Developing Single Turnover Active Fluorometry (STAF) to monitor Phytoplankton Primary Productivity (PhytoPP) on wide spatial and temporal scales

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Why are we doing this?

PhytoPP is responsible for approximately half of the carbon fixed on a planetary scale.

Satellite remote sensing…

- Operates on the widest possible spatial and temporal scales
- Includes large errors for PhytoPP

The 14C tracer method…

- Most widely used direct method for assessment of PhytoPP
- Operates on spatial and temporal scales that result in extreme undersampling

The targets for this project are to develop STAF-based instrumentation and methodology for the assessment of PhytoPP on much wider spatial and temporal scales than can be achieved using the ^{14}C tracer method, at comparable levels of accuracy and precision.

The aim of this poster is to provide an overview of STAF fluorometer systems as a cost-effective platforms for measuring PhytoPP on spatial and temporal scales that greatly relieve the existing undersampling issue.

Chelsea Technologies' LabSTAF and AutoSTAF systems were developed within the NERC-funded OCEANIDS programme (NE/P020844/1). Development of the next generation of smaller, more power efficient, deployable STAF systems is continuing within the EU-funded Technologies for Ocean Sensing (TechOceanS) programme.

Target platforms include…

- Laboratories and underway systems on research ships (**LabSTAF**)
- Large autonomous platforms, including AutoSub LR and AutoNaut (**AutoSTAF**)
- Smaller autonomous platforms, Gliders and Argo Floats (**next generation STAF systems**)

For PhytoPP assessment…

- High dynamic range for all STAF systems (from extreme oligotrophic to mesotrophic)
- Highly automated, STAF-based measurement of PSII photochemical flux (mol electrons m^{-3} s^{-1})
- Validation of STAF through ^{14}C-fixation (mol C m^{-3} s^{-1})

Overall targets…

- Minimise the errors generated when using STAF to generate values of PSII photochemical flux
- Increase our understanding of the factors defining $\Phi_{e,C}$

The basic STAF measurement [6,8]

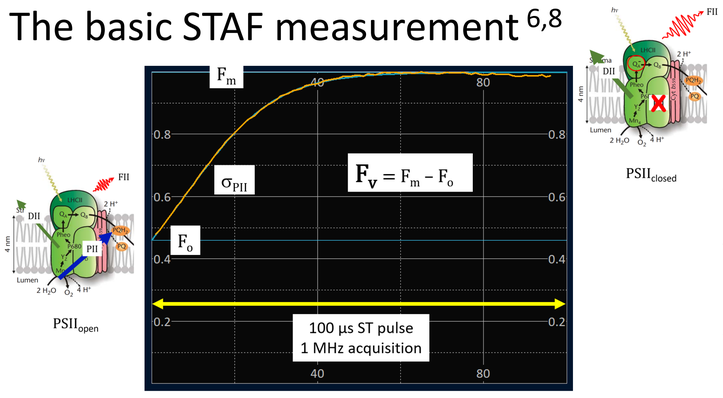

Figure 1: All STAF instruments incorporate the Single Turnover (ST) method, which typically uses 100 µs LED pulses to saturate PSII photosynthesis. The interrogated sample volume is < 5% of the total (10 to 20 mL). Passive sample exchange allows for high measurement frequency and provides high quality data in extreme oligotrophic conditions.

The ST trace image within this figure, and the images in Figures, 2, 4 and 6, are screenshot crops from the RunSTAF software used to control the LabSTAF and AutoSTAF systems.

$PSII_{open}$ and $PSII_{closed}$ graphics: Govindjee et al. (2010)

Within **Figure 1**, F_v is 'variable' fluorescence. F_o and F_m are the 'origin' of variable fluorescence and the 'maximum' level of fluorescence, respectively. σ_{PII} is the absorption cross section of PSII photochemistry. Under actinic illumination, the equivalent terms are Fq', F', F_m' and σ_{PII}'. Although unitless, all fluorescence (F) values reported by STAF systems are on a calibrated scale. Reported values of $\sigma_{PII}^{(')}$ have reported units of nm^{-2} $PSII^{-1}$.

The FLC-generated rP-E curve [1,3,4,6]

A Fluorescence Light Curve (FLC) comprises an automated set of STAF measurements made at several photon irradiance levels between darkness and saturating light. The FLC-generated dataset is comparable to the ^{14}C tracer-based photosynthesis – photon irradiance (P-E) curve.

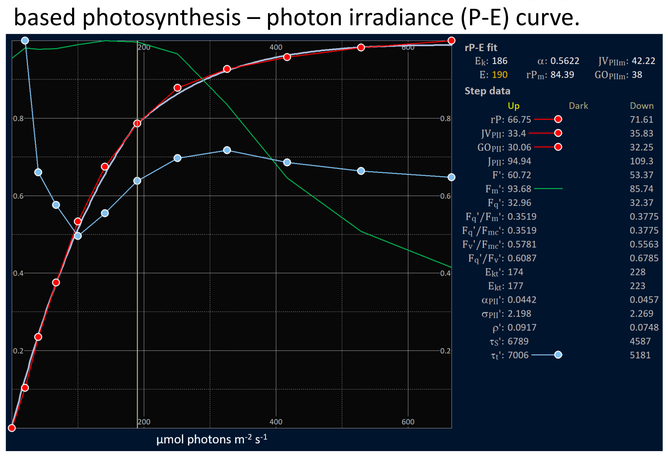

Figure 2: The first data processing step generates a relative photosynthesis-photon irradiance (rP-E) curve, where rP values are the product of incident photon irradiance and PSII photochemical efficiency (ϕ_{PII}'). The dimensionless values of ϕ_{PII}' are calculated as F_q'/F_{mc}'. This generates reported rP units of µmol photons m^{-2} s^{-1}, which translates as the rate of PSII photochemistry through a σ_{PII}' of 1 m^2.

The 'c' subscript within F_{mc}' indicates that a baseline correction has been applied, if appropriate (see **Corrections required**).

A range of other STAF-derived parameters are generated by RunSTAF at the FLC level. In this example, F_m' and τ_t' are plotted with rP. Decreasing F_m' often tracks downregulation of PSII photochemistry. The τ_t' time constant tracks the turnover time for PSII photochemistry, with reported units of µs.

PSII photochemical flux per PSII or per unit volume [2,3,4,6]

$$J_{PII} = \sigma_{PII}' \cdot \frac{F_q'}{F_v'} \cdot E \qquad \textbf{Equation 1}$$

$$JV_{PII} = a_{LHII} \cdot \frac{F_q'}{F_{mc}'} \cdot E \qquad \textbf{Equation 2}$$

Under the assumption that each PSII photochemical event results in the transfer of an electron from oxygen to plastoquinone, the SI units of rP are mol electrons m^{-2} s^{-1}. STAF systems allow for the conversion of rP values to PSII photochemical flux. Photochemical flux per PSII has SI units of electrons $PSII^{-1}$ s^{-1} and is defined by the term J_{PII}. PSII photochemistry per unit volume (of ocean or other medium) has SI units of mol electrons m^{-3} s^{-1} and is defined by the term JV_{PII}.

RunSTAF incorporates the Sigma method to generate the reported values of J_{PII} (**Equation 1**) and the Absorption method to generate the reported values of JV_{PII} (**Equation 2**).

The Sigma and Absorption methods [2,3,4]

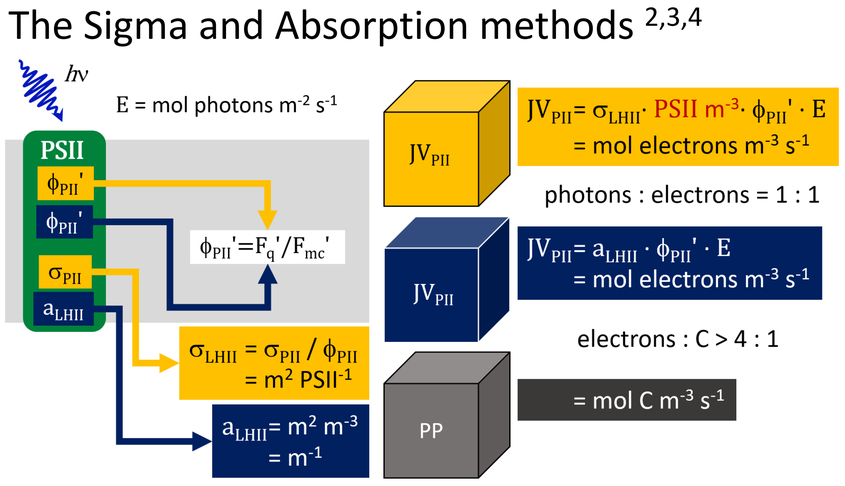

Figure 3: The Sigma method can provide J_{PII} (electrons $PSII^{-1}$ s^{-1}) from STAF data alone, but not JV_{PII} (mol electrons m^{-3} s^{-1}). The Absorption method can provide JV_{PII} from STAF data alone.

Conversion of Sigma-derived J_{PII} to JV_{PII} requires independent assessment of the concentration of photochemically active PSII m^{-3} (shown in red within the figure).

Although the Absorption method can provide PSII m^{-3} directly from STAF data, plugging this value into the Sigma equation makes it an exact equivalent of the Absorption equation (the included σ_{PII} values cancel out).

Corrections required

Generating values of J_{PII} or JV_{PII} requires spectral correction. Generating values of JV_{PII} also requires corrections for the package effect (**Figure 5**) and an assessment of baseline fluorescence (**Figure 6**).

- Spectral correction requires accurate determination of the photon flux and spectral output from the LEDs used to provide the ST measurement pulses and actinic light plus the Photochemical Excitation Profile (PEP) of the sample
- Package effect correction is achieved through dual waveband measurement of fluorescence emission at maximum and minimal reabsorption (±10 nm half bandwidth, centred at 685 nm and 730 nm)
- Baseline fluorescence correction is appropriate in situations where a low measured value of F_v/F_m can be attributed to the accumulation of photoinactivated PSII complexes

Spectral correction using a PEP [4]

The main function of the PEP is to facilitate spectral correction of J_{PII} and JV_{PII}. The PEP data are generated using different combinations of measurement LED wavebands. RunSTAF calculates both F_v and σ_{PII} values from the ST curves generated by these combinations (see Poster 2). Only the F_v values are used for spectral correction. Although generating a PEP is technically challenging, the process is fully automated and typically requires no more than two minutes of measurement.

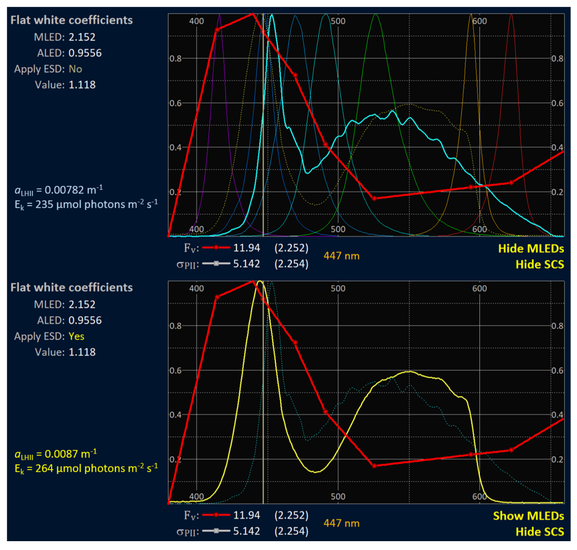

Figure 4: A PEP covers the spectral range between 416 and 622 nm. The Spectral Correction Spectra (SCS) range extends the F_v PEP to between 380 and 660 nm.

The red line in the top panel shows an extended PEP from a natural freshwater sample. The emission spectrum of the actinic LED within the LabSTAF is shown in blue. The emission spectra of the seven measuring LED wavebands are also included.

The dashed yellow line in the top panel shows an imported emission spectrum, from a photosynthetron light source. This feature has been added to facilitate comparison of data from parallel STAF and ^{14}C-fixation measurements.

In the lower panel, the spectrum from the photosynthetron light source has been applied to the LabSTAF data set (**Apply ESD**). This triggers recalculation of all affected parameter values, including a_{LHII} and E_k. The change in a_{LHII} has a proportional affect on JV_{PII} (see **Equation 2**).

Package effect correction [1,4]

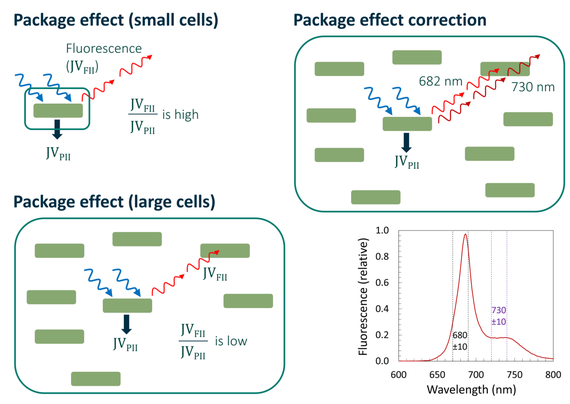

Figure 5: With the STAF method, fluorescence emission is typically measured at 685 nm. This provides the maximum spectral discrimination between emission from photochemically active PSII complexes and other sources. The downside is that reabsorption of fluorescence emission by PSII is close to maximum within this waveband. The actual level of reabsorption is dependent on the optical characteristics of individual cells (the package effect).

The ratio of F_v measured at 685 nm and 730 nm can be used to correct for the package effect. Typically, a fully automated package effect correction requires no more than 30 seconds.

Baseline correction [1,4]

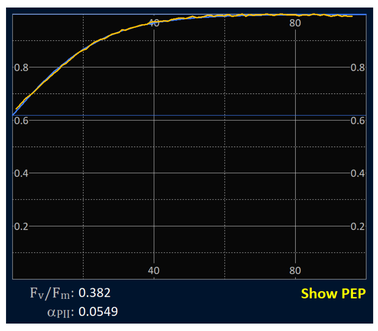

Figure 6: In this example, a ST pulse applied to a dark-adapted sample has generated an F_v/F_m of 0.382, which is significantly lower than the 0.5 to 0.65 range that is widely seen from 'healthy' dark-adapted cells. This lower value may be the result of locked-in downregulation of PSII photochemistry, or may indicate a high level of fluorescence from sources other than photochemically active PSII complexes (including photoinactivated PSII complexes) coupled with an intrinsic PSII photochemical efficiency within the healthy range. **Equation 3** can be used to calculate baseline fluorescence (F_b) at on an assumed intrinsic F_v/F_m (the F_v/F_{mc} term within the equation). Sample data are provided within Poster 2.

$$F_b = F_m - \frac{F_v}{(F_v/F_{mc})} \qquad \textbf{Equation 3}$$

Direct comparison of JV_{PII} and ^{14}C-fixation

Parallel measurements of JV_{PII} and ^{14}C-fixation are required to understand the factors controlling the variable ratio between them. To date, methodological inconsistencies, including differences in incubation lengths and light quality, have greatly inhibited practical assessment of electron to carbon ratios ($\Phi_{e,C}$ values). The image in Figure 7 shows a LabSTAF unit being used to run a 'dual incubation' measurement of JV_{PII} and ^{14}C-fixation. This measurement is facilitated by the relatively large sample chamber incorporated within LabSTAF (25 mm ID and 56 mm height). The ^{14}C-spiked sample is contained within a scintillation vial (23 mm OD). In addition to providing a single actinic light source for both measurements, this arrangement allows STAF measurements to be made over the entire ^{14}C incubation period.

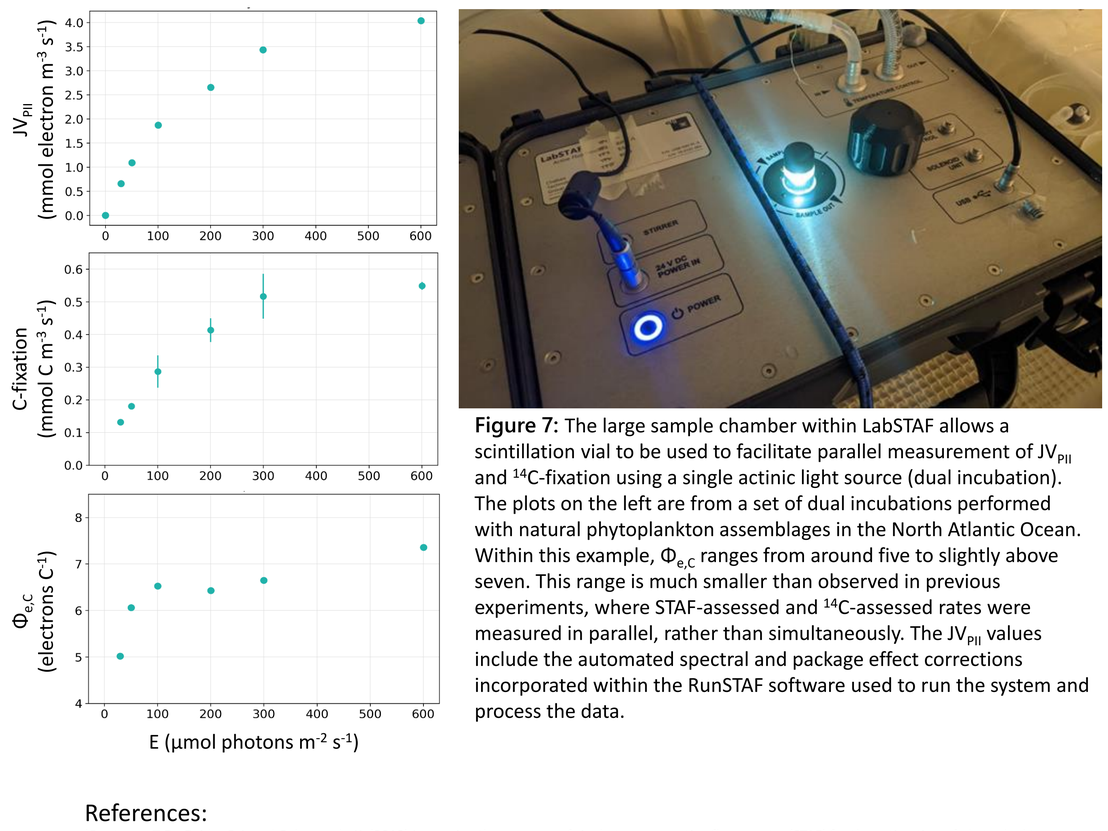

Figure 7: The large sample chamber within LabSTAF allows a scintillation vial to be used to facilitate parallel measurement of JV_{PII} and ^{14}C-fixation using a single actinic light source (dual incubation). The plots on the left are from a set of dual incubations performed with natural phytoplankton assemblages in the North Atlantic Ocean. Within this example, $\Phi_{e,C}$ ranges from around five to slightly above seven. This range is much smaller than observed in previous experiments, where STAF-assessed and ^{14}C-assessed rates were measured in parallel, rather than simultaneously. The JV_{PII} values include the automated spectral and package effect corrections incorporated within the RunSTAF software used to run the system and process the data.

References:

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