Application of FlowCAM for phytoplankton enumeration, identification and estimation of chlorophyll content per cell

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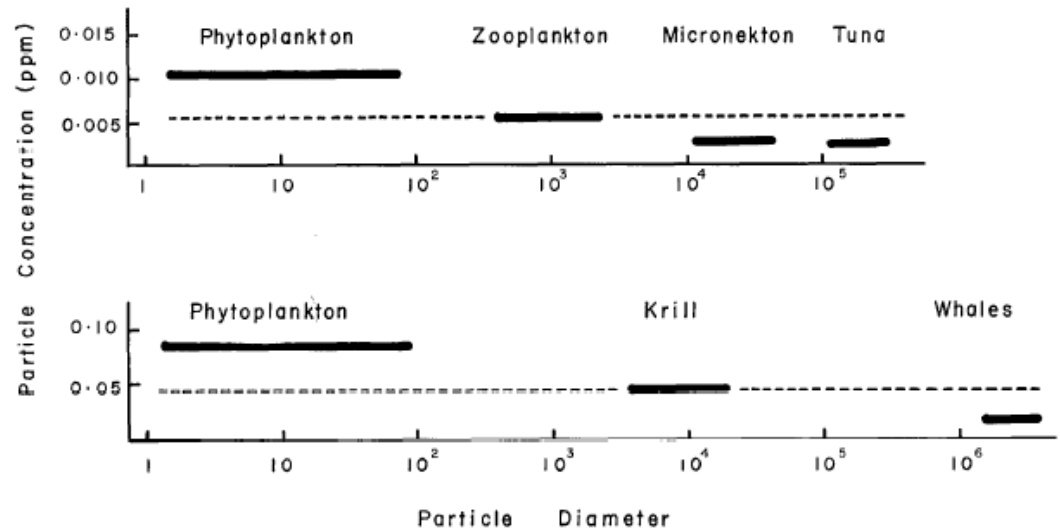Bremerhaven
Germany

Phytoplankton Ecology and Diversity

- ❑ Phytoplankton size-structure

- ❑ Taxonomical composition

- ❑ Chlorophyll content per single-cell
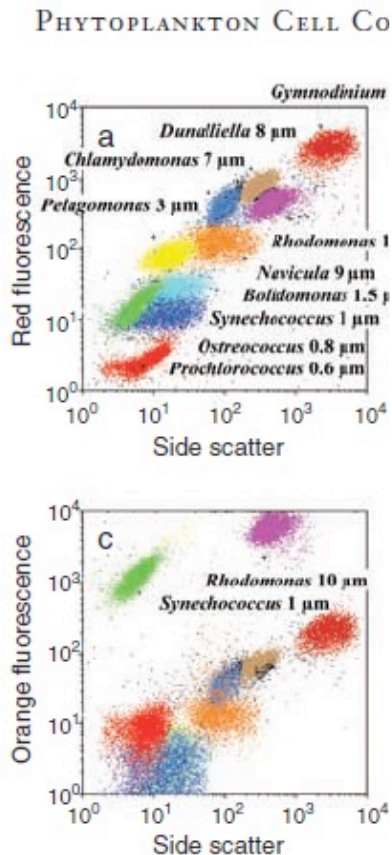
Sheldon *et al.* (1972)

Flow Cytometer and Microscope
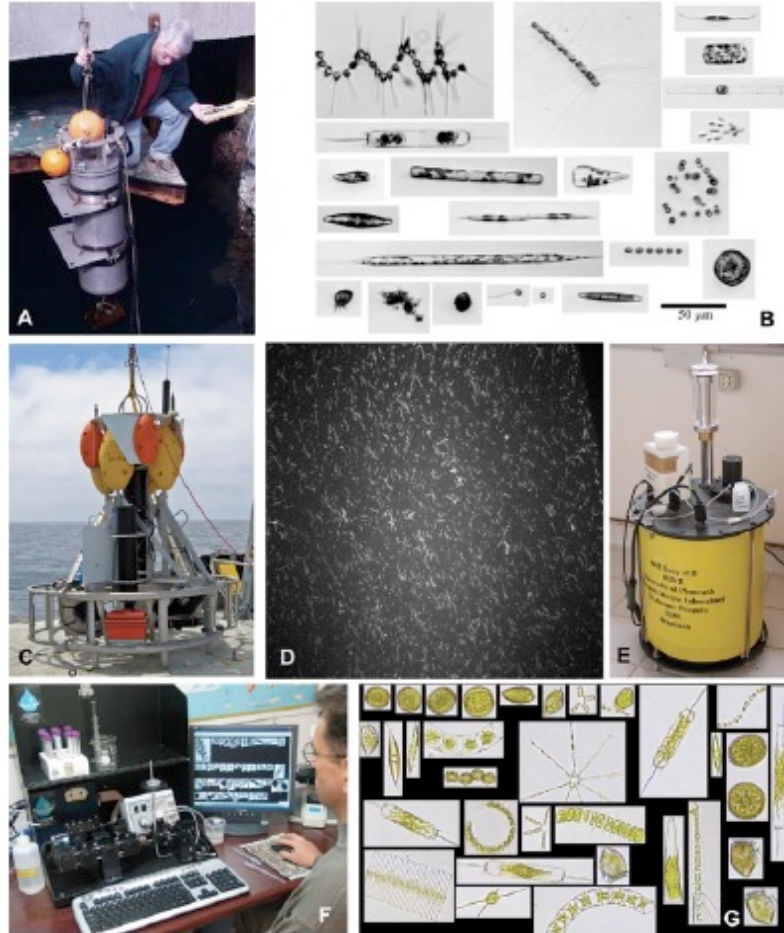
Sieracki *et al.* (1998)

Finkel *et al.* (2010)

Picoplankton (0.2-2μm)

FlowCytoBot (Sosik & Olson, 1996)

Mesoplankton (>200μm)

PHYTOPLANKTON CELL CO

Marie *et al.* (2005)
Benfield *et al.* (2007)

FlowCAM (Sieracki et al., 1998)

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Flow-cytometer And Microscope

Adapted from FlowCAM User Manual (FluidImaging Technologies)
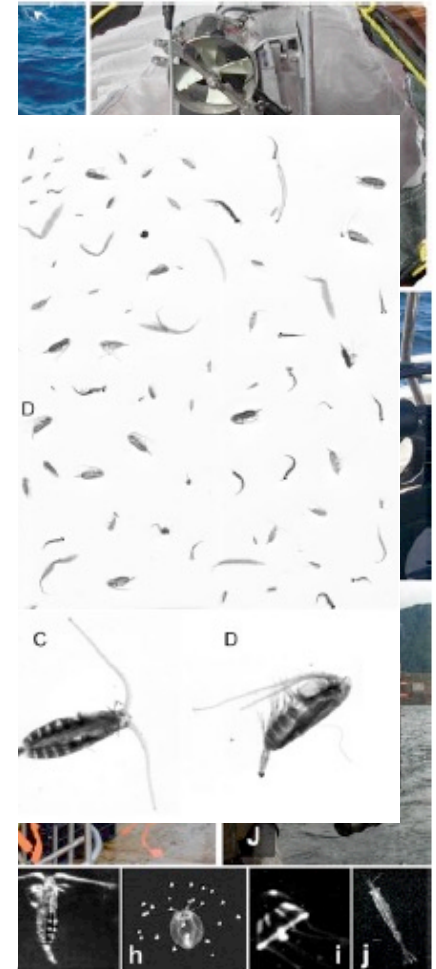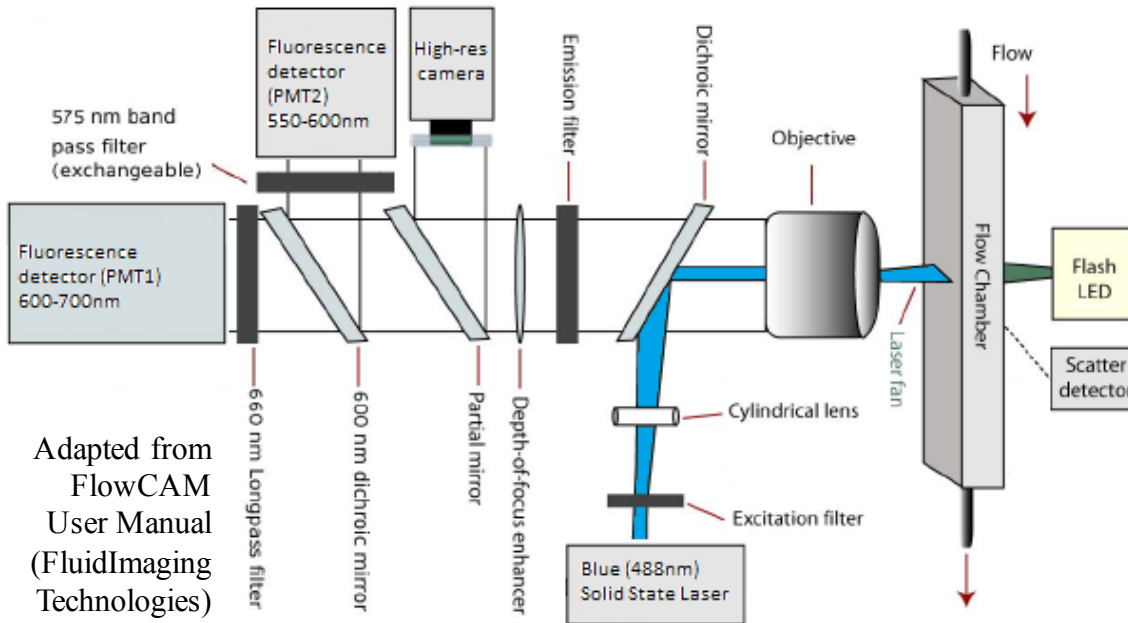
Lens	Flow Chamber	Size Range
200x	FC 50	3-50 μm
100x	FC100	15-100 μm
40x	FC100-FC600	30-600 μm

Simulations: how many cells are cells enough?

Parameters

	Magnification		
	×200	×100	×40
General characteristics			
Flow chamber depth (μm)	50	100	300
Lower limit (μm)	3	15	30
Flow chamber width (μm)	1000	2000	3000
Calibration constant (μm pixel^{-1})	0.2953	0.6151	1.6386
Field-of-view width (μm)	302.39	629.86	1677.93
Field-of-view heigth (μm)	226.79	472.4	1258.44
Pi: % sample view	30.24	31.49	55.93
Vi: volume per photo (mL)	3.43×10^{-6}	2.98×10^{-5}	6.33×10^{-4}
Max flow rate (mL min^{-1})	0.053	0.53	1.20
Min flow rate (mL min^{-1})	0.012	0.12	0.25
Max frame rate (photos s^{-1})	11	11	11
Autoimage			
Number of photos	20000	20000	10000
Time of analysis (min)	30	30	33
Frame rate (photos s^{-1})	11	11	5
Flow rate (mL min^{-1})	0.05	0.4	0.5
Triggered modes			
Flow rate (mL min^{-1})	0.04	0.12–0.4	0.4
Time (min)	25	25	25
Pre-treatment			
Filtration (μm)	40	100	200
Concentration (μm)	–	15	15

Numerical simulation

The need of sample concentration.

Concentration could be sufficient to cover an ample size range with **Autoimage** and **F-triggered** mode.
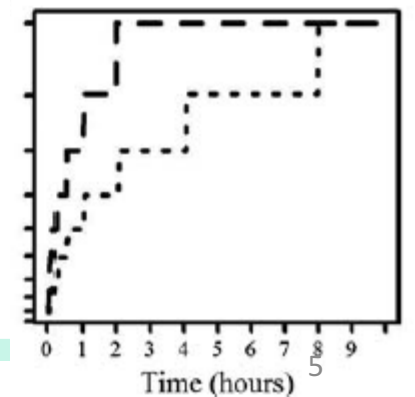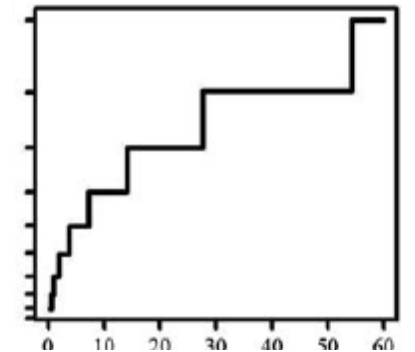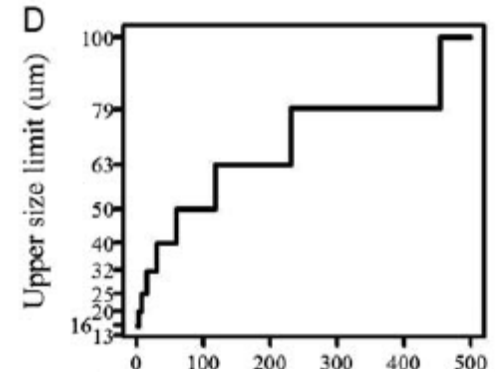
S-triggered mode has shortcommings when dealing with natural samples.


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Sample treatment
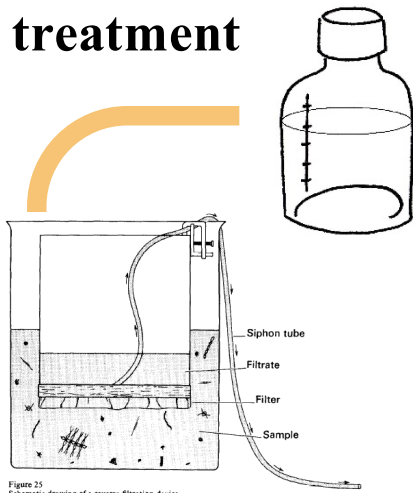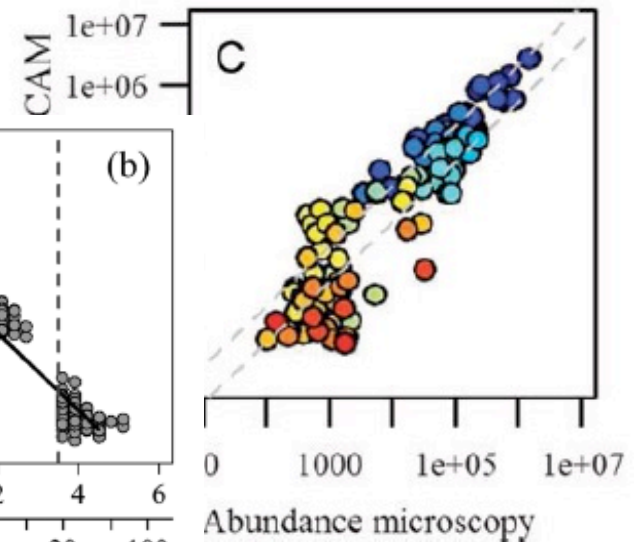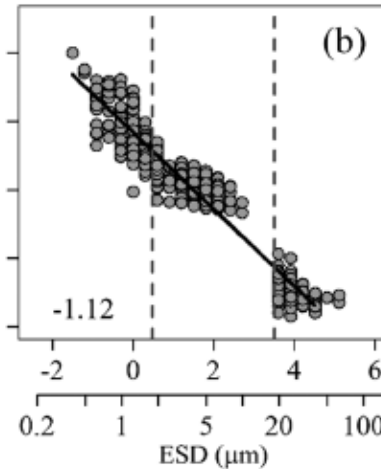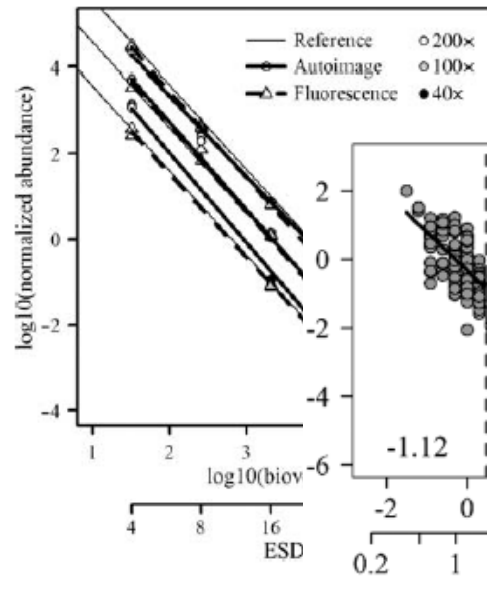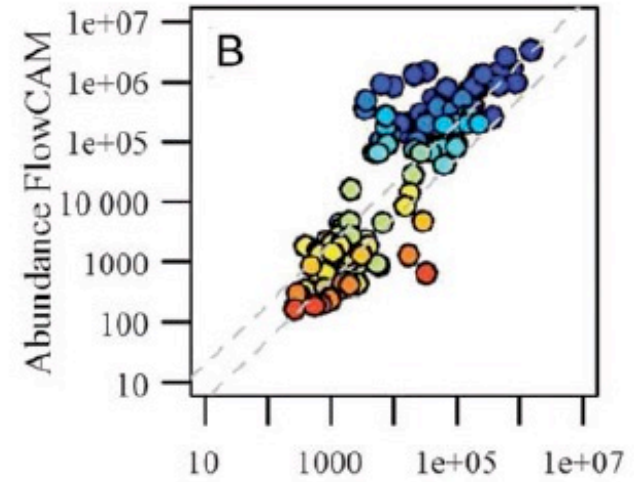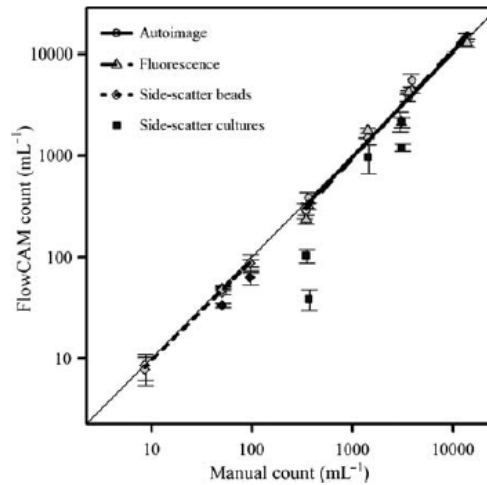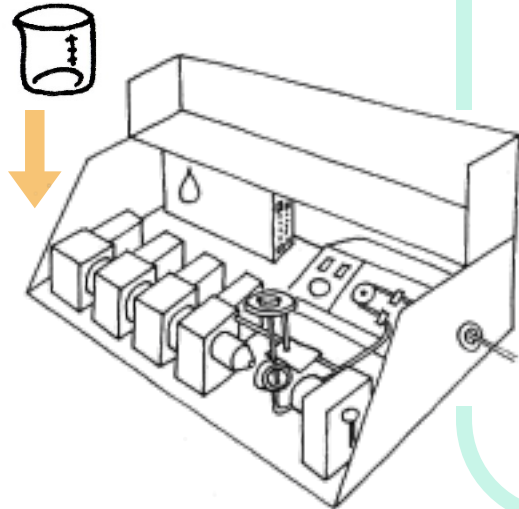
Automatic classification of images

- Describe images.


  - shape
  - texture
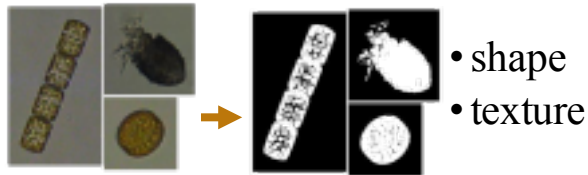
- Create Training set: group of images labelled by experts.

- Train algorithm and build classifier.
  - Linear discriminant analysis
  - Recursive partitioning tree
  - K nearest neighbour
  - Learning vector quantization
  - Neural network
  - Random forest
  - Support Vector Machine
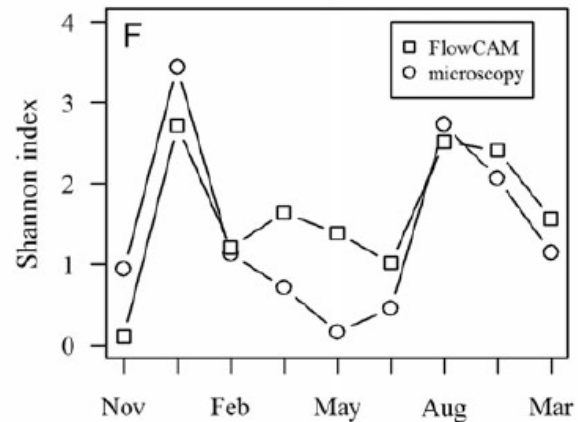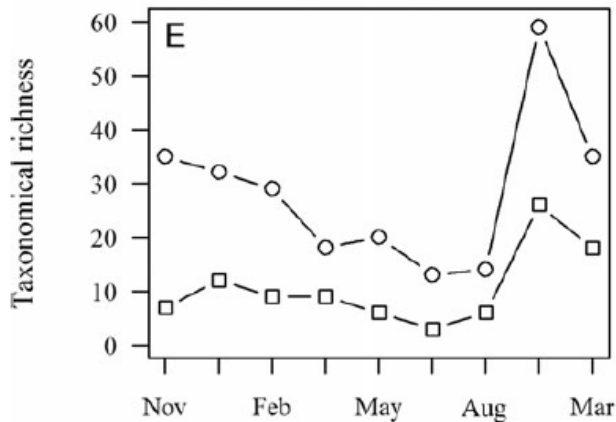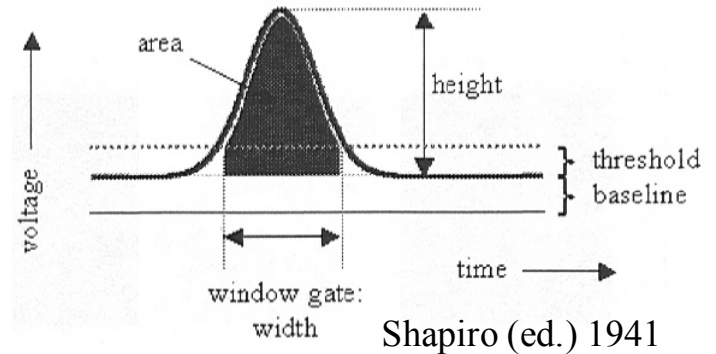
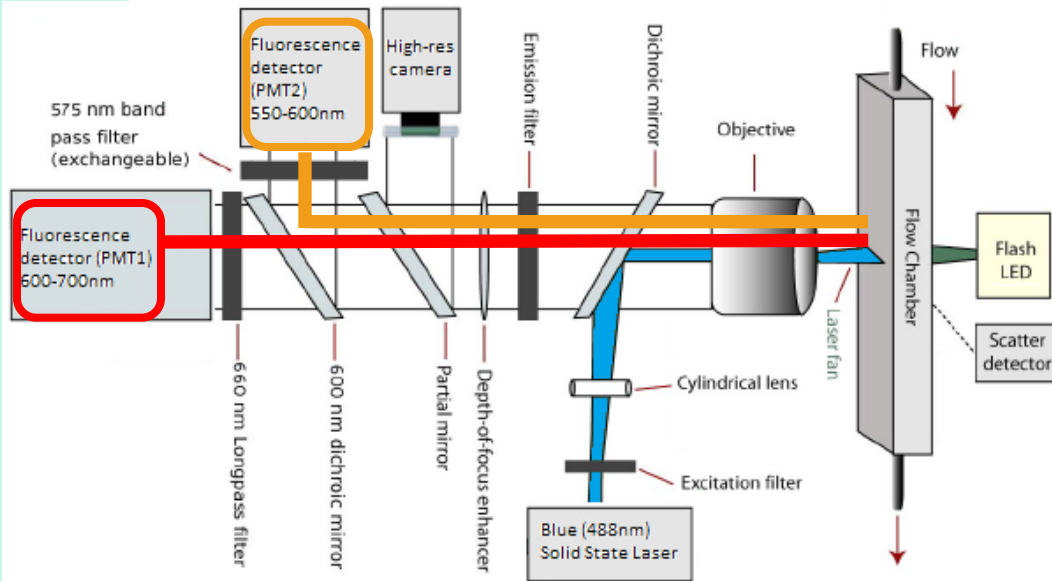- Apply classifier to new samples.
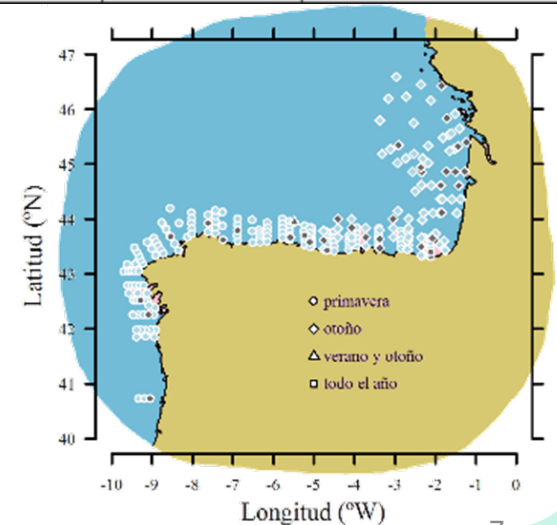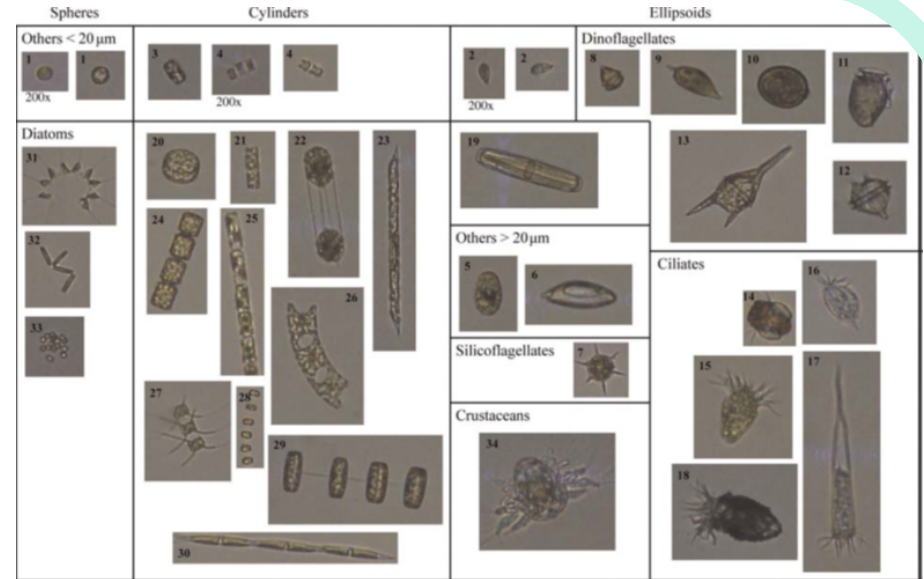
'Functional' groups

	Accuracy	Specificity	Recall
Diatoms	1.5	0.97	0.64
Silicoflagellates	1.18	0.89	0.76
Dinoflagellates	0.95	0.74	0.78
Ciliates	0.7	0.42	0.61
Crustaceans	1.25	0.75	0.6
Others	0.86	0.84	0.99

Global accuracy
0.87
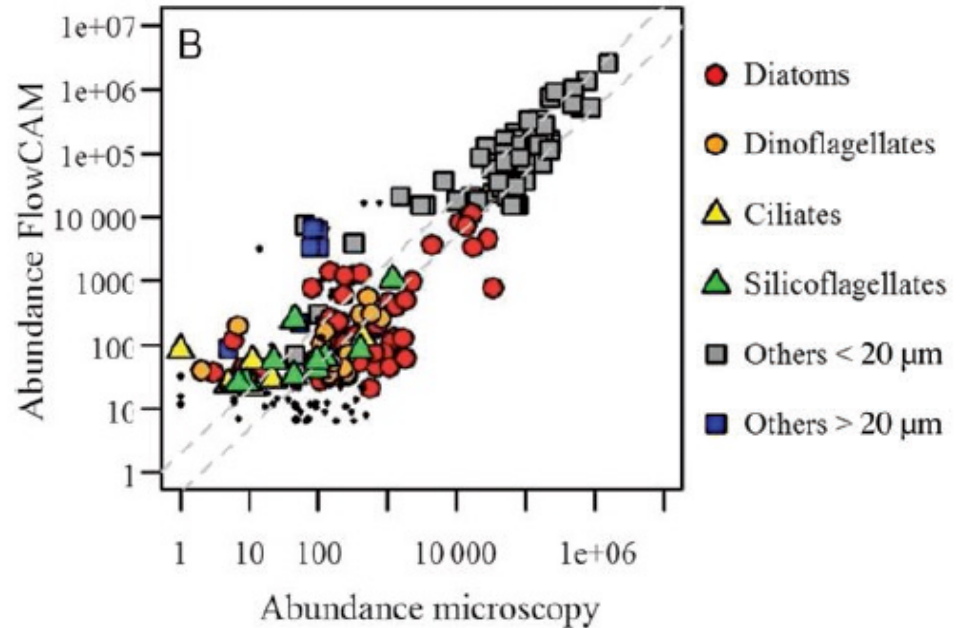
Diversity

Fluorescence signals: measurement…

Shapiro (ed.) 1941

Emiliana huxleyii
Isochrysis galbana
Rhodomonas sp.
Karlodinium micrum
Alexandrium sp.
Prorocentrum sp.

Cell abundance

Extracted chlorophyll

FlowCAM fluorescence

…and interpretation.
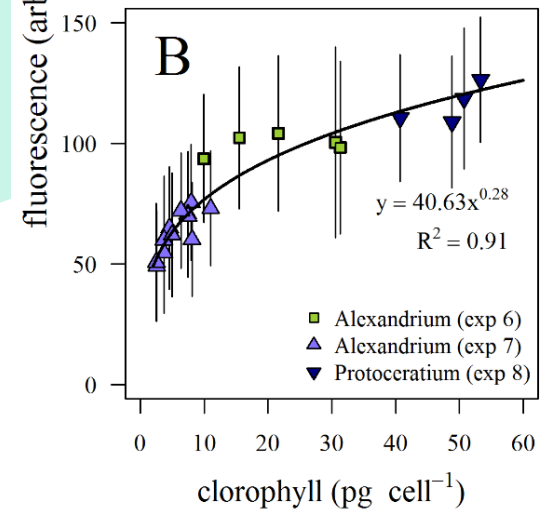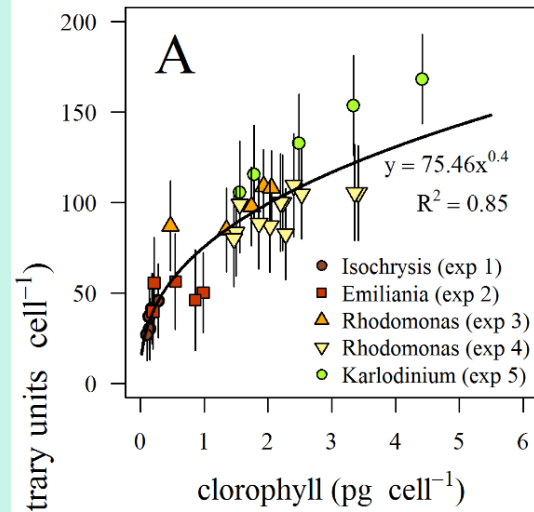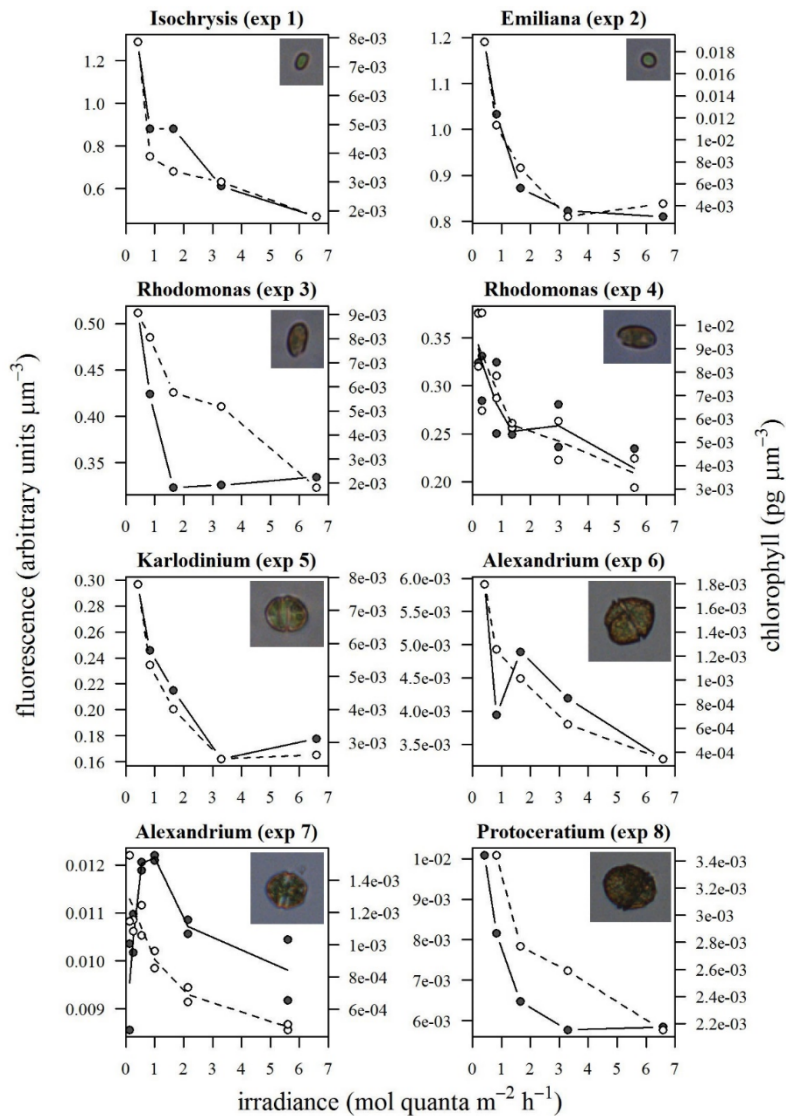
Summary

- FlowCAM is an imaging-in-flow devide applied succesfully to the analysis of plankton community, from nano to mesoplankton.

- The size structure in a 10-fold of body size-range can be estimated in a unique analysis.

- The combination of different sub-samples could cover the autotrophic community >5 µm.

- Automated classification of images could divide this community in broad 'functional' groups.

- Aditional informatic can be gathered in a single cell basis, such as macromolecules composition.

Thanks for your attention

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