Full-field optical coherence tomography: a new technology for 3D high resolution skin imaging

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Short title: Skin imaging with FFOCT

Keywords : skin imaging, optical coherence tomography (OCT)

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ABSTRACT

Background/Aims : Full-field optical coherence tomography (FFOCT) is a new imaging technology that can provide 3D micron-level resolution and is suited for high resolution imaging of biological tissue. The aim of this study is to evaluate its capacity and potential for imaging human epidermis and dermis and various skin pathologies in ex- and in-vivo conditions.

Methods: Non fixed and fixed samples of normal and pathological skin and normal in-vivo skin were imaged with a FFOCT system and compared to histological slides.

Results: The epidermis and adnexae, the collagen bundles of the dermis and the hypodermis could be identified through architectural and cellular details. The pathological structures were distinguished from the normal structures and correspond to their histopathological organization.

Conclusion: FFOCT is a novel technology in the field of skin imaging that has the potential to be a relevant complement to existing non-invasive imaging modalities for clinical and cosmetic applications.

INTRODUCTION

In dermatological examination, the non-invasive examination of skin is restrained to the capacity of the eye, the magnifying glass or the dermatoscope. The latter has gained over the years much popularity and recognition amongst practitioners, as its usefulness has been proven in particular for the detection of pigmented skin lesions [1]. However, the resolution is limited to the order of a few tens of microns, far from the resolution needed for cellular characterization of the tissue. Furthermore, all these techniques only provide information of the surface of the skin. Obtaining in-depth structural and cellular information requires performing a biopsy, then going through fixation, dehydration, clearing, infiltration, embedding, sectioning and staining, before the tissue can be observed with a microscope. This is an invasive, long and tedious process, furthermore operator dependent and not free of artifacts [2].

Various non-invasive imaging methods have been applied to the in-depth examination of skin. A possible method to see through skin tissue is by using ultrasound scanning. This imaging technique is based on the emission of an ultrasound wave and the detection of the echoes generated by the tissue. Commonly used in obstetrics, ultrasound imaging has also been widely tested in dermatology to assess the size and inflammatory processes of skin tumors [3,4]. Although this non-invasive imaging modality can provide images down to 1 cm below the surface, the resolution is limited to a few tens of microns. Therefore the practitioner does not have access to skin structure details at the cellular level. Magnetic resonance imaging is another technique proving similar resolution, of the order of 100 microns [5]. This technique relies on the nuclear magnetic resonance phenomenon, and typically has similar applications to ultrasound scanning. Although both techniques allow for non-invasive in-depth imaging of the skin, the limited resolution enables to assess only architectural changes of the skin.

Recently, as novel optical modalities have been developed to image non-invasively through scattering media, they have been applied to skin tissue high-resolution in-depth examination. The first optical sectioning technique applied to the skin, confocal microscopy, is based on the use of point illumination and an associated pinhole in the detection plane to reject out-of-focus light and select only the light of the focal plane of interest [6]. The illumination point is scanned over the sample to retrieve a 2D high-resolution image tangential to the tissue surface. Confocal imaging was first applied to skin imaging in 1993 [7] in reflectance mode (detection of the light directly back-scattered by the tissue), then in fluorescence mode (detection of the light fluoresced by the tissue) [8]. In vivo confocal microscopy allows for the examination of the epidermis and the papillary dermis at a resolution approaching histological detail, thus representing a new step towards accurate non-invasive diagnostic [9,10]. Multi-photon microscopy is another method which uses the properties of non-linear optics: in particular, second-harmonic generation emission is proportional to the square of

the excitation light, and thus limits the emitting area to the focused illumination spot in the tissue [11]. The sectioning effect is similar to that of the confocal, without the need for a detection pinhole. The resulting images show very fine skin details [12]. For both techniques, the lateral (tangential) resolution can approach 1 micron or smaller, while the axial (vertical) resolution is of a few microns, typically 3-5 µm. This resolution is obtained at the expense of very high numerical apertures, causing a high sensitivity of the systems to tissue optical defects, and a limited penetration depth (typically down to the upper dermis).

Optical coherence tomography (OCT) is another optical technique relying on light interferometry to select light from a particular depth in the sample [13]. The axial sectioning is directly related to the spectral bandwidth of the light source. Typical OCT systems use point illumination quickly scanned vertically through the tissue. The vertical scans obtained with a point detector are then repeated over a line or an area, to obtain a vertical slice or a 3D volume respectively. OCT was successfully applied to the in vivo examination of skin [14]. With a higher resolution than that provided by ultrasound or MRI (of the order of 10 microns) and a penetration depth of 1 mm, OCT is recognized as a valuable tool for skin layers thicknesses and architecture assessment [15]. In particular, the technique has been tested for epidermal thickness measurement, tumor thickness assessment, or nail disease [16,17,18].

In Full-Field OCT (FFOCT), 2D tangential optical slices are directly recorded on a camera without scanning, from a combination of interferometric images [19,20]. The biological tissue can then scanned vertically to obtain images at different depths. A source with a very broad spectrum (typically a halogen source) is chosen to yield micron-level vertical sectioning. The transverse resolution is given by the resolving power of the microscope objectives used in the interferometer. The latter can be chosen to provide appropriate resolution without the disadvantage of a system highly sensitive to the loss of focusing power in the tissue, like confocal systems. Thus the FFOCT microscope combines the optical axial sectioning and penetration capability of the OCT technique with a high transverse resolution. It can provide 3D micron-level resolution and enable to penetrate a few hundreds microns in biological tissue. The process is efficient and the light source, due to his low level of energy and a deep red wavelength range, preserves the integrity of the tissue. It has been shown to be a valuable tool to perform fast histology on fresh and fixed tissues of various organs, and well-suited for intra-operative and intra-procedural triaging and decision making applications [21].

The purpose of this paper is to present the capabilities of FFOCT for skin imaging. For this aim, images of ex-vivo and in-vivo human skin were obtained with a FFOCT system and compared when

possible to histological images, in order to fully characterize the layers and structures of the skin. Normal and pathological tissues were imaged tangentially, and reconstructed in 2D as a vertical section and in 3D, to show the different imaging modalities offered by the FFOCT technique.

METHODS

Skin tissues were obtained from subjects whose informed consent had been previously obtained. The study protocol followed the ethical guidelines of the 1975 Declaration of Helsinki as reflected in the approval by the institution's human research review committee. Three types of tissue were examined with the FFOCT system for the purpose of the present study: fresh ex-vivo tissues were analyzed without any processing, fixed ex-vivo tissues were immersed in 10% formaldehyde for 12 hours then rinsed in an isotonic solution before examination. For in-vivo examination, the arm or finger was positioned on the set-up of the microscope.

The FFOCT system used is the Light-CT Scanner, a commercial instrument from LLTech SAS, Paris, France. As shown in Figure 1a, the system consists of an upright microscope with an object and reference arm in Linnik interferometric configuration [19, 20]. A photography of the instrument can be seen in Figure 1b. The signal is extracted from the background scattered light using a combination of four phase-shifted interferometric images. The light source is a halogen lamp filtered in the red / near infrared region, providing an intensity of a few mW/mm^2 impinging on the tissue. The system provides a 0.8 mm x 0.8 mm tangential image at a rate of 35 Hz, with a 1.5 and 1 micron transverse and lateral resolution respectively. Larger fields are automatically obtained as stitches of native fields [22], and stacks of images can be obtained at the desired depths. In comparison to the previous prototype referred to in the literature [20], the present system has been optimized for higher contrast and better ergonomy / rapidity of acquisition.

For ex-vivo imaging, the tissue was placed in the purpose-made sample holder with the surface to be imaged oriented upward. An optical window was positioned on top of the tissue, and the tissue gently flattened against it [23]. Silicone oil was dropped on the window for the microscope objective to be immersed. The sample holder was set in the system, and automatically positioned first directly below a wide field camera to obtain a snapshot "en-face" image later to be used for acquisition localization. The sample was then moved under the Linnik interferometer, and the interferometer auto-adjusted for contrast optimization and automatic sample positioning. For each acquisition, the user set the position, area, depth and averaging parameters. Between 30 and 60 acquisitions were averaged for the images shown in this paper. Typical acquisitions included large field images – the

area to be acquired was selected from the snapshot image, and stacks – native field images were acquired continuously over a defined depth. The acquisition of a 10 x 10 mm large field image or a stack of 250 images took less than 5 minutes.

For in-vivo imaging, a custom-made mount was used instead of the sample holder. The mount maintained the optical window at a fixed position underneath the microscope objective. Again, the window was covered with oil to ensure immersion of the objective. The arm or the finger could be positioned directly under the window, and pressed against it. The interferometer was adjusted manually, and the same acquisition procedure was used.

The en-face 2D images acquired with the FFOCT system were visualized and exported with a medical grade viewing software; vertical as well as 3D reconstructions were obtained with that same software. No image processing was performed for the purpose of this study, except smooth filtering and adjustment of contrast and brightness.

Histological slides of normal and pathological tissues were processed for optical microscope observations. Pictures of the corresponding tissue observed with the FFOCT system were taken.

RESULTS

Observation of normal skin in vertical excision

The FFOCT technique allows the observation of the skin layers and their main structures, as shown in the "en face" FFOCT image of a vertical excision of normal aged skin, fixed in formaldehyde (Figure 2). This image was obtained as a mosaic of 84 images of 800 microns width each. The total acquisition and mosaicing time took less than 5 minutes. It must be noted that the two images shown in figure 2, namely the optical slice given by the FFOCT and histological slice, might not correspond exactly since the histological process included slicing the first layers of the sample to obtain proper section, while the FFOCT system directly imaged the flattened surface of the excision. The skin layers: epidermis, dermis, hypodermis, are clearly identified and differentiated from each other (Fig 2). The different layers of the epidermis and the nuclei of the keratinocytes are recognizable (Fig 2 inset a). In the superficial dermis a marked solar elastosis (Fig 2 inset a) is distinguished from the distribution of the bright, well defined collagen fibers of the medium and deep dermis (Fig 2 inset b). Skin structures such as pilosebaceous units (Fig 2 inset c), sweat glands (Fig 2 inset d) and blood vessels are also easily recognizable and matched to the histological features. Finally, adipocytes reveal with a clear contrast in FFOCT images (Fig 2 inset e). In conclusion, FFOCT

provides large field digital images similar to histological slides with the very high resolution enabling to magnify the observed structures. Nevertheless, it is obvious that the contrast obtained in FFOCT is different to that given by hematoxylin and eosin. In FFOCT, the signals reveal refractive index variations. Hence for example the homogeneity of refractive index inside the adipocytes results in black appearing cells. The sharpness of the images is given not only by the high transverse resolution of 1.5 µm, but also by the high axial resolution of 1 µm. Indeed, the fine axial sectioning helps to extract the features of that exact layer from a thick tissue, without having to physically slice it. This feature allows for access to the gross architecture of the tissue, as well as fine details, down to cellular features, such as nuclei.

Observation of basal cell carcinoma

Images of basal cell carcinoma (BCC) obtained with FFOCT before any fixation of the tissue demonstrate that the BCC nodules are clearly differentiated from the normal skin structures (Fig. 3). In the case shown in figure 3, this "en face" view is composed of BCC nodules surrounded by a dense peritumoral stroma, as confirmed by the histological slide. Another skin tissue revealing BCC is shown in Figure 4. At higher magnification the cellular density characteristic of BCC is visible (Fig 4, inset). As much as tumorous cells aggregations are revealed in histological slides by hematoxylin staining, they are highlighted in FFOCT by the black appearance of tumorous cell nuclei. Again, FFOCT high-resolution imaging capability and easiness of acquisition shows promise for architectural as well as cellular-level assessment of tumors.

Tangential imaging of skin and volume reconstruction

To explore the in-depth imaging capacity of the FFOCT technique, a stack of images tangential to the surface of the skin with a 1 micron step was taken in a fresh skin excision. Figure 5 shows selected images taken from the stack, averaged over 3 µm to improve signal-to-noise ratio. The different layers can be identified: the stratum corneum with anucleated bright corneocytes (Fig 5 a), the stratum granulosum and stratum spinosum with highly contrasted keratinocyte nuclei Fig 5 b and 5 c), the stratum basale with bright papillary melanin caps (Fig 5 d), and finally the imbricated bright collagen fibers and dark blood vessels of the dermis Fig 5 e). Fine details are observed here down to 100 microns below the tissue surface.

An important feature of the FFOCT technique is not only to provide high-resolution en-face images but also, thanks to the very high axial sectioning capacity, to allow reconstructing high-resolution vertical and 3D views from the en-face images. The vertical slice in Figure 6 is a reconstruction of the stack of images mentioned above.

The stack was acquired over a depth of 200 microns. The vertical reconstruction is compared to the histological slide obtained from the same excision. It must be noted that the locations of the optical FFOCT slice and the physical histological slice are not exactly matched; furthermore, the stratum corneum is probably artificially swollen by the histological process, hence it appears much thicker as compared to the equivalent layer in the FFOCT image. Again, the skin layers and features are well defined. In particular, blood vessels can be discriminated down to 200 µm below the surface. The stratum corneum or stratum spinosum thickness can be measured from the image, and the dermal/epidermal junction zone is assessed.

Finally, 3D reconstructions were obtained to investigate all the imaging possibilities offered by the FFOCT technique. The images were processed from stacks of acquisitions of in-vivo tissue (arm and finger). Again, images were acquired every micron in depth for the highest resolution. Figure 7 a shows the forearm surface. Wrinkles are well visible and can be accurately measured (the deepest here goes down to 50 µm). Figure 7 b shows a sweat gland canal imaged in the finger pulp. These examples show the capacity to image skin in-vivo and to reconstruct 3D volumes from stacks of images. This illustrates the versatility of FFOCT, and its robustness for various applications.

DISCUSSION

The skin layers and structures could be accurately identified by non-invasive observations with the FFOCT microscopy. The images obtained with this technology are very similar in their architecture to the corresponding histological slides, although the contrast modality is completely different from that given by eosin and hematoxylin staining. The stratum corneum, keratinocyte nuclei of the stratum granulosum and stratum spinosum, melanin caps of the stratum basale, collagen fibers and blood vessels of the dermis, adipocytes of the hypodermis, adnexal structures such as pilosebaceous and sweat gland units could be discriminated including the sebocytes and sweat gland epidermis fine details. Therefore architectural as well as cellular information could be retrieved from the ex-vivo "en face" images. The resolution and rapidity of acquisition make the FFOCT microscope suitable for diagnosing pathological tissues, with the example of the most current skin cancer, basal cell carcinoma, whose distinctive features such as contrasted peritumoral stroma and dense tumorous

cell aggregations were clearly visible. Therefore, the en-face resolving capability of FFOCT is similar to that offered by confocal systems, and approaches that of histological slides. The vertical and 3D reconstructions furthermore demonstrated the possibility of layer thickness and regularity measurement, similarly, but with better resolution, than standard OCT.

A quick technical review can help positioning FFOCT amongst other non-invasive imaging modalities. As compared to ultrasound imaging and MRI, optical techniques provide a much higher resolution: 1-10 μm instead of 100 μm for ultrasound and > 200 μm for MRI. The main handicap is the low penetration depth, 0.1-1 mm instead of 0.5 to > 10 cm for ultrasound and even more for MRI.

Among optical techniques, Table 1 summarizes the performance of the currently available commercial systems of OCT (Michelson Diagnostics, UK), confocal microscopy (Lucid, USA), multiphoton microscopy (JenLab, Germany), and FFOCT (LLTech, France). It can be seen that although relying on the same principles as OCT, the particular arrangement of FFOCT allows for improved resolution. The technical specifications are close to those given by commercial confocal or multiphoton microscopes, but FFOCT gives the best 3D resolution. As observed from the acquired images, this resolution allows for identification of all the skin layers and structures, and highlights architectural as well as cellular information. One advantage of FFOCT is the lack of scanning, which allows for higher acquisition speed. Similarly to the other optical techniques, FFOCT is non-invasive and non-destructive, since the light power is typically very small (about 1-2 mW/mm^2); thus, it is very save for in-vivo observations in human. Furthermore, the FFOCT technology does not require any expensive or bulky equipment and can easily be implemented in a clinical environment.

With its specifications, the FFOCT technology has potential in many fields of dermatology. The very high resolution and easiness of use allows for quick and accurate analysis of ex-vivo tissue, with or without fixation. In particular, the technique could be applied to tissue sampling and biobanking, to quickly characterize the morphology of samples before biochemical analysis or cryopreservation. The non-invasiveness ensures the tissue has not been altered. Furthermore, the BCC features observed indicate a potential use in micrographic surgery as an alternative to histological examination of surgical margins. FFOCT imaging technique could help reducing drastically the technician working time, limiting the required technical surface, and allowing for operator independent imaging capacities. Further quantitative studies would be needed to validate the pathological diagnosis based on FFOCT images.

Our studies have also shown the possibility for in-vivo FFOCT imaging of skin. Such a modality can improve skin cancer diagnosis accuracy and limit the number of required biopsies. Skin cancer early and accurate diagnosis is essential. In-vivo, new imaging techniques are thus crucial for healthcare

future. Possible applications for in-vivo FFOCT include analysis of atypical pigmented lesions selected by a dermatoscopic check-up, or definition of the excision's margins of skin cancers. As mentioned recently by Smith and MacNeil, there is still need for high-resolution techniques to assess the development of tumors through the basal layers [24]. Further development and investigation could answer the question of the capacity to fulfill this need. FFOCT can also be applied to cosmetic studies, would it be epidermis thickness measurement, wrinkle depth assessment, or dermal/epidermal junction analysis. In vivo and in situ FFOCT imaging with a rigid endoscopic probe has recently been demonstrated [25], indicating the way towards a miniaturized FFOCT in-vivo device.

CONCLUSION

In conclusion, FFOCT is a novel optical technique that combines OCT light interferometry principles with high-resolution capacity. The unique 3D micron-level resolution allows versatile viewing of thick tissues in tangential and vertical virtual slice, or in 3D full volume reconstruction. Layers and structures of normal and pathological tissues can be identified, from the architectural to the cellular level. The technology has the potential to be a relevant complement to existing non-invasive imaging modalities for clinical and cosmetic applications.

ACKNOWLEDGEMENTS

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Table 1. Comparison of optical skin imaging techniques, based on current commercial devices.

Technique	Transverse resolution	Axial resolution	Penetration depth	Analysis surface	Acquisition frequency
OCT	7.5 μm	10 μm	hypodermis	Vertical slice 5 mm x 1.5 mm	6.5 Hz
Confocal microscopy	1 μm	5-7 μm	Papillary dermis	Tangential slice 750 μm x 750 μm	9 Hz
Multiphoton microscopy	1 μm	2 μm	Papillary dermis	Tangential slice 350 μm x 350 μm	
FFOCT	1.5 μm	1μm	Dermis	Tangential slice 800 μm x800 μm	35 Hz
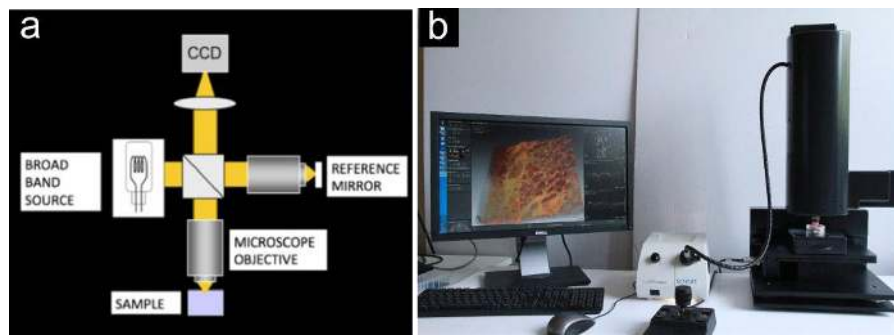
FIGURES

Figure 1. Schematic of the FFOCT microscope (a) and photography of the commercial device (b).
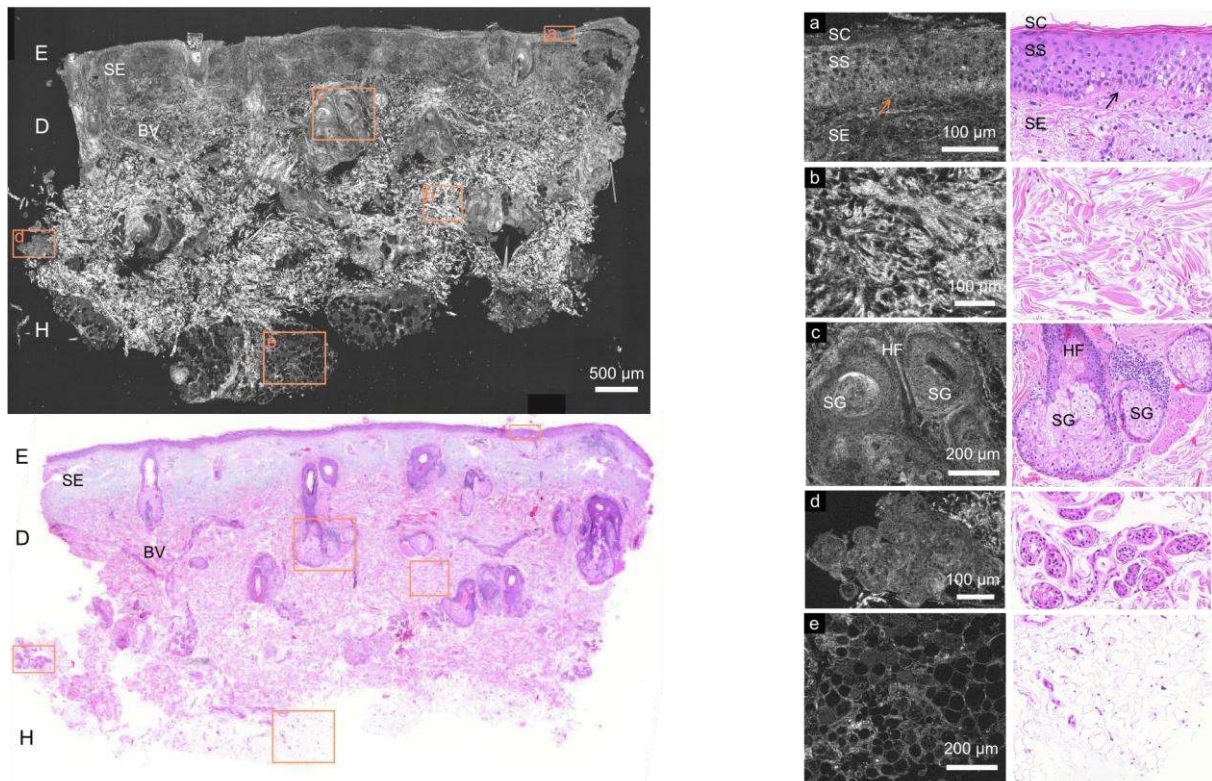
Figure 2. FFOCT image of a normal aged skin and the corresponding histological slide. The three layers of the skin, epidermis (E), dermis (D), hypodermis (H), as well as a solar elastosis region (SE) and blood vessels (BV) are clearly distinguished. At high magnification, the stratum corneum (SC) can be differentiated from the stratum spinosum (SS) (inset 2a) and the enlargement of the nuclei from the basal layer (arrows) to the upper spinous layer is highly visible. The elastotic superficial dermis (SE in 2a) contrasts with the highly refractive collagen fibers of the dermis (2b). A pilosebaceous unit with hair follicule (HF) and sebaceous glands (SG) (2c), a sweat gland unit (2d) and adipocytes with their bright cell membranes (2e) are identified.
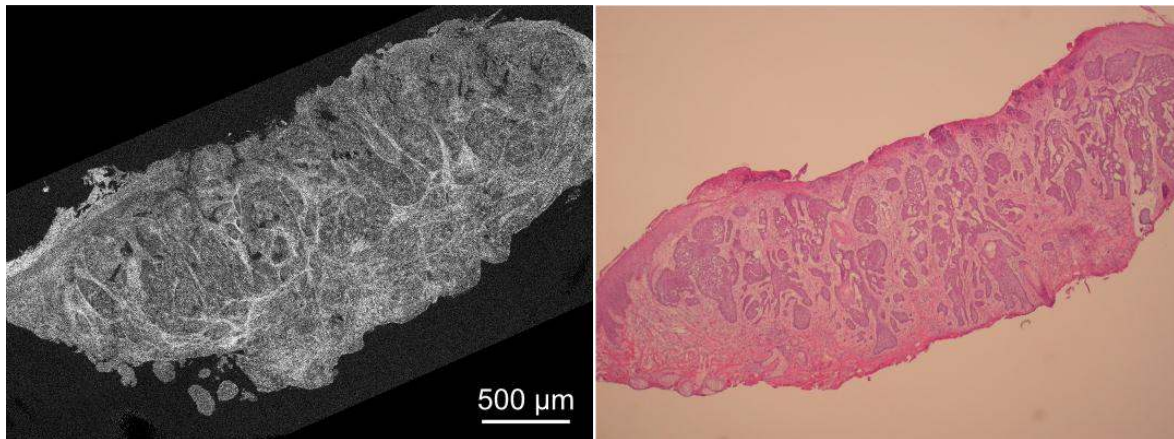
Figure 3. Comparison of the FFOCT image of a vertical skin fresh excision with nodular basal cell carcinoma, with the corresponding histological slide.
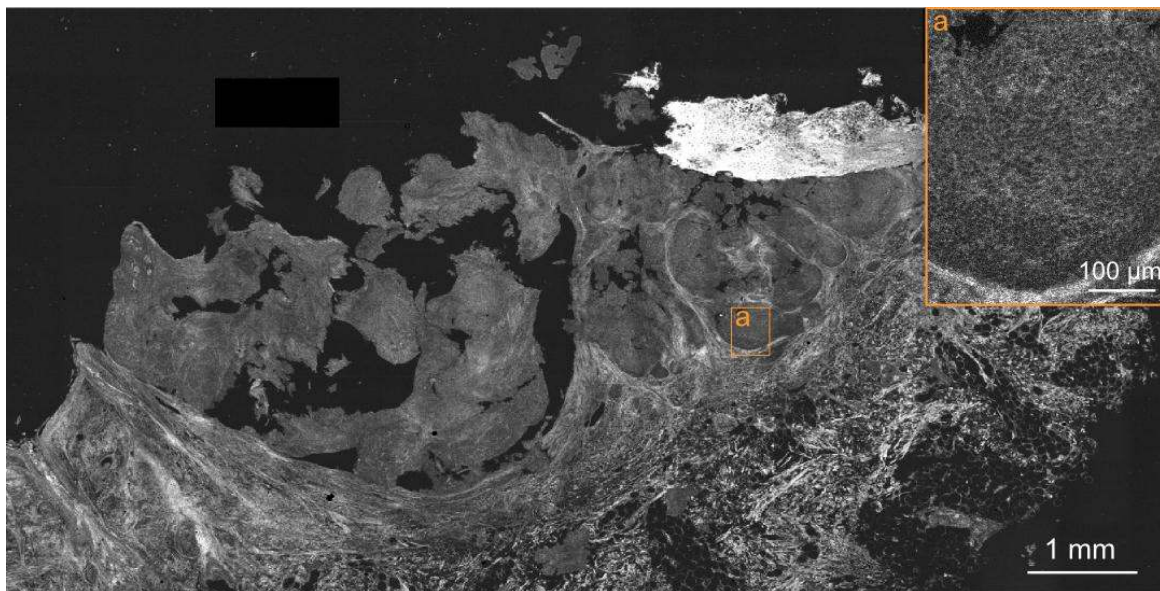

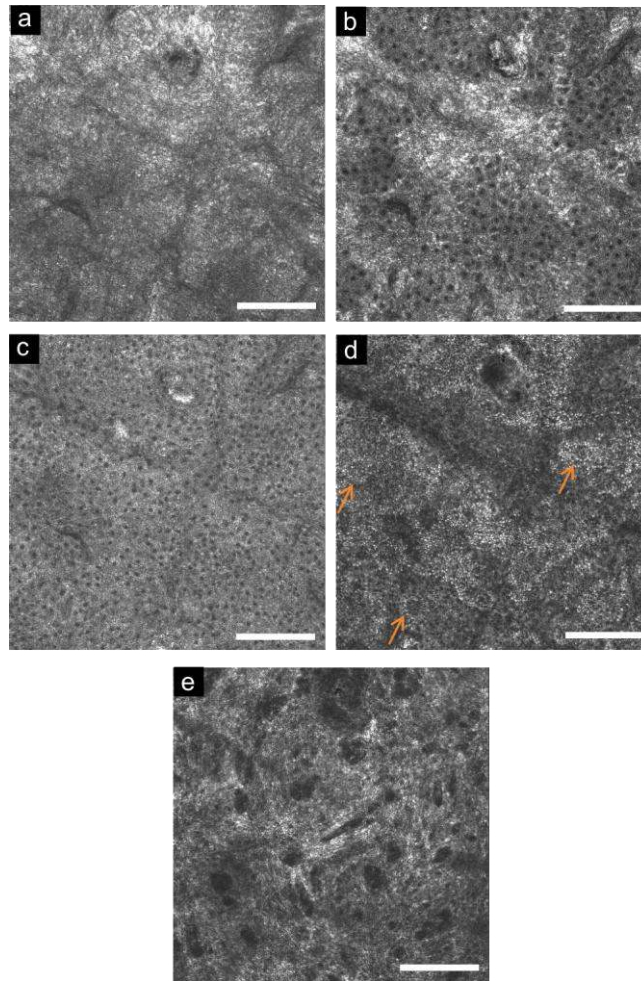Figure 4. Basal cell carcinoma in a skin vertical excision. Inset sows zoom of region a.

Figure 5. Tangential images of skin fresh excision. a: stratum corneum (depth 10 μm); b: stratum granulosum (depth 25μm); c: stratum spinosum (depth 35 μm); d: stratum basale where arrows point at some papillaries (depth 60 μm); e: dermis (depth 100 μm). Scale bar represents 200 microns.
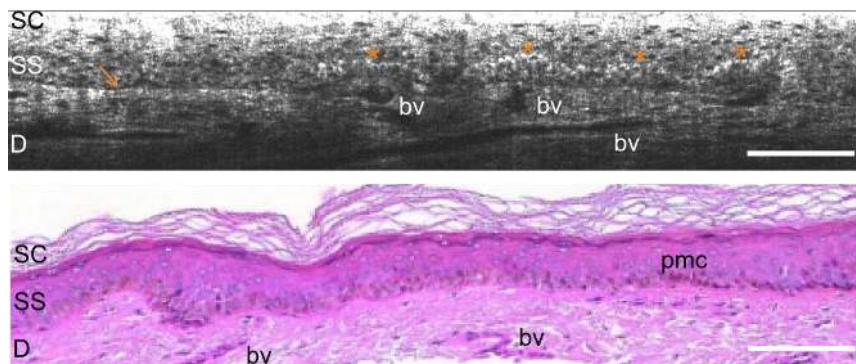

Figure 6. Reconstructed vertical slice (a) of skin tangential excision and corresponding histological slide (b).Visible layers: stratum corneum (S), stratum spinosum (SS), dermis (D). The basement membrane (arrow) and melanin caps (stars) are visible. Blood vessels (bv) can also be identified. Scale bar is 100 μm.
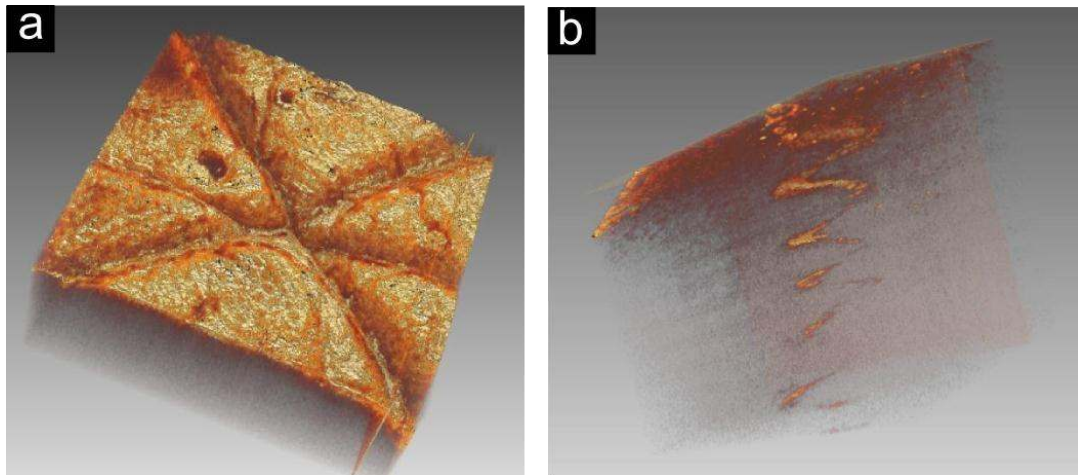
Figure 7. Reconstructed 3D volume of the skin arm surface (a) and finger sweat gland (b), from in-vivo stacks of tangential image.