



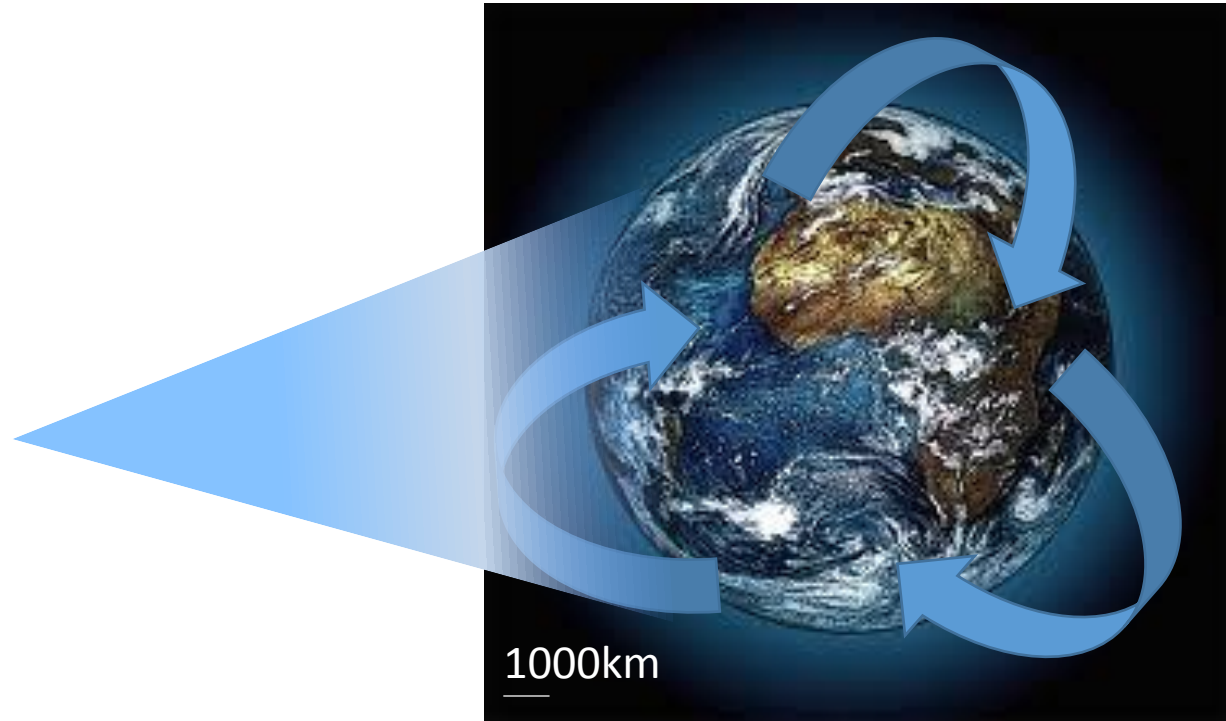
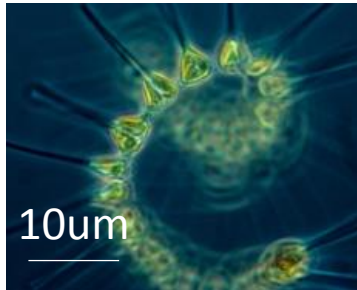
Deployment of automated flow cytometry on a FerryBox in the western Mediterranean Sea: Management and quality control of flow cytometry data

Soumaya Lahbib

M. Dugenne, M. Libes, M. Belhassen, A. Zouari, P. Marrec, N. Bhairy, G. Grégori, C. Sammari, S. Ben Ismail and M. Thyssen

Phytoplankton

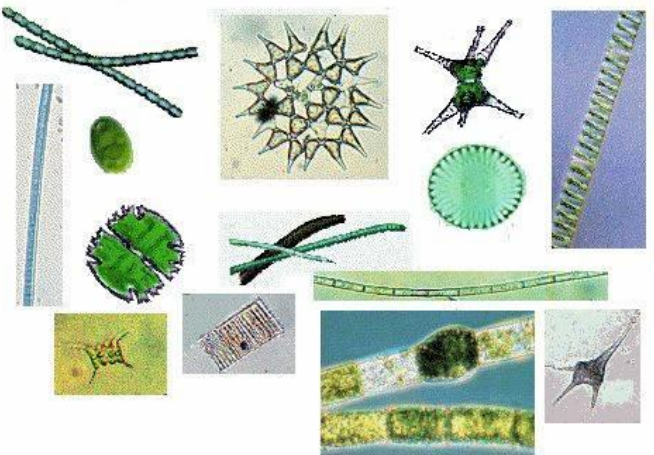
Thousands of species $< 1000 \mu\text{m}$ catalyze the most important geochemical processes for sustaining life on earth AND at a minute scale.



Phytoplankton produces between 45 and 57 Pg C Yr^{-1} of the NPP on earth ($\sim 45\%$) but represents $< 2\%$ of its biomass.
Very high turn-over rate !

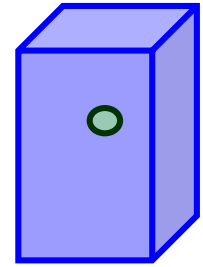
Phytoplankton observation is complex

Morphology and size

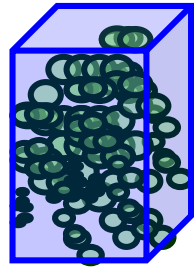


Abundances

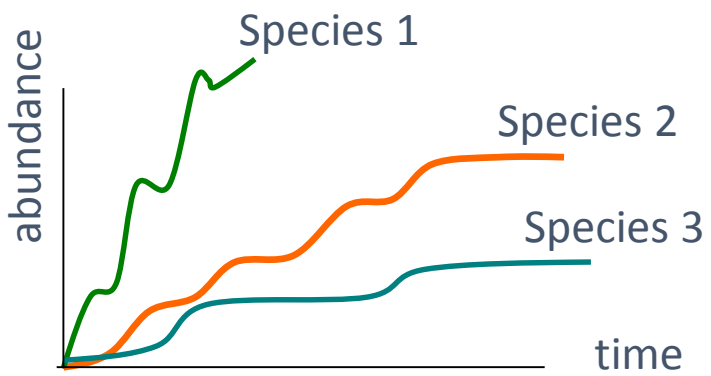
1 cell. cm^{-3}



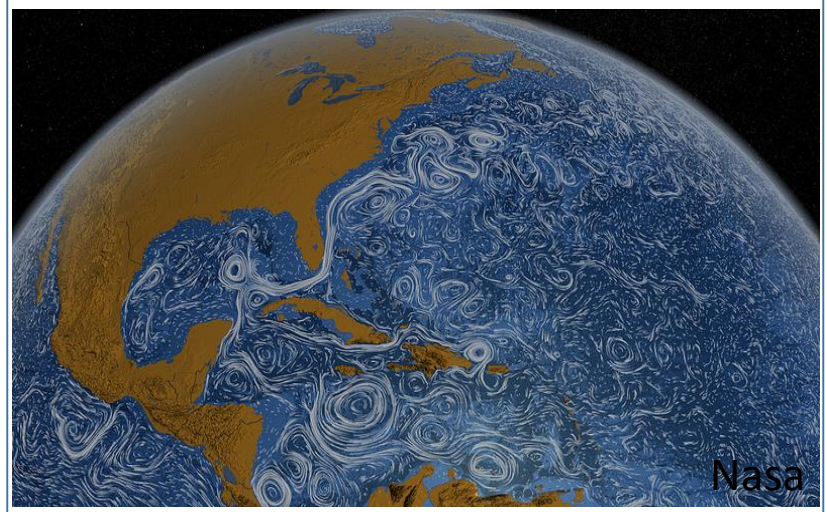
10^6 cells. cm^{-3}



Growth rates

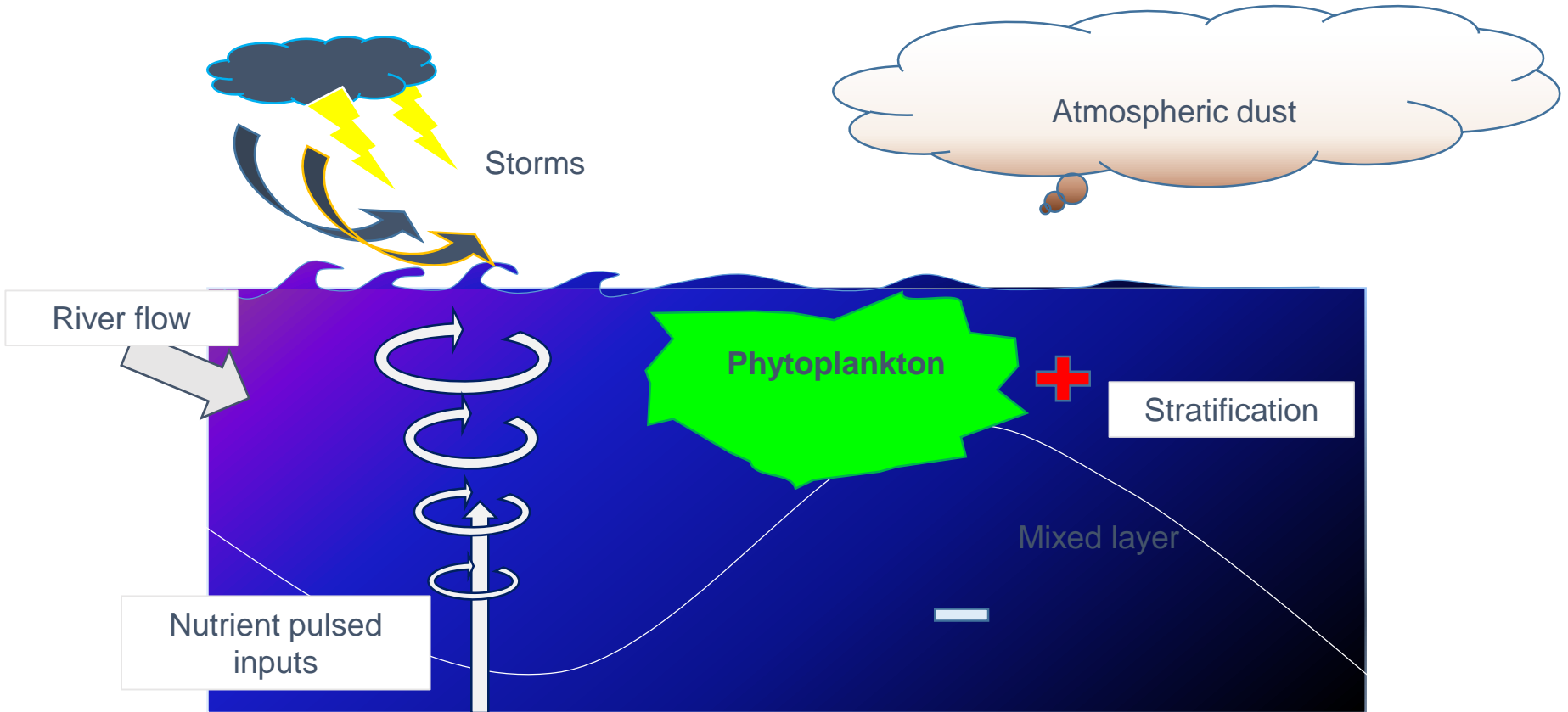


Turbulence

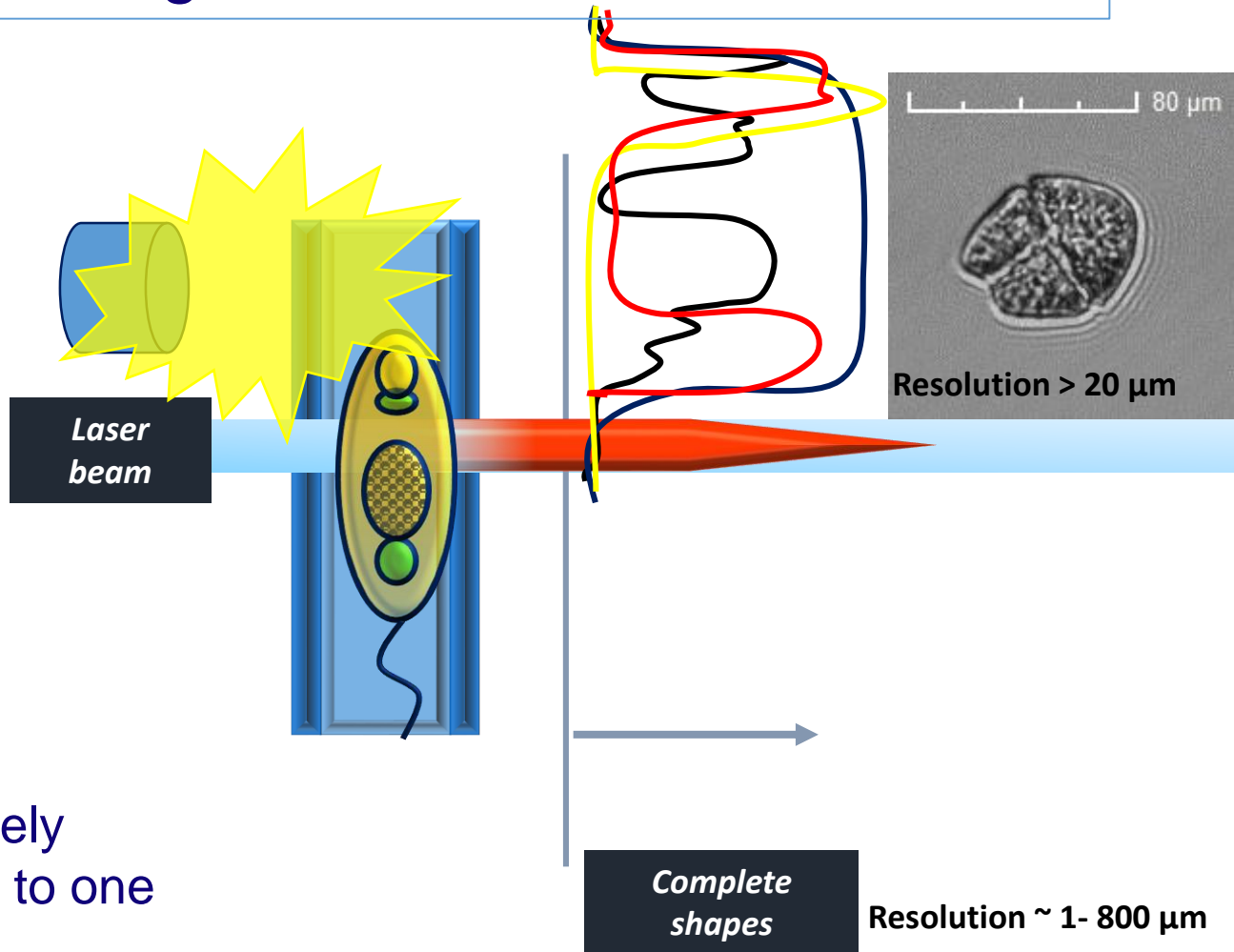
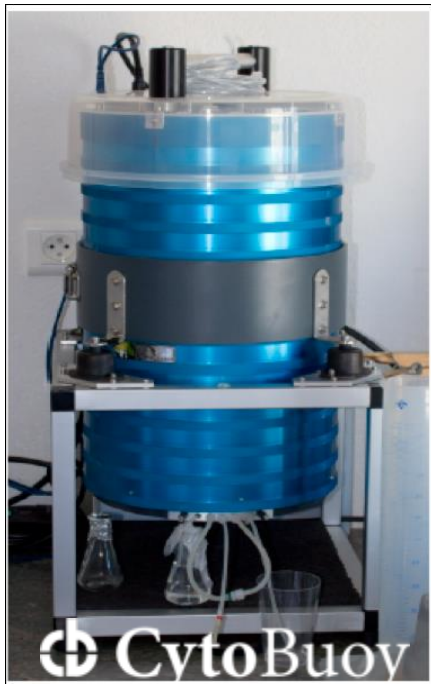


Serious lack in understanding and
quantifying the role of phytoplankton in
the biogeochemical processes

Short term variation and sporadic events impacts are nearly unknown

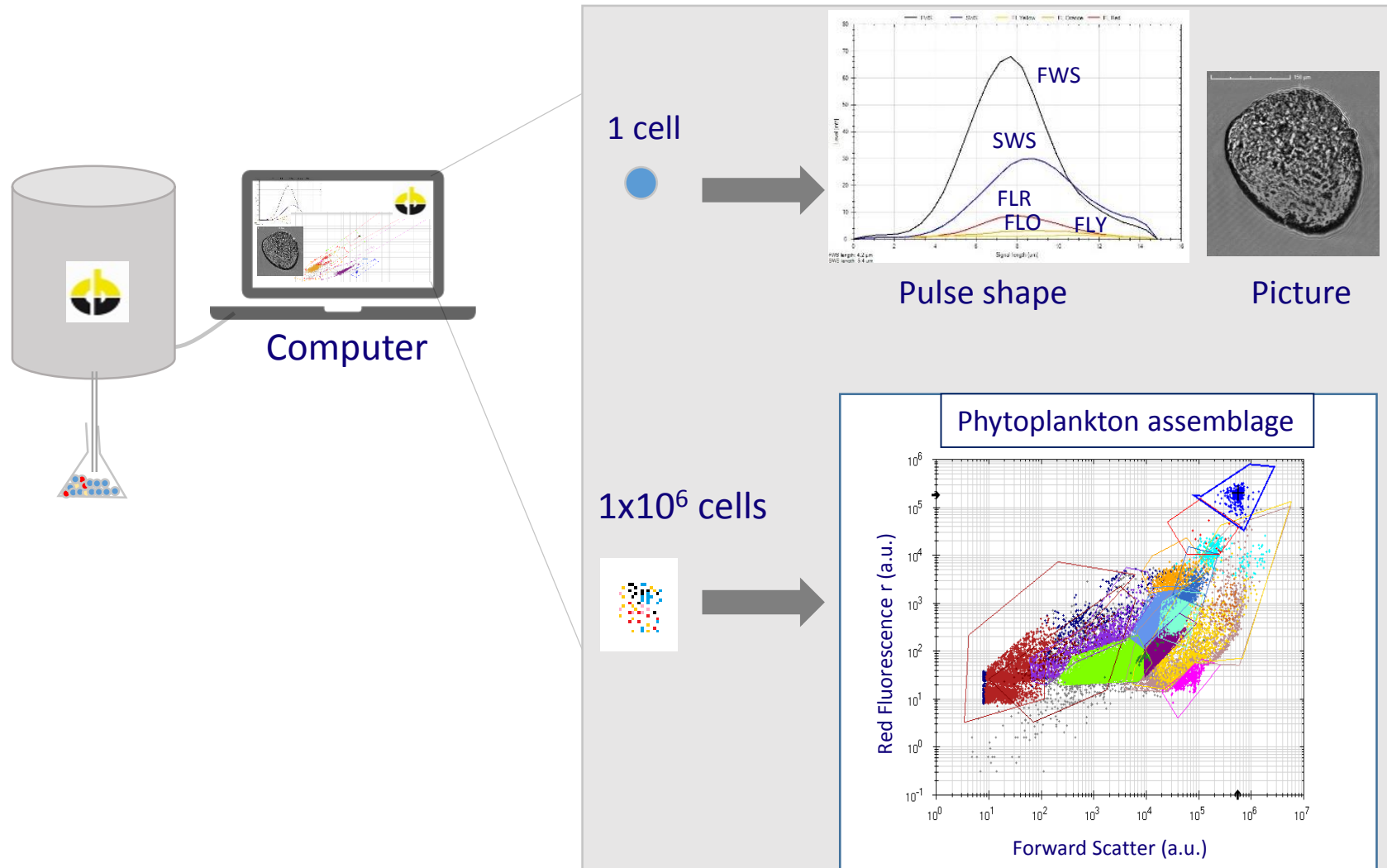


New technology for the resolution of phytoplankton functional diversity at hourly and regional scales



Automated and remotely controlled analysis up to one sample every 15 min.
Sample up to 5 cm³

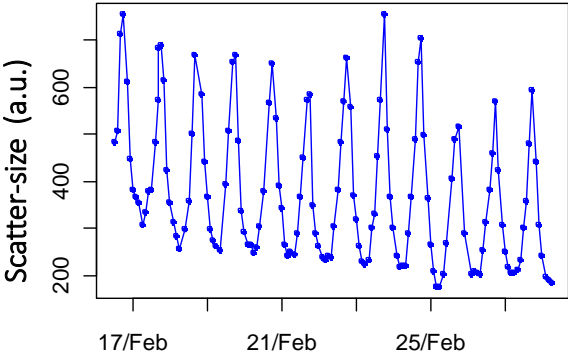
Phytoplankton functional groups resolution



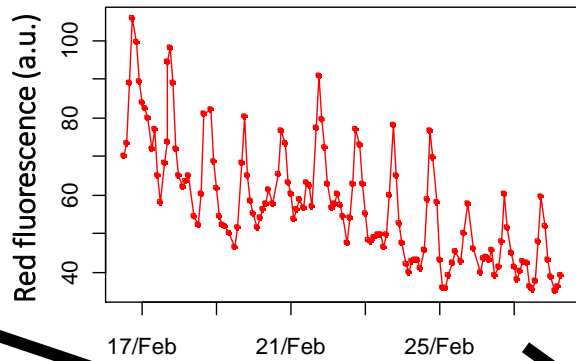
- Phytoplankton functional groups/Phytoplankton abundance per group
- Fluorescences/scatter per cell/Size estimation after calibration of scatter
- Phytoplankton images (taxonomical identification $>20 \mu\text{m}$)

Additional information extracted from the single cell approach:

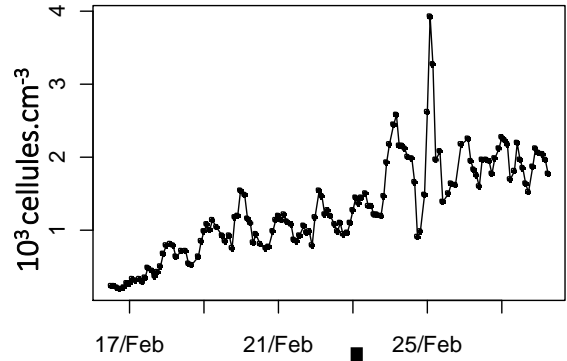
Scatter



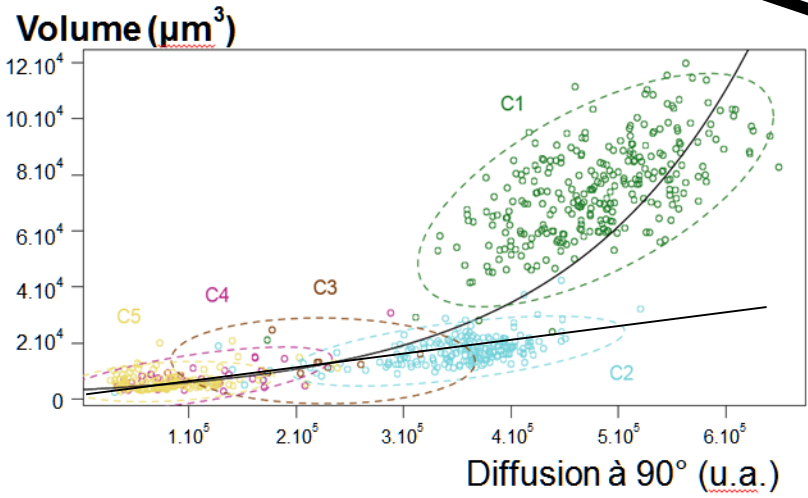
Fluorescence)



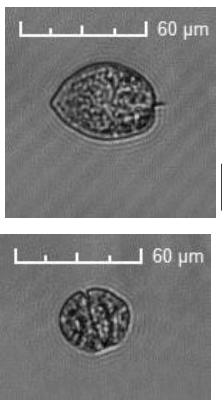
Abundances



Biovolume



Images

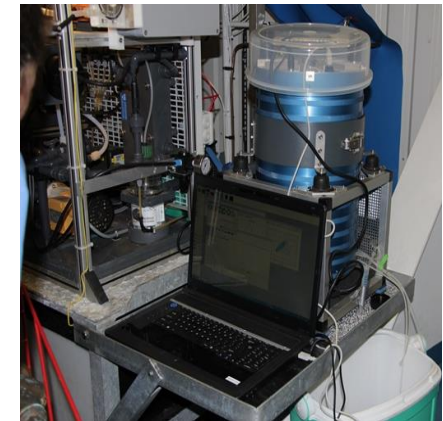
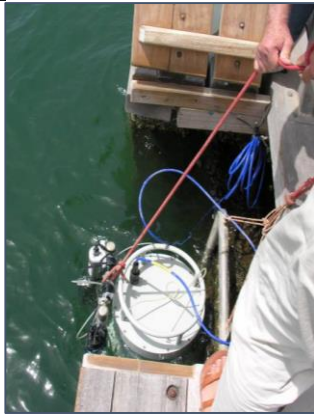


size-structured matrix population model:
In situ growth rate per phytoplankton cluster

André *et al.*, 1999; Sosik *et al.*, 2003;
Thyssen *et al.*, 2009, 2010,
Dugenne *et al.*, 2014, 2015.

Several scientific experiences were conducted with a relative autonomie up to 6 months

Scientific vessels Coastal platforms Ships of opportunity Buoys



Malkassian et al. 2011
Dugenne et al. 2014
Thyssen et al. 2008, 2009a.b, 2012, 2014, 2015





INSTM



CNRS UPMC INSU
Station Biologique
Roscoff

Institut de
Mathématiques
de Marseille,
UMR 7373



INGV

CHROME

Continuous High Resolution Observation of the
Mediterranean Sea:

<https://chrome.mio.univ-amu.fr/>

*Understanding of the ecological and
biogeochemical functioning in relation to
meso-scale dynamics at the Mediterranean
sub-basin scale and weekly scale.*



Step 1: Data Acquisition= one analysis every 30 min.

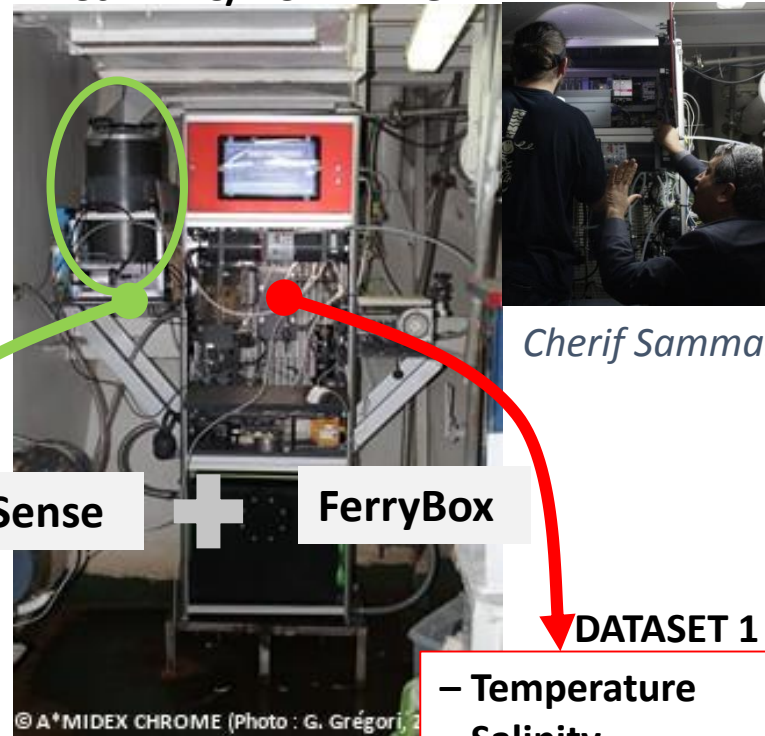
Deck 1 – C/F CARTHAGE



— C/F CARTHAGE trajectories

- Phytoplankton functional groups
- Phytoplankton abundance per group
- Fluorescences/scatter per cell
- Size estimation after calibration of scatter
- Phytoplankton images (taxonomical identification >20 μm)

DATASET 2



Cherif Sammari

CytoSense

FerryBox

DATASET 1

- Temperature
- Salinity
- Fluorescence
- Turbidity
- pH
- pCO₂
- Oxygen

Step 2: Data analysis



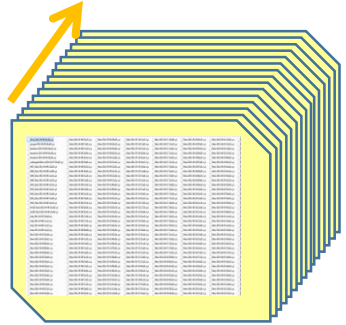
Measurements by CytoSense



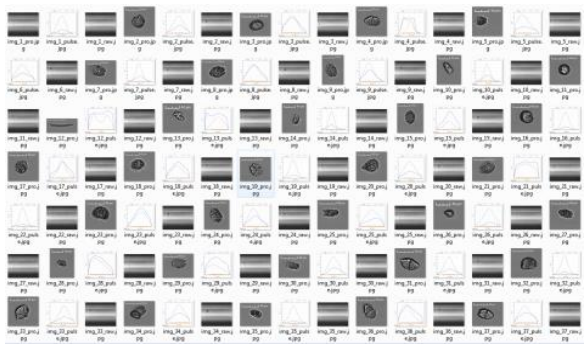
CytoClus

Output

Manual clustering



Backup



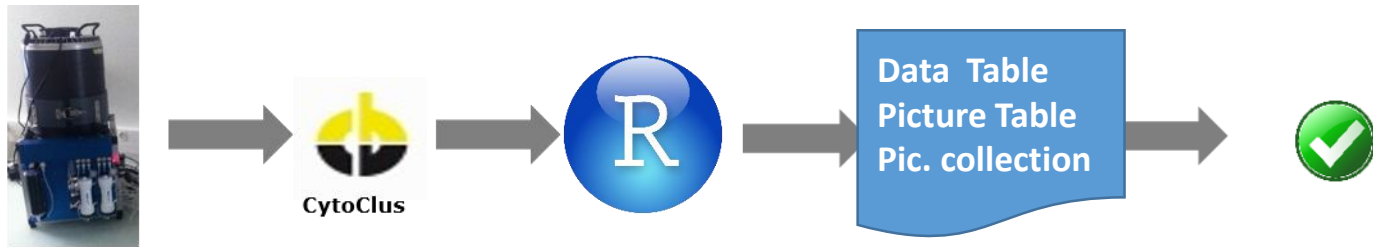
Separate statistical CSV files:
Average values of optical properties and Counts
 + Pictures

- Issues:**
1. Not a dedicated FCM database
 2. Data memory size consuming



THE CYTOBASE DATABASE

Step 3 : Data management Workflow



Data Acquisition

Batch Analyses

Data Consolidation

Expert QC



Data Integration



Step 4: Data consolidation

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 set, 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...



Cytobase input processor is a standalone software (R) by **Mathilde Dugenne** (PhD Student) in 2015. It aims to create raw data and metadata.

- ✓ Generate metadata about the project, the used instrument, the analysis methode, and the project operator
- ✓ Consolidate, aggregate and classify output data files
- ✓ Couple phytoplankton group names with standardised names
- ✓ Couple pictures with measured files
- ✓ Calculate size estimation from calibration procedure
- ✓ Generate picture and data table files interoperable with CYTOBASE



Cytobase Input Processor

2015-08-18

Parcourir... 11 fichiers sélectionnés.
Upload complete

Warning:

Metadata

Project and samples context Raw data Size conversion

Project Enter project name	Project date 2015-08-18	PI Enter PI name	Cytometer ID Enter cytometer ID
Station	Depth	Latitude	Longitude
Filename ▼	Filename model BERRE_082013_3F_FLR9 2013-12-17 13u		
Samples operator Enter name of operator	Standards reference Enter standards beads ref	Clustering method Automated ▼	Observation type 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	Picoeukaryotes 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 Cluster Beads 2 mu ▼	Trigger Channel/Level FL Red 10 ▼	PMT's amplification SWS 0 100 50	Standardized name Cluster Standard beads ▼	Associate
FLO				



Continuous and High Resolution Observation of the Mediterranean Sea



7th FerryBox Workshop

Picture selection

Project and samples context Raw data Size conversion **Image-In-Flow pictures** Stations explorer

Add samples pictures

Select file: DEWEXL2FLR10 2013-04-05 15u04.cyz

Add pictures: Choisir les fichiers 15 fichiers

Check all

Upload complete

Show 4 entries Search: _____

Stations Explorer

Project and samples context Raw data Size conversion **Image-In-Flow pictures** Stations explorer

Select station: S1

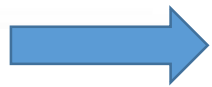
X axis: Mean Total FWS

Y axis: Mean Total FLR

Scatter plot

Leaflet | © OpenStreetMap contributors, CC-BY-SA

Download Table



Data Table
Picture Table



Step 5: Data integration into CYTOBASE

Info Tables Columns Indexes Triggers Views Stored Procedures Functions Grants Events

CytoBase_Local
cytobase_v6

Schema Details

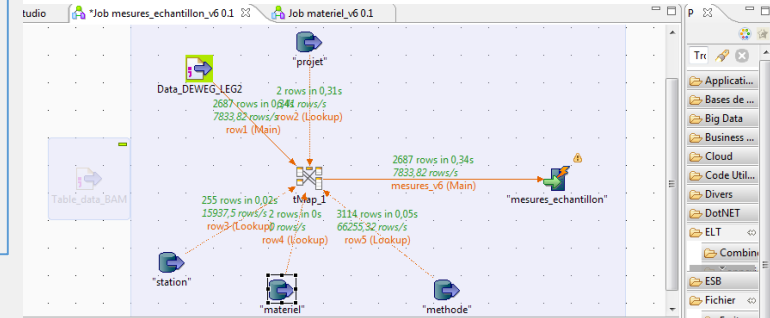
Default collation: **latin1_swedish_ci**

Default character set: **latin1**

Table count: **7**

Database size (rough estimate): **5.8 MiB**

- 7 tables
- 6 associations
- 69 columns



Designer | Code

Job(mesures_echantillon_v6.0.1) | Contexts(mesures_echantillon_v6) | Composant | Exécuter (Job mesures_echantillon_v6)

Job mesures_echantillon_v6

Exécution simple Exécution Debug Paramètres avancés Target Exec

Exécution

Exécuter Arrêter Effacer

```

tMap_1
Démarriage du job mesures_echantillon_v6 à 16:23 17/02/2016
[statistics] connecting to socket on port 3853
[statistics] connected
[statistics] disconnected
Job mesures_echantillon_v6 terminé à 16:29 17/02/2016. [Code écriture]
  
```

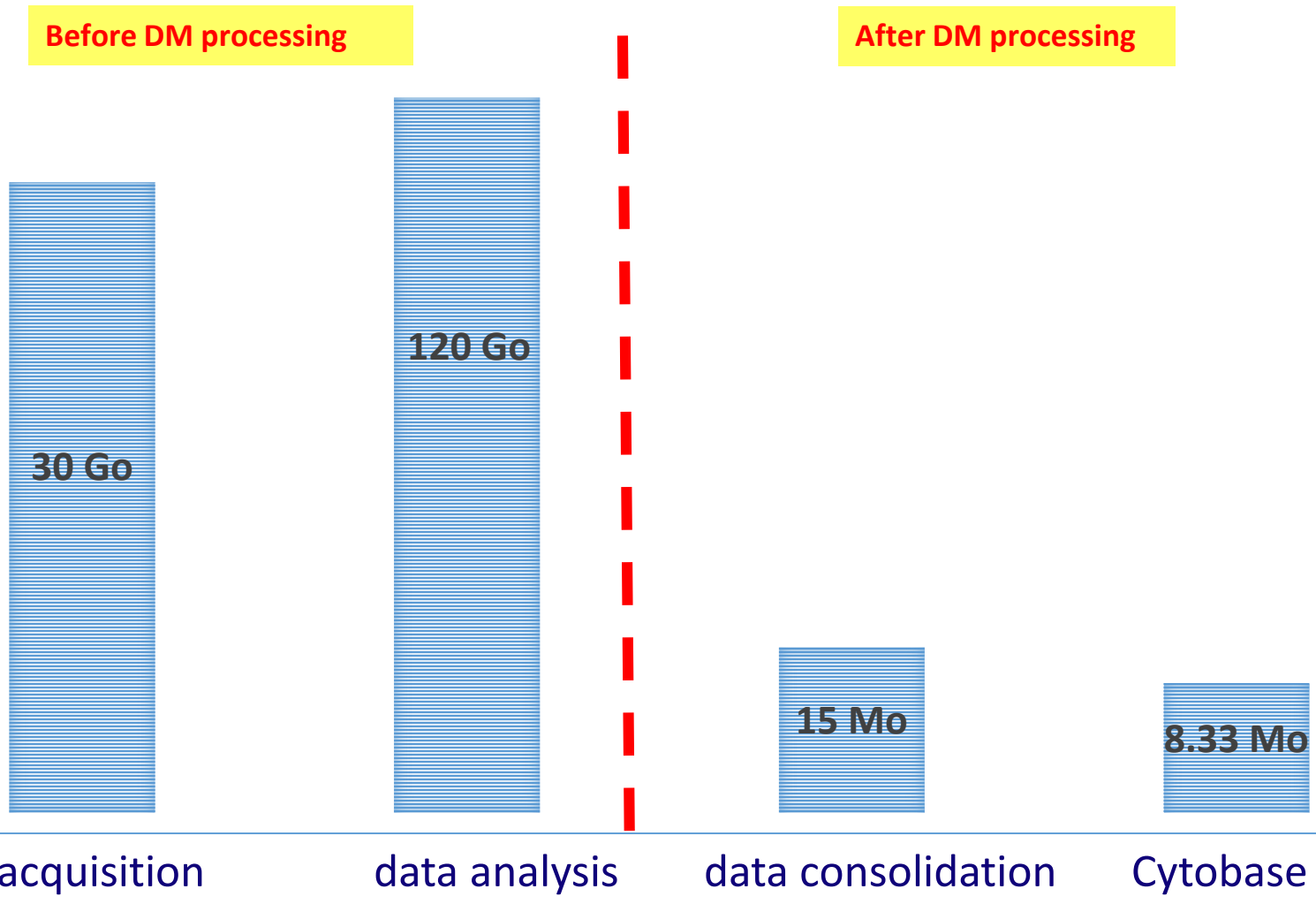
Nombre limite de lignes 100 Retour automatique à la ligne



ETL



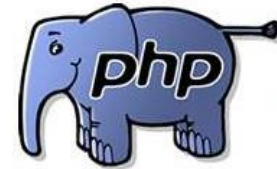
- Case of a 9 days Cruise : 1 sample / 20 min





Step 6: Data retrieval and accessibility

✓ Web Developpement



✓ Access to CYTOBASE





V. Conclusion & Perspectives

- Smart storage and sustainability → Net decrease on file size
- Dynamic and user friendly web-based interface
- Deployment of CYTOBASE (*In progress*)
- Working on international standardization and interoperability with SeaDataNet Pan-European Infrastructure.



Continuous and *High Resolution* Observation of the *ME*diterranean Sea



7th FerryBox Workshop

Thank you for your attention

Any questions?