Analytical Systems

Potential Clinical Applications of Photoacoustics

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Photoacoustic spectroscopy offers the opportunity for extending the exact science of noninvasive spectral analysis to intact medical substances such as tissues. Thermal-wave imaging offers the potential for microscopic imaging of thermal features in biological matter.

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The new fields of photoacoustic spectroscopy and thermal-wave imaging exhibit some interesting potentials as clinical tools. I shall discuss photoacoustic spectroscopy first and then proceed to the newer field of thermal-wave imaging.

Photoacoustic Spectroscopy

In photoacoustic spectroscopy (PAS) (1), the sample to be studied is placed in a closed cell or chamber. Gaseous and liquid samples generally fill the entire chamber. For solids, the sample fills only a portion of the chamber, and the rest of the chamber is filled with a nonabsorbing gas such as air. The chamber also contains a sensitive microphone. The sample is illuminated with monochromatic light that either passes through an electromechanical chopper or has its intensity modulated in some other fashion. If any of the incident photons are absorbed by the sample, internal energy levels within the sample are excited. Upon subsequent de-excitation of these energy levels, all or part of the absorbed photon energy is then transformed into heat energy through nonradiative de-excitation processes. In a gas this heat energy appears as kinetic energy of the gas molecules, whereas in a solid or liquid it appears as vibrational energy of ions or atoms. Because the incident radiation is intensity-modulated, the internal heating of the sample is periodic.

The periodic heating of a solid or liquid sample from the absorption of the optical radiation results in a periodic heat flow from the sample to the nonabsorbing gas. This in turn produces pressure and volume changes in the gas, which are detected by the condenser microphone. This method is quite sensitive, especially for samples with large surface/volume ratios such as powders, and can detect a temperature increase of 10\(^{-6}\) to 10\(^{-5}\) K in the sample, or a thermal power generation of about 10\(^{-8}\) cal \cdot cm\(^{-3}\) \cdot s.

PAS has several advantages. Because optical electromagnetic radiation must be absorbed before a photoacoustic signal can be generated, light that is transmitted or elastically scattered by the sample is not detected and hence does not interfere with the PAS measurements, which are inherently absorptive. This is of crucial importance when working with essentially transparent media such as pollutant-containing gases that have few absorbing centers. This insensitivity to scattered radiation also permits the investigator to obtain optical absorption data on highly light-scattering materials such as powders, amorphous solids, gels, colloids, etc. Another advantage is the capability of obtaining optical absorption spectra of materials that are completely opaque to transmitted light. Coupled with this is the capability, unique to PAS, of performing nondestructive depth-profile analysis of absorption as a function of depth into a material.

One of the most exciting areas for photoacoustic studies lies in the field of medicine. One can obtain optical data on medical specimens that are not amenable to conventional study because of excessive light-scattering; study specimens that are completely opaque to transmitted radiation; and perform depth-profile analysis by phase or frequency adjustments. Furthermore, it is possible to construct a photoacoustic cell to perform all of the above measurements in vivo.

Drugs in Tissue

An example of the use of PAS in medical studies is in the investigation of animal and human tissues—both hard tissues such as teeth and bone, and soft tissues, such as skin, muscle, etc. An example of a PAS experiment on soft tissue (1) is illustrated in Figure 1, which shows photoacoustic spectra of guinea pig epidermis. The top spectrum is of an epidermis that has been treated with a 20 g/L ethanolic solution of tetrachlorosalicylanilide (TCSA), a highly effective antibacterial agent known to cause photosensitivity and other skin problems. The central spectrum is of control guinea pig epidermis. The bottom spectrum, the difference spectrum, obtained by subtracting the control spectrum from the TCSA spectrum, is the absorption spectrum of TCSA bound within the epidermis. From this difference spectrum one can establish the state of the TCSA compound in situ when it is incorporated into the skin, and thus learn more of its action on and within the skin under various conditions.

Another example of an experiment to detect drugs in tissue has been reported by Campbell et al. (2). Topically applied tetracycline was detected at 380 nm, where tetracycline is strongly absorbing but skin is not. As Figure 2 shows, the PAS signal is substantially greater for the tetracycline samples than for the untreated sample, and the signal amplitude is roughly proportional to tetracycline concentration.

These examples illustrate the potential usefulness of photoacoustic spectroscopy in the study of natural, medical, and cosmetic compounds present in and on human tissue.

Human Eye Lenses

Figure 3 depicts PAS spectra taken on intact human eye lenses (1). These experiments were conducted to study the processes by which cataracts form in human eyes. Little is known about this ancient disease except that it is probably...
related to a photo-oxidative process in which the tryptophan and tyrosine residues in the protein matter of the lens form complexed compounds that are either highly light-scattering (cortical cataracts) or colored (brunescent or nuclear cataracts). Cataract studies are severely hampered by the fact that spectroscopic investigations cannot be conducted on the intact lenses. In general, these lenses must be solubilized and, except under the strongest solubilizing agents, only about one-half of the lens material goes into solution to be suitable for study. Unfortunately, the stronger solubilizing agents do too much damage to the inherent protein to be useful.

The upper spectrum in Figure 3 is of a normal intact human eye lens, the lower spectrum is of a lens with brunescent cataract. Both spectra exhibit the characteristic peak at 290 nm caused mainly by absorption of the tryptophan and tyrosine protein residues. The cataractal lens shows a much broader 280 band than does the normal lens, in agreement with the theory that the cataract is a result of conjugated tryptophan and tyrosine compounds. In addition, however, the PAS spectra indicate that the cataractal lens exhibits greater infrared absorption. This unexplained feature suggests that cataract formation impairs vision not only in the blue end of the spectrum but also throughout the visible region, with broad absorption bands impinging from both ends of the visible spectrum.

Tissue Studies

The first extensive PAS study on mammalian tissue was on the stratum corneum (3), the outermost layer of the epidermal tissue. The stratum corneum, a translucent membrane in the visible wavelength region, is a highly effective light scatterer, especially in the ultraviolet region, and thus cannot be studied readily by conventional optical absorption techniques.

The amount of water present and the role this water plays in mammalian tissue strongly determine the physicochemical properties of tissues like the stratum corneum. Because the photoacoustic effect is sensitive to the presence of water through its dependence on the thermal diffusivity of the sample, photoacoustic spectroscopy can be used for moisturization (hydration) studies, as related to the role of water in the stratum corneum (3). Figure 4 shows the photoacoustic signal at the tryptophan-tyrosine "protein" band at 285 nm,

Fig. 2. Photoacoustic signal dependence on chopping frequency for a control human stratum corneum and for stratum corneum topically treated with tetracycline (TCN)
From Campbell et al. (2)

Fig. 3. PAS spectra of intact human eye lenses: (a) a normal lens; (b) a lens with a brunescent cataract
From Rosencwaig (1)
Mammalian stratum corneum obviously serves quite different roles in the pre- and postpartum periods. Major and rapid biochemical and structural changes can be expected during the initial postpartum period, when the matrix of the stratum corneum undergoes alteration to develop its so-called "barrier" functions and adapt to its new and strikingly different environment. Rosencwaig and Pines (3) have postulated that the observed changes in the PAS spectrum of Figure 5 reflect this maturation process of the stratum corneum. In particular, the tyrosine residues in the \(\alpha\)-keratin protein of stratum corneum appear to undergo a major molecular modification, probably by enzymic action. This modification results in increased interchain hydrogen-bonding, which can play a crucial role in stabilizing the coiled-coil structure of the \(\alpha\)-keratin in the stratum corneum, enabling the stratum corneum to take on its postpartum barrier functions.

Although there is as yet no direct evidence for the appearance of a modified tyrosine (e.g., hydroxytyrosine) during the maturation or keratinization period, this hypothesis is attractive because it bears a close analogy to the known development of collagen, the structural protein in muscle tissue. As collagen forms, some of the proline residues are enzymically modified to hydroxyproline, the extra hydroxyl group of the hydroxyproline contributing significantly to the stability of the triple helix of collagen by additional hydrogen bonds. Therefore, the possibility of an enzymic molecular change in the tyrosine of a postpartum stratum corneum merits further investigation (3).

**Figure 4.** Photoacoustic signal strength at 285 nm as a function of water content for intact newborn rat stratum corneum. From Rosencwaig and Pines (3)

As a function of the water content determined gravimetrically in the stratum corneum, identical results were obtained at other spectral wavelengths. There is at first very little change in the photoacoustic signal, then a fairly rapid decrease, and then a slower decrease, with the curve apparently approaching a limiting value at high water content. The shape of this curve can be explained by the changes that occur in the thermal properties of the stratum corneum when water content increases. Such data supply information on the presence of free and "bound" water in stratum corneum.

Figure 5 shows a series of photoacoustic spectra obtained from stratum corneum membrane from newborn rats during the initial 60-h maturation period after birth (3). The times shown are the postpartum age of the rats at the time they were killed. The 280-nm band undergoes a major change, particularly in the first 10–30 h postpartum. Identical results were obtained on all sets of rats litters studied, irrespective of the period between the time of obtaining the stratum corneum and the time the photoacoustic spectra were actually taken.

**Figure 5.** Photoacoustic spectra of a series of newborn rat stratum corneum during the postpartum maturation period. From Rosencwaig and Pines (3)

**Thermal-Wave Imaging**

Thermal-wave imaging is a recent development arising from the general field of photoacoustics. In a thermal-wave microscope, a laser (4) or electron beam (5, 6) is focused and scanned across the surface of a sample. The intensity of this beam is modulated at some frequency in the 100 Hz to 10 MHz range. As the beam scans across the sample, it is absorbed at or near the surface of the sample and a periodic surface heating occurs at the modulation frequency. This periodic surface heating is the source of thermal waves that propagate from the heated region. The thermal waves interact with thermal boundaries and barriers in a manner mathematically equivalent to the scattering and reflection of conventional propagating waves. Thus any features on or beneath the surface of the sample that have thermal characteristics different from their surroundings will "reflect" and "scatter" thermal waves and thus become visible to these thermal waves. Thermal waves, however, are critically damped and travel only about one thermal wavelength, thereby limiting the imaging range.

In the example shown in Figure 6, the focused electron beam is modulated at 1 MHz, thereby producing 1-MHz thermal waves; the wavelength of these thermal waves, in the sample of Figure 6, is approximately 5 \(\mu\)m. If the subsurface flaw in this example is within 5 \(\mu\)m of the surface, thermal waves can detect it. Because the thermal waves are so highly damped, they themselves are difficult to detect. However, a fraction of the thermal wave energy is always transmitted to an acoustic wave at the same frequency, because of the local stress/strains set up by the thermal waves. Thus 1-MHz thermal waves will always generate 1-MHz acoustic waves. These acoustic waves are propagating waves, with much longer wavelengths (typically about 5 mm at 1 MHz); they travel through condensed media with ease and are readily detected with a suitable acoustic transducer placed in acoustic contact with the sample. The magnitude and phase of the acoustic waves are directly related to the interactions undergone by the thermal waves, and are measured with suitable
phase-sensitive frequency-locked electronics and recorded as a function of beam position.

Although acoustic waves are detected, thermal-wave microscopy is not acoustic microscopy. Acoustic waves with frequencies less than 100 MHz have wavelengths much too large for imaging microscopic features. In thermal-wave microscopy, the acoustic waves act solely as carriers or amplifiers of the thermal-wave imaging information, and both the imaging resolution and image range are set by the thermal waves.

The resolution obtainable in thermal-wave microscopy is determined by both the spot size of the laser or electron beam and by the thermal wavelength. The thermal wavelength, \( \lambda_{th} \), in turn, is a function of the thermal parameters of the sample, primarily the thermal conductivity, and of the frequency of which the intensity of the laser or electron beam is modulated. Thus,

\[
\lambda_{th} = \left( \frac{4\pi \kappa}{\rho C f} \right)^{1/2}
\]

where \( \kappa \) is the thermal conductivity, \( \rho \) the density and \( C \) the specific heat of the sample, and \( f \) the modulation frequency. The thermal resolution is given by \( \lambda_{th}/2 \pi \). In biological tissue the thermal resolution at 100 Hz is 20–30 \( \mu m \), at 10 kHz it is 2–3 \( \mu m \), and at 1 MHz it is 200–300 nm. The imaging or penetration range of the thermal waves is determined by the thermal wavelength and signal-to-noise criteria. With present detection systems, imaging ranges of approximately fourfold the resolution dimensions are possible. Thus at 100 Hz in biological tissue, total imaging or penetration range is approximately 100 \( \mu m \), which decreases to 1 \( \mu m \) when the modulation frequency reaches 1 MHz.

Thermal-wave imaging is thus high-resolution but near-surface imaging. Its clinical potential therefore is mainly in the research and diagnosis of cells, cell membranes, and other microscopic organisms. The principal advantage of thermal-wave imaging in biology and medicine lies in its ability to see otherwise invisible thermal features. These features are usually invisible to conventional optical imaging without special staining procedures, but should be clearly distinguishable in a thermal-wave microscope as long as they exhibit some difference in any of the thermal parameters. Of the three thermal parameters that enter into the equation above, the most important is the thermal conductivity: neither the density nor the specific heat will vary much in biological matter. However, large variations in local thermal conductivity may occur, e.g., in hydrophobic vs hydrophilic regions, thereby making these regions visible in a thermal-wave microscope.

To date, thermal-wave imaging has been confined to inorganic matter in the detection of subsurface defects and of "invisible" variations in crystallinity, composition, and material homogeneity (7, 8). These experiments have demonstrated the sensitivity of thermal-wave imaging to small changes in local thermal characteristics, and thus point the way for similar sensitivity in biological matter.

References