## Nonlinear effects in acousto-optic imaging

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Acousto-optic (AO) imaging is a promising technique that is able to reveal optical properties in the millimeter range inside scattering media by tagging the photon paths with an ultrasonic beam. To increase both the contrast and the resolution of the AO images, we have explored the possibility of using the nonlinear response of the speckle modulation. Variation of the second-harmonic signal as the square of the ultrasonic amplitude has been found, and strong reduction of the tagged zone size has been demonstrated. © 2002 Optical Society of America

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In a medium that scatters light so strongly that any incident light fills the whole sample, one way of sensing the optical properties locally consists of applying a focused ultrasound (US) beam and detecting a crossed effect of light and sound. Since the US beam usually undergoes no scattering, the size of the detected crossed, i.e., the acousto-optic (AO), signal is linked to the optical properties of the medium inside the US beam. Scanning the US beam over the sample then leads to a map of the relevant optical properties.<sup>1-9</sup> In our experiment, we shine coherent light on a CCD array and detect the light by the grain-by-grain modulation of the speckle by ultrasound.<sup>4,5,9</sup>

The size of the US beam volume is an important parameter, since it determines both the contrast for small objects and the spatial resolution. With our transducer ( $\emptyset$  45 mm; focal length, 60 mm; frequency, 3 MHz) the focal zone of the US beam is ~1.5 cm long and 1.5 mm wide. However, the whole volume of the US beam contributes to the signal. Therefore the resolution along the US axis and the contrast of objects smaller than the US volume are poor.

A possible way of increasing both the contrast and the resolution would be to use US pulses, whose length  $(\sim 2 \times$  the US wavelength) determines the axial resolution.<sup>10</sup> To our knowledge, this approach has not yet been followed for thick scattering media or biological tissues. A variant developed by Yao *et al.*<sup>11</sup> uses a frequency-swept US wave. Yet to examine thick samples one must improve the contrast and the signal-to-noise ratio.

Here we propose to explore an approach based on the nonlinear response of the system, hoping that the nonlinear signal generation will be restricted to a region of sufficiently high acoustic pressure, i.e., to a region smaller than that of the linear signal.

The samples were water-based gels (10-wt. % gelatin, 4-wt. % agar, and 0.16-wt. % latex spheres of  $\emptyset$  220 nm. The latex was a 95/5 styrene/acrylic acid copolymer (kindly supplied by T. Pith of the Institut Charles Sadron, Strasbourg, France). The agar grains acted as US reflectors; the latex spheres, as light scatterers. The resulting reduced scattering coefficient was measured to be 2 cm<sup>-1</sup>.

The light source was a coherent single-mode laser diode ( $\lambda = 840$  nm). After multiple scattering in the sample, the emerging light built a speckle pattern.

We recorded this pattern by use of a  $256 \times 256$  pixel CCD camera, whose distance we adjusted to match the speckle grain size to the CCD pixel size. The US beam (of frequency f = 3 MHz) modulated the optical paths that cross it in the sample.<sup>1,3,12</sup> This phase modulation of the partial light waves resulted in intensity modulations in the speckle (an interference pattern). For various positions of the US beam we measured the sum of the amplitudes of the modulations in all pixels, using a parallel lock-in detection method described in Refs. 4 and 5.

Let us briefly recall the detection method and explain how we apply it to the second harmonic. All the  $256 \times 256$  recorded speckle grains are equivalent and are processed in the same way; therefore we restrict the following description to a single speckle grain, i.e., to a single CCD pixel. The laser source was modulated synchronously with the 3-MHz oscillation of the US emitter. The laser was turned on for one quarter of each period of the US master clock by use of specially designed, home-made electronics. Moreover, this active quarter could be chosen to be the first, second, third, or fourth quarter of the clock period. First, for some large number N of US periods the active quarter was selected to be the first one. (The large value of N, e.g., 10<sup>5</sup>, matches the measurement to the slow frame rate of the camera:  $\leq 200$  frames/s.) Let  $S_1$  denote the intensity integrated by the CCD pixel during these *N* first quarters. Then, for an equal number *N* of US periods the active quarter is selected to be the second one. Let  $S_2$  denote the intensity integrated by the CCD pixel during these N second quarters. Similarly, the pixel then integrates N third quarters, and finally N fourth quarters, leading to respective integrated intensities  $S_3$  and  $S_4$ . Let  $I_1$  and  $I_2$  denote the weights of the first and second harmonics, respectively, in the intensity of the speckle grain. Under the assumption that no harmonics higher than the second are present, a simple computation<sup>4,5</sup> establishes the two following results:

$$[(S_0 - S_2)^2 + (S_1 - S_3)^2]^{1/2} = I_1 \frac{N\sqrt{2}}{\pi f}, \qquad (1)$$

$$|S_0 - S_1 + S_2 - S_3| = I_2 \frac{N}{f} \frac{2}{\pi} |\sin \varphi_2|, \quad (2)$$

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where  $\varphi_2$  is a phase lag between the second-harmonic modulation of the pixel intensity and the US master clock. Recalling now that the CCD camera simultaneously processes  $256 \times 256$  speckle grains, we average results (1) and (2) over these 64,000 pixels. The averaging obviously leaves result (1) unchanged. In result (2),  $\varphi_2$  is random, with a flat distribution from 0 to  $2\pi$ , so that finally

$$\langle |S_0 - S_1 + S_2 - S_3| \rangle = I_2 \frac{N}{f} \frac{4}{\pi^2}$$
 (3)

According to Eqs. (1) and (3), the weights  $I_1$  and  $I_2$  of the first and second harmonics, respectively, are easily extracted from the measurements.

We studied the dependence of the (fundamental and second-harmonic) AO signals on the voltage amplitude applied to the transducer (Fig. 1). The f signal varies linearly with the acoustic pressure. The variation of the 2f signal turns out to agree well with a quadratic fit, confirming its second-harmonic nature. At high voltage values both signals deviate from the fits and saturate. We ascribe this discrepancy to damage of the medium at high acoustic pressure, since when we turn the strong ultrasound on the signals jump high, then decrease regularly. (For 45 V applied to the transducer, the negative peak pressure at the US focus is approximately 10 bars in water).

The quadratic variation of the 2f signal does not reveal its physical origin. The 2f signal might result from purely acoustic second-harmonic generation. We thus probed our US beam in water with a hydrophone, for a voltage amplitude of 45 V, and observed the second harmonic in the US beam (Fig. 2). Yet the ratio between these acoustic 2f and f signals is equal to only 8% at the focus. This is much less than the ratio measured between the 2f and f AO components (~40%) for the same voltage applied to the transducer. Thus the acoustic effect mentioned above is not the dominant cause of the AO 2f signal.

Another possible origin is interference of optical wavelets that are phase modulated at frequency f. The phenomenon is well known and easily described for two-wave interference. The modulation of the phase  $\varphi$  of each wave at frequency f, i.e.,  $\varphi(t) = \varphi_0 + \Delta \varphi \cos(2\pi f t)$ , gives rise to first- and second-harmonic light modulation. The amplitude of the first-harmonic modulation is proportional to the Bessel function of the first order,  $J_1(\Delta \varphi)$  (which is linear when  $\Delta \varphi$  is small), and the second-harmonic modulation is proportional to  $J_2(\Delta \varphi)$  (quadratic when  $\Delta \varphi$  is small). Here the situation is more complex because of the random nature of the interference, and a detailed study would be beyond the scope of this Letter. However, we have performed crude Monte Carlo simulations that support this possibility.

To check the reduction of the AO modulation zone, we used a variant<sup>13</sup> of the setup described above: Instead of matching the speckle grain size to the pixel size, we inserted an objective in front of the camera to image the exit face of the sample on the CCD array. In the data processing we divided the CCD array into small areas (typically  $40 \times 40$ 

squares of  $6 \times 6$  pixels each), and we computed the summed modulation amplitude in each 36-pixel area separately. This yielded a map of the modulated light emerging from the sample. The US beam is buried just below the imaged face. Consequently, the light coming from the modulation zone hardly scatters before exiting the sample, so we obtain a nearly scattering-free image of the modulation zone. Figure 3 shows the image obtained for f and 2f processing of the frames after normalization by the unmodulated image, for a voltage of 45 V. The side of each imaged square is 2 cm long. One can see that the size of the modulation region is noticeably reduced when one passes from f to 2f. Taking the FWHM, we find a factor-of-2 reduction in the transverse direction and a factor-of-1.5 reduction in the axial direction. These values correspond to a reduction of the modulation volume by a factor of 6.

Then we checked that this reduction of the modulation zone improved the AO contrast of a small object. We buried a light-absorbing cylinder (diameter, 7 mm; length, 10 mm) in a 3-cm-thick gelatin phantom (as described above) made from the same gelatin with black China ink injected in it. We scanned the US beam across this sample and extracted the f and 2f signals



Fig. 1. Dependence of the normalized AO signal on the voltage applied to the transducer at the fundamental frequency (f = 3 MHz; left) and the second-harmonic frequency (2f = 6 MHz; right).



Fig. 2. Maps of the fundamental (3 MHz; top) and the second-harmonic (6 MHz; bottom) components of the acoustic pressure emitted by the ultrasonic transducer. The gray scale indicates the value of the negative peak pressure. Since the measurement was performed in water, the pressure does not undergo the attenuation of acoustic waves in tissues (1.8 dB/cm at 3 MHz).



Fig. 3. Spatial distribution of the modulated light on the exit face of the sample, showing the modulations at frequencies f (left) and 2f (right), normalized pixel to pixel by the distribution of dc-scattered light. Each side of each picture corresponds to a length of approximately 2 cm.



Fig. 4. AO profiles obtained at frequencies f and 2f on an absorbing inclusion. The scales for the two curves are different, but the baselines coincide.

(Fig. 4). For the profile at frequency f, the contrast of the absorbing cylinder is approximately 12%. The inclusion appears smaller than it actually was, probably because of a relatively high noise level. In the profile at frequency 2f, the size of the inclusion agrees with its actual size (7 mm), and the contrast ( $\sim 23\%$ ) is enhanced by a factor of almost 2.

In conclusion, we have demonstrated, for the first time to our knowledge, that second-harmonic acousto-optic detection and imaging are possible.

Use of the second harmonic led to better contrast than that yielded by the fundamental frequency by reduction of the volume of the effective modulated zone in the sample. Moreover, nonlinear response opens the door to a large variety of approaches used in signal processing, and we hope that this first study will be useful for further investigations.

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