Towards acousto-optic tissue imaging with nanosecond laser pulses

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Abstract: We present a way to generate acousto-optical signals in timovssue-like media with nanosecond laser pulses. Our method is based on recording and analyzing speckle patterns formed by interaction of nanosecond laser pulses with tissue, without and with simultaneous application of ultrasound. Stroboscopic application allows visualizing the temporal behavior of speckles while the ultrasound is propagating through the medium. We investigate two ways of quantifying the acousto-optic effect, viz. adding and subtracting speckle patterns obtained at various ultrasound phases. Both methods are compared with the existing speckle contrast method using a 2D scan and are found to perform similarly. Our method gives outlook on overcoming the speckle decorrelation problem in acousto-optics, and therefore brings in-vivo acousto-optic measurements one step closer. Furthermore it enables combining acousto-optics and photoacoustics in one setup with a single laser.

References and Links

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1. Introduction

Two hybrid techniques that combine optical contrast and the relatively high resolution of ultrasound (US) in turbid media are photoacoustics (PA) and acousto-optics (AO) [1, 2]. Both methods can be combined to achieve fluence compensated PA imaging [3, 4]. While PA already has in vivo applications, AO still suffers from so called speckle decorrelation when applied in vivo, which typically results in no or poor signals. AO imaging is performed by

injecting coherent light into the sample. The light is scattered by the sample and the exiting light forms a speckle pattern. By applying focused ultrasound on the sample the light acquires a modulated phase change in the US focus [5]. This results in a modulated speckle pattern with the frequency of the applied ultrasound (time scale ~1µs). The amount of modulation can be detected in several ways. Usually these methods require the measurement to be performed within the so-called speckle decorrelation time. Often cw lasers are used and chopped to generate relatively long pulses with long coherence length. Because of the low power a long integration time is necessary to integrate multiple pulses for different phases of the modulated speckle pattern. The total time to acquire one data point is close or even more than the speckle decorrelation time in tissue (~0.1 ms) [6], which impedes in vivo application. Here we demonstrate a novel method that brings us close to measuring acousto-optic signals far within the speckle decorrelation time of living tissue. To this end we use a laser that combines an adequately long coherence length with short (nanoseconds) high intensity pulses. In the eventual implementation of the method we will apply two nanosecond pulses within one ultrasound cycle. For now we use a laser at a repetition rate of 10 Hz, thus stable phantoms with long decorrelation time (>100 ms) are used to give a proof-of-principle.

2. Theory

Speckles are the result of interference of multiple randomly phased light waves. When the phases of these waves are modulated due to interaction of ultrasound with the medium, the intensity of the speckle is modulated. The modulation depth I_{ac} is associated with the optical and acoustical properties at the location of the ultrasound and the local fluence. The light waves that cause the intensity modulation are said to be 'tagged' by the ultrasound. The associated intensity to this tagged light is I_t where the non-tagged light has an intensity of I_{nt} and the total light illuminating a CCD pixel n at time t can under assumption of the absence of higher harmonics be approximated by:

$$I(n,t) = I_{dc}(n) + I_{ac}(n)\cos(\omega t + \varphi(n)) \tag{1}$$

Where $I_{dc}(n) = I_{nt}(n) + I_t(n)$, $I_{ac}(n) = 2\sqrt{I_{nt}(n)I_t(n)}$, ω the ultrasound angular frequency and φ the random phase for pixel n, where for the time being we neglect higher harmonic signals. Here we want to demonstrate the use of a coherent ns pulsed laser in an AO application. We let this laser illuminate the scattering medium when a US burst reaches a region of interest inside that medium, at time t. A camera detects the generated speckle pattern I_1 at the opposite end of the medium for which we write

$$I_1(n) = I_{dc}(n) + I_{ac}\cos(\varphi(n)) \tag{2}$$

A small time difference Δt later a second laser pulse is injected in the sample. This time is chosen to be half the ultrasound period so that we have a phase shift of π. At that moment a second speckle pattern I_2 is recorded, where:

$$I_2(n) = I_{dc}(n) - I_{ac}\cos(\varphi(n)) \tag{3}$$

Both speckle patterns are normalized such that the average intensity over all pixels is unity. The difference of the speckle patterns becomes larger if relatively more light has interaction with the US. We define the acousto-optic signal S_{AO} that quantifies the amount of tagged light as

$$S_{AO} = \left\langle \left(I_1(n) - I_2(n) \right)^2 \right\rangle \tag{4}$$

Where $\langle\rangle$ denotes averaging over all pixels of the speckle pattern. It can be shown that S_{AO} is proportional to the amount of tagged light by substituting Eqs. (2) and (3) in Eq. (4) and using the definition of I_{ac}. On substitution we obtain:

$$\left\langle \left(I_1(n) - I_2(n) \right)^2 \right\rangle \propto \left\langle I_{nt}(n) I_t(n) \right\rangle = \left\langle I_{nt}(n) \right\rangle \left\langle I_t(n) \right\rangle \tag{5}$$

Hence the mean square difference of the speckle patterns is proportional to the amount of tagged light. Because of the normalization and $\left\langle I_t(n) \right\rangle \ll \left\langle I_{nt}(n) \right\rangle$ we approximate $\left\langle I_{nt}(n) \right\rangle = 1$. The proportionality constant is not important for a proof of concept because signal and noise are then multiplied with the same number.

This method has some similarities with an earlier technique that uses chopped CW lasers, which however needed orders of magnitude more camera integration time than the few nanoseconds that we use [7]. We will derive Eqs. (4) and (5) in more detail in a later paper and make it quantitative instead of just qualitative. The difference between the two consecutive speckle patterns is caused by the effect of the ultrasound, speckle dynamics due to internal motion in the medium, camera noise and shot noise. By recording a large number of speckles ($\sim 10^5$) the noise is minimized and converges to a DC offset in the measured AO-signal. The DC component in the intensity of individual speckles cancels out.

Besides this difference-based method it is possible to include both light pulses in one camera exposure, which we refer to as the addition method. This results in one speckle pattern that is the sum of two instantaneous speckle patterns. This pattern has a contrast difference ΔC compared to the contrast C_0 of a speckle pattern of one pulse. For this implementation, the reduction in contrast is regarded as our acousto-optic signal. The reduction in contrast ΔC is given by:

$$\Delta C = C_0 - C \tag{6}$$

and the contrast of the integrated speckle pattern C is given by the standard deviation of the speckle pattern over its average value. For the sum of two speckle patterns this becomes:

$$C = \frac{\left(\left\langle \left(I_1(n) + I_2(n) \right)^2 \right\rangle - \left\langle I_1(n) + I_2(n) \right\rangle^2 \right)^{1/2}}{\left\langle I_1(n) + I_2(n) \right\rangle}. \tag{7}$$

This method is a variation of the speckle contrast method [8] where we now use two short pulses of light instead of a continuous wave over an entire US cycle.

3. Materials and methods

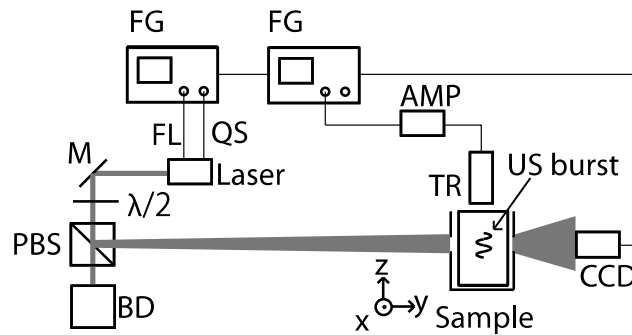For both types of signal generation we use the setup described in Fig. 1.

Fig. 1. Experimental set-up. FG: function generators, AMP: amplifier, TR: Ultrasound transducer and BD: beam dump.

The novelty is the use of a highly coherent short pulse laser. We used a frequency doubled injection seeded Nd:YAG laser (Newport Quanta Ray lab series 170) with pulse repetition rate 10 Hz, and Fourier limited pulses with a duration of 5ns, which results in a coherence

length of 1.5 m and a pulse energy of 350mJ. This system is capable of delivering enough light in a single pulse to generate a speckle pattern at the camera after transmission through a turbid medium and the energy is also sufficient for photoacoustic applications. Two synchronized function generators (FG)(Tektronix AFG 3102) give two TTL trigger signals for the laser consisting of flashlamp (FL) and Q-switch (QS) A third trigger is for the camera (CCD)(Allied Vision Technologies Manta G-145B NIR). At t = 0 the flashlamp is triggered, the QS trigger at t = 180 μs and the waveform for the US at ~162 μs depending on the depth of the ROI and the phase of the waveform. We use a simple setup for the delivery of the acoustics. The applied waveform is a sine at 5 MHz with 5 cycles. The waveform signal is amplified by ~50 dB by the amplifier (AMP) (Electronics & Innovation A075) and is connected to a focused 5 MHz US transducer (TR) (Olympus Panametrics-NDT V310). The laser light of 532 nm is attenuated with a half wave plate ($\lambda/2$) and a polarized beam splitter to reduce the pulse energy while operating the laser at its most stable settings. The excess light is collected by the beam dump (BD) so that the sample is illuminated with an optical pulse energy of approx. 3mJ. The repetition frequency of the laser is not high enough to give two pulses within one US cycle, thus we give a second US burst in time before the second laser pulse.

When the position of the US is shifted less than a wavelength the phase of the US is effectively changed. For the AO signal derived from the speckle pattern of two laser pulses the AO-signal strength depends on this phase difference. To show that the maximum signal is around π phase shift we perform a phase stepping experiment where we find the signal strength as function of the phase difference of the US bursts. Maintaining a π phase shift of the US between the two pulses of the laser it is possible to obtain an image by scanning the US focus through the object. We only use two phases for several reasons. Firstly it keeps the measurement time limited. But more importantly, in the eventual implementation we are will deliver 2 laser pulses within one US cycle, the current two phase measurement mimics this as close as possible. For the time being we need an object with tissue like properties with a longer speckle decorrelation time to overcome the problem of a low repetition rate of the laser. The phantom is a cylinder with a diameter of 20 mm and a length of 40 mm consisting of 3% agar and 3% Intralipid 20% without added background absorption. The background reduced scattering coefficient is estimated to be $\mu_s' = 0.6$ mm^{-1}. We scanned in the plane perpendicular to the ultrasound propagation direction, which is in z direction. The optical axis runs parallel to the y-axis. The optodes at the opposite ends have a diameter of 3mm. Both the subtraction and addition method were applied at the same time from the same speckle patterns. For the addition method we need to determine C_0 for every data point, however this contrast value remains the same within a small variation during the whole experiment. Therefore we assume this value to be constant and it is only measured at the start of the experiment.

We performed a measurement on the same phantom in an acousto-optic setup based on speckle contrast and a CW laser with the same experimental settings. Only the integration time of the camera is enlarged to 10 ms per speckle pattern to capture enough light, opposed to the effective 2 phases times 5ns = 10 ns integration time in our novel method. We chopped the light from a CW laser (Coherent Verdi 6, 532nm) with the use of an acousto-optic modulator to obtain pulses of 1 μs. So we only tag light from the same region as in the other experiments. Within the 10 ms exposure time we send 250 acoustic and laser pulses to capture enough light. At the opposite end of the object we capture a speckle pattern with the camera. In this way we show the relation between AO-signals generated with the speckle contrast method and the proposed technique.

But before these experiments we performed a stroboscopic measurement on a very stable phantom to test the setup. With our system we obtained snapshots of a speckle pattern for different positions of the ultrasound. By varying the delay between light and sound delivery we stroboscopically recorded the evolution of the speckle pattern while the US travels

through the medium. The ultrasound traveled along the z-axis and we placed the origin of this axis at the US focus. This experiment took several minutes and we wanted the speckle pattern to return to its original shape. This required a very stable sample material. We performed this experiment with a very stable homogeneous 5% agar phantom with paper particles of size ~300 µm as scatterers. The dimensions where the same as previously described phantom. We used the bigger paper particles in a more rigid agar matrix to maximize the decorrelation time of the speckle pattern. Brownian motion and thus speckle decorrelation is particle size dependent and the bigger scatterers result in the sample result in a decorrelation time of 10 minutes (speckle pattern cross correlation reduced to ~90%).

4. Results

The results of our stroboscopic measurement where we follow the blinking of many speckles at once are shown in Fig. 2.
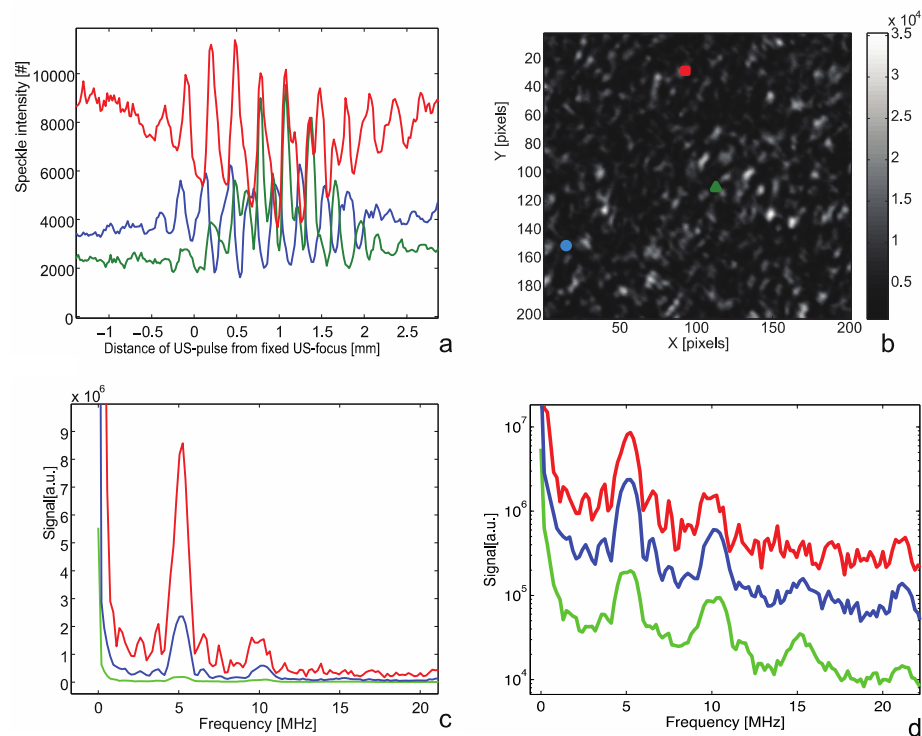

Fig. 2. (a) The intensity modulation of three randomly chosen speckles (location in b indicated by line color) as function of z-position of the US burst from the fixed US focus (b) speckle pattern and frame from the attached mov-file (Media 1) where the color scale is optimized for print. (c, d) The average power spectrum of the modulation for 3 groups of speckles: low, average and high intensity. The red (square/ upper lines) denotes a bright speckle; Blue (circle/ middle lines) denotes a speckle with average brightness. Green (Triangle/lower lines) denotes a dark speckle.

The blinking of 3 speckles, as specified in Fig. 2(b) when the US interacts with the light is shown in Fig. 2(a) and the attached mov-file (Media 1). This visualizes, to our best knowledge, for the first time the modulation of a speckle pattern while ultrasound propagates through a scattering medium opposed to following a single speckle in real-time i.e [9, 10]. The integration time for a single speckle pattern was ~5 ns. Figure 2(c) and 2(d) show power

spectra for the blinking of 3 categories of speckles. We observe that the ratio between the 5 MHz (US frequency) signal amplitude and the 10 MHz (first harmonic) signal amplitude depends on the brightness of the speckle. The brightest speckle has a ratio of ~5.7, the intermediate 4.0 and the dark speckles 2.2. We see that the phase of a speckle intensity signal is randomly related (Fig. 2(a)) to that of the US. We expect that sets of two speckle patterns with the biggest mutual differences are found at opposite phases of the ultrasound, suggesting that a π phase shift is optimal for acousto-optic signal generation. We tested this with a phase stepping experiment in which by varying the delay of the US we varied the phase difference from 0 to 2π. The normalized AO-signal amplitude from the difference method is plotted in Fig. 3 for the whole range of phase differences.
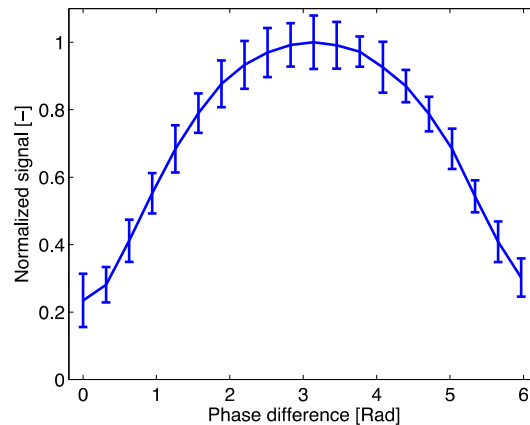
Fig. 3. Normalized AO-signal as function of US phase difference as acquired using the speckle pattern subtraction method. The size of the error bar denotes the standard deviation on the raw AO-signal. The line connects the average values for all realized phase differences.

We scanned our homogeneous 3% agar phantom 6 times, and for each scan we calculated the AO-signal and took the median for both methods to reduce the effects of noise. We scanned the plane perpendicular to the US propagation at a depth of 23 mm or approximately 0.88 inch where the transducer is focused and from light injection aperture to detection aperture. This result was verified with a speckle contrast AO measurement. The results of the three methods are shown in Fig. 4. Figure 5(a) gives a pixel-by-pixel comparison of the three images in Fig. 4 and Fig. 5(b) shows the subtraction method versus the addition method.
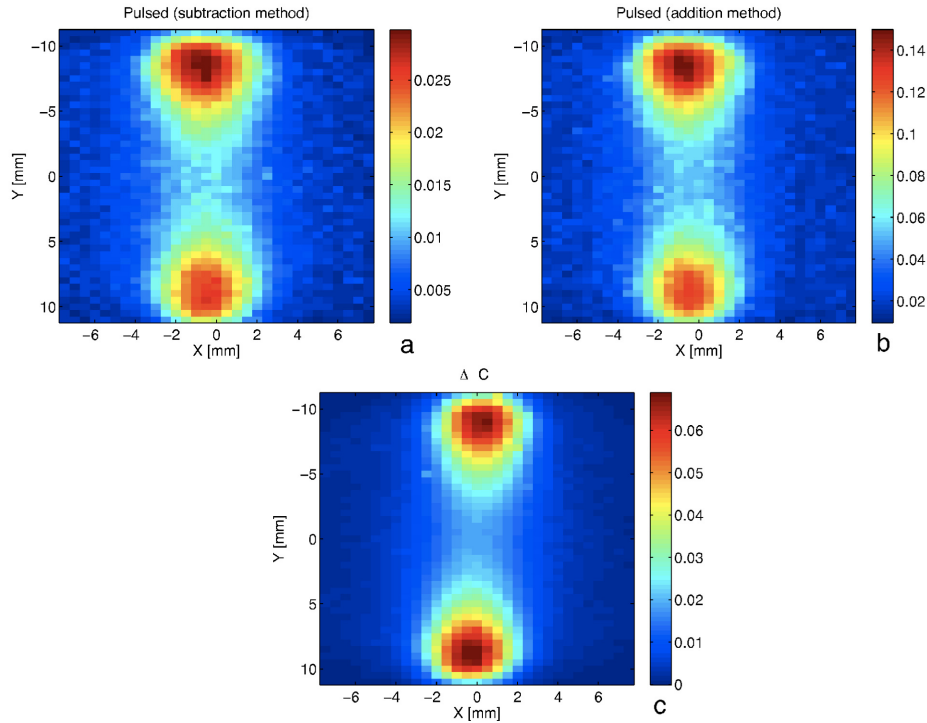
Fig. 4. AO scan results for subtracting two speckle patterns (a), adding two patterns (b) and speckle contrast method (c).
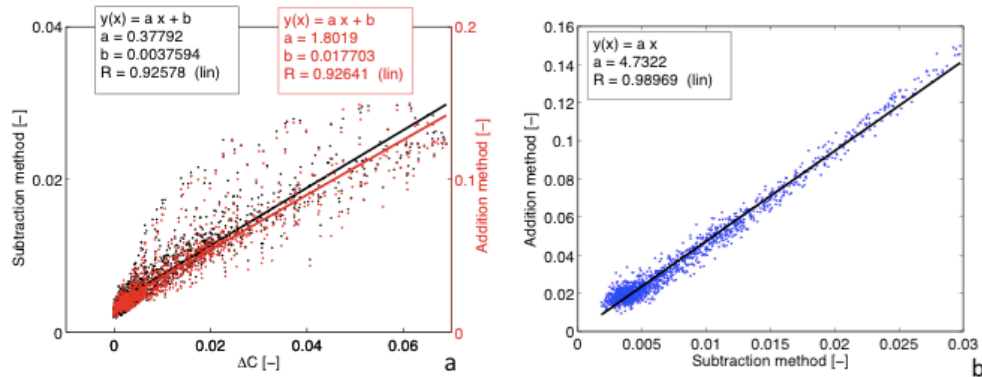

Fig. 5. Comparison of (a) pulsed methods vs. speckle contrast in CW setup, and (b) the addition method vs. subtraction method. The AO-signal from subtracting patterns (black, left axis) and addition of patterns (red, right axis).

5. Discussion and conclusions

The above experiments show that it is possible to obtain AO-signals with a ns pulsed laser. For this we used an injection seeded Nd:YAG laser with Fourier limited pulse, implying a coherence length of 1.5 m. First of all, with the used laser it is possible to generate speckles through a 2 cm thick scattering sample with a contrast of 0.43 when both polarizations are allowed on the camera. This implies that the coherence length of the pulsed laser is sufficient. The stroboscopic measurement (Fig. 2) also shows that pulse to pulse no big mode hops or other beam instabilities are observed that would lead to different speckle patterns between pulses. All the speckle patterns for which no US is applied are virtually the same. We see that

both bright and dark speckles contribute to the AO-signal, and that the bright speckles show more modulation (Fig. 2(a)). The relative amount of higher harmonics in the speckle intensity modulation depends on the brightness as well (Fig. 2(c)). This can be understood by taking the extreme case of a zero intensity region in the speckle pattern due to destructive interference: a modulation of part of the light by frequency ω will result in a 2ω frequency of the associated intensity variation since during each US cycle the local intensity will go from a minimum value (zero) to a maximum value twice. On the other hand, the brightest observed speckle is far from the maximum brightness possible, since in that case all the available energy goes into this single speckle, leaving the rest of space dark. And it is only this maximum possible intensity speckle that only can become less bright and might give higher harmonics.

The AO-signal strength depends on the US phase difference between the moments of illumination of the sample by the laser pulses. The maximum AO-signal is obtained for a phase shift of π radians. (Fig. 3) The phase stepping experiment also shows that the shift of the US pulse over 2π or 1 US cycle brings the AO-signal close to that of the 0 shift case but not entirely. This is because the shifted US burst which consists of 5 cycles only overlaps for 4 cycles with the original one. However this effect is small enough to be neglected when performing a scan.

The AO-signal strength as defined by Eqs. (4) and (6) behaves spatially very similar to speckle contrast measurements as shown by the scans for both the subtraction as addition method. (Fig. 4) Both results obtained with the pulsed light are spatially very similar (Fig. 4(a) and 4(b)). As for their quantitative agreement, in a pixel-by-pixel comparison we observe on average a factor of ~4.8 between the results of the two methods. (Fig. 5) These scans are also similar compared with the speckle contrast method. (Fig. 4(c) and 5(a)). A part of the 'noise' in Fig. 5(a) is caused by the slight tilt in the scan of Fig. 4(c).

The equivalence of results after addition or subtraction of speckle patterns initially is counter-intuitive but can be explained. (Fig. 5(b)) The bigger the difference between speckle patterns and keeping the average intensity constant for both would result in a lower standard deviation and thus lower contrast value. The fact that the addition method scales linearly with the difference method brings a great opportunity. For in-vivo applications an AO-signal should be acquired within the speckle decorrelation time of less than 0.1ms. Using two laser pulses within one US period can prevent speckle decorrelation within a measurement, e.g. a time interval of 100 ns between the two pulses for a 5 MHz US burst. To temporally resolve the speckle patterns resulting from these two pulses an ultra high-speed camera or a correctly triggered camera with sufficient short dead time is needed with high enough resolution for averaging over speckles. These cameras are expensive and large making them less desirable for future application. The addition method is equivalent to letting the camera integrate over two laser pulses. In the case of the addition method a relatively cheap and slow camera system can be used even in the case when much higher US frequencies are used and thus much shorter time between laser pulses. We see this as an important step towards in-vivo applications. To make this work we need to inject two laser pulses with sufficient wavefront matching, e.g. by splitting off light to a properly designed optical delay line. The properties of the used laser such as pulse duration and energy enable a combined performance of acousto-optic and photoacoustic measurements.