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Dosimetry for cell irradiation using orthovoltage (40-300 kV) X-ray facilities

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1 **TITLE:**

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

3

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25 **KEYWORDS:**

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

27

28 **SUMMARY:**

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

32

33 **ABSTRACT:**

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

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

49

50 **INTRODUCTION:**

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

68

69 Moreover, another crucial aspect for radiobiological studies is the complete reporting of the
70 methods and protocols used for irradiation in order to be able to reproduce, interpret and
71 compare experimental results. In 2016, Pedersen et al.¹² have highlighted the inadequate
72 reporting of dosimetry in preclinical radiobiological studies. A larger recent study from Draeger
73 et al.¹³ highlighted that even though some dosimetry parameters such as the dose, energy or
74 source type are reported, a large part of physics and dosimetry parameters that are essential to
75 properly replicate the irradiation conditions are missing. This large scale review, of more than
76 1,000 publications covering the past 20 years, shows a significant lack of the reporting of the
77 physics and dosimetry conditions in radiobiological studies. Thus, a complete description of the
78 protocol and the method utilized in radiobiological studies is mandatory in order to have robust
79 and reproducible experiments.

80

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

87 adaptations have been made as the standard recommendations from the AAPM protocol¹⁰
88 cannot be followed. The absolute dose rate measurement was then performed with the
89 ionization chamber inside the cell container used for cell irradiations, and the attenuation and
90 the scattering of the cell culture media was also quantified with EBT3 radiochromic films. As the
91 modification of only one single parameter of the protocol can significantly impact the dose
92 estimation, a dedicated dosimetry is performed for each cell irradiation configuration. Moreover,
93 the HVL value must be calculated for each voltage–filter combination. In this present work, a
94 [voltage](#) of 220 kV, an intensity of 3 mA, and an inherent and an additional filtration of 0.8 and
95 0.15 mm of beryllium and copper, respectively, are used. The cell irradiation configuration
96 chosen is on a T25 flask, where cells were irradiated with 5 mL of cell culture media.

97

98 **PROTOCOL:**

99

100 **1. Irradiation platform and determination of irradiation parameters**

101

102 1.1. Use an irradiation platform delivering low to medium energy X-rays. Determine the
103 parameters of the experiment to ensure the robustness and the reproducibility of the
104 radiobiological experiment: High voltage, Intensity, Filtration (inherent and additional), Half
105 Value Layer (HVL), Effective energy, Detector used for dosimetry measurements, Source Sample
106 Distance (SSD), Irradiation field (shape, size, geometry), Dosimetry quantity, Dosimetry method,
107 Dose rate, Cell container and Quantity of cell culture media. [All parameters used in this protocol](#)
108 [are given in table 1.](#)

109

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

111

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

114

115 2.1. Set up the equipment (support, collimator, diaphragm, ionization) inside the irradiation
116 [enclosure](#) by following the instructions in **Figure 1**. No attenuator material is used at this step.

117

118 2.2. Ensure that all the distances reported in **Figure 1** are correct. [Measure these with a tape](#)
119 [measure.](#)

120

121 2.3. Place that the ionization chamber in the horizontal position. [For this work, we used a](#)
122 [31002 \(equivalent to 31010\) cylindrical ionization chamber calibrated in air kerma.](#)

123

124 2.4. Pre-irradiate the ionization chamber for 5 min and measure background (this step can be
125 performed without a collimator).

126

127 2.5. Perform 10 measurements of 1 min each in charge [collection](#) mode corresponding to the
128 M_{raw} value (in coulombs).

129

130 2.6. Take the temperature and pressure with suitable calibrated equipment placed inside the
131 irradiation enclosure in our case (if it is not possible place it near to the experiment). Correct the
132 M_{raw} reading on the electrometer by the temperature and pressure correction factor given by
133

$$134 \quad K_{T,P} = \left(\frac{T + 273.2}{T_{ref} + 273.2} \right) \times \left(\frac{P_{ref}}{P} \right)$$

135
136 where: T (°C) and P (hPa) are the actual temperature and pressure, respectively. T_{ref} and P_{ref} are
137 the reference temperature and pressure when the ionization was calibrated by standards
138 laboratory. The pressure and temperature must be measured with calibrated instruments.
139 The obtained value in charge mode is the average reference value M (in coulombs).

140
141 NOTE: This step is not strictly necessary for HVL measurement, but it is recommended.
142

143 2.7. Place an attenuator of certain thickness above the diaphragm. The HVL set is composed
144 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
145 dimension allowing to cover the entire beam (80 x 80 mm here).

146
147 2.8. Take a measurement of 1 min (M_{raw} corrected by the $K_{T,P}$ as described before).
148

149 2.8.1. If the dose rate is divided by a factor of 2 with respect to the starting value, the HVL value
150 is found. Take 5 measurements of 1 min to estimate the average dose rate.

151
152 2.8.2. If the dose rate is not divided by a factor of 2 with respect to the starting value, increase
153 or decrease the attenuator thickness and take another measurement. Adjust the thickness of the
154 attenuator as necessary.

155
156 2.9. Once the thickness of the attenuator that decreases the intensity of the beam by a factor
157 two is found, take 5 measurements of 1 min to confirm the HVL.

158
159 NOTE: In most cases, the exact thickness of attenuator cannot be found from the foils available.
160 In this case, proceed by bisection and interpolate the HVL.

161 162 **3. Evaluation of the irradiation field (no dose estimation)**

163
164 3.1. Place an EBT3 film on the support used for irradiation.

165
166 3.2. Irradiate this film to obtain a well-marked irradiation field (at least 2 Gy).

167
168 3.3. Scan the EBT3 film using a dedicated scanner.

169
170 3.4. Plot the dose profile using Image J by using the Analyze and then plot Profile option
171 (Figure 2)

172

173 3.5. Determine the size of irradiation field usage for irradiation (homogeneous area, excluding
174 penumbra regions, see figure 2)

175

176 3.6. Make marks on the support used for irradiation to ensure that the cell container is in the
177 right position

178

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

182

183 4. Dose rate measurement with ionization chamber

184

185 4.1. Take the cell container and break a little part on the side or on the bottom (depending on
186 the particular container and ionization chamber used) to be able to place the ionization chamber
187 inside (Figure 3, upper section) or below (Figure 3, lower section) the container. Examples are
188 given in Figure 3 with different ionization chambers (cylindrical or plane parallel) and cell
189 containers. In this case, a T25 flask was used (Figure 3, red box).

190

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

192

193 4.2. Place the container inside the enclosure on the support used for irradiation (carbon plate
194 here).

195

196 4.3. Place the ionization chamber in the container (Figure 3, red box), in the correct position
197 and connect it to the electrometer

198

199 4.4. Ensure that all irradiation parameters listed in Section 1 are correct (high voltage,
200 intensity, additional filtrations, source sample distance etc.)

201

202 4.5. Pre-irradiate the ionization chamber for 5 min and perform the zeroing of the
203 electrometer

204

205 4.6. Take 10 measurements of 1 min to determine the average dose rate in air kerma ($\text{Gy}\cdot\text{min}^{-1}$).
206 The determination of the dose rate in K_{air} is calculated by:

207

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

208 Where:

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

211 $N_{K_{\text{air}}}$ and K_Q are the calibration and correction factors for radiation quality, whose values are
212 specific to each ionization chamber.

213

214 5. Measurement of cell culture media attenuation and scattering

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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
300

- 301 6.4.5. At the end of the scan, launch a timer, and wait for 90 seconds
302
303 6.4.6. At the same time, register the scan, open the image with ImageJ, trace a square ROI
304 (always the same size and in the same position), and take a measurement of the average red
305 pixel level of the area
306
307 6.4.7. At the end of the 90 seconds, repeat the procedure from Step two (without touching the
308 film inside the scanner)
309
310 6.4.8. Repeat this at least 30 times to warm up and stabilize the scanner (no variations in the
311 average red pixel level of the area selected on the non-irradiated films). If the scanner, i.e. the
312 average red pixel value, is not stabilized, continue the procedure.

313 6.5. Scanning of the EBT3 films

- 314
315
316 6.5.1. Place the first film in the center of the scanner bed. You should delimitate an area to
317 always place the film in the same place and in the same orientation
318
319 6.5.2. Launch a preview of the scan
320
321 6.5.3. Launch a timer and wait for 30 seconds
322
323 6.5.4. Launch the scan
324
325 6.5.5. At the end of the scan, launch a timer, and wait for 90 seconds. During these 90 seconds
326 change the EBT3 film
327

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

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

- 335
336
337 7.1. Convert the average dose rate obtained with the ionization chamber corrected by the
338 attenuation and scattering of the cell culture media (K) to the water kerma using the ratio of the
339 mean mass energy absorption coefficient for water to air evaluated over the photon fluence
340 spectrum (μ_{en}/ρ).

341
$$K_{water} = K \times \left[\left(\frac{\bar{\mu}_{en}}{\rho} \right)_{air}^w \right]_{air}$$

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

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

344

345 **REPRESENTATIVE RESULTS:**

346

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

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

354

355 Half value layer:

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

360

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

364

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

368

369 Dose rate measurement:

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

377

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

382

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

385

386 Cell culture media attenuation and scattering:

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

397
398 The overall uncertainty of the EBT3 film measurements was about 4% at a 95% confidence level.
399

400 Routine measurements:

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

406

407 **FIGURE AND TABLE LEGENDS:**

408

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

411

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

414

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

418

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

421

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

424

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

427

428 **Table1: List of the configuration parameters.**

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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.

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

489
490 Consequently, a dedicated dosimetry should be set up for all cell irradiation configurations.
491 [Although this is restrictive and the modification of only a single parameter requires the](#)
492 [implementation of a new configuration, we have decided to make this choice to be as close as](#)
493 [possible to the real cell irradiation conditions. This requires a close collaboration between the](#)
494 [physicists and the radiobiologist in order to set up the best design for the configuration.](#) At our
495 institute, a dozen protocols were established on our platform for a voltage range of 40 to 220 kV
496 for which T25, T75, 6- to 96-plate wells or petri dishes can be irradiated.

497
498 Although this protocol seems quite long to implement, once the configuration is established, the
499 only measurement to be taken on the day of irradiation is measurement of the dose rate with
500 the ionization chamber inside the cell container. This measurement is also a quality control that
501 enables us to ensure that the dose rate is as expected.

502
503 To ensure the reproducibility of radiobiological studies, and to be able to compare and interpret
504 experiments, it is important is to rigorously follow established protocols and report all the
505 dosimetry and configuration aspects, particularly for facilities using low or medium energy X-rays.
506 The new protocol proposed here is for cell irradiations, applicable to many X-rays facilities, and
507 takes into account all parameters influencing the dosimetry and provides a better estimation of
508 the actual dose delivered to cells.

509
510 **DISCLOSURES:**
511 The authors have nothing to disclose.

512
513 **REFERENCES**
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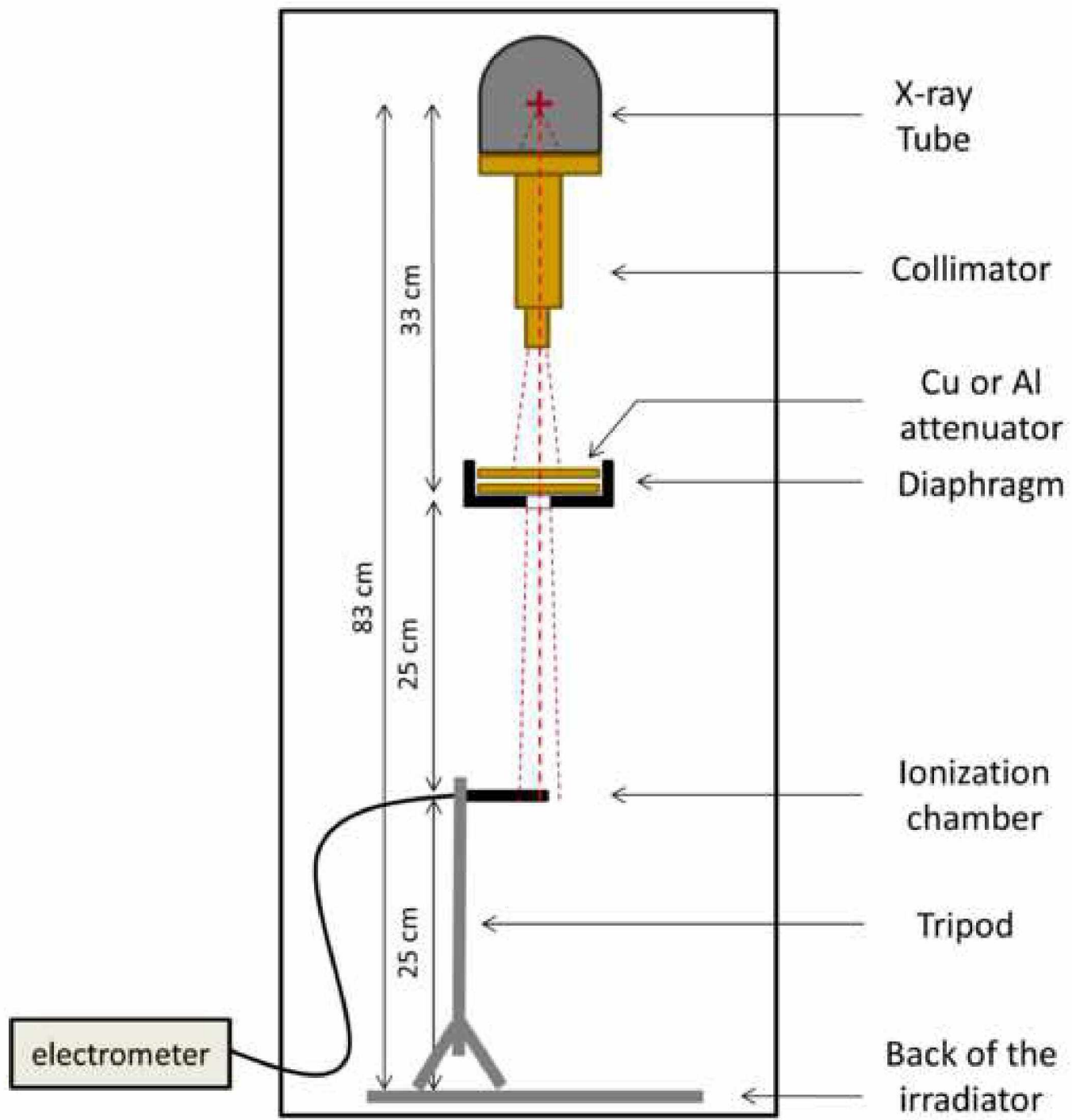
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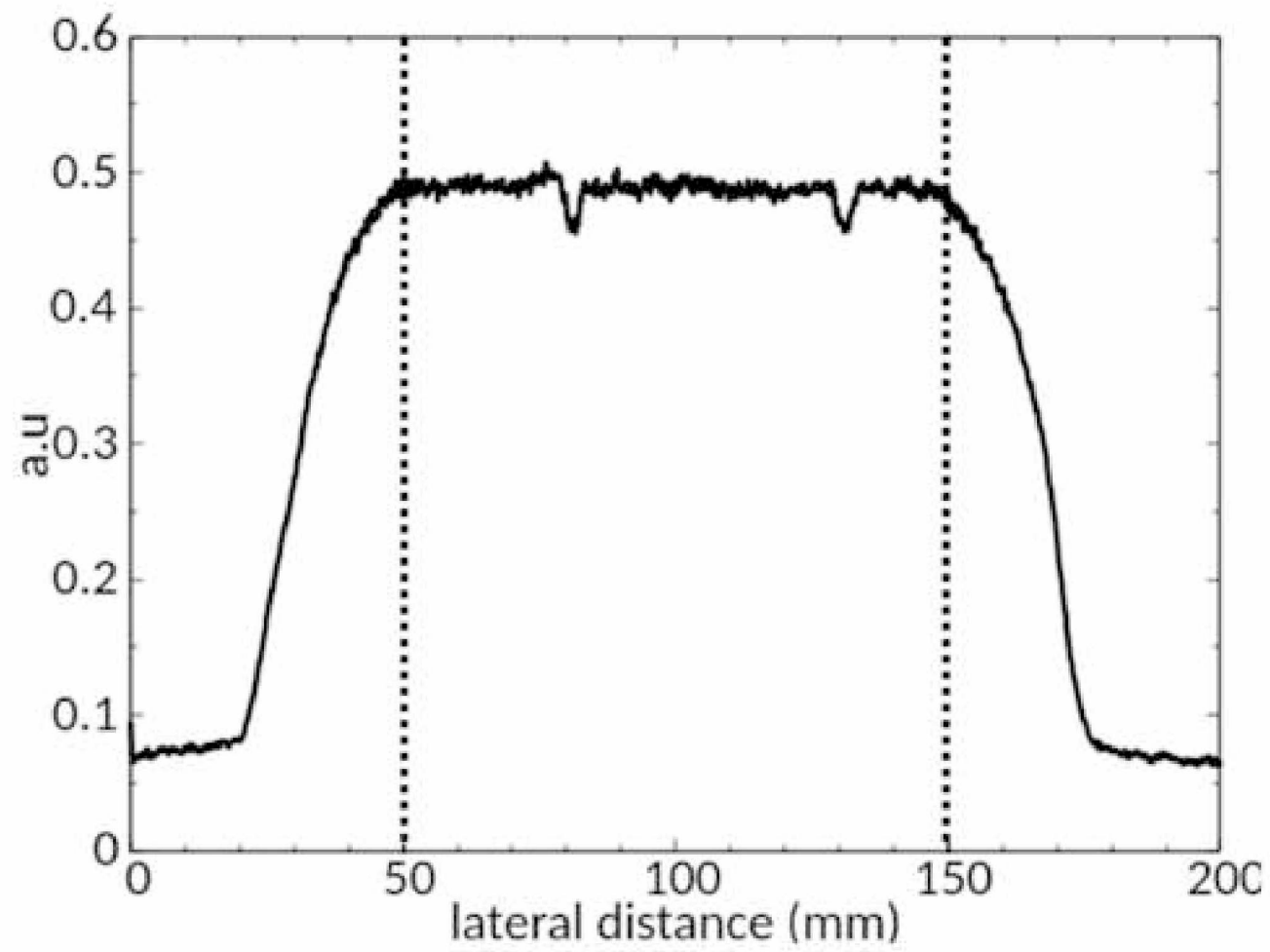
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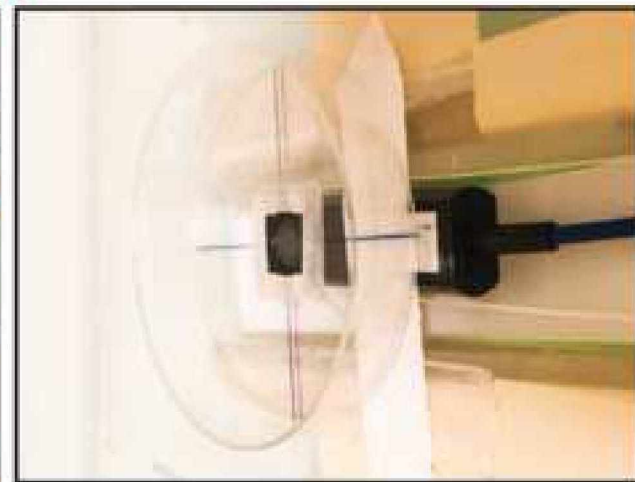
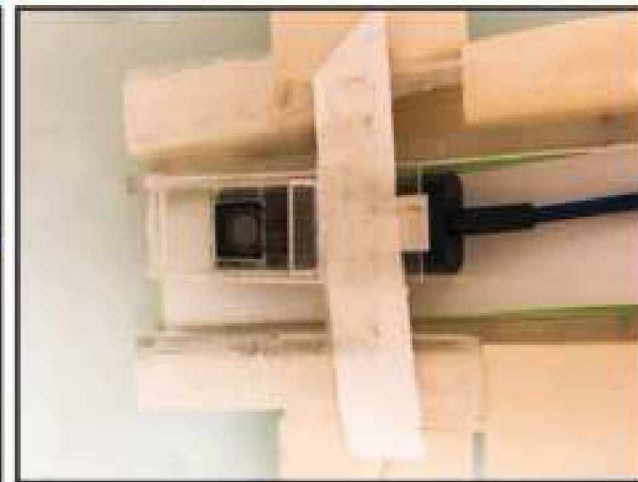
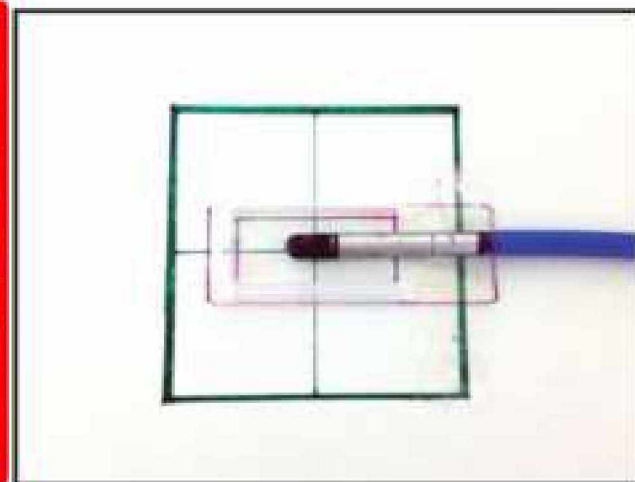
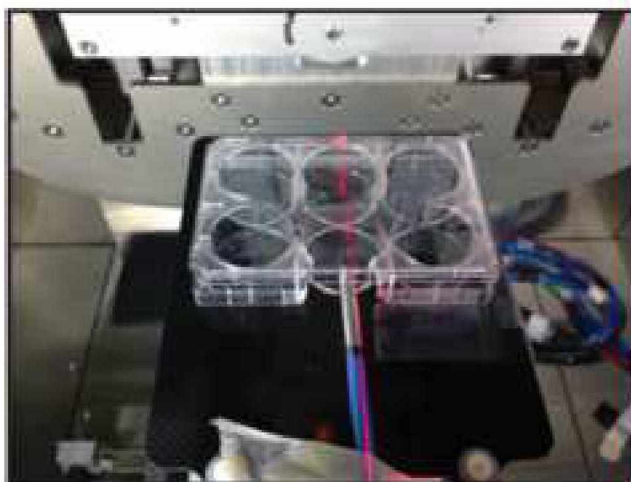
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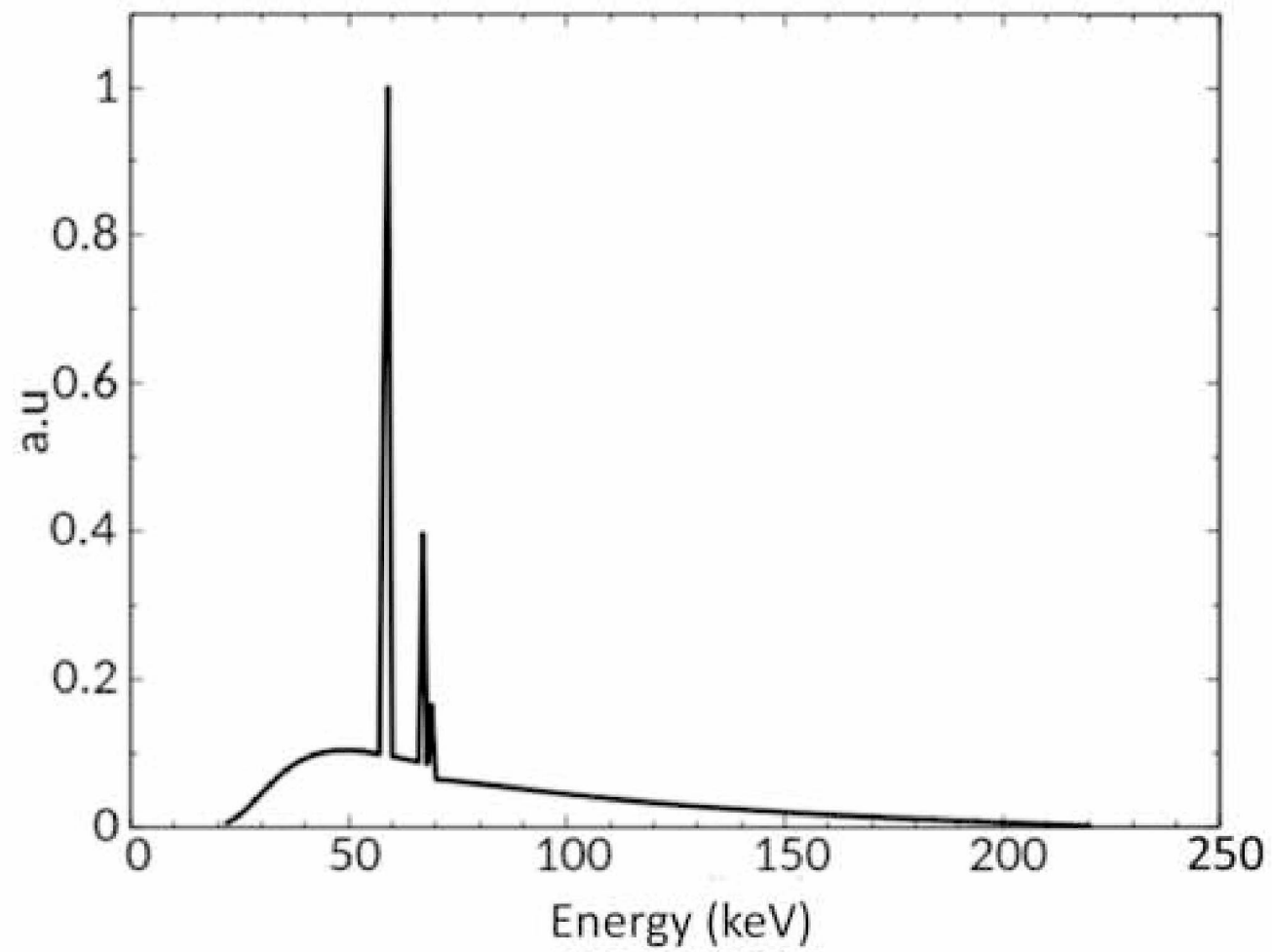
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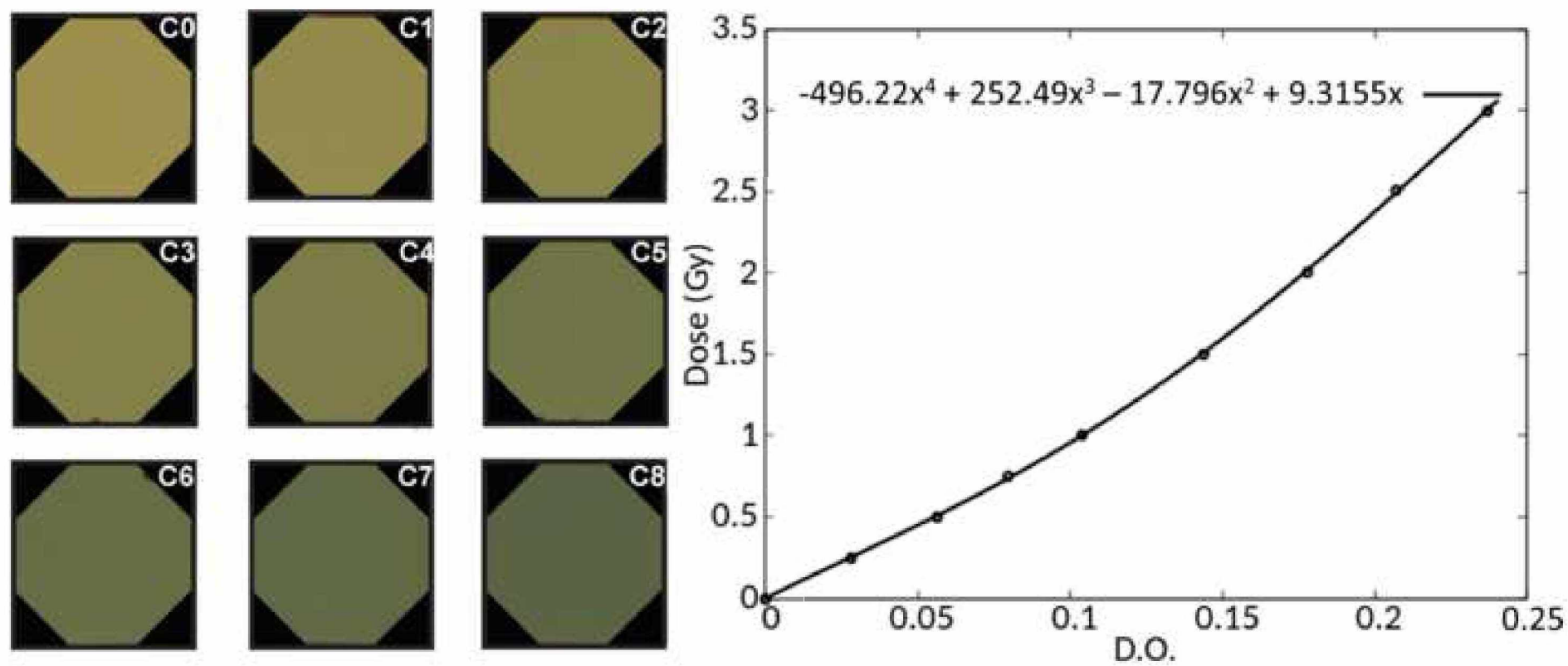












High voltage (kV)	220
Intensity (mA)	3
Filtrations (inherent and additional)	0.8 mm of Be + 0.15 mm Cu
Half value layer (mm Cu)	Determined below
Effective energy (keV)	Determined below
Detector used	Cylindrical ionization chamber + EBT3 radiochromic films
Source sample distance	35 cm
Irradiation field (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)	
reference measurements (Mref)	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	993.2
	0.564	5.651	21.7	993.2
	0.584	5.569	21.7	993.2
	0.604	5.491	21.7	993.2
0.615	5.441	21.7	993.2	
Finding of attenuator thickness (M)	0.627	5.380	21.7	993.2
	0.647	5.307	21.7	993.2
	0.667	5.240	21.8	993.2
	0.667	5.231	21.8	993.4
	0.667	5.236	21.8	993.1
	0.667	5.235	21.8	993.2
	0.667	5.236	21.8	993.2
	0.667	5.235	21.8	993.2
	0.667	5.236	21.8	993.2
	0.667	5.235	21.8	993.3
Measurments with the right attenuator (M)				

$k_{T,P}$	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				

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=6096ddc2949f8bafe5d556e931e6c865
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