Dual modality neutron and x-ray tomography for enhanced image analysis of the bone-metal interface

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Abstract

The bone tissue formed at the contact interface with metallic implants, particularly its 3D microstructure, plays a pivotal role for the structural integrity of implant fixation. X-ray tomography is the classical imaging technique used for accessing microstructural information from bone tissue. However, neutron tomography has shown promise for visualising the immediate bone-metal implant interface, something which is highly challenging with x-rays due to large differences in attenuation between metal and biological tissue causing image artefacts. To highlight and explore the complementary nature of neutron and x-ray tomography, proximal rat tibiae with titanium-based implants were imaged with both modalities. The two techniques were compared in terms of visualisation of different material phases and by comparing the properties of the individual images, such as the contrast-to-noise ratio. After superimposing the images using a dedicated image registration algorithm, the complementarity was further investigated via analysis of the dual modality histogram, joining the neutron and x-ray data. From these joint histograms, peaks with well-defined grey value intervals corresponding to the different material phases observed in the specimens were identified and compared. The results highlight differences in how neutrons and x-rays interact with biological tissues and metallic implants, as well as the benefits of combining both modalities. Future refinement of the joint histogram analysis could improve the segmentation of structures and tissues, and yield novel information about specimen-specific properties such as moisture content.

Introduction

Bone is a complex tissue that is composed primarily of an inorganic mineral phase and an organic collagen type I based matrix (Rho et al 1998, Reznikov et al 2014). The stiffness of the mineral in combination with the flexibility of the collagen, together with their intricate hierarchical arrangement from individual building blocks to organ level, result in a composite tissue with unique mechanical properties (Rho et al 1998, Van Der Linden and Weinans 2007, Isaksson et al 2010, Törnquist et al 2020). Reduced bone strength and fracture resistance due to traumas and degenerative disorders often require medical interventions adopting different types of implants (Burr 2002, Ferretti et al 2003, Van Der Linden and Weinans 2007). In such interventions, the structural integrity
of the implant fixation is of pivotal importance for the long term viability of the implant (Ryd et al 1995). One method to enhance implant stability is to use bioactive molecules that stimulate bone growth; this has been shown to improve mechanical stability when used in combination with anchoring implants (Raina et al 2019). For a complete understanding of such a treatment on the bone-implant integration, it is vital to observe and quantify the structural properties of the newly formed bone at the immediate interface.

Given the three-dimensional nature of implants and bone microstructure, tomographic imaging lends itself as an ideal approach. X-ray tomography (XRT) is the state-of-the-art in bone research because of its widespread availability and the natural contrast of the inorganic mineral in the bone tissue to the soft tissue and air. However, when implants consisting of highly attenuating metals are present, extensive artefacts occur in their vicinity. This impairs the image quality and hinders the analysis of the data (Isaksson et al 2017). Neutron tomography (NT) has shown promising results as an alternative technique to XRT for the evaluation of bone tissue in close proximity to a metallic implant, mainly due to the reduced occurrence of streaking artefacts in the neutron data (Isaksson et al 2017, Le Cann et al 2017). Neutrons interact differently with matter compared to x-rays, which translates into different attenuation coefficients for the two modalities (Strobl et al 2009). For example, hydrogen is highly visible in neutron imaging and nearly transparent in x-ray imaging, whereas metals are generally quite x-ray opaque (generating the aforementioned artefacts) but rather transparent to neutrons. Furthermore, different contrasts for the same object imaged with the two modalities make the two techniques highly complementary. For this reason, NT has been explored as an alternative to XRT to study the internal structures of, e.g. fossilised biological specimens (Schwarz et al 2005, Laaß and Schillinger 2015, Mays et al 2017, Zanolli et al 2017, Urciuoli et al 2018, Pugliesi et al 2019, Zanolli et al 2020). The complementary nature of NT and XRT has been exploited in other domains such as geomechanics and porous media (Tudisco et al 2017, Roubin et al 2019, Stavropoulou et al 2020, Tengattini et al 2020a), where the dual modality data allowed an increase in information about the imaged object.

We have previously compared structural parameters obtained from NT and XRT of one specimen (Isaksson et al 2017), and used NT to investigate bone-implant integration mechanically in two specimens (Le Cann et al 2017). These studies showed that NT offers advantages compared to XRT when studying bone structure and mechanical properties in the close vicinity of metal implants where image artefacts in the XRT data make it difficult to, e.g. track crack movement during loading of the implant. In the current study, we focus on the complementarity of the modalities to combine information from NT and XRT, with a substantially increased number of specimens. The current study explores the complementary nature of NT and XRT for applications in bone research, in particular when metallic implants are present in the specimens. Using a dual modality image registration algorithm (Tudisco et al 2017, Roubin et al 2019) to superimpose NT and XRT images of rat tibiae with titanium implants, similarities and differences between the modalities were investigated by comparing specific material phases, and contrast between these phases and the background. Additionally, visualisation accuracy for each modality was evaluated by quantifying the volume fractions of bone and soft tissue at three different locations in the specimens.

Material and methods

Animal model

Specimens consisted of proximal tibiae from male Sprague Dawley rats (N = 11, ~10 weeks old, weight 450 ± 39 g), procured from Taconic (Denmark), with a hollow titanium screw (Ø = 3.5 mm, h = 6.3 mm) implanted transversely to the longitudinal axis of the bone, as detailed in Raina et al (2019). All screws were filled with a biomaterial (calcium sulphate/hydroxyapatite). The rats were divided into two groups, a control group (Ctrl, n = 5), and a treated group (Trtd, n = 6) where the biomaterial was mixed with bioactive molecules (zoledronic acid and recombinant human bone morphogenic protein-2) to increase peri-implant bone formation (Raina et al 2019). The implants were left to integrate for six weeks before the rats were euthanised by CO₂ asphyxiation. The tibiae were dissected, cleaned of soft tissue, and sectioned approximately 25 mm from the proximal epiphysis using a Minitom (Struers), before being immersed in physiological saline solution and frozen. The animal model was carried out in accordance with the IOP ethics policy and approved by the Swedish Board of Agriculture (permit numbers: M79-15 and 15288/2019).

Neutron and x-ray tomography

Dual modality tomographic imaging was carried out at the NeXT-Grenoble beamline at Institut Laue-Langevin (ILL), Grenoble, France (Tengattini et al 2020b). Each specimen was dried in vacuum at room temperature for 11 h to remove moisture and increase the visibility of internal structures (Le Cann et al 2017), before being mounted vertically, with the proximal epiphysis facing upwards (figure 1(b)). The dual NT and XRT set-up at NeXT-Grenoble enabled each specimen to be imaged first by NT followed by XRT without moving the specimen (figure 1(a)).
For the NT, 1200 projections, covering a field of view (FOV) of $41 \times 41$ mm, were acquired over $360^\circ$ rotation using a pinhole of $23$ mm ($D$), with an exposure time of 2 sec per projection and averaging 3 projections per angle to minimise noise. The detector system included a $50 \mu m$ Li:F scintillator, optically coupled to a Hamamatsu Orca 4.0V2 sCMOS camera, with an array of $2048 \times 2048$ pixels, $6.5 \times 6.5 \mu m$ in size, by a $50 \mu m$ lens adopting an aperture of $f/1.2$, yielding a virtual isotropic voxel size of $18.6 \mu m^3$. Due to the large ($L \approx 10$ m) collimation distance, the neutron beam at the specimen position was considered to be parallel. By placing the specimen as close as possible to the detector, the measured spatial resolution was close to $46 \mu m^3$ ($L/D \approx 435$).

XRT was carried out with a divergent conical beam from a Hamamatsu L12161-07 x-ray source. 1312 projections were acquired over $360^\circ$ rotation, covering the same FOV as for the NT. The source voltage and current were $110$ kV and $160 \mu A$, respectively, in combination with a frame rate of 9 Hz, and averaging 5 projections per angle. The x-ray detector was a Varex PaxScan® 2530HE with an array of $1792 \times 2176$ pixels, $139 \mu m$ in pitch, combined with a caesium iodide scintillator, resulting in a virtual isotropic voxel size of $24.4 \mu m^3$.

Both neutron and x-ray radiographs (projections) were normalised for beam inhomogeneities and background noise in the scintillators and cameras, before being reconstructed in 16 bits using the filtered-back-projection technique (the Feldkamp (FDK) algorithm (Feldkamp et al 1984)) for the divergent x-rays and a parallel algorithm for the neutrons (commercial software, X-Act from RX Solutions, France).

In the following, all greyscale images are displayed in terms of relative attenuation, i.e. attenuation for a given material phase in relation both to other phases imaged with the same modality, and to itself and other phases imaged with the other modality.

Image registration and phase segmentation
The XRT image volumes were registered onto the NT image volumes (figure 2(a)) using the open source SPAM software (Stamati et al 2020) and the Gaussian mixture model for multi-modal image registration (Tudisco et al 2017, Roubin et al 2019). The algorithm computes a joint histogram where the grey value distributions from both NT and XRT images (figure 2(c)) are combined into a 2D map (figure 2(b)) where peaks arise from material phases in the imaged specimen. A peak located at grey values matching a specific material phase in both neutron and x-ray tomographies was considered non-spurious. Bivariate Gaussian functions were fitted to selected peaks, yielding a phase diagram (figure 2(d)) where pairs of grey values were associated with a given Bivariate Gaussian based on the Mahalanobis distance (Mahalanobis 1936). An iterative digital volume correlation based algorithm, which considers phase mapping through the Bivariate Gaussian functions, allowed the NT and XRT volumes to be registered with sub-voxel accuracy. As a by-product, the registration also yielded a phase segmentation (PS), where each phase corresponded to a different material phase. Six non-spurious peaks were identified in each specimen-specific joint histogram, corresponding to voxels associated to grey value zero, background, soft tissue, miscellaneous non-separated structures, high-density bone tissue, and the metallic implant (figure 2(e)). The voxels with a grey value of zero originated from artefacts caused by the implant in XRT, and zero-padding of one or both image volumes to obtain matching dimensions of the two. For the subsequent analyses, the registered image volumes and PSs were centred around the implant and rotated using ImageJ (v1.53c, National Institute of Health, USA) to align the implant parallel to the $y$-axis in both sagittal and transverse slices (coordinate system defined as per figure 2(a)).
Analysis of modality complementarity

To investigate how the material phases were captured in each imaging modality, peak positions, peak shapes, and contrast-to-noise ratio (CNR) were compared between modalities, treatment groups, and material phases. In addition, quantification of volume fractions of soft tissue and bone tissue were compared between treatment groups, modalities, and the PS.

Peak position and peak shape

Peak centre positions (figure 3) in each specimen-specific joint histogram (255 bins in each dimension) were quantified for the peaks described in the previous section. The peaks corresponding to background and ‘zeros’ were omitted from the analysis. User input on an initial guess of peak centre position in combination with a peak finding algorithm (findpeaks.m, Matlab, Mathworks, R2019a), were used to find the peak centre positions.
For each peak and specimen-specific joint histogram, a square (70 × 70 bins) region of interest (ROI) was centred on the identified peak centre position (figure 4(a)) and a threshold of 75% of the mean intensity inside the ROI was used to remove low intensities (figures 4(b), (d)). The intensity contributions from each modality (i.e. the ‘visibility’ of the various phases) were evaluated by projecting the peak onto the y-axis (NT) or x-axis (XRT), as defined in figure 4(b). Full-width at half-maximum (FWHM) and area-under-curve (AUC) normalised to the total-area (TA) covered by the ROI (AUC/TA) (figure 4(c)), as well as peak centre position (figure 3) were compared between modalities, specimens, and treatment groups. Low FWHM corresponds to a narrow peak, i.e. distinct grey values associated with the phase. Conversely, a high FWHM indicates higher variability of grey values. Given that a phase is free of noise and influence from other phases, a high FWHM is hence associated with a richness of information as a wider range of grey values capture more variation within the corresponding material phase. AUC/TA yields information about the number of voxels corresponding to the material phase giving rise to the peak, with a high AUC/TA corresponding to a large number of voxels and vice versa. Large differences between peak positions indicate a better distinction between phases.

**Contrast-to-noise ratio**

For each specimen, the binary PSs corresponding to the three material phases of interest (soft tissue, high-density bone tissue, and implant) and the background, $B_g$, were extracted from the dual-modality image registration, as detailed above. These PSs were used as masks to select voxels in the corresponding NT and XRT image volumes. The contrasts between the masked material phases and the background were evaluated for each modality in terms of CNR:

$$\text{CNR}_i = \frac{\text{mean}(S_i) - \text{mean}(B_g)}{\sqrt{\text{std}(S_i)^2 + \text{std}(B_g)^2}}, \quad i = [1, 3],$$

where mean($S_i$) is the mean grey value of all voxels corresponding to the segmented material phase, $S_i$, and mean($B_g$) is the mean grey value of all voxels corresponding to the segmented background, for a given modality.
Figure 4. Peak shape analysis. (a) Mean joint histogram with dashed squares indicating the ROIs used to study the peaks, colour coded to identify which peak corresponds to which material phase (soft tissue in red, miscellaneous non-separated structures in blue, high-density bone tissue in mint, and implant in black). (b) The peak corresponding to high-density bone tissue, after thresholding at 75% of the mean intensities inside the ROI. Intensity contribution from treatment groups (Ctrl and Trtd) and modalities (NT and XRT) were obtained by projecting the peak onto each of the histogram axes. The data is shown as the mean of each treatment group and modality (black line) with their respective standard deviation (shaded area). (c) Definition of AUC (shaded area under the curve) and FWHM, exemplified using an NT peak from a Trtd specimen. (d) Peaks after thresholding at 75% of the mean intensity inside the ROI, with corresponding intensity curves grouped based on modality (NT, XRT), treatment (Ctrl, Trtd), and material phase (soft tissue, miscellaneous non-separated structures, high-density bone tissue, implant).
Quantification of bone tissue and soft tissue

To evaluate each modality’s ability to capture bone and soft tissue, both tissues were isolated with the PS, and segmented based on manual thresholding of the NT images and of the XRT images. The threshold values were selected to yield the best visual segmentation for all specimens (Table 1). Due to XRT image artefacts in the vicinity of the implant, the lower XRT threshold had to be chosen differently for segmentation in this region compared to other parts of the specimens. Tissue volume fractions were compared at different locations to consider biological variation in structure and composition, and to account for the influence of image artefacts in the XRT data (Figure 5).

Table 1. Grey value intervals for segmentation of soft tissue and bone tissue. The lower threshold for the XRT data were chosen differently for the different ROIs due to image artefacts in the vicinity of the implant: * for ROI 1, and ** for ROI 2 and ROI 3.

| Soft tissue | Bone tissue |
|-------------|-------------|
| NT          | 42405–65535 | 19275–40092 |
| XRT         | 17219–28013 | *30583–65535, **27756–65535 |

Figure 5. Midline sagittal slice through a volume rendering of the segmented bone phase (based on manually defined grey values) in the NT image of a representative specimen from the Ctrl group. The three ROIs used to quantify peri-implant bone (ROI 1), cortical bone (ROI 2), and soft tissue and trabecular bone (ROI 3) are indicated in black.

a. Peri-implant bone

The amount of newly formed bone at the bone-implant interface was estimated inside a ROI defined using the PS of the implant as a mask after morphological dilation in 3D. The ROI started 1.5 mm from the tip of the dilated implant, extending 1.5 mm upwards and 0.6 mm outwards from the implant surface (ROI 1 in Figure 5). The volume fraction of peri-implant bone was calculated as the total number of segmented voxels divided by the total number of voxels inside the ROI. The results were compared between segmentation approaches and treatment groups.

b. High-density bone tissue

The volume fraction of high-density bone tissue was estimated inside a square ROI starting 2 mm distal to the implant, surrounding the cortex, and extending 2 mm along the bone shaft (ROI 2 in Figure 5). The results were compared between segmentation approaches for the specimens in the Ctrl group.

c. Trabecular bone and soft tissue

The volume fractions of soft tissue and trabecular bone were estimated in the metaphysis. The ROI started 1 mm distal to the growth plate and extended 1 mm distally, excluding the cortex (ROI 3 in Figure 5). The results were compared between segmentation approaches for the specimens in the Ctrl group.

Statistical analysis

The analysed parameters were not normally distributed, as confirmed by the Shapiro–Wilk normality test. Hence, non-parametric statistical analysis was used to compare peak centre positions, peak shapes (AUC/TA and FWHM), CNR, and volume fractions. Paired parameters obtained from the different modalities (NT and XRT) were compared using the Wilcoxon signed-rank test. Treatment groups (Ctrl and Trtd) were compared using the Mann–Whitney U-test. All statistical analyses were carried out on a significance level of $p = 0.05$, in Matlab (Mathworks, R2019a).
Results

The results of the analysis of Peak position and Peak shape are presented separately, followed by CNR, and quantification of bone tissue and soft tissue.

Peak position
For a given modality, the mean peak centre positions were significantly different ($p = 0.001$) for all the material phases ($\Delta$NT and $\Delta$XRT in figure 3(b)). For a given phase, only the XRT peak corresponding to miscellaneous non-separated structures showed statistically significant differences ($p = 0.004$) between the two treatment groups ($\delta$NT and $\delta$XRT in figure 3(c)).

Peak shape
AUC/TA and FWHM were used to evaluate the shape of the joint histogram peaks corresponding to each identified material phase, for each modality and treatment group (figure 6). Notably, the AUC/TA value for the soft tissue peak was higher in the Ctrl group than in the Trtd group ($p = 0.004$).

Contrast-to-noise ratio
CNR revealed that for both bone and soft tissues, NT gave a higher contrast against the background than XRT (table 2). For the implant, the opposite was observed.

| Table 2. Contrast-to-noise ratio (CNR). Data are shown as mean $\pm$ std. |
|---|---|---|
| Contrasting structure | CNR for NT | CNR for XRT | Statistical comparison |
| Implant | $4.3 \pm 0.8$ | $39.6 \pm 15.1$ | $p = 0.001$ |
| High-density bone tissue | $7.7 \pm 3.2$ | $5.8 \pm 1.3$ | $p = 0.019$ |
| Soft tissue | $7.5 \pm 5.4$ | $2.8 \pm 0.7$ | $p = 0.001$ |

Figure 6. Area-under-curve/total-area (AUC/TA) and full-width at half-maximum (FWHM) for the joint histogram peaks for each material phase, modality, and treatment group. Data are shown as mean $\pm$ std (bar) with specimen-specific values in black ($\times$). Statistically significant differences are indicated with ** ($p < 0.01$).
Quantification of bone tissue and soft tissue
Significantly more bone was measured around the implant (ROI 1) in the Trtd than in the Ctrl group (figure 7(e)) both for NT and XRT ($p = 0.02$ and $p = 0.01$ for NT and XRT, respectively). No statistically significant differences were found between the measurements from the two imaging modalities. The PS did not show differences between the treatment groups. All three segmentations resulted in similar volume fractions for the high-density bone tissue (ROI 2, figure 7(f)). Segmentation of soft tissue (ROI 3, figure 7(g)) resulted in similar volume fractions for NT and the PS, whereas it was higher with XRT. Bone volume fractions in ROI 3 were similar for NT and XRT, but lower for PS.

![Figure 7](image.png)

**Figure 7.** Comparison of volume fractions of bone tissue and soft tissue segmented using thresholding of NT and XRT, and phase segmentation. (a)–(d) Midline sagittal slice through volume renderings of the bone phase in NT (a), (c), and XRT (b), (d) for representative specimens from both treatment groups (Trtd (a), (b) and Ctrl (c), (d)). ROIs are indicated in black. (e) Volume fractions of peri-implant bone tissue (ROI 1). Statistically significant differences are indicated with * ($p < 0.05$) and ** ($p < 0.01$). (f) Volume fractions of high-density bone tissue in the distal shaft in specimens from the Ctrl group (ROI 2). (g) Volume fractions of soft tissue and bone tissue in the metaphysis in specimens from the Ctrl group (ROI 3).

**Discussion**

NT and XRT images of proximal rat tibiae with hollow metallic implants have been compared using joint histogram analysis and quantification of tissue volume fractions to explore the complementary nature of the two modalities. From the joint histogram analysis, three material phases of interest were isolated, namely soft tissue, high-density bone tissue, and the metallic implant. Soft tissue was more prominent in the NT images than in the XRT images, and more prevalent in the Ctrl group than in the Trtd group. High-density bone tissue was...
captured well in both modalities. Trabecular structures were not well captured by the PS but were instead segmented together with other miscellaneous non-separated structures, along with x-ray image artefacts, and void. The metallic implant gave rise to artefacts in the XRT images whilst it had no adverse effects on the NT images.

Peak centre position
The joint histograms showed five relevant peaks, corresponding to background, soft tissue, miscellaneous non-separated structures, high-density bone tissue, and the metallic implant. All peaks were visible in each specimen-specific joint histogram, at similar locations for all specimens. The mean peak centre position of each of these five peaks were clearly separated from each other in both modalities (ΔNT and ΔXRT in figure 3), indicating that the interaction between each phase and modality was different for all phases.

Statistically significant differences between mean peak centre positions for the two treatment groups (Ctrl and Trtd, /NT and /XRT in figure 3) were found only in XRT for the miscellaneous non-sorted structures peak. Further analysis is necessary to elucidate the underlying cause of this. Improving the PS could yield an additional phase corresponding to the remaining bone tissue, specifically the trabecular structures that were sorted into the miscellaneous non-separated structures phase.

For some specimens, additional peaks were seen in the joint histogram (supplementary material—figures 1 and 2). Also, some of the chosen peaks appeared to be composed of a cluster of peaks. A future investigation into the origin of these additional peaks, and the distribution of peaks in the clusters, could be beneficial to further elucidate the complementarity of NT and XRT, and to explore additional possible applications for dual modality imaging in terms of identifying sample components. However, our current assumption is that some of these differences can be attributed to biological variation such as differences in tissue composition, and slightly different states of hydration.

All specimens were dried for 11 h. However, recent data (unpublished) show that similar specimens (proximal rat tibia without a metal implant) continue to lose moisture even after 16 h of drying in vacuum. Varying moisture content in different areas of the tissues, or in different specimens, results in different distributions of grey values in the NT images and hence different peak positions and components in the joint histograms. From the current dataset it was not possible to confirm this theory, as the image artefacts in the XRT data resulted in a widening of grey value intervals.

Peak shape and CNR
Peak shape, here described through AUC/TA and FWHM (figure 6), gives an indication of how neutrons and x-rays interact with the different phases. A high AUC/TA in combination with a low FWHM means that numerous voxels in a narrow grey value interval correspond to the peak. A low AUC/TA in combination with a high FWHM means that fewer voxels correspond to the peak but has a larger spread in grey values, which can be caused by a greater variability in the attenuation of this phase, or by influence of noise or overlap with the grey-scale ranges of other phases. Hence, these parameters give an indication of the contribution from each modality to each peak.

Soft tissue
Soft tissue peaks presented lower AUC/TA in the Trtd group compared to the Ctrl. Due to the higher amount of bone tissue in the Trtd group, a lower amount of soft tissue can indeed be expected as compared to the Ctrl group. The CNR analysis (table 2) showed that soft tissue had a statistically significantly higher contrast to the background in the NT images than in the XRT images. Higher grey values seen in the NT images than in the XRT images, indicated that neutrons interact more strongly with soft tissue than x-rays do.

Miscellaneous non-separated structures
The joint histogram peak denominated miscellaneous non-separated structures (highlighted in figure 4(c)) was found to include trabecular structures, void, voxels affected by the x-ray image artefacts caused by the metallic implant, and possibly also some soft tissue. The PS (figure 2(e)) highlighted that the voxels in this phase were numerous and spread throughout the entire specimen volumes. The peak associated with this miscellaneous phase was not well-defined in XRT in the joint histogram, and hence the FWHM was not quantifiable.

High-density bone tissue
The amount of bone tissue was expected to be higher in the Trtd group than the Ctrl group due to the bioactive molecules added to the biomaterial (Raina et al 2019). However, this newly formed bone was in the form of trabecular structures, which were not fully sorted into the high-density bone tissue phase. Consequently, AUC/TA was similar for both treatment groups. FWHM was higher for XRT than for NT (not statistically significant).
indicating a wider grey value range for bone tissue in XRT than in NT. This is possibly a result of image artefacts in the vicinity of the implant in the XRT images, causing spurious grey values of higher intensities for voxels not containing any implant. The contrast (CNR, table 2) between bone tissue and background was higher in NT than in XRT. This could possibly be a result of the image artefacts in the XRT data caused by the metallic implant. Further investigation, including comparison between specimens with and without metallic implants is needed to elucidate this.

**Implant**

X-rays interact more strongly than neutrons with the metallic implant, resulting in higher voxel intensities in XRT images than in NT images (figure 2(a)). This was indicated by the higher CNR in XRT with respect to NT (table 2). For the reconstruction, the conversion from 32-bit to 16-bit (i.e. the bit depth of the reconstructed images) were determined to yield a wide dynamic range for the skeletal tissues. The selected values were $-0.5$ and 12 for NT, and $-0.5$ and 4.5 for XRT. For XRT, this meant that the intensities of the implant were cut off, causing most implant voxels to have the maximum intensity.

**Quantification of bone tissue and soft tissue**

The quantification of bone and soft tissue highlighted the strengths and limitations of the modalities and segmentation methods.

The addition of bioactive molecules was expected to yield a higher amount of newly formed bone surrounding the implant (ROI 1 in figure 7) in the Trtd group (Raina et al 2019). This was indeed observed in both NT and XRT images. No statistically significant differences were found between the modalities, indicating that both NT and XRT are viable techniques for estimating the amount of peri-implant bone. However, due to the artefacts present in the XRT images, finer analysis of bone structure in this region is challenging with XRT, and tracking damage and crack propagation close to the interface has earlier been shown difficult (Le Cann et al 2019, 2020). The lack of image artefacts in the NT data and the similar accuracy in bone volume estimation suggests NT to be a promising alternative to XRT for such analysis. Furthermore, it is important to note that the ROI used to study the bone ingrowth was not as heavily affected by the image artefacts as the region inside the implant, or at the implant threads and tip (figure 8). Should the aim be to study bone structure or volume in any of these regions, NT is therefore the better choice. For XRT, bone tissue is segmented based on mineral density, as the mineral provides the contrast against other phases. Hence, phantoms of known mineral density are often measured along with the bone specimens to determine thresholding intervals based on an objective standard. However, appropriate phantoms need to be developed for NT to enable objective comparison.

A statistically significant difference between treatment groups with regards to the amount of newly formed bone surrounding the implant ROI 1 was not captured with the PS (figure 7(e)). Furthermore, the PS resulted in a lower bone volume fraction compared to NT and XRT in the metaphyseal region (ROI 3, figure 7(g)). This was due to trabecular structures not being sorted into the high-density bone tissue phase but instead into the miscellaneous non-separated structures phase (figure 2(e)). However, in regions with mainly cortical bone (ROI 2, figure 7) PS performed similarly to thresholding (figure 7(f)).

The volume fraction of soft tissue in ROI 3 showed XRT as a clear outlier. The grey value interval for soft tissue in XRT matches that of the edges of bone tissue, resulting in an overestimated volume fraction. The grey value interval for soft tissue was more distinct in NT, which indicates that the modality is promising for characterising the amount and distribution of soft tissue. This is supported by recent work that highlighted the
potential of NT for looking at soft tissue (Guillaume et al 2021). Further investigation is needed to optimise the differentiation between bone and soft tissue in NT images.

Conclusions

The combined use of neutron and x-ray tomographic imaging of bone with metallic implants has been shown to yield more information about the material phases and their distribution in the specimens than either of these modalities individually. Three material phases of interest were captured in both NT and XRT, namely soft tissue, high-density bone tissue, and the metallic implant. After the sub-voxel registration of the dual-mode images, each of the phases was segmented, and this joint segmentation was overall a better match to the material phases, visualised in the NT and XRT images, than standard threshold-based segmentation of either dataset. Soft tissue was captured better with NT than XRT, and the metallic implant was captured more clearly with XRT than with NT. X-ray image artefacts in the vicinity of the implant negatively affected the image quality by obscuring the structures in the region. Due to the different interaction between neutrons and metal, compared to x-rays, such artefacts were not present in the NT images. Formation of new bone around the implant was captured well in both modalities for a selected ROI, where the amount of image artefacts in the XRT data was limited. Further investigation and analysis of the joint histogram and improvement of the PS could result in additional and novel information about bone and soft tissue.

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Author contributions

Study design: ET, SLC, ETu, SAH, and HI. Animal experiment: DBR, and MT. Data collection: ET, SLC, ETu, and AT. Data analysis: ET, AT, EA, NL, and JH. Data interpretation: ET, SLC, AT, EA, and HI. Drafting manuscript: ET. All authors revised the manuscript content and approved the final version. ET and HI take responsibility for the integrity of the data analysis.

Conflicts of interest

The authors declare that they have no conflict of interest.

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