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Thermal Image of Coffee-Seed Germ Obtained by Photoacoustic Microscopy

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Abstract Photoacoustic microscopy (PAM) has been shown to be a suitable technique to obtain thermal images of a wide variety of samples from semiconductors to biological material. In PAM, the incidence of a modulated laser beam on a sample within a photoacoustic (PA) cell, hermetically sealed, produces a PA signal which depends on the thermal and optical properties of the studied sample. By making a sweep of the modulated laser beam on the sample surface, it is possible to obtain the PA signal as a function of their $x-y$ coordinates, and from this signal, it is possible to reconstruct thermal images of the sample. In this study, thermal images of a coffee-seed germ were obtained, with a difference of 12 h between them, by using the PAM technique. Thermal differences observed between images give information which reflects degradation due to the fact that germ cells undergo changes as a function of time. The thermal images obtained by the PAM technique could be applied to biological materials that have a complex constitution (not homogeneous) in their structures, and thermal differences can be observed. PAM is a non-destructive technique, which is an important feature for this type of study. Other applications of this technique can be performed in the agricultural and biotechnological areas.

Keywords Coffee-seed germ · Photoacoustic microscopy · Thermal image

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1 Introduction

Characterization of materials with complex structures, such as living tissues, is important in different research areas due to their relation with other structures interacting between them, which could cause different responses to stimulation of mechanisms that produce changes into them. For example, the response of a living cell to environmental changes, which could be potentially harmful, such as an increase of temperature, pH, salt concentration, or the presence of toxins [1]. The produced changes in living tissues can be detected by several techniques and modeled by using math algorithms in order to study their response [2]. On the other hand, photothermal (PT) techniques have been used to obtain optical and thermal parameters of a wide variety of materials. PT techniques are nondestructive and non-invasive, which are important features especially for the study of living tissues. Among the PT techniques stand out photoacoustic spectroscopy (PAS), the former of the PT techniques. A theoretical model to explain the photoacoustic (PA) effect in solids was proposed for the first time by Rosencwaig and Gersho [3]. The PAS technique has already been applied to obtain the thermal and optical parameters of a wide variety of materials. Thermal parameters such as the thermal effusivity [4], specific heat, thermal diffusivity, and thermal conductivity, among others, have been obtained by the PAS technique in different configurations [5–9]. Thermal imaging by photoacoustic microscopy (PAM) has also been reported for different samples including biological specimens such as maize and wheat seeds [10].

2 Materials and Methods

2.1 Biological Material

In the present research, the germ of a coffee-seed (*Coffea arabica* L.) produced in Chiapas, Mexico, was studied. The coffee bean was 12 h in imbibition before its dissection, performed along the seed by a scalpel, in order to remove the germ entirely (see Fig. 1a).

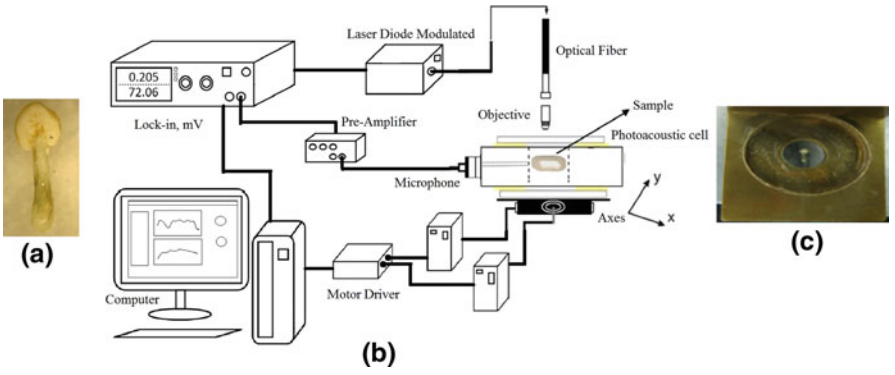

Fig. 1 (a) Recently extracted embryo, (b) PAM experimental setup, and (c) embryo inside the PA cell

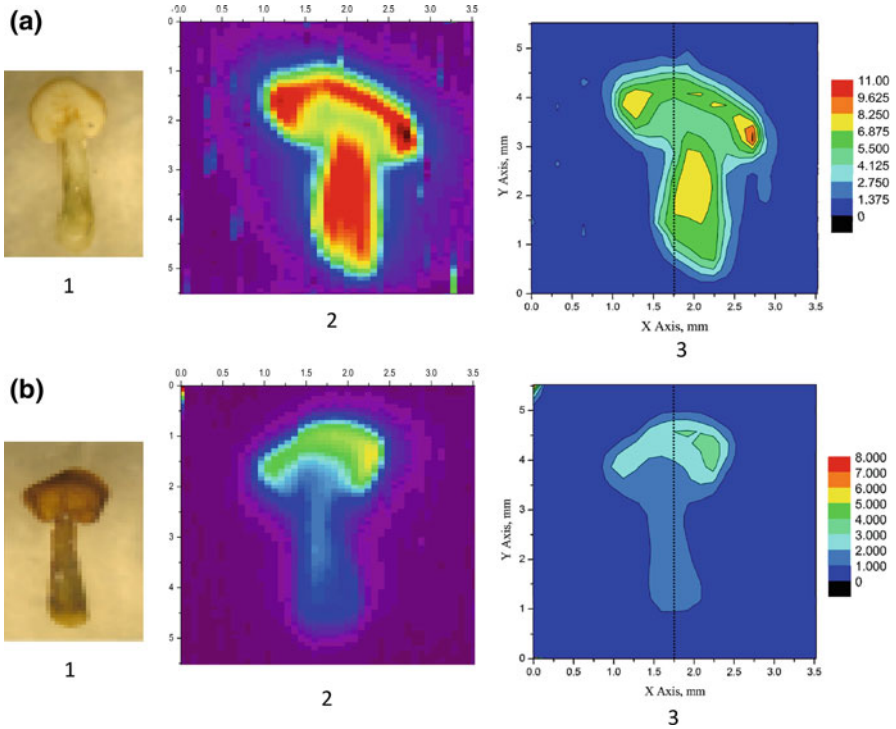

Fig. 2 (a1) Optical image, (a2) PAM image, and (a3) PA contour image of recently extracted coffee-seed embryo; (b1) optical image, (b2) PAM image, and (b3) PA contour image of embryo 12 h after its extraction

2.2 PAM Experimental Setup

The experimental setup used for the PAM technique is shown in Fig. 1b. In this setup, the PA cell and its sensor, an electret microphone, are placed on an $x-y$ motorized stage, with spatial resolution of 80 μm. The excitation source is a fiber coupled laser diode (100 mW), at 650 nm wavelength, modulated in intensity at 1 Hz frequency by the internal oscillator of a lock-in amplifier. The modulated laser beam was focused on the sample surface by using an objective of a microscope. Figure 1c shows the recently extracted embryo placed inside the PA cell. The PA signal was pre-amplified and sent to a lock-in amplifier. A personal computer was used to control the scanning of the $x-y$ stage and also to record, from the lock-in amplifier, the amplitude and phase of the experimental PA signal as a function of its position in order to obtain a PA image from this sample. The same procedure was carried out in order to obtain the PA image of the same embryo 12 h after its extraction.

3 Results and Discussion

Figure 2 shows PA images of the coffee-seed embryo, from two different conditions, i.e., the embryo recently extracted from a coffee bean with 12 h of imbibition, and the

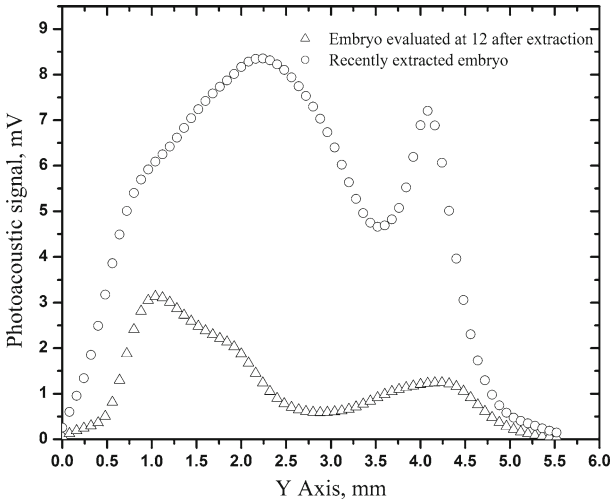

Fig. 3 PA signal amplitude as a function of the *Y* coordinate (along the *dashed line* shown in Fig. 2a3, b3) for recently extracted embryo and embryo evaluated at 12 h after extraction

same embryo after 12 h of its extraction. The scanned areas were 3.5 mm × 5.5 mm for both images of the same embryo. Figure 2 shows three columns: the first one refers to the optical image of the coffee-seed embryo (see Fig. 2a1, b1); the second one shows the PA image (see Fig. 2a2, b2); and the third one shows the PA's contour image (see Fig. 2a3, b3). The PA images, in the third column, show in a scale of colors the differences in the PA signal due to different components in the embryo. The PA contour image has advantages when compared with the optical images (see first column). That is, the PA contour image provides a clear difference in optical and thermal properties in different regions of the sample when comparing both conditions of the coffee-seed embryo. In the case of the recently extracted embryo, a more intense image was obtained when compared with the PAM image of the seed embryo after 12 h of extraction; the differences in these images would be due to different optical and thermal properties in the embryo at two different times of extraction. These differences would be due to the variation in the moisture content, which modify the thermal properties of the embryo, and also differences in the optical absorption coefficient. Some authors have reported thermal images, by PAM, of dry and wet radish and tomato seeds, in which the thermal images show different regions due to water absorption in the seeds which causes local differences in moisture content, changing their local thermal properties [11].

Figure 3 shows the PA signal amplitude as a function of the *Y* coordinate (along the dashed line shown in Fig. 2a3, b3) for (a) recently extracted embryo and (b) an embryo evaluated at 12 h after extraction. From Fig. 3, it is possible to see a profile of the embryo, along the *Y* axis, where there is evidence that the process of dehydration of the embryo also induces embryo shrinkage. In fact, water loss results in cell shrinkage and an increase of empty intercellular spaces causing a decrease in the embryo thickness along the *Y* axis.

4 Conclusions

By using PAM, thermal images of a coffee-seed embryo were obtained at different times after its extraction. Also, it can be observed with this study the differences in thermal and optical properties of the embryo due to the differences in moisture content and optical absorption coefficient. It is also possible to observe from the profile of the embryo, by the PA signal along one axis, that the process of dehydration of the embryo also induces embryo shrinkage.

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