Ingesting, validating, long-term storage and access of Flow Cytometry data

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Deliverable number	Short title
D9.13	SDN data management protocols for Flow Cytometer data

Long title

Ingesting, validating, long-term storage and access of Flow Cytometer data

Short description

This document highlight the way to integrate the flow cytometry datasets into a database that fits interoperability and meets SDC ingesting procedure in order to make the datasets available through international portals. The works presents the first steps of standardisation of vocabulary dedicated to flow cytometry variables (optical units, abundance) and resolved functional groups (phytoplankton and heterotrophic prokaryotes) thanks to a consortium of international experts. The database and workflow from the sample to the SDC portal, ODV and EMODNET are presented, as well as the first datasets available online.

Author	Working group
CNRS, NERC-BODC, VLIZ and ICES	WP9.5.2

Dissemination	Copyright terms
Public	

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1. Background

Marine microbial communities play a major role in the functioning of the global ecosystem. They are good indicators of marine health due to their sensitivity to their environment, and they play a key role in the biogeochemical cycles. Flow cytometry (FCM) is a powerful technology to investigate them. FCM measures the optical properties of single particles (cells) aligned and separated in a laminar flow stream as they cross a light source (most often, one or several laser beams). FCM enables to record various fluorescences intensities produced by the cells, the light scatter intensities per cell, and to determine the abundances of the various groups evidenced. Typically, groups of pico-, nano- and microphytoplankton, heterotrophic prokaryotes, viruses, heterotrophic nanoflagellates are defined by their inherent optical properties. Some specific and very recent instruments are also able to produce real-time, high-resolution data with pictures of each single cell as it flows, giving additional taxonomical identification of cells when larger than about 15 µm.

Flow cytometry manufacturers have harnessed the power of technology to improve their products and offer a wide range of instruments, both for conventional benchtop instruments and for automated flow cytometry deployed in the field, such as: FACS Calibur, BD Influx, CytoSense, FlowCytoBot and recently a CytoPro (with a staining module). These sensors are creating a range of new data types and data formats for which no standards or data management guidelines were available.

FCM data are processed using either the softwares provided by the manufacturers to control the cytometer, or different softwares used for data analysis only such as for instance: FlowJo, Summit, WinList, WinMDI, CytoClus, EasyClus, RtoolClus. Even though the efficiency and the conviviality of these softwares for analyzing and getting results from the acquired measurements are optimised, they deliver different output formats, file schemes and no common vocabulary. Thus, access to standardized and interoperable flow cytometry data were still challenging because of barriers in a common standardized vocabulary definition.

Within the SeaDataCloud project (WP9.5.2), Flow cytometry (FCM) data are considered as new data type that have to be ingested, validated to provide a long-term storage and easy access through SeaDataNet infrastructure. The main objectives of this WP consist of setting up an interoperable system to structure and manage FCM data and metadata (from upstream to downstream services) in coherence with international standards. This work is pioneering in both Flow Cytometry and data management fields.

2. Methodology

This work was carried out from February 2017 to May 2018 (15 Months) where teleconferences and meetings have been established between flow cytometry community and partners. A methodology was established for defining a common set of terms that could be used by a worldwide community of flow cytometry users. Then, the CNRS-MIO has adapted his FCM local data management method to SeaDataNet tools in order to fulfil the work-package 9.5.2 goals.

2.1.1. Flow Cytometry common vocabulary establishment

This part was realized thanks to the existing conjunction/interraction between SeaDataCloud and JERICO Next projects through their resepectivelly work-packages: WP9.5.2 and Task 3.1 on automated platform for the observation of phytoplankton diversity in relation to ecosystem services. In fact, both projects have a common part that deals with Flow Cytometry vocabulary standardization.

Therefore, the work held on the common vocabulary was as follow:

1. Critical Analysis of the existing NERC-BODC FCM codes
2. Captured parameters exercise
3. Literature review from 1983 to 2017
4. Questionnaire

2.1.1.1. Critical Analysis of the existing NERC-BODC FCM codes

Firstly, a general search through the NERC-BODC vocabulary system (https://www.bodc.ac.uk/resources/vocabularies/vocabulary_search/) by using 'flow cytometry' key word. The output (fig.1) revealed that there are 7 vocabulary lists that are holding FCM vocabulary.

Vocabulary search help

You searched for "flow cyto"

Found 7 records | Show (1 - 7) | 1 download results | new search | start again

Rank	Collection	Title	Definition	Governance	Url
177	P01	BODC Parameter Usage Vocabulary	Terms built using the BODC parameter semantic model designed to descibe individual measured phenomena. May be used to mark up sets of data such as a NetCDF array or spreadsheet column.	British Oceanographic Data Centre	
7	L22	SeaVoX Device Catalogue	Terms for distinct sampling or measuring devices that may be identified in the real world in terms of manufacturer and model number.	SeaDataNet and MarineXML Vocabulary Content Governance Group	
7	P09	MEDATLAS Parameter Usage Vocabulary	Terms under the content governance of SISMER used to describe measured phenomena within the MEDATLAS project.	Systèmes d'Informations Scientifiques pour la Mer	
4	S04	BODC parameter semantic model analytical method entity descriptions	Controlled vocabulary defining the terms that may be used for an analytical method entity (part of the how theme) in the BODC parameter semantic model.	British Oceanographic Data Centre	
2	C67	BODC series parameter collection names	Terms used by BODC to describe groups of related parameters brought together to form a series from the sample schema. Each term maps to multiple BODC parameter sets.	British Oceanographic Data Centre	
1	L05	SeaDataNet device categories	Terms used to classify groups of sensors, instruments, sources of algorithmically computed data (numerical models) or samplers (collectors of water, SPM, sediment, rock, air or biota samples).	SeaDataNet	
1	P10	Global Change Master Directory Instrument Keywords	Terms used to describe sensors, instruments and other pieces of scientific equipment in the NASA Global Change Master Directory metadatabase.	Global Change Master Directory	

Figure 1: The existing list before the new FCM common vocabulary establishment

Secondly, a special attention was given to P01 list about the parameter Usage vocabulary that is commonly used in SeaDataNet. After discussion with the BODC and revision, there were 34 parameter codes related to flow cytometry in the P01 vocabulary.

During the Cytobuoy workshop held in Woerden (The Netherlands) from 27 to 30 March 2017, these parameters were reviewed and discussed between the FCM users (from Euro-Mediterranean laboratories) to identify how much these codes could be helpful.

The feedback pointed out that some codes are good but there was a lot of redundancy and definitions were not clear for the FCM users and difficult to understand. Actually, these have been created over the past 30 years to mark-up datasets received at BODC. Most were created to reflect the terminology used at the source but remodelled to fit the BODC semantic model for biological parameter codes. The collection has grown and increased in diversity over the years as flow cytometry spread in marine laboratories and terminology shifted in response to new experimental applications, greater instrument performance and new scientific understanding. As a result many of these codes became ambiguous, poorly defined, or redundant. This situation is a testimony to the timeliness of agreeing on a set of

common vocabularies and on their definition in order to widely share FCM datasets and make them interoperable with one another.

2.1.1.2. Captured parameters for Automated FCM

In order to upgrade these codes at a broad level of agreement between FCM users, we worked closely with some of the JericoNext partners (CNRS/MIO, Rijkswaterstaat (RWS), the Oceanology and Geosciences laboratory (LOG), VLIZ and the Centre for Environment, Fisheries and Aquaculture Science (Cefas)) on a common exercise to identify their FCM data management method and which parameters are captured after the analysis processing. The result below (fig.2) shows common and unique captured parameters for each partner.

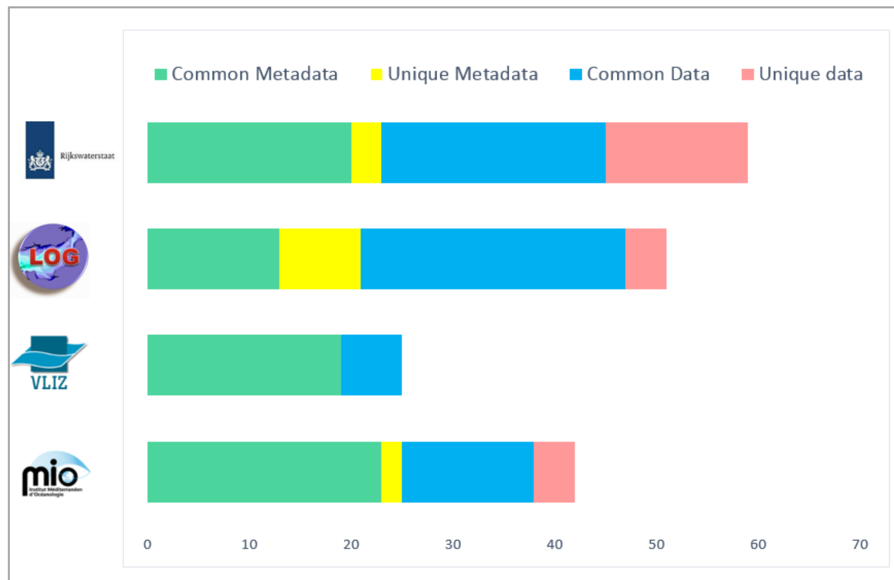

Figure 2: Synthesis of captured parameters per partner

The combination of all these parameters leads to a total of 73 captured parameters (fig.3) (metadata and data). Since we are focusing on parameter usage vocabulary, our choice was limited to the common 12 data variables found in this exercise based on 'Area' criteria (i.e., area of the collected signal). FCM scientists decided to add the same variables based on 'Height' criteria (i.e., the peak of the collected signal).

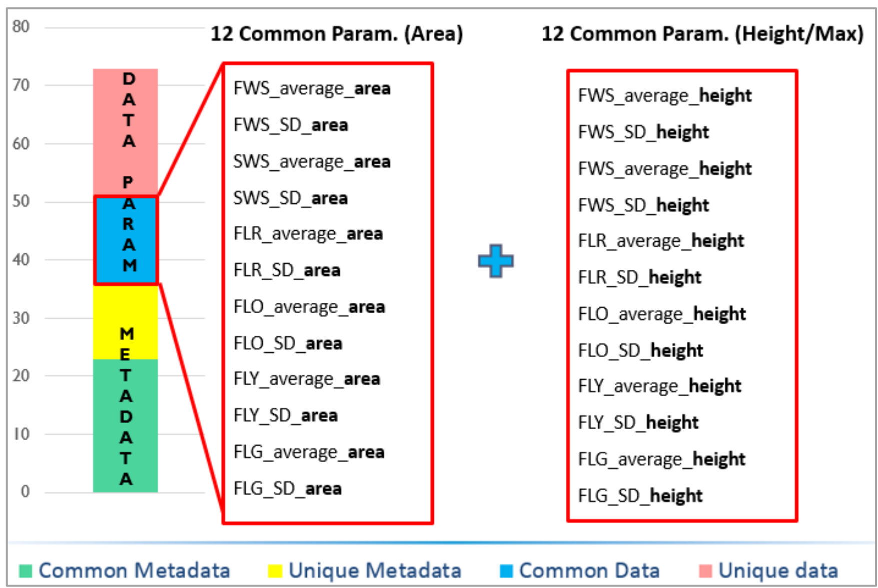

Figure 3: Common data parameters identified for P01 list

2.1.1.3. Literature analysis from 1983 to 2017

A total of 131 scientific papers (Annex 1) were read starting from the beginning of the flow cytometry technique in the 1980's till 2017. This literature review allowed to have a thorough understanding on the used instruments, analyses protocols and achieved parameters (Fig.4, 5 and 6).

There are two types of flow cytometers: (i) pulse shape with image in flow recording FCM and (ii) height and area recording FCM. Both of them have different particularities in the number of lasers used to excite the cells and or to trigger the signal emitted by the cells when the cells cross the laser beam. Some automated flow cytometers have the ability to deliver high resolution measurement, in real time. They can be deployed in situ, underwater or on a ship or a buoy. While the more conventional bench top instruments, most of them developed for the biomedical field, are only deployed in the laboratory and mostly analyse samples several days, weeks or months after sampling.

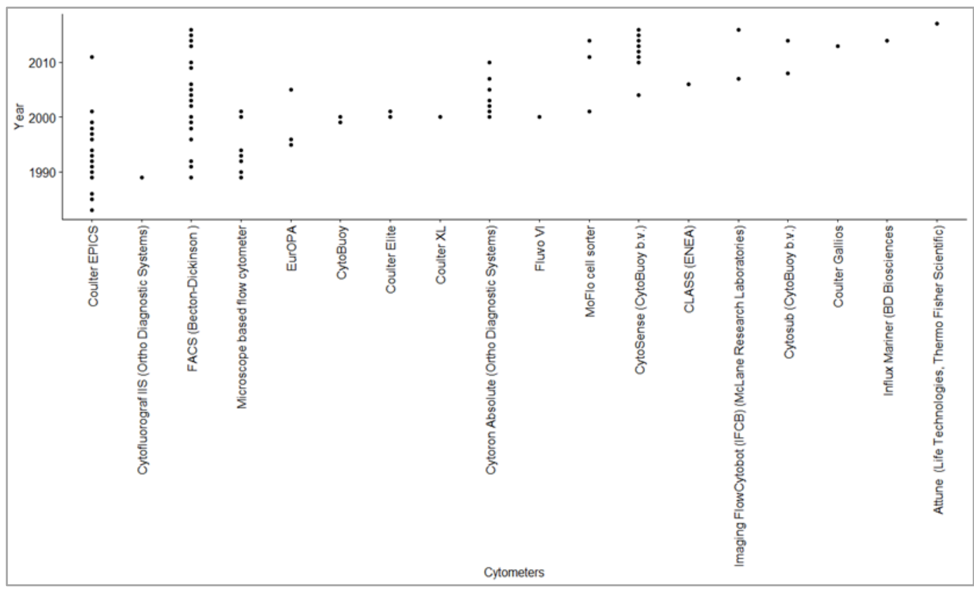

Figure 4: Evolution of the Flow cytometers

Whatever the instrument type, most of the captured parameters achieved by scientists are about the cells group names, their abundance and the statistics (means, coefficient of variation, etc…) about their optical properties related to Forward scatter, Sideward scatter and fluorescence (orange, red, green, yellow, etc..).

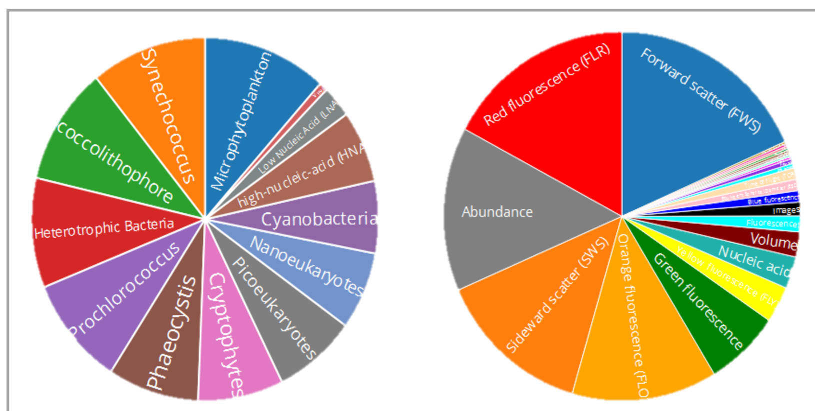

Figure 5: Captured parameters from the literature review

2.1.1.4. Flow cytometry vocabulary standardization questionnaire

In order to update and/or to create new vocabulary codes with a large consensus of FCM users, a questionnaire (58 questions) was created and submitted to 180 FCM users all over the world. It covers four main parts (fig.6 and annex 2):

Part I: Group name and definition => this part includes the captured biological group names and definition based on the literature review. User car add additional group and definition.

Part II Flow Cytometer Metadata => in this part, users were asked about the machine(s), its/their characteristics, signal, sheath fluid and quality control.

Part III: Sample Metadata => this part was related to the protocol used during the analyses: standard beads, sample, etc..

Part IV: Flow Cytometer Data => This part deals with type of particles, staining, clustering, quality control and the captured parameters.

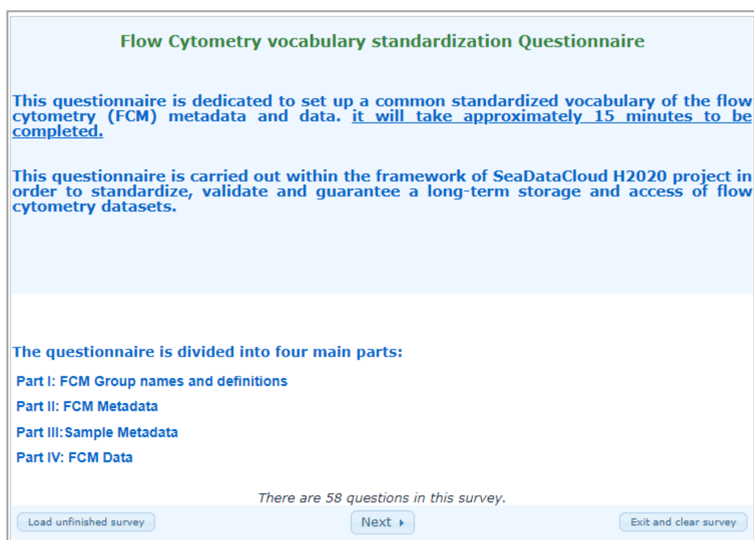

Figure 6: FCM Questionnaire

After 2 months, 38 answers were collected in which 79% were completed and 21% uncompleted but still usable.

Figure 7: distribution of FCM users who answered the questionnaire

Despite the few number of answers, 90% of the FCM user profiles were composed of researchers and engineers with confirmed to expert levels. Therefore, the collected answers (Annex 3) are so valuable that we succeeded to upgrade and define new common vocabulary codes by involving a large FCM community.

2.1.1.5. FCM Common Vocabulary setting

2.1.1.5.1. P02 and P01 lists

The P01 is related to the BODC Parameter Usage Vocabulary list hosted by the BODC and is based on semantic models whether or not the dataset is chemical or physical or biological (fig.8). In our case, FCM parameters give information on biological as well as non-biological groups of particles (i.e.: Standard fluorescent microsphere used as an internal standard for quantitative and qualitative comparisons). The physical model was chosen in order to avoid the *Organism name* which is linked to WoRMS and this value is not always guaranteed for all the groups i.e.: '*Standard beads*', *Eukaryote Picophytoplankton*, etc… 26 parameters usage vocabulary for FCM needs have been created.

Chemical model	Biological model	Physical model
Measurement Substance Measurement matrix relationship Matrix Matrix subcomponent	Measurement Organism Name Organism Specifics Measurement matrix relationship Matrix Matrix subcomponent Method	Measurement Statistical Physical entity Measurement matrix relationship Matrix Method
Concentration of carbon (total inorganic) {TCO2} per unit mass of the water body [dissolved plus reactive particulate phase]	**Abundance of Bacteria (ITIS: 202421: WoRMS 6) [Subgroup: heterotrophic] per unit volume of the water body by automated flow cytometry**	**Area mean of Forward light scatter pulse per cluster from the water body by flow cytometry**

Figure 8: Vocabulary semantic model of the BODC

The P02 is related to the SeaDataNet Parameter Discovery Vocabulary which is on the top of the P01 group of parameters. In the case of FCM vocabulary defined in the P01, they belong to the 'FCMW' code known as 'Flow cytometry parameters in water bodies' and defined as the parameters derived from flow cytometry data analysis of water samples using in situ or bench-top flow cytometers (see annex 4).

2.1.1.5.2. F02 – SeaDataCloud Flow Cytometry Standardised Cluster Names

The **F02 list** was created within the SeaDataCloud project in order to manage all the optical cluster names and definitions identified by FCM. Currently, the list contains 11 codes and can be further extended and upgraded (see annex 4).

http://seadatanet.maris2.nl/v_bodc_vocab_v2/search.asp?lib=F02

2.1.1.5.3. L22 – SeaVoX Device Catalogue

The SeaVoX device catalogue list defines and describes all the devices used for sea measurements. For FCM, we have added 2 devices to the existing list such as: **BD FACSCalibur Flow Cytometer** and **CytoSense flow cytometer**.

2.1.1.5.4. P06 – BODC data storage units

In this list we have added **the international system unit** related to Number per cubic centimetre (NCM3). Equivalent to number per millilitre.

2.1.2. Ingestion, validation and long term access of FCM data

Before ingesting the FCM data, a new **FCM SeaDataNet Ocean Data View (ODV)** data transport format was created. As both physical and biological, FCM data were not suitable for the existing standardized SeaDataNet transport formats. Here is the case of the CNRS-MIO demostrating the FCM data management method from the instrument acquisition to the SeaDataNet ingestion (fig.9) :

For a dedicated project (a cruise for instance), data files acquired by flow cytometry are analysed through a batch process clustering, converted and validated through the CytoBase Input Processor (a standalone software built on R programme by Mathilde Dugenne). Then, data integration into **CYTOBASE** (local database) is processed automatically using **Talend** (Extract Transform and Load (ETL) tool). Subsequently, CYTOBASE is connected to **MIKADO** (SeaDataNet tool for metadata production) to generate the **Common Data Index** (CDI) dataset (aggregation of measurements), the **Cruise Summary Report** (CSR), the **European Directories of Marine Environmental Datasets** (EDMED) and **Marine Environmental Research Projects** (EDMERP). Also, the connexion between MIKADO and CYTOBASE allows the generation of the coupling table which is the association of the CDIs (metadata) and the physical data files. Finally, The CDI and the coupling table are sent to SeaDataNet support team (cdi-support) for validation and ingestion into SeaDataNet infrastructure. The connexion between the data centre and the SeaDataNet Request Status Management service (RSM) is made thanks to the Download Manager (SeaDataNet java component tool) which was installed by the CNRS-MIO and has been operating since February 27th, 2018.

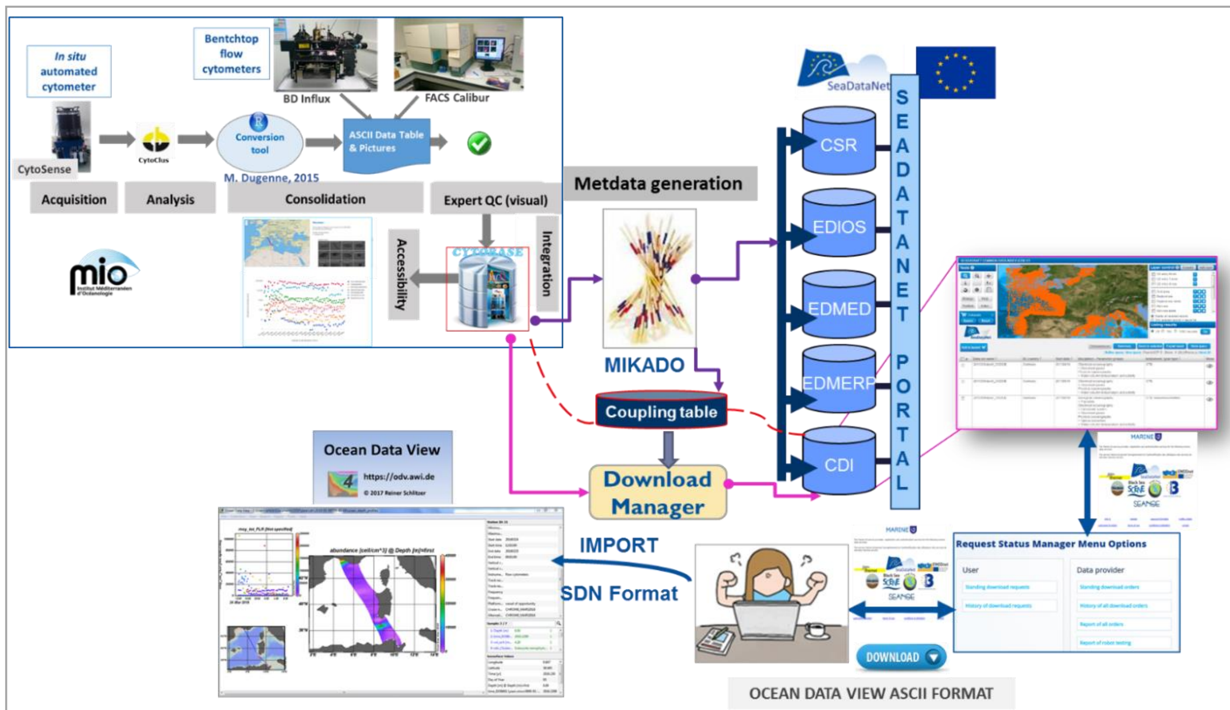

Figure 9: Flow Cytometry (FCM) data ingestion into SeaDataNet infrastructure (case of CNRS-MIO)

2.1.3. Further integration of FCM data into EMODnet Biology infrastructure

FCM data was integrated in EMODnet Biology by making use of the Darwin Core (DwC) Event schema, recently adopted by the marine biodiversity community (De Pooter et al, 2017). The DwC Event Core was implemented by OBIS through the OBIS-ENV-DATA project to respond to the growing needs to provide environmental data together with the species occurrences, and to enhance interoperability of the data through the adoption of controlled vocabularies in the extended Measurements or Facts (eMoF) extension. This schema provides the necessary flexibility to include any kind of data that can be linked either to a species occurrence (e.g. biomass or development stage), or to a sampling event (e.g. sample size, temperature of the water).

The increased flexibility allows fitting the FCM data into the DwC Event schema, by making use of the controlled vocabularies developed during the SDC project described in this deliverable. Two test datasets collected as part of the JERICO-Next JRAP actions were integrated in EMODnet Biology and are now available via the download toolbox, or in the following links:

A*MIDEX CHROME: Western Mediterranean automated flow cytometry surface sample from Ships of O/P crossing Tunis-Marseille and Tunis-Genova between October 2016-January 2017: a dataset available in SeaDataNet.

Plankton biodiversity data from a North Sea Cruise with R/V Simon Stevin in May 2017: made available directly in EMODnet Biology.

The OBIS-ENV-DATA contains a table for Extended Measurements or Facts (known as "eMoF extension") where additional data related to a sampling event or an occurrence can be provided by making use of controlled vocabularies. Using this approach, the FCM data can be provided, linking to the Event and Occurrences IDs by using the developed FCM vocabularies as follows:

Standardised cluster names:

eventID	occurrenceID	measurementType	measurementTypeID	measurementValue	measurementValueID
LL_SimonStevin_sws15flr_2uls_360sec_2017-05-08_15h01.cyz	SimonStevin_08/05/2017_111	Registered name identifier (…)	http://vocab.nerc.ac.uk/collection/P01/current/IDCLFL02	Eukaryote picophytoplankton	http://vocab.nerc.ac.uk/collection/F02/current/F0200004/
LL_SimonStevin_sws15flr_2uls_360sec_2017-05-08_15h01.cyz	SimonStevin_08/05/2017_112	Registered name identifier (…)	http://vocab.nerc.ac.uk/collection/P01/current/IDCLFL02	Eukaryote nanophytoplankton	http://vocab.nerc.ac.uk/collection/F02/current/F0200005/
LL_SimonStevin_sws15flr_2uls_360sec_2017-05-08_15h01.cyz	SimonStevin_08/05/2017_113	Registered name identifier (…)	http://vocab.nerc.ac.uk/collection/P01/current/IDCLFL02	Microphytoplankton	http://vocab.nerc.ac.uk/collection/F02/current/F0200008/

Table 1. Example of records in the eMoF extension for cluster names. MeasurementTypeID and measurementValueID contain the BODC controlled vocabularies (P01 for measurementTypeID and the developed F02 for measurementValueID). measurementType and measurementValue are free text fields but these are completed with the corresponding vocabulary preferred labels.

Optical properties:

eventID	occurenceID	measurement Type	measurementTypeID	measurement Value
CHROME_S1_2016-03-24T12:02:00.000	CHROME_S1_2016-03-24T12:02:00.000_1	Area mean of red fluorescence pulse per cluster from the water body by flow cytometry	http://vocab.nerc.ac.uk/collection/P01/current/FLRAREAA	12241.5704
CHROME_S1_2016-03-24T12:02:00.000	CHROME_S1_2016-03-24T12:02:00.000_1	Area standard deviation of red fluorescence pulse per cluster from the water body by flow cytometry	http://vocab.nerc.ac.uk/collection/P01/current/FLRARESD	6394.2

Table 2. Example of records for FCM optical properties in the eMoF extension using the P01 vocabularies developed.

Other measurements:

eventID	occurrence ID	measurementType	measurementTypeID	measurementValue	measurementValueID	measurementUnit	measurementUnitID
CHROME_MARS2016_FCMW		Sampling platform name	http://vocab.nerc.ac.uk/collection/Q01/current/Q0100001/	Carthage	http://vocab.nerc.ac.uk/collection/C17/current/88NM/		
CHROME_S1_2016-03-24T12:02:00.000	CHROME_S1_2016-03-24T12:02:00.000_1	Abundance of biological entity (…)	http://vocab.nerc.ac.uk/collection/P01/current/SDBIOL01	87.69		Number per cubic centimetre	http://vocab.nerc.ac.uk/collection/P06/current/NCM3/
CHROME_S1_2016-03-24T12:02:00.000		Volume sampled of the water body	http://vocab.nerc.ac.uk/collection/P01/current/VOLWBSMP/	0.376328		Cubic metres	http://vocab.ndg.nerc.ac.uk/collection/P06/current/MCUB
CHROME_S1_2016-03-24T12:02:00.000		Sampling instrument name	http://vocab.nerc.ac.uk/collection/Q01/current/Q0100002/	CytoBuoy CytoSense flow cytometer	http://vocab.nerc.ac.uk/collection/L22/current/TOOL1209/		

Table 3. Additional standardised measurements in the eMoF extension.

The underlying data system in EMODnet Biology stores the data following the DwC schema and associated standards, making it interoperable with (Eur)OBIS. However, to increase simplicity for users, the data is flattened when accessed via the EMODnet Biology download toolbox.

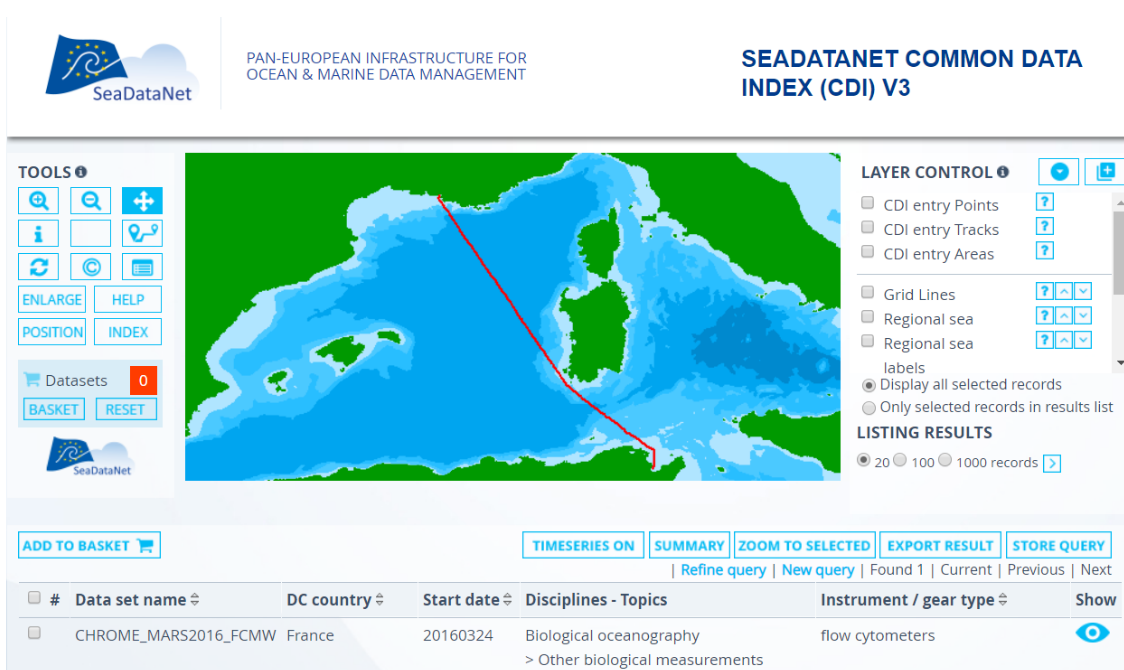

Figure 10. Screen capture of the CDI record in SDN for the MIDEX CHROME dataset.

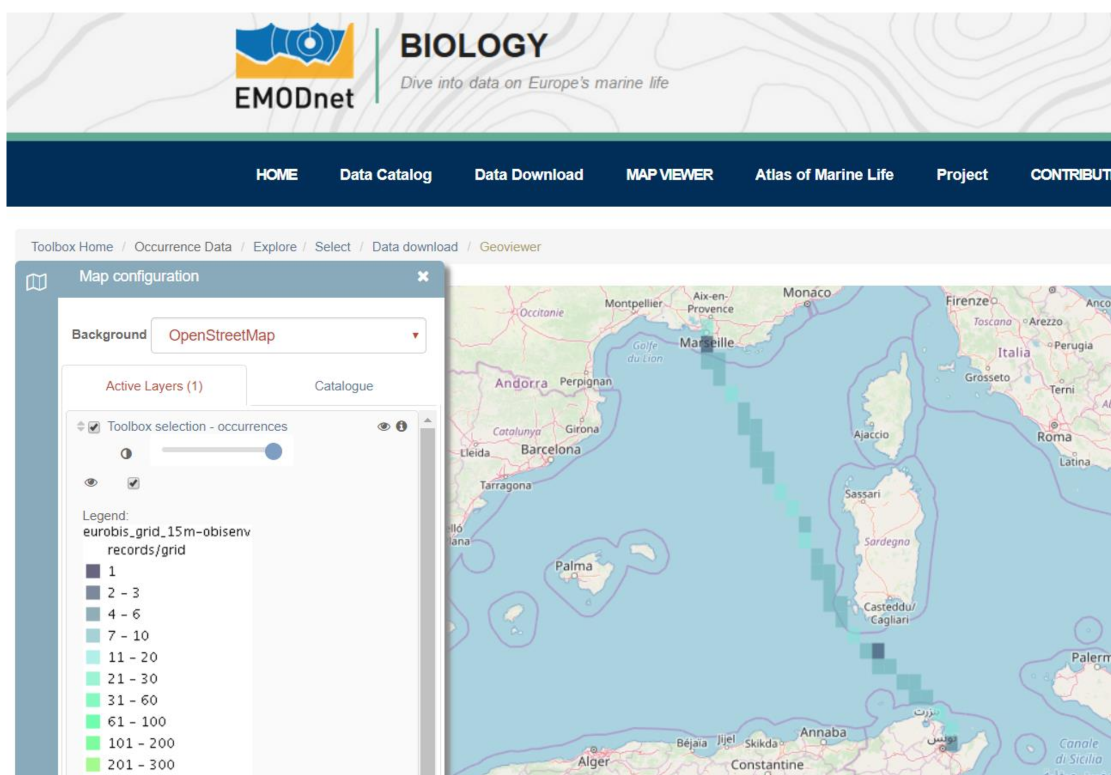

Figure 11. Screen capture of the gridded occurrences for a FCM dataset (AMIDEX-CHROME) in EMODnet Biology.

The main remaining challenge for marine biodiversity data repositories, such as EMODnet Biology and OBIS, remains how to deal with non-taxonomically resolved records in the searching interfaces. EMODnet Biology has recently launched a new version of its download toolbox, which allows searching

for data standardized to controlled vocabularies in the eMoF extension. It is therefore possible to search specifically for FCM data in EMODnet Biology. However, further investigation and consultation are recommended to find the optimal data storing and querying solutions that can meet the global biodiversity and FCM community needs in the long term.

3. Conclusion

FCM technology and mainly the automated flow cytometers are revolutionizing the biological world by acquiring high resolution (in time and space) and real-time data about the first levels of the marine food web. Making these data sustainable, accessible and standardized will be very useful for the marine community as interoperability will greatly facilitate inter-community discussions. There is still a continuous effort to update and/or define common vocabulary, add new metadata and ingest data into SeaDataNet.

This work was made with a strong interaction between FCM users and scientists from Euro-mediterranean institutes. Scientists have showed a big interest on sharing their data and put them accessible within SeaDataNet portal.

Thanks to Cytobuoy workshop (March 2017), JericoNext workshops (WP3 in 2016 and 2018) and Euormarine (March 2018) on improving the visibility of ocean data from new technologies: a case study of high frequency flow cytometry, we could disseminate SeaDataNet activity through this work-package.

4. List of acronyms

Acronym	Definition
BODC	British Oceanographic Data Centre
CDI	Common Data Index (SeaDataNet catalogue)
CEFAS	The Centre for Environment, Fisheries and Aquaculture Science
CNRS	Centre National de la Recherche Scientifique (France)
CSR	Cruise Summary Report (SeaDataNet Catalogue)
EDMED	European Directory of Marine Environmental Data sets (SeaDataNet catalogue)
EDMERP	European Directory of Marine Environmental Research Projects (SeaDataNet catalogue)
ETL	Extract Transform and Load
FACS	Fluorescence-Activated Cell Sorting
FCM	Flow Cytometry
JERICO	Joint European Research Infrastructure Network for Coastal Observatories
JRAP	Joint Research Activity Projects
LOG	Oceanology and Geosciences laboratory
MIO	Mediterranean Institute of Oceanography
NERC	Natural Environment Research Council
OBIS	Ocean Biogeographic Information System
ODV	Ocean Data View
RSM	Request Status Manager (SeaDataNet service)
RWS	Rijkswaterstaat, Netherlands
SDC	SeadataCloud
SDN	SeaDataNet
VLIZ	Flanders Marine Institute

WP	Work Package

5. ANNEX 1- References of the literature review

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6. ANNEX 2- Flow Cytometry vocabulary standardisation

Questionnaire

Flow Cytometry vocabulary standardization Questionnaire

This questionnaire is dedicated to set up a common standardized vocabulary of the flow cytometry (FCM) metadata and data. it will take approximately 15 minutes to be completed.

This questionnaire is carried out within the framework of SeaDataCloud H2020 project in order to standardize, validate and guarantee a long-term storage and access of flow cytometry datasets.

0% 100%

Presentation

In this part, participants will be asked about their position, institute affiliation and their Flow Cytometry experience.

Please fill out your first name and Last name:

* What is the full name of your Institute/organization ?

Could you please mention your gender ?

- Female
- Male
- No answer

* Please mention your experience level (from 1 to 5) on using Flow Cytometry technic ?

- 1
- 2
- 3
- 4
- 5

? (e.g.: 1= Beginner, 2= Intermediate, 3= Advanced, 4 = Confirmed, 5 = Expert)

* What is your current position ?

Check any that apply

- Researcher
- Engineer
- PhD Student
- Other level student
- Other:

0% 100%

Part I: FCM Group names and definitions

Based on litterature from 1983 to 2017, do you agree on these FCM group definitions:

* **Prochlorococcus**

Prochlorococcus cells are defined as the smallest cyanobacteria found in the marine environment. No staining is required to distinguish them by flow cytometry. Compared to any other group, their FWS and FLR signatures are the smallest recorded up to now and require sensitive PMT or high powered lasers. The cluster, when well defined (often deep water communities) is below or may overlap that of Synechococcus group, and is often partially masked by the instrument background noise. In samples stained for Heterotrophic bacteria analysis, Prochlorococcus can be distinguished using Sideward Scatter (SWS) vs Chlorophyll Red Fluorescences (FLR) cytogram. They do not emit orange fluorescence because they lack phycoerythrin.

Check any that apply

- I agree
- I do not agree

* **Synechococcus**

Synechococcus are unicellular photosynthetic cyanobacteria with flow-cytometry forward-scatter (FWS) and sideward scatter (SWS) signatures larger than those of most of the marine heterotrophic bacteria. No staining is required to distinguish them by flow cytometry. The Synechococcus cluster has higher FWS and red fluorescence (FLR) signatures than Prochlorococcus and a distinct orange fluorescence (FLO) signature from their phycoerythrin accessory pigment when excited by lasers whose wavelength is below 533 nm. Cyanobacteria may contain phycocyanin, excited by a red laser and emitting above the chl a emission wavelength. The Synechococcus cluster is well resolved in red vs green (FLR/FLG) and in red vs orange fluorescences (FLR/FLO) cytogrammes. Due to their small size (0.8-1.2 µm) as reported in the literature, Synechococcus cells exhibit a low intensity of FWS, SWS and FLR signals.They are unicellular photosynthetic Cyanobacteria with flow-cytometry forward-scatter (FWS) and sideward scatter (SWS) signatures that are larger than those of most of the marine heterotrophic bacteria. No staining is required to distinguish them by flow cytometry. The related cluster has higher FWS and red fluorescence (FLR) signatures than Prochlorococcus and a distinct orange fluorescence (FLO) signature from their phycoerythrin accessory pigment when excited by lasers whose wavelength is below 533 nm. Cyanobacteria may contain phycocyanin, excited by a red laser and emitting above the chl a emission wavelength. The Synechococcus cluster is well resolved in red vs green (FLR/FLG) and in red vs orange fluorescences (FLR/FLO) cytogrammes. Due to their small size (0.8-1.2 µm) as reported in the literature, Synechococcus cells exhibit low-intensity FWS, SWS and FLR signals.

Check any that apply

- I agree
- I do not agree

* **Eukaryotes Picophytoplankton**

The Eukaryote picophytoplankton group is defined by cells with a size range between 2-3 µm. No staining is required to distinguish them by flow cytometry. The smallest known eukaryotic picophytoplankton is Ostreococcus. Eukaryotic picophytoplankton exhibits a well-defined flow cytometry signature, with FWS and FLR signals larger than that of Prochlorococcus and Synechococcus, and smaller than that of nanophytoplankton cells, though some overlap may happen. The FWS signal of 2 µm beads is widely used as an optical standard to localize this group. It is important to keep in mind that cell cycle within this group may generate cells with size > 2 µm (2-4 µm in theory). They do not have FLO signal.

Check any that apply

- [ ] I agree
- [ ] I do not agree

*
Eukaryote Nanophytoplankton
The Eukaryote Nanophytoplankton group is defined by cells with a size range between 2-20 µm. No staining is required to distinguish them by flow cytometry. They differ from eukaryotic picophytoplankton by larger FLR, SWS and FWS signals. Eukaryote Nanophytoplankton cells are sperate from the cryptophytes due to the lack of orange fluorescence.

Check any that apply

- [ ] I agree
- [ ] I do not agree

* **Cryptophytes**

Cryptophyte cells have higher FWS and FLO signals than Synechococcus and a high FLO/FLR ratio. No staining is required to distinguish them by flow cytometry. Their FWS signal can reach values close to that of microphytoplankton. They contain high amounts of phycoerythrin and may contain phycocyanin that can be excited by a red laser. If cryptophytes cells contain both phycoerythrin and phycocyanin and are excited by a laser beam approximately < 520 nm, then they will emit a higher FLR signal compared with that of only chlorophyll a containing cells (energy transfer to FLR). Cryptophytes cluster is separated from the Eukaryote Nanophytoplankton cluster due to the presence of phycoerythrin and phycocyanin fluorescence signals.

Check any that apply

- [ ] I agree
- [ ] I do not agree

* **Coccolithophores**

Coccolithophores are nanoplanktonic cells that build calcium carbonate coccoliths. When the cells have coccolith shells, due to their $CaCO_3$ platelet covering, they are characterized by a high depolarization ratios (Horizontally polarized Forward Light Scatter (HFLS)/Vertically polarized Forward Light Scatter (VFLS)) and a high Sideward Scatter (SWS). Their FWS and FLR signals are similar to those of Eukaryote Nanophytoplankton group. No staining is required to distinguish them by flow cytometry.

Check any that apply

- [ ] I agree
- [ ] I do not agree

* **Microphytoplankton**

The microphytoplankton group is defined by cells with a size range between 20-200 µm. No staining is required to distinguish microphytoplankton by flow cytometry. This group is discriminated thanks to its FWS and FLR signals, larger than those of the other groups. Due to the low volumes analyzed by flow cytometry, this group is not always properly counted when cells are not abundant enough. When FWS is calibrated by using beads or phytoplankton cell-cultures, it enables to distinguish microphytoplankton from nanophytoplankton with size near 20 µm. Chains or colonies may outpass flow cytometry analysis depending on instrument performances (tubing size, pulse shape analysis or not). If large cryptophytes or coccolithophores are observed, they will be considered in a separate group thanks to their distinguishable optical properties.

Check any that apply

- [ ] I agree
- [ ] I do not agree

* **Heterotrophic Bacteria**

Heterotrophic prokaryotes include both bacteria and Archea. They do not contain any photosynthetic pigments and thus do not have any autofluorescence properties exploitable by flow cytometry. Thus, they require a staining with some fluorescent dye to be resolved by flow cytometry. In most studies, a nucleic acid dye is used. Staining of nucleic acids by a dye emitting in the green when excited by a blue laser enables heterotrophic prokaryotes to be distinguished in various groups thanks to SWS (or FWS) and FLG signatures : Cells with a lower FLG correspond to heterotrophic prokaryotes with a Lower Nucleic Acid content (LNA) and cells with a higher FLG correspond to a Higher Nucleic Acid content (HNA). Their scatter signals (FWS, SWS) are lower than those of Synechococcus and eukaryotic picophytoplankton and may overlap those of Prochlorococcus.

Check any that apply

- [ ] I agree
- [ ] I do not agree

* **Standard Beads**

A standard is a reference defined by a user, a laboratory, or any acknowledged authority. Properties of Standard beads are accurately known by the manufacturers (i.e: size, material, fluorescences). These fluorescent microbeads (microsphere) are used as an absolute reference for quantitative and qualitative comparisons. Standard beads are analyzed routinely in every FCM analyses in order to have confidence in the instrument performance (alignment and fluidics) and as well as in the results.

Check any that apply

- [ ] I agree
- [ ] I do not agree

Please enter other group(s) name and definition if the above list is not complete:

Group 1: ______
Group 2: ______
Group 3: ______
Group 4: ______

Resume later | Next ▸ | Exit and clear survey

0% 100%

Part II: FCM Metadata

* What model of Flow Cytometer(s) do you use?

(e.g.: CytoSense, FACS Calibur)

* Does your instrument have an image in flow device?

Yes No

(e.g.: pictures)

* What is your sample inlet internal diameter (in microns)?

(Separate multiple entries with commas)

* Which lasers wavelengths do you use (in nm)?

((e.g.:488). In case of multiple entries, separate them with commas)

* Laser beam powers (in mW)?

((e.g.:25). In case of multiple entries, please indicate the power of each of your laser separated by commas.)

* How many light scatters does your instrument record?

((e.g.:2). In case of multiple entries, separate them with commas)

* How many fluorescences does your instrument record?

((e.g.:3). In case of multiple entries, separate them with commas)

* For each laser, please indicate each optical filters configuration (light scatter, fluorescences) in nanometers?

(e.g.: For 488nm (563/30 nm), 650 LP)

* What signal do you use as trigger?

(e.g.: FLR)

* What type of signal does your instrument record ?

Check any that apply

- Pulse-width/TOF
- Area/total
- Height/max
- Other:

* What is the type/composition of the sheath fluid you use?

Check any that apply

- Natural sea water
- Artificial sea water
- Distilled water
- PBS
- Other:

* Do you perform quality control of your instrument?

Yes No

Resume later Next Exit and clear survey

0% 100%

Part III: Sample Metadata

* What Beads reference do you use?

(e.g.: brand, size, fluorescence, marterial)

* What beads diameters do you use?

(e.g.: 1, 2, 3, 6, 10 um, ...)

* What are your Beads Fluorescences?

(e.g.: Yellow)

* For what purpose do you use this instrument?

Check any that apply

- Research
- Monitoring
- Biotechnology
- Other:

* Where is your area of study?

(e.g.: North sea, North Channel, etc...)

* What type of sample do you analyze?

Check any that apply

- Sea water
- Fresh water
- Cultures
- Other:

* What is your approximate analyzed volume (mm^3)?

((e.g.:1000-5000 mm3). In case of multiple entries, separate them with commas)

* What is your sample flow rate ($mm^3.min^{-1}$)?

Resume later | Next | Exit and clear survey

0% 100%

Part IV: FCM Data

* Do you use a fluorescent Dye?

- Yes
- No

* Which type of particles do you measure?

Check any that apply

- Phytoplankton
- Heterotrophic bacteria
- Virus
- Other:

* What are the recurrent autotrophic functional groups of your area of study?

Check any that apply

- Synechococcus
- Prochlorococcus
- Picoeukaryotes
- Nanoeukaryotes
- Coccolitophore
- Cryptophytes
- Microphytoplankton
- Not concerned
- Other:

* What are the recurrent Heterotrophic groups of your area of study?

Check any that apply

- High Nucleic Acid Prokaryotes
- Low Nucleic Acid Prokaryotes
- Nanoflagellates
- Not concerned
- Other:

* What clustering method do you use?

Check any that apply

- Manual
- Automatic
- Other:

* Do you flag your data ?

- Yes
- No

? (e.g.: quality flag: good data, bad data, suspiciousdata, etc...)

* What parameters do you export after your clustering?

Check any that apply

- Functional group names
- Abundance (cell.cm-3)
- Average Side Ward Scatter (Area, length)
- Average Forward Scatter (Area, length)
- Average Red Fluorescences
- Average Orange Fluorescences
- Standard deviation Side Ward Scatter (Area, length)
- Standard deviation Forward Scatter (Area, length)
- Standard deviation Red Fluorescences
- Standard deviation Orange Fluorescences
- Other:

* What is the unit used for scatters and fluorescences ?

Check any that apply

- Arbitrary unit (a.u.)
- Other:

Resume later | Next | Exit and clear survey

0% 100%

Conclusion and recommendation

* Do you prefer to hide your name and your organization name?

- Yes
- No

Do you have any recommendations or comments you would like to add?

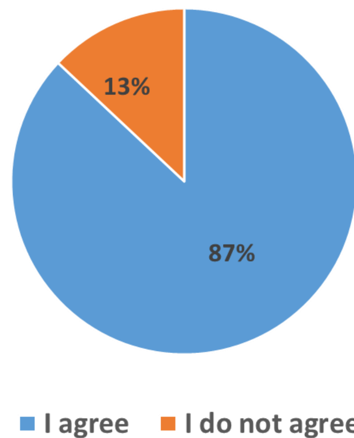
Resume later | Submit | Exit and clear survey

7. ANNEX 3- Questionnaire answers

PART I: Groups definition from the FCM point of view

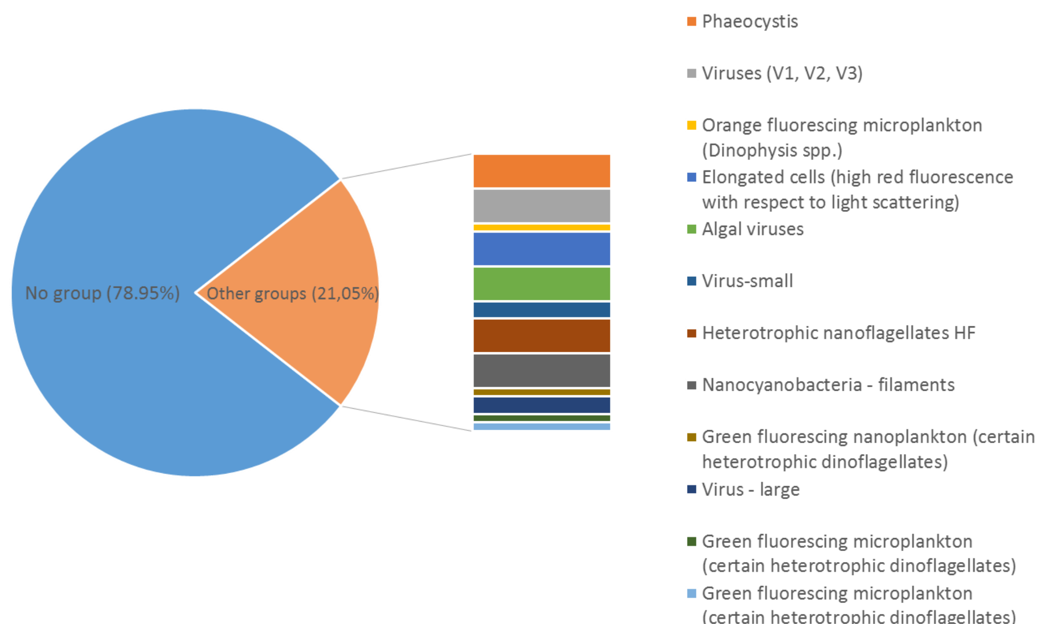
Q: Based on litterature from 1983 to 2017, do you agree on these group definitions:

Prochlorococcus, Synechococcus, Eukaryotes Picophytoplankton, Eukaryote Nanophytoplankton, Cryptophytes, Coccolithophores, Microphytoplankton and Heterotrophic Bacteria

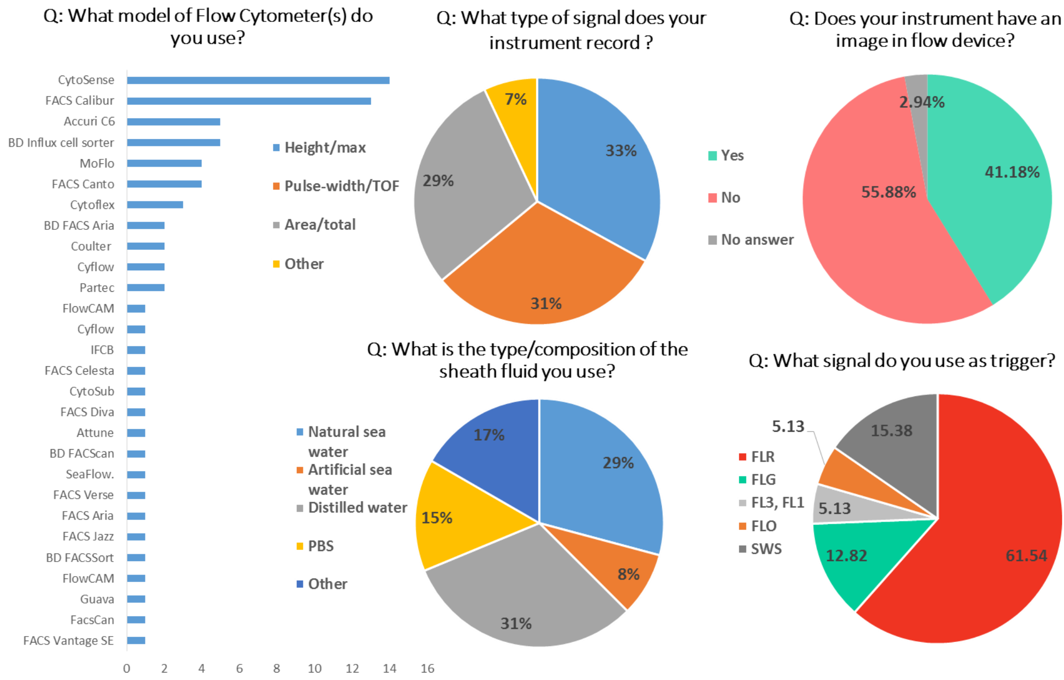


I agree I do not agree

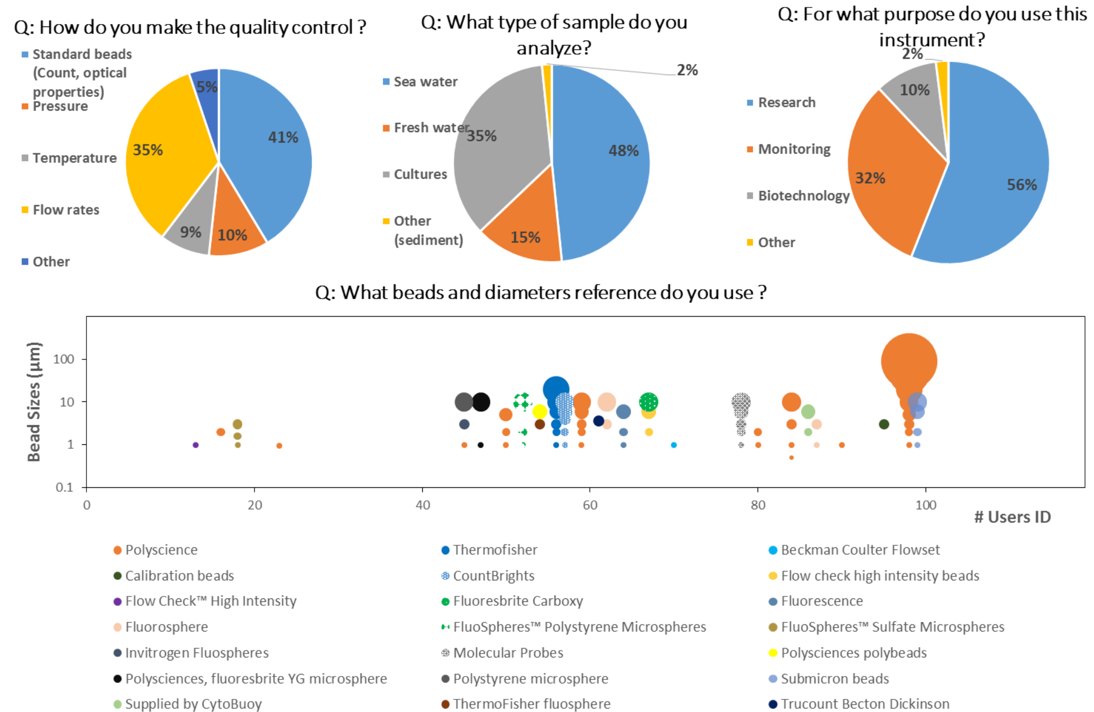
Suggestion to add other group(s) name

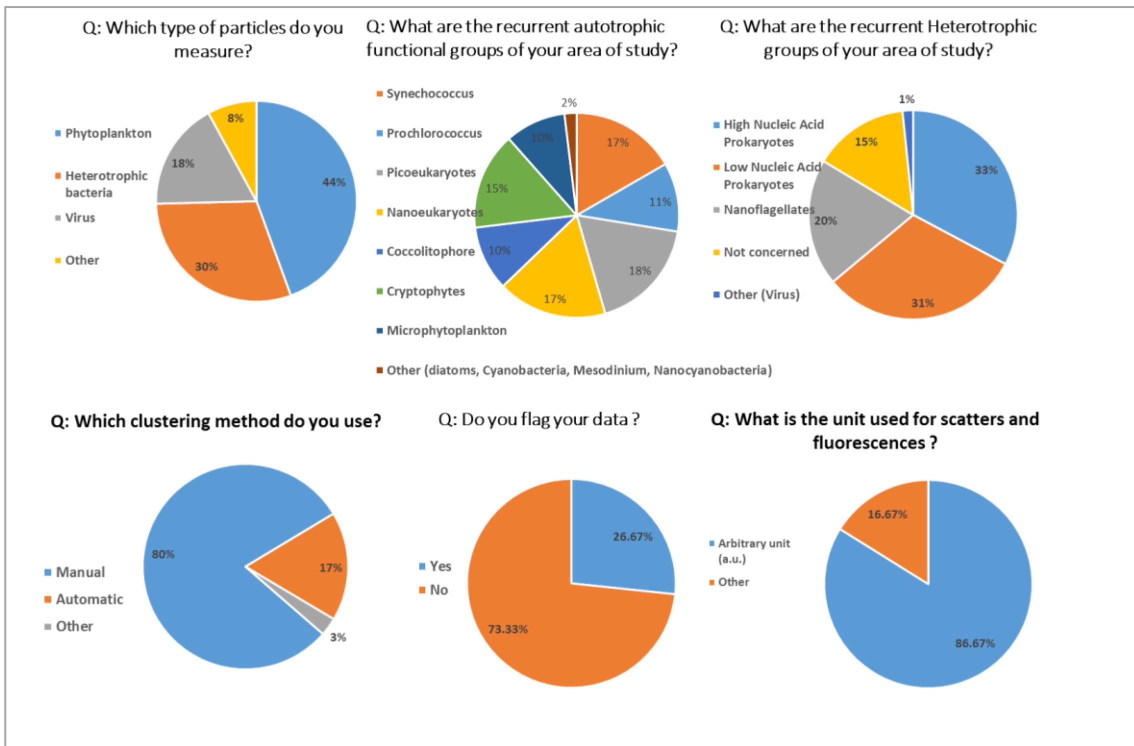


PART II: Flow Cytometer Metadata



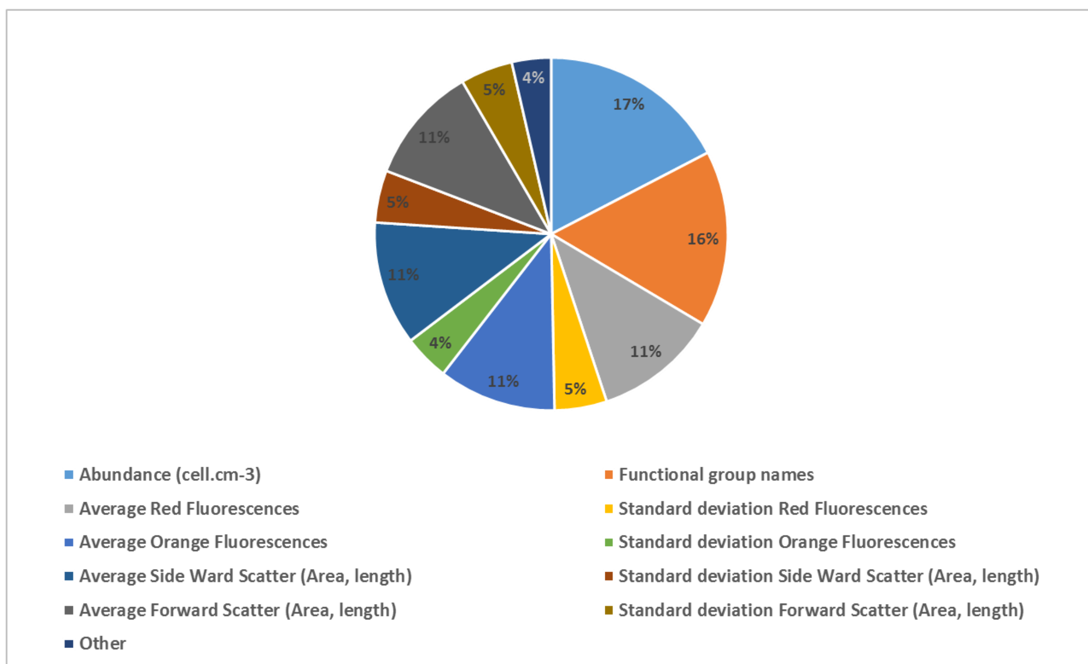
PART III: Sample Metadata





PART III: Sample Metadata

Q: Which parameters do you export after your clustering?



8. ANNEX 4- Common vocabulary P02, P01 and F02 lists

PAN-EUROPEAN INFRASTRUCTURE FOR OCEAN & MARINE DATA MANAGEMENT

BODC VOCAB LIBRARY

p02	Conceptid	Pref label
+	ASAM	Acoustic backscatter in the water column
+	GP080	Fishing by-catch
+	FEFF	Fishing effort
−	FCMW	Flow cytometry parameters in water bodies

p01	Conceptid	Pref label
	FLGAREAA	Area mean of green fluorescence pulse per cluster from the water body by flow cytometry
	FLGARESD	Area standard deviation of green fluorescence pulse per cluster from the water body by flow cytometry
	FLGMAXAA	Peak height mean of green fluorescence pulse per cluster from the water body by flow cytometry
	FLGMAXSD	Peak height standard deviation of green fluorescence pulse per cluster from the water body by flow cytometry
	FLOAREAA	Area mean of orange fluorescence pulse per cluster from the water body by flow cytometry
	FLOARESD	Area standard deviation of orange fluorescence pulse per cluster from the water body by flow cytometry
	FLOMAXAA	Peak height mean of orange fluorescence pulse per cluster from the water body by flow cytometry
	FLOMAXSD	Peak height standard deviation of orange fluorescence pulse per cluster from the water body by flow cytometry
	FLRAREAA	Area mean of red fluorescence pulse per cluster from the water body by flow cytometry
	FLRARESD	Area standard deviation of red fluorescence pulse per cluster from the water body by flow cytometry

FLRMAXAA	Peak height mean of red fluorescence pulse per cluster from the water body by flow cytometry
FLRMAXSD	Peak height standard deviation of red fluorescence pulse per cluster from the water body by flow cytometry
FLYAREAA	Area mean of yellow fluorescence pulse per cluster from the water body by flow cytometry
FLYARESD	Area standard deviation of yellow fluorescence pulse per cluster from the water body by flow cytometry
FLYMAXAA	Peak height mean of yellow fluorescence pulse per cluster from the water body by flow cytometry
FLYMAXSD	Peak height standard deviation of yellow fluorescence pulse per cluster from the water body by flow cytometry
FWSAREAA	Area mean of forward light scatter pulse per cluster from the water body by flow cytometry
FWSARESD	Area standard deviation of forward light scatter pulse per cluster from the water body by flow cytometry
FWSMAXAA	Peak height mean of forward light scatter pulse per cluster from the water body by flow cytometry
FWSMAXSD	Peak height standard deviation of forward light scatter pulse per cluster from the water body by flow cytometry

SWSAREAA	Area mean of sideward light scatter pulse per cluster from the water body by flow cytometry
SWSARESD	Area standard deviation of sideward light scatter pulse per cluster from the water body by flow cytometry
SWSMAXAA	Peak height mean of sideward light scatter pulse per cluster from the water body by flow cytometry
SWSMAXSD	Peak height standard deviation of sideward light scatter pulse per cluster from the water body by flow cytometry

ConceptID	Preferred label	Alt label	Definition	Modified
IDCLFL02	Registered name identifier of flow cytometry cluster by classification to a term from the NVS SeaDataCloud Flow Cytometry Standardised Cluster Names Vocabulary (SDN:F02::)	ClusterNameID	Opaque key term identifying the type of particles belonging to a specific flow cytometry cluster, taken from the NVS SeaDataCloud Flow Cytometry Standardised Cluster Names controlled vocabulary F02.	2/1/2018 21:53:44
NMCLFL02	Registered name of flow cytometry cluster by classification to a term from the NVS SeaDataCloud Flow Cytometry Standardised Cluster Names Vocabulary (SDN:F02::)	ClusterName	Text term identifying the type of particles belonging to a specific flow cytometry cluster, taken from the NVS SeaDataCloud Flow Cytometry Standardised Cluster Names controlled vocabulary F02.	2/1/2018 21:53:44

F02 (SEADATACLOUD FLOW CYTOMETRY STANDARDISED CLUSTER NAMES)

Overview | Export subset of list | Export full list | New query | Found 11 | Current | Previous | Next

ConceptID	Preferred label	Alt label	Definition	Modified
F0200001	Standard beads		A standard is a reference defined by a user, a laboratory, or any acknowledged authority. Properties of standard beads are accurately known by the manufacturers (i.e. size, material, fluorescence properties). These fluorescent microbeads (or microsphere) are used as an absolute reference for quantitative and qualitative comparisons. Standard beads are analyzed routinely in every flow cytometry analyses in order to have confidence in the instrument performance (alignment and fluidics) and as well as in the results.	11/20/2017 13:09:10
F0200002	Prochlorococcus		Prochlorococcus cells are defined as the smallest cyanobacteria found in the marine environment. No staining is required to distinguish them by flow cytometry. Compared to any other group, their forward scatter and red fluorescence signatures are the smallest recorded up to now and require sensitive Photo Multiplier Tube (PMT) or high powered lasers. The cluster, when well defined (often deep water communities) is below or may overlap that of the Synechococcus group, and is often partially masked by the instrument background noise. In samples stained for heterotrophic prokaryote detection, Prochlorococcus can be distinguished using the sideward scatter vs red fluorescence cytogram. They do not emit orange fluorescence because they lack phycoerythrin.	11/20/2017 13:09:10

F0200003	Synechococcus	Synechococcus are unicellular photosynthetic cyanobacteria with flow cytometry forward scatter and sideward scatter signatures larger than those of most of marine heterotrophic bacteria. No staining is required to distinguish them by flow cytometry. The Synechococcus cluster has higher forward scatter and red fluorescence signatures than Prochlorococcus and a distinct orange fluorescence signature from their phycoerythrin accessory pigment when excited by lasers whose wavelength is below 533 nanometres. Cyanobacteria may contain phycocyanin, excited by a red laser and emitting above the chlorophyll-a emission wavelength. The Synechococcus cluster is well resolved in red vs green and in red vs orange fluorescence cytograms. Due to their small size (0.8 to 1.2 microns as reported in the literature), Synechococcus cells exhibit low intensity of forward and sideward scatters and red fluorescence signals.	11/20/2017 13:09:10
F0200004	Eukaryote picophytoplankton	The eukaryote picophytoplankton group is defined by cells with a size range between 2 and 3 microns. No staining is required to distinguish them by flow cytometry. The smallest known eukaryotic picophytoplankton is Ostreococcus. Eukaryotic picophytoplankton exhibit a well-defined flow cytometry signature, with forward scatter and red fluorescence signals larger than that of Prochlorococcus and Synechococcus, and smaller than that of nanophytoplankton cells, though some overlap may happen. The forward scatter signal of 2 micron beads is widely used as an optical standard to localize this group. It is important to keep in mind that cell cycle within this group may generate cells with size greater than 2 microns (2-4 um in theory). They do not have an orange fluorescence signal.	11/20/2017 13:09:10

F0200005	Eukaryote nanophytoplankton	The eukaryote nanophytoplankton group is defined by cells with a size range between 2 and 20 microns. No staining is required to distinguish them by flow cytometry. They differ from eukaryotic picophytoplankton by their larger red fluorescence, sideward scatter and forward scatter signals. Eukaryote nanophytoplankton cells can be separated from the cryptophyte cells due to their lack of orange fluorescence.	11/20/2017 13:09:10
F0200006	Cryptophytes	Cryptophytes have higher forward scatter and orange fluorescence intensities than Synechococcus and a higher orange to red fluorescence ratio compared to other eukaryotic phytoplankton. Cryptophytes form their own cluster separated from the eukaryote nanophytoplankton cluster due to the presence of phycoerythrin and phycocyanin fluorescence signals. No staining is required to detect and discriminate them by flow cytometry. Their forward scatter signal can reach values close to that of microphytoplankton. They contain high amounts of phycoerythrin and may contain phycocyanin that can be excited by a red laser. If cryptophyte cells contain both phycoerythrin and phycocyanin, then they will emit a higher red fluorescence signal than cells containing only chlorophyll-a (energy transfer to red fluorescence).	11/20/2017 13:09:10

F0200007	Coccolithophores	Coccolithophores are nanoplanktonic cells that build calcium carbonate coccoliths. When the cells have coccolith shells,due to their CaCO3 platelet covering, they are characterized by a high depolarization ratios (Horizontally polarized Forward Light Scatter over Vertically polarized Forward Light Scatter (HFLS/VFLS)) and a high sideward scatter. Their forward scatter and red fluorescence signals are similar to those of the eukaryote nanophytoplankton group. No staining is required to distinguish them by flow cytometry.	11/20/2017 13:09:10
F0200008	Microphytoplankton	The microphytoplankton group is defined by cells with a size range between 20 and 200 microns. No staining is required to distinguish microphytoplankton by flow cytometry. This group is discriminated thanks to its forward scatter and red fluorescence signals larger than those of the other groups. Due to the low volumes analyzed by flow cytometry, this group is not always accurately quantified when cells are not abundant. It is possible to distinguish this group from nanophytoplankton (2-20 microns in diameter) when the forward scatter signal is calibrated to detect the forward scatter channel corresponding to the 20 micron limit in size. On cytometers equipped with an image in flow device, pictures are also used to measure cell size. Chains or colonies may outpass flow cytometry analysis depending on instrument performance (tubing size, pulse shape analysis or not). If large cryptophytes or coccolithophores are observed, they will be considered in a separate group thanks to their distinguishable optical properties.	11/20/2017 13:09:10

F0200009	Heterotrophic prokaryotes	Heterotrophic prokaryotes include both bacteria and Archea. They do not contain any photosynthetic pigments and thus do not have any autofluorescence properties exploitable by flow cytometry. Thus, they require a staining with some fluorescent dye to be resolved by flow cytometry. In most studies a nucleic acid dye is used. Staining of nucleic acids by a dye emitting in the green when excited by a blue laser enables heterotrophic prokaryotes to be distinguished in various groups thanks to sideward scatter (or FWS) and FLG signatures : Cells with a lower FLG correspond to heterotrophic prokaryotes with a Lower Nucleic Acid content (LNA) and cells with a higher FLG correspond to a Higher Nucleic Acid content (HNA). Their scatter signals (FWS, SWS) are lower than those of Synechoccocus and eukaryotic picophytoplankton, and may overlap those of Prochlorococcus.	11/20/2017 13:09:10
F0200010	Heterotrophic prokaryotes - HNA	Heterotrophic prokaryote cells with a Higher Nucleic Acid (HNA) content as defined by flow cytometry, based on the intensity of their fluorescence signal induced by a nucleic acid dye.	2/2/2018 15:13:20
F0200011	Heterotrophic prokaryotes - LNA	Heterotrophic prokaryote cells with a Lower Nucleic Acid (HNA) content as defined by flow cytometry, based on the intensity of their fluorescence signal induced by a nucleic acid dye.	2/2/2018 15:14:15