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Non-invasive imaging of the crystalline structure within a human tooth

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ABSTRACT

The internal crystalline structure of a human molar tooth has been non-destructively imaged in cross-section using X-ray diffraction computed tomography. Diffraction signals from high-energy X-rays which have large attenuation lengths for hard biomaterials have been collected in a transmission geometry. Coupling this with a computed tomography data acquisition and mathematically reconstructing their spatial origins, diffraction patterns from every voxel within the tooth can be obtained. Using this method we have observed the spatial variations of some key material parameters including nanocrystallite size, organic content, lattice parameters, crystallographic preferred orientation and degree of orientation. We have also made a link between the spatial variations of the unit cell lattice parameters and the chemical make-up of the tooth. In addition, we have determined how the onset of tooth decay occurs through clear amorphization of the hydroxyapatite crystal, and we have been able to map the extent of decay within the tooth. The described method has strong prospects for non-destructive probing of mineralized biomaterials.

1. Introduction

Calcium hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) is the main mineral constituent of human teeth. Dental enamel is the most mineralized, comprising approximately 96 wt.% mineral, 3 wt.% water and 1 wt.% organic matter, whereas dentine comprises approximately 70 wt.% mineral, 10 wt.% water and 20 wt.% organic matter [1]. The mineral is non-stoichiometric in that fluoride, chloride and carbonate ions can be easily incorporated. Within the enamel, hydroxyapatite (HA) crystallites form nanorods with sizes of around tens of nanometers in diameter and up to several hundreds of micrometers in length. These nanorods themselves form clusters of around 1000 crystallites (known as prisms) with diameters of around 5 μm and up to several millimeters in length. These prisms are generally orientated perpendicular to the enamel–dentine interface. This hierarchical structure has been gradually revealed over the years using a variety of microprobe techniques, including electron microscopy [2], atomic force microscopy [3] and X-ray diffraction (XRD) measurements [4,5]. Previous studies using such methods have additionally made the link between crystallite size and enamel hardness [6], as well as relating the spatial variation of, for example, the lattice parameter to localized mechanical properties [5,7]. XRD measurements on powdered human dental enamel has established the space group and lattice parameters of HA as $P6_3/m$ (hexagonal) and $a = 9.4412$ Å and $c = 6.8781$ Å [4]. Furthermore 2-D XRD mapping measurements on vertical sections has revealed the spatial variation of crystalline preferred orientation, yielding additional insight on the complex prism structure within the dental enamel [5,8].

Much of the information obtained from such microprobe techniques requires either grinding up material, e.g. for powder XRD analysis, or sawing samples into cross-sectional slices to map or image the spatial variations of such properties. Unfortunately these destructive characterization methods can distort our view of the internal crystalline structure: grinding, cutting and polishing removes a large amount of material which may be of interest, especially for localized measurements; these methods are also prone to introducing damage into the near-surface region, which may in turn change the material's mechanical properties. Furthermore, for many situations, e.g. palaeontological samples, it may not be desirable to destructively slice these into sections for study. For these reasons, a non-destructive characterization method is ideally required which can provide structural information and still probe down to the micron scale. In this paper we describe and use a method which can do just that, namely high-energy X-ray diffraction computed tomography (HEXRD-CT) [9]. High-energy X-rays

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(approximately >80 keV) are ideal for probing such high-density materials because of the large attenuation lengths involved (in the region of tens of millimeters for hard biomaterials at these energies). Recording XRD patterns in a transmission geometry and combining this with a computed tomography data acquisition, we can non-destructively image cross-sectional slices of the internal crystalline structure [10]. We have demonstrated the method by probing the complex hierarchical crystalline structure of a human molar tooth down to a resolution of 150 μm.

2. Materials and methods

Measurements were undertaken at beamline ID15A at the ESRF, France. A monochromatic X-ray beam of energy 86.7 keV and size $150 \times 150\ \mu m^2$ was horizontally scanned across the sample in a total of 100 steps with a step size of 150 μm. This was coupled with rotation around the vertical axis through 180° in a total of 80 steps, resulting in the collection of 8000 diffraction patterns per tomographic slice. Diffraction patterns were recorded in a transmission geometry using a Pixium 4700 flat panel detector with a count time of 150 ms. The total acquisition time, including scan dead time, was about 25 min per tomographic slice. Diffraction images were azimuthally integrated, yielding 1-D patterns for every translation and rotation step. Since the tooth showed strong preferred orientation, we also performed selective radial integration spanning individual diffraction peaks to produce 1-D azimuthal intensity profiles. Tomographic reconstruction was performed for every angle of diffraction covering 3000 channels using the simultaneous algebraic reconstruction technique (SART) method, with in-built absorption correction and non-negativity constraint [11]. Absorption maps were obtained by thresholding total scatter intensity images to segment enamel and dentine regions, which were correspondingly assigned X-ray attenuation coefficients of $\mu = 0.738$ and $0.470\ cm^{-1}$, respectively. These values were calculated based upon the summed weighted mass attenuation coefficients at 86.7 keV for each elemental constituent in HA (Ca, P, O and H). Attenuation coefficients were then obtained by multiplying this value by the (water-adjusted) mass density for enamel and dentine, respectively. Absorption maps were then fed back into the reconstruction algorithm to obtain the absorption-corrected tomograms. The maximum total attenuation for the tooth sample was calculated to be about 53% with a mean attenuation of about 31%.

A human adult maxillary third molar tooth (FDI World Dental Federation notation: 18) was obtained from a patient undergoing

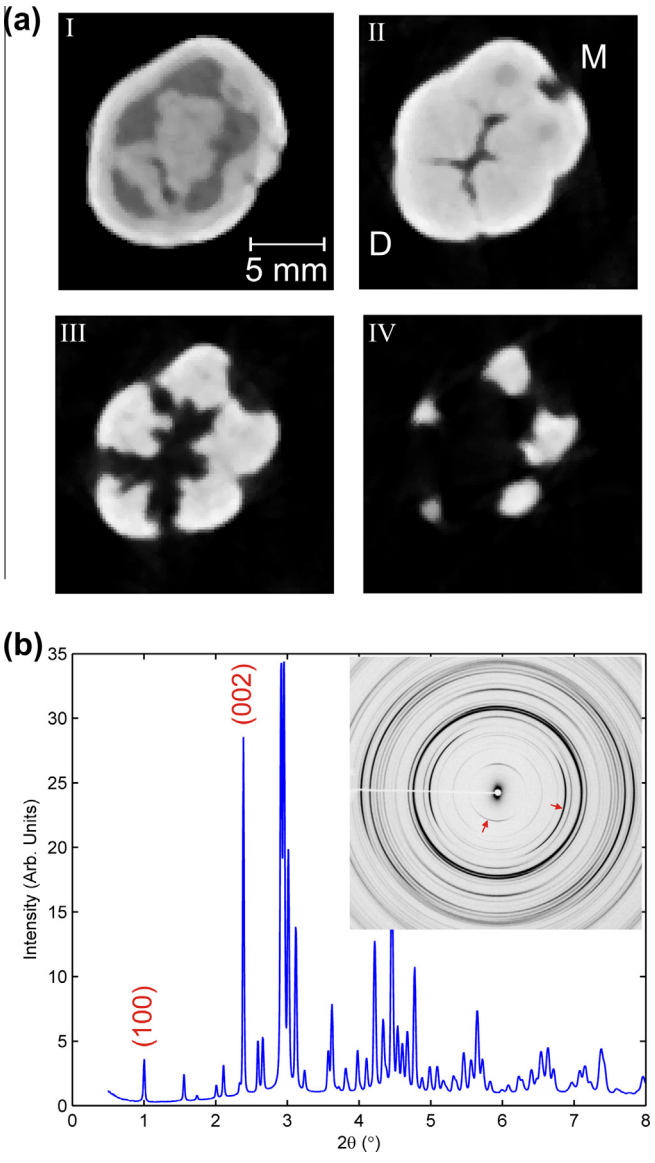

Fig. 1. XRD tomography. (a) Total scattering intensity from four horizontal tomographic slices from a molar tooth. Mesial (M) and distal (D) sides are indicated. (b) An XRD pattern from an arbitrary pixel within the tooth, showing a clear hydroxyapatite structure. The inset shows a typical 2-D area diffraction pattern exhibiting incomplete Debye–Scherrer rings (e.g. arrowed (red) showing (100) and (002) Bragg peaks) indicative of preferred orientation.

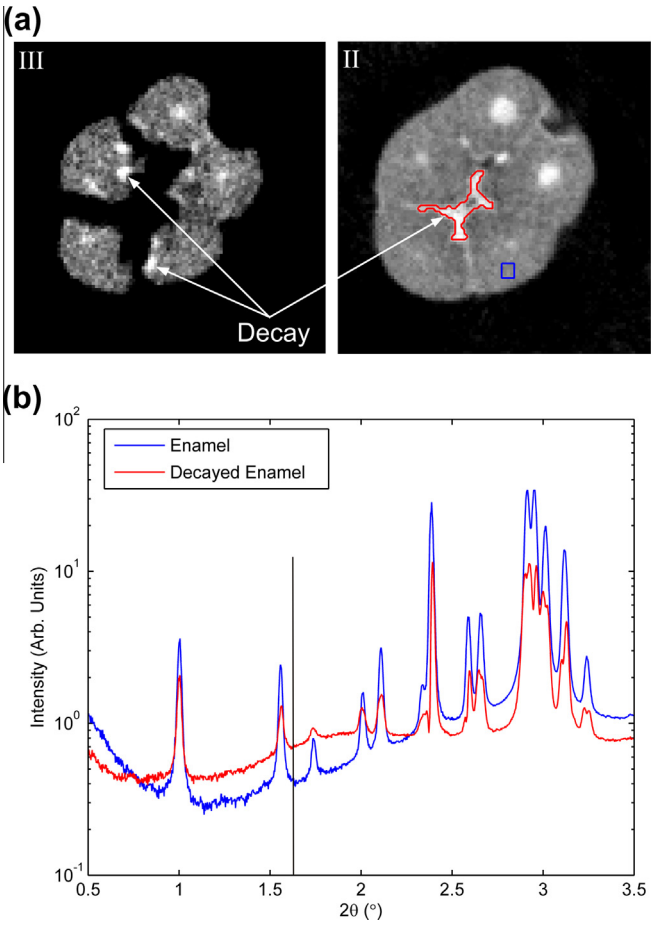

Fig. 2. Imaging tooth decay. (a) Tomographic slices III and II recorded at $2\theta = 1.65°$ showing localized regions of increased intensity corresponding to caries. (b) XRD patterns (log scale) from healthy and decayed regions.