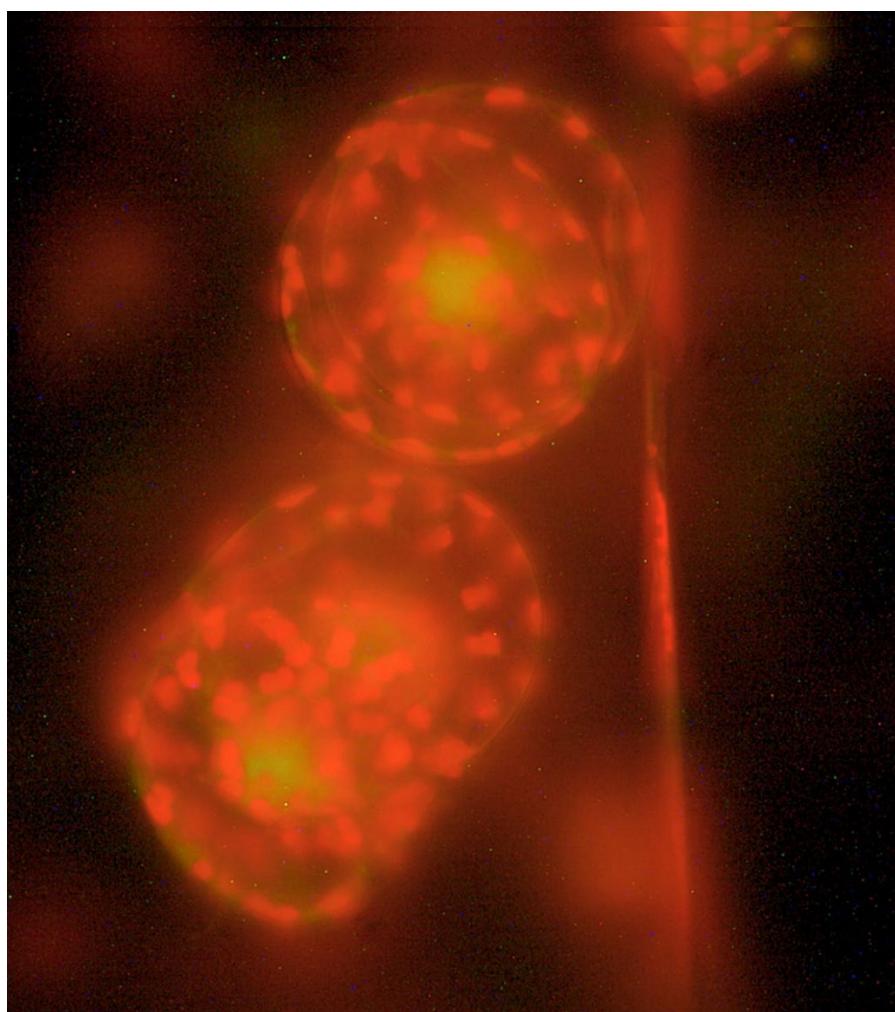# A User Guide for the Application of Single Turnover Active Chlorophyll Fluorescence for Phytoplankton Productivity Measurements

Version 1.0, June 8, 2021

SCOR Working Group 156

Centric diatoms from the Ross Sea Antarctica with red chlorophyll fluorescence visualized using epifluorescence microscopy.
Photo by P. Tortell

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Contents

Preface

This document represents the collective efforts of SCOR Working Group 156, 'Active Chlorophyll Fluorescence for Autonomous Measurements of Global Marine Primary Productivity'. The group was established in 2019, bringing together researchers and instrument manufacturers from 10 countries and 5 continents, with the goal of developing standards of best practice for the application of single turnover active chlorophyll fluorescence (ST-ChlF) to examine phytoplankton productivity. We focused our efforts on single turnover methods, which are most commonly used in phytoplankton research, while recognizing that other approaches, including Pulse Amplitude Modulation (PAM) fluorescence techniques, are also employed with macro-algae, corals and terrestrial plants. Over the past two years, our group has worked to build consensus around best practice for the collection, analysis and archiving of ST-ChlF data from a variety of aquatic environments. We aim to facilitate wide-spread use of ST-ChlF methods by the international research community, and have thus far focused our work on several key activities outlined in the Working Group's terms of reference.

1. *Develop, implement and document internationally-agreed best practice for the acquisition and analysis of ST-ChlF data to retrieve photosynthetic parameters and primary productivity estimates.*

2. *Develop, implement and document standardised ST-ChlF data output formats and archiving approaches.*

3. *Produce and distribute freely-available software and documentation to allow non-specialist users to analyze ST-ChlF data according to established best practices.*

We have recently submitted a manuscript to *Frontiers of Marine Science*, which provides a high-level synthesis of potential applications, opportunities and current limitations of ST-ChlF measurements. The manuscript outlines specific recommendations for users wishing to apply these methods and interpret the resulting data in the most robust manner possible. However, journal length restrictions precluded an in-depth treatment of many important topics (e.g. instrument calibration, data fitting and spectral correction), and the manuscript does not provide detailed step-by-step instructions for novice users. The current document is meant to expand on the material presented in the *Frontiers* manuscript, providing a hands-on guide for both experts and new users alike. Our goal is to provide both a strong theoretical background for ST-ChlF methods, and a practical handbook to inform the application of these methods across a wide range of aquatic environments.

This first version of the SCOR ST-ChlF user guide serves as a place-holder, outlining the main topics that will be addressed in the full-length document. We are currently working to refine the content of the individual sections outlined below, with the goal of releasing a first complete draft by late 2021. We are also developing a set of Python-based Jupyter notebooks (Ryan-Koegh and Robinson, 2021) to facilitate ST-ChlF data analysis from a range of different instruments and formats. We expect the contents of this User Guide to evolve significantly over the coming months, through our own efforts and with input from other experts and end-users. Once the first full draft is completed, we will solicit feedback from the international research community through open consultation and a planned town-hall meeting at the Ocean Sciences meeting, in February, 2022. Feedback on the document can be addressed to the working group chairs, Philippe Tortell and David Suggett. Revised versions of the User Guide will be posted on the Ocean Best Practices site, and interested readers are encouraged to check periodically for updates.

Acknowledgements

Dedication

We dedicate this work to memory of Jacco Kromkamp, who passed away on Oct. 5, 2020. Jacco was a pioneer in the development and application of ST-ChlF measurements to assess phytoplankton productivity. His ideas and insights contributed greatly to our field, and his warmth and friendship helped bring our community together. Much of the knowledge presented in this document was shaped by Jacco's research contributions and his passion for developing active chlorophyll fluorometry as a tool to monitor aquatic ecosystem health. He will be greatly missed.

Document History

Version Number	Date	Authors	Notes
1.0	June 8, 2021		Initial version outlining the document structure and the anticipated content of the main sections.

Document History

How to use this document

This document is intended to serve the needs of a wide range of users, from novices (first year graduate students, for example) to expert researchers with decades of experience using ST-ChlF methods. We anticipate that some readers will require only high-level information on key operational parameters needed for successful ST-ChlF measurements, while others will be interested in exploring the ideas and concepts further. For this reason, the document is built on a hierarchical structure of complexity, making liberal use of linked appendices with detailed exploration of ideas presented in the main document. The document will also include embedded links to a number of Jupyter notebooks containing tutorials and data processing tools. These software tools are meant to facilitate the inter-comparison of measurements collected by different users and instruments, helping to support the development of globally-coherent ST-ChlF data compilations.

1. INTRODUCTION

1.1. Photosynthetic Production

Photosynthetic primary productivity is the light-driven process of extracting reducing power from water to drive CO_2 reduction to carbohydrates (i.e. CO_2 'fixation'). The process is comprised of several steps, including light harvesting, stripping of electrons and O_2 from water, primary charge separation in the photosynthetic reaction center and the transfer of high potential electrons through a series of redox carriers to produce NADPH and ATP for CO_2 fixation. The reducing power generated through the photosynthetic process can be used to drive a number of other redox reactions, including the reduction of NO_3^-, assimilation of SO_4^{2-} and the dissipative photo-reduction of O_2 to water. In diazotrophic organisms, a portion of the acquired reducing power is also utilized in the process of nitrogen fixation.

Global primary productivity is a critical source of O_2 for the atmosphere and oceans, and has been responsible for setting the planetary redox state over geological time-scales. Marine primary productivity also plays an important role in carbon sequestration to the deep ocean through the so-called biological pump (e.g. Ducklow et al. 2001), while providing a critical source of organic matter to support aquatic food webs and metabolism. Global climate change has created a pressing need to better understand the environmental controls on marine primary productivity, its variability over space and time and its potential responses to various natural and anthropogenic perturbations (Behrenfeld et al. 2006, Moore et al. 2018). Addressing these questions requires consistent and coherent productivity measurements across a range of temporal and spatial scales.

1.2. Measuring Aquatic Primary Productivity

Measurements of aquatic primary productivity (PP) date back more than a century. Early approaches (Gaarder and Gran, 1927 1927) used changes in O_2 concentrations in light and dark bottles to assess gross and net productivity (GPP and NPP, respectively). These two terms quantify the total amount of carbon fixed (or photosynthetic O_2 evolved) by primary producers (GPP), and the amount of 'excess' fixed carbon (or photosynthetic O_2), after accounting for autotrophic respiration (NPP). A major advance occurred in the years post-WWII, with the increasing availability of the ^{14}C radiotracer and the development of the now classic Steeman-Nielsen incubation experiments (Steeman-Nielsen 1952). Depending on the length of the incubation period, ^{14}C measurements capture a signal somewhere between GPP and NPP, with shorter measurements more closely approximating GPP (Halsey & Jones 2015). In the decades following the advent of the ^{14}C method, other incubations approaches were developed, including $^{15}NO_3^-$ and $^{15}NH_4^+$ uptake experiments to estimate so-called 'new production' and 'regenerated' production, respectively, and $H_2^{18}O$ incubations to quantify the photosynthetic water splitting reaction as a metric of GPP. In these latter experiments, the evolved $^{18}O_2$ is diluted in a large background of unlabeled O_2 and thus not significantly affected by respiration.

Discrete incubation experiments have largely informed our understanding of oceanic PP variability, and in the case of ^{14}C measurements, have been used to benchmark satellite-based global productivity algorithms (Behrenfeld & Falkowski 1997, Bouman et al. 2018). Yet, discrete PP measurements are significantly limited in time and space, as they require dedicated personnel, the use of radioactive tracers (for ^{14}C incubations) and / or complex instrumentation (mass spectrometers for ^{15}N and ^{18}O measurements). These factors have ultimately greatly limited the coverage and sampling resolution of PP measurements (Halsey & Jones 2015). Moreover, the need for sample incubation creates potential containment artefacts, with adverse effects on some components of the plankton community.

To move beyond the limitations of discrete bottle incubation methods, a number of approaches have been developed to infer PP from mixed layer chemical tracers, including nutrients, dissolved inorganic carbon

and oxygen. Over the past decade, for example, there has been increasing use of O_2/Ar as a mixed layer productivity tracer. In this approach, net community production (NCP; defined as GPP minus community-wide respiration) is assessed from the biologically-induced oxygen saturation anomaly, after accounting for air-sea O_2 fluxes. Similarly, measurements of the triple oxygen isotope composition of water (e.g. Jaurnek & Quay 2013) have provided information on gross oxygen production over mixed layer ventilation time-scales. These tracer-based estimates provide synoptic information on bulk productivity on time-scales of mixed layer ventilation (approximately one to several weeks, depending on wind-speed and mixed layer depth). However, these methods typically do not resolve shorter-term (e.g. sub-daily) PP variability resulting from a range of environmental drivers. Such measurements require near instantaneous 'snap-shots' of surface water photosynthetic activity.

1.3 Active Chlorophyll Fluorescence as a real-time primary productivity measurement tool

In the early to mid-1990s, a new approach was introduced for the measurement of aquatic photosynthesis based on analysis of single turnover chlorophyll fluorescence transients (ST-ChlF). This approach, first developed for use in terrestrial plants, was based on the inverse relationship between chlorophyll fluorescence and PP. Seminal papers described methods to quantify photosynthetic electron transfer rates (ETR), by resolving the change in ST-ChlF following rapid modulation of an excitation light source (Kolber & Falkowski 1993, Kolber et al. 1998). As a component of the photosynthetic process, photosynthetic electron transfer is inherently coupled to the rate of light-dependent water splitting, O_2 evolution and ATP and NADPH production, thus providing an estimate of gross primary productivity. Indeed, early studies demonstrated that ETRs derived from ST-ChlF correlated well with parallel measurements of ^{14}C-uptake (Kolber & Falkowski 1993) and gross O_2 evolution (Suggett et al. 2003). Other studies demonstrated important applications of ST-ChlF data to understand the physiological status of phytoplankton in situ, including cellular responses to iron limitation (e.g. Kolber et al. 1994).

In the decade following the pioneering applications of ST-ChlF in aquatic research, the field expanded significantly, with improved instrumentation and increased physiological understanding of underlying photosynthetic processes. The first commercially available Fast Repetition Rate Fluorometers (FRRf; and derivative Fluorescence Induction and Relaxation (FIRe) fluorometers, Gorbunov & Falkowski 2005) for ST-ChlF were released in the early 2000s (Chelsea Technologies Group Ltd., Satlantic Inc.) and quickly found increasing use in laboratory experiments and on oceanographic vessels. Significant efforts were aimed at further reconciling ETRs with ^{14}C-uptake (e.g. Corno et al. 2006, Suggett et al. 2006, Moore et al. 2006), and growing data sets repeatedly demonstrated strong covariance between parallel ETRs and ^{14}C-uptake measurements in marine and freshwater systems. Results from this work also demonstrated that the relationship between ST-ChlF and other productivity metrics varied depending upon the prevailing phytoplankton taxa and/or environmental conditions (see Suggett et al. 2009, Lawrenz et al. 2013). Within a decade, ST-ChlF instruments became standard on many large-scale oceanographic programs (e.g. Atlantic Meridional Transect; Suggett et al. 2006, Hawaii Ocean Time-Series; Corno et al. 2006), and biogeochemical studies of ocean productivity (e.g. Behrenfeld et al. 2006).

Over the past decade, as ST-ChlF measurements have become increasingly common, new approaches have been developed to derive ETR estimates from the resulting data (Oxborough et al. 2012, Boatman et al. 2019, Gorbunov & Falkowski 2020). At the same time, instrument sensitivity has increased significantly, permitting robust measurements in the most oligotrophic waters, while also incorporating multi-spectral measurements to better resolve the signatures of different phytoplankton taxa (see Silsbe et al. 2015). These critical developments have further stimulated interest in the use of ST-ChlF for oceanographic and freshwater research (e.g. Schuback et al. 2017, Zhu et al. 2017, Hughes et al. 2020), while technological advances in light sources and detectors have stimulated a growing number of 'home-made' instruments (e.g. Hoadley & Warner 2017, Fujiki et al. 2020) and deployment of autonomous sensors on a range of sampling platforms, including ships, gliders, mooring and floats. These theoretical and operational advances, coupled with the advent of satellite-based passive chlorophyll fluorescence measurements, present an exciting opportunity for global-scale ST-ChlF measurements to revolutionize our understanding productivity and its response to climate change.

1.4 Opportunities and Challenges.

The field of ST-ChlF measurements now sits at a critical crossroads. As the application of these methods continues to grow, conceptual, operational and computational approaches to collect and interpret ChlF parameters are rapidly diverging (Hughes et al. 2018). An increasing number of sensors, protocols and data processing algorithms are now being used to obtain primary productivity estimates from ST-ChlF measurements, yet there has been little direct inter-comparison of methods and approaches, and no standards of best practice have been adopted by the international research community. As a result, the advantages and limitations of different methods are presently unclear, as is the influence of operational context and key environmental and taxonomic variables on the choice of optimal ST-ChlF protocols. Rapidly growing data sets may soon become increasingly difficult (perhaps impossible) to reconcile, thus limiting our ability to build global ST-ChlF compilations and examine large-scale patterns in ETR and its response to environmental forcing.

SCOR working group 156 was created to address this fundamental challenge. Our goal is to bring together world-leading experts from across the globe to produce recommendations for best-practices in the acquisition, interpretation and archiving of ST-ChlF data. This User Guide represents a key deliverable of our efforts, providing a framework for the development and expansion of ST-ChlF measurements for aquatic primary productivity research. In the text that follows, we highlight critical considerations in the use of ST-ChlF methods, presenting the current state of knowledge and gaps in understanding that must be addressed going forward. Based on this analysis, we provide recommendations to support robust application and interpretation of ST-ChlF methods under a wide range of conditions. We also provide practical instructions, tutorials and software tools to give users hands-on experience with the processing and analysis of ST-ChlF data. Finally, we discuss the necessary requirements for data fidelity, analysis and archiving, with the goal of establishing a framework for the global synthesis of ST-ChlF measurements. Such a synthesis of observations will be of significant value in understanding broad-scale patterns in aquatic productivity, while also supporting the development of satellite-based passive fluorescence measurements.

The goals of SCOR WG 156 reflect back to one of the first SCOR Working Groups (WG3, 'Biological Production of the Sea', 195x – 195y), whose primary objective was to '*appraise and recommend methods for world-wide comparisons of organic productivity and standing crops*'. Today, more than half a century later, this remains a critical challenge facing marine scientists, and one that has taken on increasing urgency in light of global climate change and other anthropogenic pressures impacting marine and freshwater ecosystems. With the United Nations Decade of Ocean Science for Sustainable Development (2021-2030), now is the time to stimulate international collaboration and cooperation in understanding global-scale patterns in critical ocean properties, such as primary productivity. Our efforts very much align with these initiatives.

Improved monitoring and managements of aquatic ecosystems is critical to achieving the UN's Sustainable Development Goals over the coming decade. Towards this end, the World Meteorological Organization established the Global Climate Observing System, defining a series of Essential Climate Variables (ECVs) that are needed to understanding the state of Earth's biophysical systems. At present, phytoplankton biomass is classified as an ECV. This variable is, no doubt, critical to understanding ocean health, but without information on physiological rates, biomass measurements alone do not provide a full understanding of photosynthetic production. For this reason, it is critical to develop standardized methods that can be employed to assess the rates of photosynthetic processes in aquatic environments. We believe that ST-ChlF methods have the potential to achieve this goal, and that ETR, in particular, is a good candidate for an ECV. However, much work remains to be done before we can apply these measurements with confidence on a global scale. It is our hope that the material presented in this User Guide provides an important step in this direction.

2. CONCEPTS AND FOUNDATIONS

2.1. Table of terminologies

Throughout this document, we will refer to a significant number of physiological variables and derived photosynthetic parameters. The literature contains numerous synonymous terms describing these properties, and previous authors have discussed the parallel nomenclature systems that have evolved over the past two decades (Kromkamp & Forster 2003, Cosgrove & Borowitzka 2011). Building on this previous work, we will provide a comprehensive table outlining all of the variable and parameter definitions used throughout this document, along with a 'thesaurus' of synonyms used in previous publications. In addition to reviewing current and past nomenclature, we will provide a series of recommendations to guide future practice, with the goal of increasing the clarity and precision of terminology. We will also address present misconceptions around the inconsistent use of terms describing fluorescence methods.

2.2. Primary Parameters from fit of ST-ChlF transients - physiological models

Determining electron transfer rates from chlorophyll fluorescence measurements begins by fitting photo-physiological models to ST-ChlF data to retrieve a number of primary ChlF parameters. This section considers the basis of these model fits, as well as the uncertainties and caveats associated with parameter determination. We will describe a number of alternative model-measurement approaches to characterize the induction phase of the fluorescence transients – when fluorescence increases from minimum to maximum values as PSII reaction centers progressively "close" due to photochemistry – and the subsequent relaxation phase – where fluorescence returns to basal levels as carrier molecules transfer electrons "downstream" of PSII, enabling reaction centres to "re-open". Changes in the fluorescence yield during these transients are determined to a significant extent by changes in the level of reduction of the first stable electron acceptor, Q_A. Here we will present conceptual and mathematical frameworks to explain the influence of different physiological variables in determining the shape of fluorescence induction – relaxation transients. These variables include the absorption cross section of photochemistry in PSII, the extent of connectivity between PSII reaction centres, the kinetics ofelectron transfer downstream of Q_A, and a range of processes regulating the excited state of chlorophyll a associated with PSII (carotenoid and p680$^+$ quenching). Retrieval of these variables will be considered alongside the actual model fitting procedures. To this end, we will provide access to a [Jupyter notebook](#) that can be used to process raw ST-ChlF data from different instruments using a range of physiological models. This open-source software tool was developed as a means of reconciling data sets using a common analysis platform, thereby eliminating sources of variability associated with different model fitting approaches. The tool can be used to directly compare results from different instruments and users, and for sensitivity studies examining the effect of different modelling approaches on the resulting parameter derivations.

2.3 Electron Transfer Rate (ETR) algorithms and estimates, and associated second order parameters

The photo-physiological models described in section 2.1 are used to derive primary ST-ChlF parameters characterizing key attributes of PSII. Primary productivity estimates are then retrieved by integrating these parameters into equations that quantify ETR. Various approaches have been developed for these calculations, including the 'sigma algorithm' (Suggett et al. 2011), the 'absorption algorithm' (Oxborough et al. 2012, Boatman et al. 2019), and the 'kinetic' approach (Gorbunov & Falkowski 2020). All of these approaches rely on accurate retrieval and interpretation of primary ChlF parameters from ST-ChlF, and all have advantages and disadvantages. This section will consider the relative advantages and limitations of various ETR derivations, particularly in the context of field measurements, and will also describe how ETR can be quantified in absolute, biomass-specific terms through the quantification of PSII reaction centre content.

2.4. Light-dependence of ETR: Protocols, curve fits and derived fit parameters

The Photosynthesis-Irradiance response curve (PvE) is fundamental to parameterizing adaptive photosynthetic performance over space and time (e.g. Sakshaug et al. 1997, Bouman et al. 2018), and various mathematical formulations of this function have been used in empirical models predicting environmental controls on primary productivity (e.g. Behrefeld & Falkowski 1997). Traditionally, the light-response function of aquatic primary producers has been characterized in terms of carbon fixation or O_2 evolution. However, ST-ChlF measurements also hold significant potential in this respect, by retrieving rapid and non-invasive information on the light-dependence of ChlF parameters, including ETR (Silsbe & Kromkamp 2012). This section will consider the various experimental protocols (e.g. sequence and duration of successive light steps) and modelling approaches that can be applied to obtain and interpret ETR versus E data sets. The section will also include links to Jupyter notebooks providing hands-on tutorials and analysis tools to process ST-ChlF data from a variety of instruments, thus permitting users to examine the effects of different model fits on derived parameters.

2.5. Data analyses and error quantification / propagation

Numerical model fitting is the critical step by which primary and secondary physiological parameters are obtained from ST-ChlF data (sections XX-XX). Having introduced key elements of the model fitting procedures and the associated Jupyter notebooks in previous sections, this section will specifically consider the nature and sources of error and uncertainty in raw data and derived model fits. Among other topics, we will discuss the question of signal-to-noise ratio in the raw ST-ChlF transients, error propagation and quantification of model goodness of fit. These topics will be addressed in the context of instrument sensitivity in low biomass regions, and under high light conditions where measurement errors increase as a result of fluorescence quenching processes.

3. OPERATION

Earlier sections of this document address theoretical and conceptual considerations relevant to the derivation of primary ST-ChlF parameters and secondary variables, such as ETR. This section discusses a number of critical operational issues that must be considered to deploy instruments and collect data in the most reliable, robust and reproduceable manner possible.

3.1. Instrument characterization, calibration and standardization

As the number of different ST-ChlF instruments and protocols increases, it is critical to ensure that common standards and benchmarks are available to facilitate comparison of data across research groups. In this respect, robust and traceable calibration of the intensity and spectral properties of excitation light sources and detectors is critical. Unfortunately, these such calibrations are not trivial, with current practice highly instrument-specific and often seen as the responsibility of manufacturers rather than end-users. Going forward, users will require practical field methods to periodically check instrument calibrations, and access to reference standards against which data can be reported. In this section, we discuss the use of commercially-available fluorescent dyes, including Rose Bengal, Rhodamine B, Nile Blue and CPN680, as potential calibration standards. We also outline protocols to assess the intensity and spectral properties of light sources and detectors, discuss the necessary ancillary equipment needed for these calibrations, and provide recommendations on the desirable range of specifications and tolerances for instrument performance. This latter information will be particularly useful for advanced users who wish to develop custom-built ST-ChlF instruments (discussed further in section xx.xx).

3.2 ST-ChlF Induction Protocols

The quality and integrity of derived ST-ChlF parameters depends, to a significant extent, on the use of appropriate protocols to achieve a single-turnover closure of PSII reaction centers and the subsequent re-oxidation through downstream electron transfer. Key elements discussed in this section will include the need to achieve minimum levels of Q_A reduction during the saturation phase of fluorescence transients, and the appropriate length, frequency and intensity of either single or multiple excitation light pulses during the saturation phase of ST-ChlF transients. Further, different approaches to resolve the relaxation phase kinetics will be described.

3.3 Blank & Baseline corrections

One of the significant, though often overlooked, challenges of phytoplankton ST-ChlF measurements is the proper quantification of blanks and baseline fluorescence, and the correction of raw measurements for these complex and poorly defined background signals. In this section, we will present current understanding of different sources of baseline fluorescence (non-variable fluorescence emanating from phytoplankton), and discuss how these can be treated in data processing to ensure accurate interpretation of true photochemical signatures and ETR derivations from ST-ChlF measurements. The section will also address analytical blanks resulting from hardware-specific properties (e.g. optical cross-talk between excitation light and detectors), and fluorescence from detrital and dissolved materials. For blank and baseline fluorescence, we will recommend approaches for quantification and data correction.

3.3. Non-photochemical quenching (NPQ) and dark relaxation

This section will discuss the complex issue of non-photochemical quenching (NPQ), a diverse range of taxonomically-variable physiological processes that directly affect the retrieval of key photosynthetic parameters from ST-ChlF measurements. To date, most work on NPQ has focused on vascular plants, with correspondingly less information available for phytoplankton. We will briefly review current understanding of NPQ in phytoplankton, and describe current approaches to estimating NPQ processes from ST-ChlF data (also section 2.5). We will also provide recommended operational procedures to achieve NPQ relaxation to enable dark-regulated ST-ChlF measurements. An important component of this discussion is an explicit focus on taxonomic diversity and environmental effects on the apparent mechanisms of NPQ and their impact on ST-ChlF data. Whereas this section will focus on the quantification of NPQ and the time-scales of NPQ relaxation, section x.x, addresses the use of NPQ to constrain various secondary parameters useful for productivity estimation from ST-ChlF data.

3.4. Spectral correction & multi-wavelength measurements

As with all photosynthetic properties, ST-ChlF exhibits a strong wavelength (i.e. spectral) dependence, which must be accounted for explicitly in the interpretation of data and the comparison of ST-ChlF measurements with other productivity metrics (e.g. [14]C incubations). In this section, we will discuss the importance of spectral correction for ST-ChlF data and provide recommended approaches and software tools that can be used to achieve this. We will provide conceptual background on the inherent variability of subsurface spectral light fields, and describe the necessary ancillary information needed for spectral correction (e.g. instrument and ambient irradiance spectra and photosynthetic pigment absorption profiles). Linked Jupyter notebooks will provide users with hands-on tutorials in spectral correction, and software tools to apply appropriate spectral corrections for their data. The section will also include a discussion of multi-wavelength ST-ChlF measurements for automated spectral correction and spectral deconvolution approaches to examine taxonomically-distinct phytoplankton groups.

3.5. Operational constraints of underway/field deployments

This section will consider operational constraints encountered during field-based ST-ChlF measurements, either from ship-based observations, coastal observatories or truly autonomous in situ deployments (e.g. on gliders, floats or moorings). Key issues include uncertainty regarding the light history of cells prior to measurements, the challenge of achieving proper dark-regulated measurements during continuous data acquisition, the potential for biofouling, and the impacts of water pumping systems on cell physiology. Appropriate sampling and analysis protocols will be recommended to address particular research questions. As in sections above, optimal approaches will vary based on environmental conditions and the taxonomic composition of phytoplankton assemblages.

3.6. Recommended specifications for custom-built instruments (including autonomous platforms)

Based on the discussion presented in the preceding sections, this section will provide a series of recommendations aimed at potential future developers of custom-built ST-ChlF sensors. The discussion will include suggestions for minimum desirable hardware specifications (e.g. required spectral bandwidth and time-resolution of instrument excitation channels and detectors), along with suggested calibration protocols. Some consideration will also be given to the requirements associated with extended autonomous deployments of in situ instruments.

4. DATA REPORTING, AND ARCHIVING

4.1. Data, metadata, levels of data reporting

The full potential of ST-ChlF measurements as a regional and global monitoring tool can only be realized through the compilation of coherent and inter-comparable data across the international research community. This, in turn, requires the adoption of standard reporting procedures for ST-ChlF data and associated metadata. Taking inspiration from the remote sensing research community, this section will describe an approach to data reporting based on a series of clearly-define data processing 'levels'. In this approach, raw measurements remain accessible in archived formats to facilitate down-stream re-processing as data processing algorithms evolve. This ensures traceability of processed data products (e.g. Level 3, in the case of ocean color measurements), and allows these to be updated with new conceptual developments. Within this framework, we will recommend essential metadata needed to accompany ST-ChlF measurements at each data processing level.

4.2. Data archiving

Efficient information exchange and communication among research groups requires a robust system to archive quality-controlled ST-ChlF data, including raw data and derived ChlF parameters, using widely-readable and stable file formats. In this section, we will provide detailed recommendations to guide such archiving efforts, building on established protocols and conventions, where possible. We will suggest appropriate file formats and naming conventions that can be widely adopted by the research community to ensure consistency and clarity in compiled data repositories.

5. INTERPRETATION & INTEGRATION

5.1. Comparing ETR to other photosynthetic currencies

A primary motivation for ST-ChlF measurements is the acquisition of high resolution, real-time measurements of aquatic PP. At best, ST-ChlF data can be used to provide a robust measure of photosynthetic ETR, which scales stoichiometrically with gross oxygen evolution from PSII (4 electrons are extracted from every water splitting reaction). In practice, however, a number of taxonomic and environmental factors complicate the relationship between ETR, O_2 and other PP metrics, including carbon

fixation. This section will discuss how electron transfer rates (ETR) relate to these other (more commonly reported) measures of phytoplankton photosynthesis, with a particular focus on O_2 evolution and carbon fixation. We will discuss the underlying physiological processes that can decouple photosynthetic electrons, O_2 and carbon, briefly reviewing the existing information on ETR : O_2 : C stoichiometry and providing recommended procedures for direct comparison of ETR estimates against other productivity measures. We will discuss the use of ETR as an empirical proxy for ^{14}C / O_2 dynamics, while also considering the physiological insights that can be gained by examining differences between these productivity metrics.

5.2. Ancillary parameters extracted from ST-ChlF to constrain downstream productivity estimates

Although the main focus of this document is on the use of primary ST-ChlF parameters to derive ETR estimates, these parameters can also be used to assess other important aspects of photosynthetic physiology. This section will discuss such applications, including the relationship between NPQ metrics, ETR : C stoichiometry, environmental controls on photosynthetic quantum yields and diagnostics of physiological stress, including iron and macro-nutrient limitation. Ultimately, these relationships may be useful in further constraining productivity estimates, but they also hold intrinsic value in understanding phytoplankton photo-physiology.

5.3. Integration of ST-ChlF with other in situ optical information (e.g. underway systems)

With the expanding use of ST-ChlF measurements in field-based research campaigns, there will be new opportunities to collect real-time multi-sensor data capturing physical, chemical and biological variability across a range of spatial and temporal scales. This section will consider the conceptual and practical issues associated with the integration of ST-ChlF instruments with other measurements systems (e.g. dissolved gas and optical sensors) to provide automated and high throughput retrieval of key biogeochemical variables in aquatic systems.

5.4. Passively-induced ChlF, fluorescence quantum yields and Sun Induced ChlF

This section will discuss potential synergies between ST-ChlF measurements from induction fluorometers, direct measurements of photosynthetic quantum yields from fluorescence life-time measurements and the sun induced fluorescence (SIF) signal detected by satellites. We will briefly outline the general principals of SIF and fluorescence lifetime measurements and their similarities and differences with ST-ChlF approaches. The discussion will focus on how these three different methods can collectively provide a richer understanding of the photosynthetic process, and how ship-based ST-ChlF and fluorescence lifetime measurements can be used to validate the interpretation of satellite-based SIF signatures. This will, in turn, open new possibilities to derive physiological information at regional and global scales, helping to inform numerical models of ocean productivity.

6. Decision Tree

6.1. A decision tree for execution and interpretation of active fluorescence measures of productivity

This final section of the document will provide a synthesis of material covered in previous chapters, in a manner that supports informed user selection of application-specific protocols to collect and analyze ST-ChlF data under a range of scenarios (e.g. different environmental conditions and taxonomic composition of phytoplankton). We will develop a decision tree to guide study design and execution, based on the research goals of a given study and the influence of key operational factors (e.g. sensor configuration, method protocols and data treatment). Each point in the decision tree will build on (and cross-reference) the detailed discussion in earlier sections of the document.

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