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**Fourier Transform of Delayed Fluorescence as an Indicator of Herbicide Concentration**

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Abstract

It is well known that delayed fluorescence (DF) from Photosystem II (PSII) of plant leaves can be potentially used to sense herbicide pollution and evaluate the effect of herbicides on plant leaves. The research of using DF as a measure of herbicides in the literature was mainly conducted in time domain and qualitative correlation was often obtained. Fourier transform is often used to analyze signals. Viewing DF signal in frequency domain through Fourier transform may allow separation of signal components and provide quantitative method for sensing herbicides. However, there is a lack of an attempt to use Fourier transform of DF as an indicator of herbicide. In this work, the relationship between the Fourier transform of DF and herbicide concentration was theoretically modelled and analyzed, which immediately yielded a quantitative method to measure herbicide concentration in frequency domain. Experiments were performed to validate the developed method.

Keywords: Fourier Transform; Delayed Fluorescence; Herbicide
1. Introduction

Extensive herbicide application has resulted in water pollution (Lambrev and Goltsev, 1999; Dewez et al., 2002; Guo and Tan, 2010). Herbicides may persist for decades in soil because of low rate of degradation (Capriel et al., 1985; Lesan and Bhandari, 2003), which may cause harm to human health and herbicide-sensitive crops when herbicides are transported to water reservoirs by runoffs (Bowmer, 1991). The conventional measurement of herbicides often requires complex instruments and it is often performed in the laboratory; therefore, there is a need to develop portable technique to measure herbicides. It is well known that the emission of delayed fluorescence (DF) from Photosystem II (PSII) of plant leaves couples with forward photosynthetic activities (Lambrev and Goltsev, 1999). Herbicides affect photosynthesis and the emission of DF. Herbicides thus can be potentially measured through DF signal (Guo and Tan, 2009; Guo et al., 2010; Guo and Tan, 2010). Katsumata et al. (2006) studied the effects of Simazine (CAT) and 3,5-dichlorophenol (3,5-DCP) on the growth of a green alga and a DF-based method for measuring the chemicals could be developed. In Li and Xing (2006), the effect of DCMU [3-(3,4-dichlorophenyl)-1,1-dimethylurea] on plant photosynthesis and DF was investigated, and a DF-based biosensor for measuring DCMU was proposed. Guo and Tan (2010) modeled the processes of herbicide diffusion into plant leaves and binding to the active sites, which led to a biophotonic method to measure the concentrations of herbicides. All the efforts of using DF as a measure of herbicides in the literature were conducted in time domain and some of them only provide qualitative measurement for herbicide.

Because signals have different frequency characteristics, Fourier transform is commonly performed in many research fields to view the signal from another angle, which allows separating signal components and analyzing the frequency characteristics of signal energy.
distribution (Frigo and Johnson, 1998; Sitnikova et al., 2009; Cunha and Richter, 2012; Sharma et al., 2013). The energy of a signal (or dynamics) relatively distributes more in the lower frequency band if the signal (or dynamics) changes slowly; otherwise, the energy relatively distributes more in the higher frequency band if the signal (or dynamics) changes rapidly. Herbicides modify the photosynthetic system and thus change the activities or paces of the system. The DF energy distribution at different frequencies as affected by herbicides can be analyzed through Fourier transform. Analysis of DF signal in frequency domain thus may provide quantitative plant-physiological-based method for sensing herbicides. However, there is no attempt to use the Fourier transform of DF signal to measure herbicides in the literature. In this work, the relationship between the Fourier transform of DF signal and herbicide concentration was theoretically analyzed and experimentally validated. This yields a biosensor to measure herbicide concentration based on the Fourier transform of DF signals. This work also provides an example of using Fourier transform of DF to sense plant physiology and environmental changes in frequency domain.

2. Fourier Transform of DF as a Measure of Herbicide Concentration

An electron of P680 (PSII reaction center) will be excited to a higher energy level when P680 absorbs a photon from its antenna complex (Goltsev et al., 2003). The excited electron is not stable and may return the ground state through the emission of heat or prompt chlorophyll fluorescence (PF). PF has a very short lifetime because the process of an electron relaxing from the high energy level to the ground level is very fast (Guo and Tan, 2011; Guo and Tan, 2014). The excited electron may also be transferred forward for the production of ATP along electron transport chain (ETC), which includes the primary electron acceptor in photosystem II,
pheophytin, and the first and the second quinone electron acceptors, plastoquinone A (QA) and plastoquinone B (QB), respectively (Goltsev and Yordanov, 1997; Blankenship, 2002). Because of the existence of reverse chemical reactions, there is a chance for the electron on the ETC to come back and recombine with a PSII chlorophyll molecule, which will re-excite the chlorophyll molecule and lead to chlorophyll fluorescence emission again. Because the reverse electron transport is slow, this type of fluorescence has a long lifetime and is thus called as DF (Goltsev et al., 2003; Guo and Tan, 2009; Guo and Tan, 2013a).

Herbicides typically function by binding to certain sites on the ETC and impeding electron transport (Oettmeier, 1992; Lazar et al., 1998; Oettmeier, 1999; Lambrev and Goltsev, 1999, Guo and Tan, 2009; Guo et al., 2010). For example, around one half of the commercial herbicides function by binding to QB sites (Oettmeier, 1992; Oettmeier, 1999). When a herbicide molecule binds to a site on the ETC, electrons cannot be transferred to the site. This will shorten electron transport pathways and influence DF emission in time domain, which unavoidably leads to the change of DF spectrum in frequency domain. This is evident from the system eigenvalues of the DF model in Guo et al. (2010).

If a leaf is submerged in a herbicide solution, herbicide molecules will diffuse into the leaf and bind to the corresponding sites. Assumed that the test herbicide concentration is \( C_0 \), the binding rate of DCMU to QB sites is \( k \), the concentration of reaction centers (RC) without herbicide binding is \( r \), and submerging time is \( t \). At a later stage of submergence, the diffusion process can be considered as close to steady state. The herbicide concentration inside the leaf can therefore be treated as the same as the concentration of herbicide solution. The RCs without herbicide binding can thus be represented as an exponential function at a later stage of submergence as shown in Eqn. 1.
\[ r(t) = r_s e^{-k_C(t-t_s)} \quad (t \geq t_s) \]  \hspace{1cm} (1)

where \( r_s \) is the concentration of RCs without herbicide binding at submerging time \( t_s \).

According to Eqn. 1, the concentration of RCs without herbicide binding (active RCs) at \( t = t_s, t_s + \Delta t, \cdots, t_s + (N-1)\Delta t \) is:

\[ r_{n\Delta t} = r_s e^{-k_C n \Delta t} \quad n = 0, 1, \cdots, N-1 \]  \hspace{1cm} (2)

where \( n \) is an index of submerging time, \( \Delta t \) is a fixed time interval, \( r_{n\Delta t} \) is the concentration of active RCs at \( t_s + n\Delta t \).

Assume that a short pulse is used to excite the submerged leaf at time \( t = t_s, t_s + \Delta t, \cdots, t_s + (N-1)\Delta t \), and assume that the pulse width and the DF record time following the pulse are much shorter than \( \Delta t \). These assumptions can be easily guaranteed in experiments by setting a larger \( \Delta t \). The DF emission following any of the short excitation pulses has two components: emissions from active RCs \( f_{ub}(t_i) \) (\( ub \) stands for “unbound RC”) and that from herbicide-bound RCs \( f_b(t_i) \), where \( t_i \) is the time variable for DF recording time (starting at the end of the excitation pulse) following any of the short excitation pulses.

If \( F_{ub}(w) \) and \( F_b(w) \) are used to denote the Fourier transform of \( f_{ub}(t_i) \) and \( f_b(t_i) \), they can be expressed as Eqns. 3 and 4 according to the definition of Fourier transform (Harris and Stocker, 1998).

\[ F_{ub}(w) = \int_{-\infty}^{\infty} f_{ub}(t_i)e^{-iwt} dt_i = F_{ub}^R(w) + iF_{ub}^I(w) \]  \hspace{1cm} (3)

\[ F_b(w) = \int_{-\infty}^{\infty} f_b(t_i)e^{-iwt} dt_i = F_b^R(w) + iF_b^I(w) \]  \hspace{1cm} (4)

where \( i \) is imaginary unit, \( F_{ub}^R(w) \) and \( F_{ub}^I(w) \) are the real part and imaginary part of \( F_{ub}(w) \), \( F_b^R(w) \) and \( F_b^I(w) \) are the real part and imaginary part of \( F_b(w) \), respectively.
The DF emission is proportional to the concentration of RCs because one RC works as a unit for photochemical reaction (Guo and Tan, 2009). If \( v \) denotes the leaf sample volume under investigation and \( R_0 \) denotes the initial RC concentration, the DF emission from all the active and DCMU-bound RCs following the \( n^{th} \) pulse excitation is:

\[
f_{n,\Delta} (t_i) = vr_s e^{-kC_\alpha n \Delta} f_{ub} (t_i) + v (R_0 - r_s e^{-kC_\alpha n \Delta}) f_b (t_i)
\]  
(5)

The Fourier transform for \( f_{n,\Delta} (t_i) \) is:

\[
F_{n,\Delta} (w) = \int_{-\infty}^{\infty} f_{n,\Delta} (t_i) e^{-i\omega t_i} dt_i = \int_{-\infty}^{\infty} \left[ vr_s e^{-kC_\alpha n \Delta} f_{ub} (t_i) + v (R_0 - r_s e^{-kC_\alpha n \Delta}) f_b (t_i) \right] e^{-i\omega t_i} dt_i
\]

\[
= vr_s e^{-kC_\alpha n \Delta} F_{ub} (w) + v (R_0 - r_s e^{-kC_\alpha n \Delta}) F_b (w)
\]

Eqn. 6 can be further written as:

\[
F_{n,\Delta} (w) = vr_s [F_{ub} (w) - F_b (w)] e^{-kC_\alpha n \Delta} + vR_0 F_b (w)
\]  
(7)

The real part and imaginary part of \( F_{n,\Delta} (w) \) can thus be expressed as:

\[
F_{n,\Delta}^R (w) = vr_s [F_{ub}^R (w) - F_b^R (w)] e^{-kC_\alpha n \Delta} + vR_0 F_b^R (w)
\]  
(8)

\[
F_{n,\Delta}^I (w) = vr_s [F_{ub}^I (w) - F_b^I (w)] e^{-kC_\alpha n \Delta} + vR_0 F_b^I (w)
\]  
(9)

The difference of the real part of \( F_{n,\Delta} (w) \) and \( F_{(n+1),\Delta} (w) \) is:

\[
F_{(n+1),\Delta}^R (w) - F_{n,\Delta}^R (w) = vr_s [F_{ub}^R (w) - F_b^R (w)] (e^{-kC_\alpha n \Delta} - 1)e^{-kC_\alpha n \Delta}
\]  
(10)

The difference of the imaginary part of \( F_{n,\Delta} (w) \) and \( F_{(n+1),\Delta} (w) \) is:

\[
F_{(n+1),\Delta}^I (w) - F_{n,\Delta}^I (w) = vr_s [F_{ub}^I (w) - F_b^I (w)] (e^{-kC_\alpha n \Delta} - 1)e^{-kC_\alpha n \Delta}
\]  
(11)

According to Eqns. (10) and (11),

\[
\log |F_{(n+1),\Delta}^R (w) - F_{n,\Delta}^R (w)| + \log |F_{(n+1),\Delta}^I (w) - F_{n,\Delta}^I (w)| = 2 \log |v_r s| + 2 \log |e^{-kC_\alpha n \Delta} - 1|
\]

\[
+ \log |F_{ub}^R (w) - F_b^R (w)| + \log |F_{ub}^I (w) - F_b^I (w)| - 2kC_\alpha n \Delta t
\]  
(12)
Let
\[ F_{LOG} = \log|F_{(n+1)\Delta t}^R (w) - F_{n\Delta t}^R (w)| + \log|F_{(n+1)\Delta t}^I (w) - F_{n\Delta t}^I (w)|, \]

\[ B = 2 \log|v_2| + 2 \log|e^{-kC_0n\Delta t} - 1| + \log|F_{ub}^R (w) - F_{h}^R (w)| + \log|F_{ub}^I (w) - F_{h}^I (w)| \]

Eqn. (12) can be written as:
\[ F_{LOG} = B - 2kC_0n\Delta t \quad (13) \]

The normalization of Eqn. 11 by a constant value at each frequency only changes the \( B \) value in Eqn. (13) and does not affect the slope \(-2kC_0\). Obviously, for any given frequency, \( F_{LOG} \) has a linear relationship with \( n\Delta t \) and the slope is \(-2kC_0\), which provides quantitative information for determining herbicide concentration \( C_0 \). The determined slope does not include the initial RC concentration, leaf size, and the detailed DF emission kinetics (\( F_{ub} (t) \) and \( F_{h} (t) \)).

This means the indicator based on Eqn. 13 will not be affected by those sample variations. From the linear dependence of \( F_{LOG} \) on \( n\Delta t \), the slope \(-2kC_0\) can be estimated. To cancel the binding rate \( k \), a reference herbicide solution with known concentration can be used. Binding rate \( k \) of two segments of one leaf may be assumed the same if they are treated with the test and the reference herbicide solutions at the same time. From the reference measurement, \( k \) can be calculated. The \( F_{LOG} \) at multiple frequencies could be used to determine the value of \(-2kC_0\) for enhancing signal-to-noise ratio by summarizing Eqn. 13 at multiple frequencies. For measurements of leaf pairs, the estimated concentration will not be affected if they are normalized by the same factor because the ratio of the two slopes maintains the same value.

The validity of the developed theory depends on whether the linear relationship in Eqn. 13 can be observed in experiments. The experimental results in this work clearly show the existence of the linear relationship.
3. Experiments

Leaves of garden bean and spinach plants were used. The garden bean plants were grown in pots with a 10-cm height and a 9-cm diameter in the laboratory. The laboratory had a constant room temperature 25°C. All the garden bean plants were kept in open air and under fluorescent light (Model F20T12/CW, Philips, New York, USA. Color temperature: 4100 K. Wavelength range: 410 – 720 nm). The fluorescent light was controlled in 12:12-hour on-off cycles. Water was regularly applied to irrigate the plants. The garden bean plants were 30-day old when the experiments were conducted. The spinach plants were purchased from a local store.

The experiments were conducted in the laboratory and the experimental setup is shown in Fig. 1. Test samples were placed in a light-tight chamber. A red LED with peak value at 680 nm (Marubeni L680-06AU) was used to illuminate the sample. The illumination light was delivered through an 8-mm liquid light guide (ORIEL 77628). DF photons were collected through a second light guide and fed into a channel multiplier tube (CMT, PerkinElmer MH1373P).

CMT was gated electronically. The photoelectric output pulses from the CMT were recorded with a gated photon counter/multiscaler card (PMS-400, Becker & Hickl GmbH) plugged in a host computer. The computer also controlled the LED illumination, CMT gating, and data acquisition timing.

![Fig. 1. Experimental setup](image-url)
Leaf discs were kept in water for 20 minutes to reduce the influence of water diffusion before submerged in DCMU solutions. After dark adaptation, an illumination pulse was applied to the sample every 15 minutes. The pulse duration was 0.5 s and DF photons were recorded for 120 s after the illumination pulse was turned off. The sampling frequency was set as 100 Hz. The initial part of the DF signal is very strong compared to those in second or minute range. In order to protect CMT and have a good resolution for the signal in minute range, DF recording was started 5 ms after the excitation light was turned off. Twenty-five leaves or leaf segments were measured. In this work, the DF spectrum in the low frequency range (< 10 Hz) was used to compute the slope through Eqn. 13.

4. Results

An example of measured signals from control and DCMU-stressed leaves following a pulse excitation is shown in Fig. 2. As expected, the DF signal can last for minutes. The amplitude spectra of the signals are shown in Fig. 3. According to the Nyquist–Shannon sampling theorem, the maximum effective analyzed frequency was 50 Hz (half of the sampling frequency 100 Hz) as shown in Fig. 3. Although the exponential/multiple exponential curves in Figs. 2 and 3 contain the information of plant physiology and environmental changes, the information is not directly available from the raw data.
Fig. 2. DF signal in time domain

Fig. 3. DF amplitude spectrum

Fig. 4 (A) shows the pattern of the real spectrum of DF from control and DCMU-stressed leaves, Fig. 4(B) shows the pattern of the imaginary spectrum of DF from control and DCMU-stressed leaves, which are expressed in Eqns. 8 and 9. Because the large dynamic range of the data and the existence of noise, only the data for the first 10 Hz were shown. The real spectrum and the imaginary spectrum have different shapes. The real spectrum is always positive and monotonically decreases with frequency, but the imaginary spectrum is negative and the absolute value increase with frequency first and then decreases with frequency. The shape difference implies they play different roles in the signal composition in frequency domain. DCMU binds to Q_B sites, which makes Q_A not able to pass electrons to Q_B. The DF signal from control samples reflects the dynamics of the whole electron transport chain, but the DF signal from DCMU-
stressed samples only reflects the electron transport before $Q_B$ sites. This explains the spectrum differences for the DF from control leaves and DCMU-stressed leaves.

![Graph](image)

Fig. 4. Real spectrum (A) and imaginary spectrum (B) for the first 10 Hz of control and DCMU-stressed leaves

As stated in Section 2, whether the developed method can be used to sense herbicide concentration quantitatively is determined by whether the linear relationship in Eqn. 13 holds. Fig. 5 shows an example of the $F_{LOG}$ vs. submerging time from garden bean leaves in 80-μM DCMU solution. It clearly shows a straight line as predicted by Eqn. 13. The error bars are the standard deviation of the results computed at different frequencies. For all the test samples, experimental data could be fitted with a straight line.
Fig. 5. An example of the linear relationship between $F_{LOG}$ and submerging time $t$ for garden bean leaves in 80-μM DCMU solution.

Fig. 6 shows the results of leaf segment pairs both in 80-μM DCMU solution. If the second one is control solution, the concentration of the test solution will be determined as 69.33 μM with a relative error of 13.33% (=1-69.33/80). Fig. 7 shows the results of leaf segment pairs in 80-μM DCMU solution and 40-μM DCMU solution. If the concentration of 80-μM DCMU solution is known, the concentration of the 40-μM DCMU solution will be determined as 31.51 μM with a relative error of 21.22% (=1-31.51/40). Because the two pairs of a leaf were not measured at a time, the error was expected considering plant biological clock and injury effect. If an instrument with two channels is developed to measure the two pairs at the same time, the error is expected smaller.
Fig. 6. The linear relationship between $F_{LOG}$ and submerging time $t$ for half-leaf pairs while both were in 80-μM DCMU solution

![Graph](image)

$$F_{LOG}(t) = -0.0026t + 0.0033 \quad (R^2=0.960)$$

$$F_{LOG}(t) = -0.0030t - 0.0002 \quad (R^2=0.999)$$

Fig. 7. The linear relationship between $F_{LOG}$ and submerging time $t$ for half-leaf pairs while one half is in 80-μM and the other half is in 40-μM DCMU solutions

![Graph](image)

$$F_{LOG}(t) = -0.0033t + 0.0063 \quad (R^2=0.982)$$

$$F_{LOG}(t) = -0.0013t + 0.0327 \quad (R^2=0.919)$$

Because developed method in Eqn. 13 senses the accumulated herbicide molecules diffused into the leaf rather than herbicide concentration directly, the effect of low concentration can be compensated by longer diffusion time and higher binding rate. The developed method is thus expected to be able to sense herbicide with very low concentration. When spinach leaves are used, the linear relationship predicted by Eqn. 13 could be clearly observed in 1 ppm (4.3 μM) DCMU solution as shown in Fig. 8. This further proves the effectiveness of the developed DF-Fourier-transform-based method.
Fig. 8. The linear relationship between $F_{LOG}$ and submerging time $t$ for spinach leave in 1 ppm (4.3 μM) DCMU solution

5. Discussion

Whether the assumption of diffusion process can be considered as close to steady state can be easily verified in experiments. When a leaf is submerged in herbicide concentration, the initial dynamics includes diffusion process and herbicide binding process, which is higher order nonlinear dynamics and implies that a simple linear relationship cannot fit $F_{LOG}$ through Eqn. 13. Later on, the diffusion process closes to steady state, the binding process will dominate and a straight line will be able to fit the data as predicted by Eqn. 13. The experiments in this work were performed enough long and $F_{LOG}$ appears as a straight line as shown in the Figures.

DF from PSII can be potentially used to sense photosynthesis rate, herbicide, metal pollution, drought stress, plant senescence, nutrients, chilling stress, heat stress, salt stress, acid rain, and plant circadian (Guo and Tan, 2013b). The research of using DF as a biosensor in the literature was conducted in time domain. Viewing signal from frequency domain provides a different angle to analyze the signal; however, there is a lack of theoretical framework of utilizing DF signal from PSII in frequency domain. The method developed in this work is an attempt of using the Fourier transform of PSII DF; therefore, the method is not only meaningful
for sensing herbicide, but also serves a preliminary example of making use of DF spectrum in frequency domain.

Because each RC of PSII functions as a unit, other chemicals diffuse into the leaf and change the emission of DF from corresponding RCs may follow similar kinetics in mathematics. The developed method should be directly applicable for detection such chemicals from theory. In future research, experiments are needed to valid the method for measuring these chemicals as well as herbicides, especially with extreme low concentration. An instrument with two channels is expected to be developed to improve the accuracy of the method.

6. Conclusions

The relationship between DF Fourier transform and herbicide action was theoretically modelled and analyzed. The analysis yields a linear relationship between a variant of DF Fourier transform and herbicide concentration. Experiments were used to validate the linear relationship. This yields a DF-based method to measure herbicide concentration in frequency domain. Future research and experiments are needed to extend this method to measure other chemicals, especially with low concentrations.

References


