NON-DESTRUCTIVE 3D STRUCTURAL STUDIES BY X-RAY MICROTOMOGRAPHY

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ABSTRACT

X-ray microtomography (XMT) is an evolving form of 3D X-ray microscopy that gives quantitative information on linear attenuation coefficients at a scale of 2 to 100 µm. The method is non-destructive, so that local changes in microscopic structure can be studied between successive applications of externally induced changes, e.g. mechanical or chemical processes. Applications of our laboratory-based XMT systems are illustrated by studies of biological mineralized tissues and in materials science. As regards 1st generation XMT systems, the ability to record attenuation spectra of elements with accessible absorption edges is exploited in studies of the ingress of KI into calcified tissues. Our novel high definition 4th generation cone-beam XMT scanner can image specimens up to 60 mm diameter at resolutions up to 8 µm. Applications include studies of host response to implants in bone, crack opening as a function of load in compact tensile samples of Al-Li 2090 using an in situ loading stage, and in vitro acid demineralization in whole teeth.

INTRODUCTION

XMT (X-ray microtomography), a miniaturized form of the well-known medical CT, gives quantitative 3D information at a scale of 2 to 100 µm. The amplitude of voxels in an XMT image is proportional to the linear attenuation coefficient in the corresponding object voxels (neglecting artefacts), which are determined by the voxel densities and elemental compositions. This evolving form of 3D X-ray microscopy is finding increasing application in materials science and in studies of bones and teeth [1, 2]. The method is non-destructive so that local changes in microscopic structure (formation of cracks, changes in crack width, changes in density, etc.) can be studied between successive applications of externally induced changes, for example, mechanical or chemical processes. A variety of laboratory XMT systems have been developed [3], and many synchrotron X-ray sources have XMT facilities (see for example [4]).

METHODS

1st generation XMT system

A detailed description of our 1st generation system has been given previously [5]. In a novel implementation of multiple-energy X-ray absorptiometry (MEXA) for elemental quantitative analysis [6], species in XMT images are resolved on the basis of their differential attenuation across a wide energy range, ideally including absorption edges. The attenuation spectrum is recorded for each point in each projection. For each point, the elemental composition is determined that gives the best fit in a least squares sense between the observed attenuation spectrum and that calculated from the determined composition using published mass attenuation coefficients (Fig. 1).
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practice, only one or two elements in the beam will have accessible absorption edges, so the composition expressed as elements is replaced by one chemical species without an absorption edge plus another species for each element with an absorption edge. The least squares analysis then resolves the total attenuation into contributions from each species which may then be individually reconstructed.

Fig. 1 Experimental (dots) and calculated (line) attenuation for aqueous CsI (1.0 mol l⁻¹) in a 5 mm diameter plastic vial (diametral ray). The upper and lower absorption edges are due to I (33.17 keV) and Cs (35.98 keV). A W target, 45 kV accelerating voltage, 1.5 mA tube current, and 15 µm X-ray beam were used. Data collection time ~1.8 s. Species used for the calculated spectrum were Cs, I and H₂O [6].

4th generation XMT system

Our 4th generation system employs a laboratory X-ray source (160 kV max, 5 µm Ultrafocus, X-Tek, UK), with a tungsten target. A thallium-activated caesium iodide scintillator is optically coupled (two 50 mm f1.2 lenses in tandem) to a cooled 16 bit slow CCD scan camera. In order to achieve artefact elimination and large specimen capability, the CCD camera is scanned across the image whilst the recorded image is clocked out of the CCD in synchrony (time-delay integration) [7]. The advantage of this system is that it provides uniform detection characteristics across all elements in each row of the projection because all CCD/scintillator pixels contribute in the same proportion to each element in a row of the recorded projection. Image pixels of 67.5 µm at the detector are created by binning together 3×3 22.5 µm CCD pixels. Movement of the specimen towards or away from the source varies the pixel size at the specimen from between 30 and 5 µm. The maximum projection width is 2400 pixels, giving a maximum specimen diameter of ~6.5 cm at the lowest magnification. The specimen can be moved along the rotation axis and blocks of data recorded which are concatenated after reconstruction, giving a maximum size of 20 cm along this axis. Correction for polychromatic radiation is made by deriving a calibration curve from a projection of an aluminium step wedge. The corrected image gives the equivalent linear attenuation coefficient at an arbitrary monochromatic energy (assuming change in attenuation versus energy is the same as for aluminium). The ability to use long X-ray exposures without problems of detector non-linearity, mechanical drift (X-ray enclosure is temperature controlled), thermal noise or detector saturation enables us to generate 3D images with a high signal to noise ratio (data acquisition times vary from an hour to several days per block). This high contrast resolution enables small differences in attenuation coefficient (less than 1%, depending on the specimen) to be resolved.

Images are reconstructed using a cone-beam version of the Cartesian axes pre-projection algorithm [8], which can reconstruct full 3D data sets typically in half the data collection time (500 MHz PC, with 2 GBytes of standard PC100 specification memory).

Specimens are mounted kinematically on the rotation stage so they can be removed and accurately repositioned. This allows longitudinal studies of changes induced externally, for example by mechanical or chemical processes. Alternatively, changes can be followed in situ, for example by using specially designed load cells.
APPLICATIONS

1st generation XMT system
An application of MEXA is given in Fig. 2. Notice that the KI concentration is elevated in the surface regions exposed to KI solution whilst the HAp concentration is uniform.

![Fig. 2 Microtomographic concentration maps (g cm$^{-3}$) for KI (left) and hydroxyapatite (HAp) (right) following diffusion of aqueous KI (3 mol l$^{-1}$) through the surface of a root of a human tooth for 10 days. Scale bar is 1 mm. A silver target, 45 kV accelerating voltage and 1.5 mA tube current were used. Spectra were recorded at 128 positions, 35 µm apart, for each of 251 projections. Data were processed using energy ranges below (27.28-31.60 keV) and above (35.06-37.36 keV) the I absorption edge (33.17 keV) [6].](image)

4th generation XMT system
Bone. XMT can be used to study host response to implant materials. Both examples are at 15 µm resolution. The first example (Fig. 3) is from a sheep hip, in which a stainless steel implant had been cemented. The measured attenuation coefficients of the larger lighter areas on the left and the small bright spots (off the white scale in this image) matched, within experimental error, those calculated for HAp and stainless steel respectively. Thus, they are identified as HAp fragments and as particles that probably originated from tooling of the prosthesis before fitting. With this level of resolution, the slices allow the tissues to be examined in a number of planes and without destroying the block. This means that numbers of animals can be significantly reduced without compromising on quality of data. The second example (Fig. 4) is of an implant in a rabbit tibia. Details in host bone show formation of woven bone both periosteally and endosteally. The implant is shown not to be ‘osteo-conductive’.

Dental caries models. Whole teeth, coated with acid-resistant varnish leaving either a ~1 mm band or spot window on the enamel surface, were scanned periodically during 84-107 days of demineralization in acetic acid buffer (100 mmol l$^{-1}$, pH 4.5, degree of saturation with respect to HAp = 0.2). Features found in natural 3D caries lesions were observed in these model systems. A relatively well mineralized surface layer of enamel was retained, despite loss of deeper mineral (Fig. 5). The influence of the anisotropy of the tissue structures on the 3D spread of demineralization can be seen in enamel (right) and dentin (left) and at the enamel-dentin junction.
Fig. 3 Mid-region from sheep hip in which a stainless steel implant had been cemented into an impact graft bed of a mixture allograft morcellised bone and a synthetic particulate mixture of HAp and tricalcium phosphate. Image 2.33 × 2.09 cm.

Fig. 4 Hydroxyapatite Reinforced Polyethylene (HRP) implant in the medial tibial plateau of a rabbit (surgery by Dr L. Wolfe). Image 12.7 × 9.1 mm.
These XMT results allow, for the first time, measurement of the volume of tissue progressively "demineralized" (defined as voxels with greater than 12.5% mineral loss) in a 3D system (Fig. 6). These new quantitative longitudinal studies of the development of extensive demineralization in 3D contrast with traditional methods employing 2D models using cut sections.

Fig. 5 Artificial lesion in human tooth after 84 days demineralization. Natural surface is on the right. Image 2.25 × 2.25 mm, 15µm resolution.

Fig. 6 Increase in lesion volume with time.

Materials. The example we give is from an in situ study of crack opening as a function of applied load for a preformed crack in an Al-Li 2090 compact tension test sample (31.9×30.6×2.7 mm). Unlike in a previous study [9,10], the plane of the specimen was perpendicular to the rotation axis so the maximum path of the X-ray beam through the specimen was 44 mm. Such studies enable, for example, the crack opening to be determined over the crack face as a function of applied load [10]. This entailed the development of a crack surface detection algorithm, which could be applied to the 3D data set to locate opposing crack surfaces, hence local crack opening.

Fig. 7 Change in one plane of a 3D data set during opening preformed crack in Al-Li 2090 with in situ load. Each image is 9.28 mm wide.
CONCLUSION

The studies of the ingress of KI into teeth, structural and particle identification in bone implants, quantitation of mineral loss within the structure of enamel and in situ images of crack opening as a function of load demonstrate that XMT is a powerful method for the non-destructive study of 3D structural features and dynamic processes (chemical, transport, mechanical etc.) in a variety of materials at a microscopic scale.

REFERENCES

[2] www.smd.qmul.ac.uk/dental/bip

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