CytoBuoy
flow cytometry solutions

FLOW CYTOMETRY DATA MANAGEMENT

CytoBuoy Meeting 2017

Woerden (Netherlands) – March 27th, 2017

Soumaya Lahbib, Mathilde Dugenne, Pierre Marrec, Maurice Libes, Gérald Grégori and Melilotus Thyssen

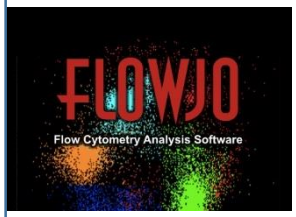
MIO/CNRS

Cytobuoy FCM MIO deployment

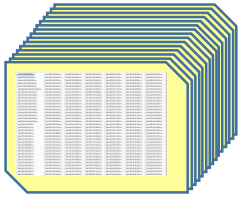
CytoBuoy Meeting – 27- 29 March 2017

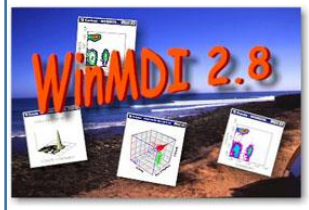
FCM tools processing

Benchtop FCM

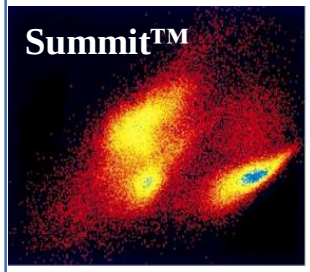


Output 1

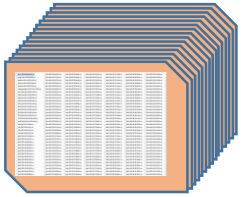




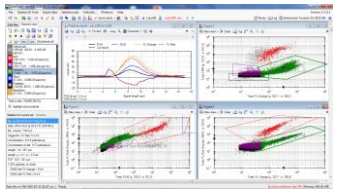
Output 2



Output 3

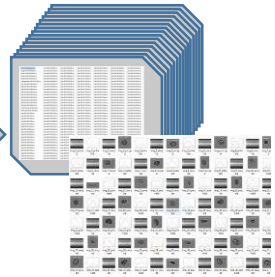


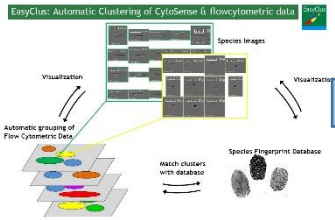
Automated FCM



CytoClus

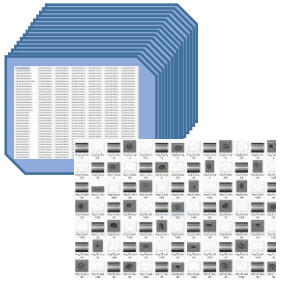
Output 1

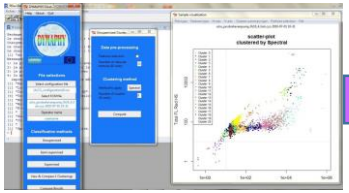




EasyClus

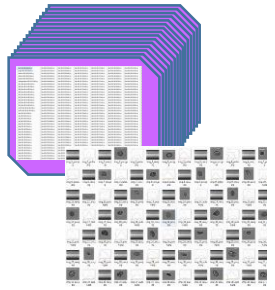
Output 2





R

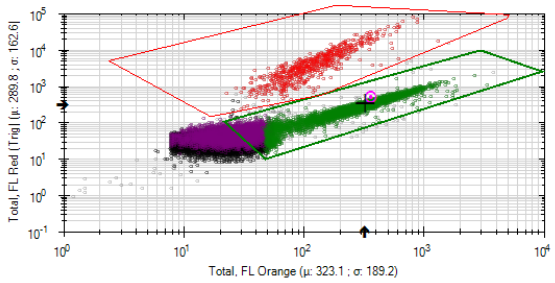
Output 3



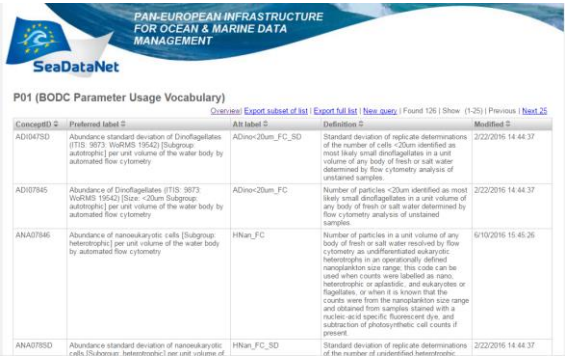
Barriers to exchange/re-use FCM data

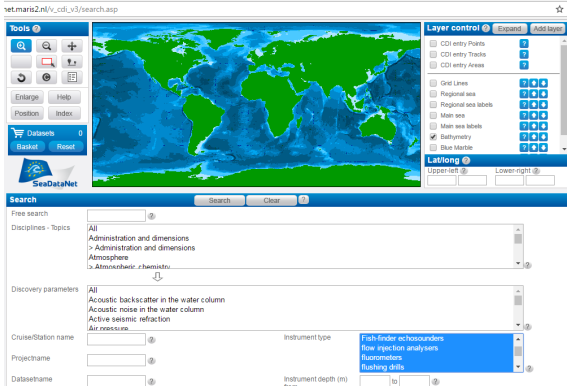
- Functional group names definition
- Metadata missing: Standard Beads, trigger, etc..
- Standard data file schemas and formats
- FCM data quality control (QC)
- FCM standard vocab (34 codes)

➔ FCM instrument and data are missing in EU portals



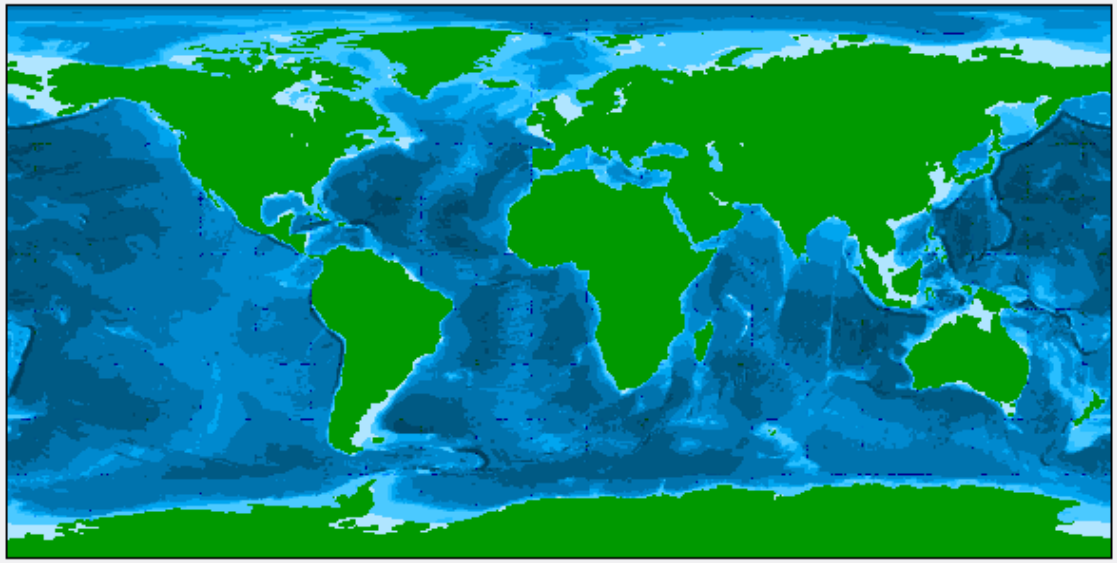
Output data files	Format	Number	Parameters
Average avgprops_process_xx-xx-xxxx avgprops_process_xx-xx-xxxx avgprops_process_xx-xx-xxxx etc..	csv	1 or many	Selection se, File name, Date, Time, Total Analyzed Volume [mL], Number of Particles Min. TOF, Max. TOF, Mean TOF, etc...
Count counts_process_xx-xx-xxxx counts_process_xx-xx-xxxx counts_process_xx-xx-xxxx counts_process_xx-xx-xxxx etc...	csv	1 or many	File name, Volume [mL], Trigger chn, Level [mV], Date, Particles, Beads 2 mu - Number, Beads 2 mu - pct/Tot Beads 2 mu - N/mL, Microphytoplankton - Number, etc...





Barriers to exchange/re-use FCM data

net.maris2.nl/v_cdi_v3/search.asp



Tools | Layer control | Expand | Add layer

Enlarge | Help | Position | Index

Datasets 0 | Basket | Reset

SeaDataNet

CDI entry Points
CDI entry Tracks
CDI entry Areas

Grid Lines
Regional sea
Regional sea labels
Main sea
Main sea labels
Bathymetry
Blue Marble

Lat/long
Upper-left | Lower-right

Search | Search | Clear

Free search

Disciplines - Topics
All
Administration and dimensions
> Administration and dimensions
Atmosphere
> Atmospheric chemistry

Discovery parameters
All
Acoustic backscatter in the water column
Acoustic noise in the water column
Active seismic refraction
Air pressure

No FCM Instruments

Cruise/Station name

Projectname

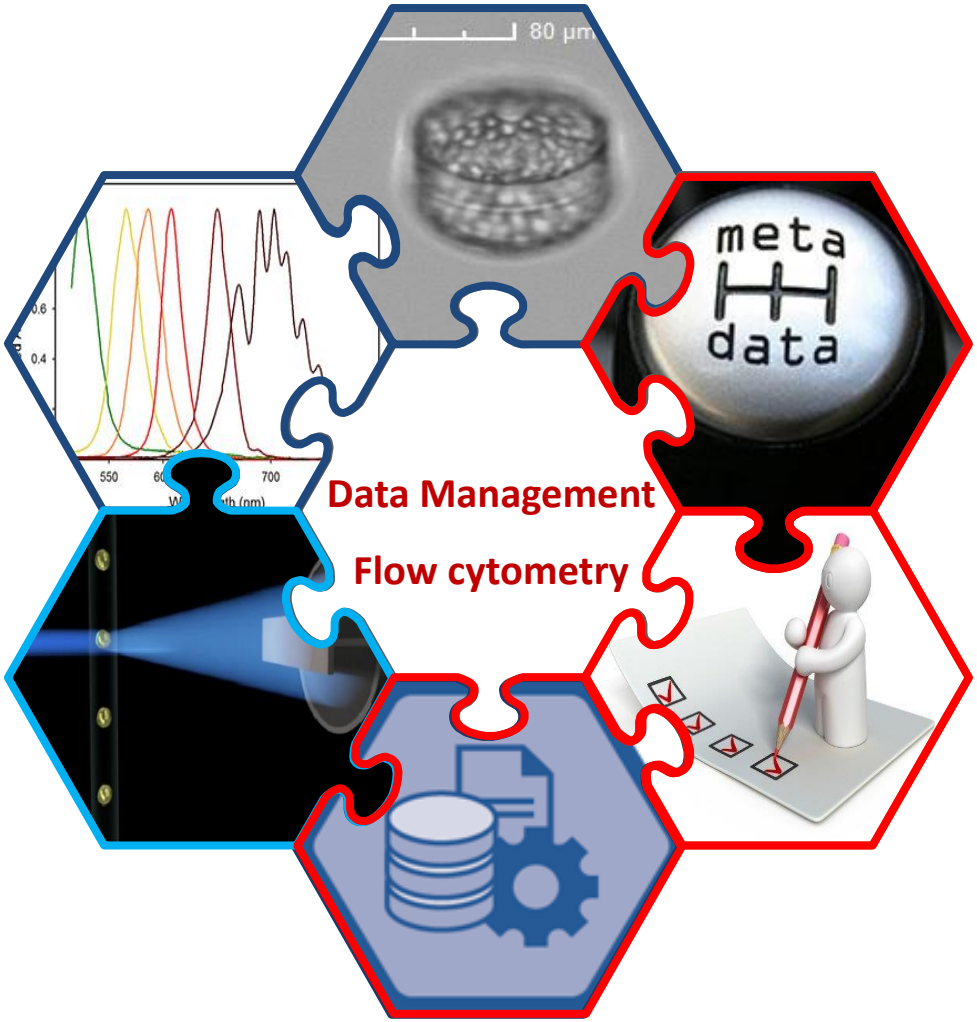
Datasetname

Instrument type
Fish-finder echosounders
flow injection analysers
fluorometers
flushing drills

Instrument depth (m) to

Data Management?

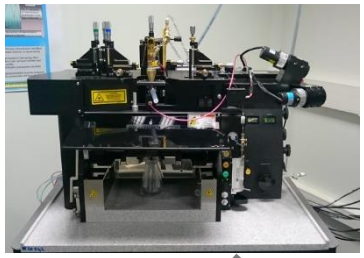
"Data Resource Management is the development and execution of architectures, policies, practices and procedures that properly manage the full data lifecycle."



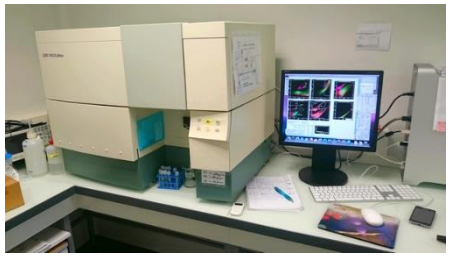
MIO FCM Data management
(*working on interoperability with SeaDataNet*)

In situ **automated cytometer**

Bentchtop flow cytometers



BD Influx



FACS Calibur

CytoSense

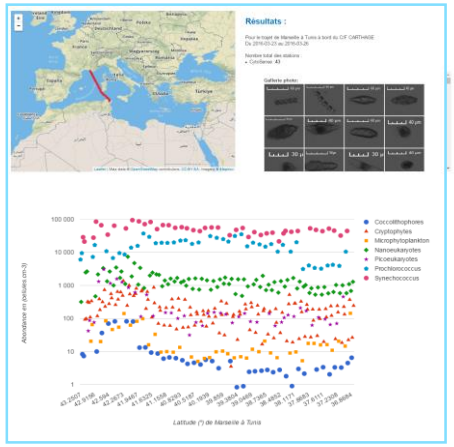
CytoClus

R **Conversion tool** *+ Metadata entry*

M. Dugenne, 2015

ASCII Data Table & Pictures

Acquisition | **Analyses** | **Consolidation** | **Visual QC**

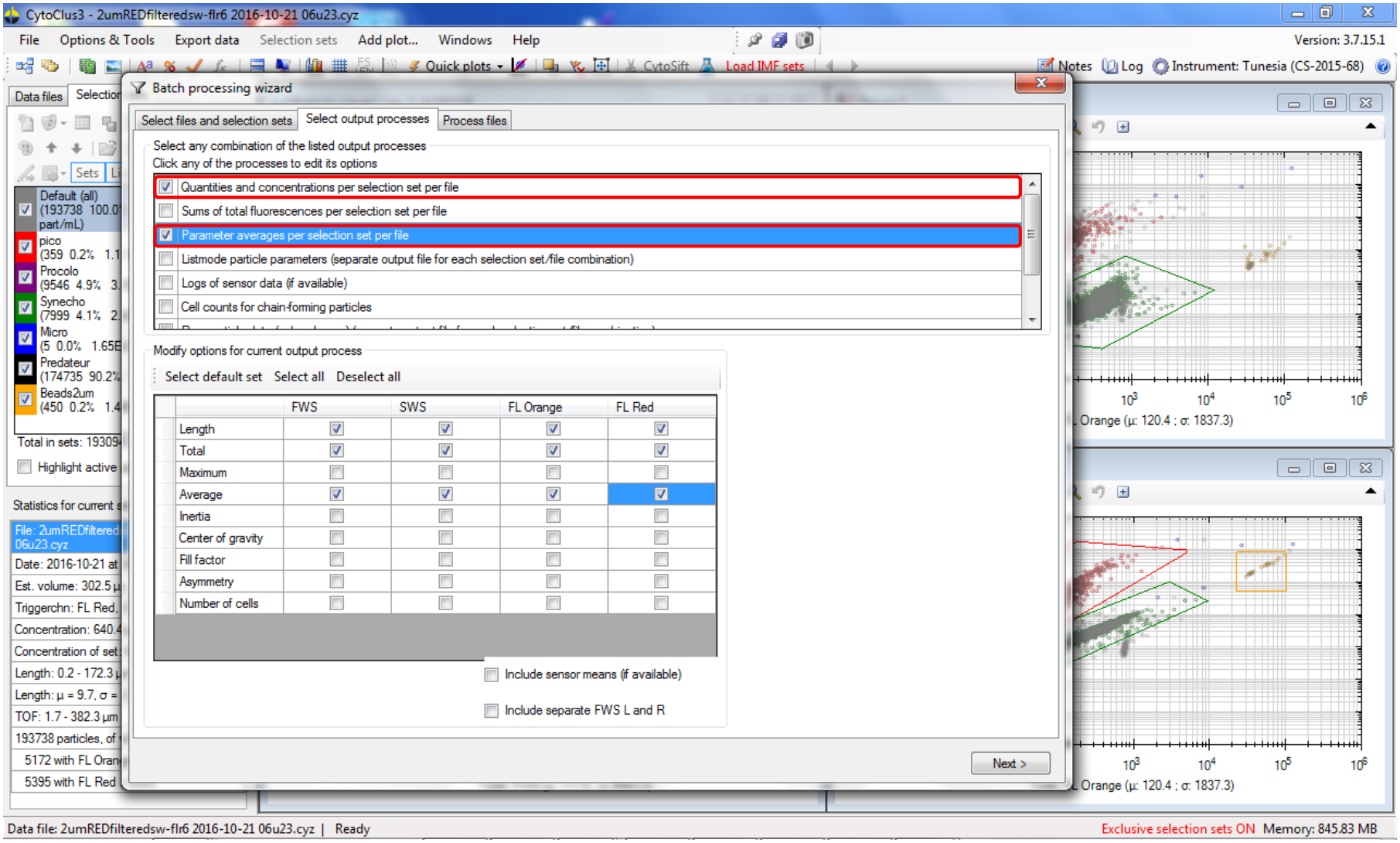


Accessibility

CYTOBASE

Integration

→ Selected CytoClus output by MIO



→ Data Consolidation

Cytobase Input Processor (Mathilde Dugenne, 2015)

mathilde.dugenne@mio.osupytheas.fr

Parcourir... 11 fichiers sélectionnés.

Upload complete

Warning:

Upload successful

Create Inputs folder

Project and samples context | Raw data | Size conversion

Metadata

Project	Project date	PI	Cytometer ID
Enter project name	2015-08-18	Enter PI name	Enter cytometer ID
Station	**Depth**	**Latitude**	**Longitude**
Filename	**Filename model**		
	BERRE_082013_3F_FLR9 2013-12-17 13(		
Samples operator	**Standards reference**	**Clustering method**	**Observation type**
Enter name of operator	Enter standards beads ref	Automated	In situ

Data

2013-12-17T14:17:00Z	2013-12-17T14:17:00Z	16,056.60		Synechococcus	BERRE_082013_12S_FLR9 2013-12-17 14u17.cyz	1.99	FL Red	10
2013-12-17T14:17:00Z	2013-12-17T14:17:00Z	16,056.60		Cryptophytes	BERRE_082013_12S_FLR9 2013-12-17 14u17.cyz	1.99	FL Red	10
2013-12-17T14:43:00Z	2013-12-17T14:43:00Z	16,056.61		Beads 2 mu	BERRE_082013_16F_FLR9 2013-12-17 14u43.cyz	2.04	FL Red	10
2013-12-17T14:43:00Z	2013-12-17T14:43:00Z	16,056.61		Microphytoplankton	BERRE_082013_16F_FLR9 2013-12-17 14u43.cyz	2.04	FL Red	10
2013-12-17T14:43:00Z	2013-12-17T14:43:00Z	16,056.61		Picooeukaryotes 2	BERRE_082013_16F_FLR9 2013-12-17 14u43.cyz	2.04	FL Red	10
2013-12-17T14:43:00Z	2013-12-17T14:43:00Z	16,056.61		Picoeukaryotes 1	BERRE_082013_16F_FLR9 2013-12-17 14u43.cyz	2.04	FL Red	10

Please associate each selection set to trigger, PMt's amplification and standardized phytoplankton category

NB: All incompatible entries will be removed

Expert name	Trigger	PMT's amplification	Standardized name	
Cluster	Channel/Level	SWS	Cluster	
Beads 2 mu	FL Red 10	0 50 100	Standard beads	Associate
		FLO 0 50 100		

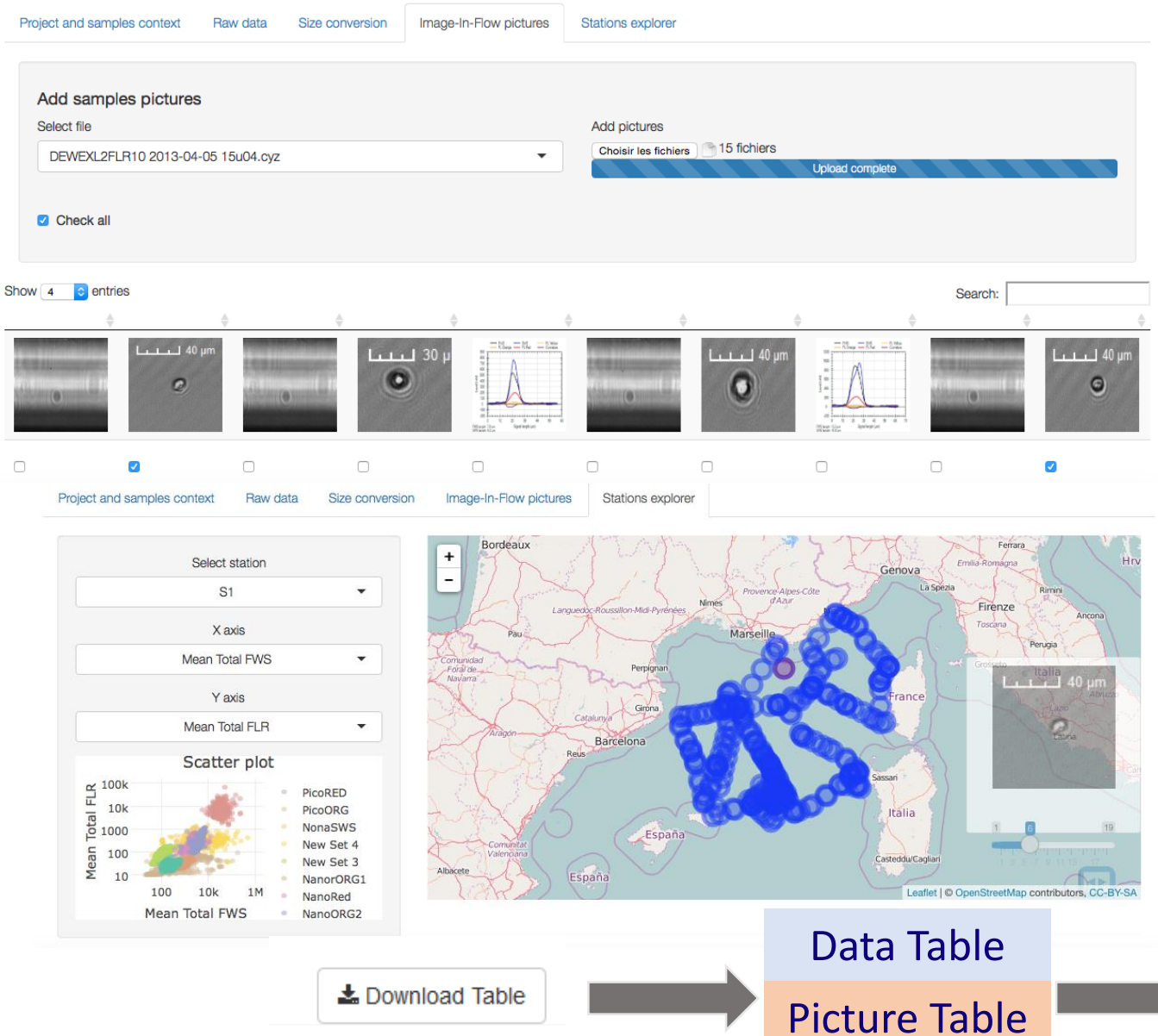
Example of standardization

SELECTION SET	STANDARDIZED NAMES
Cryptophytes	Cryptophytes
Microphytoplank	Microphytoplankton
largephyto3 - N/mL	Microphytoplankton
My favorite group	Microphytoplankton
largephyto1 - N/mL	Microphytoplankton
Nano1	Nanoeukaryotes
Nanoeukaryote 2s	Nanoeukaryotes
……	

→ Data Consolidation

Picture selection

Stations Explorer



→ High resolution flow cytometry data visualisation

CYTOBASE

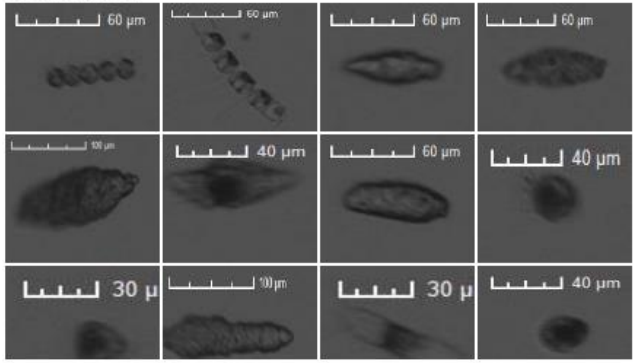
FLOW CYTOMETRY DATABASE

Pour le trajet de Marseille à Tunis à bord du C/F CARTHAGE
Du 2016-03-23 au 2016-03-26

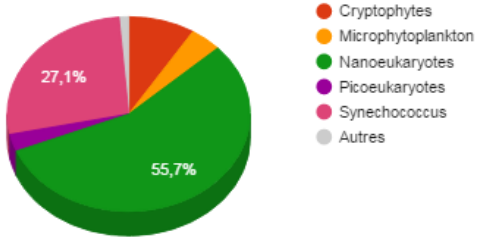
Nombre total des stations :

- CytoSense :**43**

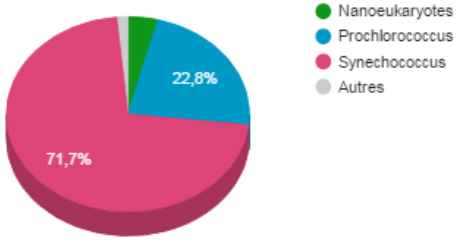
Gallerie photo:



Total Fluorescence Rouge (u.a..cm^{-3})



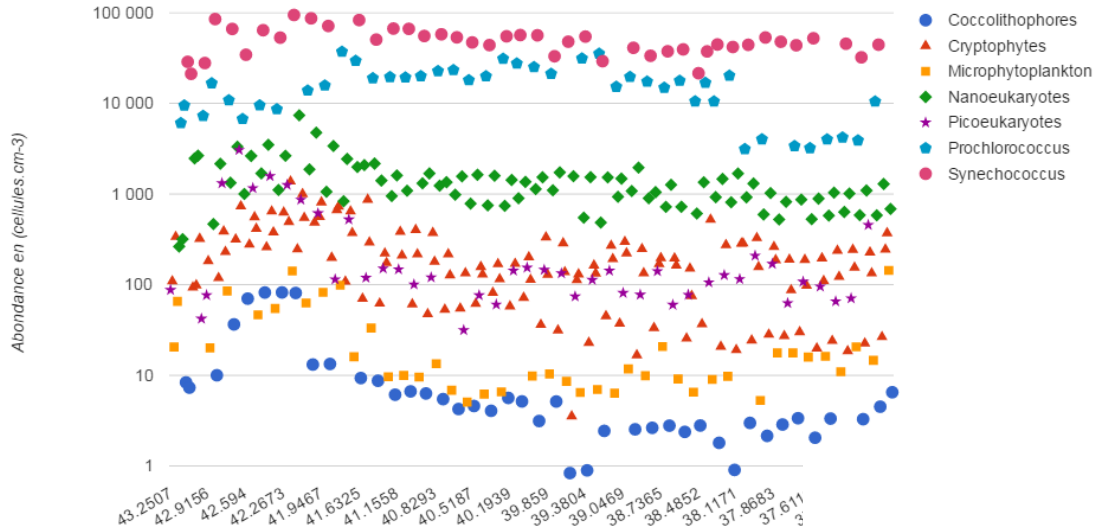
Total Abondance (cell.cm^{-3})

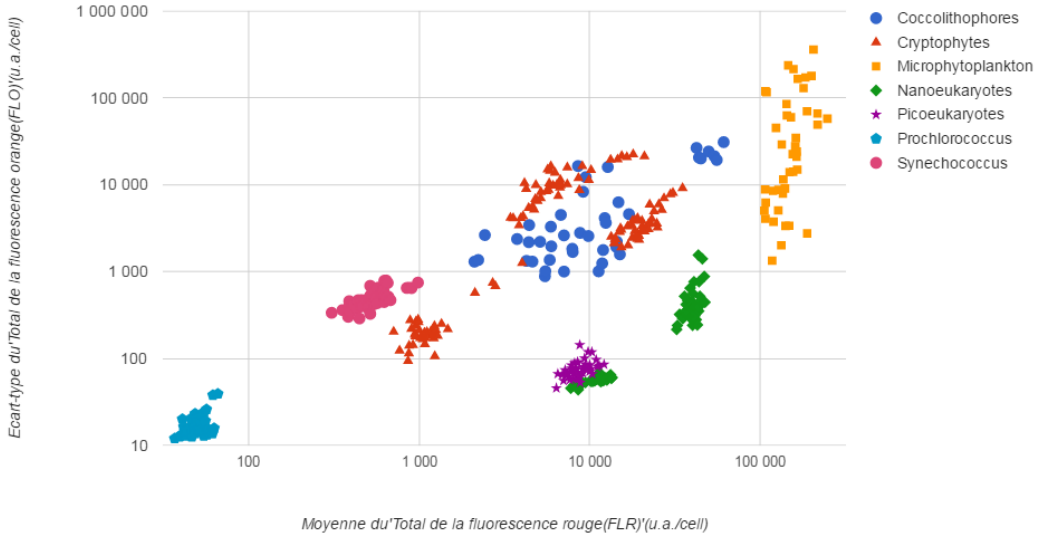


→ High resolution flow cytometry data visualisation

CYTOBASE

FLOW CYTOMETRY DATABASE





Toward EU standardisation of FCM data

PI: Patrick FARCY (IFREMER)

PI: Michèle FICHAUT (SISMER/IFREMER)

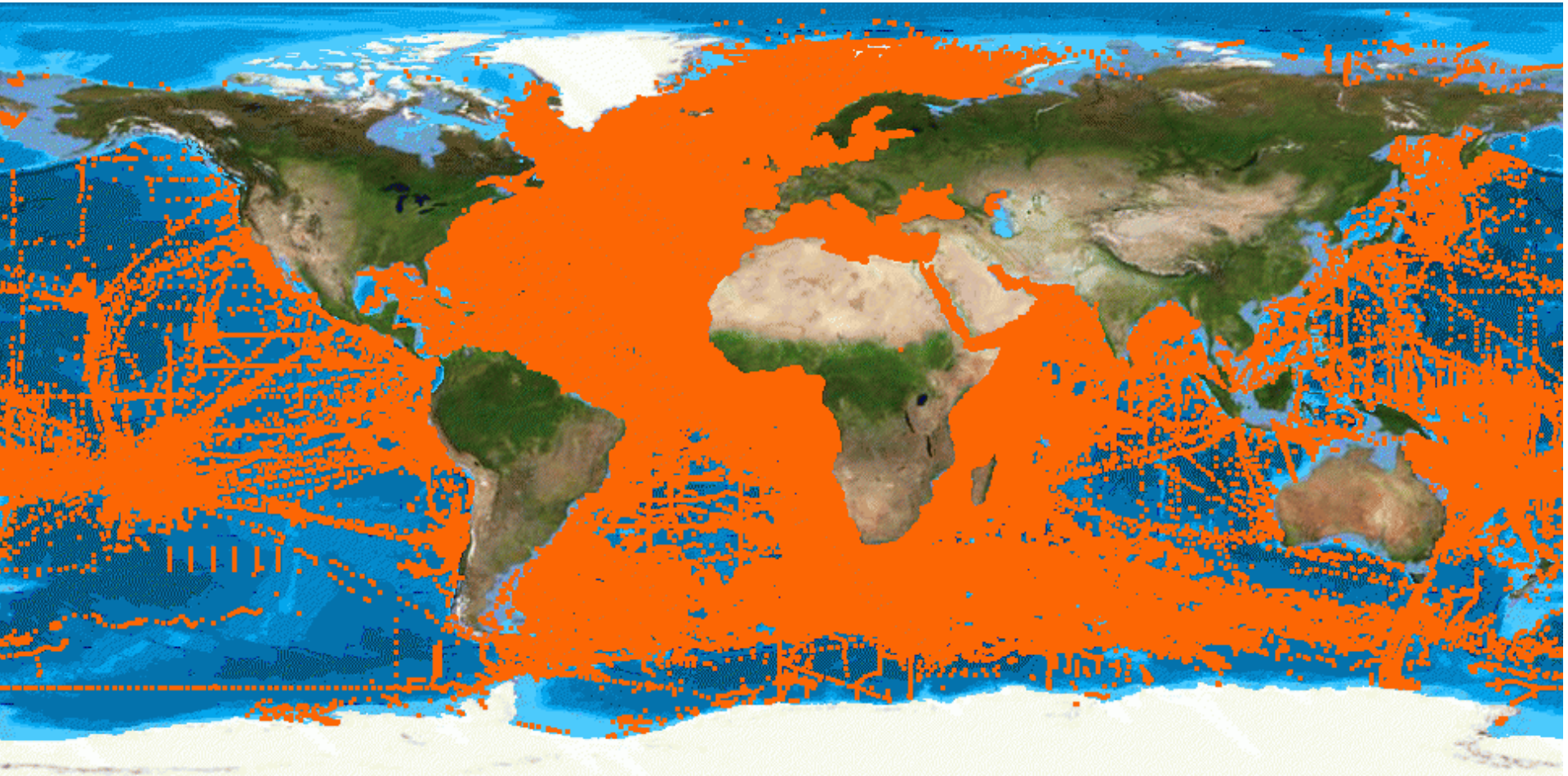
Task 3.1: Automated platform for the observation of Phytoplankton diversity in relation to ecosystem services

WP9.5.2: Ingesting, validating, long-term storage and access of Flow Cytometer data

Leader: Felipe ARTIGAS (CNRS-ULCO)

Leaders: VLIZ, CNRS-MIO, NERC-BODC and ICES

SeaDataNet



1.87 million *CDI entries from* ***34*** *countries,* ***102*** *data centres and* ***597*** *originators for physics, chemistry, geology, geophysics, bathymetry and biology; from* ***1805 to 2016****;* ***86%*** *unrestricted or under SDN License*

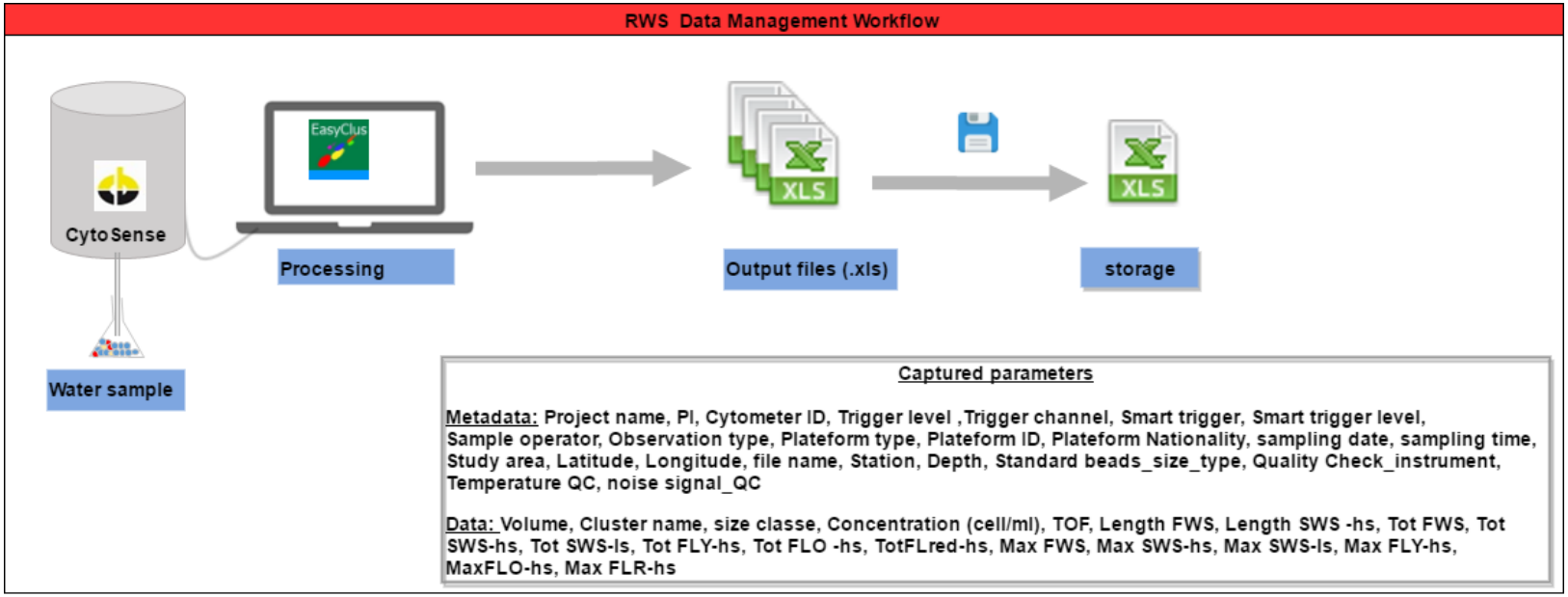
General tasks toward FCM standardization

1. Build FCM (High and low resolution) standard common vocabularies
2. Set up a common FCM data management protocols and methods
3. Connect FCM datasets to SDC infrastructure

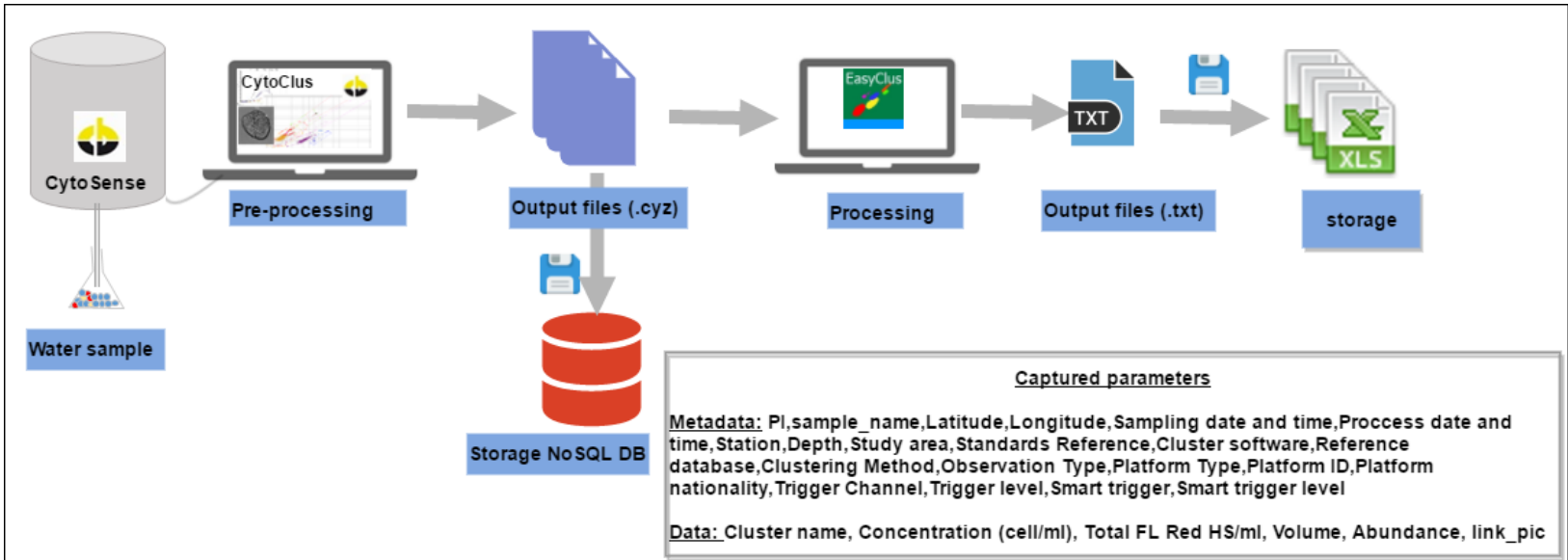
Steps1: Build standard vocabularies

➔ Set up a captured parameters for HR FCM

Rijkswaterstaat

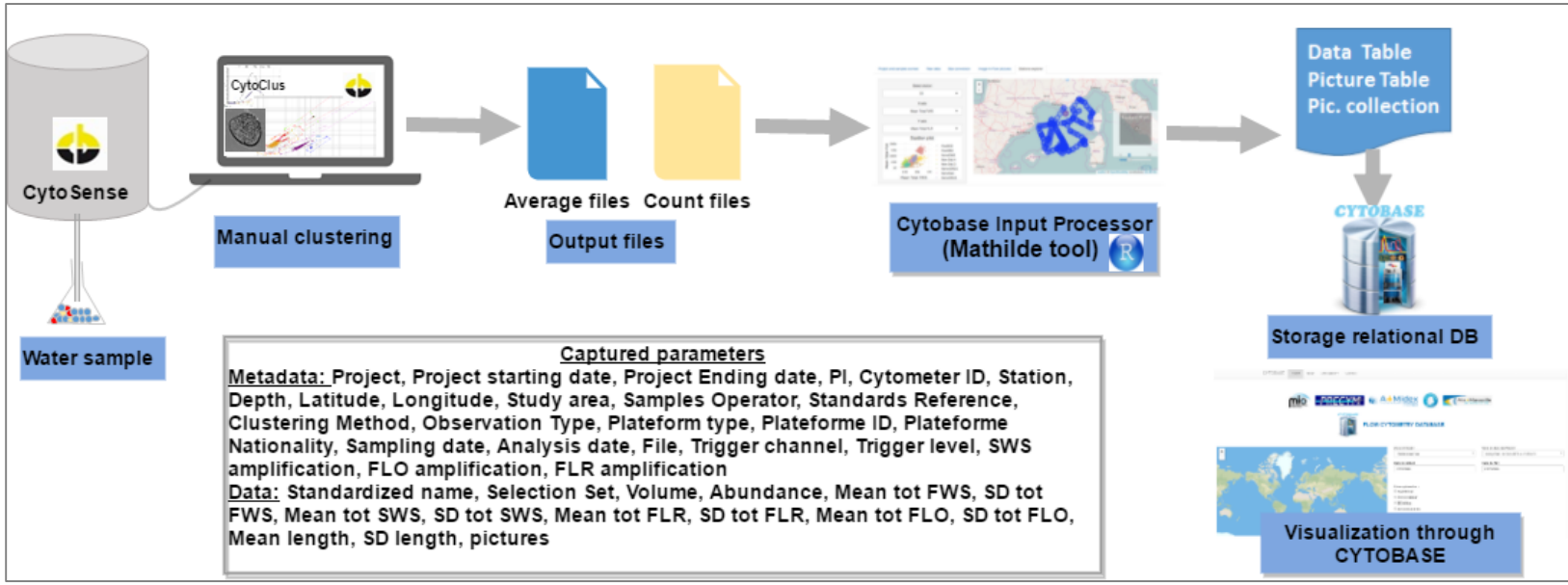


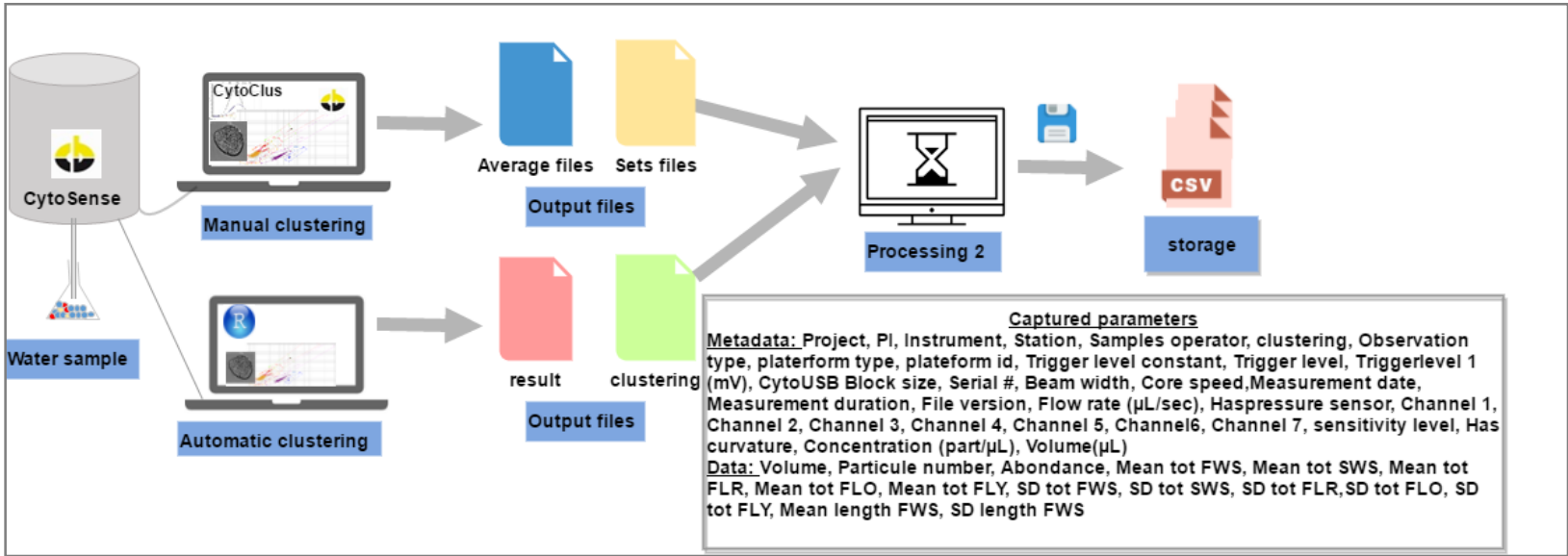
VLIZ



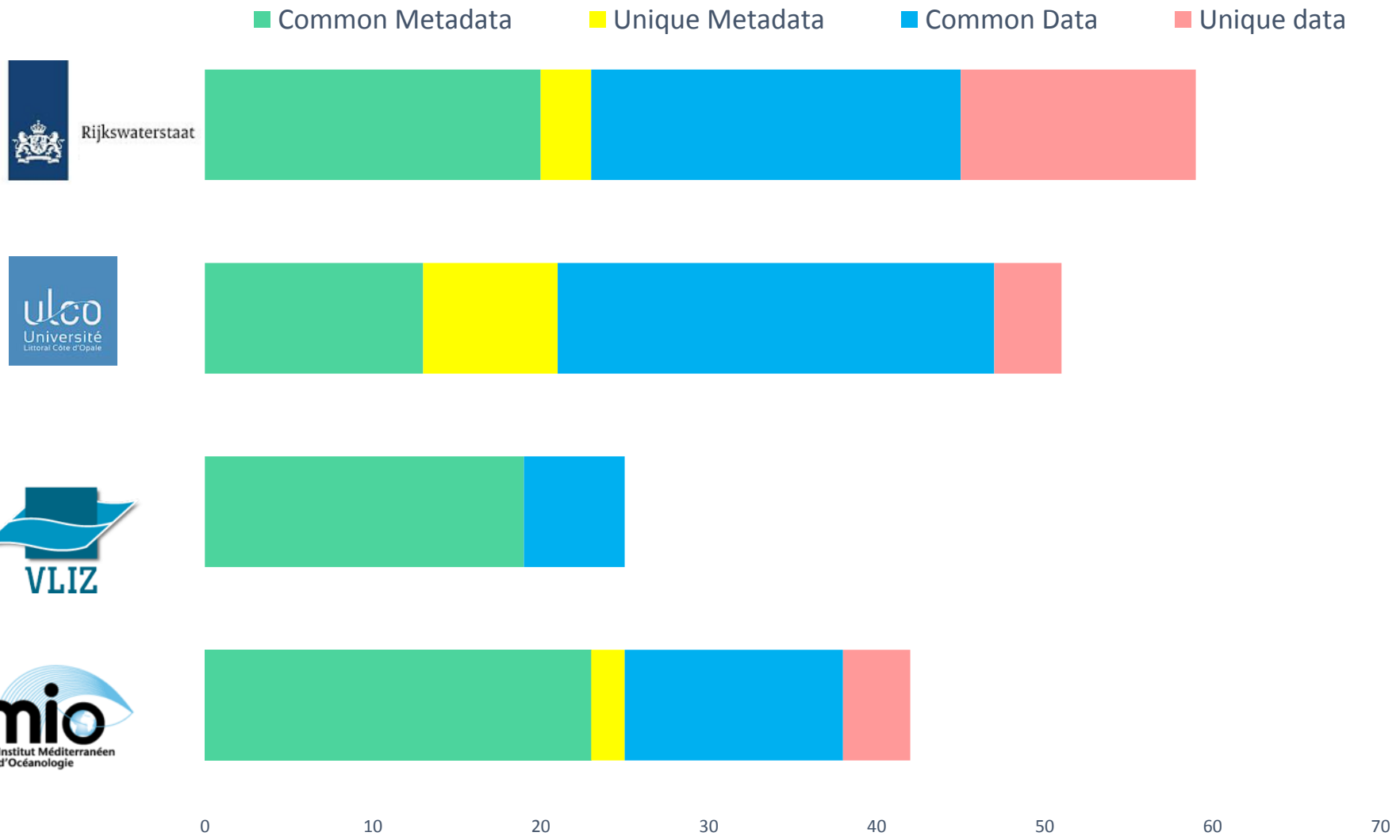
Steps1: Build standard vocabularies

➔ Set up a captured parameters for HR FCM





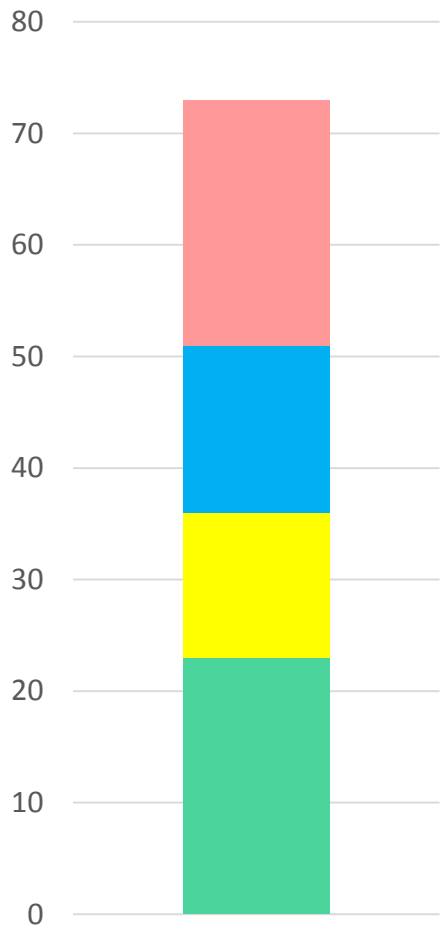
Steps1: Build standard vocabularies



➔ 73 Captured parameters (Metadata+Data)

	Captured PARAM
METADATA	Project
	Project starting Date
	Project ending Date
	Person of Interset
	Cytometer ID
	Beam width
	Core speed
	Instrument name
	CytoUSB Block size
	Quality check instrument
	Temperature QC (instrument
	noise signal QC (instrument)
	Station
	Depth
	Latitude
	Longitude
	Study area
	Samples Operator
	Standards Reference
	Clustering Method
	Observation Type
	Platform Type
	Platform ID
	Platform nationality
	Sampling Date
	Analysis Date
	Measurement duration
	File version
	Flow rate (µL/sec)
	file name
	Trigger Channel
	Trigger level
	amplification#
	Has curvature
	Smart trigger
	Smart trigger level

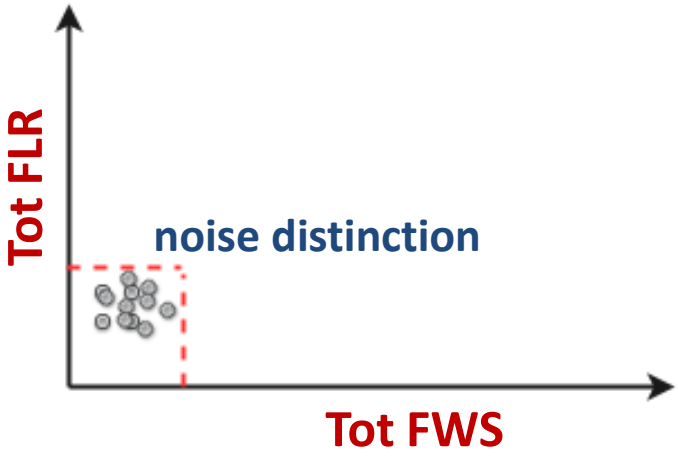
DATA	Standardized name
	Selection Set
	Volume
	Abundance (cell/cm-3)
	Particule number
	Mean tot FWS
	SD tot FWS
	Mean tot SWS
	SD tot SWS
	Mean tot FLR
	SD tot FLR
	Mean tot FLO
	SD tot FLO
	Mean tot FLY
	SD tot FLY
	Mean length FWS
	SD length FWS
	Length FWS
	Length SWS
	Tot_FWS
	Tot_SWS - hs
	Tot_SWS - ls
	Tot_FLY -hs
	Tot_FLO-hs
	Total FL Red HS/ml
	Max FWS
	Max SWS - hs
	Max SWS - ls
	Max FLY - hs
	Max FLO - hs
	Max FLR - hs
	TOF
	Beta 0 (coef intecept)
	Beta 1 (coef (slope)
	Mean Length
	SD Length
	link_pic



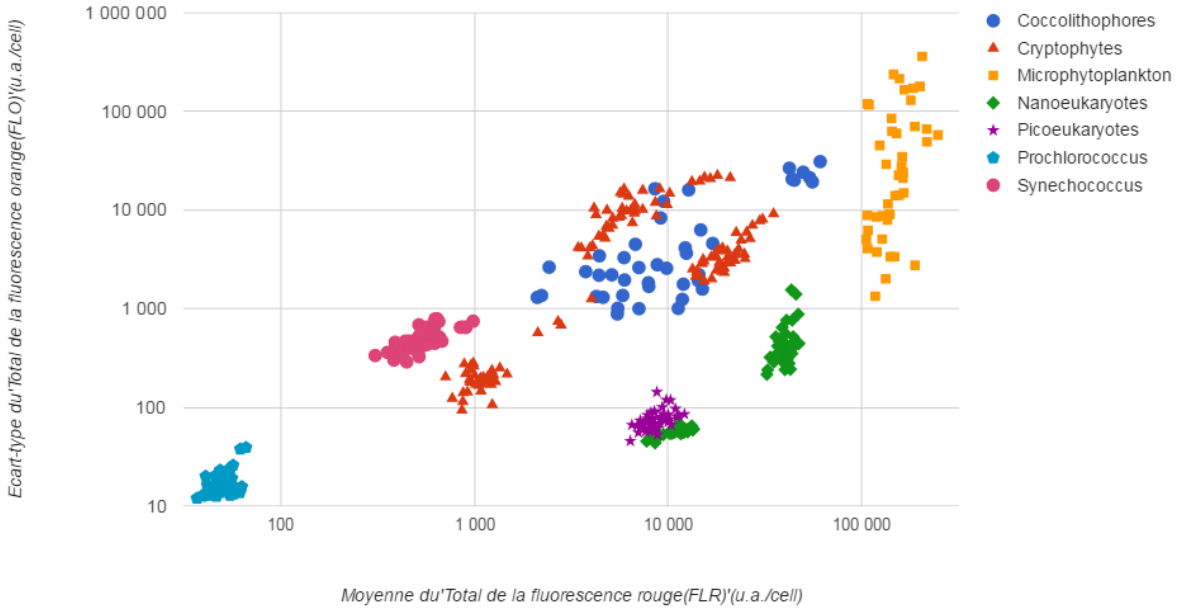
➔ Some propositions for standardization

1. Provide an average cytograms/region and techniques (2 or 3) with these combinations such as: Tot FLR/ Tot FWS or/and Tot FLR/Tot FLO

➔ Determine the cytogram of a BLANCO sample (using sea water or sheath fluid) with identical trigger for phyto. analysis



➔ Determine the average cytogram « conceptual cyto » of the phytoplankton groups in (arbitary unit/cell)



2. Define phytoplankton groups in the point of view of the Cytometrist

SELECTION SET	STANDARDIZED NAMES	DEFINITION
Cryptophytes	Cryptophytes	A group characterized by an orange fluorescences cells with similar FWS as Nanoplancton. The specific definition depends on the laser wave bands. i.e: red laser will also excite phycocyanine. The Cryptophytes are also Nanoeukaryotes
Microphytoplankton	Microphytoplankton	A group where the cluster is above Nanoeukaryotes based on average cytogram
largephyto3 - N/mL	Microphytoplankton	
largephyt2 - N/mL	Microphytoplankton	
largephyto1 - N/mL	Microphytoplankton	
Nanoeukaryotes 1	Nanoeukaryotes	It is a group above Picoeukaryotes in terms of FWS and FLR
Nanoeukaryote 2s	Nanoeukaryotes	
.....		

➔ Some propositions for standardization

3. Check the 34 existing codes (NERC vocabulary server)

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

conceptid	preflabel	modified	altlabel	definition	Deprecate Y/N	Alternative code
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_PSB1_auto	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.		
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_PSB2_auto	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.		
P18318A9	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_BE006316	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.		
C804B6A6	Abundance of Bacteria (ITIS: 202421: WoRMS 6) [Subgroup: heterotrophic; low nucleic acid cell content] per unit volume of the water body by flow cytometry	4/17/2016 15:50:07	Abund_BE006317	Number of particles resolved as heterotrophic bacteria cells from the low nucleic acid content cluster (LNA) 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.		

Conclusion

- MIO has been deploying Cytobuoy instruments for the last 10 years that allowed us to work on data management
- FCM processing tools have various output structures and formats ➔ There is a need to build a conversion tool. Mathilde's tool is a good example and can be developed to be more common.
- We need to check the 34 existing FCM standardized codes.
- 73 Captured parameters (VLIZ, MIO,RWS and ULCO) ➔ An urgent need to focus on functional group names definition : An average cytogram
- Standardizing FCM vocabulary will increase the visibility on EU plateform (SeaDataNet infrastructure) and join other datasets to make your data usefull to the community.


Thank you for building together
FCM standards
cnrs
mio
Institut Méditerranéen d'Océanologie
Aix*Marseille université

Discussion Group1: Harmonisation of FCM use and data

- The classification of functional group: Prochlorococcus, Picoeukaryotes, Synechococcus, coccolitophore, Cryptophytes, Nanophytoplankton, Microphytoplankton, Dinoflagellates, Cyanobacteria.
- Definition of group: There is no need for that because it is already done. Also, because of the different clustering and optical properties. For the moment, we can't define the group on size criteria (since it is not clear yet).
- Clustering defnition and method depend on the operator if he is using manual or auto. Thus, the intercomparaison exercice within the JERICO Next to determine the discrepancy of the output of the clustering by manual/Auto.
- Standardization of the protocol depends on regions
- Standardization of sensor by beads and by chlorophyll
- Existing stand. FCM PARAM (NERC): some are good but there is a lot of redundancy and definition are not clear. Very difficult to understand and not helpful at all.
- Another forum to discuss about standardization.