Flow cytometry and imaging in flow methods facilitate automated observations and monitoring of algal blooms and phytoplankton abundance and diversity in automated platforms

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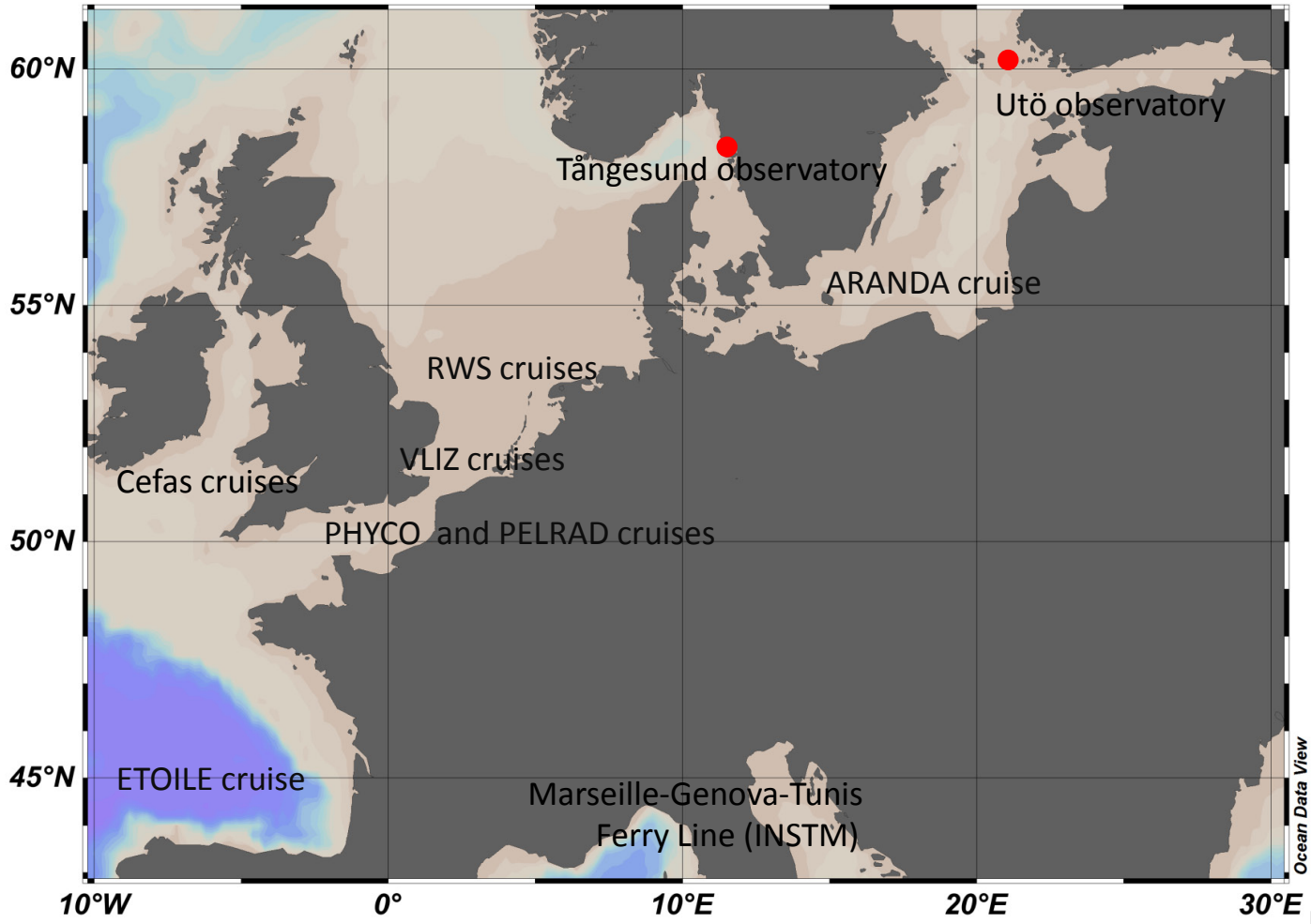
[14]Finnish Meteorology Institute, Helsinki, Finlandnish Meteorology Institute, Helsinki, Finland

Use of automated multi-spectral fluorometer and/or automated flow cytometry in JERICO-NEXT



Note: not all ferries, cruises and fixed systems used are included on map

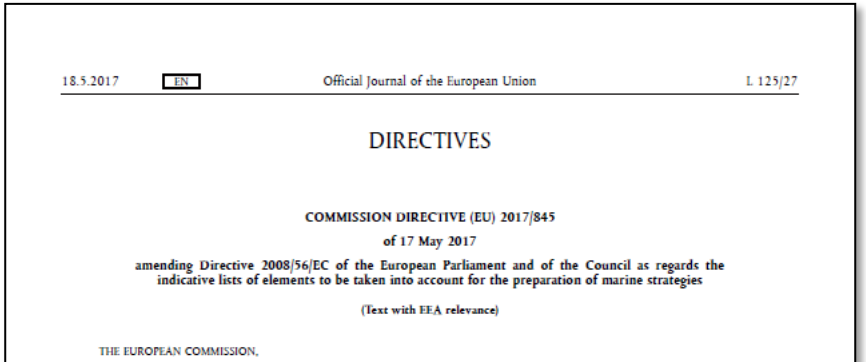
Essential Ocean Variables

PHYSICS	BIOGEOCHEMISTRY	BIOLOGY AND ECOSYSTEMS
Sea state	Oxygen	Phytoplankton biomass and diversity
Ocean surface stress	Nutrients	Zooplankton biomass and diversity
Sea ice	Inorganic carbon	Fish abundance and distribution
Sea surface height	Transient tracers	Marine turtles, birds, mammals abundance and distribution
Sea surface temperature	Particulate matter	Live coral
Subsurface temperature	Nitrous oxide	Seagrass cover
Surface currents	Stable carbon isotopes	Macroalgal canopy
Subsurface currents	Dissolved organic carbon	Mangrove cover
Sea surface salinity	Ocean colour (Spec Sheet under development)	Microbe biomass and diversity (*emerging)
Subsurface salinity		Benthic invertebrate abundance and distribution (*emerging)
Ocean surface heat flux		

The Essential Ocean Variables as defined by UNESCO Global Ocean Observing System
http://www.goosocean.org

The Marine Strategy Framework Directive was updated in May 2017

Habitats	Broad habitat types of the water column (pelagic) and seabed (benthic) (Note 5), or other habitat types, including their associated biological communities throughout the marine region or subregion	Per habitat type: — habitat distribution and extent (and volume, if appropriate) — species composition, abundance and/or biomass (spatial and temporal variation) — size and age structure of species (if appropriate) — physical, hydrological and chemical characteristics Additionally for pelagic habitats: — chlorophyll a concentration — plankton bloom frequencies and spatial extent



18.5.2017 EN Official Journal of the European Union L 125/27

DIRECTIVES

COMMISSION DIRECTIVE (EU) 2017/845

of 17 May 2017

amending Directive 2008/56/EC of the European Parliament and of the Council as regards the indicative lists of elements to be taken into account for the preparation of marine strategies

(Text with EEA relevance)

THE EUROPEAN COMMISSION,

18.5.2017 EN Official Journal of the European Union L 125/43

COMMISSION DECISION (EU) 2017/848

of 17 May 2017

laying down criteria and methodological standards on good environmental status of marine waters and specifications and standardised methods for monitoring and assessment, and repealing Decision 2010/477/EU

(Text with EEA relevance)

THE EUROPEAN COMMISSION,

Having regard to the Treaty on the Functioning of the European Union,

Having regard to Directive 2008/56/EC of the European Parliament and of the Council of 17 June 2008 establishing

What are harmful algae?

Main types

- Fish killers
- Toxin producers – affecting human health trough fish, shellfish, aerosols etc.
- Nuisance blooms – affecting tourism etc.
- High biomass blooms connected to eutrophication – results in low oxygen conditions

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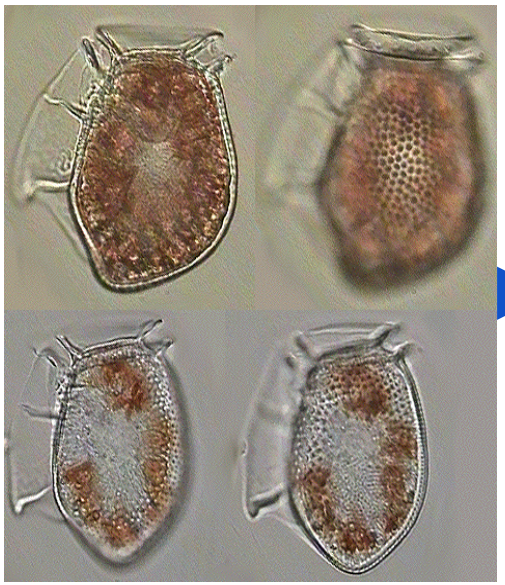
Shellfish may transfer algal toxins to humans

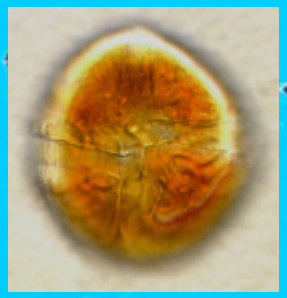


Examples of harmful algal bloom species

Note: Approximately 2000 species of phytoplankton are found in samples analysed using microscopy, meta barcoding of rDNA indicates that this is an underestimate by a factor of 20

Photos: Bengt Karlson, Ann-Turi Skjevik, Lars Edler, Jahn Throndsen and Wenche Eikrem

Dinophysis spp.

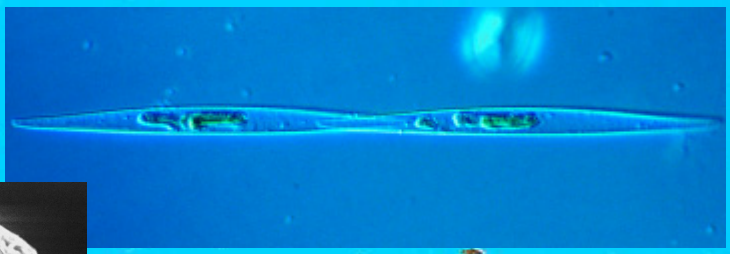


Alexandrium tamarense

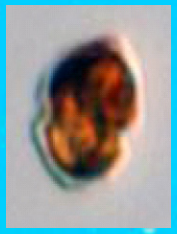
Nodularia spumigena

Prymnesium polylepis

Pseudochattonella farcimen



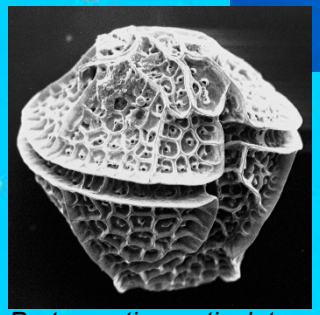
Pseudo-nitzschia sp.



cf. Azadinium spinosum

May-Oct

Max 10 000 cells l^{-1}



Protoceratium reticulatum

Karenia mikimotoi

Chaetoceros concavicornis

Observing the phytoplankton - ongoing methods

Traditional phytoplankton sampling and analysis

- Sampling devices
  - Niskin bottles
  - Tube sampling
  - ISCO-samplers
  - Etc.
- Microscopy
  - Utermöhl method
  - Fluorescence microscopy
  - Etc.

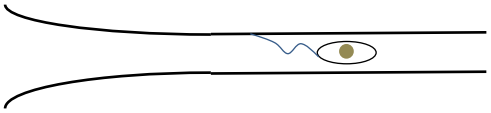
Bulk measurements based on pigment content

- HPLC
- Fluorescence
  - Chlorophyll
  - *Phycocyanin*
  - *Phycoerythrin*
  - *Multi spectral*
- Absorbtion
  - Single wavelength
  - *Multi spectral*

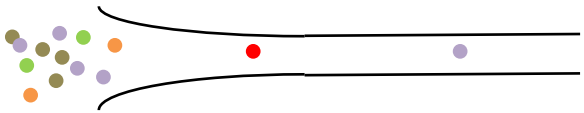
Beyond the impediment of discrete sampling

Observation of phytoplankton in near real time

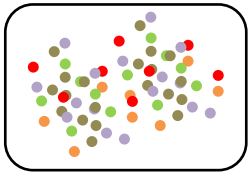
Imaging flow cytometry
Single cells – size and morphology of organisms



Flow cytometry
Single cells – fluorescence –pigment content and scattering (size, shape)

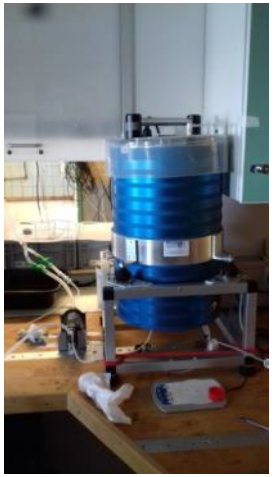


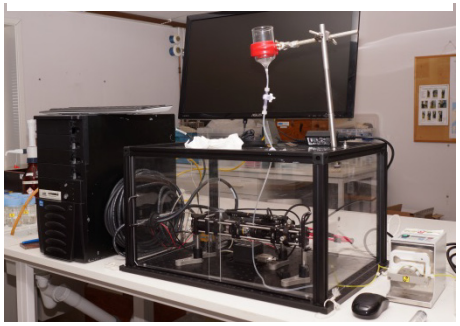
Fluorescence and absorption (multi-spectral)
Pigment based methods – bulk properties



Instruments for imaging in flow

- Imaging FlowCytoBot
  - McLane Inc., USA
- CytoSense and CytoPro
  - CytoBuoy, the Netherlands
- FlowCAM
  - Fluid Imaging Tech., USA
- FastCAM (prototype)
  - Ifremer-LDCM
- ImageStream

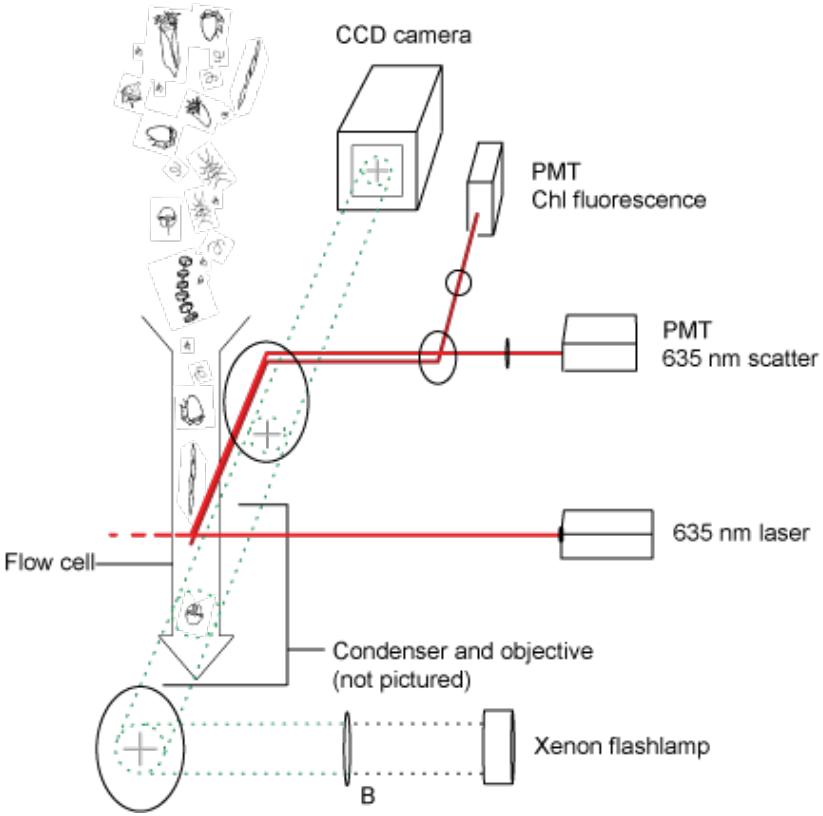


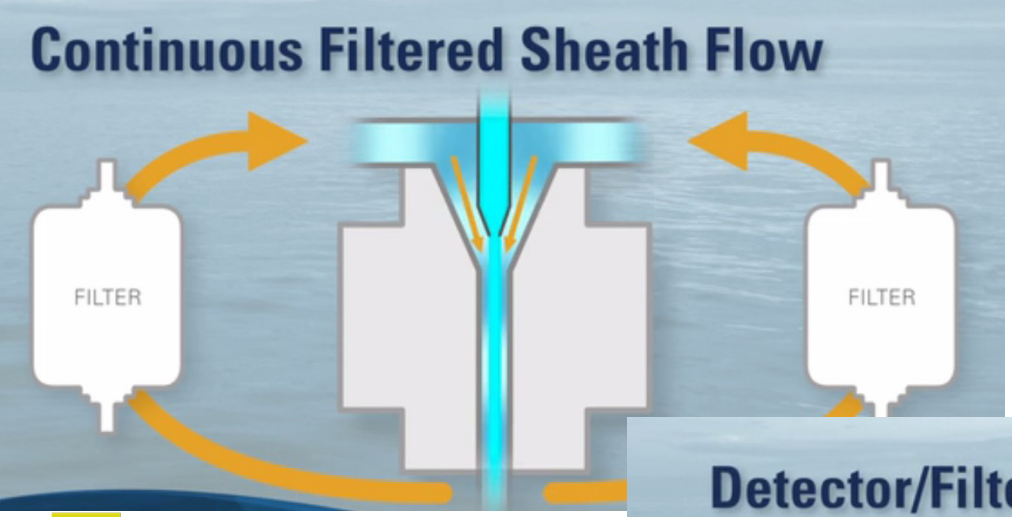


Some of the images from Dashkova et al 2017

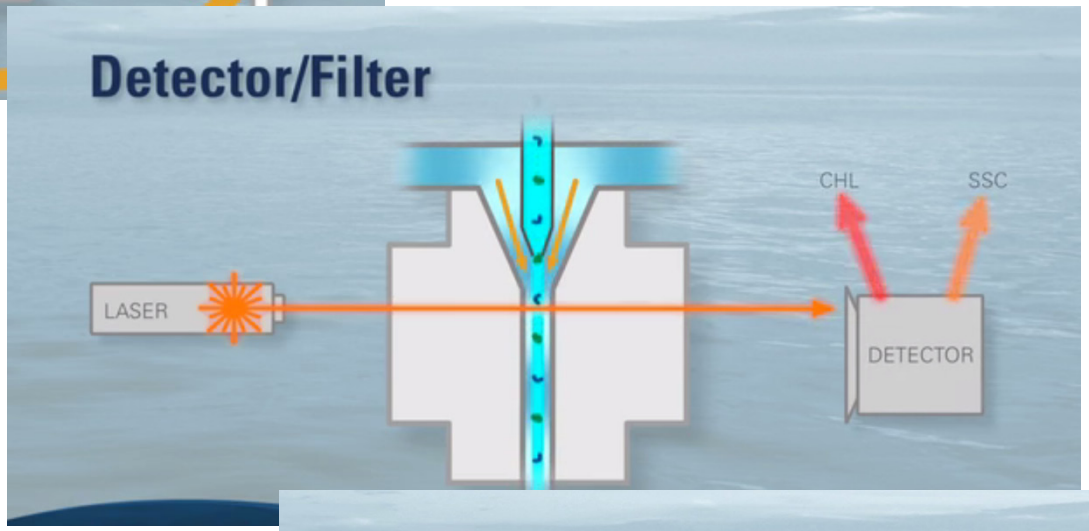
Imaging FlowCytoBot (IFCB) principle

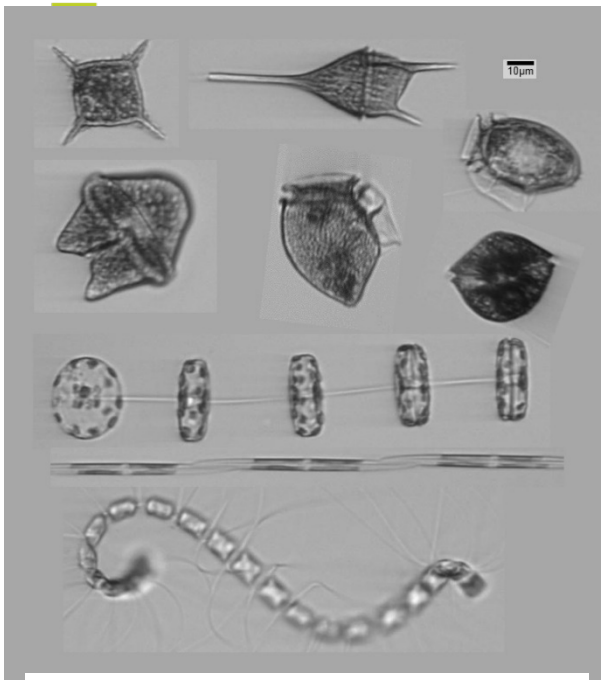
- Images of all organisms ~10-150 µm
- Sampling every 20 min.
- Several thousand images per sample of 5 mL
- Fluorescence and scattering mainly used for triggering camera
- Morphology-based
- > 200 parameters measured on each organism
- Random forest based automated classifiers



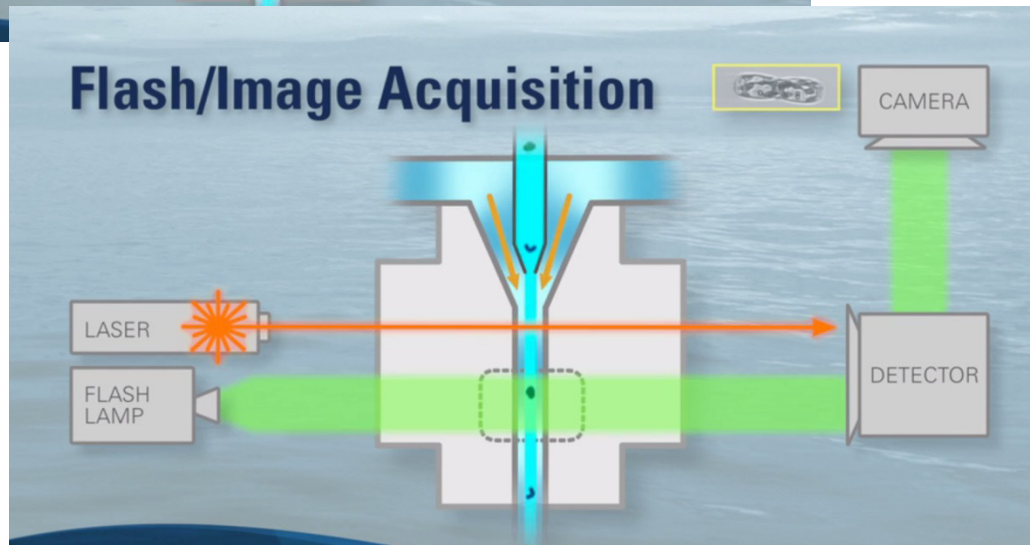


http://mclanelabs.com/



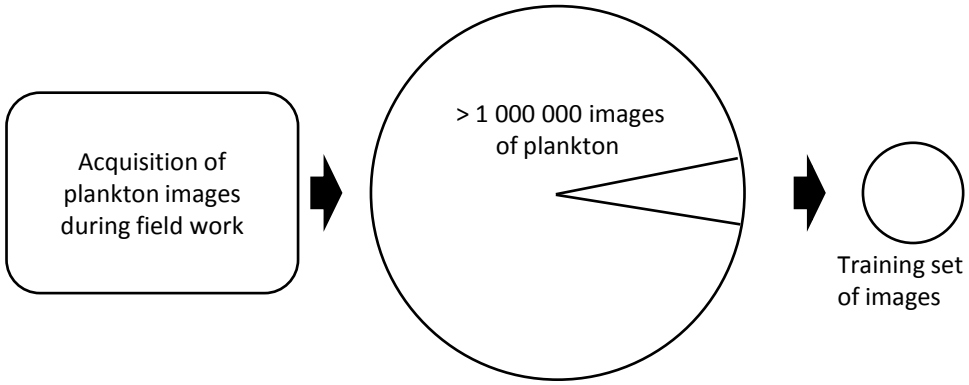


B. Karlson & M. Brosnahan

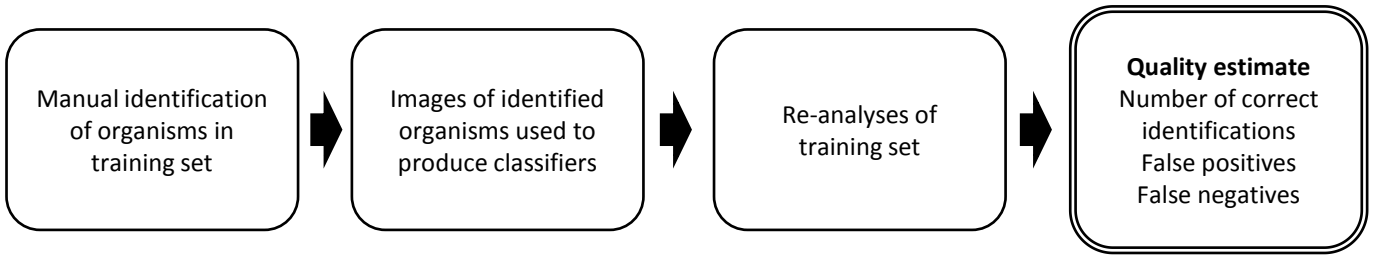


Data flow and production of classifiers

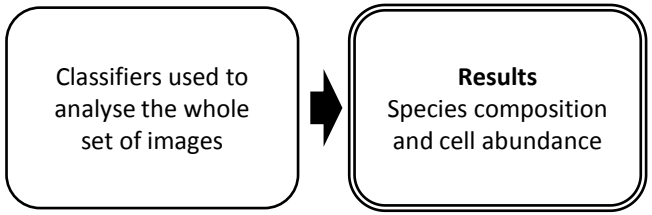
Step 1
In situ instrument (IFCB) used



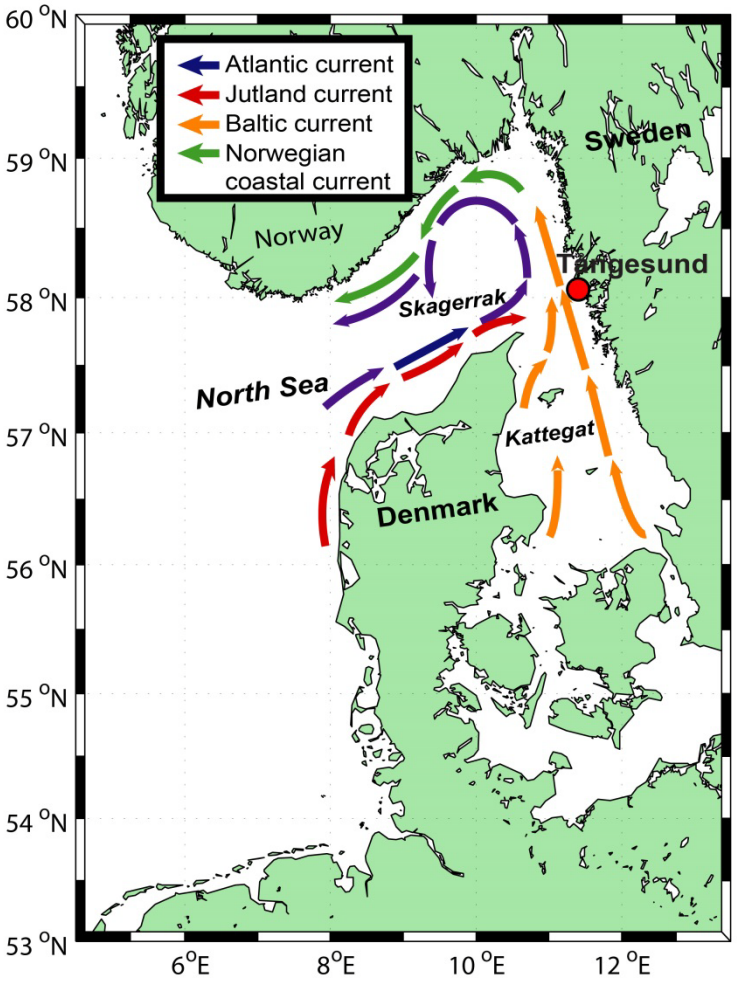
Step 2
Phytoplankton identification specialist needed

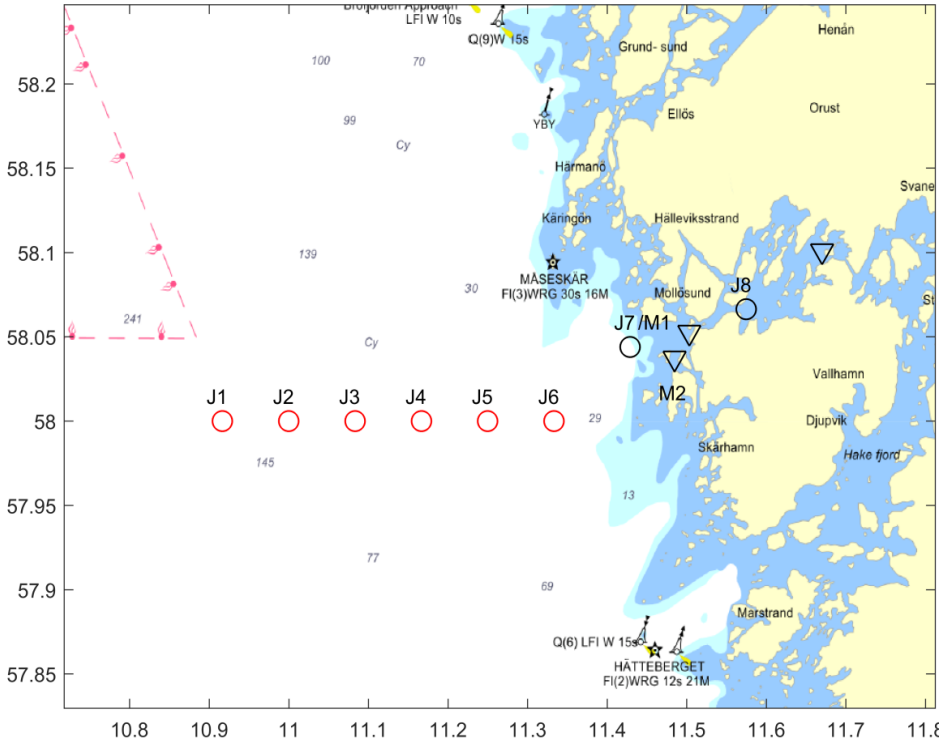


Step 3
Analysis of the whole data set – final results produced

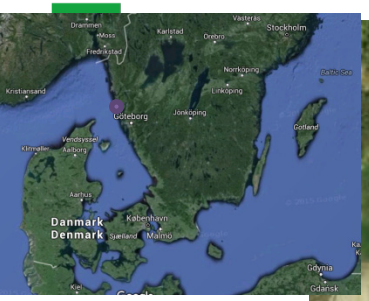


Study area


Atlantic current
Jutland current
Baltic current
Norwegian coastal current
Sweden
Norway
Tärnsesund
Skagerrak
North Sea
Kattegat
Denmark
60 °N
59 °N
58 °N
57 °N
56 °N
55 °N
54 °N
53 °N
6°E
8°E
10 °E
12 °E


LFI W 10s
Q(9)W 15s
Grund- sund
Henán
Orust
Ellös
YBY
Härmanö
Svane
Käringön
Hälleviksstrand
MASESKÄR
Fl(3)WRG 30s 16M
Mollösund
J8
J7 /M1
M2
J1
J2
J3
J4
J5
J6
Vallhamn
Djupvik
Skärhamn
Hake fjord
Märstrand
Q(6) LFI W 15s
HÄTTEBERGET
Fl(2)WRG 12s 21M
58.2
58.15
58.1
58.05
58
57.95
57.9
57.85
10.8
10.9
11
11.1
11.2
11.3
11.4
11.5
11.6
11.7

Tångesund observatory

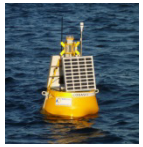


Electricity

Raft+
IFCB

Mussel
farm

Scanfjord
office and
factory

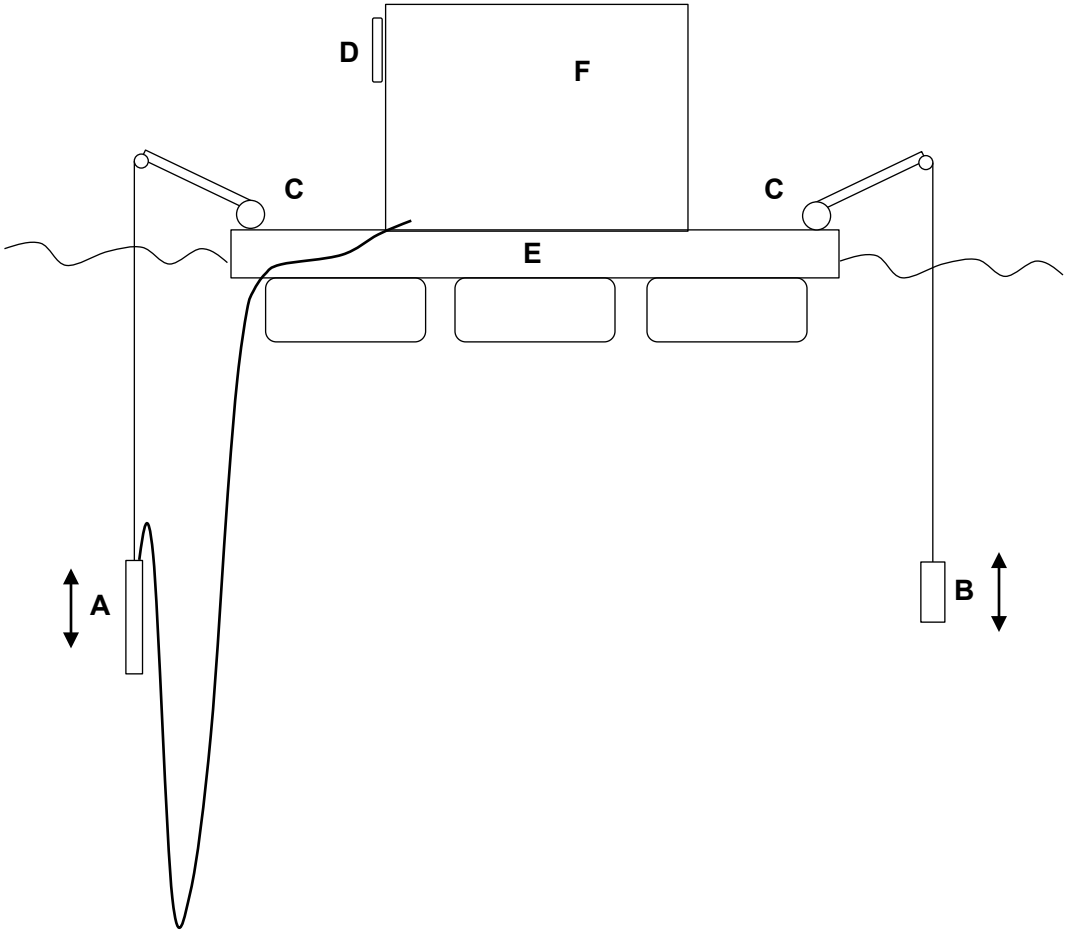


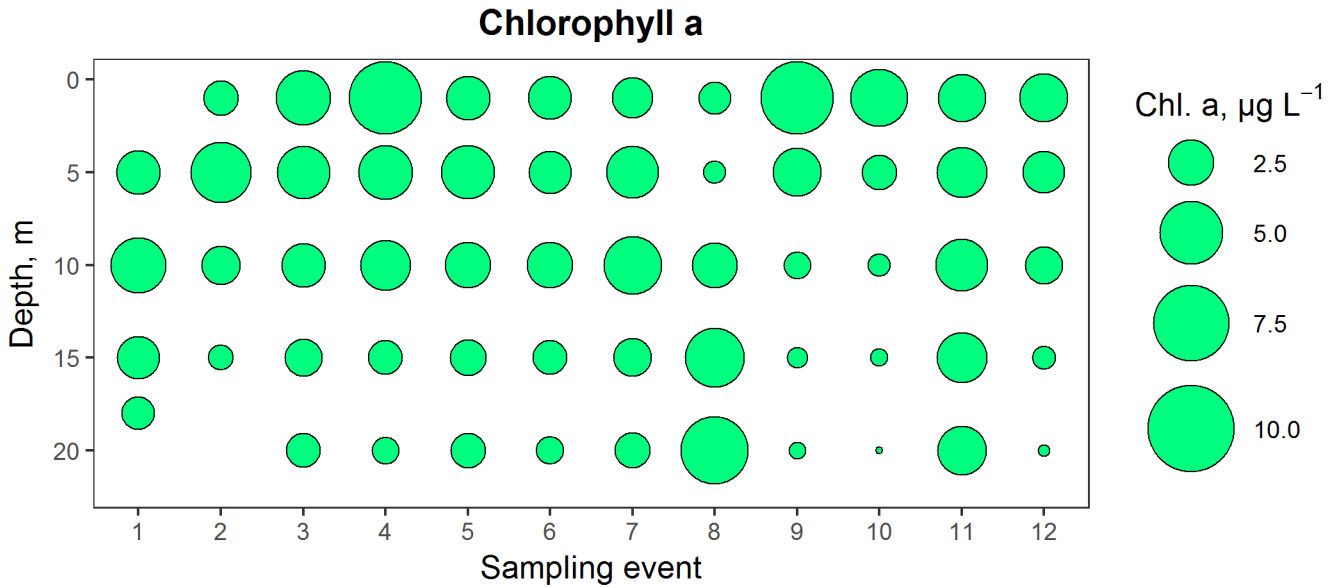
Buoy

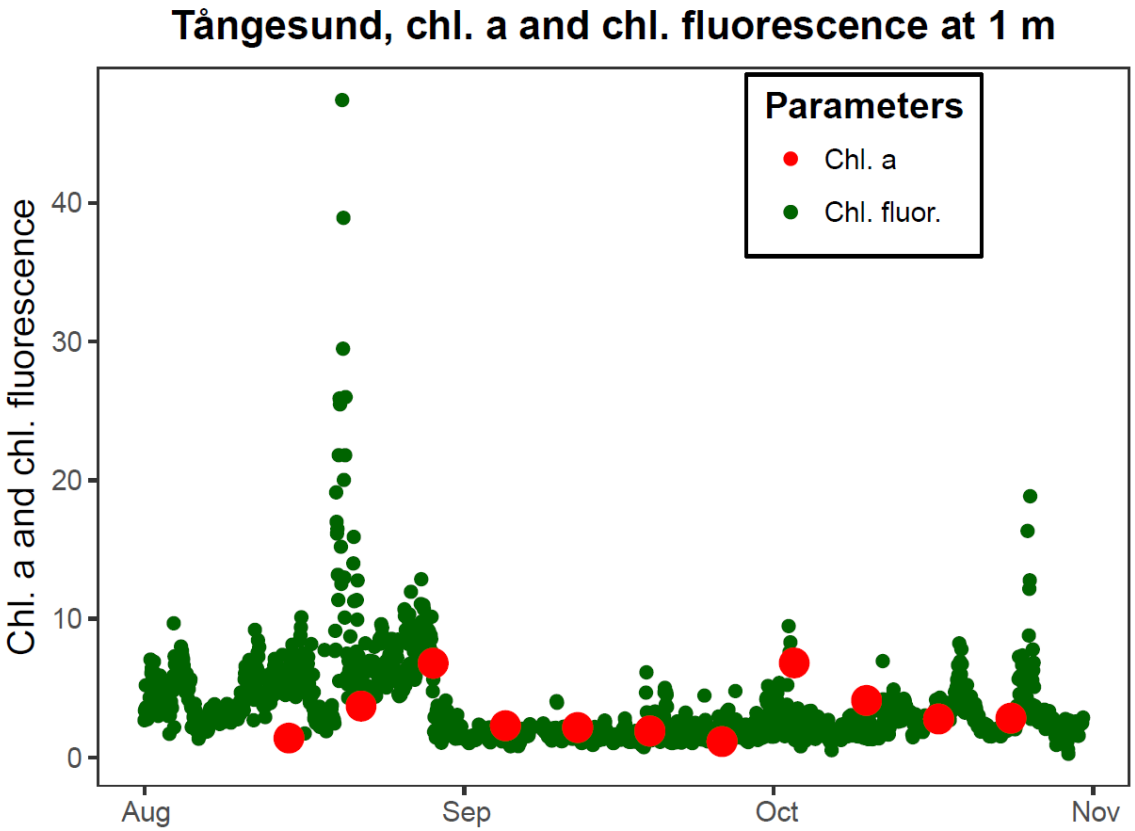
Approx. 100 m

The raft at the Tångesund observatory

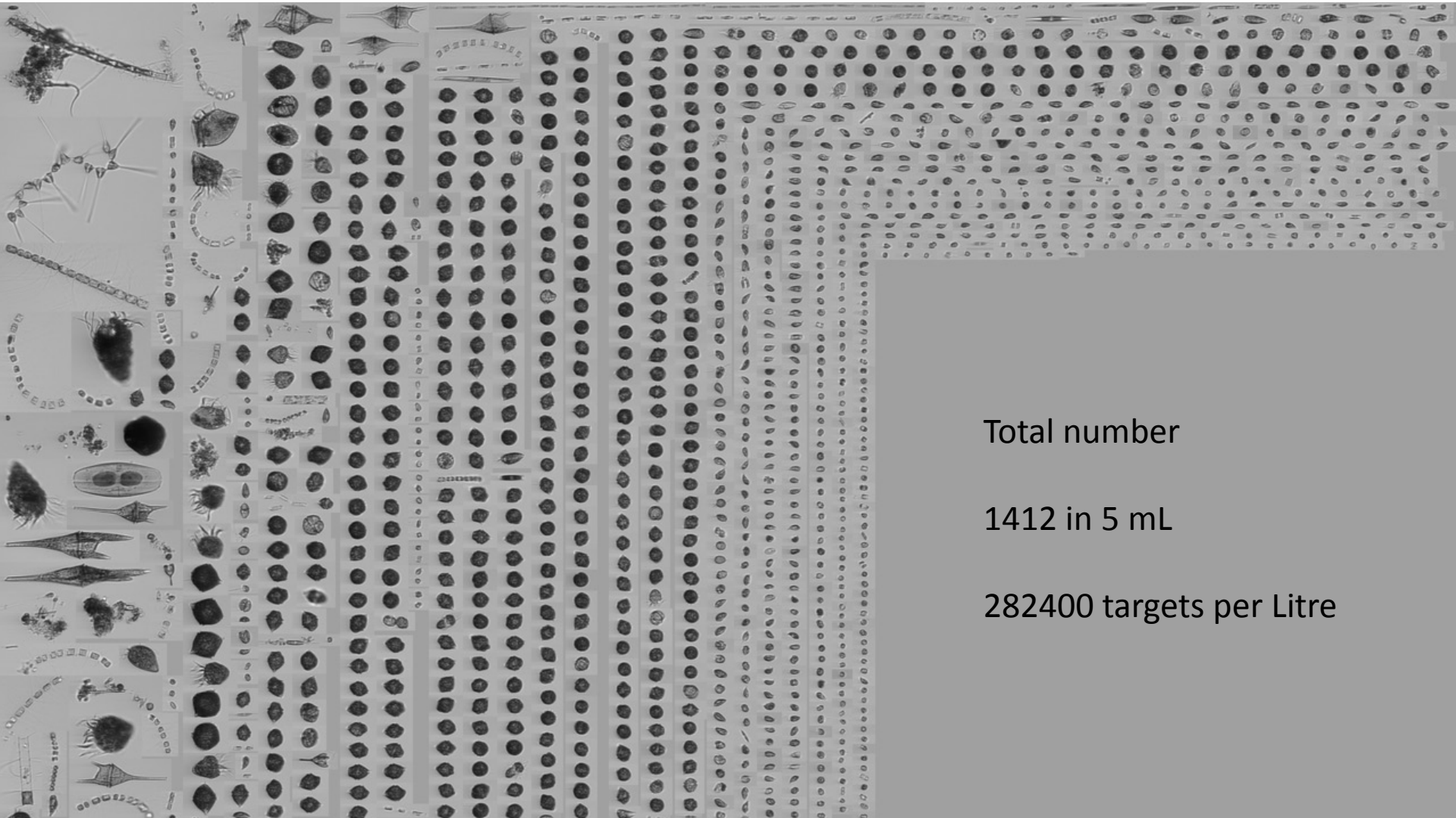
A simplified view of the Tångesund coastal ocean observatory




Chlorophyll a
Depth, m
0
5
10
15
20
1
2
3
4
5
6
7
8
9
10
11
12
Sampling event
Chl. a, µg L^{-1}
2.5
5.0
7.5
10.0


Tångesund, chl. a and chl. fluorescence at 1 m
Parameters
Chl. a
Chl. fluor.
Chl. a and chl. fluorescence
0
10
20
30
40
Aug
Sep
Oct
Nov

IFCB results Tångesund 28 Sep. 1313 UTC



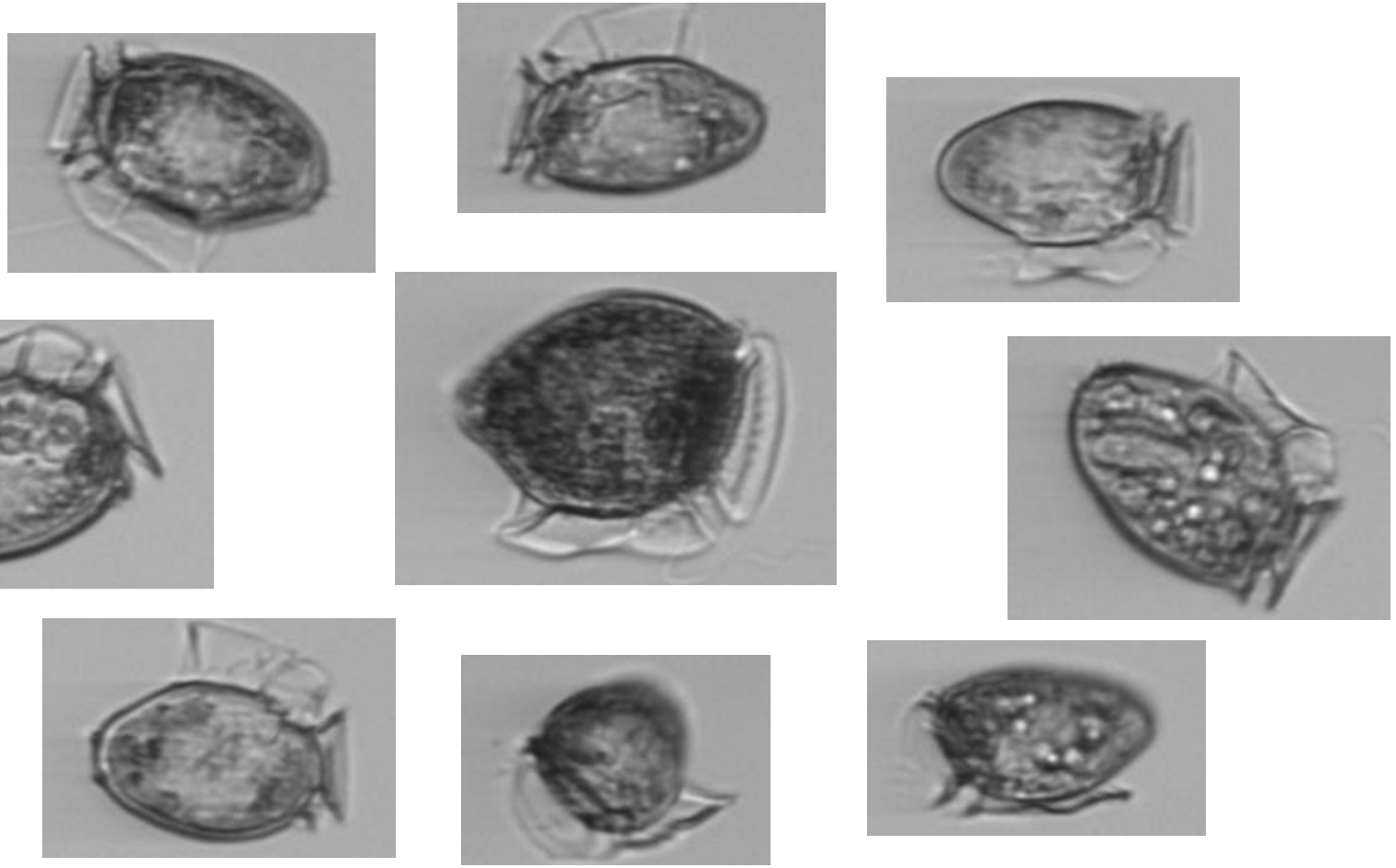
Total number

1412 in 5 mL

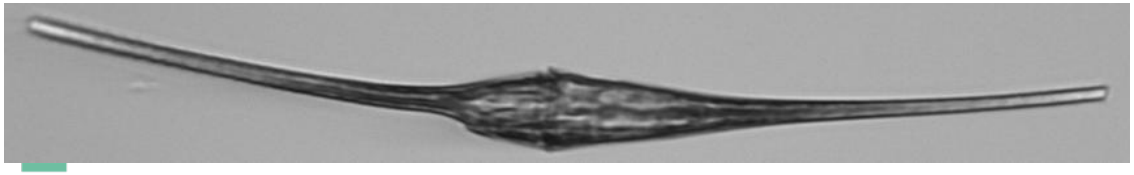
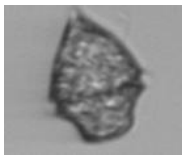
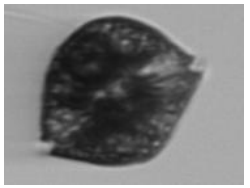
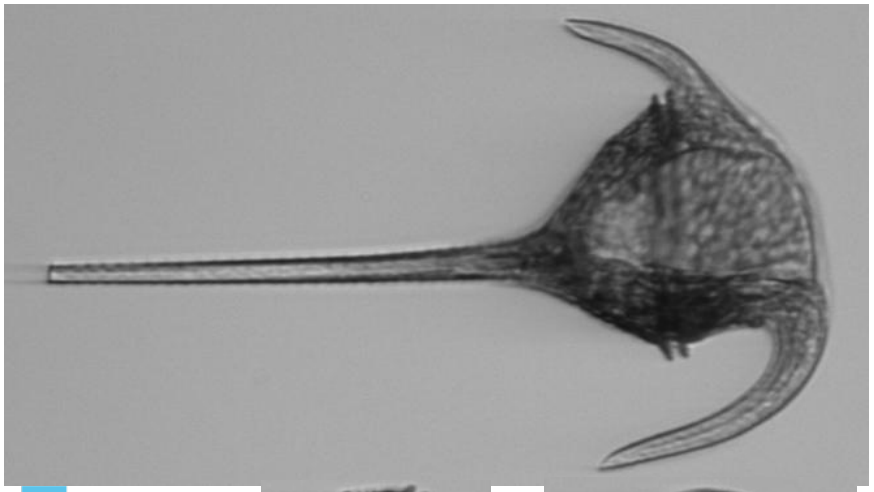
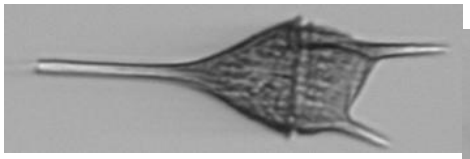
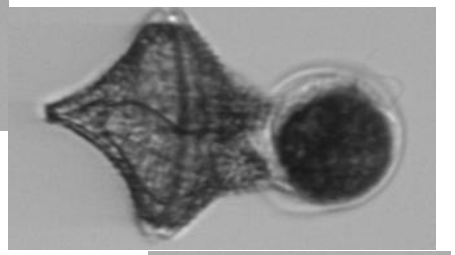
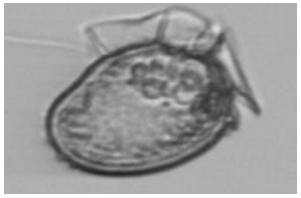
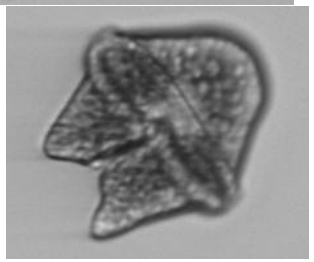
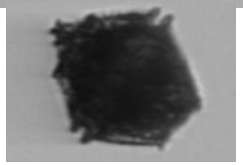
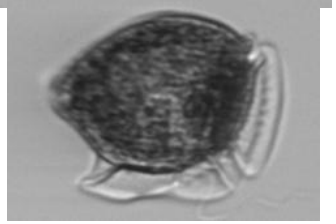
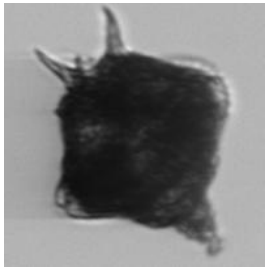
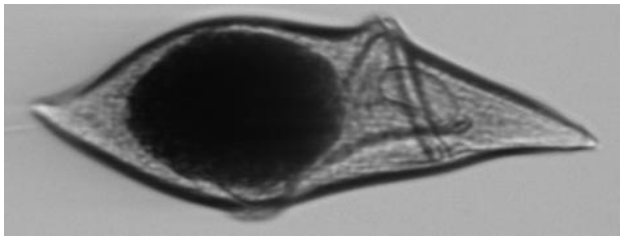
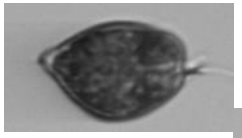
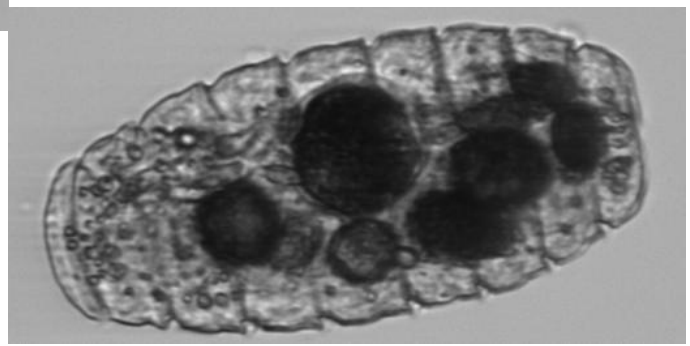
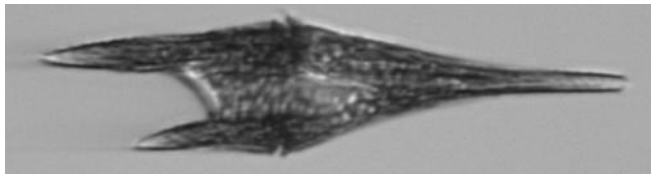
282400 targets per Litre

Examples of *Dinophysis* spp. images from IFCB

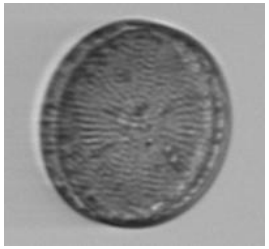
producer of Diarrhetic Shellfish Toxins (DST)

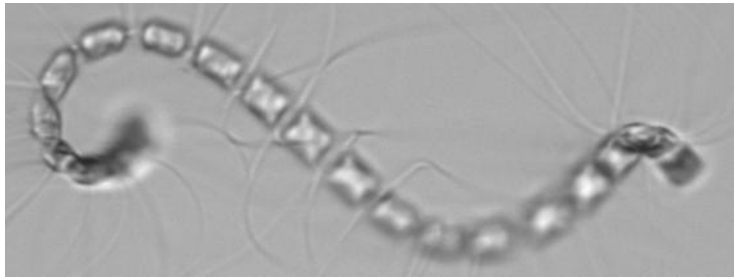


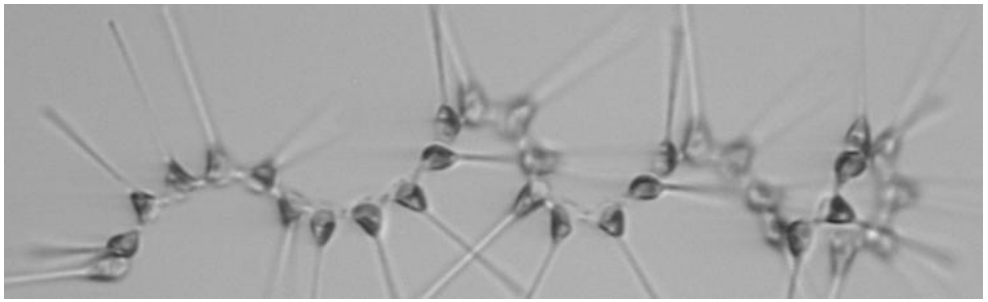
Examples of dinoflagellates

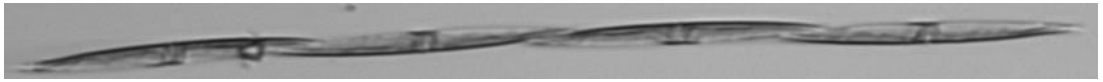
Examples of diatoms

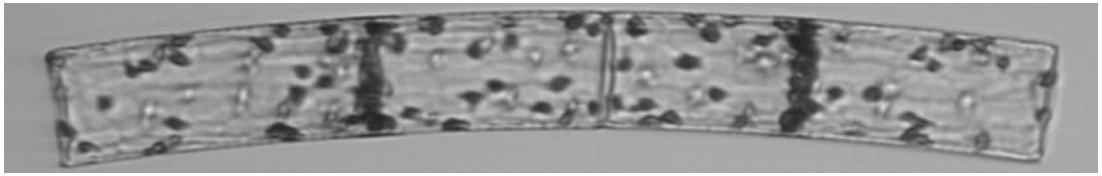




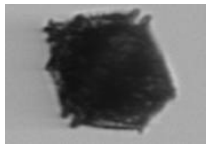


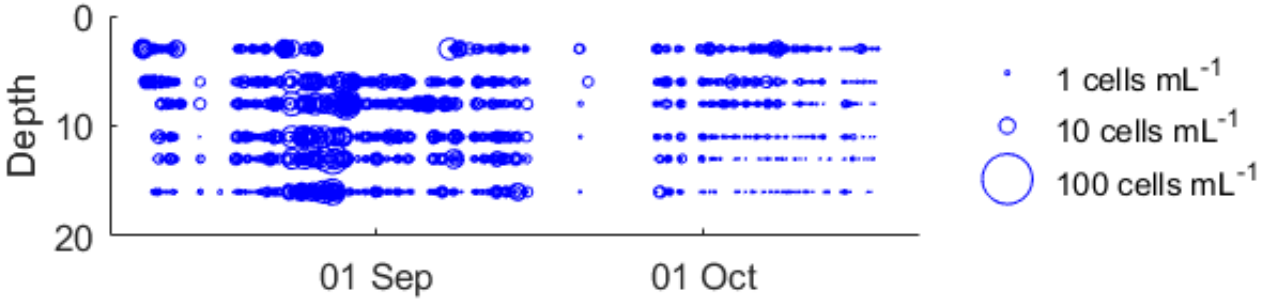
Pseudo-nitzschia sp. producer of domoic acid

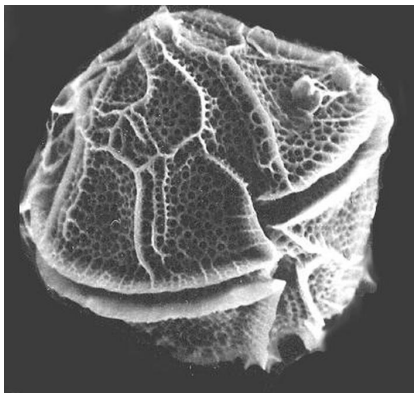




Example of results – *Lingulodinium polyedrum*



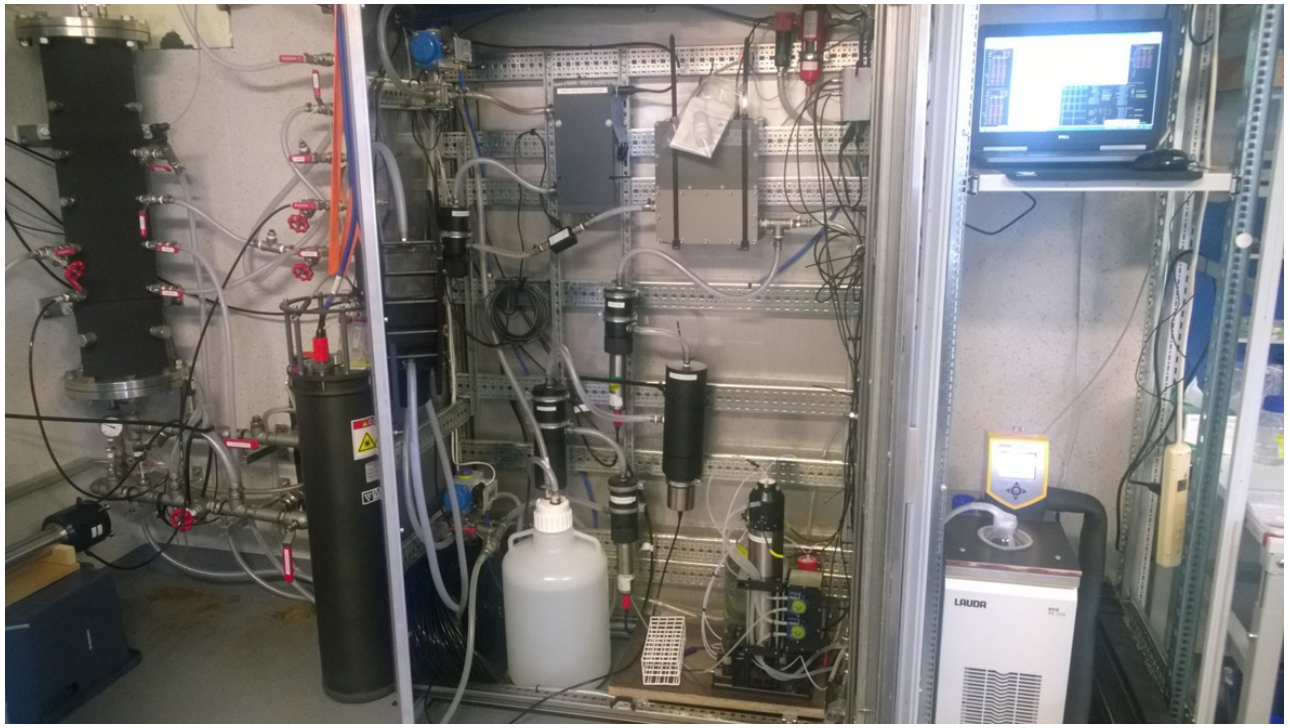




SEM photo by: Mats Kuylenstierna
Source: http://nordicmicroalgae.org

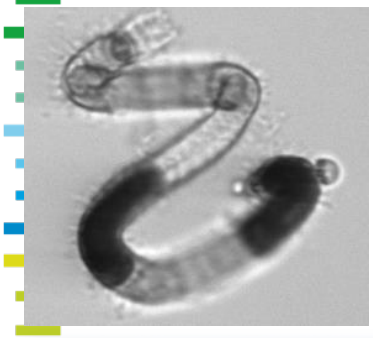
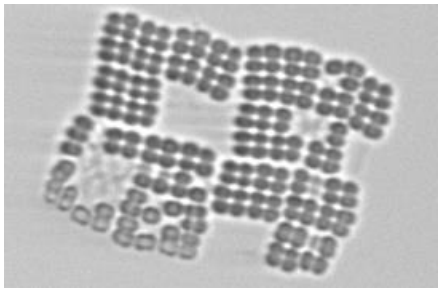
Imaging FlowCytoBot at SYKE

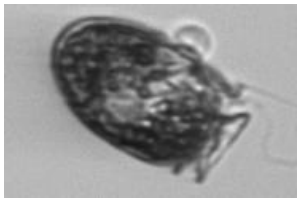
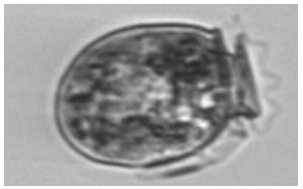
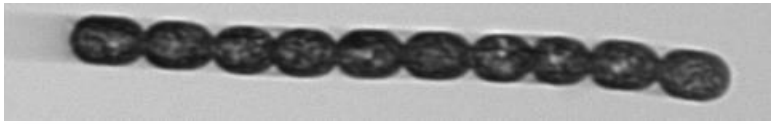
- Imaging FlowCytoBot purchased 2016
- primed, tested and run in the SYKE lab in winter 16/17; team trained by experienced user, Sílvia Anglès from the US , using test samples from Alg@line ferrybox
- part of team travelled to the US (McLane and WHOI) for further training
- deployed successfully at Utö 03/2017, connected to flow through system inside research hut

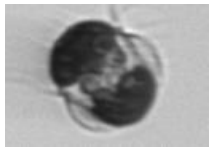


Imaging FlowCytoBot at SYKE

- At Utö IFCB samples every 20 min and transfers raw images to Helsinki in real time
- Automated cleaning cycle, but due to clogging issues (due to large cells) this is now supplemented by remotely operated one
- Some issues with pump, electronics and camera recently; although a lot can be done by operator, device will be send for the first service late 2017
- Creation of image library for the further training of the automated image classifier is in progress and funding seeked to compare IFCB data with trad. cell counts, optical data and phys-chem data collected at Utö.



Newsletter

Phytoplankton community in Utö, northern Baltic proper on 20.7.2017

Sirpa Lehtinen, Marine Research Centre of the Finnish Environment Institute (SYKE)

Phytoplankton community in Utö, northern Baltic proper, is dominated by cyanobacteria *Aphanizomenon flosaquae* and *Dolichospermum* sp. Only some filaments of the hepatotoxin producing cyanobacterium *Nodularia spumigena* have been observed. These three species are able to N_2-fixing, which may give them competitive advantage when there is plenty of phosphorus available in the sea water.

Dinoflagellates *Dinophysis spp.* and *Heterocapsa triquetra*, diatom *Chaetoceros* spp., and nanoflagellates including e.g. crypto-, prasino-, and prymnesiophytes were the other most common phytoplankton taxa (Fig. 1).

Surface temperature is ca. 15°C and chlorophyll *a* concentration ca. 5-6 µg/l in the northern Baltic proper, based on the Alg@line FerryBox data collected from the route of M/S Finnmaid.

Data sources

Phytoplankton community is observed continuously using the Imaging FlowCytoBot (IFCB, https://www.finmari-infrastructure.fi/?x118281=189689), owned by the SYKE Marine Research Centre. IFCB is situated in the Utö Atmospheric and Marine Research Station of the Finnish Meteorological Institute (59º 46'50N, 21º 22'23E). Utö Island is located at the outermost edge of the Archipelago Sea, facing the Baltic proper (Fig. 2).

IFCB, Utö Atmospheric and Marine Research Station, and the Alg@line FerryBox network are parts of the Finnish Marine Research Infrastructure FINMARI (https://www.finmari-infrastructure.fi/).

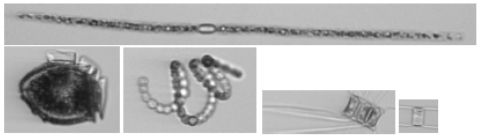
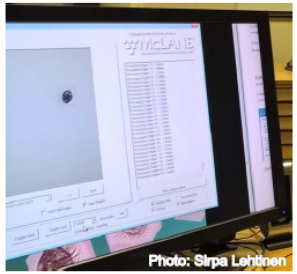


Fig. 1. Selected images taken by the Imaging FlowCytoBot (IFCB) on 20.7.2017 at Utö. Images from left to right: *Aphanizomenon flosaquae* (upper), *Dinophysis norvegica, Dolichospermum* sp., *Chaetoceros* cf. *wighamii, Chaetoceros* cf. *similis*.



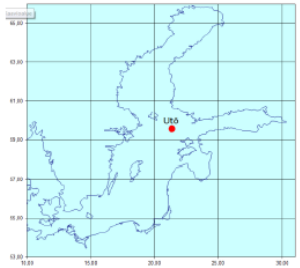
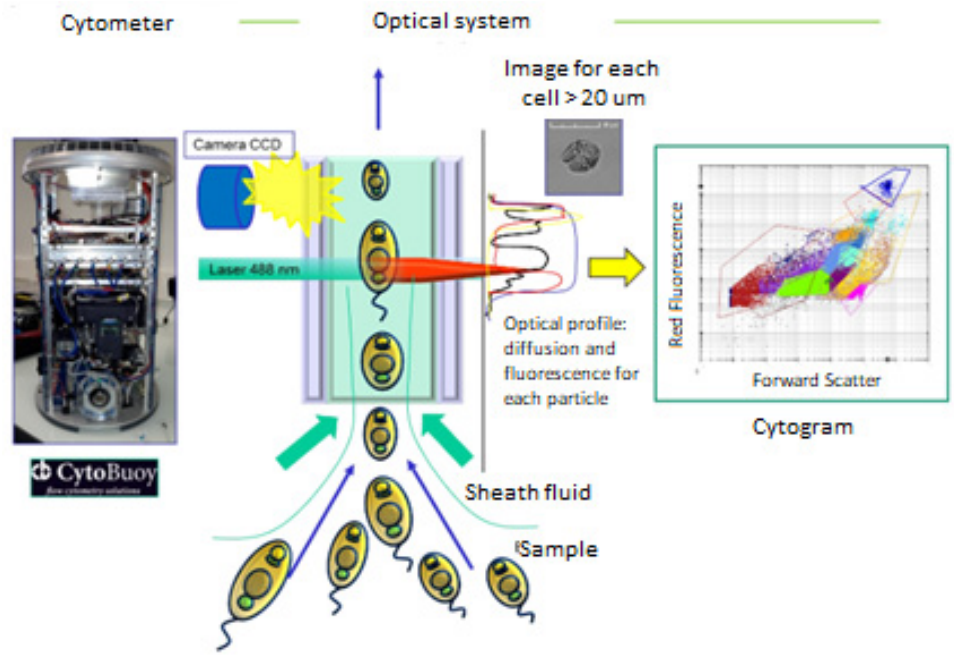
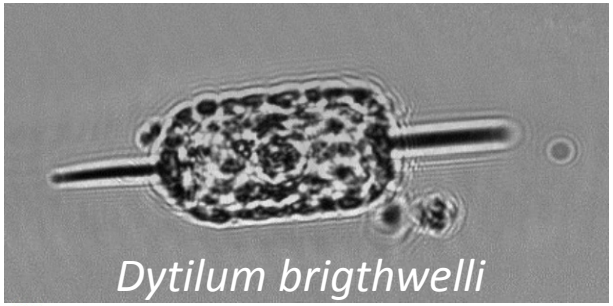


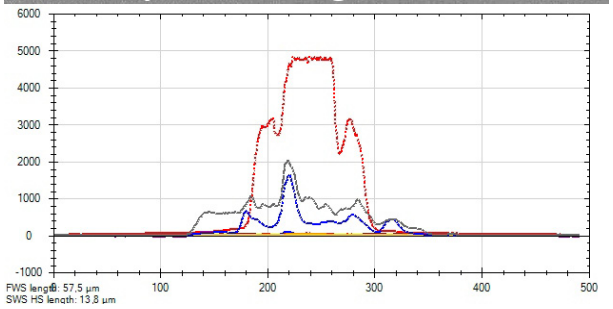
Fig. 2. Phytoplankton cells passing the flow-through system of the Imaging FlowCytoBot (IFCB) can be seen in real time in the Kumpula laboratory in Helsinki (left). IFCB is owned by the Marine Research Centre of the Finnish Environment Institute (SYKE), and it is situated in the Utö Atmospheric and Marine Research Station of the Finnish Meteorological Institute (FMI). Utö island is located at the outermost edge of the Archipelago Sea, facing the Baltic proper (right).

CytoSense/CytoPro principle

- Similar to IFCB <u>but different</u>
- Fluorescence and scatterring are the main parameters
- Optical pulse-shape profiles are recorded as signatures
- A limited number of organisms can be imaged



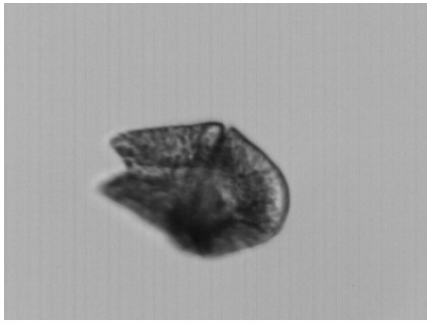


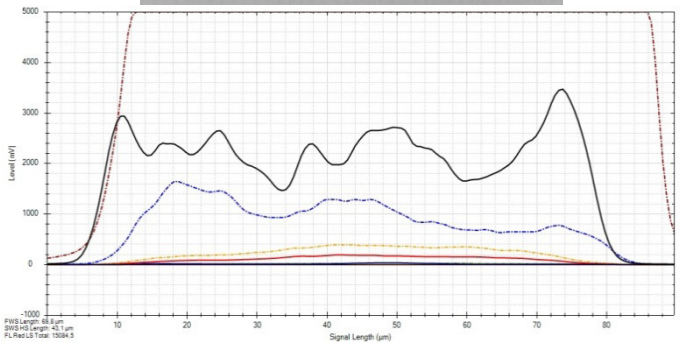


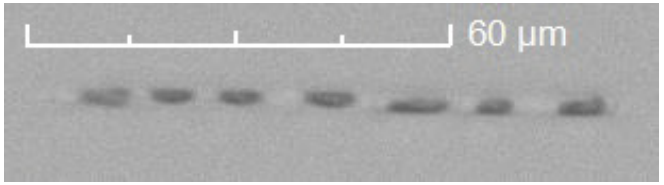
Standardized output:

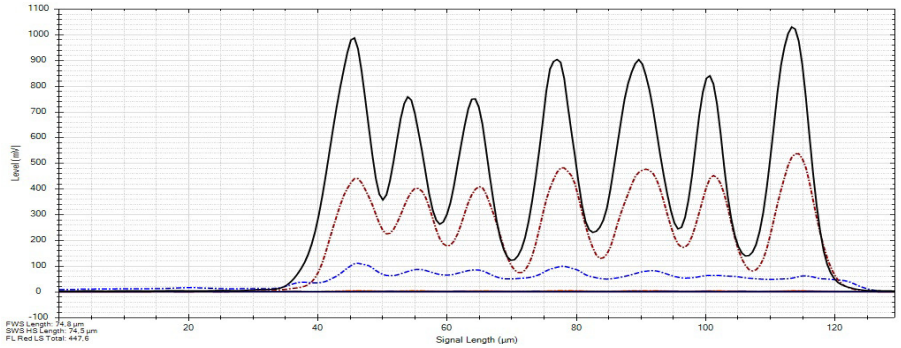
- *Synechococcus* (pico-cyanobacteria)
- Eukaryotic picoplankton
- Nanoplankton
- Microplankton

Optical signatures



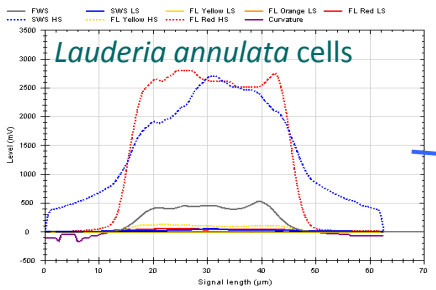


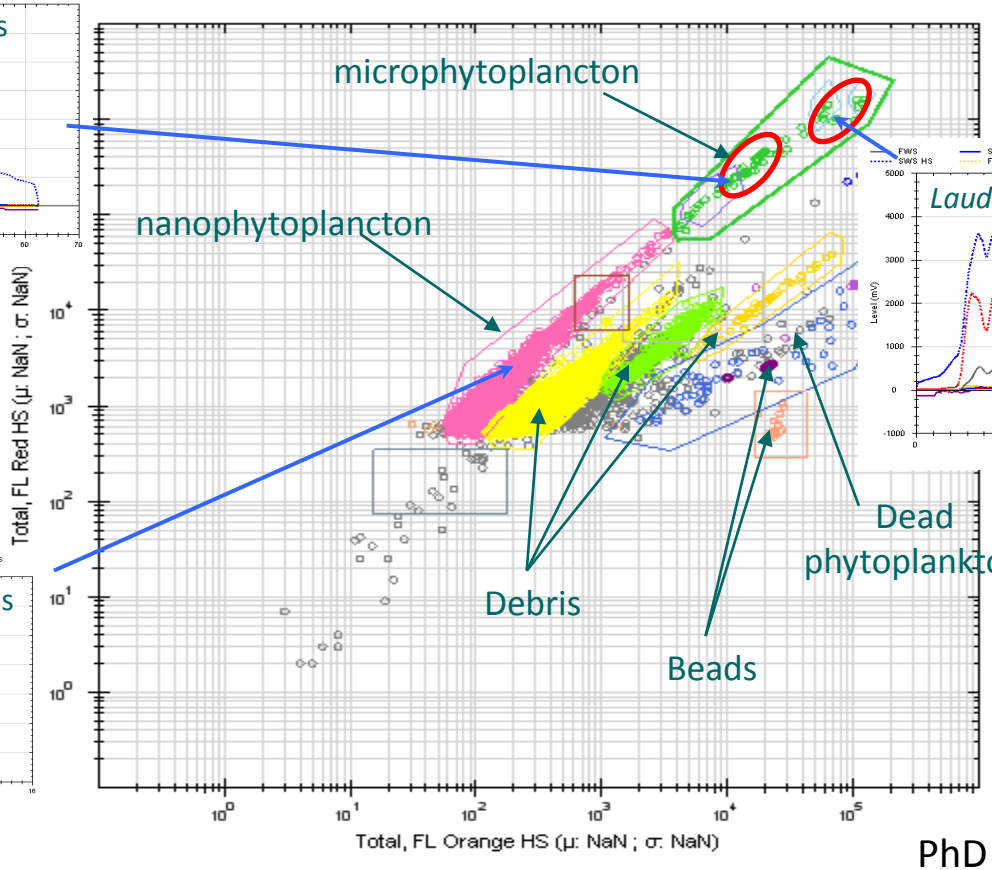


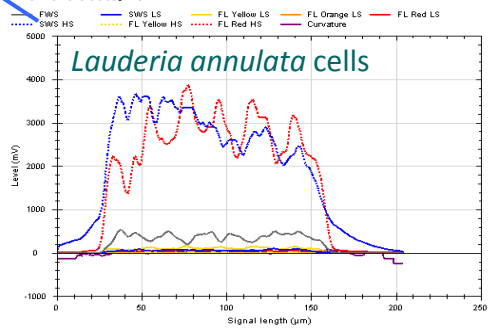


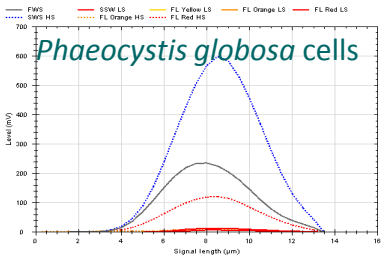
Pictures: Machteld Rijkeboer

Data processing softwares









PhD Natacha Guiselin, 2010

Subgroups can be discriminated based on **similar optical properties**.

- Manual clustering software: **CytoClus** (CytoBuoy)
- Automated clustering softwares:
  - **EasyClus/EasyClus LIVE** (Thomas Rutten projects): supervised, unsupervised analysis
  - **RclusTool** (LISIC, CNRS-LOG ULCO): supervised, unsupervised, semi-supervised analysis

INTERREG IVA "2 Seas" DYMAPHY project (2010-2014)

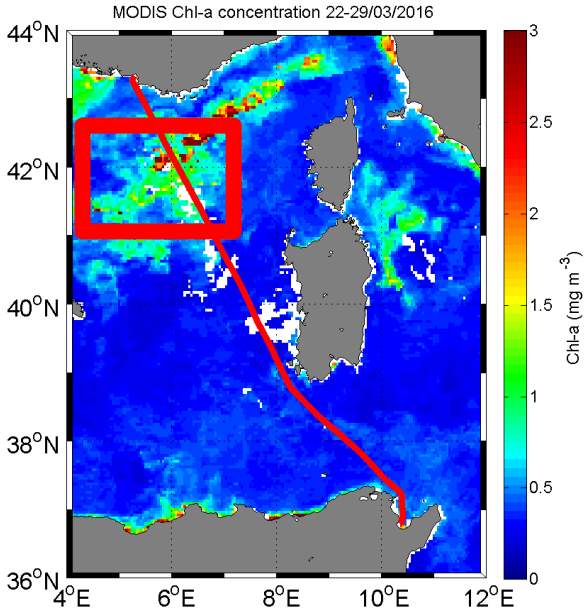
Continuous and High Resolution Observation of the MEditerranean Sea

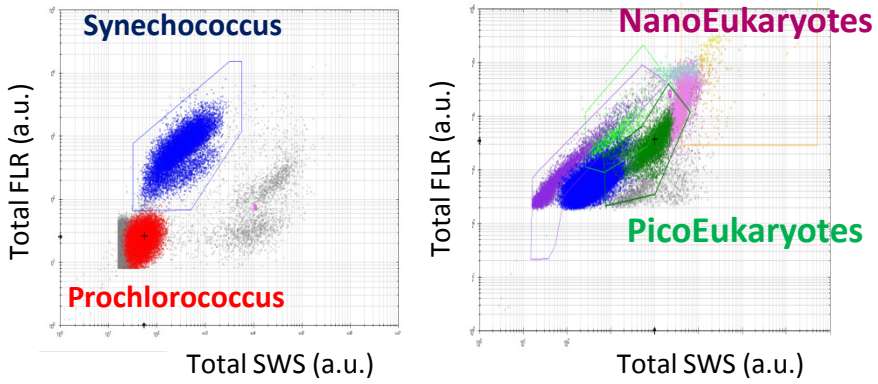
P. Marrec, G. Grégori, C. Sammari, S. Lahbib, N. Bhairy, S. Ben Ismail, M.Thyssen

The open Mediterranean sea is dominated by pico-nanoeukaryotes, even during spring blooms

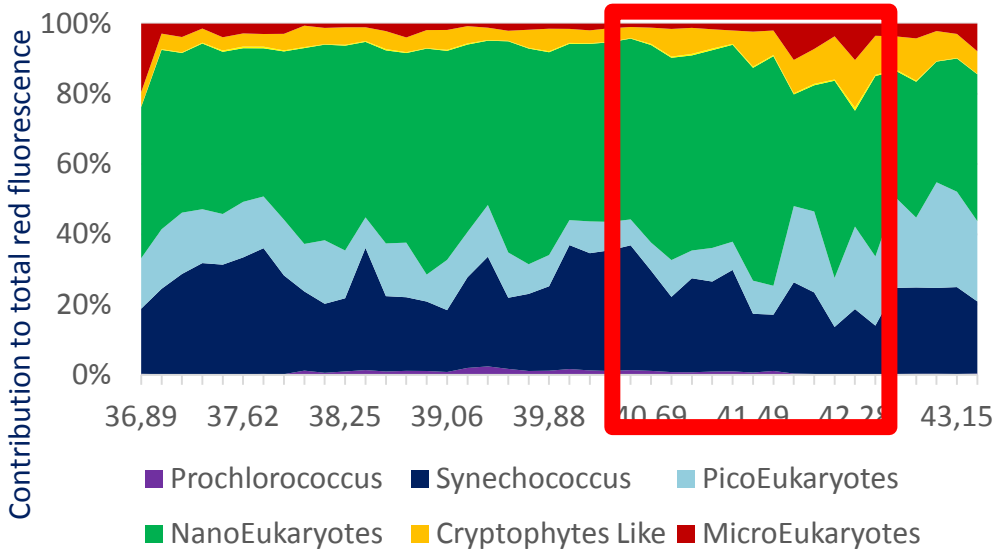
- Oligotrophic sea, max ~ 1 µg/L Chla a
- Spring bloom is often dominated by <20 µm cells

and mostly nanoeukaryotes sie classes





Cytosense flow cytometer was improved to cover <1µm and > 500 µm: Cytogrammes depicting reccorded cells



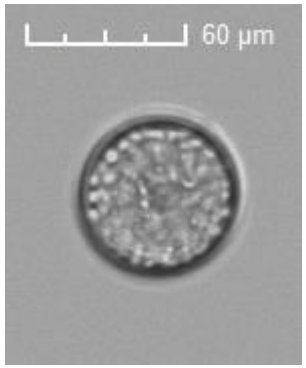
SeaDataCloud

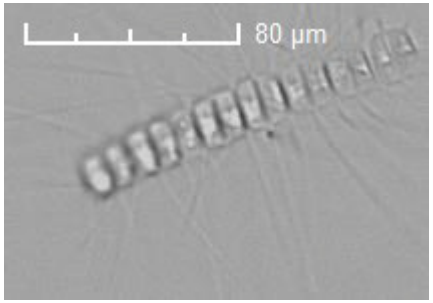
Vocabulary standardisation/database under progress within seadatacloud

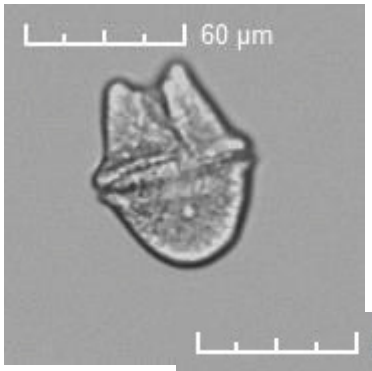
https://chrome.mio.univ-amu.fr/chrome-cytobase/

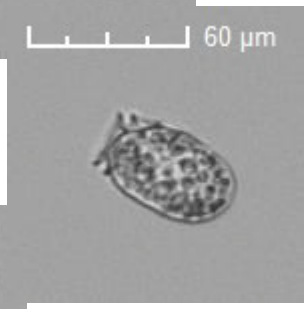
http://www.mio.univ-amu.fr/cytobase/

Images from CytoPro

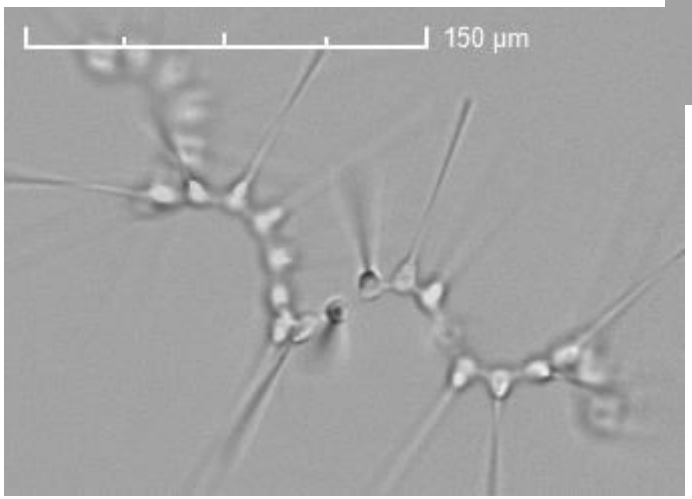


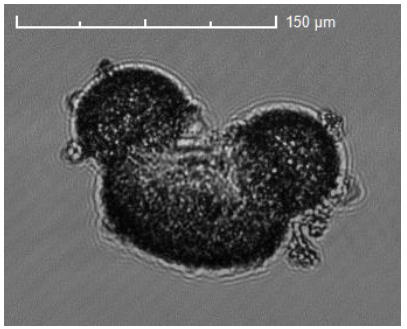






https://precym.mio.univ-amu.fr

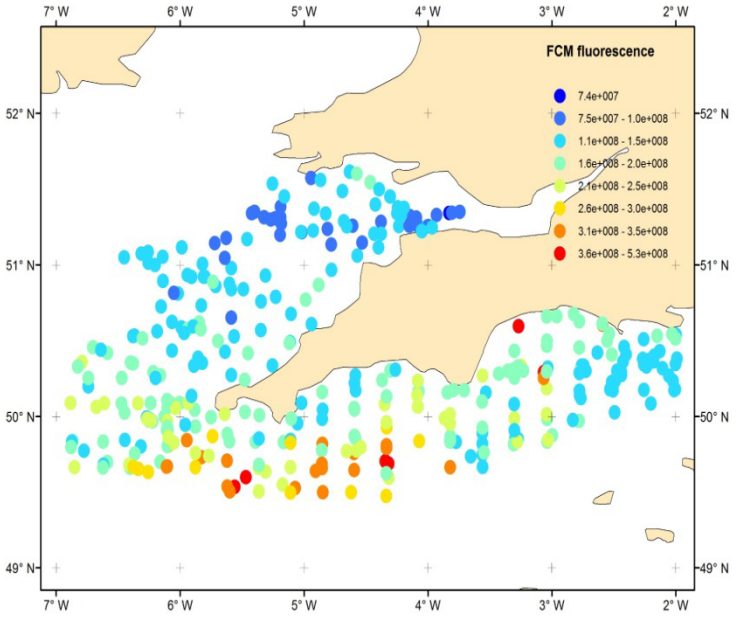




Photos: G. Gregori and M. Thyssen

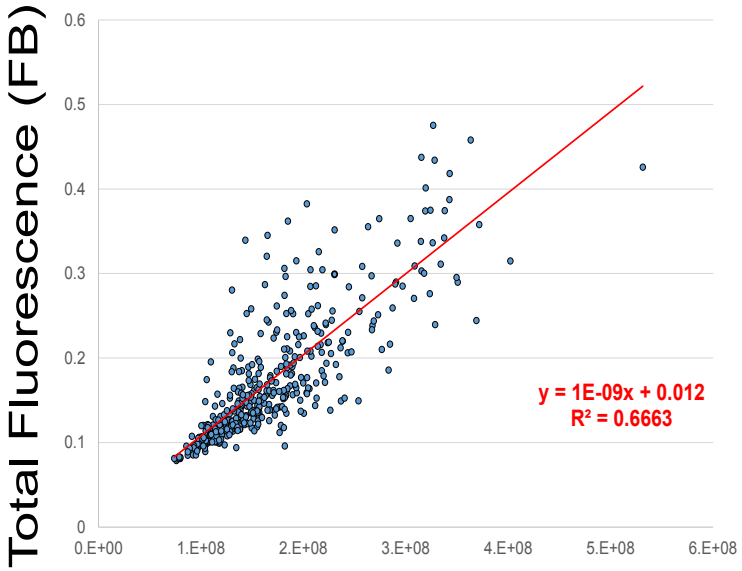
R/V Endeavour

Véronique Creach



Total FLred (FCM)

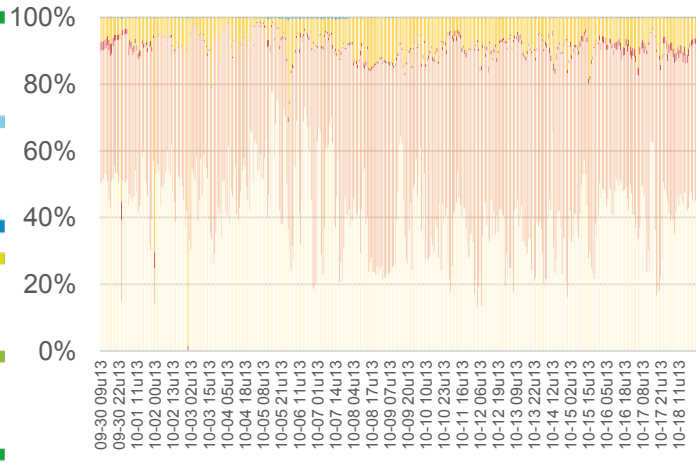
Relationship between total Fluorescence by Flow cytometry and FerryBox



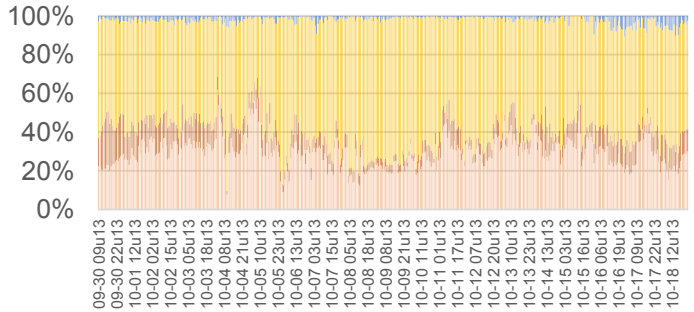
Total FLred (FCM)

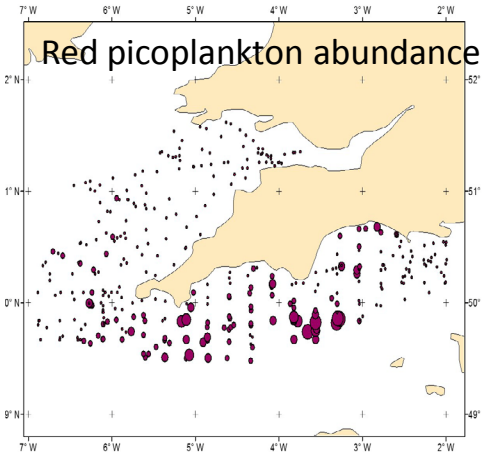
Phytoplankton functional types

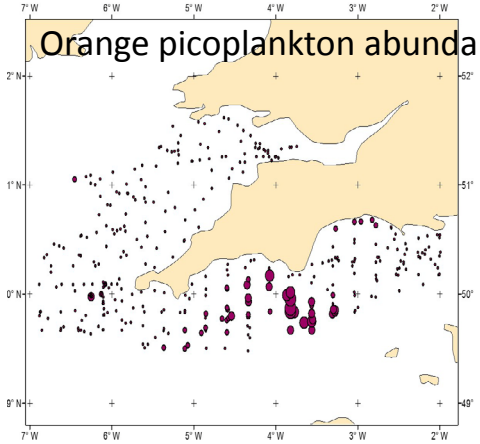
Abundance



Red fluorescence as a proxy for biomass

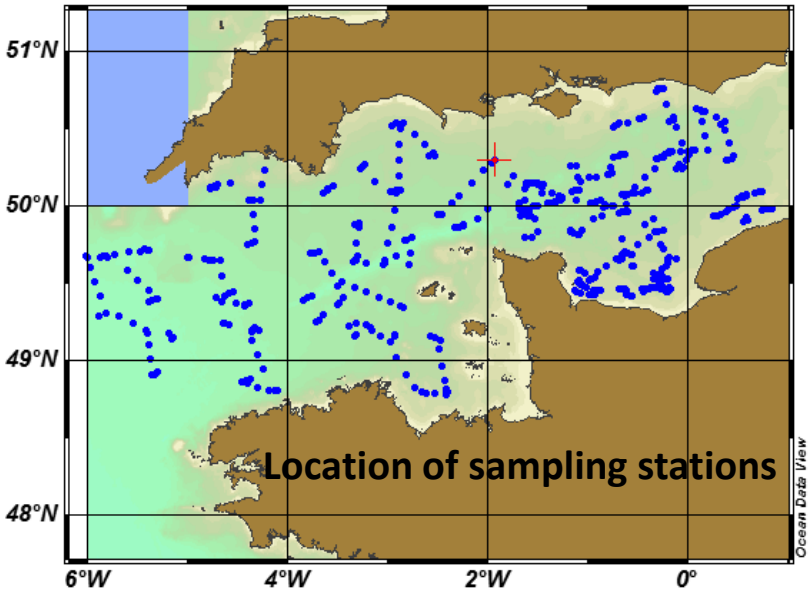






Innovative technologies on board Research Vessels

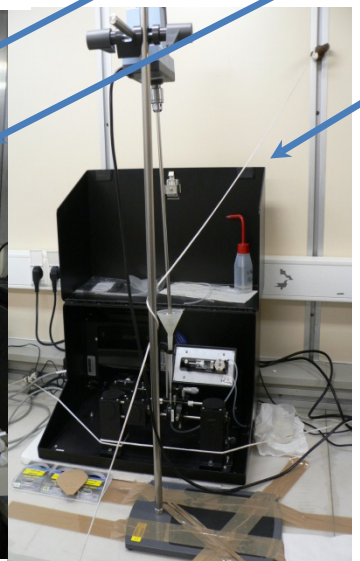
Lefebvre A., Wacquet G., Colas F., Louchart A., Artigas L.F.

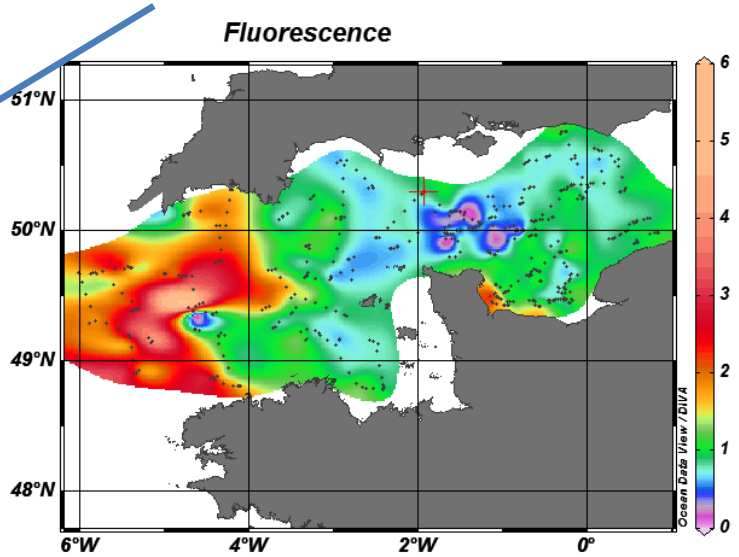


Objective: Towards the operational implementation of HF resolution techniques during ecosystemic cruises (fish stock assessment, research, MSFD purposes)

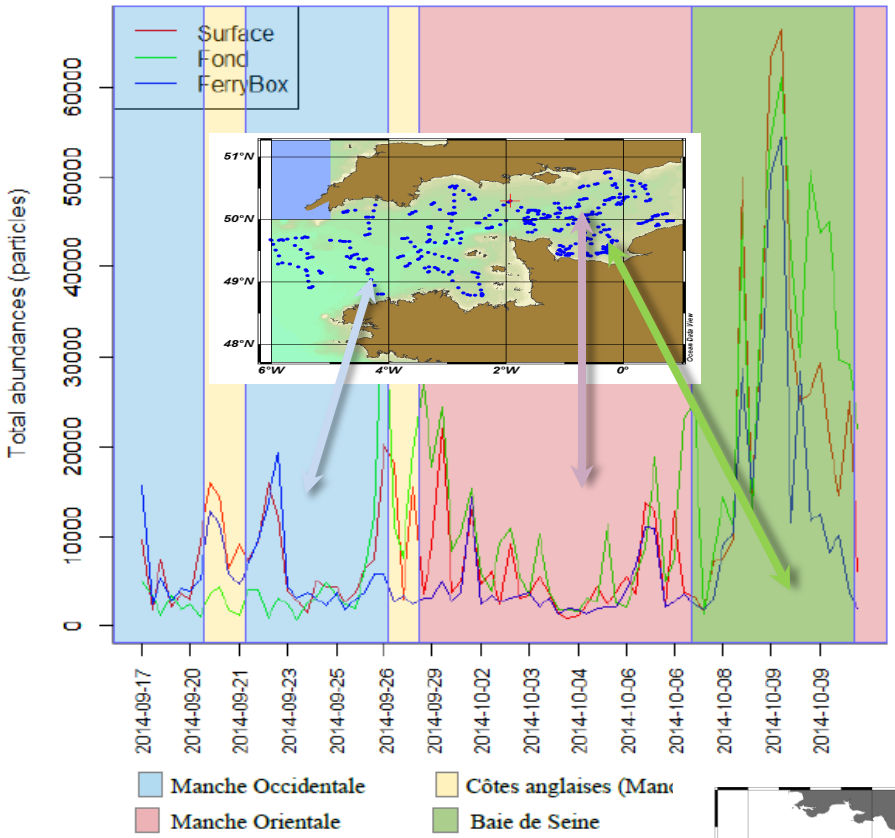
Example of combination of conventional low resolution monitoring strategy with high resolution Analysis by in vivo recording innovative technologies:

- Ferry-Box
- Spectral fluorometry
- Flow cytometry
- FlowCAM





Example of water masses discrimination based on phytoplankton abundance/discrimination using a new training set for the English Channel

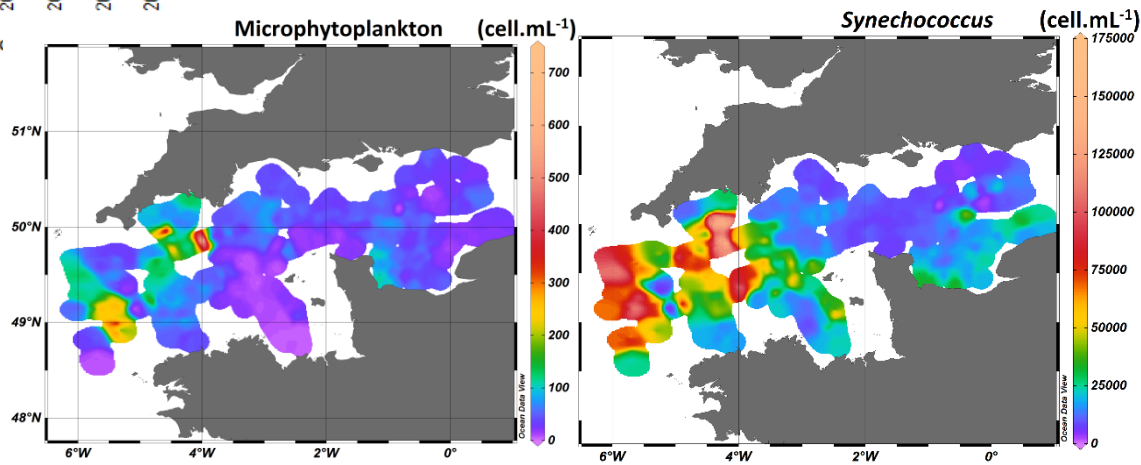


FlowCAM
***in vivo* analysis**

High variability of phytoplankton abundance between water masses on a relatively short time period (< 1 month). For each area, automated discrimination of up to 28 (image analysis) and 8-10 phytoplankton groups (automated flow cytometry).

Now available (English Channel):

- Automated Classification
- New variables
- High resolution strategy
- Early warning systems
- Quantifiable errors
- Data base to secure raw data

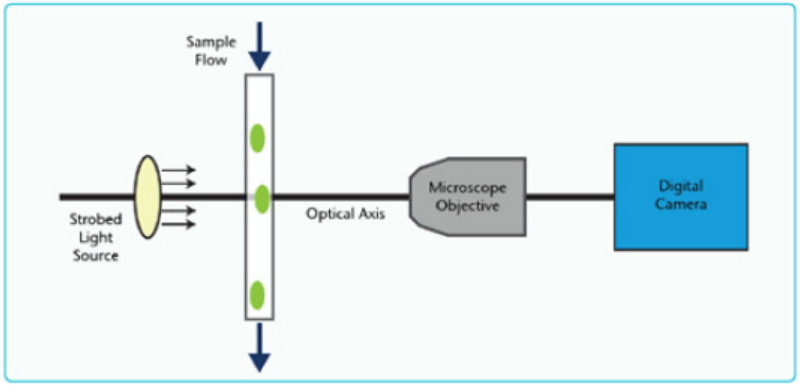


Continuous recording automated FCM

FlowCAM principle

- Similar to IFCB but different
- No sheath fluid - not a flow cytometer
- Images (in colour or black & white) of all organisms
- Fluorescence and scattering mainly used for triggering camera
- Morphology-based
- Automated classifiers (as ZooImage package in R)
  - Recognition tools build from training sets
  - Development of analytical modules like active learning, partial validation of predictions

- Dynamic imaging-in-flow system
- Camera: 8 to 22 frames per second

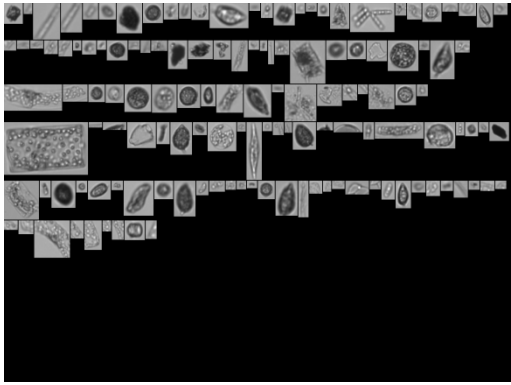


FlowCAM software: Visual Spreadsheet

- 1 frame can contain multiple particles
- Pattern recognition software:
  1. Segregate particle from background
     - Grayscale pixel ≠ grayscale background → particle pixel
     - Binary image created
     - Each particle = tiff file
     - .lst created = collage of particles

Frame

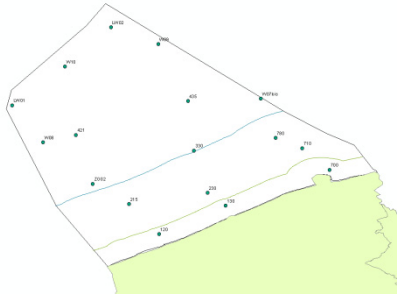
Binary image collage



Particle collage = .lst file

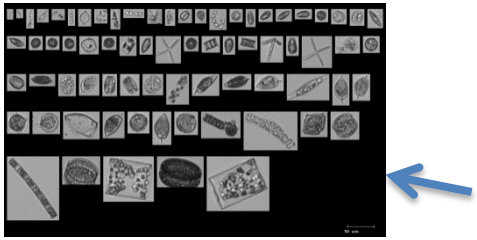
Sampling to identification

1. Monthly sampling campaigns



2. 9 Samples (2% lugol)

3. Monthly after campaigns



4. Digital copy of samples (.lst files)

5. Semiautomatically identify plankton
Visual spreadsheet

6. Offer validated data via online interface

Klaas Deneudt et al. VLIZ

FastCAM: a prototype

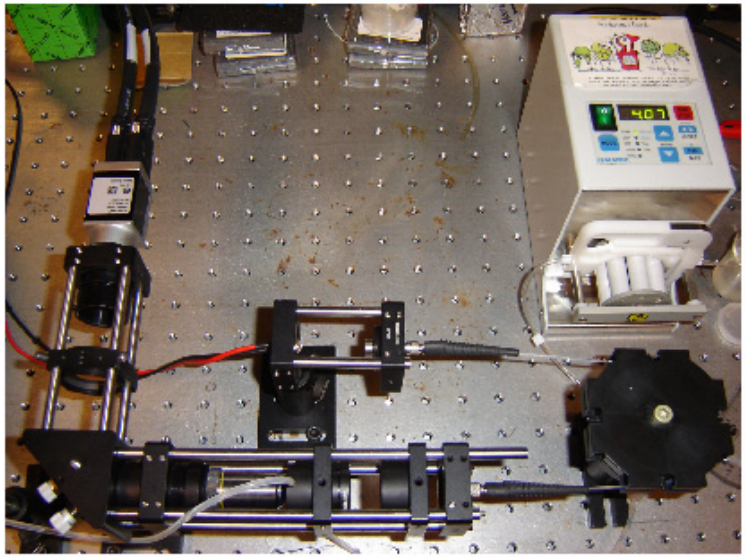
Main objectives:

- Speeding up digital images acquisition
  (340 vs 22 images/sec)
- Use of a high resolution Camera (1024 x 2048)
- Use of an autofocus mode

⇒ **13 min.** for 1 sample (10x / 100 µm)
vs 143 min. with the FlowCAM

FlowCAM performances

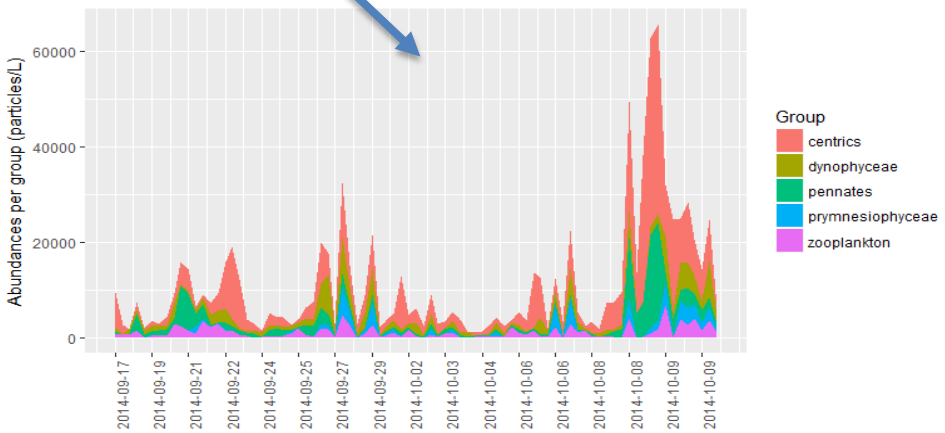
	4X	10X
ϕ_{num} (mL/mi)	1,2	0,07
t_{10mL} (min)	8,3	143



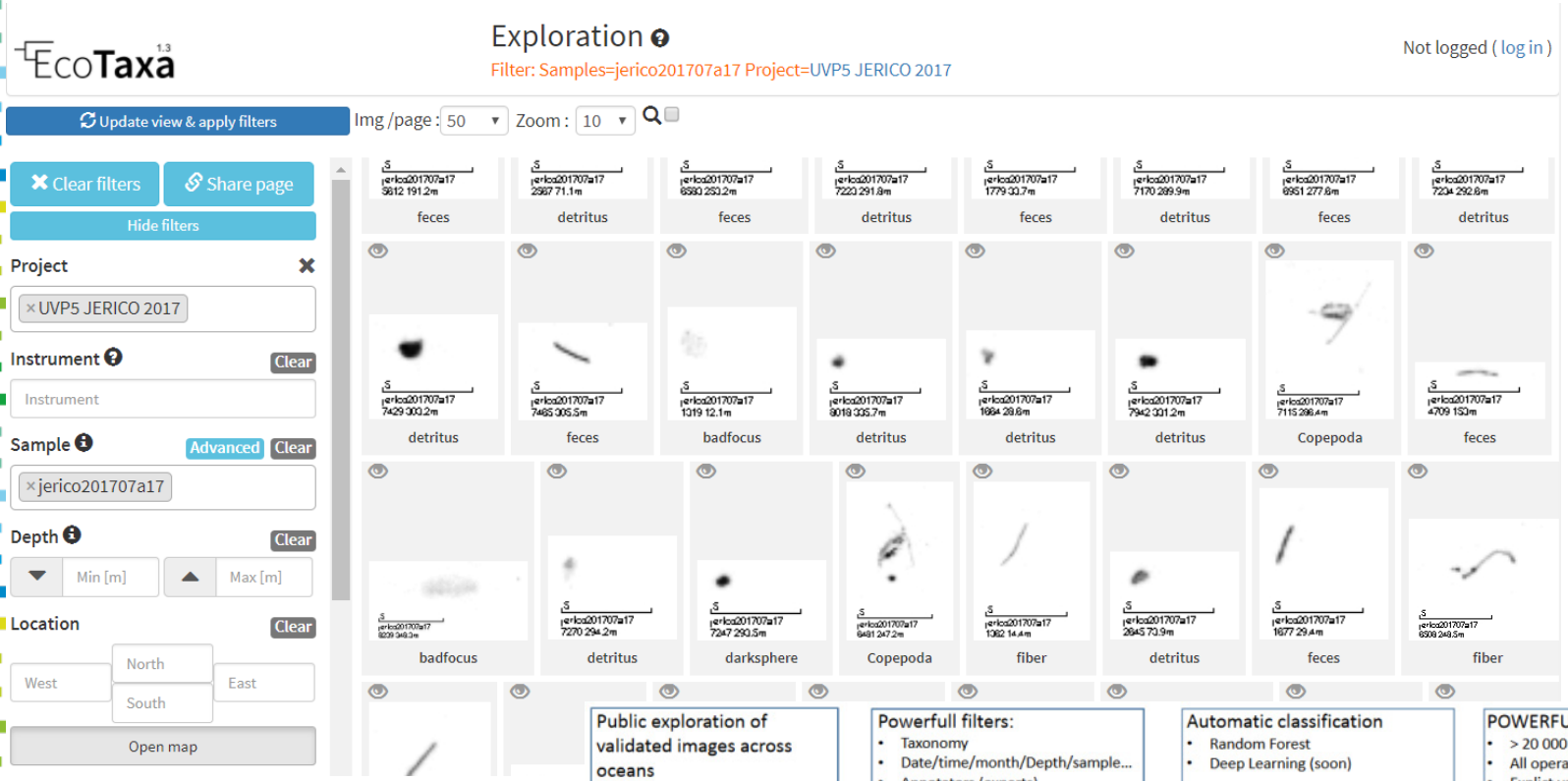
About image analysis data

1. Transfer and storage of millions of small images (3 gigabyte per 3 months)
2. Automated analysis of images
3. Results:
    1. At the species/genus level
        1. Abundance per litre
        2. Cell volume per litre
    2. Harmful taxa
    3. Aggregating data to higher taxonomic levels
        1. Class level
            1. Bacillariophyceae (diatoms)
            2. Dinophyceae (dinoflagellates)
            3. Cyanophyceae (cyanobacteria)
            4. Haptophyceae
            5. Etc.

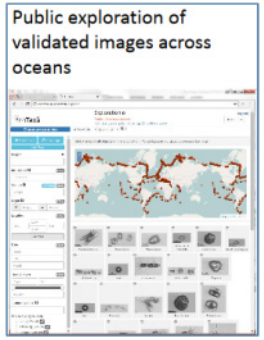
Example: FlowCAM *in vivo* analysis

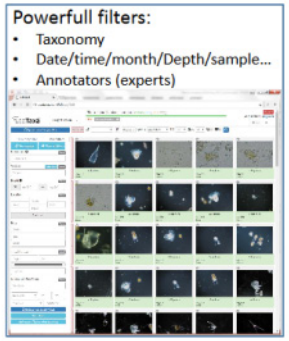


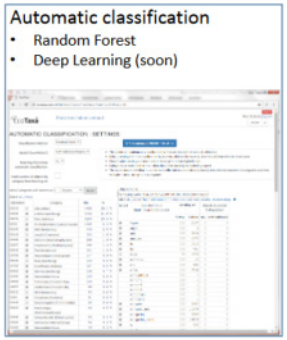
EcoTaxa a system for storage of millions of images and automated classification (species identification)

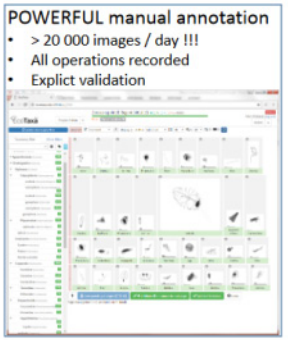


http://ecotaxa.obs-vlfr.fr/explore/









Picheral M., Stemman L., MIW 2017

Conclusions

- Imaging flow cytometry is a reliable method for sustained, automated observation of phytoplankton biodiversity and biomass, complementing manual methods for sampling and microscope analyses.
- Development of classifiers for automated identification/discrimination of organisms is time consuming and requires specific skills on signal analysis and on taxonomy.
- Automated flow cytometry has proven to be a useful tool for counting phytoplankton and for describing the phytoplankton community as size based classes and functional groups, four main functional groups were selected for inter-comparison exercises:
  - *Synechococcus* (pico-cyanobacteria)
  - Eukaryotic picoplankton
  - Nanoplankton
  - Microplankton.
- New classification tools are being defined and tested which should allow improved discrimination of phytoplankton functional groups.

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