How It Works: Chlorophyll Fluorescence the Basics

Photosynthesis is my favorite metabolic pathway. I love everything about it. Today's post is going to look at one really neat, non-invasive way scientists can monitor photosynthesis: chlorophyll fluorescence.

During photosynthesis, light excites electrons in the chlorophylls found in antenna and photosystem cores. These excited electrons are passed along and can be used in photosynthesis, however, very rarely is 100% of this energy used in this manner. Imagine for a moment that you are photosystem II and your mouth is the special chlorophyll that can pass the electrons along. Now, you have a few friends who hang out with you, they get to be the antennas (light harvesting complexes). Instead of electrons, your friends are going to pass popcorn at you. Only the popcorn that you catch in your mouth, gets to be used in photosynthesis. If your friends slowly toss one piece of popcorn at a time, you can probably catch 100% of the popcorn. But what happens when they all throw a piece at you? Or when they each start throwing handfuls? Think you could catch 100% of the popcorn? Probably not. Neither can the photosystems, which means this energy has to be released other ways.

There are 3 fates this energy can undergo: used in photosynthesis (photochemical quenching), emitted as heat (non-photochemical quenching), or released as a light particle (fluorescence). The fluorescence part can be measured and then used to calculate photochemical and non-photochemical quenching. This is done with a fluorometer. There are 2 main types of fluorometer's utilized in the study of photosynthesis: Fast Repetition Rate (FRR) and Pulse Amplitude Modulated (PAM). The difference is in the fashion the light is emitted but the data one can gather is similar. I spent my master's degree using PAM so I'll be using it as my reference.
Examples of PAM fluorometers. Walz Diving and bench top models.

The main function of the fluorometer is to provide light and measure the amount of fluorescence. Some quick and important photobiological terms:

- Open reaction centers - Photosystems are ready to accept electrons
- Closed reaction centers - Photosystems have accepted electrons and cannot take any more at the moment.

The state of the reaction centers are important. If they are all open, not a lot of energy is going to be reflected back (fluorescence). Conversely, if they are all or mostly closed, the amount of fluorescence will go up. With that in mind, let's look at mock up chlorophyll fluorescence trace and link each part to the corresponding photobiology.

Diagram of a PAM fluorometry trace. Orange stars indicate when light pulse occurs.

Usually the sample plants have been dark adapted for at least 10 minutes prior to reading (if you look at the diving PAM image above you'll note the gray circles attached to the coral, those were our homemade dark adapters). This dark adaption allows all of the electrons in PSII to be passed through to the end of the electron chain, rendering all of the reaction centers open. The amount of
fluorescence detected in the dark is the background level when all reaction centers are open and is designated F0 or minimal fluorescence.

The saturating pulse is then fired (orange star). This saturating pulse is a very strong light, providing a plethora of photons and rendering all of the reaction centers closed. The top of the peak, when all of the reaction centers are closed, is labeled Fm or maximal fluorescence.

At this point what is known as the actinic light is activated. The actinic light is a non-saturating, steady beam of light that allows photosynthesis to occur but does not saturate (close) all of the reaction centers. Each saturating pulse now results in a small peak that corresponds to photochemical quenching or the amount used in photosynthesis. The difference from the top of photochemical quenching peak (Fm') to the original saturation pulse (Fm) is accounted for by non-photochemical quenching.

Non-photochemical quenching (NPQ) can be separated out into 3 different types. These can also be measured by fluorescence. The fastest, occurring in seconds to minutes, is called qE which involves the xanthophyll cycle which I've reviewed before. The second type, taking minutes to hours, called qT occurs when the light harvesting complexes attached to photosystem II move to photosystem I in an attempt to balance light capture and electron flow through the two systems. The slowest takes hours to days and is known as qI, or photoinhibition. Photoinhibition occurs when the reaction centers start becoming damaged and must be broken down to be repaired.

From the image above it is impossible to sort out the 3 types of NPQ. However, by dropping the sample back into darkness with precisely timed saturating pulses, one can calculate the fraction of NPQ coming from each of the 3 types. Observe the diagram below. Notice that as time advances the peaks get higher and higher. The differences in the height accounts for the fractions of each type of NPQ.

Diagram of a PAM fluorometry trace with NPQ parameters.

The final measurement I want to highlight from a chlorophyll trace is that of the photochemical efficiency of photosystem II, Fv/Fm. This is one of the most, if not the most, common fluorescence measurement I run across in the literature. Basically, this is a measure of the health
of the photosystems. For example, an Fv/Fm of 0.986 would suggest that the photosytems are running at 98.6% efficiency. A high efficiency means everything inside photosystem II is working properly and precisely. In contrast, when Fv/Fm is low, say 0.687 or 68.7% efficient, the photosystems are most likely stressed and/or damaged. Fv/Fm is calculated by taking the variable fluorescence (Fv) and dividing it by the maximal fluorescence (Fm). The variable fluorescence is simply Fm - F0. As a note, if you ever come across it in the literature as Fv'/Fm' that simply means it was calculated from samples in the light. In the field, it is not always possible to allow a 10 minute dark acclimation period and the ’ equals collected without dark adaption.

And that's the basics of how chlorophyll fluorescence works! There are a host of other parameters that can be calculated but the ones above are the ones I encounter the most in the literature. To sum up:

- qP = amount of energy used in photosynthesis
- NPQ = amount of energy sent into qE + qT + qI
- Fv/Fm = efficiency ("health") of photosystem II

Chlorophyll fluorescence is a powerful tool for photobiological research. There are many different models that can be used in the lab or in the field, the prices range from not bad for lab equipment to wow ouch, and the best part it does not harm the plant in anyway.

References