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

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Author Questionnaire

1. **Microscopy**: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or similar? **N**

2. **Software:** Does the part of your protocol being filmed demonstrate software usage? **Y**

   Videographer: Authors unable to provide screen capture files, please film

3. **Interview statements:** Considering the Covid-19-imposed mask-wearing and social distancing recommendations, which interview statement filming option is the most appropriate for your group? **Please select one**.

   ☒ Interviewees wear masks until the videographer steps away (≥6 ft/2 m) and begins filming. The interviewee then removes the mask for line delivery only. When the shot is acquired, the interviewee puts the mask back on. Statements can be filmed outside if weather permits.

4. **Filming location:** Will the filming need to take place in multiple locations (greater than walking distance)? **N**

Protocol Length
Number of Shots: **37**

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Morgane Dos Santos**: This protocol was developed to allow dosimetry measurements to be performed as close as possible to real cell irradiation conditions for radiobiological studies **[1]**.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera Vid NOTE: last one

REQUIRED:

1.2. **Morgane Dos Santos**: With this protocol, we can determine the exact dose received by cells, making it applicable to many X-rays facilities, and can take into account all of the parameters influencing the dosimetry **[1]**.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera Vid NOTE: last one

OPTIONAL:

1.3. **Vincent Paget**: All irradiation parameters must be set upstream to allow an optimal acquisition of the dosimetry measurements, requiring a close collaboration between physicists and radiobiologists **[1]**.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera Vid NOTE: last one

Protocol

2. Irradiation Field Evaluation (No Dose Estimation)

2.1. To perform an irradiation field evaluation, place a self-developing dosimetry film onto the support used for irradiation **[1-TXT]** and irradiate the film with at least 2 Grays to obtain a well-marked irradiation field **[2]**.

2.1.1. WIDE: Talent placing film onto support

2.1.2. Talent starting irradiation Vid NOTE: CU at the end

2.2. Scan the self-developing dosimetry film using a dedicated scanner **[1]** and use **Analyze** and **Plot Profile** to plot the dose profile in ImageJ **[2]**.

2.2.1. Talent scanning film
2.2.2. Talent at computer, plotting dose profile

2.3. Then mark the irradiation support surface to ensure that the cell container will be placed in the correct position **[1]**.

2.3.1. Talent marking surface Vid NOTE: CU + MED on ruler

3. Dose Rate Measurement

3.1. To perform a dose rate measurement, place a modified cell container into the irradiation support enclosure **[1-TXT]** and place the ionization chamber into the container in the correct position according to the marks made on the support surface **[2]**.

3.1.1. WIDE: Talent placing container into enclosure *Videographer: Important step* **TEXT: See text for container modification details**
3.1.2. Talent placing chamber into container *Videographer: Important step*

3.2. Confirm that all of the irradiation parameters have been appropriately set **[1-TXT]** and pre-irradiate the ionization chamber for 5 minutes **[2]**.

3.2.1. Talent checking parameters, with monitor visible in frame *Videographer: Important step* **TEXT: See text for irradiation parameter setup details** Vid NOTE: with 2.3.2 MED

3.2.2. Talent starting irradiation *Videographer: Important step* Vid NOTE: CU screen, with 2.3.1

3.3. Zero the electrometer **[1]** and obtain ten, 1-minute measurements **[2]**. Then use the formula to determine the average dose rate in air kerma **[3-TXT]**.

3.3.1. Talent zeroing electrometer *Videographer: Important step*
3.3.2. Talent using electrometer to measure dose rate *Videographer: Important step*
3.3.3. Talent at computer, determining dose rate *Videographer: Important step* **TEXT:** $K_{air} = M \times N_{Kair} \times K_Q$

4. Cell Culture Medium Attenuation and Scattering Measurement

4.1. At least 24 hours before the irradiation, cut pieces of self-developing dosimetry film according to the size of the cell container **[1-TXT]**. NOTE: This step goes after 4.2

4.1.1. WIDE: Talent cutting film **TEXT: 3 pieces/calibration dose or time point + 3 pieces/attenuation point** Vid NOTE: CU + MED

4.2. Number all the films for their downstream identification **[1]**.

4.2.1. Film being labeled Vid NOTE: MED + CU

4.3. To construct a calibration curve, set aside three pieces of film for the non-irradiated controls **[1]** and place the first film inside the cell container **[2]**.

4.3.1. Talent setting aside 0 Gy films
4.3.2. Talent placing film into container

4.4. Then irradiate the film to obtain the first dose points **[1-TXT]**.

4.4.1. Talent starting irradiation **TEXT: Irradiate 3 films/dose point** Vid NOTE: use 3.2.1 or 3.2.2

4.5. When all of the films have been irradiated in triplicate at the appropriate selected doses, a calibration curve can be generated **[1]**.

4.5.1. LAB MEDIA: Figure 6 graph

4.6. To measure the cell culture medium attenuation and scattering, select the same irradiation time for all of the irradiations **[1]** and irradiate three pieces of self-developing dosimetry films in the container without water **[2]**.

4.6.1. Talent setting irradiation time
4.6.2. Talent placing films into container

4.7. Next, place a single piece of film into the container **[1]** and fill the container with the same volume of water as the volume of medium that will be irradiated **[2]**. Using small pieces of tape as necessary, position the container within the enclosure **[3]**.

4.7.1. Talent placing film into container *Videographer: Important/difficult step*
4.7.2. Talent adding water to container *Videographer: Important/difficult step*
4.7.3. Talent placing container into enclosure *Videographer: Important/difficult step*

4.8. When the irradiation is complete, dry the films with absorbent paper **[1]** and store the films protected from light **[2]**.

4.8.1. Talent drying film(s) *Videographer: Important step*
4.8.2. Talent storing films protected from light *Videographer: Important step*

5. Self-Developing Dosimetry Radiochromic Film Analysis

5.1. Twenty-four hours after their irradiation, on the scanner **[1]**, set the **tiff format** to 48 bit red-green-blue and the **transmission mode** to 150 dots per inch and select no image correction **[2]**.

5.1.1. WIDE: Talent opening scanning parameters, with monitor visible in frame
5.1.2. SCREEN: Tiff format being set, Transmission mode being set, no image correction being selected

5.2. To warm up the scanner, place a non-irradiated film on the scanner **[1]** and launch a preview of the scan **[2]**.

5.2.1. Talent placing film onto scanner
5.2.2. SCREEN: Preview being launched Vid NOTE: use take 2

5.3. Start a timer and wait 30 seconds before starting the scan **[1]**.

5.3.1. SCREEN: Timer being started, then scan being started

5.4. At the end of the scan, start a 90-second timer. At the same time, register the scan **[1]** and open the image in ImageJ **[2]**.

5.4.1. SCREEN: Timer being started and scan being registered
5.4.2. SCREEN: Image being opened Vid NOTE: use take 2

5.5. Trace a square region of interest within the scan and measure the average red pixel level of the area **[1]**.

5.5.1. SCREEN: ROI being drawn, avg red pixel level being measured Vid NOTE: use take 2

5.6. At the end of the 90 seconds, repeat the scan preview at least 30 times to warm up and stabilize the scanner **[1]**.

5.6.1. Talent repeating scan preview, with monitor visible in frame

5.7. Once the scanner has been stabilized, place a film into the center of the scanner bed **[1-TXT]** and launch a preview of the scan **[2]**.

5.7.1. Talent placing film into center of scanner **TEXT: Place all films in same space and same orientation**

5.7.2. Talent starting scan preview, with monitor visible in frame Vid NOTE: slated 5.8.1

5.8. Wait 30 seconds before starting the scan **[1]**.

5.8.1. Talent checking timer then starting scan Vid NOTE: slated 5.9.1

5.9. At the end of the scan, wait 90 seconds before starting the next scan **[1]**.

5.9.1. Talent switching films on scanner bed Vid NOTE: slated 5.10.1, scan + CU on timer

Protocol Script Questions

A. Which steps from the protocol are the most important for viewers to see? Please list 4 to 6 individual steps.
3.1.-3.3., 4.7., 4.8.

B. What is the single most difficult aspect of this procedure and what do you do to ensure success? Please list 1 or 2 individual steps from the script above.
4.7. where we measure the influence of the cell culture medium (attenuation and scattering). We have to be sure that the film is correctly placed and immerged with the exact quantity of water. As his step has to be repeat three time, we have to be very precise.

Results

6. Results: Representative Irradiation Analyses

6.1. Measurements to estimate the attenuator thickness were then performed **[1]** and the different attenuator thicknesses were tested to find the thickness that decreased the beam intensity by a factor of two **[2]**.

6.1.1. LAB MEDIA: Table 2 *Video Editor: please emphasize reference measurements rows*

6.1.2. LAB MEDIA: Table 2 *Video Editor: please emphasize Finding of attenuatior thickness rows*

6.2. When this thickness was determined, five measurements were taken to evaluate the average M-raw value corrected by the temperature and pressure correction factor **[1]**.

6.2.1. LAB MEDIA: Table 2 *Video Editor: please emphasize Measurements with the right attenuator rows*

6.3. For this configuration, a half value layer of 0.667 millimeter of copper was found **[1]**.

6.3.1. LAB MEDIA: Table 2 *Video Editor: please emphasize 0.667 grey highlighted data row*

6.4. In this representative analysis, a self-developing dosimetry film was irradiated to determine the surface on which the irradiation field is homogeneous, allowing correct placement of the cell container **[1]**.

6.4.1. LAB MEDIA: Figure 2 *Video Editor: please add/emphasize dotted lines*

6.5. To determine the exact dose for the cells, the measured air kerma dose rate was converted to water kerma **[1]**, which for this analysis was determined to be to 0.659 Grays/minute **[1]**.

6.5.1. LAB MEDIA: Figure 5

6.5.2. LAB MEDIA: Figure 5 *Video Editor: please emphasize data line*

6.6. In this cell culture medium attenuation and scattering analysis, the self-developing dosimetry radiochromic films were first calibrated between 0 and 3 Grays **[1]** with 0.25-Gray steps between 0 and 1 Gray **[2]** and 0.5-Gray steps between 1 and 3 Grays **[3]**.

6.6.1. LAB MEDIA: Figure 6 images *Video Editor: please emphasize C0 and C8 images*
6.6.2. LAB MEDIA: Figure 6 images *Video Editor: please emphasize C0-C4 images*
6.6.3. LAB MEDIA: Figure 6 images *Video Editor: please emphasize C5-C8 images*

6.7. The dose points were then fitted with a fourth-degree polynomial curve **[1]**.

6.7.1. LAB MEDIA: Figure 6 *Video Editor: please emphasize data line*

6.8. Here a representative table used for the daily measurement can be observed **[1]**.

6.8.1. LAB MEDIA: Table 3

Conclusion

7. Conclusion Interview Statements

7.1. **Morgane Dos Santos**: Make sure that all of the configuration parameters are respected, that the dose rate is measured inside the right cell container, and that the influence of the cell culture medium is evaluated **[1]**.

7.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera (3.1., 4.6.) Vid NOTE: last one

7.2. **Vincent Paget**: Several protocols exist to establish a dose reference measurement. The key point is to select the appropriate protocol for a specific application, especially when using low X-rays facilities **[1]**.

7.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera Vid NOTE: the one before last