STANDARDS AND BEST PRACTICES FOR REPORTING FLOW CYTOMETRY OBSERVATIONS

A Technical Manual

Authors:

Aimee R. Neeley, Inia Soto-Ramos, and Christopher Proctor

Affiliations:

NASA Goddard Space Flight Center
Science Systems and Applications, Inc.
Morgan State University

Version 1.0
December 2022

Table of Contents

1.0 Abstract 2

2.0 Introduction 3

3.0 Creating a data file from flow cytometry data 4

3.1 Metadata headers for flow cytometry 4

3.2 The data table 6

4.0 Submitting the data to SeaBASS 10

4.1 Required supporting files for data submission 10

4.1.1 The protocol document 10

4.1.2 The checklist 11

4.1.3 Assessed IDs list 11

4.2 Submitting raw files and additional metadata 11

Appendix A: Example Data File 13

Appendix B: Example Protocol Document 17

Appendix C: Example Checklist 21

Appendix D: Example Assessed IDs List 22

References 23

Document Data Sheet 24

1.0 Abstract

This technical manual guides the user through the detailed process of creating a standardized data table for the submission of taxonomic and morphological information collected by flow cytometry to long-term data repositories. Guidance is provided to produce documentation that describes data collection and processing techniques and outlines the creation of a data file. Field names that are required include `scientificName` that represents the lowest level taxonomic classification (e.g., genus if not certain of species, family if not certain of genus) and `scientificNameID`, the unique identifier from a reference database such as the World Register of Marine Species or AlgaeBase. The data table described here also includes the field names `volume_analyzed_ul`, `measurementValue`, `measurementValueID` and `abun`. The field names `measurementValue` and `measurementValueID` are recommended terms developed by NERC to describe morphological properties of cells. Data producers are required to submit their source data (.fcs files) as bundles and may optionally submit data plots as image files. Following these steps for standardization will help optimize the interoperability and reuse of these important data sets.

We acknowledge the contributions of Ivona Cetinić, Nicole Poulton, Jason Graff, Michael Lomas, and Stace Beaulieu to the development and review of this technical manual.

2.0 Introduction

The purpose of this manual is to describe the procedures for reporting data derived from standard flow cytometry methods following the framework established in "Standards and Practices for Reporting Plankton and Other Particle Observations From Images" (Neeley et al., 2021). Standard flow cytometry is a technique used in aquatic sciences to quantitate cells that are not detected by microscopic or in-flow-imaging methods, particularly cells that are less than ~20 µm in size, such as *Synechococcus*, *Prochlorococcus*, picoeukaryotes and nanophytoplankton. Standard flow cytometry uses lasers and optical filters (Figure 1) to separate the cells into populations based on their optical properties (fluorescence) and morphological characteristics (scattering, a proxy for size).

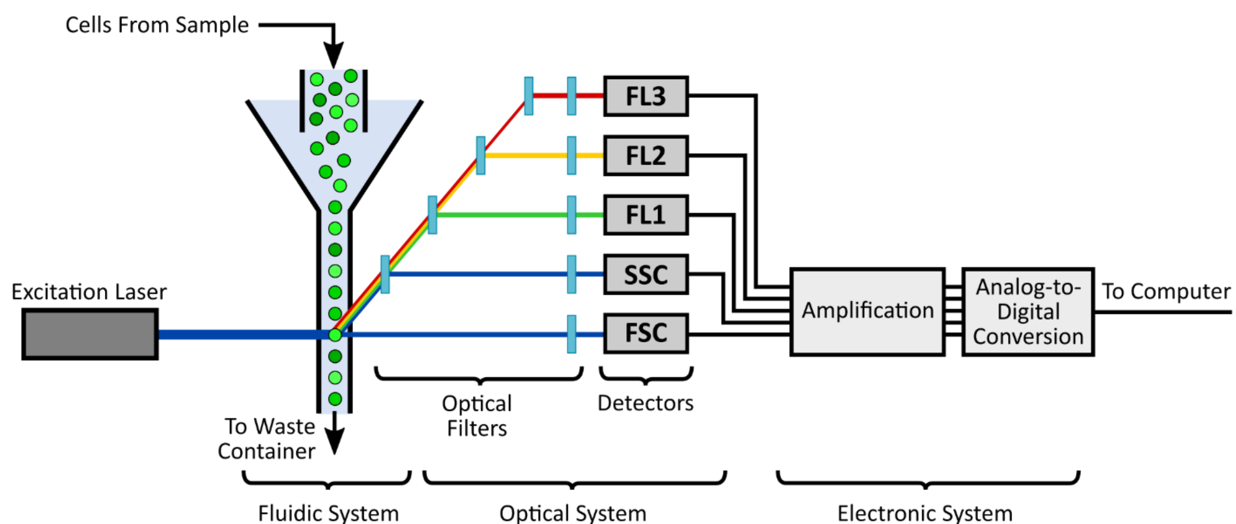

Figure 1. Schematic of a common flow cytometer, illustrating the fluidic, optical, and electronic systems. Image Source: AAT Bioquest, Inc.

NASA's upcoming ocean color mission Plankton, Aerosol, Cloud, ocean Ecosystem (PACE) will collect hyperspectral ocean color data that will provide the capability to resolve different spectral signatures of phytoplankton from space (Werdell et al., 2019). Advanced algorithms and models developed to derive phytoplankton community composition from PACE data require complete information about the entire community, including the contribution of phytoplankton and cyanobacteria that are <2 µm in size. In preparation for NASA's PACE mission, the team in charge of NASA's SeaWiFS Bio-optical Archive and Storage System (SeaBASS) has been working diligently on mechanisms to ingest biological data, in particular phytoplankton community data, collected using various methods, including in-flow-imaging, flow cytometry and, eventually, microscopy. These mechanisms include the creation of data tables with parameter-specific field names and headers for each data type. The goal is to have a complete repository of phytoplankton community composition that includes the entire size spectrum of the community that could be used for algorithm development and validation.

By developing a set of common reporting practices for observations of phytoplankton and bacteria using flow cytometry, we enable users of the data to (1) make informed decisions about which products can be integrated or compared across datasets, instruments, etc., and (2)

reproduce or reprocess products to standardize them across datasets or instruments in such a way that products can also be updated if processing approaches improve or become more standardized. The data table and documentation prototypes described in this manual were reviewed by subject matter experts in the field of flow cytometry.

The objectives of this document are to:

- Prescribe a standardized data table format for *in situ* data contributors that addresses the need for machine-readable taxonomic and morphological information from flow cytometry measurements, including ingestion of essential provenance information for reusability
- Recommend standardized vocabulary important for interoperability and reusability of data
- Explore options for how a repository could:
    - ❖ Store and serve the contributed data and metadata
    - ❖ Produce, store, and serve higher-level data products

We hope these standards and best practices for reporting flow cytometry data may be adopted and adapted for other data repositories.

3.0 Creating a data file from flow cytometry data

The format described herein allows for Level 2 submissions of flow cytometry data, meaning sums of total abundances of each phytoplankton or bacterial group. This approach differs from image datasets where each individual image or particle is reported separately. As with image data, it is critical and required for submissions to include documentation that clearly describes the methods and data analysis protocols used to obtain the final data products. In Subchapter 3.1, we define the metadata headers required for flow cytometry submissions. In Subchapter 3.2, we describe the format of the data table. In Chapter 4 and the subsequent subchapters we outline the required files and documentation that must be submitted with the data file.

3.1 Metadata headers for flow cytometry

Important metadata information must be stored in the header of every data file using keywords and values. Standard SeaBASS metadata headers include project information, spatial and temporal information, and lists of data fields and units. Metadata headers also link to the file names of special external documentation and ancillary data files. As part of a submission of flow cytometry data, SeaBASS requires several forms of external documentation: the SeaBASS flow-cytometry checklist text document, a user-created document describing data collection and processing methods, and a list of assessed identifications. These core document names are listed using the standard `/documents` header. Besides those documents, SeaBASS requires flow cytometry instrument data files (.fcs) must be submitted (described in more detail in the following list.) Optionally, submitters may include information on gating from scatter plots in the form of image files.

The following metadata headers are applicable to the submission of flow cytometry data to SeaBASS. They include important values and links to external file names:

1) `associated_archives:`
(Required) A list file of names for all external raw data (e.g., a bundle of .fcs files) and/or scientific files (e.g., a bundle of scatter plot image files.) Tar bundles with gzip compression (.tgz) are used to group data so as few as one file per type needs to be named. Multiple file names must be comma separated, no spaces allowed in the names or list. These associated file names should include info about the experiment name, cruise, and data type. For example, /associated_archives=EXPERIMENTNAME_CRUISENAME_flow_cytometry_raw_files.tgz, EXPERIMENTNAME_CRUISENAME_flow_cytometry_raw_images.tgz.

   Contact the SeaBASS data manager or consult the SeaBASS website for assistance.

2) `associated_archive_types:`
(Required) This header must be used in conjunction with `associated_archives`. Provide a value or list of text terms to describe the contents of each `associated_archive` file. Pick one per file, comma-separated, no spaces. Commonly used types are 'raw' for the bundle of flow cytometry (.fcs) files, 'ancillary_media' for images, or 'metadata' for other supporting files or code. e.g., `/associated_archive_types=`raw, ancillary_media.

3) `associated_files:`
(Required if applicable) The value is the name of the specific source file used for the scientific analysis. Include this header if all rows of data in this file came from the same sample, or .fcs file. (e.g., `/associated_files=`0000.fcs). However, if multiple samples were used, then skip this header and instead use the equivalent field `associated_files` so you can name the specific file on each data row.

4) `associated_file_types:`
(Required if applicable) This header must be used in conjunction with `associated_files`. The entry here should describe the data type within the `associated_files`. For the purposes of .fcs files, the format would be ‘raw’, images would be ‘ancillary_media’ and any other supportive information would go under ‘metadata’.

5) `eventID:`
(optional) a unique identifier associated with the sample as an event.

6) `volume_analyzed_ul:`
(required) Volume that was processed through the flow cytometer in units of microliters (µl).

If a submission has a **single raw (.fcs) file** associated with it**,** then all four headers must be included. The headers `associated_archives` and `associated_archive_types` should include the name of the bundle of all raw flow cytometry files (and, optionally, image/metadata files) associated with the flow cytometry data submitted as part of an experiment or cruise and the data type, respectively. The headers `associated_files` and `associated_file_types` should include the name of the **individual** raw file associated with the individual data submission and the data type, respectively. If a submission has **more than one raw (.fcs) file** associated with it, then only the headers `associated_archives` and `associated_archive_types` are required.

The header `/documents` must include associated documentation, such as the protocol document, checklist, and any other documents that provide additional information about the experiment or cruise. Supplementary lists of which taxonomic categories were assessed by manual classification are required as part of data submissions and must be included in the `/documents` header. The header `/calibration_files` must list documentation associated with the calibration of the instrument, e.g., use of microbeads or cultures to relate forward scatter to cell size or the calibration of the flow rate. Additional requirements for SeaBASS can be found online or modeled from the data file example in Appendix A.

3.2 The data table

In Neeley et al. (2021), we developed field names (i.e., measurement labels) to accommodate the submission of plankton and particle data collected by imaging-in-flow cytometry. In addition to the standard SeaBASS field names, we have created additional field names specific to the submission of flow cytometry data. See Appendix A for examples of each field name. Unlike the image data, the data table for flow cytometry data must be organized so that each row includes the total abundance of each cell type within a given sample.

The following field names must be included in the data table:

1) `Volume_analyzed_ul:`
(Required) Volume that was processed through the flow cytometer in units of microliters (µl). **This field name can also be used as a header when a single sample is included per submission.**
2) `measurementValue:`
(Recommended but optional) Vocabulary that describes a cluster of cells by morphological characteristics (e.g., size and fluorescence) rather than by taxonomy and is recognized by the Natural Environment Research Council (NERC). The alternative label must be used here.
3) `measurementValueID:`
(Recommended but optional) The URI associated with the NERC vocabulary defined in `measurementValue`.
4) `data_provider_category_manual:`

(Recommended but optional) A category used by the data provider to name the organism for a manual identification, not necessarily a scientific name.

5) `scientificName_manual`:
   (Required) A scientific name from a recognized taxonomic reference database (e.g., World Register of Marine Species, AlgaeBase) at the lowest level that matches the data provider's category, for a manual identification matched to `scientificNameID`.

6) `scientificNameID_manual`:
   (Required) A Life Science Identifier (LSID) from a recognized taxonomic reference database (e.g., World Register of Marine Species, AlgaeBase) at the lowest level that matches the data provider's category for a manual identification.

7) `abun`:
   (Required) Concentration of cells observed in units of cells per liter (cells/L).

Most notable from the above list is the additions of the field names `measurementValue` and `measurementValueID`, which were added to remain interoperable with OBIS and align with Darwin Core terminology when possible. `measurementValue` and `measurementValueID` must include the appropriate SeaDataCloud Flow Cytometry Standardised Cluster Names[1] developed by the SeaDataCloud working group as part of NERC to standardize naming conventions of organisms identified by flow cytometry. The purpose of the paired `measurementValue` / `measurementValueID` is to provide information about the functional or morphological group assignment based on size and fluorescence characteristics rather than taxonomic terms, e.g., a `measurementValue` may be RedPico followed by the `measurementValueID` `http://vocab.nerc.ac.uk/collection/F02/current/F0200004/`. Note that the `measurementValueID` is the fundamental reference. SeaBASS data submitters must check the NERC website for the most up-to-date alternative label (`measurementValue`) at the time of submission.

The purpose of the paired `scientificName` /`scientificNameID` (which would go into the Occurrence records in global data systems) is to provide the taxonomic assignment for the organism. In the case of flow cytometry, the taxonomic assignment is likely to be coarser than the functional group assignment that went into the `measurementValue`. The field name `data_provider_category_manual` is recommended when the lowest level of the taxonomic assignment for the living cells cannot be established e.g., picoeukaryotes or nanoeukaryotes. In such an instance, `scientificName` would be Eukaryota and `scientificNameID` would be `urn:lsid:algaebase.org:taxname:86701.`

The following table is an abbreviated version of the vocabulary found on the NERC website as of December 2022. For complete details, please refer to the NERC vocabulary server.

ID	Preferred Label	Definition

[1] This work is licensed under the Creative Commons Attribution 4.0 International License. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/ or send a letter to Creative Commons, PO Box 1866, Mountain View, CA 94042, USA.

F0200010	**HetHNA** Heterotrophic prokaryotes with relatively high nucleic acid	Heterotrophic prokaryotes include both Bacteria and Archaea. When they do not contain any photosynthetic pigments and thus do not have any autofluorescence property exploitable by flow cytometry, they require staining with a fluorescent dye. In most studies a nucleic acid dye is used. Cells with a higher green fluorescence correspond to heterotrophic prokaryotes with a relatively high nucleic acid content.
F0200011	**HetLNA** Heterotrophic prokaryotes with relatively low nucleic acid	Blue laser excitation enables heterotrophic prokaryotes to be distinguished in various groups using side or forward scatter and green fluorescence signatures. Cells with a lower green fluorescence correspond to heterotrophic prokaryotes with a relatively lower nucleic acid content.
F0200007	**HsNano** High sideward scatter nanophytoplankton	This group consists of eukaryotic cells that have a high sideward scatter signal when excited by a blue laser, compared to the RedNano and OraNano groups, but similar forward scatter and red fluorescence signatures. These cells also display a high depolarization ratio (horizontal/vertical polarized forward light scatter). This optical property is very useful to discriminate Coccolithales that are covered with coccoliths ($CaCO_3$ platelets), as well as some autotrophs and armored dinoflagellates. Staining is not required to distinguish them by flow cytometry due to the presence of fluorescent photosynthetic pigments.
F0200016	**OraMicro** Orange fluorescing microphytoplankton	This group consists of cells > 20 µm. They display higher forward scatter and orange fluorescence signals than OraNano and a higher orange fluorescence/red fluorescence ratio compared to the RedMicro group. Staining is not required to distinguish them by flow cytometry. Cells have a high phycoerythrin content and may also contain phycocyanin that can be excited by a red laser. This group can include >20 µm cryptophytes, colonial cyanobacterial filaments, but also dinoflagellates and some ciliates.
F0200006	**OraNano** Orange fluorescing nanophytoplankton	This group belongs to the arbitrary size class of nanoplankton (3-20 µm). They contain phycoerythrin and may contain phycocyanin that can be excited by a red laser (615-640 nm). When excited by a blue laser, they bear similar forward scatter as the RedNano with a high orange fluorescence signature. Staining is not required to distinguish them by flow cytometry due to the presence of fluorescent photosynthetic pigments. This group is represented by some cyanobacteria, red algae, or cryptophyte classes.
F0200014	**OraPico** Orange fluorescing picophytoplankton	This group consists of prokaryotes and eukaryotes that contain the pigment phycoerythrin and are <3 µm. When excited by a blue laser, their sideward scatter and forward scatter signatures are larger than those of OraPicoProk but with a red fluorescence signature similar to the RedPico. This group belongs mostly to cyanobacteria and Cryptophyceae.
F0200003	**OraPicoProk** Orange fluorescing prokaryote picophytoplankton	Often identified as *Synechococcus* cells, this group consists of unicellular photosynthetic cyanobacteria with forward-scatter and sideward signatures larger than those of most of marine heterotrophic bacteria. Staining is not required to distinguish them by flow cytometry due to the presence of photosynthetic pigments. When excited by a blue laser, the OraPicoProk cluster displays higher forward scatter and red

		signatures than RedPicoProk at any given depth. They contain phycoerythrin and have a distinct orange fluorescence signature which can be excited by any <575 nm wavelength laser.
F0200015	**RedRedNano** Red and red only fluorescing nanophytoplankton	This group belongs to the nanoplankton size class (3-20 µm) and cells emit in the red when excited by a red laser due to phycocyanin content in the cell, but not in the orange when excited by a blue laser. This group of cells contains phycocyanin with no or little phycoerythrin. Forward scatter and red fluorescence signatures are like RedNano. They include cryptophytes or cyanobacteria classes, especially when they form colonies.
F0200013	**RedRedPico** Red and red only fluorescing picophytoplankton	This group is mainly identified as *Synechococcus* cells that express phycocyanin. This groups can only be resolved using a red laser as excitation source of PC. These PC-rich species are expected to be more abundant in turbid estuaries, as PC is optimized for the orange-red light conditions in these waters, whereas phycoerythrin pigments present in some other *Synechococcus* are optimally tuned to the blue-green light in clear waters. These RedRedPico overlap the RedPicoProk on the cytogram orange vs red fluorescence induced by the blue (488 nm) laser beam.
F0200005	**RedNano** Red only fluorescing eukaryote nanophytoplankton	This polyphyletic group is defined by eukaryotic phytoplankton cells with a size between 3 µm and 20 µm. Staining is not required to distinguish them by flow cytometry due to the presence of fluorescent photosynthetic pigments. On cytograms and when excited by a blue laser, they differ from RedPico by higher red fluorescence, sideward scatter, and forward scatter signatures. Red only fluorescing nanophytoplankton cells are distinguished from phycoerythrin containing cells by their lack of orange fluorescence.
F0200004	**RedPico** Red only fluorescing eukaryote picophytoplankton	This is a polyphyletic group defined by eukaryotic phytoplankton cells with a diameter <3 µm. Staining is not required to distinguish them by flow cytometry due to the presence of fluorescent photosynthetic pigments. The smallest eukaryotic picophytoplankton known belongs to the genus *Ostreococcus*. Red only fluorescing eukaryotic picophytoplankton exhibits a well-defined flow cytometry signature when excited with a blue laser, with forward scatter and red fluorescence signals larger than those of RedPicoProk, but smaller than those of the RedNano.
F0200008	**RedMicro** Red only fluorescing microphytoplankton	This group is a polyphyletic group defined by cells > 20 µm in size. Staining is not required to distinguish microphytoplankton by flow cytometry due to the presence of fluorescent photosynthetic pigments. When excited by a blue laser, this group is discriminated by its forward scatter and red fluorescence signals that are the largest observed by flow cytometry in the microbial community.
F0200002	**RedPicoProk** Red only fluorescing prokaryote picophytoplankton	Commonly identified as the unicellular photosynthetic cyanobacteria *Prochlorococcus* and are the smallest marine photosynthetic microorganisms known today. Due to their photosynthetic pigments staining is not required to detect them by flow cytometry. Red auto-fluorescence is induced by a blue laser.

4.0 Submitting the data to SeaBASS

Submission of flow cytometry data requires the specialized headers and field names described in this manual. Multiple entries in each header must be comma separated and must not include any blank spaces. File names must not contain spaces or special characters except for hyphens, underscores, and periods and must end in ".sb" suffix. Data file names must be unique within a submission, and, ideally, should be completely unique in SeaBASS. It is strongly recommended that they are formed using descriptive patterns incorporating information or abbreviations of the measurement type, cruise name, date, depth, or other information. For example: <EXPERIMENT>-<CRUISE>-<DATATYPE>_<YYYYMMDDHHMM_<R#>.sb, where R stands for the release number that is determined by the submitter.

In the proceeding sections, we describe the additional submission requirements, which includes all documentation that must accompany a submission of flow cytometry data to SeaBASS and the submission of the raw flow cytometry data files (i.e., .fcs). See Appendices for examples of a data file (A), protocol document (B), checklist (C), and assessed IDs list (D). Additional and updated information can be found on the SeaBASS website.

4.1 Required supporting files for data submission

4.1.1 The protocol document

The protocol document provides a detailed account of data collection and analysis both in the field and in the laboratory. The protocol document must include the following sections:

1. Description of the instrument
2. Instrument calibration and maintenance
3. Sample collection method (e.g., by Niskin or flow through)
4. Sample analysis method
5. Notes on data post processing (e.g., software)
6. Additional data cleaning and quality assurance (if applicable)
7. Key method references

Detailed documentation of the software and hardware configurations will not only allow for duplication of such measurements but will also inform data users and interpretation. Moreover, information regarding sample collection technique, e.g., by Niskin or flow through, sampling depths, and any pre-filtering must be included in the documentation. Instrument calibration and maintenance information should include details on the calibration method used to estimate size from forward light scatter (beads, cultures, etc.). Details on any fixatives or dyes (e.g., LysoTracker Green, paraformaldehyde) and final concentrations (if applicable) used during sample measurements must also be included. Lastly, details on post-processing (including software used) and information on data cleaning and quality control (if applicable) must be provided. The recommended file name for the protocol documentation is as follows: protocol_flow_cytometry_<EXPERIMENT_CRUISE>.txt.

4.1.2 The checklist

The submission checklist, an abbreviated version of the protocol document, is designed to standardize and preserve critical methods and analysis details that are needed for intercomparison, reprocessing, and to assist in evaluating the data for satellite validation or inclusion in algorithm development datasets. The checklist will also provide guidance as to which fields and headers to include essential information that must be added to the comments section of the data file, how to arrange data matrices, and determine the critical documentation that must be included with the data submission. If multiple formats are offered for download (e.g., rich text and plain text), choose one and fill out the necessary sections. Rename the file in a relevant way to make it unique (e.g., add the cruise name to the end of the file name), and add it to the other documents and calibration files that are part of the submission. The recommended file name for the checklist is as follows:
checklist_flow_cytometry_<EXPERIMENT_CRUISE>.txt.
The checklist must also include the following:

1. A link to the most current NERC vocabulary used for the data file. (e.g., http://vocab.nerc.ac.uk/collection/F02/current/)
2. The date on which the submitter referenced the vocabulary list.

4.1.3 Assessed IDs list

Supplementary lists of which taxonomic categories and associated NERC terms that were assessed during data analysis are required as part of data submissions. Here we consider the classification method to be ‘manual’ as we assume that the cell populations in each sample were annotated manually. The submitter must provide the names assessed IDs lists in the `/documents` header as a comma separated list (no spaces) and include instrument, cruise, and experiment in the file name. The recommended file name for the assessed IDs list is as follows: manual_assessed_id_<EXPERIMENT_CRUISE>.txt. For example: `/documents=manual_assessed_id_NAB08_KN193-03.txt`. The same associated file names may be referenced in multiple data files, so it is only necessary to create additional files if different categories were assessed for different data files.

4.2 Submitting raw files and additional metadata

Additional information, such as raw files, images, data collection and processing codes (when applicable) and metadata will be archived under “associated” data. The file size of this information can often be very large; therefore, they are not included in our traditional ordering system. However, users can order the associated files along with the data if desired by clicking the “download associated files” checkmark when requesting the dataset. Associated files (raw files (.fcs), images (targeted cell scatter plots), and metadata (software and codes) can be submitted in a folder called “associated” with individual subfolders separated by data type (e.g., raw, images, metadata). Alternatively, submitters can compress the folder into individual .tgz files. If the submission is extremely large (above 5 GB), then data should be subdivided in subsequent folders or compressed files. Submitters can use their own scheme to subdivide the data, such as by year or date for long time series. The submission of raw instrument files (.fcs) with the data is required. We also recommend the submission of image files that capture the

gating structure for targeted cell populations in the scatter plots. The information would be useful to data users who may want to review and possibly reprocess the raw files. If the classification algorithms or codes are not publicly available, we recommend submitting those as well.

Each SeaBASS file must include the header `/associated_archives` and `/associated_archive_types`. The `/associated_archives` header will include the filenames of each compressed file. Your filename must include the experiment, cruise, and the type of data (e.g., flow_cytometry_raw). The header `/associated_archive_types` indicates the types of data in each compressed file, such as raw, ancillary_media (for images of scatter plots), and metadata. To specify which raw (.fcs) or image file pertains to each SeaBASS file (one sample per file) or data row (multiple samples per file) the user can use the `/associated_files` header or field respectively. If that header contains multiple values, then list them in a comma-separated format with no spaces. Either the `/associated_file_types` header or field, should be included to identify the type of file (e.g., raw, ancillary_media, metadata). Submitters may contact SeaBASS for additional assistance using the following email address: seabass@seabass.gsfc.nasa.gov.

Appendix A: Example Data File

Special notes about associated_archives:

- Bundle all your source data files (.fcs) into one file using the tar gzip (.tgz) format. If you need help, submit those files in a subfolder and the SeaBASS data manager will finish converting it into .tgz
- The following example data file is for a situation where different .fcs files are named on different data rows via the fields called `associated_files` and `associated_file_types`.
- You have the option to simplify your data file if all rows refer to the same .fcs file. In that case you do not need to include the fields `associated_files` and `associated_file_types`, but instead include them as headers (it is implied that they apply all rows of the data matrix)

THIS IS AN EXAMPLE, NOT TO BE CONSIDERED REAL DATA

```
/begin_header
/investigators=ivona_cetinic
/affiliations=NASA_Goddard_Space_Flight_Center
/contact=ivona.cetinic@nasa.gov
/experiment=NAB08
/cruise=KN193-03
/station=-999
/data_file_name=NAB08_KN193-03_flow_cytometry_20080501_R1.sb
/eventID=NAB08_KN19303
/data_type=cast
/data_status=final
/documents=protocol_flow_cytometry_NAB08_KN193-
03.txt,checklist_flow_cytometry_NAB08_KN193-
03.txt,manual_assessed_id_NAB08_KN193-03.txt
/calibration_files=NAB08_FACScan_calibration.txt
/start_date=20080502
/end_date=20080504
/start_time=17:04:00[GMT]
/end_time=20:44:00[GMT]
/north_latitude=61.1628[DEG]
/south_latitude=60.853[DEG]
/east_longitude=-25.3522[DEG]
/west_longitude=-26.637[DEG]
/water_depth=-999
/measurement_depth=-999
/instrument_model=FACScan
/instrument_manufacturer=Becton_Dickinson
/associated_archives=NAB08_KN193-03_flow_cytometry_raw_files.tgz
/associated_archive_types=raw
```

```
/volume_analyzed_ul=NA
/missing=-9999
/delimiter=comma
!
! Comments
! NERC Vocabulary:
http://vocab.nerc.ac.uk/collection/F02/current/
! List referenced on 12/13/2022
!
/fields=Sample (station or
R2R_event),cast,bottle,lat,lon,date,time,depth,water_depth,volum
e_analyzed_ul,measurementValue,measurementValueID,data_provider_
category_manual,scientificName_manual,scientificNameID_manual,ab
un,associated_files,associated_file_types
/units=none,none,none,degrees,degrees,yyyymmdd,hh:mm:ss,m,m,ul,n
one,none,none,none,cells/L,none,none
/end_header
1,1,1,61.1628,-25.3522,20080502,20:04:00,4.646,1892,500,
RedPico,http://vocab.nerc.ac.uk/collection/F02/current/F0200004/
,picoeukaryote,Eukaryota,urn:lsid:algaebase.org:taxname:86701,24
04,0001.fcs,fcs
1,1,1,61.1628,-25.3522,20080502,20:04:00,4.646,1892,500,
OraPicoProk,http://vocab.nerc.ac.uk/collection/F02/current/F0200
003/,Synechococcus,Synechococcus,urn:lsid:marinespecies.org:taxn
ame:160572,3136,0001.fcs,fcs
1,1,1,61.1628,-25.3522,20080502,20:04:00,4.646,1892,500,
OraNano,http://vocab.nerc.ac.uk/collection/F02/current/F0200006/
,Cryptophyceae,Cryptophyceae,urn:lsid:marinespecies.org:taxname:
17639,134,0001.fcs,fcs
1,1,1,61.1628,-
25.3522,20080502,20:04:00,4.646,1892,500,HetHNA,http://vocab.ner
c.ac.uk/collection/F02/current/F0200010/,
heterotrophic_prokaryote_HNA,Prokaryota,urn:lsid:algaebase.org:t
axname:86700,541315,0001.fcs,fcs
1,1,2,61.1628,-25.3522,20080502,20:10:00,10.503,1892,500,
RedPico,http://vocab.nerc.ac.uk/collection/F02/current/F0200004/
,picoeukaryote,Eukaryota,urn:lsid:algaebase.org:taxname:86701,30
81,0002.fcs,fcs
1,1,2,61.1628,-25.3522,20080502,20:10:00,10.503,1892,500,
OraPicoProk,http://vocab.nerc.ac.uk/collection/F02/current/F0200
003/,Synechococcus,Synechococcus,urn:lsid:marinespecies.org:taxn
ame:160572,3284,0002.fcs,fcs
1,1,2,61.1628,-25.3522,20080502,20:10:00,10.503,1892,500,
OraNano,http://vocab.nerc.ac.uk/collection/F02/current/F0200006/
```

```
,Cryptophyceae,Cryptophyceae,urn:lsid:marinespecies.org:taxname:
17639,170,0002.fcs,fcs
1,1,2,61.1628,-
25.3522,20080502,20:10:00,10.503,1892,500,HetHNA,http://vocab.ne
rc.ac.uk/collection/F02/current/F0200010/,
heterotrophic_prokaryote_HNA,Prokaryota,urn:lsid:algaebase.org:t
axname:86700,523587,0002.fcs,fcs
1,1,3,61.1628,-25.3522,20080502,19:56:00,30.356,1892,500,
RedPico,http://vocab.nerc.ac.uk/collection/F02/current/F0200004/
,picoeukaryote,Eukaryota,urn:lsid:algaebase.org:taxname:86701,22
89603,0003.fcs,fcs
1,1,3,61.1628,-25.3522,20080502,19:56:00,30.356,1892,500,
OraPicoProk,http://vocab.nerc.ac.uk/collection/F02/current/F0200
003/,Synechococcus,Synechococcus,urn:lsid:marinespecies.org:taxn
ame:160572,3002,0003.fcs,fcs
1,1,3,61.1628,-25.3522,20080502,19:56:00,30.356,1892,500,C
OraNano,http://vocab.nerc.ac.uk/collection/F02/current/F0200006/
,Cryptophyceae,Cryptophyceae,urn:lsid:marinespecies.org:taxname:
17639,146,0003.fcs,fcs
1,1,3,61.1628,-25.3522,20080502,19:56:00,30.356,1892,500,
HetHNA,http://vocab.nerc.ac.uk/collection/F02/current/F0200010/,
heterotrophic_prokaryote_HNA,Prokaryota,urn:lsid:algaebase.org:t
axname:86700,553356,0003.fcs,fcs
1,1,3,61.1628,-25.3522,20080502,19:53:00,50.27,1892,500,
RedPico,http://vocab.nerc.ac.uk/collection/F02/current/F0200004/
,picoeukaryote,Eukaryota,urn:lsid:algaebase.org:taxname:86701,25
84,0004.fcs,fcs
1,1,4,61.1628,-25.3522,20080502,19:53:00,50.27,1892,500,
OraPicoProk,http://vocab.nerc.ac.uk/collection/F02/current/F0200
003/,Synechococcus,Synechococcus,urn:lsid:marinespecies.org:taxn
ame:160572,2828,0004.fcs,fcs
1,1,4,61.1628,-25.3522,20080502,19:53:00,50.27,1892,500,
OraNano,http://vocab.nerc.ac.uk/collection/F02/current/F0200006/
,Cryptophyceae,Cryptophyceae,urn:lsid:marinespecies.org:taxname:
17639,132,0004.fcs,fcs
1,1,4,61.1628,-
25.3522,20080502,19:53:00,50.27,1892,500,HetHNA,http://vocab.ner
c.ac.uk/collection/F02/current/F0200010/,
heterotrophic_prokaryote_HNA,Prokaryota,urn:lsid:algaebase.org:t
axname:86700,563614,0004.fcs,fcs
2,2,1,60.853,-26.637,20080504,17:46:00,4.78,1556,500,
RedPico,http://vocab.nerc.ac.uk/collection/F02/current/F0200004/
,picoeukaryote,Eukaryota,urn:lsid:algaebase.org:taxname:86701,18
09,0005.fcs,fcs
```

```
2,2,1,60.853,-26.637,20080504,17:46:00,4.78,1556,500,
OraPicoProk,http://vocab.nerc.ac.uk/collection/F02/current/F0200
003/,Synechococcus,Synechococcus,urn:lsid:marinespecies.org:taxn
ame:160572,2722,0005.fcs,fcs
2,2,1,60.853,-26.637,20080504,17:46:00,4.78,1556,500,
OraNano,http://vocab.nerc.ac.uk/collection/F02/current/F0200006/
,Cryptophyceae,Cryptophyceae,urn:lsid:marinespecies.org:taxname:
17639,117,0005.fcs,fcs
2,2,1,60.853,-
26.637,20080504,17:46:00,4.78,1556,500,HetHNA,http://vocab.nerc.
ac.uk/collection/F02/current/F0200010/,
heterotrophic_prokaryote_HNA,Prokaryota,urn:lsid:algaebase.org:t
axname:86700,506082,0005.fcs,fcs
2,2,2,60.853,-26.637,20080504,17:44:00,10.52,1556,500,
RedPico,http://vocab.nerc.ac.uk/collection/F02/current/F0200004/
,picoeukaryote,Eukaryota,urn:lsid:algaebase.org:taxname:86701,19
97,0006.fcs,fcs
2,2,2,60.853,-26.637,20080504,17:44:00,10.52,1556,500,
OraPicoProk,http://vocab.nerc.ac.uk/collection/F02/current/F0200
003/,Synechococcus,Synechococcus,urn:lsid:marinespecies.org:taxn
ame:160572,2897,0006.fcs,fcs
2,2,2,60.853,-26.637,20080504,17:44:00,10.52,1556,500,
OraNano,http://vocab.nerc.ac.uk/collection/F02/current/F0200006/
,Cryptophyceae,Cryptophyceae,urn:lsid:marinespecies.org:taxname:
17639,117,0006.fcs,fcs
2,2,2,60.853,-
26.637,20080504,17:44:00,10.52,1556,500,HetHNA,http://vocab.nerc
.ac.uk/collection/F02/current/F0200010/,
heterotrophic_prokaryote_HNA,Prokaryota,urn:lsid:algaebase.org:t
axname:86700,506082,0006.fcs,fcs
```

Appendix B: Example Protocol Document

THIS IS AN EXAMPLE

Document author and contact info:

Nicole Poulton (npoulton@bigelow.org)

Bigelow Laboratory for Ocean Sciences, W. Boothbay Harbor, ME

Version 1.0: December 2022

Description of the instrument

Samples were analyzed on a Becton Dickinson FACScan using a 488 nm (40 mW) blue excitation laser. Emission filters used were relative green fluorescence (515 ± 30 nm Band Pass filter), right angle light scatter (side scatter – SSC), Forward scatter (FSC), relative orange fluorescence (575 ± 25 nm Band Pass filter), and relative red fluorescence (670 Long Pass filter). All detectors were PMTs (photomultiplier tubes), except FSC which was a photodiode.

Instrument calibration and maintenance

Mean cell sizes of heterotrophic and phototrophic nanoplankton were determined from flow cytometric forward light scatter (FSC). The relationship between FSC and size was determined using a set of standard microbeads (1, 2, 2.5, 4, 6, 10, 15 and 20 µm). These microbeads were run at the beginning and end of the cruise and at sea when any adjustment was made to the flow cytometer. These results were used to create calibration curves relating forward scatter to "bead diameter" (µm) - see Figure 1. After the cruise, in the laboratory, a set of 14 cultures ranging in size from 2 to 30 µm (see Table 1) were analyzed along with the microbeads on both a Coulter Counter (Beckman Coulter Multisizer) and on the BD FACScan to determine mean forward light scatter. Cultures of cells too small for the Coulter Counter (*Synechococcus* and *Micromonas*) were measured by image analysis using a Zeiss epifluorescence microscope and a Diagnostic Research Instruments color camera. Bead and Cell size vs. forward light scatter calibration curves were determined (Figure 2) and these results were used to establish a standard curve relating "bead diameter" to "cell diameter" (µm) using forward light scatter - see Figure 3.

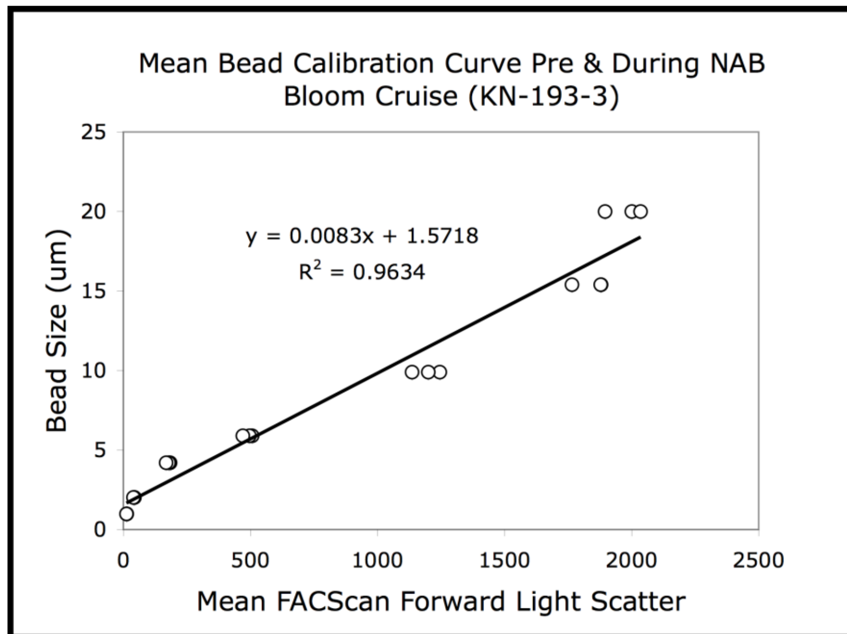

Figure 1. Relationship between Bead Size and Mean Forward Light Scatter (FSC) just prior and during the KN-193-3 2008 North Atlantic Bloom Research Cruise.

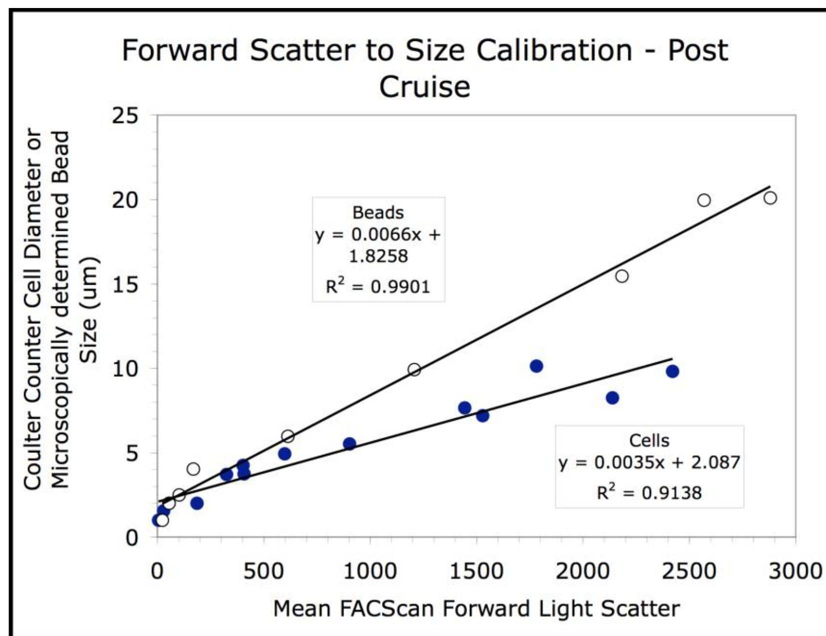

Figure 2. Bead and culture cell calibration curves relating bead or cell diameter to mean forward light scatter (FSC) – determined in the laboratory (after cruise).

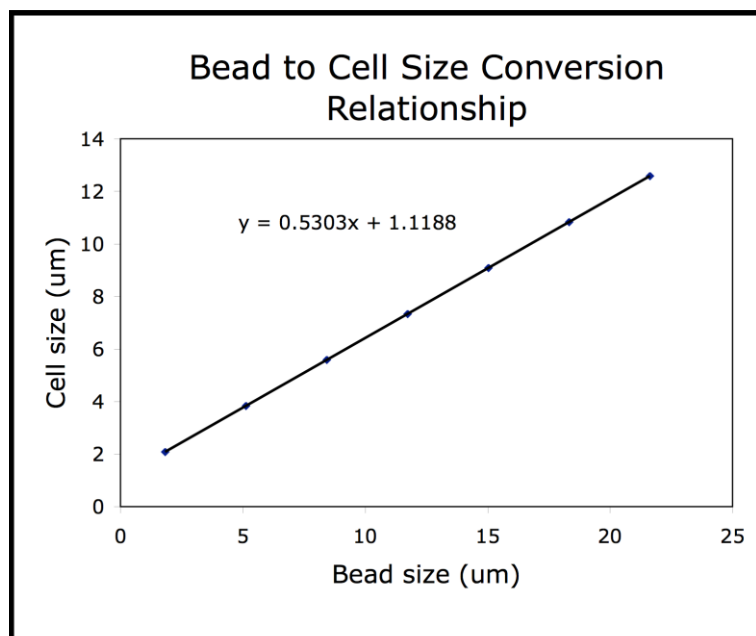

Figure 3. Standard Curve relating Bead Size to Cell Size.

Table 1. Cultures used for size calibration of forward light scatter from the BD FACScan and Coulter Counter. CCMP=Culture Center for Marine Phytoplankton.

Species Name	Stain ID – if available	Approximate Size (microns)
Alexandrium fundyense	CB-501	30
Pycnococcus provasolii	CCMP 1203	2-4
Brachiomonas		10x18
Dunaliella tertiolecta	CCMP-1302	8-13
Isochrysis galbana	CCMP 1324	4-8
Emiliana huxleyi		3-5
Thalassiosira pseudonana	CCMP 1335	4-6
Amphidinium carterae	CCMP 1314	12-18
Pleurochrysis carterae	CCMP 645	8-12
Thalassiosira weissflogii	TW	14-18
Phaeocystis globosa	CCMP 2754	5-7
Synechococcus sp. (cyanin)	8c-1k	1-2
Micromonoas pusilla	CCMP 494	1-2
Rhodomonas salina	3C / CCMP1319	9-15

Sample collection method:

Water subsamples from CTD Niskin bottles were collected for analysis. Samples were collected at depths aligning with the surface, mixed layer, and chlorophyll maximum. Samples were prefiltered with 200 μm nylon mesh screen prior to measurement.

Sample analysis method:

The volume of sample analyzed was determined by a "time" method. The flow rate of the instrument was monitored twice a day using volume standards. These were tubes containing deionized water that had been weighed and sealed ashore and stored refrigerated. Approximately twice a day a volume standard tube was opened and run on the instrument for about 5 minutes at high flow rate. Tubes were then resealed, stored in the refrigerator, and weighed upon return to the lab. From these results it was determined that the flow rate was constant over the first 2 days of the cruise, then declined over the middle portion of the cruise, and then was higher again and constant for the last 2 days. The trend was fit to a linear regression and the resulting flow rate was used to calculate cell abundances for samples run on those days, while a constant rate was used for the first and last periods. The high flow rate averaged 51.0 µL/min[1] (2.4 sd, 4.6% cv, range: 46.8 – 55.7), over the whole cruise.

Samples for heterotrophic bacteria were preserved with 10% paraformaldehyde (0.5% final), 100 uL of preserved sample was stained with 10 µL PicoGreen (Invitrogen - 1:10 dilution in deionized) for 24 hours, diluted with 900 µL of filtered seawater and analyzed by flow cytometry (Veldhuis et al. 1997).

Live samples for phototrophic picoplankton and nanoplankton were run undiluted at high flow rate as per standard flow cytometric procedures (Marie et al. 2005). Live samples for heterotrophic nanoplankton were stained with LysoTracker Green (Invitrogen). A 1mM stock of LysoTracker Green was diluted 1:10 in 0.2 µm-filtered seawater added to live seawater samples (75 nM final concentration) and incubated at in-situ temperatures for 10 minutes and enumerated by flow cytometry (Rose et al. 2004).

Notes on data post processing:

Data were analyzed using FlowJo 9.8 Software (Becton Dickinson, San Jose, CA).

Cell diameters from the cruise were determined using the bead to cell standard curve relationship in Figure 3, all heterotrophic and phototrophic nanoplankton biovolumes (μm^3) were then calculated.

Key method references:

Marie, D. N. Simon and D. Vaulot. 2005. Phytoplankton cell counting by flow cytometry. In: Algal Culture Techniques. Ed. R. A. Andersen. Elsevier. pp. 253-267.

Rose, J. M., Caron, D. A., Michael E. Sieracki, M. E., Poulton, N. 2004. Counting heterotrophic nanoplanktonic protists in cultures and aquatic communities by flow cytometry. Aquat Microb Ecol. 34: 263–277.

Veldhuis M. J. W., Cucci T. L., Sieracki M.E. 1997. Cellular DNA content of marine phytoplankton using two new fluorochromes: taxonomic and ecological implications. Journal of Phycology 33:527-541

Appendix C: Example Checklist

CHECKLIST FOR SeaBASS SUBMISSION: Flow cytometry data
V20221213
Please fill out the sections below. When finished, rename this file to be specific for your data, e.g., "checklist_flow_cytometry_<EXPERIMENT-OR-CRUISE>.txt"

Experiment Name: _______
Cruise Name: _______
Assessed ID list(s) for manual classification submitted and referenced in /documents metadata headers? ________

- SAMPLE COLLECTION METHOD -
----
1. How were the water samples collected? (Niskin bottle, bucket etc.)
2. Standard depths of sample collection (surface, chl max etc.)
3. Was the sample prefiltered? If so, type of filter (e.g., nitex, pore size)

- SAMPLE ANALYSIS METHOD -
----
1) List the instrument make, model and accessories (if applicable):
2) List instrument calibration and maintenance performed (including date):
3) Measurement mode/wavelengths (forward scatter, side scatter, fluorescence):
4) Method for measuring volume analyzed (e.g., gravimetric, syringe, etc.):
5) Sampling Flow rate:
6) Method of calibration (e.g., Culture, beads):
7) Size range of particles analyzed:
8) Were samples run live or preserved? If preserved, list method/preservative:
9) List any stains used:

- DATA POST-PROCESSING METHOD -
----
1) Data processing software used:
2) Taxonomic authority used:
3) Link to NERC vocabulary:
4) Date submitter referenced the NERC vocabulary:
5) Are lists of all Life Science Identifiers and NERC vocabulary assessed included in your submission?

Appendix D: Example Assessed IDs List

```
measurementValue,measurementValueID,data_provider_category_manual,scie
ntificName_manual,scientificNameID_manual
RedPico,http://vocab.nerc.ac.uk/collection/F02/current/F0200004/,picoe
ukaryote,Eukaryota,urn:lsid:algaebase.org:taxname:86701
OraPicoProk,http://vocab.nerc.ac.uk/collection/F02/current/F0200003/,S
ynechococcus,Synechococcus,urn:lsid:marinespecies.org:taxname:160572
OraNano,http://vocab.nerc.ac.uk/collection/F02/current/F0200006/,Crypt
ophyceae,Cryptophyceae,urn:lsid:marinespecies.org:taxname:17639
HetHNA,http://vocab.nerc.ac.uk/collection/F02/current/F0200010/,hetero
trophic_prokaryote_HNA,Prokaryota,urn:lsid:algaebase.org:taxname:86700
```

References

Neeley, A., Beaulieu, S., Proctor, C., Cetinić, I., Futrelle, J., Soto Ramos, I., Sosik, H., Devred, E., Karp-Boss, L. & Picheral, M. 2021. Standards and practices for reporting plankton and other particle observations from images. Technical Manual.

Werdell, P. J., Behrenfeld, M. J., Bontempi, P. S., Boss, E., Cairns, B., Davis, G. T., Franz, B. A., Gliese, U. B., Gorman, E. T. & Hasekamp, O. 2019. The Plankton, Aerosol, Cloud, ocean Ecosystem mission: status, science, advances. *Bull. Am. Meteorol. Soc.* **100**:1775-94.

Document Data Sheet

Language: Enter the language of the full text deposit, If the language does not appear in the list below, please enter 'Other'. If the content does not really have a language (for example, if it is software, a dataset or an image) please enter 'N/A'. English || Chinese || French || German || Italian || Japanese || Spanish || Other || N/A ||	English
Methodology type: Enter the type of methodological document you are submitting. Please enter all that apply. Separate entries N/A **Guidelines & Policies** Guidelines & Policies: A set of conventions and options to advice action; an indication or outline of conduct. Policies are generally high-level guidelines on expected or acceptable behaviour, especially of a governmental body **Method** Method: A documented procedure, a step-by-step set of instructions for accomplishing a task. Examples include manuals, scientific/medical protocols, standard test methods and standard practices (e.g. standard operation procedures) **Methodological commentary/perspect** Methodological commentary/perspective: Narrative reflections on or discussion of a methodological document **Description of a metrology standard** description of a metrology standard: Documentation of a physical standard used for metrology (e.g. a manufactured object used to calibrate sensors) **Specification of criteria** Specification of criteria: a description of requirements (e.g. a technical, quality assurance and inclusivity requirements) that a methodology should comply with in order to fulfil the expectations of a community or organisation **Reports with methodological relevance** Reports with methodological relevance: a report of any activity which has relevance to methodology (e.g. a set of existing methods were compared, a report on a field expedition where new technology was tested, or a report on a computational benchmarking experiment) **Training/Educational material** Training/Educational material: Documents designed specifically for training and/or educational activities, rather than to accomplish a task in an operational context. with a semicolon (;)	Method

Adoption level: Please indicate how broadly the uploaded methodology is used and/or adopted; please select all that apply. **Novel** (no adoption outside originators) **Validated** (tested by third parties) **Organisational** **Multi-organisational** **National** **International** **N/A**	
Endorsement (author declared): Please enter if your submission (in its entirety) has been endorsed by an organisation or community as one or more of the following: **De jure standard:** A methodology that an official authority has legally declared as a reference or authoritative model. **De facto standard:** A methodology that has become a reference or authoritative model through wide adoption and common use in at least one community of practitioners. **Good practice:** A methodology that has repeatedly produced reliable, fit-for-purpose results with regard to its stated objectives. **Recommended practice:** A methodology that has been recommended for use by an authority, organisation, community, or other group. **Best practice:** A methodology that has repeatedly reproduced superior results relative to other methodologies with the same objective and which has been adopted and employed by multiple organisations.	
Endorsement (external): Please indicate whether this submission (in its entirety) has been endorsed by an organisation or community. Please name the organisation or community that performed the endorsement above.	
Author Last, First Name(s) ** Separate multiple entries with a semicolon (;) e.g.: Smith, Joseph; Jones, H.; (enter the name/s as it appears in the document in the correct order)	Neeley, Aimee; Soto-Ramos, Inia; Proctor, Christopher

Author ORCID(s) eg. 0000-0002-4366-3088 Separate multiple entries with a semicolon (;) Visit https://orcid.org/ to register The order of these entries should correspond to that of the names above	0000-0001-5701-0953; 0000-0001-7974-857X; 0000-0002-6715-4026
Editor Last, First Name(s) Where there is no personal author list the editor/s. Separate multiple entries with a semicolon (;) (enter the name/s as it appears in the document in the correct order) eg: Buttigieg, Pier Luigi; Simpson, Pauline;	
Editor ORCID(s) e.g.: 0000-0002-4366-3088 The order of these entries should correspond to that of the names above. Separate multiple entries with a semicolon (;)	
Corporate Author Where there is no personal author or editor enter the organization, project or team name responsible for creating the best practice, eg. CleanSea Project	
Date of Issue (yyyy-mm-dd) ** e.g. 2018-05-21	2022-12-15
Recommended Next Content Review Date (yyyy-mm-dd) Please indicate the date which you believe the document should be revised and updated	
English-language document title ** Entries should be in English. If applicable, include a sub-title after a colon (:) and version number after the title text (e.g. Version 3.2).	Standards and Best Practices for Reporting Flow Cytometry Observations
Alternative or Non-English document title If the title was not originally in English, please include it in its original form here. If applicable, include a sub-title after a colon (:) and version number after the title text (e.g. Version 3.2).	
Recommended Next Content Review Date (yyyy-mm-dd) Please indicate the date which you believe the document should be revised and updated	
Place of Publication e.g.: Plouzane, France This should correspond to the publisher name(s) provided below.	Greenbelt, Maryland

Publisher Name(s) ** e.g.: Institut Français de Recherche pour l'Exploitation de la Mer (IFREMER) Please state the Institute's (Issuing Organization) name as it is specified in official communications. Separate multiple publisher entries with a semicolon (;)	NASA Goddard Space Flight Center
Pages or Extent e.g.: 57pp. Use straight through pagination of document e.g. 39pp. & Annexes Use pagination of the document body text e. g. 12 mins (for video)	32pp.
Series and/or Document Number(s) If applicable, list creator document identifiers, e.g.: SIP Protocol Series 6; JERICO-NEXT-W2-D2.1.-24112016-V2.0 Separate multiple entries with a semicolon (;).	
External identifiers e.g. DOI:xxxxxx ; ISBN: xxxxxx Separate multiple entries with a semicolon (;).	
Official location of document Enter one URL for te document: of organization; publisher, projects; Code Repository; Dataset; Other.	Organisation, publisher or project - Code Repository - Dataset - Other -
Contact person - Last, First names e.g. Smith, Joseph	Neeley, Aimee
Contact person - Email **	Aimee.neeley@nasa.gov
Abstract/Summary ** Free text, Please provide a brief summary of your best practice including, as appropriate, a brief descriptions of what techniques your best practice is about, which ocean environments or regions it targets, the primary sensors covered, what type of data/measurements/observing platform it covers, limits to its applicability and note the community of practice that developed the best practice.	This technical manual guides the user through the detailed process of creating a standardized data table for the submission of taxonomic and morphological information collected by flow cytometry to long-term data repositories. Guidance is provided to produce documentation that describes data collection and processing techniques and outlines the creation of a data file. Field names that are required include scientificName that represents the lowest level taxonomic classification

	(e.g., genus if not certain of species, family if not certain of genus) and scientificNameID, the unique identifier from a reference database such as the World Register of Marine Species or AlgaeBase. The data table described here also includes the field names volume_analyzed_ul, measurementValue, measurementValueID and abun. The field names measurementValue and measurementValueID are recommended terms developed by NERC to describe morphological properties of cells. Data producers are required to submit their source data (.fcs files) as bundles and may optionally submit data plots as image files. Following these steps for standardization will help optimize the interoperability and reuse of these important data sets.
Refereed Status** Has this document been peer reviewed/refereed? Please enter YES, NO or UNKNOWN	YES
Maturity Level If applicable, enter the maturity level of the methodology in the document **N/A**: where maturity level not applicable **Mature**: Methodologies are well demonstrated for a given objective, documented and peer reviewed; methods are commonly used by more than one organization (TRL 7-9) **Pilot or Demonstrated:** Methodologies are being demonstrated and validated; limited consensus exists on widespread use or in any given situation (TRL 4-6) **Concept:** A methodology is being developed at one institution(s) but has not been agreed to by the community; requirements and form for a methodology are understood (TRL 1-3)	
Spatial Coverage If applicable, please specify the region where the best practice is applied. For regional term guidance use the following link: https://www.nodc.noaa.gov/worlddatacenter/regions.html. e.g. SW Pacific Ocean	

Sustainable Development Goals, Targets, and Indicators ** If applicable, please specify if the best practice has application for a sustainable development goal. Target number is required and should be entered e.g 14.1 Add Indicator if applicable eg. 14.1.1 Refer to this page for more information: https://sustainabledevelopment.un.org/ Separate multiple entries with a semicolon (;) **Enter N/A if not applicable**	
Essential Ocean Variables (EOV) Copy and paste standard variable names from the list on this link. Separate multiple entries with a semicolon(;) **Enter N/A if not applicable**	Phytoplankton biomass and diversity
Essential Biodiversity Variables (EBV) Copy and paste names from this link Separate multiple entries with a semicolon(;) **Enter N/A if not applicable**	Species distributions; Species abundances; Community abundance; Morphology
Essential Climate Variables (ECV) Copy and paste standard variable names from the list on this link (e.g for atmospheric variables not already under EOVs) Separate multiple entries with a semicolon(;) **Enter N/A if not applicable**	Phytoplankton
Other Variables Please list here any other variable relevant to your document that are not included as EOVs, ECVs , EBVs or supporting variables above, (e.g. ice accretion, anthropogenic carbon) Separate multiple entries with a semicolon(;) **Enter N/A if not applicable**	
Sensors If applicable, please list here the type of sensor/s and manufacturers that are mentioned in the best practice, e.g. Water sampler General Oceanics. Separate multiple entries with a semicolon (;). **Enter N/A if not applicable**	
Other Keywords Add any other key words, e.g. Melt pond; Diatoms; Absorption coefficient	Flow cytometry; repository; data management

Separate multiple entries with a semicolon (;).	
Bibliographic Citation ** Enter the form in which you would like your article cited. For example, consider this report citation format: Author/Editor (Year) Title. Place of Publication, Publisher, Pages. (Series Document ID). DOI:	Neeley, A., Soto-Ramos, I., and Proctor, C. Standards and Best Practices for Reporting Flow Cytometry Observations. Greenbelt, Maryland, USA. NASA Goddard Space Flight Center, 31pp.
License ** (click to view license) Choose one of the following: • All rights reserved © • Public Domain (CC0) • CC BY-NC-SA 4.0 • CC BY-SA 4.0 • CC BY 4.0 • Other (please specify) • No Creative Commons License CC=Creative Commons	CC BY_SA 4.0

Version history metadata

We recommend including a revision history with your document. Please order your revisions such that the earliest is at the bottom of the table.

Revision	Date	Note on modifications	Lead Author
1.0	2022-12-09	This document is the first version of its type.	Neeley, Aimee