



### Harmful Algal Bloom studies in JERICO-NEXT

The presentation takes about 5 minutes, it will repeat.

#### Bengt Karlson and Felipe Artigas

Contributions from: Hedy Aardema, Michael Brosnahan, Reinhoud de Blok, Pascal Claquin, Florent Colas, Veronique Créach, Klaas Deneudt, Wenche Eikrem, Gérald Grégori, Jacco Kromkamp, Soumaya Lahbib, Alain Lefebvre, Sirpa Lehtinen, Fabrice Lizon, Arnaud Louchart, Pierre Marrec, Klas Möller, Emilie Poisson-Caillault, Machteld Rijkeboer, Thomas Rutten, Suvi Rytövuori, Jukka Seppälä, Lars Stemmann, Melilotus Thyssen, Lennert Tyberghein, Guillaume Wacquet and Pasi Ylöstalo.





"The complexity of the coastal ocean cannot be well understood if interconnection between physics, biogeochemistry and biology is not guaranteed. Such an integration requires new technological developments allowing continuous monitoring of a larger set of parameters." (The JERICO-RI consortium, 2014)

#### Harmful algae in focus

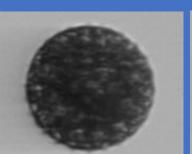
IFCB images

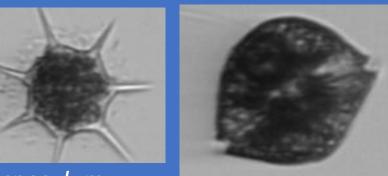




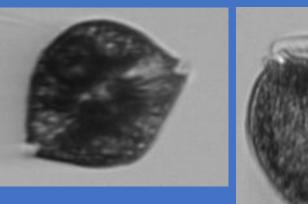


Lingulodinium polyedra

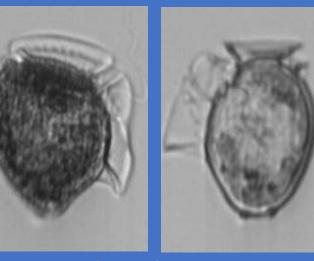




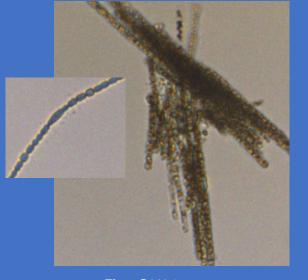
Dictyocha speculum



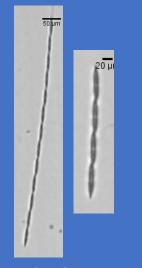
Alexandrium pseudogonyaulax



Dinophysis spp.



FlowCAM images Cyanobacteria



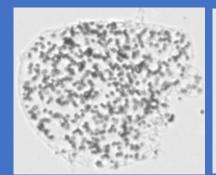
FlowCAM images (overall magnification: 40X)

Pseudo-Nitzschia spp.



FlowCAM images (overall magnification: 40X)

Dinophysis spp.



FlowCAM images (overall magnification: 40X)

Phaeocystis spp.





## Novel methods for automated in situ observations of HAB, phytoplankton abundance and diversity FlowCAM Benchtop B2 Series





FRRF



Multi wavelength absorbtion





Multi-wavelength fluorometry



Imaging Flow Cytometry
Imaging Flow Cytobot



Pulse Shape Flow Cytometry CytoSense



In flow imaging FlowCam

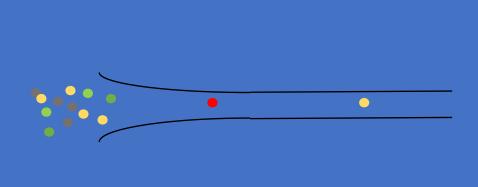
## In vivo/in situ automated approaches for observing phytoplankton

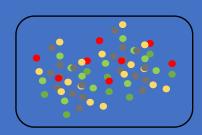


Imaging/in flow
Single cells size and
morphology
of organisms

Automated flow cytometry (pulse shape-recording)
Single cells - fluorescence -pigment content and scattering (size, shape)

Fluorescen ce and absorption (multi-spectral)
Pigment based methods
- bulk properties +
Variable fluorescence (photosynthetic parameters)









#### Case study areas

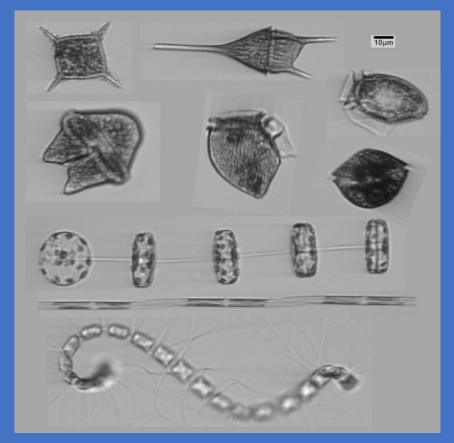
Baltic Sea
Kattegat-Skagerrak
North Sea – English Channel – British Isles
Western Mediterranean

#### The Tångesund observatory



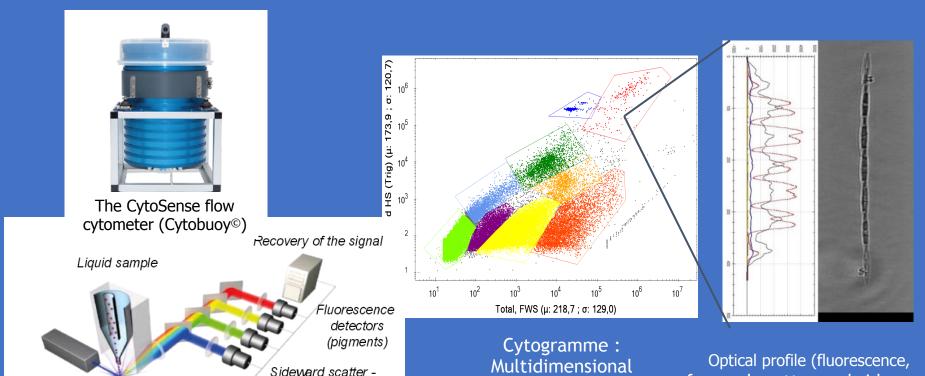






Contact persons:
Bengt Karlson and Mike Brosnahan
bengt.karlson@smhi.se
mbrosnahan@whoi.edu

# Single-cell analysis: Pulse-shape recording flow cytometry + imaging



SWS detector

(structure)

orward scatter-FWS

detector (size)

Laser beam

Cytogramme:

Multidimensional
representation of all cells
analysed according to their
optical features.

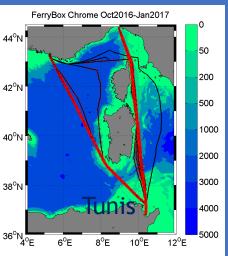
Optical profile (fluorescence, forward scatter and sideward scatter) of a particle (colony of Pseudonitzchia sp.) and associated image

Contact persons:
Felipe Artigas
Machteld Rijkeboer
Veronique Creach
Melilotus Thyssen
Klaas Deneudt

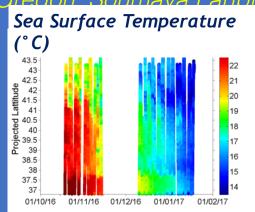
#### High resolution phytoplankton community structure in the Mediterranean= MIO/INSTM

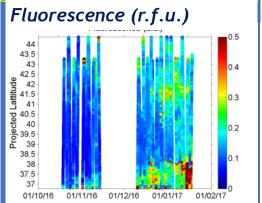
Pierre Marrec, Gérald Grégori, Soumava Labbib, Sana Ben Ismail, Cherif Sammari, and Melilotus Th

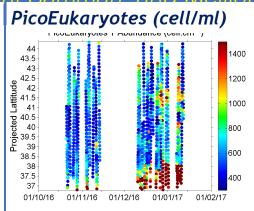


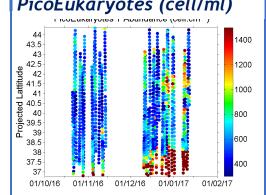


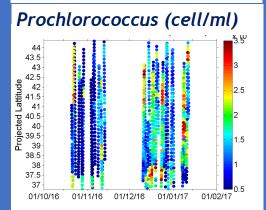


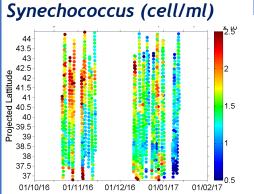


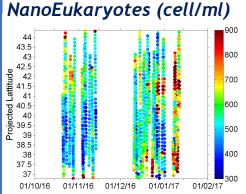












32 crossings from October 2016 to January 2017 with the flow cytometer.

= 1091 analyses performed resolving up to 10 functional groups.

Continuous and High Resolution Observation of the MEditerranean Sea









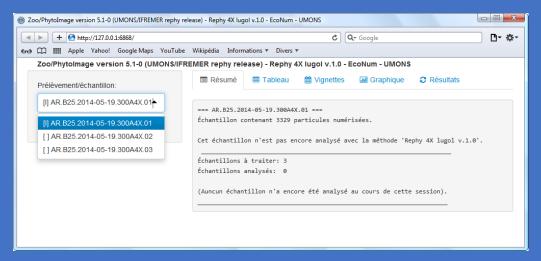
Contact person: Melilotus Thyssen

E-mail: melilotus.thyssen@mio.osupytheas.fr



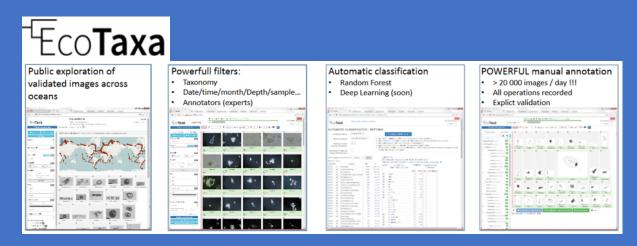
## Open source software for automated analysis of large amount of images of microalgae

Zoolmage: a R-package for image analysis and classification



- ✓ Free software written in R, and specialized in classification of digital images of zoo- or phytoplankton.
- ✓ For any kinds of plankton images, i.e. from FlowCAM, ZooSCAN, micro- or macrophotography.

EcoTaxa: a web-based application for collaborating on large plankton image datase



- ✓ It handles images of individual organisms and proposes identifications using « machine learning » algorithms.
- ✓ Today, it is considered as the most important worldwide dataset of an Abtated Plankton images. Guillaume Wacquet and Lars

Stemmann

#### Phytoplankton groups defined by Fluoroprobe





Chlorophyll a equivalents (total and per group) addressed by multispectral fluorometry (Fluoroprobe bbe): discrimination of 4 pigmentary groups (Haptophytes, brown algae, phycocyanin and phytcoerythrincontaining cells) during the 2017spring blooms



Contact persons:
Felipe Artigas
felipe.artigas@univ-littoral.fr
Arnaud Louchart
arnaud.louchart@etu.univlittoral.fr

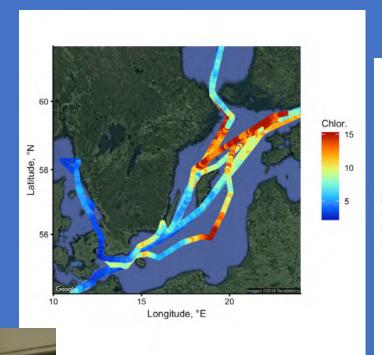








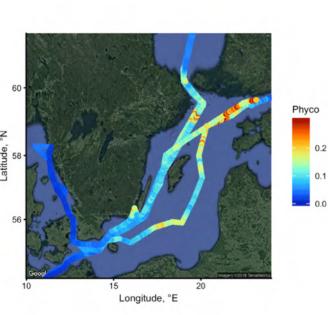
Photo by the Swedish Coast Guard



Chlorophyll fluorescence

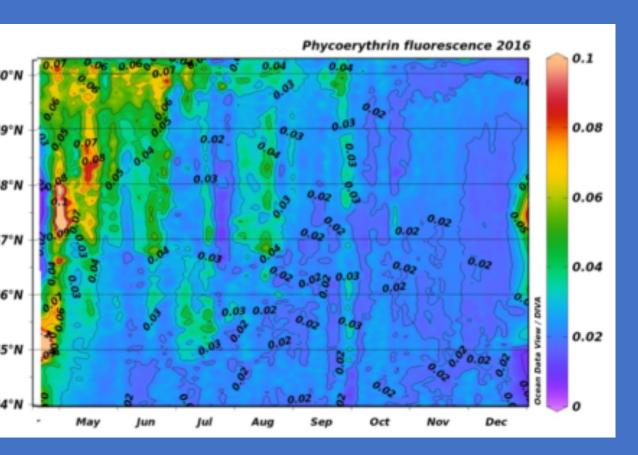


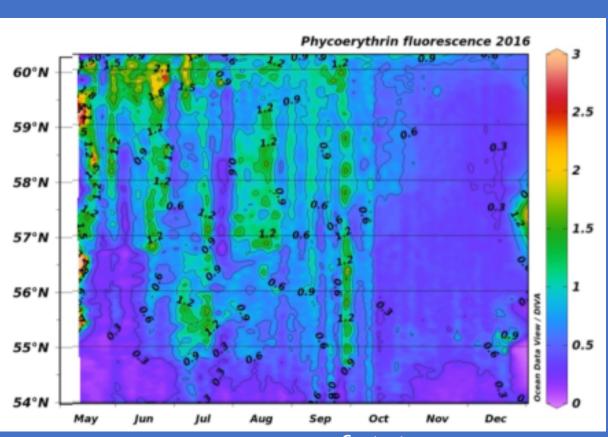
Phycocyanin fluorescence



Contact persons: Bengt Karlson bengt.karlson@smhi.se Jukka Seppälä jukka.seppala@ymparisto.fi

## Phycoerythin fluorescence - a tool for observing HAB with distinct pigments





Variability of phycoerythrin fluorescence measured with microFlu Red (left) and unilux (right fluorometers). Limits of quantification (LoQ) for instruments were 0.025 V and 0.5 fluorescence units, respectively. The values below these LoQ threshold are to be considered as background noise. Higher values indicate abundance of PE containing species.

Contact person:
Jukka Seppälä
jukka.seppala@ymparisto.fi





#### FastCam

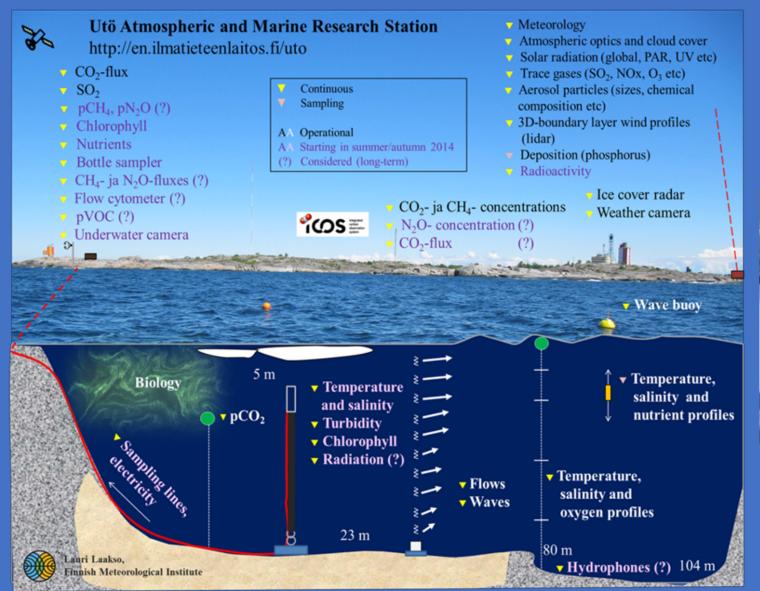


Contact person:
Florent Colas
Florent.Colas@ifremer.fr











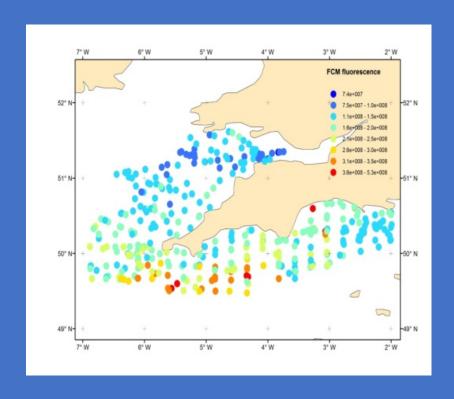


Contact person:
Jukka Seppälä
jukka.seppala@ymparisto.fi



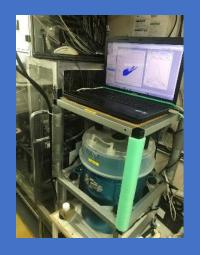


#### On board of the RV Cefas Endeavour as a platform











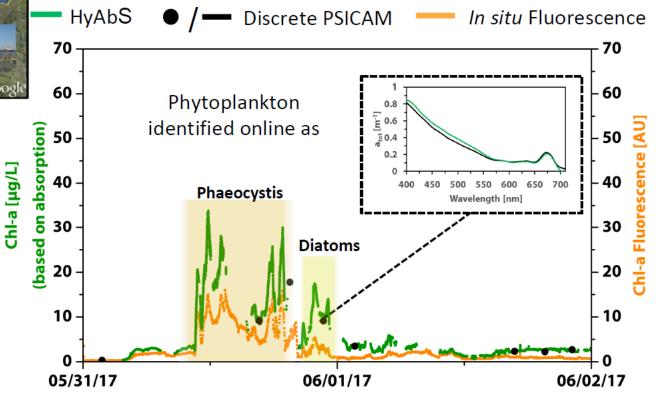
Contact person: Veronique Créach veronique.creach@cefas.co.uk







#### Multi wavelength absorption



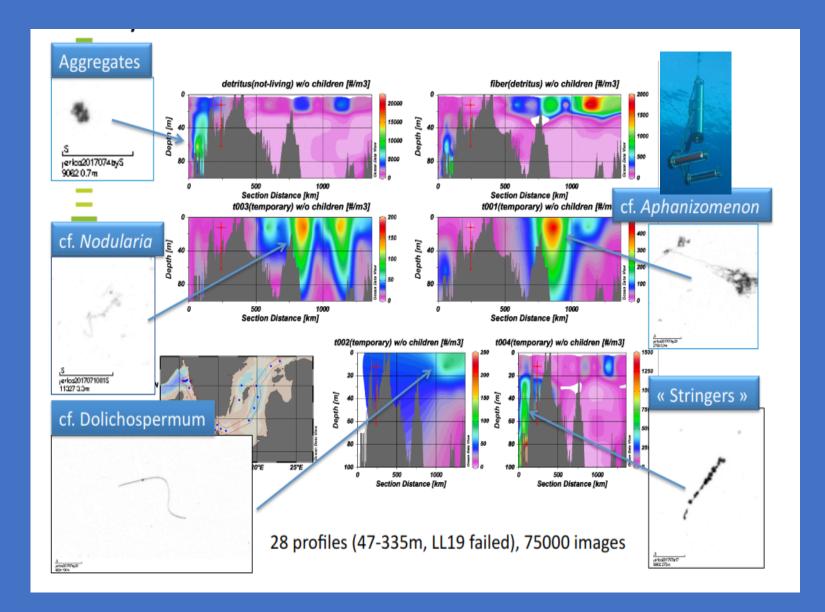
- Good agreement of discrete and continuous absorption spectra
- Good qualitative agreement of absorption-based biomass estimates with fluorescence
- Online phytoplankton identification is in accordance with on-board microscopy





Contact person: Klas Möller E-mail: klas.moeller@hzg.de

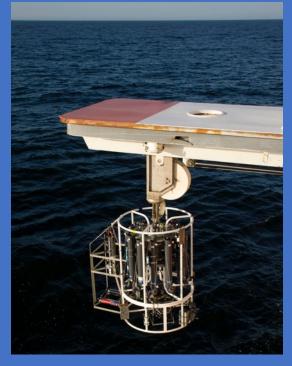
#### Underwater Vision Profiler UVP 5











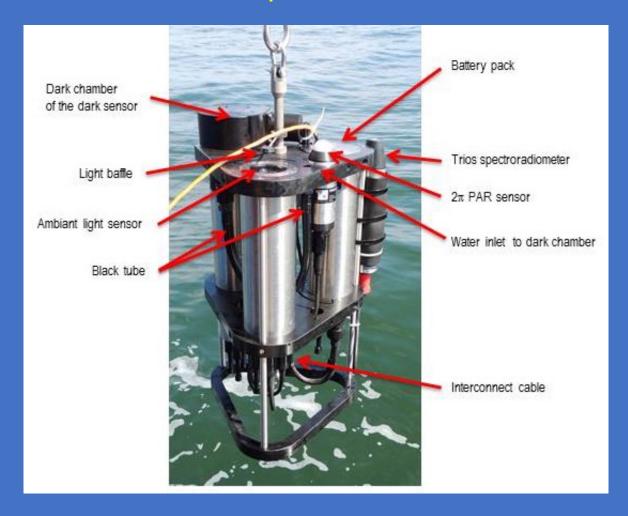
Contact person: Lars Stemman stemmann@obs-vlfr.fr







#### Fast Repetition Rate Fluorometry



Contact persons:
Fabrice Lizon
fabrice.lizon@univ-lille.fr
Jacco Kromkamp
jacco.kromkamp@nioz.nl



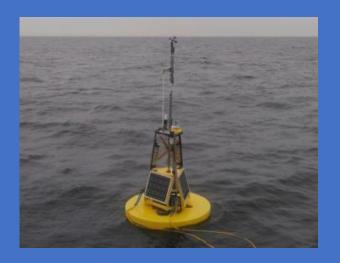


#### Other instrument platforms









#### Some persons involved

























# JERICO-NEXT is EU-funded through the Horizon 2020 program see http://jerico-ri.eu for more information







This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 654410.