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TITLE:

Dosimetry for Cell Irradiation Using Orthovoltage (40-300 kV) X-Ray Facilities

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KEYWORDS:

dosimetry, low energy X-rays, radiobiology, irradiation protocol, cell irradiation, X-ray facility

SUMMARY:

This document describes a new dosimetry protocol for cell irradiations using low energy X-ray equipment. Measurements are performed in conditions simulating real cell irradiation conditions as much as possible.

ABSTRACT:

The importance of dosimetry protocols and standards for radiobiological studies is self-evident. Several protocols have been proposed for dose determination using low energy X-rays facilities, but depending on the irradiation configurations, samples, materials or beam quality, it is sometimes difficult to know which protocol is the most appropriate to employ. We, therefore, propose a dosimetry protocol for cell irradiations using low energy X-rays facility. The aim of this method is to perform the dose estimation at the level of the cell monolayer to make it as close as possible to real cell irradiation conditions. The different steps of the protocol are as follows: determination of the irradiation parameters (high voltage, intensity, cell container etc.), determination of the beam quality index (high voltage–half value layer couple), dose rate measurement with ionization chamber calibrated in air kerma conditions, quantification of the

attenuation and scattering of the cell culture medium with EBT3 radiochromic films and determination of the dose rate at the cellular level. This methodology must be performed for each new cell irradiation configuration as the modification of only one parameter can strongly impact the real dose deposition at the level of the cell monolayer, particularly involving low energy X-rays.

INTRODUCTION:

The aim of radiobiology is to establish links between the delivered dose and the biological effects, dosimetry is a crucial aspect in the design of radiobiological experiments. For over 30 years, the importance of dosimetry standards and the harmonization of practices have been highlighted¹⁻⁵. To establish a dose rate reference, several protocols exist⁶⁻¹⁰, however, as shown by Peixoto and Andreo¹¹, there can be differences of up to 7% depending on the dosimetric quantity used for the dose rate determination. Moreover, even if protocols exist, it is sometimes difficult to know which protocol is the most suitable for a particular application, if any, because the dose rate for the cells depends on parameters such as the cell container, quantity of cell culture media or beam quality, for example. The scattering and the backscattering for this type of irradiation is also a very important parameter to take into account. Indeed, for low and medium energy X-rays, in the AAPM TG-61 reference protocol¹⁰, the absorbed dose in water is measured at the surface of a water phantom. Taking into account the very specific cell irradiation conditions, small volume of cell culture media surrounded by air, that are therefore closer to kerma conditions, *so in air*, than those defined for absorbed dose with large water equivalent phantom as in TG-61 protocol. Therefore, we have chosen to use the kerma in water as dosimetric quantity for our reference rather than the absorbed dose in water. Thus, we are proposing a new approach to provide a better determination of the actual dose delivered to cells.

Moreover, another crucial aspect for radiobiological studies is the complete reporting of the methods and protocols used for irradiation in order to be able to reproduce, interpret and compare experimental results. In 2016, Pedersen et al.¹² have highlighted the inadequate reporting of dosimetry in preclinical radiobiological studies. A larger recent study from Draeger et al.¹³ highlighted that even though some dosimetry parameters such as the dose, energy or source type are reported, a large part of physics and dosimetry parameters that are essential to properly replicate the irradiation conditions are missing. This large scale review, of more than 1,000 publications covering the past 20 years, shows a significant lack of the reporting of the physics and dosimetry conditions in radiobiological studies. Thus, a complete description of the protocol and the method utilized in radiobiological studies is mandatory in order to have robust and reproducible experiments.

Taking into account these different aspects, for the radiobiological experiments carried out at IRSN (Institute of Radiation Protection and Nuclear Safety), a stringent protocol was implemented for cell irradiations in an orthovoltage facility. This dosimetry protocol was designed in order to simulate the real cell irradiation conditions as much as possible and thus, to determine the actual dose delivered to cells. To this end, all the irradiation parameters are listed, and the beam quality index was evaluated by measuring the half value layer (HVL) for which some

adaptations have been made as the standard recommendations from the AAPM protocol¹⁰ cannot be followed. The absolute dose rate measurement was then performed with the ionization chamber inside the cell container used for cell irradiations, and the attenuation and the scattering of the cell culture media was also quantified with EBT3 radiochromic films. As the modification of only one single parameter of the protocol can significantly impact the dose estimation, a dedicated dosimetry is performed for each cell irradiation configuration. Moreover, the HVL value must be calculated for each voltage–filter combination. In this present work, a voltage of 220 kV, an intensity of 3 mA, and an inherent and an additional filtration of 0.8 and 0.15 mm of beryllium and copper, respectively, are used. The cell irradiation configuration chosen is on a T25 flask, where cells were irradiated with 5 mL of cell culture media.

PROTOCOL:

1. Irradiation platform and determination of irradiation parameters

1.1. Use an irradiation platform delivering low to medium energy X-rays. Determine the parameters of the experiment to ensure the robustness and the reproducibility of the radiobiological experiment: High voltage, Intensity, Filtration (inherent and additional), Half Value Layer (HVL), Effective energy, Detector used for dosimetry measurements, Source Sample Distance (SSD), Irradiation field (shape, size, geometry), Dosimetry quantity, Dosimetry method, Dose rate, Cell container and Quantity of cell culture media. All parameters used in this protocol are given in table 1.

2. Beam quality index: Determination of the half value layer

NOTE: The HVL is defined as the thickness of an attenuator (usually copper or aluminum) to reduce the intensity of the beam by a factor of two compared with the original value.

2.1. Set up the equipment (support, collimator, diaphragm, ionization) inside the irradiation enclosure by following the instructions in Figure 1. No attenuator material is used at this step.

2.2. Ensure that all the distances reported in Figure 1 are correct. Measure these with a tape measure.

2.3. Place that the ionization chamber in the horizontal position. For this work, we used a 31002 (equivalent to 31010) cylindrical ionization chamber calibrated in air kerma.

2.4. Pre-irradiate the ionization chamber for 5 min and measure background (this step can be performed without a collimator).

2.5. Perform 10 measurements of 1 min each in charge collection mode corresponding to the M_{raw} value (in coulombs).

2.6. Take the temperature and pressure with suitable calibrated equipment placed inside the irradiation enclosure in our case (if it is not possible place it near to the experiment). Correct the M_{raw} reading on the electrometer by the temperature and pressure correction factor given by

$$K_{T,P} = \left(\frac{T + 273.2}{T_{ref} + 273.2} \right) \times \left(\frac{P_{ref}}{P} \right)$$

where: T (°C) and P (hPa) are the actual temperature and pressure, respectively. T_{ref} and P_{ref} are the reference temperature and pressure when the ionization was calibrated by standards laboratory. The pressure and temperature must be measured with calibrated instruments. The obtained value in charge mode is the average reference value M (in coulombs).

NOTE: This step is not strictly necessary for HVL measurement, but it is recommended.

2.7. Place an attenuator of certain thickness above the diaphragm. The HVL set is composed of foils with different thicknesses (0.02, 0.05, 0.1, 0.2, 0.5, 1, 2, 5 and 10 mm of copper) with a dimension allowing to cover the entire beam (80 x 80 mm here).

2.8. Take a measurement of 1 min (M_{raw} corrected by the $K_{T,P}$ as described before).

2.8.1. If the dose rate is divided by a factor of 2 with respect to the starting value, the HVL value is found. Take 5 measurements of 1 min to estimate the average dose rate.

2.8.2. If the dose rate is not divided by a factor of 2 with respect to the starting value, increase or decrease the attenuator thickness and take another measurement. Adjust the thickness of the attenuator as necessary.

2.9. Once the thickness of the attenuator that decreases the intensity of the beam by a factor two is found, take 5 measurements of 1 min to confirm the HVL.

NOTE: In most cases, the exact thickness of attenuator cannot be found from the foils available. In this case, proceed by bisection and interpolate the HVL.

3. Evaluation of the irradiation field (no dose estimation)

3.1. Place an EBT3 film on the support used for irradiation.

3.2. Irradiate this film to obtain a well-marked irradiation field (at least 2 Gy).

3.3. Scan the EBT3 film using a dedicated scanner.

3.4. Plot the dose profile using Image J by using the Analyze and then plot Profile option (Figure 2)

3.5. Determine the size of irradiation field usage for irradiation (homogeneous area, excluding penumbra regions, see figure 2)

3.6. Make marks on the support used for irradiation to ensure that the cell container is in the right position

NOTE: In this step, the size of the irradiation field is determined, and the dose is not estimated. The complete procedure for film reading and analysis is given in Section 5. Also, take margins in order to avoid errors due to the cell container positioning.

4. Dose rate measurement with ionization chamber

4.1. Take the cell container and break a little part on the side or on the bottom (depending on the particular container and ionization chamber used) to be able to place the ionization chamber inside (Figure 3, upper section) or below (Figure 3, lower section) the container. Examples are given in Figure 3 with different ionization chambers (cylindrical or plane parallel) and cell containers. In this case, a T25 flask was used (Figure 3, red box).

NOTE: a soldering iron or heated scalpel is a good alternative to make holes in plastic ware

4.2. Place the container inside the enclosure on the support used for irradiation (carbon plate here).

4.3. Place the ionization chamber in the container (Figure 3, red box), in the correct position and connect it to the electrometer

4.4. Ensure that all irradiation parameters listed in Section 1 are correct (high voltage, intensity, additional filtrations, source sample distance etc.)

4.5. Pre-irradiate the ionization chamber for 5 min and perform the zeroing of the electrometer

4.6. Take 10 measurements of 1 min to determine the average dose rate in air kerma ($\text{Gy} \cdot \text{min}^{-1}$). The determination of the dose rate in K_{air} is calculated by:

$$K_{\text{air}} = M \times N_{K_{\text{air}}} \times K_Q$$

Where:

M is reading of the dosimeter corrected by temperature, pressure, polarity effect, ion recombination and electrometer calibration.

$N_{K_{\text{air}}}$ and K_Q are the calibration and correction factors for radiation quality, whose values are specific to each ionization chamber.

5. Measurement of cell culture media attenuation and scattering

NOTE: Handle EBT3 films with gloves throughout the procedure.

5.1. Preparation of the experiment

5.1.1. Cut small pieces of EBT3 films at least 24 h before irradiation.

5.1.2. Determine the size of the films as a function of the cell container used for radiobiology experiments (4 x 4 cm for a T25 flask, for example).

5.1.3. Cut two sets of radiochromic films must be cut:

5.1.3.1. One set for the calibration curves composed of three pieces of EBT3 radiochromic film by dose or time point. Nine points in total for this work.

5.1.3.2. One set for the quantification of the cell culture media attenuation, also three pieces per point

5.1.4. Number all the films for identification (upper right corner here) and scan them on the same position on the scanner

5.1.5. Keep the films away from light

5.1.6. Prepare the cell container used for the EBT3 film measurements and, if necessary, cut a part to put the film inside (an example with a T25 is given in Figure 4)

5.2. Dose rate estimation

5.2.1. Measure the dose rate for the configuration as described in the previous section

5.2.2. Keep this configuration in place for the irradiation of the EBT3 radiochromic films and use the same type of cell container

5.3. Construction of the calibration curve

5.3.1. Take the pre-cut EBT3 films for the calibration curve

5.3.2. Three pieces are not irradiated (0 Gy)

5.3.3. Place the first film inside the cell container, in the same configuration as for cell irradiation

5.3.4. Irradiate it to obtain the first dose points

258 5.3.5. Repeat this operation to obtain three pieces of EBT3 films irradiated with the same dose

259
260 5.3.6. Perform this for each dose point (Nine dose points in this work (0, 0.25, 0.5, 0.75, 1, 1.5,
261 2, 2.5 and 3 Gy) as illustrated in Figure 5)

262
263 5.4. Evaluation of the attenuation of cell culture media and scattering

264
265 5.4.1. Chose the same irradiation time for all irradiations (60 seconds, for example)

266
267 5.4.2. Irradiate three pieces of EBT3 films in the container without water

268
269 5.4.3. Irradiate three pieces of EBT3 films in the container with water as follows:

270
271 5.4.3.1. Place the film inside the container

272
273 5.4.3.2. Fill the container with the exact quantity of water to represent the cell culture
274 media (5 mL here). Use small pieces of tape if the films do not remain submerged properly.

275
276 5.4.3.3. Place the cell container inside the enclosure and ensure that the film is correctly
277 immersed

278
279 5.4.3.4. When the irradiation is complete, take the EBT3 films, dry them with absorbent
280 paper, and store them away from light

281 282 6. Reading of EBT3 radiochromic films

283
284 6.1. Read EBT3 films at least 24 h after irradiation

285
286 6.2. Scan the films on an dedicated scanner

287
288 6.3. Set the scanner parameters as: 48 bit red-green-blue tiff format, 150 dpi in transmission
289 mode and no image correction

290
291 6.4. Perform warm up of the scanner by following the steps below

292
293 6.4.1. Place a non-irradiated film on the scanner

294
295 6.4.2. Launch a preview of the scan

296
297 6.4.3. Launch a timer and wait for 30 seconds

298
299 6.4.4. Launch the scan

6.4.5. At the end of the scan, launch a timer, and wait for 90 seconds

6.4.6. At the same time, register the scan, open the image with ImageJ, trace a square ROI (always the same size and in the same position), and take a measurement of the average red pixel level of the area

6.4.7. At the end of the 90 seconds, repeat the procedure from Step two (without touching the film inside the scanner)

6.4.8. Repeat this at least 30 times to warm up and stabilize the scanner (no variations in the average red pixel level of the area selected on the non-irradiated films). If the scanner, i.e. the average red pixel value, is not stabilized, continue the procedure.

6.5. Scanning of the EBT3 films

6.5.1. Place the first film in the center of the scanner bed. You should delimitate an area to always place the film in the same place and in the same orientation

6.5.2. Launch a preview of the scan

6.5.3. Launch a timer and wait for 30 seconds

6.5.4. Launch the scan

6.5.5. At the end of the scan, launch a timer, and wait for 90 seconds. During these 90 seconds change the EBT3 film

Note: An analysis of the EBT3 radiochromic films was performed using a self-programmed C++ program. Different methods can be used for the EBT3 film analysis, such as the red channel method or the three channels method^{14,15}. In this case, we have used the red channel method with no background subtraction, and the images were converted to optical densities and then to the dose using our program. As this method is already well defined, our C++ program was not included here. Moreover, dedicated software¹⁶ can also be used for EBT3 film analysis.

7. Determination of the dose rate at the level of the cell monolayer

7.1. Convert the average dose rate obtained with the ionization chamber corrected by the attenuation and scattering of the cell culture media (K) to the water kerma using the ratio of the mean mass energy absorption coefficient for water to air evaluated over the photon fluence spectrum (μ_{en}/ρ).

$$K_{water} = K \times \left[\left(\frac{\bar{\mu}_{en}}{\rho} \right)_{air}^w \right]_{air}$$

A dedicated software¹⁷ was used to calculate the photon energy spectrum in air with no

phantom, and we used the NIST table¹⁸ to calculate the mean mass energy absorption coefficient.

REPRESENTATIVE RESULTS:

In this work, we used a platform dedicated to small animal irradiation¹⁹, however this platform can be used to irradiate other types of samples such as cells. The irradiation source is a Varian X-ray tube (NDI-225-22, NDI, Washington, DC) having an inherent filtration of 0.8 mm of beryllium, a large focal spot size of 3 mm, a high voltage range about 30 to 225 kV and a maximal intensity of 30 mA.

The parameters used for this study are reported in Table 1. We have chosen to show an example of the use of this protocol for cell irradiation in a T25 flask with 5 mL of cell culture media.

Half value layer:

Table 2 reports the measurements performed to estimate the attenuator thickness needed to decrease the intensity of the beam by a factor of two. For this, 10 reference measurements were taken to estimate the **average** M_{raw} reading on the electrometer (in coulombs), corrected by the temperature and pressure correction factor ($K_{T,P}$).

Different thickness of attenuator were then tested to find the thickness that decreased the beam intensity by a factor of two. When this thickness was found, five measurements were taken to evaluate the **average** M_{raw} value corrected by $K_{T,P}$.

For this configuration, a half value layer of 0.667 mm of copper was found. From the HVL measurement, we can calculate the effective energy of the beam, which is about 69 keV in our case.

Dose rate measurement:

Before to these measurements, an EBT3 film was irradiated in order to determine the surface on which the irradiation field is homogeneous, allowing us to correctly place the cell container. This area is about 10 x 10 cm² excluding penumbra regions shown by dotted lines in figure 2. Then, dose rate measurement was performed using a 31002 (equivalent to 31010) cylindrical ionization chamber calibrated in air kerma. For this configuration, with an open field irradiation field at 35 cm to the source in a T25 cell container placed on a carbon plate, the dose rate was about 0.626 Gy.min⁻¹ in K_{air} .

To determine the exact dose on cells, the measured K_{air} was converted in water kerma. Figure 5 shows the X-rays energy spectrum obtained with dedicated software¹⁷. From this energy spectrum and the NIST table, we can convert the dose rate in K_{air} to K_{water} , which was 0.659 Gy.min⁻¹.

The overall uncertainty of the absolute dose rate measurement was about 3% at a 95% confidence level.

Cell culture media attenuation and scattering:

For the quantification of cell culture media attenuation and scattering, dosimetry measurements with EBT3 radiochromic films were performed at room temperature. From the measurement with the ionization chamber, the dose rate was determined. Calibration films were then irradiated at the same position. EBT3 radiochromic films were calibrated between 0 and 3 Gy with 0.25 Gy steps between 0 and 1 Gy and 0.5 Gy steps between 1 and 3 Gy (nine dose points to construct the calibration curve) as shown in Figure 6. The dose points were fitted with a 4th-degree polynomial curve. The EBT3 films were then irradiated with and without the exact quantity of cell culture media inside the cell container to evaluate the attenuation and the scattering due to the cell culture media. For this configuration, the attenuation of the cell culture media was about 1.5%.

The overall uncertainty of the EBT3 film measurements was about 4% at a 95% confidence level.

Routine measurements:

Before performing the cell irradiations, the dose rate was measured each time in the same container used for irradiation. Thus, we used the daily dose rate to estimate our irradiation time. If we closely follow the protocol and do not change any parameters, the HVL measurement and the attenuation due to the cell culture media do not need to be repeated. As an example, the table used for our daily measurement is given in Table 3.

FIGURE AND TABLE LEGENDS:

Figure 1: Scheme of the configuration take in place on the SARRP enclosure for HVL measurements.

Figure 2: Evaluation of the irradiation field size. Dose profile obtained at 35 cm to the source without collimator. Dotted lines show the area considered for the irradiation.

Figure 3: Photograph of cell containers with the ionization chamber for dose rate measurement. Upper part: example for measurement with a 31002 cylindrical ionization chamber. Lower part: example for measurement with a TM23342 ionization chamber.

Figure 4: Photograph of the T25 used for the measurement of the cell culture media attenuation. The upper part of the T25 was cut out to be able to place the film inside the flask.

Figure 5: Simulated energy spectra for a 220 kV high voltage with 0.8 mm of Be and 0.15 mm of Cu filtrations¹⁷.

Figure 6: EBT3 films irradiated to construct the calibration curve and the corresponding calibration curve.

Table1: List of the configuration parameters.

Table 2: Measurement for the Half Value Layer determination.

Table 3: Daily dose rate measurements for cell irradiation.

DISCUSSION:

This work presents the protocol used and implemented for cell irradiations using low energy X-rays facility. Nowadays, many radiobiology experiments are performed with this type of irradiator as they are easy to use, cost effective and with very few radioprotection constraints, compared to cobalt source for example. Although these set-ups have many advantages, as they use a low X-ray energy source, a modification of only one irradiation parameter can significantly impact the dosimetry. Several studies have already highlighted the importance of dosimetry standards and protocols for radiobiology studies^{2,5,20,21}. Even though several protocols have already been well defined in the literature^{1,5}, we decided to develop a new protocol to perform dosimetry measurements to simulate real cell irradiation conditions as much as possible and take in account all the parameters that can influence the physical dose, especially for low energy X-rays^{21,22}. Thus, we have chosen to implement a stringent protocol to minimize uncertainties. To this end, irradiation parameters were set (Table1). Three steps are then necessary: i) determination of the beam quality index, ii) measurement of the absolute dose rate with an ionization chamber and iii) measurement of the attenuation and scattering due to the cell culture medium with EBT3 radiochromic films.

The beam quality index corresponded to the voltage–half value layer (HVL) couple used to characterize low energy X-rays beams. The HVL is a practical indicator to describe poly energetic radiation and is defined as the thickness of an attenuator (usually copper or aluminum) to reduce the air kerma dose rate by a factor of two from the original value. HVL measurements were performed using the following recommendations of the AAPM protocol for a 40-300 kV X-ray beam¹⁰. However, some adaptations had to be made because in the irradiator enclosure it is not possible to achieve a distance a 1 meter between the source and the ionization chamber. Therefore, in the present work, we used a distance of 58 cm between the source and the detector for HVL measurements, as illustrated in Figure 1. We decided to let 25 cm after the ionization chamber because a lot of electronical material, support and metallic element are present in the bottom of the enclosure to limit the backscatter effect of these elements. Measurement of the HVL is one of the critical aspects of this protocol. Indeed, for many X-rays irradiators, the inside of enclosures is very restricted and these are not the optimal conditions to perform the measurements or it becomes impossible. Although experimental measurements are the best way to evaluate the HVL, when these measurements were too difficult or even impossible to perform, dedicated software¹⁷ can be used to provide a good estimation for the HVL or a Monte Carlo simulation can be used²³. In the present work, we used a dedicated software to obtain the X-ray energy spectrum (Figure 5). We were also able to compare the measured and calculated HVL, which was the same, and to also compare the effective energy.

For dosimetry measurements, we then chose to simulate real cell irradiation conditions as much as possible. For this, we directly performed the absolute dose rate measurements with the ionization chamber inside the cell container used for cells irradiation (Figure 3). However, as we used a cylindrical ionization chamber calibrated for beams over 100 kV, we were not exactly at the same position as the cells because of the thickness of the ionization chamber. For lower beams (15-70kV), where plan parallel chamber can be used, we can be even closer to the real cell irradiation conditions. Then, relative dosimetry measurements were performed to evaluate the attenuation and the scattering due to the cell culture medium. The results presented on this work do not highlight a significant variation in the dose deposited with or without the exact quantity of cell culture media as we used a voltage of 220 kV, an additional filtration of 0.15 mm of Cu and we only had 5 mL of cell culture medium. However, in our previous study²¹ carried out at 80 kV, we pointed out that a variation of the cell culture media and filtration significantly impacts the physical dose, up to 40% compared to the reference configuration when we used a 1 mm aluminum filtration. This impact was also demonstrated in terms of biological effects by measuring the surviving cell fraction using a clonogenic assay^{21,24}. Thus, depending on the voltage, additional filtration, the container and quantity of cell culture media, the dose deposited on the cells can be different if the protocol is not closely followed for all irradiations.

Consequently, a dedicated dosimetry should be set up for all cell irradiation configurations. Although this is restrictive and the modification of only a single parameter requires the implementation of a new configuration, we have decided to make this choice to be as close as possible to the real cell irradiation conditions. This requires a close collaboration between the physicists and the radiobiologist in order to set up the best design for the configuration. At our institute, a dozen protocols were established on our platform for a voltage range of 40 to 220 kV for which T25, T75, 6- to 96-plate wells or petri dishes can be irradiated.

Although this protocol seems quite long to implement, once the configuration is established, the only measurement to be taken on the day of irradiation is measurement of the dose rate with the ionization chamber inside the cell container. This measurement is also a quality control that enables us to ensure that the dose rate is as expected.

To ensure the reproducibility of radiobiological studies, and to be able to compare and interpret experiments, it is important to rigorously follow established protocols and report all the dosimetry and configuration aspects, particularly for facilities using low or medium energy X-rays. The new protocol proposed here is for cell irradiations, applicable to many X-rays facilities, and takes into account all parameters influencing the dosimetry and provides a better estimation of the actual dose delivered to cells.

DISCLOSURES:

The authors have nothing to disclose.

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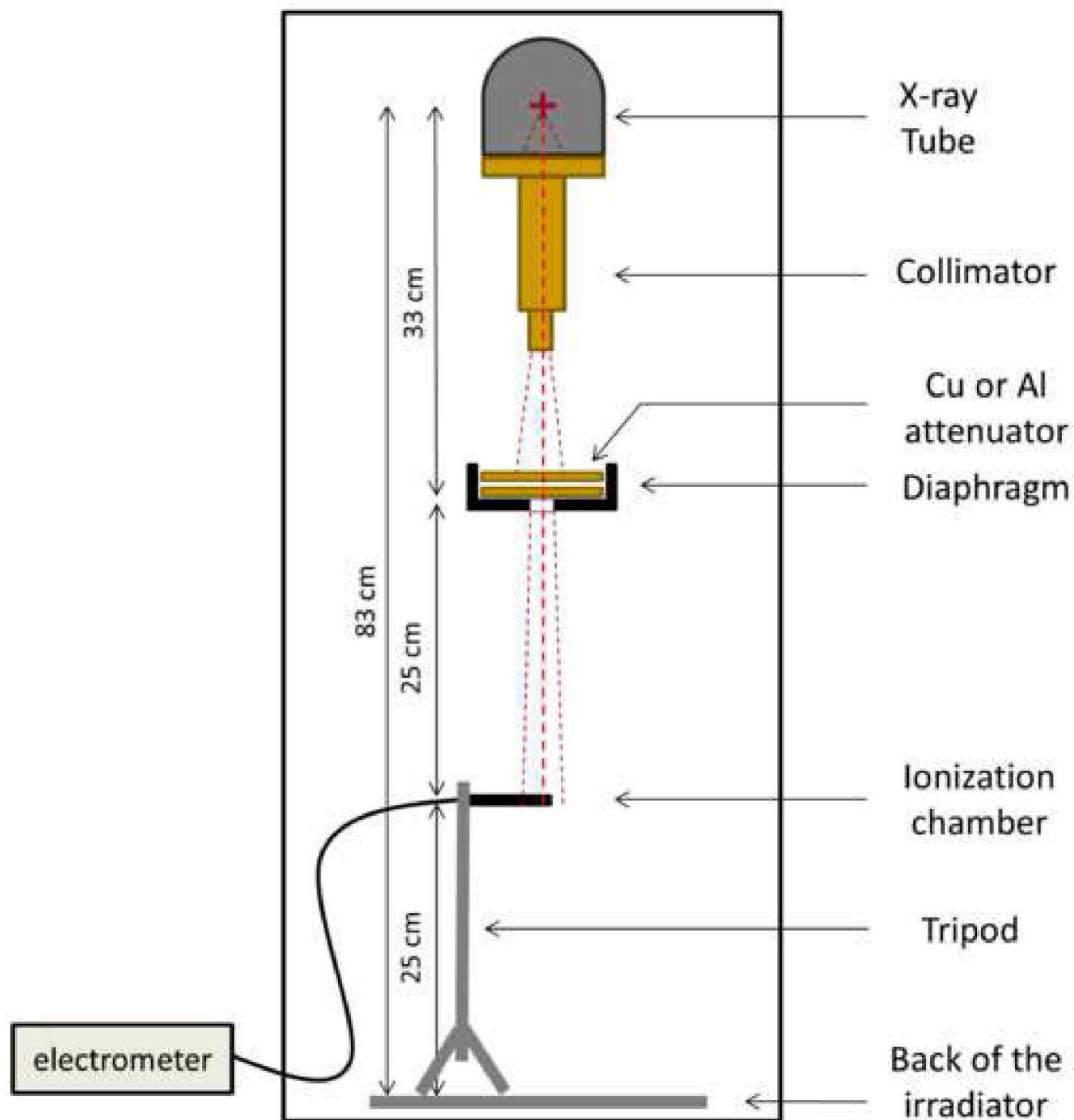


Figure2

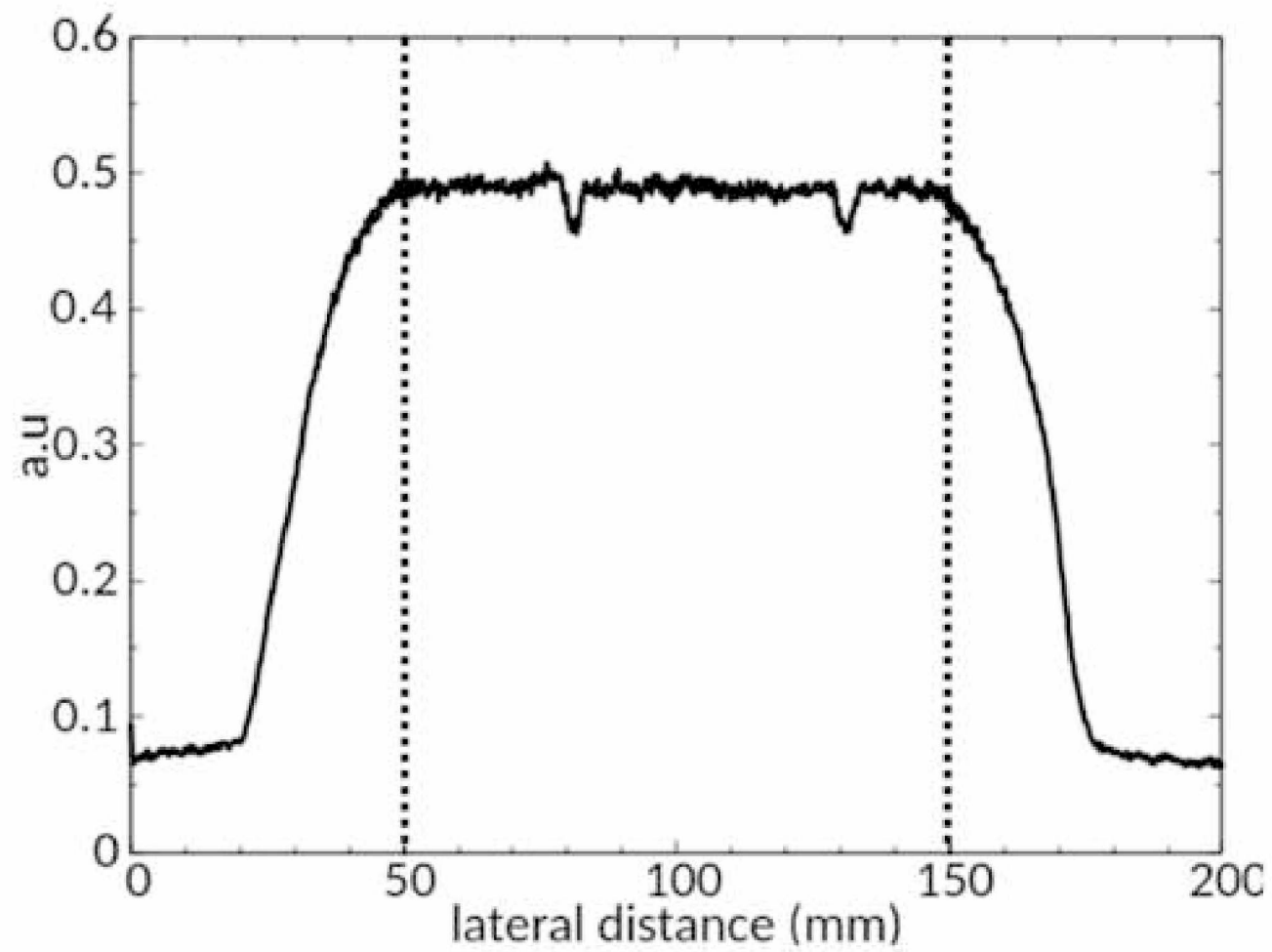
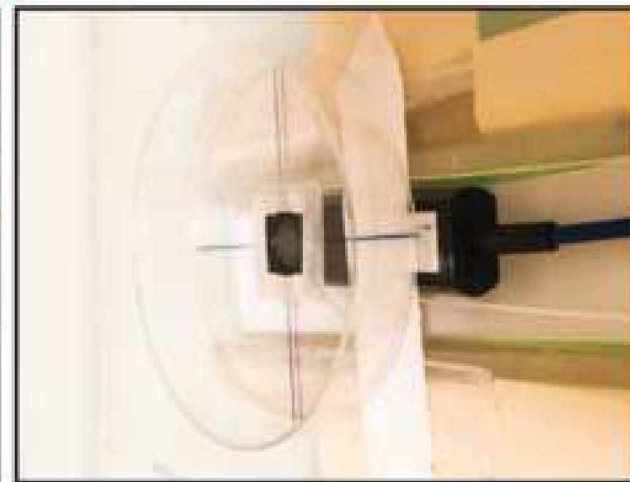
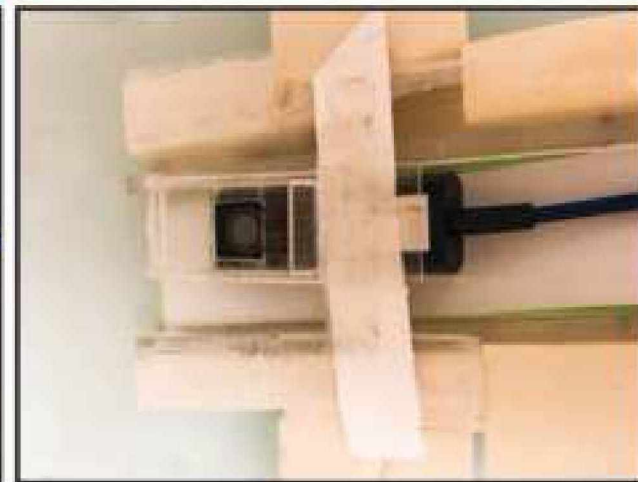
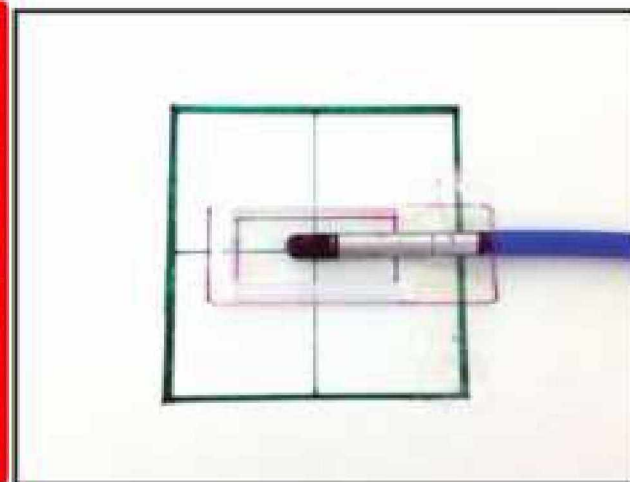
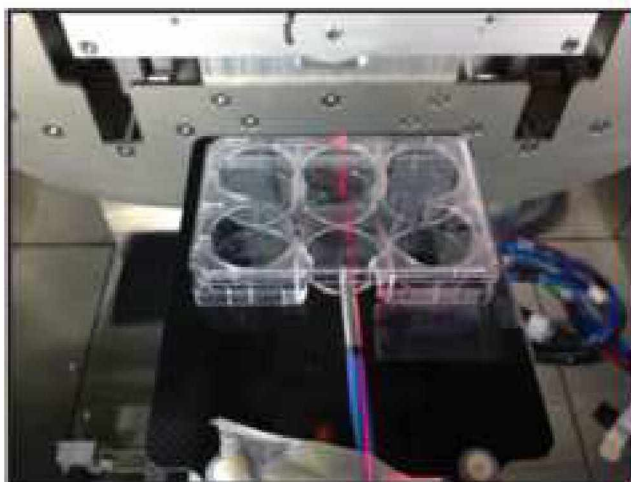
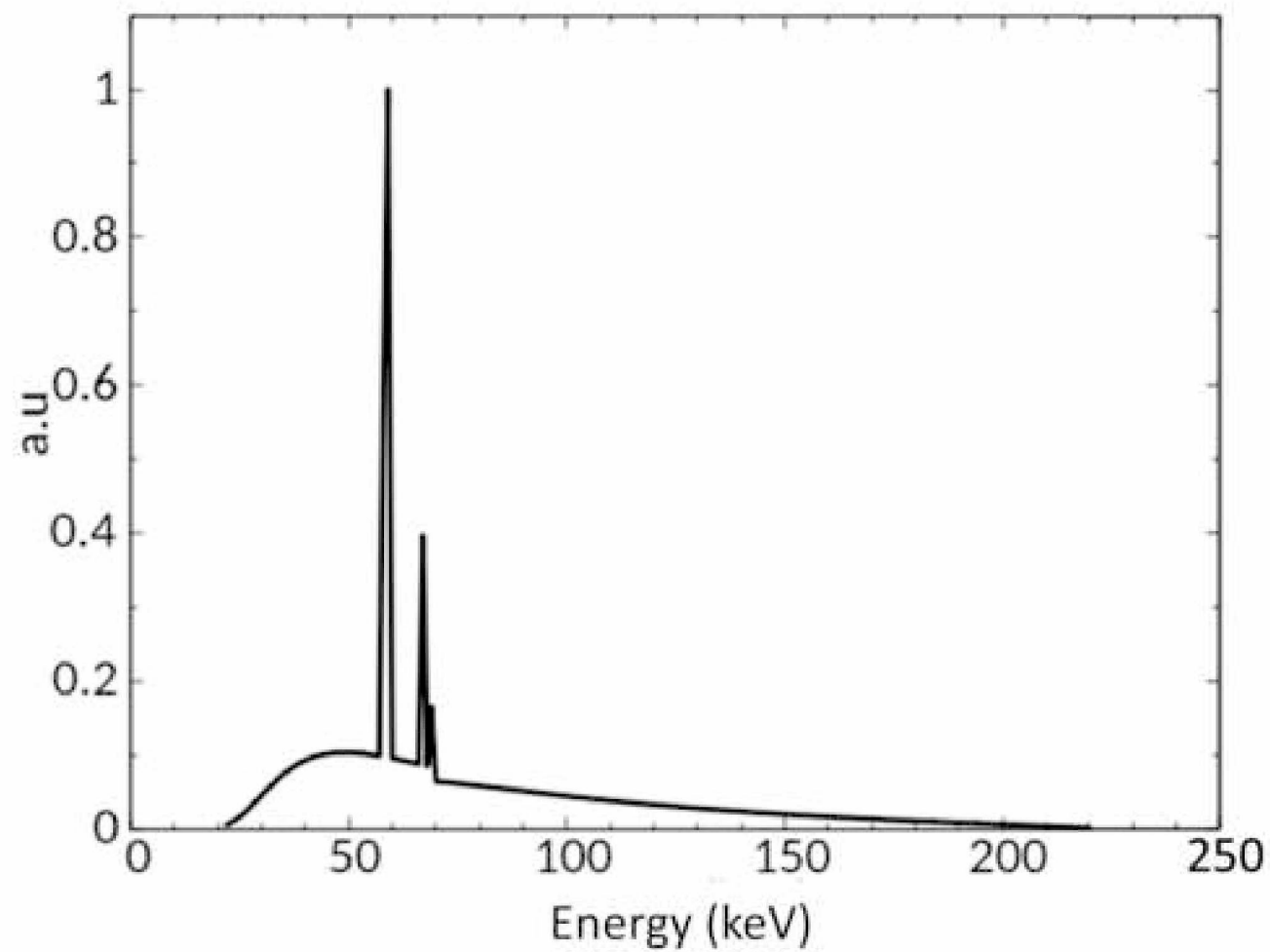


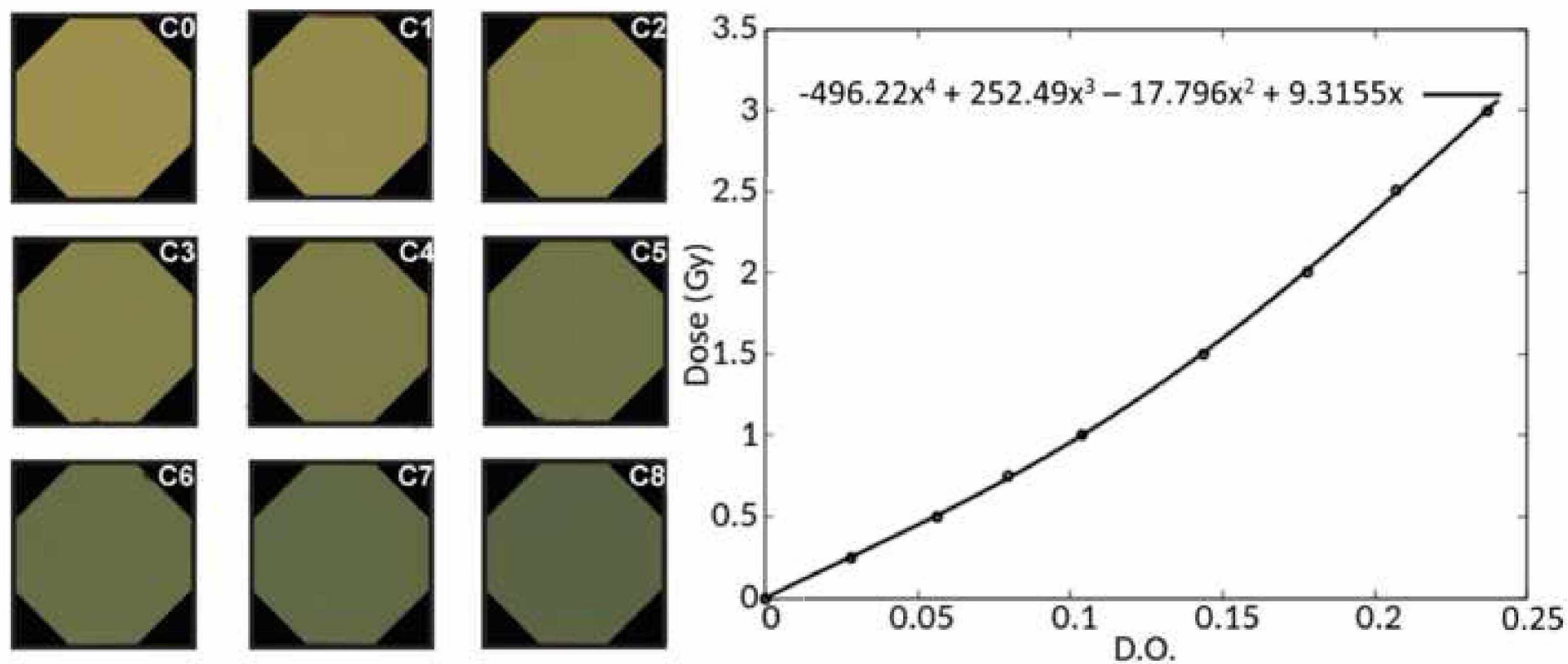
Figure3

[Click here to access/download;Figure;Figure_3.TIF](#)









High voltage (kV)	220
Intensity (mA)	3
Filtrations (inherent and additional)	0.8 mm of Be + 0.15 mm Cu
Half value layer (mm Cu)	Determined below
Effective energy (keV)	Determined below
Detector used	Cylindrical ionization chamber + EBT3 radiochromic films
Source sample distance	35 cm
Irradiation field (shape, size, geometry)	Open field (no collimator), square, 20 x 20 cm
Dosimetry quantity	Kair and Kwater
Dosimetry method	As described on the protocol section
Cell container	T25
Quantity of cell culture media	5 ml
Dose rate (Gy/min)	Determined below

Attenuator (mm Cu)	IC measure (nC)	Temperature (°C)	Pressure (hPa)
0	10.480	21.6	993.2
	10.480	21.6	993.1
	10.490	21.6	993.1
	10.490	21.6	993.1
	10.490	21.6	993.2
	10.490	21.6	993.2
	10.490	21.6	993.1
	10.490	21.6	993.2
	10.490	21.6	993.2
	10.490	21.6	993.1
	0.514	5.840	21.7
	0.564	5.651	21.7
	0.584	5.569	21.7
	0.604	5.491	21.7
	0.615	5.441	21.7
Finding of attenuator thickness (M)	0.627	5.380	21.7
	0.647	5.307	21.7
	0.667	5.240	21.8
	0.667	5.231	21.8
	0.667	5.236	21.8
Measurments with the right attenuator (M)	0.667	5.235	21.8
	0.667	5.236	21.8
	0.667	5.235	21.8
	0.667	5.235	21.8

$k_{T,P}$	IC measure corrected by $k_{T,P}$ (nC)	Corrected Mean value (nC)	ST deviation
1.026	10.752	10.761	0.005
1.026	10.752		
1.026	10.763		
1.026	10.763		
1.026	10.763		
1.026	10.763		
1.026	10.763		
1.026	10.763		
1.026	10.763		
1.026	10.763		
1.026	5.992	-	-
1.026	5.798	-	-
1.026	5.714	-	-
1.026	5.634	-	-
1.026	5.582	-	-
1.026	5.520	-	-
1.026	5.445	-	-
1.026	5.376	-	-
1.026	5.368	5.373	0.003
1.026	5.375		
1.026	5.373		
1.026	5.374		
1.026	5.373		

Attenuation estimation (M / Mref)
-
0.557
0.539
0.531
0.524
0.519
0.513
0.506
0.500
0.499

IC measure (nC)	Temperature (°C)	Pressure (hPa)	k _{T,P}
2.495	22.3	1001	1.020
2.496	22.3	1001	1.020
2.497	22.3	1001	1.020
2.498	22.3	1001	1.020
2.496	22.3	1001	1.020
2.495	22.3	1000.9	1.020
2.494	22.3	1000.9	1.020
2.495	22.3	1000.9	1.020
2.496	22.3	1000.9	1.020
2.496	22.3	1000.9	1.020

IC measure corrected by $k_{T,P}$ (nC)	Corrected Mean value by $k_{T,P}$ (nC)	ST deviation	Corrected mean value by all correction factors	Dose rate in air kerm (Gy/min)
2.545	2.546	0.001	2.536	0.626
2.546				
2.547				
2.548				
2.546				
2.545				
2.544				
2.545				
2.546				
2.546				
2.546				

Dose rate at cell level in K_{water} (Gy/min)
0.659

Name of Reagent/ Equipment	Company	Catalog Number
31010 ionization chamber	PTW	ionization Radiation, Detectors including code of practice, catalog 2019/2020, page 14
electrometer UNIDOSEwebline	PTW	online catalog, quote request
temperature and pressure measurements, Lufft OPUS20	lufft	quote request
EBT3 radiochromic films	meditest	quote request
HVL material (filter, diaphragm)	PTW	online catalog, page 70, quote request
scanner for radiochromic films	epson	quote request

Comments/Description
https://www.ptwdosimetry.com/fileadmin/user_upload/DETECTORS_Cat_en_16522900_12/blaetterkatalog/index.html?startpage=1#page_14
https://www.ptwdosimetry.com/en/products/unidos-webline/?type=3451&downloadfile=1593&cHash=6096ddc2949f8baf5d556e931e6c865
https://www.lufft.com/products/in-room-measurements-291/opus-20-thip-1983/
https://www.meditest.fr/produit/ebt3-8x10/
thickness foils: 0.02, 0.05, 0.1, 0.2, 0.5, 1, 2, 5 and 10 mm of copper, https://www.ptwdosimetry.com/fileadmin/user_upload/Online_Catalog/Radiation_Medicine_Cat_en_58721100_11/blaetterkatalog/index.html#page_70
Epson V700, seiko Epson corporation, Suwa, Japan