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 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 O₂ 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.

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 \textit{in vivo} and \textit{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 \textit{et al} [18] used it to detect x-rays, while Nery \textit{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 \textit{Laboratory of Photothermal Spectroscopy} by Marquezini \textit{et al} [19], who monitored the injurious effects of aluminum on maize through spectroscopic OPC measurements. Still working with maize leaves, Pereira \textit{et al} [20] investigated differences in the spectra of mutants and wild maize types.

Theoretical – Marquezini \textit{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 1: Scheme of the Open Photoacoustic Cell (OPC).](image-url)
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 \(\beta\) 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.

### 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.

3 OPC and *in Vivo* Plant Photosynthesis Research

Let us recall here some basic notions about photosynthesis. It is well known that plants assimilate \(\text{CO}_2\) and \(\text{H}_2\text{O}\), producing carbohydrates and \(\text{O}_2\). 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 \(\text{O}_2\) 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 \(\text{O}_2\) evolution is limited in time by electron transfers with rate constant of about \(10^{-3}\) s \([6]\). The reaction chain linked to \(\text{CO}_2\) absorption is limited by a \(10^3\) s rate constant; therefore, even for \(f \sim 10\) Hz, modulated \(\text{CO}_2\) 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 \(\text{O}_2\) 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

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\(^{1}\) A sample is called *thermally thick* when it is thicker than the *thermal diffusion length* \(\mu = [\alpha/\pi f]^{1/2}\), where \(\alpha\) 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.

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 O₂ 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 O₂ 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 O₂ 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°C and 25°C. Results indicated that Eucalyptus plants adapted at 10°C 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.
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/m², 680 nm. Continuous light: 500 W/m².

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/m², 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 O₂ 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

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

where \( S_0 \) is the initial value of the photoacoustic signal, \( \Delta S \) is the signal excursion to the saturation value which takes place during the interval \( \Delta t \) 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 O₂ evolution than inbreds. Furthermore, both \( t_0 \) and \( \Delta t \) had a tendency to be shorter in hybrids than in inbreds. This implies that hybrids reach a higher O₂ 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, \( \Delta t \) and \( t_0 \) can be very short for eucalyptus in comparison with plants like maize and coffee. Besides, \( \Delta S \) 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 \( \Delta S \) 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 \( \Delta S \) 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 O₂ 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 reforestation 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 electrolyte 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

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