Effect of irradiation distance on image contrast in epi-optoacoustic imaging of human volunteers

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Abstract: In combined clinical optoacoustic (OA) and ultrasound (US) imaging, epi-mode irradiation and detection integrated into one single probe offers flexible imaging of the human body. The imaging depth in epi-illumination is, however, strongly affected by clutter. As shown in previous phantom experiments, the location of irradiation plays an important role in clutter generation. We investigated the influence of the irradiation geometry on the local image contrast of clinical images, by varying the separation distance between the irradiated area and the acoustic imaging plane of a linear ultrasound transducer in an automated scanning setup. The results for different volunteers show that the image contrast can be enhanced on average by 25% and locally by more than a factor of two, when the irradiated area is slightly separated from the probe. Our findings have an important impact on the design of future optoacoustic probes for clinical application.

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References and links

1. P. Beard, “Biomedical photoacoustic imaging,” Interface Focus 1(4), 602–631 (2011).
2. M. Xu and L. V. Wang, “Photoacoustic imaging in biomedicine,” Rev. Sci. Instrum. 77(4), 041101 (2006).
3. X. Wang, X. Xie, G. Ku, L. V. Wang, and G. Stoiica, “Noninvasive imaging of hemoglobin concentration and oxygenation in the rat brain using high-resolution photoacoustic tomography,” J. Biomed. Opt. 11(2), 024015 (2006).
4. H. F. Zhang, K. Maslov, G. Stoiica, and L. V. Wang, “Functional photoacoustic microscopy for high-resolution and noninvasive in vivo imaging,” Nat. Biotechnol. 24(7), 848–851 (2006).
5. S. Hu and L. V. Wang, “Photoacoustic imaging and characterization of the microvasculature,” J. Biomed. Opt. 15(1), 011101 (2010).
6. J. Laufer, D. Delpy, C. Elwell, and P. C. Beard, “Quantitative spatially resolved measurement of tissue chromophore concentrations using photoacoustic spectroscopy: application to the measurement of blood oxygenation and haemoglobin concentration,” Phys. Med. Biol. 52(1), 141–168 (2007).
7. J. J. Niederhauser, M. Jaeger, and M. Frenz, “Comparison of laser-induced and classical ultrasound,” Proc. SPIE 4960, 118–123 (2003).
8. J. J. Niederhauser, M. Jaeger, R. Lemor, P. Weber, and M. Frenz, “Combined ultrasound and optoacoustic system for real-time high-contrast vascular imaging in vivo,” IEEE Trans. Med. Imaging 24(4), 436–440 (2005).
9. R. G. M. Kolkman, P. J. Brands, W. Steenbergen, and T. G. van Leeuwen, “Real-time in vivo photoacoustic and ultrasound imaging,” J. Biomed. Opt. 13(5), 050510 (2008).
10. A. Guirre, P. Guo, J. Gamelin, S. Yan, M. M. Sanders, M. Brewer, and Q. Zhu, “Coregistered three-dimensional ultrasound and photoacoustic imaging system for ovarian tissue characterization,” J. Biomed. Opt. 14(5), 054014 (2009).
11. M. Jaeger, L. Siegenthaler, M. Kitz, and M. Frenz, “Reduction of background in optoacoustic image sequences obtained under tissue deformation,” J. Biomed. Opt. 14(5), 054011 (2009).
12. J. C. Bamber, “Acoustical Characteristics of Biological Media,” in Encyclopedia of Acoustics (John Wiley & Sons, Inc., 2007), pp. 1703–1726.
1. Introduction

In optoacoustic (OA) imaging, the tissue is irradiated by pulsed laser light, leading subsequently to the generation of thermo-elastic pressure transients inside optically absorbing structures. The detection of these pressure transients by an acoustic probe allows a reconstruction of the absorbing structures deep inside biological tissue [1, 2]. Moreover,
spectral OA imaging enables spatially-resolved measurement of the local blood oxygenation level, due to the different optical absorption spectra of oxygenated and deoxygenated hemoglobin thus providing both, structural as well as functional information [3–6]. The combination of OA imaging with conventional B-mode echo ultrasound (US) offers the potential of a multi-modal functional imaging modality [7–11]. The preferred setup for this modality is based on epi-mode, which implies that tissue irradiation and OA signal detection take place at the same tissue surface. This allows imaging of body parts, where in transmission mode bones or attenuating soft tissue would obstruct ultrasound propagation from the irradiated tissue region to the acoustic probe [12, 13].

An important requirement for many clinical applications is an adequate imaging depth of several centimeters. Such imaging depths are theoretically predicted as feasible, taking into account the optical penetration depth of near-infrared laser radiation into tissue and the electronic noise of the scanning system [14]. However, in practice it turned out that such an imaging depth can hardly be achieved in epi-OA imaging. One important limiting reason is that clutter strongly degrades the signal-to-background contrast of the image and therefore considerably lowers the imaging depth. Clutter emerges from strong OA transients generated by superficial optical absorbers such as melanin and microvasculature that are directly exposed to the laser light at the irradiated area. These transients propagate to the acoustic probe over the shortest possible path, generating direct clutter, but also after being scattered by echogenic structures deeper inside the tissue, causing echo clutter. Both types of clutter can obscure weak signals from deep inside the tissue [15, 16]. Even though different techniques for clutter reduction are available [16–18], optimization of image contrast prior to applying such methods is highly desirable for clinical applications of OA imaging. In acoustic-resolution photoacoustic microscopy (AR-PAM) of superficial tissue structures it was shown that a dark-field illumination leads to superior signal-to-background contrast in comparison to bright-field illumination [19, 20]. The improvement was contributed to the reduced interference of strong optoacoustic waves emitted from optical absorbers near the surface, such as hair follicles and melanin, whose acoustic reverberations potentially overshadow the much weaker OA signals from structures deep inside tissue, where the relative fluence is much smaller [21]. For the case of deep clinical OA imaging, we have previously proposed based on simulations and phantom studies that the location of irradiation considerably influences the signal-to-clutter contrast, and that an increased separation distance between irradiation and detection can lead to an improved image contrast [15]. However, a final conclusion can only be drawn after systematically study the effect of the irradiation geometry on the signal-to-background contrast in deep clinical imaging. Such a study is important because, in the past, significant effort was put in optimizing the design of combined OA and US probes, e.g. using infrared-transparent silicon CMUT arrays or optically transparent acoustic reflectors, with the goal to maximize the fluence and therefore the amplitude of the OA signal by in-line illumination with the acoustic imaging plane ([22–26]). Although phantom experiments have shown that illumination in the acoustic imaging plane in fact maximizes the fluence leading to an increase in OA signal amplitude, a high image contrast, however, does not depend on a high fluence alone. It is a trade-off between fluence, clutter and system noise, and needs to be determined in a clinical setting.

The goal of the present study was to provide insight into the influence of the irradiation geometry on image contrast in deep clinical epi-OA imaging using a linear array probe. By scanning the forearms of human volunteers, we investigated how the distance between the irradiation area and the acoustic imaging plane of the linear transducer (2D plane of maximum sensitivity of the linear array probe) influences the local image contrast within the upper 20 mm below the skin surface. We focused on this depth because it is roughly the range that can be achieved without applying clutter reduction techniques. Out of the various irradiation parameters (distance, area, or illumination angle) we focused on the irradiation distance because preliminary experiments have shown that the irradiation distance has the
most significant influence. Rather than providing an optimum irradiation distance for the given setup, the scope of this paper is to highlight a trend, that within the depth range of the first 20 mm, the contrast of clinical epi-OA imaging as function of irradiation distance follows a single peak behavior where the optimum contrast is obtained when not as intuitively assumed irradiating below the transducer aperture where the fluence gets maximum, but well outside the imaging plane. Although this study is limited to one specific anatomical site and to a limited number of volunteers, its goal is to point out a significant trend of an improved signal-to-background contrast at an increased irradiation distance, which goes along with previously proposed simulations and phantom studies, and is in agreement with findings in OA microscopy.

2. Materials and methods

2.1. Equipment and setup

For the systematic scanning of volunteers’ forearms, we designed an automated epi-illumination scanning setup combining OA and US imaging, as depicted in Fig. 1(a). With the imaging plane perpendicular to the longitudinal axis of the arm, the goal of this setup was to reproducibly scan a 3D volume of the forearm by stepwise moving the combined probe along the arm using a linear translational stage. In addition the setup offered the possibility to scan the forearm with different irradiation distances. Image acquisition was implemented on an ultrasound research system (V-1-64, Verasonics Inc., Redmond, WA), which allowed parallel RF-data readout of 64 channels and continuous data transfer for real-time processing on a host PC. This system facilitates the acquisition and reconstruction of a full OA image with each single laser pulse, at a frame rate corresponding to the laser pulse repetition rate. The software of the ultrasound research system was programmed in order to alternately acquire US and OA images, which offers a direct one-to-one comparison between the two modalities. The US and OA pressure transients were detected by a linear array transducer (ATL L7-4, Philips N.V., NL), containing 128 elements at a pitch of 0.298 mm with a center frequency of 5 MHz and fractional bandwidth of 80%. A thin cylindrical ultrasonic lens in front of the linear array provides collimated detection in elevation with a narrow angular sensitivity and a focus at approximately 20 mm distance. This transducer selectively detects signals out of a thin 2D tissue slab, which is defined as the imaging plane. For OA signal generation, a Q-switched Nd:YAG laser (Quanta Ray, Newport, CA) was used, delivering laser pulses at a wavelength of 1064 nm with a pulse duration of 10 ns and a repetition rate of 10 Hz. The output of the laser was coupled into an 8 mm diameter optical fiber bundle (Volpi AG, Switzerland), with a rectangular distal fiber terminal (20 mm x 1 mm) aligned parallel to the linear array. The transducer array and the optical fiber terminal were fixed on two separate, parallel-aligned motorized linear translational stages (T-series, Zaber Technologies Inc., CA), which allowed automated and independent displacement and thus scanning with different irradiation distances. The output of the fiber terminal was positioned at a height of 6 mm above the skin surface and irradiated the skin under an angle of 30 degree relative to the transducers axial direction (Fig. 1(a)). The illuminated area on the skin was 3 mm by 23 mm, leading to a radiant exposure of 70 mJ/cm², well below the limit of the maximum permissible exposure (MPE) for skin. To avoid that diffusely backscattered light from the tissue get absorbed by the transducer or the probe housing causing noise, the front part of the transducer was optically shielded by an aluminum foil, which was acoustically coupled to the aperture with a thin layer of ultrasound gel.
Fig. 1. (a) Automated scanning setup for US and OA imaging, (b) Scan region with anatomical map of forearm vasculature. Anatomical structures are shown: radial artery (ar), the median nerve (nm) and the radial veins (vr). Position 1 and 2 marks the position the images of Fig. 2(a) and 2(b), respectively were taken.

2.2 Acquisition procedure

The forearm of the volunteer was immobilized inside an arm holder to avoid motion artifacts, as seen in Fig. 1(a). In a first set of experiments, the transducer was brought in direct contact to the skin using ultrasound gel for coupling. The US mode was used to adjust the transducer position such that the vessel of interest (i.e. radial artery) was located in the field-of-view of the imaging plane (dimension x and z). Starting at the distal end of the radius close to the transition to the wrist, the scan was then performed by step-wise moving the probe in direction to the elbow with a step size of 2 mm. During the scanning procedure the optical fiber terminal was shifted simultaneously with the transducer in order to maintain a constant irradiation distance. At each position, both an US image and an OA image were acquired, resulting in two three dimensional image matrices of stacked 2D images. The scanning was repeated for three different irradiation distances - 11 mm, 15 mm and 19 mm. The US images were reconstructed with a Verasonics Inc. proprietary pixel based reconstruction software. In order to reduce the presence of any non-systematic background, such as thermal noise, ten OA images were averaged at each scan position. The OA images were reconstructed using a frequency domain algorithm [27].

Scanning with direct contact of the transducer with the skin surface is the most practical and cost effective implementation of epi-mode imaging because it avoids the problem of acoustic mismatch that would be introduced by any standoff material. On the downside, the smallest possible irradiation distance is limited by the geometrical restrictions of the transducer housing, in our case to 11 mm. To provide a comparison of contrast also for smaller distances down to 0 mm (illumination in the imaging plane), a second set of experiments was performed with a transparent water bag placed between the skin surface and the transducer. The water provided acoustic coupling while allowing a spacing of 10 mm between the transducer and the skin surface and thus an illumination directly below the transducer aperture. Whereas the transducer was lifted in comparison to the first set of experiments, the fiber terminal position was kept 6 mm above the skin surface to keep the illumination area constant.

3. Results

Figure 2(a) and 2(b) show B-mode OA images of the right forearm of a volunteer at two different imaging positions separated by 1 cm (position 1 and 2 in Fig. 1(b)). The irradiation distance of 15 mm was the same for both imaging positions. The images are displayed after envelope-detection and logarithmic compression, covering an intensity range of 35 dB
starting at an identical level. Figure 2(c) shows a superposition at position 1 of the OA and the US image. Prominent anatomical structures were identified based on B-mode US and anatomy references. We in particular focused on the upper and lower vessel wall of the radial artery and on the median artery. A qualitative comparison between Fig. 2(a) and 2(b) reveals that the clutter background level varies significantly between the two positions. The high clutter level in Fig. 2(b) leads to a strong degradation of image contrast, which considerably limits the imaging depth (see for example the contrast difference of the median artery).

Fig. 2. (a) Transversal OA image at the position 1 indicated in Fig. 1(b). (b) Transversal OA image at position 2. (c) Superposition of OA (in color) and B-mode US at position 1.

In order to investigate the on-average influence of the irradiation distance on the signal-to-background contrast, long forearm scans (160 mm total scan length) were performed on four different volunteers. In order to limit the total scanning time to an acceptable level, this study was limited to only three different irradiation distances, i.e. 11 mm, 15 mm and 19 mm. Figure 3 illustrates the influence of the irradiation distance on contrast, for two different imaging positions separated by 3 cm in scan direction on the forearm of the same volunteer. For a serious visual comparison of the contrast, all pixel values were normalized relative to the intensity of the lower vessel wall of the radial artery. A comparison of (a), (b) and (c) indicates an improved signal-to-background contrast in (b) and (c), relative to the smallest irradiation distance of 11 mm, allowing for example an improved visualization of the upper and lower part of the vessel wall of the radial artery (solid arrows). The irradiation distance 15 mm (Fig. 3(b)) visualizes the median artery at a depth of 11 mm (dashed arrow) in addition to the radial artery. Similar results were obtained at the second imaging position. Figure 3(e) and 3(f) show the upper and lower wall of the same vessel at a depth of around 10 mm, which can in (d) hardly be distinguished from the background.
Fig. 3. OA transversal images indicating the influence of different irradiation distances on the image contrast at two different forearm positions, (a)-(c) and (d)-(f), of the same volunteer. The distance between the two positions was 3 cm; the solid arrows indicate the upper and lower wall of the radial artery; the dashed arrow indicates the median artery.

In order to enable a qualitative comparison of signal-to-background contrast for the entire scan region, a maximum intensity projection of the transversal OA images along the x-direction onto the z-y plane was calculated. Figure 4 shows the maximum intensity projection for the two volunteers, Fig. 4(a)-4(c) and Fig. 4(d)-4(f), respectively, as a function of the imaging position y. They visualize the sagittal profile of the vasculature, over the total length (160 mm) of the scan region.

Fig. 4. Maximum intensity projection in x-direction of OA images of two different volunteers, (a)-(c) and (d)-(f), respectively, at irradiation distances of 11 mm, 15 mm, and 19 mm; the solid arrows indicate the lower vessel wall of the radial artery; the dashed arrows show the outlines of the median artery.
The maximum intensity projection visualizes the radial artery (double-line structure with 2 to 3 mm diameter) on its trajectory along the human forearm, ranging from 2 mm to 11 mm in depth. An additional longitudinal structure, which ranges from 4 mm to around 14 mm in depth and from 0 mm to 90 mm in y-direction, is the thin median artery running parallel to the median nerve. A qualitative comparison of (a), (b) and (c) indicates an improved overall signal-to-background contrast in (b), allowing i.e. the improved visualization of the lower vessel wall of the radial artery at a depth of around 10 mm (indicated by solid arrows). In addition, Fig. 4(b) illustrates the improved visibility of the section of the median artery at a depth of 12 mm (indicated by dashed arrow), which can hardly be seen in (a) and (c). Figure 4(d), 4(e), and 4(f) show similar results for the second volunteer. With an intermediate irradiation distance, several anatomical details, such as the lower radial artery wall and the median artery, are more pronounced (indicated by arrows) than with the close or the distant irradiation.

For a quantitative comparison of image contrast, a signal-to-background analysis was performed, of the pixel intensity values (reconstructed squared envelope) in a region of interest (ROI) around the lower wall of the radial artery. See Fig. 5 for an example of the definition of the ROI. For this quantitative analysis, a threshold was defined to distinguish between signal and background, i.e. the mean value of the intensity plus two times the standard deviation. Values of the mean pixel intensity, which are higher than this threshold, were counted as signal level, whereas the mean pixel intensity below the threshold was considered as background level. The ratio between signal and background levels (signal-to-background ratio SBR) was defined as a measure of the image contrast. Note that in this definition, the background not only includes clutter and system noise, but may also contain an unknown fraction of real signal. This definition may thus lead to an offset in the value of the SBR, however, the trend of changing SBR with changing irradiation distance is preserved. For comparison to the background level, the noise level was determined, as the mean of the squared envelope within the same ROI but of an image that was acquired without laser irradiation. No normalization was used for the quantitative analysis.

Figure 6 shows the signal level (blue) in [dB] and the corresponding background level (red) as a function of imaging position y for one volunteer scan. For comparison, the noise level is displayed as a horizontal black dashed line.

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Fig. 5. Example of the definition of the ROI for contrast analysis around the lower vessel wall of the radial artery.

Figure 6 shows the signal level (blue) in [dB] and the corresponding background level (red) as a function of imaging position y for one volunteer scan. For comparison, the noise level is displayed as a horizontal black dashed line.
Fig. 6. Signal and background analysis of the ROI around the lower vessel wall (indicated in Fig. 5) as a function of the imaging position $y$ for different illumination distances for the same volunteer as shown in Fig. 4(a)-4(c); points correspond to measured data; blue solid lines indicate the moving-average of the signal level (lower vessel wall). The red dashed lines describe the moving average of the background level and the black dashed lines represent the noise level.

The scattered points represent the measured values ("raw data") at each single position along the scanning region. Strong signal and background variations between consecutive imaging positions were observed. For a better visualization, a moving average of the raw data, with a window size of 10 samples (corresponding to 20 mm scanning distance) is also displayed as solid lines. Figure 6 shows that the background level of all investigated irradiation distances is at most positions significantly above the noise level, but more and more approaches the noise level when increasing the irradiation distance.

The SBR as function of imaging position $y$ is shown in Fig. 7 for two different volunteers. The different colors blue, red and black correspond to the different irradiation distances 11 mm, 15 mm and 19 mm, respectively. The scattered points again represent the signal-to-background ratio at consecutive imaging positions whereas a moving average is displayed as lines. The horizontal lines indicate the overall average of the SBR over the entire scanning region.

Fig. 7. Local signal-to-background ratio [dB] inside the ROI (indicated in Fig. 5) as function of imaging position $y$, for two different volunteers, (a) and (b), (same volunteers as shown in Fig. 4(a)-4(c) and 4(d)-4(f)) and three irradiation distances (11 mm = blue, 15 mm = red and 19 mm = black). The scattered points correspond to raw data; lines show the moving average of the raw data. The horizontal lines indicate the average signal-to-background ratio over the entire scanning region.
The quantitative contrast analysis confirms the results of the qualitative comparison. Figure 7(a) shows an improvement of average SBR of around 1 dB at the intermediate irradiation distance compared to the closest distance, corresponding to a 25% increase in contrast. Due to the strong variations in signal and background levels, locally a contrast improvement of more than 3 dB (factor 2) can be obtained (see vertical arrows in Fig. 7). The distant irradiation seems to result on average in slightly less contrast (−0.3 dB or −8%), compared to the close irradiation distance. Similar results were obtained with the second volunteer as shown in Fig. 7(b). An overall SBR improvement of 0.9 dB (23%) was achieved for the intermediate compared to the closest irradiation distance. A distant irradiation also resulted in a slightly improved contrast by 0.3 dB (7%), relative to the close irradiation position. Not only did an intermediate irradiation position provide superior image contrast on average, but also consistently in the local moving average for the majority of imaging positions. Locally (raw data) the contrast improvement was even as large as 3.4 dB (120%) and 3.7 dB (130%) for the first and the second volunteer, respectively.

| Volunteer | avg. improvement [%] | σ [%] | avg. improvement [%] | σ [%] |
|-----------|----------------------|-------|----------------------|-------|
| 1         | 23.3                 | 23.9  | 32.2                 | 27.5  |
| 2         | 25.7                 | 23.7  | 36.7                 | 20.6  |
| 3         | 23.1                 | 24.2  | 13.9                 | 24.6  |
| 4         | 25.9                 | 21.1  | 17.7                 | 24.2  |

The quantitative analysis is summarized for the total of four volunteers in Table 1. It describes the average and standard deviation of the SBR improvement [%] at an intermediate irradiation distance (15 mm), relative to the closest distance (11 mm, Table 1 (left)) and relative to the large distance (19 mm, Table 1 (right)). The results confirm for all volunteers a strong local variation of the SBR improvement depending on the imaging position (compare to Fig. 7). In addition, Table 1 shows that for all volunteers the image contrast was improved by around 25% when increasing the irradiation distance by 4 mm relative to an irradiation directly next to the linear transducer array. A distant irradiation, however, led again to a lower contrast. These experimental results are consistent over all volunteers with good inter-subject comparability and thus suggest that there is an optimum separation distance between imaging plane and irradiation area at which image contrast is maximized.

In order to keep the scanning time for the test person at an acceptable level, only three distances were investigated in the multi-volunteer study. However, to give more evidence to the suggested trend of an optimized SBR, a larger range of irradiation distances was investigated on one volunteer using the same setup (first set of experiments), but only scanning a total length (2 cm). Figure 8 shows the resulting maximum intensity projections for irradiation distances ranging from 11 mm to 21 mm with a step size of 2 mm. The images visualize a 2 cm long sagittal profile of the radial artery as a curved double-lined structure at a depth of 7-10 mm. For a fair visual comparison of contrast, all pixel values were normalized relative to the intensity of the lower vessel wall of the radial artery (indicated by solid arrows). In agreement with the first set of experiments, the contrast increases with growing irradiation distance until an optimum at an irradiation distance of around 15 mm. For larger separation distances image contrast degrades eventually. The same trend is found for the median artery at a depth of around 15 mm (as indicated by the dotted arrow).
Fig. 8. Maximum intensity projection in x-direction of OA images for different irradiation
distances varying from 11mm to 21mm with a step size of 2mm. The solid arrows indicate the
lower vessel wall of the radial artery and the dashed arrow points at the median artery.

The quantitative analysis of the contrast is shown in Fig. 9. The bars indicate the average
local contrast and the standard deviation of the lower radial artery wall as a function of
irradiation distance, in percent of the average contrast obtained at the smallest irradiation
distance. Despite the high standard deviation of the contrast, the mean value follows the
qualitatively suggested single peak behavior. An optimum in SBR is obtained at an
irradiation distance of 15 mm, supporting the choice of irradiation distances that were used in
the first set of experiments.

Fig. 9. Bar plot showing the average and the standard deviation of the contrast of the lower
radial artery wall (indicated by solid arrows in Fig. 8) for various irradiation distances. All
values are expressed relative to the closest irradiation distance of 11mm.

Figure 10 shows the qualitative results (MIP) of the second set of experiments where the
transducer was placed inside a water bag for irradiation distances ranging from 0 mm to 18
mm with a step size of 2 mm up to 10 mm distance, and then 4 mm up to 18 mm distance. The figure visualizes a 2 cm long sagittal profile of the upper vessel wall of the radial artery at an image depth of 17 mm (indicated by an arrow), corresponding to around 7-8 mm depth in the tissue. The skin surface is indicated by a dashed blue line. For a fair visual comparison of contrast, all pixel values were normalized relative to the intensity of this vessel wall. In agreement with the previously presented results, the figure illustrates the rise of the local contrast of the vessel wall with increasing separation distance. After reaching a maximum at around 8 mm distance, the contrast decreases slowly remaining significantly above the reference level of the 0 mm irradiation distance.

Fig. 10. Maximum intensity projection in x-direction of OA images for different irradiation distances varying from 0 mm to 18 mm; for an irradiation below the transducer aperture a transparent water bag was used as spacer, leading to a spacing of around 10 mm between skin surface and transducer aperture; the dashed line in (a) indicates the skin surface; the solid arrow shows the upper vessel wall of the radial artery at a depth of 7-8 mm inside the tissue.

Fig. 11. Bar plot showing the average and the standard deviation of the contrast of the upper radial artery wall (indicated by a solid arrow in Fig. 10) for various irradiation distances. All values are expressed relative to the reference irradiation distance of 0 mm (irradiation below transducer).
The quantitative analysis is presented in Fig. 11. The bars indicate the average local contrast and the standard deviation of the upper radial artery wall as a function of irradiation distance in percent relative to the average contrast obtained with an irradiation distance of 0 mm (irradiation below the transducer aperture). The trend of the mean value confirms the qualitatively suggested single-peak behavior of the SBR, but the optimum is now obtained at an irradiation distance of 8 mm where it is improved by 80% relative to the zero irradiation distance. Beyond the optimum the SBR decreases again, but by only about 10% up to 18 mm irradiation distance.

4. Discussion and conclusion

This study demonstrates in clinical epi-OA scanning of the human forearm using a linear transducer, that the separation distance between the irradiation area and the acoustic imaging plane significantly influences the image contrast. A small separation distance, thus an irradiation close to the transducer array, corresponds to a high fluence in the imaging plane, generating strong OA signals. Increasing the irradiation distance lowers the local fluence below the transducer array and therefore the signal amplitude. When considering only fluence maximization, an optimum image contrast would therefore be expected at the closest irradiation distance. Contrast, however, is a trade-off between OA signal, clutter and system noise. For high image contrast it is not only important to maximize the fluence and therefore the OA signal within the imaging plane, but also to take background signals into account, such as clutter, which degrade the SBR and thus the image contrast. Previous simulations and phantom studies suggested that, in clinical epi-OA imaging using a linear array probe, the location of irradiation considerably influences this ratio, and that an increased separation distance can increase the signal-to-background contrast [15].

The present study confirms these findings in epi-OA imaging of the forearm, where an irradiation in or as close as possible to the imaging plane does not result in the maximum image contrast despite the fact of measuring the highest OA signal amplitudes. The results of the first set of experiments (with direct contact between transducer and skin surface) demonstrate that increasing the separation distance from 11 mm to 15 mm leads, on average, to an increase of local contrast of about 25%. Our explanation for the inferior contrast at 11 mm is the strong contribution of clutter to the background level, degrading image contrast in addition to noise. This clutter decreases more rapidly with increasing distance than the signal itself, leading relatively to an improved SBR. The OA images reveal that the contribution of direct clutter seems to be negligible since no strong background was found at a depth corresponding to the distance of irradiation [15]. Therefore, significant clutter mainly emerges from acoustic reverberations of OA transients generated by optical absorption below the irradiated area (e.g. pigmentation, micro vascularization). At a close irradiation distance when the local fluence below the transducer array is high, these reverberations interfere significantly with the OA signal from structures inside the tissue, reducing image contrast. By increasing the irradiation distance, the local fluence directly below the transducer aperture decreases more rapidly than the fluence within the underlying tissue. In agreement with the simulations and experimental results in AR-PAM [28], the relative contribution of echo clutter relative to the signal level is reduced, resulting in an improved signal-to-clutter contrast. With a further increase of the irradiation distance to 19 mm, however, contrast again decreases. A possible explanation for this observation is that the background signal approaches the noise level at a large irradiation distance (see Fig. 6(c)), such that image contrast is determined rather by the ratio of signal to noise than signal to clutter, and thus the SBR decreases with the decreasing fluence in the imaging plane. Assuming noise to be the dominant contributor to the background signal at a distant irradiation, averaging over more than 10 frames per imaging position could possibly further increase the image contrast, however, this would limit the capability of real-time imaging. This trade-off between the
contribution of clutter and noise to the background level results in an optimum contrast obtained at an intermediate irradiation distance.

The trend of an increasing SBR with growing irradiation distance was confirmed in the water bag experiment for small distances including irradiation directly below the transducer aperture, supporting the single-peak behavior of the SBR. However, in comparison to the first set of experiments with direct skin contact of the transducer, the SBR reaches an optimum at a smaller irradiation distance (8 mm instead of 15 mm). The shift of the SBR peak towards a smaller irradiation distance might be related to the different setup that was used for this experiment. Because the irradiated skin surface was located below a layer of water, back-scattered light could be multiply reflected between the water-skin and the water-air interfaces, leading to a component of persistent illumination at the skin surface below the transducer and thus to an offset of the echo clutter that was roughly independent from the irradiation distance. Such a persistent component of echo clutter would have acted like an increased noise background and could explain the generally poorer contrast in Fig. 10 compared to Fig. 8 as well as the earlier decrease of the SBR with increasing irradiation distance.

A further observation of our study is the strong variability of the observed clutter level, and therefore image contrast, depending on the imaging position, as it is indicated by the relatively large standard deviations. This variation was shown qualitatively in Fig. 2(b). It indicates the strong variation of the clutter level, limiting the imaging depth and thus the visibility of the median artery depending on scan location. The quantitative SBR analysis of a defined region of interest (Fig. 7, Fig. 9 and Fig. 11) confirms the observed contrast variations. This variation of the background might be related to a locally varying distribution of absorbing structures (i.e. pigmentation, micro vascularization in the skin below the transducer), which are considered as sources for clutter generation.

In this study we were focusing on the irradiation distance because preliminary experiments indicated that this parameter had a main influence on the SBR. Although the inclination of the beam was regarded an important parameter in AR-PAM of superficial structures [21], experiments of deep imaging with a changing irradiation angle did not show any significant influence (data not shown). This result is supported by Monte Carlo (MC) simulations of our setup, using an advanced MC software that was developed in our group [29]. The forearm was modeled by a 50 mm x 50 mm x 50 mm cuboid composed of two layers: A skin layer (thickness of 2 mm), whose optical properties (refractive index n = 1.32; absorption coefficient $\mu_a = 0.02 \text{ mm}^{-1}$; reduced scattering coefficient $\mu_s^* = 1.0 \text{ mm}^{-1}$; anisotropy factor $g = 0.9$; and transport mean free path $l^* = 1 \text{ mm}$) were chosen according to the average values that can be found in the literature for skin, dermis, and epidermis at the wavelength of interest [30–35]. Below we assumed a muscle layer (thickness of 48 mm), whose optical properties (refractive index n = 1.32; absorption coefficient $\mu_a = 0.05 \text{ mm}^{-1}$; reduced scattering coefficient $\mu_s^* = 0.45 \text{ mm}^{-1}$; anisotropy factor $g = 0.93$; and transport mean free path $l^* = 2.2 \text{ mm}$) correspond to average values found in literature [31, 36].
In the MC simulations Fresnel refraction/reflection processes were taken into account at all boundaries. The simulation results for the given set of optical properties are shown in Fig. 12. The irradiation distance was chosen to be 15 mm and the illumination area was identical to that used in the experiments. It can be seen that a variation of the inclination angle only affects the fluence distribution in the imaging plane up to a depth of 7-8 mm; light that propagates deeper into the tissue (propagation length \( \gg l^* \)) is subject to the diffusion regime. Similar to the angle, the preliminary investigation of the irradiation area revealed that this parameter, although the influence was discernible, played an inferior role compared to the irradiation distance (data not shown). Because we were limited by experimental constraints (acquisition time per single scan, availability of volunteers) the scope of this study focused on the question if a dark field illumination will result in an improved contrast compared to a bright field illumination, similar to what was previously found in AR-PAM. Based on the results of this study, it can be concluded that also in deep epi-OA imaging using a linear array probe, image contrast can be optimized by locating the irradiation site not directly below or close to the linear transducer array, but at a slightly enlarged irradiation distance.

In this study we focused on obtaining images from a tissue depth of up to 20 mm. This is approximately the imaging depth that could be achieved in our forearm scans without using clutter reduction techniques. Within this depth, and using our system, the outcomes suggest that the optimum irradiation distance was around 15 mm with good inter-subject reproducibility. A future study will focus on the investigation of optimum irradiation distance for deeper tissue where displacement-compensated averaging (DCA) is able to reduce clutter by around 10 dB [16]. However, clutter reduction techniques are only being investigated and slowly adopted by system manufacturers, and on the other hand many clinical applications using handheld probes focus on the integration with easily portable diode laser sources. Although such systems may compensate for the typically low pulse energy by a high pulse rate, the overall signal-to-noise ratio is reduced compared to bulky solid-state laser systems,
resulting in a reduced noise-limited imaging depth of typically less than 2 cm. For such applications we believe that, independent of implementing clutter reduction techniques, our findings have a strong impact on future attempts of optimizing handheld probes for clinical combined epi-OA and US imaging.

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