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Clinical optical coherence tomography combined with multiphoton tomography for evaluation of several skin disorders

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ABSTRACT

The first clinical trial of optical coherence tomography (OCT) combined with multiphoton tomography (MPT) and dermoscopy is reported. State-of-the-art (i) OCT systems for dermatology (e.g. multibeam swept source OCT), (ii) the femtosecond laser multiphoton tomograph DermaInspect™, and (iii) digital dermoscopes were applied to 47 patients with a diversity of skin diseases and disorders such as skin cancer, psoriasis, hemangioma, connective tissue diseases, pigmented lesions, and autoimmune bullous skin diseases. Dermoscopy, also called 'epiluminescent microscopy', provides two-dimensional color images of the skin surface. OCT imaging is based on the detection of optical reflections within the tissue measured interferometrically whereas nonlinear excitation of endogenous fluorophores and the second harmonic generation are the bases of MPT images. OCT cross sectional "wide field" image provides a typical field of view of 5 x 2 mm^2 and offers fast information on the depth and the volume of the investigated lesion. In comparison, multiphoton tomography presents 0.36 x 0.36 mm^2 horizontal or diagonal sections of the region of interest within seconds with submicron resolution and down to a tissue depth of 200 µm. The combination of OCT and MPT provides a synergistic optical imaging modality for early detection of skin cancer and other skin diseases.

Keywords: OCT, multiphoton tomography, skin, SHG, molecular imaging, two-photon, skin cancer, optical biopsy

1. INTRODUCTION

The most important techniques of non-invasive skin imaging are ultrasound imaging in the frequency range of 7.5 MHz to 100 MHz and optical systems in the ultraviolet (Wood lamp), visible (magnifying glass, dermoscope) and near infrared (NIR) spectral range. The advantage of NIR systems is the high light penetration depth enabling 3D imaging. For the clinical trial reported here, optical coherence tomographs based on broadband superluminescent diodes (SLD) and tunable lasers, the confocal reflectance microscope *Vivascope* (Lucid Inc., Rochester, USA) based on laser diodes, as well as the multiphoton tomograph *DermaInspect* (JenLab GmbH, Jena, Germany) based on a tunable femtosecond 80 MHz titanium:sapphire laser were applied as NIR systems. In contrast to MPT, OCT, reflectance microscopy, and dermoscopy are based on reflected/backscattered light owing to intratissue differences of the refractive index. OCT generates vertical images within milliseconds using typically powers of the incident NIR light of some milliwatt. The penetration depth in skin tissue extends to hundreds of micrometers; in low-scattering ocular tissue even millimeters are possible. The axial resolution depends on the bandwidth of the light source and the lateral resolution on the laser spot size. Time-domain OCT (TD-OCT), spectral domain OCT (SD-OCT, spectral RADAR OCT) and swept-source OCT

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(SS-OCT) are employed for clinical diagnostics [1]. SS-OCT is equipped with a very fast tunable frequency domain mode-locking (FDML) laser with a typical tuning range of up to 200 nm. The fast alteration (swept) of the laser wavelength leads to an increase of the imaging speed of up to 250 000 axial scans per second [1–3]. The multibeam swept source OCT EX1301 (Michelson Dignostics Ldt., Orpington, UK) utilizes a novel optical build-up and bases upon the idea to partition the depth of field into sub-fields and to provide a separately focused beam for each sub-field. The laser beam in the SS-OCT EX1301 is split into 5 'beamlets' using an etalon-type 'rattle plate' prior to the interferometer beamsplitter. Four of these beams are used to scan the skin and are relayed back to an array of photodiodes where they interfere with four reference beams in the conventional manner. The fifth beam is imaged onto a photodiode to generate a balance signal.

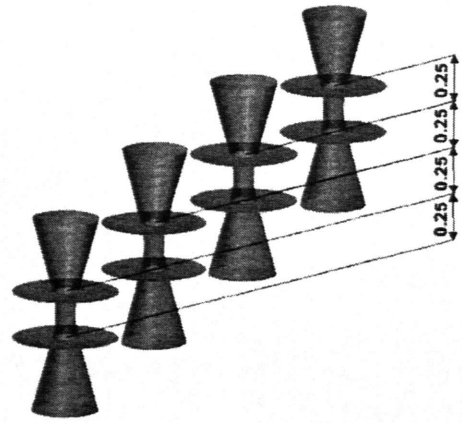

Figure 1. Four beamlets cover a total focal range of 4x0.25µm=1mm for OCT with a high resolution and contrast.

Since the 90s, OCT systems have been used in dermatology for experimental and clinical studies. Thereby, the OCT images contain information on the morphology and the epidermal thickness of normal skin [5]. OCT systems have been employed to characterize bullous diseases [5], psoriasis, contact dermatitis [6], melanocytic lesions [7], and vascular lesions [8]. Compared to ophthalmology, the technique is not yet established in dermatological routine diagnostics.

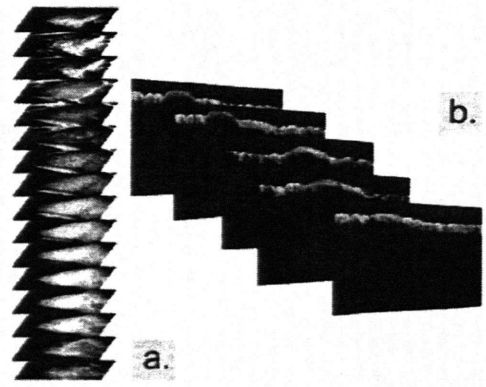

Figure 2. a. MPT stack down to depth of 100 µm (image size: 360x360 μm^2); b. OCT image sequence (image size: 5x1.9 mm^2).

Multiphoton laser scanning tomography [9–17] is based on the non-linear excitation of endogenous fluorophores such as NAD(P)H, flavoproteins, keratin, lipofuscin, elastin, collagen, melanin, and metal-free porphyrins as well as on second harmonic generation (SHG) of collagen structures. Furthermore, single photons can be counted with picosecond time resolution (time-correlated single photon counting, TCSPC) to pixelwise record the signal decay. To image an area of 512 x 512 pixel (360 x 360 μm^2) 1–8 s are required. Multiphoton tomographs provide horizontal optical sections of a particular region of interest with submicron resolution down to 200 mm tissue depth. Fields of study are early detection of melanoma and other skin diseases, to detect *in vivo* sunscreen nanoparticles and pharmacological compounds as well

as the evaluation of the skin age and the effects of anti-aging products. No clinical skin imaging trial has been conducted based on OCT in combination with multiphoton tomography before.

2. MATERIALS AND METHODS

The trial has been performed on 47 patients and the investigated dermatological disorders included epithelial skin cancer, pigmented skin lesions, inflammatory skin disorders, autoimmune diseases, seborrheic keratosis, T-cell lymphoma, hemangioma and tattoos. In selected cases the diagnosis was confirmed by histopathology.

Two OCT systems have been employed for this study. The swept-source multibeam EX1301 high resolution OCT microscope from Michelson Diagnostics (Orpington, UK) is based on the application of laser beamlets focused to different depths separated by 0.25 mm. The HSL-2000-10 MDL light source (Santec Corporation, Komaki, Japan) with a peak power of 15 mW at the laser center wavelength of 1305 nm provides a sweep range of 150 nm (at 10 kHz) that enables real-time monitoring. The acquisition time of a single 5 x 1.9 mm^2 scan is approximately 100 ms. The scan width can be enhanced up to 7 mm [19]. The EX1310 SS-OCT was mounted to a flexible arm and modified to be adapted to the interface used by the multiphoton tomograph *DermaInspect*. The single beam spectral domain fiber-optic interferometer Callisto (Thorlabs HL AG, Lübeck, Germany) is based on an SLD at a center wavelength of 930 nm, a spectral bandwidth of 50 nm, an A-scan frequency of 1.25 kHz, and an output power of 3 mW. The two-dimensional images were recorded with a lateral dimension of 4 mm to 6 mm.

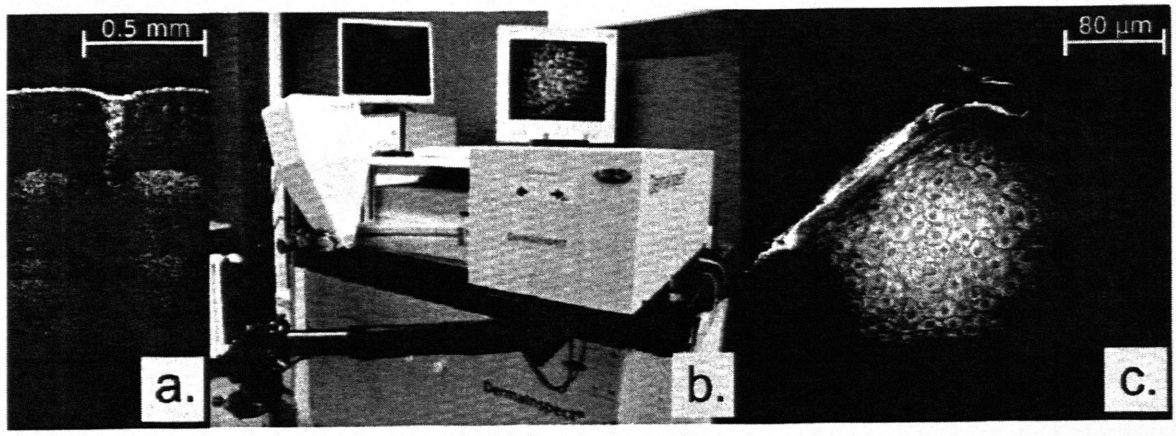

Figure 3. **a:** OCT image demonstrates a sweat gland within the finger tip [6]; **b.** Photograph of the multiphoton tomograph DermaInspect with flexible mirror arm and the multibeam EX1301 swept-source OCT system; **c:** multiphoton optical section with subcellular resolution shows fluorescent organelles.

The CE-marked clinical multiphoton tomograph DermaInspect (JenLab GmbH, Jena, Germany) with a flexible mirror arm was employed [14]. The laser source is a tunable 80 MHz femtosecond laser with a tuning range of 690–950 nm, a pulse width of 80 fs, and a maximum power of 2 W (MaiTai, Newport/Spectra Physics, Mountain View, USA). The laser beam was focused to a submicron spot onto the sample by NA 1.3 focusing optics and moved by fast x, y galvoscanners and z piezoactuators [9].

Two dermoscopes from the company *Fotofinder Systems* (Bad Birnbach, Germany) were used: The video camera Medicam 500 with a resolution of 768 x 576 pixel and the digital camera Powershot G9 from Canon with a resolution of 4,000 x 3,000 pixel. The field of view in microscopic imaging was 11 x 14 mm^2. A glass tube with a length of 30 mm assures a constant distance between the camera and the skin surface.

For matching optimization a special interface consisting of a metal ring and a marked glass coverslip was developed fitting into the *DermaInspect* as well as into the OCT and dermoscopes in order to guarantee the measurements of the same skin area.

3. RESULTS

3.1 OCT and MPT of healthy normal skin

The cross-sectional OCT images provided information on the thickness of the entire epidermis, the composition of the upper dermis as well as the location of hair follicles and sweat glands. However, the visualization of cells or cell compartments was not possible. Hence, a differentiation between the different epidermal tissue layers was also not possible. The epidermis is demarcated as homogenously scattering and the subjacent dermis has several signal-poor structures such as vessels and skin appendages. Sufficient OCT signals could be obtained down to a depth of about 1 mm. Well defined spiral structures could be imaged especially at the fingertips due to the presence of sweat glands.
The OCT technique enables imaging through a marked 160 µm thick coverslip. This enables the matching between all optical techniques used in the study. The resolution of the multibeam SS-OCT exceeded the resolution of the single beam SD-OCT system despite the higher wavelength.
Multiphoton tomography enabled the two-photon excitation of keratin in the outermost layer (stratum corneum), of the reduced coenzyme NAD(P)H in the mitochondria inside living cells, of melanin clusters, and of elastin in the upper dermis. The technique provided the visualization of the morphology of single cells and even single mitochondria.

3.2 Optical biopsies of a hemangioma

OCT sections of a capillary hemangioma of a 48-year-old mal patient revealed a signal-poor oval, well demarcated structure (0.97 x 0.51 mm^2) in the center below a slightly elevated epidermis. The 45° diagonal MPT images provided a more detailed view into the architecture of the hemangioma (Fig. 4c,d).

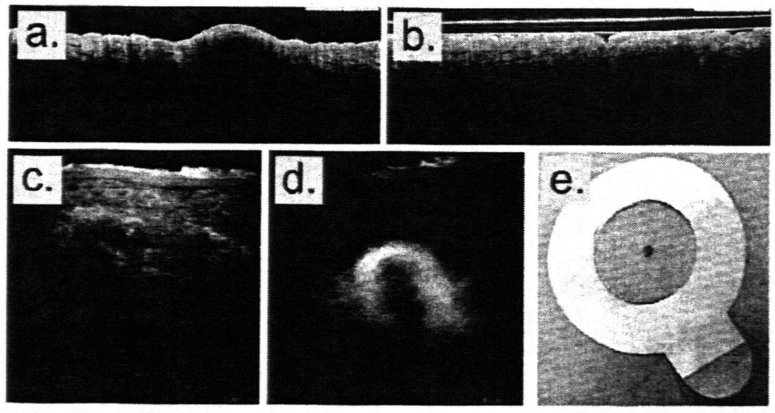

Figure 4. Optical biopsies of a capillary hemangioma: **a:** OCT image; **b:** OCT section with cover slip on top of the lesion; **c, d:** diagonal MPT images; **e:** dermoscopic image.

3.3 Optical biopsies of a patient with Pemphigus vulgaris

A 46-year-old male patient with *Pemphigus vulgaris* who suffered from intraepidermal acantholytic blisters was investigated. Several clefts ("black cavities") (Fig. 5a) were observed with the OCT systems. MPT can also be used to image blisters and epidermal splits. The image (Fig. 5d) demonstrates less fluorescent signals in the area of clefts likely due to the absence of cell material. In part, surrounding cells exhibited highly fluorescent organelles. The origin of this emission is not clear.

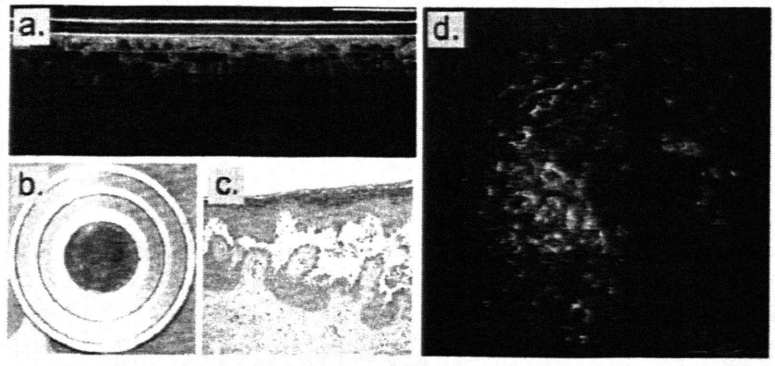

Figure 5. Optical biopsies of a Pemphigus vulgaris: **a:** OCT section with coverslip; **b:** dermoscopic image; **c:** histopathological image; **d:** MPT image.

The histopathological image fig. 5d shows an acantholytic blistering and some Tzanck-cells, typical for *Pemphigus vulgaris*.

3.4 OCT and MPT imaging of a melanocytic lesion

OCT can provide information on the thickness of a melanocytic lesion. OCT sections (Fig. 6a) of a 31-years-old female patient with a malignant melanoma showed the modified epidermal junction and highly scattering globules compared to normal pigmented skin. In contrast, MPT sections (Fig. 6d) showed a more detailed architecture including fluorescent melanocytes. Furthermore, single dendritic cells were monitored.

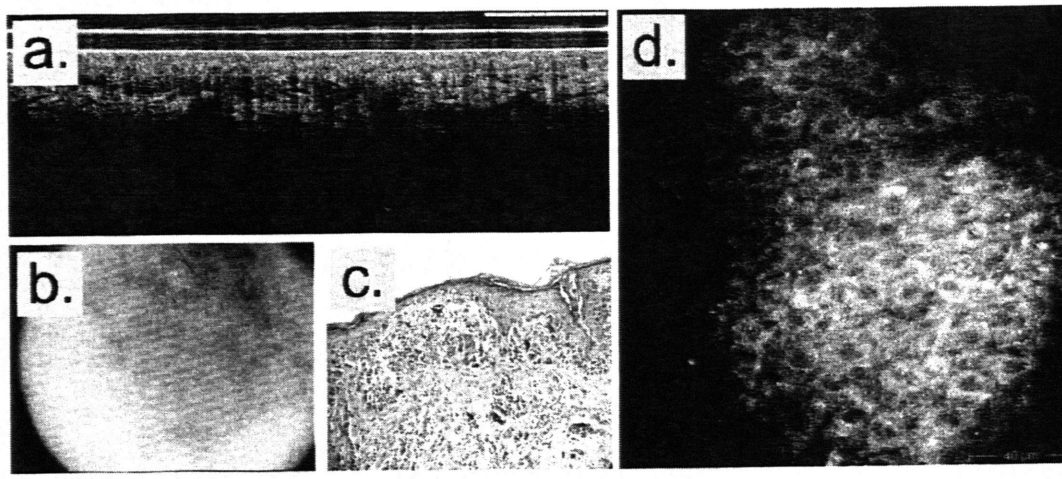

Figure 6. Optical biopsies of a malignant melanoma: **a:** OCT image with cover slip; **b:** dermoscopic image; **c:** histopathological image; **d:** MPT section.

4. CONCLUSION

The combination of OCT and MPT offers a unique tool for early non-invasive skin cancer diagnostics and the evaluation of treatments. OCT provides fast wide-field cross-sectional images (5 x 2 mm^2) of the region of the interest but no cellular resolution. MPT has an excellent submicron spatial resolution whereas it is relatively slow with the disadvantage of a small volume of view of about 0.3 x 0.3 x 0.2 mm^3. The combination offers both (i) the wide-field image with depth information up to 2 mm and the possibility to identify a particular skin area of interest and (ii) the high-resolution stack of horizontal multiphoton images of this particular region. Compared to time-domain and single beam spectral-domain OCT, multibeam swept-source OCT has significant advantages concerning resolution and scanning speed. MPT provides the best resolution in non-invasive in-vivo skin imaging and enables also functional imaging based on the ratios of (i) free to bound NAD(P)H, (ii) NAD(P) to NAD(P)H, and (iii) NAD(P)H to flavoproteins. A variety of skin diseases were investigated such as hemangioma, Pemphigus vulgaris, and malignant melanoma [4].

5. ACKNOWLEDGEMENTS

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