How Can Physicists Study Photosynthesis ?
History and Applications of the Photoacoustic Technique

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Abstract

This short review presents the photoacoustic technique applied to the study of photosynthesis. It is known that plants kept in the dark for several hours stop photosynthetic activity. After this dark-adaptation, light incidence gives rise to the so-called *photosynthetic induction* (restart of the photosynthetic processes). The Open Photoacoustic Cell described here has been used at the ***Laboratory of Photothermal Spectroscopy*** (IFGW, UNICAMP) to investigate the kinetics of the photosynthetic induction. Recent work developed at this laboratory includes *in vivo* and *in situ* measurements in eucalyptus leaves from different species and subjected to different conditions. With the photoacoustic technique, we can analyze the parameters that influence photosynthesis, such as temperature and intensity of the incident radiation.

1 History

Graham Bell discovered the photoacoustic effect in 1880, when he noticed that the incidence of modulated light on a diaphragm connected to a tube produced sound [1]. Thereafter, Bell studied the photoacoustic effect in liquids and gases, showing that the intensity of the acoustic signal observed depended on the absorption of light by the material.

In the nineteenth century, it was known that the heating of a gas in a closed chamber produced pressure and volume changes in this gas. However, there were many different theories to explain the photoacoustic effect. Rayleigh said that the effect was due to the movement of the solid diaphragm. Bell believed that the incidence of light on a porous sample expanded its particles, producing a cycle of air expulsion and reabsorption in the sample pores. Both were contested by Preece, who pointed the expansion/contraction of the gas layer inside the photoacoustic cell as cause of the phenomenon. Mercadier explained the effect conceiving what we call today *thermal diffusion mechanism*: the periodic heating of the sample is transferred to the surrounding gas layer, generating pressure oscillations.

The lack of a suitable detector for the photoacoustic signal made the interest in this area decline until the invention of the microphone. Even then, research in this field was restricted to applications in gas analysis up to 1973, when Rosencwaig started to use the photoacoustic technique in spectroscopic studies of solids and, together with Gersho, developed a theory for the photoacoustic effect in solids – the Rosencwaig-Gersho Model [2].

In the last two decades, the utilization of the photoacoustic technique in photosynthesis studies has been successful. Modulated light absorption in plant leaves accounts for the release of heat and oxygen, generating a photoacoustic signal presented on both amplitude (magnitude) and phase (delay with respect to the light absorption). As scientists recognized the potentiality of this technique in the photosynthesis field, several lines of research were open. Therefore, Malkin and Cahen [3] worked on photoacoustic spectroscopy and, together with Poulet [4], determined the O_2 diffusion coefficient in plants.

In the eighties, researchers began to use the modulated photoacoustic technique to detect the photosynthetic activity stimulated by controlled light incidence. First measurements were performed on suspensions of chloroplasts [5] or with the leaf being cut and enclosed in the cell [6]. Both alternatives represented important steps in the use of photoacoustics to study photosynthesis [7]. However, such experiments had some disadvantages. In suspensions of chloroplasts, measurements were performed under conditions far away from those of living samples; on the other side, a leaf detached from the plant has its water supply interrupted, suffering dehydration.

Things were about to change in 1987, with the development of the Open Photoacoustic Cell (OPC). The OPC was a simple and cheap device that used a commercial electret microphone as the detector. Its open chamber was closed by the sample itself, so the new tool allowed measurements in whole leaves still attached to the plant. Perondi and Miranda described the OPC as "an inexpensive detector sensitive to any radiation, ranging from microwave to x-ray" [8, 9]. After that, Nery *et al* [10] used this cell to perform spectral analysis of plant leaves. In the following years, the OPC was introduced in the photosynthesis field by researchers at the ***Laboratory of Photothermal Spectroscopy*** (IFGW, UNICAMP) [11]. Since then, several studies on photosynthesis were carried out with the OPC technique [12, 13]. Today, the use of photoacoustics in the study of photosynthesis is well described in extensive review articles [14, 15, 16].

In the following sections, we present the OPC technique and follow the historic path of its applications in the study of plants, emphasizing projects performed at the ***Laboratory of Photothermal Spectroscopy.***

2 Open Photoacoustic Cell (OPC)

The photoacoustic detection occurs in the following way: the sample absorbs incident modulated light, producing heat and therefore thermal waves. According to the Rosencwaig-Gersho Model [2], such waves generate the pressure oscillation detected as the photoacoustic signal.

Spectroscopy – In 1990, photoacoustic spectroscopy was fully incorporated to the roll of useful photothermal techniques [17]. This technique has some advantages over other spectroscopy techniques: *a)* it can directly measure the absorption (transmitted and reflected light do not interfere in the measurements); *b)* it allows the study of optically opaque and highly scattering samples which could not be analyzed by conventional spectroscopy; and *c)* as the thermal diffusion length depends on the modulation frequency, it is possible to analyze the depth-profile of multi-layered samples using photoacoustics.

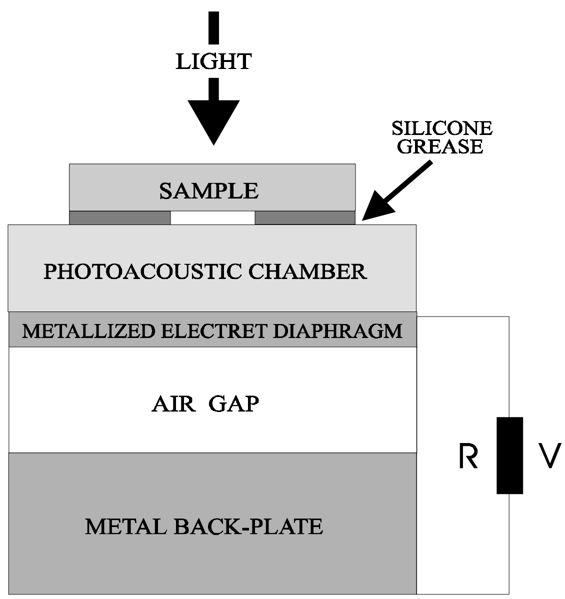

Figure 1: Scheme of the Open Photoacoustic Cell (OPC).

The OPC device and its applications – This compact device is formed by a commercial electret microphone that uses its own chamber as the acoustic cell. In this arrangement (see Fig.1), the sample itself closes the photoacoustic chamber, with the help of vacuum grease around the microphone hole to get the chamber hermetically sealed. Therefore, it is not necessary to detach the leaf from the plant for measurements: the OPC allows *in vivo* and *in situ* monitoring of the photosynthetic activity in plants, avoiding dehydration of the sample.

The microphone used as the photoacoustic detector in the OPC has a sensitivity of 10 mV/Pa. It is composed of a metallized electret diaphragm and a metal plate separated by an air gap and connected by a resistor. Pressure oscillations in the air chamber deflect the membrane, generating a voltage across the resistor. Such voltage is supplied to a FET pre-amplifier in the microphone capsule.

Perondi and Miranda [8] initially employed the OPC to measure the thermal diffusivity of solids. After that, Bento *et al* [18] used it to detect x-rays, while Nery *et al* [10] applied the OPC in spectroscopic studies to monitor herbicide activity in leaves.

The response of plants to toxic substances was studied at the ***Laboratory of Photothermal Spectroscopy*** by Marquezini *et al* [19], who monitored the injurious effects of aluminum on maize through spectroscopic OPC measurements. Still working with maize leaves, Pereira *et al* [20] investigated differences in the spectra of mutants and wild maize types.

Experimental setup – Figure 2 shows the basic setup utilized in OPC experiments. There are two light sources: a Xenon arc lamp and a Tungsten lamp. A chopper and a monochromator are placed in front of the xenon lamp to obtain modulated light of a determined wavelength. The tungsten lamp is used for photosynthesis saturation and has its emission limited (by optical filters) to the visible part of the spectra. A double-branched optical cable guides each light beam up to the OPC. The chopper and the photoacoustic cell microphone are connected to a lock-in amplifier that measures amplitude and phase of the signal.

Theory – Marquezini *et al* [21] present the theory of the OPC detection considering that (for optically transparent samples) there are two signal sources: part of the light is absorbed and part is transmitted, being absorbed by the metallized diaphragm of the microphone. These authors use the Gauss' law to show the equivalence between the electret microphone and a RC circuit in parallel with a current source proportional to the rate of change of the diaphragm deflection. This rate of change is related to the heat flow by the Rosencwaig-Gersho Model, so the OPC voltage can be expressed as a function of the temperature fluctuation obtained through the thermal diffusion equation.

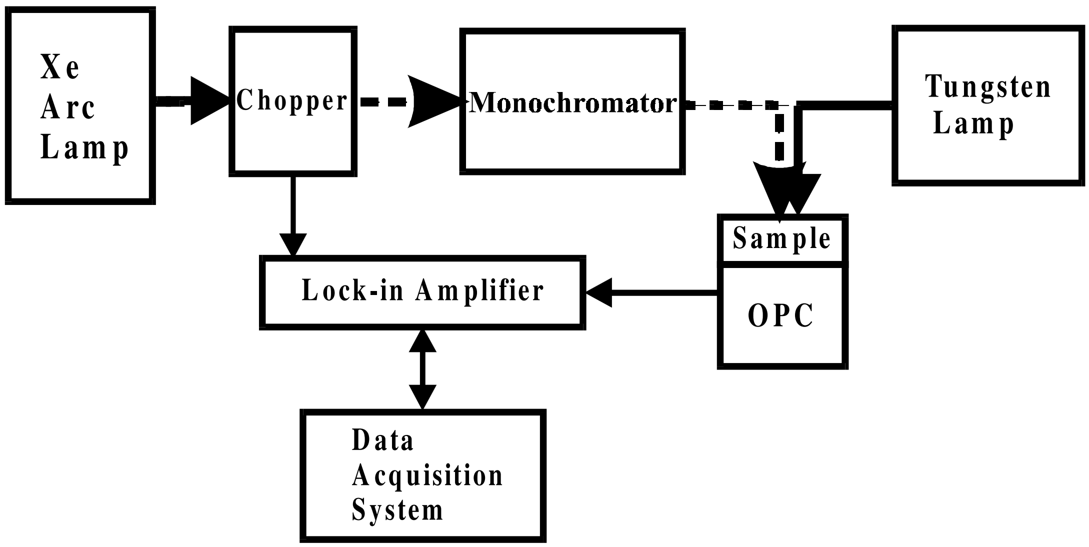

Figure 2: Diagram of the experimental setup for OPC measurements. Thin solid line indicates cable connections; thick solid line, non-modulated light; dashed line, modulated light.

Marquezini *et al* studied leaf samples, optically transparent and thermally thick[1] for the modulation frequencies f utilized. For this kind of sample, the OPC signal has a f^{-1} dependency and is proportional to the transmitted power density, $I_0.e^{-\beta.l_s}$, where β is the optical absorption coefficient and l_s, the thickness of the sample. The optical absorption coefficient of the leaf was found by studying the frequency dependence of the photoacoustic signal.

3 OPC and *in Vivo* Plant Photosynthesis Research

Let us recall here some basic notions about photosynthesis. It is well known that plants assimilate CO_2 and H_2O, producing carbohydrates and O_2. This process is induced by sunlight, absorbed by the chlorophylls along with other pigments. Photosynthesis occurs within the mesophyll cells, at the chloroplasts, which contain chlorophyll. Actually, photosynthesis is a complex process that involves a large electron transfer reaction chain and several enzymes. In modulated photoacoustic measurements, the O_2 produced has to diffuse up to the intercellular spaces in order to generate pressure waves transmitted to the microphone chamber and detected.

Photosynthetic induction – If a plant stays in the dark for a certain time, its photosynthetic capacity eventually stops. After the plant is dark-adapted, light incidence gives rise to the ***photosynthetic induction***, that is the restart of the photosynthetic processes. Photosynthetic activity achieves a steady state after a transient period that depends on parameters such as atmosphere composition, dark adaptation time, nutrients availability and temperature.

Gas component of the photoacoustic signal – When photosynthesis is stimulated by modulated light, the photoacoustic signal presents a contribution related to the gas evolution by the leaf. This contribution is observed for modulation frequencies f in the range of 10^2 Hz or lower, for O_2 evolution is limited in time by electron transfers with rate constant of about 10^{-3} s [6]. The reaction chain linked to CO_2 absorption is limited by a 10^{-1} s rate constant; therefore, even for $f \sim 10$ Hz, modulated CO_2 absorption is heavily damped and does not contribute to the gas evolution signal observed.

Photosynthesis saturation – The gas evolution component is superimposed to the thermal component of the photoacoustic signal and can be eliminated through simultaneous incidence of intense continuous light. Under this saturating light, the conversion efficiency of the modulated light in chemical energy goes to zero and the O_2 produced by the leaf starts to be liberated continuously. The change in the phase angle of the photoacoustic signal when modulated photosynthesis is eliminated confirms that the total signal has both thermal and gas evolution components. Actually, besides eliminating the gas evolution component of the photoacoustic signal, incidence of continuous saturating light also enhances the thermal contribution.

At sufficiently low frequencies, this produces a net ***negative effect*** [6]: the photoacoustic signal decreases when the saturating light is added to the modulated one. As the modulation frequency increases, gas diffusion is more

[1] A sample is called *thermally thick* when it is thicker than the *thermal diffusion length* $\mu_s = [\alpha/\pi f]^{1/2}$, where α is the thermal diffusivity and f, the modulation frequency.

attenuated than thermal diffusion, so the reduction of the gas evolution component is more pronounced than the reduction of the thermal one. In fact, for measurements performed at high modulation frequencies, the gas evolution component may become undetectable or so reduced that addition of a saturating light produces a ***positive effect***: the photoacoustic signal increases due to the enlarged conversion of the modulated light in heat. Figure 3 illustrates a net negative effect, as observed for measurements at low frequencies.

In 1992, Pereira and co-workers showed that the OPC could detect both the negative and the positive effects expected, respectively, at low and high modulation frequencies [11]. They also observed dehydration effects in the photosynthetic activity of a detached leaf, verifying that the possibility of performing measurements with the leaf attached to the plant was an essential feature of the OPC method.

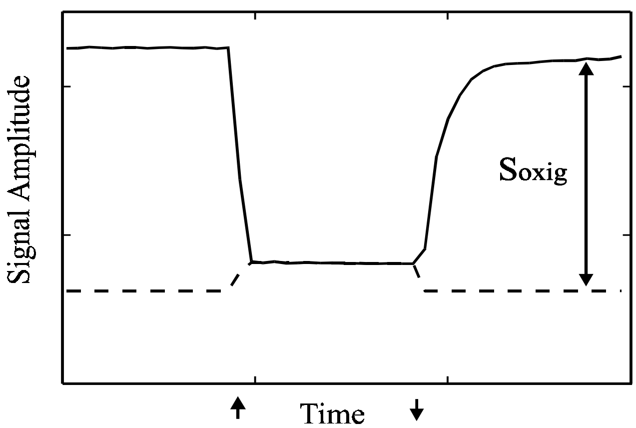

Figure 3: Solid line represents total photoacoustic signal amplitude and shows a negative effect (modulated light: 680 nm, 10 W/m^2, f = 17 Hz). Dashed line represents the thermal component (estimated) of the signal and shows a positive effect. Addition of intense continuous light (200 W/m^2, arrow up: on; arrow down: off) increases the thermal contribution, eliminating the modulated gas evolution. At high frequencies, the net effect becomes positive.

Using the OPC, in 1994 Pereira *et al* [20] showed the correlation between the photosynthetic activity and the chlorophyll content of the maize leaves. Oxygen evolution rates indicated that green leaves have a photosynthetic activity up to three times superior to that of chlorophyll-deficient samples.

Calibration – Photosynthetic activity measurements obtained with the OPC do not reveal absolute values for O_2 evolution. They are useful to compare the behavior of plants from different species or subjected to different conditions. However, it is necessary to calibrate the photoacoustic cell in order to get quantitative results. This was done by Pereira and co-workers [22], using a Clark-type O_2 electrode. To establish the correlation between photoacoustic and Clark electrode measurements, Pereira *et al* applied photosynthetic active radiation in maize leaves and waited for the achievement of a steady state photosynthesis rate. Then they measured O_2 evolution successively with the Clark electrode and the OPC, repeating the procedure for different light intensities in order to find a relationship between the two methods. We observe that, when comparing plant species with very different morphologies (which may affect the gas evolution amplitude), this calibration must be done for each species separately.

Effects of temperature and irradiance on the photosynthetic induction studied by the OPC – Recent research at the ***Laboratory of Photothermal Spectroscopy*** employed the OPC to investigate the photosynthetic activity for several Eucalyptus species.

Eucalyptus is economically important in Brazil, mainly due to paper industry. When plants grown in a greenhouse are transplanted to the field, a low adaptability to sunlight irradiance and non-controlled temperature causes damages to the seedlings. The photosynthetic performance of Eucalyptus seedlings gives evidence about their productivity and adaptability to field conditions. To analyze the influence of the temperature in plant photosynthesis, we studied Eucalyptus samples previously adapted for 24h at 10^0C and 25^0C. Results indicated that Eucalyptus plants adapted at 10^0C presented faster induction reaching lower steady-state photosynthetic activity.

Photoinhibition – Incidence of continuous saturating light (400 < λ < 700 nm) allows the study of photoinhibition (defined here as the decrease in photosynthetic activity due to exposition of the plant to high irradiance) in eucalyptus plants. Figure 4 illustrates the typical procedure for such measurements: after dark adaptation, the sample is exposed to modulated light and undergoes a normal induction period.

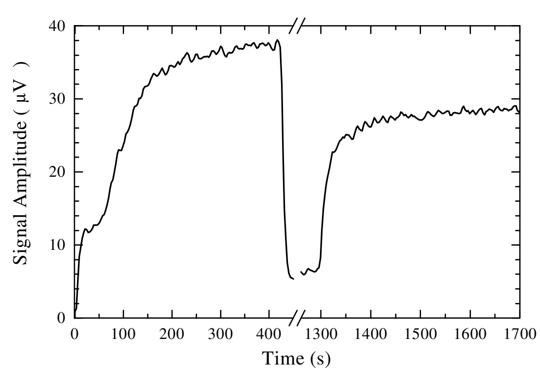

Figure 4: Photoinhibition measurement (*E. grandis* sample). Photosynthetic induction reaches steady state in about 300 s. After that, the leaf is subjected to continuous

saturating light for 15 minutes; next, this light is removed and the recovery of the gas evolution signal is monitored. Signal amplitude reduction after incidence of continuous light characterizes photoinhibition. Modulated light: 17 Hz, 8 W/m^2, 680 nm. Continuous light: 500 W/m^2.

After achieving the steady state, the leaf is subjected to fifteen minutes of non-modulated light with various intensities. Photoinhibition is monitored by the ratio between the photosynthetic activity after and before incidence of saturating light. Data shows that, for all species studied, the photosynthetic activity is substantially lowered for light intensities above 350 W/m^2, which corresponds to full sunlight incidence in open field.

4 Can We Distinguish Different Plant Species Through Photoacoustic Measurements ?

Maize hybrids – In 1994, Silva *et al* were able to differentiate maize inbreds and their hybrids by measuring their photosynthetic O_2 evolution with the OPC [12]. This was an important achievement, for until then researchers had been trying to characterize the performance of maize plants in field experiments before selecting inbreds for hybrid production. Photosynthetic activity was considered an appropriate parameter to evaluate the performance of inbreds and hybrids, and the OPC proved to be a suitable technique to study it.

The OPC signal time evolution of previously dark-adapted maize leaves describes an S-shaped curve that can be adjusted by the logistic function

$$S(t) = S_0 + \Delta S \frac{\exp[(t - t_0)/\Delta t]}{[1 + \exp[(t - t_0)/\Delta t]]} \qquad (1)$$

where S_0 is the initial value of the photoacoustic signal, ΔS is the signal excursion to the saturation value which takes place during the interval Δt and t_0 is the time at which $(S - S_0)/\Delta S = 0.5$. Data obtained by Silva *et al* indicated that hybrids present a higher O_2 evolution than inbreds. Furthermore, both t_0 and Δt had a tendency to be shorter in hybrids than in inbreds. This implies that hybrids reach a higher O_2 evolution faster than inbreds, thus expressing some genotypic advantage.

Eucalyptus – The research with maize inbreds and hybrids suggested that the OPC technique could establish distinctions between plants. Subsequent work at the ***Laboratory of Photothermal Spectroscopy*** explored this potentiality. Comparing the induction parameters (defined in Eq.1) of eucalyptus, maize and coffee leaves reveals important differences among them. In particular, Δt and t_0 can be very short for eucalyptus in comparison with plants like maize and coffee. Besides, ΔS is higher for eucalyptus plants than for the other samples studied. Calibration with the Clark-electrode, made independently for eucalyptus and maize leaves, indicated that the same gas evolution signal amplitude corresponded to a higher activity of the eucalyptus leaves. Relating this to the values of ΔS obtained for these plants, one can indeed observe a higher photosynthetic activity for eucalyptus plants.

A similar investigation compared three Eucalyptus species (*E. urophylla*, *E. grandis* and *E. urograndis*). Photoacoustic measurements revealed that these species could be distinguished in terms of their ΔS and S_0 values; furthermore, they could also be discriminated by the dependence of their gas evolution and thermal signal components with light intensity. The so-called photosynthesis saturation curves can be constructed by measuring the gas evolution as a function of the light intensity. Data shows that *E. urograndis* leaves present higher photosynthetic activity than *E. urophylla* and *E. grandis* leaves.

Seedlings x Cuttings – Recently, we used the OPC to investigate distinctions between the photosynthetic behavior of eucalyptus seedlings and cuttings (the so-called *clones*). Experimental results were interpreted considering that plants with larger photosynthetic activity are usually more vigorous and resistant when transplanted to the field. Comparison between seedlings and cuttings comprised the competition between O_2 absorption and evolution during the photosynthetic induction, the time needed for achievement of a steady state and the amplitude of the steady state gas exchange component. This study is relevant for clones are gradually replacing seedlings in reforesting practice. This research has also been performed at the ***Laboratory of Photothermal Spectroscopy***. Our results indicate a superior steady state photosynthetic activity for the clones; this can explain the better performance of clones in open field. In a similar study at the same laboratory, Silva *et al* [12] had already shown that maize hybrids were more photosynthetically active than maize inbreds.

Photosynthetic energy storage – We already know that, for optically resolved measurements (optical absorption length greater than the thermal diffusion length) the OPC can be used in spectroscopic studies. However, a normalization is required to compensate the photoacoustic signal due to light absorption by the electret membrane. Actually, in many applications (photosynthetic induction, for example) the exact value of the photoacoustic signal is not important. Besides, at low modulation frequencies the contribution of the microphone membrane to the total signal is around 1%. Nevertheless, the membrane component must be taken into account in the determination of the energy storage of plants. The energy storage (ES), measured at high modulation frequencies, is the fraction of absorbed energy stored for the photochemical reactions of photosynthesis. Barja and Mansanares [13] presented a simple methodology to solve this problem. Analyzing the influence of the membrane signal in the determination of ES in plant leaves, they showed that the energy storage calculus would be more affected by a residual O_2 signal than by a residual membrane signal. Corrected measurements in different plants achieved ES values according to those reported in literature.

Photosynthetic activity and root stress – Another research developed with the OPC at the ***Laboratory of Photothermal Spectroscopy*** was the investigation of the photosynthetic induction in eucalyptus plants as a function of their age. Plants were maintained in small pots (50 ml) up to 120 days after seedling. Measurements performed in previously dark-adapted leaves show that the gas exchange component of the photoacoustic signal can be correlated to the occurrence of a root stress: the low volume of the pot imposes a mechanic restriction to the growing of the plant roots.

5 Conclusions and Perspectives

In this article, we presented the Open Photoacoustic Cell as a convenient tool to perform *in vivo* and *in situ* plant measurements. This is important because suspensions of chloroplasts cannot reproduce the behavior of plant leaves and the response of detached leaves can be distorted by dehydration. If, years ago, the OPC was employed to monitor spectroscopic responses to herbicide application and soil toxicity, today we can use it to study photosynthetic induction and saturation, gas exchange, energy storage, photoinhibition and the responses to changes in parameters like temperature, atmospheric conditions and nutrient availability.

The OPC has shown to be a suitable technique in comparative analysis of the photosynthetic activity parameters of different samples. Evaluation of the photosynthetic performance of seedlings and cuttings of eucalyptus opens many perspectives of study, such as the analysis of leaves after transference to open field, the investigation of whether the high photosynthetic rate of the cutting compensates its slow induction and so on. Measurements already performed also provide evidence of a gas absorption component in the photoacoustic signal, which is a subject of particular interest for our research group.

In parallel to the investigation on the gas absorption, other important perspectives of work in the photosynthesis field using the OPC are related to the possibility of performing simultaneous measurements of the photosynthetic activity and chlorophyll fluorescence *in vivo* and *in situ*. Preliminary tests have been performed using the experimental setup described, with the bifurcated optical cable being replaced by a trifurcated one and a fluorescence detector system installed at the exit of the third branch. In fact, many researchers have already established correlation between chlorophyll fluorescence and the photoacoustic signal [23, 24, 25]. According to Malkin and Puchenkov [16], in the study of plant responses to environmental stresses, which requires non-invasive methods, "particular advantage would be to a combined photoacoustic and fluorimetric cell setup".

Now we can answer the question that opens this article: physicists can study photosynthesis using the photoacoustic technique. And, if our current understanding of the photosynthetic processes is far from absolute, it is also true that the association between photoacoustics and photosynthesis still can be very fruitful for a long time.

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7 References

[1] Bell, A. G., 1880. Am. J. Sci. 20: 305.
[2] Rosencwaig, A., and Gersho, A. 1976. Theory of the photoacoustic effect with solids. J. Appl. Phys. 47: 64-69.
[3] Malkin, S., and Cahen, D. 1979. Photoacoustic spectroscopy and radiant energy conversion: theory of the effect with special emphasis on photosynthesis. Photochemistry and Photobiology 29: 803-813.
[4] Poulet, P., Cahen, D., and Malkin, S. 1983. Photoacoustic detection of photosynthetic oxygen evolution from leaves: quantitative analysis by phase and amplitude measurements. Biochimica et Biophysica Acta 724: 433-446.
[5] Lasser-Ross, N., Malkin, S., and Cahen, D. 1980. Photoacoustic detection of photosynthetic activities in isolated broken chloroplasts. Biochimica et Biophysica Acta 593: 330-341.
[6] Bults, G., Horwitz, B. A., Malkin, S., and Cahen, D. 1982. Photoacoustic measurements of photosynthetic activities in whole leaves: photochemistry and gas exchange. Biochimica et Biophysica Acta 679: 452-465.
[7] Carrier, P., Chagvardieff P. and Tapie P. 1989. Comparison of the Oxygen Exchange between Photosynthetic Cell Suspensions and Detached Leaves of *Euphorbia characias* L. Plant Physiology 91:1075-1079.
[8] Perondi, L. F., and Miranda, L. C. M. 1987. Minimal-volume photoacoustic cell measurement of thermal diffusivity: effect of the thermoelastic sample bending. J. Appl. Phys. 62: 2955-2959.
[9] Silva, M. D. da, Bandeira, I. N., and Miranda, L. C. M. 1987. Open-cell photoacoustic radiation detector. J. Phys. E 20: 1476-1478.

[10] Nery, J. W., Pessoa Jr., O, Vargas, H., Reis, F. A. M., Gabrielli, A., Miranda, L. C. M., and Vinha, C. A. 1987. Photoacoustic spectroscopy for depth-profile analysis and herbicide monitoring in leaves. Analyst 112: 1487-1490.

[11] Pereira, A. C., Zerbetto, M., Silva, G. C., Silva, W. J., Vargas, H., Neto, G. de O., Cella, N., and Miranda, L. C. M. 1992. OPC technique for in vivo studies in plant photosynthesis research. Meas. Sci. Technol. 3: 931-934.

[12] Silva, W. J., Prioli, L. M., Miranda, L. C. M., Cella, N., Vargas., H., and Mansanares, A. M. 1995. Photosynthetic O_2 evolution in maize inbreds and their hybrids can be differentiated by Open Photoacoustic Cell. Plant Sci. 104: 177-181.

[13] Barja, P. R., and Mansanares, A. M. 1998. Photosynthetic energy storage and oxygen evolution determined through an Open Photoacoustic Cell technique. Instrum. Sci. Technol. 26/2&3: 209-219.

[14] Fork, D. C., and Herbert, S. K. 1993. The application of photoacoustic techniques to studies of photosynthesis. Photochemistry and Photobiology 57: 207-220.

[15] Malkin, S., and Canaani, O. 1994. The use and characteristics of the photoacoustic method in the study of photosynthesis. Annu. Rev. Plant Physiol. Plant Mol. Biol. 45: 493-526.

[16] Malkin, S., and Puchenkov, O. V. 1997. The photoacoustic effect in photosynthesis. In *Progress in Photothermal and Photoacoustic Science and Technology: Life and Earth Sciences* (Mandelis, A., and Hess, P., editors), Washington: SPIE, chapter 2.

[17] Vargas., H., and Miranda, L. C. M. 1988. Photoacoustic and related photothermal techniques. Phys. Rep. 161: 43-101.

[18] Bento, A. C., Aguiar, M. M. F., Vargas, H., Silva, M. D. da, Bandeira, I. N., and Miranda, L. C. M. 1989. Open Photoacoustic Cell X-ray detection. Appl. Phys. B 48: 269-272.

[19] Marquezini, M. V., Cella, N., Silva, E. C., Serra, D. B., Lima, C. A. S., and Vargas, H. 1990. Photoacoustic assessment of the *in vivo* genotypical response of corn to toxic Aluminium. Analyst 115: 341-343.

[20] Pereira, A. C., Prioli, L. M., Silva, W. J. da, Neto, G. O., Vargas, H., Cella, N., and Alvarado-Gil, J. 1994. In vivo and in situ measurements of spectroscopic and photosynthetic properties of undetached maize leaves using the open photoacoustic cell technique. Plant Science 96: 203-209.

[21] Marquezini, M. V., Cella, N., Mansanares, A.M., Vargas, H., and Miranda, L. C. M. 1991. Open Photoacoustic Cell spectroscopy. Meas. Sci. Technol. 2: 396-401.

[22] Pereira, A. C., Neto, G. de O., Vargas, H., Cella, N., and Miranda, L.C.M. 1994. On the use of the Open Photoacoustic Cell Technique for studying photosynthetic O_2 evolution of undetached leaves: comparison with Clark-type O_2 electrode. Rev. Sci. Instrum. 65: 1512-1516.

[23] Walker, D. A., and Osmond, C. B. 1986. Measurement of photosynthesis *in vivo* with a leaf disc eletrode: correlations between light dependence of steady state photosynthetic O_2 evolution and chlorophyll a fluorescence transients. Proc. R. Soc. Lond. B 227: 267-280.

[24] Dau, H., and Hansen, U. 1989. Studies on the adaptation of intact leaves to changing light intensities by a kinetic analysis of chlorophyll fluorescence and oxygen evolution as measured by the photoacoustic signal. Photosynthesis Research 20: 59-83.

[25] Snel, J. F. H., Kooijman, M., and Vredenberg, W. J. 1990. Correlation between chlorophyll fluorescence and photoacoustic signal transients in spinach leaves. Photosynthesis Research 25: 259-268.