

Decay of photoacoustic signals from biological tissue irradiated by near infrared laser pulses

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1 Introduction

A growing interest has been reported within the last decade concerning a variety of applications of photoacoustic (PA) or laser-ultrasound techniques for nondestructive examination (NDE) of complex materials. Some well-developed techniques that have been exploited in the industrial sector are now being further expanded for application in medical diagnosis and visualization.¹⁻⁶ In photoacoustic imaging of biological tissue, short light pulses (usually of nanoseconds of duration) are partially absorbed by a sample, leading to a local, small temperature rise (typically millikelvin). Heating of the restricted tissue volume results in its transient thermoelastic expansion. The stress-induced local deformation generates ultrasound that propagates through the sample. The initial ultrasonic pressure is (for short laser pulses) linearly proportional to the deposited optical energy. Because the amplitude and profile of a photoacoustic waveform depend strongly on tissue optical absorption coefficient μ_a , which is related to physiological (or pathological) status of the tissue, high contrast differentiation between tissue parts is possible.

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Abstract. We describe the phenomenon of a sudden decrease in the amplitude of photoacoustic signals arising from nanosecond laser pulse irradiation of biological samples, measured *in vitro*. Several dental enamel and chicken/turkey breast samples are examined. Moderate optical energy densities (i.e., about 300 mJ/cm²) are used, typical of those exploited in photoacoustic investigations. Measurements show a rapid decay of photoacoustic signals within the first few laser pulses absorbed by the sample. This phenomenon indicates that laser irradiation interacts with biological samples, causing long-term physical changes that can be attributed to a reduction of optical absorption within the samples. © 2006 Society of Photo-Optical Instrumentation Engineers. [DOI: 10.1117/1.2360690]

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Different constituents of the tissue (i.e., different muscle structures, bones, blood veins, tumour formations, etc.) may be targeted just by appropriate selection of the excitation wavelength. Photoacoustic imaging in biological tissue overcomes the low-contrast limitation of pure ultrasound imaging (low contrast in acoustic properties between different tissue sections). Simultaneously, it also helps to overcome unsatisfactory spatial resolution of all-optical techniques in those cases when optical scattering processes are dominant, such as biological tissue.²

We demonstrate for the first time the existence of an unexpected decay phenomenon of the amplitude of photoacoustic signals that we have observed during moderate laser pulse irradiation of *in-vitro* dental enamel samples, and *in-vitro* chicken or turkey breast samples.

2 Materials and Methods

2.1 Samples

Fresh samples of chicken and turkey breast tissue (skinless) were cut to small pieces, approximately 20 × 20 × 20 mm, and maintained at -20 °C in plastic bags. Samples were defrosted and equilibrated at room temperature prior to experimental examination. Six chicken breast and two turkey breast

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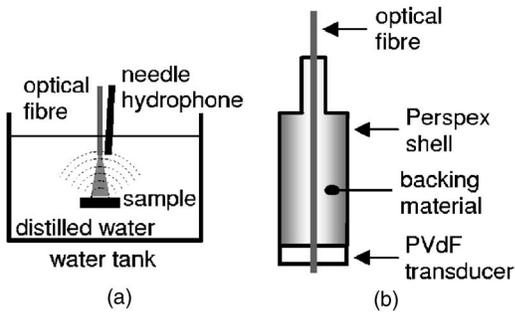


Fig. 1 Experimental arrangements (out of scale): (a) needle hydrophone detection system and (b) scheme of a compact integrated photoacoustic head used as an alternative probe system.

samples were examined. Samples were irradiated perpendicularly to the direction of muscle fibers. Several locations on each sample surface were explored in subsequent experiments. Rare samples were investigated, after which the same samples were microwave cooked and tested again.

Sterile dental enamel section samples, approximately $3 \times 3 \times 2$ mm (length, width, and thickness), were also examined. The detailed procedure of dental sample preparation is described in a paper by Lynch and ten Cate,⁷ except that the demineralization process was omitted. In total, 25 human and 6 bovine samples have been investigated. Enamel section samples were stored in air, at room temperature, in tight plastic vials and immersed in distilled water for at least 30 min before photoacoustic measurements were made. Prior to laser irradiation, care was taken to remove any tiny air bubbles emerging initially from porous dental samples. If necessary, any visible air bubbles were removed by either gently shaking the sample under water using hand tweezers, or by sweeping the sample surface with a piece of lens cleaning tissue. Each sample was irradiated several times as described later.

It should be noted here that alternative storage conditions were also considered. Two sets of control samples were stored in either distilled water or a desiccator. In all cases, we did not obtain any experimental evidence that different storage conditions significantly changed the measured photoacoustic waveforms.

2.2 Experimental Arrangement

A multimode, Q-switched Nd:YAG laser (Ultra CFR, Big Sky Laser Technologies, Incorporated) was used to irradiate samples. The laser provided pulses of wavelength $\lambda = 1064$ nm with a duration of 8 ns ($1/e$ fall). The laser was operated at a 1-Hz repetition rate, delivering pulses of approximately 11-mJ energy. Actual pulse energy was measured with a laser energy monitor (Nova, Ophir Optronics, Limited, Israel). Laser pulses were delivered via a 600- μ m-diam (core) glass fiber (PCS). The laser beam profile and its divergence were examined in air with a charge-coupled device (CCD) camera (BeamPro 2320, Photon, Incorporated). This beam divergence was next recalculated to take into account that actual experiments were conducted in distilled water. During all experiments, the optical fiber tip was kept about 7 mm above a sample surface, which led to beam size at the sample of approximately 2 mm in diameter ($1/e^2$). The cor-

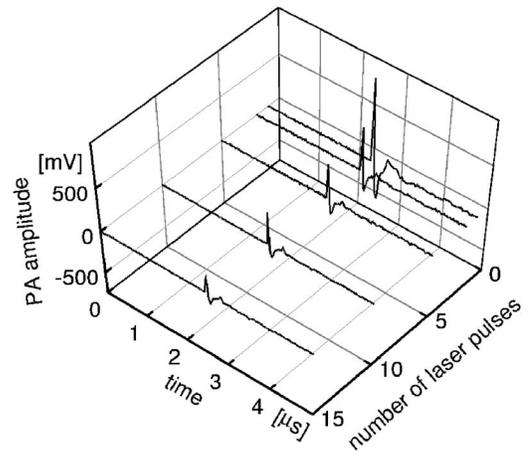


Fig. 2 Typical bipolar photoacoustic waveforms delivered by a human dental enamel section sample under distilled water. Data were taken using the arrangement shown in Fig. 1(a), with additional electronic amplification.

responding optical energy density was about 300 mJ/cm² at a sample surface using laser pulse energies of 11 mJ.

We exploited two different irradiation-detection systems, as seen in Fig. 1. In the first arrangement, Fig. 1(a), a needle hydrophone detector was used to measure photoacoustic signals. We used a 1.47-mm (outer diameter) needle hydrophone (HPM1/1, Precision Acoustics, Limited, United Kingdom) with a submersible preamplifier (HP1, Precision Acoustics) which was aligned along the optical fiber at a small angle of about 2 deg. In the second arrangement, Fig. 1(b), the hydrophone and optical fiber were replaced by a submersible homemade compact photoacoustic head. This construction was designed by our group in 1987⁸ and has been successfully developed since that time.⁹ The actual probe receiver had a 6-mm outside diameter and utilized a 28- μ m-thick polyvinylidene difluoride (PVDF) piezoelectric film, with vacuum metallized nickel-copper electrodes. Both photoacoustic receivers had an upper frequency response of approximately 15 MHz and gave similar experimental results.

Electrical signals coming from the ultrasonic receiver were captured by a 200-MHz digital oscilloscope (TDS 640A, Tek-

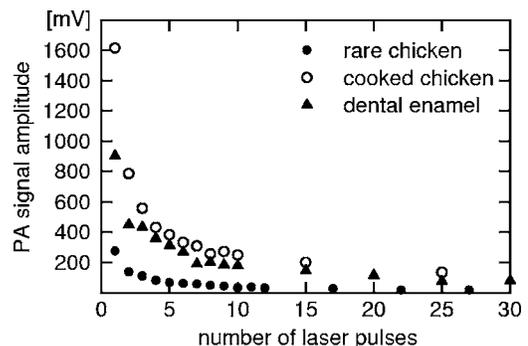


Fig. 3 Decay of the photoacoustic signal amplitude during sample irradiation: optical energy density 300 mJ/cm². Data were taken using the arrangement shown in Fig. 1(a), with additional electronic amplification.

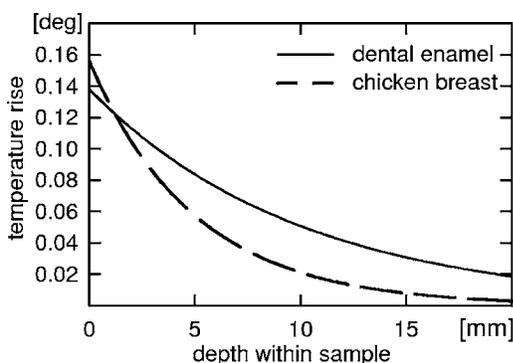


Fig. 4 Predicted temperature rise distribution within samples above ambient temperature due to a single laser pulse of 11-mJ energy. The incident optical energy density was 300 mJ/cm².

tronix, Incorporated) and transferred to a personal computer via a GPIB/IEEE488 interface. Due to the nature of our investigation, no signal averaging procedures could be applied. Transistor-transistor logic pulses from the laser's Q-switch were utilized to trigger the scope, and special software, developed under a LabView® (National Instruments) platform, was used to acquire and monitor photoacoustic waveforms.

A sample under investigation was placed on a flipped Petri dish immersed in distilled water, in a 1 litre glass beaker. Small dental samples were clamped between two microscope slides to secure their steady-state position during examination.

3 Results and Discussion

Typical photoacoustic waveforms delivered during the first few laser pulses by a human dental enamel section are presented in Fig. 2. Similar (yet different in shape) bipolar transients were generated also by bovine enamel and chicken/turkey breast samples (rare or cooked). We focused on investigating the variation in peak-to-peak amplitude of these bipolar signals when consecutive laser pulses irradiated the sample.

Figure 3 shows a graph of the peak amplitude of photoacoustic waveforms versus the consecutive laser pulse count when a sample was irradiated. The samples considered were human dental enamel, and rare and cooked chicken breast tissue. The figure shows a rapid decay of the photoacoustic signal amplitude to be clearly visible, confirming the phenomena shown in Fig. 2. An analogous decay phenomenon was also observed during experiments with bovine and turkey samples. Figure 3 reveals one more important detail: cooked chicken tissue produced initial photoacoustic signal amplitudes that were much higher than ones corresponding to an uncooked sample. This fact was consistent during all our tests, and was also confirmed in the case of turkey breast samples. Thus, the decay phenomenon itself could not be attributed to "laser cooking" or to thermal damage of the samples investigated.

These observations have been supported by theoretical calculations we performed of the temperature rise at a sample surface during laser irradiation. As a first approximation, we employed a simple 1-D model (not involving scattering) to estimate the surface temperature increase resulting from a single laser pulse^{1,10}

$$T(z) = \frac{\mu_a \cdot \exp(-\mu_a \cdot z)}{\rho \cdot C} \cdot \Phi, \quad (1)$$

where $T(z)$ is a transient temperature profile as a function of depth (z) within a sample, ρ and C are the density and specific heat capacity of a sample, respectively, while Φ stands for the incident laser energy density at a sample surface ($z=0$).

The parameter values used for human dental enamel were: $\mu_a=1 \text{ cm}^{-1}$,¹¹ $\rho=2.9 \text{ g/cm}^3$, and $C=0.75 \text{ J/(g}\cdot\text{K)}$.¹² Corresponding values assumed for rare chicken breast tissue were $\mu_a=2 \text{ cm}^{-1}$, $\rho=1.1 \text{ g/cm}^3$, and $C=3.5 \text{ J/(g}\cdot\text{K)}$, which are typical for muscle tissue.^{13,14} In both cases, an average laser energy density $\Phi=300 \text{ mJ/cm}^2$ was used in calculations. The optical absorption coefficient of water, μ_a , at $\lambda=1064 \text{ nm}$ was taken to be 0.12 cm^{-1} .¹⁵ The resulting analytical predictions are presented in Fig. 4, which shows that the expected surface temperature rise above ambient temperature for each laser pulse is predicted to be well below $0.25 \text{ }^\circ\text{C}$. In later calculations, we have considered optical scattering processes in a 3-D model. Introduction of these processes do not lead to more than a doubling of predicted temperatures at the surface of the sample.

All photoacoustic signal amplitudes varied on a shot-to-shot basis due to the fluctuations in laser pulse energy. As a consequence, an error of about $\pm 10\%$ is associated with the experimental data points shown in Fig. 3. Additionally, it is worth noting that similar decay of photoacoustic signal amplitudes was also observed during laser irradiation of dental enamel samples immersed in high performance liquid chromatography-grade methanol. Repeat measurements of signal amplitude also showed that the samples did not "recover" within several hours after laser irradiation.

Laser light can result in various processes such as tissue charging, coagulation, dehydration, ablation, or even trigger a synthesis of lethal complexes. Fried et al.¹⁶ for example, mentioned a decay phenomenon of photoacoustic signals delivered by CO₂ laser ($\lambda=9.6 \mu\text{m}$) irradiated dentin samples. These authors, however, used fluences exceeding 1 J/cm^2 which, together with a very high optical absorption coefficient of dentin at $\lambda=9.6 \mu\text{m}$, $\mu_a=6500 \text{ cm}^{-1}$,¹⁶ led to sample surface temperature rises exceeding $1000 \text{ }^\circ\text{C}$ and, as a consequence, to ablation processes. Thus, it is difficult to say whether there is any direct correlation between their findings and ours, since under our experimental conditions there is no ablation. On the other hand, Sasaki et al.¹⁷ have provided evidence based on Fourier transform infrared spectroscopy (FTIR) that laser irradiation of dental samples may result in critical changes of their chemical structure. They investigated the influence of Er:YAG ($\lambda=2.94 \mu\text{m}$) and CO₂ ($\lambda=10.6 \mu\text{m}$) laser irradiation of extracted human teeth sections. Their data showed that laser radiation results in the formation of two additional bands in FTIR spectra of irradiated samples, at 2010 cm^{-1} ($4.98 \mu\text{m}$) and also at 2200 cm^{-1} ($4.55 \mu\text{m}$). The first peak was recognized as a cyanamide (NCN²⁻) band, while the second one was attributed to cyanate (NCO⁻) ion formation. (Both compounds may be toxic.)

Neither of these interaction mechanisms at laser wavelengths extending into the infrared is likely in our present experiments. Our results at a near-infrared laser wavelength imply that the samples exhibit a decrease in the optical ab-

sorption coefficient from consecutive laser pulses. As a consequence, the samples undergo some optical transparency. Further studies are therefore required with infrared spectroscopy, and with detailed surface examinations of the samples using scanning electron microscopy. These studies may help to reveal the nature of the laser-matter interaction that results in a reduction of samples' optical absorption coefficient, leading to a photoacoustic signal decay as shown in Figs. 2 and 3.

4 Conclusions

We report on a new decay phenomenon in photoacoustic signal amplitudes from successive near-infrared laser pulse irradiation of biological tissue. The phenomenon is observed and investigated using moderate laser energy density (300 mJ/cm^2) when irradiating biological materials at a laser wavelength of 1064 nm. Laser pulse durations were of the order of 8 ns. We recognize that this phenomenon arises from a direct consequence of changes to the sample's optical properties when the sample is irradiated with such laser pulses. Future studies using analytical techniques will be required to gain further insight into these processes. A detailed understanding of the photoacoustic decay phenomena is necessary, and may be crucial for safe *in-vivo* biomedical applications of laser techniques for diagnostic and therapeutic purposes.

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