



Marseille, from 19 to 22 March 2018



JOINT EUROPEAN RESEARCH INFRASTRUCTURE NETWORK FOR COASTAL OBSERVATORIES

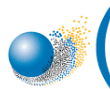
# The setting up of new flow cytometry standardized and common vocabulary



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Michèle FICHAUT (IFREMER), Dick SCHAAP (MARIS) and Melilotus THYSSEN (MIO)**

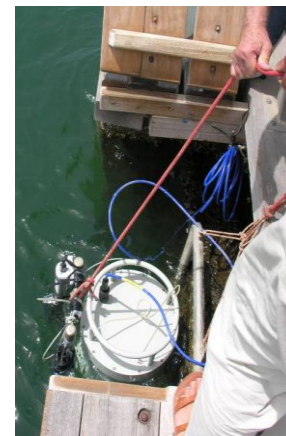


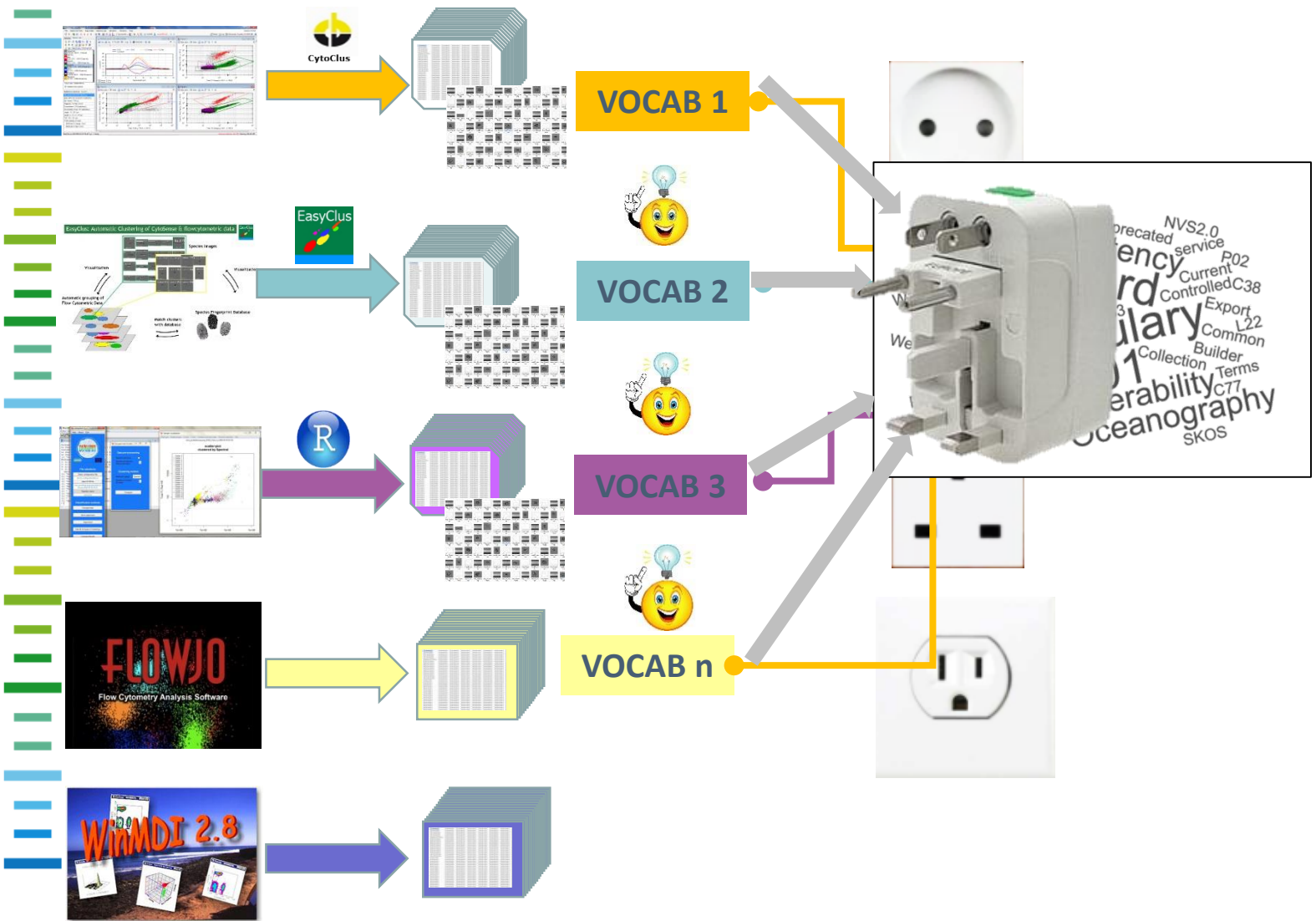
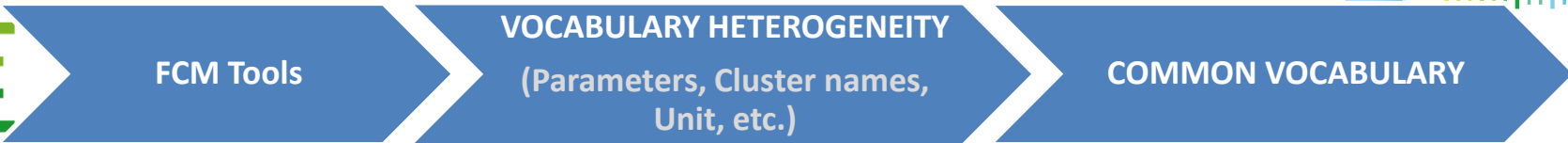
Institut Pythéas  
Observatoire des Sciences de l'Univers  
Aix-Marseille Université



*Third JERICCO-NEXT Workshop on Phytoplankton Automated Observation/ MIO/  
Marseille/ France/ 19th to 21st March 2018*

# Introduction



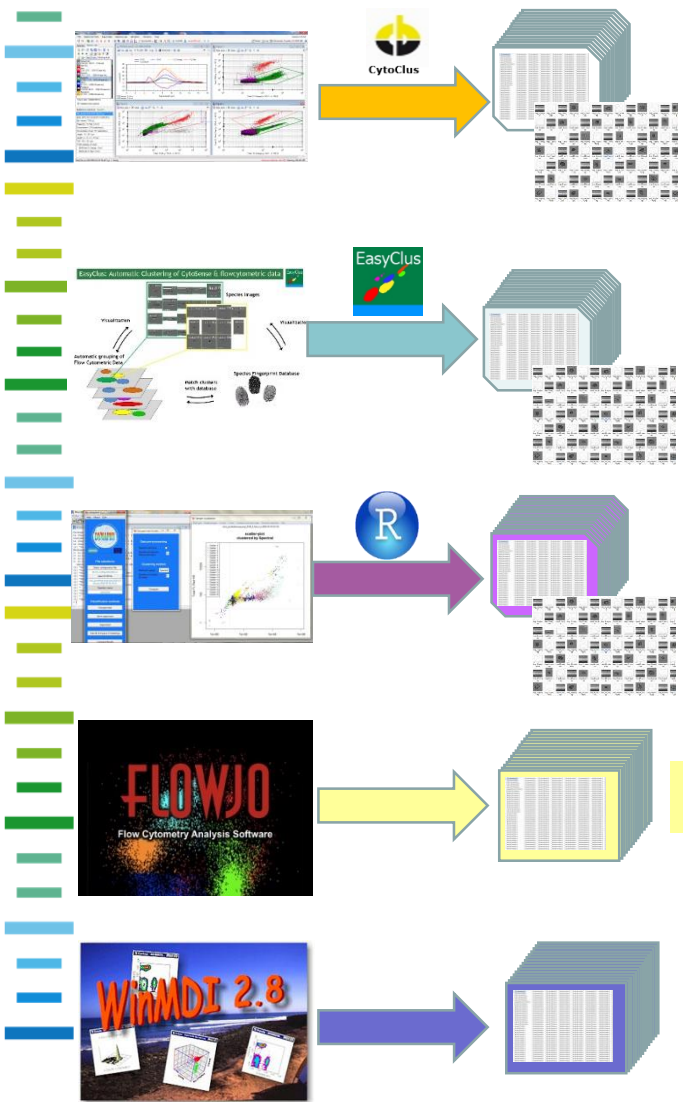




**VOCABULARY HETEROGENEITY**  
(Parameters, Cluster names, Unit, etc.)

**COMMON VOCABULARY**

FCM Tools

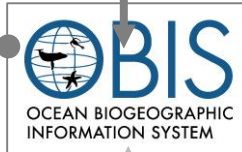
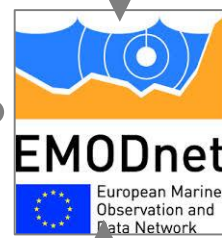


**VOCAB 1**

**VOCAB 2**

**VOCAB 3**

**VOCAB n**



Other ISO Portals



# FCM Common Vocabulary



1. Analysis of the existing FCM Common vocabulary
2. Captured parameters (Cefas, MIO, RWS, ULCO and VLIZ)
3. Setting up FCM Standardized common vocabulary
4. Questionnaire

# 1. Analysis of the existing Common vocabulary

**Cytobuoy Meeting, March 2017** → Parallel session – Harmonisation of flow cytometry use and data (protocol, standardisation, definition of functional types, quality control)

→ 34 FCM codes existed in the BODC VS



P01 (BODC Parameter Usage Vocabulary) -existing vocabulary for flow cytometry

conceptid	prelabel	modified	altlabel	definition	Deprecate Y/N	FCM Community Feedback
PYPKAFB1	Abundance of Bacteria (ITIS: 202421: WoRMS 6) [Subgroup: group PSB1 autotrophic] per unit volume of the water body by flow cytometry	4/17/2016 15:50:07	Bact_PS B1_au	Number of particles resolved as photosynthetic bacteria cells from the uncharacterised cluster PSB1 in a unit volume of any body of fresh or salt water determined by flow cytometry analysis of unstained samples.		Prochlorococcus they don't know what it is. It is not clear we need to separate syno and proclo and give definition for each one: difference by size and pigment.
PYPKAFB2	Abundance of Bacteria (ITIS: 202421: WoRMS 6) [Subgroup: group PSB2 autotrophic] per unit volume of the water body by flow cytometry	4/17/2016 15:50:07	Bact_PS B2_au	Number of particles resolved as photosynthetic bacteria cells from the uncharacterised cluster PSB2 in a unit volume of any body of fresh or salt water determined by flow cytometry analysis of unstained samples.		Synechococcus (1 - 2 um)
P1B318A9	Abundance of Bacteria (ITIS: 202421: WoRMS 6) [Subgroup: heterotrophic; high nucleic acid cell content] per unit volume of the water body by flow cytometry	4/17/2016 15:50:07	Abund_B E006316	Number of particles resolved as heterotrophic bacteria cells from the high nucleic acid content cluster (HNA) in a unit volume of any body of fresh or salt water determined by flow cytometry analysis of samples stained with a nucleic acid-specific fluorescent dye, and subtraction of cyanobacteria cell count if present.		Not concerned
AD107845	Abundance of Dinoflagellates (ITIS: 9873: WoRMS 19542) [Size: <20um Subgroup: autotrophic] per unit volume of the water body by flow cytometry	2/22/2016 14:44:37	ADino<2 Dnm_FC	Number of particles <20um identified as most likely small dinoflagellates in a unit volume of any body of fresh or salt water determined by flow cytometry analysis of unstained samples.		How can you define them by FCM??
P200A0Z	Abundance of Dinoflagellates (ITIS: 9873: WoRMS 19542) per unit volume of the water body by flow cytometry	2/18/2016 15:18:01	P200AD 0Z	This code was updated on 18-feb-2016 so that the name Dinoflagellates is used instead of Pyrrophytophyta in the preferred label field. The vernacular term 'dinoflagellates' maps to Pyrrophytophyta in the ITIS taxonomy and Dinophyceae in the WoRMS taxonomy. In the		not agree and not clear
PU00A02A	Abundance of eukaryote picophytoplankton per unit volume of the water body by flow cytometry	4/17/2016 15:50:07	UndiffEu PicoPhyt Abund	Number of particles resolved as photosynthetic eukaryote cells in the picoplankton size range in a unit volume of any body of fresh or salt water determined by flow cytometry analysis of unstained samples.		not agree and not clear

# 1. Analysis of the existing Common vocabulary

**Cytobuoy Meeting, March 2017** → Parallel session – Harmonisation of flow cytometry use and data (protocol, standardisation, definition of functional types, quality control)

→ 34 FCM codes existed in the BODC VS

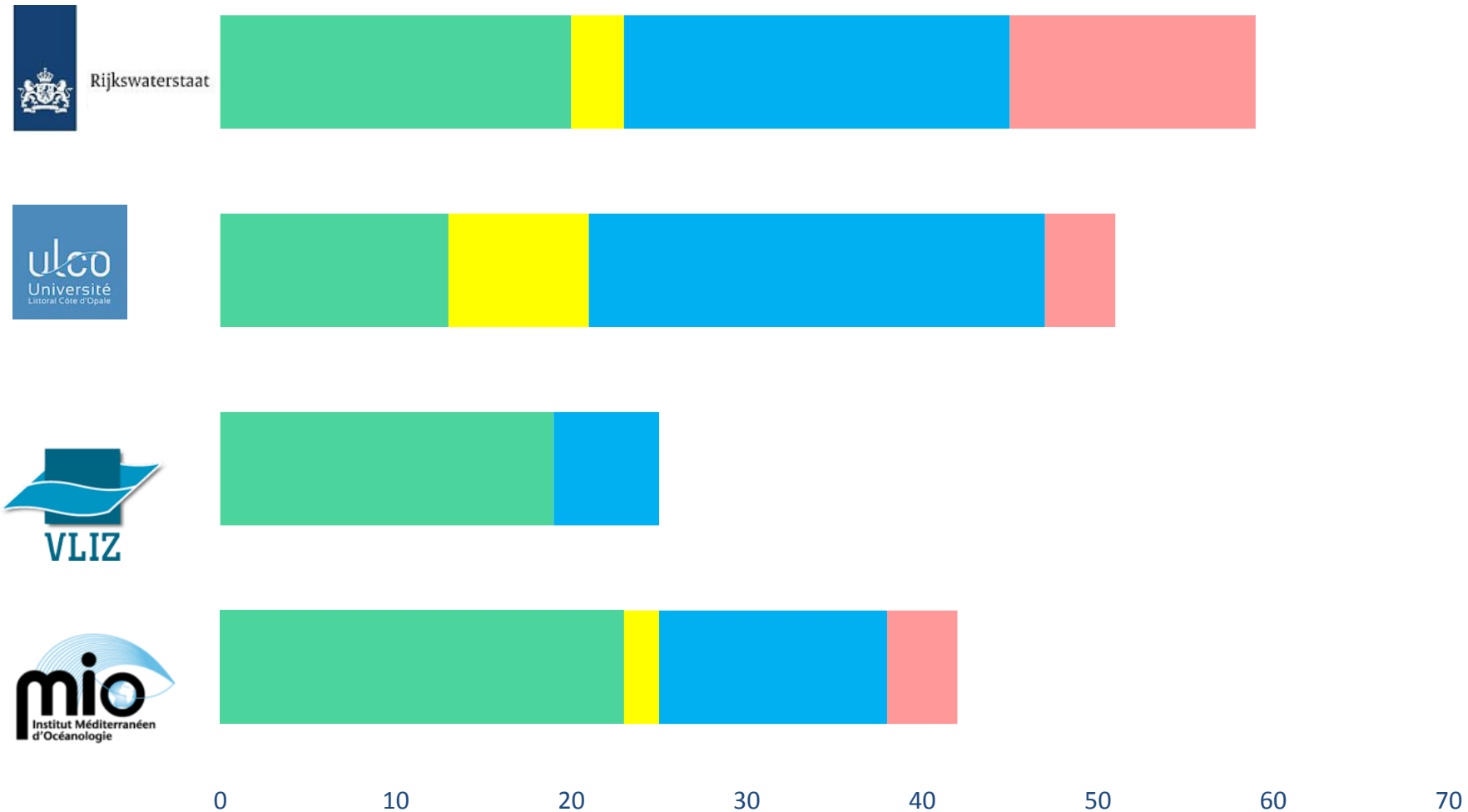


- Some codes are good
- Redundancy
- Definitions are not clear for FCM users and difficult to understand

→ Build a new FCM Common vocabulary based on the measured quantities

## 2. FCM captured parameters

Common Metadata    Unique Metadata    Common Data    Unique data

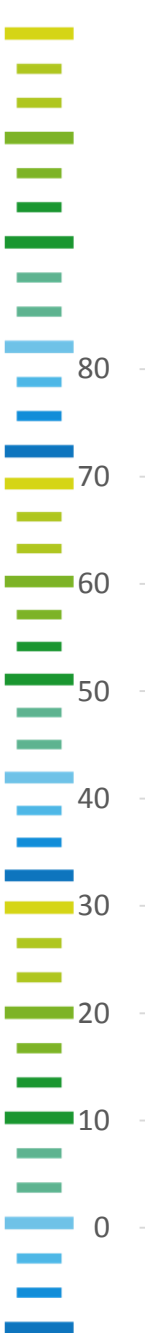




# P01 (BODC PARAMETER USAGE VOCABULARY)

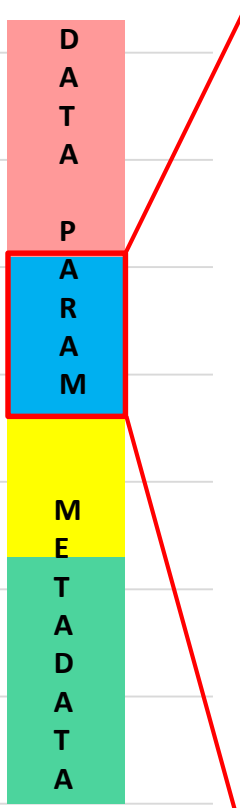


→ 24 Captured Common Data parameters sent to BODC



## 12 Common Param. (Area)

## 12 Common Param. (Height/Max)



- FWS\_average\_area
- FWS\_SD\_area
- SWS\_average\_area
- SWS\_SD\_area
- FLR\_average\_area
- FLR\_SD\_area
- FLO\_average\_area
- FLO\_SD\_area
- FLY\_average\_area
- FLY\_SD\_area
- FLG\_average\_area
- FLG\_SD\_area



- FWS\_average\_height
- FWS\_SD\_height
- FWS\_average\_height
- FWS\_SD\_height
- FLR\_average\_height
- FLR\_SD\_height
- FLO\_average\_height
- FLO\_SD\_height
- FLY\_average\_height
- FLY\_SD\_height
- FLG\_average\_height
- FLG\_SD\_height

# 3. Setting the FCM Standardized Common Vocabulary

- Semantic model (BODC)

Chemical model	Biological model	Physical model
<p>Measurement Substance Measurement matrix relationship Matrix Matrix subcomponent</p>	<p>Measurement Organism Name Organism Specifics Measurement matrix relationship Matrix Matrix subcomponent Method</p>	<p>Measurement Statistical Physical entity Measurement matrix relationship Matrix Method</p>
<p>Concentration of carbon (total inorganic) {TCO<sub>2</sub>} per unit mass of the water body [dissolved plus reactive particulate phase]</p>	<p>Abundance of Bacteria (ITIS: 202421: WoRMS 6) [Subgroup: heterotrophic] per unit volume of the water body by automated flow cytometry</p>	<p>Area mean of Forward light scatter pulse per cluster from the water body by flow cytometry</p>

Area

Mean of

Forward light scatter pulse

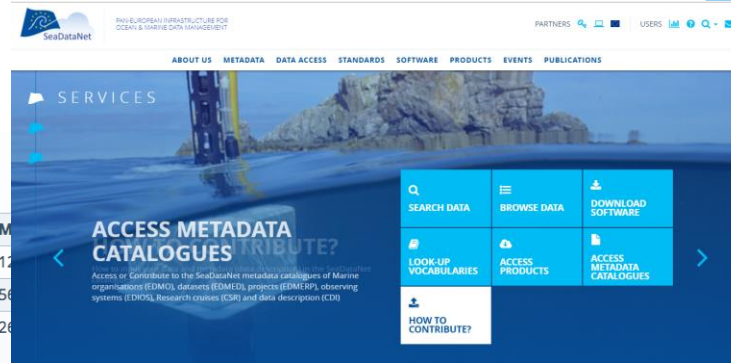
per cluster from the

Water body by flow cytometry

The cluster name is managed in a separate vocabulary list

- FCM Standardized Common Vocabulary

➔ <https://www.seadatanet.org/>



### BODC WEBSERVICES V2 (LIBRARIES) CL12

Library	Thesaurus	Title	Alt Title	Version	M	
C16		SeaDataNet sea areas	SDN sea areas	9	12	
C17		ICES Platform Codes	ICES Platforms	712	56	
C19		SeaVoX salt and fresh water body gazetteer	SeaVoX water bodies	17	26	
C32		International Standards Organisation countries	ISO countries	7	251	1/14/2016 2:00:02 AM
C34		Activity purpose categories	Purpose categories	4	22	8/27/2011 3:00:05 AM
C35		European Nature Information System	EUNIS3 Habitats	1	56	2/19/2010 2:01:37 AM

<b>F02</b>	SeaDataCloud Flow Cytometry Standardised Cluster Names	SDC flow cytometry cluster names	2	11	2/3/2018 2:00:02 AM
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<b>P01</b>	BODC Parameter Usage Vocabulary	BODC PUV	800	37732	3/14/2018 2:00:03 AM
<b>P02</b>	SeaDataNet Parameter Discovery Vocabulary	SeaDataNet PDV	107	435	2/13/2018 2:00:03 AM

<b>L22</b>	SeaVoX Device Catalogue	SeaVoX Device Catalogue	324	1280	3/6/2018 2:00:04 AM
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<b>P06</b>	BODC data storage units	BODC units	99	346	2/16/2018 2:00:02 AM
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## 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		<p>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.</p>	11/20/2017 13:09:10
F0200002	Prochlorococcus		<p>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</p>	11/20/2017 13:09:10



## P01 (BODC PARAMETER USAGE VOCABULARY)

[Overview](#) | 
 [Export subset of list](#) | 
 [Export full list](#) | 
 [New query](#) | 
 Found 2 | 
 Current | 
 Previous | 
 Next

ConceptID ↕	Preferred label ↕	Alt label ↕	Definition ↕	Modified ↕
SCNAME01	Taxon of biological entity specified elsewhere	Name_BE007117	The scientific name of the biological object.	1/21/2016 13:55:16
SNANID01	Identifier (LSID) of biological entity specified elsewhere	LSID_BE007117	A global unique identifier for the nomenclatural details of the scientific name of a biological object (urn:lsid:marinespecies.org:taxname:ID)	1/21/2016 13:55:16



**Example: SCNAME01= *Akashiwo sanguinea* and SNANID01= urn:lsid:marinespecies.org:taxname:232546**

ConceptID ↕	Preferred label ↕	Alt label ↕	Definition ↕	Modified ↕
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
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

**Example: NMCLFL02 = *Eukaryote nanophytoplankton* and IDCLFL02 = SDN:F02::F0200005**

# 3. FCM Questionnaire

- Literature review from 1983 till 2017

**COMMENT**

© 1989 Alan R. Liu, Inc. Cytometry 10:629-635 (1989)

**Flow cytometry and cell sorting: A technique for analysis and sorting of aquatic particles!**

**HETEROGENEITY IN FRAGILITY AND OTHER BIOCHEMICAL AND BIOPHYSICAL PROPERTIES**

**A Simple Method to Preserve Oceanic Phytoplankton for Flow Cytometric Analyses**

**D. Vaulot, C. Courties, and F. Partensky**  
CNRS, Station Biologique, 29211 Roscoff, France

*M. Thysen et al. / Journal of Experimental Marine Biology and Ecology 416 (2011) 95-107*

performed daily at noon in each mesocosm with a HANNA multi-parameter water quality meter (model HI9142B). These measurements showed that the water column was homogeneous during the whole experiment.

Samples for phytoplankton analysis using flow cytometry were collected every 4 h from 14:00 on August 24 to 14:30 on August 29 (sampling times were 2:30, 8:30, 14:30 and 20:30). Collecting data every 6 h is the minimal sampling frequency accepted in order to observe a 12:00 cell cycle (Thysen, 1983), or two cell-division per day, for any of the observed phytoplankton groups, which are commonly observed in natural environments (Bridger and Durand, 2002; Jacquet et al., 2002; Thysen et al., 2008). Samples for nutrient and chlorophyll *a* (chl *a*) analysis were collected once a day at 8:00.

**2.2. Flow cytometry**

Samples were collected using 1 dm<sup>3</sup> dark containers and directly transferred in 12 cm<sup>3</sup> vials for the EPICS<sup>®</sup> analysis, and 5 cm<sup>3</sup> vials for the APCX<sup>®</sup> ALTRA flow cytometer analysis, both perfilled with glutaraldehyde (0.18% final concentration). The samples were immediately stored at -80 °C (shorter than a month). Flow cytometry analyses were conducted using two different types of instruments in order to achieve accurate estimations of cell counts from the smallest phytoplankton to the largest microzooplankton, and to collect cellular information using their light scattering properties (forward light scatter (FWS) and sideward scatter (SWS)) and their auto fluorescence properties (red fluorescence from chlorophyll (FLR) and orange fluorescence from phycoerythrin (FLO)). The phytoplankton cells (Pico, diameter < 2 μm) and the smallest heterotrophic bacteria (Nano, < 2 μm) were analysed using an Epics Altra flow cytometer (Beckman Coulter) equipped with a 488 nm laser operated at 15 mW. Samples were thawed at room temperature and analysed immediately. Fluorescent beads (100 nmol/litre of fluorescein isothiocyanate (FITC) and 10 μmol/litre of phycoerythrin (PE)) were automatically added to each sample as an internal standard, in order to normalise the fluorescence emission and light scatter signals obtained from the Epics Altra flow cytometer. Abundance estimations were derived from the cell counts and the corresponding analysis algorithms defined by the acquisition time and sample flow rate. The flow rate was obtained from weighing the vials before and after analysis and dividing the mass uptake by the sample density. Size was estimated by analysing bead suspensions of different bead sizes and determining the relationship between size and forward scatter (Venkatasubramanian et al., 2006). The FLR (0.75–10 nm) and the FWS of the cells were recorded as the signal peak, thus giving little information on their shape, although the instrument is able to analyse the time of flight which gives an indication of their length. FLR and FWS peak values from the EPICS ALTRA are further defined as FLR<sub>0</sub> and FWS<sub>0</sub>.

Cells larger than 2 μm were analysed using a CytoSense flow cytometer from CytoBuoy Inc. equipped with a 488 nm laser operated at 15 mW. The pulse shape of FLR (668–734 nm), FLO (501–668 nm) and the FWS signals from the cells were recorded, allowing complex cells to be differentiated and chain-forming cells to be accounted for. Integrated values of the CytoSense FLR and FWS signals are further defined as FLR<sub>int</sub> and FWS<sub>int</sub>. Abundance was directly estimated from the analysis of the samples through a stable peristaltic pump, routinely tested by using bead suspensions of known concentration. Heterotrophic polymeric beads (Intevigorin), namely 2 μm red fluorescent and 10 μm orange fluorescent beads, were used as an internal standard to normalise scatter and fluorescence signals. Test protocols were used to optimise the abundance estimation of the small and large cells respectively. Cells < 10 μm were analysed with a peristaltic pump speed of 1.08 cm<sup>3</sup> min<sup>-1</sup> and a trigger level of 10% on FWS. Cells > 10 μm were analysed with a peristaltic pump speed of 1.08 cm<sup>3</sup> min<sup>-1</sup> and a trigger level of 10% on FWS.

**2.3. Chlorophyll *a* and nutrient analysis**

Chlorophyll *a* (chl *a*) content was determined by High Performance Liquid Chromatography (HPLC). A volume of 400–600 cm<sup>3</sup> was filtered onto a 25 mm Whatman G17 filter. Filters were stored at -80 °C. Pigments were then extracted and analysed by HPLC after Zupat et al. (2000). Nitrate + nitrite (NO<sub>3</sub><sup>-</sup> + NO<sub>2</sub><sup>-</sup>), phosphate (PO<sub>4</sub><sup>3-</sup>) and silicic acid (Si(OH)<sub>4</sub>) concentrations were determined from 25 cm<sup>3</sup> pre-combusted G17 filtered seawater samples collected (40 cm<sup>3</sup> triplicate) from each mesocosm at 8:00 am. An equal sample volume was discarded prior to storing the sample in plastic acid cleaned 100 cm<sup>3</sup> bottles, kept frozen at -20 °C until analysis at EMER within 1 month, using a Bran Luebbe AutoAnalyzer 3 system based on the method by Grasshof et al. (1983).

**2.4. Statistical analysis**

Statistical analyses were run under R software (<http://cran.r-project.org/>). For each phytoplankton cluster, abundance, average FWS<sub>int</sub> and FLO<sub>int</sub> were calculated and values per cell were calculated. In order to identify difference between treatments during 3 different stages of phytoplankton development, a set of statistical analysis was run. For each defined phytoplankton stage, a normality test (Shapiro test) followed by a test of sphericity (Mauchly test) was run in order to define the best variance test. When data followed a normal distribution and sphericity was observed, a RM-ANOVA (repeated measures) was used. When normality was validated but not sphericity, or when normality was not validated, a Friedman rank test was run. Relative phytoplankton average abundances, relative average FWS<sub>int</sub> and FLO<sub>int</sub> average values were calculated to show the differences between NUV1V (control) and the treated mesocosms (HN1V, N1V1V and H1V1V) during the 3 different stages of the phytoplankton development, while considering the respective NUV1V and N1V1V running post-hoc tests for each cluster and each phytoplankton stage, would lead to complex interpretations. Significant differences were identified using a paired Wilcoxon signed-rank test. Periodic processes in the dynamics of abundance, average FWS<sub>int</sub> and FLO<sub>int</sub> were verified using comparing periodograms with a fast Fourier transformation smoothing the results with a series of modified Daniell smoothers (moving averages giving half weight to the end values, Dunnett, 1966), generating spectral plots. These algorithms were compared on the average values between duplicate.

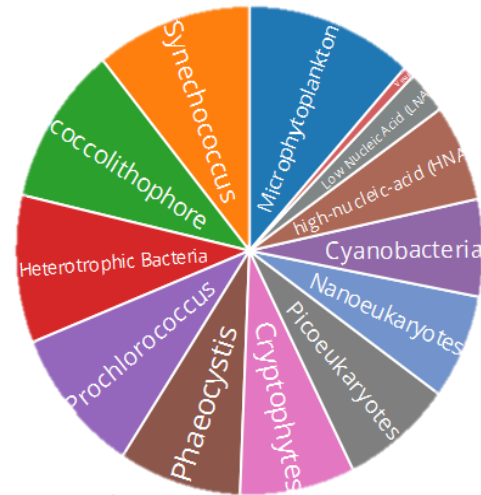
**3. Results**

**3.1. INR temperature, nitrate, chlorophyll *a* and nutrient concentrations**

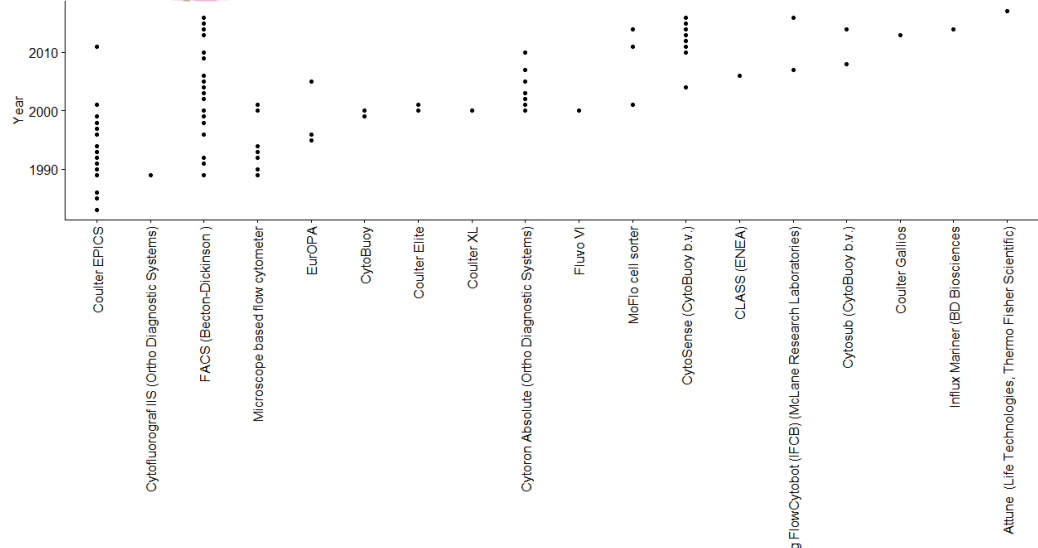
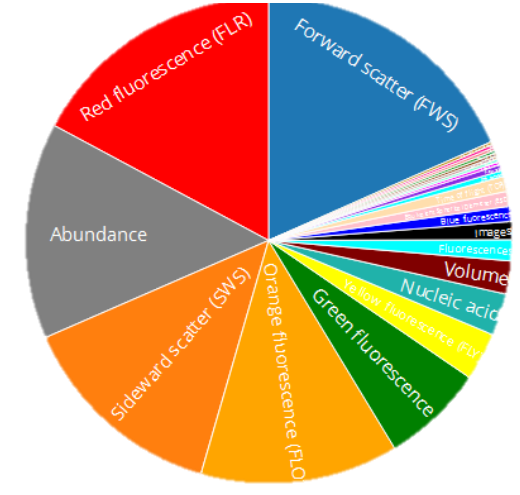
The photic depth (*Z*<sub>ph</sub>, 10% of surface incident light) represents the depth at which INR has significant biological effects (Neale et al., 2003). *Z*<sub>ph</sub> reached depths between 27 and 57 cm and between 26 and 36 cm for radiations at 305 nm and 313 nm, respectively (Fig. 1A, B). Fig. 1C and 1D shows the 305 nm and 313 nm average irradiances in the water column from surface to *Z*<sub>ph</sub>, calculated according to Madhuprat and Galois (1996). Average water column INR irradiance increase in the INR mesocosms were 77.4 ± 10.7% and 45.4 ± 14.8% for 305 and 313 nm, respectively (Fig. 1C, D), as compared to NUV1V treatments.

The initial temperature in all the mesocosms was -13 °C and was increased by 2 °C from day 2 to day 4. At day 4, temperature stabilised at +15 °C in the normal temperature and at -18 °C in the high temperature treatment mesocosms on day 5 (Fig. 2A). Salinity values varied between 24.14 in HN1V on day 6 and 25.19 in H1V1V on day 1 (data not shown). Chlorophyll concentrations increased from day 1 up to day 5 in HN1V and N1V1V mesocosms (mean value of 0.94 ± 0.16 and 0.9 ± 0.16 mg chl *a* m<sup>-3</sup>, respectively).

## Clusters



## Measured parameters



## Instruments

## Flow Cytometry vocabulary standardization Questionnaire

This flow cytometry vocabulary standardization questionnaire is dedicated to identify your metadata and data vocabulary that you use during your measurements. it will take approximately 10 to 15 minutes to complete.

This questionnaire is carried out within the framework of JERICO NEXT and SeaDataCloud (H2020 projects) so as to build a common vocabulary in order to standardize, validate and guarantee a long-term storage and access of flow cytometry datasets.

It is divided into four main parts:

- Part I : Group name and definition
- Part II : Flow Cytometer Metadata
- Part III : Sample Metadata
- Part IV : Flow Cytometer Data

*There are 56 questions in this survey.*

Load unfinished survey

Next ▶

Exit and clear survey

- Questionnaire sent to 180 FCM users all around the world

38 Answers to questionnaire from Oct. 30, 2017 to Jan. 15th 2018



**Uncompleted answers (21%)**

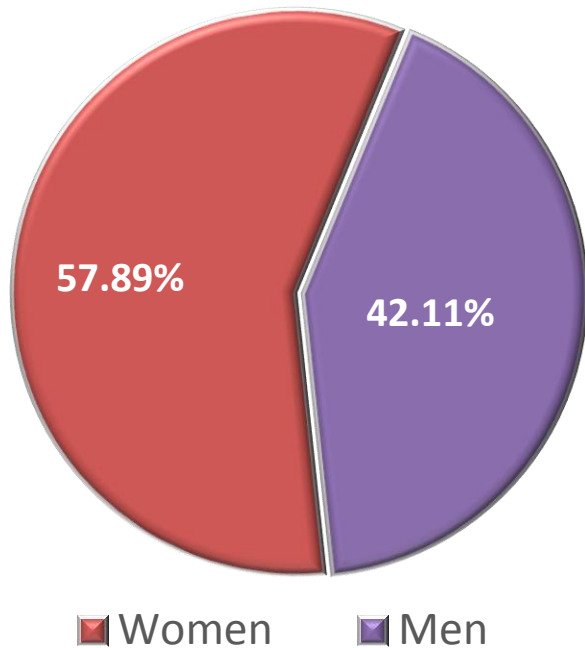


**Completed answers (79%)**

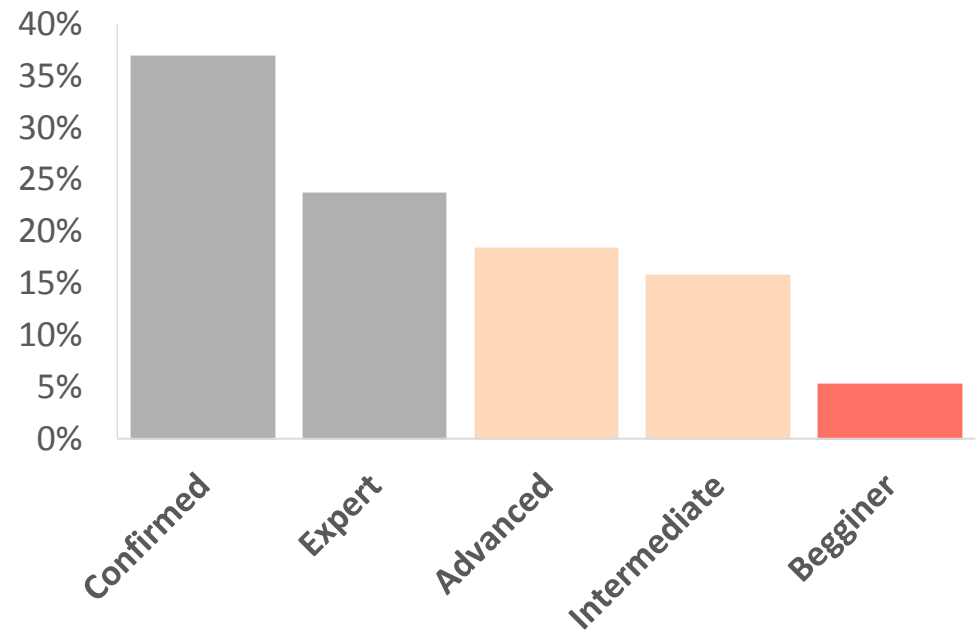
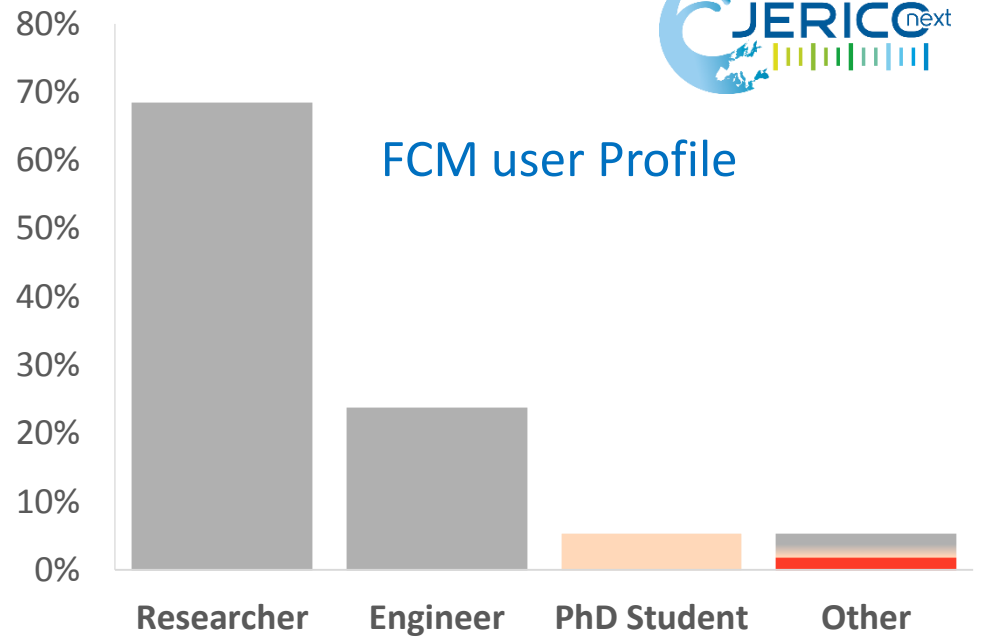




### Gender participation



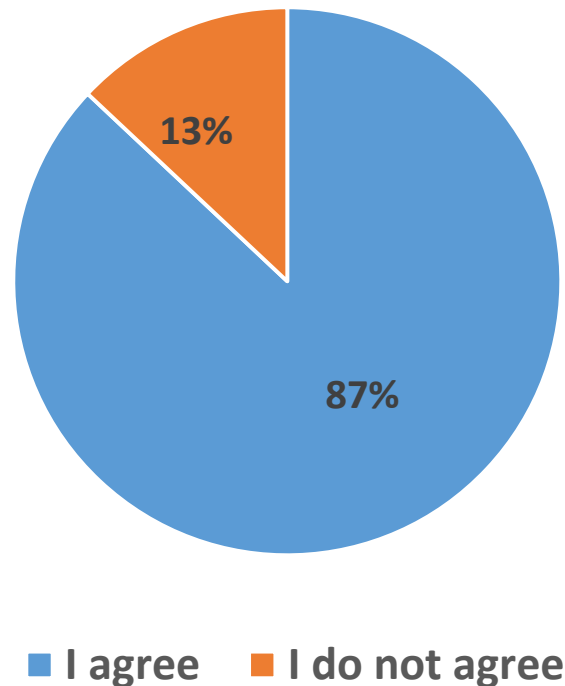
### FCM user Profile



## PART I: Groups definition from the FCM point of view

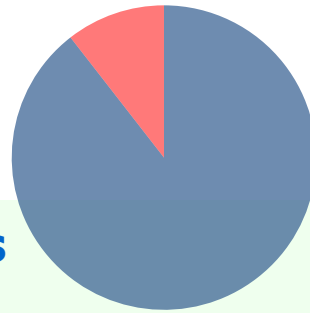
**Q: Based on literature from 1983 to 2017, do you agree on these group definitions:**

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





## Suggestion



■ I agree (90%)

■ I do not agree (10%)

### \* Prochlorococcus

Prochlorococcus are defined as the smallest cyanobacteria found in marine environment. No staining is required to distinguish them by flow cytometry. 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.

### \* Prochlorococcus

Prochlorococcus are defined as the smallest cyanobacteria found in marine environment. No staining is required to distinguish them by flow cytometry. FWS and FLR signatures are the smallest recorded **for any photosynthetic organisms** 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 **NOT masked by the instrument background noise. When the cluster is not well defined, that is when it is partially masked by the noise.**

In samples stained for Heterotrophic bacteria analysis, Prochlorococcus can be distinguished, **particularly deep communities (but not surface communities),** using Sideward Scatter (SWS) vs Chlorophyll Red Fluorescences (FLR) cytogram. They do not emit orange fluorescence because they lack phycoerythrin.

#### NOTE:

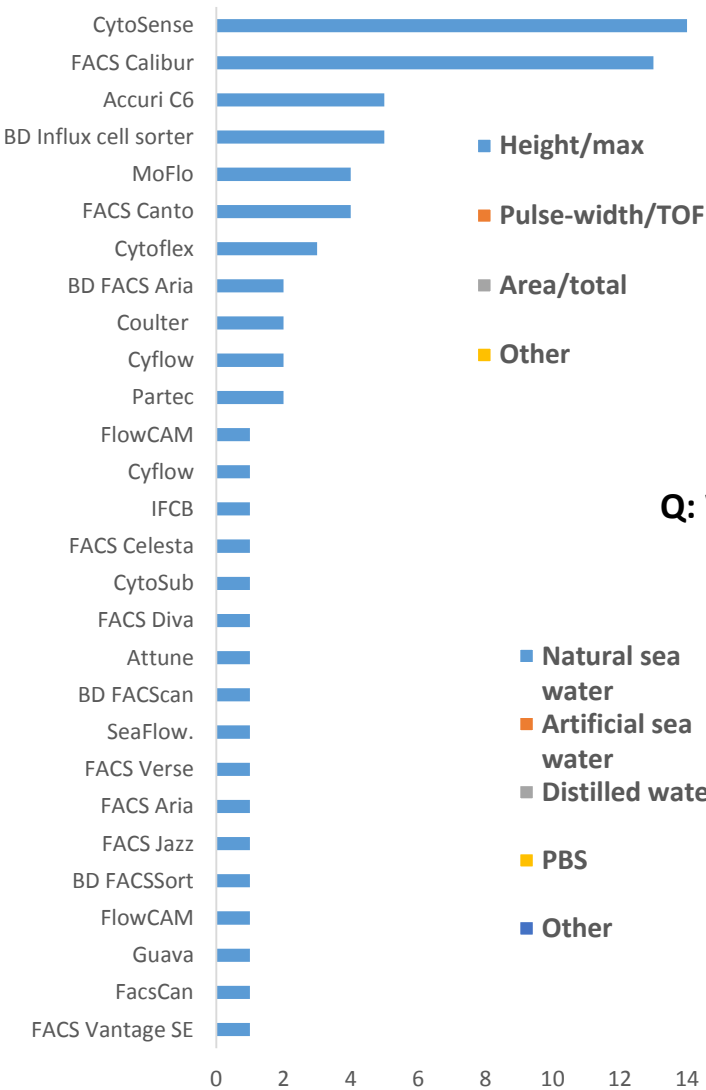
- *often the background noise is mostly NOT due to the instrument. It is due to heterotrophic bacteria, if the instrument is a good one.*



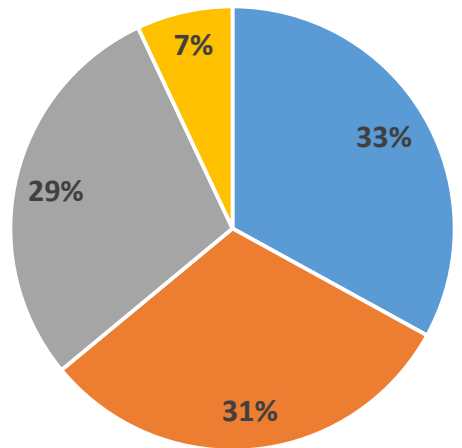
## PART II: Flow Cytometer Metadata



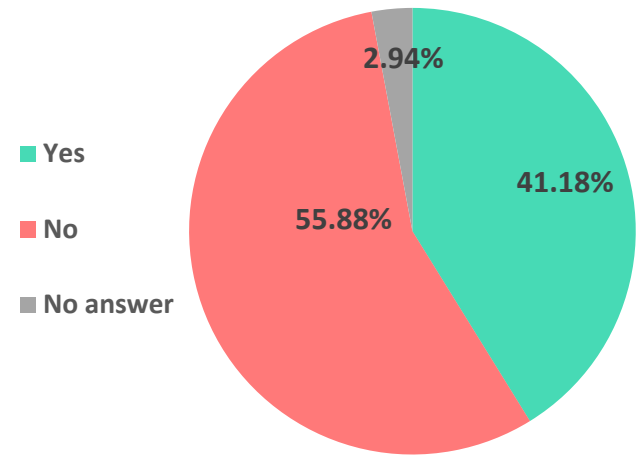
**Q: What model of Flow Cytometer(s) do you use?**



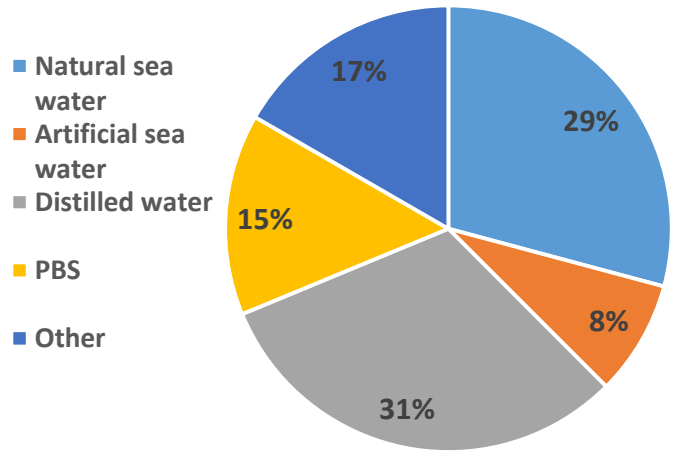
**Q: What type of signal does your instrument record ?**



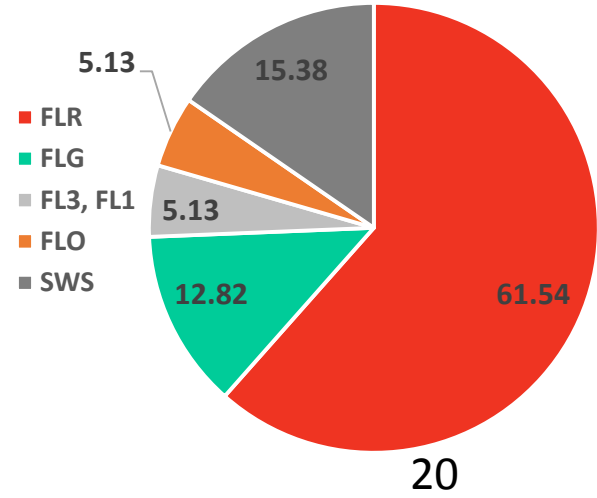
**Q: Does your instrument have an image in flow device?**



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



**Q: What signal do you use as trigger?**



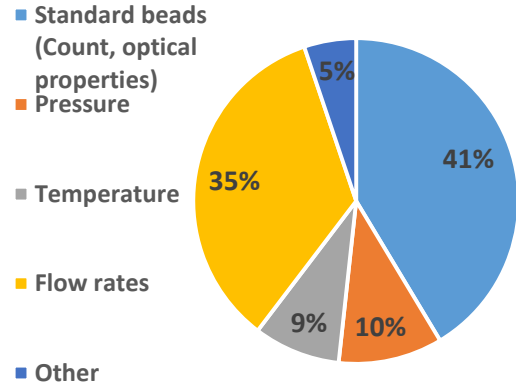




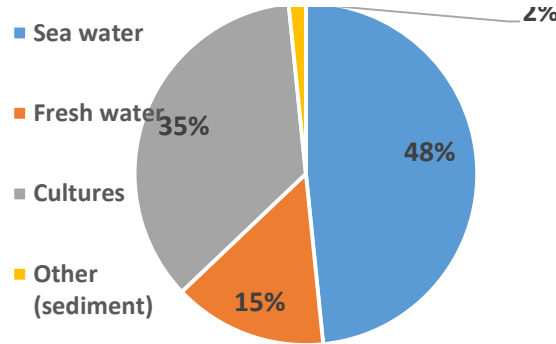
# PART III: Sample Metadata



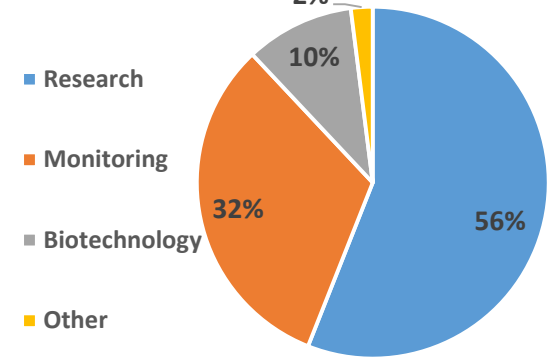
### Q: How do you make the quality control



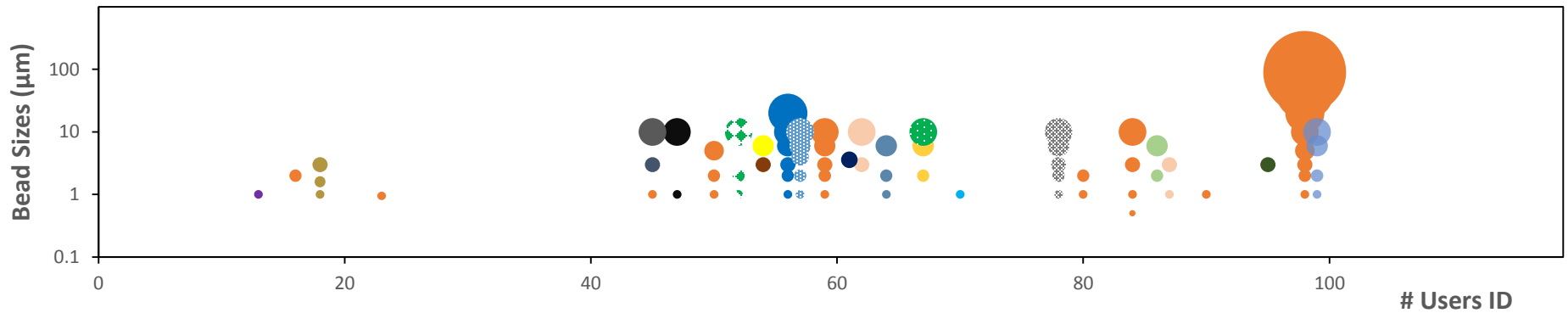
### Q: What type of sample do you analyze?



### Q: For what purpose do you use this instrument?



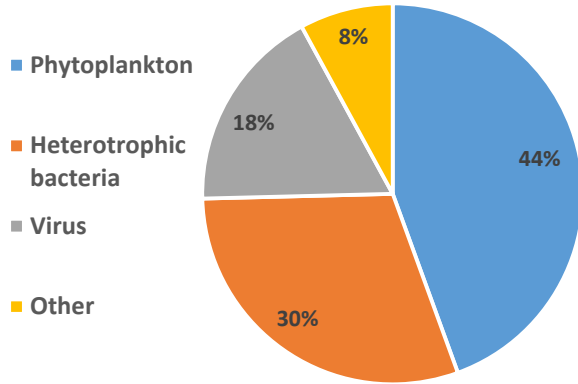
### Q: What beads and diameters reference do you use ?



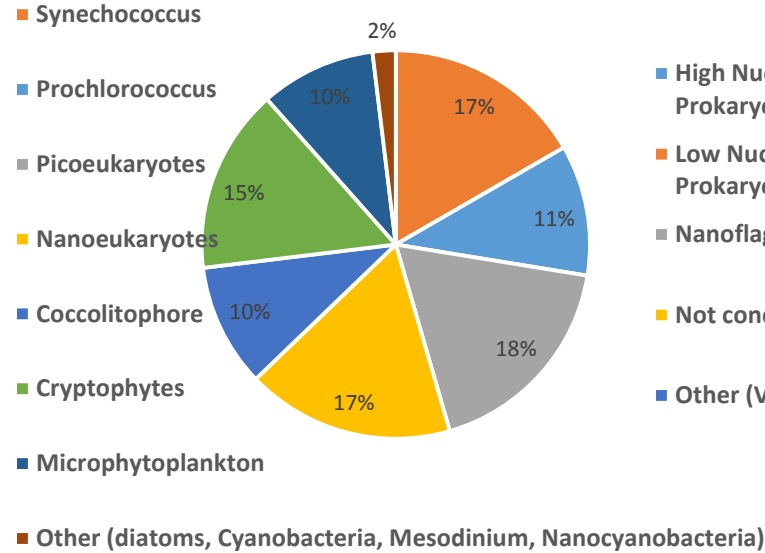
- Polyscience
- Calibration beads
- Flow Check™ High Intensity
- Fluorosphere
- Invitrogen Fluospheres
- Polysciences, fluoresbrite YG microsphere
- Supplied by CytoBuoy
- ThermoFisher
- CountBrights
- Fluoresbrite Carboxy
- FluoSpheres™ Polystyrene Microspheres
- Molecular Probes
- Polystyrene microsphere
- ThermoFisher fluosphere
- Beckman Coulter Flowset
- Flow check high intensity beads
- Fluorescence
- FluoSpheres™ Sulfate Microspheres
- Polysciences polybeads
- Submicron beads
- Trucount Becton Dickinson

## PART III: Sample Metadata

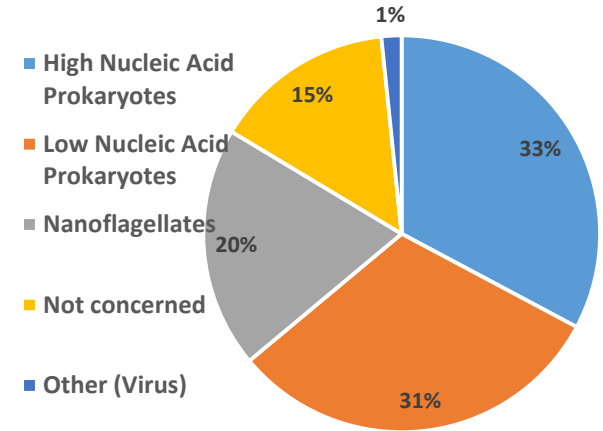
**Q: Which type of particles do you measure?**



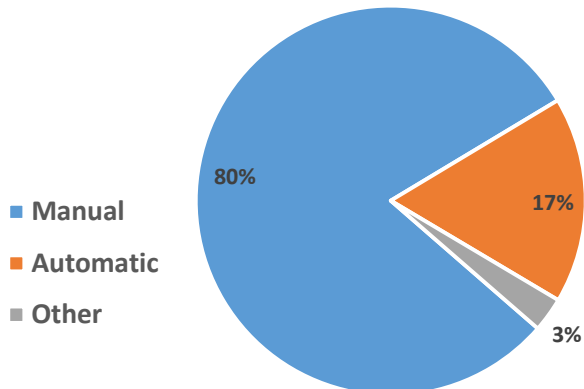
**Q: What are the recurrent autotrophic functional groups of your area of study?**



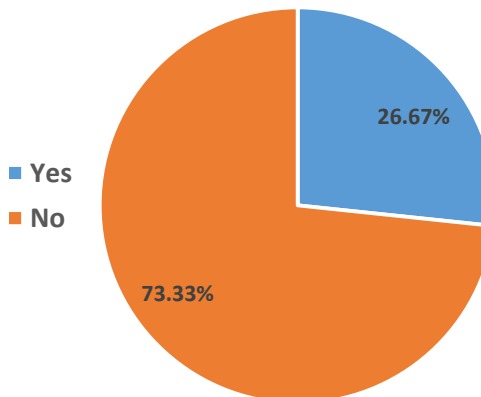
**Q: What are the recurrent Heterotrophic groups of your area of study?**



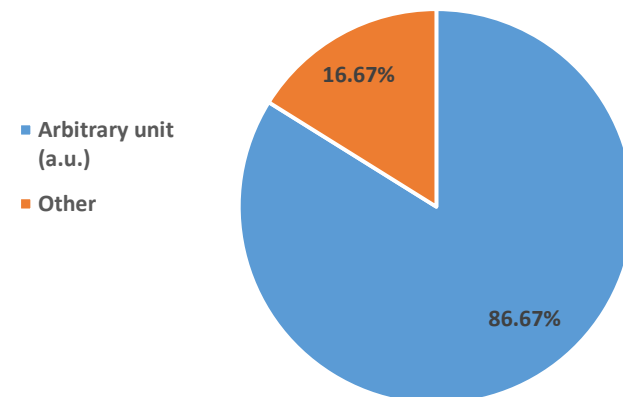
**Q: Which clustering method do you use?**



**Q: Do you flag your data ?**



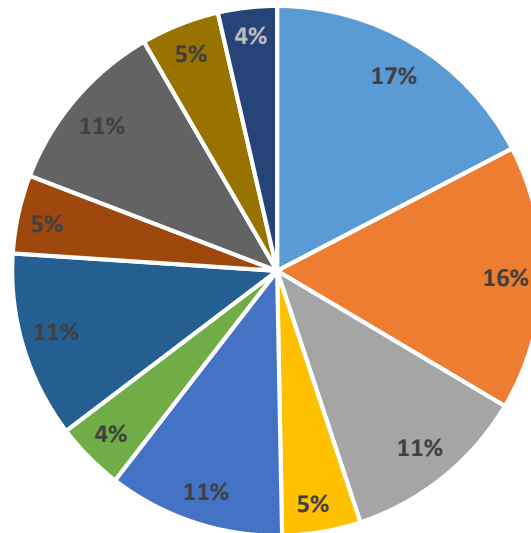
**Q: What is the unit used for scatters and fluorescences ?**



## PART III: Sample Metadata



Q: Which parameters do you export after your clustering?



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

# First FCM ingestion to SDN



[http://seadatanet.maris2.nl/v\\_cdi\\_v3/search.asp](http://seadatanet.maris2.nl/v_cdi_v3/search.asp)

The screenshot displays the SeaDataNet search interface. At the top, it features the SeaDataNet logo and the text 'PAN-EUROPEAN INFRASTRUCTURE FOR OCEAN & MARINE DATA MANAGEMENT' and 'SEADATANET COMMON DATA INDEX (CDI) V3'. The main area contains a world map and a search section. The search section includes a search bar with 'flow cytometry' entered, a 'SEARCH' button, and a dropdown menu showing 'flow cytometers' selected. Other search criteria include 'Cruise/Station name', 'Projectname', 'Datasetname', 'Instrument type', and 'Instrument depth (m)'. The 'Instrument type' dropdown is open, showing 'elemental analysers', 'expendable CTDs', 'Fish-finder echosounders', and 'flow cytometers'. A red arrow points to the 'flow cytometers' option in the dropdown.

# First FCM ingestion to SDN



[http://seadatanet.maris2.nl/v\\_cdi\\_v3/search.asp](http://seadatanet.maris2.nl/v_cdi_v3/search.asp)

SeaDataNet logo and PAN-EUROPEAN INFRASTRUCTURE FOR OCEAN & MARINE DATA MANAGEMENT. SEADATANET COMMON DATA INDEX (CDI) V3.

TOOLS: ENLARGE, HELP, POSITION, INDEX, Datasets (0), BASKET, RESET.

LAYER CONTROL: CDI entry Points, CDI entry Tracks, CDI entry Areas, Grid Lines, Regional sea, Regional sea labels. Display all selected records, Only selected records in results list.

LISTING RESULTS: 20, 100, 1000 records.

ADD TO BASKET | TIMESERIES ON | SUMMARY | ZOOM TO SELECTED | EXPORT RESULT | STORE QUERY | Refine query | New query | Found 2 | Current | Previous | Next

#	Data set name	DC country	Start date	Disciplines - Topics	Instrument / gear type	Show
1	OSCAHR_FCMW	France	20151030	Biological oceanography > Other biological measurements	flow cytometers	Show
2	CHROME_MARS2016_FCMW	France	20160324	Biological oceanography > Other biological measurements	flow cytometers	Show

DOWNLOAD

SeaDataNet logo and PAN-EUROPEAN INFRASTRUCTURE FOR OCEAN & MARINE DATA MANAGEMENT. Operated in cooperation with Black Sea SCENE, EMODnet.

User: s130e05

Downloads

Zipfile name	Download	Remove from this list, once download complete
users20e04-data_centre3078-2016-03-05_result.zip	Download	Today ZIP file can not be removed to avoid technical issues.

Visualizations

Approved requests for data sets are combined in zip files that you can download. These are prepared by the local data centre, that is responsible for managing and distributing the selected data sets. The zip file builds up during the day and each new approved request is added.

OCEAN DATA VIEW ASCII FORMAT

```

//<subject>SDN:LOCAL:vol_ech</subject><object>SDN:P01::VOLWBSMP</object><units>
SDN:P06::MCUB</units><instrument>SDN:L22::TOOL1209</instrument>
//<subject>SDN:LOCAL:sdn_ClusterName</subject><object>SDN:P01::NMCLFL02</object>
><units>SDN:P06::UUUU</units><instrument>SDN:L22::TOOL1209</instrument>
//<subject>SDN:LOCAL:abundance</subject><object>SDN:P01::SDBIOL01</object><units>
>SDN:P06::NCM3</units><instrument>SDN:L22::TOOL1209</instrument>
//<subject>SDN:LOCAL:moy_tot_SWS</subject><object>SDN:P01::SWSAREAA</object><
units>SDN:P06::USPC</units><instrument>SDN:L22::TOOL1209</instrument>

```

```

//<subject>SDN:LOCAL:sd_tot_FLO</subject><object>SDN:P01:FLOARESD</object><units>SDN:P06:USPC</units><instrument>SDN:L22:TOOL1209</instrument>
//<subject>SDN:LOCAL:moy_tot_FWS</subject><object>SDN:P01:FWSAREAA</object><units>SDN:P06:USPC</units><instrument>SDN:L22:TOOL1209</instrument>
//<subject>SDN:LOCAL:sd_tot_FWS</subject><object>SDN:P01:FWSARESD</object><units>SDN:P06:USPC</units><instrument>SDN:L22:TOOL1209</instrument>
//<subject>SDN:LOCAL:moy_tot_SWS</subject><object>SDN:P01:SWSAREAA</object><units>SDN:P06:USPC</units><instrument>SDN:L22:TOOL1209</instrument>
//<subject>SDN:LOCAL:sd_tot_SWS</subject><object>SDN:P01:SWSARESD</object><units>SDN:P06:USPC</units><instrument>SDN:L22:TOOL1209</instrument>

```

Cruise	Station	Type	YYYY-MM	Longitude [E]	Latitude [de]	LOCAL_CD	EDMO_coc	Bot. Depth	DEPTH [m]	GV:SEADA	time_ISO86	GV:SEADA	vol_ech [m]	GV:SEADA	sdn_Cluster	GV:SEADA	sdn_Cluster	GV:SEADA	abundance	GV:SEADA	moy_tot_F	GV:SEADA	FLR_TOTA	GV:SEADA	moy_tot									
CHROME_I S1	S1	C	2016-03-24	5.26124	43.2507	FA880320	3078	0	6	1	2016-03-24	1	0.376328	1	Eukaryote p	1	SDN:F02:F	1	87.63	1	1224157	1	6334.2	1	247.434									
									6	1	2016-03-24	1	0.376328	1	Synechococ	1	SDN:F02:F	1	21194.4	1	364.505	1	540.018	1	1207.7									
									6	1	2016-03-24	1	0.376328	1	Prochloroc	1	SDN:F02:F	1	3437.06	1	39.4288	1	18.0969	1	27.705									
									6	1	2016-03-24	1	4.21779	1	Cryptophyt	1	SDN:F02:F	1	741.38	1	24594.84	1	17783.15	1	10133.7									
									6	1	2016-03-24	1	4.21779	1	Microphyto	1	SDN:F02:F	1	65.67	1	123771	1	151218	1	20281.									
									6	1	2016-03-24	1	4.21779	1	Coccolitho	1	SDN:F02:F	1	7.35	1	43755.3	1	15892.1	1	24191.									
									6	1	2016-03-24	1	4.21779	1	Eukaryote n	1	SDN:F02:F	1	2970.52	1	53946.7	1	21944.63	1	618.44									
									6	1	2016-03-24	1	0.402186	1	Prochloroc	1	SDN:F02:F	1	6069.34	1	42.2521	1	17.3289	1	28.887									
									6	1	2016-03-24	1	0.402186	1	Synechococ	1	SDN:F02:F	1	28735.5	1	876.007	1	492.296	1	1045.7									
									6	1	2016-03-24	1	0.402186	1	Eukaryote p	1	SDN:F02:F	1	42.27	1	10255.1	1	8190.031	1	254.343									
									6	1	2016-03-24	1	0.402186	1	Standard bc	1	SDN:F02:F	1	2.49	1	21157.3	1	3.7525	1	38109.									
									6	1	2016-03-24	1	4.16354	1	Cryptophyt	1	SDN:F02:F	1	526.44	1	25208.31	1	16832.4	1	3793.14									
									6	1	2016-03-24	1	4.16354	1	Microphyto	1	SDN:F02:F	1	20.63	1	145903	1	180243	1	10625.									
									6	1	2016-03-24	1	4.16354	1	Coccolitho	1	SDN:F02:F	1	8.39	1	42357.2	1	17269.2	1	27263.									
									6	1	2016-03-24	1	4.16354	1	Eukaryote n	1	SDN:F02:F	1	2738.19	1	51546.6	1	19162.42	1	615.48									
									CHROME_I S2	S2	C	2016-03-24	5.37174	43.092	FA880320	3078	0	6	1	2016-03-24	1	0.377151	1	Prochloroc	1	SDN:F02:F	1	7288.88	1	43.0467	1	17.4221	1	28.38
																		6	1	2016-03-24	1	0.377151	1	Eukaryote p	1	SDN:F02:F	1	76.89	1	12170.35	1	6714.12	1	263.97
																		6	1	2016-03-24	1	0.377151	1	Synechococ	1	SDN:F02:F	1	21866.3	1	902.762	1	488.755	1	1066.3
CHROME_I S3	S3	C	2016-03-24	5.4981	42.9156	FA880320	3078	0	6	1	2016-03-24	1	3.77561	1	Cryptophyt	1	SDN:F02:F	1	691.82	1	25864.58	1	18194.95	1	9524.25									
									6	1	2016-03-24	1	3.77561	1	Microphyto	1	SDN:F02:F	1	20.13	1	108890	1	107858	1	46748.									
									6	1	2016-03-24	1	3.77561	1	Coccolitho	1	SDN:F02:F	1	10.06	1	44887.5	1	21092.5	1	23305.									
									6	1	2016-03-24	1	3.77561	1	Eukaryote n	1	SDN:F02:F	1	2629.51	1	41808.22	1	17680.85	1	5110.									
									6	1	2016-03-24	1	0.324117	1	Prochloroc	1	SDN:F02:F	1	16694.6	1	40.9643	1	20.7749	1	29.830									
									6	1	2016-03-24	1	0.324117	1	Eukaryote p	1	SDN:F02:F	1	1317.43	1	8307.87	1	5209.686	1	228.773									

FA88032016\_00001\_FCMW\_20180302\_



users\30e06-data\_centre\3078-2018-03-08\_result.zip  
Archive WinRAR ZIP

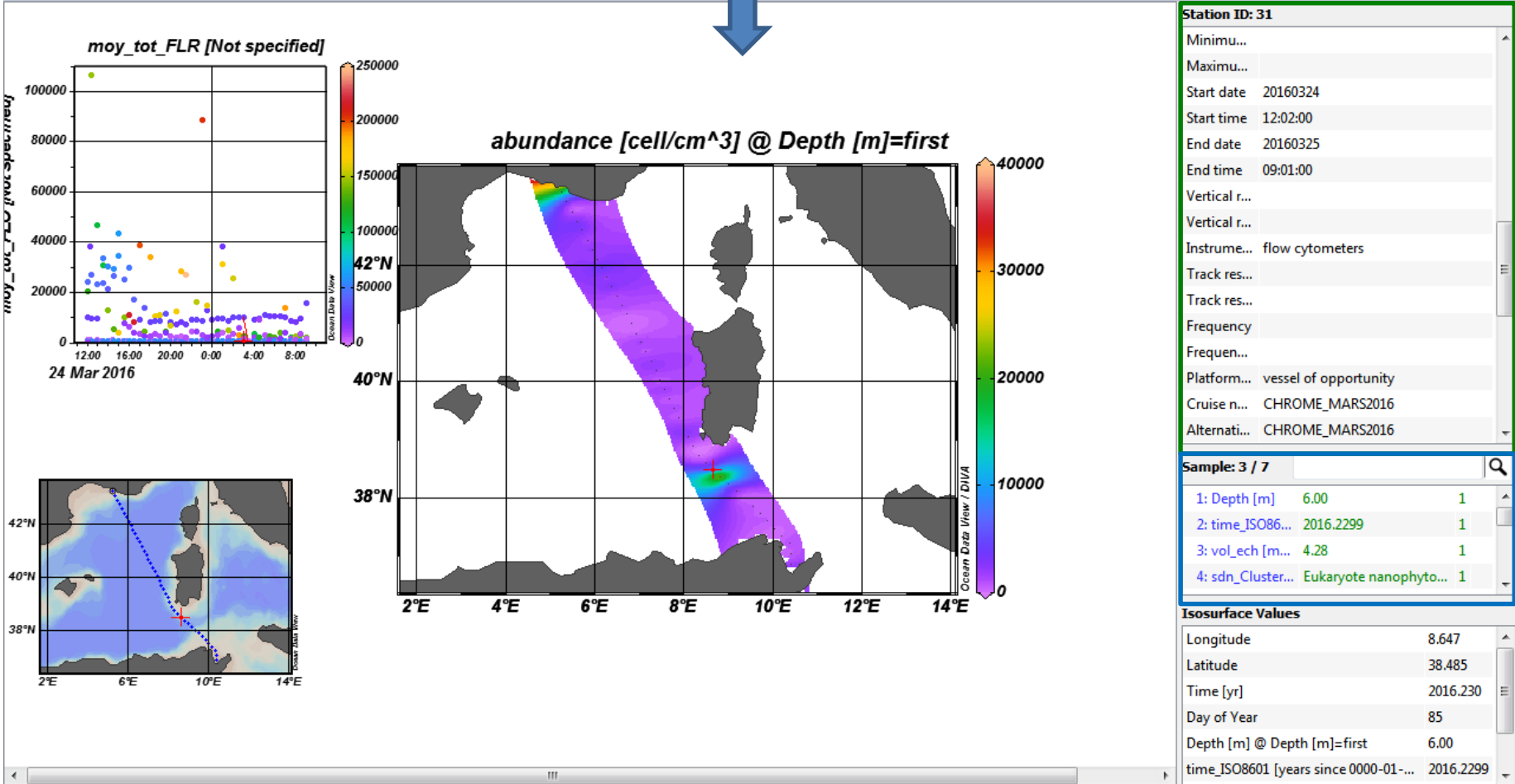
Ocean Data View

<https://odv.awi.de>

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Ocean Data View - C:\Users\lahbib\Documents\ODV\data\sdn\2018-03-09T09-48-04\ocean\_depth\_profiles

File Collection View Import Export Tools Help



# Conclusion

- Whatever the instrument used → Common Vocabulary (CV)
- We created 44 FCM CV → European portals
- Decide on a group of experts interested in contributing to the vocabulary work and decide on a co-ordinator
- Update is possible/The BODC Vocabulary Editor webpage: [https://www.bodc.ac.uk/resources/vocabularies/vocabulary\\_editor/](https://www.bodc.ac.uk/resources/vocabularies/vocabulary_editor/)
- BODC is setting up some repositories on GitHub for each individual collection and F02 will have its own too. So this could be used to share and discuss issues more widely.
- Valuable information are gathered from the questionnaire
- Set up a Quality control protocol for FCM data (70% no flag)



“Needs for standardisation ,  
Needs for automated clustering ”

*Thank you for your attention*





This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 654410.