



# SeaDataCloud

Progress made on standardization  
of flow Cytometry data

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Gwena lle Moncoiff  (BODC) and Lennert Tyberghein (VLIZ)

Plenary Meeting, Athens, 18th October 2017

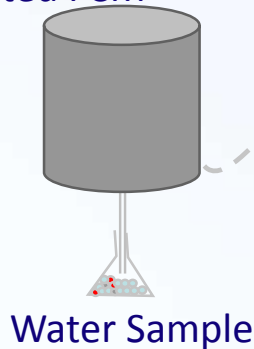
[sdn-userdesk@seadatanet.org](mailto:sdn-userdesk@seadatanet.org) – [www.seadatanet.org](http://www.seadatanet.org)

# 1. What is Flow Cytometry (FCM) Data ?

Benchtop FCM



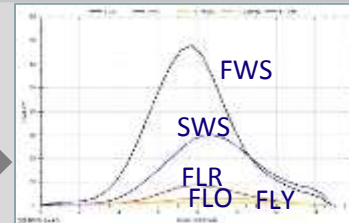
Automated FCM



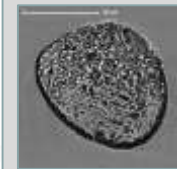
Computer



1 cell

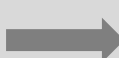


Optical prop.

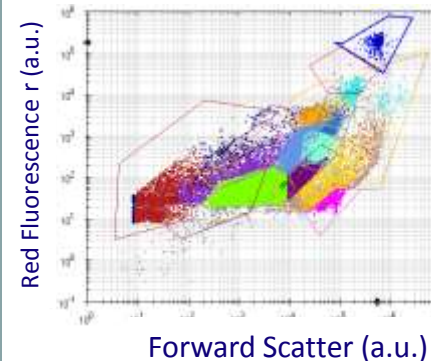


Picture

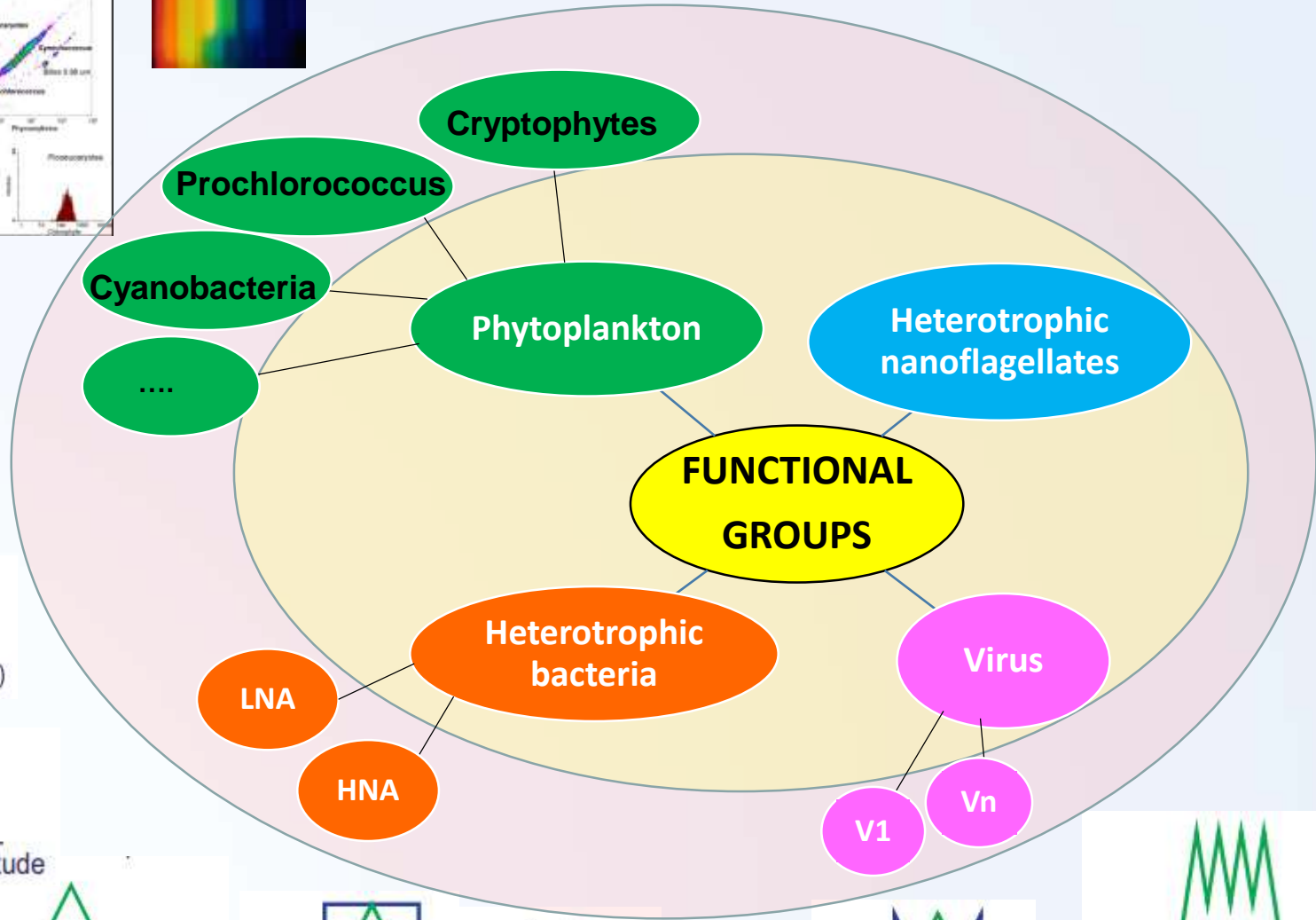
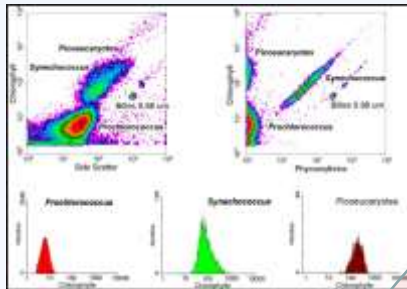
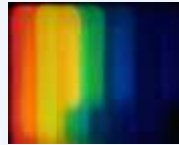
$1 \times 10^6$  cells



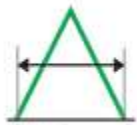
Phytoplankton assemblage



- Functional groups, abundance per group
- Fluorescences per cell, scatter per cell, Size estimation (after calibration of scatter) and Images (taxonomical identification  $>20 \mu\text{m}$ )



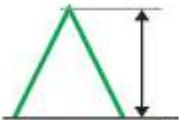
**Abundance**



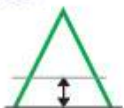
1- particle length



2- integrated signal (area)



3- maximum amplitude



4- average signal strength



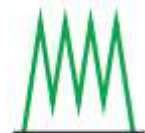
5- the fill factor



6- the asymmetry



7- the inertia



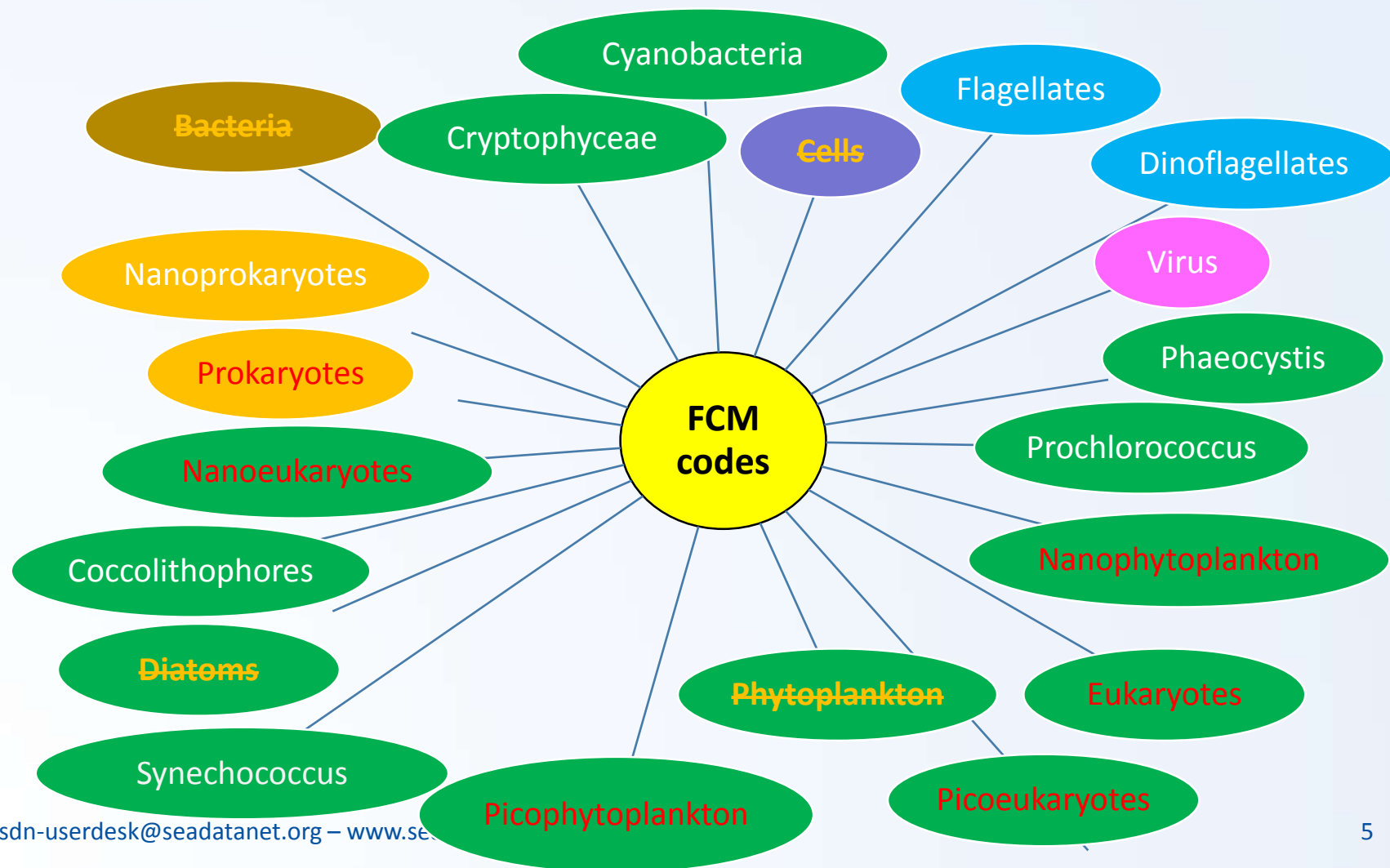
8- nr. of "humps"

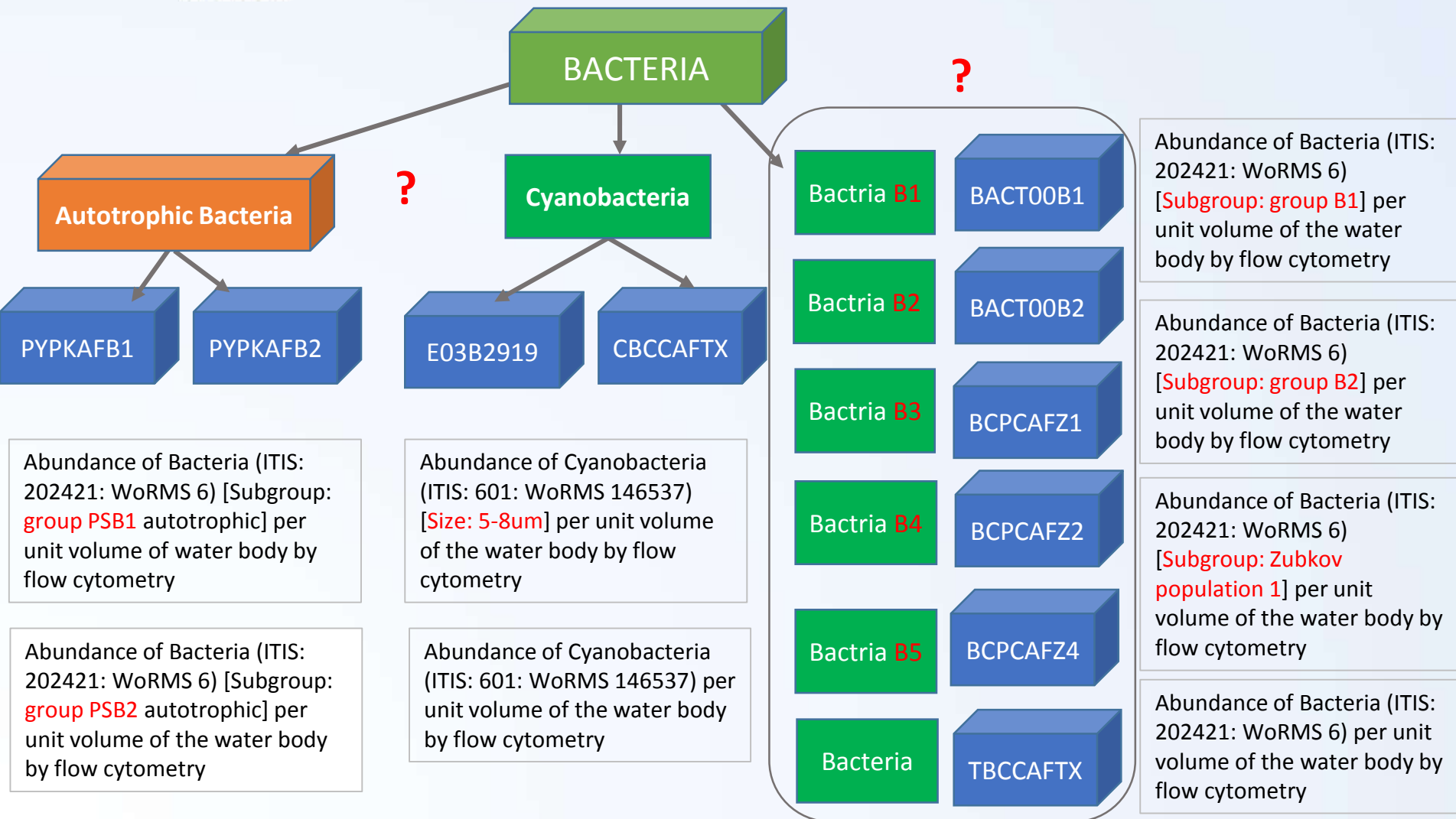


## 2. FCM Common vocabulary progress

- a) Analysis of the existing codes (P01 list)
- b) FCM captured parameters (JericoNext)
- c) Literature review and bibliography (1983-2017)

## a) Analysis of the 34 existing codes (P01 list)





## Picoeukaryotes

Group	Code BODC	Description
Picoeukaryotes	PU00A02A	Abundance of eukaryote picophytoplankton per unit volume of the water body by flow cytometry
Picoeukaryotes P1	PHYT00P1	Abundance of picoeukaryotic cells [Subgroup: group P1] per unit volume of the water body by flow cytometry
Picoeukaryotes P2	PHYT0P10	Abundance of picoeukaryotic cells [Subgroup: group P10] per unit volume of the water body by flow cytometry
Picoeukaryotes P3	PHYT00P2	Abundance of picoeukaryotic cells [Subgroup: group P2] per unit volume of the water body by flow cytometry
Picoeukaryotes P4	PHYT00P3	Abundance of picoeukaryotic cells [Subgroup: group P3] per unit volume of the water body by flow cytometry
Picoeukaryotes P5	PHYT00P4	Abundance of picoeukaryotic cells [Subgroup: group P4] per unit volume of the water body by flow cytometry
Picoeukaryotes P6	PHYT00P5	Abundance of picoeukaryotic cells [Subgroup: group P5] per unit volume of the water body by flow cytometry
Picoeukaryotes P7	PHYT00P6	Abundance of picoeukaryotic cells [Subgroup: group P6] per unit volume of the water body by flow cytometry
Picoeukaryotes P8	PHYT00P7	Abundance of picoeukaryotic cells [Subgroup: group P7] per unit volume of the water body by flow cytometry
Picoeukaryotes P9	PHYT00P8	Abundance of picoeukaryotic cells [Subgroup: group P8] per unit volume of the water body by flow cytometry
Picoeukaryotes P10	PHYT00P9	Abundance of picoeukaryotic cells [Subgroup: group P9] per unit volume of the water body by flow cytometry
Picoeukaryotes	PYEUA00A	Abundance of picoeukaryotic cells per unit volume of the water body by flow cytometry

One Code	One description
PYEUA00A	Abundance of picoeukaryotic cells per unit volume of the water body by flow cytometry



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



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





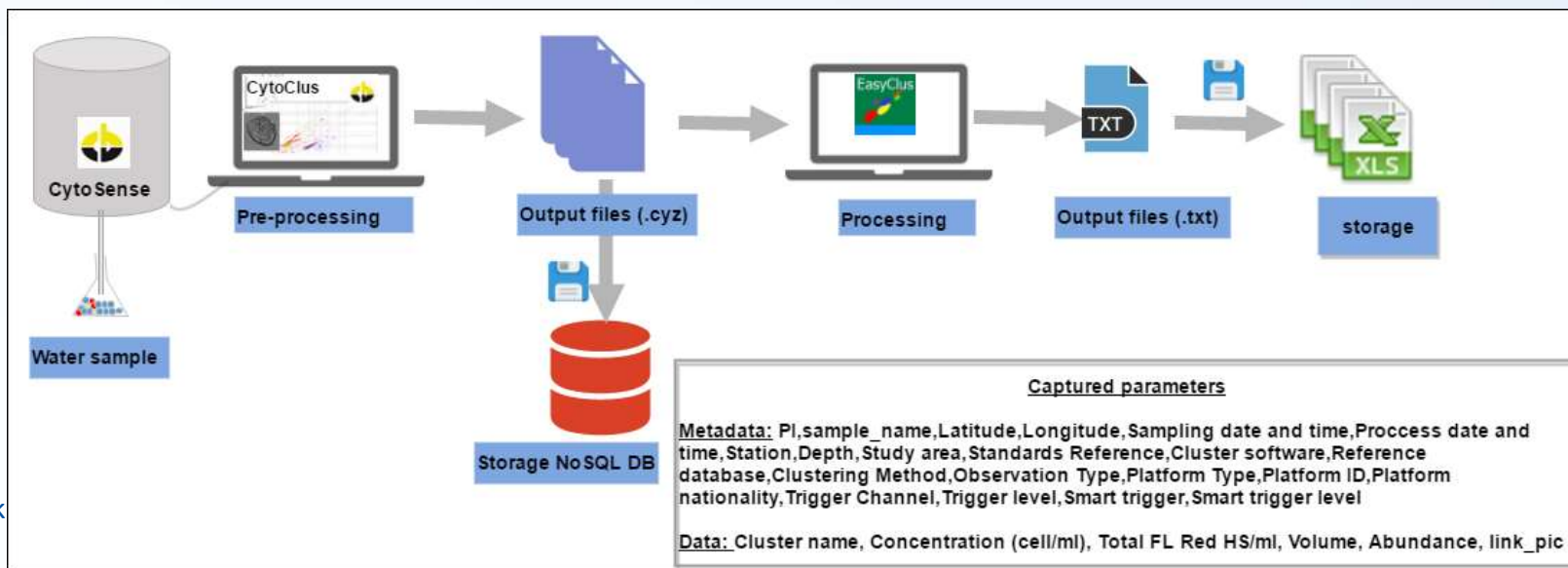
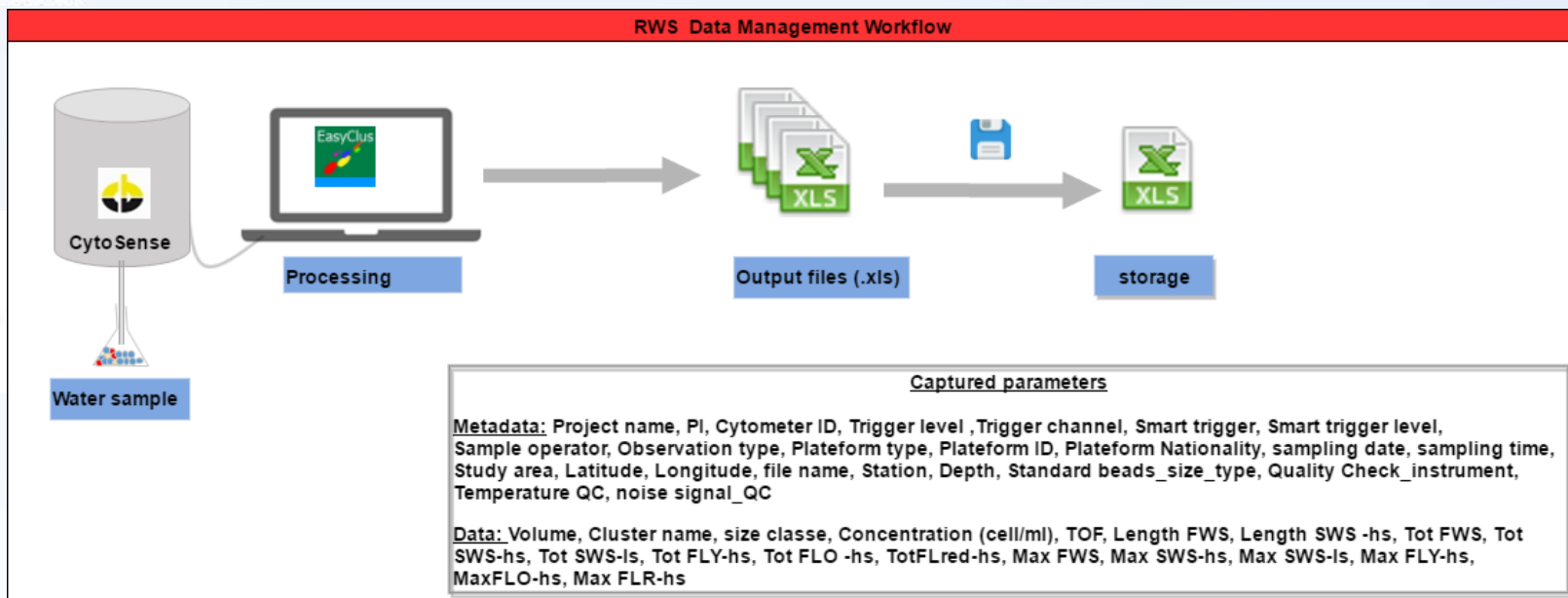
## b) FCM captured parameters

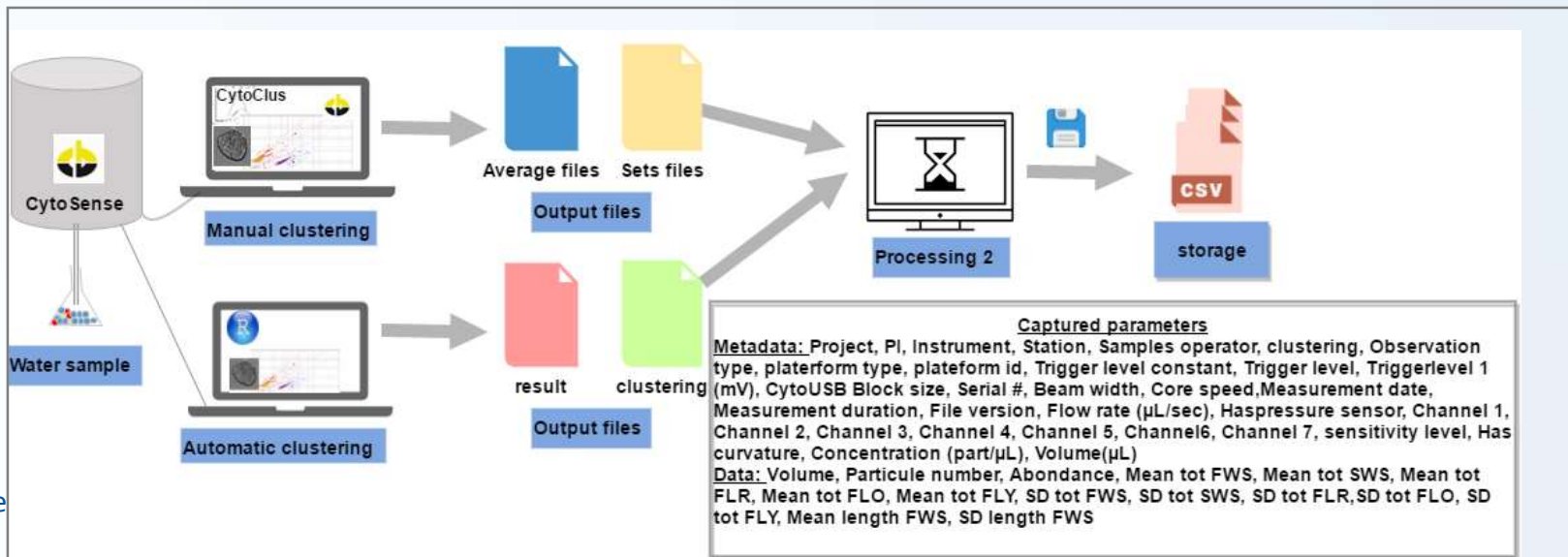
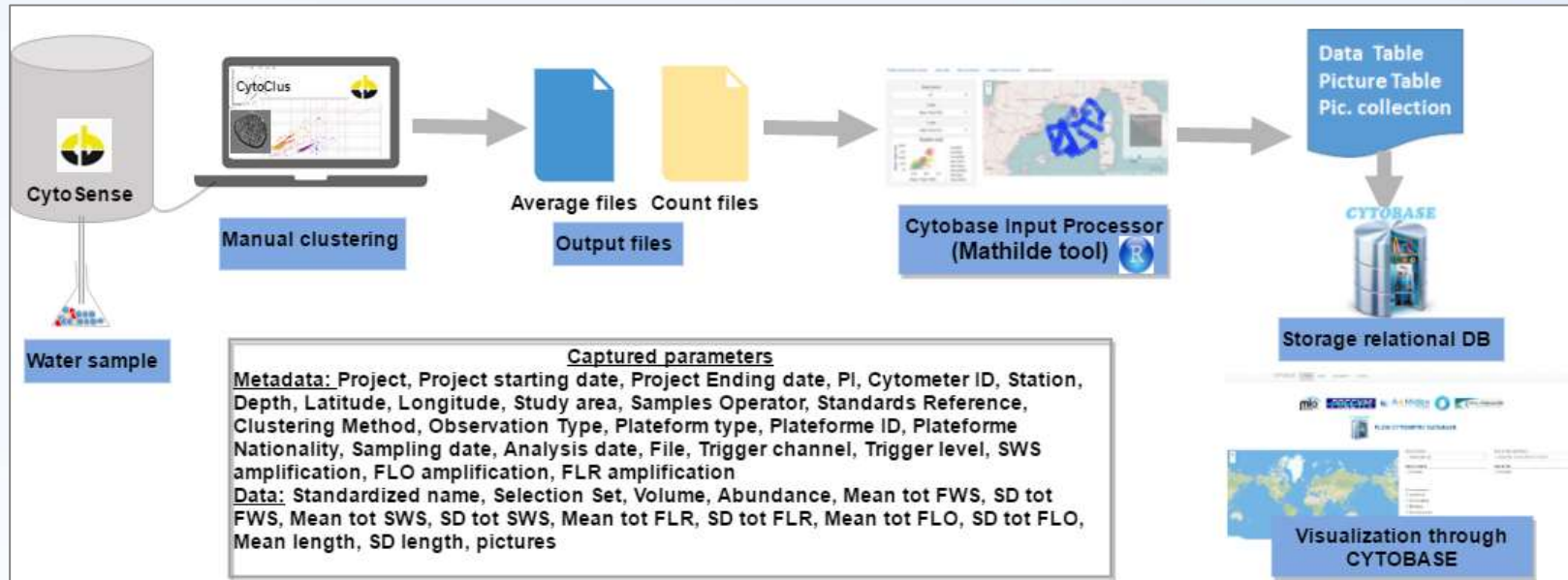
**Task 3.1:** Automated platform for the observation of Phytoplankton diversity in relation to ecosystem services

Leader: Felipe ARTIGAS (CNRS-ULCO)

### Exercise of FCM captured parameters

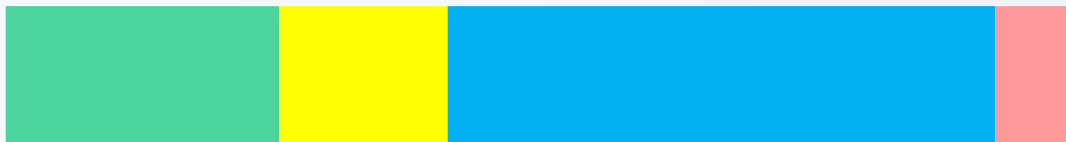




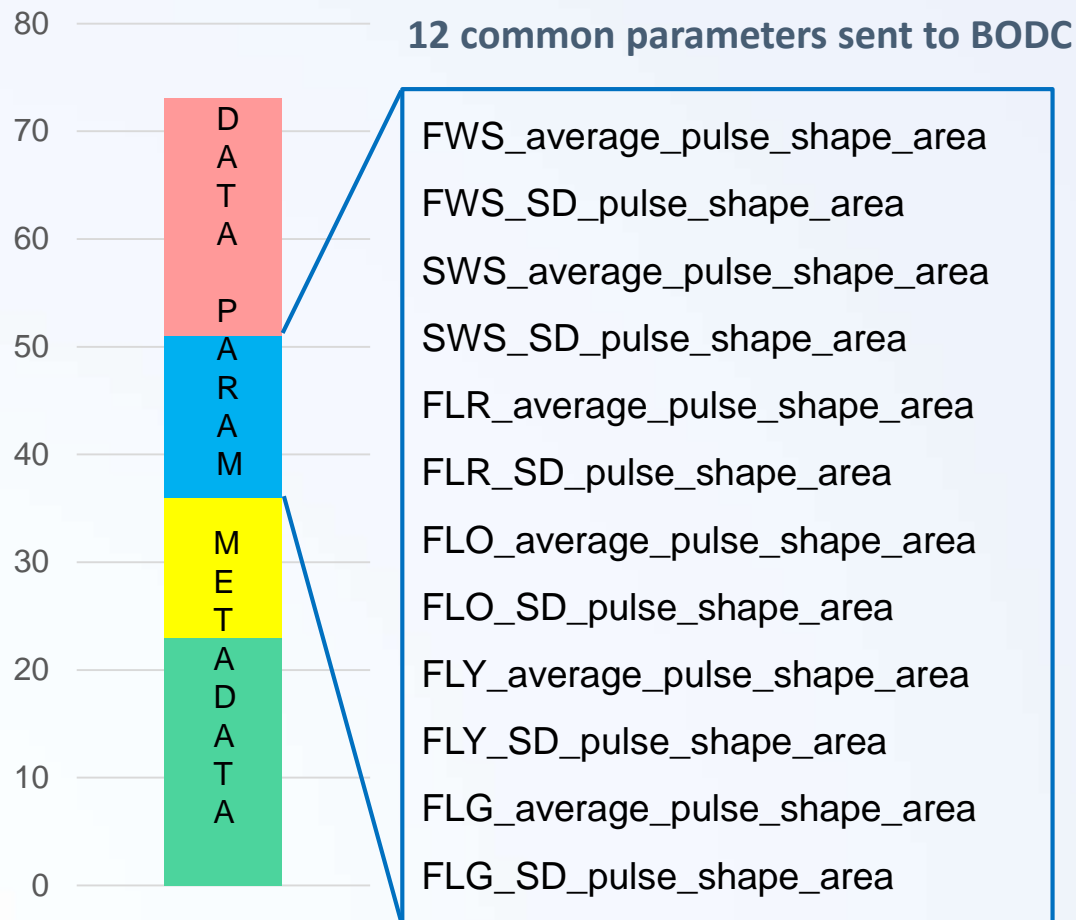




Common Metadata Unique Metadata Common Data Unique data




0 10 20 30 40 50 60 70



# Semantic model (BODC)

Chemical model	Biological model	Physical model	
<b>Measurement</b> <b>Substance</b> Measurement Matrix Relationship Matrix Matrix Subcomponent	<b>Measurement</b> <b>Organism Name</b> <b>Organism Specifics</b> Measurement Matrix Relationship Matrix Matrix Subcomponent Method	<b>Measurement</b> <b>Statistical</b> Measurement Matrix Relationship Matrix Method	<b>Forward scatter pulse shape area</b> Average per cluster in the <b>Water body</b> automated flow cytometry
<b>Concentration</b> of carbon (total inorganic) {TCO2} per unit mass of the water body [dissolved plus reactive particulate phase]	<b>Abundance of Bacteria</b> (ITIS: 202421: WoRMS 6) [Subgroup: heterotrophic] per unit volume of the water body by automated flow cytometry	<b>Forward light scatter pulse</b> shape area average per cluster in the water body by automated flow cytometry	The cluster name is managed in a separate vocabulary (and separate column in ODV).





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- ▼ Vocabularies
  - NVS search tool
  - NVS editor
  - NVS vocabulary builder
  - BODC parameter codes
  - SeaVoX
- ▶ Delivery formats
- ▶ Products
- ▶ Help and hints
- ▶ Portals and links
- Search

## P01 Physical Entity and Other Parameter Code Builder

[help](#)

Preferred label

Forward light scatter pulse shape area standard deviation of the water body by Automated flow cytometer

[Create](#)

[reset all](#)

Found 0 exact matches

- ✓ Select a measurement property
- ✓ Select a statistical parameter
- Select a physical entity (if applicable)
- ✓ Select a measurement-matrix relationship
- ✓ Select a matrix
- Select a sample preparation (if applicable)
- ✓ Select an analytical method (if applicable)
- Select a post-analysis processing (if applicable)



## Flow Cytometry Standardized cluster names

- Standard beads
- Prochlorococcus
- Synechococcus
- Eukaryote Picophytoplankton
- Eukaryote Nanophytoplankton
- Cryptophytes
- Coccolithophores
- Microphytoplankton
- Heterotrophic Bacteria

**New  
Vocab.  
list**



COMMENT

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

© 2009 Wiley Periodicals, Inc.

† *Experiments 1a* and *1b* were conducted on the same day.

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

D. Yuzhat, C. Courtney, and F. Partowsky

(2002), *Station Biologique*, 20211 Brest, France

All figures are of natural logarithmic scale excepted and scaled into 1000.

performed daily at noon in each reservoir with a YSI® multi-parameter water quality meter (model 9000). These measurements showed that the water column was homogeneous during the whole experiment.

### 3.1.1. Micrographical and narrow-angle analysis

Decomposed weight is commonly determined by high performance liquid chromatography (HPLC) analysis of 800 µm sieved soil (Shen et al. 2005). We used 200 µm sieved soil as a 25 mm Whatman G3-25 filter (Shen et al. 2005) was used to separate the particles that were extracted and analyzed by HPLC. All 200 µm sieved soil ( $n = 10$ ) from 10 sites (M1 + M2), plus three 1992 + 1993 and three 2002 + 2003 ( $n = 3$ ) measurements were deposited from 25 cm pre-combusted (200 µm) filtered aluminum samples collected (1992 + 1993) from each measurement site (M1 and M2). Inorganic carbon content was measured prior to storing the sample in plastic and cleaned 800 µm sieved large litter ( $n = 20$ ) was analyzed in 2002/2003 (3 years), using a Shimadzu Total Carbon 3 system based on the method by Davidson et al. (1993).

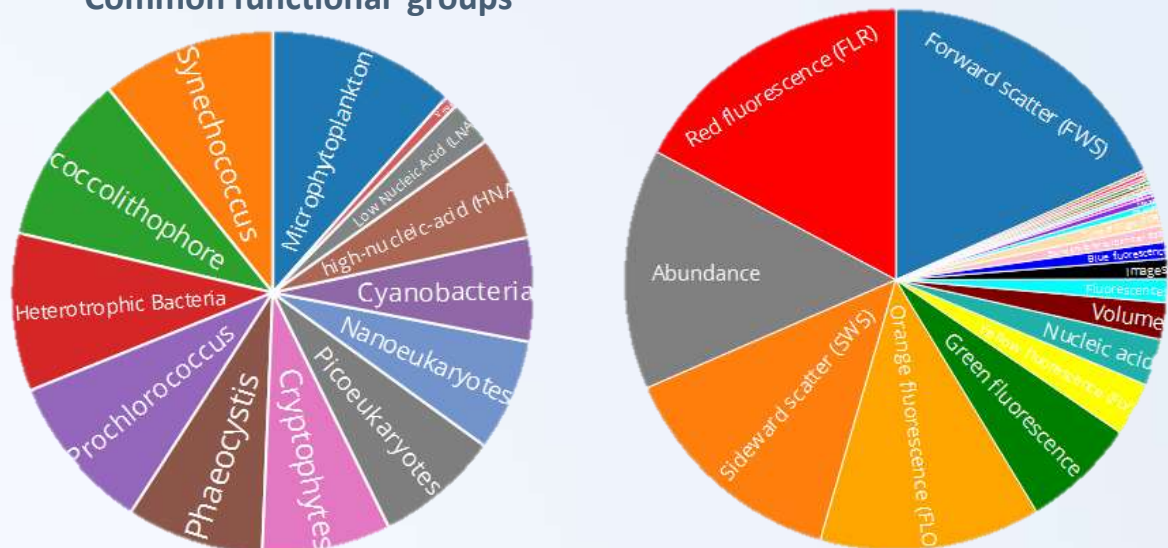
#### 4.4. Numerical solution

[illegible]

The initial temperature of the microcosms was  $-17.7^{\circ}\text{C}$  and increased  $2.9^{\circ}\text{C}$  from day 1 to day 4. At day 4, temperature subsided at  $-17^{\circ}\text{C}$  for the normal temperature and  $-18.7^{\circ}\text{C}$  for the high pressure in the temperature measurement on day 4 (Fig. 3A). Lactate values varied between 0.4–1.4 mM on day 0 and 2.0–3.0 mM on day 4, 3 days not shown. The change in microcosms occurred from day 1 to day 4, and

[illegible]

## Parameters



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:

The photosynthetic depth ( $Z_{\text{ph}}$ ) of all nuclear stations (light) was determined as the depth at which 1% of the light intensity was available (Frost & Taylor 1980).  $Z_{\text{ph}}$  reached depths between 21 and 77 m and decreased 20 and 25 m over the cruise at 305 m and 327 m, respectively (Fig. 18, K). Fig. 19, and Edwards for 205 m and 317 m are average transects in the water column from surface to  $Z_{\text{ph}}$  calculated according to Matyszevski and Collier (1986). Average water column (WVC) fluorescence is the 0.05 fluorescence value (5.0 and 10.7% and 6.0 and 15.0% for 205 and 317 m, respectively (Fig. 19, J), as compared to 10.0%

The initial temperature is at the minimum value ( $-17^{\circ}\text{C}$ ) and is increased by  $2^{\circ}\text{C}$  from day 1 (day 1, 2) day 2 (temperature constant at  $-15^{\circ}\text{C}$ ) to the normal temperature (day 3,  $-10^{\circ}\text{C}$ ) as the high program is conducted (temperature day 5) (Fig. 3A). Activity values varied between 0.4–0.6 in MINUS on day 0 and 0.0–0.2 in MINUS on day 5 (data not shown). (Observing all measurements completed from day 1 up to day 5 in MINUS for the temperature program is shown in Table 1.)

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.

**Journal of Management Inquiry** 20(1) 3-14  
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10

**Self and other ratings.**



0% 100%

**Part I: Groups definition from the FCM point of view**

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

• **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 PHT 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 Hete Scatter (SWS) vs Chlorophyll they lack phycoerythrin.

Check any that apply

☐ I agree

☐ I do not agree

• **Synechococcus**

They are unicellular photos scatter (SWS) signatures th required to distinguish them signatures than Prochlorococcus accessory pigment when ex phycoerythrin, excited by a r cluster is well resolved in re Due to their small size (0.8-1 SWS and FLR signals.

Check any that apply

☐ I agree

☐ I do not agree

• **Eukaryotes Picophytoplankton**

The common definition of th by flow cytometry. The picophytoplankton exhibits a Prochlorococcus and Synechococcus may happen. The FWS signa important to keep in mind t

**Part III: Sample Metadata**

• **What Beads reference do you use?**

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

• **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:



### 3. Conclusion/Workplan

- Standardization of 12 FCM vocabulary parameters December 2017
- Injection of FCM CDI to SDN infrastructure Jan-Feb 2017
  - ➔ Download Manager is already installed in 3 CNRS centers (Bordeaux, Roscoff and Marseille)
- Identifying/Updating FCM vocabulary (Questionnaire) Feb-March 2018
  - ➔ 38 answers (since 9 october 2017))
- Deliverable D9.5.2 : *Ingesting, validating, long-term storage and access of flow cytometry data* May 2018



Thank you for your support

Any questions?