

Journal of Biomedical Optics

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Optical coherent tomography: promising *in vivo* measurement of hair shaft cross section

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Abstract. Variations in hair shaft morphology reflect ethnical diversity, but may also indicate internal diseases, nutritional deficiency, or hair and scalp disorders. The measurement and the follow-up of the hair shaft thickness over a defined period of time would be a valuable diagnostic tool in clinical practice. Standard light microscopy (LM) measurements require the epilation of hair shafts and frequently yield inaccurate values caused by the elliptic geometry of human hair shafts. Optical coherence tomography (OCT) is a noninvasive investigation method based on the principles of Michelson interferometry with a detection depth of approximately 1 mm in human skin. Two-dimensional images of the cross sections of tissue samples at a resolution of approximately 10 μm are produced, which allows convenient calculation of hair shaft thickness. To evaluate this new methodology for hair shaft thickness measurements, hair shafts taken from 28 healthy volunteers were analyzed by *in vivo* OCT and compared to standard *in vitro* LM measurements of hair shaft thickness. OCT yielded highly reproducible measurements of hair shaft thickness with a distinctly reduced variation compared to standard LM. This technique offers a unique opportunity for *in vivo* measurement and a follow-up of the kinetics of hair shaft thickness in humans during medical therapy. © 2011 Society of Photo-Optical Instrumentation Engineers (SPIE). [DOI: 10.1117/1.3626210]

Keywords: hair shaft morphology; cross section; hair shaft measurement; optical coherence tomography; thickness; diagnostic tool.

Paper 11079R received Feb. 22, 2011; revised manuscript received Apr. 20, 2011; accepted for publication Jul. 6, 2011; published online Sep. 21, 2011.

1 Introduction

The human hair shaft reflects individual characteristics, e.g., ethnical background.¹ However, variations in morphology and thickness of the hair shaft also occur intraindividually as a sign of internal diseases, nutritional deficiencies, or hair shaft anomalies.²⁻⁵ The monitoring of hair shaft thickness over the course of a disease and during treatment may provide a valuable diagnostic tool.⁵ It may also help to evaluate the efficacy of new therapeutic compounds and strategies. The accurate measurement of the hair shaft thickness is, however, complicated by the elliptic geometry of the human hair shaft.^{3,6,7} Furthermore, standard methods for the determination of hair thickness, such as light microscopy (LM) of longitudinally embedded hair shafts and of cross sections are time consuming and require the cutting and processing of the hair shafts. Additionally, a monitoring of the kinetics of shaft thickness of one distinct hair cannot be realized, as the hairs have to be removed for investigation purposes. The availability of a reliable, noninvasive technique, which allows *in vivo* measurement of the thickness of human hair shafts, would greatly facilitate the evaluation of the hair quality in clinical practice and in clinical studies.⁵

Optical coherence tomography (OCT) is a noncontact, noninvasive coherent imaging technique based on interferometry, which is used to obtain high resolution cross-sectional images of biological tissue.⁸ The use of OCT for hair visualization and

measurement was pioneered by Wang et al., and has been successfully applied in various research fields including dermatology, ophthalmology, cardiovascular research, and others.⁸⁻¹³ In the present study, hair thickness measurements on healthy volunteers were performed using OCT and standard LM, respectively, in order to evaluate the applicability of OCT technology in hair research.

It was found that OCT measurements are well suited to determine the hair morphology. In contrast to microscopic measurements on removed hairs, the *in vivo* OCT analysis can be used to evaluate the kinetics of hair morphology influenced by diseases, medical therapy, or cosmetic treatment.¹⁴

2 Material and Methods

Three different methods were used to determine the cross section of hairs. The first two methods, based on light microscopic investigations, are invasive *in vitro* methods, requiring the removal of the hairs. The third method, based on OCT measurements, can be used *in vivo*. This method is noninvasive and can also be applied to analyze the kinetics of changes in the cross section of the hairs. The experiments started with *in vivo* measurements; the hairs were analyzed by OCT. Afterwards, the hairs were removed, embedded and the diameters of the hairs were determined by means of light microscopic images.

3 Volunteers

The investigations were carried out on 28 healthy volunteers, whereof 23 volunteers were of photo skin type II and 5

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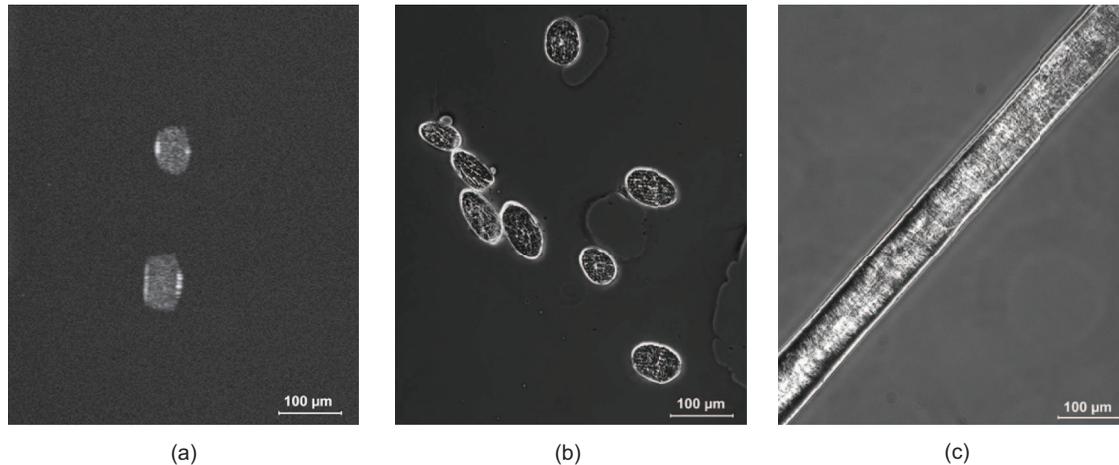


Fig. 1 Cross sections of hairs: (a) OCT method. A microscopic analysis of transversely embedded hairs (b) and longitudinally embedded hairs (c).

volunteers were of photo skin type IV.¹⁵ The volunteers were aged between 25 and 45 years. Approval for the experiments had been obtained from the local Ethics Committee of the Charité-Universitätsmedizin Berlin.

4 *In Vivo* Determination of Hair Shaft Cross Section with OCT

OCT is a noninvasive optical method based on interferometry.^{14,16} Cross-sectional images are produced using infrared light, which depict the internal microstructure within the biological tissues. The data are converted into a two-dimensional image. The employed commercial OCT system (“SkinDex 300”, ISIS optronics, Mannheim, Germany) achieves a depth and lateral resolution in tissue in the region of 3 to 5 μm , respectively. At a center wavelength of 1300 nm, a broadband light source with a bandwidth of 110 nm was employed. The detection depth is approximately 1 mm. A detailed description of the OCT system is given by Lademann et al.¹⁶ The “analySIS” imaging software was applied to determine the cross section of the hair shaft (SIS, Münster, Germany). Hair shafts from 28 volunteers were analyzed. The measurements were performed in triplicates of 10 hair shafts each. To ensure that the cross sections of the hairs were always measured perpendicular to the hair shaft, the hairs were gently stretched with a comb avoiding tension.

5 Light Microscopic Determination of the Hair Shaft Cross Section

5.1 *Determination of the Cross Section of Longitudinally Embedded Hair Shafts*

After OCT measurement, the hair shafts of volunteers were removed and divided. The proximal part of the hair was embedded in mounting medium (Eukitt®, Kindler GmbH, Freiburg, Germany).^{2,17} Five measurements in 1-mm intervals were performed on each hair shaft starting at 1 cm from the proximal end of the hair fiber. The diameter of the hair shaft was determined on LM pictures (SIS ColorView camera, analySIS, Münster, Germany) at a 100-fold magnification (IX50 micro-

scope, Olympus GmbH, Hamburg, Germany). The area of the cross section, which was proposed to be a circle, was calculated by πr^2 .

5.2 *Determination of the Cross Section of Transversely Embedded Hair Shafts*

The distal part of the hair samples was embedded as reported earlier.¹⁸ Ten micrometer cross sections of the hairs were prepared using a MICROM HM 560 cryosectioner kryostat (Microm GmbH, Walldorf, Germany). Applying the IX50 photomicroscope and analySIS imaging software (SIS, Münster, Germany), the diameter and the surface area of sectioned hair shafts were determined at a 20-fold magnification.

6 Results

The different optical images, which were obtained using the three different measuring methods, are presented in Fig. 1. The cross section of the hairs can be well recognized in the case of OCT [Fig. 1(a)] and transverse microscopic measurements [Fig. 1(b)]. Cross sections of the hair shafts illustrate that human hair shafts provide an elliptic geometry, not only in volunteers of African descent, but also in Caucasians. In Fig. 1(c), a longitudinally embedded hair shaft is presented. Here, the cross section has been calculated by πr^2 assuming that the hair is of round and not elliptical shape. The determination of the cross section by OCT measurements revealed intra- and interindividual differences. The hairs obtained from the same volunteer show different cross sections. The distribution of the cross sections from 10 hairs of a 28-year-old woman (skin type II, blond hair) measured by OCT is shown in Fig. 2(a) (volunteer 1). The average value of the cross section was $0.041 \pm 0.005 \text{ mm}^2$. Figure 2(b) presents the distribution of the cross sections obtained from a 26-year-old woman with skin type IV (black hair) (volunteer 2). In this case, the average cross section determined was $0.055 \pm 0.007 \text{ mm}^2$.

The standard deviation of the cross sections of 10 hairs determined with all three methods obtained from all 28 volunteers is quantitatively compared in Fig. 3. It can be seen that the

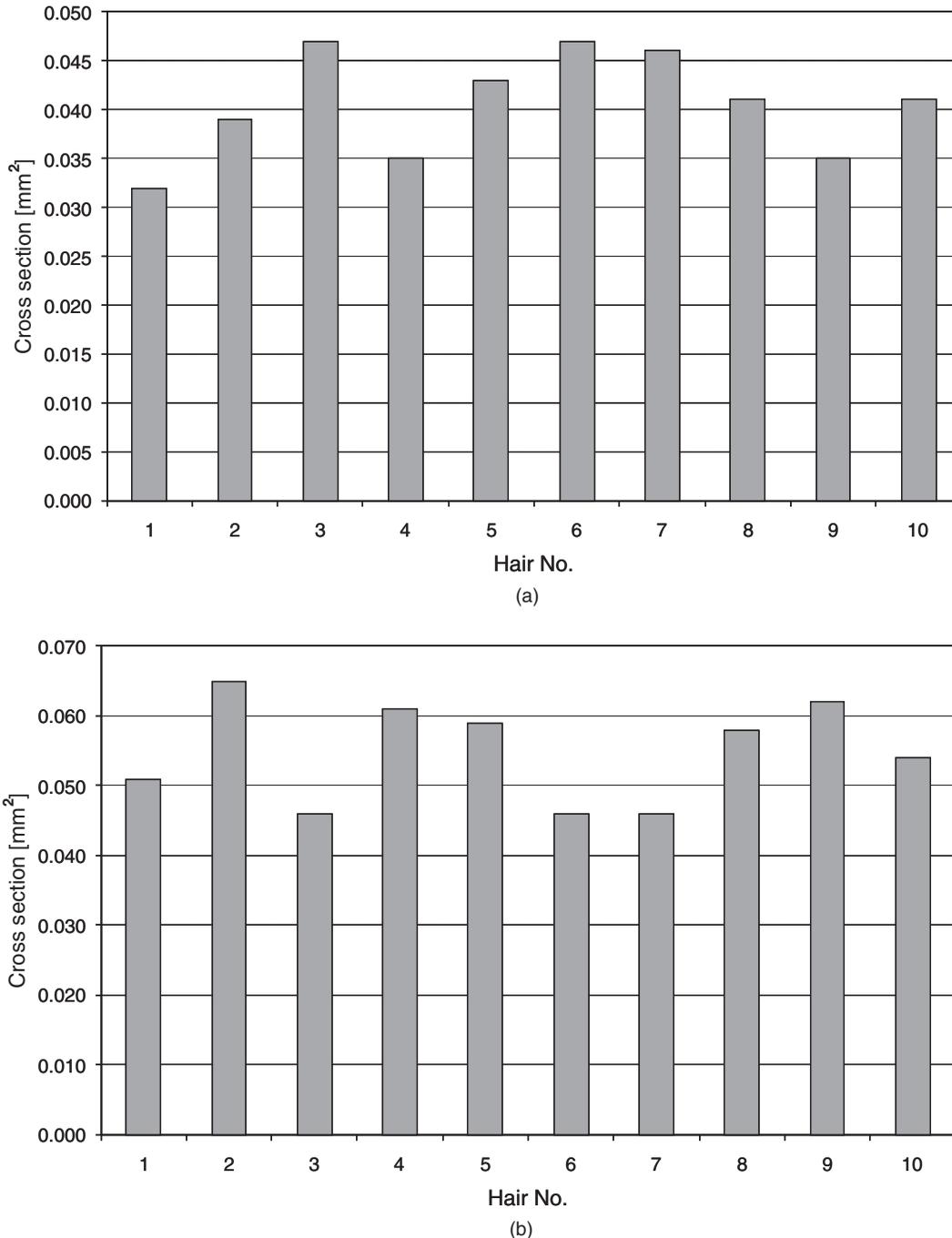


Fig. 2 Distribution of cross sections from ten hairs measured by OCT: (a) 28 year old woman, blond hair and (b) 26 year old woman, black hair.

standard deviation in the case of the OCT measurements (15%) and the microscopic analysis of the transverse sections of the hair shaft (22%) are similar, while the standard deviation of the calculated cross sections determined by measurements only of the diameter of the longitudinal hair shaft was significantly higher (84%).

7 Discussion

In several studies, it could be demonstrated that the cross sections of the hairs reflect metabolic processes of the human

organism.^{14,19,20} Recently, it could be shown that systemically applied steroids increased the cross sections of the hairs in patients suffering from various inflammatory diseases, while the shape characterized by the relation of the minimum and maximum diameters of the hairs was not influenced.¹⁴

Lademann et al. proposed that the determination of the cross section of hairs should be applied also for screening of doping substances in athletes.¹⁴ In another study, it was demonstrated that the cross sections of hairs also lend themselves to screening of narcotics in humans.²⁰ The different studies demonstrated that the analysis of the thickness and morphology of the hairs is an

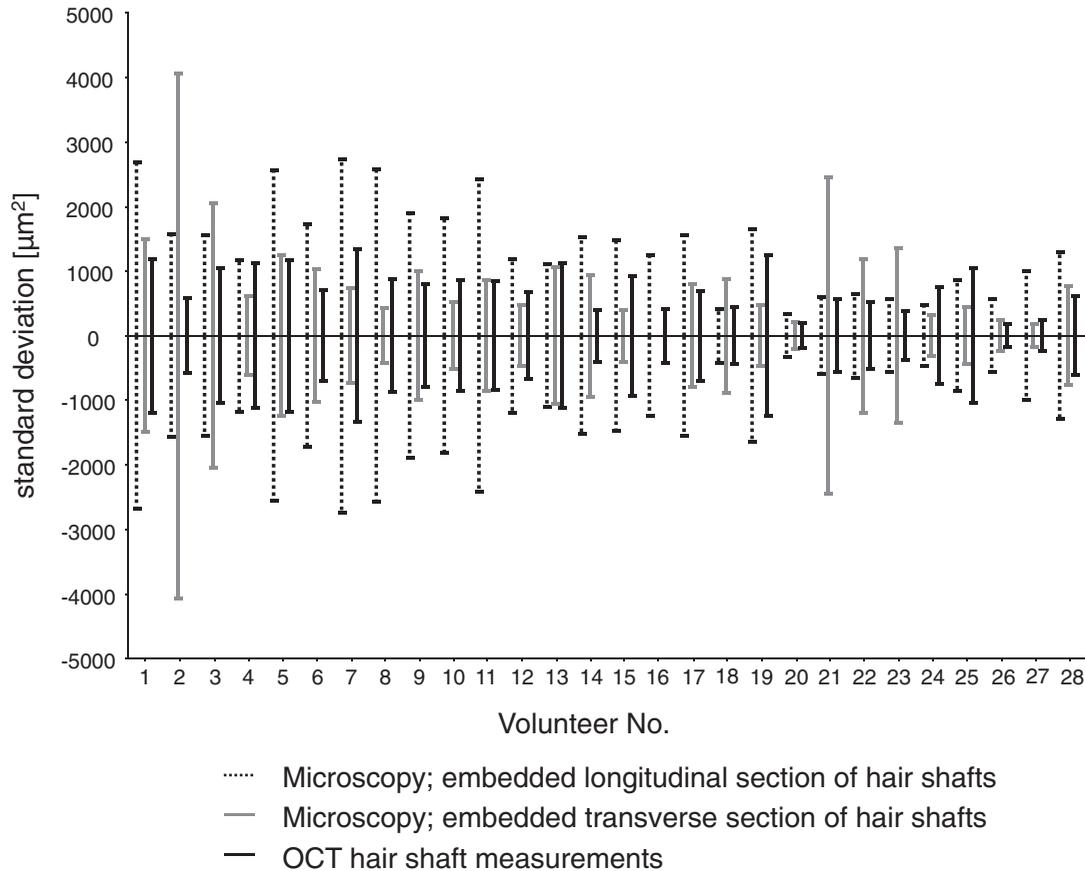


Fig. 3 Standard deviation of cross section determined by OCT, microscopic analysis of transverse section and of longitudinally embedded hairs; 10 hairs from $n = 28$ volunteers.

efficient tool for medical diagnostic and drug screening. Therefore, it is necessary to investigate the kinetics of the changes in the cross sections of the hairs also in healthy volunteers. This procedure was performed *in vivo*, because the kinetics cannot be followed, when the hairs have been removed. OCT is an efficient tool for *in vivo* measurements. This method has been successfully used up to now to determine the cross sections of human tissue *in vivo*. Applying this method in hair research, the shape, the cross sections, and the morphology of the hairs can be easily determined. The color of the hairs does not influence the measurements.¹⁶

In Fig. 1(a), the cross sections of the hairs and the medullas can be well recognized. The results obtained with OCT measurement are almost identical to the *in vitro* microscopic determination of the cross sections (Fig. 3). In contrast to the OCT measurements, for an *in vitro* analysis of the cross sections the hairs must be removed. The cutting and embedding procedures are time consuming and may change the characteristics of the hair samples. This could also be the reason for the minor differences observed in the standard deviations determined by OCT and LM measurements of hair cross sections of transversely embedded hairs. For the determination of the cross section of longitudinally embedded hairs, the hairs were assumed to have a regular circular structure, the cross section of which was determined by πr^2 . As this assumption does not reflect the actual situation, the standard deviation determined by LM measurements of longitudinally embedded hairs was increased.

Summarizing the results, it can be established that OCT measurements are well suited to determine the morphology and the thickness of human hairs *in vivo*. The method can be successfully used for clinical diagnostics and practical screening.

8 Conclusion

While standard light microscopic techniques require the cutting of hair, OCT provides a unique opportunity to perform *in vivo* measurements of the hair shaft thickness in humans. OCT yields highly reproducible measurements of the hair shaft thickness, allowing variations of the hair shaft thickness to be evaluated. With this technique, the same hair fibers can be monitored *in vivo* over a fixed period of time. OCT therefore represents a promising diagnostic tool for the classification of different hair types and for the evaluation of topical and systemic treatments of hair disorders. Once established as a routine method, OCT can be applied in clinical practice as well as in clinical studies and for therapy control.

Acknowledgments

We would like to thank Dr. Alexander Knüttel from ISIS Optonics GmbH Mannheim, Germany, for his experimental support and useful discussions. This study was supported by the European Commission under the EFRE program, by the Berlin Senate and by the Investitionsbank Berlin (IBB).

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