RESEARCH REPORTS
Biomaterials & Bioengineering

A. Kienle*, R. Michels, and R. Hibst

Institut für Lasertechnologien in der Medizin und Meßtechnik, Helmholtzstr. 12, D89081 Ulm, Germany; *corresponding author, alwin.kienle@ilm.uniulm.de

J Dent Res 85(10):955-959, 2006

Magnification—a New Look at a Long-known Optical Property of Dentin

ABSTRACT

Light propagation in human dentin exhibits a strong directional dependence featuring the long-known optical magnification property. We hypothesized that this anisotropic effect is caused by multiple scattering at the dentin tubules, and not by fiberoptic effects, as had been previously assumed. We performed measurements of the transmitted intensity from dentin disks and compared them with Monte Carlo simulations of light propagation in dentin, considering the scattering by the tissue's microstructure. We found that the optical anisotropy of dentin can be fully explained with this model. We concluded that the magnification property of dentin is due to multiple scattering by the dental microstructure.

KEY WORDS: (AQ)

Received December 22, 2005; Last revision May 23, 2006; Accepted June 14, 2006

INTRODUCTION

An understanding of light propagation in biological tissues is essential for the effective applications of light in medical diagnostics and therapeutics. In dentistry, an understanding of light propagation in the tooth is important not only for therapeutic laser applications or for diagnosis—for example, of caries or pulp vitality—but also for the precise matching of the visible appearance of restoration materials and teeth.

Recently, investigations of the dependence of light propagation in tissue on its microstructure have gained increasing attention, due to possible applications in the early detection of tissue alterations (Perelman *et al.*, 1998; Backman *et al.*, 2000; Mourant *et al.*, 2002). For example, it was found that biological tissue which has an aligned microstructure shows pronounced anisotropic effects of remitted or transmitted light (Nickell *et al.*, 2000; Dagdug *et al.*, 2003; Heino *et al.*, 2003; Kienle *et al.*, 2003, 2004; Sviridov *et al.*, 2005). Here, the term 'anisotropic' states that the light propagation in the tissue is different for different directions relative to the aligned microstructure.

In the case of dentin, it has long been known that anisotropic light propagation is especially pronounced. Three decades ago, it was shown that disks of dentin taken from extracted human teeth "demonstrated the optical property of the magnification/reduction effect" (Walton *et al.*, 1976). For an explanation of the magnification effect, it was proposed that the image transmission is a 'lightpipe or fiberoptic phenomenon', due to the arrangements of the dentin tubules (Walton *et al.*, 1976). In the following decades, several authors investigated the light propagation in dentin as such (Altshuler, 1995; Vaarkamp *et al.*, 1995), or to measure its optical properties (Zijp and ten Bosch, 1993; Fried *et al.*, 1995), to explore applications of laser Doppler measurements of the blood flow in the pulp (Ramsay *et al.*, 1991; Vongsavan and Matthews, 1993; Odor *et al.*, 1996), or for measurements of the dentin's microstructure by optical coherence tomography (Wang *et al.*, 1999). Although it was found that the tubules are the main scatterers in dentin, the anisotropic effects were usually explained as waveguide or optical fiber effects. It was reasoned that total reflection, which is necessary for optical fiber effects, occurs in the peritubular dentin, due to its high refractive index, compared with its neighboring media, the tubules and the intratubular dentin.

In this study, using a Monte Carlo model that considers the microstructure of biological tissue (Kienle *et al.*, 2003, 2004), we examined the hypothesis that the magnification effect in dentin is caused by multiple scattering by the dentin tubules, and not by optical fiber or waveguide effects. The clarification of this hypothesis is not only of fundamental theoretical importance, but also has a significant impact on a variety of optical methods in dentistry. For example, the fiber optic theory allows for only the calculation of the light propagation in dentin, if the light is coupled into the fibers. All light incident at larger angles than the aperture of the fibers cannot be described by this theory. Our method, however, enables the light propagation to be calculated with no such restriction.

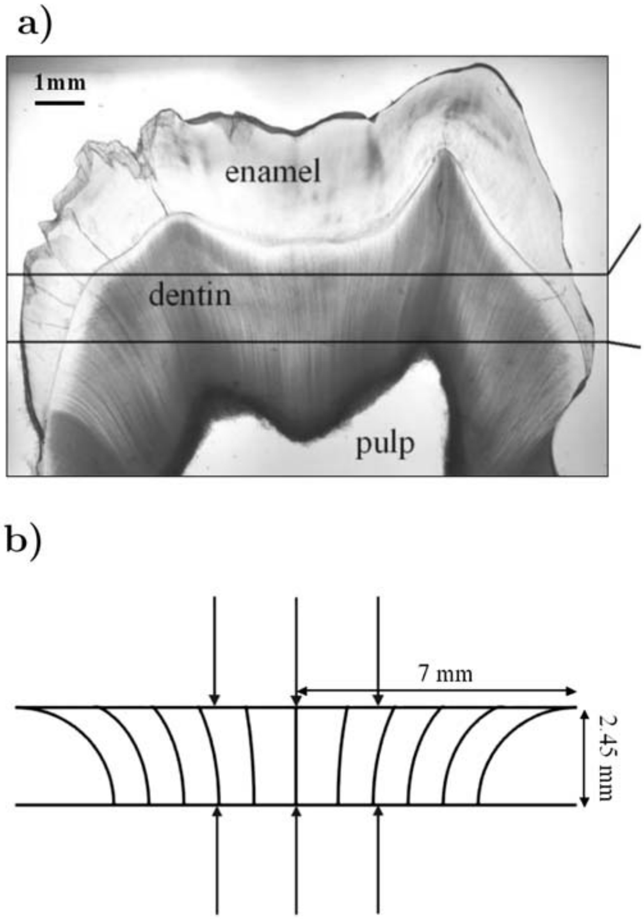

Figure 1.(AQ) (a) Microscopic image of a human tooth showing the course of the tubules in the dentin. The positions of the disks cut from the coronal dentin are also shown. **(b)** Model of the course of the tubules used in the simulations (illumination with 3 laser beams either in the occlusal-to-pulpal or in the pulpal-to-occlusal direction).

MATERIALS & METHODS

Sample Preparation and Experimental Set-up

Dentin disks were prepared from the coronal dentin of extracted human molars (Fig. 1a), used according to a protocol approved by the University of Ulm. Informed consent for the use of human subjects was obtained. The teeth were embedded in cold-curing resin, and the disks were cut with a diamond saw. Six disks having a thickness between 1 and 3 mm were manufactured.

For a qualitative demonstration of the effect of optical magnification, we repeated the measurements performed by Walton *et al.* (1976), with slight modifications. A dentin disk with 1-mm thickness was placed on a grid with a quadratic line pattern illuminated by a white light source (Xe lamp). We obtained images of the transmitted light by illuminating the disk in the occlusal-to-pulpal direction and in the pulpal-to-occlusal direction.

To investigate the anisotropic light propagation more quantitatively, we illuminated the disks by 3 collinear beams of a HeNe laser having the same intensity (wavelength λ = 633 nm; beam diameter, 0.65 mm). The beams were aligned in one row, separated from each other by 2 mm. The centers of the 3 beams were approximately in the centers of the disks (Fig. 1b). The transmitted light was imaged onto a cooled 16-bit CCD Camera.**(AQ)** Then, the disks were turned by 180°, illuminated at the corresponding positions at the other side of the disks by the 3 beams, and the transmitted light was once again imaged by the CCD Camera. During the imaging process, the teeth were kept in water to avoid scattering changes due to drying of the sample.

Monte Carlo Simulations

The Monte Carlo method is a standard technique for calculating the light propagation in biological tissues. It considers multiple scattering, but does not account for interference effects. Usually, it is assumed that the involved tissues have isotropic optical properties, which means that they do not depend on the incident direction of the light beam. However, for biological tissues, which have an aligned tissue structure, as, for example, dentin, the anisotropic light propagation has to be considered (Kienle *et al.*, 2003). The main scatterers in dentin are the tubules. For the calculation of tubular scattering, they can be regarded as infinitely long cylinders, because their length is much larger than their diameter and the light wavelength. We implemented the scattering function derived by solving Maxwell's equations for an infinitely long cylinder (Yousif and Boutros, 1992) and applying them to the Monte Carlo method (Kienle *et al.*, 2003). In our code, the concentration and the direction of the tubules can be arbitrarily chosen.

Using an optical microscope, we studied the course of the tubules from the pulp to the enamel-dentin junction of several thin tooth slabs (Fig. 1a). We found that a mathematical distribution described approximately the average distribution of the tubules in the dentin (Fig. 1b). At the center of the disk, the tubules were assumed to be perpendicular to the planes of the slab. At a distance of 7 mm from the center, we approximated the course of the tubules as a quarter circle. For the region in between, we assumed that the course of the tubules continuously alters from the quarter circle at the edge to the vertical line in the center of the disk (Fig. 1b). The concentration of the tubules was chosen to be 30,000 mm^{-2}, a typical value found in dentin. The diameter of the tubules was assumed to be 2 μm. The refractive indices inside and outside the tubules were chosen as 1.33 and 1.52, respectively (Zijp and ten Bosch, 1993).

Besides the tubules, the influence of collagen fibers, which form a network between the tubules, must be considered in any calculation of light propagation in dentin. We assumed that the collagen fibers were randomly aligned, so that the light propagation due to the fibers was isotropic. On the basis of the involved refractive indices and concentrations of the collagen fibers, we estimated the reduced scattering coefficient as $\mu'_s = 0.5$ mm^{-1}. A Henyey-Greenstein phase function with an anisotropy factor of $g = 0.8$ was used in the Monte Carlo code. The absorption coefficient was set to $\mu_a = 0.01$ mm^{-1}, which is typical for a low-absorbing biological tissue in the red-wavelength range. For the refractive index of the disks, we used $n = 1.5$ (dentin) and, for the surrounding medium, $n = 1.33$ (water).

RESULTS

We obtained magnified and demagnified images by illuminating a dentin disk, which was laid on a quadratic grid, with a white-light source (Fig. 2). The disk was illuminated in the occlusal-to-pulpal direction (Fig. 2a) and in the pulpal-to-occlusal direction (Fig. 2b). The grid was demagnified in the first case and magnified in the latter.

The imaging of the 3 laser beams transmitted through the dentin disks was performed on 6 samples. In general, we found that the thicker the samples, the larger the magnification and demagnification effects. However, qualitatively, the same effects could be seen for all samples. Thus, in the following, we present only the measurements for 1 dentin disk having a thickness of 2.45 mm. The transmitted light was imaged with the CCD Camera for cases where the disk was illuminated from the occlusal-to-pulpal direction (Fig. 3a) and for reverse illumination (Fig. 3b). The 3 laser beams could be clearly resolved in the images, and the distance between the beams decreased from 2 mm to about 1.5 mm (Fig. 3a) and increased from 2 mm to about 2.6 mm (Fig. 3b). Analogously, the diameters of the transmitted beams decreased and increased (Figs. 3a, 3b, respectively). In addition, we observed that the intensity of the beams incident from the occlusal-to-pulpal direction was higher than for the reverse direction. Due to the heterogeneity of the course of the tubules in the dentin, the transmitted light patterns of the 3 laser beams were no longer in a row. Three of the 6 samples investigated had a similar thickness: 2.13 ± 0.28 mm. For these samples, we calculated the distance of the transmitted beams for the occlusal-to-pulpal direction as 1.36 ± 0.14 mm, and that for the reverse direction as 2.71 ± 0.24 mm, confirming that the magnification effect was similar for disks with similar thickness.

We used the Monte Carlo method to calculate the transmitted light from a disk having a thickness of 2.45 mm, using the geometrical arrangement of the tubules described above (Figs. 3c, 3d). The Monte Carlo program calculates the probability that an incident photon is transmitted *per* unit area at a certain position. Thus, the units of the contour lines of the transmitted light are given in mm^{-2}. The transmitted data from the measurements in Figs. 3a and 3b were normalized to those from the Monte Carlo simulations, which means that they were multiplied by the same factor. All 3 main effects found in the experiment could also be seen in the Monte Carlo simulations. In addition, within the heterogeneity of the disk sample, the experimental and theoretical data also agreed quantitatively. For example, the transmitted pattern from the middle of the beams had a higher intensity than those of the other 2 beams.

The described effects are illustrated as follows. The scattering by the tubules can be described as the scattering by a long cylinder. Solving Maxwell's equation for an infinitely long cylinder demonstrated that the incident light was scattered solely in a cone around the cylinder axis (Bohren and Huffman, 1983) (Fig. 4a). If the angle between the direction of the light beam and that of the tubules, ξ, was small, the light was scattered in a cone with a small apex angle (also ξ). The next scattering took place by a tubule nearby; again, the light was scattered in a direction close to that of the tubule. Thus, the photons propagated approximately along the directions of the tubules, due to multiple scattering (Fig. 4b).

DISCUSSION

Inspired by the findings three decades ago (Walton *et al.*, 1976), and driven by the emerging field of investigation of the dependence of light propagation on the microstructure of biological tissues, we examined the physical cause for the magnification properties of dentin disks cut horizontally from the crown of a human molar. We first repeated the experiments by Walton *et al.* (1976) using a grid that consisted of squares (2D grid) instead of a grid consisting of lines (1D grid). Similar to the earlier results, we found a magnification of the grid, when the dentin disk was illuminated from the pulpal-to-occlusal direction, and a demagnification, when the illumination direction was reversed. We noted that the 2D grid gave more information on the inhomogeneity of the magnification effect than did the 1D grid.

a)

b)

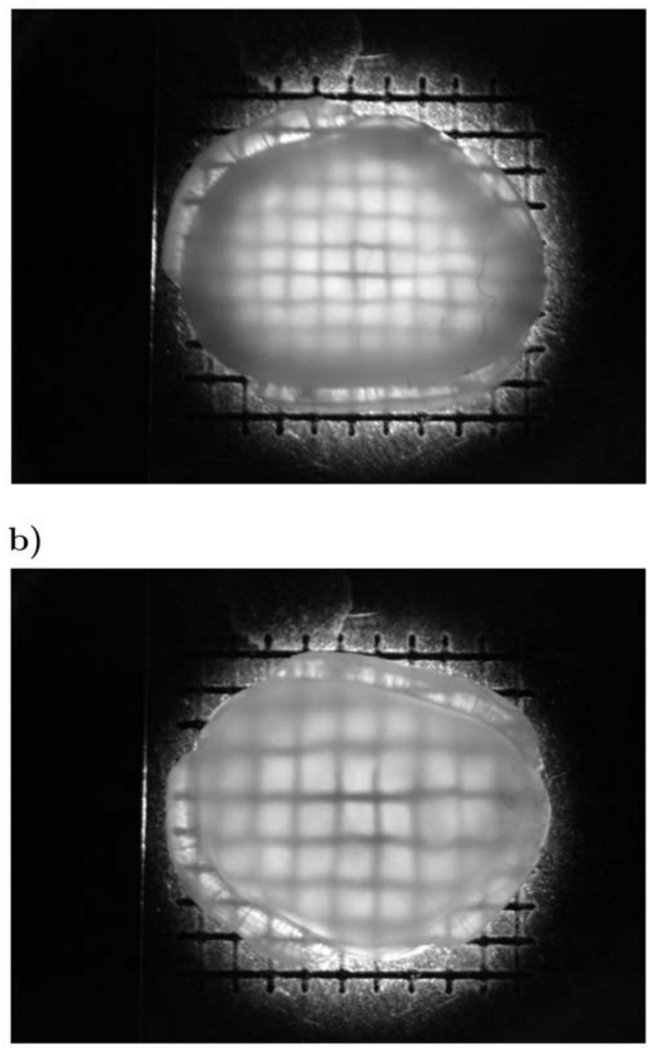

Figure 2. Image of a dentin disk (thickness: 1 mm) placed on a grid with a quadratic line pattern. **(a)** Occlusal-to-pulpal illumination; **(b)** pulpal-to-occlusal illumination. The dentin is partly surrounded by enamel. No magnification effects can be seen in the enamel. The distance between the lines of the grid outside the tooth is 1 mm.

In addition, we noted that, near the enamel-dentin junction, there was almost no light transmitted from the dentin in the case of the occlusal-to-pulpal illumination. This non-illuminated region became larger the thicker the dentin disks. This should be kept in mind for therapeutic and diagnostic applications, when the tooth is illuminated from the occlusal direction—for example, for dental caries detection or vitality studies of the pulp.

As had been found three decades ago, the microstructural reason for the magnification property of dentin is the

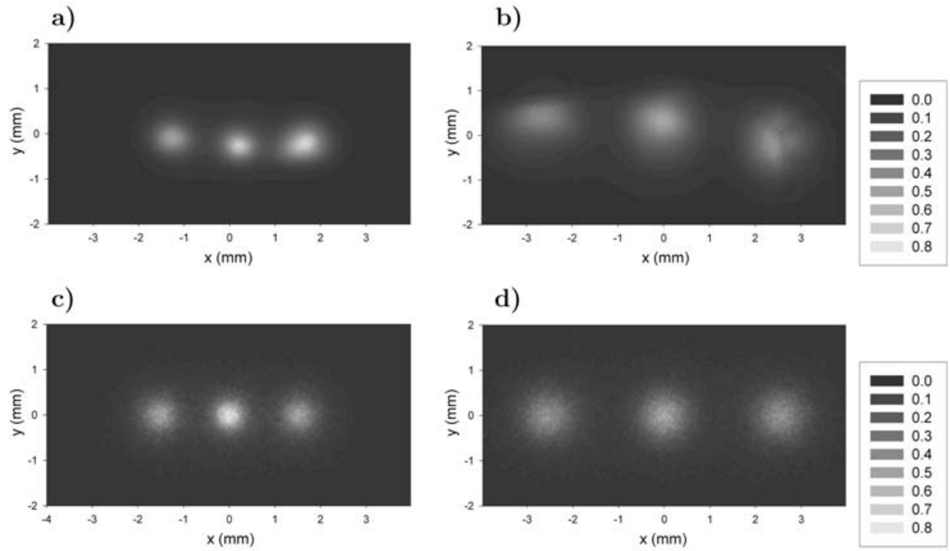

Figure 3.(AQ) Upper row: Image of the transmitted light (normalized intensity, see text) from a dentin disk, which was illuminated by 3 laser beams. Incident direction: **(a)** occlusal-to-pulpal; **(b)** pulpal-to-occlusal. **Lower row:** Monte Carlo simulations of the transmitted light (units: mm^{-2}) from a dentin disk illuminated by 3 laser beams. Incident direction: **(c)** occlusal-to-pulpal; **(d)** pulpal-to-occlusal.

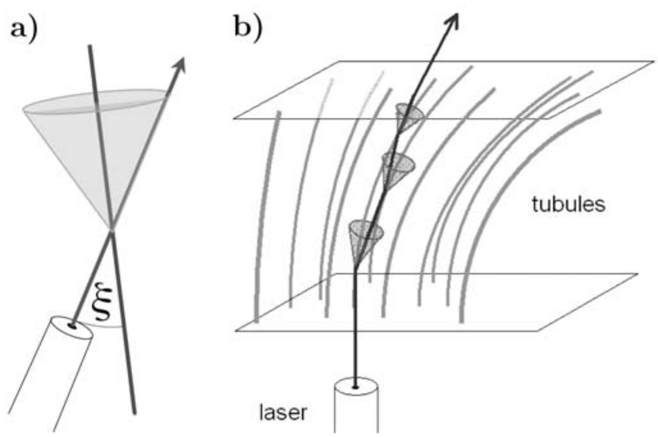

Figure 4.(AQ) **(a)** Light which is incident onto an infinitely long cylinder is scattered in a cone around the cylinder. **(b)** Principle of the magnification effect in dentin: The light is multiply scattered by the tubules approximately along the tubules' directions.

divergence of the dentin tubules (Walton *et al.*, 1976). They and the authors of subsequent publications on this subject**(AQ)** usually assumed that the optical cause of the magnification effect was a wave-guide or fiber-optic phenomenon. In contrast to this, we have showed, in this study, that the magnification can be fully explained by multiple light-scattering by the tubules, whereby light is guided along the tubules due to scattering by the tubules.

In addition to the anisotropic optical properties due to the scattering by the tubules, we took into account the scattering by the collagen fibers in the dentin. We assumed a uniform distribution of the directions of the collagen fibers. Thus, we considered the scattering contribution of the collagen by isotropic optical properties. With this modification, we could explain not only the transmitted but also the remitted light from the dentin disks (not shown). Without the scattering contributions of the collagen fibers, almost no light would be remitted from the dentin disk, due to the forward-scattering pattern of the tubules.

To confirm our findings, we used an optical microscope to investigate the transmitted light from thin disks cut horizontally from the coronal dentin (thickness between 20 μm and 1 mm). We could not find any light fiber effects, which should have been seen as light regions in the peritubular dentin. Instead, especially for thick samples, the tubules and the peritubular dentin appeared darker than the surrounding dental tissue. In addition, regarding the three-dimensional structure of the tubules, the possibility of a fiber-optic effect seems to be low, due to its inhomogeneity and the ramifications of the tubules. Finally, with the Monte Carlo method, which considers the microstructure of dentin, it is, in principle, possible to calculate light propagation through the whole tooth, if a similar model is developed for the dental enamel (and the pulp). Preliminary investigations showed that the presented model can also be used to calculate light propagation in enamel. The quantitative understanding of whole-tooth optics would have a strong impact on a variety of light applications in dentistry—for example, for caries detection based on fluorescence or on scattering alterations, tomographic transillumination methods in the visible and infrared wavelength range, vitality studies of the pulp by laser Doppler measurements—or determination of the optical properties of the tooth—for example, for dental restoration manufacturing. We emphasize that the understanding of light propagation in the tooth is a prerequisite for explaining its color, just as the knowledge of skin optics was a requirement for explaining the blue color of veins (Kienle *et al.*, 1996).

ACKNOWLEDGMENTS

We thank Julia Schaumeier, Thomas Schneck, Daniel Simon, and Andrea Böhmler for their help in performing the experiments. We acknowledge the support of the German Research Society (DFG).

REFERENCES

Altshuler GB (1995). Optical model of the tissues of the human tooth. *J Opt Technol* 62:516-520.**(AQ)**

Backman V, Wallace MB, Perelman LT, Arendt JT, Gurjar RS, Müller MG, *et al.* (2000). Detection of preinvasive cancer cells. *Nature* 406:35-36.

Bohren CF, Huffman DR (1983). Absorption and scattering of light by small particles. New York: John Wiley & Sons.

Dagdug L, Weiss GH, Gandjbakhche AH (2003). Effects of anisotropic optical properties on photon migration in structured tissues. *Phys Med Biol* 48:1361-1370.

Fried D, Glena RE, Featherstone JDB, Seka W (1995). Nature of light scattering in dental enamel and dentin at visible and nearinfrared wavelengths. *Appl Opt* 34:1278-1285.

Heino J, Arridge S, Sikora J, Somersalo E (2003). Anisotropic effects in highly scattering media. *Phys Rev E Stat Nonlin Soft Matter Phys* 68(3 Pt 1):031908.

Kienle A, Lilge L, Vitkin IA, Patterson MS, Wilson BC, Hibst R, *et al.* (1996). Why do veins appear blue? A new look at an old question. *Appl Opt* 35:1151-1160.

Kienle A, Forster FK, Diebolder R, Hibst R (2003). Light propagation in dentin: influence of microstructure on anisotropy. *Phys Med Biol* 48:N7-N14.

Kienle A, Forster FK, Hibst R (2004). Anisotropy of light propagation in biological tissue. *Opt Lett* 29:2617-2619.

Mourant JR, Johnson TM, Carpenter S, Guerra A, Aida T, Freyer JP (2002). Polarized angular dependent spectroscopy of epithelial cells and epithelial cell nuclei to determine the size scale of scattering structures. *J Biomed Opt* 7:378-387.

Nickell S, Hermann M, Essenpreis M, Farrell TJ, Krämer U, Patterson MS (2000). Anisotropy of light propagation in human skin. *Phys Med Biol* 45:2873-2886.

Odor TM, Watson TF, Pitt Ford TR, McDonald F (1996). Pattern of transmission of laser light in teeth. *Int Endod J* 29:228-234.

Perelman LT, Backman V, Wallace M, Zonios G, Manoharan R, Nusrat A, *et al.* (1998). Observation of periodic fine structure in reflectance from biological tissue: a new technique for measuring nuclear size distribution. *Phys Rev Lett* 80:627-630.

Ramsay DS, Artun J, Martinen SS (1991). Reliability of pulpal blood-flow measurements utilizing laser Doppler flowmetry. *J Dent Res* 70:1427-1430.

Sviridov A, Chernomordik V, Hassan M, Russo A, Eidsath A, Smith P, *et al.* (2005). Intensity profiles of linearly polarized light backscattered from skin and tissuelike phantoms. *J Biomed Opt* 10:14012.

Vaarkamp J, ten Bosch JJ, Verdonschot EH (1995). Propagation of light through human dental enamel and dentine. *Caries Res* 29:8-13.

Vongsavan N, Matthews B (1993). Experiments on extracted teeth into the validity of using laser Doppler techniques for recording pulpal blood flow. *Arch Oral Biol* 38:431-439.

Walton RE, Outhwaite WC, Pashley DF (1976). Magnification—an interesting optical property of dentin. *J Dent Res* 55:639-642.

Wang XJ, Milner TE, de Boer JF, Zhang Y, Pashley DH, Nelson JS (1999). Characterization of dentin and enamel by use of optical coherence tomography. *Appl Opt* 38:2092-2096.

Yousif HA, Boutros E (1992). A FORTRAN code for the scattering of EM plane waves by an infinitely long cylinder at oblique incidence. *Comput Phys Commun* 69:406-414.

Zijp JR, ten Bosch JJ (1993). Theoretical model for the scattering of light by dentin and comparison with measurements. *Appl Opt* 32:411-415.