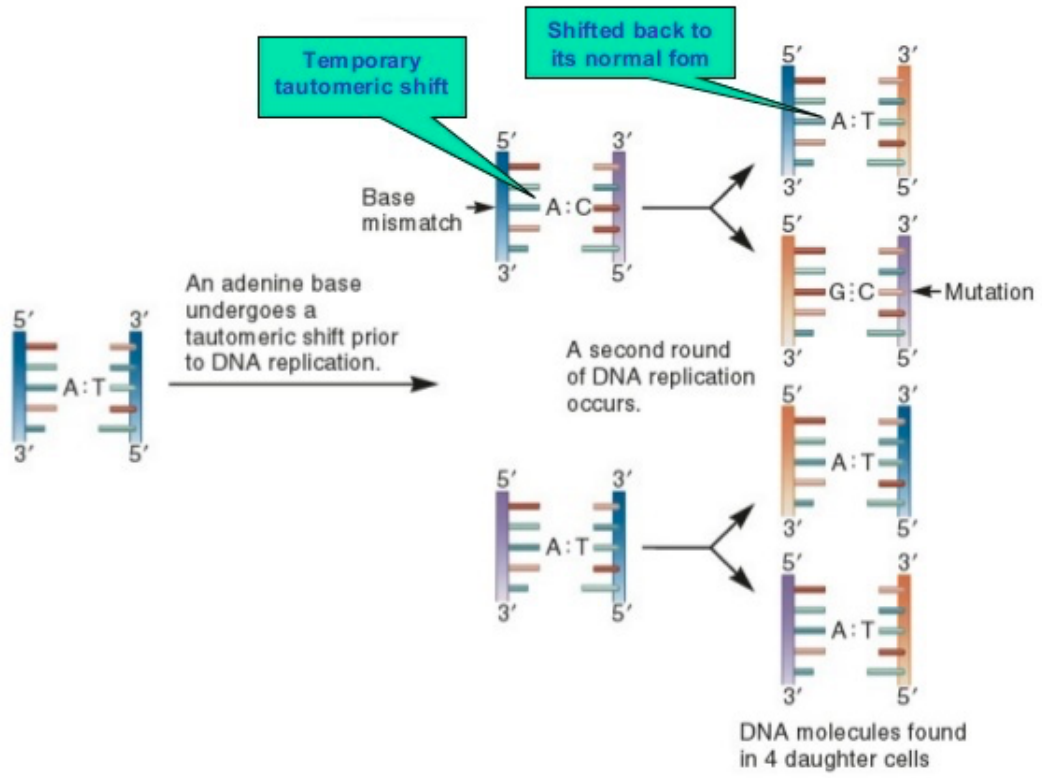


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Figure 10: *Mutation in DNA due to Tautomeric Shift.*

guanine undergoes another shift back into its keto form the mismatch error is noticed and can be fixed, but in other cases this mutation will be passed down to the next generation of daughter DNA. At which point another mutation will occur when the out of place thymine is paired with its correct counterpart adenine. This will result in an adenine-thymine pair where there once occurred a cytosine-guanine pair. Once this pairing results there is now a mutation in the DNA sequence which will continue to be passed down. Analogously the adenine-thymine pair will mutate into an adenine-cytosine pair under the less common tautomeric shifted forms.

One cause of these tautomeric shifts can be a change in pH levels within the system [35]. A change in pH will cause tautomeric shifts as well as protonation of the DNA nucleotides [27]. This protonation can be controlled by tuning the pH of the system. Single stranded DNA can only be protonated below a pH of 4.3 [12]. Protonation and deprotonation is the addition and removal of a proton from an atom, molecule, or ion [59]. Protonation can change many chemical and structural properties such as hydrophilicity and bond lengths [37]. DNA will undergo protonation in a specific order; cytosine, adenine, guanine, where purines bases are the first to become protonated [44]. By picking up an extra charge more damage is sure to result.

CHAPTER 2:

BACTERIA

Bacteria have lived on Earth a very long time, in this time they have constantly undergone evolution due to genetic mutation. These single celled organisms have developed many ways to proliferate in almost every environment found on Earth. There are many different shapes, sizes, and structures of bacteria, because of this multiple ways to differentiate them had to be developed. When it comes to DNA reproduction, repair processes, and damage assessment prokaryotes and eukaryotes share some similarities and have some very stark differences. One example is the ability of the eukaryotes, more specifically a multicellular organism, to cull problematic cells either due to extreme damage to DNA or other issues such as mutations or the loss of function of the cell, known as apoptosis. As a prokaryote, a unicellular organism, apoptosis is simply not an option, the cell must repair any issues as best as possible and then continue to divide.

Because of the ease of working with bacteria there has been and continues to be a large amount of research done using them. Certain strains of bacteria are very well studied and a lot is known about them. A large part of the ease of working with bacteria is their portable and manageable size, their growth rate, and their reproduction turnaround. In this manner bacteria becomes a great candidate for studying the effect that surface plasmons have on living organisms and biomolecules.

Bacteria Shape and Gram Staining

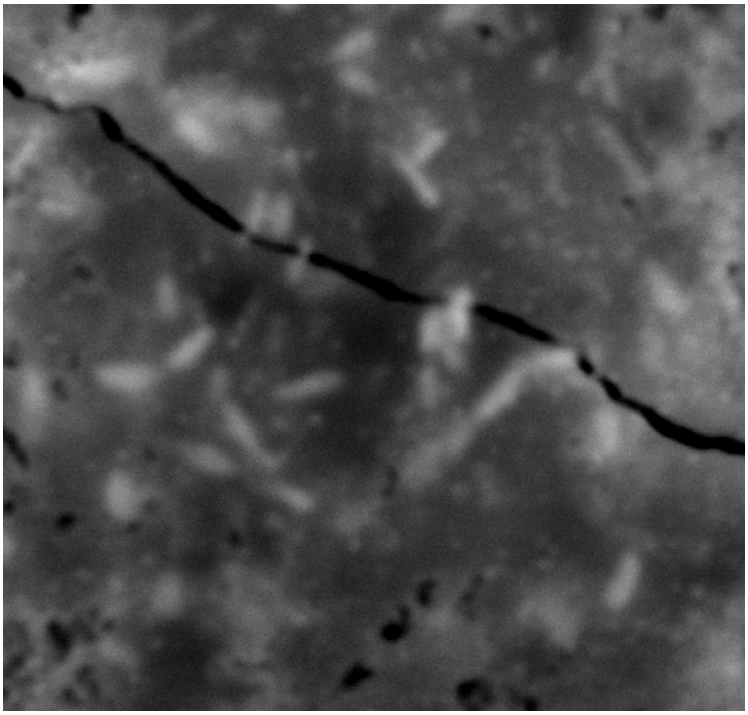


Figure 11: *Escherichia coli (E. coli) is a cylinder shaped bacteria not in the class Bacilli. This image was taken using an SEM at UHCL.*

Bacteria can be identified and categorized in a number of ways such as their cell wall, shape, genetic makeup, and Gram staining. There are three basic shapes bacteria can come in which can often be reflected in their names. The first is in the shape of a cylinder (also known as rod shaped), these capsule shaped bacteria are known as bacillus and are almost exclusively a Gram-positive bacteria. The second are called cocci, these bacteria are round in shape. Finally there is spiral shaped bacteria called spirilla. Bacteria can also come in these shapes and different shapes than those listed above and not be a part of those same taxonomic classes of bacteria. An example is Escherichia coli (E. coli) Figure 11, this is a cylinder shaped bacteria which

can be described as "bacillus" but it is in the taxonomic class Gammaproteobacteria rather than Bacilli and stains Gram-negative.

Bacteria are divided into two major groups when it comes to Gram staining; Gram-positive and Gram-negative. In 1884 Hans Christian Gram developed a technique for identifying bacteria by the composition of their cell wall. This process is known as the Gram stain test and uses a crystal violet dye to stain the bacteria. The test can identify Gram-positive bacteria, if it becomes colored violet, or Gram-negative bacteria if the bacteria does not stain and becomes red. Most other properties of the cell wall are very similar, although their shape and external topography can be different. An example of this is that for both groups of bacteria particles of roughly 2 nm can can pass through the peptidoglycan [8], which is the part of the cell wall which give it its strength and firmness. Peptidoglycan is made up of alternating N-Acetylmuramic acid (NAM) and N-acetylglucosamine (NAG) residues in even amounts and form a structure which has the appearance of a chain link fence.

The staining is a consequence of the thickness of the peptidoglycan in the cell wall and is also dependent on if the cell does or does not have an outer membrane. Bacteria that have outer membranes will also have thinner cell walls and therefore a thinner peptidoglycan layer. Some bacteria have thicker cell walls and therefore will have multiple layers of peptidoglycan, these bacteria do not have outer membranes. If the bacteria does not have an outer membrane the bacteria will tend to have a thicker peptidoglycan layer that will hold onto the crystal violet dye and will stain, Gram-positive. If the bacteria does have an outer membrane then the peptidoglycan layer tends to be much thinner and when the outer membrane is degraded with alcohol,

during the Gram staining process, the crystal violet dye will flush out and not stain, Gram-negative. [41].

Bacteria Life Cycle

Bacteria are asexual and reproduce via binary fission. A bacteria cell will start out small when newly separated from its parent cell. At this time the cell will begin to consume nutrients and grow larger gaining mass. There are many important parameters to consider when a cell is in its growing phase. All of these important parameters can effect how fast a cell grows and when the cell is ready to divide. Environmental parameters are the largest influencers in the bacterias growing stage these can include the abundance of nutrients in the vicinity, the chemical state of the local environment, and surrounding temperature.

The bacteria's life cycle can be divided into four phases, lag phase, log phase, stationary phase, and the death phase. In the lag phase the bacteria do not grow, they spend their time looking for food, adjust to their current environment, and replicate the DNA. the length of the lag phase depends very much on the outside environment, with an overabundance of nutrients the lag phase is very short. Next, in the log phase, the bacteria begin to divide very quickly. In this phase the rate of replication is dependent on the type of bacteria replicating, this is binary fission. In the stationary phase the replication slows, this is due to the new lack of nutrients and space in the surrounding area for the bacteria to grow into. If moved to a new location the log phase can continue almost uninterrupted. Finally, the death phase

the bacteria lose all ability to reproduce and begin to die. The rate at which the death phase takes place can be just as fast as the log phase.

Plasmon Effect on Bacteria

Now more detail will be provided on one of the potential causes of mutation in DNA and more specifically sources of plasmonic effects. Plasmons will arise on the surface of metals and can interact with objects on that surface. A plasmon is a quantum plasma oscillation in a noble metal and can be thought of as mechanical oscillations of the electron gas in metals. The plasmon energy 3 can be found using the free electron model.

$$E_p = \hbar\sqrt{\frac{ne^2}{m^*\epsilon_0}} = \hbar\omega_p \tag{3}$$

where n is the conduction electron density, e is the elementary charge, m^* is the effective electron mass, ϵ_0 the permittivity of free space, $\hbar$ the reduced Planck constant, and ω_p the plasmon frequency. A more thorough derivation will be given in a later section. There are three types of plasmons, on the surface of the metals plasmons are called surface plasmons and their strength is dependent on the angle of incidence of light, within the volume of the metals they are called bulk or volume plasmons, and when confined to the surface of a nanoparticle they are called localized surface plasmons as in Figure 12. The oscillating electric field of the incoming plane wave is what excites the surface plasmons. The higher the angle of incidence the higher the

efficiency of the photon coupling to the plasmon. In order to create a localized surface plasmon the incoming light's wavelength needs to be of comparable size to that of the nanoparticle it is interacting with, this will greatly enhance the magnitude of the electric field near the surface but falls off quickly with distance [16].

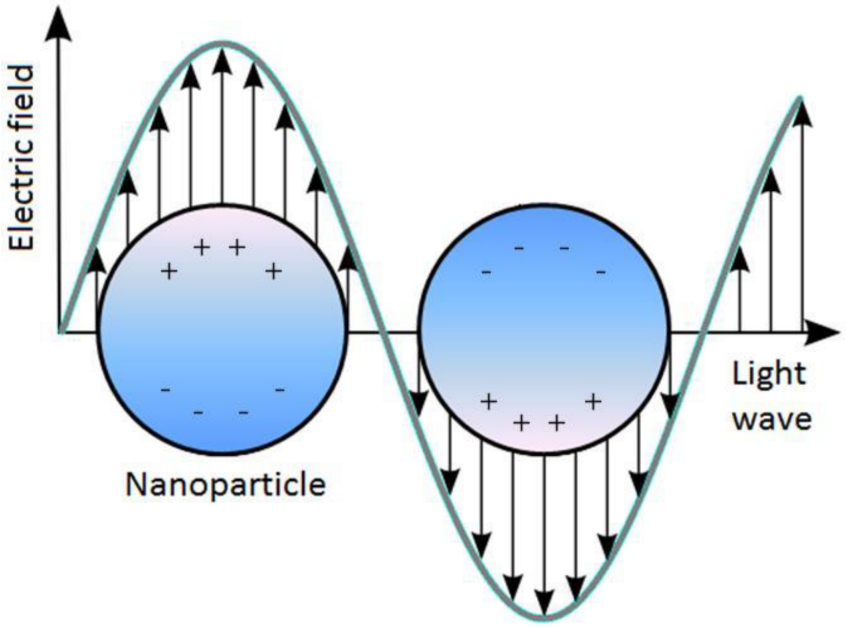


Figure 12: *Localized Surface Plasmon Resonance*

With a recent interest in nano biosensors an increase in the usage of different physical phenomena has been implemented to understand their potential [19]. Surface plasmon resonance has many attributes working in its favor from low cost and high sensitivity [49] to real time detection and tunability. Applications of these optical nano-biosensors have proven useful in areas such as DNA recognition, drug screening, and biomolecular detection [56]. At the current moment the most common method used for nano-sensor technology is a functionalized optically transparent surface to support chemically immobilized metal nanoparticles. The biomolecular interaction

is analyzed using an optical transducer which provides a quantifiable and detectable signal. In his papers [39][40] Saleem demonstrated a gold nano-ripple biosensor, a type of localized surface plasmon resonance sensor, oscillations of charge density on metallic nanoparticles and nano structures, that is produced in a single step using a gas cluster ion beam. In this nano-ripple biosensor the incident light is scattered from the metal showing a peak intensity at the resonant wavelength of the nano-ripples which is dependent on the dimensions of the ripples. Immobilized biomolecules on the surface of the sensor alter the resonant wavelength of the reflected light which acts like a probe and those specific shifts in the peak act as biomarkers that can help identify specific biomolecules. This can also be interpreted as an interaction of the biomolecules with the surface plasmon which then creates these specific shifts in the peak. How these plasmon interactions affects or alters the biomolecules is unclear.

Surface plasmons, when excited by an electromagnetic radiation (light), will be expressed as absorption bands, enhanced electromagnetic fields, and scattered light. Material type, in our case gold but also typically silver or platinum, and other factors such as particle size and shape will be big influencers in the frequency and intensity of the surface plasmon characteristic absorption bands. These surface plasmon sensors are commonly used to detect and analyze biological samples and determine certain characteristics of them. It is known that light [51][47] and magnetic fields [28] can cause changes in biomolecules which are formed in human cells and bacteria. It is also known that the nanoscale electromagnetic fields, confined to the surface and localized, can interact with cells, large biomolecules, and small biomolecules in three different ways [58]. These three ways that the nanoscale electromagnetic fields can interact are optically, mechanically, and thermally. Researchers have been using these

methods to interact with biomolecules in such ways as manipulation, trapping, and transportation. We want to see and measure how these different ways of interacting with the bacteria, cells, and biomolecules affects them. Exposing bacteria to these different forms of interaction may cause a noticeable change in some aspect of the bacteria. In the future these changes could be traced back to mutations in DNA caused by these surface plasmon resonances as well as alterations and disruptions in proteins and protein structures within the bacteria.

CHAPTER 3:

PHYSICAL PHENOMENA UNDER INVESTIGATION

Radiation

Radiation can be categorized into two types; ionizing radiation and non-ionizing radiation. Ionizing radiation is radiation that interacts with matter to form ions, radiation with enough energy to knock electrons out of their orbits in the atoms. Non-ionizing radiation is radiation that does not have enough energy to remove electrons from their orbits in atoms, that does not form ions. These radiation types will be further broken down into two forms: waves and particles, waves includes all electromagnetic radiation whereas particle radiation includes alpha particles, beta particles, and cosmic rays. Non-ionizing radiation will only be composed of low frequency waves such as radio waves, microwaves, infrared, and visible light. Ionizing radiation can be in both forms: waves and particles. Some examples of ionizing radiation are high energy ultraviolet light, alpha particles, beta particles, gamma rays, and x-rays.

Ionizing radiation can have a direct or indirect action on atoms or molecules. Direct action refers to direct interaction with the electrons in the atom or molecule due to coulomb interactions. Indirect action is when the radiation interacts with some initial object, such as the interaction of radiation with water molecules to produce reactive oxygen species or free radicals, the outcome of which causes the ionization

of atoms or molecules. Or simply stated does not interact with the atomic electrons through coulomb interactions.

Electromagnetic Radiation

Electromagnetic radiation is a form of radiation that comes in discrete wave packets called photons. These photons exhibit particle and wave like properties and a better understanding of them was brought about by James Clerk Maxwell as a theory of waves, and Max Plank and Albert Einstein in the energy quantization of electromagnetic radiation. The electromagnetic spectrum has a very large range from very low frequency, long wavelength, to very high frequency, short wavelength. The electromagnetic spectrum is broken into different bands with each frequency band denoted by the differences in transmission, emission, and absorption as well as their different practical applications. These frequency bands, measured in wavelengths, are as follow, in order from longest to shortest wavelength, respectively.

Radio waves ($> 10cm$)

Microwaves ($10cm - 1mm$)

Infrared ($1mm - 750nm$)

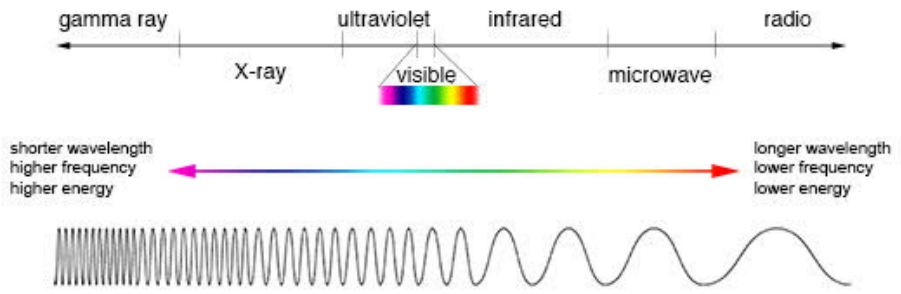
Visible light ($380nm - 760nm$)

Ultraviolet ($380nm - 1nm$)

X-rays ($1nm - 1pm$)

Gamma rays ($< 1pm$)

There is no discrete boundary for the different frequency bands and some overlap does occur, Figure 13. In theory the longest wavelengths can be as long as the universe



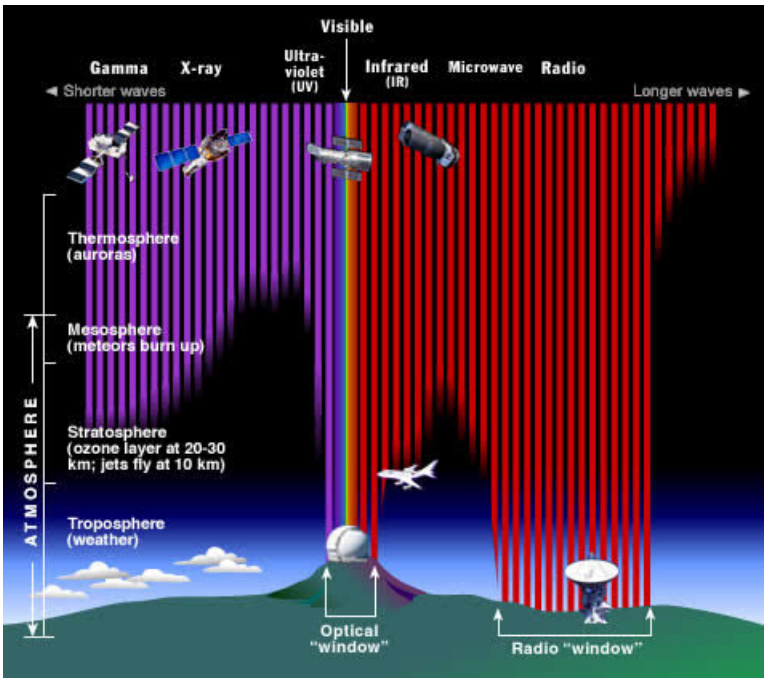
https://imagine.gsfc.nasa.gov/science/toolbox/emspectrum1.html

Figure 13: *Full electromagnetic spectrum with approximate frequency bands.*

and the shortest as short as the Planck length. Not all electromagnetic radiation is ionizing, low frequency radiation like radio waves, microwaves, infrared, visible, and low ultraviolet is non-ionizing. Ionization energies start at around the high ultraviolet range and continue through x-rays and gamma rays.

Many sources are responsible for the production of electromagnetic radiation, some main sources will be listed with their corresponding output. The sun is a major source and produces radiation over the full spectrum. The sun emits radiation most strongly in the visible spectrum with ultraviolet and infrared as close seconds. The sun also emits microwaves, radio waves and x-rays but not with the same intensity of visible light. Although the sun does produce gamma rays in the nuclear fusion process, from the proton-proton chain, these gamma rays are continuously captured and reemitted as lower energy photons before they leave the sun's surface. In space, gamma rays are produced by high energy objects such as pulsars, supernova explosions, neutron stars, and blackholes accretion disks. On earth some sources of gamma rays can be from lightning, radioactive decay, and nuclear explosions. X-rays are emitted from multiple sources as well, and can be divided into natural and artificial. Some natural

sources are the accretion disks surrounding blackholes, supernova remnants, galaxy clusters, and some binary star systems. Artificial sources can be particle accelerators, x-ray lasers, and medical devices such as x-ray machines. Earth's atmosphere does an excellent job at protecting all living organisms from the more harmful types of electromagnetic radiation as can be seen in Figure 14. The Earth's atmosphere blocks electromagnetic radiation in many ways which are dependent



https://imagine.gsfc.nasa.gov/science/toolbox/emspectrum1.html

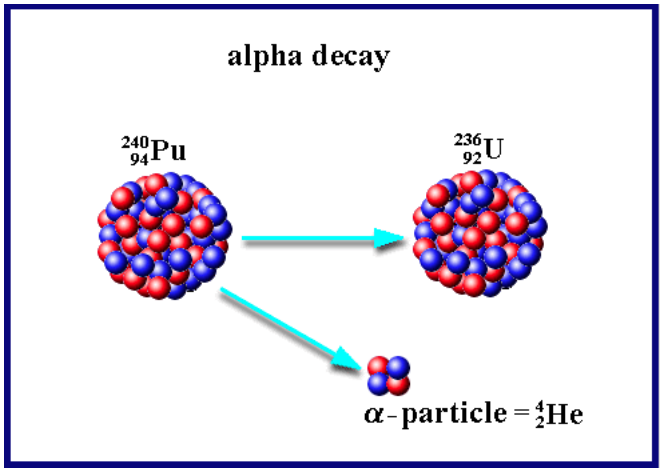
Figure 14: *Approximate depth at which the different frequency bands penetrate the Earth's atmosphere.*

on the different compositional components of the atmosphere. The most well known is the Ozone (O_3) layer. The Ozone layer absorbs light very strongly in the UV band of the spectrum, reducing the amount of UV radiation that would reach the surface of the Earth significantly if it were not there. Gamma rays and X-rays are also absorbed

by different atoms and molecules in the atmosphere which keeps them from reaching the surface of the Earth. We also see 20% of radiation being reflected by clouds and particles floating in the air. About 20% - 25% of radiation is also absorbed by water vapor (h_2O) and carbon dioxide (CO_2). Water vapor will absorb in the 5.5 μm - 7 μm and $>$ 27 μm wavelength. And carbon dioxide will absorb in the mid to far infrared range.

Particle Radiation

Particle radiation comes in the form of atomic or subatomic particles, the sources of which can be from radioactive decay, cosmic rays, and solar events. All particle radiation is ionizing radiation and includes alpha-particles, beta-particles, neutrons, protons, muons, etc.. Charged particles will interact directly whereas uncharged particles will interact indirectly with atoms or molecules. Alpha-particles are produced in radioactive decay in which a larger atomic nucleus emits a helium nucleus and decays into a smaller atom, Figure 15. This happens for elements heavier than nickel where the nuclides are unstable for a spontaneous fission type process
due to the overall binding energy per nucleon no longer being a minimum. Alpha decay usually only occurs in the heaviest elements and is fundamentally a quantum tunneling phenomenon. Beta-particles, high energy electrons, and positrons are also produced by radioactive decay in which an atomic nucleus emits a beta-particle, that causes the nuclide to transform a neutron to a proton with the additional emission of an antineutrino, or neutrino going from proton to neutron, Figure 16. The weak force is responsible for beta-decay, it allows for the quarks within hadrons to change flavor

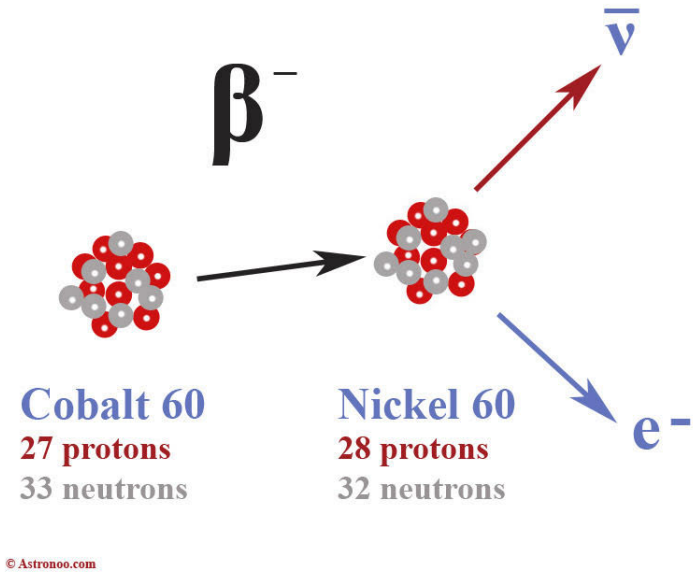


http://www.nuceng.ca/igna/radioactivity.htm

Figure 15: *When an atom undergoes alpha decay it emits a helium atom and loses mass.*

via the emission of a W-boson that, in consequence, creates an electron/antineutrino or positron/neutrino pair.

Cosmic rays are yet another source of particle radiation that shower down on earth in a constant and consistent flow. Cosmic rays can be differentiated into primary cosmic rays and secondary cosmic rays and consist of a large mixture of particles with a wide range of energies, Figure 17. Primary cosmic rays consist mainly of protons and alpha-particles but do include a small amount of heavier nuclei. Secondary cosmic rays, which are created when the primary cosmic rays collide with atoms and molecules in the earth's atmosphere, are made up of photons, electrons, and hadrons such as muons and pions. The sources of primary cosmic rays are still somewhat of a mystery but some known sources are the sun, supernova explosions, active galactic nuclei, and quasars. From this list of sources, it can be seen that cosmic rays originate from both galactic and extragalactic sources. Cosmic rays interact with and are shielded by the earth's magnetic field and atmosphere, thus living organisms



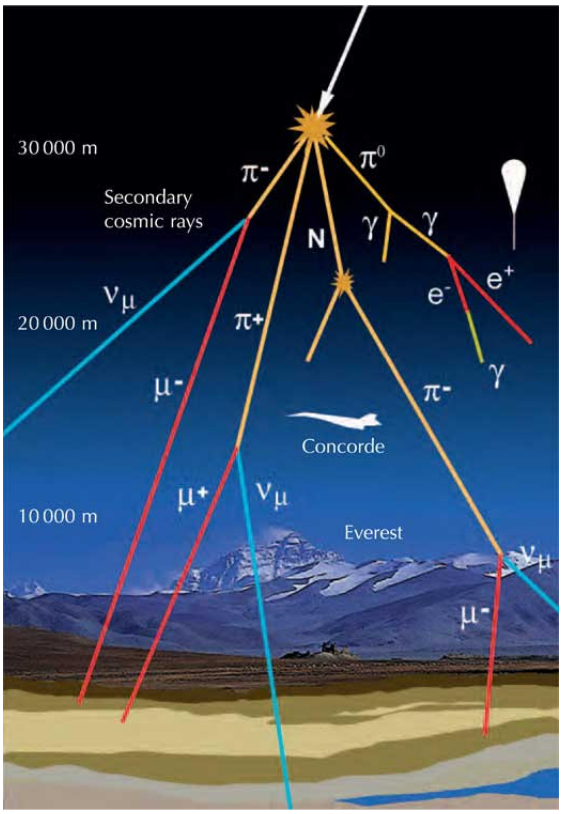
http://www.astronoo.com/en/articles/neutrino.html

Figure 16: *The process of beta decay turns a neutron into a proton.*

taken into space will start to see many negative effects due to this radiation compared to those living on earth.

Plasmons

Next we visit the field of plasmons. Plasmons, which are equivalent to photons or phonons, but whereas photons are oscillations of the electromagnetic field and phonons are mechanical oscillations, plasmons are plasma oscillations. Plasmons are in the same field as phonons categorized as quasiparticles since they are both just a quantization of plasma and mechanical oscillations, respectively. The frequency of light allowed to penetrate a metal is dependent on the plasma frequency, below the plasma frequency and the light is reflected, above and the metal becomes transparent



https://www.extremetech.com/extreme/207353-cosmic-ray-induced-radiation-powered-life-in-subsurface-pockets-of-the-universe

Figure 17: *Depiction of a primary cosmic ray colliding with and becoming a secondary cosmic ray.*

to the light and is transmitted, equal to and it develops a resonant frequency. The transmission, reflection, and resonant frequencies are different for every metal but the underlying cause is the same, the oscillating electrons in the metal shield the electric field component of the incoming electromagnetic wave. Here plasma frequency refers to the oscillation of electrons in a metal in which the coulomb force acts as a restoring force when the charge distribution is not in equilibrium. For most metals, the plasma

frequency is in the ultraviolet region which is why they appear shiny and reflect visible light.

In order to derive the plasmon frequency we need to start with the *plasma model*, this model will provide insight into optical properties of metals over a large frequency range. In this model it is assumed that an electron gas of number density n_e, with an effective mass m^*, is free to move against a positively charged background of atoms. The electrons are free to oscillate under an applied electromagnetic field but their motion will be dampened by collisions which can be characterized by collision frequency $\gamma = \frac{1}{\tau}$. Here τ is the relaxation time of the free electron gas, which is around 10^{-14} s at room temperature. Modeling this oscillation with an equation of motion subject to an external electric field $\mathbf{E}$(t) we get:

$$m^*\ddot{\mathbf{x}} + m^*\gamma\dot{\mathbf{x}} = -e\mathbf{E}(t) \tag{4}$$

Now assuming a harmonic time dependence of the electric field $\mathbf{E}(t) = \mathbf{E}_0 e^{-i\omega t}$ and that all electrons move in phase we can solve this second order differential equation and obtain:

$$\ddot{\mathbf{x}} + \gamma\dot{\mathbf{x}} = \frac{-e\mathbf{E}(t)}{m^*}$$

$$\left[\frac{d^2}{dt^2} + \gamma\frac{d}{dt}\right]\mathbf{x} = \frac{-ee^{-i\omega t}\mathbf{E}_0}{m^*}$$

and letting $D^2 = \frac{d^2}{dt^2}$ and $\omega^2 = \gamma\frac{d}{dt}$ we get,

$$[D^2 + \omega^2]\mathbf{x} = \frac{-ee^{-i\omega t}\mathbf{E}_0}{m^*}$$

$$[(D + i\omega)(D - i\omega)]\mathbf{x} = \frac{-ee^{-i\omega t}\mathbf{E}_0}{m^*}$$

Then taking the correct term we get

$$(D - i\omega)\mathbf{x} = \frac{-ee^{-i\omega t}\mathbf{E}_0}{m^*}$$

$$\left(\frac{d}{dt} - i\omega\right)\mathbf{x} = \frac{-ee^{-i\omega t}\mathbf{E}_0}{m^*}$$

$$\frac{d\mathbf{x}}{dt} - i\omega\mathbf{x} = \frac{-ee^{-i\omega t}\mathbf{E}_0}{m^*}$$

From here we can multiply by dt and integrate the full equation

$$d\mathbf{x} - i\omega\mathbf{x}dt = \frac{-ee^{-i\omega t}\mathbf{E}_0}{m^*}dt$$

$$\int d\mathbf{x} - i\int \omega\mathbf{x}dt = -\int \frac{ee^{-i\omega t}\mathbf{E}_0}{m^*}dt$$

$$\mathbf{x} - i\omega\mathbf{x}t = \frac{-ee^{-i\omega t}\mathbf{E}_0}{m^*}\left(\frac{-1}{i\omega}\right)$$

$$\mathbf{x}(1 - i\omega t) = \frac{-e\mathbf{E}(t)}{m^*}\left(\frac{-1}{i\omega}\right)$$

$$\mathbf{x}(t) = \frac{-e\mathbf{E}(t)}{m^*}\left(\frac{-1}{i\omega}\right)\left(\frac{1}{(1 - i\omega t)}\right)$$

$$\mathbf{x}(t) = \frac{e\mathbf{E}(t)}{m^*}\left(\frac{1}{(i\omega - i^2\omega^2 t)}\right)$$

We finally achieve the solution

$$\mathbf{x}(t) = \frac{e}{m^*(\omega^2 + i\gamma\omega)}\mathbf{E}(t) \tag{5}$$

From the displacement of the electrons a polarization $\mathbf{P} = -n_e e\mathbf{x}$ is thus developed and equation 5 becomes:

$$\mathbf{P} = \frac{n_e e^2}{m^*(\omega^2 + i\gamma\omega)}\mathbf{E}(t) \tag{6}$$

Now that we have the polarization in terms of the electric field we can find the dielectric displacement and the complex dielectric function $\epsilon(\omega) = \epsilon_1(\omega) + i\epsilon_2(\omega)$. From the dielectric displacement we can find the plasma frequency and show that the charge density oscillation is equal to the plasma frequency:

$$\mathbf{D} = \epsilon_0\mathbf{E}(t) + \mathbf{P} = \epsilon_0\epsilon\mathbf{E}(t) \tag{7}$$

plugging equation 6 into equation 7:

$$\mathbf{D} = \epsilon_0\mathbf{E}(t) + \frac{n_e e^2}{m^*(\omega^2 + i\gamma\omega)}\mathbf{E}(t) \tag{8}$$

$$= \left(\epsilon_0 + \frac{n_e e^2}{m^*(\omega^2 + i\gamma\omega)}\right)\mathbf{E}(t) \tag{9}$$

$$= \epsilon_0\left(1 + \frac{n_e e^2}{\epsilon_0 m^*(\omega^2 + i\gamma\omega)}\right)\mathbf{E}(t) \tag{10}$$

$$= \epsilon_0 \left(1 + \frac{\omega_p^2}{\omega^2 + i\gamma\omega}\right) \mathbf{E}(t) \tag{11}$$

and

$$\epsilon(\omega) = 1 + \frac{\omega_p^2}{\omega^2 + i\gamma\omega} \tag{12}$$

here we have derived the dielectric function, or better known as the dielectric constant in electrostatics and the relative permittivity in electrodynamics, of the free electron gas. This is because ϵ is dependent on the frequency ω since $\epsilon(\omega)$ changes when the frequency changes. We can now introduce the *plasma frequency* $\omega_p^2 = \frac{n_e e^2}{m^* \epsilon_0}$ of the free electron gas, here n_e is number density of electrons, e is the charge of the electron, m^* is the effective mass of the electron, and ϵ_0 is the permittivity of free space. Plasma frequency can thus be recognized as the natural frequency of a free oscillation of the electron fluid. When the frequency of light is below the plasmon frequency $\omega < \omega_p$ the light wave is not permitted. When $\omega > \omega_p$ the waves will penetrate the metal and will propagate with a group velocity of $v_g = \frac{d\omega}{dK} < c$, where the individual components of the plane wave are the wave vector K and the angular frequency ω. When $\omega = \omega_p$ we get resonance.

The assumption of a uniform background of charge with an electron fluid is only valid at a length scale that is large in comparison to the interparticle spacing of the medium. The motion of the charge will always create an electromagnetic field both inside and outside of the metal. The interface of two materials is where surface plasmons exist, this is where the real part of the dielectric function changes signs across the interface. The imaginary part of the dielectric function can be attributed to the phase shift of the polarization $\mathbf{P}$ relative to the electric field $\mathbf{E}$(t) which, when

passing through the medium, leads to the dampening of the electromagnetic waves. The plasma frequency of most metals is around $5eV - 15eV$.

Using the free electron model and knowing that the energy of a quantized oscillation is proportional to the frequency the plasmon energy can be calculated using:

$$E_p = \hbar\sqrt{\frac{n_e e^2}{m^*\epsilon_0}} = \hbar\omega_p \tag{13}$$

where $\hbar$ is the reduced Planck's constant. The free electron model, developed by Arnold Sommerfeld, is a simple model for how charge carriers act in a metallic solid. This model also assumes the metals are composed of a quantum electron gas [45].

Jaynes-Cummings Model

In its most basic form the Jaynes-Cummings Model is a model describing the interaction of electromagnetic waves with matter. The description is that of an atom with two entangled states, the ground state and the excited state, both interacting with the quantized electromagnetic field to investigate the phenomena of absorption and emission of a photon in a lossless cavity. Microscopic deviations from the Jaynes-Cummings master equation with cavity losses has also been investigated [42]. The phenomenological masters equation describing cavity losses is

$$\dot{\rho} = -i[H_{JC}, \rho] + \gamma(a\rho a^\dagger - \frac{1}{2}a^\dagger a\rho - \frac{1}{2}\rho a^\dagger a) \tag{14}$$

where ρ is the density matrix of the atom-cavity system, H_{JC} is the Jaynes-Cummings Hamiltonian, and γ represents the rate of loss of photons from the cavity, here $\hbar = 1$. This theory was proposed as a way to clarify the quantum theory of radiation and

the semiclassical theory [43]. The semiclassical theory can be extended to take into account the field acting on the molecules and the action of the molecules on the field, which will produce very near the same coherence properties and energies as that of quantum electrodynamics, but the quantum theory still has certain properties which the semiclassical lacks such as the combined states (superposition) of both molecules and fields, which cannot be described by the classical theory [21].

In DNA each of the four nucleotides have two tautomers, for guanine and thymine they are the keto and enol form and in cytosine and adenine they are the amino and imino form. These tautomers differ via the location of hydrogen atoms and bonds. In this thesis we will propose a future investigation of the entanglement of the different tautomers of the four nucleotides and the use of a modified Jaynes-Cummings Model to model the interaction of radiation with the hydrogen atoms. In this case the different tautomer forms are analogous to the ground state and the first excited states and the incoming radiation will be responsible for the bumping of the tautomers into their different states/forms.

$$H_j = \hbar\omega_j(N_j + \frac{1}{2}) + \frac{1}{2}E_j\hat{\sigma}_{zj} + \hbar\kappa_j(\hat{a}_j^+\hat{\sigma}_{-j} + \hat{a}_j\hat{\sigma}_{+j}) \tag{15}$$

The first term in the above hamiltonian will describe the unperturbed energy of the photon mode in the system. The second term describes the unperturbed energies of the individual nucleotide molecules, either A, T, C, or G. Examining the third term in the hamiltonian, which is the term for the photon-molecule interaction, we observe that this term describes the transitions from one tautomeric form to another, the different states of the nucleotide. In this case the keto or amino tautomer will absorb a photon and will transition into the enol or imino tautomer and the

emission of the photon mode will result in an enol/imino tautomer transitioning into the keto/amino tautomer. Under this investigation we can apply a theoretical model to a commonly occurring phenomena and potentially extract a more complete and thorough understanding of just one of the many processes ongoing within DNA.

Lennard-Jones Potential

The hamiltonian we will be using for the description of tautomeric shifts within DNA will also include a description of the interaction between the two nucleotides. These nucleotides, within the DNA, and all the other proteins share a very small space and are constantly interacting with one another, this interaction between nearby nucleotides should be taken into account. For this interaction term we will propose the use of the Lennard-Jones Potential (LJP). The LJP is a mathematically simple potential that is used widely and gives an acceptable approximation when it comes to interatomic and molecular interactions.

$$V = 4\epsilon[(\frac{\sigma}{r})^{12} - (\frac{\sigma}{r})^{6}] \tag{16}$$

The first term in this equation describes the Pauli repulsion while the second term describes the long distance attraction. While the Jaynes-Cummings model will handle the interaction in terms of matter interacting with radiation, the Lennard-Jones potential will take into account the interaction of matter with itself. This will be helpful in developing a more full model of all of the processes happening simultaneously. With this interaction term we may be able to observe when certain

processes are more matter or radiation dependent which depends upon which is the dominating term in the process.

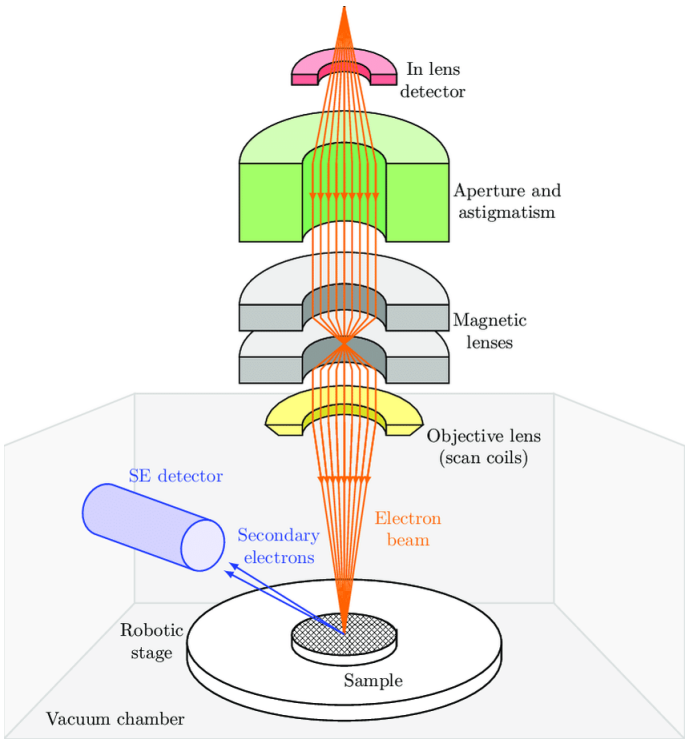
CHAPTER 4:

MATERIALS AND INSTRUMENTATION

Scanning Electron Microscopy (SEM)

The Scanning Electron Microscope (SEM) is extremely versatile in function and uses, some of which are imaging, measurements, elemental analysis, and chemical analysis. The use of the SEM requires the implementation of various techniques dependent on the type of sample being observed and measured. Care must be taken when using the SEM otherwise damage to the sample or equipment can occur. In this experiment observations of morphological changes in the structure and size of bacteria, exposed to electromagnetic fields, are to be implemented. Exposure to light 24 hours a day and exposure to plasmonic effects from gold will be the sources of electromagnetic fields. Once exposed, and techniques for observations are implemented, any changes in morphology will be noted.

An introduction on how the SEM works will now be given. When a sample is initially loaded into the SEM, the first step is to reach a complete vacuumization of the sample chamber. Once the chamber is completely evacuated a current is run through a tungsten tip which in turn heats up, at the top of the column, until it begins emitting electrons Figure 18. This tungsten tip is now effectively a cathode. The intensity of the electrons emitted can be controlled by the strength of the current which in turn controls the intensity of the heat the tip reaches. After emission, the



https://www.researchgate.net/figure/Internal-structure-of-SEM-Scale-of-elements-is-not-respected_fig5_329138904

Figure 18: *Representation of the column used by SEM to focus electron beam.*

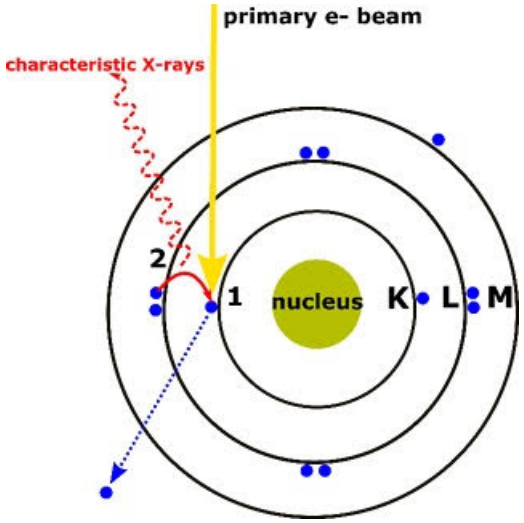
electrons travel down the column past an oppositely charged anode which is shaped like a donut. The electrons pass through the center of the anode and are accelerated towards the sample. The electrons of which this initial beam is composed are called the primary electrons. Accelerating past the anode the electron beam next encounters a strong electromagnetic lens to help focus the beam. After the focused beam passes through the electromagnetic lens it will pass through an electromagnetic deflector, which can control the direction of the focused electron beam. This beam of primary electrons then hits the sample in a focused beam and knocks out the electrons from the sample material, these knocked out electrons are called secondary electrons.

In order to produce an image the beam is focused on a single point which will produce a certain amount of secondary electrons. A secondary electron detector will then measure the amount of secondary electrons emitted from that point and fills in a pixel of the monitor somewhere in a gradient from white to black, white being above a certain threshold and black being the absence of electrons. The monitor is thus filled in pixel by pixel creating an image of the sample. To make imaging much quicker multiple electron beams are used at once.

The primary electrons will interact with the sample in a way which generates a variety of signals. These signals are in the form of secondary electrons, X-rays, transmitted electrons, cathodolumanescence, and backscattered electrons. These secondary electrons are what give the topological information. Using these other signals, analysis of the sample can be taken. The elemental and chemical identification component is run by a program containing the application that will allow for the measurement of the elemental composition and each individual elemental concentration of the sample. This is done by measuring the X-rays emitted from the sample and using the characteristic X-ray energy to identify the atom. In this process an inner electron is knocked out of the atom, becoming a secondary electron, by the primary electron. At this point an electron from a higher energy shell transitions to a lower energy shell Figure 19. The amount of energy lost in this transition is the characteristic energy and is emitted in the form of an X-ray, all elements have a characteristic energy dependent on the atomic number.

In this way we can use the SEM to image and collect valuable data from our samples. In conjunction with these methods, screen shots of the objects we are viewing can be taken with time, date, and scale (the size of the screen magnification)

is all included. An approximate size of objects being imaged on the screen can be taken and recorded as well. The SEM in UHCL's modern physics lab is a Phenom Pro Desktop SEM and can measure objects down to a scale of about 10 μm very clearly or about 500 nm with some patience, time, and effort.



https://blog.phenom-world.com/edx-analysis-sem

Figure 19: *Emission of characteristic X-ray from atom.*

Atomic Force Microscopy (AFM)

The Atomic Force Microscope (AFM) is a high precision microscope that allows the user to translate atomic forces into high quality images, the AFM gives us a window into the nanoscale world. These images can tell us a great deal of information about the shape, size, structure, and mechanical properties of the objects being measured and observed. In terms of scale, the AFM will provide a very detailed image of the surface topography, down to the order of nanometers, of any object placed inside. This information can then let the user know if the surface is extremely smooth and

flat or if the surface is full of nano ridges, which could potentially capture and hold samples in place during an experiment. The AFM also comes with both data taking and data analysis softwares, SmartScan and XEI, respectively, which can then be used to interpret and manipulate the gathered data.

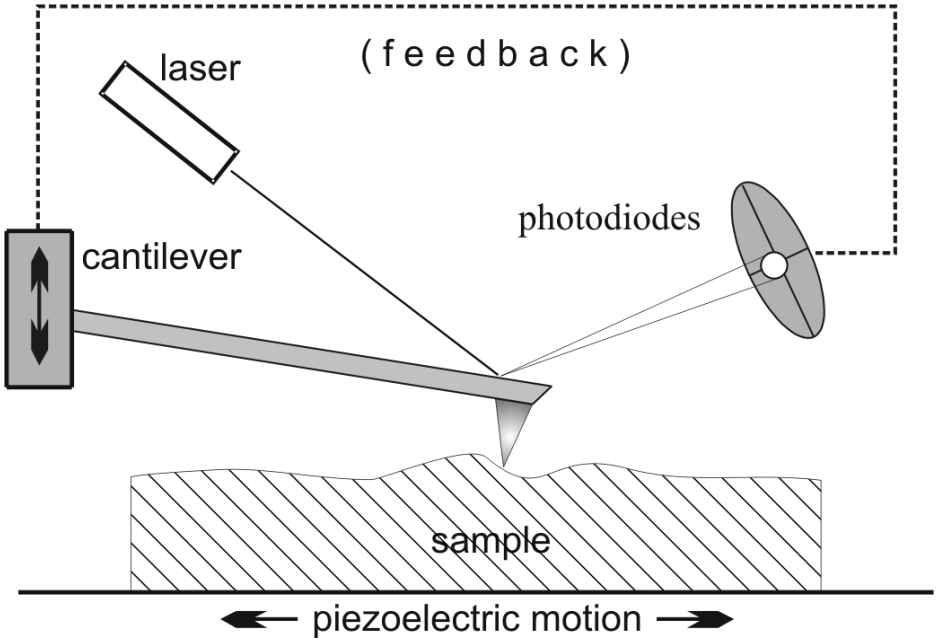


Figure 20: *Diagram of the cantilever deflecting laser while imaging a sample.*

The AFM consists of a microscope which is isolated from vibrations within a sealable insulated box connected to a computer in which all data can be acquired using SmartScan. The AFM uses an extremely small and sensitive cantilever to measure the atomic forces produced by the object being measured, Figure 20. The atomic forces are measured by deformation of the cantilever, this deformation can be modeled by Equation 17, Hooke's law. This deformation is detected via a laser reflected off of the back of the cantilever and onto a photodiode array. Hooke's Law

is a first order linear approximation in which the forces needed to compress or extend a spring is directly proportional to the distance the spring is deformed

$$F = kx \tag{17}$$

where F is the force applied, k is the spring constant, and x is the distance the spring is deformed. The forces used to deflect this cantilever are extremely small, so caution needs to be taken when handling the NX10 head of the AFM.

While the cantilever uses atomic forces to measure the deflection it can also measure other properties of the materials which are dependent on the specific atomic forces of those materials. In this way, once captured using the SmartScan software, the XEI software can be used to determine more information about the materials themselves.

Materials and Instrumentation

Surface topography and metal properties are gathered from the use of the AFM. The stainless steel plates are placed into the AFM prior to inoculation of bacteria and a full analysis is run using the SmartScan program. The AFM scans a small 5 μm x 5 μm area on the surfaces of multiple stainless steel plates in order to measure and ensure that on average the surfaces of the stainless steel plates will be very similar in topography. Scans with the AFM provide us with various other bits of information about the stainless steel plates which are all viewed using the XEI analysis program. Similarly multiple stainless steel plates are placed in the SEM in order to image their surfaces and to take an elemental analysis of the make-up of the stainless steel plate.

Once this step is complete it is repeated with stainless steel plates sputter coated with a thin gold film.

The 108 manual sputter coater is used to apply a thin gold coat to the stainless steel plates in order to generate plasmonic effects. The coater applies a coat of 9 nm in thickness at a working distance of 50 mm using 30 mA of current for 20 seconds in argon, see Table 1. Argon is sometimes used to provide a smoother uniform coat with smaller gold particle grain size and application at a faster rate. We used air at a working distance of 50 mm to apply the gold coating at a slower rate and to generate larger gold particle sizes. One drawback to this method is that this makes it very difficult to measure the thickness of the gold coating. The next step is to use an autoclave to sterilize all of our equipment to provide a clean growing environment and eliminate all other organic molecules on the surface of the stainless steel plates and the sample plate. The autoclave was used in a dry setting allowing a higher heat for a shorter amount of time and then taking the equipment through a drying cycle prior to removal. Finally, once sterilized, double sided conductive tape was placed on the sample plate and desiccated in the vacuum chamber to eliminate any living cells on the surface of the tape. This was done before the steel plate was placed on top of the sample plate and inoculated with bacteria. These steps were done in this order because the double sided tape could not survive a treatment within the autoclave without losing some of its conductive capabilities due to the heat, see Table 1.

Preparing the bacteria was completed prior to the measurements of the sample plates and left in a refrigerator, which slows the bacteria's growth, so they would be ready once all other measurements were taken. To prepare the bacteria we started with the nutrient broth LB Broth Ultrapure, from Affymetrix USB, we then used the

30 mm WORKING DISTANCE									
	0.02 mbar			0.05 mbar			0.08 mbar		
	20 s	40 s	60 s	20 s	40 s	60 s	20 s	40 s	60 s
20 mA	12 nm	24 nm	36 nm	10 nm	21 nm	31 nm	7 nm	14 nm	21 nm
30 mA	17 nm	35 nm	53 nm	16 nm	33 nm	50 nm	13 nm	25 nm	38 nm
40 mA	22 nm	48 nm	67 nm	25 nm	51 nm	77 nm	19 nm	39 nm	57 nm
50 mm WORKING DISTANCE									
	0.02 mbar			0.05 mbar			0.08 mbar		
	20 s	40 s	60 s	20 s	40 s	60 s	20 s	40 s	60 s
20 mA	12 nm	24 nm	36 nm	10 nm	21 nm	31 nm	7 nm	14 nm	21 nm
30 mA	17 nm	35 nm	53 nm	16 nm	33 nm	50 nm	13 nm	25 nm	38 nm
40 mA	22 nm	48 nm	67 nm	25 nm	51 nm	77 nm	19 nm	39 nm	57 nm
70 mm WORKING DISTANCE									
	0.02 mbar			0.05 mbar			0.08 mbar		
	20 s	40 s	60 s	20 s	40 s	60 s	20 s	40 s	60 s
20 mA	12 nm	24 nm	36 nm	10 nm	21 nm	31 nm	7 nm	14 nm	21 nm
30 mA	17 nm	35 nm	53 nm	16 nm	33 nm	50 nm	13 nm	25 nm	38 nm
40 mA	22 nm	48 nm	67 nm	25 nm	51 nm	77 nm	19 nm	39 nm	57 nm

Table 1: Thickness Reference Chart
Results were compiled using a Gold target with Argon gas.
Working distance is measured from sample table to target.
MTM Monitor settings: Density = 19.3, Tooling Factor = 1.4
All thickness values are approximate and are intended for reference only. Actual results may vary.

Sample Plates	Resistance Ω
Steel Plate	0.5
Sample Plate	0.8
Conductive Dot	0.8×10^6
Combind_{BA}	0.5×10^6
Combind_{PA}	N/A
Conductive Strip	2.22×10^6
Combind_{BA}	0.31×10^6
Combind_{PA}	15×10^6
BA=Before Autoclave	PA=Post Autoclave

Table 2: Conductivity of sample plates.

recommended amount of 20 gm/L in a flask and set it in the autoclave. The nutrient broth is then autoclaved to sterilize it, which should eliminate all other bacteria living in the broth, flask, and deionized water. After being autoclaved the flask was allowed to cool. Once cooled we used a micropipette to fill 12 test tubes with the nutrient broth. From here four different strains of bacteria were inoculated from their mother cultures into the 3 test tubes each: Escherichia Coli (E. Coli), Staphylococcus Aureus (S. Aureus), Pseudomonas Aeruginosa (P. Aeruginosa), Bacillus Spizizenii (B. Spizizenii). These test tubes were placed in an incubator for twenty-four hours before being placed in the refrigerator to slow their growth.

Inoculating the sample plates with bacteria was the next step in the process, once all other preliminary measurements were taken. Using a micropipette, one test tube of E. Coli was used to inoculate all twelve sample plates we had prepared, three stainless steel and three gold coated stainless steel plates to be placed within the magnetic field and three stainless steel and three gold coated stainless steel as the control to be left outside of the magnetic field. The static magnetic field was generated by a solenoid

and maintain at the value of $\mathbf{B} = 10$ gauss or 0.001 T. A static magnetic field using a solenoid can be calculated using

$$\mathbf{B} = \mu_0 I n \tag{18}$$

where $\mu_0 = 4\pi \times 10^{-6}$ H/m is the permeability of free space, I is the current, and $n = N/l$ number of turns per unit length. These samples were then placed underneath the fume hood and allowed to grow and dry for the next forty-eight hours. Under the fume hood the control group was placed about thirty centimeters from the solenoid. The other group of six sample plates were placed into the center of the solenoid which was producing a magnetic field at 10 Gauss (0.001 T). After forty-eight hours the bacteria was fully desiccated in the vacuum chamber for 1 minute and loaded into the SEM for imaging, analysis, and measurement.

Each stainless steel and gold coated plate were imaged in three different locations and the length measurements of twelve bacteria were taken in order to find an average bacteria length. On top of acquiring images and length measurements, one location on each sample plate underwent a full elemental analysis. An image of each location was taken and the bacteria on the images were numbered one through twelve. Each bacteria's length was measured and corresponds to its number on a spreadsheet. An average length for the bacteria at each location was taken for each image. After this an average bacteria length was then calculated per sample plate.

CHAPTER 5:

RESULTS AND DISCUSSION

In the following section all of the data gathered in the experimental process and the results, which can be extrapolated from that data, will be presented. Different tools and methods were used to take all data and compile it into a comprehensive fashion. A summary of the data's significance will be expressed in this section as well as other concluding remarks.

Data and Results

A discussion of the following results from the data gathered while growing the bacteria on top of the two different surfaces, gold coated stainless steel and stainless steel, will proceed. As was discussed in a previous chapter, changes to the growth of the bacteria may come from a combination of plasmon effects as well as the structure of the surfaces they are grown on. In order to ensure that these could be the only contributions care was taken in setting up the experiment and making all other parameters equal. All gold coated stainless steel plates were placed inside of the sputter coater for 30 seconds and the vacuum was allowed to reach a pressure of 43 millibars before coating was initiated. Initially galvanized steel and steel sheet metal were tested under the same conditions as the stainless steel but they both did not survive the autoclave process without developing a high level of oxidation after one cycle of

sterilization. This oxidation is not ideal for our experiment and so we did not use these materials. The fact that stainless steel did not oxidize after multiple cycles in the autoclave and the cost per sample plate were the two main motivating factors behind choosing stainless steel above other materials.

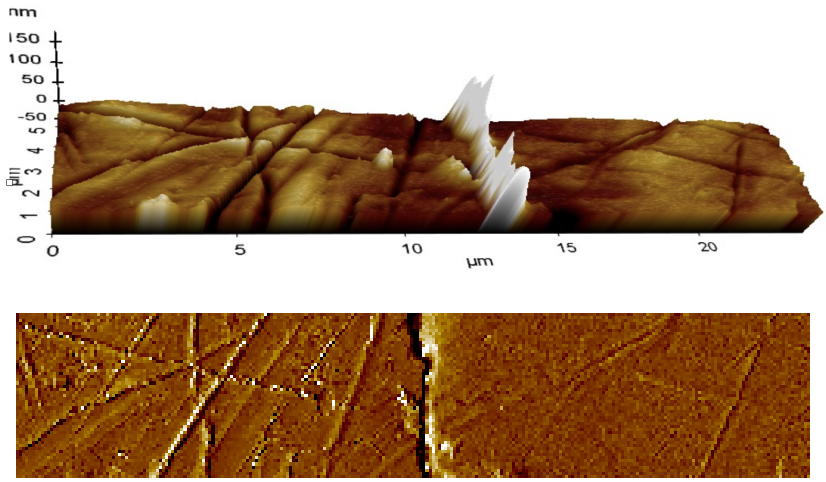


Figure 21: *To the left of the center dividing line the features are sharp and fine, no coating, and to the right they are smooth and filled in, gold coating.*

The structure of the growing surface is full of small and large disruptions, some large enough to trap multiple bacteria and others much smaller. The stainless steel plates, when viewed under the AFM, are full of scratches and lacerations on the scale of tens of nanometers to the scale of tens of micrometers. After the surface of the stainless steel plate was coated in gold the surface took on a much smoother topography. To show this relative smoothness we took a stainless steel plate and coated one half of it in gold and scanned across the interface to image both sides side by side. As can be seen in Figure 21 the left side of the image has very sharp fine details and to the right, of the center dividing line, the features have become more

smooth and filled in. This can be an important feature in what may have allowed the bacteria on the gold surface to grow larger than those grown on the steel surface.

The following data was gathered after exposing the bacteria to a constant weak magnetic field and letting them grow and live until the sample plates had dried out and were ready for processing, about 48 hours. Once ready full scans were taken using the SEM for elemental analysis, measurements of the bacteria length were also taken. In order to gather this data each sample plate was removed from the magnetic field and placed in the vacuum chamber to desiccated the bacteria and remove any liquids from the sample plate, to complete the drying process. After a satisfactory amount of time in the vacuum chamber the sample plate was placed on the SEM sample holder and placed into the SEM. Each individual sample plate was imaged in three separate locations. The length of twelve bacteria were taken and recorded for each image at each location. Finally, one image location from each sample plate underwent a full elemental analysis in order to compare elemental distribution and concentrations this also allowed us to view the distribution of the nutrient broth and composition of bacteria.

The following graph shows the difference in average length of the bacteria grown on the different surfaces, steel and gold coated. The graph in Figure 22, shows an average size difference of about 24% for the bacteria grown in the magnetic field and about 33% for the control group of bacteria. These percent differences in size are noticed in both bacteria groups, always in favor of the bacteria grown on the gold coated sample plate, the bacteria grown on gold plates are larger. The first conclusion we can asses from this data is that the gold coated sample plates had a larger affect on the bacteria. What we may be able to deduce from this is that the surface plasmons

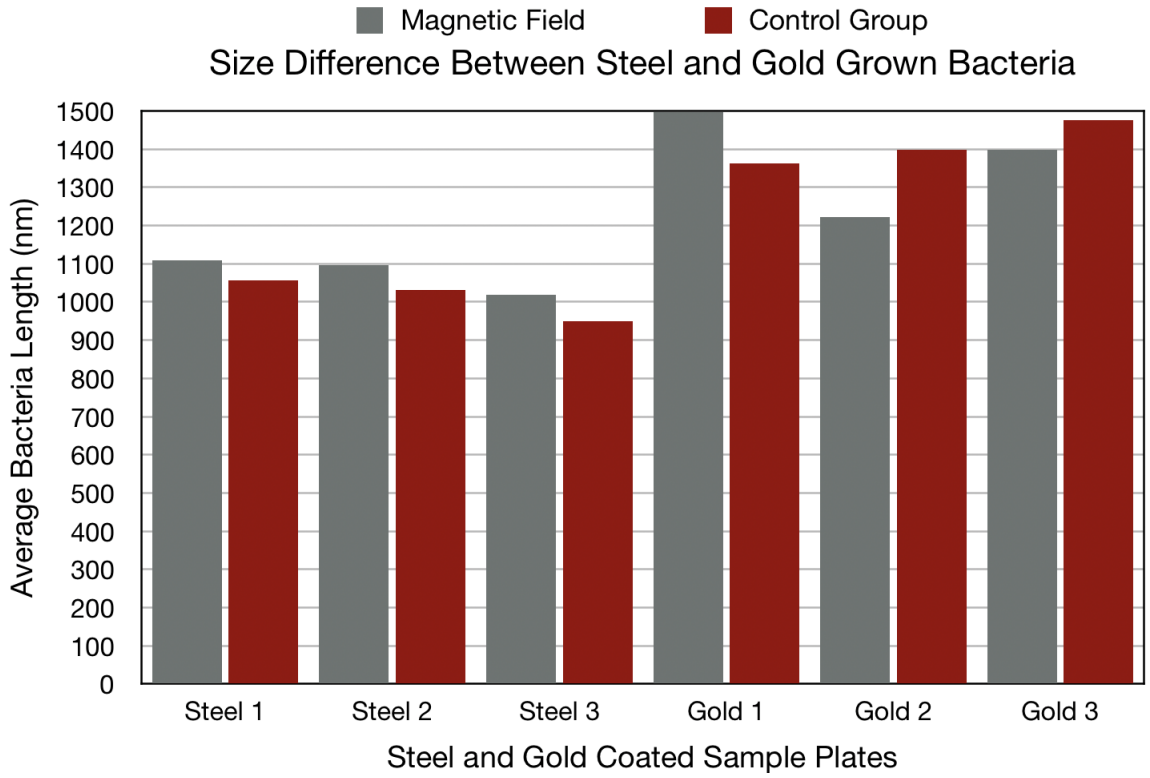
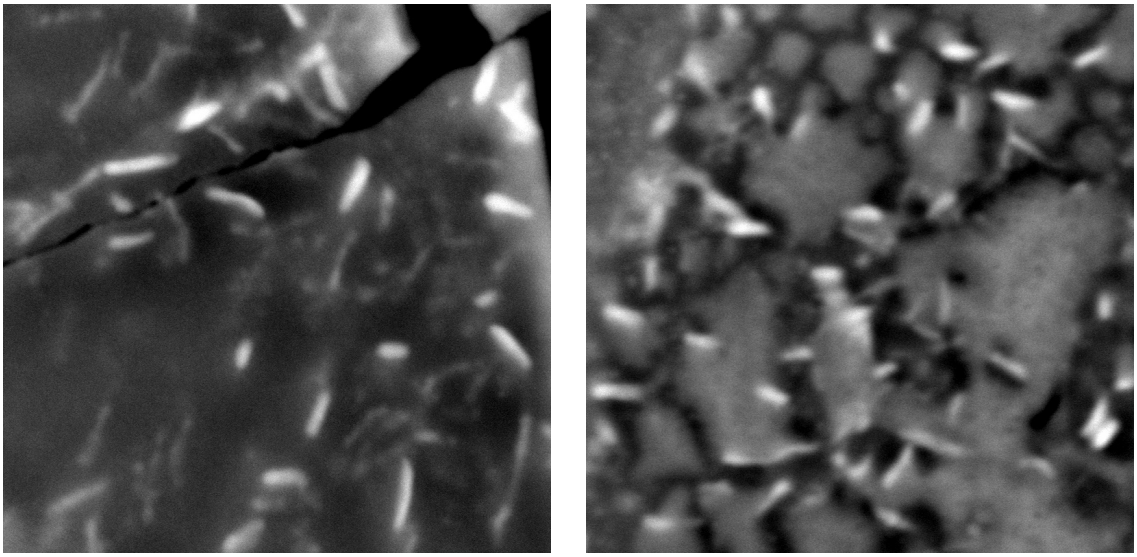


Figure 22: *An average size difference of 24% in the magnetic field grown bacteria and 33% in the control group bacteria.*

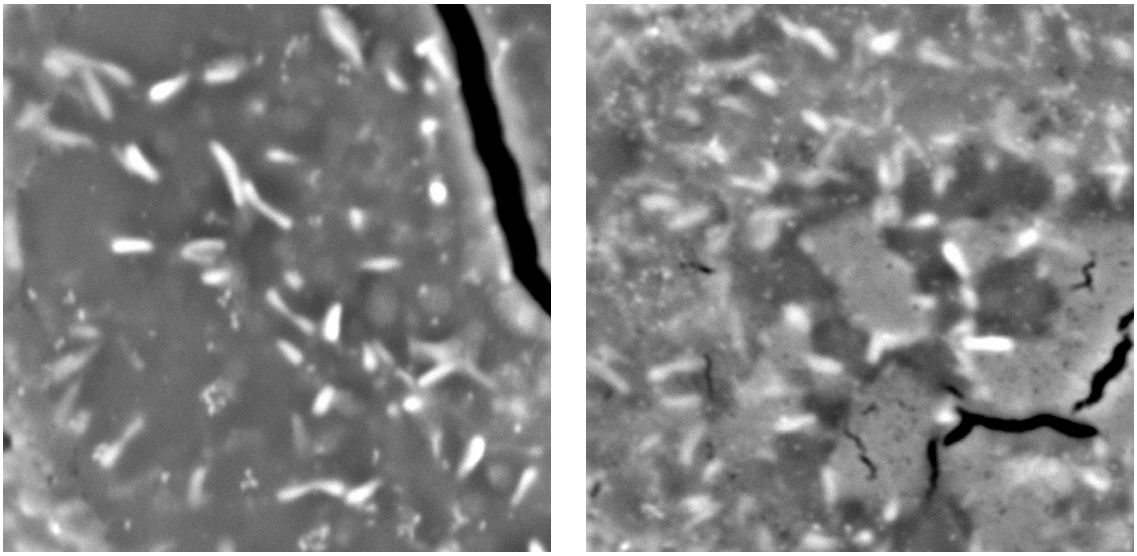
generated on the gold surface had an overall dominating effect. This means that the electromagnetic fields produced by the surface plasmons were the dominating force, even more so than any effects produced by the magnetic fields. In this scenario we may expect that the electromagnetic fields generated by the surface plasmons were also affecting the bacteria on the surface since, as mentioned before, the motion of the charge will create an electromagnetic field inside and outside of the metal surface.

The following figures 23 and 24 are side by side comparisons of the bacteria, these are taken on the same magnification level for all photographs. This is to ensure accurate and precise length measurements as well as provide visual aid in viewing



(a) *Bacteria grown on gold coated plate.* ***(b)*** *Bacteria grown on Stainless Steel.*

Figure 23: *A side by side comparison of (a) bacteria grown on the gold coated plates and (b) bacteria grown on stainless steel plates in the magnetic field.*



(a) *Bacteria grown on gold coated plate.* ***(b)*** *Bacteria grown on Stainless Steel.*

Figure 24: *A side by side comparison of the control group bacteria (a) grown on gold coated plates and (b) grown on stainless steel plates.*

the E. coli. An imaging screen width of 52.7 μm was used to allow enough space to show the full circular area containing the bacteria as well as to be magnified enough to show detailed bacteria shape and morphology. As can be seen in the Figure 23 the bacteria in the image on the left are noticeably large than the bacteria on the right. The images in Figure 23 are of bacteria grown within the static 10 Gauss magnetic field, gold grown on the left and steel on the right. As can be seen in Figure 24 the bacteria within the image on the left are significantly larger than the bacteria on the right. Again, the bacteria in the left image of Figure 24 are the bacteria grown on the gold plates and the bacteria on the right side are grown on the steel plates. After these images were taken a full elemental analysis was taken and the results appear very interesting. The results from this analysis can be viewed in the next section.

Elemental Analysis

Using the SEM we could take a highly precise elemental analysis of bacteria. It was seen that the basic composition remains unchanged, however the comparative study shows that small changes take place in different elements. We have included elemental data for gold coated and stainless steel surfaces, with and without the magnetic field. What these results indicate is that the atomic concentration between the different groups of bacteria did not change significantly. These results, Figure 25, show a comparison between the bacteria grown within the magnetic field on both gold coated and stainless steel plates. What we see is bacteria growing to different sizes while not much else is changing. The control group of bacteria, Figure 26, on both gold coated and stainless steel plates, also show very similar results to those

on the previous figure. Next, Figure 27, shows bacteria grown on gold coated plates within and outside of the magnetic field, to show a comparison of the differences and similarities for all bacteria grown on gold. Finally, Figure 28, shows bacteria grown on stainless steel both within and outside of the magnetic field. What we can see is that the concentration levels for neutral atoms was relatively the same, but for atoms which easily form ions there is a bit more variance. This slightly larger amount of variation may come from the ions interacting more easily with the magnetic field, created by us, or the electromagnetic field produced from the plasmons themselves. This slight shifting of ions could be a contributing factor in the size differences observed.

Summary

It is a well known fact that many different forms of radiation can have a deleterious effect on DNA within the cell [26] [53] [6]. Well known and studied are non-ionizing and ionizing radiation. UV light, an ionizing form of radiation, can cause pyrimidine dimers to develop, this in conjunction with genetic factors can lead to the development of melanoma, the deadliest form of skin cancer [57]. An area where not much work has been done is in the study of quasiparticles or collective excitations and the effects they have on living organisms and their DNA, some examples are plasmons and phonons. Since DNA is such an important aspect of all living organisms it is crucial that research and understanding continue to be pursued.

As was noted in previous sections we noticed an average size difference of 24% and 33% in physical size of the E. coli grown on the gold coated plates and the stainless steel plates. E. coli grown on the stainless steel sample plates were, on average, 24%

Element Number	Element Symbol	Element Name	Atomic Conc.	Weight Conc.
6	C	Carbon	55.12	47.39
7	N	Nitrogen	24.82	24.88
8	O	Oxygen	14.60	16.72
11	Na	Sodium	3.06	5.04
17	Cl	Chlorine	1.19	3.03

Element Number	Element Symbol	Element Name	Atomic Conc.	Weight Conc.
6	C	Carbon	55.94	48.22
7	N	Nitrogen	23.76	23.89
8	O	Oxygen	15.10	17.33
11	Na	Sodium	2.87	4.74
17	Cl	Chlorine	1.21	3.08

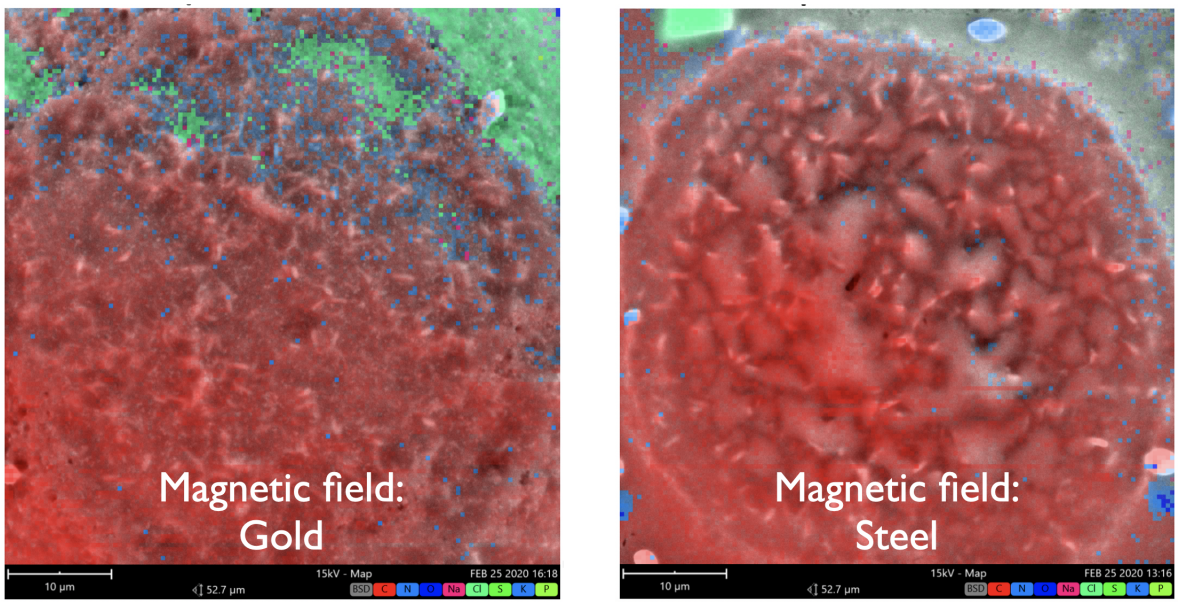


Figure 25: *Side by side comparison of bacteria grown on the gold coated and stainless steel plates within the magnetic field.*

and 33% smaller than those grown on the gold. Another notable aspect about this difference in size is the fact that this change in size may appear to be related to being grown within or outside of a static magnetic field, which in our case was produced by a solenoid. Although work has been done on E. coli grown in magnetic fields [9] [30], a physical size difference was seen between both the control and the magnetic field grown bacteria. Some of the effects that may be attributed to this size difference may be due to the fact that the gold coated surface was overall smoother than the stainless steel surface, perhaps allowing for easier motility along a smoother surface for the E.

Element Number	Element Symbol	Element Name	Atomic Conc.	Weight Conc.
6	C	Carbon	54.72	45.60
7	N	Nitrogen	24.34	23.65
8	O	Oxygen	14.69	16.31
11	Na	Sodium	2.82	4.49
17	Cl	Chlorine	1.39	3.43
26	Fe	Iron	0.78	3.03

Element Number	Element Symbol	Element Name	Atomic Conc.	Weight Conc.
6	C	Carbon	54.75	46.40
7	N	Nitrogen	24.90	24.60
8	O	Oxygen	14.80	16.71
11	Na	Sodium	2.69	4.36
17	Cl	Chlorine	1.16	2.90
26	Fe	Iron	0.58	2.30

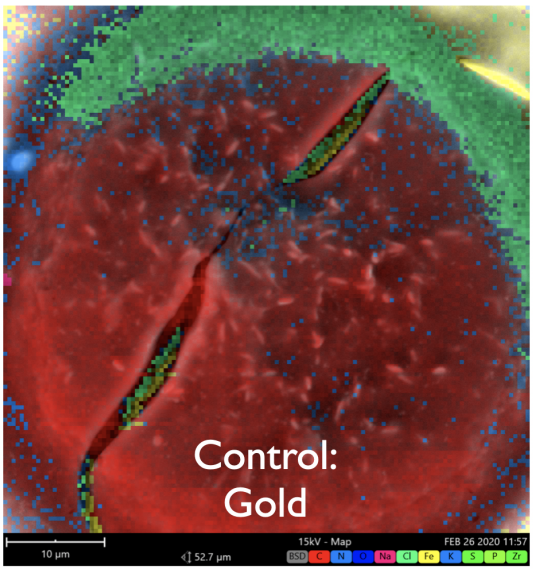


Figure 26: *Side by side comparison of the control group of bacteria grown on the gold coated and stainless steel plates.*

Element Number	Element Symbol	Element Name	Atomic Conc.	Weight Conc.
6	C	Carbon	55.12	47.39
7	N	Nitrogen	24.82	24.88
8	O	Oxygen	14.60	16.72
11	Na	Sodium	3.06	5.04
17	Cl	Chlorine	1.19	3.03

Element Number	Element Symbol	Element Name	Atomic Conc.	Weight Conc.
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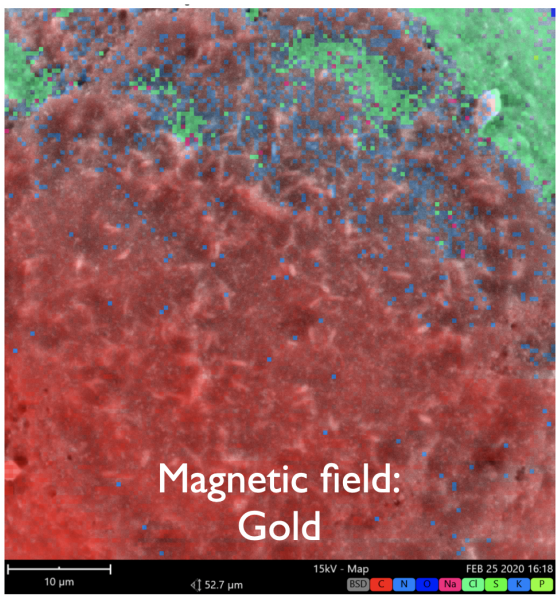
11	Na	Sodium	2.82	4.49
17	Cl	Chlorine	1.39	3.43
26	Fe	Iron	0.78	3.03



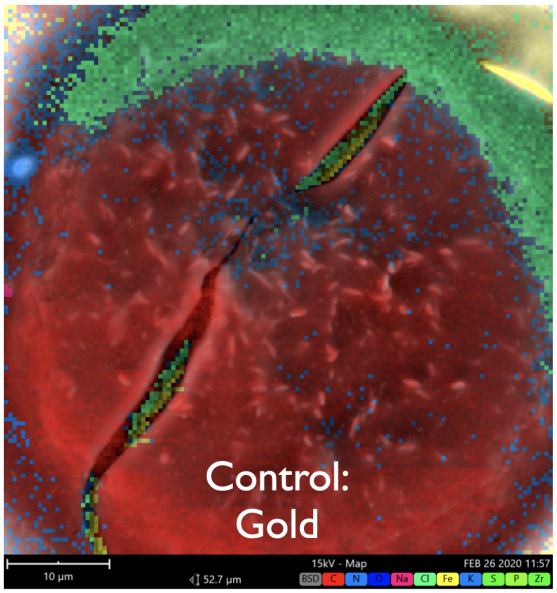


Figure 27: *Side by side comparison of bacteria grown on the gold coated plates in and out of the magnetic field, respectively.*

Element Number	Element Symbol	Element Name	Atomic Conc.	Weight Conc.
6	C	Carbon	55.94	48.22
7	N	Nitrogen	23.76	23.89
8	O	Oxygen	15.10	17.33
11	Na	Sodium	2.87	4.74
17	Cl	Chlorine	1.21	3.08

Element Number	Element Symbol	Element Name	Atomic Conc.	Weight Conc.
6	C	Carbon	54.75	46.40
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8	O	Oxygen	14.80	16.71
11	Na	Sodium	2.69	4.36
17	Cl	Chlorine	1.16	2.90
26	Fe	Iron	0.58	2.30

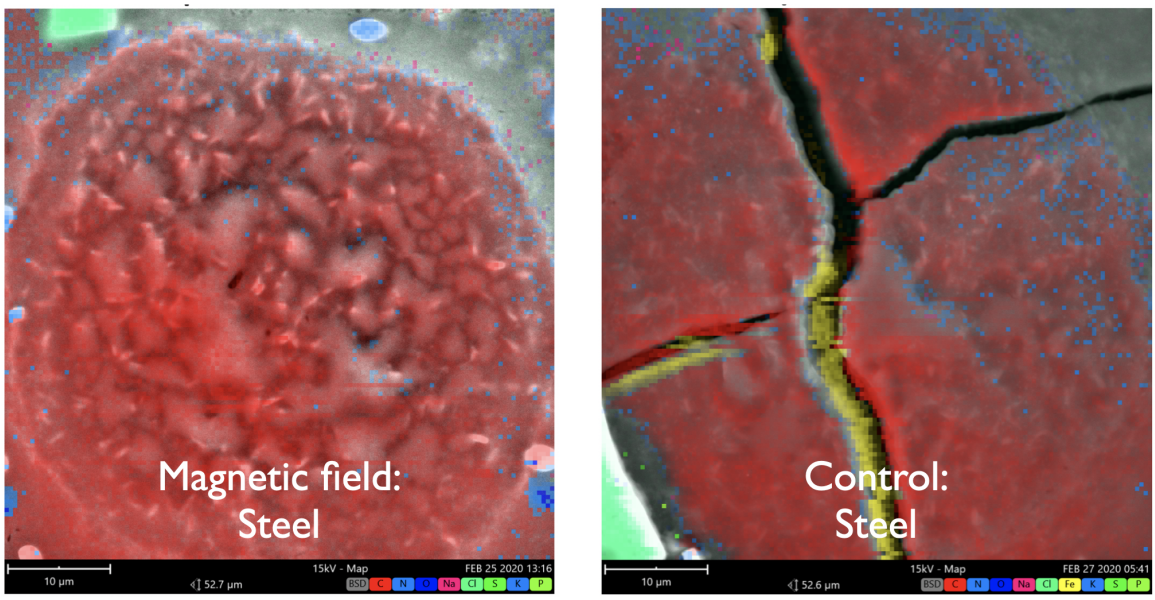

Figure 28: *Side by side comparison of bacteria grown on the stainless steel plates in and out the magnetic field.*

coli to locate nutrients. The elemental analysis of both bacterial systems provided very interesting and notable results. These results may indicate that the ions in the nutrient broth could be shifted by the magnetic field of the electromagnetic field being created by the plasmons themselves. This plasmon electromagnetic field may also be causing a slight increase in ions by bumping electrons out of their orbits. A more in depth study of the elemental analysis should be continued to better understand how these results affected our bacteria's growth.

Overall a noticeable difference in size was measured and observed. As an initial study on the effect plasmons have on bacteria, cells, biomolecules, and DNA we made some interesting discoveries. Continued work on the effects that the electromagnetic fields, that plasmons generate, have on biomatter and how it might be causing damage to the DNA should be developed further to provide a more concrete understanding of this phenomena.

Future Work

Unfortunately due to the Covid-19 experience there is still much work left to be done in developing this research at an even more meaningful depth. As was seen in previous sections we researched the effects that plasmons had on E. coli grown on top of gold coated steel plates and steel plates. In order to continue this study in the future the use of three other bacterias: Pseudomonas Aeruginosa (P. aeruginosa), Bacillus Spizizenii (B. spizizenii), and Staphylococcus Aureus (S. aureus); all grown on top of these same three surfaces within the same conditions would need to be done. Other variations of the same experiments would also need to be done.

Exposing the surfaces and bacteria to the full visible spectrum of light and growing a control group in a dark environment could help develop a distinction between effects created via surface plasmons and any effects created by other properties of the metals themselves. Exposing the substrate and bacteria to very specific frequencies of light, frequencies which excite plasmons, can remove any effects produced by certain frequencies of light favored by the bacteria or which may be harmful to the bacteria. Finally, changing the material of the surface itself, we use gold and stainless steel in this experiment but the use of other metals such as silver, copper, aluminum, and iron, would be a great way to observe the effect of plasmons at different energy levels and with their own unique plasma frequency. Observing these effects and placing them in the context of how individuals interact with and are in constant contact with a wide variety of metals daily can lead to a more in depth understanding of how, interacting which these metals, can and does have positive and negative consequences in our lives.

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