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Dentinal tubules revealed with X-ray tensor tomography

Christoph Jud[a,*], Florian Schaff[a], Irene Zanette[a,1], Johannes Wolf[a], Andreas Fehringer[a], Franz Pfeiffer[a,b,c]

[a] Lehrstuhl für Biomedizinische Physik, Physik-Department & Institut für Medizintechnik, Technische Universität München, 85748 Garching, Germany

[b] Institut für diagnostische und interventionelle Radiologie, Klinikum rechts der Isar, Technische Universität München, 81675 München, Germany

[c] Institute for Advanced Study, Technische Universität München, 85768 Garching, Germany

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ABSTRACT

Dentin is a mineralized material making up most of the tooth bulk. A system of microtubules, so called dentinal tubules, transverses it radially from the pulp chamber to the outside. This highly oriented structure leads to anisotropic mechanical properties directly connected to the tubules orientation and density: the ultimate tensile strength as well as the fracture toughness and the shear strength are largest perpendicular to dentinal tubules. Consequently, the fatigue strength depends on the direction of dentinal tubules, too. However, none of the existing techniques used to investigate teeth provide access to orientation and density of dentinal tubules for an entire specimen in a non-destructive way. In this paper, we measure a third molar human tooth both with conventional micro-CT and X-ray tensor tomography (XTT). While the achievable resolution in micro-CT is too low to directly resolve the dentinal tubules, we provide strong evidence that the direction and density of dentinal tubules can be indirectly measured by XTT, which exploits small-angle X-ray scattering to retrieve a 3D map of scattering tensors. We show that the mean directions of scattering structures correlate to the orientation of dentinal tubules and that the mean effective scattering strength provides an estimation of the relative density of dentinal tubules. Thus, this method could be applied to investigate the connection between tubule orientation and fatigue or tensile properties of teeth for a full sample without cutting one, non-representative peace of tooth out of the full sample.

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1. Introduction

Human teeth are complex biomaterials consisting of four main materials: enamel, cementum, dentin and pulp. Enamel covers the crown of the tooth and is highly mineralized, making it the hardest material in the human body. The root of the tooth is covered by cementum, a bone-like material which connects the tooth to the bone. It has one or several openings in the tooth apex which allows blood vessels and veins to

* *Corresponding author at*: Boltzmannstr. 11, 86748 Garching, Germany. Tel.: +49 89 289 10905.
E-mail address: christoph.jud@tum.de (C. Jud).
[1] Present address: Diamond Light Source, Harwell Science and Innovation Campus, Didcot OX11 0QX, United Kingdom.

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enter the pulp chamber, the organic innermost tooth part [1]. In between the pulp chamber and enamel there is a layer of dentin, the most abundant material in teeth. Being less mineralized than enamel it serves as elastic foundation, shapes the root and protects the pulp chamber [2]. The most striking feature of dentin are the dentinal tubules that start at the pulp–dentin border and transverse it straight (in the root) or in an S-shape (in the crown) toward the dentin-enamel junction [3]. Their diameter varies from 2.5 μm near the pulp wall to 0.8 μm at a distance about 3.3 mm away from it. At the same time, their density decreases from about 45,000 mm^{-2} tubules to 19,000 mm^{-2} tubules. The density decreases from the crown to the root as well [4]. Dentinal tubules are surrounded by a layer of highly mineralized peritubular dentin and collagen fibrils which form a felt-like intertubular dentin-matrix in the plane orthogonal to the dentinal tubules [5]. The highly oriented dentin microstructure leads to anisotropic mechanical properties, which has been confirmed by many studies investigating, e.g., the fracture properties of dentin [6–13]. It was found that dentin fractures more easily orthogonal to dentinal tubules than parallel to them. The ultimate tensile strength has been investigated as well [14–19]. In agreement with the fracture properties it has been found to be highest orthogonal to the tubules (52.9 ± 4 MPa) and lowest parallel to them (42 ± 5.7 MPa). The shear strength measured in the central crown region yielded a similar result [20]. Consequently, the fatigue strength of dentin varies with the orientation of dentinal tubules [21,22]. In addition to the tubule orientation, mechanical properties vary with location within the dentin: the tensile strength in the crown was found to be greater than in the root [17,23] due to the difference in tubular density [23], although different mineral compositions play a role, too [18].

However, only destructive methods are capable to visualize the orientation of dentinal tubules for a whole tooth, e.g., visible light microscopy, scanning electron microscopy or recently X-ray ptychography and nanotomography [24]. Here we present a measurement method based on X-ray dark-field images that reveals the orientation and density of dentinal tubules for a whole tooth, in three dimensions, and without the need of sectioning it [25–28]. This contrast modality is accessed with a Talbot-Lau grating interferometer (see Fig. 1). A phase grating (denoted by G_1) periodically shifts the X-ray wavefront by a defined amount, creating an interference pattern downstream its position known ad Talbot-carpet. By analysing this interference pattern, i.e. with an absorption grating (G_2), the differential phase-contrast and the dark-field contrast are retrieved in addition to the conventional absorption. The interference effect is only visible if enough spatial coherence is ensured by a third absorption grating (G_0). The dark-field image is a measure for small and ultra-small angle scattering orthogonal to the grating orientation [26]. This directional dependency led to the development of X-ray vector radiography (XVR) [29,30] and, in three dimensions, X-ray tensor tomography (XTT) [31]. XTT yields the directional dependent effective scattering strength in 3D. In other words, a scattering tensor is reconstructed as mean value for each voxel. Since fibrous structures scatter predominantly orthogonal to their orientation [31], XTT can be used to detect their orientation by investigating the direction of least scattering. In addition, the mean effective scattering strength

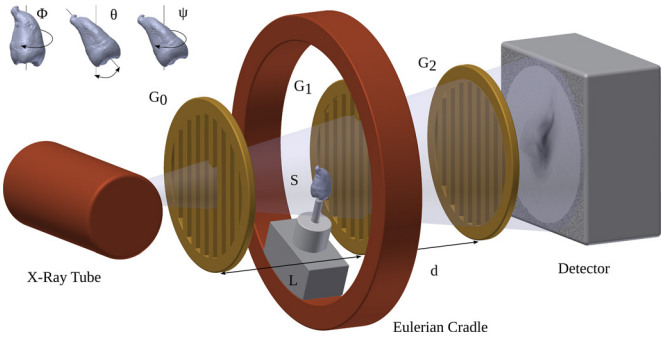

Fig. 1 – Talbot-Lau grating interferometer setup. A conventional tube generates X-rays with energies up to 160 keV. Source grating G_0 provides enough spatial coherence for the Talbot effect to appear after the phase grating G_1. The interference pattern is recorded by stepping the absorption grating G_2 and by analysing the resulting contrast curve. In this way, absorption, differential phase and dark-field contrast images are obtained.

provides complementary information about the density of scattering structures. In contrast to micro-CT, XTT reveals information about the sample morphology without the need of directly resolving the structures of interest. Hence, it overcomes the limited sample size in micro-CT when measuring high-resolution features. This makes XTT a well suited tool for the measurement of dentinal tubules.

2. Materials and methods

The sample was an upper left third molar tooth of an adult female which was routinely extracted and cleaned for several hours in a mouthwash (Coolmint Listerine, Johnson & Johnson Consumer, UK). The sample had been fully erupted and was considered caries-free. It had a small damage on the buccal side of the enamel caused by the mechanical extraction.

2.1. *Sample preparation*

For the XTT and the micro-CT, the tooth was glued with two component glue on a plastic sample holder with the crown pointing down toward the sample holder. To examine tooth slices with the confocal visible light microscope, it was embedded in polyurethane, a mixture of isocyanate and polyol in a one-to-one ratio. Afterwards it was cut with a diamond saw (EXACT 300 CL, EXAKT, Germany) into an axial and a sagittal slice which were polished to get a flat surface.

2.2. *X-ray tensor tomography*

The setup was located at the Zentralinstitut für Medizintechnik in Garching. The source was a conventional X-ray tube (MXR-160HP/11, Comet AG, Switzerland) with a focal spot size of 0.4 mm^2. The tube voltage was 60 kV and the current 13.3 mA. The spectrum was filtered with 2 mm of aluminum. A flat panel detector (Varian PaxScan2520D, Varian Medical Systems, USA) recorded the images with a pixel pitch of 127 μm and a 600 μm CsI scintillator. The grating interferometer was

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