

Leaf Fluorescence: Macroscale FLIM and Microscopic FLIM of Mobile Chloroplasts

Fluorescence



Application Note
Life Science
FL252201

Abstract

Photosynthesis is a key component of life on earth. Studying the steps involved in the photosynthetic process from the absorption of light through to its conversion to energy and gas is essential in many aspects. These range from agricultural to fundamental biology and the renewable energy area. The leaf can be thought of as a scaffold for supporting the chloroplasts containing the photosynthetic reaction centers. Fluorescence is particularly apt for the study of photosynthesis and fluorescence lifetime imaging (FLIM) is particularly suited as it provides spatial information. The FLIMera™ widefield TCSPC camera can be equipped with a lens and filter enabling the whole of the leaf to be rapidly imaged, providing macro FLIM capability. More typically, when attached to a microscope, its ability to collect over 24,000 fluorescence decays simultaneously at rates up to ~12.5 kfps, allows it to capture lifetime data from moving samples. This is demonstrated in this note observing whole leaf fluorescence.

Introduction

Fluorescence is particularly suitable for following the light induced photosynthetic process and has been used to distinguish photosystems I and II [1], illustrated in Fig. 1. Imaging the whole leaf is advantageous as it looks at photosynthesis in its native environment.

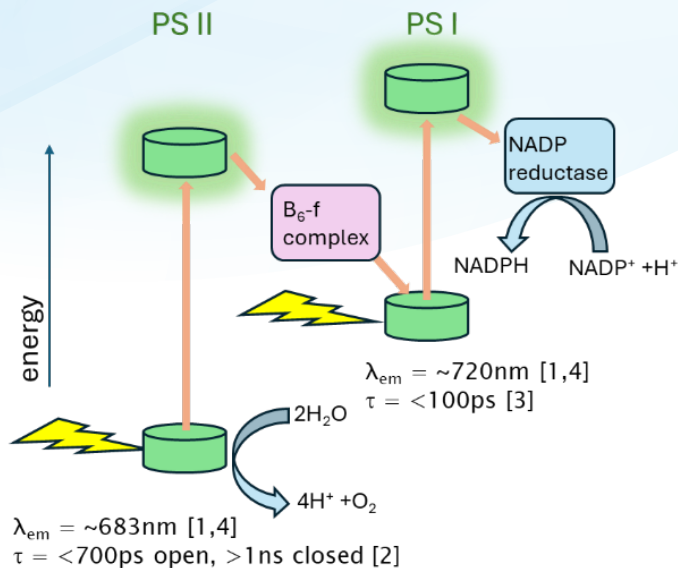


Figure 1. Schematic representation of the light induced processes in the two photosystems

The light incident on the leaf can be absorbed by the chlorophyll to start the photosynthetic cycle. Excess energy is either given off as heat or by fluorescence. This emission can be used to access the efficiency of the process and can have obvious applications in the study of plant health. Time-resolved fluorescence is apt for studying kinetic processes [2,3], and fluorescence lifetime imaging (FLIM) can provide

related spatial information [4]. However, the fluorescence emission is not constant, thus a means is needed to study this kinetic process. Indeed, although in terrestrial plants the chloroplasts are fixed in their leaf “scaffold,” the same is not always true for aquatic plants. Here, water assists in supporting the plant structure and the chloroplasts can be mobile within the cell.

Therefore, rapid fluorescence imaging is advantageous in the study of whole leaf fluorescence. The HORIBA FLIMera (see Fig. 2) is a widefield fluorescence lifetime camera based on time-correlated single-photon counting (TCSPC).

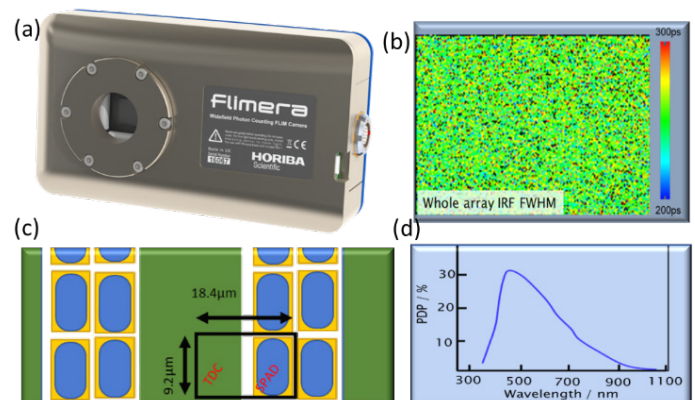


Figure 2. (a) The FLIMera camera, (b) IRF image over the whole array, (c) schematic of the sensor construct and (d) the wavelength response.

This method is considered the “gold standard” in fluorescence lifetime determination. The FLIMera consists of a 192 x 126 pixel array, where each pixel has its own SPAD detector and TCSPC timing electronics. This means that it can capture more than 24 thousand fluorescence

decays simultaneously, with a readout rate of ~12.5 kfps. This makes it well suited to a fluorescence lifetime imaging (FLIM) approach to visualise mobile samples and kinetic events. Although typically microscope based, when equipped with an appropriate lens system it can be used as a TCSPC camera to obtain macroscale images.

Methodology

The application of the FLIMera is demonstrated in a “macro configuration” equipped with a lens (not supplied, see Fig. 3 below) and longpass filter (>630nm).



Figure 3. FLIMera equipped with a lens for macro imaging.

Light from a DeltaDiode (emitting at 494 nm) with a diffuser placed in front of it to produce a wide excitation beam, sufficient to illuminate a whole leaf was used. In this example a ~8.5 cm long leaf from a variegated rubber plant (*Ficus elastica tineke*) was chosen. Images were taken using the EzTime™ Image software. This software is used to control the camera, acquire and analyze data.

To observe motion within the cell, the FLIMera was attached to the side port of a Nikon® Ti2-U inverted microscope for the study of pond weed (*Vallisneria*), a small section of which was mounted in a water filled cavity slide with a coverslip. Excitation in this case was via a DeltaDiode (emitting at 475 nm) coupled to the microscope’s epi-port. The data was “photon-streamed” to an HDF5 file format. The photon events were “tagged” in terms of pixel position, macro, and micro time. “Frames,” initially at 4fps, were then reconstructed using the EzTime Image software, which was also used for basic image processing. The images (frames) obtained were then exported to Fiji (ImageJ) to display (a montage taken from a “movie” is shown here).

Results

The FLIM measurements of the whole of the variegated leaf under subdued lighting for different measurement times (frame rates) along with a photo are given in Fig. 4. In this custom set up, it can be seen that even at a relatively low collection time (reasonable frame rate) the different regions of the leaf (i.e. those containing more chlorophyll) can be distinguished using the fluorescence lifetime.

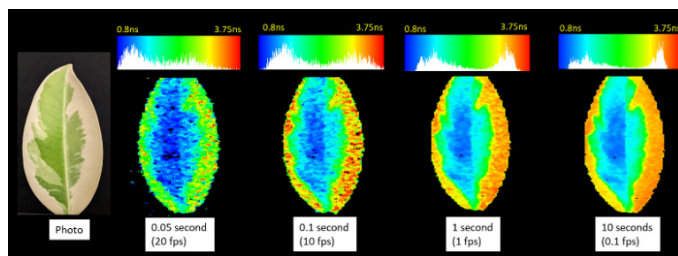


Figure 4. Photo of a variegated leaf along with lifetime images taken with different data collection times.

Knowing the fluorescence behaviour over the whole leaf can be important in the area of plant health, as is the ability to collect FLIM data in a short time. Delving into the leaf to monitor the fluorescence from individual chloroplasts is also a major area of interest. In a terrestrial leaf, the structure of the leaf acts as a support to hold the chloroplasts in position. In aquatic plants, support is also provided by the surrounding water. In fact, in some leaves the chloroplasts are mobile which is the case in a pond weed, *Vallisneria*. Using the “photon-streaming” functionality of the FLIMera enables the raw photon data, in terms of pixel, macrotime and microtime to be streamed to disk in a HDF5 file format. This can be accessed using EzTime Image or third-party software.

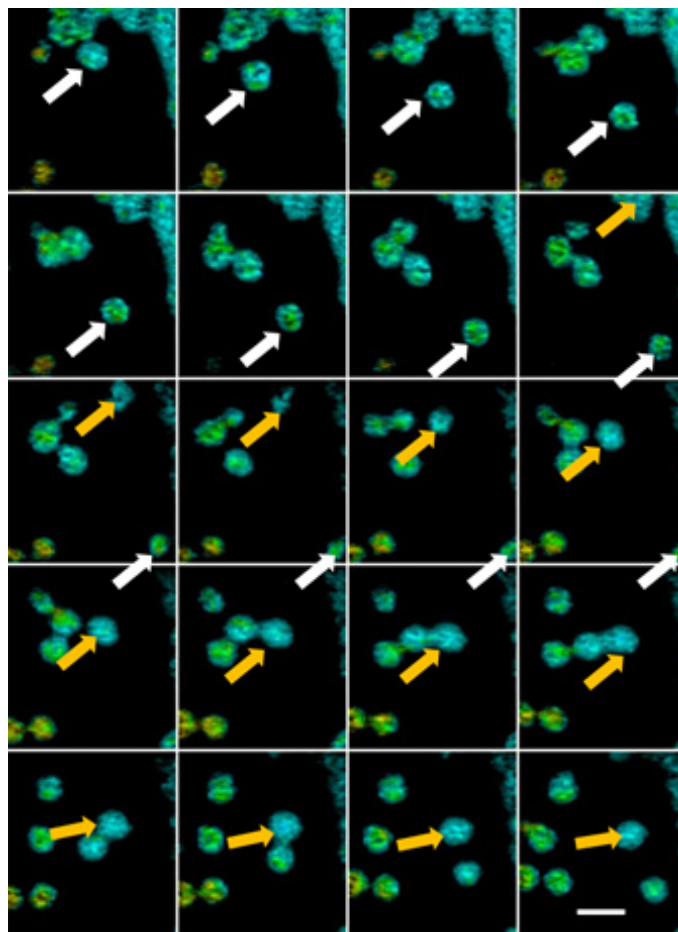


Figure 5. Frames taken from a “movie” showing the motion of chloroplasts within a *Vallis* leaf. Two chloroplasts (white and yellow arrows) are indicated to show movement. The scale bar, bottom right, is 10 µm.

The addition of the FLIMera to the side port of a microscope using the epi-port to couple a pulsed laser diode for excitation simplifies FLIM measurements. The photon streaming approach, and the use of EzTime Image to construct “frames” from the HDF5 file, means that images can be manipulated prior to exporting into ImageJ. A montage with frames every ~1.25s from a “movie” is shown in Fig. 5. Here two chloroplasts are indicated so that their motion can be followed. There is a difference in lifetime, as indicated by the color, which is indicative of one having a closed reaction center (green) and the other open (blue), thus, showing the capability of the FLIMera to distinguish the lifetimes from moving samples.

Summary

This note shows the capability of the FLIMera, both off and on a microscope, to be applied in the study of photosynthesis. Its flexibility can be used for macro imaging and to monitor the fluorescence lifetime of a whole leaf in a custom set up, as well as following moving samples, in this case, monitoring the status of the photosynthetic reaction center with time on a moving sample.



References

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- [2] K. Amarnath et al., 2012, *PNAS*, 109, 8405
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- [4] I. Iermak et al., 2016, *Biochim. Biophys. Acta*, 1857, 1473

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