Biophotonic *in situ* sensor for plant leaves

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Received 12 August 2009; revised 26 February 2010; accepted 1 March 2010; posted 2 March 2010 (Doc. ID 115397); published 22 March 2010

Knowledge of the water concentration of plants can be helpful in several environmental and agricultural domains. There are many methods for the determination of water content in plant leaves; however, most of them give a relative moisture level or an analytical measure after a previous calibration procedure. Even for other biochemical compounds such as dry matter or chlorophyll, the measurement techniques could be destructive. For this reason, a nondestructive method has been developed to measure the biochemical compounds of a plant leaf, using an infrared spectroscopy technique. One important advantage is the simplicity of the device (RAdiomètre portatif de Mesure In Situ, RAMIS) and its capability to perform measurements *in situ*. The prototype is a leaf-clip configuration and is made of LEDs at five wavelengths (656, 721, 843, 937, and 1550 nm), and a silicon/germanium photosensor. To compute the water content of vegetative leaves, the radiative transfer model PROSPECT was implemented. This model can accurately predict spectral transmittances in the 400 nm to 2500 nm spectral region as a function of the principal leaf biochemical contents: water, dry matter, and chlorophyll. Using the transmittance measured by RAMIS into an inversion procedure of PROSPECT: A Model of Leaf Optical Properties Spectra, we are able to compute the values of water contents that show an agreement with the water contents measured directly using dry weight procedures. This method is presented as a possibility to estimate other leaf biochemical compounds using appropriate wavelengths. © 2010 Optical Society of America

**OCIS codes:** 280.1415, 120.0120.

1. Introduction

NIR spectroscopy is a useful tool for many applications in diagnostic and biochemical estimation on plant tissues [1]. Today, for various application domains such as precision agriculture, global-scale ecology [2], and the validation of satellite remote sensing products [3], it is very important to assess the leaf biochemical composition: water content ($C_w$, also called equivalent water thickness) [4], dry matter content ($C_m$, also called leaf mass per area), and total chlorophyll content ($C_{ab}$). Estimations of these biochemical compounds are usually performed by laboratory procedures, and most of them are lengthy, complex, and destructive. For these reasons there is a necessity for new procedures that make leaf biochemical measurements *in situ* possible.

Several techniques have been explored for this purpose, based on the interaction of electromagnetic radiation in the internal components of a plant leaf [5,6]. These physical interaction models take into account transmittance, reflectance, and fluorescent measurements on the vegetation tissue. This idea sets a practical method to estimate leaf biochemical levels in a nondestructive way [7]. For example, the estimation of total chlorophyll on a plant leaf, which normally involves analytical chemical techniques, can be measured by instruments that exist today: the SPAD-502 (Konica Minolta Sensing Inc, Osaka, Japan) [8,9] and the CCM-200 (ADC BioScientific Ltd, Hertford, UK) [9]. Those instruments use selected wavelengths from the visible domain to compute relative measurements of chlorophyll. Others instruments such the Dualex FLAV and the Dualex HCA, which have been developed by FORCE-A (Orsay, France) [10] and are based on the fluorescence emission by chlorophyll after exposure to ultraviolet
(UV) radiation, give relative measurements of chlorophyll and analytical measurements of other biochemical compounds. Our main purpose is to present an instrument that can perform the analytical estimation of leaf water content via biophotonic measurements in situ, using a nondestructive technique that can be easily applied.

To achieve the goal of the estimation of water content, an inversion procedure of the radiative transfer model PROSPECT is performed on the RAMIS measurements.

This work involves two research activities: instrumentation and model inversion. The instrumentation part describes the RAMIS prototype, including a quick description of the source system, sensor system, and the signal acquisition procedure. Next, the inversion procedure of the PROSPECT model is described and how it is adapted to the output data from the RAMIS system is described. Last, the result of water contents measured from several leaf samples are compared with the computed water contents from the inversion procedure.

2. Physical Principles

The interaction of electromagnetic radiation with plant leaves can be computed from knowledge of the spectral variation of the complex refractive index \[1,11\]. This phenomenon is directly related to different processes such as the transmission, reflection and absorption of light, and electronic orbital interactions with the leaf structure in the visible and infrared domain. The absorption phenomenon allows the definition of a spectrometric method to perform analytical measurements of the principal leaf biochemical compounds. In order to accomplish this objective, it is essential to know the spectral absorption characteristics of each compound: water, chlorophyll, and dry matter. Figure 1 shows the specific absorption coefficients of these three important biochemical components.

The spectral transmittance and reflectance of a plant leaf can be predicted from the spectral response of its biochemical constituents (Fig. 1) and the behavior of light in the internal leaf structure. This relationship between the spectral leaf transmittance–reflectance and the concentration of biochemical compounds presents a useful tool to relate external optical measurements to its internal components such as water, dry matter, and chlorophyll (principal A and B). Therefore, a spectrometric method could be implemented in a portable device in order to perform in situ transmittance measurements using a selected set of wavelengths. More specifically, the wavelengths must be selected as a function of the appropriate biochemical leaf compounds considering their sensitivity response at their respective concentration levels.

All these characteristics have been implemented in the development process of the RAMIS prototype. In addition, the relationship between optical measurements and chemical species concentrations has been established by comparison with regular laboratory measurement methods and by inversion of the PROSPECT model.

A. Radiative Transfer Model PROSPECT

The radiative transfer model PROSPECT \[12\] is an optical model of a plant leaf that is capable of

![Fig. 1. (Color online) Specific absorption coefficients of water, chlorophyll, and dry matter determined after a chemical extraction \[14\].](image)
computing its spectral transmittance and reflectance from 400 nm to 2500 nm. PROSPECT is based on a dielectric model of a plant leaf that could be composed of one or several layers and is able to account for multiple internal reflections within them [13]. Furthermore, the attenuation of electromagnetic radiation due to spectral absorption by biochemical compounds is considered for each internal reflection. For this reason, it is important to know the spectral refractive index of leaf material and the specific absorption coefficients for each biochemical compound [14]: chlorophyll, water, and dry matter (Fig. 1). Finally, the formalism could be extended to a continuous layer [15], with the possibility of describing different structures of plant leaf mesophyll, namely, monocotyledon leaves (unique compact layer) and dicotyledon leaves (several layers).

As a result, the radiative transfer model PROSPECT incorporates three biochemical parameters: chlorophyll content $C_{ab}$, water content $C_w$, dry matter content $C_m$, plus an additional parameter $N$, to simulate spectral transmittance and reflectance. The parameter $N$, called also parameter of structure, describes the mesophyll structure of a plant leaf and it could be used to differentiate one species from another [16, 17].

For this reason, PROSPECT can be used as a forward model that allows us to apply inverse theory modeling [18]. It can be performed in order to estimate all parameters or a selected biochemical parameter from the transmittance and the reflectance measured by RAMIS.

3. Description of RAMIS

The prototype RAMIS [19] consists principally of two functional parts: a source system of photonic radiation to illuminate the adaxial faces of the leaf and a photonic sensor system with the function of performing transmittance measurements.

A. Source System

In order to justify the spectral band position of the RAMIS source system, several transmittance spectra were computed using PROSPECT, varying each time one of the biochemical parameters (Fig. 2). This indicates where the spectral sensitive zones are located in relation with each biochemical leaf compound. Consequently, we are able to determine the optimal wavelength positions on the spectral transmittance. However, some of the ideal wavelengths were shifted to other values as a result of limitations on the nominal values of LED wavelengths available in the market. The bands chosen for the source system are centered on 656, 721, 843, 937, and 1550 nm.

However, in our application of determination of water and dry matter contents, it is just necessary to use 843, 937, and 1550 nm wavebands. The capability of RAMIS to determine leaf chlorophyll content, using the wavelengths of 656 nm and 721 nm, will not be discussed in the present paper.

Light emission diodes (LEDs) (Roithner Lasertechnik, Vienna, Austria) were used with a characteristic band-centered spectrum of nominal values (Fig. 2). Their respective spectral emissions were measured in order to verify and use them in the adaptation
of the PROSPECT model. Finally, they are arranged in a compact formation (Fig. 3) to obtain an approximation of an incident light source that illuminates the plant leaf sample.

B. Detector System

The RAMIS detector system is formed by a Si/Ge double layer photodiode (Judson Technologies, Philadelphia, Pennsylvania, USA) designed with a responsivity range of 500 to 2000 nm. It is composed of two spectral zones, situated between 500 and 1200 nm, corresponding to the photodiode layer of Si that it is sensitive to the spectral emission of 843 and 937 nm photodiodes. The second zone is situated between 1000 and 2000 nm, corresponding to the photodiode layer of Ge, and is sensitive at 1550 nm (Fig. 4).

The mechanical mount of the Si/Ge double layer photodiode sensor is a cylindrical compartment with the sensor situated at the center, aimed toward the source system on the opposite jaw of the RAMIS prototype (Fig. 5). The prototype is assembled in a pincer configuration, and the sample of plant leaf is clamped between the photonic source and sensor.

The electronic systems that drive the source and the sensor are placed in each respective jaw. The source controller circuit is composed of a current driver circuit with the functionality to activate each photodiode independently and to control the current stability of each one. This circuit is controlled by a data acquisition universal serial bus (USB) module using its digital input/output (I/O) parallel port. The activation procedure of each photodiode is controlled by the state of the digital I/O port bits; in this case the source is controlled by five bits, one bit for each photodiode.

For the sensor, a current-to-voltage circuit and an amplifier stage were implemented with the functionality to produce an output voltage level $V$. 

Fig. 3. (Color online) Source system of the RAMIS prototype conformed of five LEDs: (a) transversal view, (b) top view of the close compartment to avoid external light interference in the measurement procedure, and (c) mechanical support for the source.

Fig. 4. (Color online) Spectral responsivity of Si/Ge sensor (Judson Technologies).

Fig. 5. (Color online) Detector system of the RAMIS prototype composed of Si/Ge double layer photodiode sensor: (a) transversal view, (b) top view of mechanical configuration, and (c) mechanical support for the photodiode.
proportional to intensity $I$ of the radiation incident on the sensor. Two circuits were implemented, one for each channel of the Si/Ge photodiode. These two output voltage lines are connected to the analog-to-digital converter (ADC) port of the USB data acquisition module. The retrieved values are recorded on the computer that controls the data acquisition USB module (Fig. 6).

The procedure to perform the measurement consists of activating each photodiode for an activation time $t_{on}$. This time $t_{on}$ should be small enough to avoid an increase of the temperature of each photodiode; consequently it could affects their radiated power while the measurement procedure is taking place. At the same time the ADC port acquires the signal for a sampling time $t_s$. The sampling time $t_s$ is shorter than the activation time $t_{on}$ and centered to avoid the rising and the falling edges of the activation signal (Fig. 7).

4. Signal Treatment
In order to achieve transmittance measurements with the RAMIS prototype, the transmittance measurement intensity $I_0$ must be defined as a measure performed by the source and detector system without any leaf sample inside. This intensity $I_0$ is transduced as a proportional output voltage $V_0$ from the electronic circuit of the sensor. If the air attenuation of the source signal is neglected, then the intensity $I_0$ serves as a reference signal that is related to a transmittance equal to unity. However, because such an intensity is a function of the photodiode of the source system (843, 937, and 1550 nm), it is important to define the reference intensity $I_0(\lambda_{C,i})$, where the wavebands are centered at each specific $\lambda_{C,i}$. Consequently, we must define a sequential activation for each diode of the source system.

After $I_0(\lambda_{C,i})$ has been determined, the system is ready to measure the signal intensity of incident radiation from the source, with a plant leaf sample placed between the source and sensor system. The same sequential procedure of LED activations must be used at this point also, due to the monochannel characteristic of the Si/Ge sensor in the wavelength range of 500 to 1200 nm, where all spectral LED emissions are situated. The intensities $I(\lambda_{C,i})$ are then defined for each specific $\lambda_{C,i}$ from the source that goes through the plant leaf sample (Fig. 8). In the same way as $I_0(\lambda_{C,i})$, the intensities $I(\lambda_{C,i})$ are transduced as proportional output voltage $V$ for each diode wavelength $\lambda_{C,i}$.

At this point, with the measurements of $I_0(\lambda_{C,i})$ and $I(\lambda_{C,i})$, the transmittance measured by the RAMIS system can be defined for each photodiode waveband as follows:

$$T^R(\lambda_{C,i}) = \frac{V(\lambda_{C,i})}{V_0(\lambda_{C,i})} = \frac{I(\lambda_{C,i})}{I_0(\lambda_{C,i})}.$$  \hspace{1cm} (1)

It is important to note here that the Si/Ge sensor gives a response signal that is proportional to the incident intensity $I$, integrated along the waveband centered at a specific $\lambda_{C,i}$. This aspect is considered in computing the plant leaf transmittance in the radiative spectral model. Furthermore, the spectral responsivity of the Si/Ge detector is not constant.
through the interval of 500 to 2000 nm and the spectral emissions of each LED have a characteristic shape (Fig. 9). Last, the transmittance measured by RAMIS is not a hemispherical quantity. For these reasons, two important procedures must be carried out: spectral treatment and hemispherical correction.

A. Spectral Treatment

Based on the characteristics of the photonic components used in the RAMIS prototype, and considering the theoretical method that describes the phenomena of leaf light transmittance, it is necessary to consider the spectral response of the sensor and the spectral emission of the source. For this reason, the spectral emission of each light-emitting diode is defined as $E(\lambda, \lambda_C)$, where its characteristic spectrum is centered at a wavelength $\lambda_C$ (Fig. 9). We also define $S(\lambda)$ as the spectral responsivity of the Si/Ge double layer photodiode (Fig. 4).

Next, the hemispherical transmittance spectrum of a plant leaf is defined as $T_H(\lambda)$, defined for the
500 to 2000 nm wavelength interval. It is then possible to express the intensity measured by the RAMIS prototype by only regarding the spectral behavior of the photonic components [20]. The intensity measured by RAMIS can be written as

\[ I_H(\lambda_{C,i}) = k \int_0^\infty E(\lambda, \lambda_{C,i}) T_H(\lambda) S(\lambda)d\lambda, \]  

where the intensity \( I_H(\lambda_{C,i}) \) is a function of the source spectral band centered at \( \lambda_{C,i} \) (Fig. 9) and \( k \) is a factor of proportionality. The case where there is no material between the source and sensor must be considered. Here, the spectral transmittance \( T_H(\lambda) \) should be equal to unity for all \( \lambda \). The reference intensity can then be defined as

\[ I_{0,H}(\lambda_{C,i}) = k \int_0^\infty E(\lambda, \lambda_{C,i}) S(\lambda)d\lambda, \]  

assuming provisionally that the totality of the transmitted light enters into the detector. Finally, using Eqs. (2) and (3), the hemispherical transmittance is defined as

\[ T^{R}_H(\lambda_{C,i}) = \frac{I_H(\lambda_{C,i})}{I_{0,H}(\lambda_{C,i})} = \frac{\int_0^\infty E(\lambda, \lambda_{C,i}) T_H(\lambda) S(\lambda)d\lambda}{\int_0^\infty E(\lambda, \lambda_{C,i}) S(\lambda)d\lambda}. \]  

This expression represents the hemispherical transmittance for each photodiode wavelength \( \lambda_{C,i} \) implemented into the RAMIS prototype.

B. Hemispherical Correction

Equation (4) only describes the spectral characteristics of an ideal hemispherical sensor with similar spectral characteristics to those of the RAMIS Si/Ge sensor. The idea implicit in this formulation is to have a relationship between RAMIS measurements and a physical model of transmittance. In this case, we are interested in the implementation of the radiative transfer model PROSPECT. However, PROSPECT computes hemispherical transmittances, which are geometrically different from the transmittances measured by RAMIS. For this reason, it is necessary to perform a geometrical correction, specifically, to find a relationship between the RAMIS transmittance and the computed spectral hemispherical transmittance.

For this reason, reference measurements of transmittance were performed, especially of hemispherical leaf transmittance. For this purpose, a spectrometer (Analytical Spectral Device ASD from FieldSpec-FR) with an integrating sphere (LICOR) was used to perform the reference measurements of hemispherical transmittance.

In this case, the hemispherical correction can be described, and it is possible to find a relationship between the transmittance measurement of the RAMIS prototype and the hemispherical measurements. Using Eq. (1) as a definition of RAMIS transmittance and regarding the Lambertian characteristic of the sources, we define

\[ T^R_H(\lambda_{C,i}) = \xi_{\lambda_{C,i}} T^R(\lambda_{C,i}), \]  

where \( T^R(\lambda_{C,i}) \) is the transmittance measured by RAMIS, \( T^R_H(\lambda_{C,i}) \) is the hemispherical transmittance measured by the ASD, and \( \xi_{\lambda_{C,i}} \) is the correction coefficient associated with the geometrical differences between RAMIS and the ASD system.

It is observed that the correction parameter \( \xi_{\lambda_{C,i}} \) does not depend strongly on the sampled leaf species; they have thereafter been assumed independent of these.

5. Material and Methods

Two electronic systems are incorporated into RAMIS. The first drives each LED sequentially, while the second makes a current-to-voltage conversion of the output signal from the detector. Both electronic systems work in synchrony during the measurement procedure.

In order to have a portable instrument, an automatic data acquisition module was incorporated into the electronic system of the prototype. This consists of a NI USB-609 module from National Instruments with the following characteristics: a 14 bit ADC input and I/O digital ports. Thus, we can control the LED activation and the data acquisition of the prototype electronic system. Consequently, this module enables the connection of RAMIS to a laptop and uses any G programming language such as LabView to control the acquisition and processing data.

The measurement procedure on a plant leaf sample consists in performing two transmittance measurements. The first one, a measurement without a plant leaf sample, determines the reference intensity \( I_0(\lambda_{C,i}) \). The second determines the intensity that passes through the plant leaf sample \( I(\lambda_{C,i}) \). The transmittance measured by RAMIS can then be defined by Eq. (1). The distance between the sample and the sensor must be constant for all measured samples, as well as the distance between the sample and the source.

The objective of the experiment was to verify the performance of the RAMIS prototype for estimating water and dry matter contents. In order to validate its performance, a comparison was made between RAMIS estimations and the values of water and dry matter contents measured by a direct procedure of the drying process. A variety of plant leaves was used Table 1.

The experimental water thickness (\( C_w \)) of each plant leaf sample can be defined as the water content per unit leaf surface, denoted as

\[ C_w = \frac{M - M_{\text{dry}}}{S}, \]  

where \( M \) is the mass of the sample leaf when the transmittance measurement is performed, \( M_{\text{dry}} \) is the dry mass of the leaf at the end of the drying process, and \( S \) is the area of the leaf (in cm²). Using the density of water, it is possible to express \( C_w \) as a
water thickness that it is related to an equivalent optical thickness of water.

At the same time the mass \( M \) is measured, the measurements of \( I(\lambda_{C_1}) \) and \( I_0(\lambda_{C_1}) \) were performed by RAMIS, and finally the transmittance \( T^R(\lambda_{C_1}) \) was computed using Eq. (1) for each wavelength.

### 6. Results and Discussion

The respective values of measured transmittances \( T^R(\lambda_{C_1}) \) are denoted as the input parameters for the inversion procedure.

Into the inversion procedure, the source spectral emission \( E(\lambda, \lambda_{C_1}) \) and the sensor spectral responsivity \( S(\lambda) \) are included for PROSPECT model adaptation. Then, the output parameters of the inversion procedure are the water thickness \( C_w \), the dry matter content \( C_m \), and the internal structure parameter \( N \) (Fig. 10).

The three values of measured transmittance \( T^R(\lambda_{C_1}) \) could be used for inverting the set of biochemical parameters at the same time (\( N, C_w, \) and \( C_m \)); this implies the use of three wavelengths implemented into the RAMIS prototype. Furthermore, it is possible to invert each parameters individually if they are not strongly correlated; this is the case of the dry matter \( C_m \) at 843 nm wavelength transmittance. However, the leaf structure parameter \( N \) must be inverted at the same time with all or selected biochemical parameters; then for inverting one parameter we must use two wavelength at least.

For the inversion procedure of water estimations, we must deal with the correlation of the dry matter at the spectral zone where the photodiodes wavebands were placed. For this reason, the leaf structure parameter \( N \) must be inverted together with the water \( C_w \) and the dry matter \( C_m \) content, so three wavelengths must be used in this case: 843, 937, and 1550 nm.

The result of water estimation content, from different leaves samples, shows a fair agreement with the water content measured by the drying process, as is shown in Fig. 11.

For dry matter estimation \( C_m \), only two wavebands were used: 843 and 937 nm. The values of water

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**Table 1. Leaves of Different Plants Species Measured by RAMIS for Water and Dry Matter Estimations**

<table>
<thead>
<tr>
<th>Latin Name</th>
<th>Common Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prunus avium</td>
<td>Sweet Cherry</td>
</tr>
<tr>
<td>Hedera helix</td>
<td>Common Ivy</td>
</tr>
<tr>
<td>Davidia involucrata</td>
<td>Dove Tree</td>
</tr>
<tr>
<td>Quercus agrifolia</td>
<td>Coast Live Oak</td>
</tr>
<tr>
<td>Ficus benjamina</td>
<td>Weeping Fig</td>
</tr>
<tr>
<td>Ginkgo biloba</td>
<td>Ginkgo</td>
</tr>
<tr>
<td>Fagus sylvatica purpurea</td>
<td>Purple Beech</td>
</tr>
<tr>
<td>Ilex aquifolium</td>
<td>European Holly</td>
</tr>
<tr>
<td>Laurus nobilis</td>
<td>Laurel Tree</td>
</tr>
<tr>
<td>Syringa vulgaris</td>
<td>Common Lilac</td>
</tr>
<tr>
<td>Platanus hispanica</td>
<td>London Plane</td>
</tr>
<tr>
<td>Rosa eglanteria</td>
<td>Eglantine Rose</td>
</tr>
<tr>
<td>Tilia platyphyllos</td>
<td>Large-Leaved Linden</td>
</tr>
</tbody>
</table>

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Fig. 10. (Color online) Inversion procedure of the transmittances \( T^R(\lambda_{C_1}) \), measured by RAMIS in order to compute the biochemical leaf parameters \( N, C_w, \) and \( C_m \) using the adaptation of the radiative transfer model PROSPECT.
contents $C_w$ previously computed were used as a priori information as a result of its small correlation at 937 nm (Fig. 2(b)) for inverting the dry matter content $C_m$. Also, the structure leaf parameter $N$ must be inverted as well. The results obtained do not agree with those obtained by measuring the dry mass directly, as is shown in Fig. 12.

The dispersion of the estimated values could be due to different factors such as uncertainty of the digital acquisition system and the geometrical variation of source–sensor configuration.

Also, the reasons for the discrepancies are linked to the small dependence of the transmittance with $C_m$, as can be seen in Fig. 2(b). In fact, a change of
3% of the measured transmittances values produces a variation by more than 100% on the computed dry matter estimation $C_m$.

7. Conclusions

Our aim in developing RAMIS was to present an instrument capable of performing measurements of the main biochemical compounds of plant leaves, with the principal characteristic of being a portable instrument able to perform measurements in situ. The capability of RAMIS to determine water content $C_w$ was demonstrated by computing the inversion of transmittance measurements. The choice of wavelength used in the developing process of the prototype is based on the results of the spectral response of plant leaf transmittance, verifying the spectral sensitivity zone at each biochemical compound and avoiding spectral zones with high correlation between them and the LED wavelength availability.

The estimation of dry matter, regarding its small effect on spectral variations (Fig. 2), is affected by the limited accuracy of transmittance measurements; consequently, dry matter estimation was not accomplished.

The implementation of the radiative transfer model PROSPECT into the RAMIS prototype shows a good result regarding water estimation.

The capability of the radiative transfer model PROSPECT to compute also the plant leaf reflectance as a function of its biochemical contents could be used to improve the accuracy of RAMIS by setting a reflectance sensor in an adequate position [21].

These possible upgrades have been considered based on the conception of a portable instrument capable of performing multi-biochemical measurements using different wavelengths [22] and using an optical leaf model. This device could perform biochemical leaf estimations without a previous calibration procedure for any internal leaf structure.

We thank the Centre Régional d’Innovation et de Transfert Technologique—Conception Circuits Spéciaux et Télématique (CRITT-CCST), Force A Company, and Diderot Valorisation Bureau of the Paris Diderot University. We thank also Natacha Vendola for her contribution on this work. This project received the 2006 Environmental Innovative Technology Award of ADEME (French Agency for Environment and Energy Management), Contribution No. IPGP 2636.

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