“What is the purpose”? you may ask.   
“Grid”, I answer

[Go Grid, or go home]

Not to toot my own horn, but

[Jacob]

Logo

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# Abbreviations and explanations

Apoptosis – A mechanism allowing damaged cells to initiate self-destruction (*Apoptosis | Cytology | Britannica*, 2013).

ATM – A protein activated by DNA damage. Its purpose is to phosphorylate (activate) numerous proteins related to cellular response after exposure to ionizing radiation (Samuel et al., 2002).

ATP – Adenosine triphosphate a molecule, which provides cells with energy for the cell and phosphate groups for protein activation. Through the process of hydrolysis, ATP is converted to ADP (adenosine diphosphate) (*Adenosine Triphosphate | Definition, Structure, Function, & Facts | Britannica*, 2020).

BM – Biomolecule. There are four types of biomolecules: carbohydrates, lipids, nucleic acids and proteins (*Biomolecule | Definition, Structure, Functions, Examples, & Facts | Britannica*, 2020). These are molecules important for normal cell functioning, and damage to them can have harmful consequences.

CDK – Cyclin dependent kinase

CT – Computed Tomography is an image modality using an X-ray source that creates an X-ray beam, which rotates around the patient, which lays between the source and a detector array. Attenuation values in different tissues are found and used to create an image (Villarraga-Gómez, 2016).

Cytokines – A category for signaling molecules that mediate immune responses by enabling cell to cell communication (Mandal Ananya, 2019). The cytokines relevant to this thesis are different variants of IL (interleukin) cytokine, TNF- and TGF-. They are all important in regulating cell division (Najafi et al., 2014).

DNA – Deoxyribose nucleic acid

DNA transcription – A process of creating a messenger ribonucleic acid (mRNA), which is used to create specific proteins. A transcription factor binds to the DNA, telling the enzyme RNA-polymerase to read a gene sequence of interest. As each base (Adenine, Cytosine, Thymine and Guanine) in the sequence is read, a complementary nucleotide is attached to form the mRNA strand (*Transcription | Definition, Steps, & Biology | Britannica*, 2019). In the DNA Adenine binds to Thymine, but during transcription, Thymine is replaced with Uracil (Mason et al., 2020, p.48).

EBT – External Beam Therapy

GD – Gradient Descent

GLM – Generalized Linear Models

GN – Gaussian-Newton

HR – Homologous recombination

IAEA – International Atomic Energy Agency

LET – Linear energy transfer

LINAC – Linear accelerator is a device that accelerates charged particles using an alternating electric field. They are used in radiation treatment to accelerate electrons into a high atomic-number target, creating high energy (MV) bremsstrahlung (Philip Mayes et al., 2007).

LM – Levenberg-Marquardt algorithm

MLC – Multileaf collimators are individual metal (often Wolfram) blocks used to shape a radiation beam coming from an external radiation therapy machine (Galvin et al., 1993).

MLE – Maximum likelihood estimator

NHEJ – Nonhomologous End-Joining

OD – Optical density

P53 – A protein that is bound to another protein called mdm2. When DNA damage occur, it is released from mdm2, and it will bind to a gene for transcription of the p21 protein. This protein will in turn inactivate a protein complex responsible for promoting cell division (Alberts et al., 2014, p.1014).

Phosphorylation – Is the process of transferring a phosphate group (PO3) to a molecule. Proteins are activated by phosphorylation, and the addition of a phosphate group might change the proteins in different ways: Extra charge from PO3 can attract amino acid chains that connect to the existing protein, or the structure of the binding cite can change (Alberts et al., 2014, p.153-154). Both processes regulate the activity level of the protein.

PMF – Probability Mass Function

Proteins – Chains of amino acids with different shapes, sizes and functions. One main function is catalyzing certain chemical reactions. These proteins are called enzymes and have binding cites where specific molecules fit. The enzymes can both break and form chemical bonds (Mason et al., 2020, p.38).

RNA-primer – Ribonucleic acid primers are short nucleotide sequences generated by RNA-polymerase. They exist as a starting point for DNA-polymerase, because they are only able to continue an existing strand (Mason et al., 2020, p.292). The RNA-polymerase will attach Uracil instead of Thymine (see 1.7.1), and they must be removed and replaced by a different DNA-polymerase (Cooper, 2000b).

ROS – Reactive Oxygen Species is a collective term for highly reactive molecules based on oxygen. It has been shown an increase in ROS after exposure to radiation, and it has been hypothesized that they mediate DNA damage (Narayanan et al., 1997).

SDD . Source to Detector Distance

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# Theory

## Ionizing Radiation

Radiation is transfer of energy. The main categories are ionizing and non-ionizing radiation. I.e., it either has enough energy to liberate an electron from the atom, or it doesn’t. Non-ionizing radiation consists of low-energy electromagnetic (EM) waves such as UV-light and microwaves. When we move further right in the EM spectrum, we get ionizing X-ray and particles. To clarify: The EM waves do not become particles, but we know from the discovery of the photo-electric effect that you might interpret EM waves as “showers” of photons (Einstein & Infeld, 1938). Together with neutrons, they make up a group called uncharged particles. They are highly penetrating because they need to interact directly with a target.

Charged particles are different, they consist of particles with either positive or negative charge. Some examples are protons (+), electrons (-), and -particles (+2). Their interaction probability is greater compared to uncharged particles. A photon must be close to either a nucleus or an electron for an interaction to occur. A charged particle may interact at a distance. Their Coulomb field interacts with the Coulomb fields of other electrons, causing a “Continuous Slowing Down” (Attix, 1986, p. 160).

The path of a charged particle through a medium is highly dependent on the particle at hand. What is the charge, its velocity, and its weight?   
In medical physics, we’re primarily interested in ionizing radiation and using its properties to identify or treat medical lesions. This is further examined in the radiobiology section (1.7).

### Photon interaction in matter

Photons are energy-carrying particles, without mass, traveling at the speed of light. They interact with the surrounding medium in several ways. The main interactions are Rayleigh Scattering, Photoelectric effect, Compton Scattering, pair/triplet production, and photonuclear interactions (Attix, 1986, p.124-125). Which interaction you’ll have is highly dependent on the atomic number of the photon absorber and the energy of the incoming photon. The probability of interaction is defined as interaction cross-section, with the unit . In Figure 1‑1 we see which interaction type dominates for specific energies and atomic number Z. Pair production is the annihilation of photons in the presence of a nucleus’s Coulomb field, producing a positron () electron () pair. The interaction demands, at minimum, the rest energy of two electrons () (Attix, 1986, p.146-148). We will use photons with energies in the kV region, where the photoelectric effect and Compton scattering dominate, and these interaction types will naturally be our focus.

Rayleigh scattering is a relevant interaction type for lower energy photons. The photons are deflected from their path, but no energy transfers occur; hence, Rayleigh scattering does not contribute to the absorbed dose in the medium. However, it is still an important interaction type because it gives a complete picture of the photon’s path.   
Diagram

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Figure 1‑1. Photon interaction probability (defined as interaction cross-section [] as a function of atomic number Z and photon energy [MeV] The curves represent the area where two interactions have the same probability (Attix, 1986, p.125).

#### Photoelectric effect

The photoelectric effect is when an incident photon’s energy is absorbed by an electron bound to an atom. The kinetics is illustrated in Figure 1‑2. If the energy is equal to or larger than the binding energy of the electron it will ionize the electron. The energy transferred from the photon to the electron depends on its initial energy and the electron’s binding energy (Attix, 1986, p. 139).

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The photon might liberate an inner shell electron with higher binding energy (K- or L-shell electron). A looser bound electron will deexcite, filling the vacancy. The energy difference is either emitted as characteristic X-rays or by ionization of a valence electron (outer shell electron). The latter is called the auger effect, and the ionized electrons are known as auger electrons (Attix, 1986, p.143).   
The cross-section per atom for the photo-electric effect is proportional to atomic number and incident energy of the photon

The expression confirms Figure 1‑1, as photoelectric effect increases with atomic number, and decreases with energy.

Diagram

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Figure 1‑2. Illustration of the photoelectric effect. A bound electron absorbs all the energy of incoming a photon. If the energy is high enough, it will ionize the electrons (Attix, 1986, p.138). The kinetic energy of the electron is dependent on the initial energy of the photon and the binding energy of the electron . Recoil energy is given to the atom, but it is approximately .

#### Compton Scattering

The scattering process differs from the photo-electric effect in that the electron orbiting the nucleus is assumed free, which results in an inelastic collision. It is illustrated in Figure 1‑3. The errors from this assumption have proved negligible, as the errors don’t become substantial until we have a high atomic number Z and low initial energy . When these conditions are reached, the photoelectric become the dominating interaction type (Attix, 1986,p. 125).

Diagram

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Figure 1‑3. Illustration of Compton scattering, where the electron is assumed free.

The second difference compared to the photo-electric effect is that the photon only transfers part of its energy. The energy of the scattered photon follows this expression, which is derived in Appendix A

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We observe a strong correlation with the energy of the incident photon, and the scattering angle. And a maximum energy transfer to the electron for .   
The Compton cross section per electron was derived by Klein and Nishina. They improved on the existing theory of Thomson scattering. Thomson’s cross section was independent of incident photon energy, and assumed (Attix, 1986, p.130). This is correct for low energies. However, in Figure 1‑4 we see the energy of the scattered photon starts decreasing for increasing scattering angle around , and reaches its minimum at .

Chart, line chart

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Figure 1‑4. Compton scatter energies in . is energy of incident photon, is energy of scattered photon.

The Klein-Nishina cross section is represented by the symbol .

Each interaction has its cross section. The photo-electric effect has , Compton scatter has pair production has and Rayleigh scattering has . It is practical to sum each cross section to create a combined interaction variable. It is defined as the attenuation coefficient

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However, it is more insightful to normalize it with density . This way, we get , which represents how a photon is attenuated in a medium. This is called the **mass attenuation coefficient**.

For a polyenergetic photons, you must average the coefficient to include the full energy spectrum of the photons, but for simplicity we’ll assume monoenergetic photons.   
Now that we have the probability of interaction, we can include the fraction of kinetic energy transferred from the incident photon to a secondary electrons within a volume of interest. This is called the **mass energy-transfer coefficient**

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Where is the energy of the liberated electron after Compton scatter, is the energy of either K- or L-shell (highest binding energy) characteristic X-rays generated after the Auger effect. These X-rays carry energy away from the volume of interest and we therefore subtract their energy.  
 is the rest energy of the electron-positron pair created by pair production. We subtract because it’s the energy needed to create the electron-positron pair, leaving us with the kinetic energy (Attix, 1986, p.155).  
  
The last expression we want is the **mass energy-absorption coefficient** . It represents the energy absorbed by the volume. It relates to the mass energy-transfer coefficient by

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Where g is the fraction of energy lost to radiative loss (Attix, 1986, p.155-156).

#### Photon range

(Might remove)

Mean free path is the expected distance a photon will travel before interacting with the medium (*Mean Free Path | Physics | Britannica*, 2007). In Appendix A we derived an expression for mean free path for a photon traversing a slab of material with infinitesimal area and thickness

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The pathlength of the photon decreases with increasing attenuation. This is an important result, because it allows us to predict the path of the photon. It is especially useful when performing Monte Carlo simulations, which we’ll come back to in (ref here).

### Charged Particle interaction in matter

A charged particle has either positive or negative net electrical charge, such as electrons (), protons () and ions (an atom with a surplus of electrons or protons). Because of their charge, the particles will interact with other charged particles through their Coulomb fields. Two equal charges will repell and two opposite charges will attract. A charged particle traversing a medium is surrounded by Coulomb fields of positive (nuclei, positive ions) and negative (electrons, negative ions) charges, enabling the charged particles to interact at a distance. This distance is known as the impact parameter and is defined as the distance from the charged particle to the closest nucleus. We have three main categories of interactions:  
Soft collisions, hard collisions and Coulomb interactions with the nucleus.

#### Soft collisions

Soft collisions are small Coulomb interactions between the Coulomb fields of an atom and a charged particle. The impact parameter is much larger than the atomic radius (distance from valence electrons to nuclei), and small amounts of energy are transferred to the orbiting electrons of an atom, mainly causing excitations. Even though the energy transfers are very small, the interaction is highly probable, therefore contributing to half of the energy loss of the charge particle (Attix, 1986, p.161).

#### Hard collision

Hard collisions happen when the impact parameter has the same order of magnitude as the atomic radius. The result is a significant kinetic energy transfer to an assumed free and stationary electron. These electrons are called -rays, which undergo the same charge particle interactions. The hard collisions might also result in liberation of an inner shell electron as described in 1.1.1.1, resulting in emission of characteristic X-rays (Attix, 1986, p.162).

#### Radiative transfer

Radiative transfer, also known as bremsstrahlung, is a process where a charged particle interacts with the nucleus’s Coulomb field. The impact parameter must be much lower than the atomic radius for this to happen. The interaction mainly occurs with electrons and will therefore be the focus point (Attix, 1986, p.163). The radiative transfer refers to an inelastic collision between the nucleus and the electron (See Figure 1‑5). The electron with its negative charge is attracted to the nucleus’s positive charge, causing a deacceleration and deflection of the electron from its incident path. The decrease in kinetic energy is converted to a photon, thus conserving energy.   
The probability of radiative transfer Is much lower compared to elastic scattering (2-3%) and is proportional to (Grieken & Markowicz, 1993, p.3), where q is the charge, Z is the atomic number of the atom, T is the kinetic energy of the electron and is the rest mass of the electron. With a larger Z, the atom has a higher proton count, resulting in the nucleus having a larger Coulomb field attracting the electron. The kinetic energy of the electron is important because the electron needs to penetrate the electron cloud surrounding the nucleus.  
Radiative transfer is used when creating X-rays inside an X-ray tube, but we will come back to this in the section covering the X-ray tube (1.2.1).

**Diagram, schematic

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Figure 1‑5. Illustration of radiative transfer, where an electron inelastically collides with an atom’s nucleus deflecting it from its path. The result is an emitted photon with energy equaling the energy loss of the electron (Hapugoda, 2017).

#### Stopping Power

Stopping power is how much energy we expect the charged particle to lose per unit length. It can be found by integrating differential energy loss per length over possible energy transfers.   
As discussed, the charged particle might lose its energy by colliding or by radiative transfer, we therefore separate these contributions (Attix, 1986, .165)

Energy lost to radiative transfer does not contribute to dose, because of the larger range of the bremsstrahlung. Radiative stopping power is still important to accurately describe the range of the charged particle.   
Collision stopping power is split into two parts: for soft and hard collisions . Energy loss is dependent on the material it penetrates, we therefore introduce **mass stopping power** by dividing the stopping power by the material density .   
The total collision mass stopping power is

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Where is electrons per gram, is classical electron radius, , I is mean excitation potential in the medium, z is electron charge, and is shell correction. The collision stopping power assumes the electron’s velocity to be much greater than that of the orbiting electrons in atoms. As the electrons slow down the assumption becomes untrue, and the shell correction accounts for this. Stopping power is inversely dependent on the square of the velocity and electrons with high kinetic energy will lose more energy further into the medium.

Mass collision stopping power is closely related to absorbed dose (energy absorbed per mass). When CPE is achieved absorbed dose is expressed as

is electron fluence in a radiation field (1.3.1) (Seuntjens et al., 2005).

Stopping power is useful because we can estimate the range of the charged particle, but we also need to know how much of that energy is absorbed by the medium. Linear Energy Transfer (LET) represents this quantity with the unit . It is also known as restricted stopping power (Attix, 1986, p.179). When high energy electrons experience hard collisions, they liberate secondary electrons. A cutoff energy is introduced, because some electrons might have high enough energy to escape the volume of interest. If none of the secondary electrons can escape, we have CPE (1.3.1) and

LET is especially important in radiobiology, because it measures how damaging a radiation type might be. A higher density of energy depositions results in higher dose absorbed and more damage. A typical percentage depth dose curve from photon beams of various energies is shown in Figure 1‑6. As photons attenuate through the medium, they dissipate their energy by interactions mentioned in 1.1.1. For high-energy photons we see a build-up of dose. Because of the high energies of the photons, the generated electrons will also have high energies. As seen in equation 1‑7 the stopping power is lower for charged particles with high kinetic energy. So, the secondary electrons will lose their energy further into the medium, causing the buildup of dose we see in Figure 1‑1.

Chart

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Figure 1‑6. Percent Depth Dose curve for photon beams of different energies. As the photons attenuate through the medium they lose energy to electrons, which in turn lose energy through various interactions. A build-up region is seen for high energy photons before the maximum relative dose is reached. The same effect is not seen for photons of kV energy (Photon Dose Distributions | Oncology Medical Physics, n.d.).

#### CSDA

If we assume that the electrons are continuously slowing down (i.e., neglecting fluctuations in energy loss) as they interact, we can integrate total mass stopping power to get an approximate range called the continuously slowing down approximation (CSDA) range

This lets us describe the path of secondary charged particles following an ionization event. It is different to **projected range**, as it describes the total range of the particle including all twists and turns. Projectedrange is a measure of how deep into the medium the particle penetrates (Attix, 1986, p.181).

## Creating the radiation beam

When treating or diagnosing patients using radiation, you need a stable and reliable beam. Various techniques are used for this purpose, but for our case we will focus on the X-ray tube.

### X-ray tube

The X-ray tube’s purpose is to convert electron energy into X-rays. As mentioned in 1.1.2 we have two interaction types generating X-rays from electrons interacting with matter. The first is characteristic X-rays generated after ionization of an inner-shell electron and the second is generation of bremsstrahlung from deacceleration of electrons traversing close to the atomic nucleus. In Figure 1‑7 we see an illustration of the tube. The first component of the X-ray tube is the cathode, which is a spiraled wire called the filament. The filament is often placed inside a glass chamber called the envelope (Nadrljanski, 2021b). The filament is heated through resistance heating of a wire running through the cathode (Goel, 2021a). When the cathode reaches the right temperature, it will emit electrons through thermionic emission. A process where the heating energy surpasses the binding energy of electrons, and they are emitted from the metallic structure (*Thermionic Emission | Physics | Britannica*, 2021). The electrons are released into an evacuated tube with a high voltage. The potential difference accelerates the electrons toward a positively charged anode/target. Most anodes in x-ray tube targets are made of tungsten, because of their high atomic number and high melting point (Nadrljanski, 2021a).

![Diagram

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Figure 1‑7. Coolidge hot cathode X-ray tube with heated cathode, that releases electrons into vacuum sealed tube with potential difference causing acceleration of the electrons. The electrons are focused using a magnetic lens before hitting a target of high density material image ref. (Aksnes, 2020).

The electrons generate bremsstrahlung at different impact parameters (see 1.1.2), we therefore get a spectrum of possible X-ray energies, which is decided by Kramer’s rule. The essence of this rule is that when electrons hit a thick target the probability of direct impact with the nucleus, i.e., the impact parameter (see 1.1.2) is 0, is small. It is more probable that the electron experiences many smaller energy transfers and gradually deaccelerates, creating X-rays with less energy (Attix, 1986, p.211-214). X-ray tubes usually produce photon energies between 20 and 100 kV (Potts, 2005). The intensity of the beam follows the inverse square law where intensity decreases with the square of the distance away from the source following the equation (Attix, 1986, p.44)

where is a proportionality constant.

### X-ray filtering

It is often desirable to remove the smaller X-ray energies from the X-ray beam. This is done by inserting a filter inside the X-ray beam. The filter attenuates lower energy X-rays and creates a more homogeneous energy spectrum (Goel, 2021). Figure 1‑8 illustrates the difference between an unfiltered and filtered X-ray beam created in an X-ray tube with tungsten as target. The first peak is the low energy photons such as background radiation, the second and third peak is X-rays emitted after ionization of K-shell electrons (Amiri et al., 2021).

**Chart, line chart

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Figure 1‑8. Unfiltered vs filtered X-ray energy spectrum created from a Tungsten anode with 2.5 mm aluminum filtering (Amiri et al., 2021).

## Dosimetry

Taking any medicine requires knowledge about the correct dosage. Medicine is often administered through pills with carefully measured ingredients to give the right effect. Radiation does not have that advantage. Radiation dose is dependent on the energy, exposure time, distance from source, material of the absorber and quality (e.g., photons, protons or neutrons) of the radiation.   
The solution has been to use the effects of radiation on different substances such as discoloring (film dosimeter), temperature change (calorimeter) and light emission (thermoluminescence). To understand all the intricacies of dose measurement we need to define important quantities.

### Quantities

#### Ionizing radiation field

We wish to find number of ionizations in a point P inside a field of ionizing radiation. A ray cannot interact with a 0-dimensional point, we therefore define a finite area around the point. The incident angle of the ray might not be parallel to the area , we therefore need to account for all possible angles. This results in a sphere around P as seen in Figure 1‑9**.** with infinitesimal volume dV, mass dm and cross-sectional area da (Attix, 1986, p.5-6).   
The number of traversing rays per cross sectional area da is defined as **fluence** .

If the number of rays differs over time, the fluence needs to be integrated over time to get the fluence rate.   
The radiant energy of the rays is equally as important as the number of rays, for a polyenergetic beam with energies E, we have **energy fluence**  **.**

**Diagram

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Figure 1‑9. Ionizing radiation field defined as a sphere encapsulation a point P with infinitesimal volume dV, mass dm, with a cross sectional area da (Attix, 1986, p.6).

#### KERMA

With the energy fluence we have the energies traversing the sphere, but to get a dose we need the rays to interact and release energy. This is where KERMA comes in. Kinetic Energy Release per Mass. It describes the process where uncharged particles (photons and neutrons) enters a defined volume and transfers some or all of their energy to electrons in the volume. The energy transfer is expressed by

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Where is the expected energy transferred from uncharged radiation with energy into the volume minus the energy of the uncharged radiation leaving the volume without interacting.   
RL stands for radiative losses and represents interactions where charged particles generate photon energy after the initial ionization. If these photons leave the volume, it does not matter because we’re only interested in the energy transferred by the incident particles entering the volume.  
The final term is conversion of rest mass to energy or energy to rest mass e.g., pair production (see 1.1.1) where a photon annihilates creating an electron positron pair.   
With we can define KERMA

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For monoenergetic photons, KERMA is related to energy fluence using the expression

Where is the mass energy transfer coefficient (see 1.1.1), which represents the probability of the photons transferring energy to the volume (Attix, 1986, p. 21-22).

Until now, we’ve neglected how the electrons have spent their energy after they’ve received it from the incident photons. Accounting for radiative loss gives net energy transfer represented by this expression

represents the energy of the electrons, not lost to radiative transfer. We can now separate KERMA into two parts: collision KERMA and radiative KERMA , where for monoenergetic photons is expressed as

We can relate and energy fluence to another known quantity: mass energy-absorption coefficient (see 1.1.1) (Attix, 1986, p.24-25)

#### Absorbed dose

When describing KERMA, we’re interested in how energy is transferred from the photon to the medium. But photons aren’t the only contributors to dose; charged particles also transfer energy to the volume. We therefore define total energy transfer as

Where we include the energy transferred by charged particles entering the volume.   
We can now define dose as (Attix, 1986, p.26-27)

The unit is the same as KERMA, but it is called Gray or Gy. The unit is especially important in radiobiology, because the amount of absorbed energy affect an organism’s chance of repairing the damage caused by the radiation (see 1.7.3).

#### Exposure

Exposure is defined as total charge Q of ions of one sign (+ or -) produced by X-rays or -rays per mass m of air when all secondary electrons are stopped in the air and charged particle equilibrium (see next paragraph) is achieved (Attix, 1986, p.29-30). The expression for exposure in an infinitesimal air volume is

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We can relate exposure to mass energy absorption coefficient () and energy fluence by introducing the variable . is the mean energy required to create an ion pairs (Podgorsak, 2016, p. 744). The resulting expression for monoenergetic photons is

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is the elementary charge.

#### Charged Particle Equilibrium (CPE)

If the energy distribution of charged particles entering the volume is equal to the energy distribution of charged particles leaving the volume (visualized in Figure 1‑10) (Attix, 1986, p.65). This situation is called **charged particle equilibrium** (CPE), and if satisfied,  
reduces absorbed dose to (Attix, 1986, p.69).

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CPE essentially demands constant photon fluence everywhere around and within the smaller volume v in Figure 1‑10. If the fluence dropped significantly when entering the large volume V, more secondary electrons would be generated at the entrance of v, compared to the exit and we don’t have CPE (Attix, 1986, p.67).

When CPE is achieved, we can easily find the dose ratio between two volumes. This is a practical metric because we often need to relate dose to mediums of different densities.   
CPE is not necessarily easy to achieve. If the volumes are near the source, we’ll have much higher fluence on the side closest to the source (Attix, 1986, p.72). This causes more ionizations closer to the surface of V, compared to the surface of v, and CPE fails.   
For larger photon energies, the range of the liberated charged particles will increase compared to the range of the photons. Therefore, charged particles ionized near the surface of V have larger ranges than the charged particles ionized near the surface of v. The generated charged particles will therefore enter volume v but doesn’t exit, and CPE fails.

Chart

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Figure 1‑10. Charged particle equilibrium visualized, where photon energy enters a volume transferring energy to charged particles (electrons in this case), that traverses a smaller volume . The electrons exiting are of same type and energy distribution as the electrons entering, and we have CPE.

**Transient Charged Particle Equilibrium (TCPE)**

CPE demands no significant attenuation of photons within the smaller volume in Figure 1‑10. As mentioned, this would require an infinitesimal small volume. A more realistic approach is that the photons ionize electrons inside the smaller volume. As the photons traverse, they lose energy, causing a decrease in dose contribution. Instead of dose being equal to we assume proportionality, and the expression for dose becomes

where f is the dose contribution from attenuation of photons through the volume.

## Cavity Theory

When measuring dose, we use a dosimeter. A very popular dosimeter is the ionization chamber, which we’ll discuss in detail (1.5). A more fundamental description of an ion chamber is a gas filled chamber connected to an electrometer. Radiation ionizes the gas, and the electrometer measures a charge proportional to absorbed dose.   
However, dose (a.k.a., energy absorbed in the medium) is not equal between mediums of different density. We therefore need to relate the dose absorbed by the gas to the medium we’re interested in. The situation is visualized in Figure 1‑11.

### Bragg-Gray cavity

In Bragg-Gray cavity theory, dose to water is related to dose to air by

where is the mass stopping power ratio between water and air. Dose to water is often used because radiation beams in the clinic are calibrated with the assumption that all tissue is “water-like” (Andreo, 2015). We see that the electron fluence is assumed constant, but for this to be true two conditions needs to be fulfilled (Attix, 1986, p. 232):

1. The fluence of charged particles should not be perturbed in the cavity
2. Only charged particles crossing the cavity contributes to the dose.

The second condition requires no significant attenuation of photons inside the cavity. Which in turn requires an infinitesimally dimensioned cavity. Gray calculated the size that satisfies these conditions and found that for unfiltered -radiation you would need a air volume, while X-rays require for air filled cavity with graphite walls (Alm Carlsson, 2001).

Graphical user interface

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Figure 1‑11. A volume with material w (e.g., water), with an air-filled cavity inside. Energy traverses the volume releasing energy contributing to dose inside the cavity.

### Bragg-Gray-Laurence

The ideal Bragg-Gray scenario is not possible, as it would require an infinitesimally dimensioned dosimeter not to alter the charged particle fluence. It also demands the stopping power ratio to be independent on energy, which is not the case for increasingly different mediums (Alm Carlsson, 2002). The theory was improved by introducing CSDA of the charged particles. This allows the charged particles to slow down inside the cavity, and we need to integrate over the stopping power ratio

Bragg-Gray-Laurence in its simplest form requires CPE to accurately calculate the energy distribution of the electrons (Alm Carlsson, 2001). It might seem like a contradiction to incorporate CSDA, because CPE requires the energy of entering charged particles to be equal to the energy of the exiting charged particles. However, the cavity is still assumed to be small compared to the charged particle range and the energy loss inside the cavity is negligible. And because of the small size of the cavity, even small energy transfers will result in a significant dose.

The Bragg-Gray-Laurence cavity theory does not account for secondary electrons (-rays) generated inside the cavity. The **Spencer-Attix** cavity theory accounts for these electrons, and sets a cutoff energy , where all -rays with energy higher than escapes the cavity. As a result, more low energy electrons are added to the fluence.

### Burlin

All previous cavity theories concern small cavities. Burlin cavity theory tries to make a general theory for small, intermediate and large cavities (Attix, 1986, p.248). Looking at Figure 1‑12, we see that the dose contribution from charged particles generated inside the cavity g outweighs the dose contribution from charged particles generated in w. The dose is therefore collision KERMA ratio .   
For intermediate sized cavities we have a mix of charged particles that are generated inside and outside the cavity. We also have charged particles that are either stopped inside the cavity or they escape.

The expression for dose ratio using the same air-water example

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where d is a factor that becomes 0 for large cavities and 1 for small cavities.

Diagram

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Figure 1‑12. From left to right we see increasing cavity sizes, where dose contribution differs. W is the medium surrounding the cavity, g is the medium of the cavity. e is charged particles released in either w or g and is incoming photons. Burlin cavity is a general cavity theory which includes small, intermediate and large cavity sizes.

## Dosimetry methods

As mentioned in 1.3 different techniques are used for dose measuring. However, this thesis will focus on three different dosimeters: Ionization chamber, gafchromic film and Alanine EPR (Electron Paramagnetic Resonance) dosimeter. These dosimeters fall into one of two categories: Absolute and relative dosimeters. The volume of an absolute dosimeter responds to radiation in such a way, that we’re able to directly measure the received dose and does not require calibration in a known radiation field (Attix, 1986, p. 277). E.g., a free-air ionization chamber is an ionization chamber without walls, where the goal is to measure exposure (1.3.1) in a known mass of air. However, for photon energies surpassing 1.5 MeV the secondary electrons have such a large range, that the free-air ionization chamber can’t fulfill CPE without becoming unpractically large (Podgorsak, 2016, p. 737). The ionization chambers we’ll discuss here are relative dosimeters.

### Parallel- Plate Ionization Chamber

The purpose of an ionization chamber is measuring ionizations inside a gas-filled cavity. A fundamental type of ionization chamber is the parallel plate chamber illustrated in Figure 1‑13  
The chamber is connected to a polarizing (negative cathode) and measuring electrode (positive anode). The polarizing electrode is directly connected to the power supply and creates the voltage difference, which in turn generates an electric field. Adjacent to the measuring electrode you have the guarding ring/electrode with the purpose of preventing leaking current from being measured by the electrometer (Podgorsak, 2016, p. 702). Looking at Figure 1‑13 you see that the electrometer is connected to the power supply. Ideally the impedance (a circuit’s opposition to charge) inside the electrometer is high enough (typically ) and no charge moves through it (*High Accuracy Electrometers for Low Current/High Resistance Applications | Tektronix*, n.d.). However, some leakage will occur, and the guard rings are there to prevent this from being measured. The second purpose is to help define the effective collection volume of the chamber (see 1.5.2), by ensuring straight electric field lines (Podgorsak, 2016, p.703).

Diagram

Description automatically generatedWhen the electrons are ionized one of two things may happen: If the cavity is filled with electronegative gas with high electron affinity, the electrons will bind to the gas to create an ion. The ion will move toward the positively charged plate and the charge is “collected” by a measuring electrode connected to the electrometer. If the cavity does not have electronegative gas the ionized electrons moves directly to the measuring electrode (Podgorsak, 2016, p.705). The electrometer measures the accumulated charge, which is converted to dose.   
If the applied voltage is too low the negative ion will tend to recombine with the positive ion. The solution is increasing the voltage until the measured current is no longer dependent on voltage, this is called the saturation region (Attix, 1986, p.330-331). The measured output in this region is known as the saturation charge, or .

Figure 1‑13. The schematics of a parallel plate ionization chambers. An electric field is established on the gas-filled cavity, so when the gas is ionized electrons will move toward the positively charged side, where they’re collected by a measuring electrode connected to an electrometer.

### Thimble Ionization Chamber

In Figure 1‑14 we see a thimble (a.k.a. farmer type) ionization chamber. The chamber differs from the parallel-plate type by having a graphite thimble surrounding the gas-filled cavity. The graphite is chosen because it protects the sensitive volume and it is air equivalent, therefore minimizing the density difference between cavity and wall. This achieves charged particle equilibrium (Shortt et al., 2002). The protection cap is not shown in Figure 1‑14 but surrounds the wall and ensures that we’re passed the buildup region when the secondary electrons reach the wall and the cavity (see 1.1.2.4). The chamber has a central electrode connected to the electrometer.   
In a thimble ionization chamber, we want to find the exposure (see 1.3.1) to measure absorbed dose in the sensitive volume. Rearranging equation 1‑11 we see that collision KERMA might be written as

From equation 1‑10 we have the expression for exposure. Replacing the infinitesimal with the saturated charge and total air mass , we get collision KERMA for air

CPE is achieved in a thimble ionization chamber, so absorbed dose can be found using equation 1‑12. But the challenge arises when finding . is found using the effective volume, which is not necessarily the same as the geometrical volume of the chamber. The effective volume is defined by the electric field lines running from polarizing electrode to measuring electrode (Podgorsak, 2016, p.745). The field lines tend to bend outward away from the center, and this affects the fate of the ionized electron, thereby affecting the effective volume where electrons can be collected.   
To ensure most accurate dose measuring, a primary standards laboratory is given the job of calibrating the ionization chamber and finding its effective volume.

The calibration of a thimble ionization chamber is performed by measuring exposure using a free-air chamber, then replacing it with a thimble chamber and measure the air KERMA . The measurements are performed under specific reference conditions such as temperature, air pressure and humidity, using a reference beam energy and quality (typically 60Co photons). From this we get a calibration factor that relates output in nC to dose Gy (Podgorsak, 2016, p.744). The thimble chamber is then inserted into a water phantom, still in the known radiation field, where the is measured again and converted to dose to undisturbed water (as if the ionization chamber wasn’t present) using a conversion factor and correction factors ) (P.Andreo et al., 1996, p.48).

A picture containing text, device

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Figure 1‑14. Schematic of thimble ionization chamber, where the sensitive air-filled volume is encapsulated in a thimble (Podgorsak, 2016, p. 741).

### Radiochromic film

Radiochromic film is a self-developing film, which reacts when exposed to radiation (McLaughlin & Chalkley, 1965). The film is typically made ­up of a protective layer and an active layer (see Figure 1‑15). The active layer consists of one or two layers of monomers called diacetylene. Monomers are molecules that interact with other monomer molecules to create polymer chains (*Monomer | Definition & Facts | Britannica*, 2022). When diacetylene is exposed to radiation it polymerizes to create polydiacetylene, changing both the chemical and optical characteristic of the active layer (McLaughlin et al., 1996).   
The color of the film darkens and optical density (OD) can be measured either by measuring light transmitted through the film, or light reflected by the film (Andreo et al., 2017, 562). Radiochromic film is an absolute dosimeter, as OD can be directly related to dose if there is an established conversion of the film response to dose deposited in a reference medium (Devic et al., 2016). It demands calibrating the films and accounting for factors that might change the response, such as time waited after irradiation before scanning the films, temperature and light exposure. (ikke ferdig, snakk om directional dependency)

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Figure 1‑15. Different radiochromic film structures used for external beam therapy (EBT) (Devic et al., 2016). The active layer is made from monomers that polymerize when exposed to radiation, causing a darkening of the film. The optical density (OD) is measured and related to dose

## Statistics

### Non-linear curve fit

Regression is a tool used for predicting data. In traditional linear regression you have a dataset containing response/dependent variables y and explanatory/independent values x. Linear regression tries to fit the equation

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using the method of least squares. I.e. find the line that minimizes deviation between the true response variables and the predicted values  . The deviation is defined as the cost function, and both linear and nonlinear regression uses the sum of squared residuals (RSS)

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| where is the individual weight of the ith residual, which is used when the assumption of approximately equal residual for every is not satisfied. | 1‑15 |

For linear regression, one can find differentiate the RSS w.r.t. both coefficients and and find a closed form expression for both coefficients (Bingham & Fry, 2010, p.3-5).   
Not all response variables are linearly dependent on the explanatory variable, which might lead to the expression in RSS not having a closed form solution when differentiated. The solution is to guess the values of the unknown parameters, then iteratively tune them to find the minimum RSS (Kirkup, 2012, p.335-337).

#### Levenberg-Marquardt algorithm

This section is based on (Gavin, 2020).

Levenber-Marquardt algorithm (LM) combines two minimization methods known as the Gradient descent (GD) and the Gaussian-Newton (GN) method. **GD** uses the derivative of the RSS to update the parameters towards the steepest descent towards the minimum of RSS. For all n parameters we have a weighted RSS of

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is a diagonal matrix with shape m x m for m datapoints, with all the weights on the diagonal.   
Using the second binomial formula we remove the parentheses and get

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Differentiating the RSS w.r.t. all parameters we get

where is the m x n jacobian matrix containing the partial derivatives of , 2 comes from the fact that RSS is squared, and the minus sign is from . The partial derivatives becomes 0 because does not vary with changing parameters.

Rearranging the expression using the fact that is symmetrical, the theorem and introducing a constant we get the value that updates the parameters in the direction of steepest descent

is chosen and decides how fast we’re moving. Choosing an too large we risk passing the minimum but choosing one too small we might never reach the minimum.

**GN** is an expansion of the Newton’s method (Cavazzuti, 2013, p.85). Newton’s method assumes that the RSS function is quadratic near the minimum. This is reasonable because if the RSS function has a minimum, the value will sink to the minimum then increase when passing the minimum like a quadratic function. The quadratic function around the minimum is Taylor expanded and becomes

Inserting the approximation for into the RSS in equation 1‑17, we get

Again, using the second binomial formula and the theorem we get

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Newton’s method differs compared to GD, because it also accounts for the curvature of the RSS function. We therefore differentiate equation 1‑18 w.r.t. to find the one that minimizes RSS. As mentioned, is the jacobian and differentiating w.r.t. we get a matrix called the hessian matrix. It can be written as the sum (Chen, 2011)

In the GN method the second derivative term is assumed to be 0. Going back to matrix notation we are left with

This results in the derivative becoming

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Setting equation 1‑19 equal to 0 and rearranging using the same theorem from before we get

The **LM** method introduces a dampening link , which is scaled according to the diagonal elements of the hessian matrix

The dampening factor punishes updated parameters that does not contribute to reduced RSS by increasing , and encourage updated parameters that reduce RSS by decreasing . The result is a method that acts as GD far from the minimum but becomes GN when approaching the minimum.

### Akaike’s Information Criteria

### Poisson Regression

Poisson regression is a way of predicting discrete count data. It is like linear regression (see 1.6.1) in that it tries to fit a model to data, but the method is very different.   
In Poisson regression you assume that your response variables are Poisson distributed. I.e. it follows the probability mass function (PMF)

where it finds the probability of getting counts when is the mean. In the Poisson distribution the mean and variance is assumed to be equal (Legler, 2021).   
Poisson regression tries to estimate the mean using the model

The model can of course be expanded to include more than one regressor with adjoining coefficient . The estimation of the coefficients is done using a Maximum Likelihood Estimation (MLE) of the likelihood function

Maximum likelihood estimator

## Radiobiology

Cells are the building blocks of all living things. Animals are made from eukaryotic cells, which contain a nucleus, and the purpose of a cell is converting the nutrients we consume into energy needed to perform a task. Such tasks might be to provide structure (bone cells, muscle cells), sensory signaling (nerve cells) or transportation (blood cells). These cells are highly specialized and stem from an unspecialized cell (stem cell) that have finished their chain of cell divisions.   
The process of cell division (proliferation) is sensitive and damaged DNA (deoxyribose nucleic acid) might lead to cancer if not repaired.

### DNA basics

DNA or deoxyribose nucleic acid contains the complete genetic information needed to produce necessary proteins. The DNA is made up of two antiparallel strands (3 to 5 and 5 to 3, see further down for explanation) with a nitrogenous base pair connecting the strands (see Figure 1‑16).  
The strands consist of nucleotides, which again is broken into a sugar-phosphate backbone and the DNA nitrogenous base (*Nucleotide | Biochemistry | Britannica*, n.d.). There are five bases: Adenine, Guanine, Cytosine, Thymine and Uracil. The first four are in our DNA, and Uracil replaces Thymine in the mRNA (*Abbreviations and explanations*). The bases are divided into two categories: Adenine and Guanine are known as pyrimidines, while Cytosine, Thymine and Uracil are purines.   
If three adjacent bases code for an amino acid, it’s called a codon (see proteins in Abbreviations and explanations). Gene sequences, which includes codons are called exons, but if they do not include codons, they’re called introns.   
The bases form hydrogen bonds to create a base pair, but only with dedicated “partners”. Because of the the chemical structure adenine only binds with thymine (or Uracil during DNA transcription), while guanine only binds with cytosine (Mason et al., 2020, p.48). The hydrogen bonds are the first of two bonds between nucleotides. The second bond is the phosphodiester bonds between each sugar-phosphate (see Figure 1‑16).

Looking at Figure 1‑17 we have a closer look at an individual nucleotide. The carbon atoms in the deoxyribose molecules are marked with numbers 1-5 (Mason et al., 2020, p.47). When a new nucleotide is bound to the existing nucleotide, they can only be connected to the 3 carbon because they are able to chemically interact to create the phosphodiester bond (Mason et al., 2020, p.284).

The DNA strands are twirled around its own axis to form a DNA double strand helix, then they’re coiled around proteins known as histones to form a nucleosome. The nucleosomes are folded to produce fibers called chromatin. When a cell is preparing for cell division, the chromatin is tightly coiled into what’s known as a supercoil (Mason et al., 2020, p.210). However, during interface (1.7.2) the DNA needs to be accessible for DNA replication and DNA transcription (Abbreviations and explanations). Therefore, the chromatin is a dynamic structure that condense and decondense according to the needs of the cell (Alberts et al., 2002).   
When chromatin is folded it forms a chromosome. The chromosome has a centromere which allows for linkage between chromosomes to create chromosome pairs. When chromosomes are connected, we refer to the chromosomes as a sister chromatids (*Centromere | Biology | Britannica*, 2012).

Diagram

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Figure 1‑16. Schematic of DNA double strand (Nucleotide, n.d.)

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Figure 1‑17. Three molecules making up a nucleotide. The positions of each carbon in the sugar molecule are marked by a number (Mason et al., 2020, p.47).

Figure 1‑18. Chromosome structure (The Structure and Function of Chromatin, 2017).

### Cell Cycle and Checkpoints

The cell cycle consists of four phases: G1, S, G2 and M (see **Error! Reference source not found.**). G1 and G2 are the gap phases where cell growth occurs. G2 is the last phase before cell division where all organelles, but the DNA, replicates. During S-phase DNA is replicated. This happens by a series of initiator proteins that open the DNA double helix, creating a replication fork (Figure 1‑20) (Mason et al., 2020, p.293). Two DNA-polymerase enzymes are recruited to the DNA strands, with the purpose of generating two new complementary DNA strands. DNA-polymerase is only able to continue an existing complementary strand, therefore RNA-primers (Abbreviations and explanations) are created as a starting point for the DNA-polymerase (Mason et al., 2020, p.293). As mentioned in 1.7.1 new nucleotides can only attach to the 3 carbon. Since the two strands are antiparallel one of the DNA-polymerases needs to build a complimentary strand away from the replication fork. Therefore, the DNA-polymerase must jump back and forth, creating smaller bites of the new strand called Okazaki fragments. Then the RNA primers are removed and the bites are glued together by an enzyme called ligase ­­(Mason et al., 2020, p.292).

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Figure 1‑19. DNA replication schematic, where DNA is opened, and two complementary strands are created. Nucleotides only binds on 3’-OH group, which affects how the DNA-polymerase connects new nucleotides, leading to a leading strand and a lagging strand (Mason et al., 2020)

Figure 1‑20. Cell cycle schematic, G1, S and G2 phase is known as interphase, where the cell grows and replicates its organelles to prepare for cell division in Mitosis. In Mitosis the cell separates the chromosomes before the cytoplasm is separated, and we’re left with two identical daughter cells (Mason et al., 2020, p.212).

M-phase is mitosis and the process of separating the replicated chromosomes before the cytoplasm is separated to create two identical daughter cells. If the cell’s external environment isn’t favorable the cell might enter a resting phase known as G0, where it’s still able to perform its task, but does not prepare for cell division (Mason et al., 2020, p.212).

A cell’s progression through the cell cycle is highly regulated. The three checkpoints G1- G2- and M-checkpoint has the task of controlling that the adjoining phase has had enough time to complete its tasks (Alberts et al., 2014, p.967). If the cells were to enter cytokinesis before completely separating the chromosomes, or if the DNA weren’t replicated correctly before entering Mitosis, it would have disastrous consequences.

One very important enzyme in cell cycle progression (see protein in Abbreviations and explanations) is the cyclin dependent kinase (CDK). cdk’s are inactivated unless bound to another protein called cyclin (Mason et al., 2020, p.219). There are three main categories of cyclins: G1/S- S- and M- cyclins. Their levels rise and fall as the individual cyclins are needed (Mason et al., 2020, p.219). A cdk bound to a cyclin is called a cdk complex.   
  
For a cell to pass the G1-checkpoint there is a threshold of external and internal signaling levels that needs to be passed, for the cell to enter S-phase. A complex system of positive feedback loops drive the cell over a point called the restriction point (Johnson & Skotheim, 2013). E.g., when enough cdk’s are activated, they will phosphorylate (see Abbreviations and explanations) a protein called Rb (retinoblastoma). If the Rb protein receives two phosphors it will release itself from a protein called E2F (Alberts et al., 2014, p.1013), which in turn induce transcription of new cyclins (Werner & Jumaa, 2015). E2F also targets a protein on the SCF protein complex, which is important in degradation of the p27 protein, a protein that binds to a cdk complex inhibiting phosphorylation (Johnson & Skotheim, 2013).

The S-cyclin activated cdk’s phosphorylate proteins responsible for activation of the DNA replication process (Masumoto et al., 2002).

The G2-checkpoint assures that the necessary proteins for mitosis are present and the DNA as well as all the organelles has been correctly replicated in S-phase and G2-phase. Both G1- and G2-checkpoints are able to arrest the cell cycle if DNA-damage is spotted (Studzinski & Danilenko, 2005).

The M-checkpoint is the final checkpoint, and ensures correct separation of chromosomes before cytokinesis (Studzinski & Danilenko, 2005). Another set of positive feedback loops kicks in. A protein called Cdc25 activates M-cdk complexes, which was inactivated by another protein called wee1. These M-cdk complexes begins inhibiting the Wee1 protein, while also induce important events in mitosis (Alberts et al., 2014, p.978).

All checkpoints are controlled by genes that either promote or demote the cell cycle. Cell cycle promoting genes are called proto-oncogenes. If mutated, they become oncogenes and might lead to uncontrolled cell division and cancer (Heidi Chial, 2008). In the event of oncogene activation, tumor suppressors kick in. They code proteins responsible for inhibiting the cdk complexes from operating. Tumor suppressors are also responsible for cell cycle arrest when DNA-damages are detected (Alberts et al., 2014, p.1015), or if DNA-damage is unrepairable it might kill the cell through apoptosis (see Abbreviations and explanations) (Delbridge et al., 2012).

### DNA damage and repair

Ionizing radiation damage the DNA in two ways: Either directly depositing its energy in a biomolecule (see Abbreviations and explanations), or by ionizing molecules surrounding the biomolecules, creating highly reactive radicals that oxidize (loss of electrons) the biomolecules (*RADIATION BIOLOGY: A HANDBOOK FOR TEACHERS AND STUDENTS*, 2010).   
DNA is also damaged without being exposed to ionizing radiation. Especially during DNA replication and building of Okazaki fragments (see 1.7.2). About one mistake is made for every nucleotide (Alberts et al., 2014, p.242). The mistakes might also be spontaneous such as deamination (cytosine become uracil) and depurination (loss of either Adenine or Guanine) (Cooper, 2000a). Even UV-light might cause adjacent pyrimidines to glue together creating a pyrimidine dimer (Mason et al., 2020, p.364)  
  
The mentioned damages are discontinuities in the DNA strands and might be classified as either single strand breaks (SSB) or double strand breaks (DSB). A single strand break only occurs in one DNA strand. If two breaks happen, one on each strand and close in time and space, they’re called double strand breaks (Hall & Giaccia, 2012, p.11).   
Damages might also be classified as sublethal, potentially lethal and lethal. Sublethal damages are not lethal, potentially lethal damages are lethal unless repaired and lethal damages are of course lethal.   
The cell has many DNA repair pathways, it can remove deaminated bases through base excision or remove whole nucleotides in a pyrimidine dimer with nucleotide excision repair (Hall & Giaccia, 2012, p.16). However, for more complicated damages like DSB’s, more intricate repair mechanisms are needed.   
  
Nonhomologous end-joining (NHEJ) is a repair mechanism activated in G1. The DNA is yet to be replicated so no sister chromatid is available. The solution is to simply reattach the strands as seen in Figure 1‑22. The result is loss of the damaged nucleotides, which might result in removing important exons (see 1.7.1) (Hall & Giaccia, 2012, p. 18).

Homologous recombination (HR) is another repair mechanism, but it is activated late S/G2 with available sister chromatid. The cell searches for a DNA segment on the unharmed sister chromatid, that is similar or identical to the damaged chromatid. This search is performed by one of the damaged strands by invading an unharmed strand and sampling base sequences. When homology is found DNA-polymerase is recruited to extend the damaged strand. The unharmed strand is released and the newly extended strand is used as a template to fix the other damaged strand (Alberts et al., 2014, p.278-279). Compared to NHEJ, HR is less efficient, but no DNA is lost during HR (see Figure 1‑22) and DNA is accurately restored (Liu et al., 2019). This underlines the importance of the G2 checkpoint.

Diagram

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Figure 1‑21. (A) Nonhomologous end-joining is DNA repair happening in the G1-phase of the cell cycle, before DNA is replicated. The strands are connected, but the nucleotides that existed on the strand before damaged occurred, are gone. (B) Homologous Recombination during late G2/S phase where sister chromatid is available after DNA replication and completely restores the broken DNA (Alberts et al., 2014, p.275).

Failure of repairing DNA damage might result in severe consequences. An unbound base caused by a DSB easily forms new hydrogen bonds with unbound bases elsewhere on the strand where a DSB has occurred. If enough DSB’s are created, they can cause severe abnormalities known as chromosomal aberrations (Iliakis et al., 2004). Lethal chromosomal aberrations affect the separation of replicated chromosomes in metaphase. The cell is unable to correctly separate the chromosomes and the cell dies. Worse is non-lethal chromosomal aberrations. Examples are symmetrical translocation and small deletions. Translocation is when a piece of chromosomes breaks and reattach to another chromosome, while deletion is removal of a piece of chromosome without reattaching (Hall & Giaccia, 2012, p.31). These damages might lead to activation of oncogenes (Zheng, 2013) or inactivation of a tumor suppressor gene (Mao et al., 2011).

### Cell Survival Curves

A cell survival curve is a staple in radiobiology and explains the relationship between cell survival S and radiation dose D. A cancer cell divides indefinitely if there is enough space and nutrients. The cancer cell is therefore defined as dead if it has completely lost its ability to divide and form colonies, thereby the name clonogenic survival (Hall & Giaccia, 2012, p.35).   
Cancer cells response to irradiation is often studied by in vitro (in cell dish) cell survival experiments. The cells are seeded in monolayers at the bottom of a cell dish with a medium containing all necessary nutrients. The cells are kept in an incubator with ideal growth temperature, and after x number of days they’re counted. The cells in a non-irradiated cell dish have a probability of growing into colonies, which is affected by the external environment as well as cell division errors (Hall & Giaccia, 2012, p.36). This efficiency is called the plating efficiency and is represented by the formula

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Plating efficiency is used as a normalization factor to accurately compare different experiments with different conditions. The survival fraction of an irradiated cell dish is found using the formula

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In Figure 1‑23 we see a typical cell survival curve, with the natural log of survival as a function of radiation dose. However, it is worth noting that each irradiated dish’s survival constitutes a point on the curve, and the curve is a result of interpolation. The interpolation is decided by a model known as the linear quadratic (LQ) model, which is discussed in 1.7.5.

Diagram

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Figure 1‑22. Typical cell survival curve with low and high LET (see 1.1.2) which is typically expressed in the natural log of survival (Giridhar & Rath, 2020).

### LQ-model

The LQ-model tries to explain the shape of the cell survival curve as a function of increasing dose. From Figure 1‑23 we see that the survival curve becomes less linear as LET (see 1.1.2) decreases. The increased curvature of the low LET region is known as the “shoulder” of the survival curve. The formula explaining this shape is

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where D is dose. and represents radiosensitivity of the cell (McMahon, 2018). The model assumes that there is a critical molecule crucial for a cell’s ability to divide, and that this molecule is the DNA. It also assumes that DSB is the critical damage type, but that the cell has repair mechanisms that might repair the damage (Chadwick & Leenhouts, 1973). It accounts for two different DSB events (breakage of chemical bonds in the DNA molecule).

1. One event causing one DSB
2. Two SSB’s close enough in time and space to create one DSB

represents mode 1, and represents mode 2. In and we have the probability the chemical bonds, that received dose D, are broken. Represented by

where k is the probability per bond per unit dose that the bond is broken. is the proportion of dose that breaks the bonds with mode 1, and is the proportion of dose for mode 2. Chadwick & Leenhouts showed that the mean number of DSB’s from mode 1 was (Chadwick & Leenhouts, 1973)

where A is the number of bonds on the DNA double helix, where mode 1 DSB’s occur.  
A first order Taylor approximation around 0 for gives

For mode 2 they showed that mean number of DSB’s was

where B is number of SSB’s not repaired on both strands, that are also close enough in time and space to constitute a DSB. Doing the same Taylor expansion gives

Combining the number of DSB’s from each mode and multiplying with the probability of the DSB’s being repaired f, we get

From here they introduced a proportionality constant to relate number of DSB’s to cell death and assumed Poisson distribution to achieve probability of cell survival per dose. Which results in the equation 1‑22. This shows why we have a linear and a quadratic link in the LQ-model, which does fit well with the curves we see in Figure 1‑23, where the high LET radiation has a denser energy deposition, creating more mode 1 DSBs. And for low LET there is a shoulder caused by accumulation of SSB’s for higher doses creating more mode 2 DSB’s.

The ratio is a typical measurement of radiosensitivity, where you find the dose of equal contribution between and (see Figure 1‑24). The ratio indicates whether it is reasonable to split the dose you want to give into fractions to allow time for repair (van Leeuwen et al., 2018). The ratio is found like this (Hall & Giaccia, 2012, p.39)

Chart

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Figure 1‑23. Typical cell survival curves for low (blue) and high (red) LET, with ratio illustrated (Hall & Giaccia, 2012,p.39).

Figure 1‑24. Early (high ) vs late (low ) responding tissues. Early responding cells are typically cancer cells because they are always dividing, and (Hall & Giaccia, 2012, p. 395).

### Radiation Induced Bystander Effect

As mentioned in 1.7.3, cells are normally damaged either directly or indirectly. However, it has been showed that non-irradiated cells might exhibit the same biological endpoints (e.g. apoptosis, generation of micronuclei or DNA strand breaks) as irradiated cells (Marín et al., 2014). This phenomenon is called the radiation induced bystander effect (RIBE). The theory is that irradiated cells signal to non-irradiated cells, either by intracellular communication trough protein channels (Gap junctions) between cells, or intercellular communication by excreting soluble factors into the surrounding medium (Daguenet et al., 2020). Although the existence of RIBE’s is indisputable, the characteristics of the biological parameters constituting an RIBE is not well defined (Blyth & Sykes, 2011).

It has been shown that a multitude of pathways are involved in RIBE’s, such as p53 (ref here), ATM (Ataxia telangiectasia mutated protein, ref here), reactive oxygen species (ROS ref here) and various cytokines (ref here) (Kirolikar et al., 2018).   
We will not discuss the various pathways in detail, but for shorter explanations see ***Abbreviations***.

Another complicating factor is all the factors that influence what bystander effect we get. It has been shown that for low doses with high LET (ref here) we have protective bystander effects, where rats inhaling uranium dust lived longer than the control rats (Mitchel, 2004).   
Dose received is also important, as it was shown that bystander response was almost as effective in killing cells compared to direct effects. But as dose increased the bystander effect saturated (Prise & O’Sullivan, 2009). What type of signaling you get is decided by what cell line you perform your experiments on.

Because of the bystander effect saturation for increasing dose, you neglect the effect for normal open field radiotherapy, but the effect has been established for SFRT (Spatially Fractionated Radiotherapy), where you have decreased toxicity in low-dose regions and increased survival in high-dose regions compared to open field (Asur et al., 2015).   
Better understanding of the biological mechanisms behind these results are important and has the potential of improving how SFRT (spatially fractionated radiotherapy) is used clinically.

### Spatially Fractionated Radiation Therapy

Spatially fractionated radiation therapy (SFRT) aims to achieve non-uniform dose distribution. It is a stark contrast to traditional radiotherapy where dose uniformity in the target is desirable. GRID therapy is a SFRT method where a photon beam is collimated into a specific pattern with low and high intensity areas, as is shown in Figure 1‑26. Traditionally a GRID block was used create high (peak) and low (valley) dose areas (Billena & Khan, 2019). GRID therapy arose from the need of treating deep seated or bulky tumors (Yan et al., 2019). The external beam therapy (EBT) machines of the early 20th century were typically X-ray tubes (1.2.1), and couldn’t create photon beams with energies surpassing kV (Gianfaldoni et al., 2017). Therefore, few photons penetrated deep enough into the skin to reach the tumor. A GRID block was necessary to increase the dose but keep skin toxicity at a tolerable level. When MV photons were introduced with the linear accelerator (LINAC see Abbreviations and explanations) and photon ranges increased, the need for GRID decreased (Yan et al., 2019).  
Today there are many ways to apply SFRT. Linear accelerators have preinstalled MLC’s (see Abbreviations and explanations) which can modulate the GRID pattern in a way the static GRID block can’t (Billena & Khan, 2019). Tomotherapy is a treatment method, that combines a CT machine (see Abbreviations and explanations) with a linear accelerator to give radiation treatment in degrees (Sterzing et al., 2009). The CT image is used to adjust the position of the patient to ensure that accurate dose is given. Modulation of beam intensity and direction can create a GRID shaped dose distribution (Zhang et al., 2016).   
3D SFRT methods have been developed, where the linear accelerator is rotated and the beam is modulated to create a sphere-like dose distribution (Wu et al., 2010).

A screenshot of a computer

Description automatically generated with low confidence

Figure 1‑25. (A) An example of a dose plan for GRID therapy from anteroposterior view of the lung area. The GRID pattern is clearly visible with dose gradients out from the center of the circles. (B) A transversal view of dose plan. Here we also see gradients in dose into the patient as the radiation attenuates into the body (Billena & Khan, 2019)

From the 1990’s to today, SFRT has primarily been used for palliative (treatment meant as pain relief rather than curing the patient) treatment of bulky (larger than 8 cm) tumors (Yan et al., 2019). However, (Asur et al., 2015) showed cells located in the valley region had overexpressed genes related to DNA-repair, cell cycle arrest and apoptosis. He also showed that cells located in peak areas had increased survival, leading it to believe that inter- and intracellular communication happen between cells (see 1.7.6). There is also evidence of immune system activation by recruitment of T-cells (a type of white blood cell) (Kanagavelu et al., 2014).  
These results indicate the potential of SFRT as a curative treatment.

# Materials and Methods

## Dosimetry

We want to establish a 2D analysis method of X-ray irradiated A549 cells through a GRID block. Accurate measurements of dose received by the cells were necessary because of the non-uniform dose distribution. The dosimetry methodology was made by Bjørg Vårlig Håland in her master thesis (Bjørg Vårli Håland, 2020). Gafchromic EBT3 film (Ashland, USA) was chosen as dosimeter for reasons we’ll get into (ref here). Using the films as an absolute dosimeter (see 1.5) we needed to make a calibration curve that related the optical density (OD) to dose. Therefore, we needed to perform X-ray dosimetry on an air-filled thimble ionization chamber (ion chamber) (FC65-G, IBA, Germany).

### X-ray dosimetry

An X-ray beam was generated using a PMC 1000 X-ray unit (PANTAK, USA) operating at 10 mA and 220 kV for sufficient electron fluence from the cathode with high enough energy to generate photons of kV range. We used filtering of 0.7 mm Cu and 1.52 mm of Al. This is a standard in the X-ray lab at medical physics department at the University of Oslo to achieve a more homogeneous energy distribution. A source to detector distance (SDD) of 60 cm was used to ensure the right beam divergence and high enough intensity. The experimental setup can be seen in Figure 2‑1.  
  
When cells are irradiated, they’re put inside a cell flask holder made from polymethyl methacrylate (PMMA), that holds four cell flasks in position A, B, C and D. To simulate the same conditions, the ion chamber was placed inside a T25 cell flask (Nunc, Denmark) and measurements were performed in all positions as seen in Figure 2‑2. The protective cap of the ion chamber slightly lifts the sensitive volume above the bottom of the cell flask (see Figure 2‑1), closer to the radiation source, but the cells are seeded in monolayers at the bottom. It is therefore necessary to account for this height difference when prescribing radiation.

Diagram, engineering drawing

Description automatically generated

Figure 2‑1. Ion chamber inserted into a T25 cell flask, that has been cut open. The sensitive volume is surrounded by a graphite wall within the protective cap, and we observe the height difference between the bottom of the cell flask and the cell flask. During experiments efforts were made to position the ionization chamber as flat as possible inside the cell flask. Figure is only for illustration purposes.

Because intensity follows the inverse square law (see 1.2.1), the ratio between intensity at the sensitive volume of the ion chamber and the intensity at the bottom of the cell flask is

Using a caliper (ikke ferdig ta med usikkerheter), the thickness of the cell flask holder and the cell flask bottom was found to be cm and cm, respectfully. The diameter of the protective cap, also measured with a caliper, was cm. Giving a radius of cm. The ratio becomes

­The intensity in the sensitive volume of the ion chamber is higher compared to the bottom of the cell flask, and we need to increase the dose accordingly when irradiating the EBT3 films.

A picture containing desk

Description automatically generated

Figure 2‑2. An overlook of the experimental setup for X-ray dosimetry. The cell flask holder is placed inside an irradiation chamber made in-house.

A machine on the counter

Description automatically generated with low confidence

Figure 2‑3. A closer look at the experimental setup of X-ray dosimetry. The ionization chamber is inserted into the cell flask, which is placed into the cell flask holder. Ionizations is measured in all four positions.

A max 4000 electrometer (Standard Imaging, USA) was used to measure the ionization current from the ion chamber. Converting the output of the electrometer, from nC in air to [] in water, was done using the formalism established by the International IAEA explained in 1.5.2

|  |  |
| --- | --- |
|  | 2‑1 |

is dose to water, is the calibration factor relating ion chamber output to dose in water and is given by the calibration lab (Statens strålevern, Norway). is the output from the electrometer in nC, is correction factor accounting for change in ion chamber resulting in a shift in spectral distribution when moving from air to water. corrects for effects caused by the ion chamber displacing the water. is mass energy absorption coefficient (see 1.3.1) ratio between water and air averaged over the photon energy spectrum. is when air pressure and temperature conditions are different, than that of the calibration lab. is found using the formula

where and is temperature and air pressure during measurements, and and is temperature and air pressure during calibration using reference conditions. , . Updated Correction factors and mass energy absorption coefficient rates were found in (Waldeland et al., 2010) (see Table 1).

Table 1. Calibration factor with correction factors and mass energy absorption coefficient ratio. Two experiments were performed using dotted and striped GRID configurations with different temperature and air pressure conditions.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  |  |  | (310821, stripes) | (131021, dots) |  |
|  |  |  |  |  |  |

The ionization chamber was irradiated 5, 10, 15, 20 and 60 seconds 3-4 times in each position. We wanted the calibration films to receive doses that correlated with doses the cells would receive during GRID irradiation. Hence, dose points of 0 (control), 0.1, 0.2, 0.5, 1, 2, 5, 10 Gy were chosen. Because of potential delay in beam production from the X-ray machine, we needed to make sure that there was a linear relationship between irradiation time and the lowest doses (0.1, 0.2, 0.5 Gy). Using linear regression (see 1.6.1) we find a regression model for each position, then find the mean of these to get

where are mean coefficients and D is dose.

For doses above 0.5 Gy we assumed linearity. So, we only made one 60 second measurement found the doserate and extrapolated to 1, 2, 5 and 10 Gy.

We assumed that the uncertainties were connected to and , respectively. Using the standard equation for error propagation with independent variables for a function

The uncertainty for from 2‑1 becomes

Gafchromic film

1. Protocol follows (Devic et al., 2016), for EBT3 films. EBT3 chosen because they give 2D dose distribution, which is perfect considering the cells are seeded in monolayers at the bottom. And can be cut to fit neatly inside the cell flask. They’re chemical composition makes them tissue equivalent, so they give accurate measurements of the dose to the cells. Because of their intrinsic low sensitivity to visible light, they do not need developing.   
   The films might change their optical densities from other external factors such as temperature, UV light and humidity. Control films of equal size to calibration films were used and handled the same way to account for these uncertainties.
2. The film’s chemical composition can be seen in Figure 1‑15. EBT3 was chosen because of its symmetry. You don’t have to worry about flipping the film. EBT3 also has matte polyester substrate compared to EBT2’s smooth polyester, which removes formation of the newton rings artefact when scanning the films. EBT3 also has the right working range of 0.1 – 10 Gy. It has an active layer of 28 m and two protective layers of 125 m polyester. A slab of Nylon 6 was used as an electron buildup layer to ensure that we’re passed the buildup region (ikke ferdig, why?).
3. How will the photons interact in the nylon 6 slab, and the polyester layer before entering the active layer ?
4. The calibration was performed by …

Used caliper with 0.0001 cm uncertainty

Used Nylon 6 to have equality between ion chamber and film irradiation

Irradiated x seconds to achieve 0.1 0.2 … 10 Gy For calibration curve all four positions twice

Measurement films shaped 5 Gy four positions four times

Open and GRID stripes

131021   
Only GRID

Scanning 48 hours later, transmission mode, name , 300 dpi, no image correction or adjustment, only raw data, pixel resolution

48 hours to ensure long enough time

Husk å se på tidligere artikler

Image registration pystackreg based on turboreg in imagej which is based on a pyramid approach

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# Appendix

## Appendix A

### Compton Scattering

Here we derive the photon energy scattered after interaction with a free electron at rest. We will use the four-momentum vector   , which consist of a time component and three space components .   
  
First, we use conservation of energy.

The energy of the incident photon is . Because of the assumption of an electron at rest, we only have rest energy . The energy of the photon after the interaction is simply , and the energy of the electron after the interaction is unknown. We end up with this equation

|  |  |
| --- | --- |
|  | 4‑1 |

Finding requires that we introduce conservation of momentum. We need to find the components of the four-momentum vector for the photon and the electron. We’ll use Figure 1‑3 as a basis for our calculations.

For the incident photon, the time component become . We only have momentum in the x-direction. Using the relation

|  |  |
| --- | --- |
|  | 4‑2 |

and the fact that the photon is massless, we get .

The electron at rest does not have momentum, but it does have rest energy. The time component become . Where is the rest mass of the electron.

We use trigonometry to find the spatial components of the momentum for the electron and photon after the interaction. Combining all the results, we get four four-momentum vectors

|  |  |
| --- | --- |
|  | 4‑3 |

With all the components in place, we use conservation of momentum.

First, we separate the and , then we square both sides of the equation. For simplicity, we remove the vector sign above our four-vectors. We get this equation

|  |  |
| --- | --- |
|  | 4‑4 |

The product of two four-vectors is , we see that becomes . And becomes .   
We use the dot product to find , , , and .

If we use equation 4‑2, we can exchange with . We get

Finally, we put all our calculations together and rewrite equation 4‑4 to get

solving for we get

Inserting this expression into equation 4‑1 and solving for photon energy after the interaction we get

|  |  |
| --- | --- |
|  | 4‑5 |

Which is the expression for the energy of the photon after the interaction, with scattering angle .

### Mean free path

Here we derive the mean free path of a photon.

Assume that you have incoming photons hitting a slab of material with infinitesimal area dA and width dx (see **Error! Reference source not found.**). The total probability of N photons hitting the slab, with an interaction probability of is

|  |  |
| --- | --- |
|  | 4‑6 |

Where n is atoms per unit volume

The probability of **not** interacting ­is of course , then number of photons after the slab becomes

Rearranging and inserting our expression for P we get

If we divide by and let approach , we get

Solving the differential equation, we get

where is the number of photons entering the slab. Now we have the fraction of photons that doesn’t interact in the slab. If we multiply Q with P, we get a binomial looking probability density function, which describes the probability of an interaction happening somewhere between and

Integrating over possible pathlength from to we get an expected pathlength of

Solving the integral using partial integration we get

Using L’Hôpital’s rule we see that

For the second term we get . This results in a mean free path of

We know that is atoms per unit volume, and from 1.1.1 that has the unit . Multiplying them, we get the attenuation coefficient , resulting in the expression

|  |  |
| --- | --- |
|  | 4‑7 |

Schematic

Description automatically generated with low confidence

Figure 4‑1. A thin slab of material with atoms that might interact with an incoming photon (“Mean Free Path,” 2021)