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Radiochromic Film Dosimetry in Kilovoltage X-ray Beams: A Pre-Investigation for In Vitro Studies of Bystander Effects

Zeinab Hossein

Supervision

Crister Ceberg, Lund

Department of Medical Radiation Physics,
Clinical Sciences, Lund
Lund University
www.msf.lu.se

Summary in Swedish

Inom strålbehandling innebär den s.k. åskådareffekten ("bystander effect") ett relativt nytt tankesätt som betonar de biologiska effekterna av strålning på icke-bestrålade celler i närheten av bestrålade celler. Denna effekt har visat sig påverka inte bara de celler som angränsar till det bestrålade området utan även celler på större avstånd. I Lund pågår just nu ett projekt där man vill undersöka dessa effekter närmare. Användning av intensitetsmodulerad strålning är en av flera viktiga faktorer som måste utvärderas i undersökningar av hur celler påverkar varandra när de exponeras för strålning med låga och höga doshastigheter.

Syftet med denna studie är att etablera en bra och praktisk dosimetrisk metod för mätning av den absorberade dosen till cellkulturer i olika geometriska uppställningar med bestrålade och skärmade celler. För att kunna ta fram förbättrade strålningsgeometrier med mer noggrann dosimetri krävs specifika dosimetriska genomförbarhetsstudier under motsvarande förhållanden. Inför sådana mätningar måste man noggrant överväga lämplig metod och val av dosimeter.

Alla bestrålningsexperiment i denna studie genomfördes med en röntgenapparat vid Skånes universitetssjukhus i Lund vid röntgenspänningar på 120 kV och 200 kV. Film av typen Gafchromic EBT3 användes för mätning av den absorberade dosen. Olika mätningar utfördes för att förstå filmens struktur och konstruktionsegenskaperna och hur dessa faktorer påverkade strålningsresponsen. För analys av filmmätningarna utvärderades tre olika program, d.v.s. OmniPro program, ett eget tillverkat Matlab-program kallat FILMGUI och FilmQA Pro program. Efter att ha säkerställt en procedur för noggrann mätning av den absorberade dosen bestrålades cellkulturer.

Filmdosimetri är en pålitlig metod med hög spatial upplösning som fungerar bra för mätning av absorberad dos. Olika analysprogram undersöktes i detta arbete, och resultaten av denna jämförelse visade att man kan mäta den absorberade dosen med stor noggrannhet. Flera fördelar erhölls vid användning av FilmQA Pro, som också rekommenderades av tillverkaren av EBT3-filmerna. En väsentlig orsak till att detta program ger hög noggrannhet är att det använder sig av flerkanalsdosimetri.

Abstract

Purpose

Intensity modulation of radiation is one of several important conditions to evaluate for full understanding of how cells involved in the bystander effect influence each other when cells are exposed to radiation at low and high dose rates. The aim of this study was to develop and apply a useful and practical dosimetric method for measurement of the absorbed dose to cell cultures exposed to intensity modulated radiation in investigations of the bystander effects.

Material and methods

All irradiations in this study were performed using an orthovoltage x-ray unit at Skåne University Hospital in Lund using 120 kV and 200 kV. Film dosimetry using Gafchromic EBT3 type film was the method of choice for measurement of the absorbed dose. A number of measurements were performed to understand the basic properties of the film with respect to radiation response, including inter- and intra-individual film sheet variations. Furthermore, the effect of different scattering conditions was investigated, including the use of different calibration phantom materials and the amount of nutrition medium in the cell culture flask. Dose calibrations were carried out and to evaluate if the film calibration datasets returned the expected absorbed dose at different dose levels, a depth dose measurement was performed which was compared with ion chamber measurements. For dosimetric analysis of film measurements, three different software tools were evaluated, i.e., (i) the OmniPro software, (ii) a locally developed MATLAB program called FILMGUI and (iii) the FilmQA Pro software.

Results

Generally, the variations in measured absorbed dose between film sheets, between film pieces within one sheet and between pixels in a ROI was highest for the OmniPro software and the lowest for FilmQA Pro. Evaluation of the inherent properties of EBT3 film showed that the relative deviations under different measurement conditions were ≤ 1 %. In measurement with different calibration phantom materials, i.e., a solid water phantom slab versus a plastic phantom, in which the plastic phantom returned 5 % higher absorbed dose. Finally, the absorbed dose measured under the same conditions as a cell flask exposed to 98 MU (corresponds to 1.0 Gy at the surface) was measured to 1.05 Gy when the flask was fully filled with water and 0.94 Gy when the flask was filled with 5 ml water.

Conclusions

Film dosimetry is a reliable method with high spatial resolution which is expected to work well for measurement of absorbed dose in cell exposure applications. The basic tests were satisfactory, and the inherent variations in radiation response were modest. The depth dose measurements compared to ion chamber measurements implied that film measurements returned the expected absorbed dose using at 200 kV, and this was validated by Monte Carlo simulations. The use of FilmQA Pro appeared to be optimal in this context, and this program is also recommended for analysis by the EBT3 manufacturer due to the use of accurate multi-channel dosimetry. After ensuring accurate measurement of the absorbed dose the cell irradiation experiments was started.

Abbreviations

PMMA: Polymethylmethacrylate

FSD: Focus-to-surface distance

Dpi: Dots per inch

ROI: Region of interest

OD: Optical density

NetOD: Net optical density

ADC: Analog-to-digital converter

RGB: Red-green-blue

MU: Monitor units

Gy: Gray, unit of absorbed dose

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I. Introduction

Radiation therapy for cancer was initiated soon after the discovery of x-rays, and has become an important treatment modality. Today, about half of all cancers are treated by radiotherapy. However, the attempts to eliminate a tumour by radiation can also cause adverse biological effects to normal tissue, and the understanding of biological effects, both in tumours and in normal tissue, is crucial for efficient therapy. The general view of these biological effects is about to change. Historically, the focus has always been on the direct effects on irradiated cells and side effects after radiotherapy. The bystander effect is a relatively new approach that emphasizes the biological effects of the radiation on non-irradiated cells in the close proximity of irradiated cells. Cells affected by this bystander effect exhibit symptom (as well as the exposed cells) such as chromosomal instability, telomere aberrations, cell death and micronucleation (Prise et al., 2003), (Nasir et al., 2014).

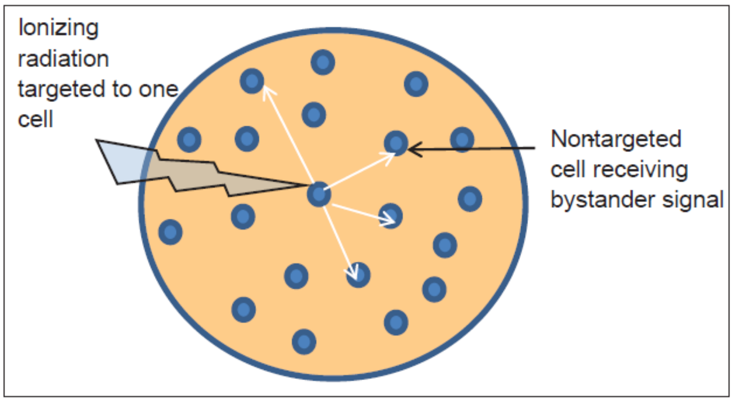

Figure 1: The principle of the radiation-induced bystander effect (Nasir et al., 2014).

Radiation-induced bystander effect influences not only the neighbouring cells, but it also affects cells far from the irradiated region. One study has shown irradiation effects in cells up to 1 mm away from an irradiated region consisting of 2 µm of skin tissue (Belyakov et al., 2005), and similar results of the radiation-induced bystander effect on cells in other regions than the irradiated region have been reported also by others (Hu et al., 2006), (Lyng et al., 2002), (Wang et al., 2011). In a cell cultured bottle the radiation-induced bystander effect is given in the whole bottle, up to about 4 cm from the irradiated area (Olsson et al., 2010).

The bystander effect is believed to involve various chemical substances, and this provides possibilities to find new ways of combining radiotherapy with the body's own chemical processes and to develop combination therapies with chemotherapy agents. Closer examination of the bystander effect is intriguing in the search for new treatment strategies, in order to evaluate whether these observations can be exploited clinically, and the present work aims to follow this track, with emphasis on the dosimetric aspects. Careful dosimetric calculations are important to determine whether different levels of absorbed dose may cause different bystander effects on the cells, but still the observed effects are not just an effect of inaccurate dosimetry.

Dosimetric challenges

The intensity modulation of the radiation is one of several important properties to evaluate for full understanding of how cells involved in the bystander effect influence each other when cells are exposed to low and high dose rates. One important aspect of this work is to develop and apply a useful and practical dosimetric method for measurement of the absorbed dose in this context.

Hence, in the present study, preparations were made for experiments with cell cultures were irradiated using different geometrical sets with irradiated and shielded cells. X-rays with a beam quality corresponding to a voltage of 200 kV were used to produce a sharp delineation between irradiated and shielded cells at different dose rates. Specific dosimetric feasibility studies were required in order to accurately determine the absorbed dose in the various geometries used in the experiment. Such measurements need careful considerations about appropriate methodology and choice of dosimeter. The need for high spatial resolution in these difficult geometries with modulated fields tends to rule out the use of an ion chamber or a diode. A reliable, robust and high-resolution alternative in this context is to use film dosimetry, and Gafchromic EBT3 film was the method of choice for measurement of the absorbed dose and for verification of the dose distribution of the intensity modulated radiation. Dose calibrations were carried out at 200 kV x-rays and various film analysis strategies were evaluated. The main reasons for choosing EBT3 film for dose verification (Fuss et al., 2007) were high spatial resolution, near-tissue equivalence (Niroomand-Rad et al., 1998) and weak energy dependence (Butson et al., 2006), (Chiu-Tsao et al., 2005), (Lindsay et al., 2010).

The initially available software for the analysis of the results (OmniPro from iba Dosimetry), previously used in the clinic, was known to show insufficiently accurate response in this kind of applications. Hence, an important aspect of the present work was to develop and evaluate a more robust and accurate analysis method. To optimize the dosimetric accuracy in this project, three different software tools were evaluated and compared for analysis of the results: (i) the OmniPro software which was available at the clinic, (ii) a locally developed MATLAB program called FILMGUI and (iii) the FilmQA Pro software created by the manufacturer of Gafchromic EBT3. Finally, Monte Carlo simulations were used to validate some of results.

II. Material and methods

a. Equipment

1. Film dosimetry

Radiochromic film is a practical and accurate dosimeter for quality assurance (QA), especially in intensity modulated radiation therapy (IMRT). The advantages of using radiochromic film are, for instance, the ability to perform measurements over a wide range of doses (from 1 cGy up to 40 Gy), high spatial resolution and near tissue equivalent properties (Gafchromic dosimetry, 2013). This type of film was regarded to be the most appropriate dosimeter in this study, due to its high spatial resolution and high dose accuracy.

In this study, Gafchromic EBT3 film was used, which functions without processing (contrary to the traditional silver film) and is based on radiation-induced polymerization of diacetylene, a non-saturated hydrocarbon with four carbon atoms containing two triple- and three single bonds. During the exposure, diacetylenes are converted to a dyed polymer crystal. Similarly to silver halide film, the degree of dye darkening is related to absorbed dose and is detected optically.

2. Gafchromic films

Gafchromic EBT2/EBT3 films have been designed to save both time and cost in dosimetric measurements. The Gafchromic EBT3 film (Ashland Specialty Ingredients, NJ, USA) consists of a single active layer with a thickness of around 28 µm, containing the active component, yellow marker dye, stabilizer and other substances to ensure that the film shows very low energy dependence in water. The yellow marker dye is added to decrease light sensitivity, facilitate multichannel dosimetry and to allow for correction of non-uniformities of the film. The active layer is coated with a matte polyester layer with a thickness of 125 µm on both sides (Figure 2, left). The EBT3 surface layer of polyester substrate consists of microscopic silica particles that create a gap (of approximately 10 times the wavelength of visible light) between the film and the glass window of the scanner. The matte substrate and the size of the gap prevent formation of interference artefacts (Newton’s Rings) during scanning. In contrast to the earlier film version, EBT2, the symmetric structure of EBT3 eliminates the need to establish which side to be scanned. EBT2 consists of an active layer, but also an adhesive layer coated with clear polyester on both sides, with different thicknesses (Figure 2, right). The EBT2 film has more problems with Newton's Rings because of the thickness of the polyester layer and the characteristics of the material (Gafchromic dosimetry, 2013).

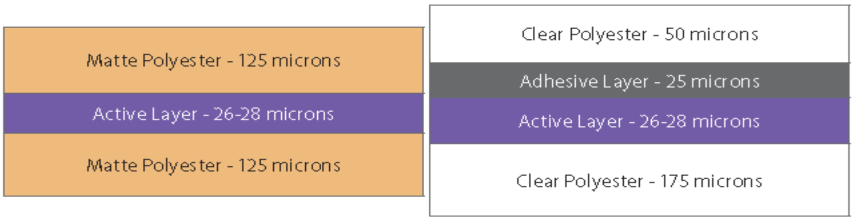

Figure 2: Left: The structure of Gafchromic EBT3 dosimetry film. Right: Gafchromic EBT2 dosimetry film for comparison.

3. Optical density of film

The radiation effect is measured as the *optical density* d_x (*OD*) of the film, which describes the change in light intensity during the passage through the film material, i.e.,

$$d_x = \log\left(\frac{I_0}{I}\right),$$

where I_0 is the intensity of the incident light before passing through the film material, perpendicularly to the film surface, and I is the intensity of light after the passage through the matter. The optical density depends on the thickness of the film, but also on the wavelength of the incident light. Radiochromic films have an inherent colour that develops during radiation exposure, and the optical absorption varies by wavelength (Devic et al., 2010). In practice, the optical density d_x is determined by using a colour flatbed scanner which digitalizes the image and measures the film response over different wavelength bands divided into red, green and blue (RGB) bands in the visible spectrum. The absorbance spectrum of the active layer for Gafchromic EBT3 has a peak at 636 nm after exposure to ionizing radiation, which means that the maximum sensitivity for optical density measurements is obtained with red colour. Figure 3 shows the absorption spectra of EBT3 film before and after irradiation to 2.0 Gy, with the post-exposure peak seen at wavelength 636 nm.

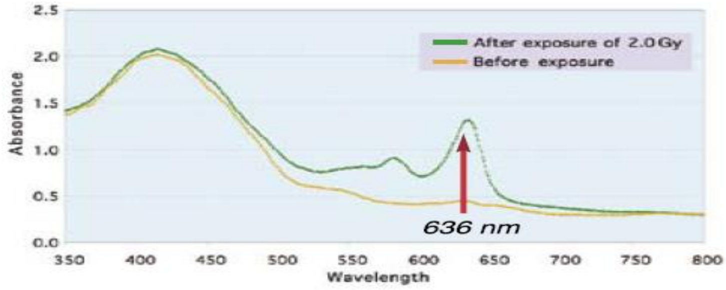

Figure 3: Absorption spectra of EBT3 film as a function of wavelength, before exposure (the yellow line) and after irradiation corresponding to 2.0 Gy (the green line).

Hence, the red colour channel provides the most sensitive response to radiation for EBT3, while the green colour channel enables high dose measurements between 8 and 40 Gy, and the blue colour channel may be used to enhance the uniformity due to a marker dye in the active layer.

4. Phantom

In radiotherapy, a phantom is often used to represent a real situation, in which the radiation interaction with a material and the corresponding absorption and scattering properties need to be described. The most convenient material for such dosimetric procedures is water, because of its suitable effective atomic number ($Z_{\mathrm{eff}} = 7.51$) and density ($\rho = 1\,\mathrm{g/cm^3}$), thereby constituting a god approximation to tissue. In this work, a versatile phantom, to be filled with distilled water was used, consisting of a PMMA basin with dimensions of 15×35×35 cm^3 and open for filling at the top. Beam calibration measurements were performed using a so-called solid water phantom. Solid water simulates the absorption characteristics of water for different energies, and this type of phantom has radiation scatter and attenuation properties similar to water (Seuntjens J1, 2005).

b. Irradiation procedure and set up

All irradiations in this study were performed using an orthovoltage x-ray machine (Gulmay Medical D3225) at Skåne University Hospital in Lund. The system generates x-rays photon beam qualities in the range 20 to 225 kV with an inherent filtration of 0.8 mm Be. This unit consists of a generator, an x-ray tube, different filters for different energies, removable applicators divided into two sets: (i) circular applicators with diameter sizes from 2 to 10 cm and 20 cm FSD for softer beam qualities, and (ii) square applicators with areas from 4×4 cm^2 to 20×20 cm^2 and 50 cm FSD, focus to surface distance, for harder beam qualities. The appropriate dosimetry protocol for this machine is the IAEA TRS-398 (Andreo et al., 2000), which is based on standards for absorbed dose to water and available for low and medium x-ray energies.

The measurements in this work (Figure 4) were performed using 120 kV/2 mm Al (kV/filter) and 200 kV/0.5 mm Cu, and applicator size 4×4 cm^2 and 15×15 cm^2 with FSD 50 cm. This FSD corresponds to the distance to water surface. The absorbed dose 1 Gy at the surface using 4×4 cm^2 and 15×15 cm^2 field size and 120 kV beam quality corresponded to the a value of 118 MU respective 97 MU, and for field size 4×4 cm^2 and 15×15 cm^2 and 200 kV beam quality the corresponding value for 1 Gy was 123 MU respective 94 MU at the surface.

Figure 4: The Gulmay Medical x-ray unit with inserted applicator 4×4 cm^2.

c. Evaluation of different properties of film

Gafchromic EBT3 film with sheet size 8"×10" was used for the initial experiments, performed to understand the structure and properties of the film as well as to become familiarized with film handling and the entire film process from exposure to data analysis.

1. Basic tests of EBT3 film

Several basic experiments were performed to establish efficient handling procedures without introducing any bias of the results. The first experiment was performed to establish whether uncut and cut film pieces show identical results. Hence, one film sheet was cut into three pieces while another sheet was left intact, and films were then irradiated to 118 MU corresponds to 1 Gy at the surface using 120 kV and 4×4 cm^2 applicator size. The second test was performed to assure that the film sheets were water resistant. A film sheet was cut into two pieces and one of the pieces was placed at the bottom of a water bath, and thereafter each piece was exposed to 118 MU. Furthermore, different calibration phantom materials were tested, since using water for calibration may not be practical in all cases. An experiment was performed using a 4 cm solid water slab on top of the film piece and a plastic phantom was placed under the film while another film piece was exposed with the applicator placed directly on the film, lying on a plastic phantom. This was compared with a set up with liquid water under the film. Finally, experiments were made to determine if the film was equally sensitive near the edge or if the radiation response was the same as near the centre of the film sheet. The existence of a potential edge effect was evaluated by placing two film pieces adjacent to each other, with one piece displaced 2 cm longitudinally relative the other, and immersing both pieces in water vertically. The film samples were irradiated with 120 kV to 94 MU and field size 15×15 cm^2, corresponding to an absorbed dose of 1 Gy. This measurement was also performed with 200 kV irradiated to 94 MU with 15×15 cm^2 applicator size.

2. Statistical analysis of EBT3 film

The response of film might be different between different film sheets, but might also depending on where the exposure occurs on a given film. Hence, one important aspect in film measurements is to check such intra- and inter-individual film sheet differences in radiation response. To investigate inter-individual film sheet variations, an experiment was performed where small parts of 10 different film sheets were irradiated with 200 kV to 94 MU corresponding to 1 Gy. To analyse the variations between different film sheets, a dose profile measurement in Gy was selected for each film piece, and the standard deviation was determined by calculating the average value for the whole dose profile values.

Another measurement was performed to determine the intra-individual variations, i.e., the variations within the same film sheet. In this experiment, a single film sheet was cut in 10 pieces and each piece was exposed to the same absorbed dose under the same conditions. Variations between different positions within the same film were analysed by choosing dose profiles over 10 film pieces from the same film sheet, and the standard deviation of the average values from each piece was calculated using three different analysing methods. Finally, for comparison, the variation between pixels in a ROI were analysed by choosing a dose profile over a film piece and calculate the standard deviation for each pixel.

d. Calibration

To obtain the relationship between optical density and delivered dose, the film system was calibrated for the beam qualities of 120 kV and 200 kV. A set of 8 small pieces of film were pre-scanned and then irradiated to dose levels of 0.2, 0.4, 0.6, 0.8, 1.0, 1.1, 1.2 Gy. Irradiations were performed with FSD 50 cm and field size 15×15 cm^2. The dimensions of each film piece were 6×10 cm^2. Each film piece was placed on top of a plastic phantom and a solid water phantom slab with thickness 4 cm was placed on top of the film piece. After irradiation, all film pieces were post-scanned to establish a calibration curve. A non-irradiated reference film piece was used to correct for intensity variations in the scanner light (Figure 5). This calibration method was applied to the OmniPro software (without using the pre-scanning data) and the FILMGUI program.

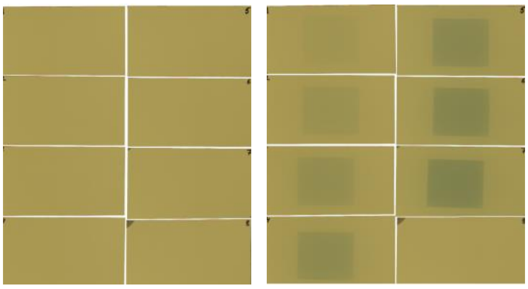

Figure 5: Illustration of film calibration methodology. Different pieces of a film sheet were exposed to eight dose levels for determination of the calibration curve. The image to the left shows film pieces scanned before exposure, and the image to the right shows the same film pieces after irradiation. In the right-hand image, beginning from the left column, the dose levels, from top to bottom in each column, are 0.2, 0.4, 0.6, 0.8, 1.0, 1.1, 1.2 Gy and the last one is the reference piece.

e. Image processing

The film scanning was performed in landscape position using an EPSON Perfection 4990 PHOTO flatbed RGB scanner. Scanning was carried out at least 6 hours after irradiation, but the result will be most reliable if scanning is performed 24 hours after the irradiation to ensure darkening effect stabilization (Cheung et al., 2005). The following settings were selected: Transmission mode (positive film mode), 72 dpi resolution, 48-bits RGB image type, without image correction. Before scanning of the film, three blank scans were performed in order to warm up the lamp of the scanner. Film pieces were marked with numbers at the top to facilitate consistency in orientation, and, for each number, orientation, dose rate and other relevant details were recorded.

In this work, three different software tools for analysis of the EBT3 films, as further described below, were evaluated with respect to statistical variations in the calculation of the absorbed dose.

1. OmniPro IMRT software

In this software, the employed RGB channels are selected manually, and the red channel was selected in this study. The image to be analysed was imported together with a background image (unexposed film) into the program.

Dose calibration: In order to determine the relative dose after an unknown exposure, it is necessary to calibrate the film scanner and the film using film pieces irradiated according to the procedure described above. The calibration curve was obtained by post-irradiation scanning of the film samples with known absorbed doses, and the values from the analog-to-digital converter (ADC) were obtained by selecting the individual film pieces and marking the centre section with a cursor. After recording of values for all of the film pieces, the calibration curve (ADC as a function of absorbed dose) was created by specifying the known absorbed dose for each piece.

Dosimetric analysis of irradiated film: Irradiated film samples were analysed by selecting the scanned image files of interest as well as a background image. The image was then cropped to optimal size, excluding the grey background region. The background image was used to correct for scanning artefacts, which take account of any uneven scan light, but not an uneven film thickness, due to a different film piece, i.e., for spatially non-uniform response of the scanner. The background correction was established, and applied to the image of interest. Thereafter, the calibration file, created according to the previous section, was loaded and a calibration graph was displayed. Finally, the selected image was converted to absorbed dose using the loaded calibration curve (see Figure 6).

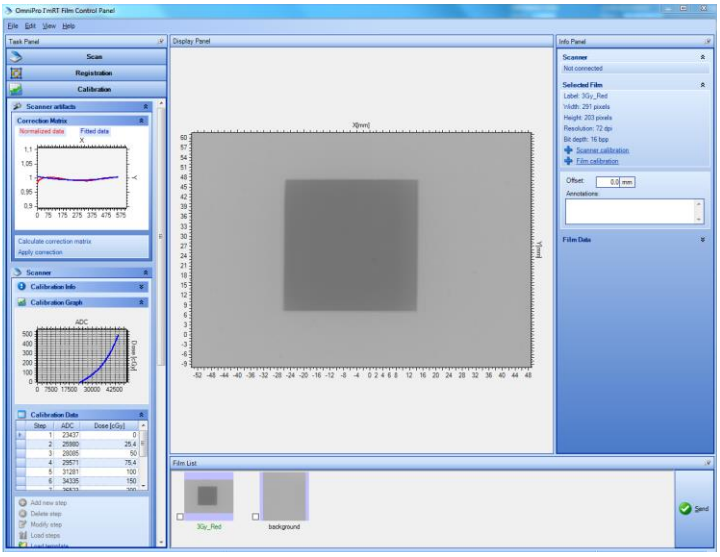

Figure 6: OmniProIMRT Film Control Panel. Loaded files appear in the film list at the bottom of the film scan panel. In this example, the background is to the right in the bottom part of the panel while the image of the irradiated film is to the left. Correction for scanner artefacts is shown in the graph at the top left and the graph at the lower left panel shows the calibration curve.

After applying the calibration data to the scanned image, the film was exported into the OmniPro-IMRT analysis panel. In the analysis panel, the dose map of the image appeared, and two dose profiles are shown in Figure 7. The top right panel shows the profile along the x-direction and bottom right panel shows the profile along the y-direction.

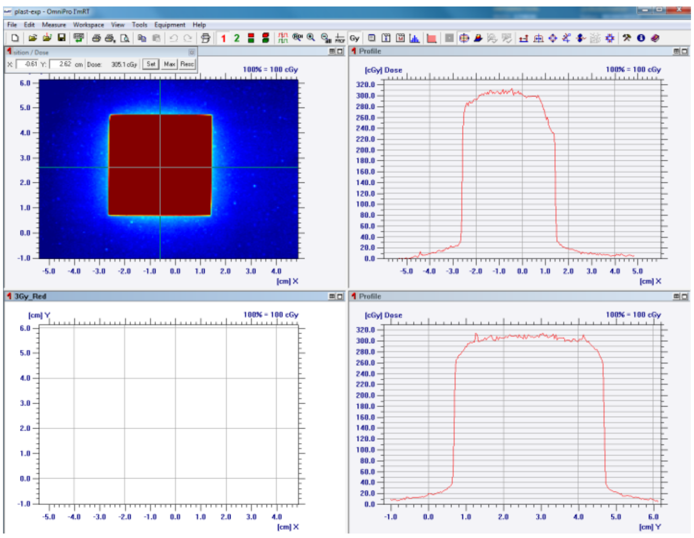

Figure 7: OmniProIMRT analysis panel. The image dose map is shown in red at the top left. The dose profiles are shown to the right (upper graph is the profile along the x-direction and the lower graph is along the y-direction).

2. Locally developed Matlab software

The second method for image processing was based on locally developed software called FILMGUI (Matlab R2014b). This program is based on a method presented by Kairn et al. (Kairn et al., 2010) for elimination of the effects of heterogeneities of Gafchromic EBT. By evaluating the net optical density, pixel by pixel, and taking the logarithm of the ratio of the pixel value for the red channel before and after irradiation these effects can be minimized. This method uses a reference image for correction of scanner variations and a measurement image with known absorbed dose. The program interface shows an uploaded unexposed film, i.e., the reference image, to the left and an exposed film, i.e., the measurement image, to the right (Figure 8). In total, two ROIs are selected and a ROI placed in one image (reference/measurement) is automatically placed at the corresponding position in the other image (measurement/reference). The two ROIs correspond to (i) reference film piece and (iii) the actual measurement ROI. The program then calculates the corrected net optical density $\mathrm{netOD_{red}}$ from each pixel (i, j) according to the following equation (Kairn et al., 2010):

$$\mathrm{netOD_{red}} = \log\left(\frac{P_{red,pre}(i,j)}{P_{red,post,C}(i,j)}\right),$$

where $\mathrm{netOD_{red}}$ is net optical density for the red channel, the $P_{red,pre}(i,j)$ is the pixel value for the red channel image in the pre-scan, and $P_{red,post,C}(i,j)$ is the pixel value for the red channel image in the scanner-output-corrected post-scan.

The program also enables positioning of x- and y-direction profiles (as shown to the right in Figure 8). The profiles are shown as $\mathrm{netOD_{red}}$, in the graphs to the right. Two curves are shown in each graph, i.e., one original (in blue) and one for which data have been subjected to a 5-pixel moving average spatial smoothing filter (in red). Saved profile files are automatically assigned file names "profileX.dat" and "profileY.dat". Data opens in Notepad and values can be copied into an Excel document. Selection of "Smooth" prior to saving, results in saving of the filtered profile (i.e., the red one).

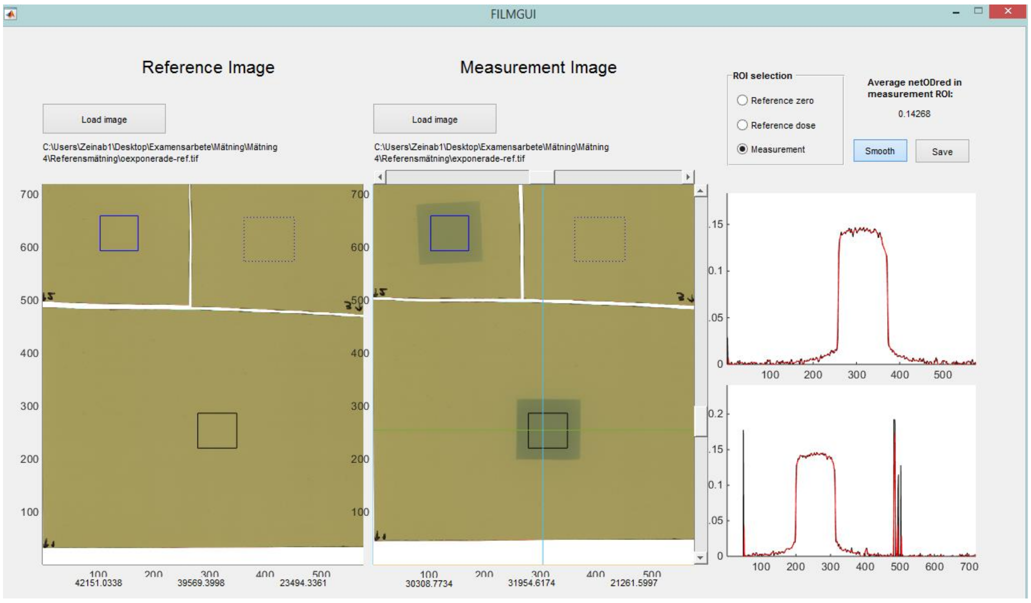

Figure 8: Illustration of the FILMGUI program interface, with the reference image to the left and the measurement image to the right, for determining the netOD. The profiles are shown in the right-hand panel, where the top graph is along the x-direction and the bottom graph is along the y-direction.

To convert scanned images to absorbed dose, a calibration curve was created using the FILMGUI. The reference image (pre-scanned films) and the measurement image (post-irradiation scanned films) were loaded to obtain a netOD value for each film piece by drawing a ROI for the reference image and another one in the measurement image with well-defined absorbed dose. The netOD values were exported to an Excel document, and the calibration curve was obtained by fitting a second-order polynomial to the netOD data versus known absorbed dose.

3. FilmQA Pro software

The FilmQA Pro (used in a 30 days trial version in this study) is a commercial software package provided by the manufacturer of EBT3, and recommended for analysis because the program applies multi-channel dosimetry. The software is based on triple-channel film dosimetry and uses the following formula for calibration:

$$d_x = \log_{10}((a + bD)/(c + D)),$$

where d_x represents optical density, D represents absorbed dose, and a, b and c are the function parameters.

Triple-channel dosimetry method

In this method, three dose values are calculated for each pixel, and the dose image is presented in three colours: red, green and blue. Furthermore, two data maps are generated from the dose image which provide information about the dose and the uniformity of the film (Micke et al., 2011). All three colours are used to calculate dose values in a consistency map, where the red channel presents the arithmetic mean, $\{|R-G| + |R-B| + |G-B|\}/3$, the green channel image data presents the geometric mean, $\{|R-G| \times |R-B| \times |G-B|\}^{(1/3)}$ and the blue channel presents the maximum of the absolute dose differences (Hoof et al., 2012). The advantage of this triple-channel dosimetry is that this method takes into account to reduce consistency values with varying a single parameter.

The optical density value $d_x(D)$ is inversely proportional to the thickness of the coated active layer of the film, $d_x(D) = d_X^D(D)\,\tau$ where the $d_X^D(D)$ is independent of the relative thickness τ which varies with the absorbed dose D.

Lateral distortions may be shown during the film scanning due to that the Gafchromic EBT2/3 polarizes the transmitted light partially (Menegotti et al., 2008), (Saur and Frengen, 2008). By using the multichannel method the lateral dependence will be reduced.

In FilmQA Pro, the film calibration part was chosen from the case object management menu. Beginning with calibration, the scanned image was loaded from the calibration tool section. A reference image and three other images, corresponding to different dose levels were selected and a ROI was place in each piece. A calibration curve was created after entering the known absorbed doses for the different images.

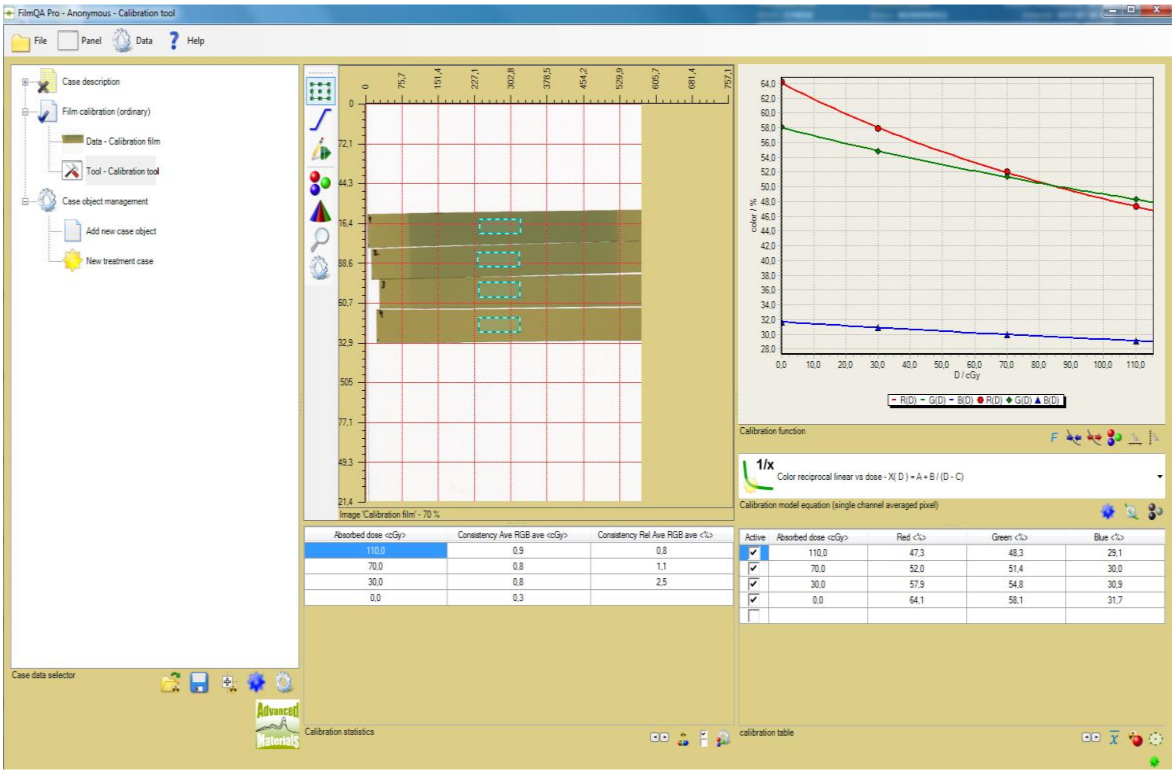

Figure 9: FilmQA Pro panel. Multichannel calibration curves where the scanned image is loaded and a calibration curve including the three RGB channels is created to the right.

To perform dose quantification on exposed Gafchromic EBT3 images, a dose map was chosen from the case object management part. The image with unknown dose was loaded and the calibration data from previous calibration (including three colour channels) were applied. In the optimized dose map, the blue colour channel was used to correct for the thickness artefacts by measurement of the absorbance of dye marker in EBT3 film. In the consistency map, a profile was drawn to obtain the absorbed dose, and dose data were saved for export to an Excel document.

f. Depth dose measurement

To evaluate if the film calibration dataset returned the expected absorbed dose at different depths up to 20 cm in a water phantom, a depth dose measurement was performed. A film sheet was cut into two smaller pieces of 8×10 cm^2, i.e., serving as one reference piece and one measurement piece and both pieces were pre-scanned. A PMMA basin with dimensions 25×25×30 cm^3 was filled with water and the film piece was immersed in water vertically. Two identically experiments were performed with 120 kV with 4×4 cm^2 applicator size exposed to 97 MU and 200 kV with 15×15 cm^2 applicator size exposed to 97 MU.

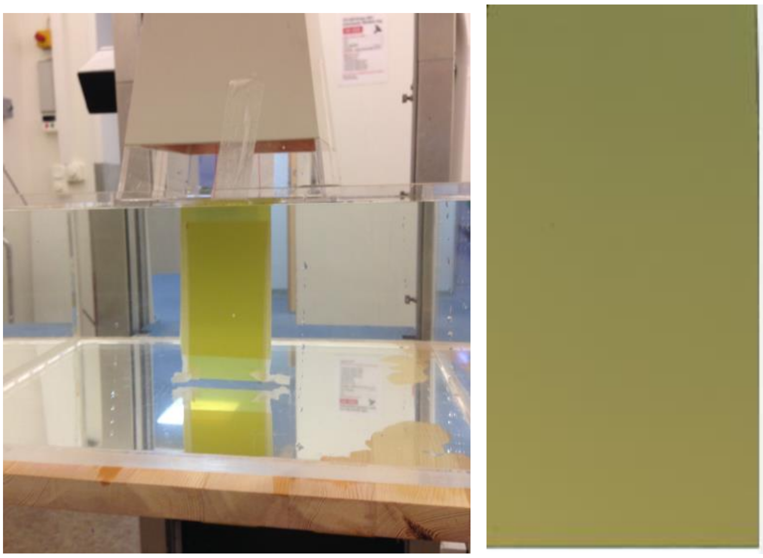

Figure 10: Left panel: The setup of the depth dose measurement, the film piece immersed in water phantom with a field size of 15×15 cm^2. Right panel: A depth dose measurement piece.

The film piece was scanned pre- and post-irradiation and a calibration curve was applied according to the three different analysis methods. For the OmniPro program, the calibration curve was used to determine the absorbed dose by applying it to the image, and then a dose profile was drawn over the whole image and the dose data was exported to Matlab for plotting of the depth dose curve. For the FILMGUI software, images before and after irradiation were loaded and a profile was drawn over the depth dose measurement image. The dose data containing net optical density were saved, after spatial smoothing, and imported to a Matlab file where the absorbed dose was calculated at different depths using the formula from the previously constructed calibration curve. The x-axis should represent the depth in mm, and the depth was calculated by converting the pixel positions of the film to mm by using the scanning resolution of 72 dpi (converted to pixels/mm). The depth dose curve was then plotted as absorbed dose as a function of depth. Finally, the same measurement was analysed by the FilmQA Pro program, where the calibration curve using three colour channels formed the basis for the depth dose calculation. The calibration curve was applied to the image, and a dose profile was drawn in the consistency map over the chosen film piece and the dose data, containing the absorbed dose and the depth in mm, were saved. The depth dose curves from all three analysis tools were compared with the corresponding dose values previously measured with an ionization chamber.

g. Preparing for irradiation of cell cultures

After ensuring accurate measurement of the absorbed dose, according to the previously described quality assurance procedure, a set up was prepared for irradiating of the cell cultures. The cell culture was plated into T25 flasks and the whole flask filled with phosphate buffered saline (PBS) to get full scatter conditions. Another liquid (serum) with smaller volume (5 ml) was also tested. To determine the absorbed dose to the cells at the same conditions, two film pieces were cut to the same shape as the flask and put into it. Two flasks with the film inside each, was filled completely with water and with 5 ml, respectively, and placed in a water bath with dimensions of 15×35×35 cm^3 and open for filling at the top. The bottom of the water phantom, where the cell flask was lying had a thickness of 16 mm for attenuating of the backscatter photons. The gantry with an applicator size of 15×15 cm^2 was rotated 180° for irradiation from below due to the short distance to the cell flask. The irradiation was performed 200 kV to 98 MU corresponding 1 Gy at surface using a uniform open field (Figure 12).

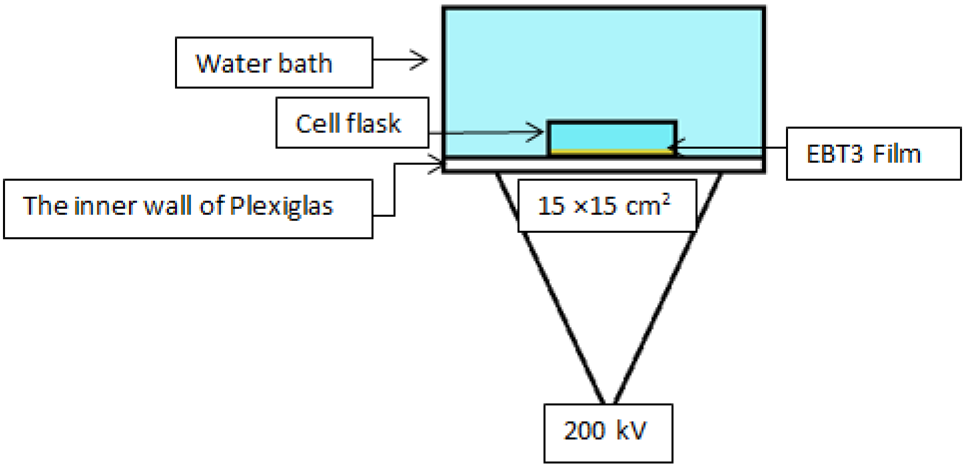

Figure 11: A schematic illustration of the experimental set up for the cell irradiations. The flask fully filled with water, containing a piece of EBT3 film (marked as yellow) in a water bath. The inner wall of the water bath consists of 16 mm Plexiglas.

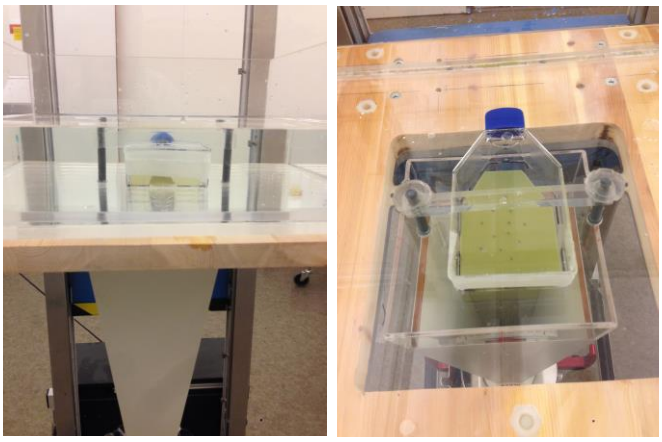

Figure 12: Left: The experimental set-up for cell irradiation. Right: The cell flask, containing a piece of EBT3 film formed as the flask, immersed in a water bath with gantry from below.

h. Monte Carlo Simulation

Some of the measurement results were validated using Monte Carlo simulations. The EGSnrc was used to simulate an orthovoltage x-ray irradiator and the BEAMnrc code was used to simulate the transport of electrons produced by the x-ray photons. These simulation data were taken from a trial in a previous study for simulation of depth dose measurements at 120 kV and 200 kV (Knöös et al., 2007).

III. Results

a. Evaluation of different properties of film

1. Basic tests of EBT3 film

Results from investigations of the dose response of EBT3 film under different basic conditions are shown in Table 1. Different measurements were performed with film pieces of EBT3 exposed to 118 MU each at 120 kV and the result was compared as relative deviation.

Table 1: Results from different measurements for evaluation of basic dose response properties of the EBT3 film at 120 kV. In the first row, cut and uncut film pieces are compared. The second row shows results from film measurement with and without water and the third row provides results regarding the orientation of the film pieces, i.e., vertically compared with horizontally. Row number 4 displays the results for measurement in a solid water phantom slab compared with a plastic phantom.

1. **Uncut versus cut film**	Relative deviation
Uncut film versus cut film	1.0 %
2. **Water surroundings**	Relative deviation
Film in water versus film without water	1.0 %
3. **Film orientation**	Relative deviation
Vertical film versus horizontal film	0.3 %
4. **Different calibration materials**	Relative deviation
With plastic phantom versus solid water phantom	4.7 %

All results in Table 1correspond to an excepted absorbed dose of 1 Gy (data taken from ion chamber measurements) at 120 kV. The first section of Table 1 shows the data for a cut versus an uncut film piece. It shows that the measured absorbed dose was equal to within 1.0 % higher for the cut film than the uncut film. In the second section of this table, it is shown that measurements in water surroundings were equal to within about 1.0 % to measurements without water. Vertical and horizontal orientations of the film resulted in very close agreement (0.3% difference). The last section shows results for calibration with a plastic phantom compared with a solid water phantom slab of 4 cm. The calibration with the plastic phantom resulted in 4.7 % higher measured absorbed dose than the corresponding exposure with a solid water phantom.

The edge effect is visualised as a depth dose curve for two film pieces, where the blue graph corresponds to the film piece placed directly at the surface and the red graph corresponds to the film piece with 2 cm longitudinal displacement (Figure 13).

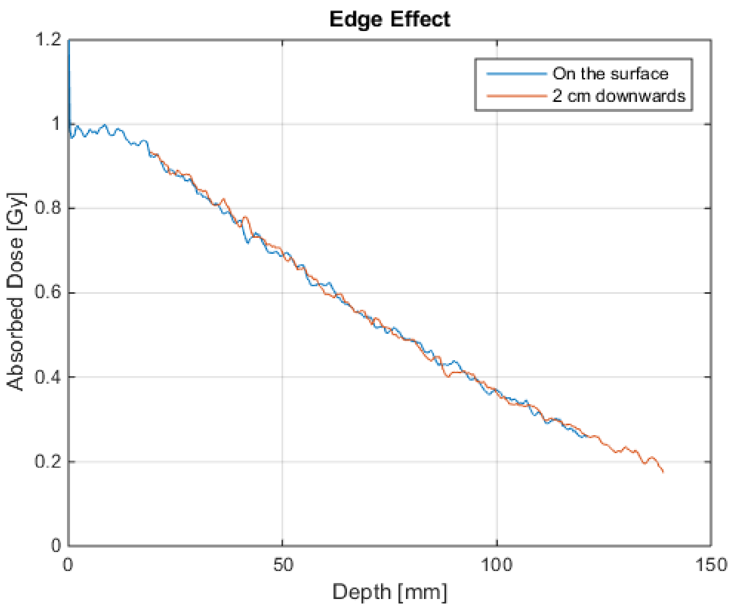

Figure 13: Investigation of the edge effect with results displayed as a depth dose curve. The blue graph corresponds to the film piece placed on the surface, directly on the applicator, and the red graph corresponds to the film piece shifted 2 cm downwards. Both pieces were simultaneously exposed with 200 to 1 Gy at the surface.

2. Statistical analysis of EBT3 film

The results for intra- and inter-individual film sheet differences in radiation response, and variations between pixels within one film piece are shown in Table 2.The film pieces were irradiated with 200 kV to 94 MU corresponds to 1 Gy at the surface. Calculated standard deviations are based on three different analysis methods, i.e., the OmniPro software, the FILMGUI program and the FilmQA Pro software. The first section in Table 2 shows the inter-individual standard deviation, i.e., the standard deviation of average values from 10 film sheets. The second section displays the intra-individual standard deviations of 10 film pieces within one sheet, and the last section gives the standard deviation of pixel values in 10 ROIs calculated by taking the average of standard deviations for 10 different dose profiles for each ROI.

Table 2: Variations in the dose response of the film. The first section shows the variation between film sheets, the second section displays the result of variations between film pieces within the same sheet and the last section shows the variation between pixel values. Corresponding results for the three different analysis methods are provided.

1. Standard deviation of different film sheets [Gy]		
OmniPro	FILMGUI	FilmQA Pro
$1.89 \cdot 10^{-2}$	$1.88 \cdot 10^{-2}$	$1.68 \cdot 10^{-2}$
2. Standard deviation of different film pieces [Gy]		
OmniPro	FILMGUI	FilmQA Pro
$3.68 \cdot 10^{-2}$	$1.39 \cdot 10^{-2}$	$1.70 \cdot 10^{-2}$
3. Standard deviation of different pixel values [Gy]		
OmniPro	FILMGUI	FilmQA Pro
$2.64 \cdot 10^{-2}$	$2.29 \cdot 10^{-2}$	$6.73 \cdot 10^{-3}$

b. Calibration curve

The dose calibration curve obtained by the OmniPro software at 200 kV is displayed in Figure 14, showing the relationship between absorbed dose and ADC for the red channel. The red circles represent the calibration patches and the purple line is the result of the quadratic fitting.

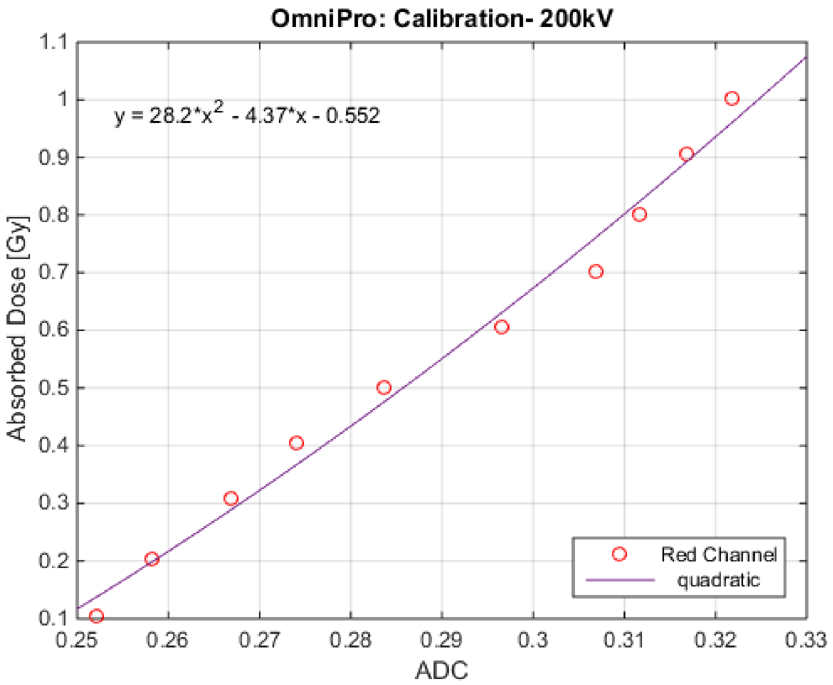

Figure 14: OmniPro calibration curve. Absorbed dose [Gy] as a function of ADC.

The calibration curve obtained with the locally developed FILMGUI software is shown in Figure 15:

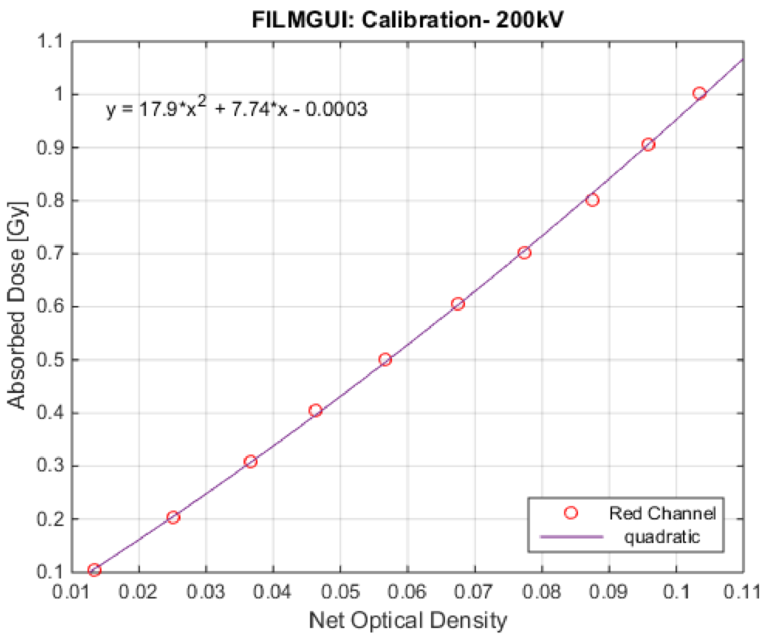

Figure 15: FILMGUI calibration curve. Absorbed dose [Gy] as a function of net optical density.

The equation of the calibration curve is: $y = 17.9x^2 + 7.74x - 0.003$, where y is the absorbed dose and x is the net optical density in a ROI in the centre of an image.

The calibration curves in Figure 16 were obtained by the FilmQA Pro software, using a multichannel calibration method at 200 kV.

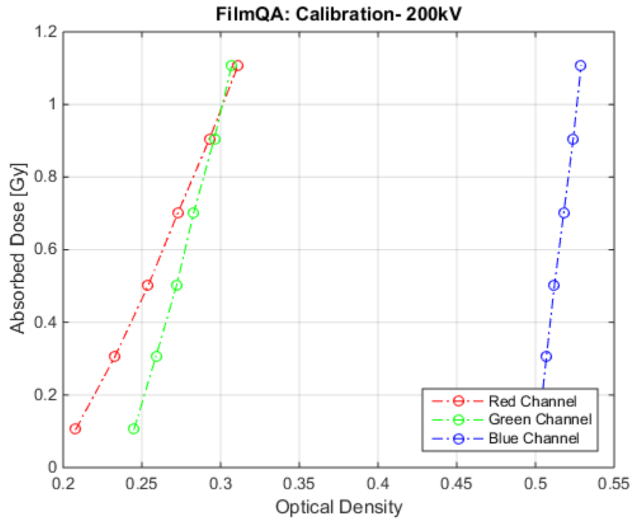

Figure 16: FilmQA Pro multichannel calibration curves. Absorbed dose [Gy] as a function of optical density for the red, green and blue channel, respectively.

Note that the calibration curves for the three different software programs are not directly comparable, due to the use of different methods of calculation.

c. Depth dose measurements

In this part the depth dose measurement are presented for different analysing methods for two different energy qualities, namely 120 kV and 200 kV.

Depth dose curves for 120 kV and 200 kV, according to the OmniPro software, are given in Figure 17:

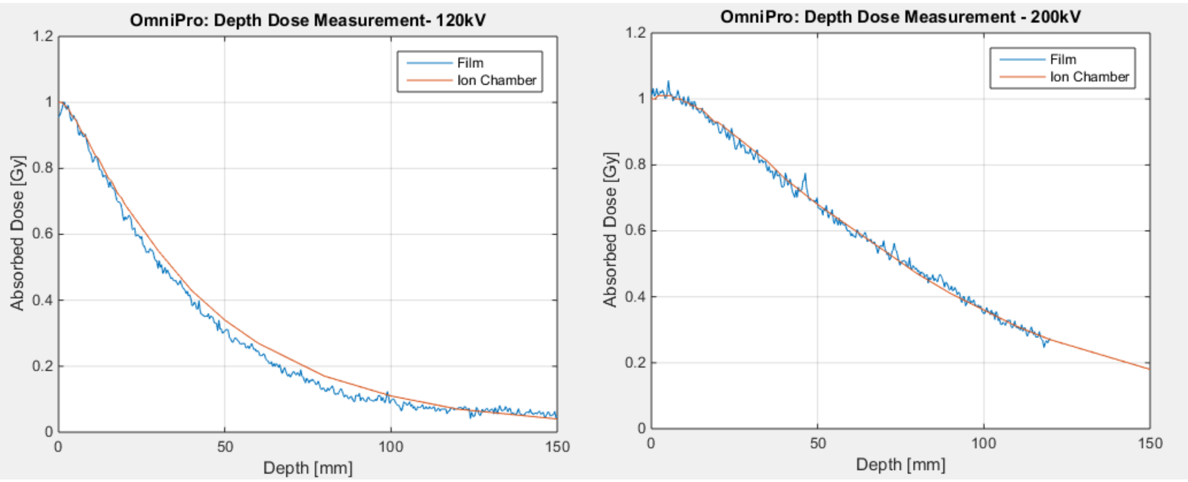

Figure 17: Depth dose measurements according to the OmniPro software: Absorbed dose [Gy] as a function of depth [mm]. The blue curve shows measurements with film and the red curve corresponds to data from the ion chamber measurements. Left panel: 120 kV 4×4 cm^2 applicator size. Right panel: 200 kV15×15 cm^2 applicator size.

In Figure 18, the 120 kV and 200 kV depth dose curves obtained by analysis using the FILMGUI software are displayed.

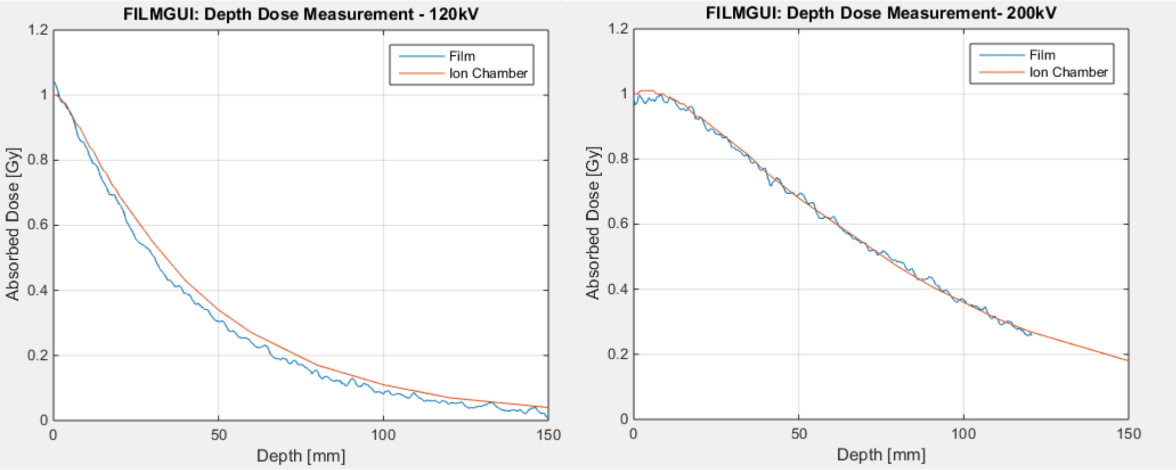

Figure 18: Depth dose measurements according to the FILMGUI analysis program. Absorbed dose [Gy] as a function of depth [mm]. The blue curve shows measurements with film and the red curve corresponds to data from the ion chamber measurements. Left panel: 120 kV, 4×4 cm^2 applicator size. Right panel: 200 kV 15×15 cm^2 applicator size.

The depth dose curves, at 120 kV and 200 kV, according to the analysis by FilmQA Pro, are given in Figure 19.

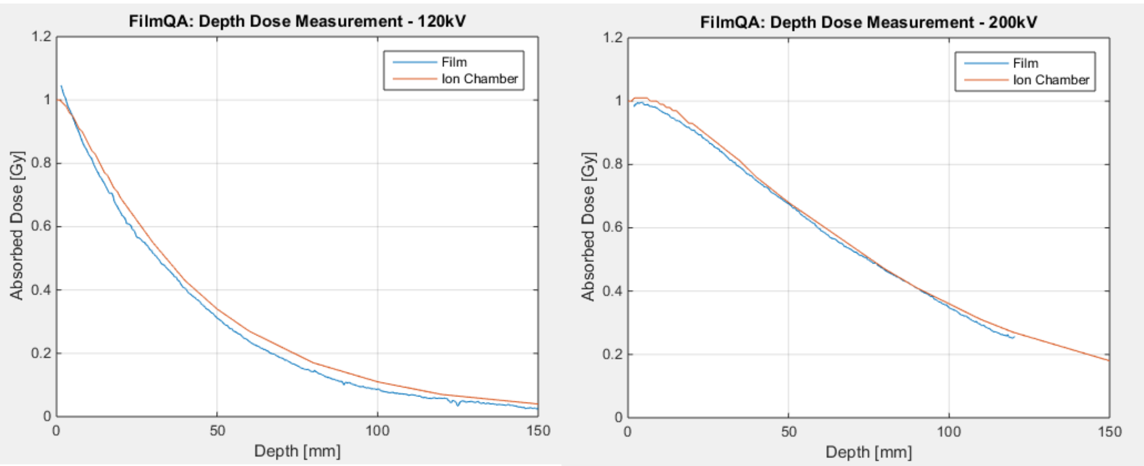

Figure 19: Depth dose measurements according to the FilmQA Pro software. Absorbed dose [Gy] as a function of depth [mm]. The blue curve shows measurements with film and the red curve corresponds to data from the ion chamber measurements. Left panel: 120 kV 4×4 cm^2 applicator size. Right panel: 200 kV15×15 cm^2 applicator size.

d. Cell irradiation conditions

The results of irradiation in the cell irradiation geometry are shown in Table 3, i.e., film in a T25 flask was irradiated (i) with the flask fully filled with water and (ii) with 5 ml water in the flask.

Table 3: The results of cell irradiation measurement exposed to 94 MU at 200 kV.

	Film measurement	Monte Carlo Simulation
	Absorbed dose [Gy]	Absorbed dose [Gy]
Fully water filled T-25	1.05	1.04
T-25 with 5 ml water	0.94	0.86

IV. Discussion

a. Evaluation of different properties of film

In this project Gafchromic EBT3 film was prepared and used for measurements of absorbed dose. To investigate potential variations in irradiation response and sensitivity of the film, the first step was to perform a number of basic measurements in order to become familiar with film properties and how the films behave under different experimental conditions.

1. Different properties of the EBT3 film

The results in Table 1 indicate that there is no significant difference between cut and uncut film pieces, and this result was important because most of the experiments in this study with this type of film were performed with cut film pieces. Furthermore, the film appeared to be water resistant in the sense that water surroundings did not influence the dosimetric response, and the finding agreed well with the manufacturer's descriptions in their user manual. The film orientation did not affect the response of the film significantly, indicating that the geometric dependence is small and results are not likely to depend on whether measurements for calibration or cell irradiation are carried out with the film in vertical or horizontal orientations relative the beam. Calibration with a plastic phantom will led to 4.7 % higher absorbed dose than the use of a solid water phantom material, and this is likely to be explained by the fact that solid water has almost the same density and the same x-ray absorption and scattering properties as water (Seuntjens et al., 2005). The plastic, on the other hand, differed from water by showing almost 5.0 % higher absorbed dose, this is likely to depend on the fact that scattered photons from the collimator head were stopped by the solid water slab, before the film surface, while the exposure without solid water led to a higher dose due to contributions from these photons. Note that the inherent variations in response between film sheets, between film pieces and between pixels in a ROI, as shown in Table 2, will be a source of additional variation in all measurements.

The graphs in Figure 13 show depth dose measurements performed with two film pieces at the same time, one shifted 2 cm downwards. The two graphs do not show any systematic differences, implying that there was no observable edge effect in this study.

2. Statistical analysis of EBT3 film

Another important preparation for accurate measurements of the absorbed dose was to determine the variations in irradiation response between different EBT3 film sheets, between film pieces within one sheet and between pixels in a ROI. These variations were quantified using the OmniPro software, the FILMGUI program and the FilmQA Pro software. Generally, FILMGUI showed consistently reasonable standard deviations, while OmniPro showed somewhat higher standard deviation for different film pieces. One of the reasons is that the calibration curve calculating by the OmniPro shows more instability and uncertainties, bur the FILMGUI take account into the scanning correction and light correction by using a pre-scanning method, which decreases some of these uncertainties. FilmQA Pro exhibited markedly lower standard deviation than other programs with regard to different pixel values. FilmQA Pro software takes the three colour channels into account, which is likely to have improved the result, due to the red colour channel provides the most sensitive response to radiation, the green colour channel enables high dose measurements, and the blue colour channel enhance the uniformity due to a marker dye in the active layer.. The method used in FILMGUI corrects for scanning effects by scanning of the film before and after irradiation, and the calculating occurs without use of the blue channel correction for minimizing of heterogeneity effects of the film. Variations between pixel values showed generally the smallest standard deviations and the largest variation was seen among different film sheets. This result is expected because the coating of the film may differ slightly between film sheets.

b. Calibration curves

The relationship between optical density and delivered dose is given by the calibration curve, and the three investigated analysis tools differed a bit in the employed methodology. When comparing the calibration curves in Figure 14 and Figure 15, they are quite similar in appearance and the fitting lines follow the data points very well for FILMGUI but with slight variations for OmniPro. The calibration curve for multichannel RGB data, calculated by the FilmQA Pro software, differs from the other methods and is not directly comparable due to the use of multichannel dosimetry.

c. Depth dose

The depth dose measurements were performed to evaluate whether the calibration data returned the correct absorbed dose results at different depths in water, for both120 kV and 200 kV at 1 Gy surface dose. Comparing the different energies, one can see that two depth dose evaluations at 120 kV returned about 5 % elevated dose at the surface (see Figure 18 and Figure 19), while one evaluation (Figure 17) showed somewhat underestimated dose at the surface. All three film calculation methods returned underestimated dose at larger depths (up to >100 mm). This implies that the 120 kV measurements seemed to have been quite unstable, due to increased absorbed dose at the surface and the film measurement did not follow the ion chamber measurement, which may due to badly conditions during calibration, such as calibration phantom materials. For the energy 200 kV, the depth dose curves obtained by film measurements were generally in better agreement with the ion chamber measurements. Note that the calibration was performed from 0.2 Gy and therefore the depth dose curves below this value are not reliable.

Comparing the three different software tools, one can observe that the OmniPro software showed the noisiest data. FILMGUI showed a relatively good and more reliable result with less noise compared with OmniPro. FilmQA Pro showed negligible noise, systematically better and the film measurements followed the ion chamber data very well.

d. Cell irradiation conditions

Based on the basic investigations of this study, the beam quality corresponding to 200 kV was selected for the irradiation of cells, as this energy spectrum seemed give the most reliable and accurate results under the current experimental conditions. Cell flasks were thus irradiated with 200 kV at 1 Gy with different contents and amounts of liquid. The first cell flask was fully filled with liquid (for full scatter conditions) and the second flask was filled with 5 ml liquid to simulate the actual cell culture irradiation. The cell irradiation conditions were reproduced, with film in the flask instead of cells, and the results in Table 3 show that the flask with less liquid received a lower absorbed dose compared to the fully filled flask. This is explained by the fact that 5 ml of liquid leads to less photon interaction due to the air compared with a fully filled flask, i.e., a lack of backscattered photons. Comparing film measurements with the Monte Carlo simulations one can see that the result for flask fully filled with liquid gives almost the same result.

V. Future work

This work constitutes an initial dosimetric foundation for planned cell irradiation measurements, with modulated fields, aimed at investigations of the bystander effect. Film irradiation with different shields of lead, with different thicknesses, needs to be performed for evaluation how much lead is required for sufficient attenuation of the incident photons. Other shielding materials with different densities, such as copper and brass, would be of interest to test in this context.

VI. Conclusion

Measurements of the absorbed dose using EBT3 film in this study ensured that film dosimetry is a reliable method with high spatial resolution that works well for measurement of absorbed dose under conditions relevant for studies of the bystander effect. The basic tests were satisfactory, and the inherent variations in radiation response were modest. The depth dose measurements compared to ion chamber measurements implied that film measurements returned the expected absorbed dose using at 200 kV, and this was validated by Monte Carlo simulations. The use of FilmQA Pro appeared to be optimal in this context, and this program is also recommended for analysis by the EBT3 manufacturer due to the use of accurate multi-channel dosimetry.

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