FPGA-based chlorophyll fluorescence measurement system with arbitrary light stimulation waveform using direct digital synthesis

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A B S T R A C T
Nowadays, it is critical to generate technology to be applied to crops in order to counteract the lack of food, which can be produced by several biotic and abiotic factors. It is well known that stress conditions affect photosynthetic activity, which is closely related to crop yields. As a result, chlorophyll fluorescence can be used as an appropriate analytical tool to investigate the physiology and stress conditions of photosynthetic organisms, and to improve them. This paper presents an FPGA-based measurement system with direct digital synthesis capabilities for Chlorophyll fluorescence measurement. This is achieved through a customizable waveform processor that is capable of implementing arbitrary waveforms to modulate light source, get new frequency components to enrich information that a photosynthetic sample can generate, and provide researchers with new ways to obtain physiological information.

1. Introduction

Recently, food production has been affected by several biotic and abiotic factors that directly impact underdeveloped countries in regards to quantity. According to the FAO for 2013, there are 842 million undernourished people, especially in African countries where 65% of the population suffers from nutrition issues [1]. Therefore, it is crucial to generate technology that can be applied to crops in order to counteract the insufficient quantity currently being produced. It is well known that stress conditions affect photosynthetic activity, which is closely related to crop yields [2]. Consequently, great efforts are now being devoted to understanding the physiology of plants. One pathway is the fluorescence generated by photosystems I (PSI) and II (PSII) which are highly susceptible to environmental stress [3]. This is due to the fact that photosynthetic organisms possess chloroplasts formed from thylakoids membranes with both photosystems. These are responsible for the production of Chlorophyll fluorescence (Chlf). However, PSI only contributes negligible

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quantities. PSII is the main contributor to variable fluorescence [4]. As a result, Chlf can be used as an appropriate analytical tool to investigate physiology and stress conditions of photosynthetic organisms [5].

In order to create new technology designed to improve the productivity of the primary sector, it is necessary to make more efficient and non-invasive measurements such as Chlf fluorometer. This basically consists of using a PAR (Photosynthetically Active Radiation) light source on a photosynthetic sample to induce chlorophyll excitation in order to obtain the fluorescence response through a photodetector device [6]. There are several Chlf fluorometer methodologies where a high rate is important in order to measure fast photosynthetic processes; for example, measuring the fast phase of Chlf, which is easier to analyze than slow phases or Delayed Fluorescence (DF). This is usually used for the phase of Chlf, which is easier to analyze than slow phases to synthetic processes; for example, measuring the fast phase of Chlf, which is easier to analyze than slow phases or Delayed Fluorescence (DF). This is usually used for the phase of Chlf, which is easier to analyze than slow phases to synthetic processes; for example, measuring the fast phase of Chlf, which is easier to analyze than slow phases or Delayed Fluorescence (DF). This is usually used for the phase of Chlf, which is easier to analyze than slow phases to synthetic processes; for example, measuring the fast phase of Chlf, which is easier to analyze than slow phases or Delayed Fluorescence (DF). This is usually used for the phase of Chlf, which is easier to analyze than slow phases to synthetic processes; for example, measuring the fast phase of Chlf, which is easier to analyze than slow phases or Delayed Fluorescence (DF). This is usually used for the phase of Chlf, which is easier to analyze than slow phases to synthetic processes; for example, measuring the fast phase of Chlf, which is easier to analyze than slow phases or Delayed Fluorescence (DF). This is usually used for the phase of Chlf, which is easier to analyze than slow phases to synthetic processes; for example, measuring the fast phase of Chlf, which is easier to analyze than slow phases or Delayed Fluorescence (DF). This is usually used for the phase of Chlf, which is easier to analyze than slow phases to synthetic processes; for example, measuring the fast phase of Chlf, which is easier to analyze than slow phases or Delayed Fluorescence (DF). This is usually used for the phase of Chlf, which is easier to analyze than slow phases to synthetic processes; for example, measuring the fast phase of Chlf, which is easier to analyze than slow phases or Delayed Fluorescence (DF). This is usually used for the phase of Chlf, which is easier to analyze than slow phases to synthetic processes; for example, measuring the fast phase of Chlf, which is easier to analyze than slow phases or Delayed Fluorescence (DF). This is usually used for the phase of Chlf, which is easier to analyze than slow phases to synthetic processes; for example, measuring the fast phase of Chlf, which is easier to analyze than slow phases or Delayed Fluorescence (DF). This is usually used for the phase of Chlf, which is easier to analyze than slow phases.

The novelty of this article is to take advantage of the fluorescence as an analytical tool, and to describe the development of a measurement system based on Direct Digital Synthesis (DDS) capabilities for Chlf measurement [8,15–18]. This was achieved through a customizable waveform processor that is capable of implementing arbitrary waveforms to modulate light source in order to improve the ON–OFF control, as was aforementioned [19–21]. The main desired characteristic is to obtain new frequency components to enrich information that a photosynthetic sample can generate, thus providing researchers with new ways to obtain physiological information. More specifically, to provide a generic and customizable measurement system with an FPGA-based (Field Programmable Gate Array) soft-core, which will have many advantages over other technologies presently being used. These include reconfiguration, high parallel computation speeds, and integration in a single chip and custom architecture, to name but a few [22]. The following four main waveforms were implemented with DDS: First, a sine waveform was implemented because is the purest, composed of a single frequency and magnitude. Then, a triangle and saw-tooth was utilized with the objective of adding high frequency components in their abrupt waveform changes. Finally, a square waveform was reached, whereupon high frequency components were increased even more. It is noteworthy that the last waveform is different as compared to an ON–OFF control because with DDS, even at a lower state, the Chlf is excited. Furthermore, all signals were implemented with respective frequency sweeps; consequently, the measurement system would be capable of acquiring both a Chlf and light source excitation signal in order to statistically compare significant deformations between both signals.

2. Theoretical background

Chlf is a red and far-red emission investigated for monitoring photosynthesis generated from photosynthetic tissues when they are excited by a PAR light source. The
tissue re-emits photons with a higher wavelength, especially in the spectral region near 685 nm, and others near 730 nm [23]. This phenomenon is a dissipative pathway of an excess of energy [15] between thermal and photochemistry dissipation, where the leaf must reject the excess energy that cannot be assimilated. Hans W. Kautsky and his collaborator A. Hirsch described Chlf, also known as the Kautsky effect, in 1931. They observed that when a Dark-Adapted (DA), photosynthetically active sample was kept in the dark for 15 min to an hour and then illuminated, it generated a Chlf increase. Moreover, this increase was compared with CO2 assimilation, resulting in a qualitative correlation [24].

All plants, from higher plants to unicellular green algae, possess chloroplasts with thylakoid membranes that contain PSI and PSII, and are connected to each other with cytochrome b6f complex. These two photosystems perform photosynthetic light reactions and associated electron transport. On a DA sample, the PSII and PSI are impaired because the dark photosynthetic apparatus is in a non-functional state. After irradiation the sample was taken again, after a few minutes to induce the cooperation between both photosystems necessary to perform the photosynthetic electron transport reaction. Thus, the proper water split, oxygen evolution, as well as NADP+ reduction and ATP were permitted [11].

As aforementioned, PSII is the major contributor of Chlf and consists of pigments and proteins in the thylakoids membrane of chloroplasts, which is the major target of stresses. The Chlf transient is divided into two parts: the prompt and delayed phase. The first occurs within seconds and is also usually called the OJIP phase to ending on T, as shown in Fig. 1. This phase can divide photo-chemicals formed by O–J and the non-photo-chemical that consists of a J–I–P transient, as was mentioned above. The photo-chemical is directly dependent on the intensity of the excitation light, and the non-photo-chemical is principally controlled by the mechanisms for the excitation-dependent thermal dissipation of energy from PSII [25]. There are mathematical analyses of OJIP curves, in order to predict the part of close PSII centers where J stage must have undergone more than one turnover. On the other hand, Joliot and Joliot on 1964 [26], reported that fluorescence yields (\( \Phi \)) derive on nonlinear functions of the fraction of open reaction centers (q). Furthermore, theoretical models show hyperbolic relationships with a q as shown below:

\[
\Phi_p = (1-p)(1-q)/(1-p(1-q)) \quad \text{with} \quad p = \omega(1-F_o/F_m)
\]

\[
\Phi_f = (1-q)/(1 + p_{2g}(F_m/F_o - 1)q)
\]

\[
\Phi_f = (1-q)/(1+q)
\]

Being \( p \), a parameter of connectivity is defined as the probability of the excitation energy transfer from a closed PSII reaction center to a neighboring one; while \( \omega \) is a value between 0 and 1, where 0 is “puddle” model and 1 the “lake” model, which means that all PSI units are assumed to be interconnected. Consequently, \( p_{2g} \) depends directly on the probabilities of excitation transfers between different PSII antenna domains. In addition, \( J = C_{IPP} = P_{2g}(F_m/F_o - 1) \) represents the sigmoidicity parameter [27].

On the other hand, the delayed phase is a much longer fluorescence phase in terms of minutes; therefore, this is a potential indicator of photosynthesis efficiency and plant stress because of the difference of the prompt phase, where a single molecule is sufficient for the generation. The delayed fluorescence is dependent on systems interactions. This is widely utilized to detect herbicide and drought issues, among others [28].

Consequently, in order to interpret the fluorescence induction kinetics several parameters were defined, the origin \( F_o \) and the maximum \( F_m \) being the most common. Based on these, the ratio \( F_m/F_o \) is utilized and results in a high value when the sample is non-stressed. However, in graphical representations, the relative variable fluorescence at the time is widely used, which is defined in Eq. (4), being the relative variable fluorescence at the time interval \( t \) between \( F_o \) and \( F_m \) [3]. Moreover, there are values to identify light-adapted fluorescence response, which is represented by \( F_m \) and is usually lower than \( F_m \), whereas \( F_o \) is represented by \( F_o \); depending on the plant species, this can vary. Nonetheless, the change can often be neglected by assuming that \( F_o = F_o \).

\[
V_t = (F_t - F_o)/(F_m - F_o)
\]

\[
F_v = F_m - F_o
\]

\[
F_v = F_m - F_o
\]

All the parameters are defined with the intention of identifying physiological issues in the sample in a quantitative form, shown in Table 1; however, not all stresses manifest themselves in the same way. In the case of a low temperature stress, metabolism is highly affected, damaging the PSII and decreasing the maximum quantum yield of photochemistry. This is represented in Eq. (5), which is represented by subtracting the maximum light-

<table>
<thead>
<tr>
<th>Table 1</th>
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<tbody>
<tr>
<td><strong>Definition of chlorophyll fluorescence nomenclature.</strong></td>
</tr>
<tr>
<td><strong>Fluorescence nomenclature</strong></td>
</tr>
<tr>
<td>( F )</td>
</tr>
<tr>
<td>( F_o )</td>
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<tr>
<td>( F_m )</td>
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<tr>
<td>( F_v )</td>
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<tr>
<td>( F_m )</td>
</tr>
<tr>
<td>( F_v )</td>
</tr>
<tr>
<td>( p_{2g} )</td>
</tr>
<tr>
<td>( \phi_f )</td>
</tr>
<tr>
<td>( Q )</td>
</tr>
<tr>
<td>( p )</td>
</tr>
<tr>
<td>( \omega )</td>
</tr>
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<td>( p_{2g} )</td>
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</tbody>
</table>
adapted fluorescence and the origin of the fluorescence on dark adapted. On the other hand, high temperatures affect the thylakoid and deactivate the PSII, causing a rise of $F_o$ affecting the maximum PSII efficiency of dark-adapted leaves. This can be seen in Eq. (6) [29]. With nutritional issues, especially lack of nitrogen (which is the main mineral nutrient for chlorophyll production [30]), there is a decrease in PSII operating efficiency and the quantum efficiency of photochemistry in open reaction centers of Chlf, as shown in Eqs. (7) and (8) respectively [31].

$$\frac{F_o - F_m}{F_m}$$

where $F_o$ is the difference between $F_m$ and $F$, where $F$ is the steady state condition of Chlf in DA.

$$\frac{F_V}{F_m}$$

3. Design of measurement system

3.1. Spectral response of Chlf

In order to design the measurement system, it is necessary to take into account the spectral response of the Chlf as mentioned above. It is well known that a blue light source fits in the chlorophyll absorption, which is why two ultrabright blue LEDs LZ4-40b200 (LED ENGIN) were utilized [32]. Furthermore, in the measurement system it was necessary to set up a colored glass band-pass filter FGB37S (Thorlabs) that works on a 335–610 nm wavelength, which was used in the light source. Thus, infrared noise superposition on the measurement of Chlf was avoided, permitting the separation of any emissions that can interfere and cause erroneous measurements. Also a FGL695 nm Colored Glass Filter Long-pass that reject wavelengths lower than 695 nm (Thorlabs) was mounted on the photodiode to acquire only the Chlf signal, as is shown in Fig. 2b. A long-pass filter is enough for this measurement system because of the light control conditions that the isolation chamber provides. This was tested using a high reflective surface in order to ensure that the light from the LEDs was minimally acquired by the Chlf photodiode. However, coupled to Chlf, there are others signals that are not easy to exclude. Nevertheless, these can provide information about the physiological state of the sample, such as the reflectance that is in the same region of the Chlf [33]. Therefore, in order to validate the proposed Chlf measurement system, a spectrograph Acton Series SP 200i (Princeton Instruments) was utilized to characterize the fluorescence system response as shown in Fig. 2a, with a comparison of the chlorophyll absorption and fluorescence, which was based to the correct selection of LEDs and photodiodes.

3.2. Experimental set-up

The measurement system was divided into two main stages: the excitation and detection stage. The first contains digital waveforms implemented in the RAM of the FPGA through DDS. This is why an interface based on MATLAB R2013a [34] was developed, which is capable of generating through DDS a Random Access Memory (RAM) able to implement arbitrary waveforms in FPGA, as can be seen in the main measurement system diagram shown in Fig. 3.

Then, in order to provide an analog signal, a DAC7565 (Digital-to-Analog Converter of Texas Instruments) was utilized. Due to a low power signal provided by the DAC, an OPA453 (Texas Instruments) high voltage operational amplifier configured as non-inverting amplifier was utilized. However, because of the current requirements of 1400 mA, it was necessary to implement a Darlington amplifier MJ1106G (ON semiconductor) working in common-collector configuration which was intended to keep almost the same voltage, increase the current and reach the nominal values in order to obtain a high brightness modulated light from LZ4-40b200 (LED ENGIN), as shown in Fig. 3.

In the detection stage, it is critical to avoid taking the reference signal directly from an electrical signal that is supplying the LED. This can generate a phase shift due to photodiode and LED latency. It is necessary that reference and fluorescence are light signals. That is why two photodiodes (OPT301, Texas Instruments) were implemented in order to make a better comparison; however, the light that each photodiode detects has a different intensity. Consequently, these need different amplification values to analyze light response. Despite the fact that these photodiodes have integrated transimpedance amplifiers in their packaged, in the case of the Chlf detector, the internal feedback resistor of 1 MΩ was utilized. Nevertheless, for a reference light source detector it was necessary to use an external resistor (RF) to set the value amplification, shown in Fig. 3. Afterwards, the signal was passed through an antialiasing filter to avoid high frequency noise; furthermore, the analog signal was converted to digital data and stored for subsequent analysis. The reference photodiode is important to compare if the plant is adding information to original signal. It is also useful to subtract the reference signal from the Chlf and to obtain only the information signal. This provides sturdiness by suppressing noise in the measurement. Photodiodes signals were passed through a conditioning stage and antialiasing filter in order to sample and store light signals in the FPGA RAM.

Finally, it is necessary to maintain controlled conditions and avoid problems generated by a sun-light measurement such as: the low fluorescence magnitude emission and the environmental noise that may cause the utilization of complex techniques to differentiate from other reflectant emissions (e.g. Fraunhofer line depth) [35]. Because of this, it was necessary to design a leaf chamber, as shown in the experimental setup in Fig. 5, taking into account that the process must avoid any stress conditions until the measurement itself has been permitted. The main problem in the chamber is temperature. LEDs are the main heat source. In order to avoid temperature issues, a ventilation system was installed, coupled with a pane of glass between the light source and the plant. The glass was used as a heat source.
filter, decreasing the effect of heat stress that the light source can cause to the sample being measured. Two photodiodes have been mounted within the chamber: light reference and Chlf. The Chlf photodiode is configured in order to be capable of generating a greater amplification because the Chlf is weaker than the light source emission, while the light reference photodiode is configured to be less sensitive to the light that is sensing with the aim to not saturate with the intense blue light. The Chlf photodiode is coupled with an optical long-pass filter in order to acquire only the sample signal and filter the excitation intense blue light. The leaf chamber fulfills the function of isolation, primarily for the ambient light to reach a DA state for the sample. The isolation chamber is designed for field measurements. As indicated, a single leaf of the plant being tested is first encased in the chamber during data acquisition, and then the leaf is released to permit the continuation of normal growth.

3.3. FPGA implementation

First, in order to design a customizable measurement system, it was necessary to use a FPGA platform (Altera Development & Education board with a Cyclone II EP2C20F484C7 N FPGA chip) to take advantage of its parallelism and high computation capabilities. This permits the user to execute different drivers such as waveform generators at the same time.

The control of the Chlf measurement system is implemented in FPGA. There are 3 control options for the user to choose from, being Start_DA_Chirp for ST_DA_Chirp and Start_SS_Chirp for ST_SS_Chirp measurement and ST_TRSM for start transmission, as shown at the top of Fig. 6. These are connected to Finite State Machine (FSM) waveform with control signals. The first measurement is started by ST_CHIRP, which consists of a measurement of a sample previously kept on the DA. However, with
The processor keeps the sample in a light-saturated lapse of time with a value OFFSET_SS, which was previously selected to set the intensity. This is related to the intensity of the saturated light and performs a measurement in a SS state. In contrast, the ENB_SS signal is switched when it is necessary to use the offset or use only the arbitrary waveform. The FSM waveform also controls the E_CHIRP that enables the signal to start the frequency sweep for the arbitrary waveform implemented in RAM. The aforementioned were initiated to transmit the waveform values to DAC driver and finally to modulate the light source. The Chlf and reference signal storage processes are performed in parallel through the LOAD_CHIRP signal. The storage of the Chlf is acquired with the ADC (Analog-to-Digital Converter) driver, which saves it in RAM storage and sweeping ADDR with the assistance of the Counter Storage. Finally, it is necessary to transmit the signal acquired for further processing to a Personal Computer (PC). The ST_TRSM signal connected to the FSM storage and transmission module, which is in charge of selecting the storage and transmission counter through TRSM signal, as well as the flow of data in the RAM storage. To carry out transmission, a similar process to that of the storage is performed; however, the data flow is contrary in order to be sent to a PC through a UART driver.

4. Experimental results and discussion

There are three phases in the experimental stage: DA, Saturated, and Prompt transient, the last being the transitory state of the DA or prompt fluorescence [36]. All stages underwent four different waveforms with 23–500 Hz frequency sweep: sine, triangle, saw-tooth and square, respectively. The frequency sweep was selected in order to exploit the various electronic devices’ performance in the measurement systems. It is noteworthy that the difference between the ON–OFF control and square waveform using DDS is that the latter, even at a low state, keeps...
the chlorophyll in excitation state which is unlike that of conventional measurements where the light source is at an OFF state.

The photosynthetic samples utilized in this experiment were *Ficus Insipida* Willd, 1.6 years old and in the vegetative growth phase. In the case of DA measurement, it was necessary keep a leaf in the isolation chamber for 10 min; subsequently, making the measurement took 1.53 s, while the transitory state was carried out in the first 0.5 s of the DA measurement [28]. On the other hand, the Saturated Sample (SS) measurement consisted of keeping the leaf exposed to intense blue light for one minute to avoid the transitory state. Measurements with modulate light were carried out immediately thereafter, as seen in Fig. 7. The SS is different from delayed fluorescence as applied in other methodologies [36].

First, it was necessary make a comparison between the reference signal and Chlf response. Therefore, it was necessary to carry out two hundred and forty measurements, thirty for each waveform resulting in one hundred and twenty measurements each for DA and SS. This was carried out to ascertain if there would be a significant difference using a photosynthetic sample such as a black box model. A blue square-form light is applied to $h(t)$, as shown in Fig. 8, resulting in a red fluorescence square-form light as $y(t)$. This represents a significant difference because $y(t)$ has a different behavior at low or high frequencies.

In order to measure the range of statistical differences between data groups [37], the change that light reference undergoes after being converted to Chlf. It was necessary do an Analysis of Variance (ANOVA). Due to that measurement, which consists of an 18,565 digital value that corresponds to a full reference and Chlf light signal, it was necessary to calculate mean and standard deviation from each dataset. Consequently, the $p$-value was utilized for statistically significant testing: where a null hypothesis occurs when $p > 0.05$. This means that the analyzed groups do not possess significant differences [38]. On the other hand, when $p \leq 0.05$ it is considered as an alternative hypothesis, which means that a significant statistical difference between the data groups exists. Following these findings there are significant statistical differences between reference and fluorescence signals. It is necessary to prove the repeatability of the measurements obtained by this system. Consequently, 240 measurements were utilized again to quantify the repeatability. Due to the above, the one-sigma and three-sigma rules were employed.
In addition to the aforementioned analysis, only a precision test was applied, because there is currently no other commercial equipment that can do a similar measurement with waveforms that use this sensor. Therefore, an accuracy test could not be performed. On the other hand, a precision test was carried out using Eq. (9) where $\sigma$ is the standard deviation. The measuring range was taken from the difference between maximum and minimum measurement that was obtained from each waveform and sample condition, SS and DA.

$$\text{PRECISION} = \frac{\sigma}{\text{MEASURING RANGE}} \times (100) \quad (9)$$

4.1. Discussion of results

The statistical analysis of the ANOVA results can be observed in Table 2. Here, it is notable that all the $p$-values are small, occurring an alternative hypothesis, which means that there is a significant statistical difference between reference and fluorescence signals. In
addition, it can be observed that the Fisher-test is a far of the unit value to reaffirm the statistical difference between the variances. It can also be observed that the sums of squares show that the variability of the each type of measurements is low from its mean. However this parameter presents higher values with SS as well as with the signal which contain high frequency components, such as square form. This can be advantageous in adding more frequency information and observing the behavior on high and low frequencies. However, it can also be a noisier signal than the purest, such as sine form. Results from the repeatability tests are shown in Table 3 and represented in graphic form in Fig. 9, with the aim to sigma rule analysis, where it should be noted that the lowest rate is 70% on sine and triangle waveform in SS. However, these values lie within permissible values for the one-sigma rule that is nearly 68.1%. The three-sigma rule was employed to prove that nearly of 99.73% of the measurements fall within acceptable limits, as can be seen in Fig. 9, where 100% of the assessments are within the rule [39]. Finally, precision results can be observed in Table 3, where it should be noted that the most precise measurement was obtained from the sine waveform within DA conditions, and the least precise one was the square waveform with SS light conditions.

Table 2
ANOVA results by comparing the reference signal with the Chlf signal.

<table>
<thead>
<tr>
<th>Reference vs chlorophyll</th>
<th>Sums of squares</th>
<th>Fisher-test</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sine DA</td>
<td>0.00395</td>
<td>2631.01</td>
<td>5.0603e-50</td>
</tr>
<tr>
<td>Triangle DA</td>
<td>0.00499</td>
<td>348.89</td>
<td>3.27351e-26</td>
</tr>
<tr>
<td>Sawtooth DA</td>
<td>0.00146</td>
<td>333.81</td>
<td>9.81793e-26</td>
</tr>
<tr>
<td>Square DA</td>
<td>0.02816</td>
<td>846.47</td>
<td>2.73042e-36</td>
</tr>
<tr>
<td>Sine SS</td>
<td>0.01523</td>
<td>841.61</td>
<td>3.19276e-36</td>
</tr>
<tr>
<td>Triangle SS</td>
<td>0.01164</td>
<td>317.36</td>
<td>3.41827e-25</td>
</tr>
<tr>
<td>Sawtooth SS</td>
<td>0.01394</td>
<td>1083.59</td>
<td>3.16887e-39</td>
</tr>
<tr>
<td>Square SS</td>
<td>0.02087</td>
<td>233.12</td>
<td>5.57613e-22</td>
</tr>
</tbody>
</table>

Table 3
Statistical analysis of assessments and one-sigma rule.

<table>
<thead>
<tr>
<th>Chlf</th>
<th>Mean</th>
<th>Std. Dev.</th>
<th>% Inside one-sigma</th>
<th>Precision</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sine DA</td>
<td>0.4751</td>
<td>0.0012</td>
<td>76.6667</td>
<td>4.9051e-05</td>
</tr>
<tr>
<td>Triangle DA</td>
<td>0.4512</td>
<td>0.0035</td>
<td>73.3333</td>
<td>1.6493e-04</td>
</tr>
<tr>
<td>Sawtooth DA</td>
<td>0.4647</td>
<td>0.0033</td>
<td>86.6667</td>
<td>1.1761e-04</td>
</tr>
<tr>
<td>Square DA</td>
<td>0.4646</td>
<td>0.0075</td>
<td>73.3333</td>
<td>2.6462e-04</td>
</tr>
<tr>
<td>Sine SS</td>
<td>0.4758</td>
<td>0.0057</td>
<td>70</td>
<td>7.1811e-04</td>
</tr>
<tr>
<td>Triangle SS</td>
<td>0.4748</td>
<td>0.0072</td>
<td>70</td>
<td>8.1209e-04</td>
</tr>
<tr>
<td>Sawtooth SS</td>
<td>0.4650</td>
<td>0.0038</td>
<td>76.6667</td>
<td>3.7893e-04</td>
</tr>
<tr>
<td>Square SS</td>
<td>0.4732</td>
<td>0.0121</td>
<td>83.3333</td>
<td>0.0011</td>
</tr>
</tbody>
</table>

Fig. 8. A plant used as a black box model and excited by a square waveform signal on DA conditions.

Fig. 9. DA and SS assessment on four different waveforms applying one-sigma rule.
5. Conclusions

This article presents a reliable measurement system that provides different ways to analyze Chlf by utilizing customizable DDS. Also, it has the capability of implementing arbitrary signals by introducing previously stored signals or functions into the developed MATLAB interface. Because of this, it constitutes an easily re-configurable FPGA-based tool that creates new ways to study photosynthetic samples by modifying the excitation light waveform. Hence, this implementation utilized basic waveforms with frequency sweep as its main contribution, but further investigations can apply new waveforms in order to obtain new Chlf related information. Furthermore, it is possible to apply spectral or statistical techniques of digital signal processing such as FFT, spectrogram, entropy, deconvolution or even ratiometric technique with reference and Chlf related information. Consequently, the proposed methodology permits researchers to investigate new signal deformation effects that cannot be noticed by using traditional measurement methods, which only distinguish fluorescence magnitude and period.

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