Standards and Best Practices for the Collection and Assessment of Operational Phytoplankton Observations

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1. Scientific Summary and Rationale

The number of particle imaging instruments (PII) for quantifying and identifying phytoplankton in aquatic environments has grown over the last decade, with several instruments now considered to be "mature technologies" with a Technological Readiness Level (TRL) between 7 and 9[1]. Such methods are greatly advancing our capacity to measure Essential Ocean Variables (EOVs) and Essential Biodiversity Variables (EBVs) such as phytoplankton biomass, community composition, functional group, and size distribution, which have traditionally required labor-intensive collection and analysis methods. By enabling automated and high throughput quantification of phytoplankton cell biomass (cell counts, biovolume) and taxonomic diversity over both ocean space and time, PIIs are poised to revolutionize our understanding of planktonic ecosystems and will allow us to effectively monitor global changes over time.

Observations of phytoplankton biomass and diversity are essential to understand the impacts of natural and anthropogenic drivers on ecosystem status and function. A number of existing internationally based data archives and repositories can accept and make available taxonomic data and the associated metadata derived from imaging instruments (e.g., SeaBASS, OBIS, BCO-DMO, MBON). Data standards, such as Darwin Core, have been established to facilitate the interoperability of biodiversity data among data sources by standardizing terminology (Wieczorek et al. 2012). Moreover, additional standards and practices for reporting instrument specific methods, image data management and data archiving have been rigorously established in multiple recent publications that also include critical details that account for the reporting of metadata (Martin-Cabrera et al., 2022; Neeley et al. 2021; Owen et al. 2022). Overall, community efforts to standardize methods of data reporting have been successful. ***Yet, to date, the best practices for the collection of phytoplankton images and downstream processing of the images have not been addressed.*** With the advancement of imaging tools for phytoplankton taxonomy and diversity, and their deployment as part of operational ocean observing networks, it is imperative that we establish and standardize data collection and processing methods across PIIs and environments.

Each PII and sampling method has inherent limitations associated with the range of particles that it can detect and image, and the environment being sampled. Best practices for phytoplankton image collection will define not only the sampling plan required to obtain quantitative phytoplankton data, but also the downstream post-processing of the data. Such standards have been established for a number of optics and biogeochemistry measurements, many of which have been reported in a series of protocol documents, including the IOCCG Protocols for Satellite Ocean Colour Sensor Validation[2].

Our goal is to develop a set of best practices for both the collection and downstream processing of phytoplankton images produced by PIIs; such best practices currently do not exist. These best practices will result in ***consistent, quantitative observations of phytoplankton taxonomy and biomass***. We propose to assemble a small working group (12 participants) at the Woods Hole Oceanographic Institution for two in-person meetings for 1.5 days in June 2023 and 2 days in February 2024 following the Ocean Sciences meeting in New Orleans, LA.

The Operational Phytoplankton Observations (OPO) Working Group will identify challenges associated with quantitative sampling phytoplankton:

1. Different oceanic provinces and biomass levels (e.g., coastal, open ocean).
2. Aquatic sampling techniques (e.g., bottle, underway), profiling or towed technologies, ocean observatories (moorings).
3. Current phytoplankton imaging tools that are most used.

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[1] Technology Readiness Level | NASA
[2] https://tinyurl.com/4aj67xjm

The OPO Working Group will develop recommendations for specific instruments and sampling techniques to ensure that phytoplankton measurements are consistent, robust, and quantitative.

2. Scientific Justification and Relevance to OCB

Phytoplankton are integral to aquatic system functioning. They form the base of the aquatic food web and provide many crucial ecosystem services, including but not limited to, biogeochemical (nutrients, carbon, etc.) cycling, regulation of atmospheric CO_2 and O_2, algal biofuels, and nutritional supplements and food for humans and livestock (Demirbas 2011; Saadaoui et al. 2021; Naselli-Flores and Padisak 2022). Moreover, harmful algal bloom (HAB) events, which can cause both animal and human mortality and be detrimental to ecosystem services such as fisheries and oyster reefs, have exhibited an increase in frequency, duration, and extent over the last 30 years (Anderson 2009; Anderson et al. 2021). The National Harmful Algal Bloom Network[3] (NHABON) has recently established a framework for the monitoring of HABs in coastal marine and freshwater environments around the United States that includes the detection of HAB species using automated systems including the IFCB[4]. The Marine Biodiversity Observation Network (MBON) Plankton Workshop identified that knowledge of taxonomy and diversity is a critical need to understand the impacts of climate change on community composition and ecosystem function (Grigoratou et al. 2022).

The impact of climate change, by way of ocean acidification, sea surface warming and enhanced stratification, on phytoplankton biodiversity, biogeography and phenology are not yet fully understood. Model predictions indicate that phytoplankton communities will likely experience shifts, changes in space, and shuffles, changes in taxonomic species, within the communities over the next century (Barton et al. 2016; Anderson et al., 2021). Ocean color satellite sensors provide global coverage of the ocean to an extent that cannot be covered by ships alone. Ocean color modeling of phytoplankton groups will provide a synoptic view of phytoplankton community composition, phenology, and changes therein. NASA's Plankton, Aerosol, Cloud, ocean Ecosystem[5] (PACE) mission observatory will include a hyperspectral ocean color instrument (OCI) providing more spectral information to distinguish phytoplankton groups by their spectral properties (Werdell et al. 2019). Moreover, global ocean biogeochemical and ecological models that derive and forecast phytoplankton biomass and community composition require high resolution taxonomy information for model development and validation, which has, to date, been lacking. ***All of these methods used to model or predict phytoplankton composition need high quality ground truth data on phytoplankton community composition and cell size distribution;*** *bulk Chl a is not enough.*

Imaging instruments have played a pivotal role in the understanding of ocean biology and carbon export for a number of recent large scale research cruises and process studies, including NAAMES, EXPORTS, the Distributed Biological Observatory, and Tara Oceans. Moreover, a number of ocean observing programs have incorporated measurements of plankton by PIIs as part of their routine sampling. Repeat hydrographic surveys, including GO-SHIP, AMT and GeoTRACES, have collected hydrographic and biogeochemical data from repeat transects around the global ocean. To varying degrees, these programs have integrated biological sampling, including measurements of phytoplankton taxonomy through flow cytometry, imaging platforms and 'omics, into their sampling routines. Notably, phytoplankton observations are being incorporated into the GO-SHIP global repeat hydrological sections as part of the Bio-GO-SHIP program (Clayton et al., 2022), and an IFCB will be deployed on the NSF-funded OOI Pioneer Array when it is relocated to the southern Mid-Atlantic Bight in 2024. The collection of information on biological and ecosystem EOVs, coincident with hydrological and biochemical data, will allow the quantification of phytoplankton abundance and diversity across various spatial, vertical, and temporal scales. Given that the number of PII currently being used in, or soon to be incorporated into, a variety of ocean observing networks and platforms has grown substantially, ***now is the time to establish***

[3] https://ioosassociation.org/nhabon/
[4] https://tinyurl.com/2kn7dbnj
[5] https://pace.gsfc.nasa.gov/

best practices for using these instruments in a quantitative way that can be used to derive robust and sustained measurements of biological and ecological EOVs. The phytoplankton ecology community is lagging behind ocean chemists in developing measurement standards and best practices. Moreover, different PII have different limitations, such as different capacities for cell size detection, differences in camera sensors (e.g., pixel resolution), and the use (or not) of lasers for the triggering of the PII camera on fluorescence or scatter. Lombard et al. (2019) note that the 'effective size range' of cell sizes detectable by different instruments is not always clear. Commonly used imaging flow cytometers, such as the IFCB, FlowCAM and Cytosense, also differ in their data outputs as well as recommended sampling procedures. Moreover, in some water types, larger volumes of water must be analyzed to quantitatively sample the population of phytoplankton. Additionally, each aquatic sampling technique and the volume of water measured will impact the quantitative rigor of the measurements.

The benefits of the proposed activity will be multifold and are aligned with OCB research priorities, which include marine organism response to environmental change and marine organism-mediated carbon cycling and export via the biological pump. The OPO Working Group members will create a set of best practices for different PIIs, water types, and sampling approaches. This activity is a follow-up to the Phytoplankton Taxonomy Working Group, which developed standards and practices for image data management and interoperability. The ultimate goal is to have these best practices used in large scale ocean observing programs, such as repeat hydrographic programs and ocean observatories, long term ecological time series, as well as funded process study research cruises. Participants will be recruited via an open call to the community describing the proposed OPO WG activities and providing an application form for interested applicants through appropriate mailing lists, including, but not limited to those supported by OCB, GOOS, OOI, MBON, and OBIS. ***Applications will be assessed based on the interests and experience of the applicants in using PII systems, as well as attempting to balance gender, career stage, and the representation of historically underrepresented groups in oceanographic research.*** *We will strive to include participants with diverse backgrounds and expertise, including phytoplankton image data collectors, data users, and modelers.*

3. Meeting Logistics

We propose to host two in-person small working group meetings that will coincide with scientific conferences. The target size for the working group is 12 participants.

1. The first meeting will take place in 2023 over a 1.5-day period. The topic will be introduced through presentations and discussions on the first day. During the subsequent 1.5 days, we will (a) outline a set of data standards and best practices and (b) identify a few existing demonstration data sets for implementation in a 'pilot study.' Prior to the first meeting, we will hold at least ONE virtual meeting for introductions and to initiate discussion.
2. Over the following year, the s*tandards and best practices* will be refined and evaluated by the working group participants, with virtual meetings every 2 months between in person meetings. We will also create an open communication channel, such as Slack or similar, to sustain discussions between synchronous virtual and in person meetings.
3. A follow-up 2-day in-person meeting will be held in February 2024 in conjunction with the Ocean Sciences Meeting in New Orleans. The OPO WG participants will reconvene to review results from the pilot study and its evaluation. If deemed necessary, additional recommendations will be made to improve the data standards and best practices.

<u>The in-person meetings could be converted to virtual meetings if pandemic-related issues arise.</u>

4. Anticipated Outcomes and Deliverables

1. Following the first meeting, a summary of the working group goals, activities, and preliminary results will be produced for the OCB Newsletter.
2. Following the second meeting, a set of recommended standards and best practices for operational phytoplankton observations will be finalized and plans for implementation, such as during Bio-GO-SHIP, will be described.

3. Within one year of the second meeting, an L&O Methods-type publication will be produced to present the resulting *standards and best practices* developed over the course of the OPO WG activity.
4. We will also work to obtain GOOS endorsement[6] for the OPO best practices through an open community review process to ensure that they are widely adopted by the international Ocean Observing community.

5. Budget

Meeting 1				**Meeting 2**		
Travel Budget	Domestic	Local[a]	International	**Travel Budget**	Domestic	International
Number of participants	7	2	1	**Number of participants**	9	1
Airfare	$600	-------	$2,200	**Airfare**	-------	-------
Transportation	$64	$250[a]	$64	**Transportation**	-------	-------
Hotel (3 nights @ $265)	$795	$795	$795	**Hotel (3 nights @ $200)**	$600	$600
Meals (Catering)[b]	$180	$180	$180	**Meals (Catering)[b]**	$180	$180
Per diem[c]	$103	$103	$103	**Per diem[d]**	$203	$203
Trip cost pp	$1,742	$1,328	$3,162	**Trip cost pp**	$983	$983
Total travel cost	$12,194	$2,656	$3,162	**Total travel cost**	$8,847	$983
Admin support	$1,000			**Admin support**	$1,000	
				Publication costs[e]	$2,400	
Total meeting budget	$19,012			**Total meeting budget**	$13,230	

a. Participants within 400 miles of Woods Hole, MA. POV mileage rate at GSA.gov
b. Meals/catering: based on estimated catering costs for breakfast, lunch, and dinner over 2 days.
c. ¾ travel days, per diem MI&E is current CONUS rate published on GSA.gov
d. Two days plus two travel days, per diem MI&E is current CONUS rate published on GSA.gov
e. Estimated for open access publication in L&O Methods

Total budget for the meeting is $32,242.

[6] https://tinyurl.com/2p89wp7v

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