Simultaneous three-dimensional photoacoustic and laser-ultrasound tomography

Gerhild Wurzinger,1,* Robert Nuster,1
Nicole Schmitner,2 Sibylle Gratt,1 Dirk Meyer,2 Günther Paltauf1

1 Department of Physics, Karl-Franzens Universitaet Graz, Graz, Austria
2 Institute of Molecular Biology, University of Innsbruck, Innsbruck, Austria
*gerhild.wurzinger@uni-graz.at

Abstract: A tomographic setup that provides the co-registration of photoacoustic (PA) and ultrasound (US) images is presented. For pulse-echo US-tomography laser-induced broadband plane ultrasonic waves are produced by illuminating an optically absorbing target with a short near-infrared laser pulse. Part of the same pulse is frequency doubled and used for the generation of PA waves within the object of interest. The laser-generated plane waves are scattered at the imaging object and measured with the same interferometric detector that also acquires the photoacoustic signals. After collection and separation of the data image reconstruction is done using back-projection resulting in three-dimensional, co-registered PA and US images. The setup is characterized and the resolution in PA and US mode is estimated to be about 85 µm and 40 µm, respectively. Besides measurements on phantoms the performance is also tested on a biological sample.

©2013 Optical Society of America

OCIS codes: (110.5120) Photoacoustic imaging; (110.7170) Ultrasound.

References and links

1. L. V. Wang and S. Hu, “Photoacoustic Tomography: In Vivo Imaging from Organelles to Organs,” Science 335(6075), 1458–1462 (2012).
2. J. Laufer, P. Johnson, E. Zhang, B. Treeby, B. Cox, B. Pedley, and P. Beard, “In vivo preclinical photoacoustic imaging of tumor vasculature development and therapy,” J. Biomed. Opt. 17(5), 056016 (2012).
3. M. H. Xu and L. V. Wang, “Biomedical photoacoustic imaging,” Rev. Sci. Instrum. 77(4), 041101 (2006).
4. P. Beard, “Biomedical photoacoustic imaging,” Interface Focus 1(4), 602–631 (2011).
5. E. Z. Zhang, J. G. Laufer, R. B. Pedley, and P. C. Beard, “In vivo high-resolution 3D photoacoustic imaging of superficial vascular anatomy,” Phys. Med. Biol. 54(4), 1035–1046 (2009).
6. 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).
7. 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).
8. S. Y. Emelianov, S. R. Aglyamov, J. Shah, S. Sethuraman, W. G. Scott, R. Schmitt, M. Motamedi, A. Karpiouk, and A. Oraevsky, “Combined ultrasound, optoacoustic and elasticity imaging,” Photons Plus Ultrasound: Imaging and Sensing 5320, 101–112 (2004).
9. T. Harrison, J. C. Ranasinghesagara, H. Lu, K. Mathewson, A. Walsh, and R. J. Zemp, “Combined photoacoustic and ultrasound biomicroscopy,” Opt. Express 17(24), 22041–22046 (2009).
10. M. Jaeger, D. Harris-Birtill, A. Gertsch, E. O’Flynn, and J. Bamber, “Deformation-compensated averaging for clutter reduction in epiphotoacoustic imaging in vivo,” J. Biomed. Opt. 17(6), 066007 (2012).
11. P. C. Beard, E. Z. Zhang, and B. T. Cox, “Transparent Fabry Perot polymer film ultrasound array for backward-mode photoacoustic imaging,” Photons Plus Ultrasound: Imaging and Sensing 5320, 230–237 (2004).
12. G. Paltauf, R. Nuster, M. Haltmeier, and P. Burgholzer, “Photoacoustic tomography using a Mach-Zehnder interferometer as an acoustic line detector,” Appl. Opt. 46(16), 3352–3358 (2007).
13. M. Jaeger, J. J. Niederhauser, M. Hejazi, and M. Frenz, “Diffraction-free acoustic detection for optoacoustic depth profiling of tissue using an optically transparent polyvinylidene fluoride pressure transducer operated in backward and forward mode,” J. Biomed. Opt. 10(2), 024035 (2005).
14. R. Nuster, S. Gratt, K. Passler, H. Grun, T. Berer, P. Burgholzer, and G. Paltauf, “Comparison of optical and piezoelectric integrating line detectors,” Photons Plus Ultrasound: Imaging and Sensing 2009, 7177 (2009).

#188775 - $15.00 USD
Received 16 Apr 2013; revised 27 Jun 2013; accepted 17 Jul 2013; published 19 Jul 2013
1. Introduction

In the field of biomedical imaging several non-invasive optical or ultrasound (US) methods have already found widespread application. With purely optical techniques high resolution images can be obtained. However, they suffer from the strong scattering of light in biological tissue, which limits the resolution in regions deeper than about 1 mm from the tissue surface [1,2]. On the other hand, acoustic waves are weakly scattered in tissue allowing for the imaging of deeply buried objects. Photoacoustic imaging (PAI), also called optoacoustic imaging, provides a novel method that combines the advantages of optical and acoustic imaging methods, yielding high resolution images with optical contrast [3,4].

The photoacoustic effect describes the excitation of an ultrasonic wave after irradiating an object with a short laser pulse. Absorption of the diffusely propagating light in tissue leads to a localized temperature rise followed by thermoelastic expansion and the launch of an US pulse. The propagating pressure waves are detected outside and after...
reconstruction an image depicting the distribution of the absorbed energy is obtained. PAI therefore mainly depicts the optical properties of an object, with absorption as the main source of contrast. Therefore it provides a promising tool mainly for imaging the vasculature, including oxygen saturation of blood cells, when light in the visible or near infrared light is used for excitation [2,5].

In conventional pulse-echo US imaging the contrast is given by the varying echogenicity of different tissue types, resulting in different average intensity levels across interfaces. Therefore, upon detection of the back scattered US waves, the resulting image mostly depicts the tissue echogenicity, which is based on short-scale impedance variations below the resolution of the transducer. As the acoustic impedance is given by the product of the density and the speed of sound the US image mainly contains information of the mechanical properties of the object. By combining these two imaging modalities, PA and pulse-echo US imaging, the information content can be drastically increased as they reveal different properties of the object under investigation. As PA and US imaging use the same detection instrumentation, they can be easily combined. Several research groups have picked up the idea of combining PA with US imaging by equipping a piezoelectric pulser/receiver device with a laser source [6–10]. These devices are optimized for ultrasound imaging and often do not provide the wide bandwidth that is used in specialized PA imaging devices. The latter include optical ultrasound detection [11,12] and thin piezoelectric film sensors [13,14]. The laser-ultrasound (LUS) method uses the photoacoustic effect for the generation of the incoming ultrasound waves. These waves have a broad bandwidth, similar to the waves generated by the PA effect in the investigated object. Therefore, detectors can be used that are equally optimized for US and PA imaging. For simultaneous US and PA imaging the same laser pulse is used for illuminating an external absorber and the object under investigation. Nuster et al. used this method for hybrid PA and US section imaging with focused detection to obtain co-registered 2D images [15]. Various researchers proposed setups for hybrid PA and LUS-transmission imaging [16–19]. Manohar et al., for example, placed a small absorber into the illuminating laser path so that still enough light fell onto the imaging object for PA excitation [16–18]. Behind the sample a transducer array was arranged to measure the transmitted US signal and the PA signals emitted by the target. Distinction between the transmitted US signals and the PA signals was possible because of their different times of flight. This setup yielded simultaneous PA, speed of sound (SOS) and acoustic attenuation images. The knowledge of the SOS distribution within a sample can be used for the improvement of PA or thermoacoustic reconstruction [20,21].

In this study, we investigate the combination of three-dimensional (3D) photoacoustic and pulse-echo laser ultrasound tomography. This is made possible by equipping an earlier developed PA tomograph based on an optical line detector [12,22] with a target for generation of plane acoustic waves for pulse-echo LUS imaging. Since the PA and LUS transients are generated at the same time by the same laser pulse and are detected by the same optical sensor, it is possible to achieve a perfect co-registration of the resulting images.

In the following sections first the experimental method is described, including the characterization of the emitted LUS field, the reconstruction and the imaging resolution in the PA and LUS modes. The results show resolution measurements on phantoms and first experiments on a zebrafish.

2. Methods

A tomographic setup for the simultaneous acquisition of PA and US images is presented. It is based on a setup for 3D PA tomography of small objects, using a thin laser beam as an interferometric ultrasound sensor. To this device we add an external absorber that is hit by a part of the same laser pulse that is used for PA excitation of the object. A single acoustic transient recorded by the optical detector contains at the same time data for photoacoustic and ultrasound images. After separation of the data, three-dimensional, co-registered images are reconstructed using back-projection [23–25].
2.1 Experimental setup

A scheme of the measurement setup is depicted in Fig. 1. Laser pulses of 1064 nm wavelength with 10 ns duration were emitted by a Nd:YAG laser with a repetition rate of 10 Hz. This near-infrared (NIR) light was split up and part of it was coupled into an optical fiber and used for illuminating an external absorber. The absorber consisted of a transparent 10 mm thick polycarbonate substrate which was coated with a thin layer of black acrylic dye. Illumination was achieved by imaging the end face of the optical fiber onto the substrate with the absorbing layer oriented towards the sample. This way homogenous plane waves were generated photoacoustically. The other part of the emitted NIR-laser pulse was frequency doubled and coupled into two optical fibers, which guided light to the object under investigation for the excitation of PA signals. Illumination of the sample was done from two opposite sides orthogonal to the acoustic propagation direction by imaging the end faces of the optical fibers onto the sample. The illuminating spots on the sample and on the external absorber had diameters of about 10 mm and 25 mm, respectively. The detection of the pulses that had been either generated in or back scattered at the object was done optically by means of a Mach-Zehnder interferometer as illustrated in Fig. 1. A detailed description of the detector can be found in [12,22]. The basic principle of such a detector is the change of the refractive index and thus of the optical path length by a pressure wave traversing the detection laser beam. Interferometric detectors are usually very broadband and in contrast to most piezoelectric detectors they are also completely transparent for traversing US waves. The maximum achievable spatial resolution of the optical detector is limited by the diameter of the detection laser beam [26]. Therefore its diameter was reduced to a beam waist of about 35 to 40 µm. The beam diameter also limited the bandwidth of the detector which ranged from about 200 kHz to ~17.5 MHz. Compared to the setup described in [12,22] where both the detection and the reference beam of the Mach-Zehnder interferometer traversed the water tank, in the current arrangement the reference beam was guided outside (see Fig. 1). The sensitivity of the sensor was determined by measuring signals from a defined photoacoustic source [14]. The noise equivalent pressure length product (NEPLP) of the current setup was 3 mbar·mm. Distances between object, sensor and LUS target were chosen in a way that the PA signals generated inside the object of interest arrived at the detector at first. Then the incoming LUS pulse traversed the detection beam and finally the back-scattered US signals were detected. That means that the distance between the absorber and the detector needed to be greater than the diameter of the sample plus the...
distance between the object and the detector (b > 2R + a in Fig. 1). After signal amplification, data acquisition was done by an oscilloscope. As 3D tomography requires gathering of signals from all directions the usage of a single detector requires scanning around the object. Since the detector was part of an interferometer, it was easier for experimental reasons to leave the detector fixed and move the sample instead. Hence the sample was mounted on a moveable stage and was rotated around an axis that was oriented in z-direction. The absorber and the optical components used for illumination were vertically moved together with the sample during measurements, keeping the relative z-position of the illuminating spots and the incoming US wave constant with respect to the object. Using a single detector leads to longer measurement times compared to the usage of arrays, which collect data from different views simultaneously. With our system the acquisition time was about 1 hour, limited by the relatively low repetition rate of the excitation laser system. Water was used as coupling medium for the propagation of sound waves and therefore the sample, the absorber and the detection beam were placed in a water tank.

2.2 Image reconstruction for the PA and US modes

Image reconstruction in the PA mode used the universal back-projection algorithm developed by Xu et al. [23], modified for the integrating line detector [25]. Both, the original 3D algorithm by Xu et al. and the modified, 2D algorithm assume constant average speed of sound in the object. In the 2D case a line sensor oriented in x-direction measures time dependent signals \( p(r_0, t) \) at positions \( r_0 = (y_0, z_0) \) on a detection curve \( C_0 \) in the y-z-plane. For the linear scan used in our experiments, \( C_0 \) is a straight line in z-direction. The back projection formula gives an estimate of the initial pressure \( p_0(b)(r) \) at point \( r = (y, z) \),

\[
p_0^{(b)}(r) = \left[ B(r_0, t) \frac{d\Omega_0}{\Omega_0} \right]_{r_0}
\]

The integration goes over the total angle \( \Omega_0 \) of the detection aperture, i.e. the total angle under which the line \( C_0 \) is seen from point \( r \). In PA tomography, we set \( t = c_s / d \), where \( d = |d|, \quad d = r - r_0 \) and \( c_s \) is the speed of sound. In this formula the initial pressure is reconstructed by back projecting a quantity \( B \) derived from the measured signals with appropriate time delays and weighted with \( d\Omega_0 \), which is the angle under which an element \( dC_0 \) on the detection line is seen from the reconstruction point. The quantities \( B \) and \( d\Omega_0 \) are given by

\[
B(r_0, t) = -2\sqrt{\frac{d}{c_s^2\tau^2 - d^2}} \frac{\partial}{\partial \tau} \left( \frac{p(r_0, \tau)}{\tau} \right) d\tau, \quad d\Omega_0 = \frac{dC_0 \cdot \hat{n}_0 \cdot \hat{d}}{d \hat{d}}
\]

where \( \hat{n}_0 \) is the normal unit vector perpendicular to \( C_0 \), pointing towards the photoacoustic source. In US imaging the basic idea is to insonify the object with plane waves and to use back projection, which is in this case equivalent to dynamic focusing in receive mode, to localize sources of ultrasound backscattering. This approach, in the same way as conventional diagnostic ultrasound imaging, assumes sound propagation along straight lines with constant average speed and single scattering. Considering that the single, scanning detector emulates a linear array, the described imaging scenario is equivalent to sending a plane wave into the object and to recording all temporal signals from all elements of the array for off-line focusing to all possible source locations of acoustic backscatter. Sound waves are generated by the light absorbing target that is hit by a laser pulse and has a distance \( b \) from the array. Accordingly, the back projection formula has to be modified by using in \( B(r_0, t) \) a delay of \( t = \frac{|d| + b + |y - y_0|}{c_s} \), which is now the total roundtrip time from the target to a point in the sample and back to a detector.
position. From all the resulting two-dimensional projection images the 3D image is reconstructed by applying the inverse Radon transform in the x-y-planes.

2.3 Characterization of the laser ultrasound field

The homogeneity of the acoustic field was analyzed by scanning it with a home-made point-like piezoelectric detector made of a 28 µm thick PVDF-film (~6 dB bandwidth, DC-22 MHz). The sensing area of the detector had a diameter of about 100 µm, more than two orders of magnitude smaller than the size of the illuminating spot, which was about 30 mm. The radiant exposure of the NIR light pulse used for US generation was about 9 mJ/cm². The detector was scanned in the x-z-plane along lines parallel to the x-axis within a distance of about 20 mm from the absorber. During the scan every 0.2 mm a signal was recorded and the total number of scan-lines was 40. The distance between the lines was 1 mm, thus the detector covered an area of 40x40 mm.

2.4 Estimation of the resolution

An estimation of the resolution of the setup was obtained from phantom measurements. For determining the US resolution, two copper wires with a diameter of 50 µm were embedded in agarose made of a 1 wt%-aqueous solution. To obtain the horizontal resolution (x- or y-direction) one of the wires was mounted vertically and for determining the vertical resolution (z-direction) the other wire containing a knot was mounted perpendicular to it. The phantom also contained several black polymer beads with a diameter of 100 µm for the PA resolution measurement. A photograph of the phantom is shown in Fig. 3(a). The total scan length was 35 mm with a z-increment of 250 µm. A full rotation of the sample around an axis parallel to the z-axis with an angular increment of 0.9 ° was performed at each sample position. The US waves were detected with the Mach-Zehnder interferometer and recorded by the oscilloscope. After recording and separating the data image reconstruction was performed using the back-projection algorithms described above.

3. Experiments on a biological sample

Zebrafish have become a widely studied model organism e.g. for studying regeneration processes [27–30], tumor biology [31] or angiogenesis [32]. Apart from various optical methods also PAT [33,34] and US [35,36] found application for imaging of zebrafish. In this study a transparent adult zebrafish was investigated. The fish that was fixed in 4% paraformaldehyde (PFA) was embedded in gelatin together with a black metallic sphere (see Fig. 4(a) and 4(b)). For tomography the sample was moved in z-direction with a step size of 0.5 mm covering a total scan length of 22 mm. Again the sample was rotated at each z-position with an angular increment of 0.9 °. After collection of the data image reconstruction was performed by applying the standard back-projection reconstruction algorithms as described above.

4. Results

4.1 Field measurements

An image of the normalized maximum amplitude values obtained at each position is shown in Fig. 2(a) revealing the appearance of plane waves emitted from the absorber. The excited waves have the same diameter as the laser spot size used for illuminating the absorber. These plane waves are very homogeneous as the mean value and the standard deviation of the measured normalized maximum pressure amplitudes are 0.63 ± 0.08. These values were calculated within a circular area with a radius of 12 mm around the center of the illuminating spot. Figures 2(b) and 2(c) depict one single signal measured at a position in the center of the illuminating spot and its corresponding spectrum. The signal contained frequencies up to 50 MHz and the full width at half maximum (FWHM) of the spectrum was about 15 MHz.
Maximum amplitude projections from different views are shown in Fig. 3(b)-3(e). An estimation of the resolution can be obtained by plotting profiles across the image. The shape of the profile was approximated with a Gaussian function (see Fig. 3(f)) and deconvolved with another Gaussian having a FWHM-value matching the known diameter of the objects. The FWHM-value of the resulting Gaussian can be regarded as an estimation of the achievable resolution. As shown in Fig. 3(b) and 3(c) the copper wires give a good contrast for pulse-echo US imaging while they are not visible in the PA image. On the other hand the black polymer beads can clearly be seen in PA imaging mode while their acoustic impedance is well matched to the surrounding agarose and therefore they do not appear in the US images (Fig. 3(d) and 3(e)). For assessing the resolution of the PA images, profiles of several spheres were extracted and the average FHWM value was taken. After deconvolution the estimated PA resolution in $x$- and $y$-direction was approximately 85 µm and about 120 µm in $z$-direction. The achievable in-plane resolution of the US images was estimated to be about 40 µm in $x$- and $y$-direction and about 140 µm in $z$-direction.

4.2 US and PA resolution
4.3 Zebrafish imaging

Figures 4(c) – 4(i) show the obtained PA and US images of selected sections of the zebrafish in different orientations. It can clearly be seen that the images are based on different contrast mechanisms. While the PA image reveals different inner structures of the fish the US image mainly illustrates its skin and bones. In the US image shown in Fig. 4(d) also detailed structures of the fins are clearly visible.

5. Discussion

With the proposed setup simultaneous imaging of the optical and acoustic properties of an object can be achieved within one tomographic measurement. This is made possible by making use of the PA effect for the generation of the incoming wave used for pulse-echo US imaging. The US and PA images are inherently co-registered, because the same detector measures at the same position both signals. Information for the two modes at one detector position can even be contained in a single time trace, as was demonstrated here. This reduces the measurement time by a factor of two compared to consecutive PA and US tomography. However, this simultaneous acquisition is not necessary. Even if the PA and LUS images were acquired sequentially with the same detector, resulting images would be inherently co-registered, as it has been demonstrated for combined PA and US transmission tomography [19]. In full 3D tomography an exact reconstruction requires the collection of signals from all directions around the object. This is usually done by rotating and scanning with the detector around the object or vice versa. However, with our setup only scanning in vertical (z-) direction is possible. Therefore, for an exact 3D-reconstruction an infinite scan length would be necessary for obtaining the necessary information. In literature the loss of information due to an incomplete scan is called the limited view problem [24,37]. This could be the reason why the resolution in horizontal planes is much better than the vertical resolution. Another possible explanation could be that the chosen step size, which was selected in order to limit the acquisition time, was too large leading to grating lobes and thus to the appearance of artifacts in the reconstructed images. However, after having done several measurements on spherical or cylindrical objects with varying scan length and z-increments, it turned out that this effect has only minor influence on the resolution. Thus we conclude that the main limitation for the resolution was indeed the limited aperture of the system, at least for the investigated objects.

The factor two between the resolution values in PA and US modes is in accordance with the different image generation mechanisms: in PA mode, two sources separated by a distance $d$ will lead to signals arriving at the detector with a maximum temporal separation of $d/c_s$. In the US backscatter mode, owing to the roundtrip of the US waves from the target to the sample and back to the detector, the temporal separation is $2d/c_s$, allowing for a better distinction of sources with the same temporal resolution of the detector.
Fig. 4. (a) and (b) Photographs of the investigated transparent zebrafish. The dotted lines indicate the positions of the displayed sections. (c) Coronal (dorsal to ventral) PA (left) and US (right) maximum amplitude projection images over 10 adjacent slices, indicated by the shaded area in (a). (d) Sagittal (left to right) maximum amplitude projection images over five adjacent sections. The PA image is shown on the left, the corresponding US image on the right. The white dotted lines indicate the positions of the displayed transverse (head to tail) sections in (e) – (i), where an overlay of PA (green) and US (red) images is shown.

The measured zebrafish had already been fixed in a PFA solution for several days which could have led to changes in the optical and acoustic properties of the fish. Normally the swim bladder is gas-filled and thus acoustically intransparent owing to the strong impedance mismatch, leading to strong US signals. However, it appears that the swim bladder had collapsed after the death of the zebrafish leaking the gas. In the US images the shape of the swim bladder can still be seen, which leads to the suggestion that it was filled with some acoustically better matched liquid instead. The fish images provide a good example for the advantage of combining PA and US imaging. Due to the absence of any skin pigmentation in the white fish there is no simple anatomic reference to identify what the inner structures that give PA contrast are from their location. However, the strong US contrast of skin and bones and the exact overlap of the two images facilitate localization of structures in the fish. Furthermore, the extra information provided by the US image can be used to improve the PA image reconstruction. It has been demonstrated that acoustic scattering has an effect on the quality of PA reconstruction, which normally assumes an acoustically homogenous medium [38]. With prior knowledge of the distribution of scatterers the reconstruction can be modified, leading to a reduction of the acoustic scattering related artifacts [38]. The PA
reconstruction in presence of a strongly echogenic, macroscopic structure such as the swim bladder in a living zebrafish will be subject of further investigations.

6. Conclusion

The presented purely optical setup for simultaneous PA and US imaging allows for the co-registration of three-dimensional PA and US images. The same laser pulse is used for the excitation of PA waves within the sample and for PA generation of incoming US pulses with high bandwidth. For the detection of both, the backscattered US and the PA signals, a broadband interferometric detector is used. The obtained images contain different information about the object under investigation as they reveal different properties. This technique is well suited for tomography measurements of small animals as its resolution in both, the US and PA mode is sufficient to image objects smaller than 100 μm.

Acknowledgment

This work has been supported by the Austrian Science Fund (FWF), projects No. S10502-N20, S10508-N20 and S10509-N20.