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SMALL ANIMAL IGRT SPECIAL FEATURE: FULL PAPER

Development of a dynamic phantom and investigation of mobile target imaging and irradiation in preclinical small animal research

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Objective: Progress made in preclinical radiotherapy makes respiratory gating reachable. Nevertheless, technical means are still needed, as well as accurate investigations of the effect of motion on small animal treatment plans.

Methods: An animal-scaled dynamic phantom (0.3–11.1-mm motion peak-to-peak amplitude, 30–120 cycles per minute) was developed and characterized. It was used to evaluate respiratory monitoring and high resolution imaging (μ PET/CT scans). The width and position variations of a fluorine-18 solution were measured for various motions and gating configurations. The phantom was finally used to measure the impact of motion on dose distribution for vertical irradiation using 2.5- and 5-mm collimations.

Results: Phantom motions accurately reproduced original waveforms with good rate and amplitude linearity ($R^2 = 1$ and $R^2 = 0.9995$, respectively). μ PET/CT acquisitions

showed an increase of 92% of the target size caused by a 4.9-mm sine motion and reduced to <12% by gating. Target motion measurements showed consistency better than 18% between modalities. Irradiations showed that motions >0.8 and 1.1 mm (for the 2.5- and 5-mm collimations, respectively) significantly impact dose homogeneity in the target.

Conclusion: The phantom allowed studying motion in small animal imaging and irradiation. It showed the important impact of motions >2 mm and provided accurate data to improve the management of mobile tumour irradiation. The implementation of gated irradiation, associated with motion-compensated imaging, is currently under progress.

Advances in knowledge: Small animal irradiation gating is not yet used in preclinical studies. As few solutions are under development, tools and accurate studies are highly needed.

INTRODUCTION

Cancer concerns >3,400,000 patients each year in Europe.¹ Many clinical issues still require radiobiological studies to improve the treatment efficiency or decrease secondary effects. Preclinical animal radiobiology is of prime interest to investigate the effect of treatments on tumours as well as healthy tissues. Yet, the reliability of these studies strongly depends on their similarity to clinical practice.

Until a few years ago, animal irradiations were more simplistic than advanced radiotherapy treatments such as intensity-modulated radiation therapy or radiosurgery. They often consisted of a single irradiation delivered without collimation, thus preventing clear transfer of preclinical results obtained with animals to radiotherapy protocols. Recently, major progress has been made with the development of micro-irradiators such as the X-RAD 225Cx distributed by PXI

Company or the SARRP (Small Animal Radiation Research Platform) system commercialized by Xstrahl Ltd.² These systems are specially designed to decrease the irradiation scale to the size of the animal, thanks to orthovoltage energies (X-rays generated at tube potentials between 100 and 300 kV) and 0.5-mm diameter fields. In addition, a stage and a dedicated cone-beam CT imager allow an accurate positioning of the animal with a 200- μ m targeting accuracy.^{3–5} Consequently, dose distributions as complex as clinical ones can now be achieved in preclinical studies. Nevertheless, these advances are not sufficient in some cases and particularly in the case of mobile tumours situated in the thorax or the abdomen.

Respiration gating on freely breathing animals is widely used in motion-compensated imaging. Prospective^{6,7} and retrospective techniques, with intrinsic or extrinsic gating,⁸ were implemented and investigated to improve spatial

resolution. In prospective gating, data acquisition is restricted to a specific cardiac or respiratory phase given by a transitor–transitor logic (TTL) signal produced by an external system (a pneumatic cushion, for example).^{6,7} In retrospective gating, acquisition is performed continuously without considering the respiratory or cardiac motions. Acquisitions are sorted afterwards with regard to a synchronized extrinsic or intrinsic gating signal. Contrary to extrinsic triggers, intrinsic gating directly uses spatial variations of the different acquisitions (diaphragm position, for example) to sort them and therefore, no external monitoring is needed.^{9,10}

Nevertheless, irradiation gating does not address the same issues as motion-compensated imaging. It implies to deliver the right dose at the right place and at the right moment.¹¹ That is why only prospective gating, delivering irradiation during a specified respiratory phase, can be applied to irradiation gating. This requires an important evaluation of the spatial and dosimetric

accuracy, especially at small animal scale, which necessitates dedicated tools. To answer this need, we developed a small animal dynamic phantom compatible with cone-beam CT, μ PET cameras and small animal irradiators. Its performance was evaluated; it was then used to investigate the effect of motion during small animal imaging and irradiation.

METHODS AND MATERIALS

We developed a dynamic phantom producing longitudinal movement of variable amplitude range (between 0.3 and 11.1 mm) and motion frequency from <30 to >120 cycles per minute (cpm). It is composed of three main parts represented in Figure 1: a motor section (a), controlled by a function generator and driving a polymethyl-methacrylate (PMMA) probe, which is imaged and irradiated (b). The system was adapted to a pneumatic respiratory monitoring system (c) and was tested with the X-RAD 225Cx irradiator (PXi) as well as the INVEON μ PET/CT camera (Siemens).

Figure 1. Parts constituting the phantom: the motor section (a), a polymethyl-methacrylate (PMMA) probe (b) and the positioning of a respiratory monitoring system (c)—the mechanical part is composed of a servo motor commanded by a numerical function generator and a gear unit generating the linear motion. The system produces two different outputs: a customizable motion of the probe and a respiratory/motion monitoring output provided by the monitoring system. The PMMA probe includes four holes drilled through the entire width and containing various contrast or active agents (shown as 1–4) and one hole drilled to half of the width to contain a point dosemeter (shown as 5). TTL, transitor–transitor logic.

Phantom description

Motor section

The motion of the phantom is generated by a servomotor composed of a DC rotary motor, a potentiometer providing an angular position feedback and a controller comparing the measured position of the system with the position commanded by a numerical function generator (Pc-Lab 2000 LTTM; Velleman Instruments) delivering various waveforms such as sine, triangle or custom-made functions. The motion is then converted into linear motion by a gear unit thus producing periodical and millimetre movements comparable with mice and rats respiratory motions.

Polymethyl-methacrylate probe

A PMMA probe of $30 \times 30 \times 90$ mm was realized to evaluate imaging as well as irradiation accuracy and is represented in Figure 1. Five holes of about 1.5-mm diameter (shown as 1–5 on Figure 1) were drilled perpendicularly to the motion direction. Four of these holes were drilled over the entire width of the phantom and can receive water, contrast agent or fluorine-18 (¹⁸F) solution. The fifth one (shown as 5 on Figure 1) was drilled to the half of the width and can receive a point dosimeter such as a scintillating fibre dosimeter, its sensitive volume being at the centre of the phantom. In addition, the square section of the phantom is perfectly suitable to the use of a dosimetric film placed on its upper surface. Finally, a piece of higher density polymer was placed on top of the phantom during the preliminary phantom characterization to perform semi-automatic analysis of the phantom motion.

Respiratory monitoring

To mimic small animal gating conditions, our phantom was adapted to the 1028 Monitoring and gating system (SA Instruments Inc.), already used in the laboratory. This monitoring system relies on the measurement of the pressure exerted by the animal on a pneumatic pillow sensor during the respiratory

cycle. A control/gating module delivers TTL gating signals with adjustable delay and width. The pneumatic sensor of the monitoring system was positioned between the extremity of the probe rod and a wedge. In this configuration, the motion of the probe exerted a pressure on the sensor similar to an animal inhalation. It can be noticed that the use of another kind of monitoring system (optical, for example) could be easily adapted.

Phantom evaluation

The first step of this study was the evaluation of the phantom performance to check its ability to correctly reproduce commanded phantom motion over a range of amplitudes and frequencies.

The reference motions were acquired, thanks to the method represented in Figure 2: fluoroscopic acquisition of the phantom every 66.6 ms and calculation of the centre of mass, providing the relative position of the phantom as a function of time.

A higher density polymer was placed on the upper surface of the phantom in order to provide a defined area with a better contrast. The phantom motion being longitudinal, it was analyzed thanks to two-dimensional fluoroscopic projections performed in free running mode (*i.e.* without respiratory monitoring) at 40 kV, 7 mA and 15 frames per second, providing 100 projections. The frame numbers combined with the image integration time of 66.6 ms provided the time scale of motion measurements.

The 100 acquisitions were then semi-automatically analyzed with the ImageJ (National Institutes of Health, Bethesda, MD) analysis software:¹²

- A spatial calibration was performed between the phantom dimensions (in pixels) measured in the acquisition and the physical dimensions (in millimetre) measured on the probe.

Figure 2. Fluoroscopic acquisition of the phantom every 66.6 ms and calculation of the centre of mass, providing the relative position of the phantom as a function of time. 2D, two-dimensional.

- The polymer paste area was automatically selected in each acquisition by pixel value threshold, leading to pixel values of 1 for the polymer paste and 0 elsewhere.
- The centre of mass coordinates (X_M and Y_M) of the polymer paste were then automatically calculated for each acquisition, thus providing the phantom relative position as a function of time.
- This relative position was finally fitted with a sine function to determine the frequency and amplitude of the phantom motion.

Several series of sine waveforms were tested to evaluate any errors between actual motion and command motion waveforms:

- In the first series, the amplitude was kept constant at 10 V and the frequency varied from 0.5 to 2 Hz (corresponding to the range 30–120 cpm) by 0.25-Hz steps.
- In the three following series, the waveform frequency was kept constant at 0.5, 1 and 2 Hz, and the amplitude varied from 0.5 to 5 V by 0.5-V steps, then from 5 to 10 V by 1-V steps.

The first series allowed evaluating frequency linearity, while the three other ones allowed evaluating amplitude linearity.

To test the phantom in more realistic conditions, respiration-like waveforms were also applied at repetition rates of 60 and 120 cpm. They were obtained from the diaphragm motion of a free-breathing mouse acquired in fluoroscopic mode with the same acquisition parameters as before.

The animal was anaesthetized using isoflurane 2.5% in a gaz mixture composed of N_2O/O_2 70%/30%. Animal investigations were performed by competent personnel under the current European directive (2010/63/EU) in authorized laboratories (E14118001) with the permission of the regional and national committees on animal ethics #3995-2016020816219034.

Monitoring evaluation

Pneumatic monitoring systems are commonly used to increase spatial accuracy in cardiac or lung imaging. The pneumatic sensor is placed against the animal so that the pressure in the sensor increases with the animal inspiration. The maximal inhalation is then used to trigger a gate whose delay time and width are adjustable to coincide with the animal resting phase (plateau of minimal motion between breaths). Nevertheless, the delay time between the maximal inhalation and the gate are known to show variations with breathing rate⁷ and care must be taken to ensure reliable gating in the absence of motion data (which is the case in most animal experiments). The phantom was thus used to characterize the monitoring system over a range of frequencies. Sine and respiratory motions were performed at rates ranging from 30 to 120 cpm. The maximal inhalation phase (corresponding to the minimal relative position of the phantom in this study) was detected and triggered a 300-ms-width gate. The function generator waveform, the pressure signal and the gate output were recorded with an oscilloscope, and the delay time between the maximal inhalation peak and the gate over a range of simulated breathing rates was determined.

Imaging evaluation

Once the phantom and the pneumatic monitoring system were characterized, the impact of motion on positron emission tomography (PET)/CT scans and the consequences on a radiotherapy treatment plan were studied with our system. For this purpose, two holes of the PMMA probe were filled with ^{18}F solutions of respective concentrations of 10,000 and 100,000 nCi/cc [low activity (LA) and high activity (HA)]. Sine waveforms of amplitudes ranging from 1.1 to 4.9 mm and frequencies of 60 and 120 cpm were applied to the phantom. PET/CT scans were performed with and without gating and processed, as shown in Figure 3. The ^{18}F solutions were not visible in CT scans because they generate Hounsfield units too close to

Figure 3. μ PET/CT acquisition of the dynamic phantom: the low activity (LA) and high activity (HA) distributions are fitted by a two-dimensional (2D) Gaussian function, providing the dimensions and the relative position of each distribution. Similarly, the air-filled cavity is fitted in the motion direction, providing its width. FWHM, full width at half maximum.

Figure 4. Relative position as a function of time in X and Y directions at 1 Hz (a) and 2 Hz (b).

the PMMA ones. Consequently, gated acquisitions were studied by measuring the air-filled cavity width in the motion direction.

The acquisitions were performed with the INVEON μ PET/CT camera and synchronized to the TTL signal provided by the pneumatic monitoring system coupled to the phantom. PET scans were acquired for 1800 s with a voxel size of $776 \times 776 \times 796 \mu\text{m}$, and motion compensation was performed thanks to four or eight time windows (TWs) of equal width dividing the whole motion cycle. For four TWs, it corresponds to 250- and 125-ms widths at 60 and 120 cpm, respectively, whereas for eight TWs, it corresponds to 125 and 62.5 ms widths at 60 and 120 cpm, respectively. For this study, an arbitrary additional gate delay time was set. CT scans were performed at 80 kV and 0.5 mA, with a voxel size of $220 \times 220 \times 220 \mu\text{m}$, and motion compensation was performed by limiting the acquisition time to 100 or 250 ms after the gating TTL signal.

The LA and HA distributions measured by the PET camera were fitted with ROOT analysis software¹³ by two-dimensional

Gaussian functions, thus providing the widths in X and Y directions, as well as their relative positions. The air-filled cavity profile (in the motion direction), acquired with the CT camera, was also fitted by a Gaussian function to determine its apparent width.

Irradiation evaluation

The phantom was finally used to measure the effect of motion on dose deposition during irradiation delivered by the X-RAD 225Cx, without motion compensation. An EBT3 gafchromic film was placed on the upper surface of the phantom, at a source-to-axis distance of 30.7 cm. Vertical irradiations (perpendicular to the phantom motion) of 90 s were performed at 225 kV and 13 mA, with 2.5- and 5-mm circular fields. The dose distributions were compared without motion and with respiratory motions of amplitudes ranging from 0.6 to 4.2 mm generated at 60 cpm. A quantitative analysis of the dose distributions was performed thanks to dose-volume histograms (DVHs) computed inside the irradiation field, illustrating the target volume, and outside the irradiation field, to illustrate the surrounding tissue irradiation.

Figure 5. (a) Motion amplitude as a function of signal frequency for a signal amplitude of 10 V and (b) motion amplitude as a function of signal amplitude for waveform frequencies of 0.5, 1 and 2 Hz.

RESULTS AND DISCUSSION

Phantom evaluation

The first step of this study was to provide reference measurements of the phantom motions, to evaluate the phantom performance and suitability for imaging and irradiation applications.

Spatial calibration

Preliminary to the actual performance evaluation, the spatial calibration factor of the fluoroscopic acquisitions was determined. It is the ratio between the real dimensions of the probe (in millimetre) and the dimensions measured on the acquisition (in pixels). It depends on the source-phantom distance, and in our configuration (phantom at the isocentre), this coefficient was found to be $9.8 \times 10^{-2} \pm 0.3 \times 10^{-2} \text{ mm/px}$.

Experimental motion consistency with numerical waveform

As shown in Figure 4, which represents two acquisitions of the phantom motion at 1 and 2 Hz, the consistency between waveforms delivered by the function generator and the

phantom experimental motion is excellent. The figure also displays small displacements in the Y direction (perpendicular to the motion), originating from a resonance-driven parasitic motion and a slight misalignment of the phantom with the imager referential. This amplitude does not exceed $200 \mu\text{m}$ for the largest motion amplitudes. It will generate a slight increase of the apparent size of the target volume in the Y direction in imaging and a slight dose deposition outside the target volume in irradiation.

Motion linearity

In the first measurement series, the signal voltage was kept constant at 10 V (corresponding to about 11 mm), while the frequency was varied from 0.5 to 2 Hz (corresponding to frequencies ranging from 30 to 120 cpm). The frequency of the measured motion was found to be within 0.33% of the command frequency. On the other hand, the experimental motion amplitude was found to depend on the frequency with a decrease of 11% between 30 and 120 cpm, as shown in Figure 5a. Thus, the extent of phantom travel should be calibrated at each command frequency.

Figure 6. Relative position of the phantom measured at a frequency of 30 cpm for different signal amplitudes (10, 5, 2.5 and 0.5 V) and the corresponding sine fits.

Motion amplitude linearity

The motion amplitude linearity was then studied at three constant frequencies of 30, 60 and 120 cpm (Figure 5b). Linear fits of these data showed R^2 correlation coefficients higher than 0.9995. Nevertheless, the sine fit amplitudes showed a better linearity ($R^2 = 1.0000$) and systematic discrepancies with experimental amplitudes of 344, 272 and 390 μm at 30, 60 and 120 cpm, respectively.

This bias was investigated by decreasing the signal amplitude. The phantom response is illustrated in Figure 6 at a frequency of 30 cpm. It shows a mechanical damping at the beginning and at the end of the motion, which becomes significant at low signal voltage. This phenomenon can be attributed to the rotary-to-linear conversion by the gear unit or a possible inadequate motor speed and could be improved by other technical choices (use of a linear motor, for example). However, this damping

Figure 7. Evaluation of the phantom submitted to respiratory waveforms: the diaphragm motion of a free-breathing anaesthetized mouse is measured on a line from coronal acquisitions performed in fluoroscopic mode (a). The diaphragm movements are then fitted by Gaussian functions (b). This respiratory waveform has been finally applied to the phantom at 60 and 120 cpm rates (c and d, respectively).

a) Diaphragm relative position at a given time

b) Diaphragm position as a function of time

c) Respiratory , 2.5 mm, 60 cpm

d) Respiratory, 2.5 mm, 120 cpm

remains below 450 μm and does not constitute a limitation, even for small amplitudes, as long as the calibration between signal voltage and motion amplitude is accurately determined for each motion frequency.

However, the motion frequency remained very stable and did not vary $>0.05\%$, whatever the applied voltage.

Respiratory waveforms

To complete the evaluation with sine waveforms, the phantom was tested with respiratory waveforms obtained from the diaphragm motion of an anaesthetized mouse acquired in fluoroscopic mode, as shown in Figure 7a. The mouse was freely breathing at a mean rate of 62 bpm and the diaphragm movement was fitted by a Gaussian of 2.62-mm amplitude and 223-ms full width at half maximum (FWHM), as represented Figure 7b. Figure 7c,d show that the phantom presents no difficulty in reproducing such waveforms, even if in this case the fits were more limited by the fluoroscopy time resolution (15 fps).

The evaluation of the phantom proved its ability to produce motions scaled to small animal size (0.3–11 mm) with a very good linearity. The phantom motion was accurately characterized at relevant frequencies (between 30 and 120 cpm) and was then perfectly suitable to study gated imaging and irradiation in controlled conditions.

Monitoring evaluation

In order to determine the reliability of the pneumatic monitoring system for imaging and irradiation applications, its response was evaluated as a function of the frequency. The variation of the inherent delay time between the minimal relative position of the phantom and the gate was evaluated for sine and respiratory motions at different rates. The measurement of the phantom motion, the pressure variation in the sensor and the gate output performed at 60 cpm are represented in Figure 8a,b. They show similar monitoring signals despite different original motions and significantly different

delay times (Figure 8c). In this study an 180-ms delay time was added to the 44-ms inherent delay time measured at 60 cpm, so that the gate matches the resting phase (Figure 8a). As shown Figure 8c, the inherent delay times significantly decrease between 30 and 120 cpm: 107 ms with the respiratory motion and 165 ms with the sine motion. Nevertheless, these variations have a limited impact on the gate adjustment made at 60 cpm (180-ms additional delay time and 300-ms width). Indeed, for lower frequencies, the increase of the delay time is compensated by the longer resting phase. On the other hand, at higher frequencies, the gate width should be shortened to match the resting phase duration (of the order of 200 ms at 120 cpm in our study).

So, pneumatic respiratory systems, carefully positioned to avoid compression of the lungs or pressure signal saturation, are suited to perform reliable gating, especially in the case of deeply anaesthetized animals whose breathing rate varies slowly (70–37 bpm/45 min in our study). Even if irregular breaths can occur, thus shifting the gate location with regard to the resting phase, they represent a small proportion of the acquisition or irradiation time.⁷

Nevertheless, the breathing rate of an animal fully anaesthetized varies slowly with variations of about 20 bpm (70–37 bpm/45 min) in our study (if one excludes the few fast breathing cycles which can occur but represent a small fraction of time).⁷ Consequently, a careful adjustment of the gate parameters can ensure that the resting phase is correctly targeted.

Imaging evaluation

Once the phantom and the monitoring system were characterized, the phantom was used to perform quantitative evaluation of PET scans and CT scans. Sine motions of 1.1, 3.2 and 4.9 mm were performed at a rate of 60 cpm and sine motions of 1.2 and 3 mm were applied at 120 cpm. The LA and HA distributions were analyzed with and without gating to determine the consequences of motion on the position and the size of the target, which will be considered to compute the irradiation plan.

Figure 8. Comparison of motion waveforms (respiratory in a and sine in b), pressure measurements and gate outputs performed at a frequency of 60 cpm and the delay between the minimum position of the phantom (corresponding to the maximal inspiration) and the gate beginning is represented in c as a function of frequency.

Figure 9. Low activity (LA) and high activity (HA) distributions, with and without motion, and the corresponding two-dimensional Gaussian fits. FWHM, full width at half maximum.

PET scan

Without motion compensation The upper part of Figure 9 shows the LA and HA distributions in a given slice, when the phantom is immobile, as well as their widths in the X and Y directions. These dimensions are directly related to the physical

size of the holes. As expected, the LA distribution is noisier, which results in larger uncertainties on the LA distribution width measurements. The fits also showed that the HA distribution amplitude was about 10.6 times higher than that of the LA distribution, which is consistent with the solution concentrations.

Figure 10. High activity distributions acquired in each time window (TW) (four) and the phantom being submitted to a 4.9-mm sine motion at a rate of 60 cpm. FWHM, full width at half maximum.

Figure 11. Low activity (LA) and high activity (HA) distribution widths averaged on the number of time windows (TWs) vs motion amplitude, compared with the widths without gating. FWHM, full width at half maximum.

The lower part of Figure 9 shows the distributions acquired in the same conditions (without motion compensation) but with a 4.9-mm sine waveform performed at a rate of 60 cpm. The figure clearly shows the widening of the distributions in the motion direction; FWHMs were increased by 59% for the LA distribution and by 92% for the HA distribution in the motion direction. The FWHMs in the Y direction remained stable within 6% compared with measurements performed with the phantom immobile.

Distribution widths measured with all the waveforms showed an overestimation of the target size >20% for motion amplitudes >2 mm, whatever the frequency applied. This clearly confirms the need to study motion-compensated imaging in the context of preclinical mobile tumour irradiation.

Motion compensation with four time windows The PET acquisitions of the mobile phantom were then synchronized

with the pneumatic respiratory monitoring, and the motion cycle was divided into four TWs, corresponding to a 250-ms acquisition time for 60-cpm frequency.

Figure 10 shows the HA distribution corresponding to each TW, the phantom being submitted to the 4.9-mm sine motion performed at 60 cpm. The reduction of the distribution width in the motion direction, compared with Figure 9, is clearly visible, whatever the TW. Nevertheless, widths measured in some TWs remain larger than widths obtained when the phantom is immobile. It is due to large motion gradients of the sine waveforms compared with the acquisition time.

The mean LA and HA distribution widths (averaged on the four TWs) measured for the other waveforms are compared with the non-compensated measurements in Figure 11. The dispersion between the different TWs is given by the error bars. They show

Figure 12. High activity distributions acquired in each time window (TW) (eight) and the phantom being submitted to a 4.9-mm sine motion at a rate of 60 cpm. FWHM, full width at half maximum.

Figure 13. High activity distribution relative position vs time (represented by t for 1-Hz motions and t' for 2-Hz motion) with eight time windows during one motion cycle.

that gating allows reducing the maximum overestimation (reached with the largest motion amplitude) from 59 to 18% for the LA distribution and from 92 to 33% for the HA distribution. However, large discrepancies exist between the different TWs.

Motion compensation with eight time windows The same analysis was performed with eight TWs. The HA distribution, corresponding to the 4.9-mm sine motion performed at 60 cpm, is represented in Figure 12 for each TW. Similar to the reconstruction using four TWs, the figure shows the reduction of the distribution width, whatever the TW.

The mean width in the motion direction (averaged on the eight TWs) measured for all the waveforms are displayed in Figure 11 with the ones obtained with four TWs and without gating. As previously, the dispersion between the TWs is given by the error bars. With this gating condition, the maximum overestimation is further reduced to 7% for the LA distribution and to 11% for the HA distribution. Besides, the dispersion between the TWs can be reduced by a factor >2 for the larger motions.

As with four TWs, the relative position of the phantom could be followed in each TW. As shown in Figure 13, the consistency with sine waveforms could be verified for the larger motions and

the measured amplitudes were in good agreement with the reference motions measured in fluoroscopic mode, as summarized in Table 1 (discrepancies between 3 and 12% for the HA distribution and between 10 and 18% for the LA acquisition). As expected, the lower the considered activity (and consequently the measured signal), the larger the uncertainty.

CT scan

The air-filled cavity profiles in the motion direction, acquired with the different gating conditions, are displayed in Figure 14 for the 4.9-mm sine motion. The respective widths without motion, without gating, with a 250-ms acquisition time and with a 100-ms acquisition time are 1.52, 3.23, 2.15 and 1.63 mm, respectively. They confirm the results of the PET scans.

The evaluation of gated μ PET/CT scans showed that motions >2 mm lead to an important overestimation of the target volume, which necessitates the use of larger beams. Nevertheless, motion compensation allowed measuring the right size of the target with reasonable precision, as well as its position with TWs adapted to the motion. These data would allow improvement in the treatment with corresponding gating tools for irradiation.

Irradiation evaluation

Finally, the consequence of motion on irradiation without compensation was studied with respiratory motions ranging from 0.6 to 4.2 mm performed at 60 cpm. Figure 15a shows the dose distributions delivered to the film by 2.5- and 5-mm-diameter irradiation fields, when the phantom was immobile and for the different motions. A horizontal profile of the different distributions is represented in Figure 15b and shows the increasing dose heterogeneity inside the target volume (corresponding to a 1.8-mm-diameter area for the smallest beam and a 4.2-mm-diameter area for the largest one), as well as dose deposition outside of the initial target volume with motion amplitude.

The DVHs normalized to the prescribed dose, computed inside the target volume and in the surrounding volume are represented in Figure 15c. They show the important reduction of target volume receiving the prescribed dose, especially with the 2.5-mm-diameter irradiation field. Indeed, a quality criterion of 90% of the volume receiving $>90\%$ of the prescribed dose ($0.90V > 0.90 D_{\text{presc.}}$) would only be fulfilled for motions

Table 1. Motion amplitude measured with the different acquisition modes

Waveform	Amplitude measured by fluoroscopy (mm)	Amplitude measured on the HA distribution motion (mm)	Amplitude measured on the LA distribution motion (mm)
(1 Hz, 4.5 V)	4.9	4.4	4.5
(1 Hz, 3 V)	3.2	3.0	2.8
(1 Hz, 1.25 V)	1.1	1.1	0.9
(2 Hz, 3.25 V)	3.0	2.7	2.6
(2 Hz, 1.5 V)	1.2	1.2	1.1

HA, high activity; LA, low activity.

Figure 14. Profiles of the air-filled cavity in the motion direction acquired with the different gating conditions: the different profiles have been shifted relative to each other for clarity. a. u., arbitrary unit.

smaller than 0.45 mm for the 5-mm irradiation field. This criterion was too drastic to be evaluated for the 2.5-mm-diameter irradiation field. Even a less selective criterion of 90% of the

volume receiving $>80\%$ of the prescribed dose ($0.90V > 0.80 D_{\text{presc.}}$) is only fulfilled for motions smaller than 1.1 mm and 0.8 mm, respectively, for the 5-mm and 2.5-mm irradiation fields. On the other hand, the largest motions can lead to an average dose increase of 54% (5-mm irradiation field) and 38% (2.5-mm irradiation field) in the surrounding volume.

In this illustrating case, the irradiation field should be changed for larger rectangular fields (or of more elaborate shapes) to compensate the motion and get back a satisfying target DVH, the surrounding tissues inevitably suffering a higher dose.

This investigation demonstrated the ability of the phantom to determine the consequences of motion on a treatment plan, to take this motion into account, and to adapt the irradiation plan, by enlarging the irradiation fields.

CONCLUSION

We developed and characterized a dynamic phantom mimicking small animal respiratory motion, thanks to a programmable motion waveform, amplitude and frequency. A preliminary

Figure 15. Dose–volume histograms computed inside the target volume (1.8- and 4.2-mm-diameter areas for the 2.5- and 5-mm diameter irradiation fields) and in the surrounding volume (outside respective areas of 2.9- and 5.7-mm diameter for the 2.5- and 5-mm irradiation fields) (c).

characterization of the phantom showed a very good frequency response and good linearity between actual and commanded motion.

The phantom was used to study the consequences of motion on a treatment. Imaging and irradiations of a mobile target were performed with different motion waveforms and amplitudes. The results showed that motions >2 mm generate large overestimations of the target volume and lead to an alteration of the dose distribution, with a lower dose in the target volume and higher dose in the surrounding volume. For smaller amplitudes, motion compensation is limited by the spatial resolution of the PET camera. This study provided accurate measurements to improve the management of mobile tumour irradiation, with an adapted beam widening, for example. It also highlighted the improvement which could be achieved with gated irradiation associated with motion-compensated μ PET/CT acquisitions.

Nevertheless, motion management in preclinical radiotherapy requires many investigations either preclinical or technical.

Indeed, even if motion compensation was extensively studied in preclinical imaging, it was mostly in terms of image contrast and spatial resolution. Consequently, rather limited information is available about animal organ motion amplitude to determine the locations which could benefit from motion compensation. Similarly, investigations on organ position reproducibility would be highly desirable to evaluate dose delivery uncertainty, especially when relying on respiratory monitoring. Finally, new irradiation protocols and techniques (such as motion encompassing, breath-hold technique, respiratory gating) must be developed and compared to optimize irradiation of mobile tumours at the animal scale.

In this context, our dynamic phantom represents a very useful tool to design and optimize protocols in controlled conditions, preliminary to effective small animal study. To complete this investigation, gated irradiation implementation is currently under progress, thanks to the development and characterization of a fast shutter adapted to the X-RAD 225Cx, as well as *in vivo* experiments to complete phantom measurements.

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