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

Soumaya Lahbib
Phytoplankton

Thousands of species < 1000 µm 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 (~45%) but represents <2% of its biomass. Very high turn-over rate!
Phytoplankton observation is complex

Morphology and size

Abundances

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

Resolution > 20 µm

Resolution ~ 1-800 µm

Laser beam

Complete shapes
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 µm)
Additional information extracted from the single cell approach:

- Scatter
- Fluorescence
- Abundances
- Images
- Biovolume

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,
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
Continuous High Resolution Observation of the Mediterranean Sea:

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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.

- Phytoplankton functional groups
- Phytoplankton abundance per group
- Fluorescences/scatter per cell
- Size estimation after calibration of scatter
- Phytoplankton images (taxonomical identification >20 µm)
Step 2: Data analysis

Measurements by CytoSense

Output

Manual clustering

Backup

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

THE CYTOBASE DATABASE

Issues:
1. Not a dedicated FCM database
2. Data memory size consuming
Step 3: Data management Workflow

- Data Acquisition
- Batch Analyses
- Data Consolidation
- Expert QC

Data Table
Picture Table
Pic. collection

CytoClus

Data Integration

CYTOBASE
## Step 4: Data consolidation

<table>
<thead>
<tr>
<th>Output data files</th>
<th>Format</th>
<th>Number</th>
<th>Parameters</th>
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<td>csv</td>
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<td>Selection set, File name, Date, Time, Total Analyzed Volume [mL], Number of Particles, Min. TOF, Max. TOF, Mean TOF, etc...</td>
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<tr>
<td>avgprops_process_xx-xx-xxxx</td>
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<tr>
<td>etc...</td>
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<tr>
<td><strong>Count</strong></td>
<td>csv</td>
<td>1 or many</td>
<td>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...</td>
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<td>etc...</td>
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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 methods, 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

**Metadata**
- **Project**: Enter project name
- **Project date**: 2015-08-18
- **Station**: Enter station name
- **Depth**: Enter depth
- **Latitude**: Enter latitude
- **Longitude**: Enter longitude
- **Filename model**: BERRE_022013_3F_FLR9 2013-12-17 13
- **Samples operator**: Enter name of operator
- **Standards reference**: Enter standards beads ref
- **Clustering method**: Automated
- **Observation type**: In situ

**Data**

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</tbody>
</table>

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

**NB**: All incompatible entries will be removed.

- **Expert name**: Cluster
- **Trigger**: Channel/Level
- **PMT's amplification**: SWS
- **Standardized name**: Cluster

Associate
Step 5: Data integration into CYTOBASE

- 7 tables
- 6 associations
- 69 columns

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

Before DM processing

30 Go

120 Go

After DM processing

15 Mo

8.33 Mo

Data acquisition data analysis data consolidation Cytobase

https://chrome.mio.univ-amu.fr/
Step 6: Data retrieval and accessibility

- Web Developpement
  - HTML
  - CSS
  - JavaScript
  - PHP
  - MySQL
  - AJAX
  - jQuery

- Access to CYTOBASE

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V. Conclusion & Perspectives

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

https://chrome.mio.univ-amu.fr/
Thank you for your attention

Any questions?

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