

Thermal Images of Seeds Obtained at Different Depths by Photoacoustic Microscopy (PAM)

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Abstract The objective of the present study was to obtain thermal images of a broccoli seed (*Brassica oleracea*) by photoacoustic microscopy, at different modulation frequencies of the incident light beam ((0.5, 1, 5, and 20) Hz). The thermal images obtained in the amplitude of the photoacoustic signal vary with each applied frequency. In the lowest light frequency modulation, there is greater thermal wave penetration in the sample. Likewise, the photoacoustic signal is modified according to the structural characteristics of the sample and the modulation frequency of the incident light. Different structural components could be seen by photothermal techniques, as shown in the present study.

Keywords Modulation frequency · Photoacoustic microscopy · Thermal images

1 Introduction

Alternative techniques to characterize materials with diverse structures are very important nowadays. These techniques are valuable to study biological materials such as tissue; also, it is important to not produce damage to the specimens. Among the alternative techniques, the photothermal (PT) techniques that have different applications in a wide range of disciplines [1–4] stand out. Among the PT techniques, photoacoustic spectroscopy (PAS) and the photoacoustic and photopyroelectric microscopies (PAM

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and PPEM, respectively) stand out for their applications in a wide range of systems [5–9]. Some theories have been developed for a one-dimensional model depth profile of the thermal waves in a sample and also for the thermoelastic response beneath the surface; moreover, the mathematical formalism describing the thermal wave propagation in solids, using the photoacoustic (PA) technique, has been reported. Some applications of PAS have been carried out to study biological materials of interest in agriculture [10]. The study of inhomogeneous materials such as biological samples requires more care, since the material consists of several structures, such as the case of agricultural seeds, whose characterization involves sometimes fractional destruction or decomposition. Always, it is necessary to look for techniques that prevent the destruction of and invasion into the material to explore.

PT techniques have been applied to study optical and thermal phenomena in materials, such as optical absorption and transmission and reflection of light; these phenomena induce thermal effects on the surface and also inside materials. By using the PA technique, it is possible to obtain the optical and thermal properties of a wide range of materials. The PA effect occurs when a sample, confined in a cell with gas, is illuminated with modulated light causing pressure fluctuations inside the cell [11]. A mathematical model to describe the PA effect in solids was developed by Rosencwaig and Gersho (RG) [12]. The RG model has been used to obtain the optical absorption coefficient of several materials including biological samples [13]. On the other hand, the PAS technique, based on the PA effect, has been applied to obtain the characteristic absorption peaks of several natural pigments such as flavonoids, carotenoids, anthocyanins, and chlorophyll among others. Also, the absorbed modulated light in the sample can produce a thermoelastic effect in the studied materials and the PA effect allows exploration of the interior of the sample based on this effect [14].

The study of thermal wave propagation, by using a three-dimensional model, in a sample for the response beneath the surface was developed by Mandelis [15] who developed a formalism, on the basis of the Hamilton–Jacobi theory, to provide analytical expressions for the distribution of temperature and heat flux in materials with inhomogeneous thermal properties. Also, in 1985, Iravani et al. proposed a scattering matrix approach to thermal wave propagation in layered structures [16]. The theoretical description of thermal waves in inhomogeneous materials gives support to the photoacoustic microscopy (PAM) technique, which is based on the photoacoustic phenomenon. By using the PAM technique, it is possible to obtain thermal imaging of a sample by scanning a focused modulated light beam on the sample, which is placed in a PA cell. Thermal characteristics above and below the surface of a sample can be detected as a microscope image by using thermal waves [17, 18]. The low-frequency excitation can be used to allow more depth range which is relevant for different applications [19]. The PAM technique has included a correlation between the photoacoustic signal and the surface microstructure making it possible to detect inhomogeneities that are not visually detected under a microscope [20]. By using PAM, it is possible to evaluate inhomogeneous materials [21].

The PAM technique, applied to obtain thermal images of seeds, was used for the first time, to our knowledge, by Suzuki et al. [22] who used an argon laser as a light source to evaluate rice quality, where a grayscale image of a rice seed was obtained to analyze its entire structure. Xu and Wang [4] developed a biomedical

application of PAM and radiometric IR techniques on biological samples to obtain very high resolution results in superficial images, taking into account the theory and modeling performed on the penetration and propagation of the thermal wave from the work of Rosencwaig and Gersho [12] and Mandelis [15]. Rosencwaig [23], in their work with thermal wave microscopy, relates PA absorption processes of energy generation and propagation of thermal and elastic waves; also, Busse and Rosencwaig [24] obtained sub-surface images with PAM. Rosencwaig [18] proposed that the PAM technique can be imaged at different depths by varying the modulation frequency.

In the present study, PAM was applied to obtain inner thermal images of a seed without destroying it. In particular, several thermal images of a broccoli seed (*Brassica oleracea*) were obtained by photoacoustic microscopy at different modulation frequencies to look inside the broccoli seed. The inside of the broccoli seed can reveal some problems, such as the presence of mycobiota and bad storage, which could be related to low seed germination and poor sanitary quality.

2 Materials and Methods

2.1 Biological Material

Broccoli seeds (*B. oleracea*) of Waltham 29 type, from the Mexico central zone, were studied. The seeds were randomly selected to obtain thermal images. The measurements were carried out one by one placing the sample in the PAM experimental setup.

2.2 PAM Experimental Setup

The PAM experimental setup is shown in Fig. 1a. In this setup, the photoacoustic cell and its sensor, an electret microphone, are mounted on an x - y motorized stage, with a spatial resolution of $80\ \mu\text{m}$. The excitation source is a fiber coupled laser diode, at $650\ \text{nm}$ wavelength, modulated in intensity at $f = (0.5, 1, 5, \text{ and } 20)\ \text{Hz}$ frequency by the reference oscillator of a lock-in amplifier. By using a microscope objective, the laser beam was focused on the seed surface. The PA signal was pre-amplified and sent to the lock-in amplifier. A personal computer was used to control the x - y stage, in order to scan the focused laser beam on the sample surface and also to record, from the lock-in amplifier, the experimental PA signal amplitude and phase. A broccoli seed was placed inside the PA cell, and the PA signal was recorded as a function of its position in order to obtain a PA image of each seed. This procedure was repeated for each light modulation frequency.

3 Results and Discussion

Figure 1b shows the optical images of the photoacoustic cell (Fig. 1b.1), the entire broccoli seed (Fig. 1b.2), and the broccoli seed cross section (Fig. 1b.3). The scanned area for each image, obtained by PAM, was $3\ \text{mm} \times 3\ \text{mm}$. Each thermal image of the

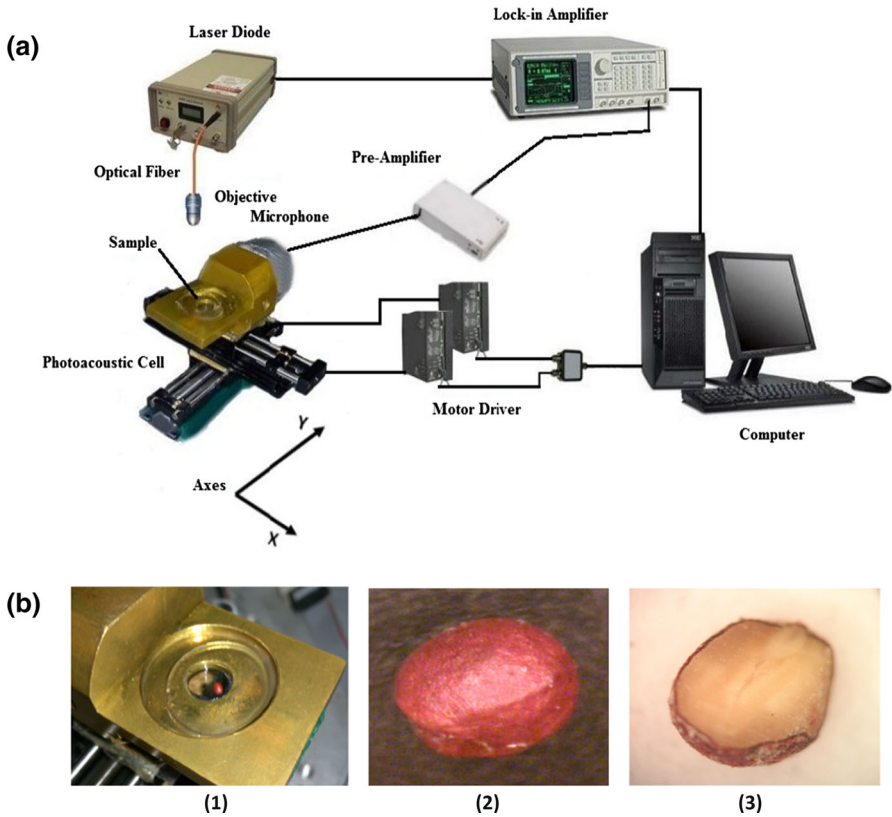


Fig. 1 (a) PAM experimental setup, (b1) optical images of the photoacoustic cell, (b2) broccoli seed, and (b3) broccoli seed cross section

broccoli seeds was obtained at a different modulation frequency of the incident light, in order to have in each image a different thermal diffusion length ($\mu_s = [\alpha/\pi f]^{1/2}$, where α is the sample thermal diffusivity) of the thermal wave propagating through the seed.

Figure 2 shows three columns, the first and second (1 and 2) columns refer to the thermal images on a gray scale and in color obtained by PAM, respectively, and the third (3) column shows the PA contour image. The thermal images (first and second columns) and the PA contour image (third column) have advantages when compared with an optical image (Fig. 1b.2). The PA images in the third column show, on a scale of colors, the differences in the PA signal due to different components in the seed. The PA contour image has advantages when compared with the optical images (Fig. 1b.2, b.3), because the PA contour image provides clear differences in thermal properties in different regions of the sample. It was possible to observe, when comparing the thermal images of the broccoli seed (cases a, b, c, and d) that the PAM technique allowed more definition of structures in the thermal contour image. On the other hand, it is possible to observe that when the modulation frequency decreases, the thermal diffusion length (μ_s) of the thermal wave increases, so that it is possible to observe thermal imaging of

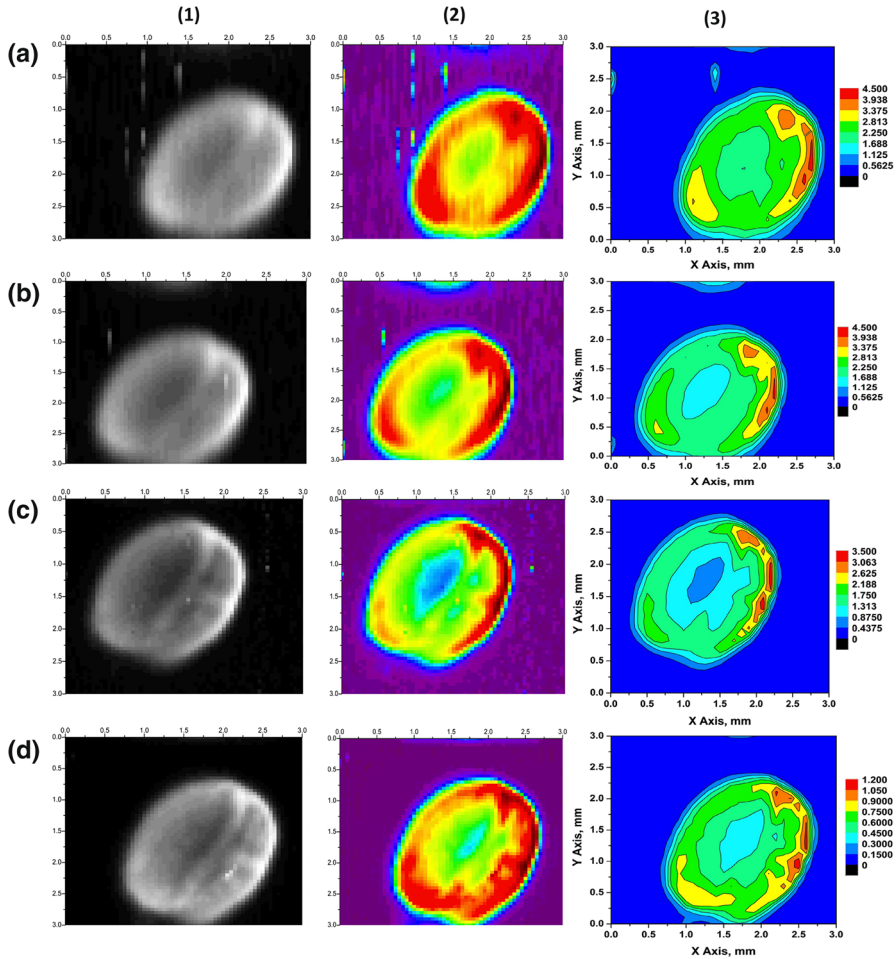


Fig. 2 Columns (1) grayscale, (2) thermal, and (3) contour seed images; rows show the obtained images at light modulation frequencies of (a) 0.5 Hz, (b) 1 Hz, (c) 5 Hz, and (d) 20 Hz

several layers inside the seed, where the thermal image at $f = 0.5$ Hz is mainly sensed at a depth of μ_s (figures on row 2a). By considering $\alpha = 4.44 \times 10^{-3} \text{ cm}^2 \cdot \text{s}^{-1}$ [25] (α for starch, which is the main component in broccoli seeds), the value of the thermal diffusion length for this frequency is $\mu_s = 531 \mu\text{m}$. The obtained image inside the seed is comparable with the optical image of the seed cross section (Fig. 1b.3), i.e., in the PAM images, it is possible to observe areas with different structures, and it is also possible to see the increase of these areas by varying the modulation frequency of the incident light.

At $f = 20$ Hz light modulation frequency, as shown in Fig. 2d, the value of the thermal diffusion length, by considering once again the α value for starch, is $\mu_s = 84 \mu\text{m}$; then the thermal wave is sensing close to the sample surface. At lower frequencies more layers of the seed will be sensed, and areas of the structures appear in Fig. 2a–c with a

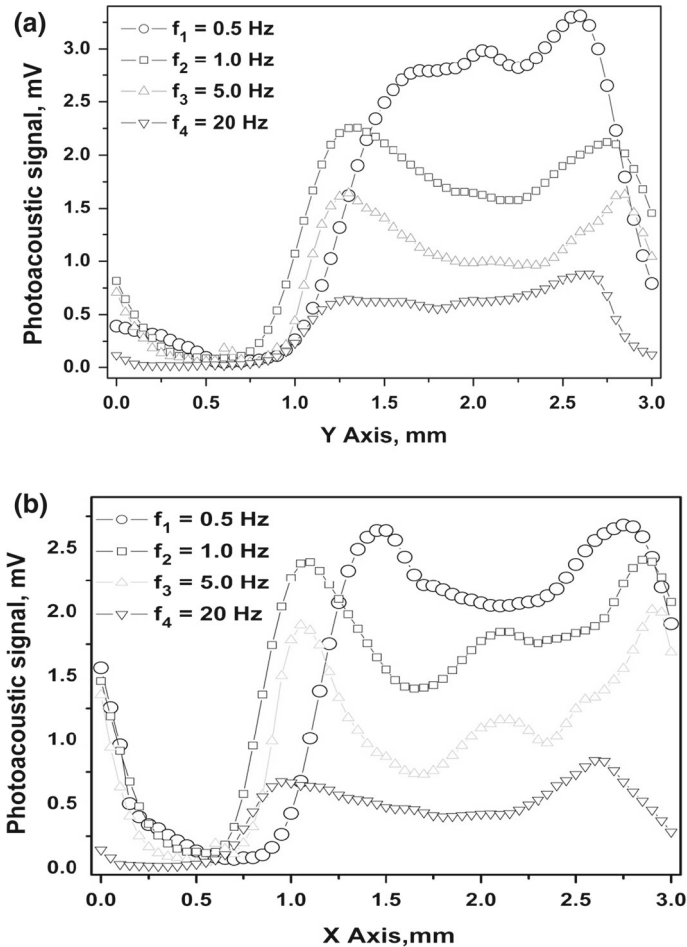


Fig. 3 (a) PA signal with x at a constant position ($x = 1$) and y ranging from 0 mm to 3 mm and (b) PA signal with y at a constant position ($y = 1.5$) and x ranging from 0 mm to 3 mm (in the same region of the sample)

light modulation frequency of (0.5, 1, and 5) Hz, respectively. A line on the horizontal ($y = \text{constant}$) and vertical ($x = \text{constant}$) axes is taken in the scanning of the sample, at different frequencies, i.e., a scan ranging from $x = 1.0, y = 0$ to $x = 1.0, y = 3.0$ (Fig. 3a) and another scan ranging from $x = 0, y = 1.5$ to $x = 3.0, y = 1.5$ (Fig. 3b). It is possible to observe that at a higher frequency, the PA signal decreases, and conversely at a lower frequency, the PA signal increases, but also the PA signals show variations, as a function of their position, which indicates the presence of structures (with different thermal properties) inside the seed at different depths, depending on the light modulation frequency of the incident beam. The observed layers correspond to different materials which have different values in their thermal properties such as the thermal diffusivity, whose α_s value for pericarp is $\alpha_s = 2.4 \times 10^{-4} \text{ cm}^2 \cdot \text{s}^{-1}$ [26]

and for starch is $\alpha_s = 4.44 \times 10^{-3} \text{ cm}^2 \cdot \text{s}^{-1}$ [25], with perceptible differences between the layers making up the contour image of the seeds.

4 Conclusions

Photothermal images of a broccoli seed (*B. oleracea*) were obtained by using the PAM technique. It is possible to observe differences in the sample images by scanning the modulated laser beam on the sample, at different modulation frequencies. The studied broccoli seed showed that the thermal images obtained by PAM, at different light modulation frequencies, allow exploring different depth levels of the seeds, i.e., the obtained images from complex materials, constituted by different internal structures, can be characterized by the PAM technique to obtain surface and below surface information from different depth layers.

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