GlobalHAB symposium on automated in situ observations of plankton
Kristineberg Marine Research Station, Fiskebäckskil, Sweden
August 22-27, 2022
Session 1

Using the Cytosense automated imaging flow cytometer for HAB observations

Luis Felipe Artigas, Arnaud Louchart, Zéline Hubert, Clémentine Gallot, Simon Bonato, Alexandre Epinoux

Laboratory of Oceanology and Geosciences
CNRS – UMR 8187 LOG – ULCO
Wimereux (France)

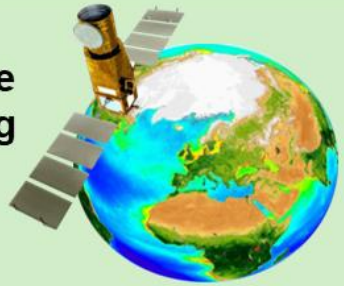
Approaches for phytoplankton observation

Research / Opportunity Vessels

Moorings

Gliders

Remote sensing

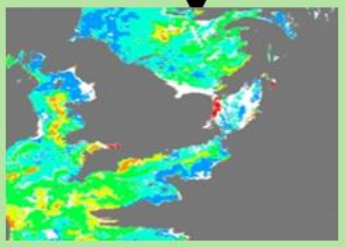


Monitoring strategy

Discrete sampling

Profiles

Continuous pumping/recording



Synoptic/regional

Sampling strategy

At the laboratory/ On board

Real time

Real time

Real time

Analysis

Microscopy
Pigment analysis
Total/Spectral Fluorometry
Flow cytometry
Image Analysis
Molecular tools

In vivo
Total/Spectral Fluorometry
Optic probes
Automated Flow cytometry
Image Analysis

In vivo
Total/spectral Fluorometry
Automated Flow cytometry
Image Analysis
Molecular tools

Ocean optics from satellites
Development of new Algorithms
Comparison with *in situ* data

Techniques

www.dymaphy.eu

Phytoplankton / HAB monitoring challenges

Changes in **phytoplankton abundance, biomass and composition** usually occur at **short-time and fine spatial scales** : need for **high resolution automated sensors** implemented in **autonomous platforms** (buoys, automated stations, research vessels, ships of opportunity)

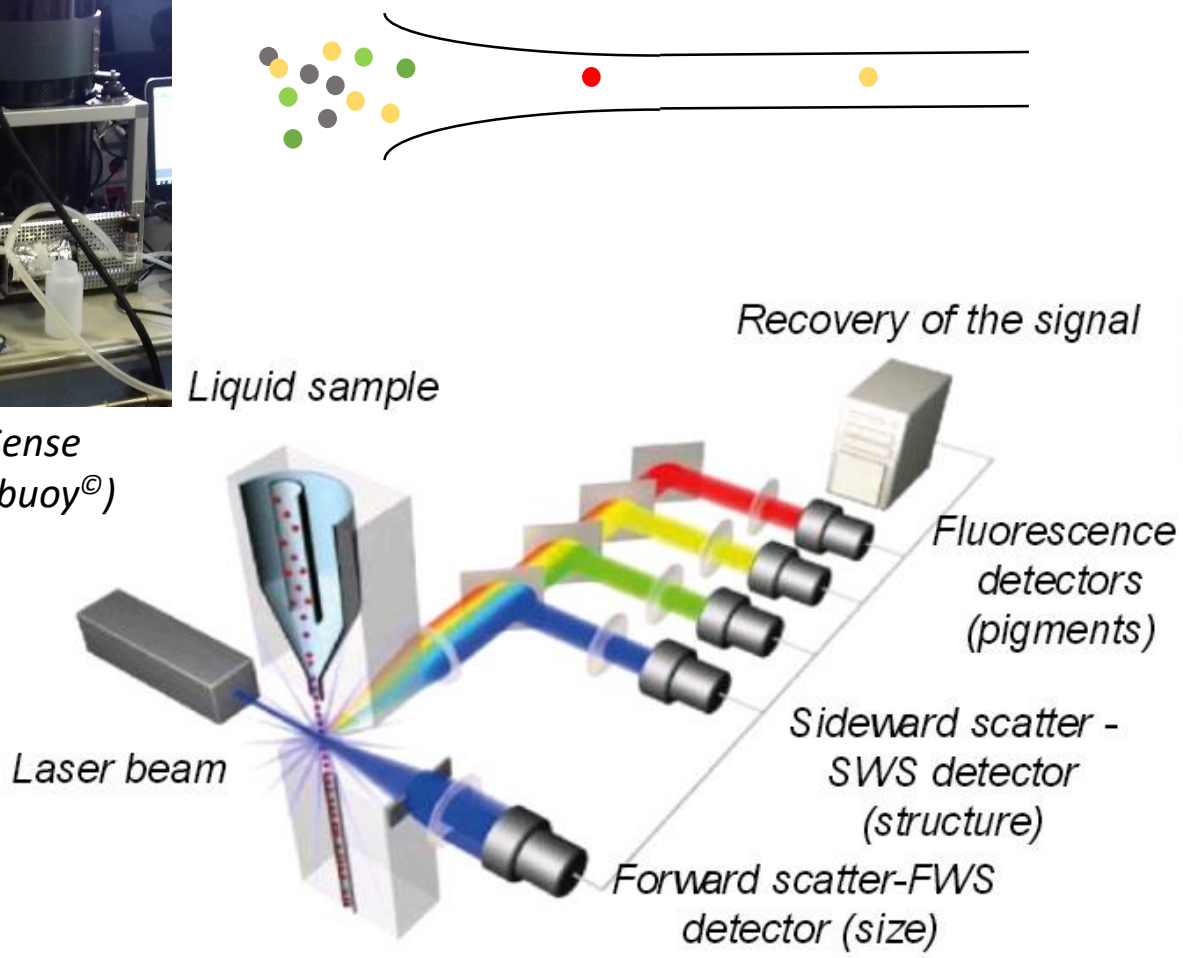
Critical gaps :
- lack of definition of the **most appropriate methodologies** and approaches (platforms)
- deficiencies in the **spatio-temporal distribution of observations**
- not adopting **FAIR principles in data distribution**, including using adequate QA/QC measures

An **international network of experts is essential to work on** :
- best suitable **combination of *in vivo* automated sensors** for each marine system considered
- harmonizing operational practices for defining common **best practices**
- **defining common vocabulary** and data quality control, **data charts & flows**

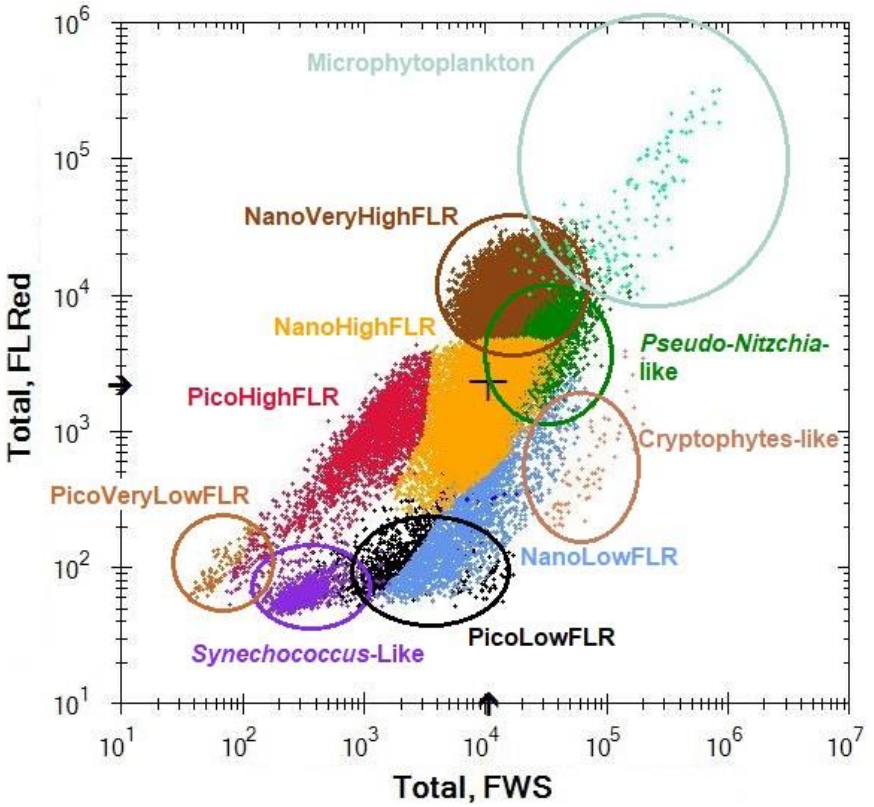
Investigation *carried out in the frame of national and regional marine & coastal programmes in Europe, including past projects INTERREG IVA « 2 Seas » **DYMAPHY** (2010-2014), H2020 **JERICO-Next** (2015-2019) & CPER **MARCO** (2016-2021), Pan-European infrastructure for ocean & marine data management (**SeaDataNet),** as well as ongoing ones **JERICO S3** (Science, Service, Sustainability - 2020-2024), **JERICO DS,** for building an European Research Infrastructure for Joint Coastal Observatories **(JERICO RI)** and recently approved projects as **OBAMA Next** (H2020, 2023-2026), amongst others.*

Pulse shape-recording automated imaging flow cytometry CytoSense®/CytoSub® (Cytobuoy©)

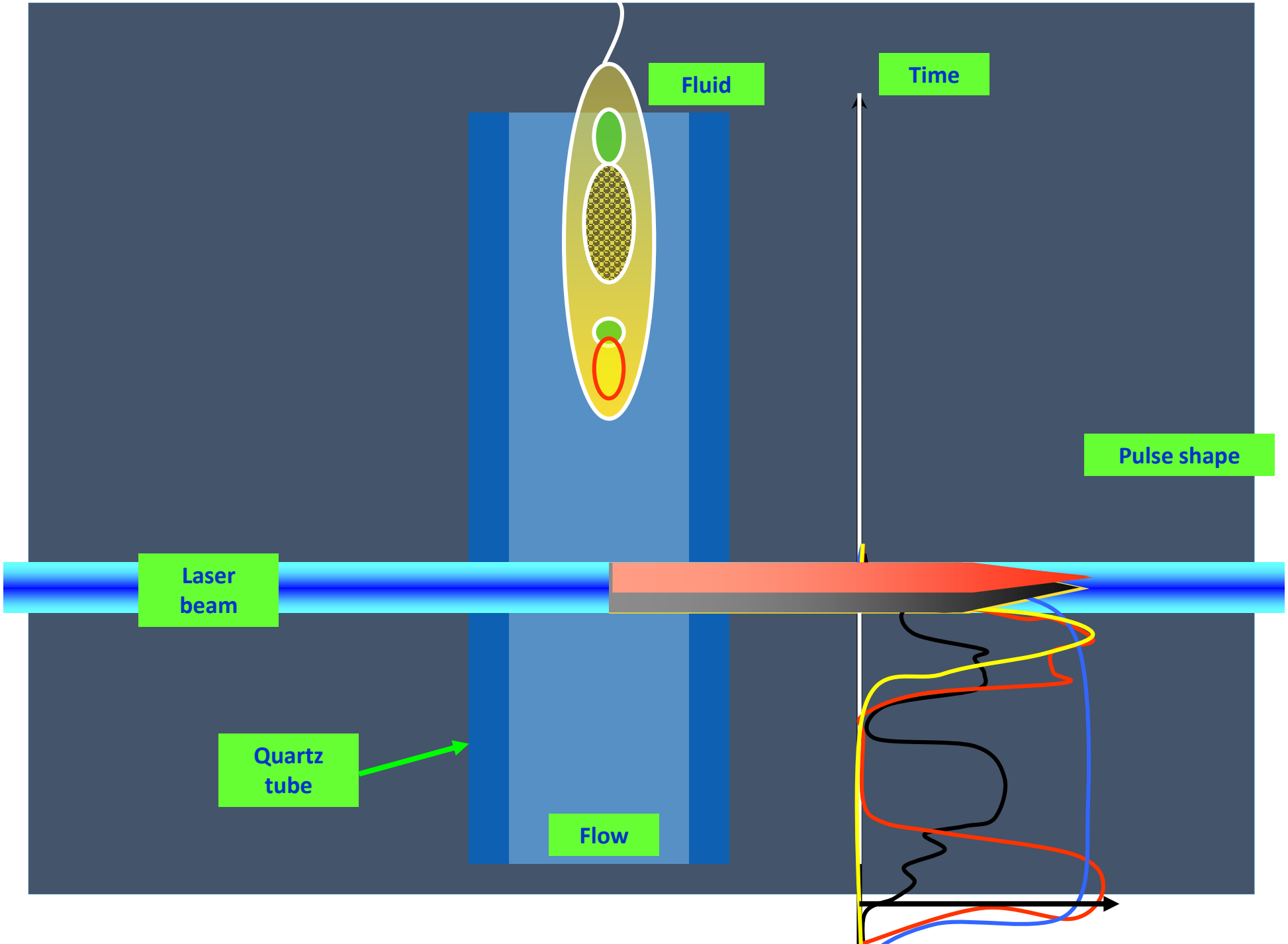
CytoSense (Cytobuoy©)



Principle of the PSFCM: from 1 to 800 µm width… and few mm

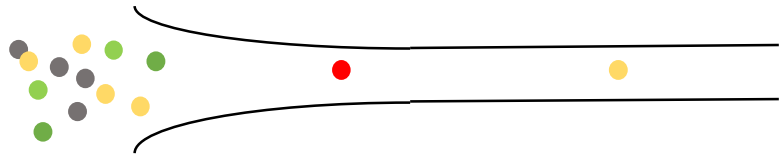


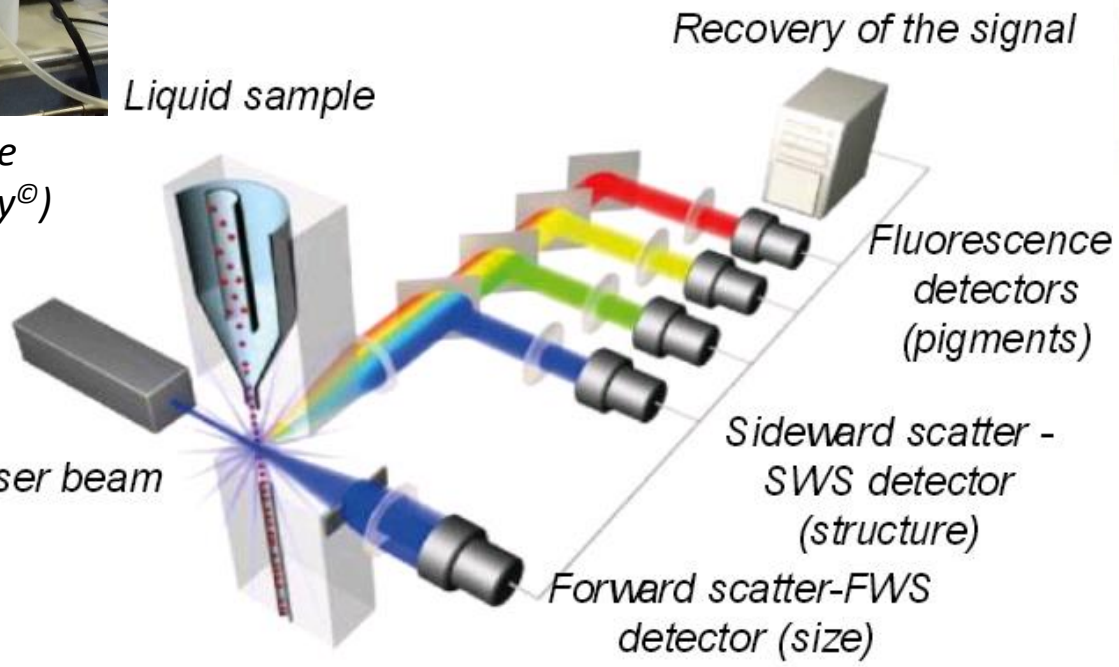
Example of Cytogramme from the PHYCO cruise (EEC, April 2017) with CytoClus 3 software by manual gating


Fluid
Time
Pulse shape
Laser beam
Quartz tube
Flow

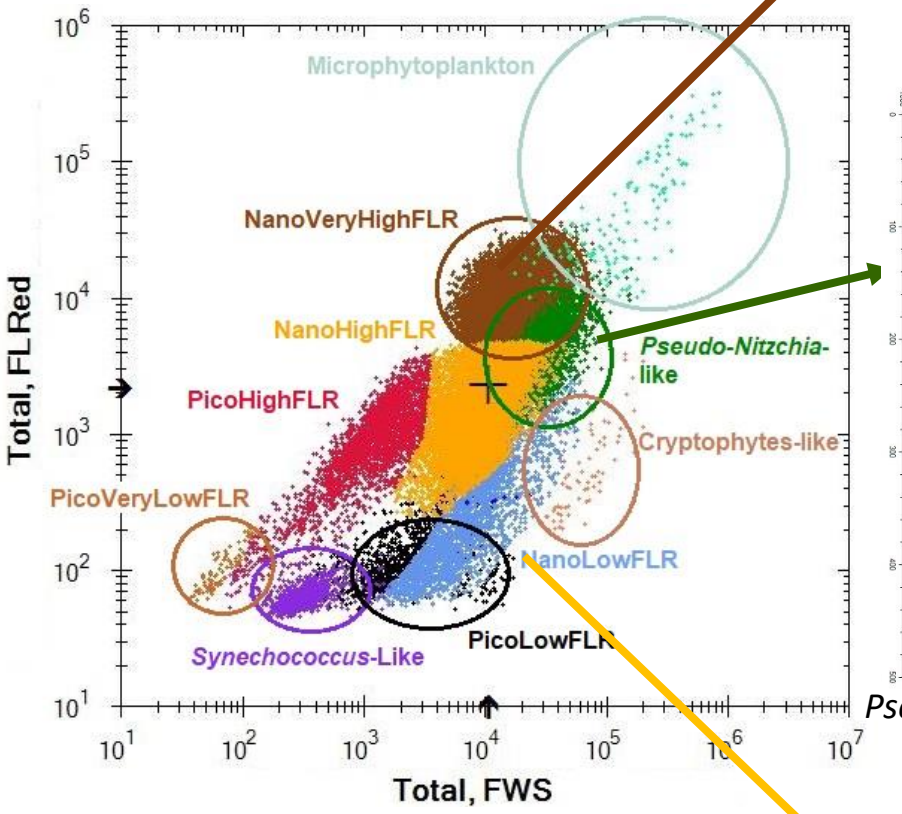
Pulse shape-recording automated imaging flow cytometry CytoSense®/CytoSub® (Cytobuoy©)

CytoSense (Cytobuoy©)

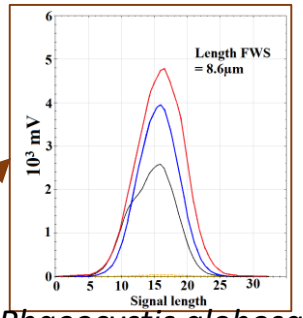




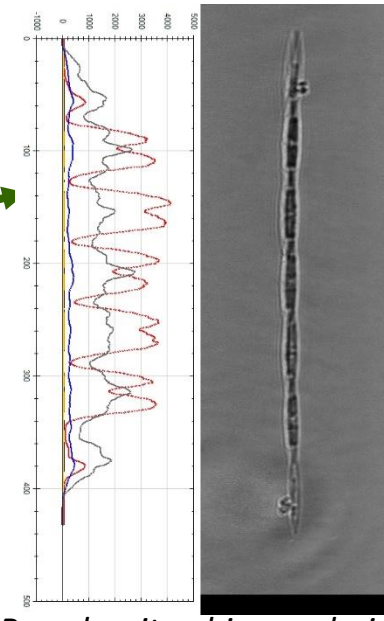
Principle of the PSFCM: from 1 to 800 µm width… and few mm length



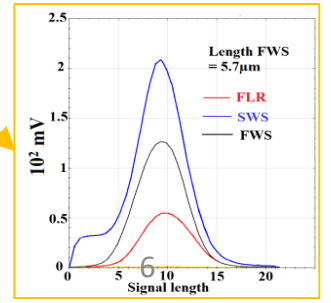
Example of Cytogramme from the PHYCO cruise (EEC, April 2017) with CytoClus 3 software by manual gating



Phaeocystis globosa diploid stage



Pseudo-nitzschia sp. chair

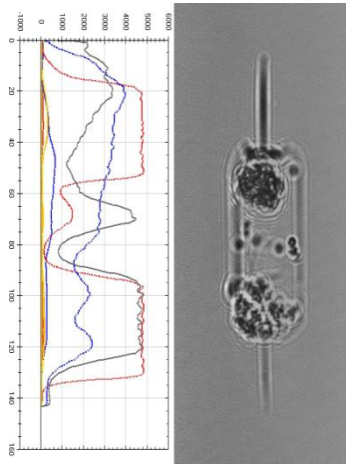


Phaeocystis globosa haploid stage

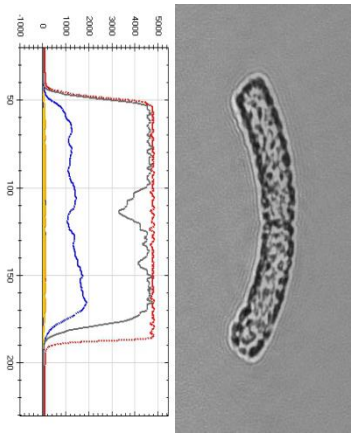
A. Louchart (PhD, 2020)

Integration of the image acquisition system, for the improvement of the detection of phytoplankton species

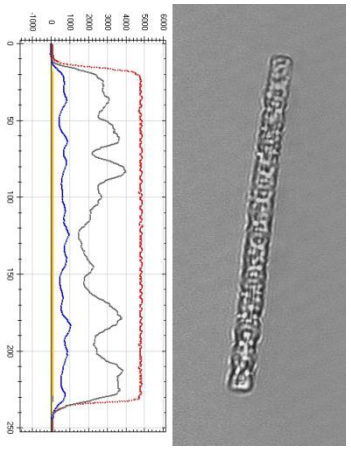
→ Library of cytometric scanned profiles and images : example of diatoms



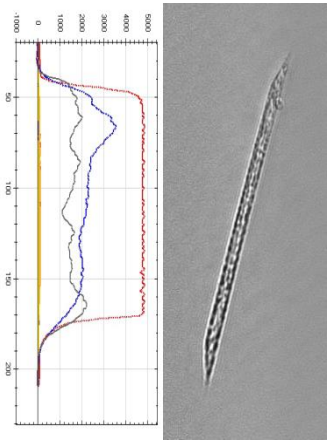
Ditylum brightwellii



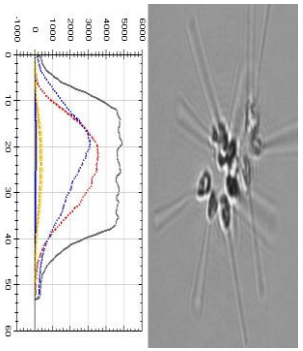
Guinardia striata



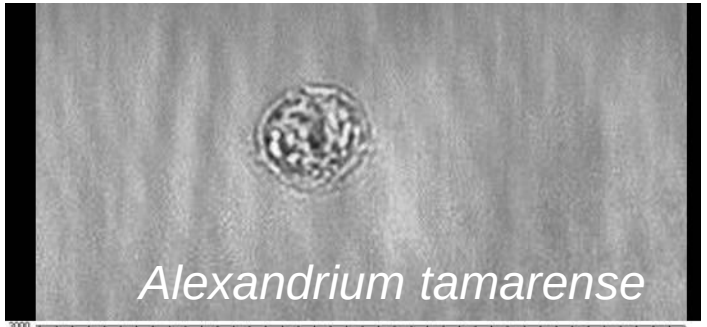
Guinardia delicatula

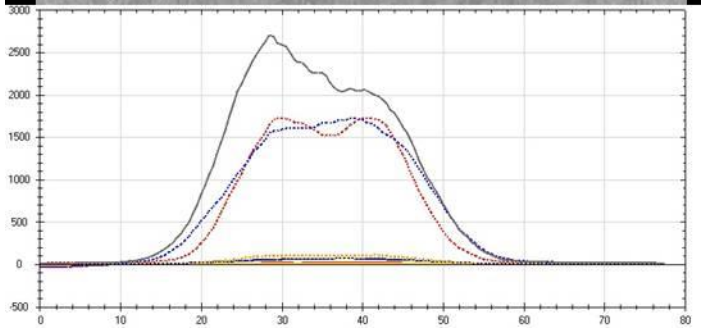


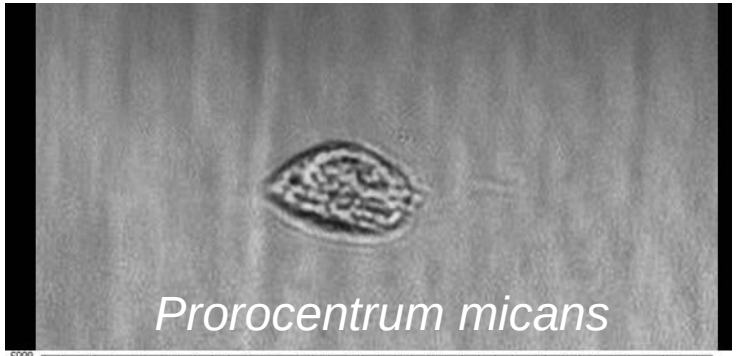
Rhizosolenia imbricata var. *shrubsolei*

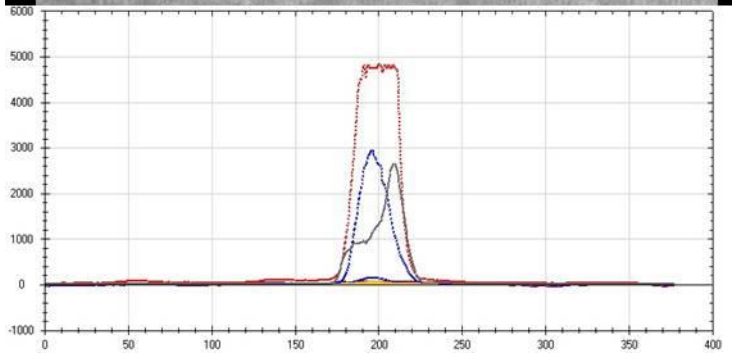


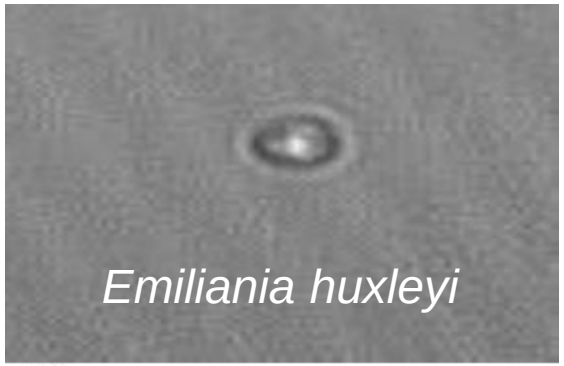
Asterionellopsis glacialis

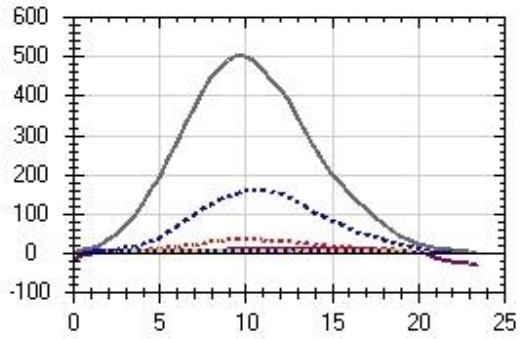

Alexandrium tamarense

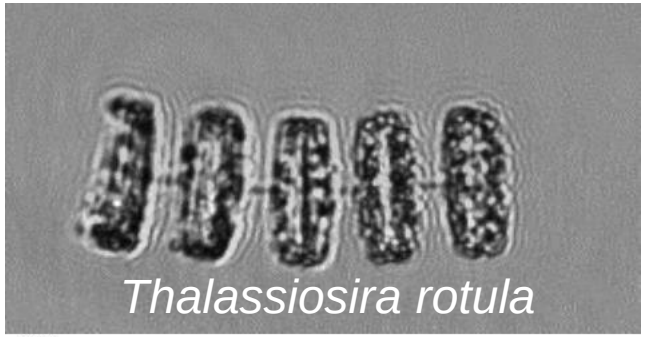


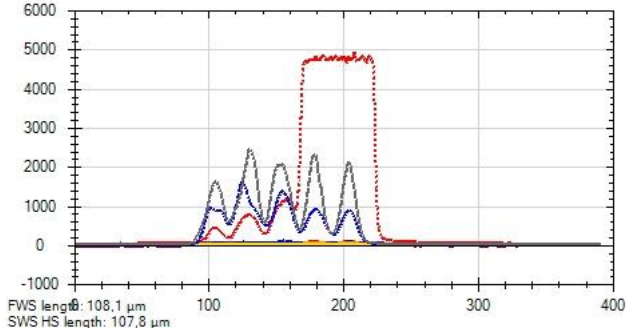

Prorocentrum micans

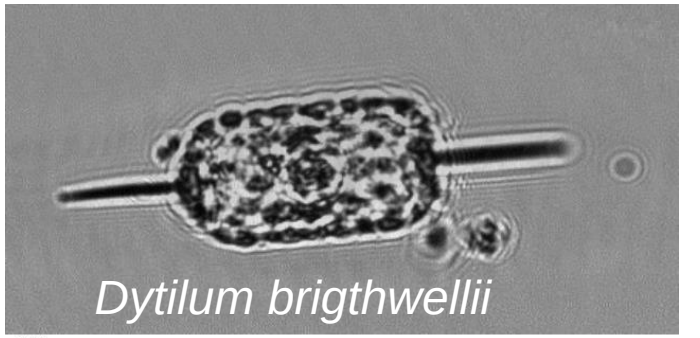


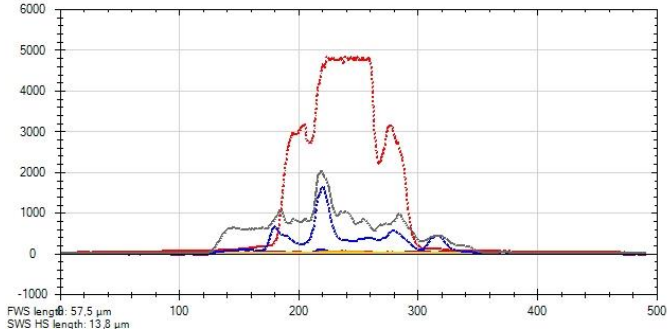

Emiliania huxleyi



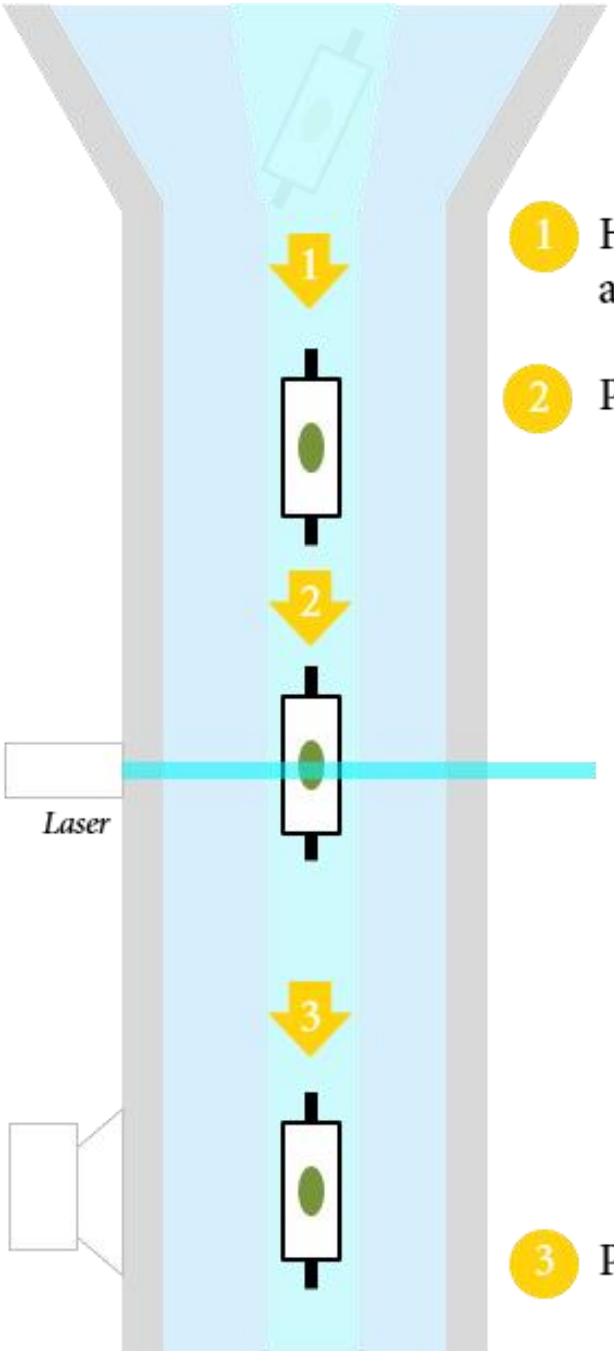

Thalassiosira rotula


FWS length: 108,1 µm
SWS HS length: 107,8 µm


Dytilum brigthwellii

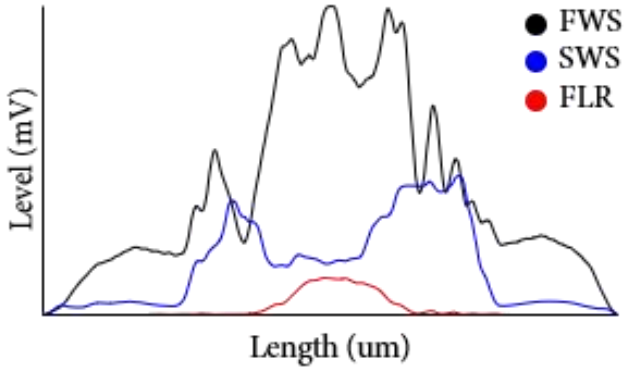

FWS length: 57,5 µm
SWS HS length: 13,8 µm

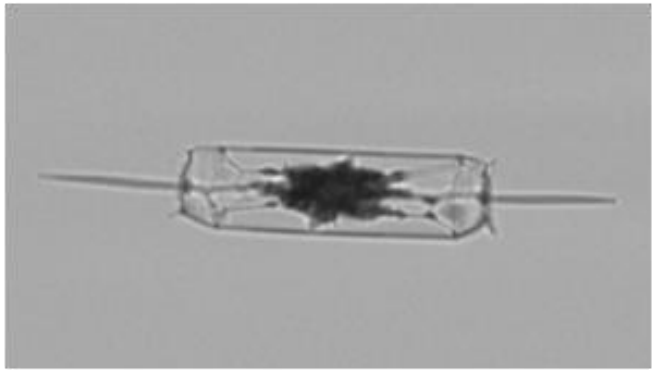
Imaging In Flow (IIF)



1 Hydronamic alignment of analyzed particles

2 Particle is scanned by the laser



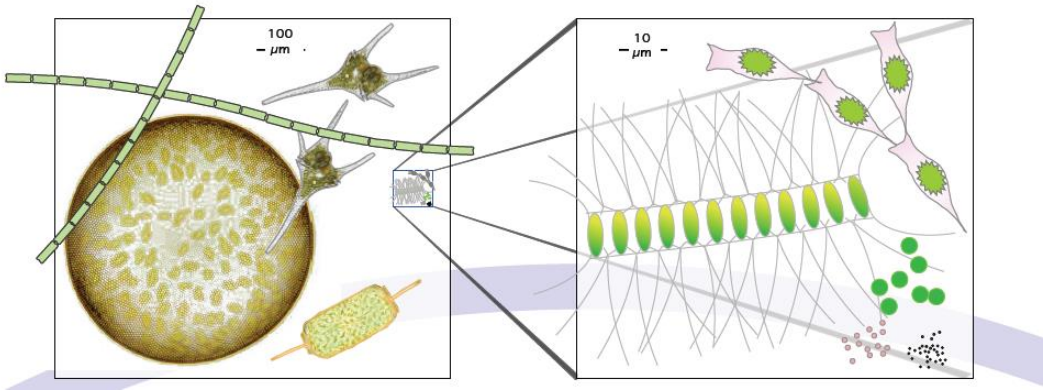


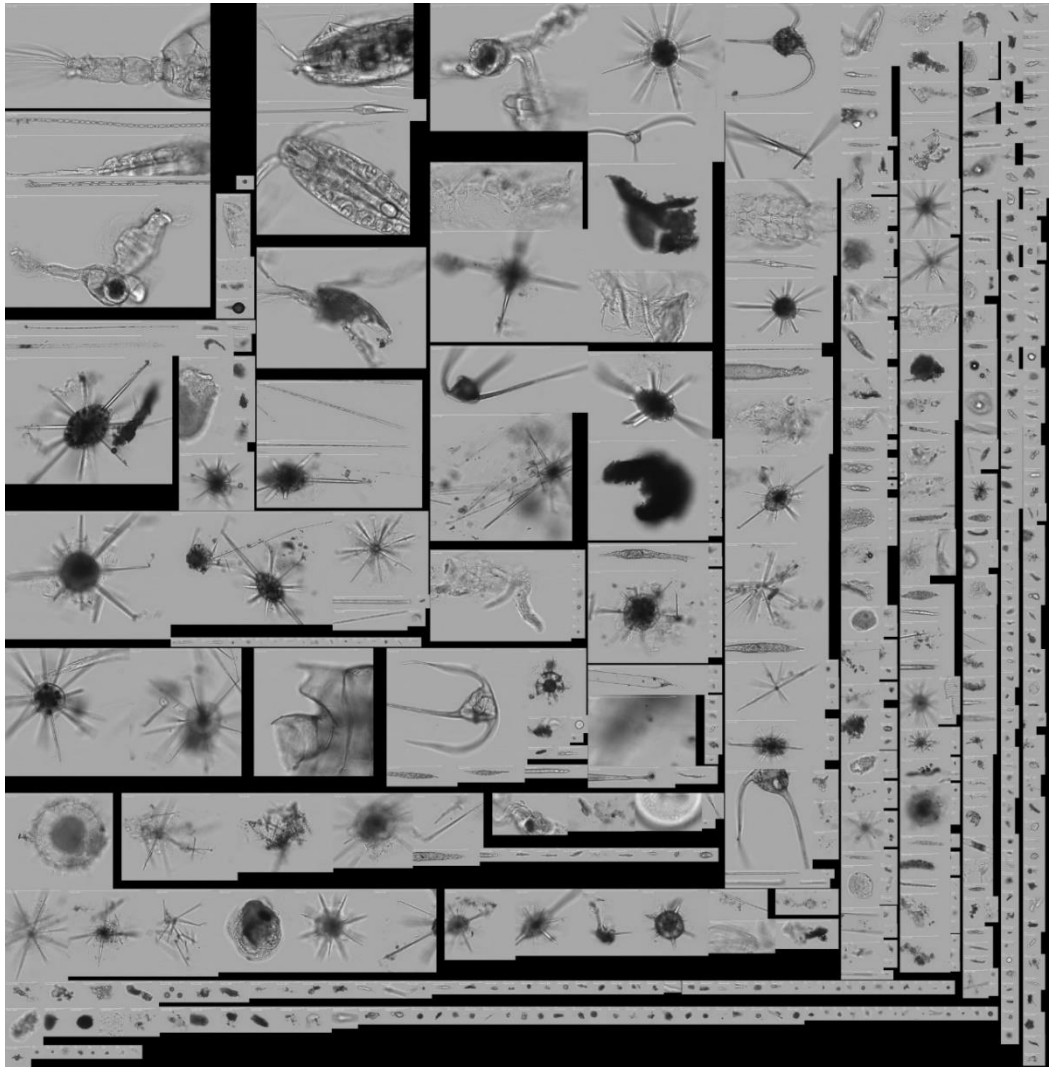
3 Particle is imaged by the camera

CytoBuoy

Imaging In Flow (IIF)

- High quality images at 16x magnification with a very big flow cell
- The CytoSense image resolution is <1µm, with ‘only’ 16x magnification for the whole particle size range
- This 16x imaging system is mounted on a fixed flow cell of 1000 x 1200 µm cross section and has very large field of view
- Optical resolution is ca. 0.8 µm as confirmed by photographing small beads doublets.

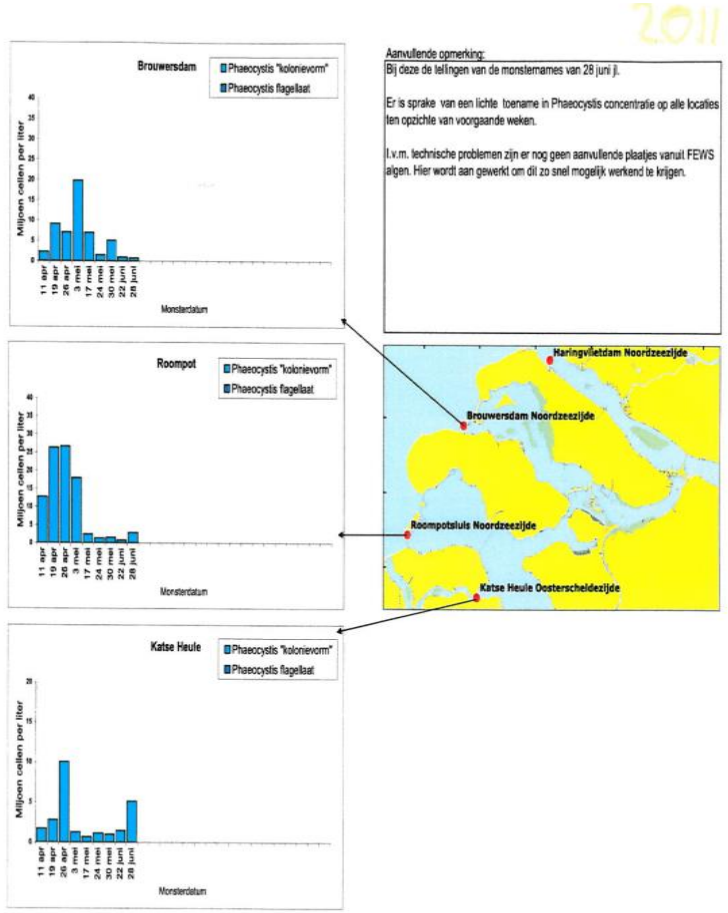


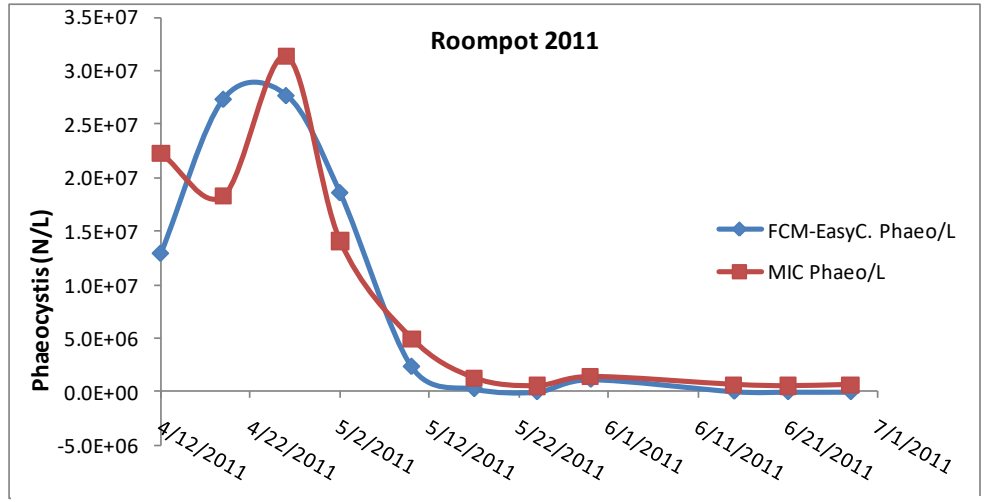


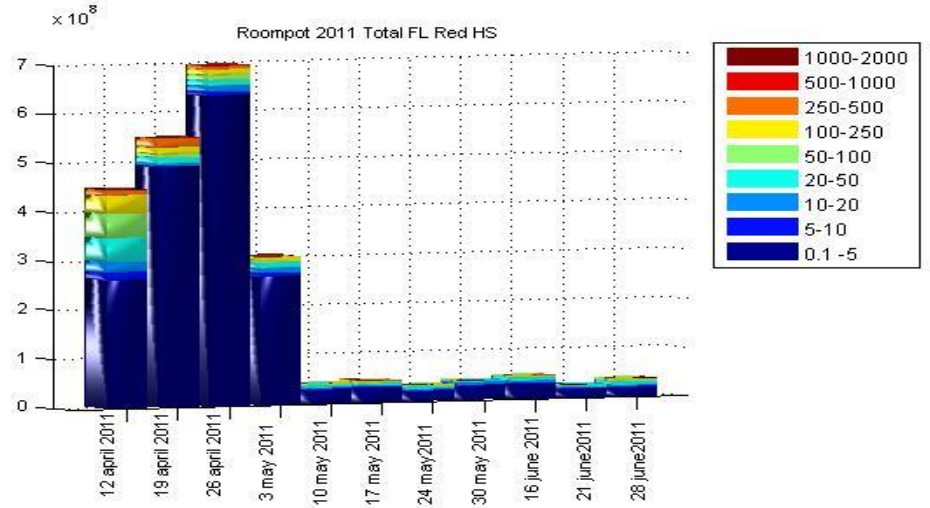
Case study in estuarine waters monitoring : Monisnel (NL)

Flowcytometry for routine long term monitoring: Daily practice

- Standard operation procedure
- Long term stability control
- Shewart charts
- Suitable software for objective data analysis



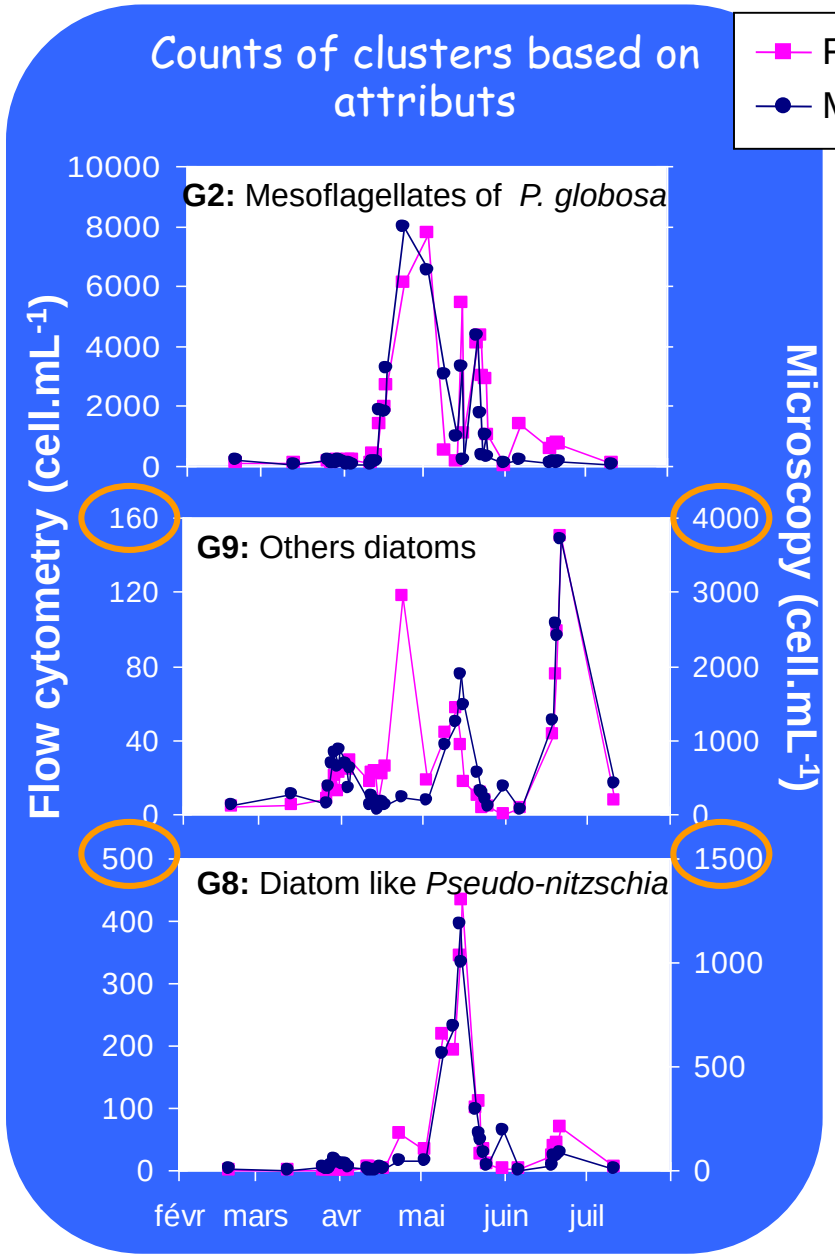


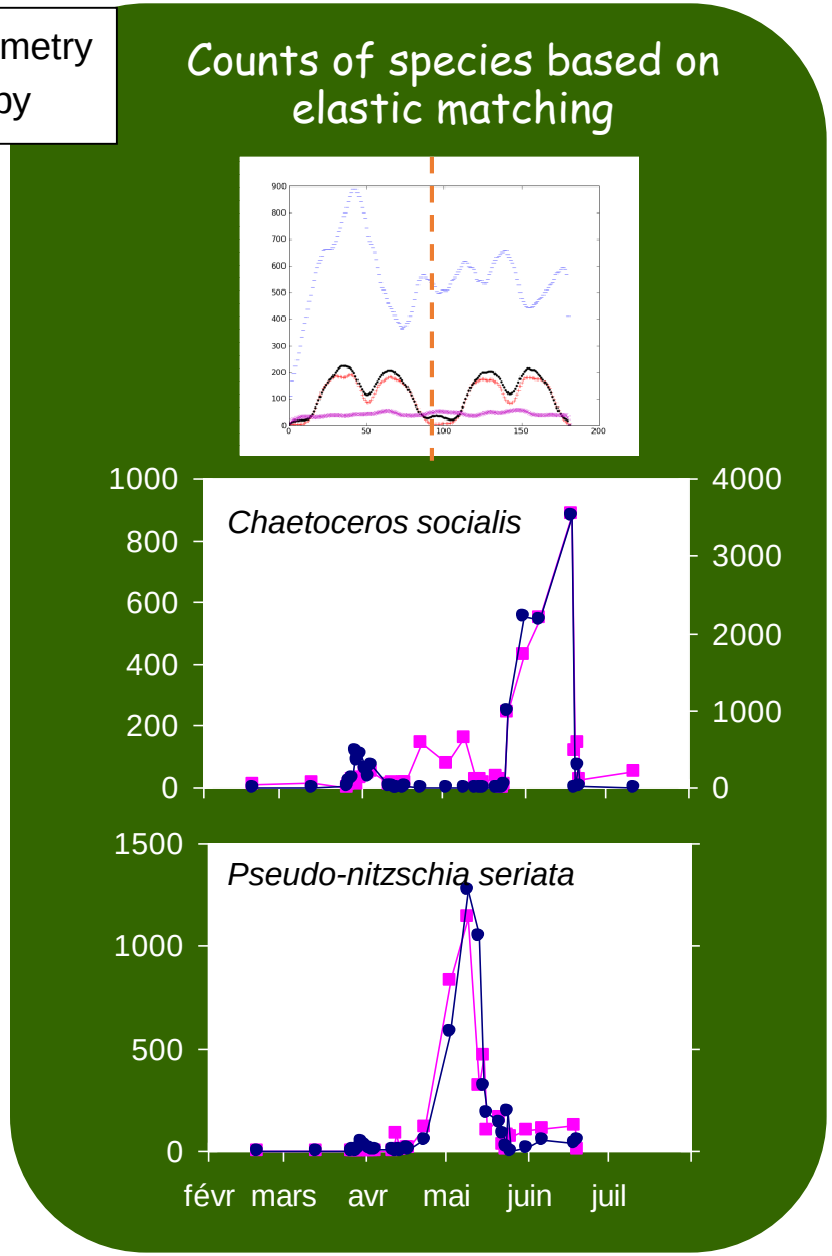


Rijkswaterstaat
Ministry of Infrastructure and the Environment

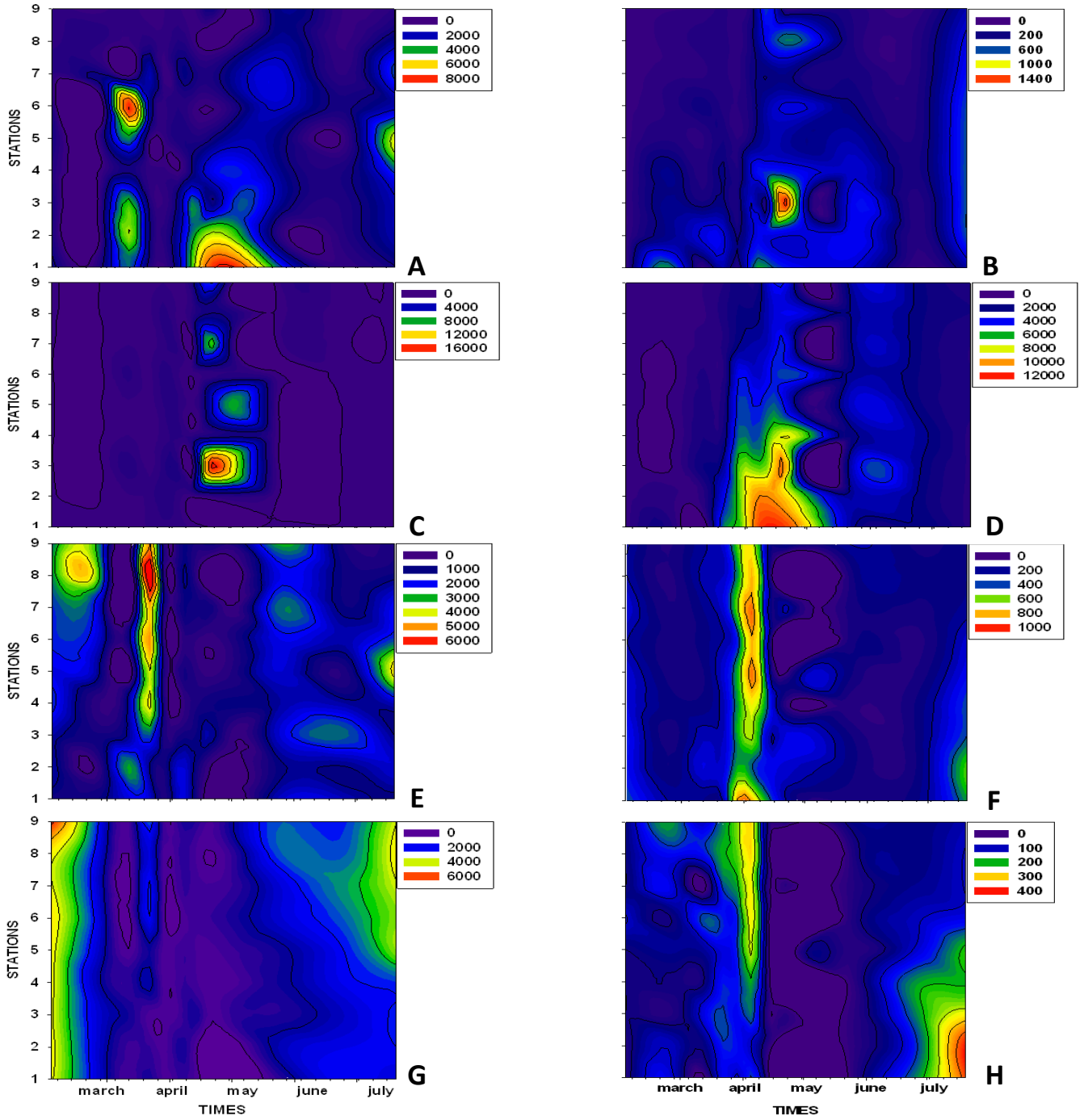
Rijkeboer et al., Symposium on phytoplankton blooms 2011

PSFM flow cytometry vs microscopy : manual and automated methods





N. Guiselin (PhD, 2010) ; G. Wacquet (PhD,2011) ; Caillault et al., 2009



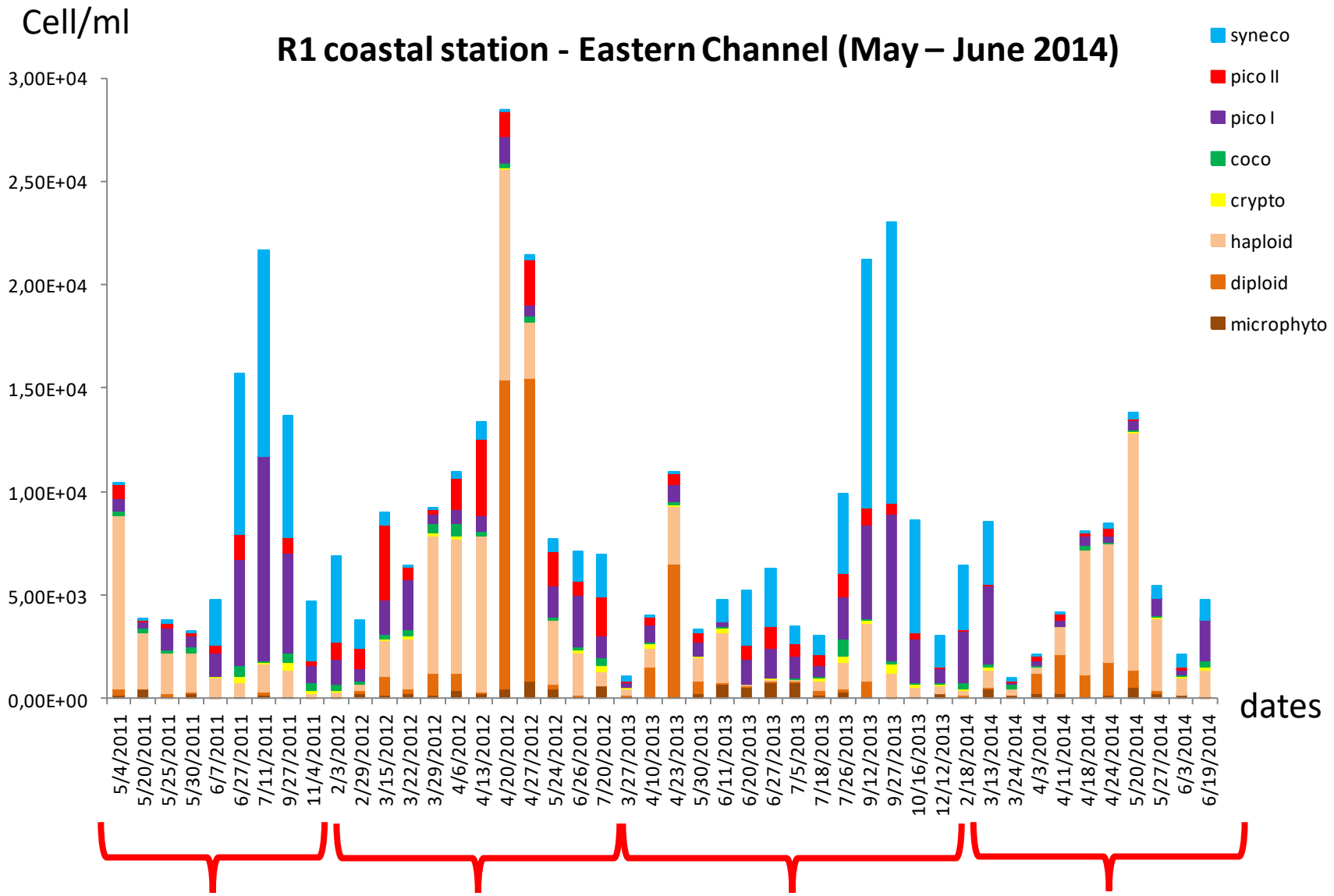
Spatio-temporal abundance distribution (cell ml^{-1}) of

A) picoeukaryotes II,
B) microphytoplankton,
C) *Phaeocystis globosa* diploid,
D) *Phaeocystis globosa* haploid,
E) picoeukaryotes I,
F) coccolithes,
G) *Synechococcus* spp.,
& H) cryptophytes in surface waters.

Bonato et al. JSR 2016

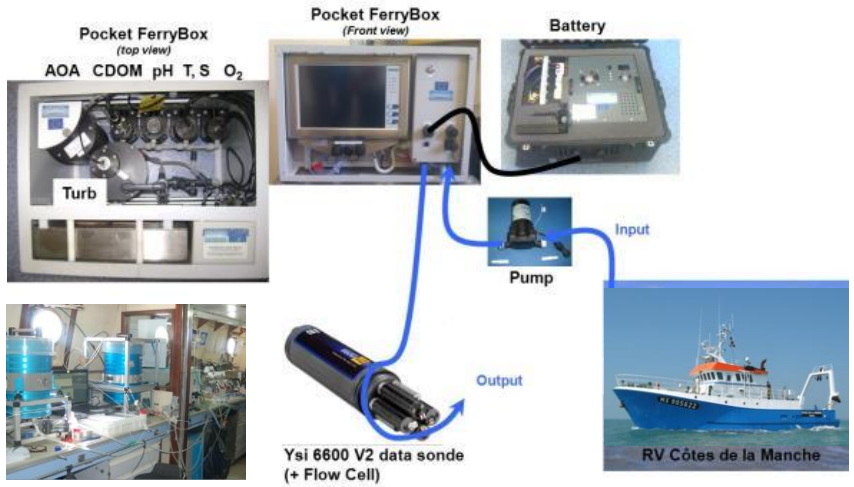
S. Bonato (Ph.D. 2011-2015)

Seasonal and short inter-annual variability : cell abundance

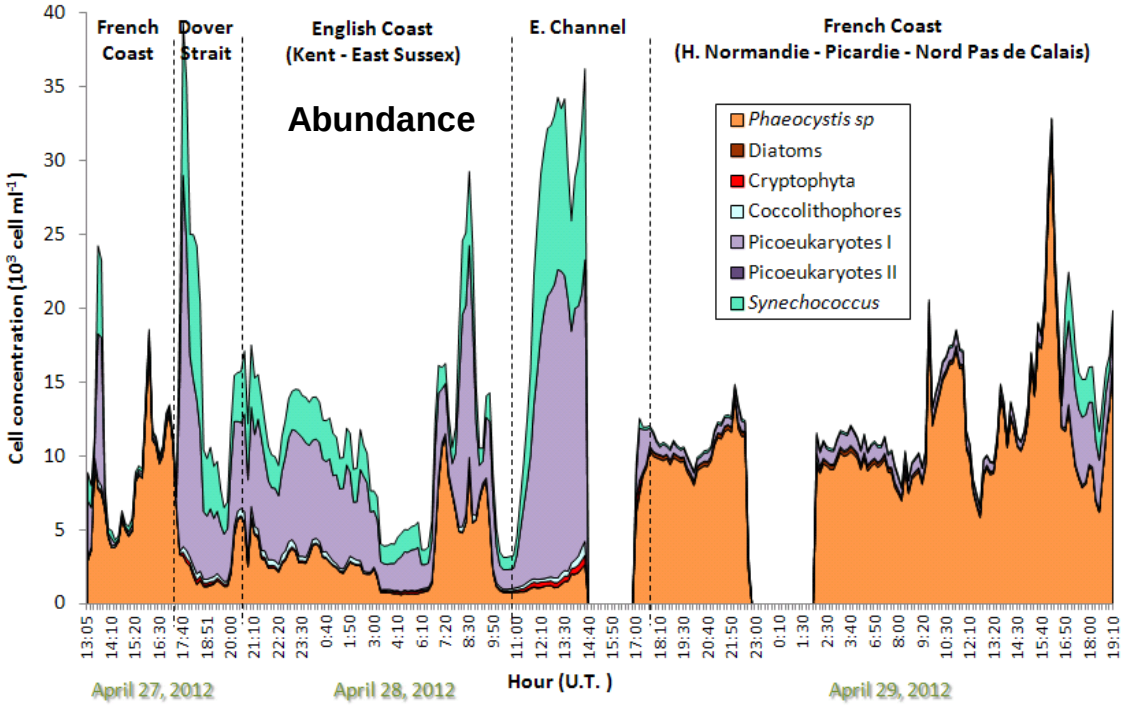


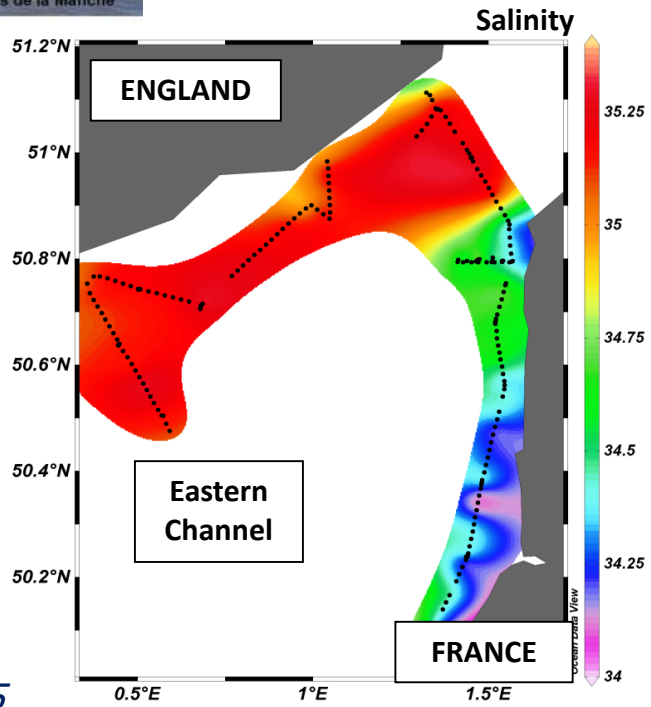
S. Bonato (Ph.D. 2015)

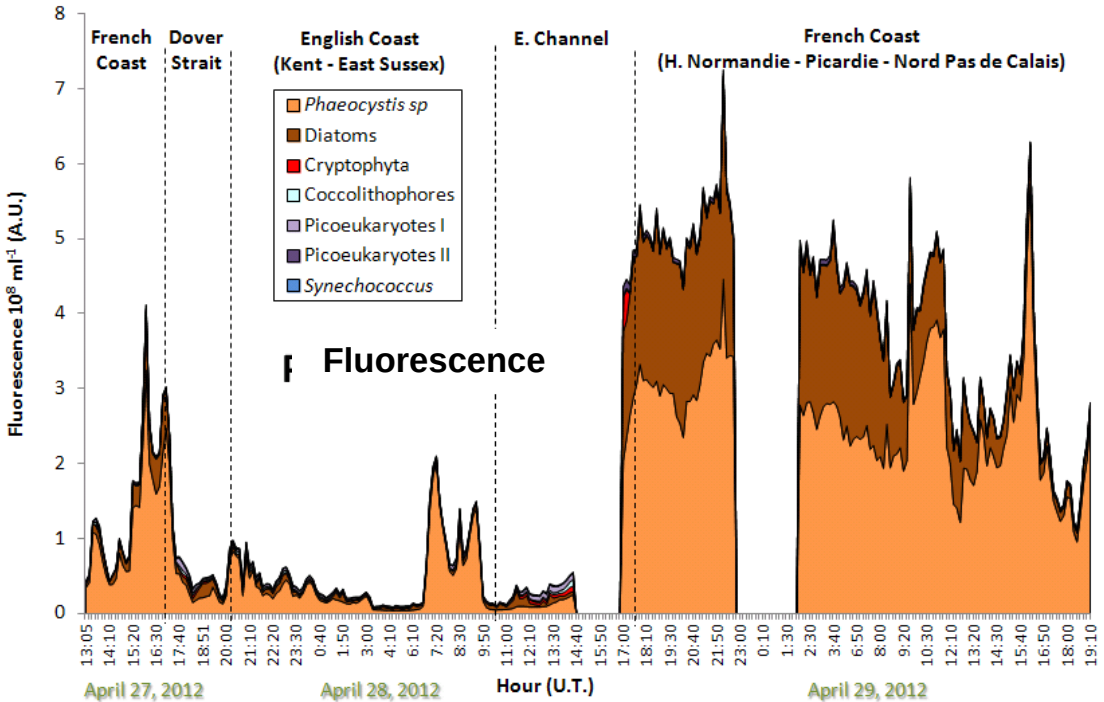
Continuous recording of phytoplankton in eastern Channel coastal waters DYPHYMA Cruise (Spring 2012)



CytoSense (CytoBuoy©)



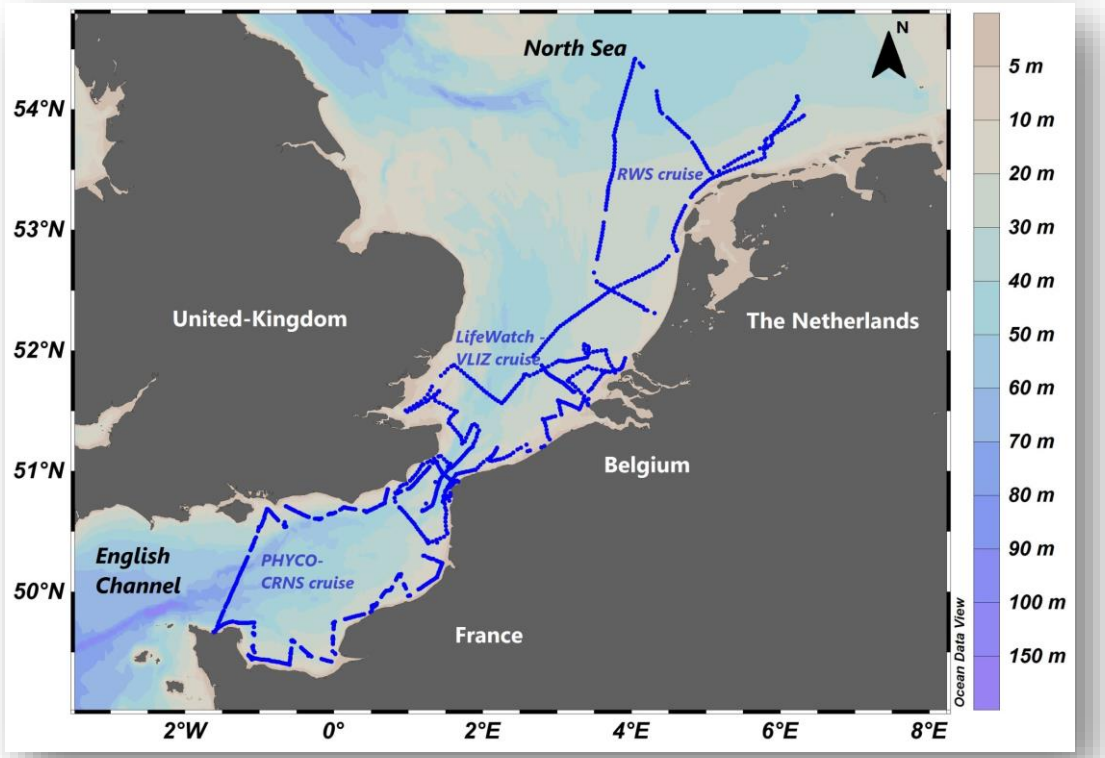


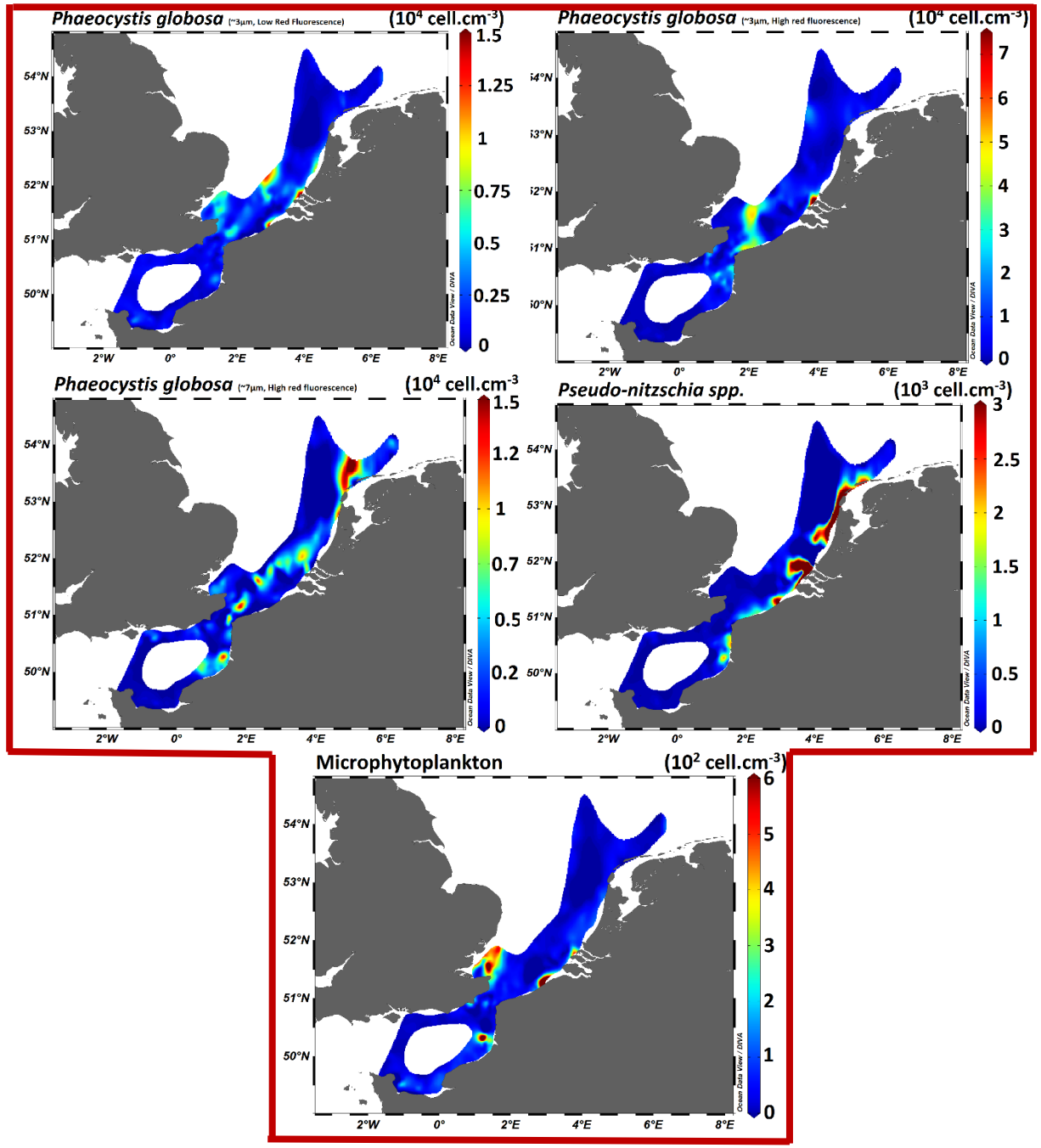


S. Bonato (Ph.D. 2015)

Bonato et al., Estuar.Coast.Shelf.Sc. 2015

Tracking *Phaeocystis globosa* life stages, *Pseudo-nitzschia* spp. and big microphytoplankton in spring from EEC to SNS (PHYCO - CNRS, JERICO/LifeWatch - VLIZ and Monisnel-RWS cruises April-May 2017)

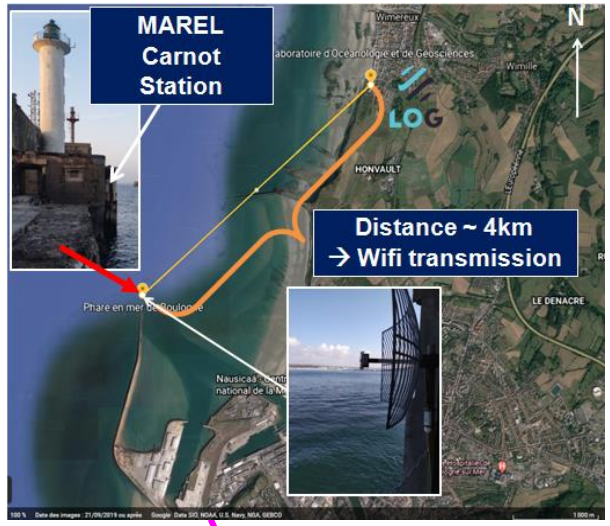


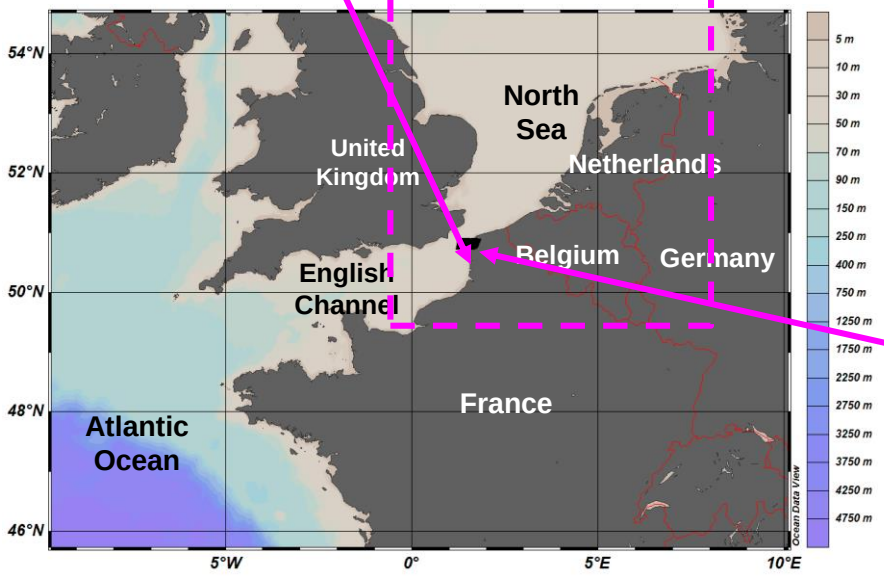


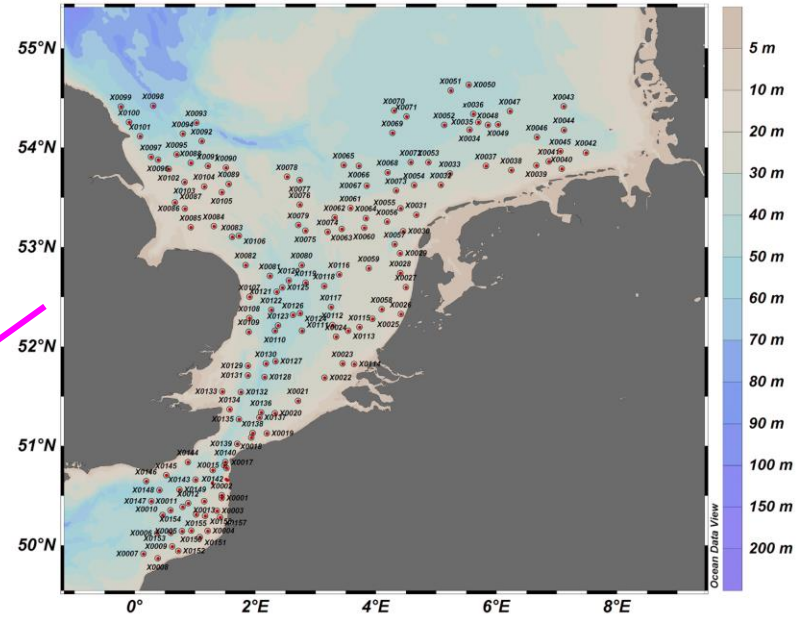
Louchart et al., 2020 (Proceedings of ICHA 2018)

Automated monitoring of phytoplankton abundance, biomass and diversity - Channel and North Sea Pilot Super Site (2019-2021)

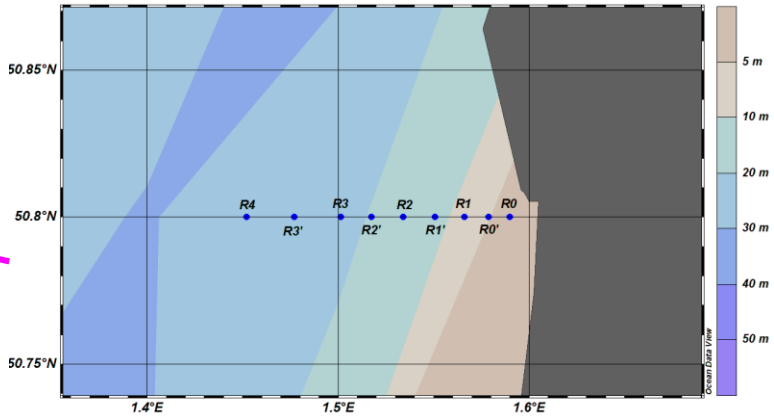
Zéline Hubert (PhD), Clémentine Gallot, Alexandre Epinoux, Kevin Robache, Marie Bruaut, Elise Caillard, Vincent Cornille, Jessica Delarbre, Claire Dédécker, Muriel Crouvoisier, Emeline Lebourg, Eric Lécuyer, Arnaud Louchart, Jean-Valery Facq, Alain Lefebvre, Luis Felipe Artigas







IBTS Fisheries cruise January 2020



DYPHYRAD transect by the Strait of Dover

Phytoplankton Monitoring by High Performance Flow Cytometry: A Successful Approach?

Thomas P. A. Rutten,* Ben Sandee, and Angelo R. T. Hofman

Cytometry Part A 64A:16–26 (2005)

Monitoring of a Potential Harmful Algal Species in the Berre Lagoon by Automated *In Situ* Flow Cytometry

Mathilde Dugenne, Mélilotus Thyssen, Nicole Garcia, Nicolas Mayot, Guillaume Bernard, and Gérald Grégori

H.-J. Ceccaldi et al. (eds.), *Marine Productivity: Perturbations and Resilience of Socio-ecosystems*, DOI 10.1007/978-3-319-13878-7_13, © Springer International Publishing Switzerland 2015

Water **2022**, *14*, 1099. https://doi.org/10.3390/w14071099

Article

Spatiotemporal Variation in Phytoplankton and Physiochemical Factors during *Phaeocystis globosa* Red-Tide Blooms in the Northern Beibu Gulf of China

Ming-Ben Xu [1,2,3,4,5], Rong-Can Zhang [5], Fa-Jun Jiang [5], Hui-Zhu Pan [5], Jie Li [5], Ke-Fu Yu [1,2,3,*] and Jun-Xiang Lai [5,*]

Front. Mar. Sci. 9:791329. doi: 10.3389/fmars.2022.791329

Novel Methodologies for Providing *In Situ* Data to HAB Early Warning Systems in the European Atlantic Area: The PRIMROSE Experience

Manuel Ruiz-Villarreal[1], Marc Sourisseau[2], Phil Anderson[3], Caroline Cusack[4], Patricia Neira[5], Joe Silke[5], Francisco Rodriguez[6], Begoña Ben-Gigirey[6†], Callum Whyte[3], Solene Giraudeau-Potel[3], Loic Quemener[7], Gregg Arthur[8] and Keith Davidson[3]*

JOURNAL OF MARINE ENGINEERING & TECHNOLOGY
https://doi.org/10.1080/20464177.2018.1525806

Taylor & Francis
Taylor & Francis Group

OPEN ACCESS Check for updates

Comparing flow cytometry and microscopy in the quantification of vital aquatic organisms in ballast water

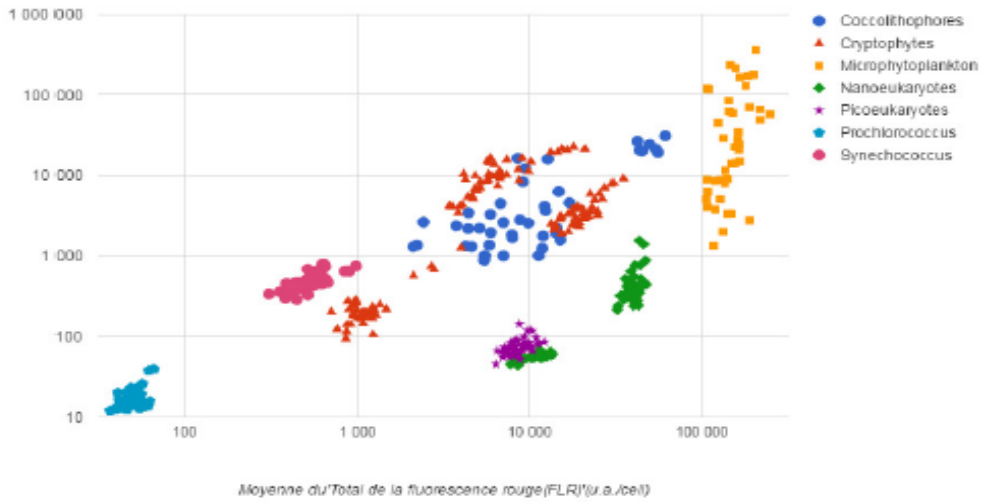
Louis Peperzak [a], Eva-Maria Zetsche [a,b*], Stephan Gollasch [c], Luis Felipe Artigas[d], Simon Bonato[e], Veronique Creach[f], Pieter de Vré[g], George B.J. Dubelaar [h], Joël Henneghien[i†], Ole-Kristian Hess-Erga[j], Roland Langelaar [k], Aud Larsen [l], Brian N. Maurer[m], Albert Mosselaar [n], Euan D. Reavie [o], Machteld Rijkeboer[p] and August Tobiesen [q]

Work on FCM common vocabulary SeaDataNet project (S. Lahbib, M. Thyssen) CNRS/MOI and JERICO S3

P01 (BODC Parameter Usage Vocabulary)				
conceptid	preflabel	modified	altlabel	definition
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.
HBCCAFTX	Abundance of Bacteria (ITIS: 202421: WoRMS 6) [Subgroup: heterotrophic] per unit volume of the water body by flow cytometry and subtraction of Synechococcus+Prochlorococcus from total bacteria	4/17/2016 15:50:07	HetBactCellNo	Number of particles resolved as heterotrophic bacteria cells 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.
.....	.....			
PYPKAFTX	Abundance of prokaryotic cells per unit volume of the water body by flow cytometry	4/22/2010 17:47:51	ProkCellNo	Unavailable

74 codes

existing FCM BODC codes



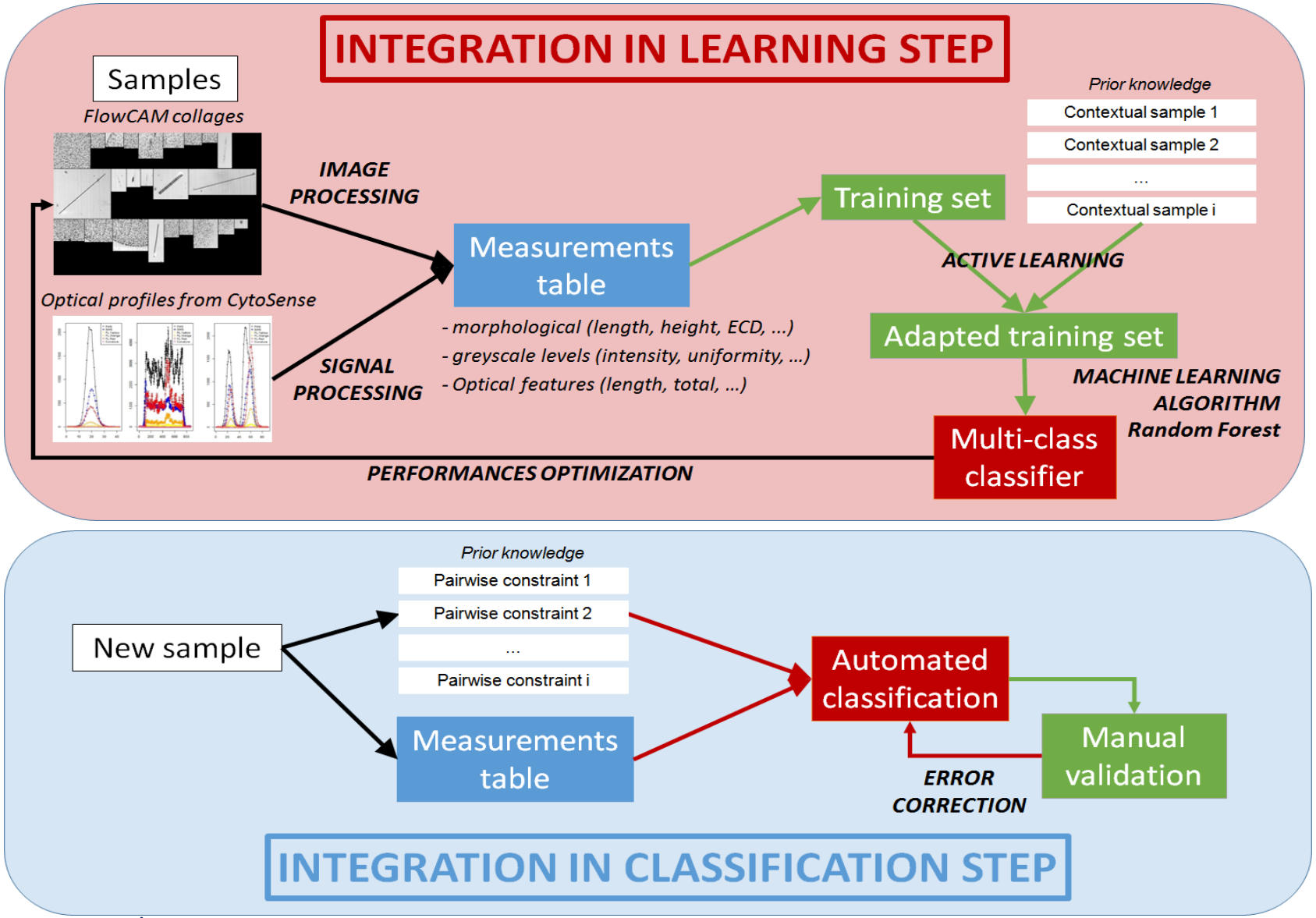
Definition of functional types

Definition of functional types and common vocabulary (Thyssen et al., 2022)

Picoeukaryotes
Nanophytoplankton
Microphytoplankton

SeaDataCloud

Towards automated analysis of phytoplankton images/optical profiles



Wacquet *et al., 2020* (ICHA 2018)

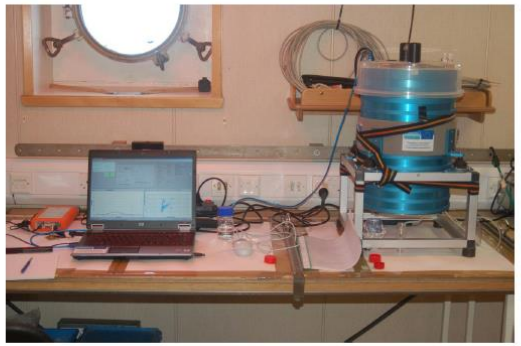
DYMAPHY deliverables Action 1

+ PhD Thesis (K. Owen, S. Bonato…) + publications : Bonato *et al.*, 2015; Thyssen *et al.*, 2015, Bonato *et al.*, 2016, ….

INTERREG IVA 2 Mers Seas Zeeën Crossborder Cooperation Programme 2007-2013

DYMAPHY
Operational Common Protocol for Pulse-shape recording Flow Cytometry

T.Rutten, M.Rijkeboer, A.Veen, M.Thyssen, S.Bonato, V.Creach, L.F.Artigas



Part-financed by the European Regional Development Fund (ERDF)

INTERREG IVA 2 Mers Seas Zeeën Crossborder Cooperation Programme 2007-2013
Part-financed by the European Regional Development Fund (ERDF)

Recommendations for the validation of custom built flow cytometers:

Guidance for operators

Thomas Rutten, Machteld Rijkeboer , Arnold Veen

JERICO NEXT deliverables D3.1 & D3.2

+ PhD Thesis (A. Louchart) + publications (Aardema *et al.*, 2019; Marrec *et al.*, 2018; Louchart *et al.*, 2020a, b)…

JERICO-NEXT

Joint European Research Infrastructure network for Coastal Observatory – Novel European eXpertise for coastal observaTories - **JERICO-NEXT**

Deliverable title	Novel methods for automated *in situ* observations of phytoplankton diversity
Work Package Title	WP 3
Deliverable number	D3.1
Description	Synthesis report after developments dedicated to the observation of th phytoplankton diversity
Lead beneficiary	SMHI
Lead Authors	Bengt Karlson, Felipe Artigas, Veronique Créach, Arnaud Louchart, Guillaum Wacquet and Jukka Seppälä
Contributors	Hedy Aardema, Michael Brosnahan, Reinhoud de Blok, Pascal Claquin Florent Colas, Klaas Deneudt, Gérald Grégori, Jacco Kromkamp, Soumay Lahbib, Alain Lefebvre, Fabrice Lizon, , Klas Möller, Emilie Poisson-Caillaul Machteld Rijkeboer, Thomas Rutten, Suvi Rytövuori, Lars Stemmann Melilotus Thyssen, Lennert Tyberghein, and Pasi Ylöstalo.
Submitted by	Bengt Karlson
Revision number	9
Revision Date	4 October 2017
Security	Public

Reference: JERICO-NEXT-WP3-D3.1, 9 Oct. Sep 2017*.

JERICO-NEXT

Joint European Research Infrastructure network for Coastal Observatory – Novel European eXpertise for coastal observaTories - **JERICO-NEXT**

Deliverable title	Novel methods for automated *in situ* observations of phytoplankton diversity and productivity: synthesis of exploration, inter comparisons and improvements
Work Package Title	WP 3
Deliverable number	D3.2
Description	Report on the technical and analytical improvements of innovative techniques and recommendations on their use
Lead beneficiary	CNRS
Lead Authors	Felipe Artigas, Véronique Créach, Emilie Houliez, Bengt Karlson, Fabrice Lizon, Jukka Seppälä, Guillaume Wacquet
Contributors	Hedy Aardema, Michael Brosnahan, Reinhoud de Blok, Pascal Claquin, Gérald Grégori, Florent Colas, Elisabeth Debusschere, Klaas Deneudt, Jacco Kromkamp, Soumaya Lahbib, Alain Lefebvre, Arnaud Louchart, Klas Möller, Emilie Poisson-Caillault, Thomas Rutten, Machteld Rijkeboer, Suvi Rytövuori, Lars Stemmann, Melilotus Thyssen, Lennert Tyberghein, Jochen Wollschläger and Pasi Ylöstalo.
Submitted by	Felipe Artigas
Revision number	1

Reference: JERICO-NEXT-WP3-D3.2, April 2019*.

JERICO S3 deliverables D5.1

The JERICO-S3 project is funded by the European Commission's H2020 Framework Programme under grant agreement No. 871153. Project coordinator: Ifremer, France.

The JERICO-S3 project is funded by the European Commission's H2020 Framework Programme under grant agreement No. 871153. Project coordinator: Ifremer, France.

DELIVERABLE TITLE:
JERICO-S3 D5.1 Catalogue and checklists for existing biological plankton sensors that will be implemented in JERICO-S3

DELIVERABLE NUMBER: **D5.1, WP5 Task 5.3, Subtask 5.3.3 – ST 7**

WORK PACKAGE N° and NAME: WP5-NA4: Harmonisation of integrated Multiplatform & Multidisciplinary systems

Authors: Gallot, C. & Artigas L.F. (CNRS-LOG)

Contributors: Créach, V., Borst, K., Brunetti, F., Cabrera, P., Cantoni, C., Deneudt, K., Eikrem, W., Frangoulis, C., Grégori, G., Karlson, B., King, A., Lefebvre, A., Lindh, M., Lizon, F., Lombard, F., Möller, K.O., Rühl, S. Lars- Naustvoll, L.J., Pfannkuchen, M., Rombouts, I., Salter, I., Seppälä, J., Steemann, L. & Vitorino J.

Involved Institution: Lead: CNRS-LOG; Partners: SMHI, SOCIB, NIVA, CNRS-MIO, CNRS-LOV, CNRS-BOREA, HCMR, CNR, AZTI, NORCE, IFREMER, CEFAS, IRB, VLIZ, Hereon (previously HZG), SYKE, IH, OGS, FAMRI.

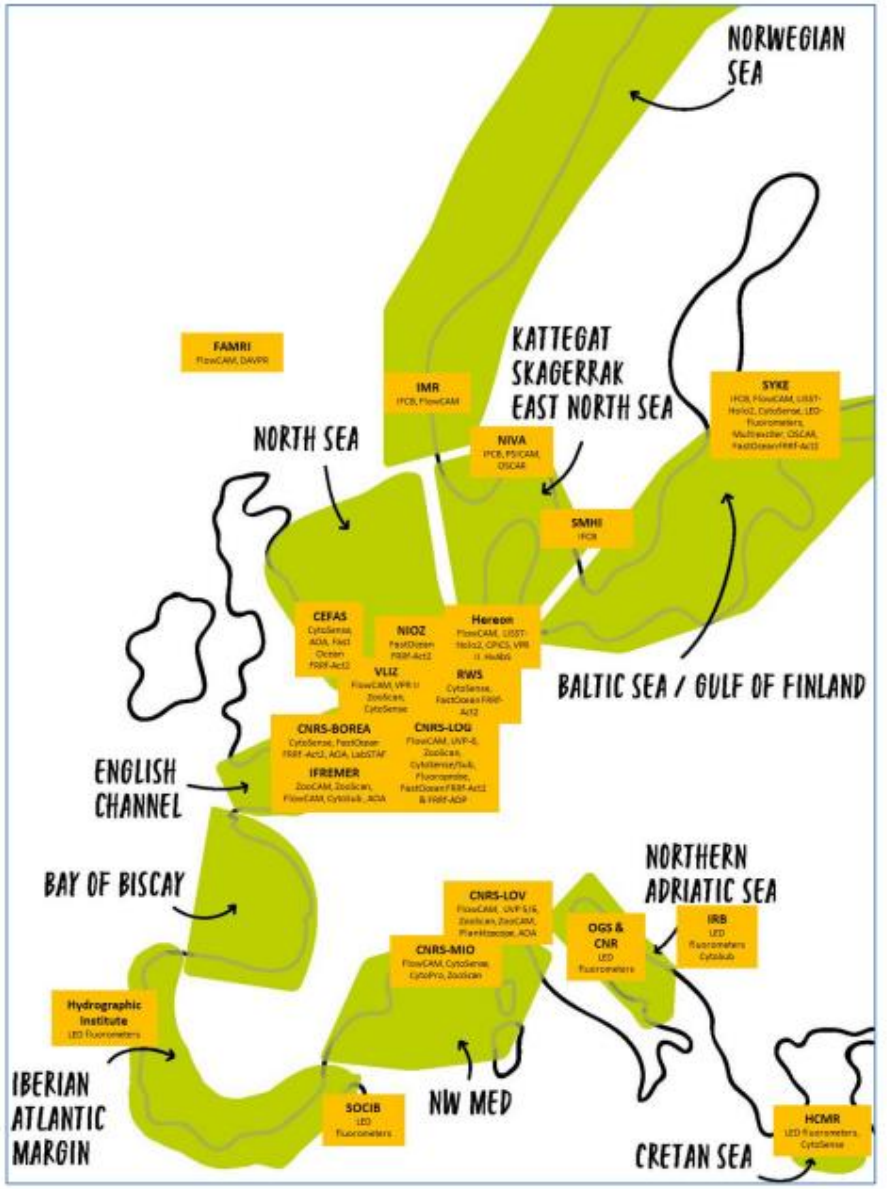
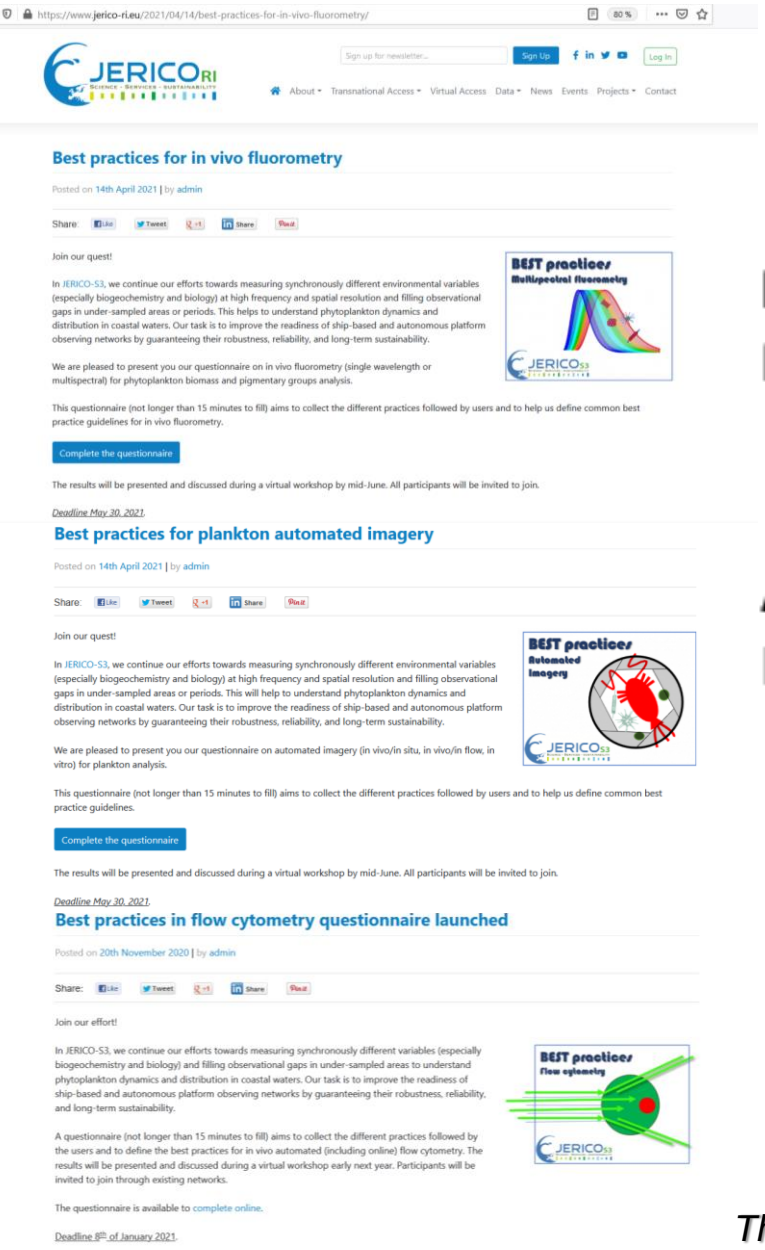


Figure 18 – Map of the localisation of sensors potentially or effectively deployed by JERICO S3 participants in the different Integrated Research Sites (IRS) and Pilot Super Sites (PSS) of European Coastal systems (based on JS3 partner's information and JS3 MS25; Artigas et al., 2021).

Best practices, Integration, validation, archives and long-term accessibility of biological (plankton) data



NEED TO GO

Findable Accessible Interoperable Reusable

Multispectral Fluorometry

Automated Imaging

Automated Flow cytometry

will be delivered in 2022

Three questionnaires were released on the www.jerico-ri.eu webpage

Summary and perspectives for the use of automated PSR flow cytometry for phytoplankton & HAB monitoring

- Automated PSR FCM brings optical chararcterization of cells and colonies beyond taxonomic resolution (functional diversity, Fontana et al., 2014; Fragoso et al., 2019; Louchart, 2020; Epinoux, 2021)
- Continuous high frequency eulerian monitoring in lagoons (i.e. the Berre lagoon, Duguenne et al. 2014, G. Grégori M.I.O.; Venice lagoon, Epinoux, 2021), coastal buoys (as in the rade de Villefranche-EoL buoy, Thyssen et al., 2014; rade of Brest, Ruiz-Villareal et al., 2022; the Bay of Naples, Epinoux 2021, MAREL fixed station in the rade de Boulogne, Robache, 2021, 2022). To be coupled with nutrients & *in vivo* fluorescence automated *in situ* analysis.
- On board continuous measurements for high spatial resolution (R.V. scientific cruises, Bonato et al., 2015, 2016; Thyssen et al. 2015; ships/cruises of opportunity, Thyssen et al., 2009; Louchart et al., 2020).
- Definition of common vocabulary and operational and data base best practices (Thyssen et al., 2022).
- Integration of optical features, optical pulse shapes and/or images automated classification (including new CNN approaches; Fuchs et al., 2022; Wacquet et al., 2020). Coupling with other high througput imaging instruments FlowCAM, Imaging Flow Cytobot.
- Physiological assessment (Thyssen et al., 2008; Duguenne et al. 2014) and coupling with physiological photosynthetic assessment (variable fluorescence PAM, FRRf) and pCO2 (Marrec et al., 2018)
- Coupling with *in situ* DNA extraction (ESP, Moore et al., 2021) in autonomous stations and/or coupled with an AUV (Yamahara et al., 2019); species-specific probes (Brosnahan et al., 2014) for tracking HABs species

Thank you very much for your attention!

Tack så mycket för er uppmärksamhet

Part of this work was supported by the JERICO-S3 project. This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement n° 871153.