

Monitoring whole leaf fluorescence using time-resolved techniques

Light incident on a leaf can be absorbed by chlorophyll to commence the photosynthetic cycle. Excess energy can be liberated as heat or by emission of fluorescence and this can be used to assess the efficiency of the photosynthetic process. Because of the Kautsky effect, the amount of fluorescence emanating from photosystem II (PSII) is not constant. There is an initial rise, upon illumination followed by a decrease caused by PSII closing down, as it is unable to transfer electrons. There can also be a contribution from PSI to the overall fluorescence. Here we employ different time-resolved fluorescence techniques to demonstrate the types of measurement that can be performed on a whole leaf.

Time-resolved fluorescence measurements

Time-correlated single-photon counting (TCSPC) was used to obtain the fluorescence decay data and performed using a *DeltaFlex* system equipped with *DeltaDiode* (DD-485L) excitation (a DD-510L is also suitable) and a *PPD-850* detector (see Fig. 1). Microscope measurements involved the use of a *Fluorescence microscope* with *DeltaHub* timing electronics and *DeltaDiode* excitation.

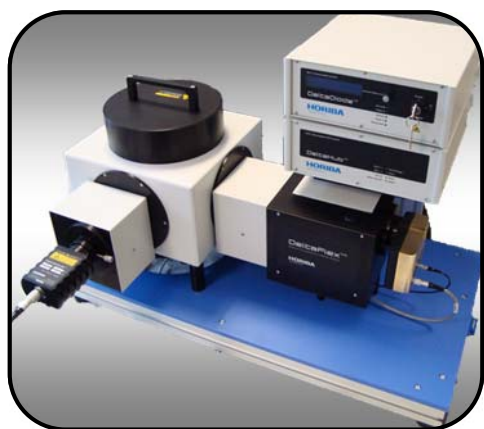


Fig. 1. DeltaFlex set up

To monitor the time-resolved decay behaviour with time, the kinetic TCSPC measurement mode was employed, this is capable of collecting up to 10,000 seamlessly consecutive decay measurements, with data collection times down to 1ms per decay. This is assisted by using a very high repetition rate excitation source (*DeltaDiode* lasers can run up to 100Mz and are well matched to the 10ns dead time of the *DeltaHub*, enabling efficient data collection – see Note TRFA-5). In order to elucidate fluorescing species time-resolved emission spectra (TRES) were measured and decay associated spectra determined (see Note TRFT-4). Measurements were made in a front face geometry using the corresponding sample holder.

Monitoring leaf fluorescence

When considering leaf fluorescence, this can also relate to the measure of chloroplast efficiency. Fig. 2, shows an image from the CCD camera, using white light illumination and filter cubes, showing the distribution of chloroplast emission from a variegated leaf.

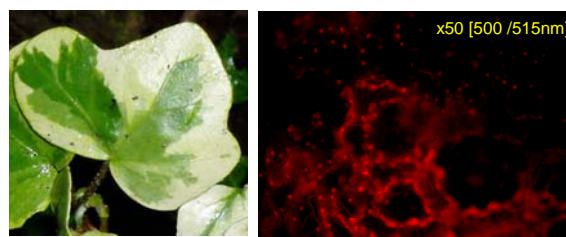


Fig. 2. Variegated leaf and microscope camera view using appropriate filters

To uncover both spectral and time-resolved data, TRES measurements were performed. The resultant TRES and the decay associated spectra, obtained from a global analysis with three common lifetimes, are shown in Fig. 3 and 4, for the leaf shown inset.

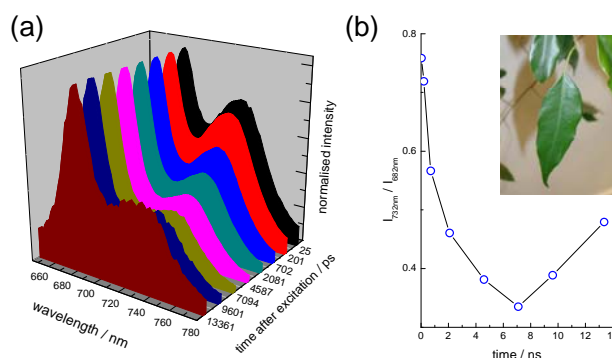
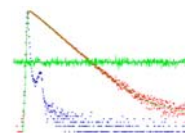


Fig. 3. (a) TRES and (b) ratio of the two main peak emissions relative to the excitation pulse from the leaf shown inset.



The TRES data show the presence of two main emissions, the relative intensities of which display a time dependency. This is depicted in Fig. 3b, showing the ratio of the peak intensities with time (relative to the excitation pulse).

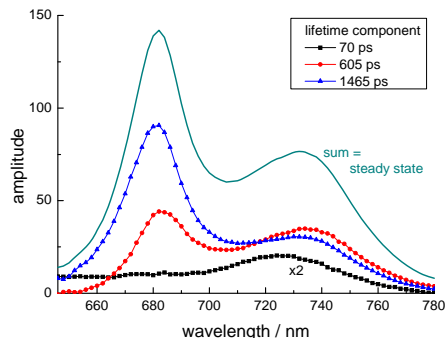


Fig. 4. Decay associated spectra from the global analysis of the TRES measurement (Fig. 3a)

By performing a global analysis it is possible to determine the decay associated spectra. The fit required the sum of three exponential decays and this is useful in revealing the kinetics of the two principal emissions, seen in the TRES (and steady state spectrum).

Making use of the kinetic TCSPC mode of the *DeltaHub* timing electronics, it is possible to follow the time evolution of the decay kinetics with time. The data were acquired sequentially for 100ms and the excitation rate used was 50MHz. The resultant data (10,000 decays) were analysed in a batch mode fitting to the sum of 3 exponentials, which also return intensity and average lifetime data. Fig. 5 shows the intensity and average lifetime dependency with time.

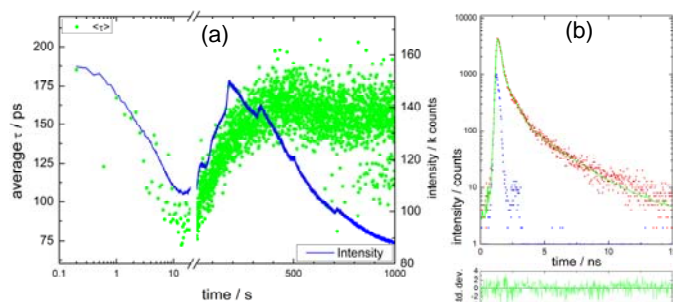


Fig. 5. (a) Change in average lifetime (o) and intensity with time (—), taken from a 3 exponential analysis, (b) example fit data from 10 seconds after start.

This shows that initially both the intensity and average lifetime data show similar trends, until longer times where

photobleaching or intensity fluctuations may have an influence. Since these data were obtained from a three exponential analysis it is also possible to see trends in both the lifetimes and their pre-exponential factors (showing their contribution to the measured fluorescence) with time. Data pertaining to this are shown in Fig. 6. The component lifetimes remain, more or less, constant and the main effect that alters the average lifetime (Fig. 5a) is the different contributions of these decay species to the emission. It is clear that the main influence on the average lifetime (and intensity) relates to changes in the relative contributions of τ_1 and τ_2 , information not available from a simple intensity measurement and unaffected by photobleaching or other causes of intensity fluctuations.

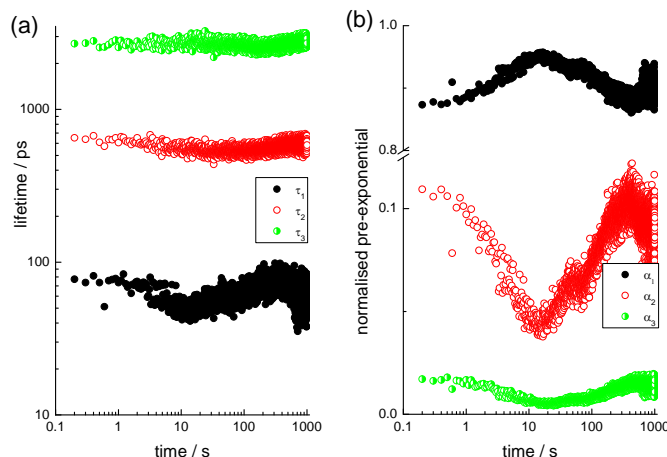


Fig. 6. Fluorescence decay data from the batch analysis showing (a) lifetime values and (b) corresponding pre-exponential values obtained from analysing the kinetic TCSPC measurement.

Summary

This note highlights some of the time-resolved fluorescence measurements that can be used in elucidating the kinetics, mainly emanating from PSII, with a contribution from PSI. The use of the fluorescence lifetime is advantageous, as it recovers more information than a simple intensity measurement. Combined time and spectral data can be obtained using the *DeltaFlex*. The heart of this system, the *DeltaHub* with its low dead time, in conjunction with the high repetition rate of the *DeltaDiodes* enables the kinetic TCSPC mode to efficiently collect decay data. This allows the fluorescence decay kinetics to be monitored with time.

