Simulation and experimental verification of the DNA damage due to X-rays interaction

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Abstract
It has been developed a model of the DNA in MATLAB, where it has been taken into account each one of the component atoms. It is possible to have a sequence length of even 10000 basis pairs with the possibility for introducing all types of sequences. It was studied the DNA damage in a single strain and in double strain, SSB and DSB respectively as a product of ionising radiation from X-rays when interacting with the DNA immersed in water. This is a theoretical and experimental study in-vitro that quantifies the SSB and DSB with respect to different Gy’s of radiation, this allows the understanding and prediction about the exact risks of the exposition. It has been developed for energies corresponding to the 17.6 KeV however the energy parameters are also variable which allows the simulation of all type of ionising radiation. For this simulation it is also taken into account the indirect damage as a product of the free electrons and radicals from the water effects. The information of the spatial distribution of the electrones is obtained form Geant4 and afterwards it is implemented in Matlab where rays are created with tri-dimensional random trajectories through Monte Carlo simulations. The experimental confirmation was developed by radiating DNA samples immersed in water with a X-rays unit with Molibdeno target and it is observed and quantified the damage level through Atomic Force Microscopy (AFM). It was obtained a direct relation between the damage and the radiation dose in the experiment and in the model and it is concluded that for 17.6 KeV the deterioration for interaction is observed very low doses but the mutagenic damage only appears until the 17 Gy.

Keywords: MATLAB model, PARTRAC, Geant4, X-rays, Free radicals, Double Strain DNA damage, Atomic Force Microscopy (AFM).

1. Introduction

When cells are exposed to ionising radiation, their DNA suffers a damage that can have as a result cell death (Sachs et al. 1992), it is important to emphasise that the X-rays have as main target the nucleus DNA. Radiation therapy (radiotherapy) is governed under this principle to counteract cancer, where it is aimed to kill tumor cells through ionising radiation.

Cancer is the main problem of public health in many countries and it is the responsible of one quarter of the mortality in the United States (Siegel et al. 2013). It is for this reason that it is invested in research about its causes, consequences and prevention through treatments among which radiotherapy has been one of the most used to counterattack its effects.

The use of radiation as a therapy against cancer had its beginning since its discovery (Burns et al. 2010). X-rays are photons with frequencies from 50 to 5000 times bigger than visible light and its wavelength is a lot shorter, it is of atomic order, and this confer them with its main characteristic that is its capacity to pass across most solids (ASRTFASRT and FASRT 2009). X-rays have the tendency to act more like particles that as waves. They are created by emission of photons at electronic layers emitted by product of the acceleration and deceleration of charged particles (Attia et al. 2011).

The study of radiation is biologically important given that as a product of the particle interaction, cells suffer around one million "normal" molecular lesions, for example the UltraViolet component of sunlight can cause more than $1 \times 10^5$ lesions in the DNA of only one cell per day (Lord and Ashworth 2012), these lesions are corrected by the DNA reparation mechanisms. But the rate of activity and efficiency of this mechanisms...
depend on the cell type, cell age and extracellular environment (Panasci and Alaoui-Jamali, 2010). For this reason, the DNA repair mechanisms would be relative to each person and not everybody repair at the same time and with the same efficiency. It is because of this that in some people it is observed that their cells accumulate big amounts of DNA damage or that their cells do not repair correctly, which generate mutations (Popanda et al., 2003).

The specific damage that is conferred to the ionising radiation as is the case of the X-rays radiation is the Single-Strand Breaks, SSBs and the Double-Strand Breaks, DSBs (Lord and Ashworth, 2012). As it was mentioned before, the damage caused in the DNA as a product of the interaction with the X-rays is relative to each person and its quantification is crucial specially for people with cancer who receive treatment by radiotherapy.

The main focus of this research is the quantification of the damage generated in the DNA after interacting with all type of ionising radiation. It is necessary to develop epidemiological studies that indicate which are the exact risks of the exposition in humans, this models are developed by simulations of the DNA interacting with the X-rays and generating statistics of the damage according to the reaction possibilities between the DNA components, photons and indirectly created particles (Friedland et al., 2011).

When X-rays photons interact with the DNA immersed in water, it is talked about energy deposition along the electron trajectories that are generated during the radiation. The X-rays deposit around the 95% of their energy through the Compton Effect, vibrations, ionisations and excitations. The Compton Effect is observed given that the photons interact directly with the atom’s electrons sharing part of their energy (Bernhardt et al., 2003). It is ignored the effects given by the interaction of the photons with the atoms of the DNA, given that we are using X-rays with energetic conditions that allow us to approach the problem in this way.

After taking into account the direct effect of the X-rays over the DNA, it is important to emphasise that the DNA is always surrounded by more molecules, mainly water, and these molecules also interact with the X-rays which generate free radicals that are harmful for the integrity of DNA (Elshaikh et al., 2006).

To know the radiation effects in alive systems, it is required the computational modelling and the experimental efforts that verify the theoretical predictions. Although it has been invested big efforts in numerical models, the statistical power is a big limiting factor for the risk estimation depending on the radiation doses. Another limiting factor is the lack of correlation between the experimental results of induction and quantification of the damage generated in the DNA with the simulations and mathematical models (Deisboeck et al., 2011).

On another hand, current DNA models have the problem that have been developed under forceful parametrisation that hide the true nature of the double strain and are centred mainly in physical phenomena of the interaction, without accounting for the strong dependency an influence of the DNA components.

It is because of this that the main achievement of this research is the simulation of the DNA with each one of its atomic components. This is also immerse in water to have into account the generation of electrons released by water which are responsible of the main DNA damage. Both the DNA and the water molecules are exposed to X-rays and the damage is quantified according to the number of DSB obtained by the DNA strain rupture and it is also obtained the total number of electrons that interact and generate an event.

According to the international system, the absorbed energy by matter is measured by Gray (Gy) units. The energies that are typically measured with this unit are the X and Γ rays. The unit is defined as follows (ASRTFASRT and FASRT, 2009):

\[ 1\text{Gy} = 1\frac{J}{Kg} \quad (1) \]

Where 1 Joule corresponds to ionising energy interacting in 1 Kg of matter. This unit is widely used in radiotherapy where it is defined important concepts like \( D_0 \), which is the dose that in average induce one letal effect for each exposed cell (Haffty Bruce G, 2009). Such a letal doses approximately corresponds to \( D_0 = 1 - 2\text{Gy} \).

Currently there are two existing simulations of these interaction processes between X-rays and DNA and they are explained in the following section:

- PARTRAC is a code developed during the last 25 years by the Helmholtz Zentrum München ISS,
Research Group Radiation Risk. The code consists of different modules that describe individual interaction processes. The module were written in FORTRAN and its components are presented in fig.1. This code has the DNA organization until their higher domains, modelling chromatin fibers and their packaged into chromosomes. Even thought this DNA model is developed under the parametrisation that the double helix is a cylinder with diameter of 2.3 nm which is fractioned into sections of 0.34 nm of thickness, these fractions represent the nucleotides. The developers also divided this cylinder in a smaller cylinder with diameter of 1 nm together with two arcs rotated by 36° that surround it. This is done for each nucleotide achieving the basis separation of the sugar and the phosphate group (Friedland et al., 2011).

Such parametrisation hides the DNA atomic composition which makes the interaction of the electrons with the DNA. The damage generation is done assuming that the energy deposition in spherical volumes or cylindrical of nanometric size, however the atomic bonds are ignored and they carry the majority of the damage generation.

This is a private software which makes its development slower given that only a reduced group of people are able to work on it. But it is the software with the highest reach in this area and it can even consider the DNA reparation rates.

- GEANT4 is a Software available in Linux Scientific, which simulates particles interactions and their trajectories effects along materials with variable characteristics. It is mainly focused in particles of very high energy given that it is part if the European Spatial Agency (ESA). A couple of years ago, it was started a project called Geant4-DNA, which consists of a Monte Carlo simulation for the passage of particles of lower energy through matter (Francis et al., 2011).

Its main objective is to know the risk of cancer after the exposition spatial radiation in humans. It is compose of a Monte Carlo for the generation of X-rays and it is analysed each one of the phenomena of its interaction with water. Due its recent development, there is not a free easy access model for the DNA yet. However, on September 2013, the PhD student Louis Olivier from the University of Paris, achieved the development of a model of the DNA at Geant4-DNA, during its interchange with the Medical physics group of the National University. This is a big advancement for the Software. The DNA is parametrised under geometrical conditions. In

![Figure 1: Diagram of the modules that make part of the PARTRAC code (Friedland et al., 2011)](image1)

![Figure 2: Model of the three organization levels of the DNA developed in Geant4 for Louis Olivier](image2)
the fig. 2 it is presented an image of the obtained result from their DNA model. This model reach the third level of organization of the double helix, the nucleosome and the chromatine fibers.

1.1. MATLAB simulation

Given the existing conditions of the codes it was decided to build the simulation of the X-rays interaction with the DNA, taking under consideration the structure and atomic composition of the DNA. This DNA was created with spatial coordinates of each one of the atoms that are joined through vectors that emulate the bonds. It is obtained from Geant4 the spatial distribution of the electrons generated after the X-rays radiation of 17.6 KeV in water. Finally it is generated a random ray with origin in the three spatial dimension through Monte Carlo, each one of this rays will create the spatial distribution of electrons under the parameters obtained in Geant4.

It is taking into account and it is assume as a relevant factor for the breaking and the damage generation, the proper sequence of the DNA, in this way, the simulation allows the variation of the sequence and its size. This is an important factor that is not possible in another simulation and open the door for research regarding the relation between the influence of basis content in the vulnerability to damage.

It is possible to simulate 10,000 pair bases with a total of 634,983 atoms. This number of bases could be increase in the simulation but for the results of this research this is the maximum considered number given the computational power constraint.

1.2. Experimental verification

In order to observe the damage in the DNA after its respective irradiation, it was used the Atomic Force Microscopy (AFM), this microscopy technic works hitting with an atomic tip the DNA sample and maintaining a constant force between the tip’s atom and the sample’s atom and sweeping fast the sample with an oscillation of 68 kHz [Eaton and West 2010], such frequency is given by a piezoelectric material of high precision.

When keeping the force between the atoms constant, the tip should change its position, this movement together with the location of the tip in the sample, provides the three spatial data points to create a surface that approximates the appearance of the sample to an atomic scale.

The process to the microscopic visualisation is not simple, but historically the first obtained image for AFM was the image of a strain of DNA, the preparation protocol of the sample and the analysis of the sample are very well documented and standardised [Cerreta et al.] 2013. The DNA sample should be reduced to the adequate concentrations, it is placed over a negatively charged surface called MICA, given that the DNA is also negatively charged, a positive buffer is added which serve as interface between both phases. The sample could be dry or in water [Eaton and West 2010].

2. Methodology

2.1. Simulation

2.1.1. DNA creation

The key piece of the simulation is a double DNA helix. In order to create in a realistic way the DNA, it is necessary to obtain the information about the location of the DNA atoms, its spatial coordinates. This information was obtained from the Protein Data Base(PDB), which provided a file with the spatial coordinates of 637 atoms that compose the double helix with 10 base pairs and allows the DNA visualisation.

The disadvantages from the file of the PDB is that can only be used for visualisation and the data extraction is difficult, to solve this problem, the spatial data was extracted from the file and they were implemented in MATLAB where the simulation can be programmed. To achieve the data extraction of the PDB file should be done with care and the objective of the simulation is to be able to introduce all types of sequences and achieve its helix. This is done with the aim to check if different DNA sequences have influences in the damage susceptibility.

First the data from the PDB is organised such that each atom has an atomic number, in order to identify each base, it is assigned a number to each base in alphabetic order. This file is then exported to MATLAB. Then the atoms that are part of each molecule are identified with the purpose of organising them and being able to compare them, finding in this way the pattern in their structure. Once the bases are identified in Matlab, it is necessary to identified the common bonds, the three more important bonds are compared between molecules depending of their order in the strain, with the aim of obtaining the way in which
the molecules rotate when shaping the DNA. In order to achieve this, we make use of three vectors that correspond to the line between each bond, these three vectors are compared between the molecules of each helix through the following rotation matrix:

$$
R = \begin{bmatrix}
C_\phi C_\theta & C_\phi S_\theta S_\phi - S_\phi C_\theta & C_\phi S_\theta C_\phi + S_\phi S_\theta \\
C_\theta S_\phi & S_\phi S_\theta + C_\phi C_\theta & S_\phi S_\theta C_\phi - C_\phi S_\theta \\
-S_\theta & C_\phi S_\theta & C_\phi C_\theta
\end{bmatrix}
$$

(2)

Where $\phi$, $\theta$ and $\psi$ are the rotation angles measured from the axis $X$, $Y$, $Z$, and $C_\phi$, $C_\theta$, $C_\psi$ are the cosines of the respective measurements and $S_\phi$, $S_\theta$, $S_\psi$ the sine of the angles. It would be necessary to use the rotation matrix and position vectors that locate the molecule in the correct place and in the correct direction. This happens for each nitrogen base with the associated location. In fig. 3 is presented the visualisation obtained from the PDB data file. The four molecules that constitute the DNA were created through the PDB file in order to develop the simulation were the desired sequence is the input and it can be build according to the atoms spatial location the results of our modelled molecules can be seen in fig. 3.

![Figure 3: Molecules visualised in Matlab](image)

In order to simulate the effect of X-rays in the DNA, the most important observation is that the DNA is irradiated in a way that can not be predicted, that is why it is necessary to use a Monte-Carlo simulation of the rays trajectories during the simulation. This trajectories can be seen in fig. 6, where the size of the strain was double and it was generated random trajectories of the x-rays, these trajectories are the ones corresponding to the photons that hit the DNA and as a product of this hitting, electrons are generated in their proximity with spatial distribution obtained in Geant4.

The X-rays have the peculiarity that generate a considerable big amount of electrons as soon as they traverse a medium with water. This is due to the fact that its energy is bigger than the ionising energy of the water and because of its high energy, the energy of its products is also higher than the ionising energy, this allows to have a long reaction of cascade effect with origin in the X-rays but mainly followed by the produced free radicals, this effect is pretty important and can not be ignored in the simulation given that is the responsible for the biggest damage of the DNA.

In order to understand how can we generate electrons in water, it is necessary to use the software Geant4, which contains all the theory of particles and experimental and theoretical information that allows to find in the most realistic way the spatial distribution of the electrons that are generated in the proximities of the trajectories of the X-rays. The theory that allows the simulation of this process has been developed for many years and can be found in many other papers, it is not the scope of this research to work on this theory.

This information about the electrons was added in MATLAB in order to simulate the damage. And the huge amount of particles that are generated around a X-ray are included in the simulation of the randomly generated X-rays trajectories. The visualisation is as follows: fig. 6

2.1.3. Visualisation and DNA damage quantification

In order to quantify the damage that the X-rays generate, after each iteration of the simulation, the state of the DNA and the distribution of each one of its atoms is saved. This means that given the reactions that happen in the DNA, it is necessary to save the events after the collisions with the X-rays or with the electrons. From this information it is analysed the SSB and DSB with respect to increased radiation.

Determining wether or not there was a SSB or DSB, it was set a boundary of interaction given by
the rule that the electron and each DNA atom should be maximum two Van Der Waals radius from the respective radius. If this condition is reach, the events counting is increase, after this event is counted, it is necessary to analyse the hits that are able to produce the disappearance of crucial bonds to the helix conformation, this mainly happens if there is a DSB more than a SSB. To find a DSB, it must be followed that the nucleotides faced right in front of each strain have a damage, this means, two SSB account as one DSB is the location is the same in each helix.

2.2. Experimental procedure

2.2.1. DNA extraction

It was followed a protocol of blood DNA extraction from a blood sample of a healthy volunteer. After the extraction the DNA was distributed into seven aliquots each one of them to a concentration of $20 \mu g$ and with a total volume of $50 \mu L$. The samples were immersed in water to simulate the cell conditions where it is compose mainly by water, the employed water was distillate, deionised and pure.

These aliquots are irradiated with the followed radiation values: 0.006 Gy, 0.025 Gy, 0.08 Gy, 1 Gy, 5 Gy and 30 Gy. Remembering that the definition of Gray is the absorption of 1 Joule of ionising radiation per kilogram of mass, this is the most used unit in radiobiology. A sample that has not been irradiated is our negative control, but under the same conditions of transportation, movement and temperature than the irradiated samples. This negative control will be the bases for comparison to show how much damage was generated in the DNA. Each one of the aliquots is keep in ice during the irradiation and then they are conserved to $4^\circ$.

2.2.2. X rays-DNA Interaction

Each one of the six aliquots were irradiated in a basic unit of X-rays of 35 KeV to different Grays (Gy). The maximum peak of the work function of this unit of this moliibdeno target is 17.6 KeV, for which this is the real energy that the samples are receiving. X-rays were obtained from a PHYWE machine as is shown in the sample in fig. 4.

The chosen Gy’s were 0.006 Gy obtained after an exposition time of 2 seconds, 0.025 Gy with 8 seconds, 0.08 Gy with 27 seconds, 1 Gy with 5 minutes 35 seconds, 5 Gy with 27 minutes 58 seconds and 30 Gy with 2 hours 47 minutes 50 seconds.

These exposition times are done by a calibration which its obtained by shooting a bean towards aluminium of different thickness and taking the photon counting with a Geiger tube for each one of the tubes. The relation between the number of photons against the thickness of the aluminium plate and an exponential regression is generated in order to obtain the total number of photons that correspond to an exposition without aluminium plate. The results of the exposition are presented in fig. 5.
The power is calculated with the number of photons per second, this power is given by the absorbed energy per second, this is obtained by the Molybdenum work function, because the X-rays emission tube is made of Molybdenum and its electrons have a maximum of energy of 20 KeV.

As it was mentioned before, the Gy have into account the mass of the exposed sample, then it is necessary to find the DNA mass, in order to do that, it is used the volume and the density of the sample. With this it is possible to find the power over mass unit.

$$\frac{\text{Potencia}}{m_{ADN}}$$  

(3)

Finally, it is possible to find the exposition time to all desired Gy values by the following equation:

$$T_{\text{exposicion}} = \text{Gys} \cdot \frac{m_{ADN}}{\text{Potencia}_{\text{absorbida}}}$$  

(4)

These exposition values were selected because they are the most used in radiotherapy applications and it is desirable to have a logarithmic sampling to generate converging results. The amount of 30 Gy is applied to patients that receive radiotherapy, but this an accumulative radiation obtained after many sessions, this to the aim that healthy cells that are affected after the irradiation have enough time to repair, while carcinogenic cells are repair at a slower rate and the time between each irradiation is not enough for them to repair, this is how is achieved their elimination. On the other hand, 0.006 Gy should be irradiated in one hour as is declared by the UNSCEAR (United Nations Scientific Committee on the Effects of Atomic Radiation).

2.2.3. Damage visualisation and quantification. Atomic Force Microscopy

A DNA sample preparation protocol is followed, this is an especial procedure for the ATM. It requires a Buffer preparation of NiCl₂, which will be useful to fix the DNA to a surface with minimum roughness and of atomic scale called MICA which has a negative charge as well as the DNA, that is why the NiCl₂ is used, its positivity allows the interface between the MICA and the DNA (Eaton and West, 2010).

Another relevant aspect for the visualisation in the AFM is the DNA concentration in the sample preparation, which should be 5 µg mL⁻¹ and then a 1 µg mL⁻¹. Finally 37µL are added to the mixture DNA-Buffer over the MICA, which acquires the shape of meniscus.

The protocol offers the option to make the sample in dry or liquid but for this research the sample was used dry. It is important to emphasise that the preparation of the sample is the most important factor to the success of the microscopy, isolation conditions are desirable.

For the damage quantification, the irradiated samples are compared with the negative control. After the search for differences given by the fragment size for both samples.

3. Results

3.1. Simulation Results

The first result obtained from our Software, is the generation of DNA helix of single or double strain with any longitude and with any desirable sequence. Our software builds the sequence with the respective atoms associated to the nucleotides and with their respective bonds as can be seen in fig. 7.
Figure 6: DNA subject to X-Rays

Figure 7: Example of a short DNA sequence obtained in our developed Software

Figure 8: DNA subject to X-Rays and obtained by Monte-Carlo and its respective particles following the spatial distribution

Many rotation matrices are obtained which cor-
respond to the rotation that happens between one molecule and the other as we walk along each DNA strain. Once we have the rotation matrices, we found the angles $\phi$, $\theta$ and $\psi$ that describe each rotation. Surprisingly, it was found that in the DNA each molecule rotate $36^\circ$ counterclockwise as it is walk along the helix, this means $\phi = 0$, $\theta = 0$ and $\psi = 36^\circ$.

The matrices are pretty similar and all of them where used to find this last result which makes a lot of sense given that after observing the DNA at the center, the basis are parallel which is an indication that the basis only can rotate over a plane. This data agrees with what is found in the literature (Sinden [1994]).

It was implemented a way to introduce any type of DNA sequence and the program has to create the helix that corresponds to such a sequence, an example is given in fig. 7, which corresponds to the sequence ACGTAG, this sequence can be freely chosen in terms of length and content.

This is a valuable characteristic that is not found in PARTRAC or Geant4, and it was the hardest to achieve feature of our software given that one of the objectives is the discovery if a sequence with a high Guanine-Cytosine content is stronger against damage in comparison to a sequence with a low Guanine-Cytosine content.

In fig. 6 is presented a visualisation of the way in which DNA is damage in a direct way by the generated random radiation. The trajectories of each photon are indicated in green. It is important to emphasise that not all of this trajectories create a SSB or DSB given that there is a constrain in atomic distance in order to have an interaction.

Given that implementing all the possible reactions in Matlab is too long and out of the reach of this research, it was employed the results from Geant4. Geant4 is the only Software available to study in great detail particle interaction with matter. In Geant4 we simulated the effect of X-rays in water for the case of many events and for the energy at which the samples were exposed in the experiment.

It was obtained a distribution of the free radicals around the X-ray trajectory, in order to appreciate the complexity of the phenomenon and the amount of particles that are created when the X-rays traverse a medium of water, it can be seen from the Geant4 simulation results in fig. 9, there we can see that the amount of particles generated is found over the trajectory of the ray, the following products from the reaction chain can be followed by means of the trajectories marked in red. Here we can see the enormous amount of free radicals in yellow that are generated by the green X-ray. Once this information is join together for each one of the X-rays trajectories generated in Matlab, it is possible to generate the visualisation of some of the particles that a ray generates. This can be seen in fig. 8, where it is observed one of the most relevant results of this simulation, the DNA helix, the ray and the created particles are all interacting and its effects computed.

Finally, it is done an analysis of the number of events or the interactions, the quantification of SSB and DSB. In order to do that, the simulation is run to the mentioned radiation values, and with an helix of 10000 pair basis. This simulation needs arounds eight hours per run given that the DNA is constantly changing according to the interaction effects, on the other hand the visualisation requires a lot of computational power. The ray is created for Monte-Carlo and with this ray it is considered a big number of photons of high energy, the trajectory of each one of this rays and it is visualised, the electrons spatial distribution is coupled and the respective constraints for interaction and damage are specified.

The total number of events for each radiation do-sis are shown in fig. 10, this graph has a linear behaviour as it was expected given that the DNA-electrons, DNA-X-ray reaction time probability is increased. On another hand, the SSB are quantified finding that there is no SSB for values of 0.006 Gy and 0.025 Gy given that this dosis are short and for dosis of 0.08 Gy, 0.3 Gy and 1 Gy there are around 5 damages.

The SSB increase exponentially for 1 Gy, 5 Gy and 30 Gy where it was found 6000 damages, this given that there are many interactions where the DNA gets weaker and the damage grow. Lastly, the DSB appear only for 17 Gy and 30 Gy where 8 DSB damages are found.
Figure 9: Free radicals generation simulation in Geant4, for a photon energy of 20keV, the green line shows the trajectory of the X-rays, the yellow particles are the free radicles created and the red lines are their respective trajectories.

Figure 10: Quantification of the events (SSB and DSB) from the whole simulation for different values of radiation (Gy)

What is indicated in fig.10 is in agreement with the analysed real number of basis from the DNA that is found in the cell nucleus, which is of the order of $10^9$, the number of DSB increase to values of the order of what is found in the literature (Friedland et al., 2011). For which it is possible to say that this is a correct simulation.

On the other hand, the variable given for the number of photons that arrive to the DNA is graduated according to the volume that is considered in our experimental case, but this can be modified letting the arrival of more photons and the damage possibility is increased. This fact will be also limited to the computational power.

3.2. Experimental Results

What was found after the irradiation of the DNA submerged in water was observed in a AFM, the data analysis is not possible by quantification of SSB and DSB given that we only have a visualisation and not an automatic measure of the conformational changes but we can see the state in which the X-rays have leave the DNA after their interaction. That is why we used a tool
analysis by fragment size. It is done a comparison of fragment size versus applied Gys.

The measure of the fragment size is developed with the Software Asylum Research, which lets the visualisation of the images and generates contrasts to its best observation (Morris et al., 1999). In this way fig. [1] is determined, where it is observed that with the increase of the applied radiation, the fragment size is decreased. With the increase in the damage the DNA gets fragmented in many parts that are harder to find and measure given that get diffused in the medium because of their weak state and size.

![Figure 11: Analysis of the DNA fragment size for different radiation dosis.](image)

In the following figures are presented the images obtained in the AFM, with they it can be corroborated the DNA fragment size found and the state in which this is found. This analysis was done based in standarised parameters of image analysis for the AFM.

First we have the negative control, that was not exposed to the X-rays and that had the same conditions of temperature and transportation as the other samples. This control is going to be crucial at the time to judge the relevance of the radiation in the DNA state for the other samples.

![Figure 12: Negative control results: a) 2D view of the AFM, b) 3D view of the AFM](image)

Under this result fig. [12] the fragment size and DNA physical conditions are compared after it is irradiated under different Gys. In fig[12] it is observed that the DNA is a long fragment and consecutive that is of easy localisation and sampling under the microscope. It has the dimensions and conditions indicated to guarantee that this is about a double strain DNA chain (Eaton and West, 2010) that works as negative control.

In the fig. [13] it is observed the fragmentation produced by the radiation at 0.006 Gy, based to the obtained in fig. [12] it is possible to approximate the damage intensity based to the average fragment size. This value of radiation was achieved with an exposition time of 2 seconds, to a constant energy value of 17.6 KeV.
Figure 13: Irradiated sample with 0.006 Gy, exposition time of 2 seconds: a) 2D view of the AFM to 5 µm, b) 3D view of the AFM to 1 µm, c) 2D view of the AFM to 1 µm.

Figure 14: Irradiated sample with 0.025 Gy, exposition time of 8 seconds: a) 2D view of the AFM to 5 µm, b) 3D view of the AFM to 5 µm.

Figure 15: Irradiated sample with 0.08 Gy, exposition time of 27 seconds: a) 3D view of the AFM to 5 µm, b) 2D view of AFM to 5 µm.

Figure 16: Irradiated sample with 1 Gy, exposition time of 5 minutes 35 seconds: a) 2D view of the AFM to 5 µm, b) 3D view of the AFM to 1 µm.
In the fig. 14, to a value that corresponds to 0.025 Gy, obtained with an exposition time of 8 seconds, it is observed the formation of higher DNA accumulations in some zones, this is possible because of the amount of particles that generate hits in this zones, this is contrasted with the zones where there is lower accumulation and also with the negative control. Of the same way, the DNA in this image stops being consecutive and organise as it is appreciated in the treatments before.

For the case of 0.08 Gy of the fig. 15, the energy is obtained with an exposition time of 27 seconds. It is observed a result pretty similar to the 0.025 Gy, it supports the results for both, for this it was desired a closer value, however in this case there was not accumulations, the sizes and visualisations of fragments are very similar. It calls the attention the linearisation that tends to appear.

In the fid. 16 it is observed the DNA generating aggregations and packaging. This fig. corresponds to 1 Gy, obtaining after an exposition of 5 minutes 35 seconds. It is observed the appearance of circles with a thickness in the image of 5 \( \mu m \), these can be the residues of some reactive in the preparation of the sample, however there is doubt that this is DNA or atomic waste organised given that when generating contrasts in the image, this circles were always uniforms to the DNA for which it could be said that they are the same material, but this can be confirmed (Cerreta et al., 2013).

The images corresponding to the fig. 17 y 18 were very difficult to find in the AFM in comparison to the lower radiations, given that this were done simultaneously to all the treatments, with the same reactive and with the same conditions and DNA concentrations, the only variables that could have caused this difficulty is that we could have not had enough DNA at the moment of mounting the sample over the MICA or that the radiation was too high to the sample’s size causing its evaporation. This hypothesis should be confirm experimentally.

Figure 17: Irradiated sample with 5Gy, exposition time of 27 minutes 58 seconds: a) 2D view of the AFM to 3.5\( \mu m \), b) 3D view of the AFM to 3\( \mu m \).

Figure 18: Irradiated sample with 30Gy, exposition time of 2 hours 47 minutes 50 seconds: a) 3D view of the AFM to 20\( \mu m \), b) 2D view of the AFM to 20\( \mu m \).

The fig. 17 was obtained to 3\( \mu m \) m, it is of 5 Gy obtained after exposition time of 27 minutes 58 seconds, thinner helices are obtained in comparison to lower
radiations. Again we observed thick circles, this are more numerous and of the same contrast to the DNA. In the [18] it is observed what was found for the sample with the higher radiation, 30 Gy. This sample was the hardest to detect and localise in the AFM.

The sample is observe in a sampling of 20 µm given its difficult localisation, from a 3D view , the DNA is hard to distinguish, but it is observed that the circles are not as uniform as could have been seen to lower radiations. In the 2D view, it is possible to see the DNA and it is observe that there are circles of the same DNA composition, the grays and others that are not of the same DNA composition, the white ones.

4. Discussion

Nowadays the real energy of the photons that are used for radiotherapy are of the order of few MeV, this to allow the Compton effect as the predominant one between the rays and the DNA, in this way, the DNA will be as focus as possible and with the less risk found [19]. This energy couldn’t be reach with the experimental setup given that the phywe instruments does not have this energies and this value could be reached but with an exposition time of the order of days nonstopping, for this reason we could not use this energy value.

For the simulation is necessary to increase the number of basis to the order of $10^8$, which corresponds to the total length of the DNA as has been sequenced. This would reflect the real value that a single exposed cell receives. Given the computational limitation, it was only possible to run the simulation with 10000 base pairs, but the program hold any sequence length.

It is necessary to develop repetitions of the experimental part in the AFM given that the protocol can take into many errors and given that at the end the damage analysis is only by fragment size, it is ideal to increase the repetition number to be able to have a bigger sample with a standard error lower in comparison to the data average.

It could have also been done a bigger sampling of each of the samples, given that zones of the sample are chosen and it is from this little zone that the image is selected. In this way, if more zones per sample are analysed, it can be possible to have a bigger consensus of the average size obtained per dosis.

The simulation and the experiment confirm that to higher dosis of radiation there is a higher number of interactions and the probability of DSB is increased. It was obtained as maximum value the dosis without visible damage to 0.025 Gy in the simulation, while in the experiment it was observe damage from the lower dosis of 0.006 Gy.

In can be conclude that low dosis of radiation also have the probability to generate damage and this damage can or cannot be lethal, if the simulation would have been iterated many times, there would have shown at least one event at low dosis given that there are not restrictions for this to happen, nor in simulation or in real life.

Finally, this simulation can have into account superior levels of organization of the DNA reaching even cromatine fibres, but given the time limitations, it was not possible to reach this characterisation, but it is possible to build it using the same principle of this first structural level. Superior packaging simulations will show the factors that protect and generate resistance to the damage, which lets to find more exact statistics to the damage [20].

5. Conclusions and perspectives

This simulation is a big advance regarding DNA modelling given that it is considered the atomic composition of the DNA and its sequence as crucial variables in the damage generation as a product of the interaction with x-rays. However, it requires a lot more statistical power for the finding of results to bigger scale.

Results were obtained and are congruent with what is found in the literature, As radiation is increased, as the number of events as the SSB and DSB increase in a linear way but with different slopes depending on the radiation.

The relation and comparison of the simulation with the experimental results and requires of time that given the time constraint they were not possible.

The perspective of this effort are to achieve a software that in the future could be implemented freely in all the radiotherapy treatments in order to predict the damage that each patient has at the exposition time with X-rays and in this way estimate which are the indicated dosis that should be received without the generation of a higher damage.
It is possible to add to the model the DNA repairation. This work is a lot harder and it should be specific to each patient or at least it should be dependent of the corporal mass specific to the irradiated zone. On the other hand, it can be followed the idea of the no parametrisation of the proteins but the modelling by coordinates and modelled movements by rotational matrices as it was generated for the case of this computational simulation.

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