

Implications of Ultrasound Frequency in Optoacoustic Mesoscopy of the Skin

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Abstract—Raster-scan optoacoustic mesoscopy (RSOM) comes with high potential for *in vivo* diagnostic imaging in dermatology, since it allows for high resolution imaging of the natural chromophores melanin, and hemoglobin at depths of several millimeters. We have applied ultra-wideband RSOM, in the 10–160 MHz frequency band, to image healthy human skin at distinct locations. We analyzed the anatomical information contained at different frequency ranges of the optoacoustic (photoacoustic) signals in relation to resolving features of different skin layers *in vivo*. We further compared results obtained from glabrous and hairy skin and identify that frequencies above 60 MHz are necessary for revealing the epidermal thickness, a prerequisite for determining the invasion depth of melanoma in future studies. By imaging a benign nevus we show that the applied RSOM system provides strong contrast of melanin-rich structures. We further identify the spectral bands responsible for imaging the fine structures in the *stratum corneum*, assessing dermal papillae, and resolving microvascular structures in the horizontal plexus.

Index Terms—Dermatology, imaging, microvasculature, nevus, optoacoustic, photoacoustic, skin.

I. INTRODUCTION

OPTOACOUSTIC mesoscopy offers label-free high-resolution imaging of melanin, and hemoglobin [1] through several millimeters of tissue [2], and comes with high potential for applications in dermatology. Due to its sensitivity in detecting hemoglobin, the method could identify a variety of anatomical features associated with skin vasculature. Blood vessels in human skin range from thin capillaries with an internal endothelial tube diameter of 4–6 μm [3], located in the dermal papillae to larger vessels with a diameter of up to 100 μm in the mid- and deep dermis [4]. Thus, the frequency content of optoacoustic signals generated in the skin is intrinsically broadband. Conversely, the attenuation of ultrasound waves in tissue increases exponentially with frequency [5]–[7] limiting the detection of high frequency components from deep tissue layers.

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The useful bandwidth available for optoacoustic imaging of the skin, and its relation to depth, is currently not known. However, it would be important that optoacoustic skin mesoscopy could resolve fine vascular structures crucial to the diagnosis of several skin diseases including systemic sclerosis [8], [9], psoriasis [10], [11], and collagenoses [12], and the infiltration depth of skin lesions such as melanoma skin cancer; the latter being a significant factor in the prognosis of recurrence and metastasis [13]–[15].

Optoacoustic tomography has been considered for the visualization of human skin since the late 1990s but originally suffered from low lateral resolution of 200 μm [16]. More recently, optoacoustic imaging has been applied for assessing the human cutaneous vascular network or the depth of thermal burns by raster-scans of a single transducer with central frequency of 50 MHz, providing lateral resolution of $\sim 45 \mu\text{m}$ [17]–[20]. The low lateral resolution was identified as a limitation in visualizing the microcirculation in the papillary dermis [21]. Moreover, it was not possible to differentiate the *stratum corneum* and the epidermal-dermal junction in hairy skin [19]. With a different setup consisting of a linear detector array at 24 MHz central frequency, several millimeters of tissue burn and of superficial skin lesions have been imaged with a resolution of $< 100 \mu\text{m}$ [22], [23]. Most recent, human skin was optoacoustically imaged using an ultra-wideband ultrasound transducer with a central frequency of 102.8 MHz, enabling the visualization of different small vascular structures at the epidermal-dermal junction together with bigger structures situated deeper in the horizontal plexus [24].

The study herein focused on identifying the frequency contributions from different skin layers. This information could be used to better understand the performance of optoacoustic imaging of skin and overall epithelial tissues and optimize the detection characteristics of mesoscopic systems designed for dermatology applications. We interrogated the relation of the frequency band attained by optoacoustic mesoscopy and the overall quality of skin imaging achieved. We particularly examined the frequency content required to reveal structures and depths implicated in the diagnosis or staging of skin diseases [25], including resolving the basement membrane at the epidermal-dermal junction or the microcirculatory network below the epidermal-dermal junction. Notably, we identify the frequency content required for imaging the *stratum corneum*, i.e., the outmost layer of the epidermis; a structure that to the best of our knowledge has never previously been visualized in hairy skin using optoacoustics.

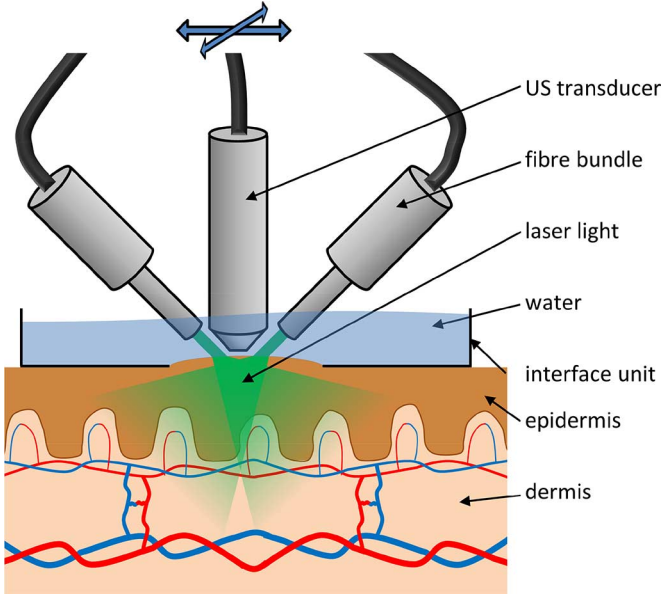


Fig. 1. RSOM schematic employed to perform *in vivo* experiments of the skin. Interface unit has an opening in the center to allow direct coupling of water with the region of interest. Ultra-wideband transducer and the fiber bundles are immersed in water. Beams are aligned in order to cross each other slightly beneath the skin surface. Two arrows indicate 2-D scanning of the illumination-transducer unit.

II. METHODS AND MATERIALS

A. Experimental Setup

To investigate the optoacoustic frequency content of skin, we employed raster-scan optoacoustic mesoscopy (RSOM) (see Fig. 1), based on a spherically focused transducer operating at 10–160 MHz with a diameter of 1.5 mm, and an f-number of ~ 1 . The transducer was connected to a 63 dB low noise amplifier (AU-1291, MITEQ Inc.) and measurements were collected with a high-speed digitizer operating at 1 GS/s (Gage Applied Technologies). Tissue excitation was based on a 1 ns pulse width laser, operating at 532 nm, pulsing with a frequency of 1 kHz and 0.5 mJ per pulse, or 2 kHz and 0.3 mJ per pulse, respectively (Wedge HB.532, Bright-solutions). Laser energy was measured at the fiber tips. The laser light was delivered to the skin using one fiber bundle with three arms, each with a core diameter of 3 mm, and angled at approximately 55° with respect to the skin normal, illuminating an area of approximately 40 mm^2 at the skin surface. Thus, the per pulse incident light fluence at the skin surface was less than 1.25 mJ/cm^2 , which is well below the ANSI laser safety limit of 20 mJ/cm^2 [26]. The maximum permissible pulse repetition rate, for repetitive pulsing on the skin surface for less than 10 s, was below the ANSI laser safety limit stated in [18].

For imaging purposes, the transducer and illumination system were raster-scanned in-tandem using two piezo-electric stages (PI-Physik Instrumente). The step size of the 2-D scan was $10 \text{ }\mu\text{m}$ in both directions. An area of $8 \text{ mm} \times 8 \text{ mm}$ was scanned in approximately 5.3 min [27], [28]. The system achieved an isotropic in-plane resolution of $18 \text{ }\mu\text{m}$ and axial resolution of $4 \text{ }\mu\text{m}$ [28].

An interface unit (IU), shown on Fig. 1, was designed to couple sound from the skin to the RSOM system. The IU consisted of acrylic glass with a $2 \text{ cm} \times 2 \text{ cm}$ square opening in the center to allow direct contact of skin and water, the latter acting as a sound-coupling medium. The holder was fixed to the skin with double-sided tape. The RSOM system was scanned through this opening over an $8 \text{ mm} \times 8 \text{ mm}$ region of interest (ROI), acquiring 641601 A-scans.

B. Image Reconstruction and Visualization

Image reconstruction was based on a 3-D beam-forming algorithm, with dynamic aperture, to account for the directivity of the detectors [27], [28]. The size of the reconstruction voxel was set to $10 \text{ }\mu\text{m} \times 10 \text{ }\mu\text{m} \times 3 \text{ }\mu\text{m}$.

To identify the influence of ultrasound bandwidth on the images, the raw-data were filtered with a Butterworth band-pass filter of the order 2. Different spectral bands were reconstructed to identify the relative influence of frequency on imaging components. Herein we present representative results from three key frequency bands. The frequency bands selected were consecutive and attained $\sim 86\%$ relative bandwidth, i.e., 10–25 MHz, 25–63.5 MHz, and 63.5–160 MHz, hereafter referred to as low, mid and high frequency ranges, respectively.

The three reconstructed volumes at low (R_{low}), mid (R_{mid}), and high frequencies (R_{high}) were summed up voxel-by-voxel to obtain a reconstruction volume covering the whole frequency range of the transducer

$$R^{xyz} = W_{\text{low}} R_{\text{low}}^{xyz} + W_{\text{mid}} R_{\text{mid}}^{xyz} + W_{\text{high}} R_{\text{high}}^{xyz} \quad (1)$$

where R^{xyz} is the reconstruction of a voxel at position (x, y, z) , and W_{low} , W_{mid} , and W_{high} are weighting factors. By setting $W_{\text{mid}} W_{\text{high}} > W_{\text{low}}$ small microvascular structures, contained in high frequencies, are amplified. However, amplifying high frequency components would introduce noise as well, since high-frequency components are attenuated more strongly in tissue and, thus, have lower signal-to-noise ratio (SNR). The SNR increases with the amount of energy deposited in the tissue per laser pulse. To balance amplification of high-frequency components and noise reduction, the weighting factors were empirically set as follows: $W_{\text{low}} = 1$, $W_{\text{mid}} = 2$, and $W_{\text{high}} = 2$ for a per pulse energy of 0.3 mJ, and $W_{\text{low}} = 1$, $W_{\text{mid}} = 3$, and $W_{\text{high}} = 5$ for a per pulse energy of 0.5 mJ.

C. Experimental Measurements and Frequency Analysis

For the frequency analysis of the optoacoustic data and the evaluation of epidermal thickness, three different areas on the hand of a healthy human male volunteer were imaged. Fig. 4(d)–(f) shows the three areas that were scanned, the first one located at the dorsum of the hand between metacarpal I and II, the second one located on the thenar, and the third one located at the finger pad of the thumb. In the following we will refer to these areas by dorsum of hand, thenar, and thumb tip areas, respectively. The regions of interest were chosen in order to cover both glabrous skin, featuring epidermal ridges, and hairy skin, also known as nonridged skin. The thenar, and thumb tip belong to glabrous skin, the dorsum of hand is part of the hairy skin. The spectral density of the data acquired was

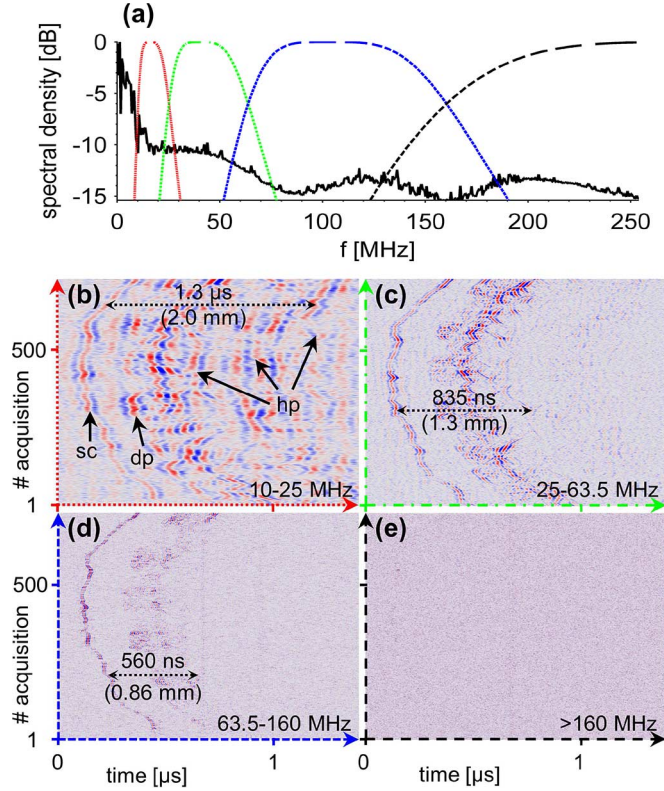


Fig. 2. Frequency content of the raw data acquired by scanning the thenar area. (a) Mean spectral density of the acoustic frequencies acquired over 681601 individual A-scans. Four different frequency bands are marked: 10–25 MHz (red, dotted), 25–63.5 MHz (green, dash-dotted), 63.5–160 MHz (blue, dashed), and >160 MHz (black, dashed). (b)–(e) Raw data of a single line-scan with 720 subsequent acquisitions (y-axis) filtered by the frequency bands depicted in (a). Depicted time interval is $1.4 \mu\text{s}$, corresponding to roughly 2.1 mm in depth for a speed of sound of 1530 m/s. Raw data filtered by the frequency ranges 10–25 MHz, 25–63.5 MHz, 63.5–160 MHz, and >160 MHz, are visualized in (b), (c), (d), and (e), respectively. sc: stratum corneum, dp: dermal papillae, hp: horizontal plexus.

inspected in the Fourier domain, by applying the Fast Fourier Transform to the 641601 A-scans collected per area imaged.

Additionally, a benign nevus located at the lower arm of a male human volunteer was imaged in order to show the potential of optoacoustics in imaging lesions of the human skin, which will be evaluated in detail in further studies. The benign nevus is shown in Fig. 6.

III. RESULTS

A. Frequency Content of Raw Data

Fig. 2 shows the frequency content of the optoacoustic signals collected from the thenar area. Fig. 2(a) depicts a graphical representation of the filters applied to analyze the optoacoustic data.

In Fig. 2(b)–(e) 720 subsequent acquisitions from a representative line-scan are plotted as a function of time demonstrating the filtered data obtained in the four frequency bands shown in Fig. 2(a). This view allows a first inspection of the strength of the signals collected from different skin layers. The dotted double-sided arrow indicates the distance between the entrance signal of the skin and signal from the deepest visible structure. Already in this “raw” view, major skin layers can be identified.

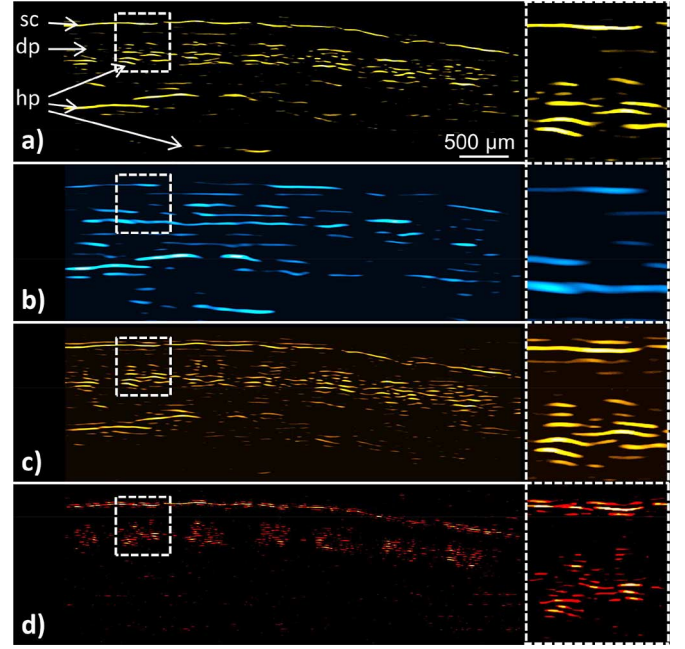


Fig. 3. Frequency content of different structures in skin. (a)–(d) Exemplary cross section through the reconstructed image of the thenar area at different frequency bands. Reconstruction bandwidths are: (a) 10–160 MHz, as described in (Section II-B). (b) 10–25 MHz. (c) 25–63.5 MHz. (d) 63.5–160 MHz. Small figures on the right-hand side show a zoom in of the ROI marked by the white dashed box in (a)–(d). sc: stratum corneum, dp: dermal papillae, hp: horizontal plexus.

The structure, marked by *sc* corresponds to signals from the stratum corneum, *dp* indicates the dermal papillae, and *hp* indicates structures in the horizontal plexus.

The images demonstrate that signals in the 10–25 MHz range reach approximately 2 mm, signals at the 25–63.5 MHz range can be detected deeper than 1 mm, and signals in the 63.5–160 MHz range can be detected from depths of less than 1 mm. The high frequency range, depicted in Fig. 2(d), carries information on the entrance signal at the skin surface and the vascular network in the dermal papillae, i.e., the fine capillary network below the epidermal-dermal junction. Higher frequencies do not appear to contain any information.

B. Frequency Analysis on the Reconstruction

To understand the influence of frequency content on RSOM skin images we reconstructed images of the thenar area using raw data at different frequency bands. Fig. 3 shows reconstructions of single cross sections through the skin at the three relevant frequency bands considered in Fig. 2(a), and an image combining all frequencies. Fig. 3(b) depicts the reconstruction obtained at the frequency range 10–25 MHz and showcases visualization of skin features from depths of at least 1.5 mm. The stratum corneum shows large discontinuities at low frequencies. The vessel network in the papillary dermis is visible, but at low resolution. Distinct neighboring vessels in the upper papillary dermis are not resolved as distinct objects. In the lower papillary dermis larger vessels of the horizontal plexuses appear. In the frequency range 25–63.5 MHz, shown in Fig. 3(c), detailed anatomical structures of the skin surface, the papillary dermis and the upper horizontal plexus of the dermis are seen down

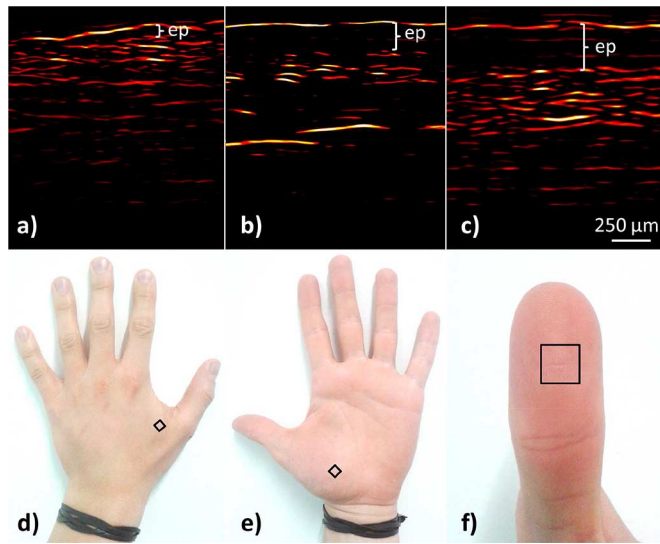


Fig. 4. Cross-section through skin at different locations. (a) Dorsum of hand. (b) Thenar. (c) Thumb tip. Locations marked in (d), (e), and (f) correspond to the measured skin area from which the cross sections in (a), (b), and (c), respectively, were taken. ep: epidermis.

to a depth of approximately 1 mm. Due to the limited relative bandwidth of $\sim 86\%$ after band-pass filtering of a broadband optoacoustic signal, the optoacoustic signal is distorted and ringing of single anatomical structures appears. The effect of signal distortion through band-pass filtering of the raw data is even more severe at high frequencies between 63.5 MHz and 160 MHz, shown in Fig. 3(d). The high frequencies contain valuable information on the skin surface and some details on the papillary dermis within the upper 500 μm . Only by combining all of the above frequency ranges from 10–160 MHz into a single image, the detailed anatomical features are seen without bandwidth related image artifacts. The *stratum corneum* appears as a thin single-layered surface with only minor discontinuities in the image. Fine structures in the papillary dermis are visualized. At the same time deep-seated vessels are still visible. As described above (Section II-B), fine structures on the skin surface and the upper papillary dermis were enhanced by amplification of mid- and high-frequency components in the reconstruction.

C. Thickness of the Epidermis

Fig. 3 shows the relative importance of mid- and high frequencies to visualize the small structures that appear in the upper layers of the skin, including the *stratum corneum*. Fig. 3(c) and (d) shows that these small structures are found to emit frequencies above 25 MHz. The mid and high frequency bands show bandwidth-limited multi-layer effects. However the combination of all three frequency bands (ultra-bandwidth RSOM) accurately reveals the *stratum corneum* by reconstructing a single-layered homogeneous surface.

To interrogate the RSOM ability to visualize variations in the epidermis thickness we performed scans at three different locations, as shown in Fig. 4. We imaged at the dorsum of hand, at the thenar, and on the thumb tip, as shown in Fig. 4(d)–(f). Exemplary cross sections of the reconstructed images at these three locations are shown in Fig. 4(a)–(c). In all skin types the

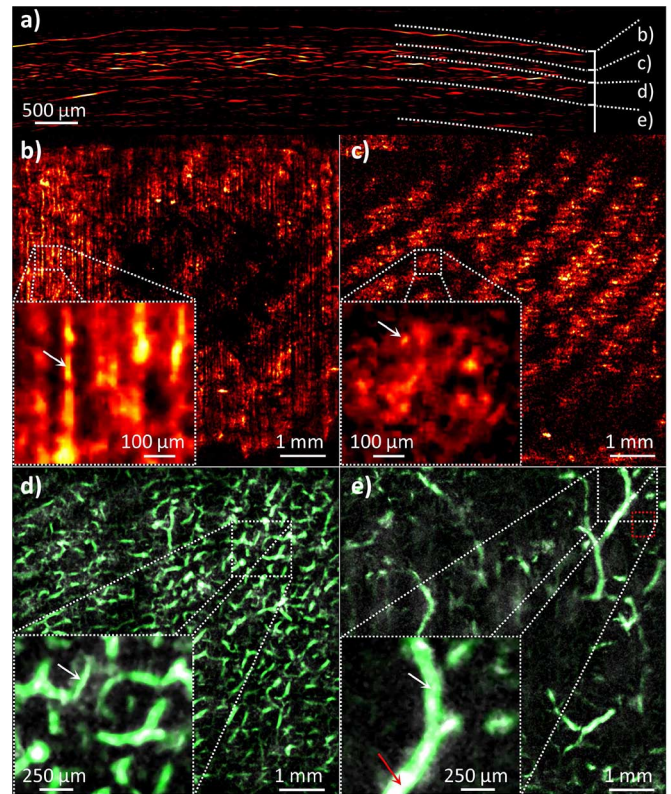


Fig. 5. Different layers of human skin. (a) Cross section through human skin. (b)–(e) MAPs along the depth direction within the regions marked in (a). (b) Epidermis (0–150 μm), (c) dermal papillae (150–300 μm), (d) subpapillary plexus (300–555 μm), and (e) deep vessels of the subpapillary plexus or the intermediate venous plexus of the dermis (555–975 μm). In (b)–(e) a zoom in into the region marked by the white dashed box is shown. FWHM of the line-like or point-like structures marked by the white arrows in each subfigure were calculated to be: 27.5 μm (b), 35 μm (c), 55 μm (d), and 87.5 μm (e). In (d), (e) the original image (white) is overlain with an image filtered for vessels in green. The SNR of the vessel marked by the red arrow with respect to the background marked by the red box in (e) in the unfiltered image is 12 dB.

stratum corneum of the epidermis is clearly visible as one continuous layer. We determined the thickness of the epidermis by measuring the vertical distance of the skin surface to the first vessels in the papillary dermis. The thickness of the epidermis varies significantly between different locations. The thickness at the dorsum of hand, the thenar, and the thumb tip measured $\sim 70 \mu\text{m}$, $\sim 190 \mu\text{m}$, and $\sim 310 \mu\text{m}$, respectively.

D. Depth and Resolution of Distinct Skin Layers

To study how the resolution of the applied RSOM system depends on depth, we segmented four different skin layers oriented parallel to the skin surface and evaluated the structure size within each layer. Fig. 5(a) shows a cross slice through the skin and the four distinct layers observed with our system. Fig. 5(b)–(e) shows maximum amplitude projections (MAP) in the direction perpendicular to the skin surface within the depth ranges marked in Fig. 5(a). The *stratum corneum* in Fig. 5(b) is located at depth $< 150 \mu\text{m}$ and is seen as a homogeneous surface with structures as small as 27.5 μm . In the center, where the distance of the skin to the transducer surface was lowest, the homogeneous skin surface has a low SNR because of weak illumination. The vascular fingerprint of the epidermal-dermal

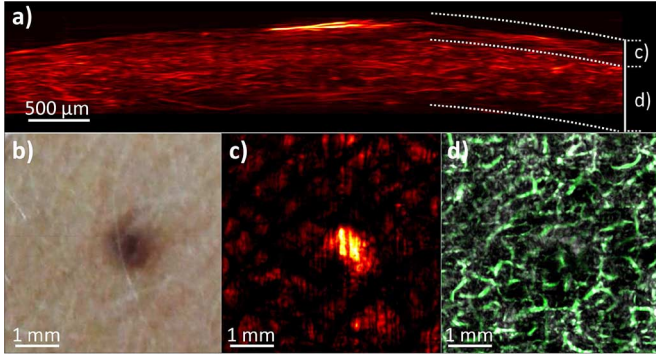


Fig. 6. Imaging of a benign nevus. (a) MAP parallel to the skin surface of the ROI shown in (b). Bright spot stems from the benign nevus. (b) ROI containing a benign nevus, which measured ~ 1.5 mm in diameter. (c), (d) MAP along the direction perpendicular to the skin surface within the limits marked in (a). In (d) the original image (white) is overlain with an image filtered for vessels in green.

junction is shown in Fig. 5(c). In this region we observe small absorbing spots arranged in a stripe pattern that are separated by approximately 0.9 mm. The zoom in Fig. 5(c) shows single anatomical structures as small as $35 \mu\text{m}$ located $150\text{--}300 \mu\text{m}$ below the surface. Fig. 5(d) shows the vast vascular network of the papillary dermis, in the lower papillary dermis. In white the original data is shown overlaid with an image filtered for vessels in green [29]. We observe vessels with a FWHM of $55 \mu\text{m}$ and higher at $300\text{--}555 \mu\text{m}$ below the skin surface. Below this network of microvasculature, we observe larger vessels that belong to either the lower parts of the sub-papillary plexus or the intermediate venous plexus of the dermis. The vessel marked in Fig. 5(e) has a FWHM of $87.5 \mu\text{m}$ and lies $555\text{--}975 \mu\text{m}$ below the skin surface. The SNR of the vessel marked by the red arrow with respect to the background, marked by the red box in Fig. 5(e), was 12 dB.

E. Imaging of a Benign Nevus

To show the potential of optoacoustic imaging in visualizing the infiltration depth of melanoma, we imaged a benign nevus of approximately 1.5 mm in diameter. Fig. 6 shows the optoacoustic images of the nevus in comparison to a photograph. Fig. 6(a) depicts the MAP of the nevus as seen from the side. The nevus itself shows strong absorbance in the superficial layers of the skin. Below the nevus the vascular network of the upper dermis is intact. Fig. 2(c) shows a MAP along the depth of the first $130 \mu\text{m}$ of the ROI around the nevus. The size and absorption amplitude of the nevus correlates well with the photograph depicted in Fig. 6(b). Fig. 6(d) shows the MAP starting at a depth of $130 \mu\text{m}$ below the skin surface. The vascular network in Fig. 6(d) is overlaid with an image filtered for vessels in green [29]. The vascular network is not affected by the benign nevus.

IV. DISCUSSION

We have imaged human skin with a raster-scan optoacoustic mesoscopy system, employing an ultra-wideband spherical transducer, and analyzed the frequency content of optoacoustic

signals emitted from skin as a function of depth. At frequencies below 25 MHz, we observe vascular structures to a depth of approximately 2 mm. Frequencies from 25 MHz to 63.5 MHz penetrate >1 mm through cutaneous tissue. Frequencies above 63.5 MHz penetrate $0.5\text{--}0.9$ mm through cutaneous tissue, capturing valuable information on the *stratum corneum* and the vascular fingerprint of the epidermal-dermal junction [24], [30], [31]. With the high frequencies single absorbing structures of $27.5 \mu\text{m}$ on the skin surface, and $35 \mu\text{m}$ in the epidermal-dermal junction were observed up to $300 \mu\text{m}$ below the skin surface. Compared to previous optoacoustic systems [17]–[20], the resolution has been significantly improved and provides sufficient resolution for the visualization of the microcirculatory network [21]. The importance of high ultrasonic frequencies in visualizing the *stratum corneum* and the capillaries of the epidermal-dermal junction is well understood by the fact that the thickness of these structures measure $\sim 5\text{--}15 \mu\text{m}$ and, thus, emit optoacoustic frequencies above 100 MHz.

We observed an increase in the size of absorbers with depth that can be attributed to two aspects. First, the attenuation of high ultrasonic frequencies penetrating through cutaneous tissue leads to a slow degradation of resolution with depth. Second, the diameter of vessels in the mid- and lower dermis is larger [32] compared to the size of the microvessels in the dermal papillae [33].

We were able to reliably visualize the *stratum corneum* of human skin in glabrous as well as in hairy skin. Consequently, we were able to determine the thickness of the epidermis to be $\sim 70 \mu\text{m}$ at the dorsum of hand, $\sim 190 \mu\text{m}$ at the thenar, and $\sim 310 \mu\text{m}$ at the thumb tip. These values correspond well to the mean epidermal thickness of $72 \pm 12 \mu\text{m}$ at the radial aspect at the dorsum of hand, $222.7 \pm 92.9 \mu\text{m}$ at the side of a finger, and $369 \pm 111.9 \mu\text{m}$ at the fingertip as reported in the literature [34].

These findings validate the need for ultra-bandwidth in RSOM measurements of skin above 60 MHz that was found necessary in order to visualize both features of the epidermis and the microvascular network of the epidermal-dermal junction. This study also provides justification for the development of clinical protocols in the future, in order to apply RSOM in clinical settings. The imaging performance revealed by imaging a benign nevus could be used to resolve the infiltration depth of skin lesions such as melanoma skin cancer beyond the basement membrane [35], which is a significant factor in the prognosis of recurrence and metastasis [13]–[15]. RSOM imaging could provide a cost-effective diagnostic tool for psoriatic skin, which is characterized by the widening of capillaries in the dermal papillae [10], [11]. Furthermore, damage to small blood vessels in systemic sclerosis [8], [9] is expected to show direct contrast on RSOM images.

Future plans will include the translation of the RSOM system into a portable device that will open up possibilities for clinical studies of skin conditions. To avoid minor discontinuities in the vascular network of the subpapillary plexus, caused by the low numerical aperture of the transducer, the system will be developed further. Future steps in the development of the system will include the design of an improved IU between the optoacoustic system and human tissue, the application of a transducer with higher numerical aperture, and improving the illumination.

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