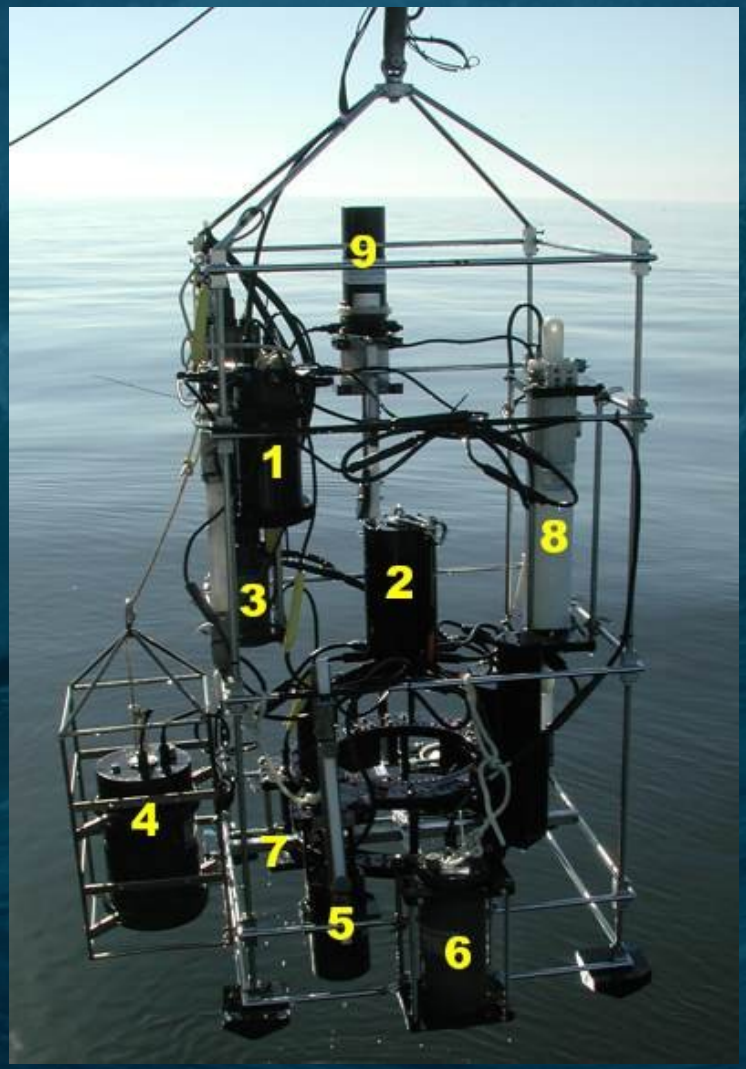
OPB 305 - Ch 7 – In situ Instrumentation



A. Petrenko – Mediterranean Institute of Oceanography

Comparing optical to classical measurements

Disadvantages of classical chemical analyses: requires taking samples, can alter the environment, intrusive, low spatio-temporal coverage

In contrast, optical measurements require no samples, no transporting to a lab, are non-intrusive (mostly)

If continuous profiles:
- excellent temporal resolution

If remote sensing:
- Excellent spatial coverage
- Good temporal coverage

"Optical Signature" of a marine water sample

for phytoplankton communities it can provide an indication of:

- their size structure (e.g., Ciotti *et al. 2002*, Ciotti & Bricaud 2006)

- the functional groups (e.g., Alvain *et al., 2005, 2008*)

- their physiological state, esp. the level of photoacclimatation (e.g., Behrenfeld & Boss 2003)

- photosynthetic parameters (Uitz et al., 2008)

- primary production (Silio-Calzada *et al., 2008*)

"Optical Signature" of a marine water sample (continued)

---

* particle size distribution (PSD), (e.g., Kitchen *et al., 1982;* Boss *et al., 2001;* Loisel *et al., 2006*)

a) optical approach, based on the spectral dependence of the scattering coefficient. Mean slope of size distribution law, over a size range of about 0.1 to 20 µm (e.g., Boss *et al., 2001*).

Boss, E., M. S. Twardowski, and S. Herring. 2001. Shape of the particulate beam attenuation spectrum and its inversion to obtain the shape of the particulate size distribution. Appl. Opt. 40: 4885 - 4893.

Note: PSD can yield phytoplankton groups (e.g., Kostadinov JGR 2009, Biogeosc 2010)

b) classical particle counting methods cannot detect particles smaller than ~1 micron (e.g., Jackson *et al., 1997*).

Note: There exists an important uncertainty for the size range ~[0.1 ; 1] µm. These sub-microscopic particles are not well known -> problems when analysing optical properties (Stramski & Kiefer 1991).

"Optical Signature" of a marine water sample (continued)

* biogenic or mineral (e.g., Twardowski *et al., 2001;* Loisel *et al., 2007*)

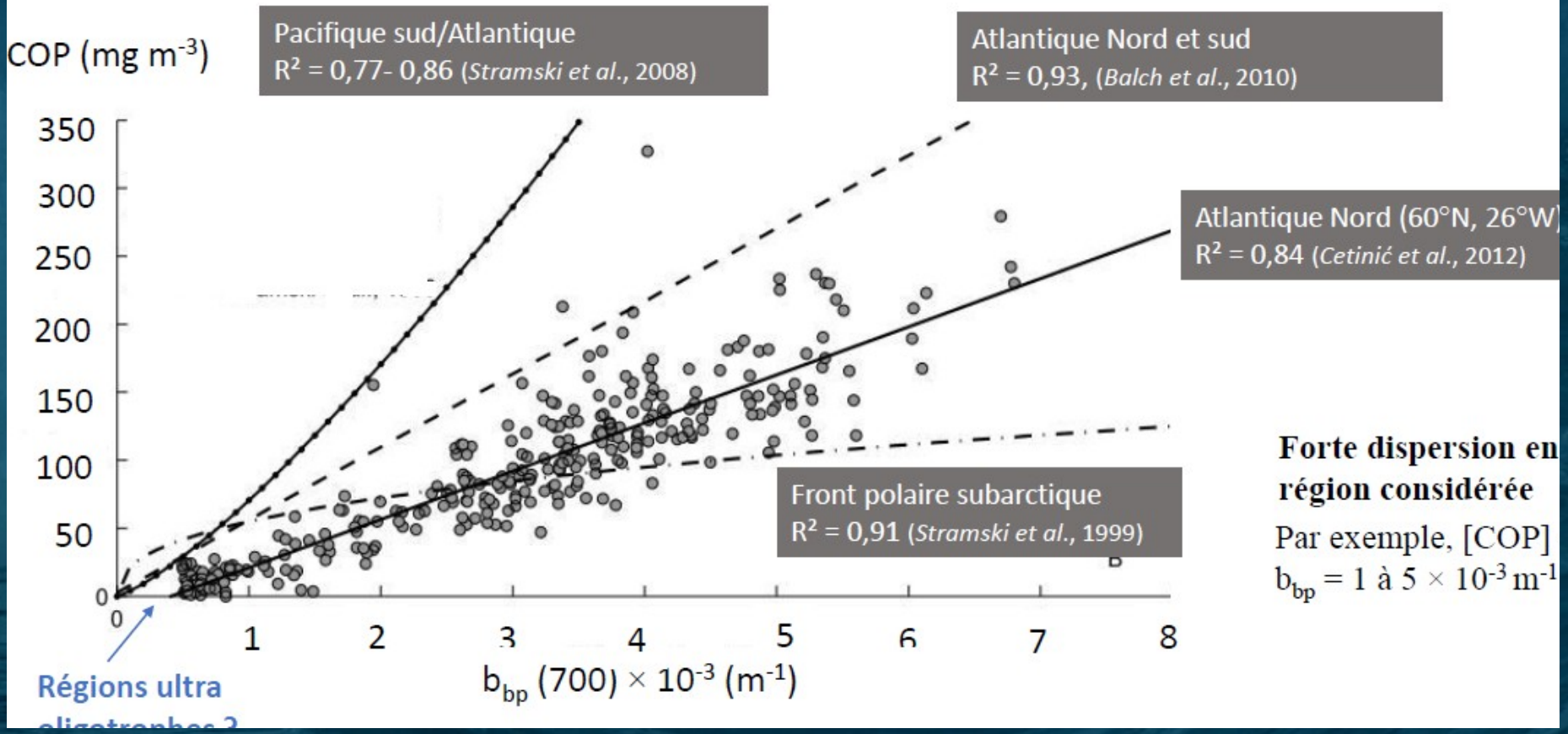
* POC can be directly quantified by measuring the scattering coefficient (Morel 1988).
From space, semi-analytical algorithms Rrs → Bbp and Chl → POC
(e.g., Loisel & Stramski, 2001; Loisel *et al.*, 2002)

And empirical
(Gardner *et al., 2006;* Stramski *et al., 2008*) with uncertainties of the order of 20%
(Stramski *et al., 2008*).

Note 1 - POC from measurements of Bbp or Cp (Stramska et al 2005)
Note 2 – There is still the problem of measuring DOC and its "invisible" parts

Relation b_{bp} *vs* COP dans la li

(Modifiée de *Cetinić et al.*, 2012)

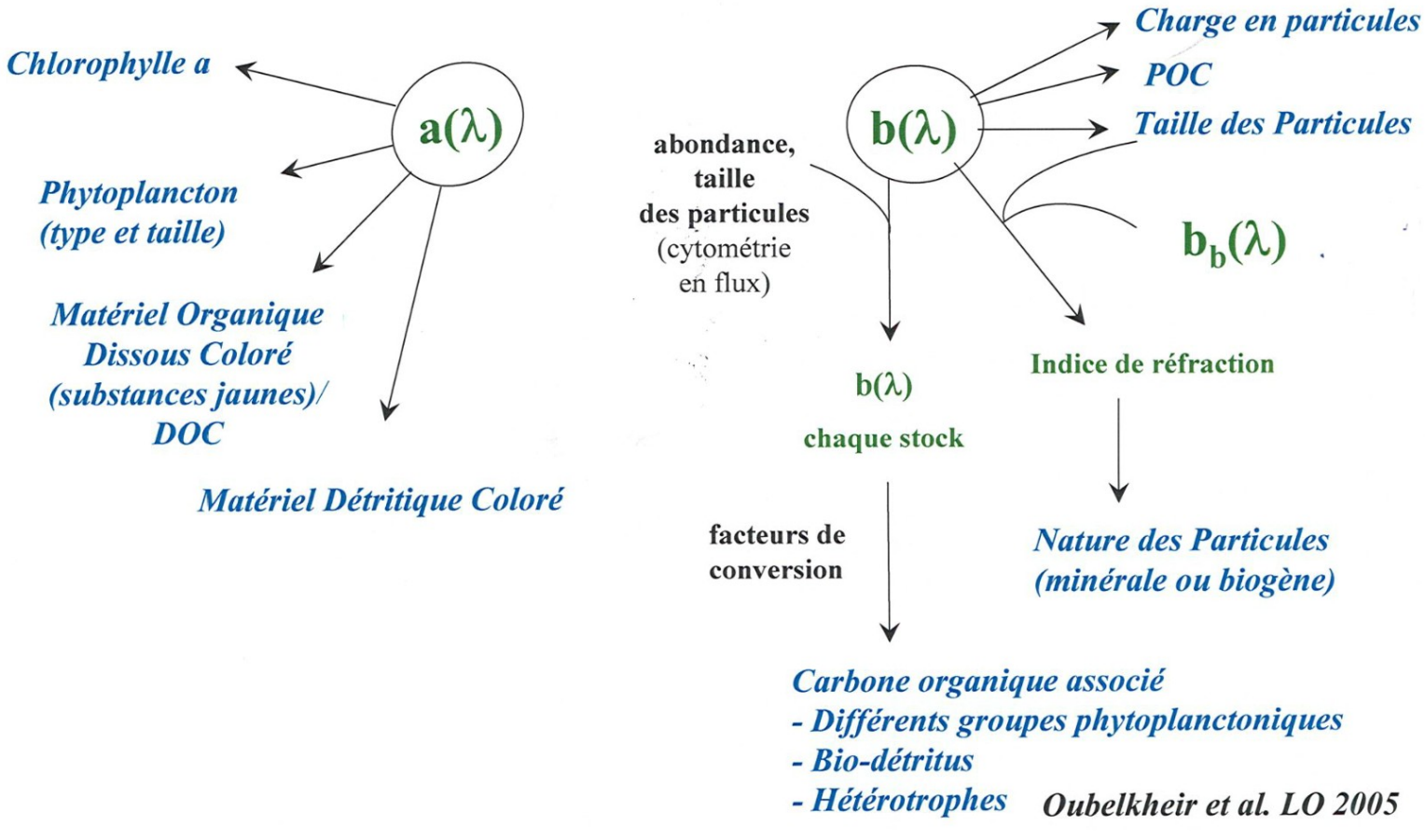


Forte dispersion en région considérée

Par exemple, [COP] $b_{bp} = 1$ à 5×10^{-3} m^{-1}

Remerciement – extrait soutenance thèse
A. Fumenia – 3 juin 2020

Quelles sont les grandeurs biogéochimiques accessibles par des mesures optiques spectrales?




Radiometric
Measurements

Radiometres

multispectral				
	OCR-500 micro-sensor	wavelengths (voir attache)	Satlantic	
	OCR-504 UV	305, 325, 340, 380 nm		
	OCR-507I	E at 7 wavelengths		
	OCR-507R	L at 7 wavelengths		
	PRR-800	Ed and Eu (or Lu) measured at 15 to 19 wavelengths + Twater + pressure	Biospherical	
	PUV	Ed at 305, 313, 320, 340, 395 nm + PAR + Twater + pressure		
hyperspectral				
	HyperOCR	256 wavelengths between 350 to 800 nm	Satlantic	
	HydroRad	Fiber optics technology between 350 and 850 nm: res=0.3 nm extended 250 to 1050 nm: res=0.4 nm	Hobilabs	about 1500 wavelengths
	Walrus	hyperspectral radiometer bouy above and below the surface	Hobilabs	

www.satlantic.com

OCR= Ocean Color Radiometer — wide field of view

SAS=Surface Acquisition Systems — narrow field of view

www.biospherical.com

PRR=Profiling Reflectance Radiometers

Boussole
(Villefranche sur mer)

http://www.obs-vlfr.fr/Boussole

25 m de haut ancrée sous tension sur 2400 m de fond
PI D. Antoine

Boussole:

Satlantic 200 series radiometers measure Es (4.5 meters above the water surface); Ed, Eu, and Lu (nadir) measured at 2 depths: 4 and 9 m.

Two-axis tilt and compass at 9 m.

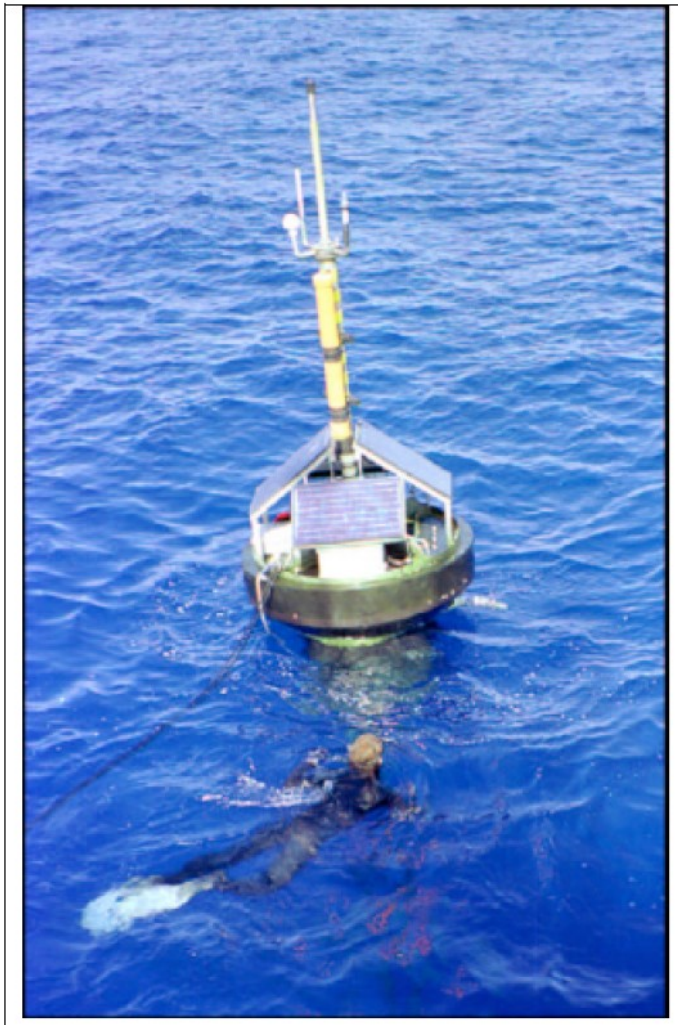
A Sea-Bird Electronics CTD at 9 m for temperature, conductivity and pressure.

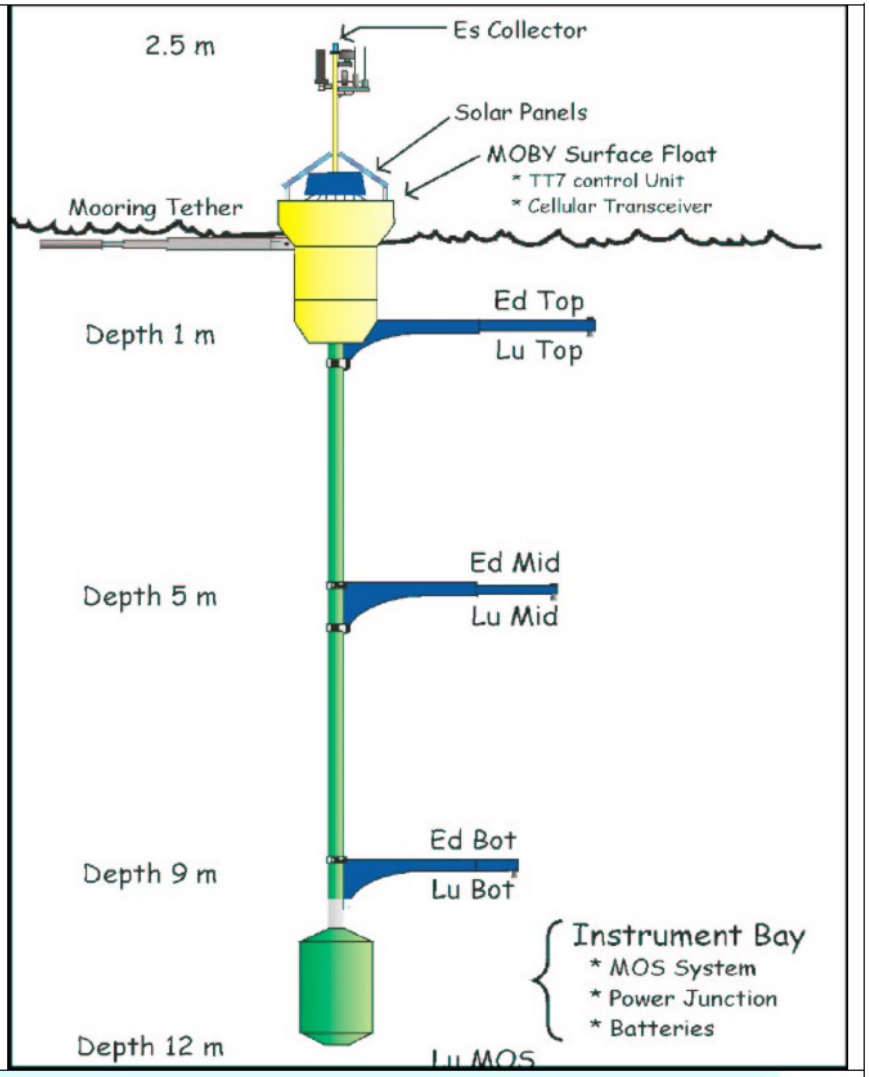
Fluorometers at 4 and 9 m to obtain a proxy for the chlorophyll *a* concentration.

Transmissometers at 4 and 9 m for a proxy of the particle load.

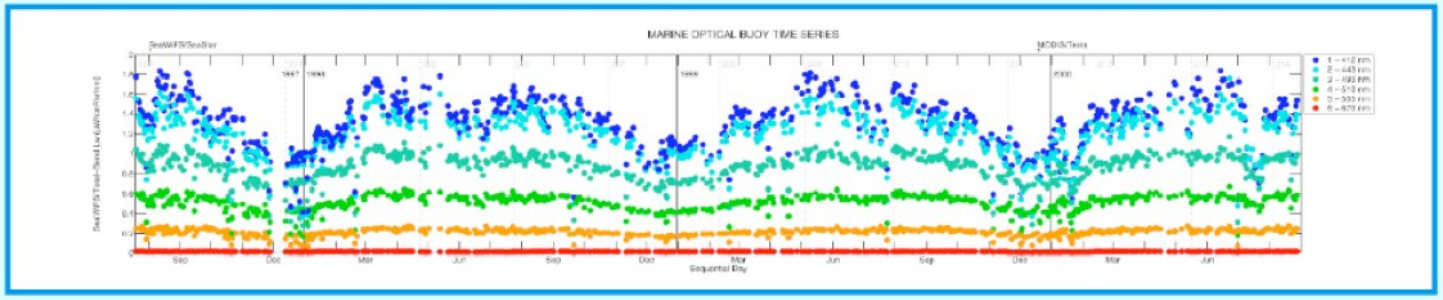
Backscattering meter at 9 m to obtain a proxy for bb at two wavelengths (442 and 560nm).

These data are collected every 15min during daylight, and every hour at night. Each data acquisition sequence lasts one minute.





350 nm
to 940 nm
Monterey
Hawaii

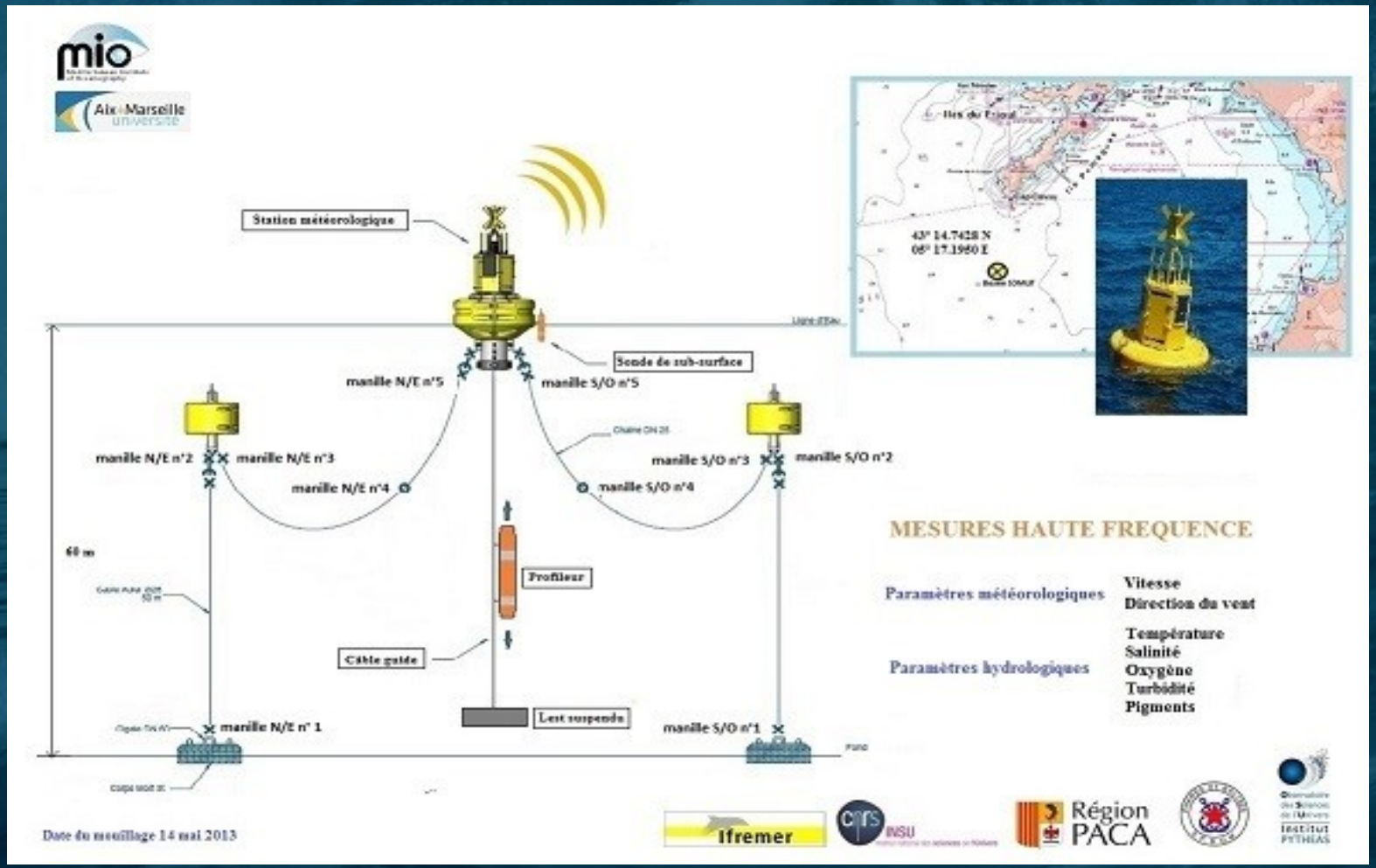


Bouée MOBY – radiomètre hyperspectral (choix des longueurs d'onde)

SOLEMIO buoy: (F. Garcia, MIO)

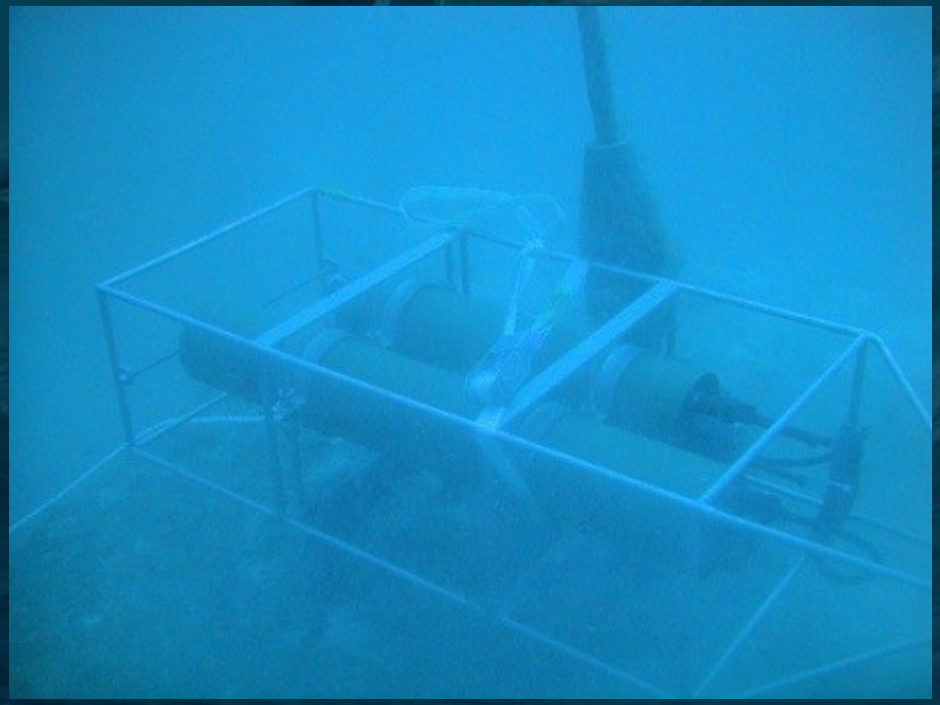
Ensures long term observation of the Bay of Marseille;

1 out of a total of 10 stations in the nation SOMLIT network (Service d'Observation en Milieu Littoral – http://www.SOMLIT.INSU.fr).



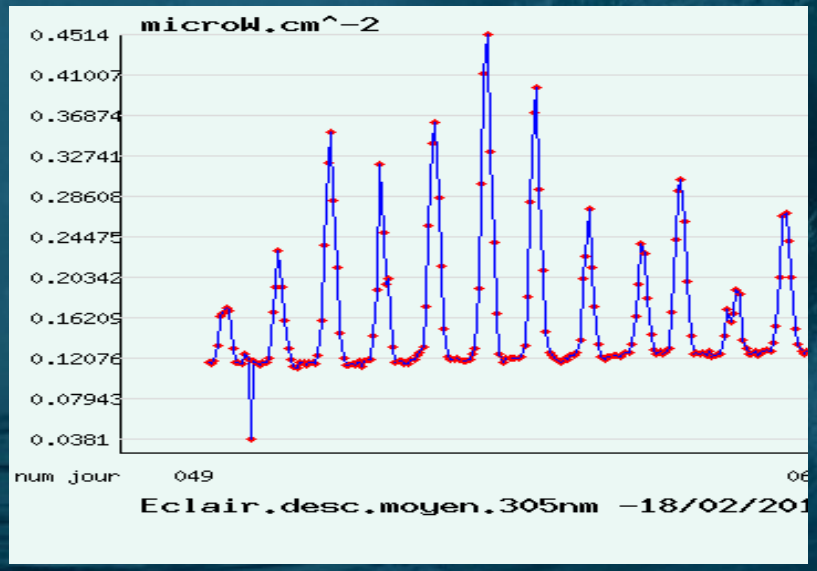
SOLEMIO buoy: (F. Garcia, MIO)

An additional autonomous nitrate sensor of the ISUS/MBARI type by Satlantic is attached to the southern dead weight at a depth of 60 m to perform high-frequency measurements (1 measurement per hour).



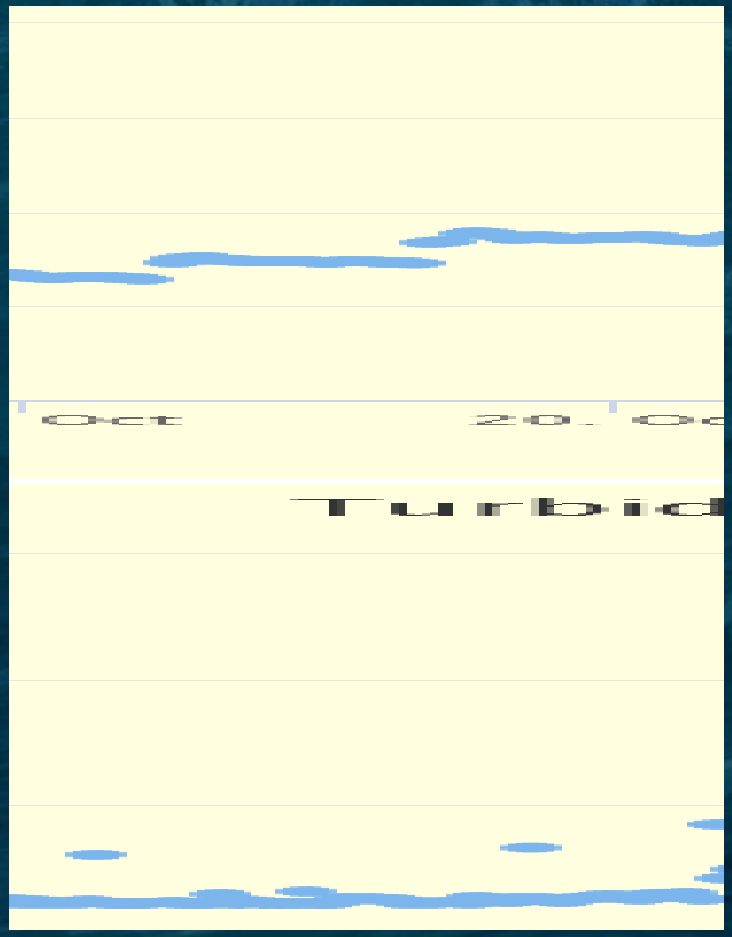
The SUNMEX line (MIO, R. Sempere and B.Charrière)

This bio-optical line houses sensors to measure radiometry (Ed & Eu), biogeochemistry (Chla, CDOM, particles), and physics (T, S, P).
It was attached to a float and has been operating in the Bay of Marseille (43°15’64 N, 05°20’01 E) since 6 April 2011.



<- 2014

2020 ->



https://www.mio.osupytheas.fr/fr/recherche/

<u>Observation activities</u>

→ Real time data from the Bay of Marseille

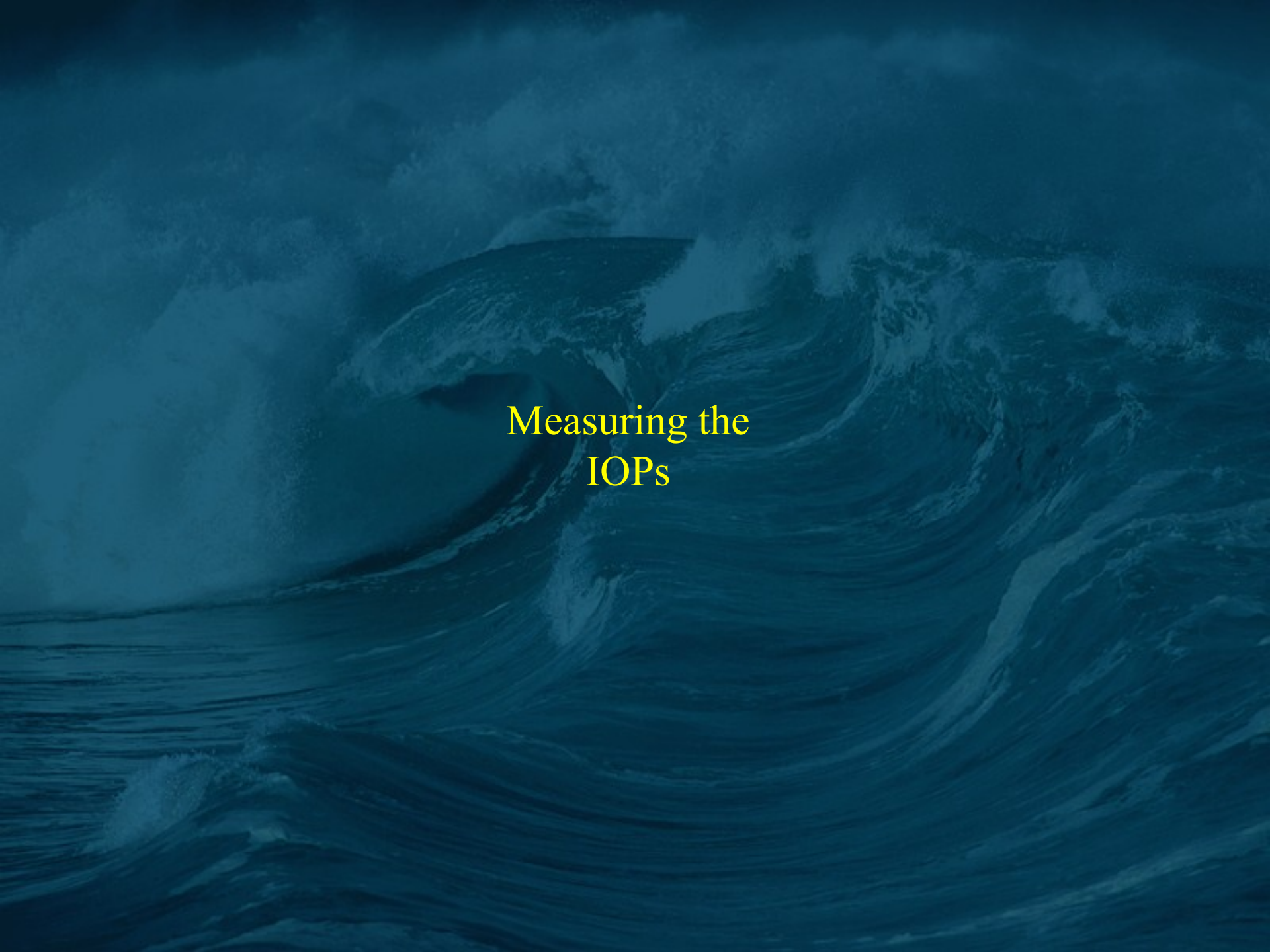
Parameters measured by the SUNMEX line:

* Atmospheric irradiance at 8 wavelengths:
305, 325, 340, 380, 412, 443, 495, and 565 nm (µW cm-2 nm-1)

* In the water column at 2 and 6 m depth

- Ed at 8 wavelengths: 305, 325, 340, 380, 412, 443, 495, and 565 nm (µW cm-2 nm-1)
- Eu at the same 8 wavelengths (µW cm-2 nm-1sr-1)
- coloured dissolved organic matter (CDOM) (ppb quinine sulfate equivalent)
- particle backscattering at 650 nm (m-1)
- chlorophyll *a* (µg l-1)
- temperature (°C)
- salinity
- pressure (bar)

“The radiometric data yields the ‘AOP’s’ (apparent optical properties) such as Kd (diffuse attentuation coefficient of downwelling irradiance) or the ‘nLW’ (normalized water leaving radiance) at 8 wavelengths throughout the year. The line also contains an inclinometer which allows to discard any irradiance measurements if the inclination of the line is >5°”
-Excerpt translated from SUNMEX site-


Measuring the
IOPs

Supplier: Wetlabs http://www.wetlabs.com

Transmissometers
(e.g., BAM, C-Star, C-Rover)

Spectrophotometer
- Multi-spectral absorption and attenuation meter

[+ Fluorometers
-Open path and flow-through fluorometers for measuring Chlorophyll, CDOM, Rhodamine, and Phycoerythrin fluorescence
-Combination fluorometer-turbidity sensor
-Custom three-parameter fluorescence/scattering meter

Water Quality
-Cycle PO4 Meter
-Water Quality Monitor]

Bioluminescence
- Underwater Bioluminescence Assessment Tool

Turbidity and Scattering meters
- Single-angle backscattering meter
- Turbidity sensor
- Three-wavelength backscattering meter
- Combination chlorophyll fluorometer–turbidity sensor
- Three-angle, single wavelength VSF meter
- Three-angle, three-wavelength VSF meter

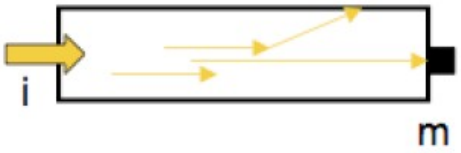
+ Photosynthetically Active Radiation sensor
+ Custom three-parameter scattering meter

Systems Mini Bio-Optics

Supplier WETLabs

Product	Instrument Description
ac-9	Absorption and Attenuation Meter ; λ =412, 440, 488, 510, 560, 630, 650, 676, and 715 nm
ac-9 Plus	Absorption and Attenuation Meter, expanded data handling capabilities
ac-spectra	Multispectral Absorption and Attenuation Meter ; 80 longueurs d'onde λ; Δλ = 4 nm ; de 400 à 730 nm
C-Star	Transmissometer ; λ = 660 nm
C-Rover	Transmissometer built for profilers
DH-4	Data Handler
ECO BB	Single-Angle Scattering Meter ; ψ = 117°
ECO BB2F	Combination Scattering Meter and Fluorometer; ψ = 117° at λ = 470, 700 nm + chlor fluor (425 – 675 nm)
ECO BB3	Three-wavelength Scattering Meter ; ψ = 117° (ou 140°); λ = 470, 532, 660 nm
ECO FL	Open-face Chlorophyll Fluorometer
ECO FLNTU	Combination Chlorophyll Fluorometer and Turbidity Sensor
ECO Pucks™	Miniature ECO for AUVs, gliders, profiling floats
ECO Triplet	Custom three-channel sensor
ECO VSF	Three-angle Backscattering Meter; ψ = 117°, 125° et 140°
ECO VSF3	Three-angle, Three-wavelength Backscattering Meter ψ = 117°, 125° et 140° λ = 450, 530, 650 nm
SAFire	Spectral Fluorescence Meter
WETPak	Battery Pack
WETStar (Chl)	Chlorophyll Fluorometer
WETStar (CDOM)	CDOM Fluorometer

Atténuation



Tube noir
Petit récepteur
m = i - a - b
c = i - m

Absorption

Tube mirroir
Grand récepteur
m = i - a
a = i - m

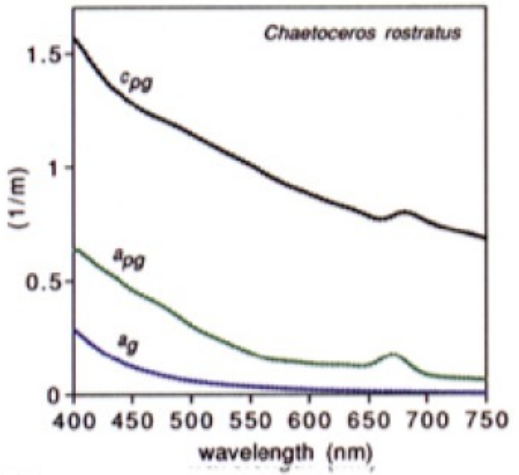
* Surestimation de a
m = i - a - bb
a + bb = i - m
donc correction
à effectuer

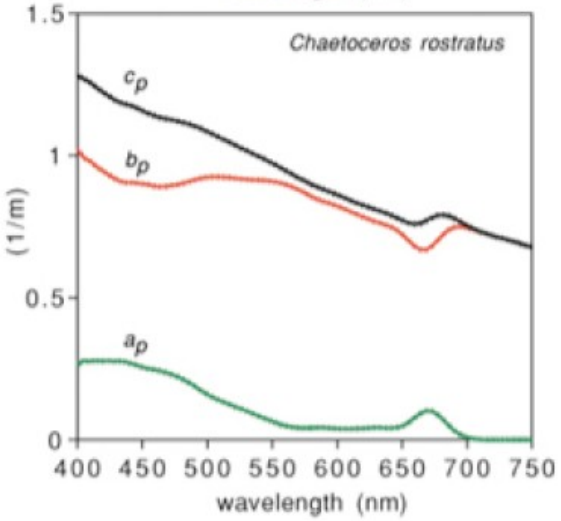
AC 9 « Absorption and attenuation meter at 8 wavelengths (WETLabs)

WET Labs ac-s - Culture of Chaetoceros rostratus.

apg = a - aw = ap + ag
cpg = c - cw
(a g measured by filtering the sample through a 0.2 mm filter).

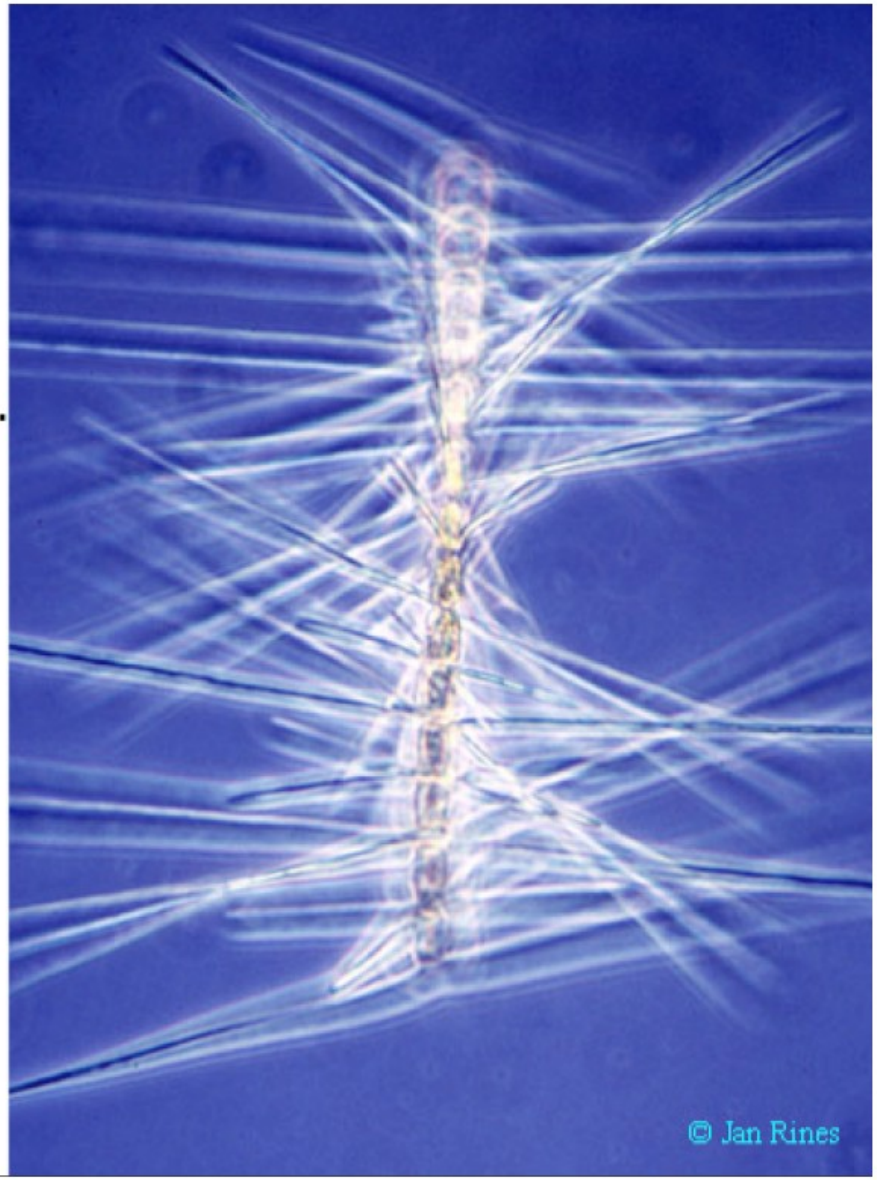
In the lower figure ap = apg - ag
bp = cpg - apg
Hyp: dissolved materials do not scatter.



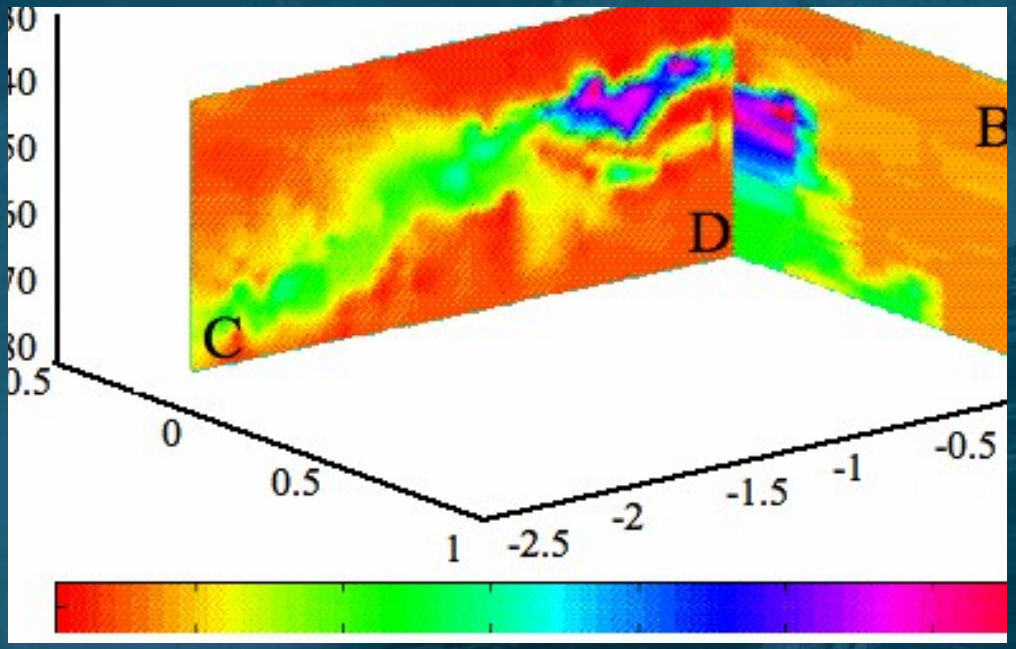


* diatoms

View photomicrographs of Chaetoceros rostratus. (courtesy Dr. Jan Rines, University of Rhode Island).



Detecting wastewater through fluorescence



Beam attenuation c660
Sand Island, HI

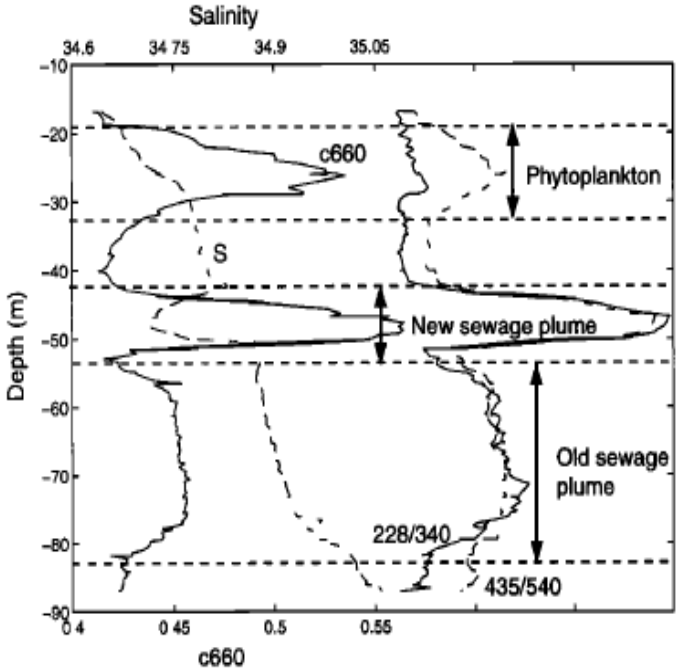
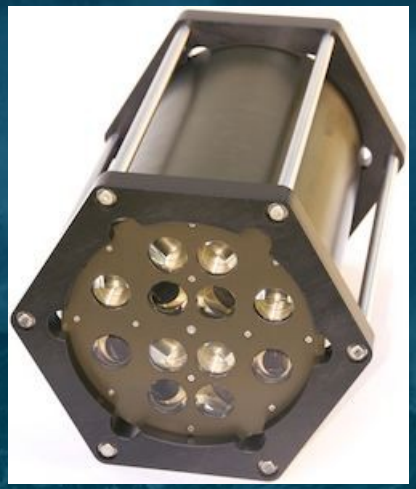


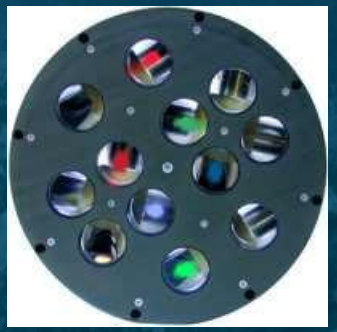
Figure 2. Profiles of salinity, beam attenuation coefficient at 660 nm (c660), and fluorescence, in arbitrary units, for Ex/Em = 228/340 nm and Ex/Em = 435/540 nm. Data are from the second downcast of towyo 32. Three layers were observed: shallow phytoplankton, new and old sewage plumes.

Fluorescence of tryptophane Ex = 228 / Em = 340 nm
[Petrenko et al., JGR 1997]

Supplier: HOBILabs

http://www.hobilabs.com





HYDROSCAT 6

Backscattering (ß[140°] and bb) at six wavelengths

Standard bb wavelengths: 420, 442, 470, 510, 590, and 700 nm

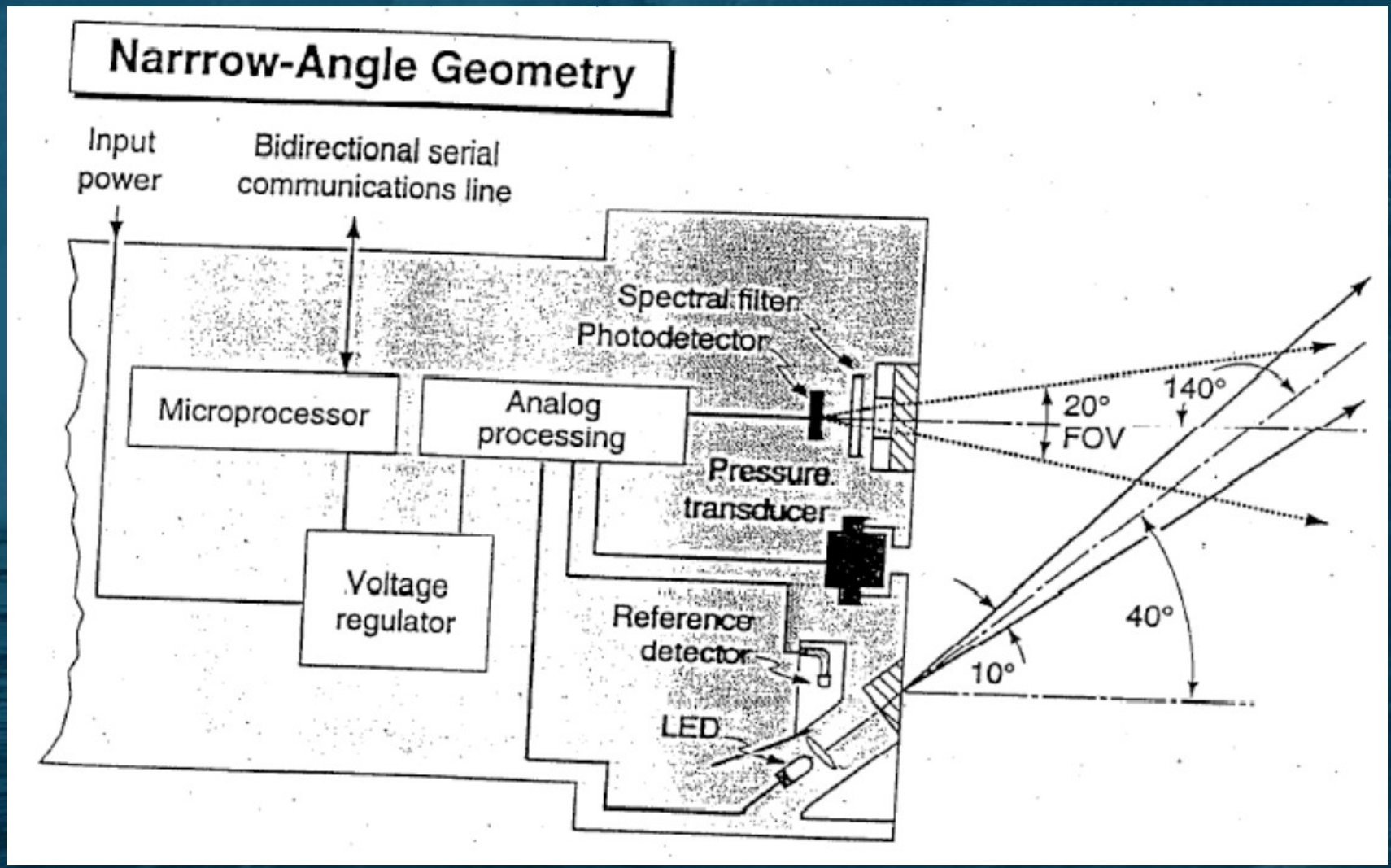
Other wavelengths available

+ Fluorescence at two wavelengths

Standard: 700 nm excited with 442 nm (chlorophyll),

510 nm excited with 420 nm

Depth (330 m standard, 500 m optional)


Narrrow-Angle Geometry
Input power
Bidirectional serial communications line
Spectral filter
Photodetector
Microprocessor
Analog processing
Pressure transducer
Voltage regulator
Reference detector
LED
20° FOV
140°
40°
10°

HydroScat Backscattering Sensor Family

Overview | Optics | Calibration | Logging & Control | Example Data | Determining $b_{bλ}$

HydroScat Optical Backscattering Sensor / Fluorometers

The **HydroScat-2**, **HydroScat-2 Abyss**, **HydroScat-4** and **HydroScat-6** are literally and figuratively the **First Family** of multi-wavelength optical backscattering sensors: not only the **first in the world**, but also preeminent in **performance.**

Measurements

- Backscattering (β[140°] and $b_{bλ}$) at multiple wavelengths
- Fluorescence (optional on HydroScat-4)
- Depth transducer, standard
- Outstanding optical performance
- Calibration—rigorous yet can be performed without elaborate equipment and techniques

Data Handling and Control

- Internal data logging
- Long-term autonomous operation
- Real-time data output
- Activate logging by magnetic switch, or software command

Batteries & Power

- Internal rechargeable batteries standard
- External 10 V to 15 V supply capability
- External battery pack available

Software

- HydroSoft included standard

Options

- Choice of wavelengths
- High-capacity, nonvolatile memory expansion up to 2 GB
- Several depth ratings
- Integrated anti-fouling shutter support
- Copper anti-fouling face plates
- Calibration fixture

HydroScat-2

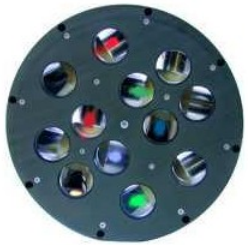
The most economical HydroScat. Measure backscattering at 2 wavelength, plus fluorescence.

HydroScat-2 Abyss

HydroScat-2 performance at 4 km depth. Measure backscattering at 2 wavelengths, plus fluorescence.

HydroScat-4

Measure backscattering, or a combination of backscattering and fluorescence, at 4 wavelengths.



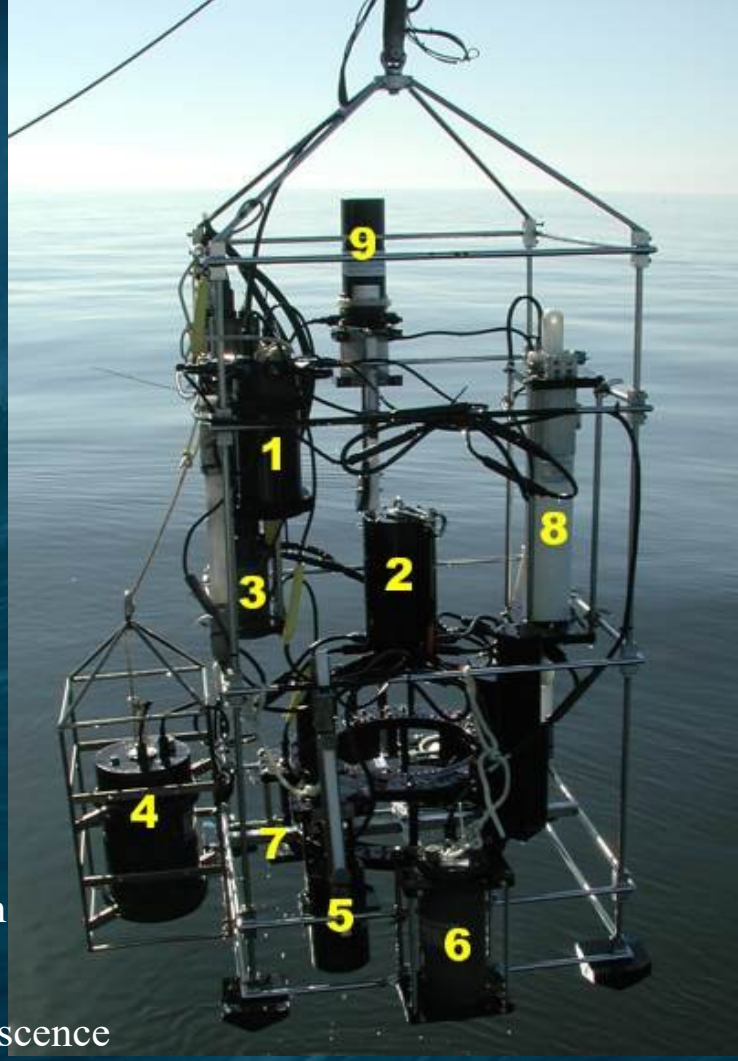
HydroScat-6

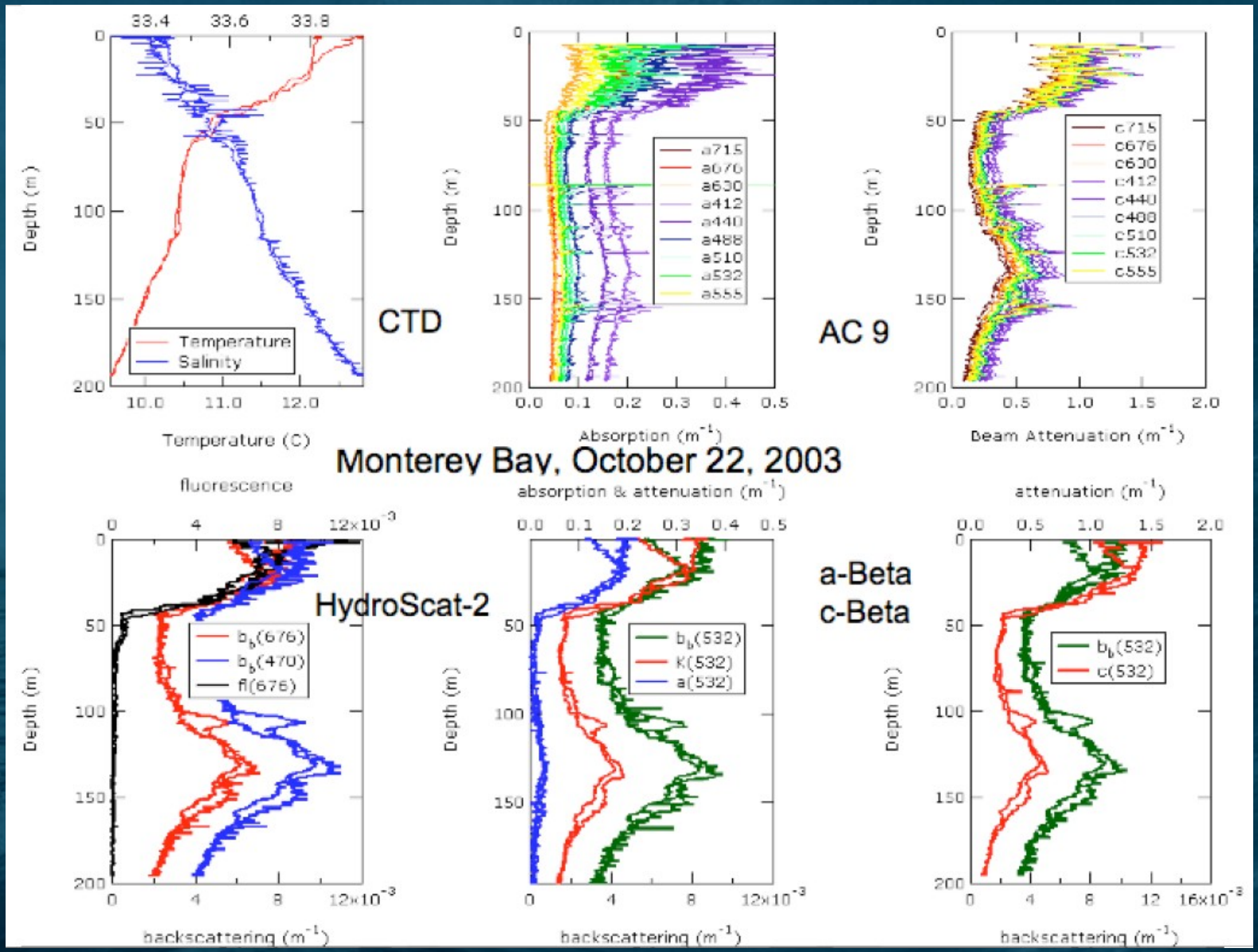
Measure 8 quantities: backscattering at 6 wavelengths, plus 2 fluorescence.

Optical measurements in the sea

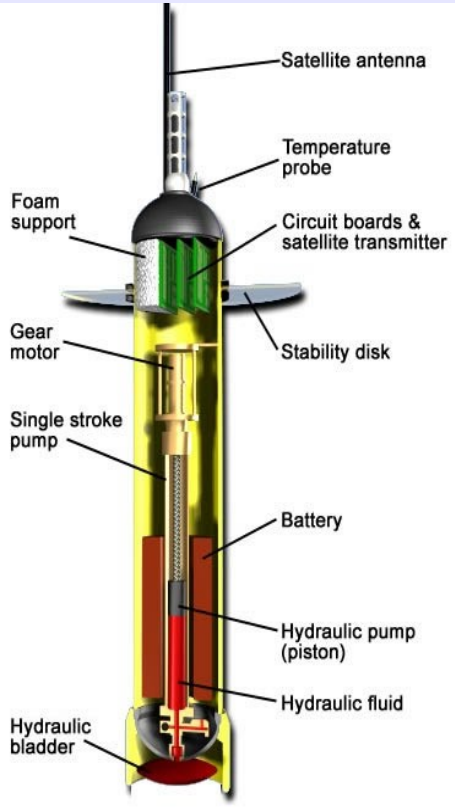
HOBI LABS HydroProfiler

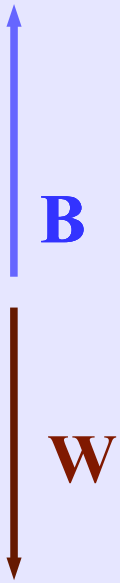
#	Instrument	Measurement(s)
•1	HydroDAS	Controller
•2	HydroBeta	volume scattering function
•3	WET Labs AC9	absorption, attenuation
•4	HydroScat-6	backscattering, fluorescence
•5	a-Beta	backscattering, attenuation, absorption
•6	HydroScat-4	backscattering
•7	HydroScat-2 (back side of profiler)	backscattering, fluorescence
•8	Seabird SBE-19	conductivity, temperature
•9	Biospherical PRR-600	downwelling irradiance (separate upwelling radiance head on bottom rear)
•10	c-Beta (back side of profiler, not visible)	backscattering, beam attenuation




CTD
Temperature
Salinity
Temperature (C)
Depth (m)
AC 9
Absorption (m-1)
Beam Attenuation (m-1)
Monterey Bay, October 22, 2003
HydroScat-2
fluorescence
backscattering (m-1)
absorption & attenuation (m-1)
a-Beta
c-Beta
attenuation (m-1)

Argo – basic system

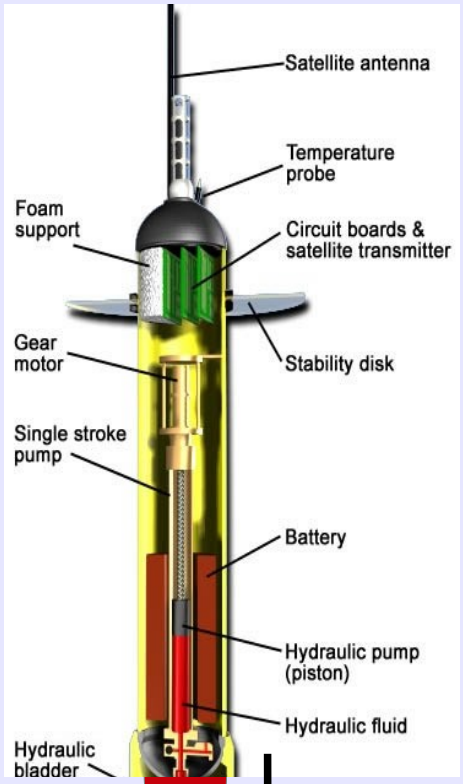


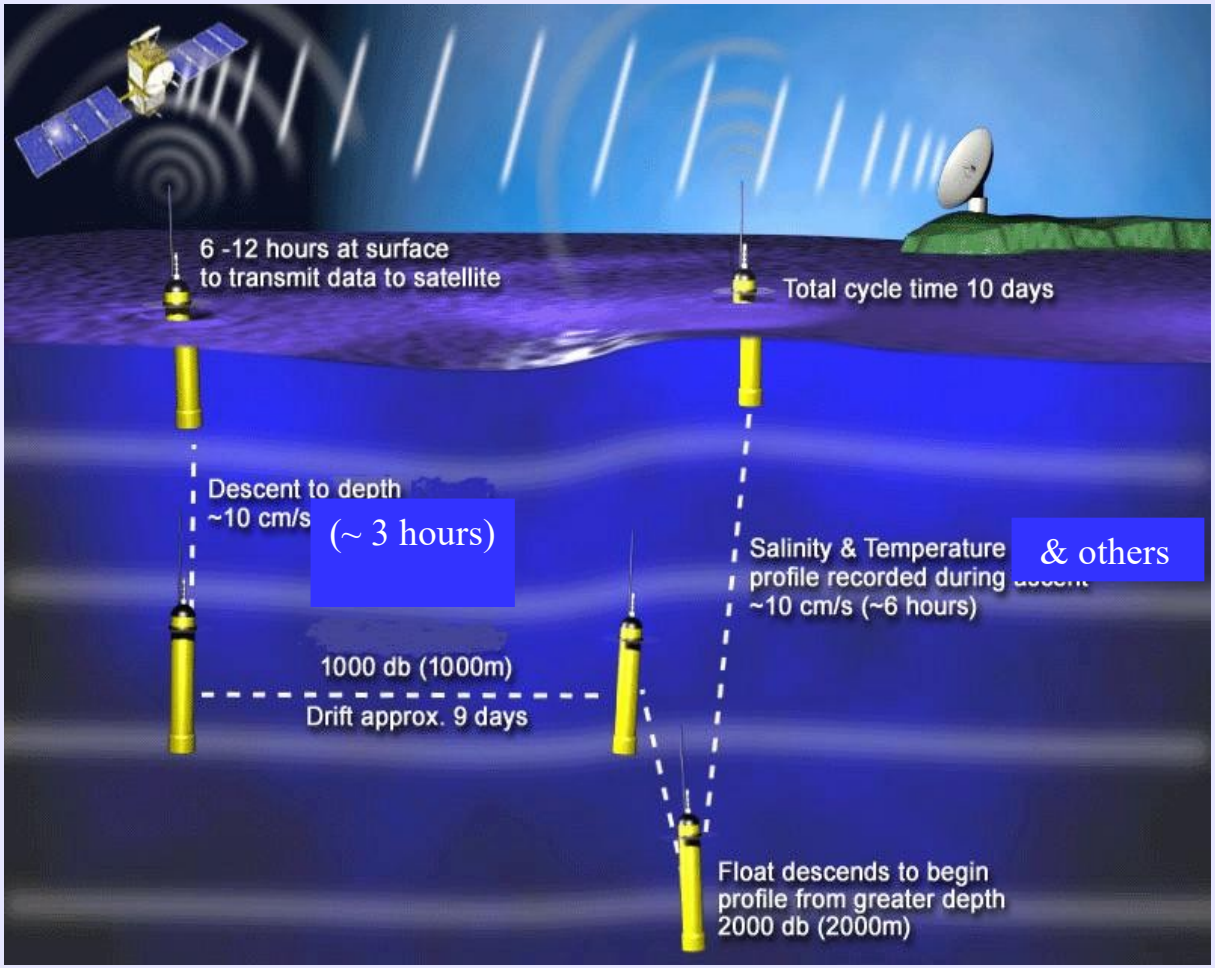


Parking depth:

Equilibrium between weight, W, and buoyancy force B.

Argo – basic system

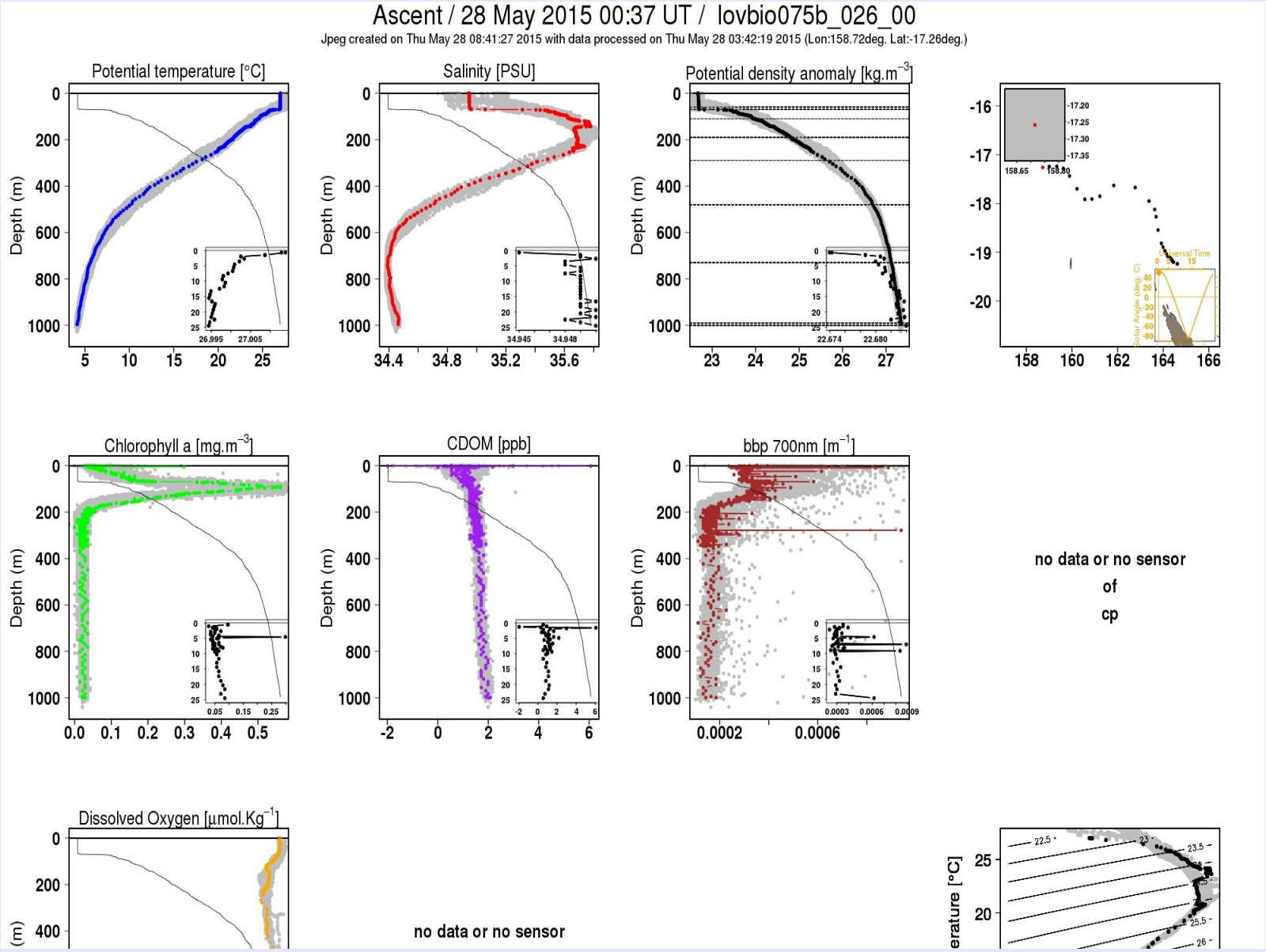




Fluid pumped into external bladder causes volume and thus buoyancy to increase (while weight remains constant)

Disruption of the equilibrium => the float rises

BGC- ARGO (BioGeoChemical)



Pressure
T
S
Chlor a
CDOM
b_{bp} 700 nm
DO (A and B)
Ed 380 nm
Ed 410 nm
Ed 490 nm
PAR

(adapted from http://www.oao.obs-vlfr.fr/bioargo/)

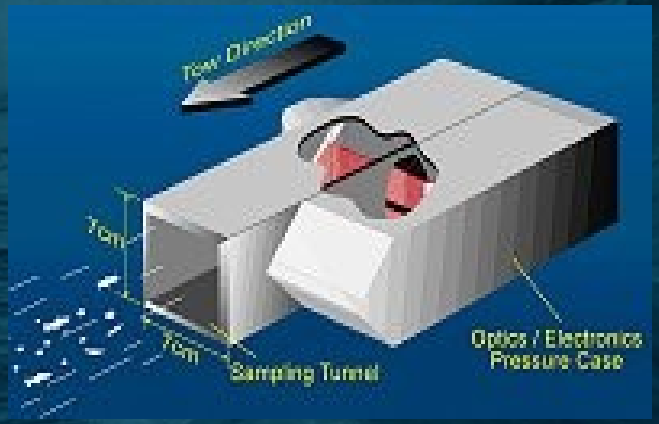
Measured parameters		Derived parameters
Pressure T S	}	Theta, CT, sigma, TS diagram-s, MLD
CDOM		
Chlor b_{bp} 700 nm	}	bp = f(bbp, Chl) (Twardowski et al., 2001) POC = g(bp) (Loisel and Stramski, 2002)
DO		
Ed 380 nm Ed 410 nm Ed 490 nm	}	Downwelling irradiance → Kd at these 3 wavelengths
PAR		Kpar

With optics or Rrs, possibility to obtain phytoplankton communities...
Work and collaborations with colleagues.

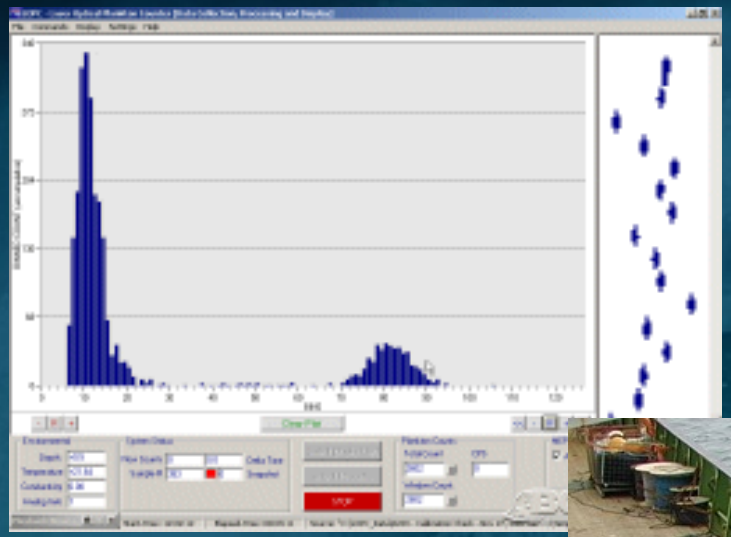

Measuring particles

LOPC - Laser Optical Particle Counter
Resolution ranges from 100µm to 35000µm.
Particles from 100µm – 1500µm (single element plankton) are binned and plotted as a histogram
Particles from 1500um – 35000um (multi-element plankton) have their shape outline displayed in real time.

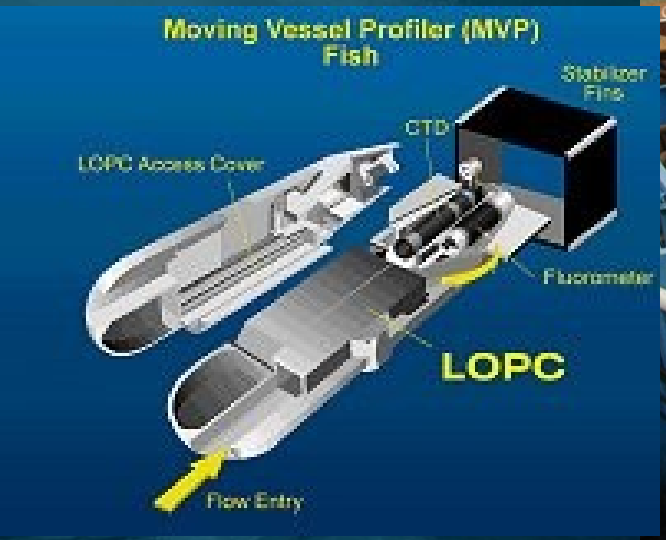
(Standard Rolls-Royce LOPC model)



http://www.brooke-ocean.com/lopc.html

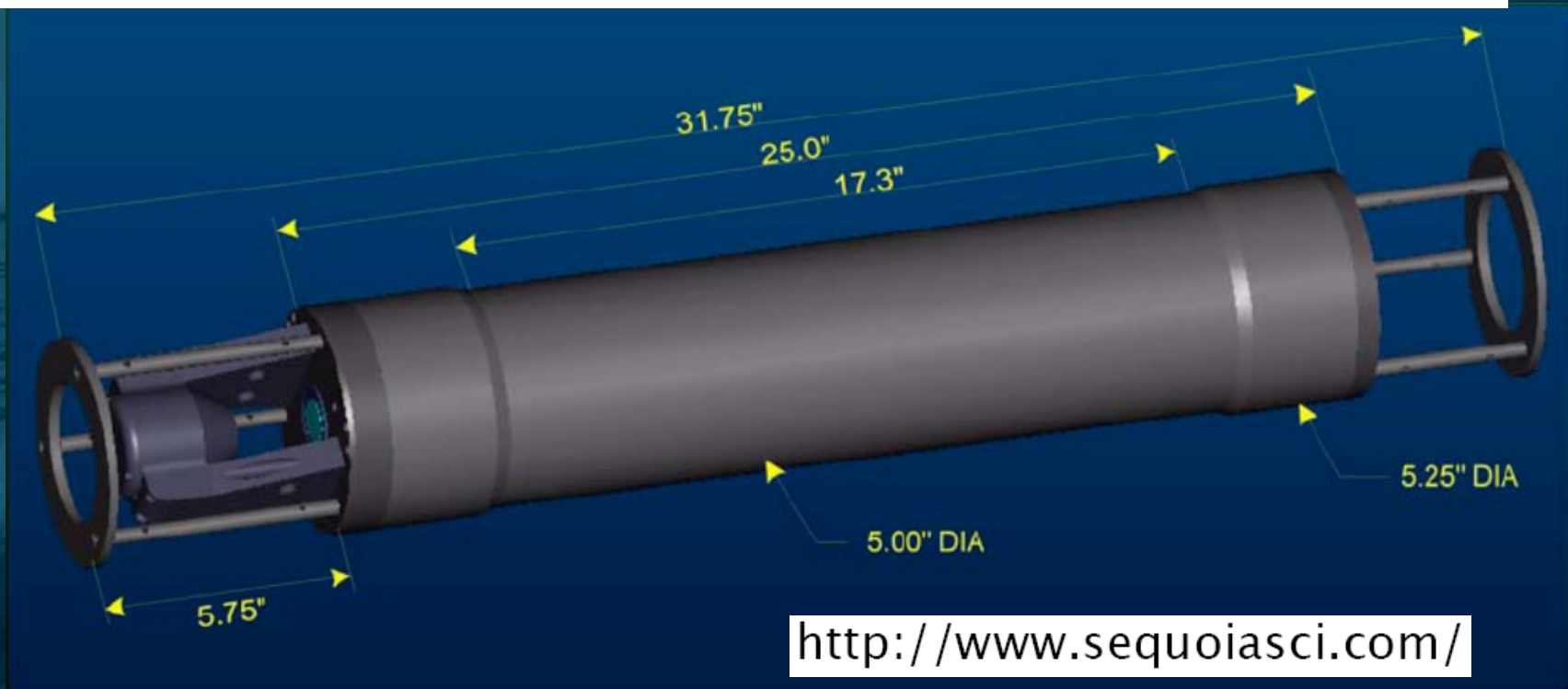


Plankton >1mm and <= 40 mm
The LOPC can operate in high particle concentrations of up to ~ < 10 ^{6}/m ^{3} with MVP towing speeds of up to 12 knots.



LISST = Laser In-Situ Scattering and Transmissometry
Particle size range: 1.25 µm – 250 µm ou 2.5 - 500 µm

Principle : At the heart of the instrument is a collimated laser diode and a specially constructed annular ring detector.
Scattering at 32 angles is the primary information that is recorded. This primary measurement is mathematically inverted to get the size distribution, and also scaled to obtain the volume scattering function (VSF). The size distribution is presented as concentration (micro-l/l) in each of 32 log-spaced size bins. Optical transmission, water depth and temperature are recorded as supporting measurements.



http://www.sequoiasci.com/

Moving Vessel Profiler with LISST – LOPC

Towing speed:
6 – 12 knots

Profile depth:
400 – 800 m

Flux Cytometry

Measures optical properties of cells (cyto-) transported by a fluid flow (flux) to a light excitation source (usually a laser).

- Light (laser or arc lamp) scattered by the particles (cells)
- Natural or induced fluorescence emitted by the particles (cells)
- Monodisperse particle flow
- Multivariate analysis of particles (cells)
- Identification of sub-populations

- 1st instrument dedicated to studying aquatic microorganisms
  → constructed in 1983

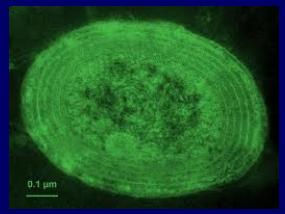
Courtesy, Gregori 2018]

Important discoveries made using flow cytometry

- *Prochlorococcus* (cyanobacteria)

= the smalles photoautotrophic procaryotes* and the most abundant on the planet

Chisholm, 1988, Nature 334

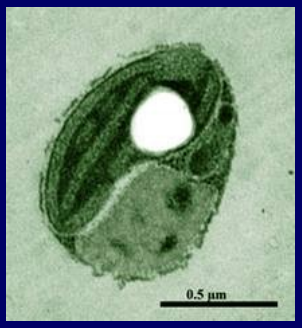


* no cell nucleus ("bacteria", archaea and eubacteria)

- *Ostreococcus tauri* (Chlorophyta, Prasinophyceae)

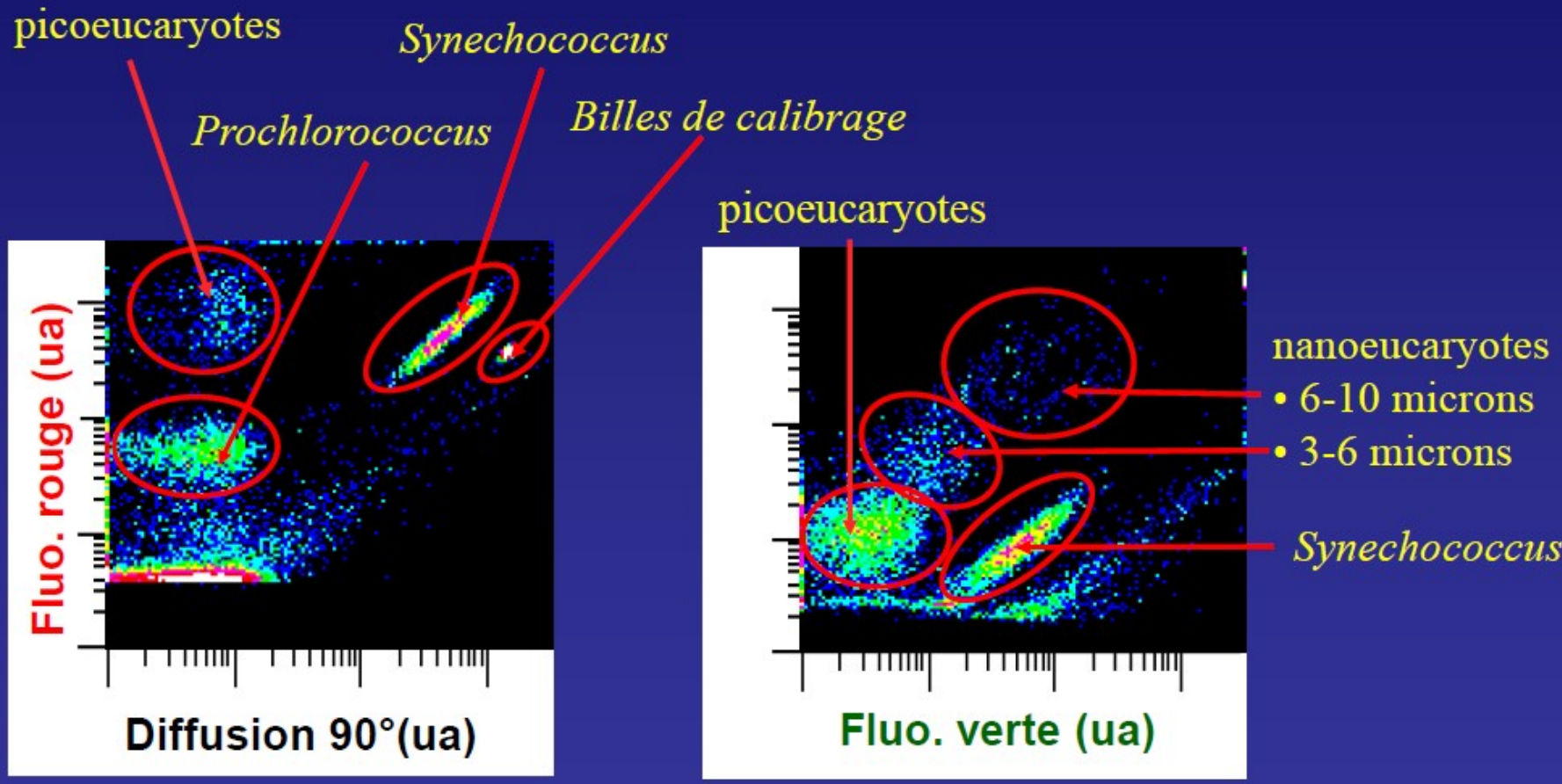
= the smallest unicellular eucaryot know today (discovered in the Thau lagoon, France)

Courties *et al*, 1998**, J Phycol 34**



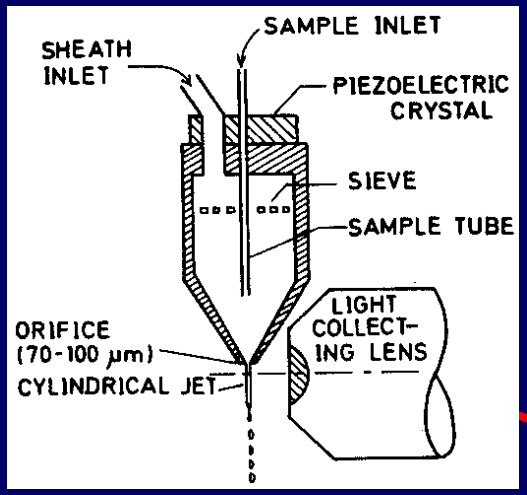
Courtesy, Gregori 2018]

Data obtained with flow cytometry

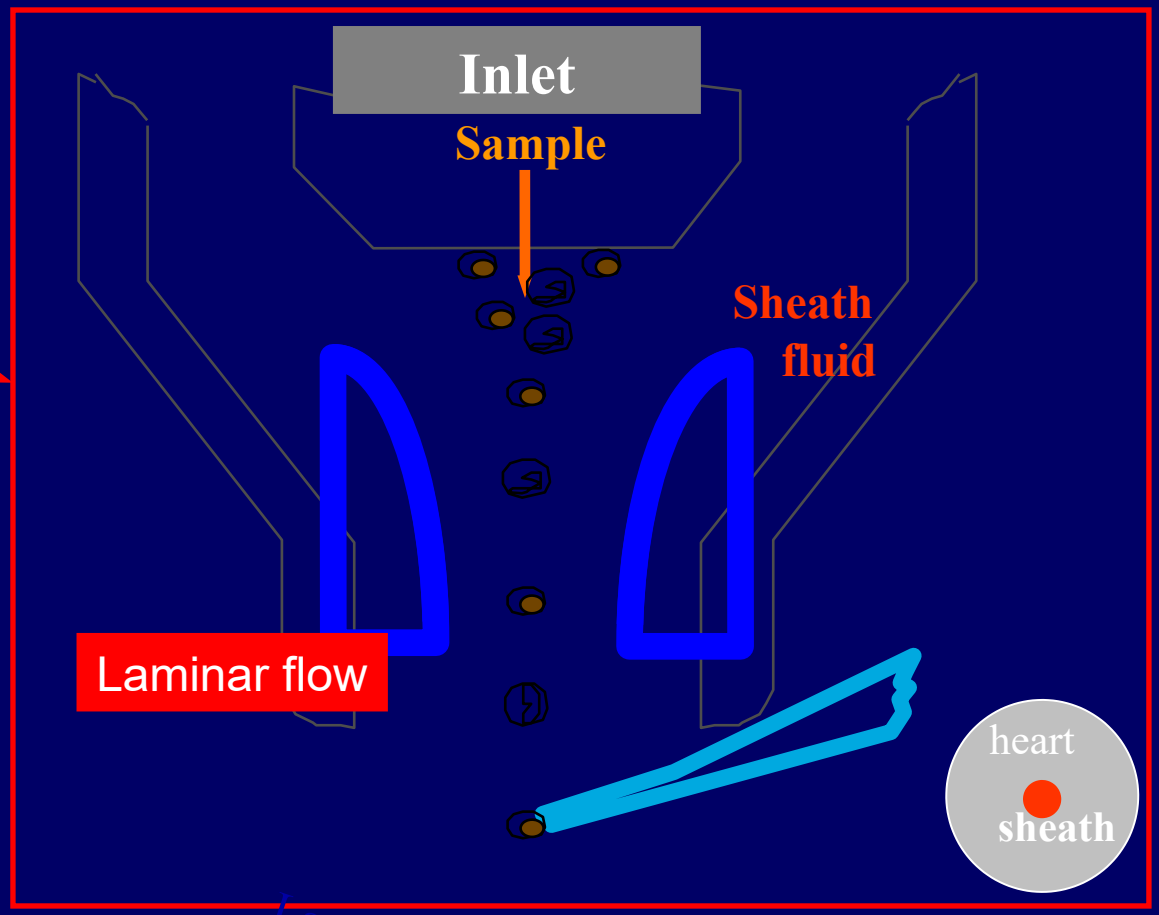


Courtesy, Thyssen 2020]

Principle of hydrodynamic focussing in a flow chamber (sheath fluid)



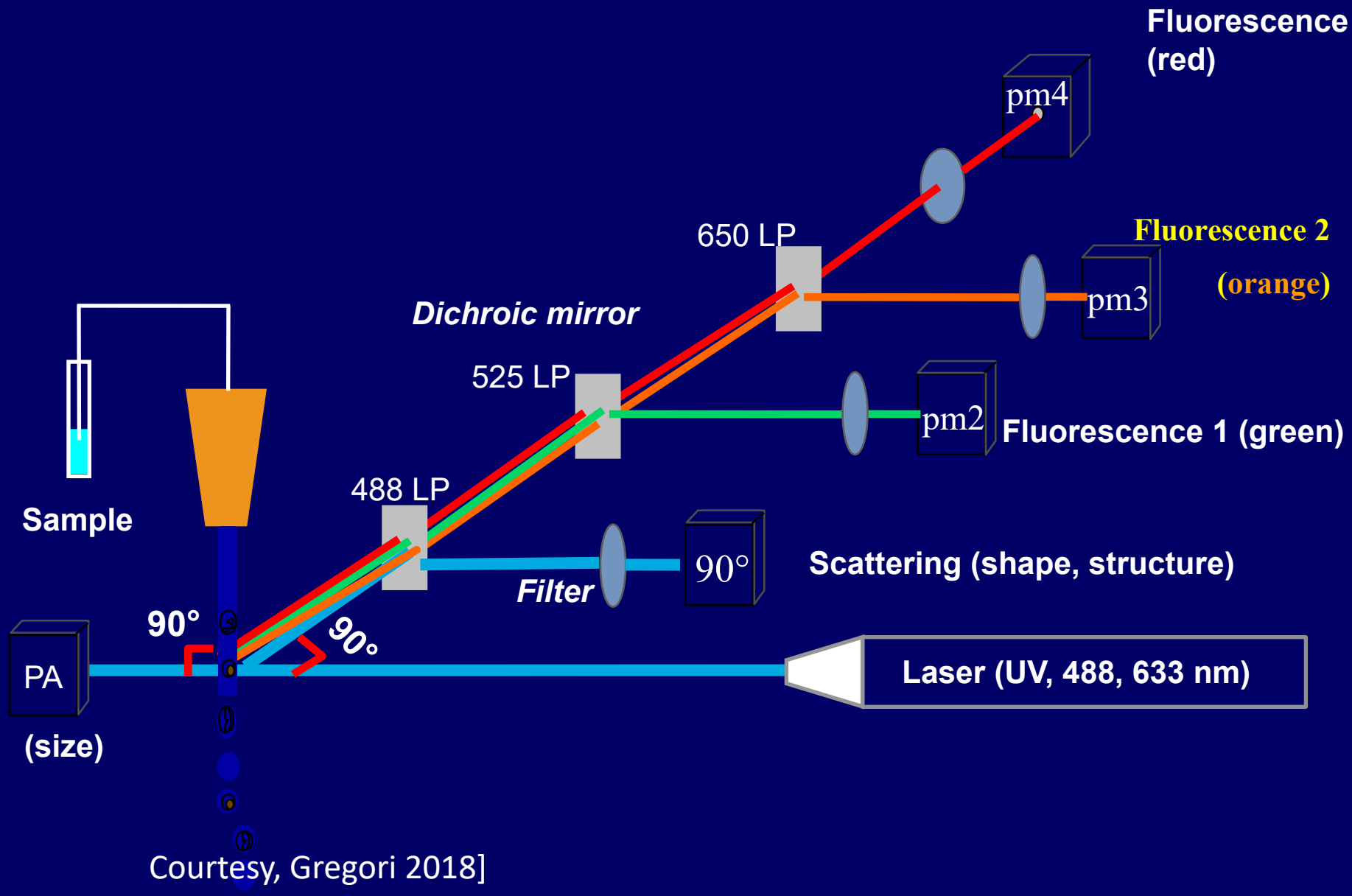
Flow chamber



- **Separation**
- **Alignment of particles**

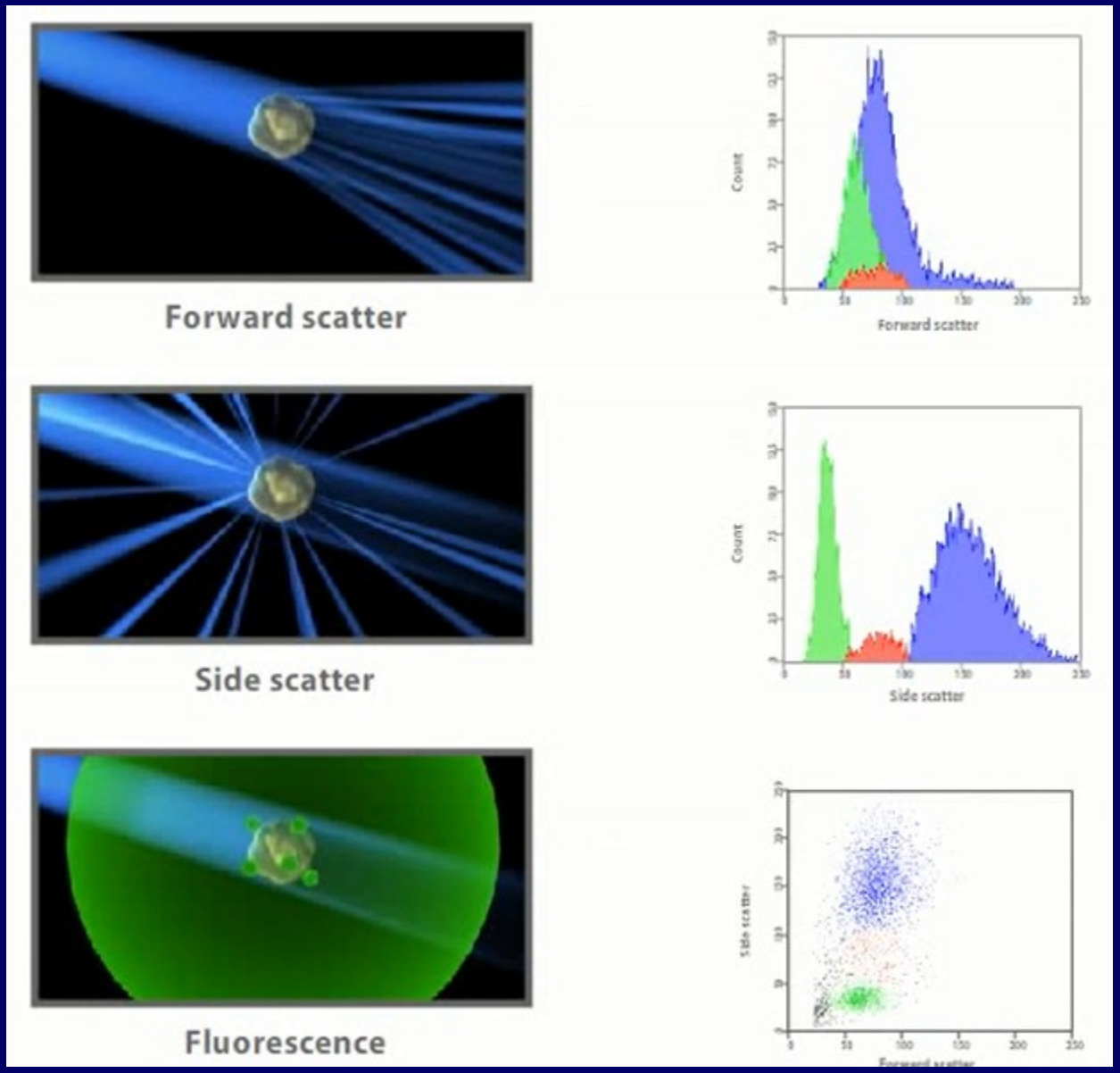
Courtesy, Gregori 2018]

Operating principle of flow cytometry



Courtesy, Gregori 2018]

Cytometry data

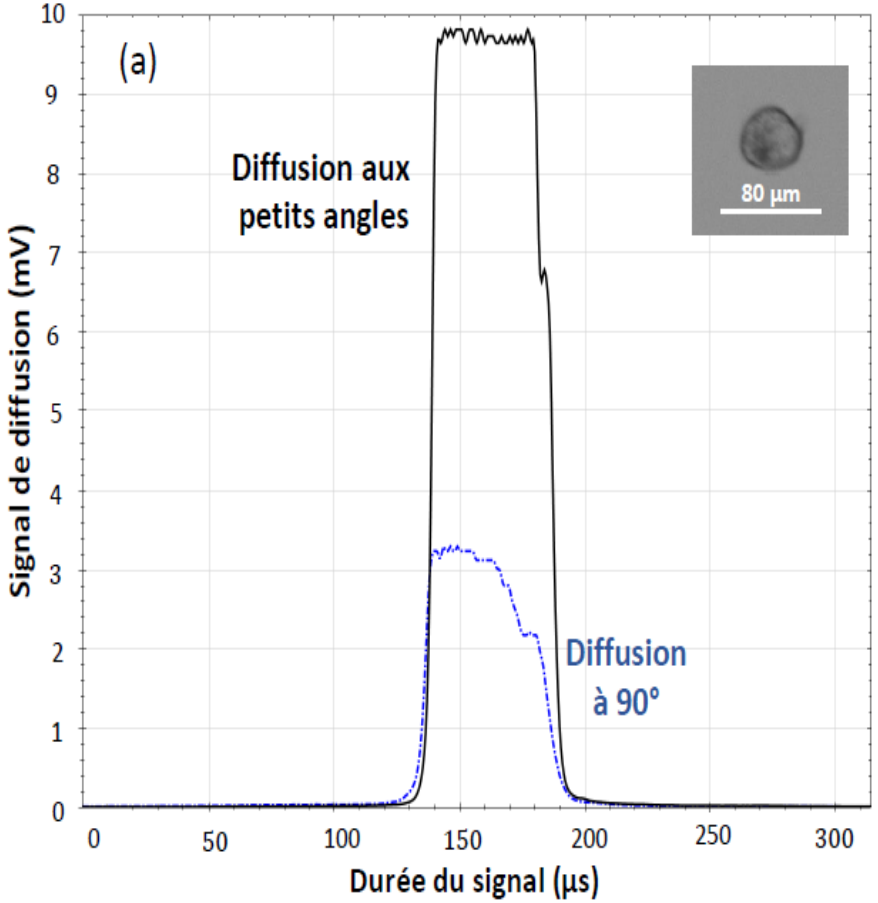


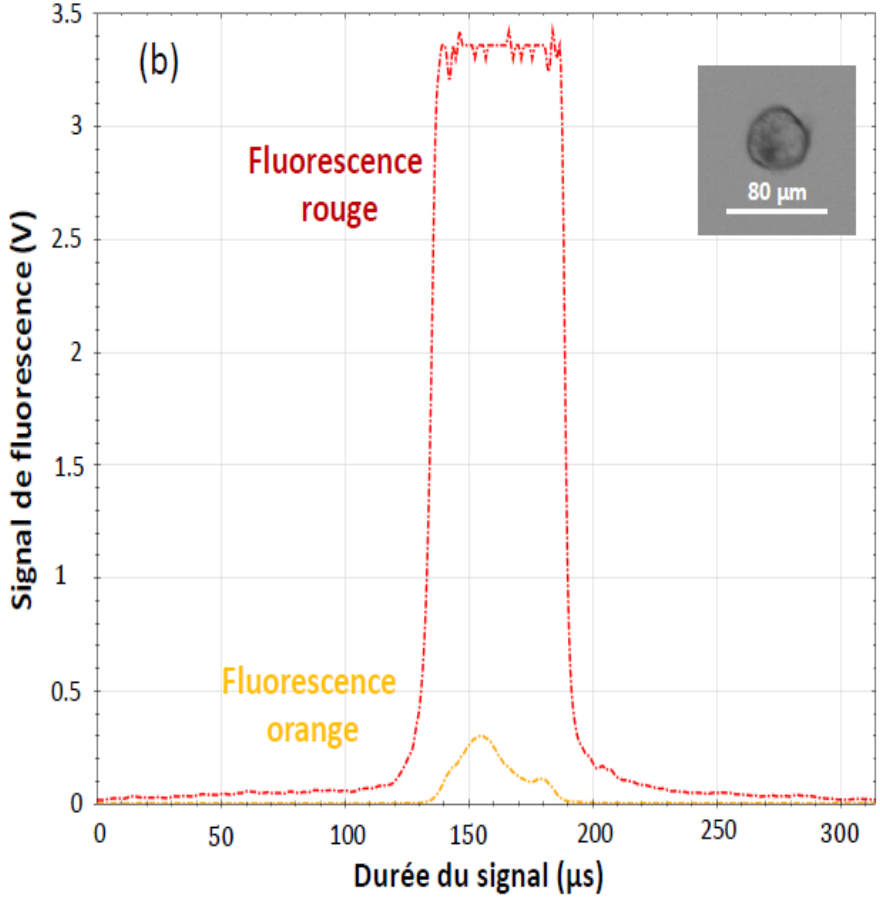
Courtesy,
Gregori 2018]

Résultat d'analyse d'UNE particule micro-phytoplanctonique d'UN échantillon d'eau de mer :

Diffusion aux petits angles : proxy de la taille de la particule.
Diffusion à 90° : proxy de la granularité/forme de la particule.

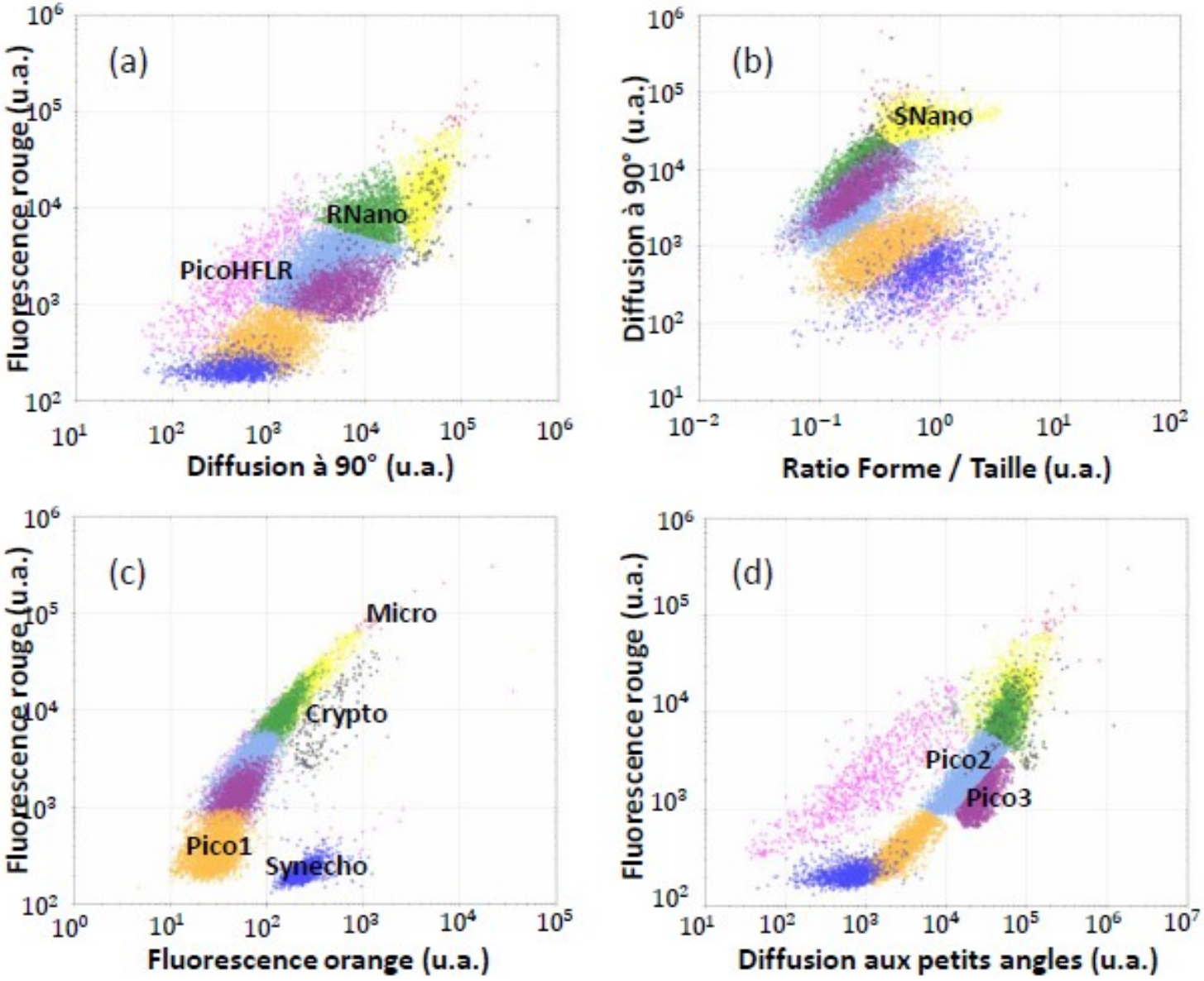
Fluorescence rouge : liée à l'excitation de la chlorophylle.
Fluorescence orange : liée à l'excitation de la phycoérythrine.





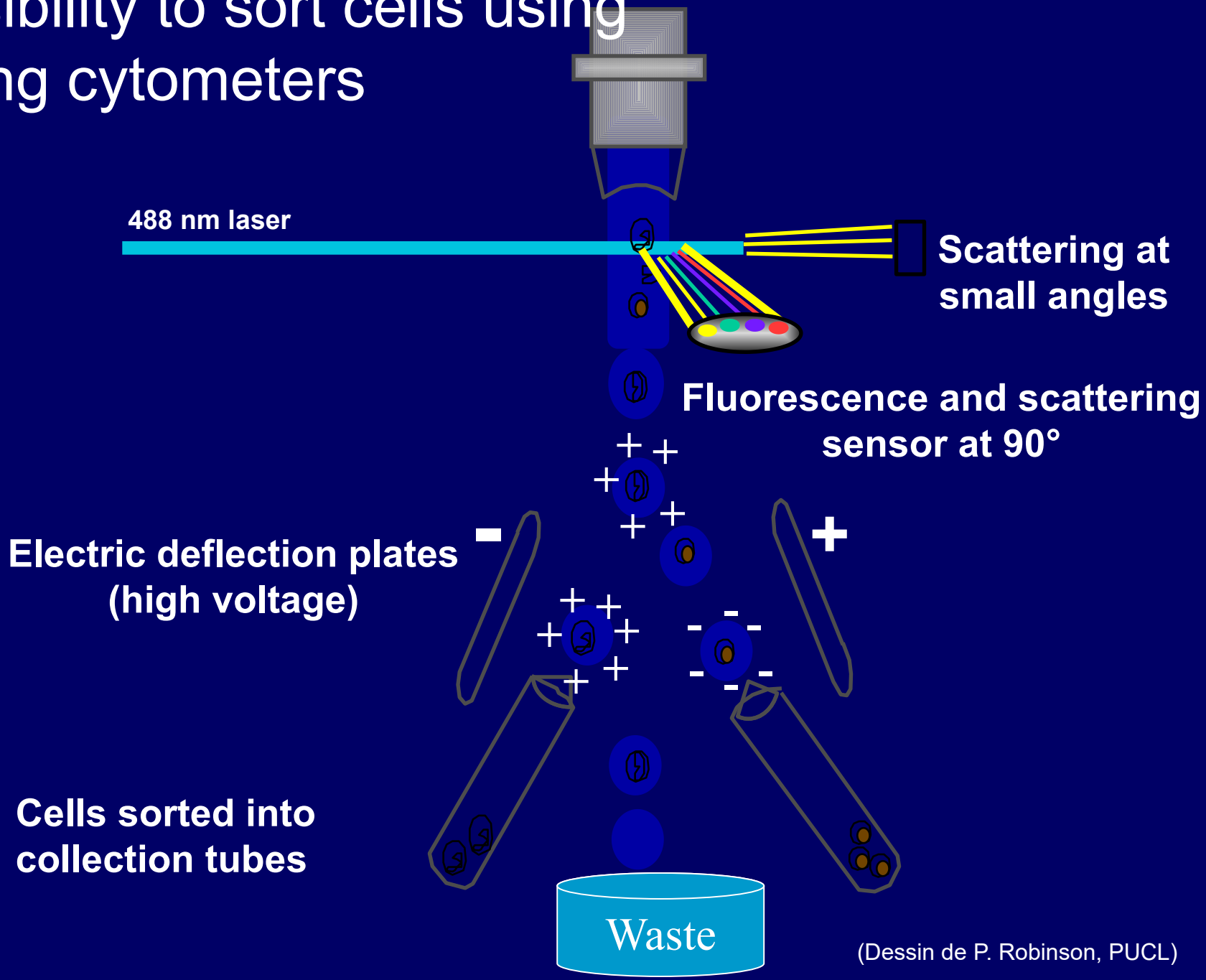
Courtesy, Izard 2019]

Représentation des mêmes particules d'UN échantillon dans 4 cytogrammes différents :



Courtesy, Gregori 2018]

Possibility to sort cells using sorting cytometers



(Dessin de P. Robinson, PUCL)

Cytosub (Cytobuoy): a flow cytometer for autonomous *in situ* measurements of phytoplankton.

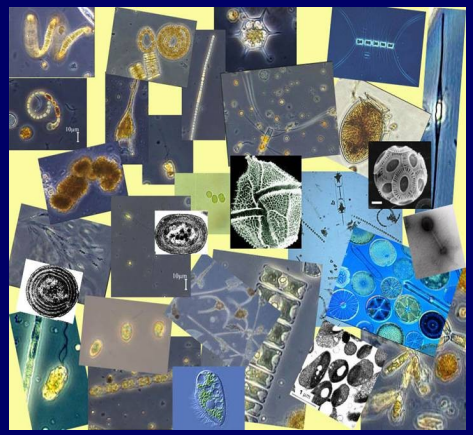
- *In situ* analysis down to a maximum depth of 200 m.
- 10 min max sampling frequency
- Can be deployed for several weeks at a time

(G. Gregori,
M. Thyssen, MIO)

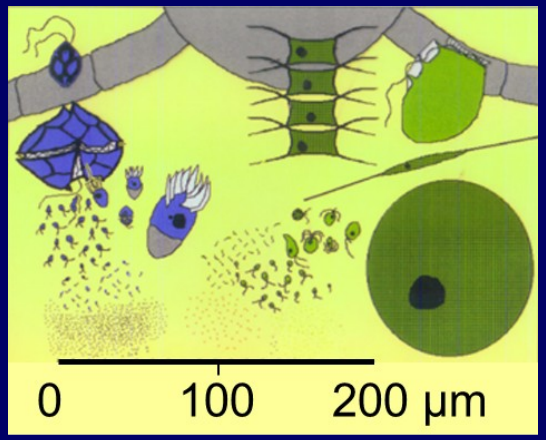
Difficulties and tools

Difficulties:

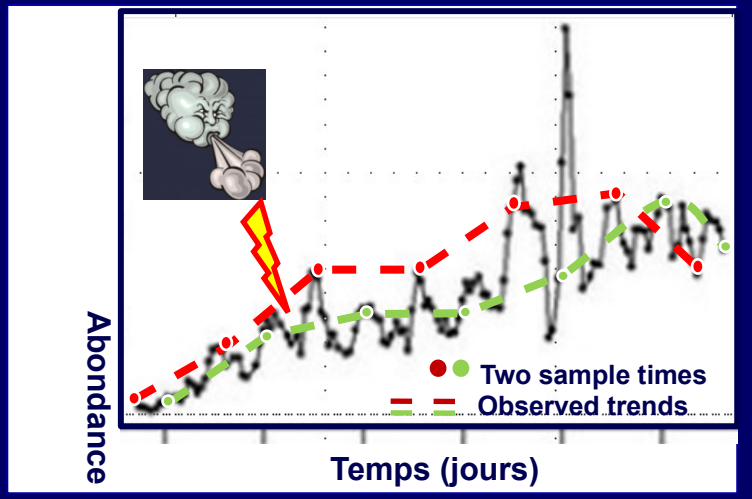
Diversity



Large size range

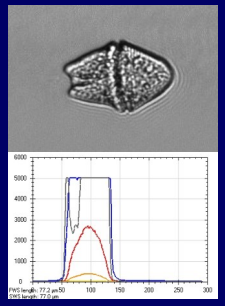


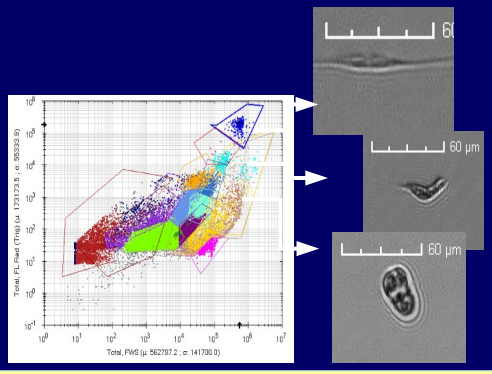
Highly dynamic



Tools:

Automated image collection using flow cytometry





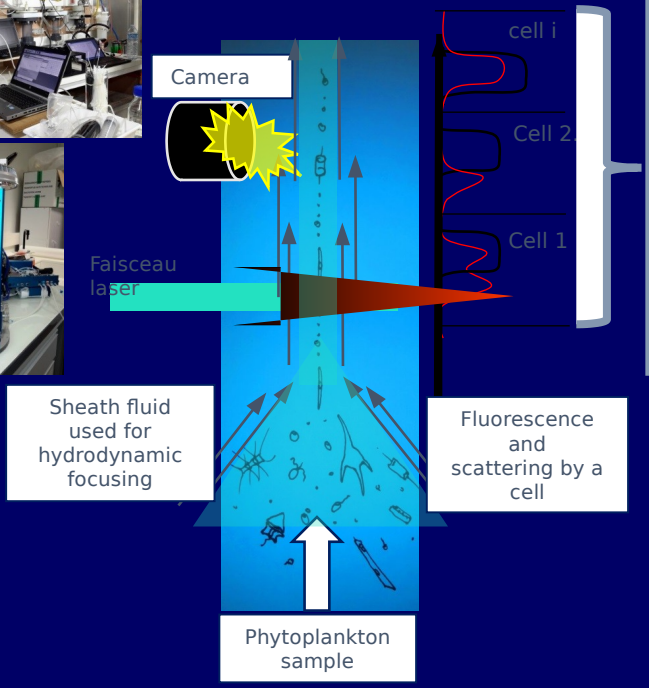
Analysis of community size structure

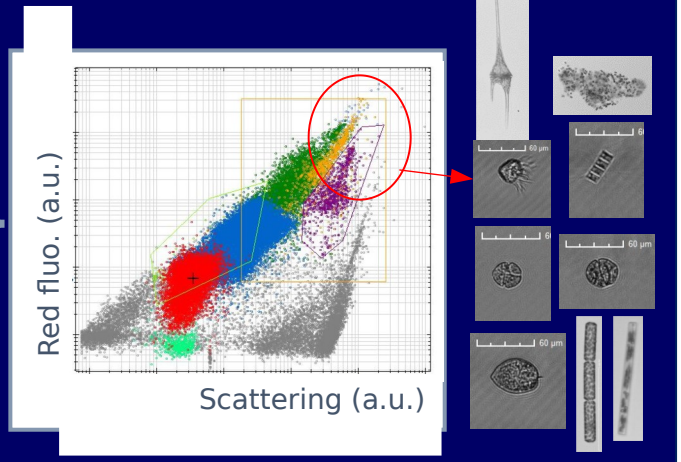
Buoys

Ships of opportunity

Research Vessels

The automated flow cytometry allows for **high-resolution observations** of different phytoplankton on a cell-by-cell basis. This can be used to **calibrate remote sensing observations** or to guide **theoretical studies** to explain/improve the interpretation of ocean colour measurements





Resolved functional groups

Prochlorococcus
Synechococcus
Picoeucaryotes
Nanoeucaryotes
Microphytoplancton
Cryptophytes
Coccolitophorideae

Images

Taxonomic diversity for organisms > 15 µm

- Sampling frequency 0.1 min^{-1}
- Autonomous sampling for several months yield time series with high temporal resolution
- Expertise at MIO

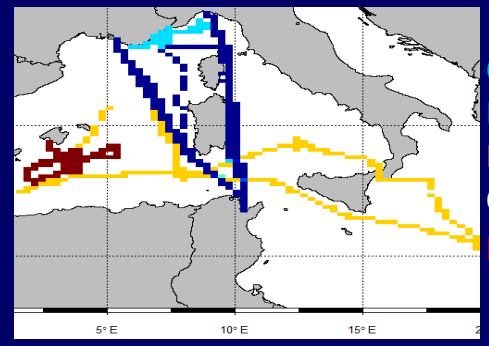
(Thyssen et al., 2015; Duforet et al. 2015; *Moutier et al., 2016, 2017)*

Courtesy, Thyssen 2020]

Links with optical properties of water and PHYSAT

Application in the Mediterranean=

>2500 flow cytometry samples collected between 2015 and 2020 during campaigns at sea.



OSCAHR
FUMSECK
PEACETIME
CHROME
preBIOSWOT

Interpretation of ocean colour measurements via PHYSAT (detection of dominant species and assemblages)
(S. Alvain, AH. Rêve)

Tosca CYTOSAT

Theoretical model. Estimating biomass and the influence of phytoplankton cell morphologies
(L. Duforet)

Best characterisation:

- of phyotplankton species composition
- of their functions in marine cycles (specifically carbon)

-Globally applicable
-Integration growth processes in the data
-Data needed by biogeochemical models
- Links with resources (food chains).

Coupled experiments within the BIOSWOT framework

Courtesy, Thyssen 2020]


Measuring nutrients

Capteurs de Nutriments (commercialisés)

= > 2 principales technologies:

• **Optique** (ex: **I**n **S**itu **U**ltraviolet **S**pectrophotometre pour nitrates): mesure du spectre d'absorption mais problème de consommation d'énergie et de sensibilité (LD = 0.5 µM) et non disponible pour tous les nutriments

• **Analyse chimique** = Méthodes d'autoanalyse standard; = la plus performante à l'heure actuelle (LD ~ 0.05 µM); durée de déploiement de plusieurs mois (> 4 mois); = sur différents types de plateformes autonomes (mouillage, AUV le subchem de Wetlabs)
Mais problème = instrumentation complexe : dispositifs **lourds** (environ 10 – 30 kg, **encombrants** 0.5 m3) ; **chers** (entre 10 et 100 k€) ; maintenance technique lourde.

• Analyseur de nutriment multiparamètres

EcoLAB (EnviroTech)

SubChemPak Analyzer
(Subchem Syst./WetLabs)

NPA (Systea)
(nutrient probe
analyzer)

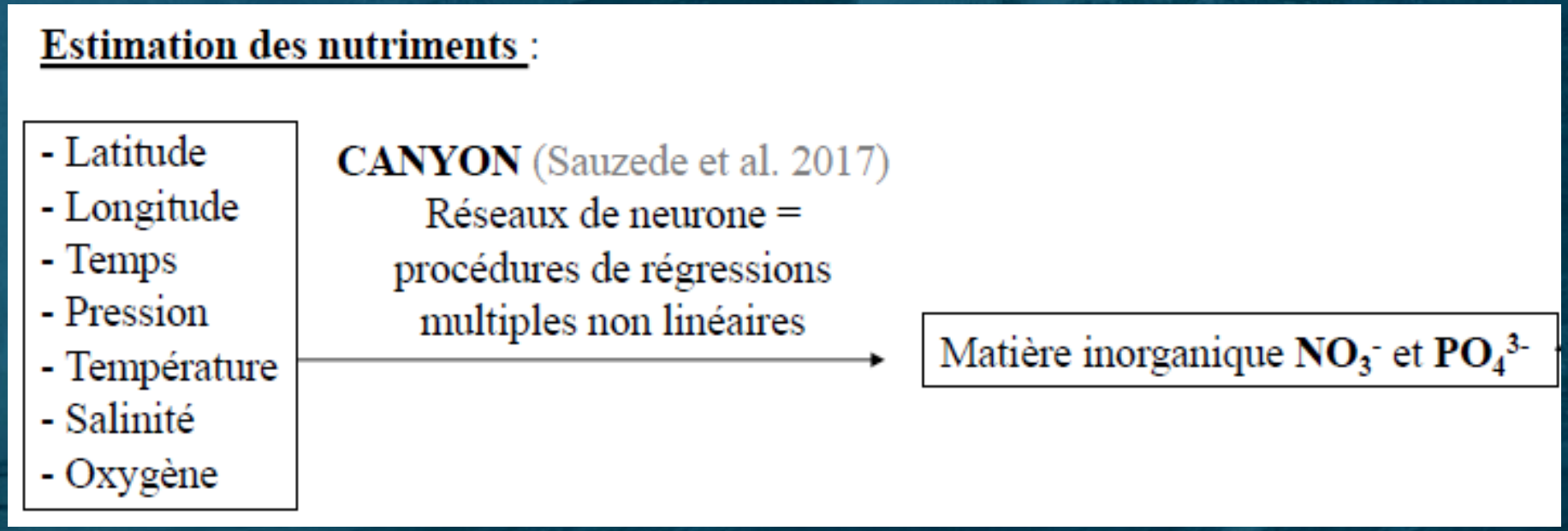
• Analyseur de nutriment mono-paramétrique

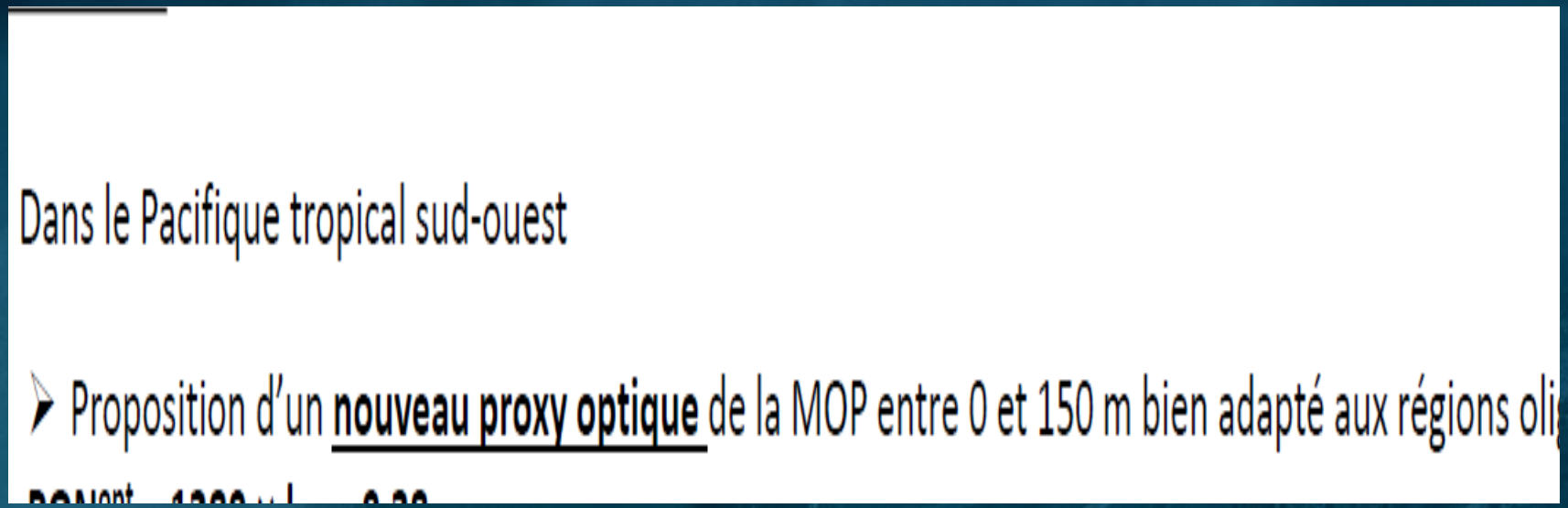
9600 Nitrate Monitor (Ysi)

Cycle-PO4 (Wetlabs)

Using the PROVBIO floats

(Below schematic courtesy A. Fumenia)





Fumenia, A., A. Petrenko, H. Loisel, K. Djaoudi, A. de Verneil and T. Moutin (2020), Optical proxy for particulate organic nitrogen from BGC-Argo floats , Optics Express, doi:10.1364/OE.395648

(Above correlation R^2=0.87
More work to do as there are 28 profiles
Few data available for oligotrophic areas of the Tropical SW Pacific
Great contribution by BGC-Argo floats)

(MOP = Particulate organig matter (POM); PON = Particulate Organic Nitrogen)

Courtesy – excerpt from thesis - A. Fumenia – 3 June 2020

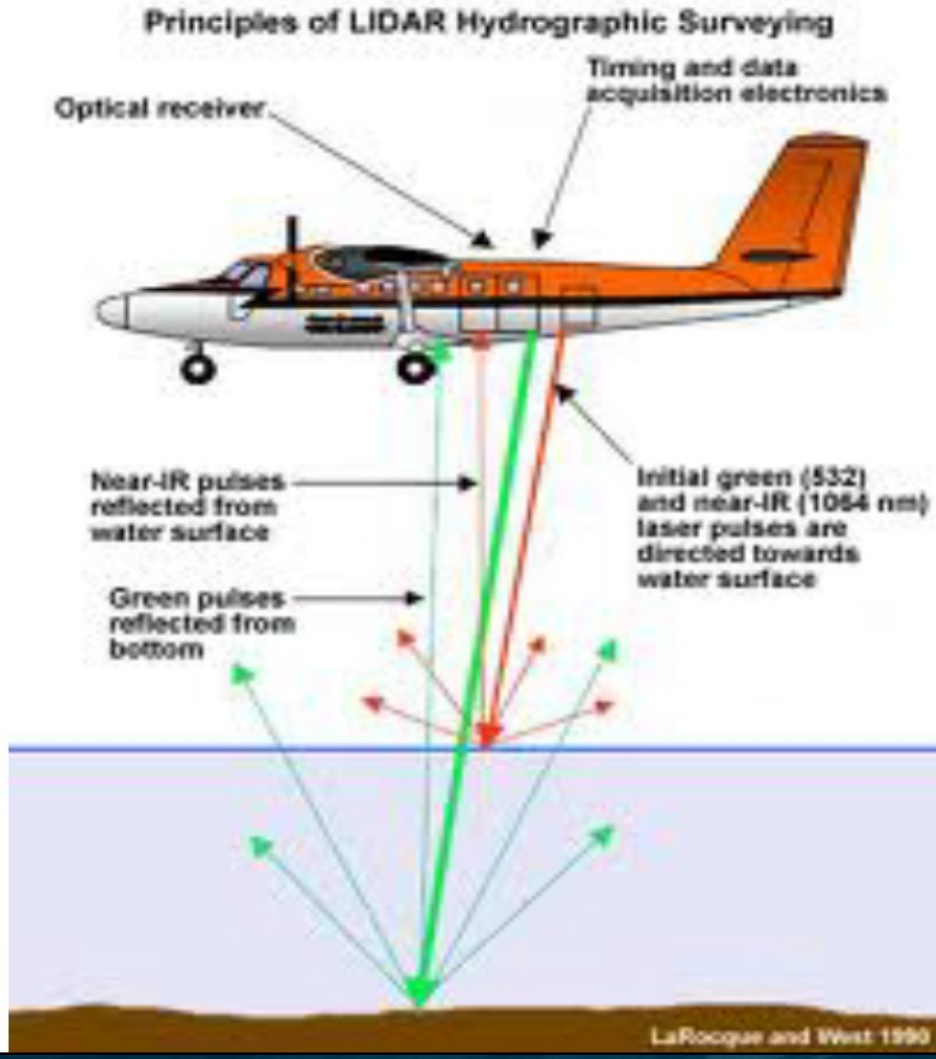
Le LIDAR (détection active)
(Light Detection And Ranging)

Utilité:

Bathymetrie et type de fond.

Poisson (sardine).

Distribution et type de particules.



Peut aussi détecter de la fluorescence – Lidar à fluorescence)

LIDAR Active Instrument ~Laser and Radar

Advantages:

- Active, thus functions also at night (detection of nycthemeral migration)
- Passing cloud cover
- Sampling in rarely samples zones; e.g., poles (no coverage by satellites)

More details than you want to know:
https://en.wikipedia.org/wiki/Lidar

Site by M. Behrenfeld
https://www.scientia.global/professor-michael-behrenfeld-advancing-satellite-technology-to-monitor-ocean-phytoplankton/?doing_wp_cron=1605025154.6465411186218261718750

MJ Behrenfeld, Y Hu, CA Hostetler, G Dall'Olmo, SD Rodier, JW Hair, CR Trepte, Space-based lidar measurements of global ocean carbon stocks, Geophysical Research Letters, 2013, 40, 4355–4360.

NASA Langley Research Centre more sophisticated lidar technique: high-spectral resolution lidar (HSRL). HSRL adds an additional detector system to independently separate scattering and absorbing components in the water.