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X-ray MicroCT Imaging of Dentin Tubules in a Human Tooth

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Dentin in human teeth contains porous dentin tubules, which play an important role during the formation of dentin, as the odontoblast process occurs within them [1]. After the dentin is formed, the opening of these tubules in healthy teeth are usually protected from exposure to external stimuli. These tubules can become exposed, however, through either gingival recession from early gingivitis or enamel loss from erosion. The tubules are filled with dentinal fluid and pass from the pulp chamber to the interfaces between the dentin-enamel junction (DEJ) and dentin-cementum junction (DCJ). When the tubules are exposed, external stimuli are able to affect the nerves in the pulp, in this case people can experience a short, sharp pain [2,3]. This is known as dentin hypersensitivity, and it often requires treatment to avoid further irritation and pain.

The overall aim of this project is to better understand the microstructural differences between healthy and hypersensitive teeth. X-ray micro computed tomography (μ-XCT) is a powerful tool for this type of project, as it can provide three-dimensional information about the orientation, size, and distribution of micron-scale features such as dentin tubules, including insight into abnormalities such as open tubules. In this project, we aim to use μ-XCT to track the tubules from the surface of the tooth, where the cause of sensitivity originates, all the way to their termination at the pulp chamber. This data can be correlated with data collected via other electron microscopy techniques to produce nano-structural information. This paper describes the application of μ-XCT within a multiscale correlative study of dentin tubules in human teeth.

All μ-XCT imaging for this project was performed using a ThermoFisher Scientific (TFS) HeliScan MicroCT. The Heliscan was operated in its high resolution/low flux mode, which uses a LaB_6 filament. Data were collected with a space filling trajectory [4]. A third human molar was used in this study, which was obtained from an ethic approved source at The Ohio State University College of Dentistry [5].

Initial μ-XCT imaging of the entire tooth resulted in an overview scan with voxel size of 6.99μm. To prevent cracking of the tooth due to it drying out during the μ-XCT scan, the tooth was wrapped in a piece of Kimwipe that had been dampened with sodium hypochlorite (NaOCl) and then wrapped in parafilm. Visual inspection of the reconstructed tomogram allowed for baseline analysis of the overall tooth structure, including assessment of any internal cracking or other damage present in the as-received sample. From this data set, the dentin and enamel were segmented as separate layers using the TFS software Avizo (Figure 1A).

Subsequently, the tooth was cut into four longitudinal cross-sections using a low speed rotary saw, with one interior piece selected for further analysis. All four pieces were stacked together and re-imaged, in this case with a 10.94μm voxel size. The main purpose of acquiring this second μ-XCT tomogram was to assist with the spatial alignment of the initial whole tooth tomogram with the higher resolution μ-XCT tomogram acquired in the next step from a small piece of the tooth. It also provided a check to ensure the pieces were not otherwise damaged by the cutting process. The sample was mounted so that its shortest edge was vertical during the scan, which reduced the total scan height and maximized the contact area between the sample and the post to which it was mounted. Loading the sample in this way also meant sacrificing some resolution, however, since the larger diameter meant it had to be moved farther from the x-ray source to keep it within the field of view. From this second μ-XCT tomogram, the selected piece was digitally isolated within Avizo and then registered to the first tomogram of the whole tooth (Figure 1B).

Since dentin tubules are three dimensional features, a key goal of this workflow is to capture three-dimensional information about the tubules as they pass from the pulp chamber to the DEJ or DCJ. To facilitate higher resolution μ-XCT scans, a small (1x2x2 mm^3) piece of the selected cross-section was further isolated from the sample. The location of the small piece was selected carefully to include a portion of the DEJ as well as the exposed surface of the pulp chamber. The sample was cleaned and dehydrated using a graded-series of ethanol baths. The sample was placed in a small container on some paper towel and once the ethanol had evaporated off the sample was glued to the end of a glass pipette for μ-XCT imaging.

A high-resolution region-of-interest μ-XCT scan was performed on the cut piece using a tube voltage of 60kV with scan time of 41 hours and 50 minutes. The reconstructed μ-XCT tomogram has a voxel size of 430 nm. Visual inspection of slices from the tomogram clearly show the presence of dentin tubules, which appear densely packed near the pulp chamber but become sparser near the DEJ (Figure 2). Visual inspection of the data also reveals superior image contrast in images acquired with a lower tube voltage of 60kV compared to images collected on previous samples at 100kV. This result suggests lower energy x-rays are preferable for extracting details from low density materials, despite the associated decrease in flux. Based on these results, further optimization of acquisition parameters appears unlikely to result in any significant improvement to image resolution or contrast.

Once fully segmented and made into 3D visualizations, these μ-XCT data will serve as a key part of a correlative multiscale workflow that can be used to identify and track microstructural differences between human teeth that are healthy compared to those experiencing dentin hypersensitivity. This will further our understanding of human teeth and provide useful insights into optimal hypersensitivity treatment protocols and be a useful data set for further modeling or experimentation [5].

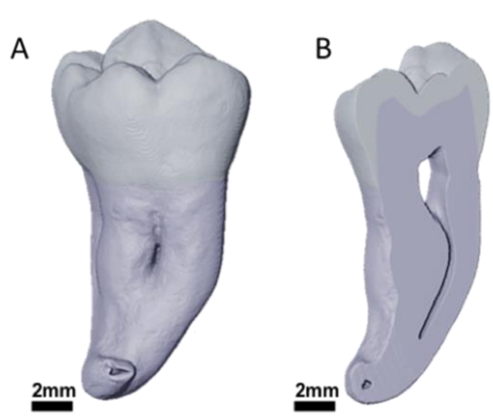

Figure 1. A) Volume rendering of segmented whole tooth tomogram. B) Volume rendering of segmented tomogram of slice of tooth after cutting.

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Figure 2. Vertical slice from the region of interest μ- XCT tomogram, with voxel size 430nm, where dentin tubules can clearly be resolved. The top of the tomogram is close to the pulp chamber, while the bottom of the tomogram encompasses a transition from dentin to enamel.

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