

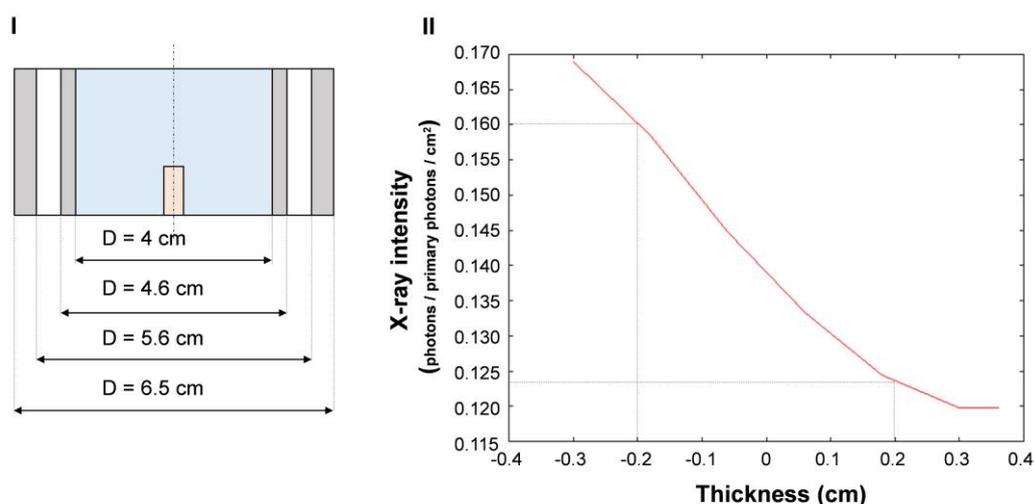
Supplementary Material S2: Estimation of radiation exposures

1. Introduction

Trabecular bone specimens in this study underwent several consecutive tomograms using high-energy synchrotron radiation, which resulted in a progressive radiation dose accumulation within the bone tissue. To the author's knowledge only two studies [1,2] addressed a similar procedure for estimating the radiation dose absorbed by bone samples subjected to SR-microCT. However, the proposed formulation considered a uniform distribution for the absorption of X-rays within the samples. Therefore, the manuscript used a simulation using FLUKA Monte Carlo code [3] of the delivered dose during the acquisition of one tomography scan, providing not only the average dose absorbed by the specimen, but also the local distribution. Nevertheless, an estimation of the delivered dose was also carried out and presented in this text following the proposed formulation in previous studies and compared to average simulated dose.

2. Simulated geometry (FLUKA Monte Carlo)

The geometry simulated in FLUKA consisted on a trabecular bone sample within the loading device as described in the manuscript. A scheme of the simulated geometry is shown in Fig. S1-I, in which a different colour is assigned to each material. The bone specimen was assumed as a cylinder (orange, 4 mm diameter, 10 mm length, density of 0.5 g/cm^3 [4]) placed in the centre of the environmental chamber (40 mm inner diameter, 3 mm thickness) made of glassy-carbon (grey, density of 1.5 g/cm^3) and filled with saline solution (blue, density of 1 g/cm^3). The absorption profile (Fig S2-II) was obtained from the simulation and the transmitted (I) and incident (I_0) X-ray intensities were calculated as the intensities after and before the bone, respectively, where the bone sample (4 mm in diameter, 10 mm in length) was positioned in the centre of the geometry.



3. Beam parameters

The average photon energy and photon flux during the experiment was estimated using SPECTRA code [5] as described in the manuscript, using a $2 \times 2 \text{ mm}$ aperture 220 m after the X-ray source. Filters (1.3 mm pyrolytic graphite, 3.2 mm aluminium and $60 \mu\text{m}$ steel) and reflectivity of the platinum mirror used during the experiment were considered. An average beam energy, $E = 28.93 \text{ keV}$ and photon flux, $\Phi = 4.9 \times 10^{13} \text{ photons/s}$ was calculated.

4. Average simulated dose (FLUKA Monte Carlo)

The delivered dose was simulated for the previously described geometry. The simulation results have an error below 15%, such an error is associated to the standard deviation of the energy deposition of the simulated X-ray when interacts with an object. In fact, it is the statistical error associated to the probability of absorption in matter. From the simulation, the absorption profile (Fig. S2-II) and the energy deposition (Fig. 5) is calculated. The average dose rate in the target volume was obtained as an output of the simulation $\dot{d}_{average,simulated} = 35 \text{ Gy s}^{-1}$ (6.4 Gy/s standard deviation).

5. Estimated dose (previous formulation)

5.1. Estimation of mass attenuation coefficient, α

The mass attenuation coefficient was calculated using the Beer-Lambert Law [6]:

$$T = \frac{I}{I_0} = e^{-\alpha \rho l}$$

Where T is the transmission of X-rays through a material, of thickness l , α is the mass attenuation coefficient ($\text{cm}^2 \text{g}^{-1}$) and ρ is the density (g cm^{-3}).

5.2. Estimation of the flux density, ψ

Due to the cylindrical geometry of the bone specimen, the X-ray path through it is not constant. Therefore, a numerical integration is performed splitting half of the cylinder (considering symmetry) in 90 steps of 1 degree. For each angle, the surface seen by the incident X-rays can be calculated as:

$$S_i = \sin \Delta\theta_i \times r \times h$$

Where S_i is the surface seen by the X-rays at each angle θ_i , r is the radius of the specimen and h is its height.

The flux at the surface, ψ_{surf} is then calculated from the value of the photon flux, Φ (photons s^{-1}), simulated using SPECTRA code [5]:

$$\psi_{surf,i} = S_i \times I_0 \times \Phi$$

The thickness of the sample, l , varies as a function of the angle θ .

$$l_i = \cos \vartheta_i \times r \times 2$$

The transmission, T , of X-rays through the cylinder is expressed using the Beer-Lambert Law:

$$T_i = e^{-\alpha \rho l_i}$$

The fraction of X-rays absorbed, A , by the samples is given as $(1-T)$. The flux absorbed $\psi_{a,i}$ (photons s^{-1}) is then calculated as:

$$\psi_{a,i} = A \times \psi_{surf,i}$$

Doing a numerical integration of the flux absorbed at each angular step, the total absorbed flux, ψ (photons s^{-1}) is obtained:

$$\psi_T = 2 \times \sum_{i=1}^{90} \psi_{a,i}$$

And the flux density, ψ (photons/s/cm³), is obtained dividing the total flux divided by the volume of the specimen, V :

$$\psi = \psi_T / V$$

5.3. Estimation of the radiation dose

To estimate the radiation dose absorbed by the sample, which is measured in grays (1 Gy \equiv 1 J kg⁻¹), the radiation flux density is converted into an energy density, E_p (J s⁻³ cm⁻³).

$$E_p = \psi \times 1.6 \times 10^{-19} J / eV \times E$$

Where E is the energy of the beam in eV and the dose rate, \dot{d} (Gy s⁻¹), can be obtained as:

$$\dot{d} = \frac{E_p}{\rho} = 43.7 \text{ Gy s}^{-1}$$

6. Comparison

The total irradiation dose, d , received during each exposure can be then found from the dose rate and the total exposure time during image acquisition.

$$d = \dot{d} \times t$$

A comparison between the accumulated dose for each specimen during the seven sequential tomographies is reported in Table S2, computed varying the exposure time.

Table S2. Nominal radiation absorbed by each specimen after each loading cycle, calculated by varying the exposure time. Values were truncated to one decimal place.

Exposure time	512 ms						
Load cycles	1	2	3	4	5	6	7
Average accumulated dose simulated (kGy)	32.9	65.9	98.8	131.8	164.7	197.7	230.6
Estimated accumulated dose (kGy)	41.1	82.3	123.4	164.6	205.7	246.8	288.0
Exposure time	256 ms						
Load cycles	1	2	3	4	5	6	7
Average accumulated dose simulated (kGy)	16.8	33.6	50.5	67.3	84.1	100.9	117.7
Estimated accumulated dose (kGy)	21.0	42.0	63.0	84.0	105.0	126.0	147.0
Exposure time	128 ms						
Load cycles	1	2	3	4	5	6	7
Average accumulated dose simulated (kGy)	8.8	17.5	26.3	35.0	43.8	52.5	61.3
Estimated accumulated dose (kGy)	10.9	21.9	32.8	43.7	54.7	65.6	76.5
Exposure time	64 ms						
Load cycles	1	2	3	4	5	6	7
Average accumulated dose simulated (kGy)	4.7	9.5	14.2	18.9	23.6	28.4	33.1
Estimated accumulated dose (kGy)	5.9	11.8	17.7	23.6	29.5	35.4	41.3

7. Discussion

The average dose simulated in this study is in well agreement to the estimated dose using mathematical formulation previously proposed [1,2]. The estimation presented herein assumed that the X-rays pierce a constant thickness of bone material (a constant value is used in the Beer-Lambert Law), corresponding to the sample diameter. However, due to the cylindrical geometry of the specimen, X-rays go through a different thickness at the centre of the specimen while off centre. Therefore, the extra

thickness assumed in this estimation overestimate the dose absorbed by the specimen compared to the simulation using FLUKA Monte Carlo.

References

- [1] H.D. Barth, M.E. Launey, A.A. MacDowell, J.W. Ager, R.O. Ritchie, On the effect of X-ray irradiation on the deformation and fracture behavior of human cortical bone, *Bone*. 46 (2010) 1475–1485. doi:10.1016/j.bone.2010.02.025.
- [2] A. Pacureanu, M. Langer, E. Boller, P. Tafforeau, F. Peyrin, Nanoscale imaging of the bone cell network with synchrotron X-ray tomography: optimization of acquisition setup., *Med. Phys.* 39 (2012) 2229–38. doi:10.1118/1.3697525.
- [3] G. Battistoni, F. Cerutti, A. Fassò, A. Ferrari, S. Muraro, J. Ranft, S. Roesler, P.R. Sala, The FLUKA code: Description and benchmarking, *AIP Conf. Proc.* 896 (2007) 31–49. doi:10.1063/1.2720455.
- [4] A. Nafei, C.C. Danielsen, A. Odgaard, F. Linde, I. Hvid, Properties of growing trabecular ovine bone. Part I: mechanical and physical properties., *J. Bone Joint Surg. Br.* 82 (2000) 910–920.
- [5] T. Tanaka, H. Kitamura, SPECTRA: A synchrotron radiation calculation code, *J. Synchrotron Radiat.* 8 (2001) 1221–1228. doi:10.1107/S090904950101425X.
- [6] F. Swinehart, The Beer-Lambert, *J. Chem. Educ.* 39 (1962) 333–335. doi:10.1021/ed039p333.