Gas Coupled Laser Photothermal Interferometry for Non-destructive and Non-contact Studies of Biological Specimens

Kay Haupt, Dane Bicanic,* Edo Gerkema,* and Angela Frandas**

Physikalisch-Astronomische-Technikwissenschaftliche Fakultät, Institut für Optik und Quantenelektronik, Friedrich-Schiller-Universität, Max Wien Platz 1, Jena 07743, Germany

*Laser Photoacoustic Laboratory, Department of Agricultural Engineering and Physics, Agricultural University, Bomenweg 4, 6703 HD Wageningen, The Netherlands

**Institute for Isotopic and Molecular Technology, 3400 Cluj-Napoca, Romania

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Photothermal signals from some biological materials exposed to modulated CO₂ laser radiation were detected using the concept of photothermal interferometry. The magnitude and a phase of the signals were found to contain qualitative information about subsurface structural changes and variations of moisture content.

During recent years photoacoustic, photothermal, and other related techniques have been used in many different areas of science.¹ ⁻³ One among the attractive features of these methods is their suitability for studies of inhomogeneous specimens as well as the potential for non-destructive, non-contact analysis of layered samples.⁴ ⁻⁵ This enables one to obtain depth-related information of the subsurface structures in opaque samples.⁶ ⁻⁷

All photothermal methods rely on the localized excitation of a sample using periodically modulated or pulsed radiation. For specimens that are opaque to incident radiation, the largest fraction of radiation is absorbed in the uppermost layer of the sample, which leads to the flow of heat into the sample. For sinusoidal modulation, the propagating heat flow can be described as a highly damped oscillation⁶ originating at the location of absorption; the “wave” length λ of such oscillation depends on a modulation frequency f and thermal diffusivity D as λ = 2π(Dπf)₁/². Thermal diffusivity, in its turn, is a function of thermal conductivity, k, density ρ, and the specific heat c at constant pressure, i.e., D = k/pc. The expression (Dπf)₁/² is generally addressed as the sample’s thermal diffusion length μ, hence λ = 2πμ (after propagating over one such “wave” length, the amplitude of the thermal wave becomes practically negligible). Because of physical structures such as for example thermal inhomogeneities at a certain depth (existing or missing interface, inclusion, structural defects, concentration depth profiles etc.) in the path of the thermal wave, some portion of the thermal energy is reflected. The superposition of incident and reflected waves produces characteristic changes in a time-dependent temperature at the sample’s surface that can be detected in several ways.

When the sample is surrounded by a gaseous medium, the resulting surface temperature will lead to a periodically varying characteristic temperature profile in a gas layer adjacent to the surface. This is due to thermal transport achieved between μs (thermal diffusion length in sample, (subscript s) and μg (thermal diffusion length in the gas (subscript g)). The amplitude of the generated signal contains information on the absorption coefficient, density, specific heat, and the thermal conductivity. On the other hand, the phase of the signal provides information of the specific heat, density and thermal conductivity. Due on the f₁/² dependence of μ, it follows that higher modulation frequencies correspond to probing of a sample closer to its surface. In an early photoacoustic depth sampling experiment, Adams and Kirkbright succeeded in discriminating (μg for waterlike samples at 300 Hz is 10 μm as compared to 33 μm at 30 Hz) between optical absorptions taking place in a top waxy layer and the underlying, pigment-containing layers of an apple.⁸

The work described here is concerned with the measurement of thermal profiles in a single bean and the shell of an egg. Detection of qualitative photothermal signals was done by a gas coupled Michelson interferometer, the operating principle of which is based on the fact that the refractive index of the gas is temperature-dependent. Each of the investigated samples was treated in some way before comparing the time dependent photoacoustic signals at some later stage. The objective in undertaking that non-spectroscopic study was to evaluate the potential of the newly proposed method for non-contact, subsurface, non-destructive, and in vivo investigation of (un) perturbed biological specimens.

Materials and Methods

The experimental set-up used in this study is shown in Fig. 1a. The radiation emitted from a home made waveguide c.w. CO₂ laser (1) tuned to any arbitrary transition (the only function of laser radiation was to provide heating) was modulated by a chopper (2,3) before reaching two plane mirrors (4) that directed the radiation through a positive ZnSe lens (5) toward the mirror (6). The infrared laser beam impinging on the platform (8) mounted to an X-Y-Z stage that served as a sample holder (and for lateral scanning if required). As absorption of laser radiation by biological sample is substantial, and the power of the CO₂ laser was high, care had to be exercised to avoid damaging the sample. This was done by focusing the heating beam to approximately 40 mm above the sample (the spot diameter at the sample itself was then 3 mm); the power measured at the sample site was typically 150 mW.

The 10 mW Uniphase 1301P He-Ne probe laser (14) passed through a λ/4 plate operating as an optical isolator (13) and reached a 45° beam divider (10) provided with the compensating plate. This laser beam was then split into two beams falling perpendicularly on plane mirrors (7) and (9) (one of beams traversed a region above the heated surface of the
sample). Returned beams reunited at the same surface of a beam divider and interfered at a plane of the photodetector (12) where a total intensity (a sum of intensities of individual beams) was measured. A fraction of the beam could be diverted towards another detector using a prism (11) for monitoring purposes, if necessary.

The periodic variation of the refractive index $n(t)$ for air caused corresponding changes in the optical pathlength $n(t)L$ (where $L$ is the geometrical pathlength) giving rise to alteration in interference pattern at the detector plane. For maximal sensitivity one has to operate the interferometer with a phase difference of $\pi/2$ (this corresponds to a half maximum of a d.c. intensity). Therefore, the plane mirror (7) was mounted to a Jodon piezoelectric crystal; position of latter was regulated automatically by a Physik Instrumenten PI-263 high voltage powder supply.

To prevent effects of air turbulence, the interferometer was covered with a Perspex cap (provided with openings for undisturbed passage of heating and probing beams). Optically induced distribution of the refractive index causes deflection of the probe beam simultaneously with the alterations in optical pathlength. However, the effects of this were neglected in this experiment. This is because the photothermal beam deflection was due to a gradient in the distribution of refractive index, while the change of optical pathlength is an integral effect. At the modulation frequencies used in this work, small refractive index gradients (large thermal lenses) are generated, so that the interferometer is more sensitive to optical changes than to the effects of photothermal beam deflection.

**Results and Discussion**

The initial experiment was done with a single bean. A tray with the single bean on it (Fig. 1b) was brought into the proper position with respect to the probe laser beam. The sample-probe beam distance is of major importance whenever attempting to obtain reliable results in any photothermal experiment. The procedure which was consistently followed to ensure a very good degree of reproducibility, consisted in adjusting manually (micrometer) the tray's vertical position in such a way that a top portion of the sample just intersected the axis of the probe axis. The final position was that corresponding to 95% of the maximal (unblocked) intensity of the probe laser (Fig. 1c).

The amplitude (Fig. 2a) and phase (Fig. 2b) of photothermal signals were recorded using the two phase lock-in amplifier model Lthaco 3691A by varying the modulation frequency. The lower plot in Fig. 2a is obtained with the intact bean. Then, the "skin" of a bean was carefully removed and the measurement repeated (upper plot in Fig. 2a). The larger amplitude of the signal observed for a bean with skin removed is probably due to a higher water content (bulk of bean) absorbing the CO$_2$ laser radiation (the shell itself is drier). Both curves display a similar trend; the ratio of the upper to lower curve (solid line in Fig. 2a) is rather flat for low modulation frequencies but increases to 2.5 for 100 Hz. As stated above, at higher frequencies the thermal diffusion length is short, which enables detection of either the "skin" or the interface. The results are in qualitative agreement with those predicted by the theoretical model based on a one-dimensional heat transfer in a two-layer system (the latter implies that the diameter of a heating beam at the focal spot is much smaller than the curvature radius of the sample's surface).

The results for phase are displayed in Fig. 2b. In case of the intact bean (lower trace), the phase changes significantly
within the range of modulation frequencies used in this study. With skin removed, a similar pattern is observed (upper trace in Fig. 2b). Subtracting the upper trace from the lower trace in Fig. 2b yields the plot of phase difference. The purpose of the next experiment was to obtain information about transport of moisture in a single bean. The bean was first cut in half along the longitudinal symmetry axis and then with its “naked” side immersed in dish filled with water. The dish was then placed on the interferometer tray with the bean’s “skin” facing the heating beam. The phase as a function of the modulation frequency \( f \) was measured at the beginning, as well as one and two hours after onset of the experiment. The difference between measured phases at each modulation frequency was computed and is plotted in Fig. 2. Note that pronounced changes are observed only for frequencies exceeding 10 Hz, suggesting that thermal properties of “skin” (or interface) differ with time, probably as a result of varying moisture content. A tentative explanation for this pattern is that the transport of moisture through the skin is more rapid than through the bulk. This is consistent with assumptions of increasing thermal diffusivity as the bulk gets wetter. The effect of a changing probe beam-sample distance that could otherwise affect the phase of the signal was eliminated by controlling the position of the sample as described above. For that reason we believe that observed changes (varying water content in uppermost layers is one of such possibilities) can be ascribed to alterations of the sample’s physical parameters. After two hours, the phase difference decreased to zero, suggesting that there were comparable amounts of thermally active water in the bulk and the shell, making detection of thermal interface more difficult. For longer intervals the initial stage of skin detachment from the bulk could be seen even with a naked eye.

Finally, an attempt was made to monitor the drying process of a shell of a hen’s egg over a period of six consecutive days. The impervious shell having numerous minor pores essential for interchange of oxygen, contains normally about 1.5 percent water. A top of a hard boiled egg (with shell facing the heating beam) was investigated first by recording phase-frequency dependence. Then, the uppermost shell layer was separated from underlying layer(s) and the bulk, before repeating the experiment within the same frequency range (Fig. 4). Similar measurements were done 1, 2, and 6 days after the beginning of the experiment (Fig. 4). Following the sixth day, shell was

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**Fig. 2a.** The Amplitude of the Photothermal Signal Obtained from Single Bean with (Lower Trace) and without (Upper Trace) the Uppermost Layer Plotted versus the Modulation Frequency. The Ratio (Upper Trace/Lower Trace) of Two Curves Increases at Higher Frequencies.

**Fig. 2b.** When Plotted versus the Modulation Frequency, the Phases of Photothermal Signal Obtained from a Single Bean with (Lower Trace) and without (Upper Trace) the Uppermost Layer Show a Similar Trend. Subtracting Lower Trace from the Upper One Produces the Phase Difference-Frequency Plot (also Shown).

**Fig. 3.** The Phase Difference (Rel ated to Initial Phase) for a Single Beam (Partially Immersed in Water), Measured 1 (●) and 2 (□) Hours after the Onset of Experiment, Plotted versus Modulation Frequency.
rewetted (with water). Inspection of plots in Fig. 4 shows some similarity between different curves; nearly systematic differences in slopes were observed only at high frequencies. In general, phase decreased (decrease of moisture content) in time and reached zero (at 100 Hz) after six days. Upon rewetting, the phase was found to approach the value observed at the beginning of the experiment.

In principle, the analysis of phase-frequency plots offers the possibility (advantage of such approach lies in the fact that phase plots do not depend on the absorption parameters and the intensity of heating beam; these affect only amplitude) to calculate directly the thickness of a layer in simple systems. In this work, layer thickness could not be calculated because of lacking values of the thermal parameters for all components of the layered system. In addition, thermal properties of porous media are not easy to interpret. It is therefore necessary to calibrate the proposed methodology using a model sample. More work on this matter is to be done in the near future.

In conclusion, gas coupled photothermal interferometry was used to qualitatively probe subsurface structures, and the time evolution of moisture content in layered structures (a single bean and egg shell) in a non-contact and non-destructive fashion. No preparation of the samples was required. The same technique might prove applicable not only to other materials, but also be useful for investigation of various mass diffusion and subsurface phenomena (examples are effects of spoilage, potential disease, wound healing, influence of water on compaction behavior, etc.) otherwise not accessible to conventional inspection methods. As such, the proposed method can be a valuable tool for assessment of vital parameters in tissues of biological and agricultural interest.

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