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Spectroscopic Study of Human Teeth and Blood from Visible to Terahertz Frequencies for Clinical Diagnosis of Dental Pulp Vitality

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Abstract Transmission spectra of wet human teeth and dentin slices, together with blood of different flow rates were investigated. The measurements carried out over a wide spectral range, from visible light down to terahertz radiation. The results make it possible to find the optimum light frequency for an all-optical determination of pulpal blood flow and, consequently, for clinically diagnosis of tooth vitality.

Keywords Infrared and terahertz transmission · Dentin · Lentic or flowing blood · Dental pulp vitality

1 Introduction

The diagnosis of human dental pulp vitality is the basis for an appropriate therapy of pulp diseases or dental traumata. The clinical methods currently used to assess pulp vitality are based mainly on the patient's perception. Ther-

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mal or electrical stimuli are applied to the respective tooth and pulp vitality is indirectly assessed by the elicited nerve response [1]. Such sensibility responses are subjective and must be communicated to the dentist. In particular with children, older, handicapped, diseased, or patients under anesthesia this method is not reliable [2]. Numerous other noninvasive technical approaches, e.g., the determination of pulpal blood flow using laser Doppler flowmetry, photoplethysmography, ultrasound or pulse oximetry have been proposed [3–6]. However, none of these methods has so far found its way into clinical applications and thus the development of a reliable method for dental pulp vitality testing remains a challenge. Optical methods appear to offer a reliable assessment of pulp vitality. Much work has already been carried out in the field of imaging and caries localization by throughput of light with an emphasis on the determination of suitable transmission windows in the IR and THz spectral ranges [7–12].

One interesting approach to the determination of dental pulp vitality by optical means utilizes the measurement of pulpal blood flow and is based on the different transmission characteristics of dentin, enamel and blood [13–15]. In this method the pulp vitality estimation results from the modulation of light transmitted through a tooth by blood flow pulsation in the pulp. Although this method is expected to be objective, reliable, contactless and does not require tooth-imaging, its realization remains a challenge. A major task in this respect is to find an optimum frequency for which light transmission, T, is sufficiently large (in order to provide a precise and reliable measurement of the light passed through a tooth), blood absorbs light and the heart frequency pulsation modulates light transmission. The latter is of particular importance, because the existence of the pulsating pulpal blood flow provides clear evidence of tooth vitality [16, 17]. So far only several single frequencies in the visible and infrared (IR) ranges have been applied to study the dental pulp vitality and pulpal blood flow [2, 5, 18–22]. While the method is very promising, suggested light frequencies have hitherto not been optimized. Moreover, data were obtained only for dry tooth specimens and not for in vivo-like conditions taking into account that teeth are localized inside the oral cavity and are invariably wet. Furthermore, in these experiments blood flow took place through relatively large cuvettes or tubes, with diameters of the order of millimeters [5, 19, 20]. Consequently, these results are not directly applicable to clinical situations, where blood flows in micrometer size pulp blood vessels [23]. As a result, investigations of transmission through wet teeth and lentic (still) and flowing blood with varying blood film thickness over a wide spectral range are needed.

Here, we present data from spectroscopic investigations of wet human teeth and dentin slices as well as of lentic/flowing blood over a wide frequency range from visible light down to terahertz radiation. These measurements make it possible to find the optimum light wavelengths for an all-optical determination of pulpal blood flow. They demonstrate that the spectral range between 0.65 and about 1.4 μm is well adapted to this purpose with the best conditions being obtainable at wavelengths ranging from 0.85 to 1.38 μm. Hereby, light

is transmitted through teeth without large losses and light absorption differs depending on the presence of lentic or flowing blood in a narrow channel of the order of 100 to 500 μm. This difference allows the application of lock-in-modulation techniques using the human pulse as a reference.

2 Experimental technique and samples

Experiments were carried out in the spectral range from 0.6 μm to 80 μm. In order to cover a large range of light frequencies Fourier Transform Infrared (FTIR) spectrometer Vertex-80, the free electron laser FELIX at FOM-Rijnhuizen in the Netherlands [24–26] as well as pulsed line-by-line tunable TEA CO_2 and molecular terahertz lasers [27–31] were utilized.

The FTIR spectrometer was used to determine light transmitted through hard tooth substances or blood (lentic and flowing) in the spectral range from 0.6 to 3 μm. For longer wavelengths the losses in the teeth substantially increase and the sensitivity of the FTIR spectrometer becomes insufficient. Note that due to the large distance between the sample and detector in the FTIR spectrometer the measured attenuation of the incoming intensity may not only be due to the sample's reflection and absorption but also be caused by the scattering processes. Therefore, values of transmission obtained in this work applying the FTIR spectrometer can not be used for determination of, e.g., sample absorption coefficients.

The measurements in the long-wavelength range from 7 to 80 μm were carried out making use of the frequency tunability of the free electron laser. The output pulses of light from the FELIX were chosen to be ≈ 6 ps long, separated by 40 ns, in a train (or "macropulse") of 7 μs duration. The macropulses had a repetition rate of 5 Hz. The radiation power was controlled by pyroelectric detectors and a THz photon drag detector [31, 32]. In this configuration a monochromatic collimated laser beam was used and a large area detector placed directly behind the sample. Due to a small distance between the sample and detector the role of scattering in this set-up is reduced compared to that of the FTIR spectrometer.

Freshly extracted caries free human teeth without any fillings were stored in 0.5% chloramine solution, cleaned and then stored in physiological saline (4°C) for a maximum of six months until use. Four molar teeth, one premolar, and one central incisor were used for measurements. In addition to this, different dentin slices were measured. For this purpose, molars were longitudinally cut using a rotating diamond saw and water cooling. By that the set of dentin slices having thicknesses, d, from 100 to 800 μm was obtained. During the measurements all samples were maintained wet.

In the present study blood in the form of standard erythrocyte concentrate was used, which is usually treated with anticoagulants and stabilizers such as sodium citrate, citric acid monohydrate and sodium dihydrogen phosphate [33]. The average erythrocyte count of such concentrates is in the order of 5.3 million/μl, see Ref. [34], being in the same range as standard erythrocyte

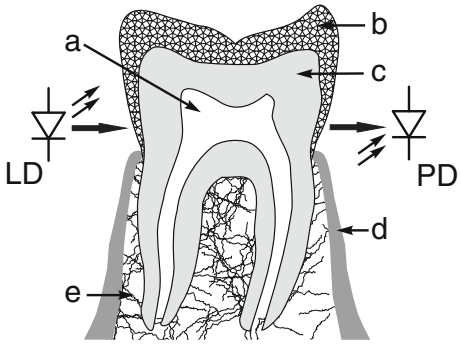

Fig. 1 Setup for the clinically diagnosis of tooth vitality. **a** dental pulp; **b** enamel; **c** dentin; **d** gum; **e** bone; *LD* semiconductor laser diode; *PD* photodiode.

counts in human blood [35]. In order to obtain a homogeneous suspension 59.4 ml erythrocyte concentrate was mixed with 600 μl of a 10% Synperonik-NaCl solution (Synperonik F68, SERVA Electrophoresis GmbH, Heidelberg, Germany) [33].

2.1 Results and discussion on the transmission spectra of wet human teeth and dentin slices

As already addressed, the optical method for the determination of dental pulp vitality utilizes the modulation of light transmission through a tooth by pulpal blood pulsation. The experimental arrangement for the clinically diagnosis of tooth vitality is sketched in Fig. 1. Consequently, the first task of optimization is to find a spectral range in which teeth are sufficiently transparent.

Figure 2 shows transmission curves of tooth dentin slices in the spectral range between 0.6 and 80 μm. Transmission spectra of dentin slices with different thicknesses were measured using the FTIR spectrometer and free electron laser. The data of Fig. 2 demonstrate that at long wavelengths ($\lambda > 3$ μm), even though small thicknesses in the order of hundreds of micrometers

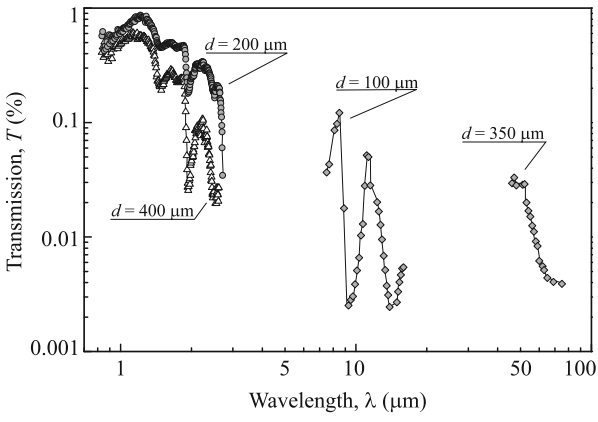

Fig. 2 Transmission spectra of tooth dentin slices of different thicknesses, *d*, measured by FTIR spectrometry ($\lambda < 4$ μm) as well as tunable radiation from the free electron laser FELIX (λ from 7 to 80 μm).

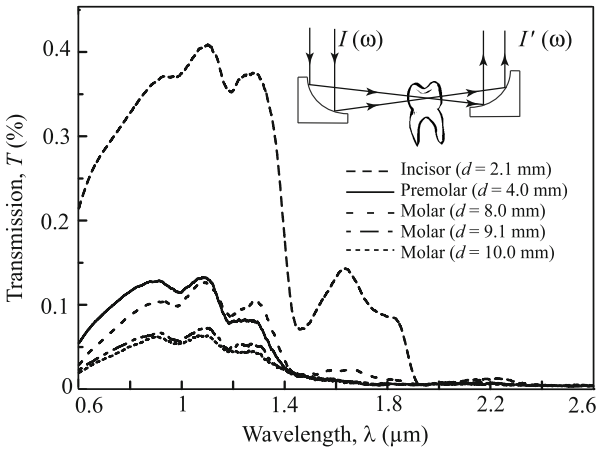

Fig. 3 Typical transmission spectra of different kinds of human teeth measured using FTIR spectrometer. *Inset* shows schematically the experimental geometry. Transmission T is determined as a ratio of $I'(\omega)$ and $I(\omega)$ corresponding to intensity of light transmitted through the sample which is collected by a detector and incoming light intensity, respectively.

were used, the light transmission through the dentin slices is very low (less than 0.1 %). It should be noted that the values of the transmission measured at $\lambda \approx 80$ μm ($f = 3.75$ THz) are much smaller than those reported for close frequencies (f about 3 THz) in Refs. [9, 12]. This result may be attributed to the fact that the samples used were wet and the well known water absorption of THz radiation inevitably results in additional uncontrollable losses. Taking this into account, together with the fact that the light transmission of thick teeth is even much smaller than that of the studied dentin slices, terahertz spectral range was excluded from further consideration. Instead, it was decided to focus on the near infrared range with $\lambda < 3$ μm.

Figures 3 and 4 show the transmission spectra of different types of human teeth—incisors, premolars and molars—and dentin slices, respectively. The spectra were obtained by FTIR spectrometry. As an important result the

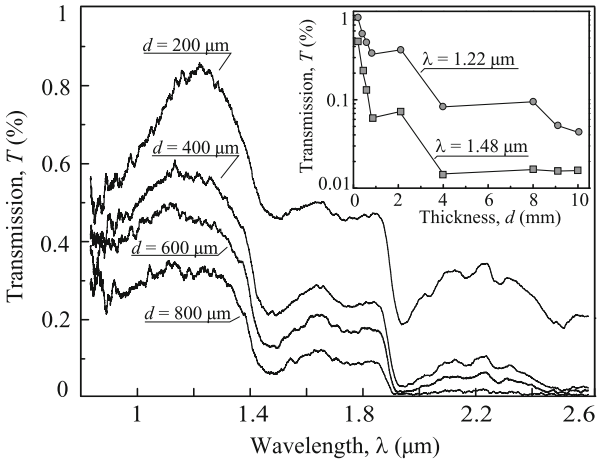

Fig. 4 Light transmission in tooth dentin slices. *Inset* shows transmission as a function of the dentin slices thickness d measured for two wavelengths. The points at $d > 2$ mm are given for complete teeth. Note, that when it comes to complete teeth the thickness varies within the *light spot* (here 6 mm in diameter) and, therefore, the average value of d is used in the *inset*. We would also like to note, that the data for $d = 2.1$ mm corresponds to the cutting edge of an incisor.

data shows that the intensity of NIR light transmitted through the teeth is decreased to a fraction of a percent. In spite of this relatively strong light attenuation (about 3 orders of magnitude) this range can be successfully implemented for all-optical detection of tooth vitality. Indeed, this attenuation does not prevent the achievement of large signal to noise ratios, particularly in an optical configuration consisting of a semiconductor laser and a sensitive detector placed immediately behind the tooth.

Comparison of the transmission curves for teeth and dentin slices demonstrates their similar spectral behavior in the spectral range between λ about 1 and 2 μm indicating that here transmission through the teeth is determined mostly by dentin rather than by enamel. A small deviation in transmission behavior of different samples could be caused by a different percentage of dentin and enamel in the tooth specimens. The best range with transmission larger than the half maximum is obtained with the wavelengths from about 0.85 to 1.38 μm.

The spectra measured on a number of different teeth reveal that dentin transmission is characterized by a maximum transmission for $\lambda \approx 1.3$ μm and local minima at 1.45 and 1.75 μm. The rather high transparency of human teeth at $\lambda = 1.3$ μm has been reported previously and this wavelength has been suggested for teeth imaging [8]. The inset in Fig. 4 shows transmission as a function of the tooth slice thickness. Note that the data for $d > 2$ mm correspond to complete teeth. The measurement demonstrates that transmission can not be simply described by Lambert–Beer–Bouger law, $T = \exp(-kd)$, where k is the absorption coefficient. This can be caused for various reasons such as light scattering or dentinal tubules. The latter is particularly relevant to thin dentin slices. The mechanisms of light attenuation in teeth are beyond the scope of this study, but, we would like to emphasize a rather weak dependence on the thickness observed for complete teeth, see Fig. 4. This fact is of importance for the discussed method because of the large variety of teeth dimensions in practice.

2.2 Results and discussion on the transmission of lentic and flowing blood

The next important issue for the realization of an all-optical clinical diagnosis of dental pulp vitality is the necessity of light modulation due to the heart frequency pulsation of blood flowing through the dental pulp. Applying FTIR spectrometry we investigated light transmission of lentic and flowing blood. To simulate the blood flow within the dental pulp we used quartz cuvettes (Starna type 48) of different thicknesses: 0.1, 0.2 and 0.5 mm. The blood flow was obtained by a flow pump. In order to imitate the pulpal blood pulsation, several measurements were carried out with different blood flow rates, Q, from 0 to 99 ml/h. The chosen cuvette thicknesses and blood flow rates were close to that known for perfusion of the dental pulp [23].

Figure 5 shows transmission spectra obtained for flowing blood pumped with a flow rate of 99 ml/h through cuvettes. The interference fringes on upper spectra in Fig. 5 were superimposed due to multiple reflections from cuvette

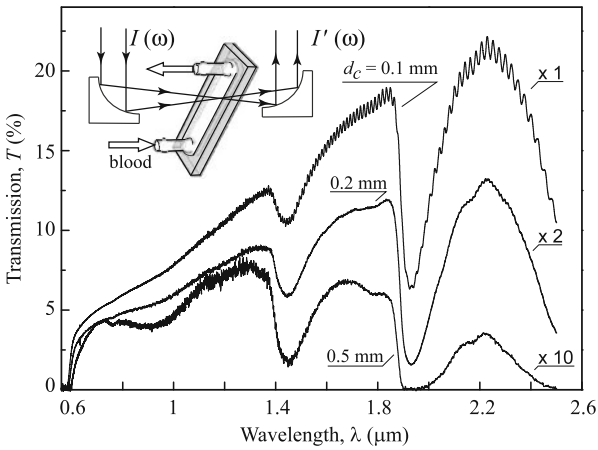

Fig. 5 Transmission of flowing blood. The data are obtained for blood pumped with a flow rate of $Q = 99$ ml/h through cuvettes of different thicknesses, d_c, equal to 0.1, 0.2 and 0.5 mm. The interference fringes on upper spectra are attributed to multiple reflections from cuvette plane-parallel sides. *Inset* shows schematically the experimental geometry (quartz cuvette—Starna type 48).

plane-parallel sides. The data demonstrate that light transmission through blood varies from fractions of a percent up to several percent, depending on the cuvette thickness and the light wavelength. Two local minima and three local maxima were measured at $\lambda = 1.45$ and 1.9 μm, and $\lambda = 1.3$, 1.7 and 2.2 μm, respectively. We also observed that in this spectral range light transmission for most wavelengths depends on the blood flow rate. This is demonstrated in Fig. 6 for a 0.2 mm cuvette. The data show that for wavelengths between 0.6 and 1.9 μm that the transmission increases with rising blood flow rate from 10 to 99 ml/h. We note that for wavelengths somewhat smaller than $\lambda = 0.6$ μm variation of blood flow rate does not significantly change transmitted light intensity. This result is in full agreement with previous studies carried out at $\lambda = 0.576$ μm, see Ref. [5].

Figure 7 shows the relative change of transmission T compared to the lentic blood, $\Delta T/T(0) = (T(Q) - T(0))/T(0)$. Dependence of the blood trans-

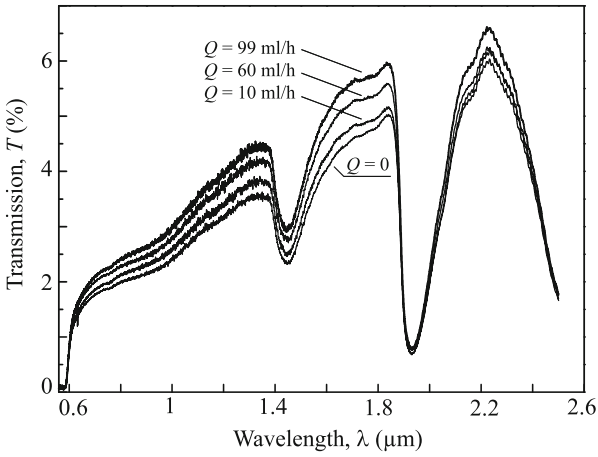

Fig. 6 Spectra of flowing blood obtained for cuvette with $d_c = 0.2$ mm and different flow rates, Q, varying from $Q = 0$ (lentic blood) up to $Q = 99$ ml/h.

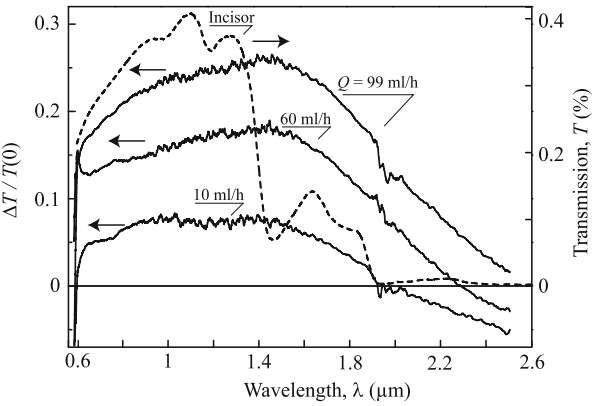

Fig. 7 The relative transmission $\Delta T/T(0) = (T(Q) - T(0))/T(0)$ measured for three values of blood flow rate. The *dashed curve* shows light transmission *curve* through incisor being representative of characteristic tooth transmission.

parency on the flow rate is a key observation for the all-optical detection of the tooth pulp vitality. Indeed, the difference between transmission of lentic and flowing blood can be used to infer the perfusion of the dental pulp: the signal of a photodetector placed directly behind a tooth should be proportional to $\Delta T/T(0)$ and, consequently, be modulated by the heartbeat frequency. Note that the observed variation of the blood transmission depending on the flow rate through thin cuvettes is of the independent interest. Exploring the underlying mechanism requires a special study and is out of scope of the present paper aimed to development of the method for clinical diagnosis of dental pulp vitality.

3 Conclusions

The measurements carried out over a wide frequency range demonstrates that the region between $\lambda = 0.65$ and 1.4 μm represent the spectral range suitable for detection of pulp vitality, because it is characterized, on the one hand, by teeth "transmission window" and by the different value of transmission for lentic and flowing blood on the other. Consequently, the difference between the lentic and flowing blood light transmission can be used as a tool for the in vivo determination and characterization of blood pulsation in the dental pulp. This facilitates the deduction of pulp vitality by measuring the light transmission. In particular, the signal modulation makes the application of lock-in-modulation techniques possible using the human pulse as a reference. The best contrast of dentin transparency and blood absorption with respect to its flow rate was found to be at wavelengths between 0.85 and 1.38 μm. This spectral range is of particular practical importance, because it includes wavelengths used for standard for optical communication systems employing well-developed technology. Therefore, for this frequency range there are a large variety of cost effective light sources, light guides, sensitive detectors and other optical components available.

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