

# CHLOROPHYLL FLUORESCENCE

## EXERCISE 16

### RATES OF ELECTRON TRANSPORT FROM CHLOROPHYLL FLUORESCENCE

#### I. INTRODUCTION

Photosynthesis involves the conversion of light energy into chemical energy mediated by light sensitive chlorophyll molecules in the leaf. Light-harvesting complexes involving numerous chlorophyll molecules intercept radiant energy and transfer this to a reaction center in Photosystem II (PSII). At the heart of the PSII reaction center a special chlorophyll molecule called  $P_{680}$  becomes excited and an electron from  $P_{680}$  is raised to a higher energy level. Under ideal conditions for photosynthetic activity this electron reduces an acceptor molecule called pheophytin and is then transferred through a series of electron carriers including QA, QB Plastoquinone (PQ) and cytochromes (Cyts). This transfer of electrons is associated with the production of a trans-thylakoid proton gradient, which may be coupled to the chemiosmotic production of ATP. This process is called non-cyclic photophosphorylation, and requires processing of electrons by  $P_{680}$ .

Like any other energy transduction system, conversion of light energy to chemical energy in photosynthesis is far from a perfect process. For various reasons, not all electrons raised to higher energy levels by irradiance are passed to electron acceptors and these electrons return to their parent chlorophyll molecules without the production of ATP and/or  $NADPH_2$ . The energy lost during the return to the ground state may be manifested as heat or light. If light is produced, it is emitted at a longer wavelength than the light that stimulated electron release from chlorophyll, and under these circumstances the chlorophyll molecule is said to **fluoresce**.

**Chlorophyll fluorescence** usually occurs maximally under conditions of high light when photosynthetic activity is inhibited. Consider, for example, a leaf that is exposed to high light after being maintained in the dark for several hours. During the dark period, several of the enzymes involved in the Calvin Cycle become deactivated and must be re-activated by light before they can operate. Also, the metabolites involved in the Calvin Cycle reactions must reach appropriate levels before  $CO_2$  fixation can occur at optimal rates. Thus, there is a "Photosynthetic Induction Period" on the transfer of a leaf from dark to light during which  $CO_2$  fixation is very slow.

During the photosynthetic induction period the electron acceptors in the leaf continue to accept electrons from excited chlorophyll molecules but have no way of dissipating the energy from these electrons because the "dark reactions" are not yet active or optimized. As a result, the number of these acceptors, which can accept electrons quickly falls to zero since all reduction sites are occupied. Consequently, the electrons released from chlorophyll must dissipate their energy in another way. This is done partly by fluorescence, in which light is emitted as the electrons return to their initial energy level, and partly by dissipation of energy as heat. Therefore, we would expect fluorescence to be high during the photosynthetic induction period, and then decline, as the  $CO_2$  fixation reactions become more active. The fluorescence yield, i.e. the

# CHLOROPHYLL FLUORESCENCE

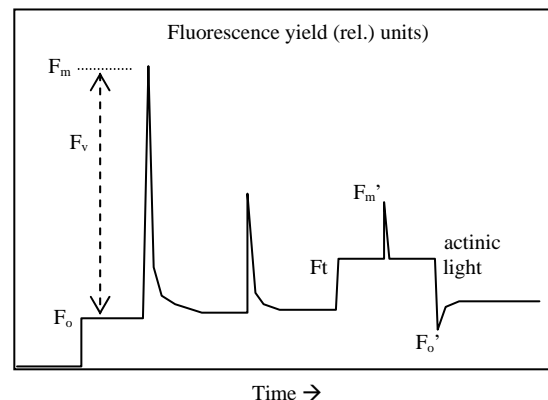
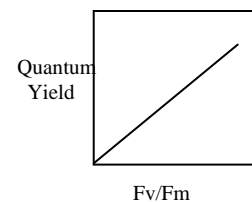
probability that the energy in an absorbed photon is re-emitted, depends on the efficiency with which light energy is transformed within the photosynthetic apparatus. Fluorescence is a powerful probe of the other dissipation mechanisms (including photosynthetic electron transport). Essentially anything that reduces photosynthesis or is stressful to the system will cause an increase in chlorophyll fluorescence.

Measurement of chlorophyll fluorescence requires a chlorophyll fluorometer with an LED light source to excite fluorescence and a detector to measure this fluorescence, and a halogen light source for activating photosynthesis. If a leaf has been maintained in the dark for a time long enough to require a photosynthetic induction period on re-illumination, then when that leaf is exposed to an extremely bright flash of light, the chlorophyll molecules within the leaf are excited and pass their excited electrons to PSII and PSI. However, because the Calvin cycle reactions are not active, the NADPH<sub>2</sub> formed as a result of this photochemistry cannot be used and the pool of NADP available for reduction is very quickly exhausted. As a consequence, within a few microseconds of illumination, the electron carriers in PSI and PSII become unable to pass on their electrons and remain in the reduced condition. Therefore, electrons released from chlorophyll during continued illumination cannot be used for photochemical reactions. As a result, during a saturating flash of light following dark-adaptation of a leaf quantum yield of photochemistry ( $P = \text{moles of O}_2 \text{ evolved} / \text{moles of photons absorbed}$ ) declines to zero and Fluorescence yield (F) and Heat dissipation reach maximal values.

One of the most widely used fluorescence parameters is  $F_v/F_m$  measured during a dark to light transition.  $F_o$  is the dark adapted minimal fluorescence due to the measurement beam (very weak light).  $F_o$  is observed when all reaction centers are "open" or oxidized (capable of receiving an electron).  $F_m$  is the maximal fluorescence yield when all reaction centers are "closed" (unable to accept an electron).  $F_m$  is produced by giving the leaf a saturating pulse (very high light to close all reaction centers). The difference between  $F_m$  and  $F_o$  is termed variable fluorescence ( $F_v$ ).

The ratio  $F_v/F_m$  is approximately 0.8 in non-stressed leaves in the dark.

$F_v/F_m$  is well correlated with quantum yield (moles of O<sub>2</sub> evolved / moles of photons absorbed) and is often used as a measure of quantum efficiency. An analogous form of  $F_v/F_m$  ( $\phi_{PSII}$ ) can also be measured in the light and is often used to estimate *in situ* rates of electron transport. We will estimate the electron transport rate for leaves exposed to different light levels (a light response curve).



# CHLOROPHYLL FLUORESCENCE

## II Methods and Materials

- (1) Your instructor will set up and calibrate the equipment prior to lab.
- (2) Turn on the light control box (actinic light) by turning the switch to the 'On' position and turn the "Intensity" potentiometer counter-clockwise until it clicks. This will maintain the leaf in the dark until the potentiometer is turned clockwise.
- (3) Place an opaque card (3x5 card provided) into the leaf clamp. Make sure the fluorometer is turned on. Observe the voltage signal from the fluorescence detector both graphically and numerically in the data box at the bottom of the computer screen. Fluorescence will have a low value because the card contains very little material that fluoresces at the wavelengths detected by the photodetector.
- (4) Press the red "Flash" button on the front of the Actinic Light Control Box. This will produce a flash of light with an intensity in excess of  $5000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ . A spike will be seen on the graph showing actinic light level. This is an event marker and the recorded level does not correspond to the light level during the flash. Note that there is little increase in the fluorescence signal during the flash of light.
- (5) Place a dark-adapted leaf in the leaf clamp. Stop data collection by clicking on the **STOP** icon. There is no need to save the data collected with the card in place.
- (6) Start data collection by clicking on the **COLLECT** Icon. Expose the leaf to the LED source, and adjust the gain control on the fluorometer, if necessary, to set the fluorescence value at an appropriate value with a low signal noise. The value you obtain at this time is the  $F_0$  value. A reading between 0.2 and 0.6 is usually optimal. At this point you may adjust the position of the LED and detector housing (and the leaf chamber if it is used) to optimize signal characteristics. If you make significant adjustments to the system it may be necessary to re-measure zero, without the leaf, at the end of the experiment.
- (7) Press the red **FLASH** button on the light control box and observe the transient increase in the chlorophyll Fluorescence signal. The peak value represents  $F_m$ . Note that the saturating flash disturbs the dark-adapted state of the leaf. Therefore, if you have any difficulty in obtaining a good  $F_m$  value and need to adjust the geometry of the system, you must dark adapt the leaf before measuring  $F_m$  again.
- (8) Having measured  $F_m$ , turn on the actinic light source and adjust the "Actinic" potentiometer on the control box by turning it clockwise slowly until a light level of approx.  $500 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  is attained. This will cause Fluorescence to increase quickly and then decline slowly to a steady value  $F_t$  when photosynthetic induction is complete. This may take several minutes due to photosynthetic

## CHLOROPHYLL FLUORESCENCE

induction and the slow opening of stomata after illumination. You may use the Auto Flash to provide periodic saturating flashes to help track steady state.

- (9) Reduce the actinic light level to approx.  $50 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ , wait until a steady  $F_t$  value is attained and then press the Flash button to measure  $F_m'$  at the new light level. Record the irradiance in Table 16.1 but do not attempt to record  $F_t$  or  $F_m'$  until the experiment is complete.
- (11) Repeat the procedure at 100, 200, 300, 400, 600, 800, 1200, 1600 and 2000  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ .
- (12) Save your data by selecting "Save As" from the FILE menu. Give your data an appropriate name other than the name of the set-up file, and save it in the location **allocated by your instructor**.

### III. DATA ANALYSIS

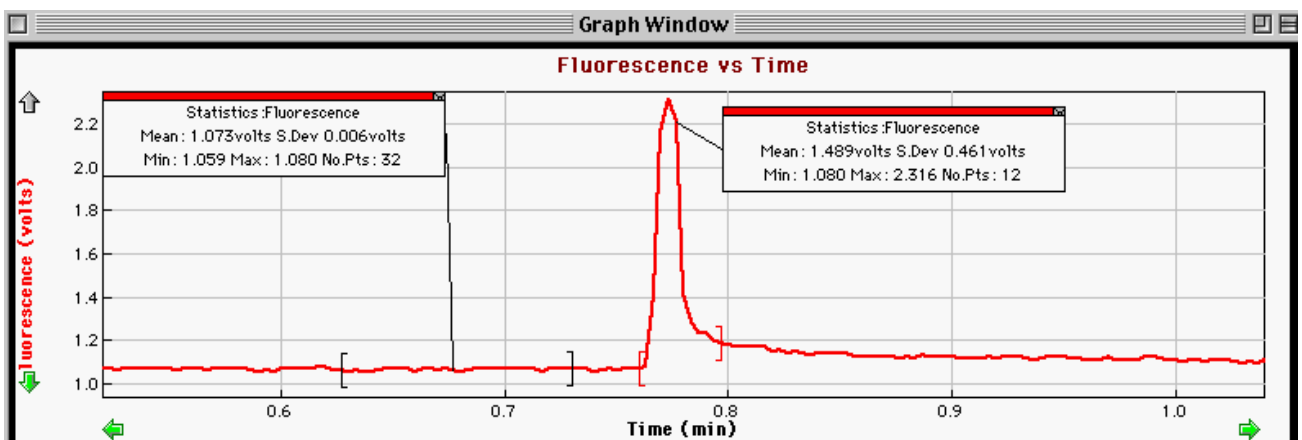
- Open the file containing data from the shade plant. Your data will appear on the screen exactly as it appeared when you saved it at the end of the experiment.
- Click on the graph showing your Fluorescence data and then select VIEW from the main menu. Click on GRAPH LAYOUT, select ONE PANE and then click on OK. The Fluorescence graph will now fill the entire screen making data analysis easier.
- Place the cursor to the left and just above the part of the trace showing your  $F_m$  value in the dark-adapted leaf. Click and hold on the mouse as you drag the cursor across your data so that a black box appears around the  $F_o$  and  $F_m$  values collected at the beginning of your experiment.
- Select VIEW and ZOOM IN. The data in the black box will now fill the entire screen.
- Select ANALYZE and then EXAMINE. A vertical line will appear on your graph that can be moved along the data points on the graph by moving the mouse. A box will also appear on each graph showing data values and time values. As you move the vertical line on the graph, the numerical display in the box will change to show you the exact data values and time value at the point on the graph where the line is situated. If the box obscures any part of the trace click on it and hold, then drag with the mouse to place the box in a convenient location.
- Scroll the cursor across your Fluorescence data and identify the values of  $F_o$  and  $F_m$  in the dark-adapted leaf. Record these data in Table 16.1. If you have difficulty identifying peak data refer to the **Statistics** section below.

# CHLOROPHYLL FLUORESCENCE

- Select VIEW and then UNDO ZOOM. Your graph will return to its original configuration. Move the cursor to the next point in which you applied the first saturating flash to the illuminated leaf and Zoom In as described above. Record the Ft value attained just before the saturating flash and the Fm' value attained during the flash. Record these data in Table 16.1.
- Repeat the procedure at each irradiance used in your experiment. At the highest light intensities it may be more difficult to determine accurate values of Ft and Fm', since these values are closer together. In these cases, use the statistics function of Logger Pro, as described below, to obtain these values precisely.
- For each leaf calculate the quantum yield of photochemistry ( $\phi_{PSII}$ ) at each light level used in your experiment. Calculate the electron transport rate (ETR) at each light level and plot this against irradiance.

## Using the Statistics Function to Obtain Values

If there is noise on the Fluorescence signal, or the Fm' value is only slightly greater than the Ft value, you may use the Statistics function of Logger Pro to obtain the best values for your Fluorescence parameters. Zoom In on your data as described above and then highlight the data you wish to examine by clicking and dragging over the data with the mouse. A black box will appear around the selected data, which will remain when you unclick on the mouse. Select "Analyze" from the main menu and then "Statistics". A box will appear on the screen showing the mean, maximum and minimum values of all the data in the box, as well as other statistical parameters. From this data you can obtain, for example, the mean value of F or Fo, before a saturating light pulse was applied. If you use the statistics function to analyze the data following a saturating pulse, the maximum value shown in the statistics box identifies the maximum Fluorescence value (Fm or Fm'). You may delete the statistics boxes from the screen by clicking on the icon in the top right hand corner of the box.





# CHLOROPHYLL FLUORESCENCE

## VI. ASSIGNMENT AND DISCUSSION QUESTIONS

NAME \_\_\_\_\_

- A. (4pts) Plot of the electron transport rate (ETR) against irradiance.
- B. (3pts) Explain the plot of ETR and irradiance. Why is it linear at low irradiance? Why does the shape of the light response curve flatten out at higher irradiances? Describe what is limiting photosynthesis.
- C. (3pts) Explain why the  $F_v/F_m$  obtained for darkened leaves differs from  $\phi_{PSII}$  obtained for leaves exposed to higher light levels (both  $F_v/F_m$  and  $\phi_{PSII}$  are quantum efficiencies)?
- D. (2pts) Explain why chlorophyll fluorescence is a powerful tool to probe the light reactions of photosynthesis and plant stress. Why does it “work?”

# CHLOROPHYLL FLUORESCENCE