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High Frequency Optoacoustic Microscopy

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Abstract—**Photoacoustic imaging - also called optoacoustic imaging - is a new hybrid modality of high tissue contrast which is based on the varying optical properties of tissue. The acoustic signal generated by pulsed laser absorption reports tissue-specific information with high spatial resolution. To increase the intrinsic contrast in tissue, absorbing particles are of great interest for optical imaging because of their considerable capacity to absorb and scatter light at visible and near-infrared wavelengths.**

The aim of the work presented here is to establish a scalable photoacoustic technology for volume imaging of biological samples down to diffraction limited microscopy. For this purpose a versatile photoacoustic microscopy platform has been developed with unmatched spatial resolution consisting of a microchip laser and a measurement cell with different transducers attached allowing generation and detection of laser-induced ultrasound signals in a frequency range up to 400 MHz.

The performance of a versatile photoacoustic microscopy platform was evaluated via 2D optoacoustic images of light absorbing microparticles (5 µm Fe_3O_4 and 1 µm black toner particles) embedded in a polystyrene matrix. High frequency signals in the frequency range of 400 MHz generated by a single 1 µm particle could be recorded with a high signal to noise ratio (SNR) of 34 dB.

I. Introduction

ULTRASOUND imaging has the advantage of low cost, rapid imaging speed, portability and high resolution. The main drawback of this method is the relatively poor image contrast. Optical imaging techniques can provide very good contrast, but limited penetration depth in tissues due to high optical scattering. Therefore it is advantageous to combine the rapid data acquisition and penetration depth of ultrasound imaging with high contrast of optics. Photoacoustic imaging (also called optoacoustic imaging) is a new hybrid and minimal invasive imaging modality which features main advantages of optical and acoustical techniques. In optoacoustic imaging a laser source is used to illuminate a tissue volume, and the absorption of light within the media results in heating, thermal expansion and acoustic wave generation. The generated acoustic waves are less sensitive to scattering than the optical field, and serve to transport the local absorption information from the detection region.

One fundamental feature of optoacoustic sound sources is their broadband performance. At high light absorption the characteristic frequency spectrum lies in the range of 10^{10}-10^{11} s^{-1} [1]. Optical excitation of short acoustic pulses was first reported in Ref.2. Sound waves were excited by a mode-locked laser in a metallic film deposited on a crystal bar. This experiment however did not record the pulse wave form, but rather measured the amplitudes of the acoustic harmonics at even multiple frequencies of the repetition rate of the light pulses. These schemes were subsequently used [3, 4] to excite sub-nanosecond acoustic pulses and these are recorded with piezoelectric and capacitive transducers. However, detection of optoacoustic transients with both axial and lateral resolution less than 10 µm remains a formidable task, particularly in a backward mode detection configuration where the source and detector are co-located on the target surface [5]. Another challenge in piezoelectric technology is the construction of 3-D images at sufficiently high frame rate and spatial resolution. For clinical applications 2-D arrays operating at frequencies higher than 50 MHz are required to construct 3-D images. Recently O'Donnell et al. reported two types of transducers operating at center frequencies around 50-100MHz [6]. Confocal photoacoustic microscopy has been proposed by Maslov et al. [7].

In order to increase the resolution and sensitivity we developed a microscopy system. The microscope is based on the SASAM acoustic microscopy system which has also been developed at the Fraunhofer Institute for Biomedical Technology [8]. In this system a focused pulsed laser is used to generate an optoacoustic signal and detection in the photoacoustic confocal setup is performed using a focused 60 MHz thin film PVDF transducer as well as ZnO based 200 and 400MHz acoustic lenses. With this newly developed photoacoustic microscope it is possible to evaluate the photoacoustic suitability of different kind of absorbing particles.

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II. Materials and Methods

Our photoacoustic microscopy system (SASAM OPTO) consists of an inverted optical microscope (Olympus IX 81, Tokyo, Japan), a laser source, a single element transducer and the SASAM 1000 electronics system (kibero, Germany). In order to enable high frequency ultrasound generation and therefore a good acoustic resolution, preferably shortpulsed lasers with sufficient pulse energy were used: a Q-switched Nd:YAG solid-state-laser (Teem Photonics, France) producing sub-nanosecond micro-Joule pulses at kilohertz repetition rates which corresponds to an ultrasound frequency response up to a cut-off frequency of

$$f_c = 1/2\tau_l \qquad (1)$$

The laser emits light in the near infrared to take advantage of low absorption and scattering of biomaterials in this spectral range (so called "optical window").

The solid state laser is coupled to the photoacoustic instrument either via single mode fiber (core diameter 5 µm) or multi mode fiber (core diameter 200 µm). Using single-mode fibers diffraction-limited focusing onto the sample is enabled. This is the upper resolution boundary in optically limited photoacoustic imaging. Using multi-mode fibers the fiber-core front-side is imaged onto the sample with approximately unity magnification, i.e. the laser spot in the image plane is also 200 µm in diameter.

In this system, the coupled light supplies incident pulse energy of 350nJ to induce photoacoustic waves within the sample. In-house constructed transducers based on PVDF and ZnO working at a frequency range between 60 MHz and 400 MHz were used for signal detection. By switching the mode there is a possibility to measure the response and the acoustic sound-field of the transducer. The transducer surface is aligned in parallel to a polished stainless steel reflector in a water bath. The reflected signal is measured as a result of the excited pulse. The sensitivity of the transducer can be measured by scanning a small spherical target in a two-dimensional pattern. Figure 1 shows the sound-field of the 60 MHz PVDF transducer. In lateral direction, the resolution is about 40 µm and in axial direction about 370 µm [8].

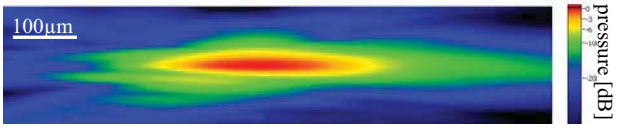

Fig. 2: Sound-field of the PVDF transducer working at 62 MHz (axial direction)

The characteristics of the used transducers are listed in the table below (Table I)

TABLE I
SPECIFICATIONS OF THE ULTRASOUND TRANSDUCERS.

Types of transducer	Center Freq. [MHz]	FWHM [MHz]	Focal Length [µm]	Axial Res. [µm]	Lateral Res. [µm]
PVDF 60MHz	62	30-95	370	370	40
ZnO 200MHz	205	145-235	500	8.3	7.3
ZnO 400MHz	375	250-440	300	3.9	4.7

An acoustic scanning unit is attached to a rotating column that allows exchanging of the transillumination condenser, which is needed for visualization and adjustment of the sample and the acoustic scanning unit. After switching to the acoustic scanning unit the acoustic lens is aligned to the optical path of the microscope. This enables imaging of the same sample position by different scanning modalities. In addition to the photoacoustic imaging mode, all common optical and acoustic modalities are implemented. An illumination stage consisting of an adjustable collimator and a fibre plug for coupling in various laser sources for optical multi wavelength excitation is attached at the backport of the microscope. Also a short pass dichroic mirror with a cut-off wavelength of 700 nm is inserted into the filter wheel to reflect the excitation light to the objective and to transmit light in the visible range for monitoring per CCD camera mounted on the sideport of the microscope. Additionally safety filters are implemented into the oculars to protect the eye of injurious laser radiation (Fig. 2).

Various lasers can be coupled into the system via an optical fiber plug at the microscope back port. The plug is mounted on appropriate beam collimators, which are preadjusted and magnetically fixed at the back port. So, different laser sources can be exchanged quickly and conveniently.

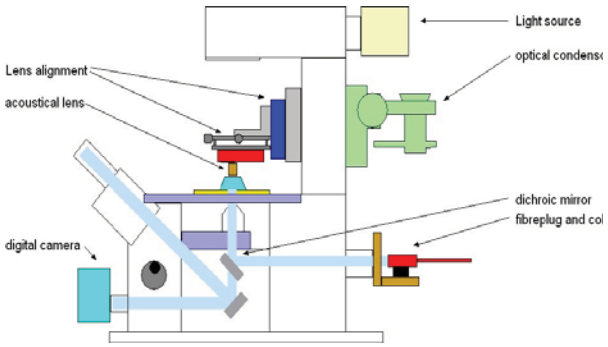

Fig. 2: Setup of the combined optical, acoustical and photoacoustical microscope

Different scanning modes are implemented. In the first scanning mode, the sample is illuminated with a focused laser beam and the generated signal is received with an unfocussed ultrasound transducer. Another possibility is to

use a focused transducer (spherical lens) and illuminate an area, which is much larger than the size of the focus spot of the transducer. In this acoustical focusing mode the lateral resolution of the system corresponds with the resolution of the transducer. To overcome these limitations optical illumination and acoustic detection focal volumes of comparable sizes are superimposed. The resolution of the system strongly depends on the focal spot. The sample is scanned mechanically line by line in a two dimensional pattern. While scanning the sample, the RF-signal is recorded, post-processed and a grey-scale image is generated. All the experiments described below were realized in the confocal photoacoustic setup.

III. Results and Discussion

The performance of the system was evaluated via 2D optoacoustic images of light absorbing microparticles (5 µm Fe_3O_4 and 1 µm black Toner particles). By projecting the peak-to-peak amplitude value of the raw RF-data of every scanning point a C-Scan image was calculated. One line of the C-Scan image was extracted to get a sectional view along the particle. The point spread function (PSF) was constructed to measure full width at half maximum (FWHM) by displaying the amplitude against the position. The FWHM-value of the PSF determines the resolution limit of the system.

To demonstrate the spatial resolution of the system phantom tests were first obtained detecting the photoacoustic signals from magnetite particles (Fe_3O_4) with 5µm in size embedded in a polystyrene matrix using the 60 MHz PVDF transducer as a receiver. In figure 3 the full width at half maximum value (FWHM) of the imaged particle is measured. On the left hand side of the figure 3, the optical image and the 2D projection of the received optoacoustic signals and on the right hand side the received signal produced by the single particle, the recorded acoustic frequency spectrum and the PSF are visualized. In this case photoacoustic signals could be recorded with a high signal to noise ratio (SNR) of 32 dB. The FWHM of the PSF is measured to 5.4 µm.

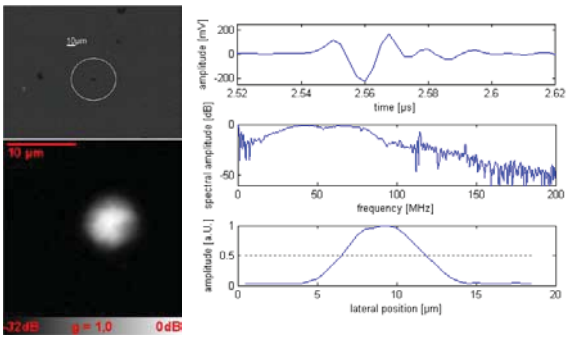

Fig. 3: Characterization of the lateral resolution of the photoacoustic microscopy system by measuring the RF-signal, frequency spectrum and point-spread-function of a single 5 µm Fe_3O_4 particle

Embedded black toner particles with size of 1 µm were also used as photoacoustic targets and imaged with various transducers working at centre frequencies of 60 MHz, 200 MHz and 400 MHz with sampling frequencies up to 4GHz (figure 4). Sample RF lines from the images acquired in figure 4 are shown in figure 5(a), and the corresponding power spectra in figure 5(b).

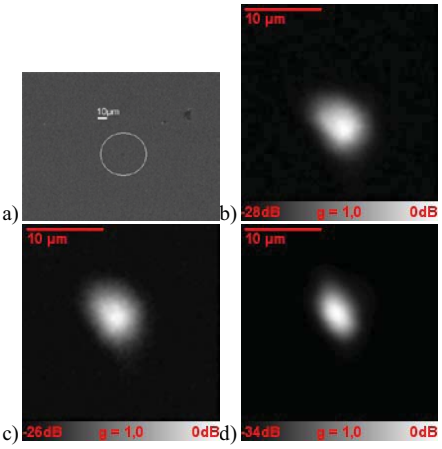

Fig. 4: optical (a) and photoacoustic image of the toner particle of size around 1 µm with 60 MHz (b), 200 MHz (c) 400 MHz

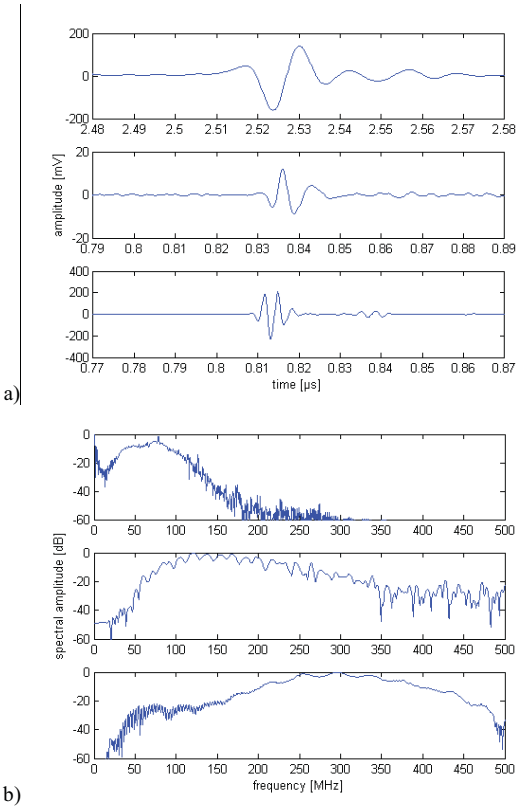

Fig. 5: Time signal and acoustic frequency spectrum of the generated optoacoustic pressure transients recorded by 60 MHz, 200 MHz and 400 MHz transducers (top down)

The normalized amplitude profile of the imaged 1 µm toner particle is calculated from a line plot through the center of the particle from figure 4c and shown in figure 6. The full width at half maximum value (FWHM) is measured to be 3.6 µm which is in reasonable agreement with the theoretical calculation using a 0.3 NA objective (4.3µm).

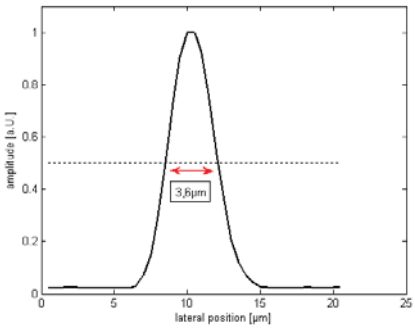

Fig. 6: Point-spread-function of the received photoacoustic signal using a 400 MHz transducer

The system resolution for the different transducers used is listed in the table below (Table II). The FWHM of the PSF shows a narrower profile for higher frequencies but it appears that in the confocal optoacoustic mode the laser spotsize determines the point-spread-function of the system almost independently of the used transducer.

TABLE II
RESOLUTION OF THE SYSTEM

Types of transducer	Center Freq. [MHz]	FWHM [µm]	SNR [dB]
PVDF 60MHz	62	4.9	28
ZnO 200MHz	205	4.8	26
ZnO 400MHz	375	3.6	34

IV. Conclusion

In this work the development and establishment of a scalable photoacoustic technology for high quality and high resolution volume imaging down to diffraction limited microscopy is presented.
A high frequency signal in the frequency range of 400 MHz of a single 1 µm particle could be recorded with a high signal to noise ratio (SNR) of 34 dB. The lateral resolution of the system is characterized better than 4 µm using a 400 MHz ZnO lens as acoustical detector.

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