



# Visualization of the pulp chamber roof and residual dentin thickness by spectral-domain optical coherence tomography in vitro

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Received: 28 March 2018 / Accepted: 7 November 2018 / Published online: 13 November 2018  
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## Abstract

The aim of this study was to evaluate the ability of spectral-domain optical coherence tomography (SD-OCT) to display the roof of the pulp chamber and to estimate the residual dentin thickness (RDT) of the pulp complex. The roots of 20 extracted human molars were embedded in epoxy resin, and crowns were longitudinally sectioned in the mesial-distal direction, exposing the pulp chamber. The coronal part of the crown was removed up to an RDT to the pulp chamber roof of 2 mm. Samples were imaged by SD-OCT from coronal view and by light microscopy (LM) in the sagittal plane. Using a microtome, dentin was subsequently removed in four levels from the occlusal aspect in steps of 250  $\mu\text{m}$ . At each level, RDT was documented and measured by both methods. The data were compared (Spearman's rho correlation coefficient, Wilcoxon signed-rank test). Using OCT, the roof of the pulp chamber was first displayed at a maximum RDT of 1.94 mm. The minimal RDT that could be imaged by OCT was 0.06 mm. Values from both methods were strongly correlated ( $r$ , 0.83–0.95;  $p_i \leq 0.05$ ) and differed significantly for large RDTs (dentin levels 1, 2;  $p_i < 0.05$ ) but not for small RDTs (levels 3, 4;  $p_i \geq 0.226$ ). The roof of the dental pulp chamber could be already visualized by SD-OCT with a RDT of 1.94 mm. Therefore, the method could be a useful diagnostic tool during the preparation of deep dentin cavities and might help to preserve the integrity of the pulp chamber.

**Keywords** Diagnosis · OCT · Deep dentin cavities · Residual dentin thickness · Dental pulp · Pulp protection

## Introduction

The dental pulp is enclosed by enamel, dentin, and cementum, offering mechanical resistance and serving as barrier against the microbial oral environment. However, if this protective shell loses its structural integrity, e.g., due to caries, fractures, and open restoration margins, a passage of bacteria toward the pulp can occur [1]. Pulpal reaction to irritation is inflammation, which can possibly progress to pulp necrosis. Since the preservation of the pulpal health should be prioritized [2], the awareness and understanding of the physiological and pathological features of the dental pulp, as well as the consequences of treatment interventions by clinicians, are important.

Avoidable opening of the pulp chamber frequently leads to root canal treatment, thus contributing to an increase in

unnecessary endodontic treatments [3]. However, already small thicknesses of residual dentin are critical concerning the intensity of pulpal response. The literature indicates that a residual dentin thickness of a minimum of 0.5 to 1.0 mm is required to prevent irreversible alterations of the pulp, depending on the restorative treatment procedure [4–6].

Additionally, in caries therapy, an estimation of the remaining distance from the pulp chamber is necessary for the treatment of dental caries lesions to avoid an opening of the pulp. In this context, it could be shown that incomplete caries removal seems advantageous, particularly in proximity to the pulp, since it significantly reduces the risks of pulpal exposure and postoperative pulpal symptoms, compared with complete excavation [7]. Therefore, preferred therapies target the preservation of carious dentin near the pulp to avoid unnecessary pulp irritation or exposure [2]. However, the question arises regarding what the correct “end-point” would be to stop this incomplete excavation procedure without opening of the pulp. The opportunity to estimate the remaining (carious) dentin to the pulp would therefore be beneficial.

In general, accurate determination of the pulp location remains difficult [8]. Conventional techniques, such as X-

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ray, are limited due to the low sensitivity and spatial resolution of the technique and the lack of a three-dimensional display. Furthermore, repeated radiographic imaging to measure residual dentin thickness during tooth or cavity preparation cannot be recommended because of ionizing beam exposure [9, 10].

In the past, electrical resistance measurement, as a potential indirect tool to determine the residual dentin thickness, was examined and shown to be reproducible. However, although it corresponds with dentin-surface permeability, no correlation exists with dentin thickness [11, 12].

Another method for investigating residual dentin thickness *in vitro* and *in vivo* is to determine the accuracy of the pulse-echo mode with an ultrasonic micrometer (USG) [13]. It could be shown that USG was limited in the detection of residual dentin thickness because of nonparallel surfaces reflecting the signal away from the transducer, thus minimizing the detectable signal amount, so ultrasound is primarily used for the assessment of soft tissue lesions or the gingiva, and it is not suitable for hard tissue detection.

In overall, to avoid accidental openings, a reliable device is desirable for measuring the remaining dentin thickness and displaying the three-dimensional extent of the pulp.

A promising technique to visualize the pulp complex is optical coherence tomography (OCT) [14–17]. This method is mainly used in ophthalmology for the diagnosis of pathological changes in retinal layers and the optic nerve [18]. Other medical fields of application include dermatology [19] and cardiology [20]. Images of human dental tissues were first presented by Colston et al. [17] and Feldchtein et al. [21], followed by numerous other authors [22, 23]. The possibility of imaging the dental pulp from rats' teeth was reported by Kauffman et al. [16]. OCT is suitable for clinical application, since it is noninvasive and avoids radiation-induced tissue damage. High-resolution cross-sectional images, generated from series of laterally adjacent depth scans at high speed, are self-explanatory [24, 25].

For the purpose of pulp imaging, it was shown that 1300 nm operates more effectively and is visible to a greater penetration depth with a reduction in absorption and scattering coefficients of the dentin [26]. Therefore, our study targeted OCT as an imaging method for the relevant dentin-pulp borderlines (roof of the pulp chamber including the pulp horns) during tooth preparation or caries removal using the center wavelength 1310 nm. There have been few studies focusing on the comparison of OCT, X-ray microtomography (micro-CT), and cone beam CT [14, 15, 27]. However, until now, OCT was not validated against the reference standard of light microscopy based on cross-sections. Therefore, the present study first evaluated the ability

of OCT to display the roof of the pulp chamber for estimating the residual dentin thickness to the pulp complex and second correlated the findings with light microscopy.

## Materials and methods

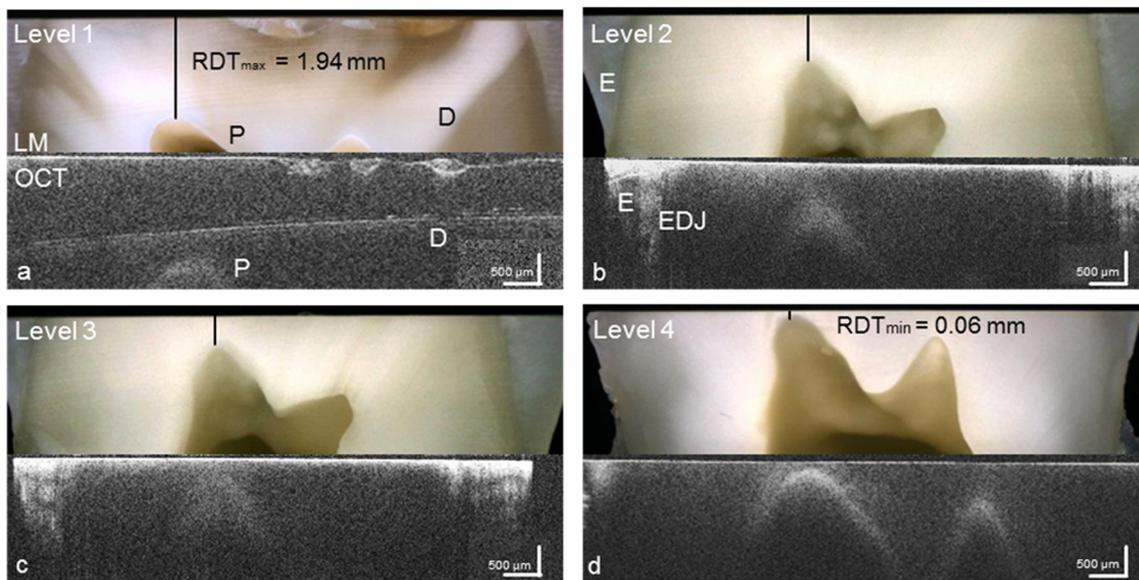
The study was conducted in accordance with the Declaration of Helsinki, and the protocols were approved by the Ethics Committee of the University of Leipzig. The extracted teeth were used on the basis of patients' approval (informed consent, protocol no. 299-10-04102010).

## Specimen preparation and imaging

Twenty extracted human molars free of caries or fractures were included. Immediately after extraction and before examination, the teeth were stored in a 0.5% chloramine solution at 4 °C. For examination, teeth were selected randomly. The roots of the teeth were embedded in epoxy resin (Stycast 1266, Emerson & Cuming, Westerlo, Belgium). Using a microtome (Leitz 1600, Ernst Leitz GmbH, Wetzlar, Germany), the crowns were longitudinally sectioned (mesial-distal) exposing the pulp chamber, and the coronal parts of crowns were removed up to a remaining distance to the pulp of 2 mm. Sagittal planes were imaged by light microscopy (x 1.25, Stemi 2000—stereo microscope, Carl Zeiss Microscopy GmbH, Jena, Germany), followed by SD-OCT from the coronal view (2D image stacks; Telesto SP5; Thorlabs GmbH, Dachau, G, Fig. 1). Residual dentin thickness (RDT) was reduced from the occlusal aspects in steps of  $250 \pm 25 \mu\text{m}$ . The smear layer produced by the microtome was removed with 10% NaOCl for 30 s. To prevent dehydration during examinations, the samples were consistently rewetted using physiological saline solution and were dried using a cotton pellet to leave samples moist with no visible water droplets (controlled hydrated condition). Each step of dentin removal was imaged by both methods, and the RDT was determined by representing levels one to four using SD-OCT (ImageJ v1.45, Wayne Rasband, National Institutes of Health, Bethesda, MD, USA) and LM (AxioVision Rel. 4.8, Carl Zeiss Microscopy GmbH) (Fig. 1).

## OCT imaging technique

OCT is based on low-coherence interferometry. In this study, an SD-OCT unit was used. The technical specifications of the SD-OCT system were as follows: the center wavelength was  $1310 \pm 100 \text{ nm}$ . The axial/lateral resolution of the system was  $7 \mu\text{m}/15 \mu\text{m}$  in air, which corresponds to  $5 \mu\text{m}/15 \mu\text{m}$  in tooth



**Fig. 1** Light microscopic and corresponding OCT cross-sectional images of pulp chamber roofs for dentin levels 1–4 (a–d) showing maximum (a) and minimum (d) RDT to the pulp. E, enamel; D, dentin; EDJ, enamel-dentin junction; P, pulp

tissue. The acquired 2D OCT image size was  $512 \times 512 \times 512$  pixels corresponding to a maximum field of view of  $12.0 \text{ mm} \times 8.5 \text{ mm} \times 3.0 \text{ mm}$ . The A-scan line rate was 91 kHz, the acquisition speed 177 frames/s and the sensitivity  $\leq 106 \text{ dB}$  (Table 1). The functional principles and device parameters are shown in Fig. 2. The light of the broadband source is split into a sample and a reference beam (Michelson interferometer configuration). The light beam scans the sample surface point by point and line by line with a penetration depth of up to 2.0–2.5 mm in enamel and dentin. The back reflected light from the sample (sample arm) and from the reference mirror interfere with each other, and the resulting signal (spectrum) is recorded by a spectrometer. After a Fourier transform of the spectrum, a depth profile of backscattering along a line perpendicular to the object surface is generated (A-scan). A series of laterally adjacent A-scans generates a 2D cross-sectional image (B-scan), and a series of 2D images creates a 3D image stack. Image contrast arises in areas with structures of different refractive indices, such as the dentin-pulp interfaces, gaps, bubbles, material cracks, or porous areas in carious lesions [28].

### Calculation of RDT

Using OCT on the basis of an average refractive index of 1.54 [27, 29–31], the RDT was determined for thickness levels one to four, as well as in parallel by LM (Fig. 1).

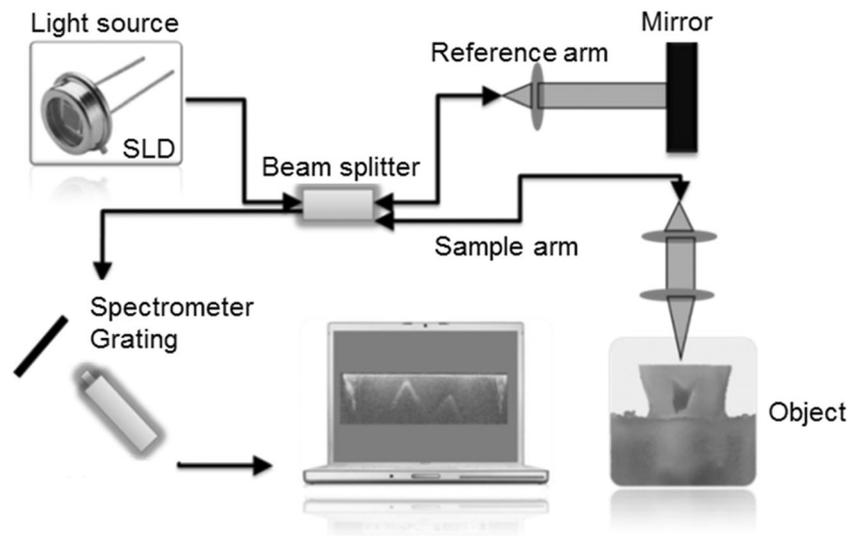
### Statistical analysis

RDT values, determined using OCT and LM, were submitted to the Kolmogorov-Smirnov and Shapiro-Wilk tests, which indicated the comparison of both methods using Wilcoxon's test. The correlation between paired values for levels one to four of RDT was calculated (Spearman's Rho,  $r_s$ ). The significance level was set at  $\alpha = 0.05$ . Statistical analysis was performed using SPSS Statistics software (SPSS Statistics for Windows 24.0, Armonk, NY, USA: IBM Corp.), and the diagrams were created using GraphPad Prism software, version 5 (GraphPad Software Inc., La Jolla, CA, USA).

**Table 1** Parameters of the Telesto SP5

Center wavelength	1310 nm
Bandwidth	100 nm
A-Scan line rate	91 kHz
Axial (depth) resolution (air)	$< 7 \mu\text{m}$
Lateral resolution	$15 \mu\text{m}$
Sensitivity	$\leq 106 \text{ dB}$
A-Scan average	1
Maximum pixels per A-scan	512
Frames (B-scans)/s	177
Power on sample	3 mW
Maximum field of view	$12.0 \text{ mm} \times 8.5 \text{ mm} \times 3.0 \text{ mm}$ ( $512 \times 512 \times 512$ pixels)

**Fig. 2** Principle of function of SD-OCT



## Results

Using OCT, the margins of the pulp chamber were displayed for the first time at a maximum RDT of 1.94 mm, whereas the minimal RDT was 60  $\mu\text{m}$ . Table 2 shows the mean values of RDT using LM and OCT for the four levels, as well as a strong and statistically significant correlation between LM and OCT (Fig. 3). The difference between  $\text{RDT}_{\text{LM}}$  and  $\text{RDT}_{\text{OCT}}$  diminished with decreasing RDT (Table 2), whereby  $\text{RDT}_{\text{LM}}$  was significantly lower at levels one and two.

## Discussion

The overall aim of our research was to evaluate the ability of OCT to display the roof of the pulp chamber to estimate the residual dentin thickness of the pulp complex and to correlate the findings with light microscopy. It could be shown that the roof of the pulp chamber was imaged using OCT already at a maximum RDT of 1.94 mm and that both methods, OCT and LM, were strongly correlated. This topic of imaging the pulp complex and/or residual dentin thickness using OCT has

already been discussed [10, 14, 15, 27, 32]. Nevertheless, this study was the first systematically comparing the OCT method with light microscopy as the laboratory gold standard.

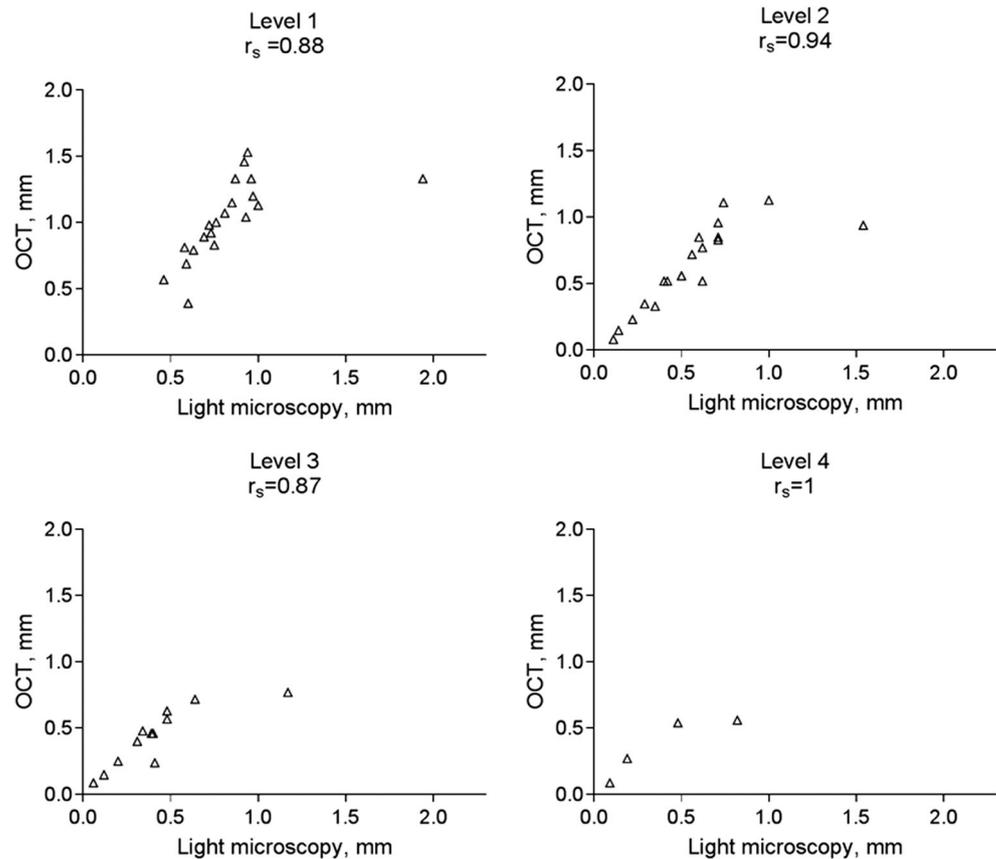
In general, our results were corroborated by previous findings, showing that the remaining dentin and margins of the pulp chamber can be visualized by OCT. Fujita et al. [15] aimed to investigate the maximum RDT and optical properties of dentin close to the pulp using ten caries-free human molars with (swept-source) OCT with a center wavelength of 1330 nm. The results were compared with micro-CT measurements. The authors demonstrated that OCT was only effective for measuring 1 mm or less of residual dentin thickness. Fonséca et al. [14] investigated RDT using SD-OCT with central wavelengths of 1280 and 850 nm and with cone beam volumetric tomography (CBVT). They showed that OCT with a wavelength close to 1280 nm presented greater penetration depth in the dentin than using 850 nm, as expected from scattering and absorption coefficients. Sinescu et al. [32] investigated residual dentin thickness in longitudinal tooth sections after cavity preparation using time-domain (TD) OCT of six teeth. TD-OCT images showed a large number of image artifacts influencing thickness measurement. Elimination of

**Table 2** Residual dentin thickness measured using light microscopy and OCT. Difference between methods (Wilcoxon,  $p_i$ ) and correlation of both

Level of imaging	$\text{RDT}_{\text{LM}}$ mm	$\text{RDT}_{\text{OCT}}$ mm	Wilcoxon's test, $p_i$	Spearman's Rho/ $p_i$
First	$0.84 \pm 0.29$	$1.02 \pm 0.29$	0.004	$0.83/<0.001$
Second	$0.57 \pm 0.34$	$0.63 \pm 0.32$	0.019	$0.94/<0.001$
Third	$0.42 \pm 0.29$	$0.46 \pm 0.23$	0.226	$0.83/0.001$
Fourth	$0.35 \pm 0.35$	$0.30 \pm 0.25$	0.815	$0.95/0.051$

Mean  $\pm$  standard deviation

**Fig. 3** Spearman's rho correlation ( $r_s$ ) between OCT and light microscopy related to RDT for levels one to four



artifacts is necessary to evaluate the images. Spatial resolution was 15  $\mu\text{m}$  and the penetration depth 1 mm. Due to limited penetration depth, the evaluations in this study suggested an RDT safety limit of 0.5 mm [32]. In a study by Majkut et al. [27], validation of OCT against micro-CT for the evaluation of RDT was performed using ten molars. The authors found a strong correlation between the used techniques and measured RDT after removal of caries-infected tissue up to 1.5 mm in thickness. In our study, an RDT up to 1.94 mm could be visualized, however showing caries-non-affected tooth samples.

Preliminary studies investigated the importance of the residual dentin thicknesses as a pulp-protective barrier. They proposed different values of dentin thicknesses as pulp-protecting barriers. Schmalz et al. suggested a dentin layer of 0.5–1 mm without pulpal reactions after application of dental adhesive materials and composites in plain cavities [33]. In this study, dentin was investigated as a protective barrier for the diffusion of different substances segregated by dental materials. It was shown that the permeability properties of dentin increase exponentially with decreasing thickness. During dental cavity preparation, odontoblast cell activity is disturbed. A

dentin layer of more than 0.5 mm might be sufficient so that no cell injury occurs, and the pulp is not damaged [33].

Hargreaves et al. [34] and Pashley [35], for example, assumed within the scope of dentin permeability, a minimum dentin thickness of 0.5 mm serving as protective barrier for the pulp. Residual dentin less than 0.5 mm leads to an increase in pulpal inflammation. Our findings demonstrated that OCT is able to image dentin thickness without an opening of the pulp, helping the dentist to perform pulp-protective actions. Stanley suggested a residual dentin thickness of 2 mm as an impermeable barrier to different toxic dental materials [36]. In an investigation by Camps [37] studying three groups with different dentin thicknesses, pulpal inflammation and injury were decreased with residual dentin thicknesses of 0.5–1 and 1 mm, respectively.

About et al. [38] investigated the degree of pulpal inflammation depending on the thickness of the dentin barrier. He found that residual dentin is highly relevant for mediating the pulpal inflammatory response. A decreased dentin barrier thinner than 0.25 mm leads to severe inflammatory activity due to the influence of bacteria. However, bacteria alone caused a moderate pulpal response in cavities with RDT >

0.25 mm. He could demonstrate that cavity restoration materials play an important role as well. Adhesive-bonded composite, for example, leads to an increase in pulpal inflammation independent of the presence or absence of bacteria. Etching substances enhance dentin permeability, leading to an increase in pulpal reactions [38]. In this context, with a residual dentin thickness of 1.94 mm, we measured our maximum residual dentin thickness by OCT, which seemed sufficient to ensure that the pulp would not be affected during dental treatment and that OCT would be sufficiently sensitive to detect the pulp at a residual dentin thickness greater than the authors above recommend, reducing pulpal response.

The penetration depth of OCT in biological structures is 2–3 mm on average [39], for teeth penetration depths of 2–2.5 were proposed [22]. In our study, the roof of the pulp chamber was imaged by OCT at a maximum RDT of 1.94 mm. However, imaging depth depends not only on the device attributes and specifications but also on the optical properties of the tissue structure. Signal attenuation through sound dentin is greater than that of sound enamel because the dentin contains more than 50 vol% of organic structures and fluid, which scatter and absorb the near-infrared light. Moreover, the presence of tubules and their orientation have great influences on the scattering and attenuation of light [30, 40, 41]. In this context, the refractive index ( $n$ ) is an important optical parameter of light propagation through biological tissues, including teeth. While the values measured by light microscope are supposed to be real values indicating the physical dimensions of the structure, the depth values measured by OCT are affected by the optical path length within the dentin and cannot directly indicate the physical dimension without prior knowledge of the refractive index [42, 43]. It has been reported that the dentin refractive index depends on its composition (e.g., mineral content) and structure, such as dentinal tubule density and orientation [30, 31]. Therefore, the refractive index varies in each region of the dentin. There have been several studies calculating the refractive indices of dentin using OCT [27, 29–31]. In an *in vitro* study by Meng et al. [29], the refractive index was measured for human dentin, and a value of 1.54 was obtained. Using OCT, Hairiri et al. [30] investigated the optical properties of human dentin in relationship to the structural orientation of the dentinal tubules. In their study, an average refractive index of 1.55 for dentin was calculated. In another study by Hairiri et al. [31] aiming to elucidate the relationship between the local refractive index and the mineral content of dentin, the authors found that the  $n$  ranged from 1.43 to 1.57. Their findings coincided with results of Majkut et al. [27], concluding that a refractive index value of 1.54 could be applied to convert optical thickness measurements of RDT to actual values over a wide range of readings. Our own unpublished data confirm these findings. Therefore, in our study, we used OCT on the basis of the refractive index of 1.54. In our study, the difference between  $RDT_{LM}$  and

$RDT_{OCT}$  diminished with decreasing RDT, whereby  $RDT_{LM}$  was significantly lower at levels one and two. This effect could be caused by light scattering in the samples and the oblique orientation of dentinal tubules from the pulp to the tooth periphery [30].

To prevent dehydration, directly after extraction and before examination, the samples were stored in 0.5% chloramine solution at 4 °C. During examination, the samples were consistently rewetted using physiological saline solution and dried using a cotton pellet to leave the samples moist with no visible water droplets (controlled hydrated condition). The results of a study investigating the influence of hydration of the enamel tooth surface on OCT imaging showed that tooth substrate hydration conditions influence OCT imaging. In their study, the OCT signal intensity was greater under wet conditions [44]. Moreover, the optical properties of tissue changed when the dentin became dehydrated. As shown for enamel and even for dentin, an increased-intensity backscattered signal at the dentin/air interface might be created by the dehydration of dentin. Furthermore, the dehydration of dentin might also decrease the relative translucency of the wet dentin substrate.

In our study, the pulp was destroyed by sectioning the teeth, and OCT imaging was performed without this water-containing tissue. Therefore, in *in vivo* situations, the pulpal tissue might affect the OCT image because of less scattering and greater absorption of the light by pulpal tissue, leading to a more decreased signal at the dentin-pulp interface.

In this study, RDT was imaged and measured using caries-free teeth. For caries therapy, it could be shown that incomplete caries removal seems advantageous, particularly in proximity to the pulp, since it significantly reduces the risks of pulpal exposure and postoperative pulpal symptoms, compared with complete excavation [7]. However, caries-infected and/or caries-affected dentin might have an impact on the imaging of the pulp chamber and RDT using OCT. Carious lesions vary in mineral density and occur as white OCT signals with underlying shadowing, which might increase the difficulty in pulp detection for thicker dentin above the pulp because light is absorbed, and the area beneath demineralized regions is not visible [45]. The results of a study by Majkut et al. [27] showed that pulp horns could be visualized and RDT measured up to 1.5 mm in thickness. However, the study was performed on six teeth after complete excavation of caries-affected dentin using a caries-detector dye. Therefore, experiments should also be performed on carious-infected dentin.

## Conclusion

The dental pulp chamber roof could be visualized by SD-OCT, and remaining dentin could be imaged within a

thickness range serving as a sufficient barrier against the microbial oral environment, as well as estimating the morphology of the pulp chamber. Therefore, this technique might help to prevent iatrogenic opening of the pulp chamber during clinical procedures, such as crown preparation, preparation of deep dentin cavities, or deep caries excavation procedures. Furthermore, OCT can contribute to maintaining the dental pulp function and vitality of teeth and could obviate root canal treatment or tooth extractions.

**Acknowledgements** The authors would like to acknowledge Thorlabs GmbH, Dachau, Germany for providing the OCT.

### Compliance with ethical standards

The study was conducted in accordance with the Declaration of Helsinki, and the protocols were approved by the Ethics Committee of the University of Leipzig. The extracted teeth were used on the basis of patients' approval (informed consent, protocol no. 299-10-04102010).

**Conflict of interest** The authors declare that they have no conflicts of interest.

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