Biospherical Instruments Inc.

APPLICATION NOTE:

Natural Fluorescence Calculations: Terminology and Units

The purpose of this document is to provide a ready reference for the equations, coefficients, and units used in the calculation of chlorophyll and instantaneous gross photosynthesis from measurements of Natural Fluorescence. Although the theoretical relationships apply to any of our Natural Fluorometers, this discussion is meant to emphasize the practical information needed to evaluate output from software, focusing primarily on the units of the measurements and the flow from measured values to calculated results. To be useful to researchers over a wide range of backgrounds, we have tried to define as many terms as possible.

The calculation of chlorophyll concentration and primary production results from the coincident measurement of two optical variables: 1) the incident irradiance (which is driving photosynthesis) and 2) the upwelling red radiance that results from fluorescence by the phytoplankton crop.

Photosynthetically Active (or Available) Radiation (PAR). For the purposes of our instruments, Photosynthetically Active Radiation (PAR) is measured over the spectral region from 400 to 700 nm using sensors with a flat quantum response (responds equally to all wavelengths). Instruments are available from Biospherical with one of two different irradiance geometries. The PNF-300 measures scalar irradiance, E_0, over PAR, in units of $\mu E \cdot m^{-2} \cdot sec^{-1}$, using a spherical collector (looking something like a table tennis ball) to achieve a constant directional response over nearly 4π steradians solid angle Part of the logic behind using the scalar collector is that many photosynthetic systems are scalar collectors - they respond to photons independently of the radiance distribution of the light field. In contrast, the PRR-600 and PUV-500 measure downwelling irradiance, $E_d(PAR)$, using a flat collector (2π steradians) specifically designed to achieve a response that is proportional to the cosine of the angle of incident light (a so-called cosine collector).

An Einstein, denoted "E," is a mole of quanta or photons and 1 µE is 6.022×10^{17} quanta.

The areal units of irradiance vary depending on the model and you should consult your calibration to confirm them. For instance, the PUV is calibrated in $\mu E \cdot cm^{-2} \cdot sec^{-1}$ so there is a difference in areal unit for the PUV from the PNF (cm^{-2}, not m^{-2}). The irradiance values resulting from measurements of $E_d(PAR)$ and $E_0(PAR)$ are not the same, but there is much literature concerning their relationship (see Van Tran and Collins, SPIE Ocean Optics X, 1302: 443-453).

Upwelling Radiance. Given an accurate measurement of available irradiance, a Natural Fluorometer is an optical sensor whose spectral responsivity is designed to measure upwelling radiance $L_u(chl)$ ($nE \cdot m^{-2} \cdot sec^{-1} \cdot str^{-1}$) specifically over the emission spectrum of chlorophyll a. If V (volts) is the output of the sensor with responsivity $R(\lambda)$ (volts per $nE \cdot m^{-2} \cdot sec^{-1} \cdot str^{-1}$) to a

radiance with the emission characteristics of chlorophyll, $L_C(\lambda)$ (nE·m^{-2}·sec^{-1}·str^{-1}), then Equation 1 can be used to measure the radiance from chlorophyll fluorescence:

$$L_u = \frac{V \int_{\lambda=400}^{700} L_c(\lambda)\, d\lambda}{\int_{\lambda=400}^{700} R(\lambda) L_c(\lambda)\, d\lambda} \qquad (1)$$

Typically, the emission spectrum of chlorophyll a is assumed to have a maximum at 683 nm *in vivo*, and it is important to distinguish between measurements of $L_u(683)$ and $L_u(chl)$. $L_u(683)$ (measured by some PRR-600s, some MERs, and the MRP-200 with a 683-nm radiance detector) is a measurement of upwelling radiance centered spectrally at 683 nm using a sensor with a 10 nm wide spectral responsivity. These sensors are calibrated using a broadband extended source and normally the units are radiance in μW·cm^{-2}·str^{-1}·nm^{-1}, which is different from those of $L_u(chl)$. Note: the calibration constants for a $L_u(683)$ sensor depend on the source bandwidth being significantly wider (spectrally flat) than the 10 nm bandwidth. For measurements of chlorophyll emission (which is not spectrally flat), the $L_u(683)$ sensor is not the best detector and the following calculations should be reviewed carefully.

A steradian (str) is a unit of solid angle describing a "slice" out of a sphere where the complete sphere has 4 str of solid angle, a hemisphere has 2 str, half of a hemisphere has 1 str, etc.

An $L_u(chl)$ Natural Fluorescence detector has a response function that is wider than 10 nm and closely follows the emission spectrum for chlorophyll. The calibration units for an $L_u(chl)$ sensor are specifically referenced to a chlorophyll-like source. Assuming that the source is a chlorophyll *a* emission, careful consideration of the bandwidths of both the source and sensor is required for proper interpretation of the data. **The PNF and PUV instruments are always fitted with detectors for $L_u(chl)$.** The PRR may have either or both types of detectors. (Note that since our radiance detectors are collecting light from a solid cone where the detector is positioned at the apex, the geometric unit describing the volume viewed is the steradian.)

$$L_u = \frac{(V - b)}{m} \qquad (2)$$

In practice, the conversion from voltage out of the sensor to L_u is done using the calibration constants listed on the calibration certificate of each instrument, which takes the responsivity and spectral source (Equation 1) into account. Equation 2 shows this relationship, where V is the output of the sensor in volts, b (volts) as the offset as listed on the calibration certificate, and m is the scale factor as listed on the calibration certificate ($L_u(chl)$ scale factor is volts per nE·m^{-2}·sec^{-1}·str^{-1}).

Natural Fluorescence. Assuming the instrument is fitted with the appropriate detector and calibrated properly, a number of intermediate variables are calculated from the actual measurements of radiance and irradiance in order to calculate chlorophyll concentration and instantaneous photosynthetic rates. The volume fluorescence flux of natural fluorescence (F_f), that is, the measure of light per unit volume assuming the source is chlorophyll fluorescence, can

be calculated from measurements of $L_u(chl)$ and knowledge of the rate of attenuation of light through the water:

$$F_f = 4\pi(k(PAR) + a(chl))L_u(chl) \quad (3)$$

In equation 3, the term 4π is a geometric constant with units of steradians (not str^{-1} as listed in Chamberlin *et al.* (1990), Deep-Sea Res. 37(6):951-973). The diffuse attenuation coefficient for PAR, *k(PAR)* (m^{-1}), is calculated from the irradiance profile, and accounts for the attenuation of the excitation irradiance as a function of depth below the sensor. Our research in the field (unpublished) has shown that there is very little difference between the attenuation coefficients for scalar and cosine PAR. Since k(PAR) is calculated *as a function of depth* from the irradiance profile, it is important to deploy the instrument at a reasonably constant rate and not to hold it at one depth. In the event that the sensor is moored and there is no significant change in depth with time, then the calculations for chlorophyll concentration and photosynthetic rates may be performed manually with an assumed value for k(PAR). The term *a(chl)* is the total absorption coefficient for water and its constituents weighted over the emission (not absorption) spectrum of chlorophyll. This term accounts for the decrease in the fluorescence signal with distance from the detector. Over this region, *a(chl)* is most strongly influenced by water absorption and may assumed to be reasonably constant at low to moderate chlorophyll concentrations (we typically assign value of 0.48 m^{-1}). Collectively, these terms are used to convert the radiance measurement ($\mu E \cdot m^{-2} \cdot sec^{-1} \cdot str^{-1}$) to volume fluorescence ($\mu E \cdot m^{-3} \cdot sec^{-1}$).

"For marine atmospheres with sun altitudes above 22°, the quanta/watt ratio for the region 400-700nm is 2.77 x 10^{18} quanta/sec/watt to an accuracy of plus or minus a few percent." This quote and a further discussion of the relationship of quanta to watts in the water column can be found in Smith and Morel (1974) Limnol. Oceanogr. 19(4):591-600.

If your instrument is fitted with a Lu(683) detector instead of a Lu(chl) detector, but you are interested in working with the fluorescence signal using the formulations of Kiefer et al., you will need to convert the radiance measurement from $\mu W \cdot cm^{-2}$ radiometric units (ε in watts) to $\mu E \cdot m^{-2} \cdot sec^{-1}$ quantum units (*F* in quanta·sec^{-1}). This conversion is wavelength dependent since the energy of a photon is determined by its wavelength:

$$F[\text{quanta sec}^{-1}] = 5.03x10^{15}\varepsilon[watts]\lambda[nm]$$

Use the center wavelength of the detector as the wavelength (i.e., λ=683). Don't forget to include the units of area in your conversion (cm^{-2} or m^{-2}). Lastly, accounting for the change in the photodetector responsivities requires the addition of a geometric constant (27) to Equation 3 (e.g., equation 8 in Kiefer et al. (1989) Limnol. Oceanogr. 34(5):868-881):

$$F_f = 4\pi 27(k(PAR) + a(chl))L_u(chl)$$

Chlorophyll. Chlorophyll concentration ($mg \cdot m^{-3}$) may be calculated from the natural fluorescence flux, F_f, and the incident irradiance using Equation 4. There are two important optical assumptions used in this: ${}^{o}a_c(PAR)$ is the chlorophyll-specific absorption coefficient (absorption normalized to chlorophyll concentration) and ϕ_f is the quantum yield of fluorescence. In our early work, these values were treated as constants, such as in the software for the PNF-300 and PUV-500, which assigned typical values of 0.04 $m^2 \cdot mg^{-1}$. and 0.045 μE

fluoresced per μE absorbed, respectively. Subsequent natural fluorescence research by the community has shown that holding these values constant is a simplification and specific applications may require verification of the values or empirical adjustments.

$$Chl = \frac{F_f}{{}^{o}a_c(PAR) * \phi_f * E_o(PAR)} \qquad (4)$$

Primary Production. The rate of photosynthesis, F_c, (ng-atoms-Carbon·m^{-3}·sec^{-1}) may be computed from the volume fluorescence (Equation 3) and the incident irradiance using Equation 5. The empirical constants ϕ_{rmax} and k_{cf} are the maximum value of the ratio of the quantum yields of photosynthesis to fluorescence and the irradiance at which this ratio is one-half the maximum. A value of 4.0 carbon atoms per photon has been assigned to ϕ_{rmax}; k_{cf} has been assigned a value of 133 μE·m^{-2}·sec^{-1}. (Note that this value was assigned units of milliEinsteins as a typographic error in Chamberlin *et al.* (1990), Deep-Sea Res. 37(6):951-973.)

$$F_c = F_f \frac{k_{cf}\phi_{r\max}}{k_{cf} + E_o(PAR)} \qquad (5)$$

These constants are empirical, and specific applications may require verification or adjustment.

As discussed in Kiefer *et al.* (1989), the accuracy of the measurement of chlorophyll from measurements of natural fluorescence decreases as concentrations increase above about 5 mg·m^{-3}, such as in the middle of a bloom. This decrease in accuracy occurs because at high concentrations, chlorophyll itself begins to absorb the red fluorescence and the flux never reaches the detector on the instrument. In addition, high concentrations of dissolved materials or small particles that absorb strongly in the red portion of the spectrum may also mask the fluorescence signal. These conditions will also reduce the fluorescent flux from the phytoplankton crop and it will not be efficiently picked up by the instruments, causing an underestimation of the actual values.

As discussed in Kiefer *et al.* and Chamberlin *et al.*, backscattered red light, typically near the surface, may reach the radiance detector and be improperly interpreted as fluorescence from the crop. This condition will tend to overestimate chlorophyll and production values.

Terminology and Units in Natural Fluorometers

Variable	Description	Units	Default Value
E_o	Scalar irradiance	$\mu E \cdot m^{-2} \cdot sec^{-1}$	Measured
E_d	Downwelling irradiance	$\mu E \cdot cm^{-2} \cdot sec^{-1}$	Measured
$L_u(chl)$	Upwelling chlorophyll radiance	$nE \cdot m^{-2} \cdot sec^{-1}$	Measured
$L_u(683)$	Upwelling red radiance	$\mu W\ cm^{-2} \cdot nm^{-1} \cdot str^{-1}$	Measured
F_f	Natural fluorescence	$nE \cdot m^{-3} \cdot sec^{-1}$	Calculated
F_c	Instantaneous gross primary productivity	Ng-at $C \cdot m^{-3} \cdot sec^{-1}$ or nmol $C \cdot m^{-3} \cdot sec^{-1}$	Calculated
chl	Chlorophyll a concentration	$mg \cdot m^{-3}$	Calculated
k(PAR)	Diffuse attenuation coefficient for PAR	m^{-1}	Calculated
a(chl)	Total absorption from water and its constituents over chl emission spectrum	m^{-1}	0.48
${}^{o}a_c(PAR)$	Chl-specific absorption coefficient	$m^2 \cdot mg^{-1}$	.04
ϕ_f	Quantum yield of fluorescence	µE fluoresced per µE absorbed	0.045
ϕ_{rmax}	Maximum value for (ϕ_c/ϕ_f) where ϕ_c is the quantum ield of photosynthesis)	carbon atoms per photon	4
k_{cf}	Irradiance at which ϕ_{rmax} is one-half the maximum	$\mu E \cdot m^{-2} \cdot sec^{-1}$	133

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