African Journal of Agricultural Research Vol. 6(12), pp. 2638-2647, 18 June, 2011
Available online at http://www.academicjournals.org/AJAR
DOI: 10.5897/AJAR10.1098
ISSN 1991-637X ©2011 Academic Journals

Review

Description of photosynthesis measurement methods in green plants involving optical techniques, advantages and limitations

Alejandro Espinosa-Calderon[1], Irineo Torres-Pacheco[1], Jose Alfredo Padilla-Medina[2], Roque Alfredo Osornio-Rios[3], Rene de Jesus Romero-Troncoso[4], Carlos Villaseñor-Mora[5], Enrique Rico-Garcia[1] and Ramon Gerardo Guevara-Gonzalez[1]*

[1]CA Ingeniería de Biosistemas, División de Estudios de Posgrado, Facultad de Ingeniería, Universidad Autónoma de Querétaro, Cerro de las Campanas s/n, C.P. 76010, Querétaro, Qro., México.
[2]Dpto. de Ingeniería Electrónica, Instituto Tecnológico de Celaya, Av. Tecnológico y Cubas s/n C.P. 38010, Celaya, Gto., México.
[3]Facultad de Ingeniería, Campus San Juan del Río, Universidad Autónoma de Querétaro, Río Moctezuma No. 249, Col. San Cayetano, C.P. 76807, San Juan del Río, Qro., México.
[4]HSPdigital Research Group, División de Ingenierías, Campus Irapuato-Salamanca, Universidad de Guanajuato, Carr. Salamanca-Valle km 3.5+1.8, Comunidad de Palo Blanco C.P.36700, Salamanca, Gto., México.
[5]División de Ciencias de la Salud e Ingenierías, Campus Celaya-Salvatierra, Universidad de Guanajuato Prolongación Río Lerma s/n, Col. Suiza, C.P. 38060, Celaya, Gto., México.

Accepted 31 January, 2011

Through photosynthesis green plants transform luminous energy, CO_2, water and ground minerals into carbohydrates with O_2 as residuum. Production in plants depends on the level of absorbed nutrients and their capability to process them through photosynthesis. Measuring this process allows predictive maintenance directed to optimize the development and production of the plants. Many photosynthesis measurement methods exist, but given the natural rapidness of this phenomenon, optical techniques appear to be the clue to quantify it in real time. This review describes different optical techniques applied to photosynthesis measurement, commenting on their principal advantages and limitations.

Key words: Infrared gas analyzer (IRGA), photosynthesis measurement, spectroscopy, fluorescence, photoacoustics, optodes, optical microscopy.

INTRODUCTION

Photosynthesis measurement is a predictive indicator of biomass accumulation in green plants. In addition, sciences like physiology, botany and biology are concerned with the photosynthetic response of the plant to climatic factors (Taiz and Zeiger, 1998).

There are different methods, invasive or not, to measure the photosynthesis at leaf, plant or group level (Millan-Almaraz et al., 2009). Invasiveness consists of physical-chemical interference with, entrance to, or reaction with the analyte. Invasiveness is undesirable because it interferes with the normal behavior of the plant. Some invasive and destructive methods are dry matter accumulation (Hodson et al., 2005), rubisco quantification (Flexas et al., 2006), and carbon dioxide isotope (Kawachi et al., 2006). Electrochemical electrode methods (Takahashi et al., 2001) are also invasive because they use gases involved in photosynthesis (CO_2 or O_2) to make the measurement, disturbing the natural environment of the sample.

*Corresponding author. E-mail: ramon.guevara@uaq.mx Tel: +52 (442) 192 1200 Ext. 6093.

Scientific progress in understanding bioprocesses, as photosynthesis, requires non-invasive methods and techniques with high-resolution scale in space and time (Gansert and Blossfeld, 2008). Thus, using light as the carrier of information, optical sensors provide the possibility of non-invasive measurement because no physical contact is required between the sensor and the monitored material. They are analytical tools that satisfy requirements as accuracy, precision and specificity in the selection of the analyte, allowing *in vivo* or *in vitro* investigations. Even though acoustic methods generate non-invasive measurements (Hermand et al., 1998), optical techniques provide a larger realm of possibilities based on properties such as absorbance, reflectance and luminescence of single elements or groups of plants (Wolfbeis, 2005). Optical sensing typically performs in three different ways (Gansert and Blossfeld, 2008):

1) An analyte is measured directly by its specific optical properties.
2) An indicator is added to the medium where it reacts with the analyte. The change of the optical properties of the indicator derived from this reaction can then be measured.
3) An indicator is embedded in a matrix. The analyte diffuses into the matrix, and changes the optical properties of the indicator. The interaction should be fully reversible.

This review aims to provide information about photo-synthesis measurement methods involved with optical techniques.

SPECTROSCOPY

Technological developments in detectors and digital electronics allow using the whole electromagnetic spectrum for detecting and quantifying ever finer details of the emitted and reflected waves that contain crucial information of the medium with which they interact (Jayaraman et al., 2007). Consequently, spectroscopy appears as a direct identification of the surface or atmospheric composition. It is based on the fact that solar radiation, which interacts with plants, is reflected, absorbed or transmitted. Hence, it can be used as a measure to derive information about the biochemical and biophysical properties of plants (Rascher and Pieruschka, 2008).

Optical spectroscopy consists of the monitoring of interactions between matter and electromagnetic radiation in the ranges of ultraviolet (UV, 100-400 nm), visible (VIS, 400-760 nm) and infrared (IR, 760-1000 µm) (Waynant and Ediger, 1994). This method can be applied to monitor plant tissues, canopies and fields in either aquatic (Zimmerman, 2006; Jayaraman et al., 2007) or terrestrial (Cui et al., 2009; Rascher and Pieruschka, 2008; Jayaraman et al., 2007) vegetation features. For more information refer to Schlodder (Schlodder, 2009).

Absorption spectroscopy

Absorption of light radiation induces electronic and vibration transitions of molecules depending on the wavelength of the light source (Schlodder, 2009). The absorption spectrum provides a spectroscopic ”fingerprint” of pigments. Such pigments absorb radiation at a specific wavelength. Thus, they can be selectively excited, suffering a photochemical or photophysical reaction. The result is a decrease of the absorbance around a specific wavelength produced by a radiation source, also called hole burnt (Reinot et al., 2001). It yields information on the dynamics of the excited state and on interactions of the excited pigment with its environment. The limitations of this technique are: self-shading, low canopy cover, vegetation height, and natural change of orientation as a response to environmental or seasonal changes in canopy structure (Rascher and Pieruschka, 2008; Olioso et al., 1998, Zimmerman, 2006).

This principle has been used since the 70’s (Thomas and Oerther, 1972). It has been used to estimate nitrogen status, derived from chlorophyll content (with high sensitivity at 550 nm) as an evaluator of crop health, photosynthesis level and predictor of crop yield (Cui et al., 2009); to retrieve canopy structure parameters as vegetation height (Olioso et al., 1998); and to distinguish tropical tree species, expressing differences in leaf and canopy biochemical properties, using an airborne imaging spectrometer (Chambers et al., 2007); among many other applications.

Variations of absorption spectroscopy, such as Circular dichroism (CD) and Linear dichroism (LD), use polarized light (Schlodder, 2009). CD consists of the difference in the absorption of left and right circularly polarized light. It yields information about secondary structure of biological macromolecules. CD has been used to analyze photosynthetic pigment-protein complexes (Cantor and Schimmel, 1980). Alternatively, LD is the difference in the absorption of parallel and perpendicular linearly polarized light. It makes the absorbance proportional to the square of the scalar product of the oscillating electric field and the transition dipole. LD is used to obtain information relative to the orientation of the pigments with respect to the membrane plane and with respect to each other (Rodger and Nordén, 1997).

Absorption difference spectroscopy

This methodology was first implemented in 1952 (Duysens, 1952) to monitor absorbance changes induced by continuous actinic light. It can detect single pigments

specifically involved with a particular process. The detection results from light-minus-dark difference absorption spectra, but requires an irreversible formed or sufficiently long product state (Schlodder, 2009).

Ultrafast time-resolved absorption spectroscopy (UTRAS)

UTRAS for photosynthesis research was developed in 1949 (Norrish and Porter, 1949).

It consists on the application of a continuous train of short and high-intensity flashes of light that generates photochemical reactions. The flash-induced absorption changes are monitored as a function of both time and wavelength. This technique provides information about the reactions of short-lived intermediates (Schlodder, 2009). Femtosecond resolution has been achieved with such technique, becoming into a powerful tool to study ultrafast relaxation and dynamics on organic and biochemical materials, kinetic studies of lightdriven reactions in photosynthesis, such as excitation energy transfer, electron transfer, lattice vibrations, molecular rotations or a combination of excited states produced by laser heating. These studies can be managed by light pulses, leaving its resolution dependency on the pulse width and the speed of response of the detection system (Leahy-Hoppa and Spicer, 2010).

Raman and Infrared (IR) spectroscopy

Raman spectroscopy, discovered in 1928 (Raman, 1928), is based on inelastic scattering of electromagnetic radiation. A Raman emission spectrum exhibits sharp lines at frequencies different from the excitation light frequency. The final energy is the change of the energy of the molecule due to a transition between energy levels of the molecule. The energy of the scattered radiation is less than that of the incident radiation. This method allows high selectivity. However, it can produce strong fluorescence derived from the difficulty to select the correct excitation light (Schlodder, 2009).

IR spectroscopy, which has achieved femtosecond resolution, is based on the absorption of electromagnetic radiation with wavelengths in the IR region, typically from 1.5 to 50 µm (Shlodder, 2009; Asakawa and Tonokura, 2010).

Infrared (IR) and Raman spectroscopy can be used to observe vibrations of molecules. IR spectrum is usually measured with a Fourier transform infrared (FTIR) spectrometer (Kumar and Barth, 2010), whereas that infrared brightness temperatures are commonly measured using thermal radiometers (Singh et al., 2006). Thanks to the small size and relatively low cost of semiconductors, near infrared (NIR) diode lasers (1,515 and 1,572 nm) have become suitable for transportable spectrometers for *in situ* measurements (Asakawa and Tonokura, 2010).

FLUORESCENCE

Molecules that have been excited to a higher energy state emit radiation when decay to ground state. The emission is called phosphorescence, if the transition occurs between electronic states of different spins; and fluorescence, if it occurs between electronic states of the same spin. Phosphorescence lifetimes are typically long (10^{-3} to 1 s), whereas fluorescence lifetimes are usually in the nanosecond to picoseconds range (Schlodder, 2009). When chlorophyll reaches a light excited state, fluoresces at wavelengths of 685 and 740 nm (Taiz and Zeiger, 1998; Rascher and Pieruschka, 2008). This phenomenon was discovered in 1931 (Kautsky and Hirsch, 1931). The intensity of the emitted fluorescence signal is inversely correlated to the energy used for photosynthesis.

Chlorophyll fluorescence emitted by a leaf under natural sunlight represents 1-5% of the total reflected light. Terrestrial atmosphere presents no, or greatly reduced, absorption (Fraunhofer lines) at 687 and 760 nm. The overlapping of Fraunhofer lines with chlorophyll fluorescence allows retrieving its spectrum from air- and space-borne platforms under daylight excitation (Rascher and Pieruschka, 2008).

Delayed fluorescence (DF), discovered in 1951 (Strehler and Arnold, 1951), consists of photon emissions which intensity depends on the rate of backward electron transport reactions in the reaction center of the photosystem II (PSII) (Zhang et al., 2007). It occurs shortly after stimulation by visible radiation (Schlodder, 2009). DF is more intrinsically related to photosynthetic process than to chlorophyll fluorescence, thus can provide more valuable information about net photosynthesis rate (PN). Its applications have reached biosensor levels, with potential use for *in vivo* real-time monitoring of plant growth regulators of processes as photosynthetic metabolism, cellular differentiation or stomatal movements (Zhang et al., 2007).

Fluorescence technology includes imaging by digital cameras. It allows *in vivo*: estimation of leaf area and growth; identification of spatial heterogeneity of photosynthetic performance, screen and image; registration of the photosynthetic activity of several thousand leaf points; indirectly recognition of physiological phenomena that interfere with the operation of photosynthesis (e.g. herbicide effects and stomatal heterogeneity); and understanding of the operation and regulation of photosynthesis (Baker, 2008; Lichtenthaler et al., 2005).

Fluorescence methods coupled with absorption spectroscopy allows *in vivo*, and sometimes *in situ*,

monitoring of: maximum apparent electron transport rate (ETRmax), photosynthetic light-use efficiency (LUE), quantum yield of PSII, capacity of non-photochemical energy dissipation or quenching (NPQ), organisms with modified photosynthetic performance (as algae), physiological plasticity of plant species, plastid and vacuolar pigments in their natural state, differences in ontogeny of the plant, spatio-temporal variations of fluorescence yield, photochemical reflectance index (PRI), and fluorescence spectrum and lifetimes of single pigment-protein complexes (Rascher and Pieruschka, 2008; Baker, 2008; Damm et al., 2010; Romanov et al., 2008). It has also delivered global distribution maps of plant stresses and photosynthetic efficiency from field, air or space-borne platforms (Rascher and Pieruschka, 2008; Rascher et al., 2009). Given the commercial instruments availability, fluorescence has become one of the most used spectroscopic techniques in plant physiological research from canopy to leaf level (Rascher and Pieruschka, 2008).

Fluorescence techniques have advantages as: high self-descriptiveness, expressiveness, non-invasiveness, non-destructiveness, high sensibility, and rapid and high-throughput screening. However, its limitations are: changing sun angle, soil background, leaf orientation, viewing angle, cells movement, species composition, and varying canopy structure and light intensity (Rascher et al., 2009). Users of this method must consider: selection and calibration of appropriate fluorescence factors; obtainment of the exactly relationship between fluorescence and the measured factor; examination of the relationship between the area of fluorescence and leaf growth; and accumulation of non photosynthetic pigments. These lasts markedly modify the proportion of incident photosynthetically active photon flux density (PPFD) absorbed by the leaf (Baker, 2008).

GAS ANALYSIS

Gas analysis is the most used technique for research and commercial applications in bioprocesses (Millan-Almaraz et al., 2009; Zhang et al., 2007). Gas analysis systems measure gas interchange of O_2 or CO_2 in gaseous or aqueous phase.

Gaseous phase

CO_2 molecule absorbs IR at the wavelengths of 2.66, 2.77, 4.26 and 14.99 µm (Millan-Almaraz et al., 2009). Infrared gas analyzers (IRGAs) utilize the light absorption characteristics of CO_2 molecule to measure the decrease in quantum flow density of infrared light as a quantitative measure of this gas. Even though CO_2 production is not an accurate measure for oxidative respiration, it is for bioprocesses as photosynthesis, stomatal conductance, mesophyll limitations, carboxylation efficiency, transpiration, oxidative respiration, fermentation, denitrification or methanogenesis (Gansert and Blossfeld, 2008).

Hoon et al. (2009) classifies gas interchange chambers in statics and dynamics. Static chambers employ an enriching method (Fang and Moncrieff, 1996), permitting a buildup of CO_2 in the chamber. However, static chambers are inappropriate for the determination of CO_2 sequestration by photosynthesis because of their underestimation of diffusional gaseous efflux. Such underestimation corresponds to the progressive reduction in the concentration gradient across the object of study and the atmosphere boundary. Dynamic gas interchange chambers of IRGAs can be closed or opened (Hoon et al., 2009). First, draw air from the chamber through a gas sensor before returning it to the chamber; secondly, draw air from the chamber and from a reference atmosphere, comparing them in the gas sensor.

IRGAs, essentially, consist of: a transparent dynamic chamber to enclose the illuminated sample (leaf or plant); two tubes for air flux, one for the sample, and other for a reference; a circulating air pump; a focused dual optical path; a radiation source at 4.26 µm; choppers, power sources, amplifiers, filters and phase detectors (Williams et al., 1982; Gürtner et al., 2005). The time taken for the CO_2 concentration to decrease by a predetermined amount (typically 30 ppm) is inversely proportional to the rate of photosynthesis of the leaf. Thermoelectric coolers were used once to ensure a good signal to noise ratio (Williams et al., 1982), but they are no more needed due to advances on solid state optoelectronics (Gürtner et al., 2005).

IRGAs have advantages as being light, portable, not affected by vibrations and free from analyte-consumption flaws. Their limiting factors are: flow systems; tubings and analyzers; non-reciprocal effects of blowing and drawing; pressure gradients; different chamber dimensions due to diverse leaf or plant morphology; mechanical stress on the enclosed leaf; seals; and ventilation in the chambers. Flow systems should be equipped with precision logic and control units which should generate constant and small gas flows (typically from 4 to 10 l/min for IRGAs) through the chambers that house the samples (Pumpanen et al., 2004). Strong ventilation in chambers is needed for minimizing the boundary layer resistance for gas exchange between the plant, or leave, and the atmosphere. Unfortunately, they can create considerably unnatural conditions, and consequently show methodical limitations for *in vivo* quantification of bioprocesses involved in plant gas exchange (Lange, 2002). Seals shadow the sample, promoting CO_2 production derived from dark respiration, generating a photosynthesis calculus error (Long and Bernacchi, 2003).

Differential O_2 in photosynthesis is much smaller than differential CO_2. In addition, O_2 is more unstable. Then, O_2 monitoring needs more accurate and expensive

sensors. Therefore, O_2 gaseous phase analyzers are not commonly used to infer photosynthesis (Hunt, 2003).

Aqueous phase

O_2 and CO_2 gases in the aqueous phase are generally electrochemically measured using Clark-type electrodes. Such electrodes consume part of the analyte, affecting the accuracy of bioprocess analysis due to an artificial gas sink formed during the invasive measuring process (Gansert and Blossfeld, 2008).

Optical fiber O_2 sensors depend on the quenching of luminescence (fluorescence and phosphorescence) produced by O_2. Such luminescence is generated from organometallic compounds embedded in an oxygen permeable polymer matrix, such as polyvinylchloride (PVC) or silicone, located at the tip of an optic light guide. A light of 580 nm is transmitted to a fluorometer. The luminescence quantum yield drops hyperbolically with O_2 concentration, following the Stern-Volmer relationship, leading to linear dependence between O2 concentration and the ratio of unquenched luminescence intensity (Tyystjärvi et al., 1998).

These sensors are limited by their strong dependence on sophisticated laboratory equipment. However, this technique does not consume analyte and responds faster than electrochemical electrodes. It also permits the use of same optics for the simultaneous monitoring of O_2 concentration and chlorophyll fluorescence, by using modulated excitation and separating both signals by their spectra (Hunt, 2003).

Optodes

Optodes are optical chemical sensors capable of continuously recording physical parameters or chemical compounds, based on the fluorescence properties of some specific analyte indicator dye (Wolfbeis, 2005). Optodes measure in either gaseous (Neurauter et al., 1999) or aqueous (Tengberg et al., 2006) phase, and do not restrict the analyte-specific sensing performance (Gansert and Blossfeld, 2008).

In optodes, analyte luminescent microparticles (luminophores) are incorporated in a specifically designed polymer matrix. This matrix defines the shape of the sensor spot, and tunes sensor properties (sensitivity, cross-sensitivity and response times) to other analytes.

Single optodes can measure temperature (Liebsch et al., 1999), CO_2 (Neurauter et al., 1999) and O_2 (Tengberg et al., 2006). Hybrid optodes quantify two analytes at the same location and time: O_2-temperature, O_2-pH, O_2-CO_2, temperature-CO_2, and O_2-chlorophyll (Gansert and Blossfeld, 2008). In general, optodes allow real-time measurements without metrological disturbance and/or contamination of media. Optodes monitor *in vivo* metabolic processes (as photosynthesis) in cells, tissues, organisms and their environments (Gansert and Blossfeld, 2008).

PHOTOACOUSTICS

In 1881, Alexander Graham Bell (Bell, 1881) discovered the photoacoustic effect and defined it as the production of sound by light. In 1972, the photothermal effect during photosynthesis was monitored (Callis et al., 1972). In 1978, the thermally-induced pressure wave was detected by a gas-coupled microphone (Cahen et al., 1978). Nowadays, photoacoustic techniques can also be applied in aqueous samples with green algae (Hou and Sakmar, 2010).

In this technique, a leaf is enclosed into a cell and exposed to a modulated light beam. The light absorption of the leaf generates both, a molecular volume change and a photoreaction enthalpy change. These changes produce pressure signals of heat and oxygen at the same frequency of the light beam and are sensed by a piezoelectric transducer (Hou and Sakmar, 2010). This signal throws information of optical and thermal properties of the sample, O_2 and CO_2 diffusion, quantum yield spectra, and time constants of the various steps in the electron transfer chain. Such data are reflected on its amplitude and its phase from the exciting beam (Barja et al., 2001).

The Open Acoustic Cell (OPC) is a photoacoustic sensor where the electrets microphone uses its own chamber as the acoustic cell. Here the sample acts as one of the walls, capturing external CO_2. OPC avoids dehydration of the sample and minimizes changes in the atmosphere of the photoacoustic chamber (Barja et al., 2001).

Photoacoustic systems can measure thermodynamic changes of volume, enthalpy and entropy. They provide critical information about photoactivation mechanisms of photosystems and photoreceptors (Delosme, 2003); reveal energy levels of reactants and products, as well as the driving forces in a chemical reaction (Hou and Sakmar, 2010); and quantify *in vitro* and *in vivo* chemical and biological reactions as photosynthetic activities of plants under chemical stress (Ouzounidou, 1996).

Limitations of this technique include extensive mathematical developments and inappropriate or non-standardized language (Delosme, 2003). *In vitro* measurements can show significant differences from *in vivo* values, maybe due to *in vivo* diversity of change factors (Hou and Sakmar, 2010). Its time resolution (nanosecond to microsecond ranges) is restricted by the duration of the laser pulse, the time response of the piezoelectric detector, and the transit time of the acoustic pulse across the diameter of the laser beam (Delosme,

2003). Finally, beyond the dependence on the temperature and light treatment of the samples, the photoacoustic signal also depends on parameters such as leaf morphology and gas atmosphere (Barja et al., 2001).

IRRADIANCE

In 1929, Emerson exposed that external factors as light, temperature and CO_2 affect the rate of photosynthesis in green plants. Photosynthesis is a quantum phenomenon. Consequently, it is the number of available photons rather than the amount of radiant energy that is relevant to the chemical transformations. For example, if a photon, of a wavelength of 350 nm, is absorbed by chlorophyll, it induces the same chemical change as does a photon of 700 nm, even though the first has twice the energy of the second. Chlorophyll utilizes photons equally, regardless of their direction of travel. Therefore, the relevant measure of the irradiance (*I*) is the photosynthetically available radiation (PAR), which consists of a wavelength range from 400 to 700 nm (Bass et al., 1995).

Relationship between *I* and P_N has different models: exponential functions (Smith and Houpis, 2004), tangent functions (Kurano and Miyachi, 2005), nonrectangular hyperbolic model (Yufeng et al., 2005), rectangular hyperbolic method (Kurano and Miyachi, 2005), binomial regression method (Wei-guo, 2006), and the Ye model (Ye, 2007). The most used models are the nonrectangular hyperbola and binomial regression (Ye, 2007). Besides irradiance, these models need environmental conditions as CO_2 and O_2 concentration, temperature and humidity to calculate a response curve of P_N. The processing of temporal averages of the named variables usually overestimates results, then, instantaneous values become preferable (Berninger, 1994). Limiting values, as dark respiration rate (negative P_N at $I = 0$), and saturation irradiance (I_{max}) must also be provided. Anyway, nonrectangular and rectangular hyperbola models cannot describe the *I* -response curve at low *I* and photo-inhibition (Yufeng et al., 2005, Kurano and Miyachi, 2005; Ye, 2007). Conversely, the Ye model can deal with low PAR, but implies a difficult mathematical computation.

INTRACELLULAR OXYGEN CONCENTRATIONS

Plants, unlike animals, do not possess an efficient distribution system for the O_2 supply to their tissues. So, if oxygen concentration is reduced, as during photosynthesis, plants reduce metabolic processes in order to diminish their energy consumption (Van Dongen et al., 2004); allowing a more efficiently O_2 use (Bologa et al., 2003); and inducing formation of lenticels and aerenchyma to improve the oxygen permeability of the tissue (Evans, 2004). Therefore, the measurement of the internal oxygen concentration of the plant tissue proves to be a direct quantifier of its photosynthetic activity. It is a pretty novel technique developed by Schmälzlin et al. (2005).

Intracellular O_2 in plants could be measured by an adapted handmade Clark-type oxygen electrode, but its continuous consumption of O_2 would affect the internal concentration of the cell (Buerk, 2004). The optical technique to measure intracellular O_2 concentrations in green plants consists of inject cells of oxygen-sensitive phosphorescent Pt(II)-tetra-pentafluorophenylporphyrin (PtPFPP) (Papkovsky, 2004) encapsulated in polystyrene microbeads. But, since such microbeds phosphoresce in red, there is spectral overlapping with the autofluorescence of chlorophyll in the cell. However, luminescent lifetime is the differentiation key point. PtPFPP phosphoresces on the range of microseconds, whereas chlorophyll does that in the nanoseconds range. Hence, excitation with an optical multyfrequency phase-modulation technique enables a precise determination of changes in phosphorescent lifetime due to oxygen-induced quenching of the signal (Schmälzlin et al., 2005). For the moment, this technique can only be applied at specialized laboratories and at tissue level.

OPTICAL MICROSCOPY

The first microscope, compounded by Janssen in the 1590s (Doetsch, 1961), used glass ball lenses. Later, Robert Hooke (Gest, 2004) perfected it, reaching many biological applications. Some modern microscopy techniques are electron, atomic force and optical microscopies. Even though the first and the second provide higher resolution, only the third one permits the examination of biological structures at the single molecule detection level, and enables investigations of functional dynamics in living cells for prolonged periods of time (Cisek et al., 2009). In optical microscopes, image formation is based on the spatial variation of light intensity recorded by a detector, whereas that the visibility of a structure against the image background is characterized by its contrast. Due to the extension of this topic and its numerous techniques and applications, this review only comments about the most popular linear and nonlinear optical microscopy techniques(Cisek *et al*., 2009).

Wide field linear microscopy

This microscopy forms the image of an object straight from the microscope optics. Such image is projected onto a two-dimensional array detector (human retina or electronic camera). Properties of light, such as phase or polarization, used as image contrast mechanisms for

these microscopes, are converted to changes in light intensity in order to permit its quantification by the detector (Bradbury and Evennett, 1996). Some examples of this kind of microscopy are mentioned ahead:

i) Bright field illumination microscopy is used for very thin pigmented photosynthetic samples because of their low absorption and scattering. It rings difficulties for control the contrast of the image when observing unstained subcellular structures (Buléon et al., 1998).
ii) Dark field illumination microscopy emphasizes interfaces between structures with high scattering coefficients. This process can lose some information because it cannot collect the transmitted rays that are unaffected by the sample (Bradbury and Evennett, 1996). It has been used to visualize chloroplasts (Higashi-Fujime, 1980).
iii) Phase contrast microscopy is used only for samples that change the phase of the transmitted light (Higashi-Fujime, 1980).
iv) Polarization microscopy uses linearly and circularly polarized light to image for birefringent objects (Bayoudh et al., 2003). Such objects have double refraction, decomposing a ray of light into two rays (Waynant and Ediger, 1994).
v) Differential interference contrast microscopy generates pseudo 3D images of samples, but without a high specificity (Cisek et al., 2009).
vi) Fluorescence microscopy offers advantages as high sensitivity and specificity (Haugland et al., 2005). Even though it is one of the most frequently used contrast methods in microscopy, its axial resolution is relatively low (Cisek et al., 2009).

Scanning linear microscopy

Scanning microscopes focus a beam of light into a tiny focal spot on the sample. Then, either the sample stage or the scanning beam is translated to obtain the full measurement of the sample. Variations of this technique have reached resolutions down to several hundred nanometers (Cisek et al., 2009), and are mentioned ahead:

i) Confocal fluorescence microscopy helps to avoid image quality impairment in fluorescence microscopy (Pawley and Masters, 2008). Some restrictions of these microscopes include poor light transmission of the setup, signal crosstalk from nearby pixels and limited speed.
ii) Polarization confocal microscopy makes very sensitive detections of samples with linear and circular dichroism (Finzi et al., 1989).
iii) Vibrational microscopy uses IR and Raman spectroscopy to map chemical composition in biological samples. It also brings high specificity imaging without the use of artificial labels. This technique is limited by the molecular structure and environmental conditions such as pH. IR absorption microscopy can be applied only to very thin samples, approximately 15 µm thick (Cisek et al., 2009). Raman microscopy should consider abolition of autofluorescence emission (Gierlinger and Schwanninger, 2007).
iv) Near-field scanning optical microscopy (NSOM) achieves 2D scanning of the topography of the sample added to its fluorescence or transmitted light signals. Its resolution achieves until 1 nm axially and 50-150 nm laterally (Brunner et al., 1997).

Scanning nonlinear microscopy

In microscopes, ultra-short pulsed laser induces nonlinear polarization in the observed sample, while keeps low the average laser power. This technique allows imaging of live specimens without damage, while providing optical sectioning (Denk et al., 1990). Each nonlinear microscopy contrast carries specific information about the structure of the sample, presenting numerous advantages over linear microscopy techniques including improved deep tissue imaging, optical sectioning, and imaging of live unstained samples. Several nonlinear processes can be induced in the sample simultaneously. However, nonlinear microscopy is still not very popular, lacking protocols, users and applications (Cisek et al., 2009). Some examples of nonlinear microscopies are mentioned ahead:

i) Multiphoton excitation fluorescence is, nowadays, the most frequently used nonlinear contrast mechanism in microscopic investigations due to the commercial availability of two-photon excitation laser scanning microscopes (Cisek et al., 2009). It can excite molecules within a tiny focal volume due to nonlinearity of the excitation process. The nonlinear confinement of excitation provides inherent optical sectioning and reduces out of focus bleaching (Denk et al., 1990).
ii) Second harmonic generation can be used only in media with non-central (non-inversion) symmetry (Cisek et al., 2009).
iii) Third harmonic generation is sensitive to the orientation of the interface with respect to the principal direction of propagation of the laser beam (Müller and Wilson, 1998).
iv) Nonlinear multicontrast microscope has its resolution limited by the laser wavelength and the numerical aperture of the objective, while the imaging depth into the sample is limited by the working distance of the focusing objective, laser radiation penetration depth and the attenuation of harmonic signals by the sample (Carriles et al., 2009).

CONCLUSIONS

Since photosynthesis process is a key factor for plants

production, its quantification has unleashed several researches. Due to the natural rapidness of this process, the fact that it can only be indirectly inferred through the measurement of related variables, and in order to improve non-invasiveness, optical techniques coupled to multidisciplinary studies have demonstrated to be a powerful tool for real time-*in vivo* quantification of photosynthesis.

Each existing method has technical, practical and physiological advantages and limitations to be considered. Such characteristics have been discussed along this review, showing that optical techniques have the potential to detect physiological and biochemical changes in plants at tissue, leaf, plant or even canopy level.

Existing techniques for photosynthesis measurement should be improved, and new methods should be developed in order to enhance, not only accurate measurements or models for knowledge of the biological environment around us, but also for production of natural fibers, pigments, construction materials, fuels and, principally, provisions for a non stopping demographic explosion.

In situ measurements allow producers and researchers to have a real time quantification of the photosynthesis made by their plants in the precise place and conditions where they are cultivated. Among all the exposed methods in this review, it has been shown that there are only a few options with the principles of infrared spectroscopy (Asakawa and Tonokura, 2010), fluorescence (Romanov et al., 2008) and gas analysis (Hoon et al., 2009; McGuire and Teskey, 2002) for *in situ* measurements of photosynthesis. This fact shows that there is still a lack of technological development in this field.

There are continuous advances on plant physiology, biochemistry, electronics, detectors, communications, computer technology, image analysis, visualization, materials, laser sources, and optical technologies such as adaptive optics and optical fibers. Such advances can help to reach or improve non-invasive-*in vivo,* and maybe *in situ,* real time detection of changes in the photosynthetic process of plants. Furthermore, such advances may be of great potential for managing crop production in a possible none far future agro-based economy.

ACKNOWLEDGEMENTS

Authors thank FOMIX-Qro, SEP-CONACyT and PROMEP. AEC thanks also goes to CONACyT for its economical support through the scholarship with Reg. Num. 239421.

REFERENCES

Asakawa TKN, Tonokura K (2010). Diode Laser Detection of Greenhouse Gases in the Near Infrared Region by Wavelength Modulation Spectroscopy: Pressure Dependence of the Detection Sensitivity. Sensors, 10: 4686–4699.

Baker N (2008). Chlorophyll fluorescence: a probe of photosynthesis in vivo. Annu. Rev., 59:89–113.

Barja P, Mansanares A, da Silva E, Magalhães A, Alves P (2001). Photosynthesis in eucalyptus studied by the Open Photoacoustic technique: Effects of irradiance and temperature. Acoust. Phys., 47(1): 16–21.

Bass M, Van Stryland E, Williams D, Wolfe W (1995). Handbook of Optics, Volumes I & II. McGraw-Hill, New York.

Bayoudh S, Nieminen T, Heckenberg N, Rubinsztein-Dunlop H (2003). Orientation of biological cells using planepolarized Gaussian beam optical tweezers. J. Modern Optics, 50(10): 1581–1590.

Bell A (1881). LXVIII. Upon the production of sound by radiant energy. Philosophical Mag., 11(71): 510–528.

Berninger F (1994). Simulated irradiance and temperature estimates as a possible source of bias in the simulation of photosynthesis. Agric. For. Meteorol., 71(1-2): 19–32.

Bologa K, Fernie A, Leisse A, Ehlers LM, Geigenberger P (2003). A bypass of sucrose synthase leads to low internal oxygen and impaired metabolic performance in growing potato tubers. Plant Physiol., 132(4): 2058–2072.

Bradbury S, Evennett P (1996). Contrast techniques in light microscopy. BIOS Scientific.

Brunner R, Bietsch A, Hollricher O, Marti O (1997). Distance control in near-field optical microscopy with piezoelectrical shear-force detection suitable for imaging in liquids. Rev. Sci. Instrum., 68: 1769-1773.

Buerk D (2004). Measuring tissue PO2 with microelectrodes. Methods Enzymol., 381: 665–690.

Buléon A, Colonna P, Planchot V, Ball S (1998). Starch granules: structure and biosynthesis. Inter. J. Biol. Macromol., 23(2): 85–112.

Cahen D, Malkin S, Lerner E (1978). Photoacoustic spectroscopy of chloroplast membranes; listening to photosynthesis. FEBS lett., 91(2): 339–342.

Callis J, Parson W, Gouterman M (1972). Fast changes of enthalpy and volume on flash excitation of Chromatium chromatophores. Biochim. Biophys. Acta (BBA)-Bioenergetics, 267(2): 348–362.

Cantor C, Schimmel P (1980). Biophysical chemistry: Part II: Techniques for the study of biological structure and function. WH Freeman and Company.

Carriles R, Schafer D, Sheetz K, Field J, Cisek R, Barzda V, Sylvester A, Squier J (2009). Invited Review Article: Imaging techniques for harmonic and multiphoton absorption fluorescence microscopy. Rev. Sci. Instrum., 80: 081101.

Chambers J, Asner G, Morton D, Anderson L, Saatchi S, Espírito-Santo F, Palace M, Souza Jr C (2007). Regional ecosystem structure and function: ecological insights from remote sensing of tropical forests. Trends Ecol. Evol., 22(8): 414–423.

Cisek R, Spencer L, Prent N, Zigmantas D, Espie G, Barzda V (2009). Optical microscopy in photosynthesis. Photosynthesis Res., 102(2): 111–141.

Cui D, Li M, Zhang Q (2009). Development of an optical sensor for crop leaf chlorophyll content detection. Comput. Electron. Agric., 69(2): 171–176.

Damm A, Elbers J, Erler A, Gioli B, Hamdi K, Hutjes R, Kosvancova M, Meroni M, Miglietta F, Moersch A (2010). Remote sensing of sun-induced fluorescence to improve modeling of diurnal courses of gross primary production (GPP). Glob. Change Biol., 16(1): 171–186.

Delosme R (2003). On some aspects of photosynthesis revealed by photoacoustic studies: A critical evaluation. Photosynthesis Res., 76(1): 289–301.

Denk W, Strickler J, Webb W (1990). Two-photon laser scanning fluorescence microscopy. Science, 248(4951): 73–76.

Doetsch R (1961). History of the Microscope. Science, 133(3456): 946–947.

Duysens L (1952). Transfer of excitation energy in photosynthesis. PhD thesis, Utrecht,

Emerson R (1929). Chlorophyll content and rate of photosynthesis. Proceedings of the National Academy of Sciences of the United States of America, 15(3): 281–284.

Evans D (2004). Aerenchyma formation. New Phytol., 161(1): 35–49.

Fang C, Moncrieff J (1996). An improved dynamic chamber technique for measuring CO 2 efflux from the surface of soil. Funct. Ecol., pp 297–305.
Finzi L, Bustamante C, Garab G, Juang C (1989). Direct observation of large chiral domains in chloroplast thylakoid membranes by differential polarization microscopy. Proceedings of the National Academy of Sciences of the United States of America, 86(22): 8748.
Flexas J, Ribas-Carbó M, Bota J, Galmés J, Henkle M, Martínez-Cañellas S, Medrano H (2006). Decreased Rubisco activity during water stress is not induced by decreased relative water content but related to conditions of low stomatal conductance and chloroplast CO_2 concentration. New Phytol., 172(1): 73–82.
Gansert D, Blossfeld S (2008). The Application of Novel Optical Sensors (Optodes) in Exper. Plant Ecol. Progress Bot., 69(4): 333–358.
Gest H (2004). The discovery of microorganisms by Robert Hooke and Antoni Van Leeuwenhoek, fellows of the Royal Society. Notes and Records, 58(2): 187–201.
Gierlinger N, Schwanninger M (2007). The potential of Raman microscopy and Raman imaging in plant research. Spectrosc. Int. J., 21: 69–89.
Gürtner M, Neumann N, Schneider F (2005). An Intelligent CO_2 Sensor for Private Homes. Key Eng. Mater., 295: 151–158.
Haugland R, Spence M, Johnson I (2005). The handbook: a guide to fluorescent probes and labeling technologies. Molecular Probes.
Higashi-Fujime S (1980). Active movement *in vitro* of bundle of microfilaments isolated from Nitella cell. J. Cell Biol., 87(3): 569-578.
Hermand J, Nascetti P, Cinelli F (1998). Inversion of acoustic waveguide propagation features to measure oxygen synthesis by Posidonia oceanica. In OCEANS'98 Conference Proceedings, Nice, France, pp. 919–926.
Hodson M, White P, Mead A, Broadley M (2005). Phylogenetic variation in the silicon composition of plants. Ann. Bot., 96(6): 1027–1046.
Hoon S, Thomas A, Linton P (2009). The design and development of a closed chamber for the in-situ quantification of dryland soil carbon dioxide fluxes. Geogr. Res., 47(1): 71–82.
Hou H, Sakmar T (2010). Methodology of Pulsed Photoacoustics and Its Application to Probe Photosystems and Receptors. Sensors, 10: 5642–5667.
Hunt S (2003). Measurements of photosynthesis and respiration in plants. Physiol. Plant., 117(3): 314–325.
Jayaraman A, Joshi P, Ramesh R (2007). Developments and achievements in atmospheric sciences and space meteorology in India. Curr. Sci., 93(12): 1779–1811.
Kautsky H, Hirsch A (1931). Neue versuche zur kohlenstoffassimilation. Naturwissenschaften, 19: 964–964.
Kawachi N, Sakamoto K, Ishii S, Fujimaki S, Suzui N, Ishioka N, Matsuhashi S (2006). Kinetic analysis of carbon-11-labeled carbon dioxide for studying photosynthesis in a leaf using positron emitting tracer imaging system. IEEE Trans. Nuclear Sci., 53(5 Part 2): 2991–2997.
Kumar S, Barth A (2010). Following Enzyme Activity with Infrared Spectroscopy. Sensors, 10: 2626–2637.
Kurano N, Miyachi S (2005). Selection of microalgal growth model for describing specific growth rate-light response using extended information criterion. J. Biosci. Bioeng., 100(4): 403–408.
Lange O (2002). Photosynthetic productivity of the epilithic lichen Lecanora muralis: long-term field monitoring of CO_2 exchange and its physiological interpretation. I. Dependence of photosynthesis on water content, light, temperature, and CO_2 concentration from laboratory measurements. Flora-Morphol. Distrib. Funct. Ecol. Plants, 197(4): 233–249.
Leahy-Hoppa MR, Miragliotta J, Osiander R, Burnett J, Dikmelik Y, McEnnis C, Spicer J (2010). Ultrafast Laser-Based Spectroscopy and Sensing: Applications in LIBS, CARS, and THz Spectroscopy. Sensors, 10: 4342–4372.
Lichtenthaler H, Langsdorf G, Lenk S, Buschmann C (2005). Chlorophyll fluorescence imaging of photosynthetic activity with the flash-lamp fluorescence imaging system. Photosynthetica, 43(3): 355–369.
Liebsch G, Klimant I, Wolfbeis O (1999). Luminescence lifetime temperature sensing based on sol-gels and poly (acrylonitrile) s dyed with ruthenium metal-ligand complexes. Adv. Mater., 11(15): 1296–1299.
Long S, Bernacchi C (2003). Gas exchange measurements, what can they tell us about the underlying limitations to photosynthesis? Procedures and sources of error. J. Exp. Bot., 54(392): 2393–2401.
Millan-Almaraz J, Guevara-Gonzalez R, de Jesus Romero-Troncoso R, Osornio-Rios R, Torres-Pacheco I (2009). Advantages and disadvantages on photosynthesis measurement techniques: A review. Afr. J. Biotechnol., 8(25): 7340–7349.
Müller S, Wilson B (1998). 3D microscopy of transparent objects using third-harmonic generation. J. Microsc., 191(3): 266–274.
Neurauter G, Klimant I, Wolfbeis O (1999). Microsecond lifetime-based optical carbon dioxide sensor using luminescence resonance energy transfer. Anal. Chim. Acta, 382(1-2): 67–75.
Norrish R, Porter G (1949). Chemical reactions produced by very high light intensities. Nature, 164(4172): 658–658.
Olioso A, Chauki H, Wigneron J, Bertuzzi P (1998). Estimation of energy fluxes and photosynthesis from thermal infrared, spectral reflectances, microwave data and SVAT modeling. IGARSS'98-1998 IEEE International Geoscience and Remote Sensing Symposium, Seattle, WA, pp. 1493–1495.
Ouzounidou G (1996). The use of photoacoustic spectroscopy in assessing leaf photosynthesis under copper stress: correlation of energy storage to photosystem II fluorescence parameters and redox change of P700. Plant Sci., 113(2): 229–237.
Papkovsky D (2004). Methods in optical oxygen sensing: protocols and critical analyses. Methods Enzymol., 381: 715–735.
Pawley J, Masters B (2008). Handbook of biological confocal microscopy. J. Biomed. Optics, 13: 029902–1–029902–3.
Pumpanen J, Kolari P, Ilvesniemi H, Minkkinen K, Vesala T, Niinistö S, Lohila A, Larmola T, Morero M, Pihlatie M (2004). Comparison of different chamber techniques for measuring soil CO_2 efflux. Agric. For. Meteorol., 123(3-4): 159–176.
Raman C (1928). A change of wave-length in light scattering. Nature, 121(3051): 619–619.
Rascher U, Agati G, Alonso L, Cecchi G, Champagne S, Colombo R, Damm A, Daumard F, Miguel E (2009). CEFLES 2: The remote sensing component to quantify photosynthetic efficiency from the leaf to the region by measuring sun-induced fluorescence in the oxygen absorption bands. Biogeosci., 6(7): 1181–1198.
Rascher U, Pieruschka R (2008). Spatiotemporal variations of photosynthesis: the potential of optical remote sensing to better understand and scale light use efficiency and stresses of plant ecosystems. Precision Agric., 9(6): 355–366.
Reinot T, Zazubovich V, Hayes J, Small G (2001). New insights on persistent nonphotochemical hole burning and its application to photosynthetic complexes. J. Phys. Chem., 105(22): 5083–5098.
Rodger A, Nordén B (1997). Circular dichroism and linear dichroism. Oxford University Press, USA.
Romanov V, Sherer V, Galelyuka I, Sarakhan Y, Skrypnyk O (2008). Smart Portable Fluorometer for Express-Diagnostics of Photosynthesis: Principles of Operation and Results of Exper. Res.,
Schlodder E (2009). Introduction to optical methods in photosynthesis. Photosynthesis Res., 101(2): 93–104.
Schmälzlin E, Van Dongen J, Klimant I, Marmodée B, Steup M, Fisahn J, Geigenberger P, Löhmannsröben H (2005). An optical multifrequency phase-modulation method using microbeads for measuring intracellular oxygen concentrations in plants. Biophysi. J., 89(2): 1339–1345.
Singh R, Oza S, Pandya M (2006). Observing long-term changes in rice phenology using NOAA-AVHRR and DMSP-SSM/I satellite sensor measurements in Punjab, India. Curr. Sci., 91(9): 1217–1221.
Smith M, Houpis J (2004). Gas exchange responses of the wetland plant *Schoenoplectus hallii* to irradiance and vapor pressure deficit. Aquat. Bot., 79(3): 267–275.
Strehler B, Arnold W (1951). Light production by green plants. J. Gen. Physiol., 34(6): 809–809.
Taiz L, Zeiger E (1998). Plant Physiology 2nd. Ed. Sinauer, Sunderland, Massachussets, pp. 111-143.
Takahashi M, Ishiji T, Kawashima N (2001). Handmade oxygen and carbon dioxide sensors for monitoring the photosynthesis process as instruction material for science education. Sensors and Actuators B:

Chemical, 77(1-2): 237–243.
Tengberg A, Hovdenes J, Andersson H, Brocandel O, Diaz R, Hebert D, Arnerich T, Huber C, Körtzinger A, Khripounoff A, Rey F, Rönning C, Schimanski J, Sommer S, Stangelmayer A (2006). Evaluation of a lifetime-based optode to measure oxygen in aquatic systems. Limnology and Oceanography: Methods, 4: 7-17.
Thomas J, Oerther G (1972). Estimating nitrogen content of sweet pepper leaves by reflectance measurements. Agron. J., 64(1): 11–13.
Tyystjärvi E, Karunen J, Lemmetyinen H (1998). Measurement of photosynthetic oxygen evolution with a new type of oxygen sensor. Photosynthesis Res., 56(2): 223–227.
Van Dongen J, Roeb G, Dautzenberg M, Froehlich A, Vigeolas H, Minchin P, Geigenberger P (2004). Phloem import and storage metabolism are highly coordinated by the low oxygen concentrations within developing wheat seeds. Plant Physiol., 135(3): 1809–1821.
Waynant R, Ediger M (1994). Electro-optics handbook. McGraw-Hill,
Wei-guo F (2006). Diurnal Photosynthetic Changes of Phragmites communis in the Wetland Lying in Beigushan Mountain of Zhengjian Prefecture. Acta Bot. Boreali-Occidentalia Sin., 3: 0496–0501.
Williams B, Gurner P, Austin R (1982). A new infra-red gas analyser and portable photosynthesis meter. Photosynthesis Res., 3(2): 141–151.
Wolfbeis O (2005). Materials for fluorescence-based optical chemical sensors. J. Mater. Chem., 15(27-28): 2657–2669.
Ye Z (2007). A new model for relationship between irradiance and the rate of photosynthesis in *Oryza sativa*. Photosynthetica, 45(4): 637–640.
Yufeng L, Langtao X, Jianhua T, Xiaobo L (2005). Primary Application on the Non-rectangular Hyperbola Model for Photosynthetic Light-Response Curve (J). Chinese Agric. Sci. Bull., 8: 76–79.
Zhang L, Xing D, Wang J (2007). A non-invasive and real-time monitoring of the regulation of photosynthetic metabolism biosensor based on measurement of delayed fluorescence *in vivo*. Sensors, 7: 52–66.
Zimmerman R (2006). Light and photosynthesis in seagrass meadows. Seagrasses: Biol. Ecol. Conserv., pp. 303–321.