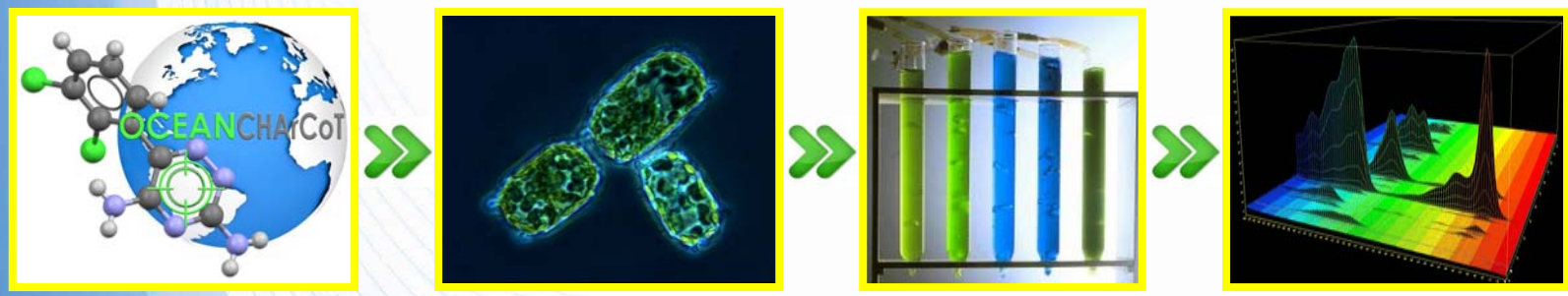
MARIE CURIE ACTIONS

CNRS UPMC
Station Biologique
Roscoff

Ifremer

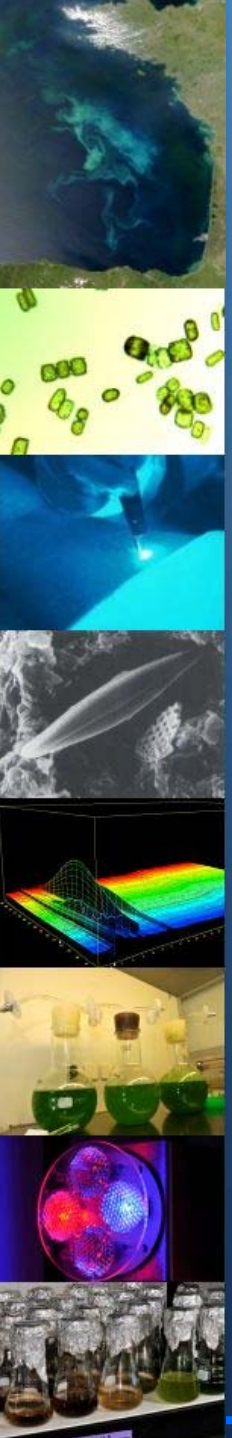
Optimized extraction of microalgae's metabolites: a crucial step in High-Throughput Screening programs dedicated to phytoplankton chemodiversity

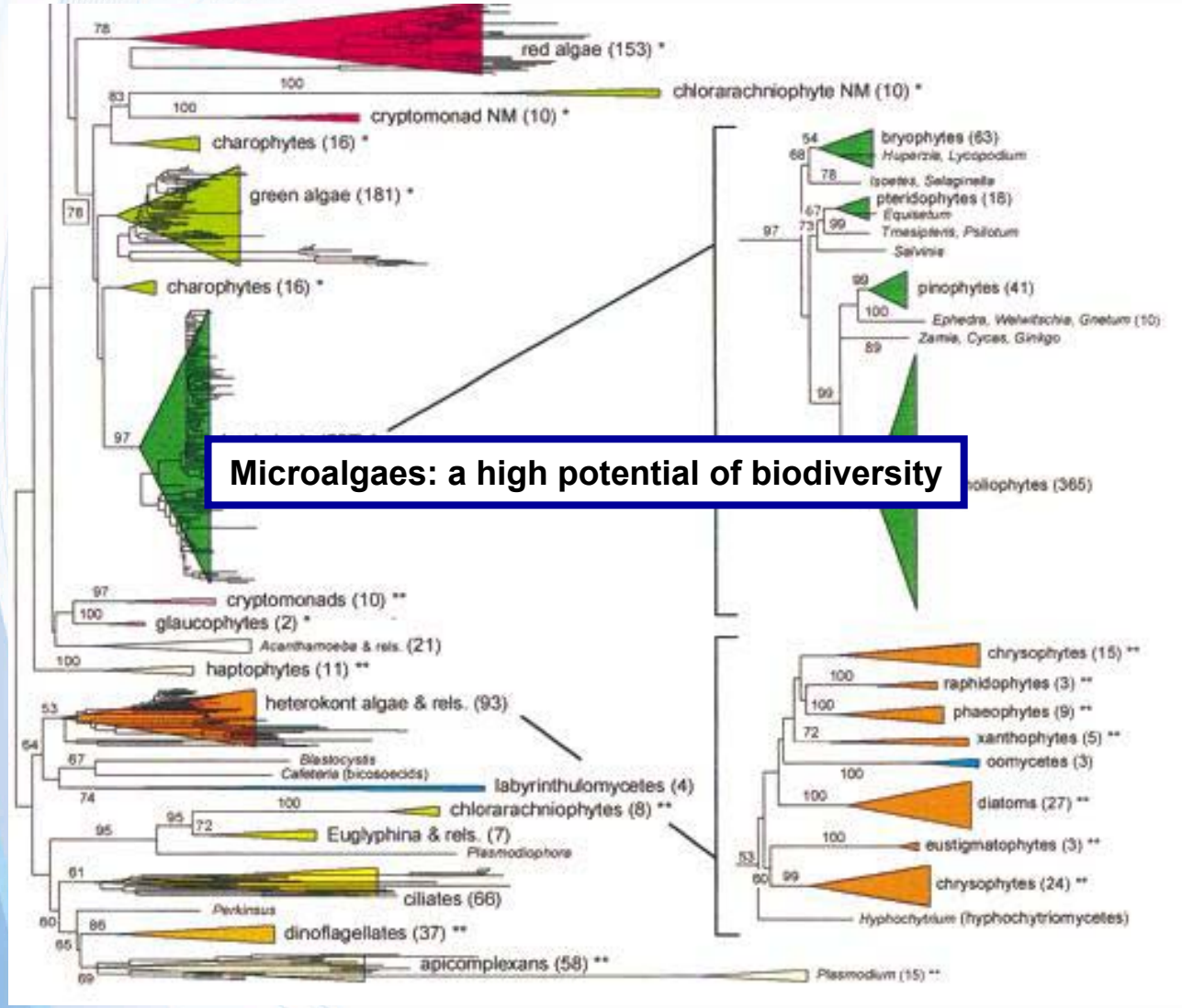


Track 12-1 Marine natural products and biomolecules

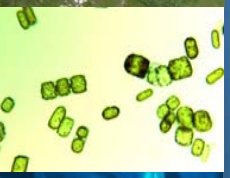
Serive B., Kaas R., La Barre S., Bach S., Cadoret J-P.

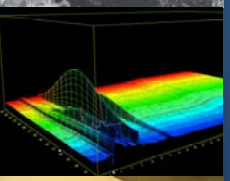
Bioresources exploration



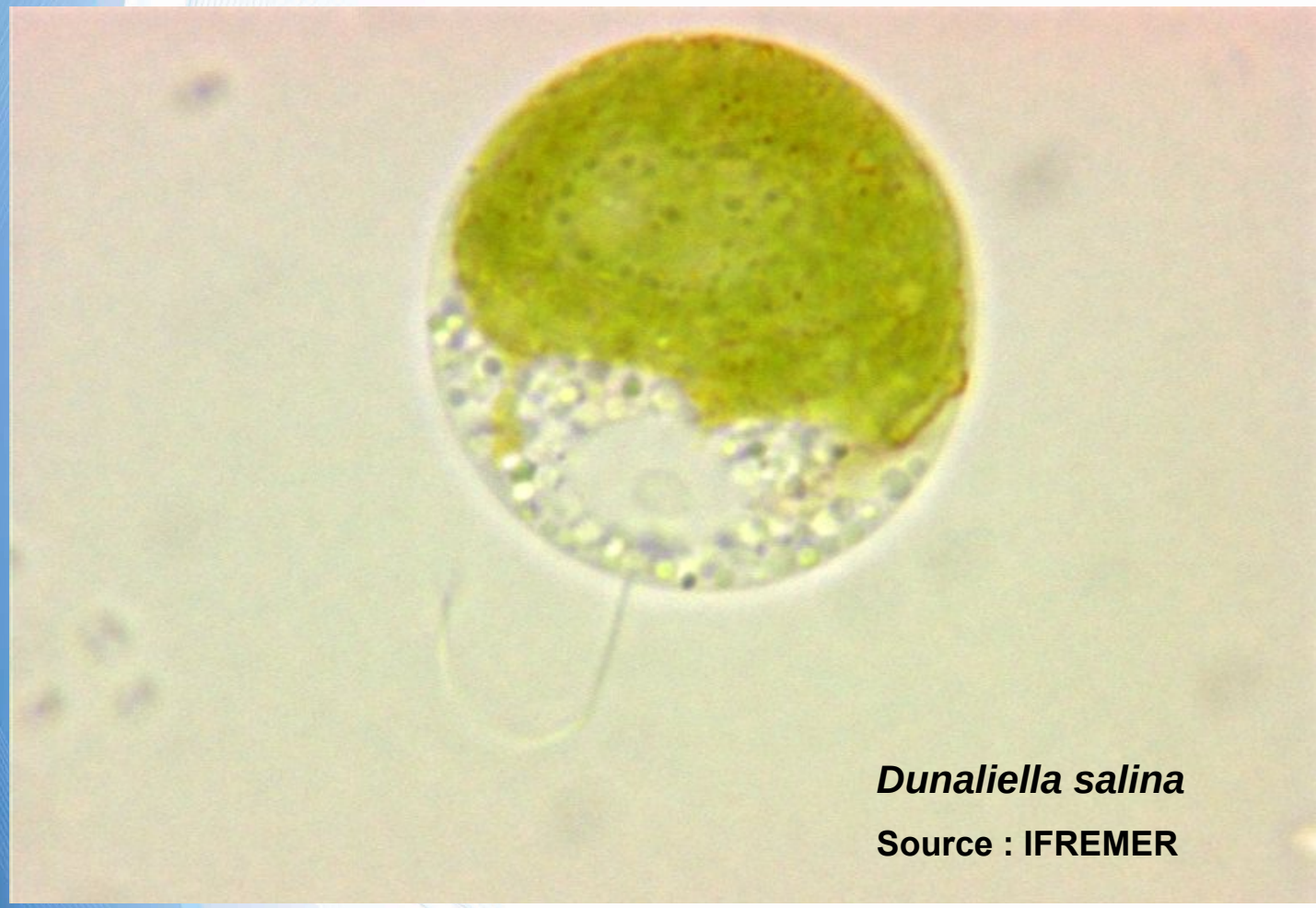


Van de Peer *et al*, 2000

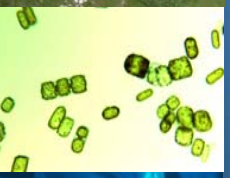



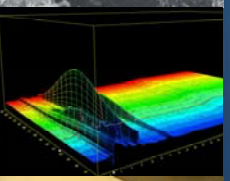

Microalgae biodiversity



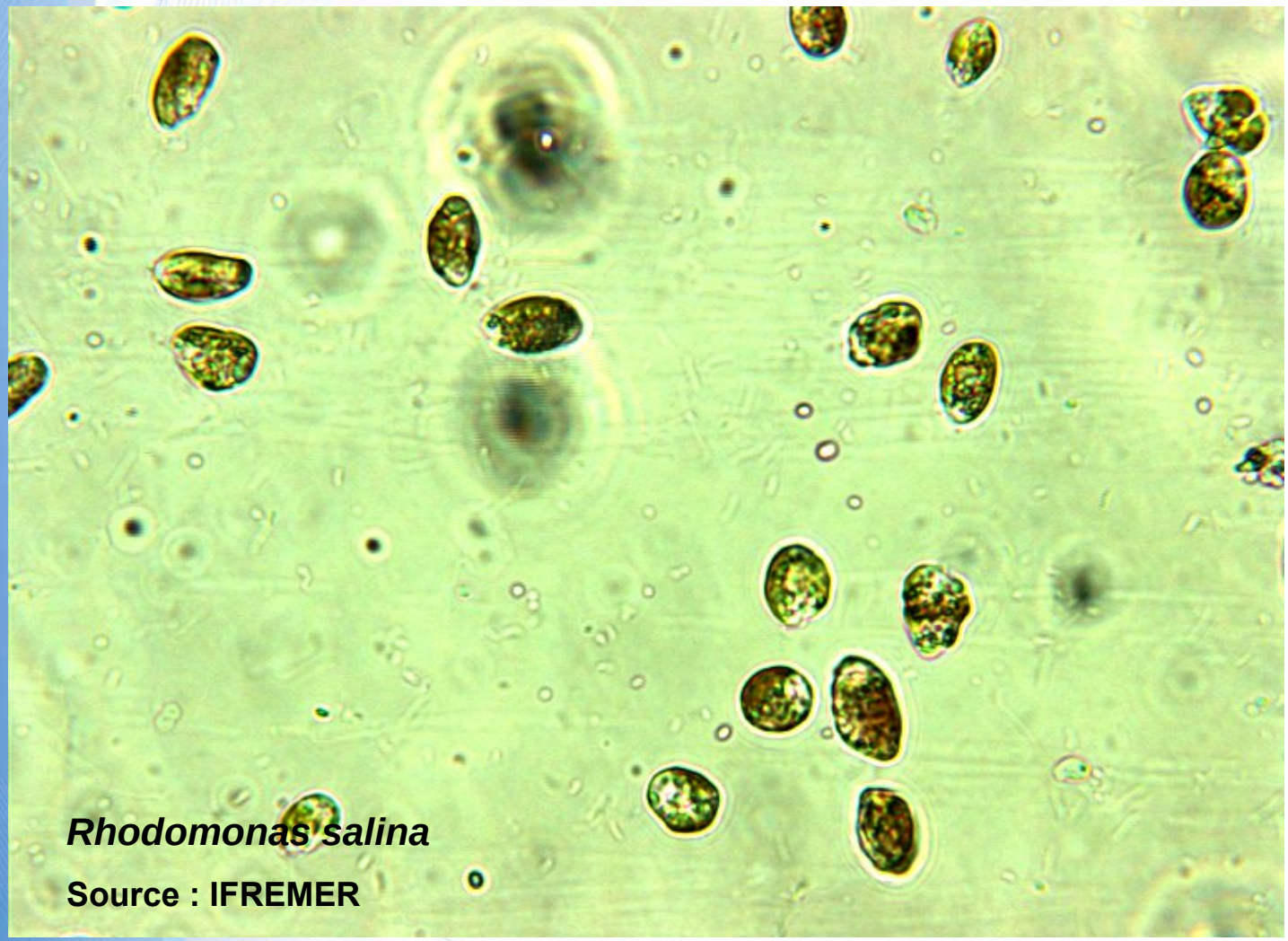
Dunaliella salina

Source : IFREMER

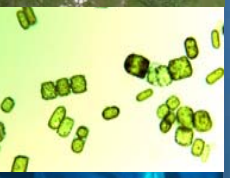



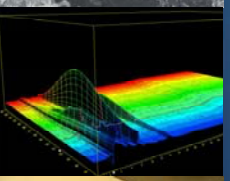

Microalgae biodiversity



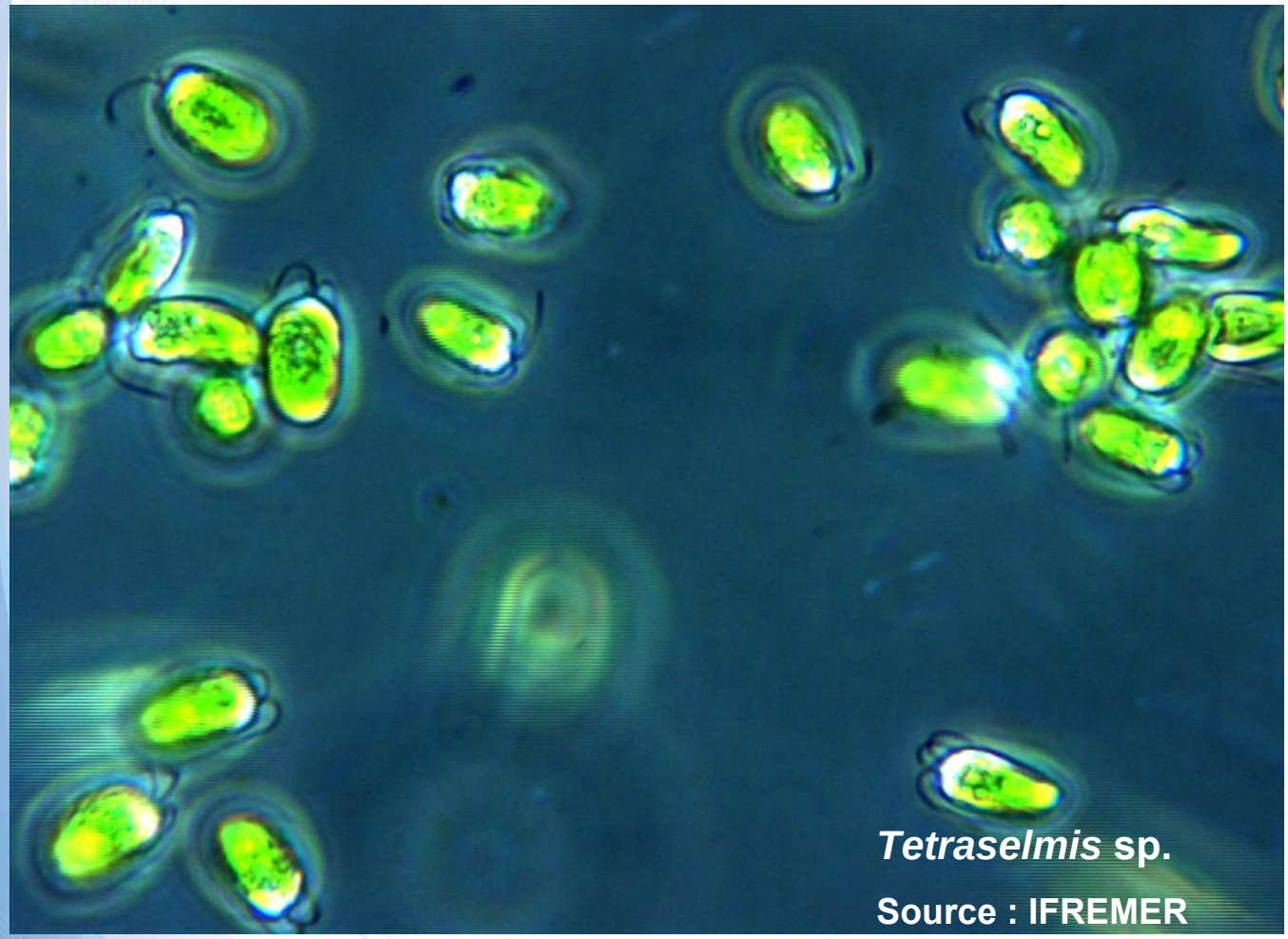
Rhodomonas salina

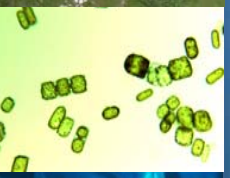
Source : IFREMER

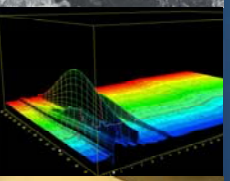



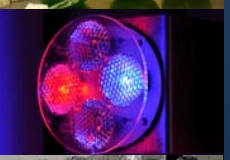




Microalgae biodiversity

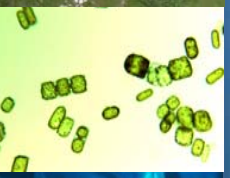


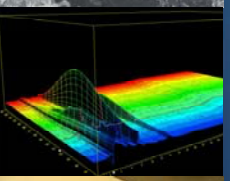



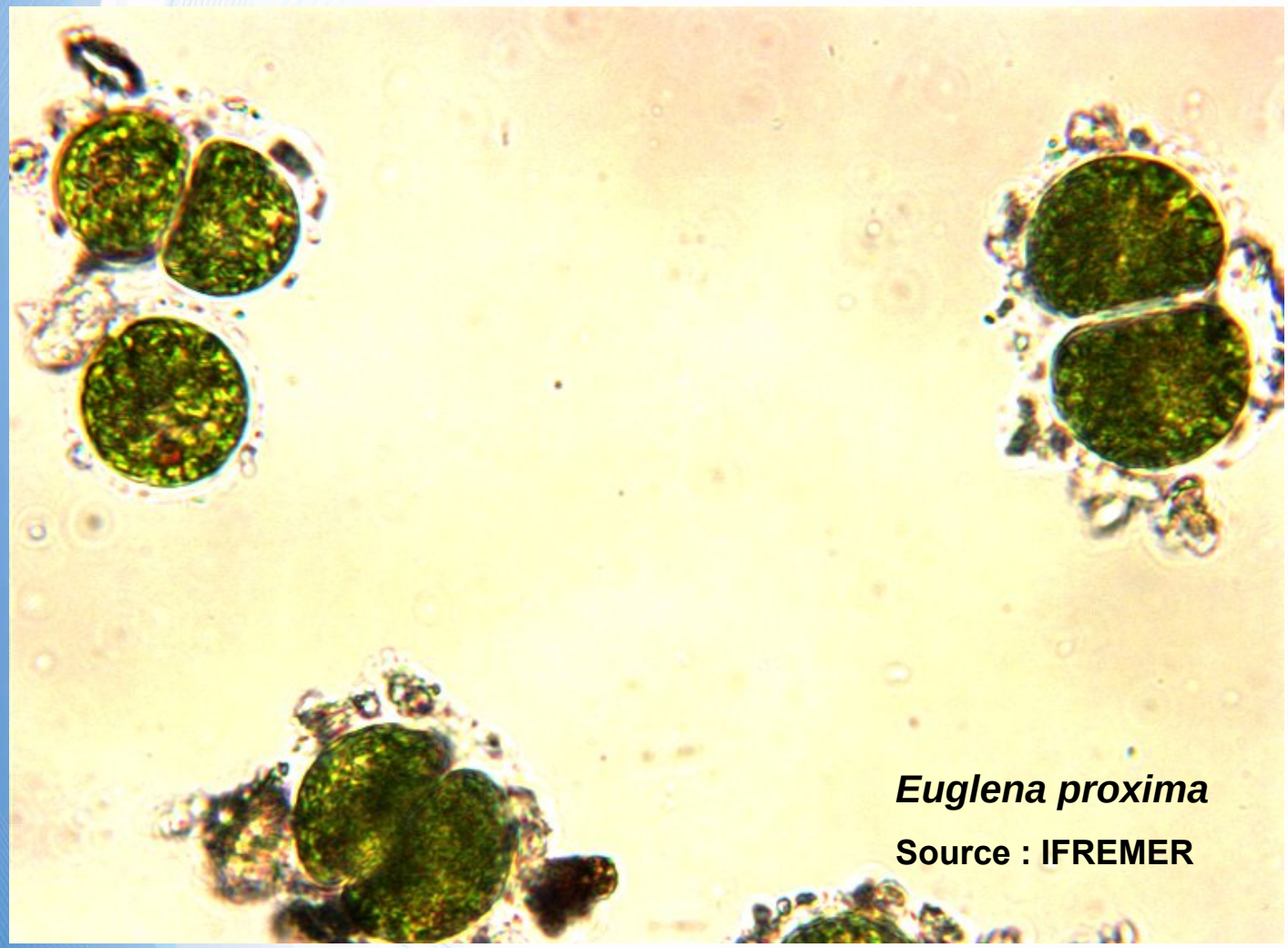



Microalgae biodiversity

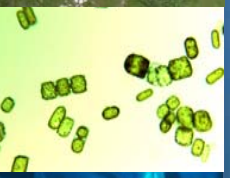



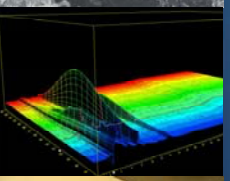

Microalgae biodiversity



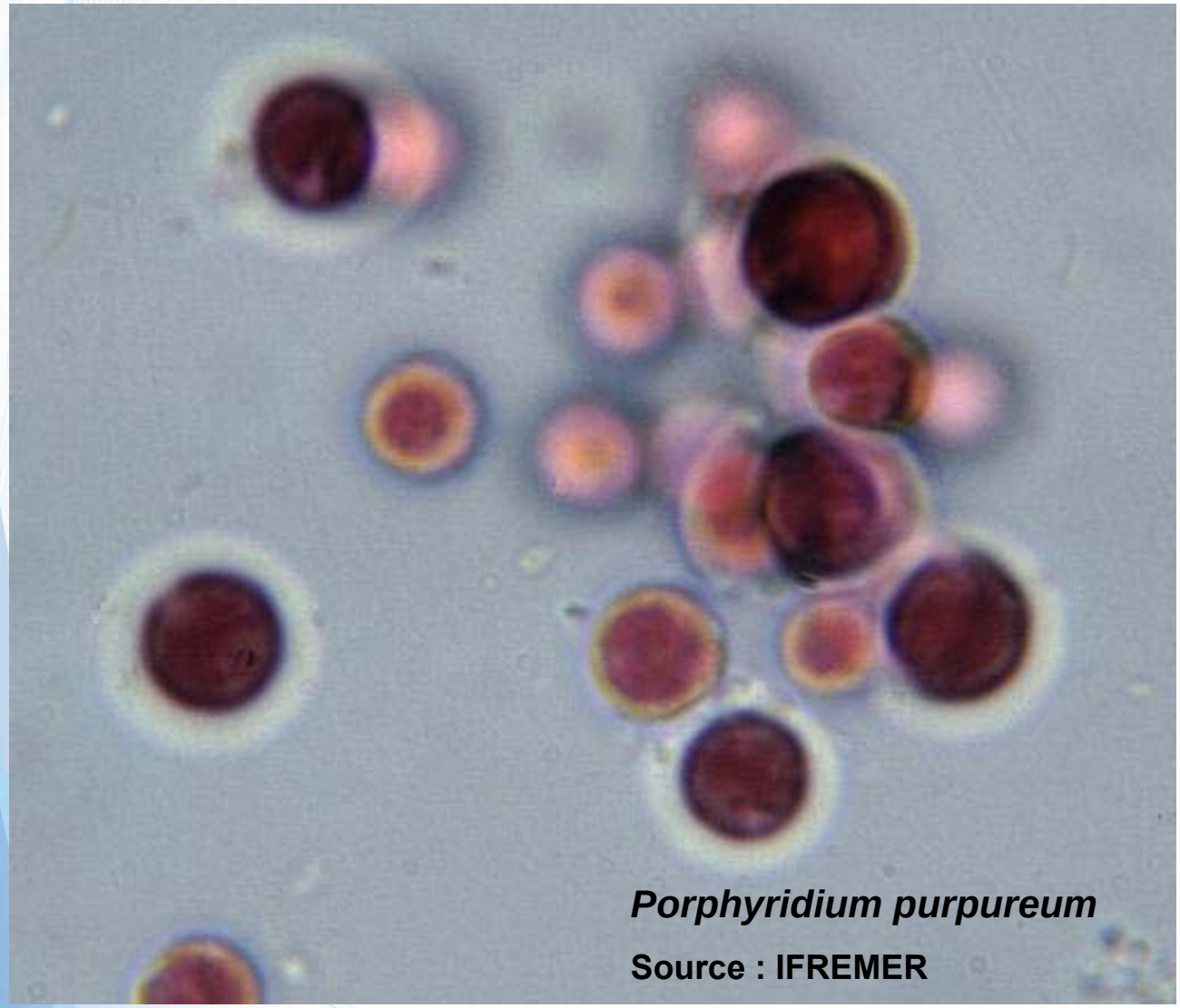
Euglena proxima

Source : IFREMER

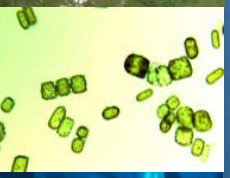



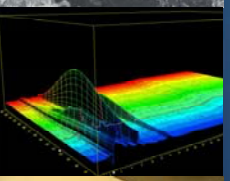

Microalgae biodiversity



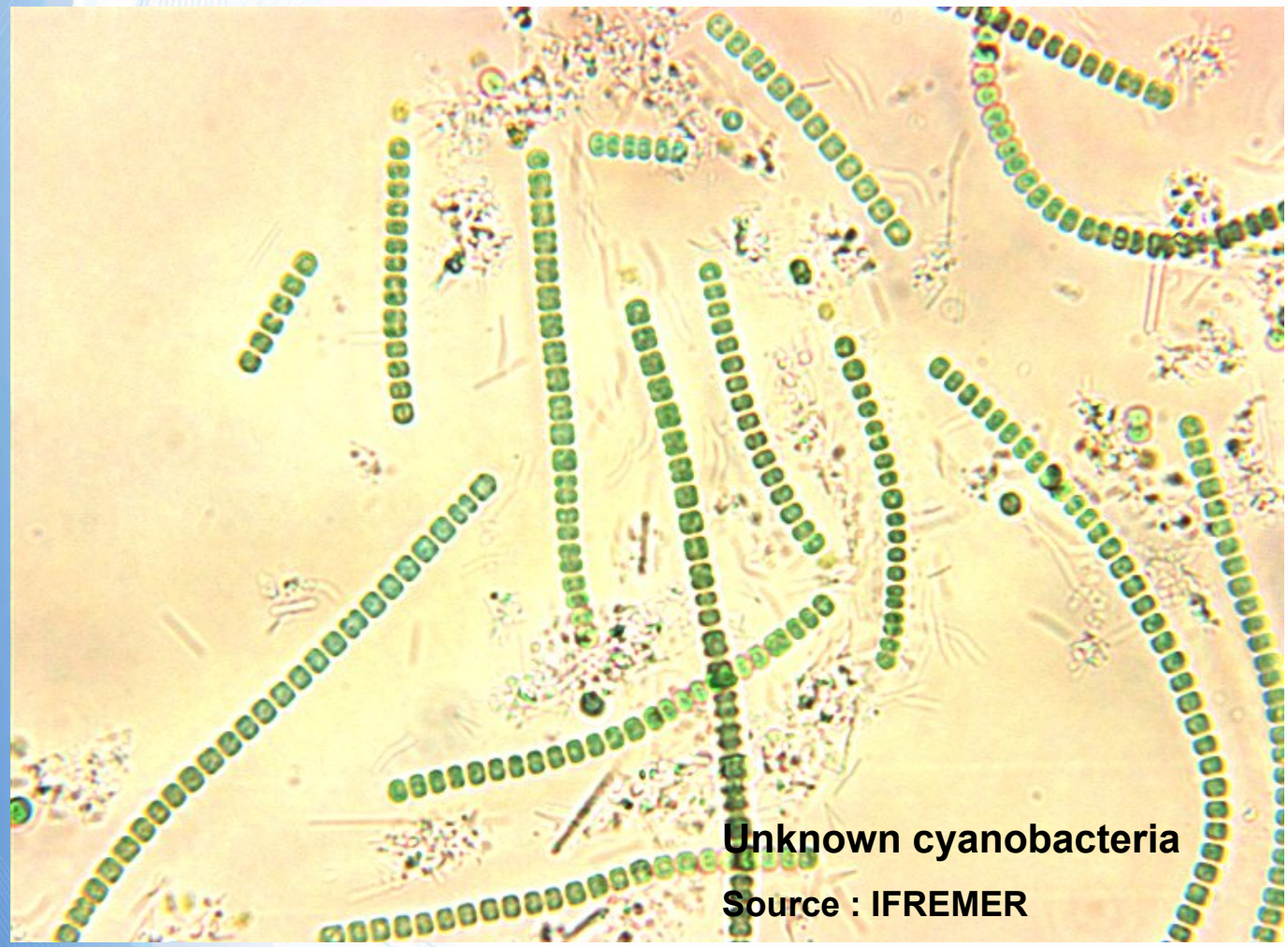
Porphyridium purpureum

Source : IFREMER

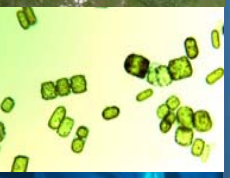



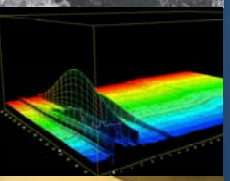

Microalgae biodiversity



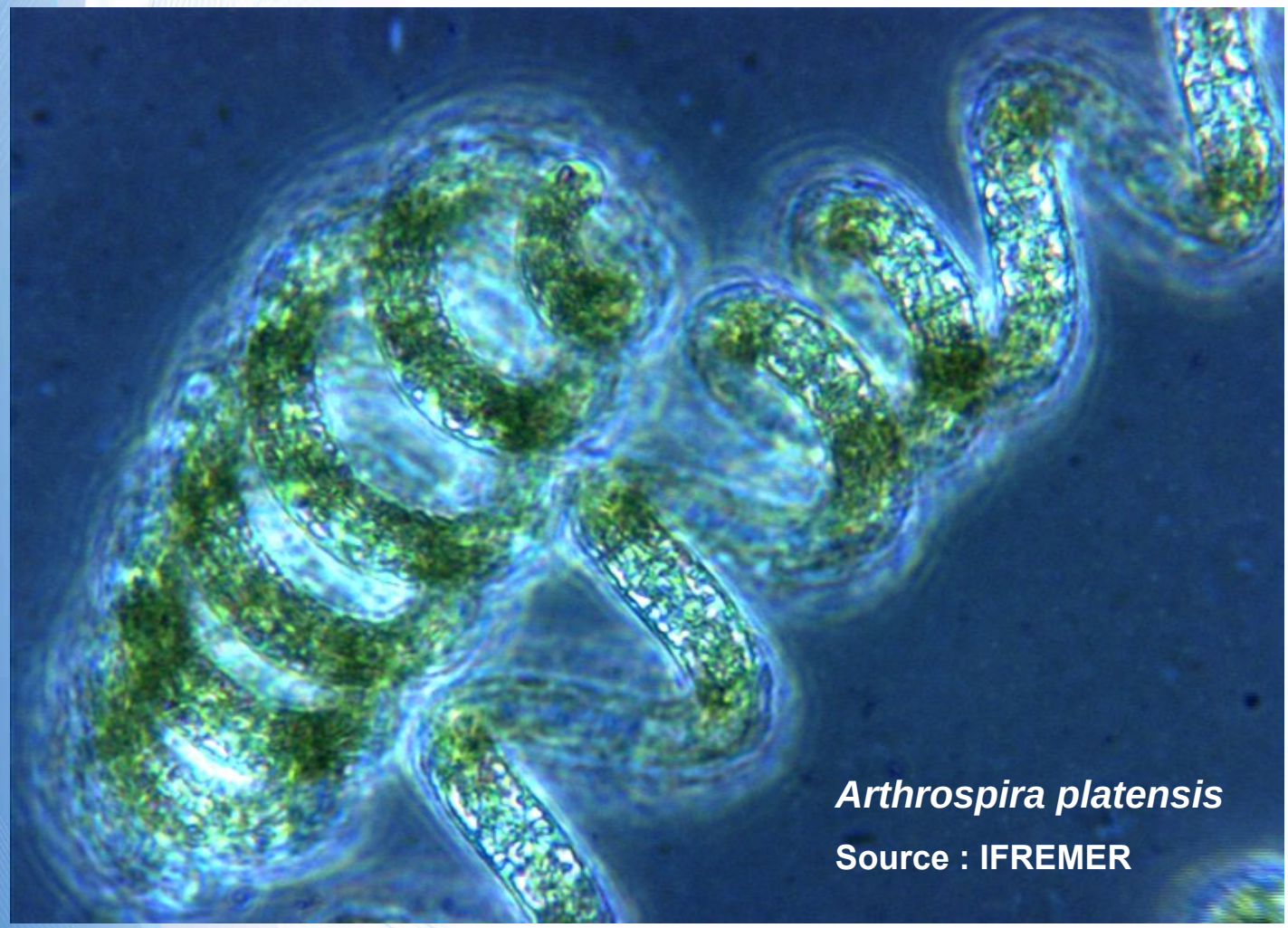
Unknown cyanobacteria

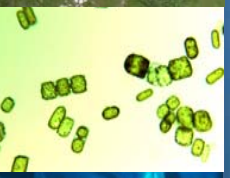
Source : IFREMER

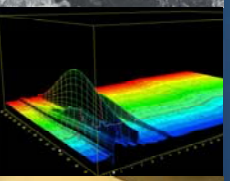



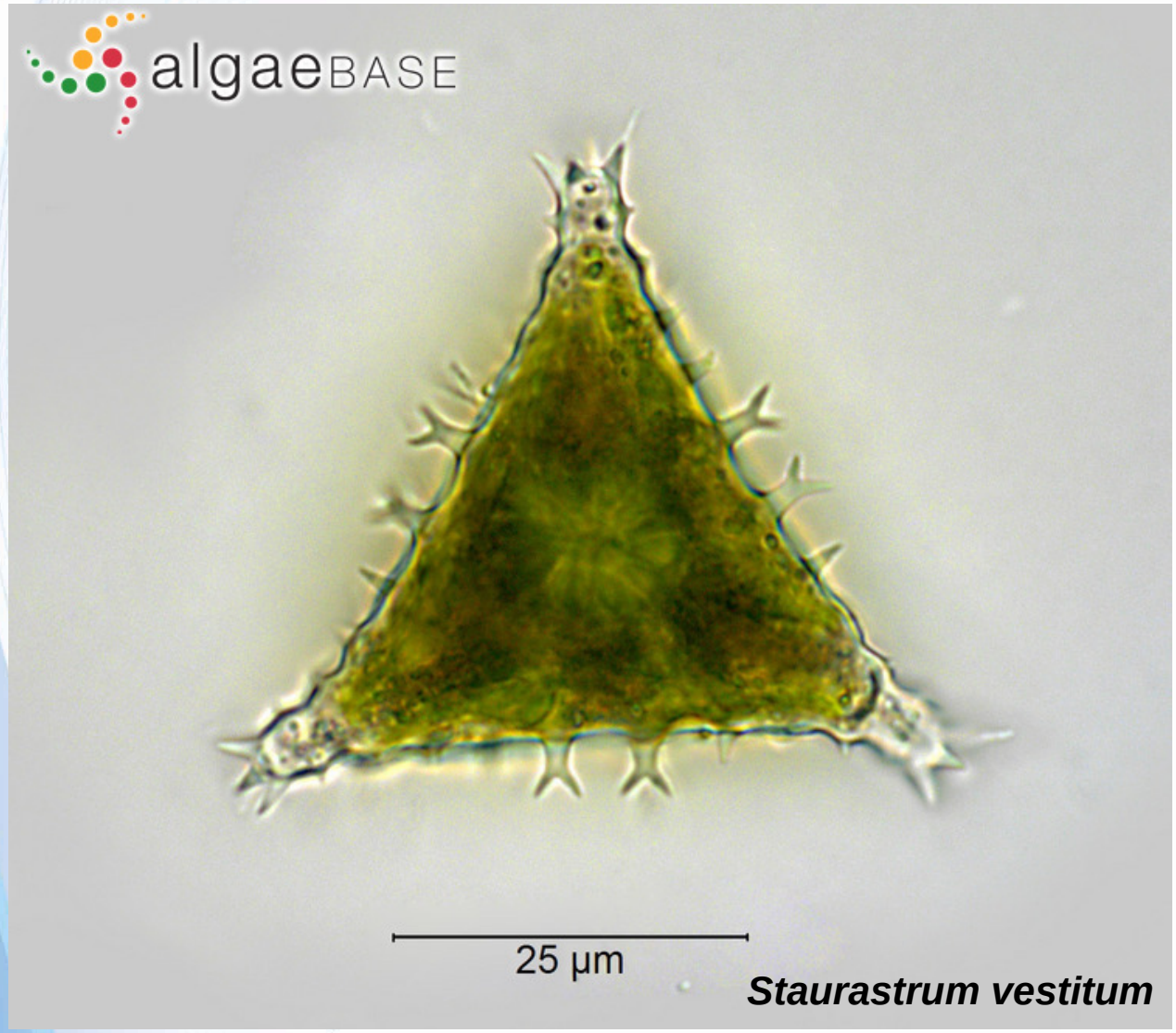

Microalgae biodiversity



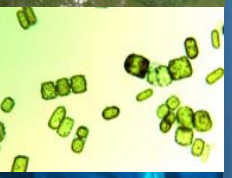

Microalgae biodiversity

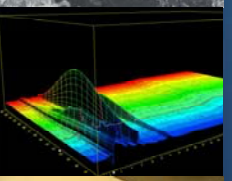


Microalgae biodiversity

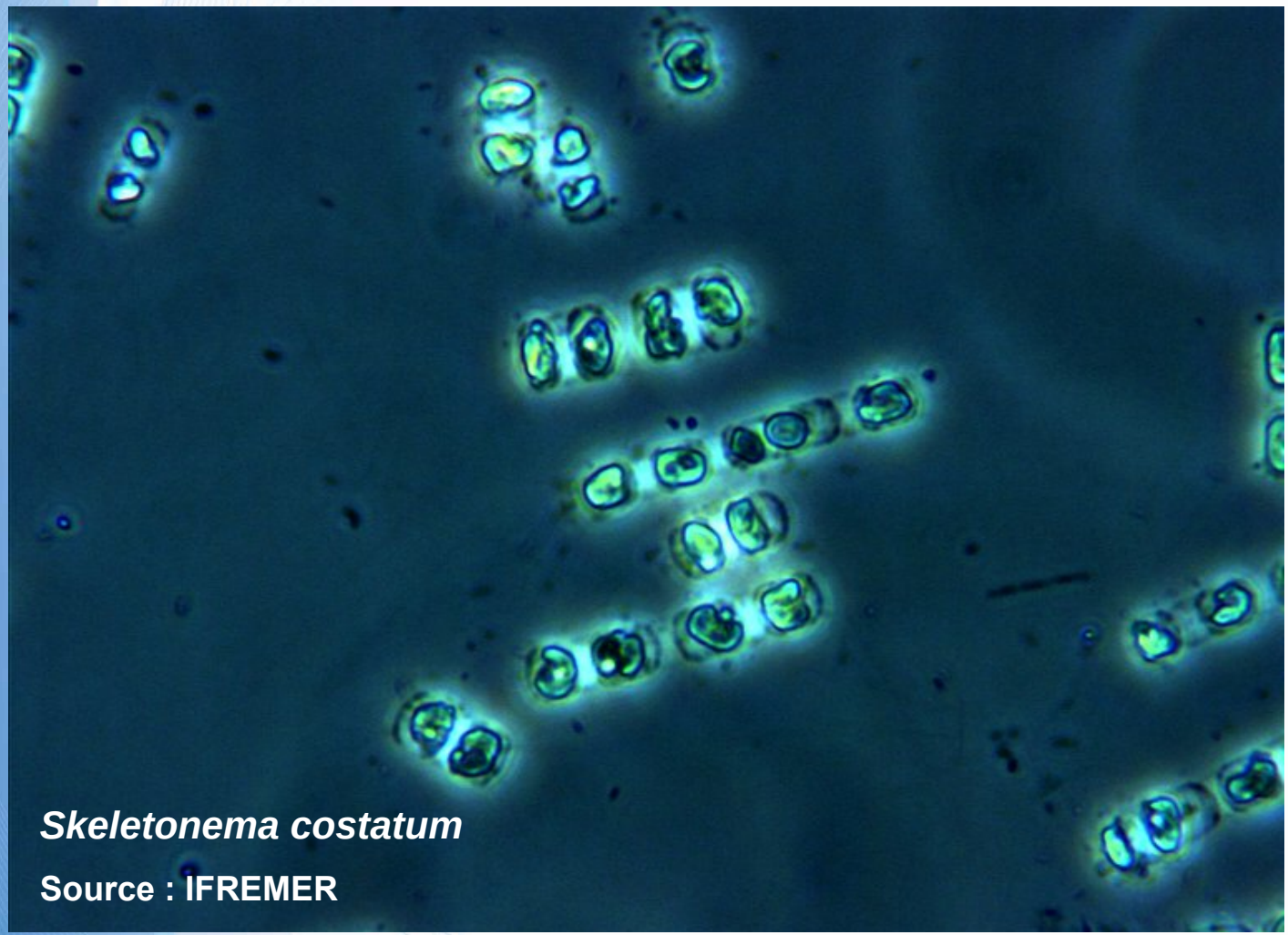
Chaetoceros muelleri

Source : gt-online.pl

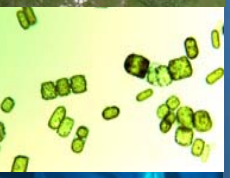



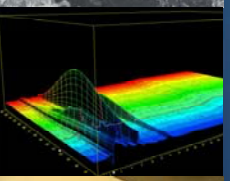

Microalgae biodiversity



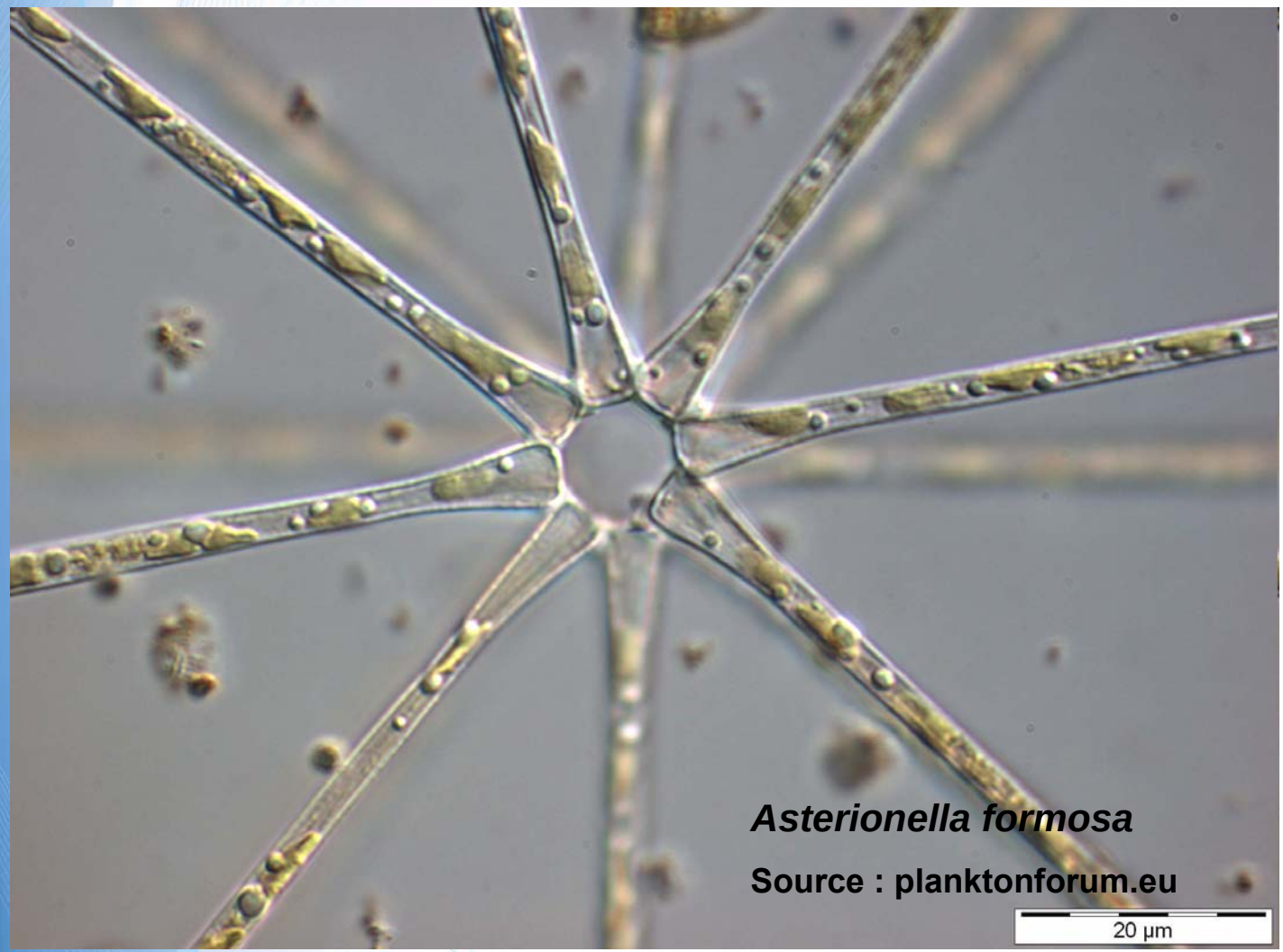
Skeletonema costatum

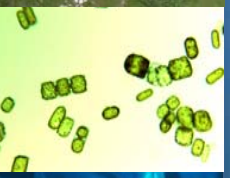
Source : IFREMER

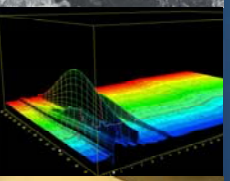



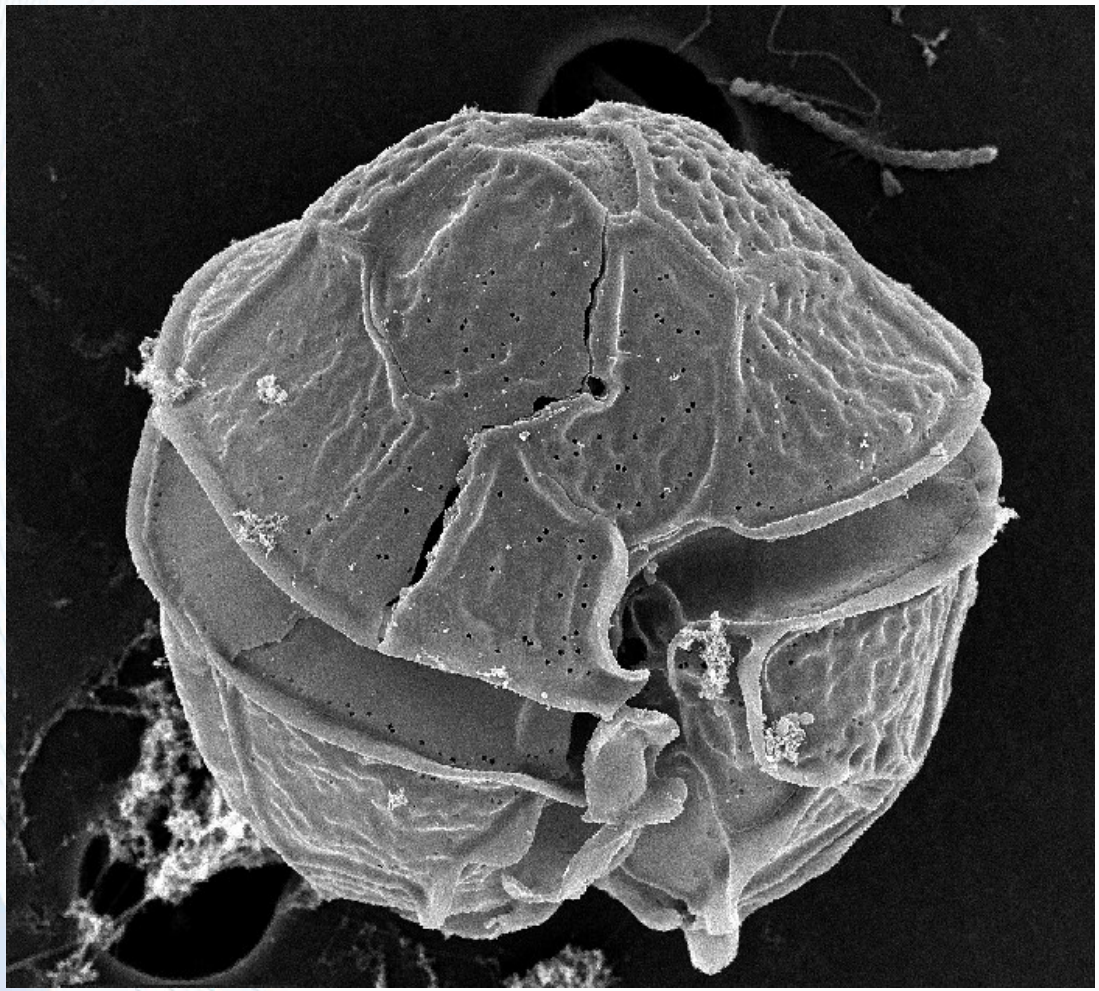

Microalgae biodiversity



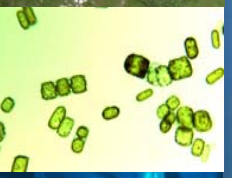



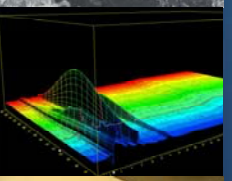

Microalgae biodiversity



Alexandrium minutum

Source : Gert Hansen

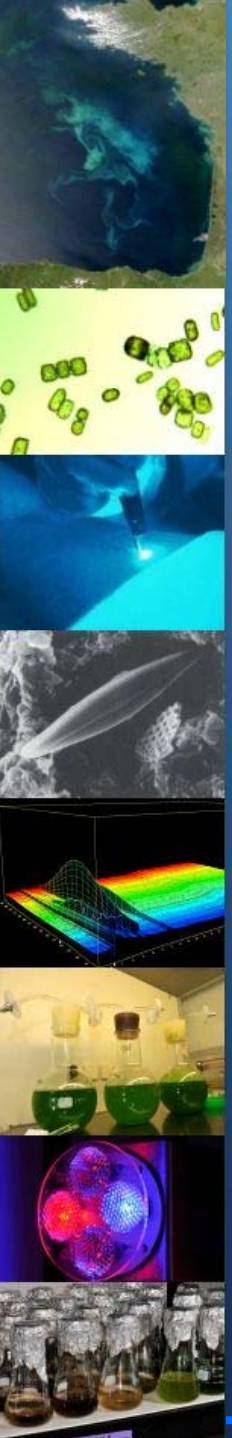



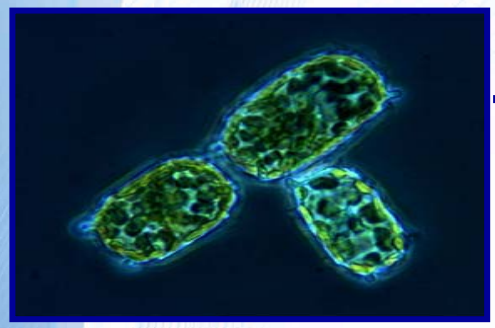




Microalgae biodiversity

Scenedesmus acutus

Bonn / nördlicher Melbweiher
29.07.2010





Biodiversity

12 phyla eucaryota 1 phylum procaryota

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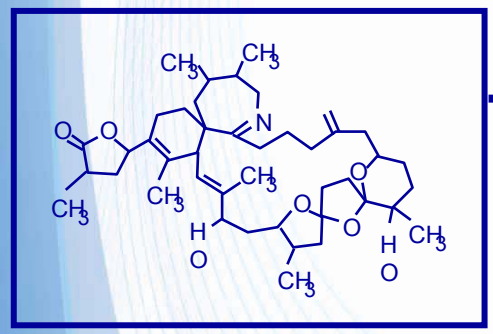

Chemodiversity

Polyketides, terpenes, alkaloides, isoprenoids, oxylipins, peptides, macrolides, microlides, lectines, glycolipids, polysaccharides, PUFAs, mycosporine-like AA, pigments

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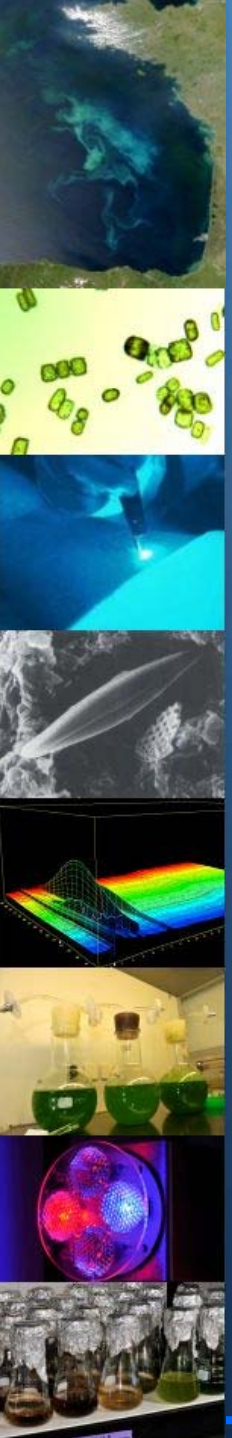
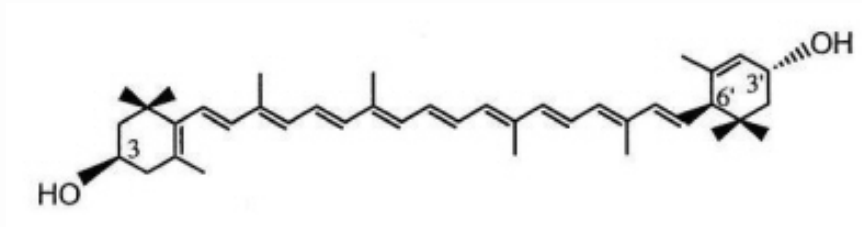
Pharmacodiversity

Potential against cancers, Parkinson, Alzheimer, neurodegenerative diseases, bacterial and viral diseases, as immunostimulant, as fluorescent probes

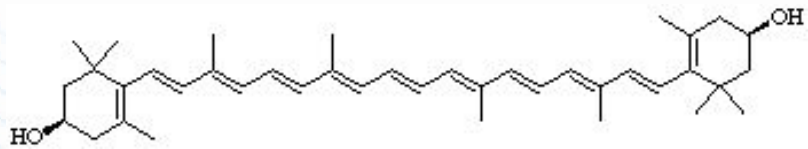
Microalgae chemodiversity and pharmacodiversity



Anti Age-related Macular Degeneration



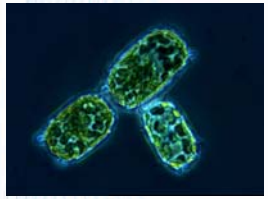
Lutein



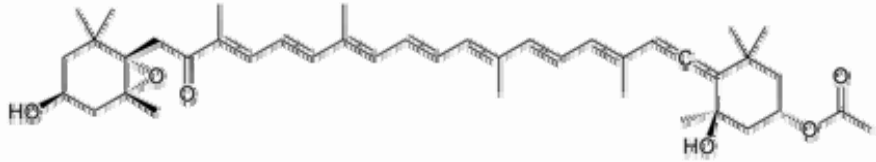
Zeaxanthin

Landrum & Bone, 2001

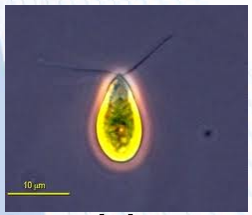
Microalgae chemodiversity and pharmacodiversity



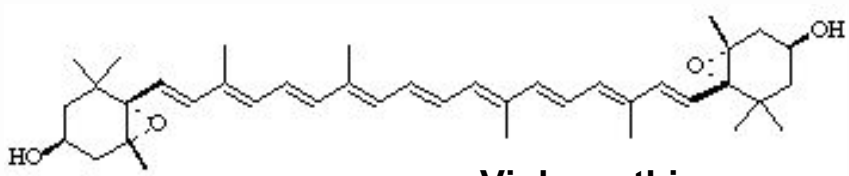
O. aurita



Moreau *et al*, 2006 **Fucoxanthine**



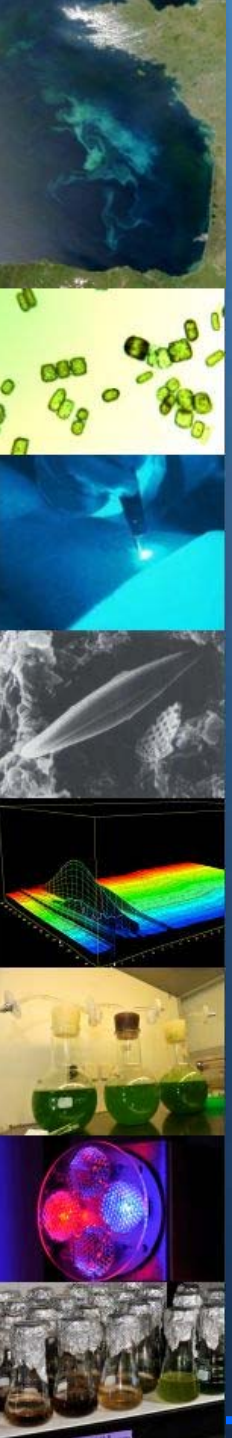
D. tertiolecta



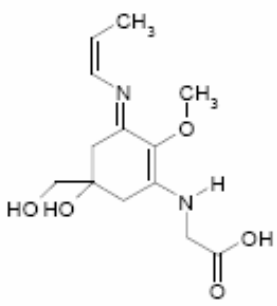
Violaxanthine

Pasquet V., Morisset P., Ihammouine S., Chepied A., Aumailley L., Berard J-B., **Serive B.**, Kaas R., Lanneluc I., Thiery V., Lafferriere M., Piot J-M., Patrice T., Cadoret J-P., Picot L., 2011.

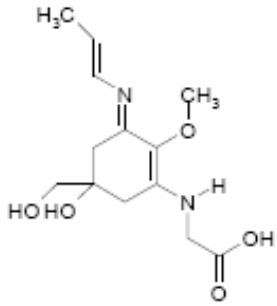
Antiproliferative activity of Violaxanthin isolated from bioguided fractionation of *Dunaliella tertiolecta* extracts. *Marine Drugs*, 9, 819-831.



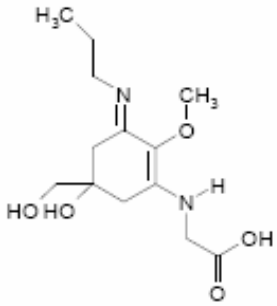
Microalgae chemodiversity and pharmacodiversity



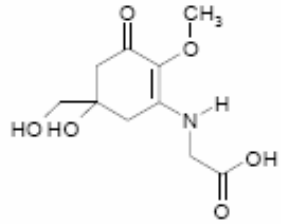
Usijirene



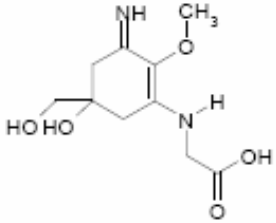
Palythene



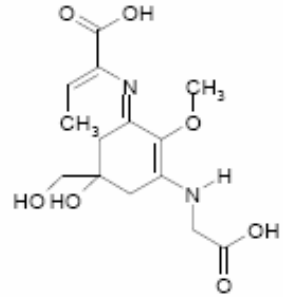
Asterina-330



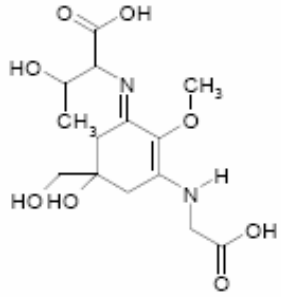
Mycosporine-glycine



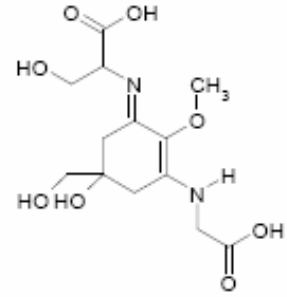
Palythine



Palythenic acid



Porphyra-334



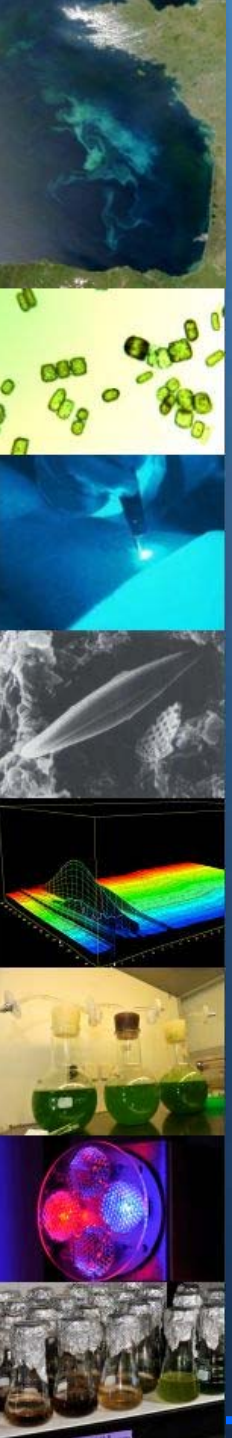
Shinorine

Photoprotectant

Antiproliferative activity

Antioxidant

Mimouni et al, 2012. The Potential of Microalgae for the Production of Bioactive Molecules of Pharmaceutical Interest. *Current Pharmaceutical Biotechnology* 13(15): 2733-2750.



Aims

- The challenge is therefore to identify molecules issued from this biodiversity which are often poorly known in terms of chemodiversity.
- For high-throughput screening of such metabolites, it is essential to reach the inner content of the cell.
- A part of our project was the research and development of a technique enabling a high extraction yield of any metabolite, taking into account the difficulty of extracting bound and or inaccessible molecules with a wide variety of polarities.
- The final aim is to compare without extraction bias the potential of microalgae.

Choice of recalcitrant biological models

For the study of the chemodiversity extraction

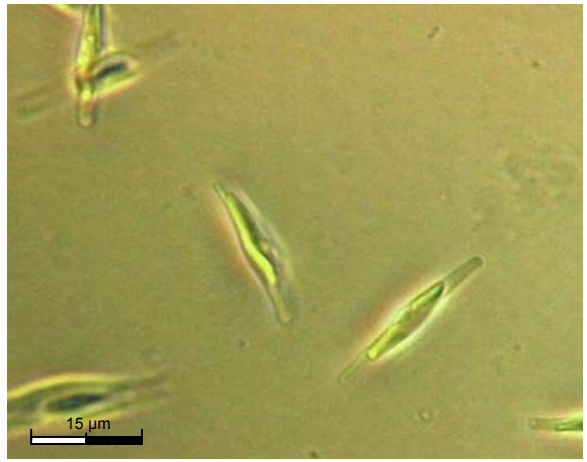
Porphyridium purpureum

Length : 5-8 µm

Phylum : Rhodophyta

Features :

- Exopolysaccharides
- Thick wall



Phaeodactylum tricornutum

Length : 3-4 x 15-20 µm

Phylum : Ochrophyta

Features :

- Silica wall

Chemodiversity extraction

Comparison of 9 disruption techniques

Main studied parameters:

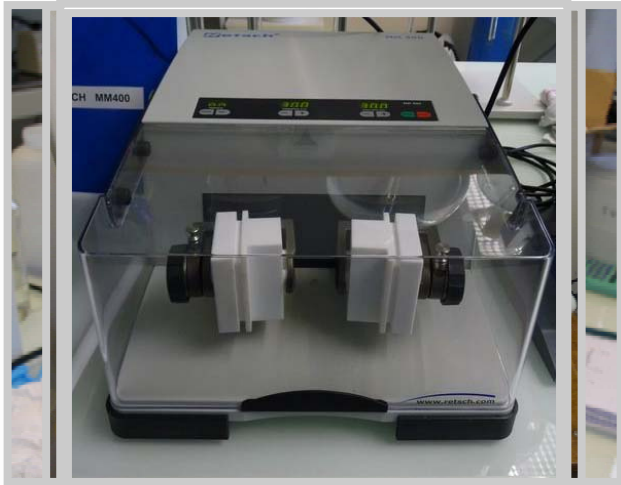
Effectiveness (1-(recalcitrant cells/total))

Reproducibility

Operating cost

Temperature stability

Easy to handling

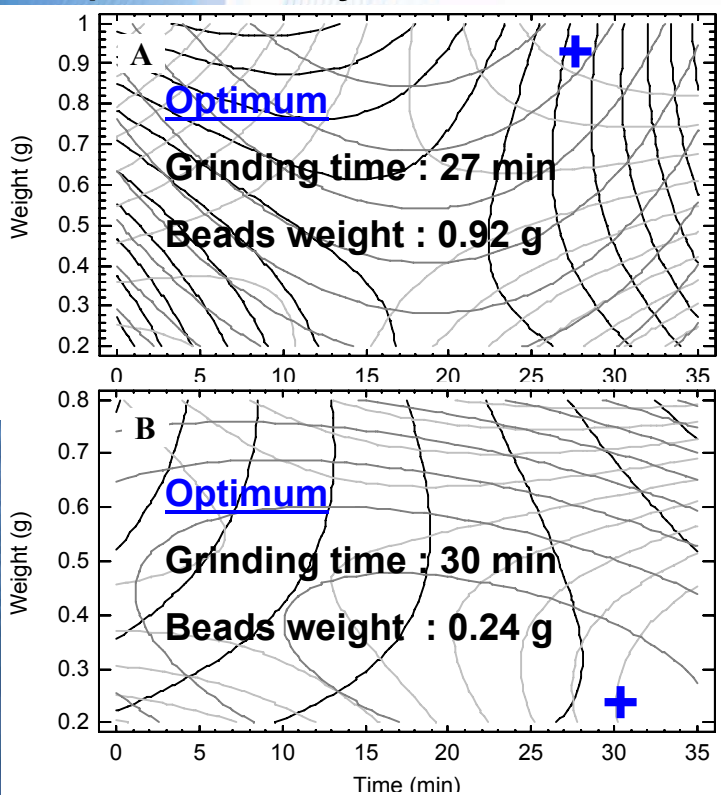


Method	Effectiveness	Reproductibility	Other considerations
Soaking	-	-	Long processing
Cryogrinding	-	-	Liquid nitrogen handling require
Potter homogeniser	-	-	n.r.
Homogeniser	+	-	Blade blunted by diatoms
Bead-beater	+	-	Particles release
Planetary micro mill	+++	+++	Particles release
Sonication	++	+	ROS release, hot spots
Mixer mill – stainless steel jars	+	+	n.r.
Mixer mill – plastic grinding tube	+++	+++	n.r.

Grinding optimisation on Retsch MM-400

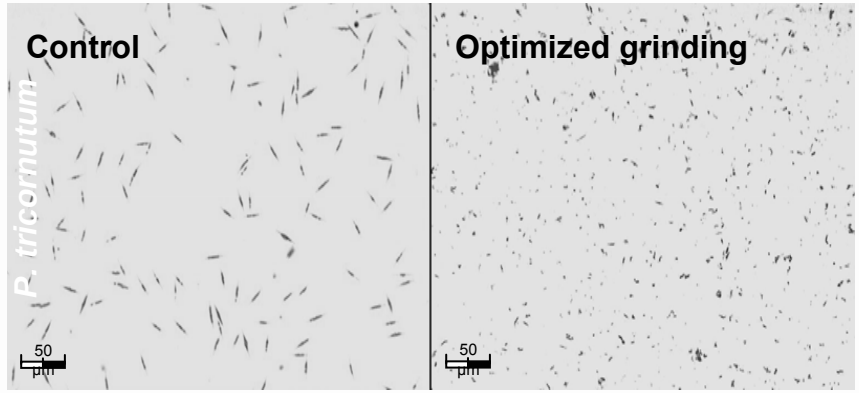
Image analysis to evaluate acurately the particle size distribution

Isoresponses overlay

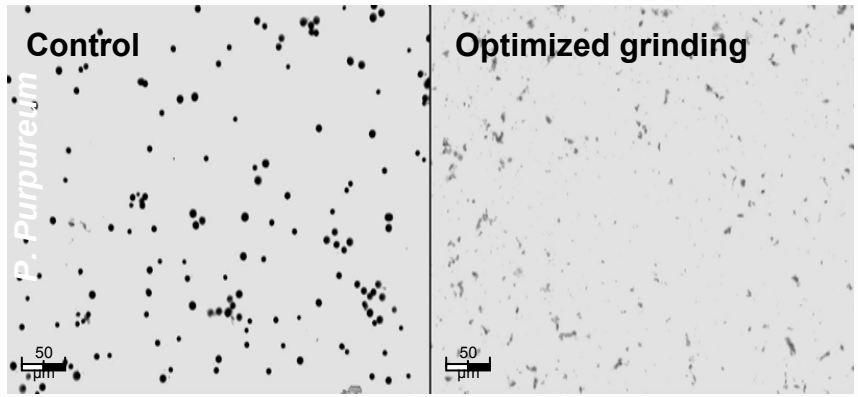


Size classes:
(·····) Fragments; (—) Whole cells ; (—) Agregates.

P. tricornutum

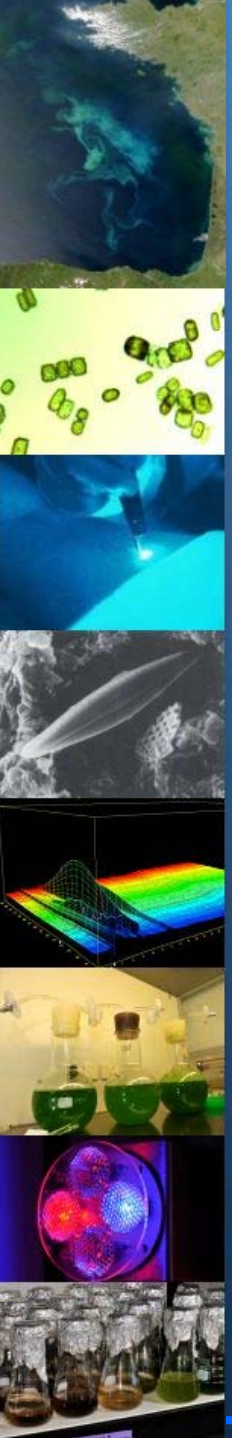


P. purpureum



Serive et al, 2012. *Bioresource Technology* 124

Optimisation of 3 studied variables by image analysis: creation of an index of desirability.



Total extract, chemodiversity and dereplication

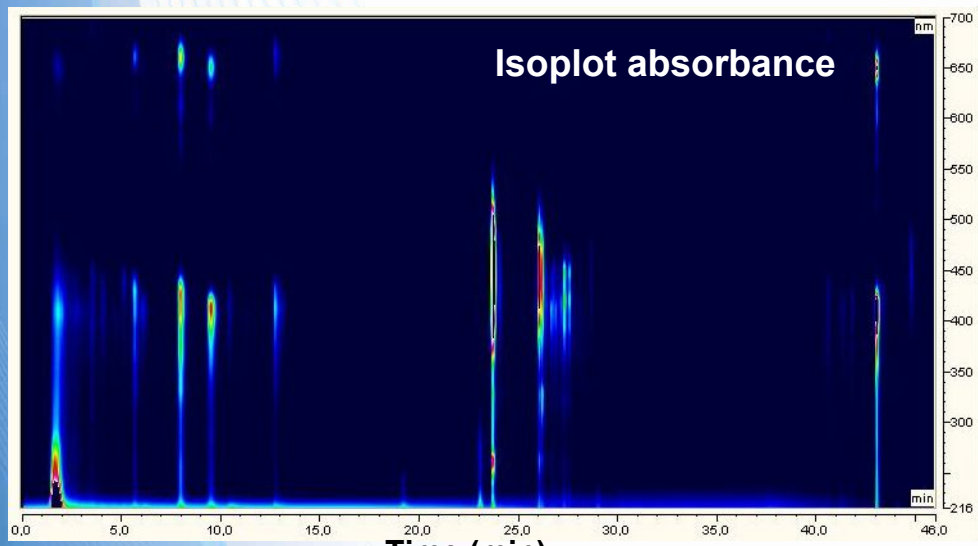
→ In a total extract, we have different kind of molecules:

Known by the scientific community and known by us

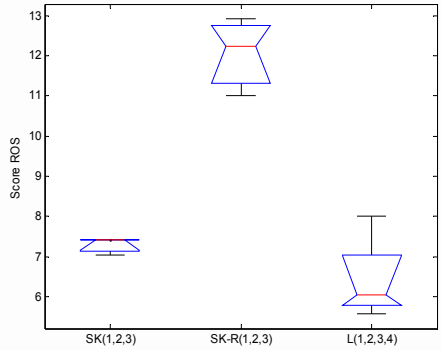
Known somewhere in the world but unknown by our database

Unknown by everyone

→ Isoplot absorbance → *metabolic snapshot* because of the microalgae cellular plasticity

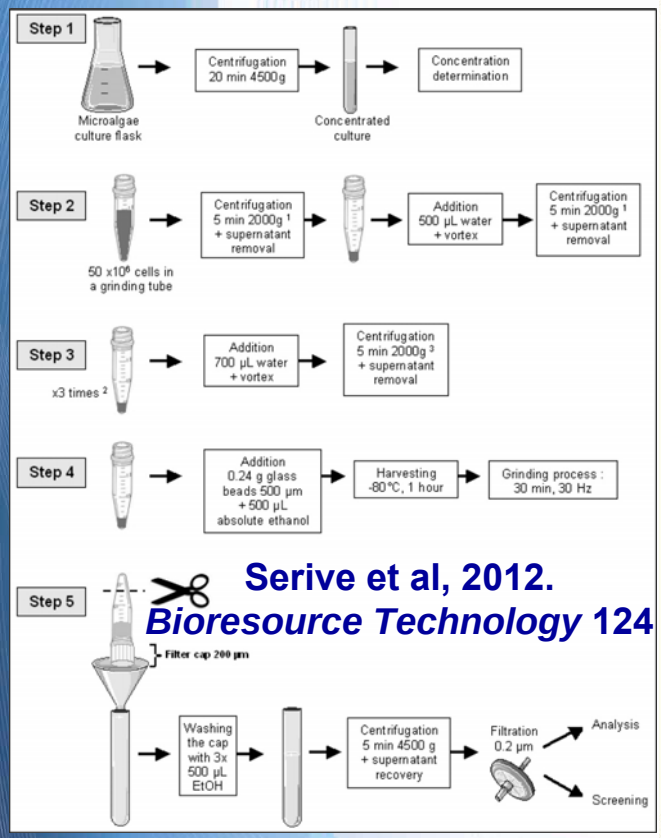


Bioactivity for a same strain and different environmental conditions

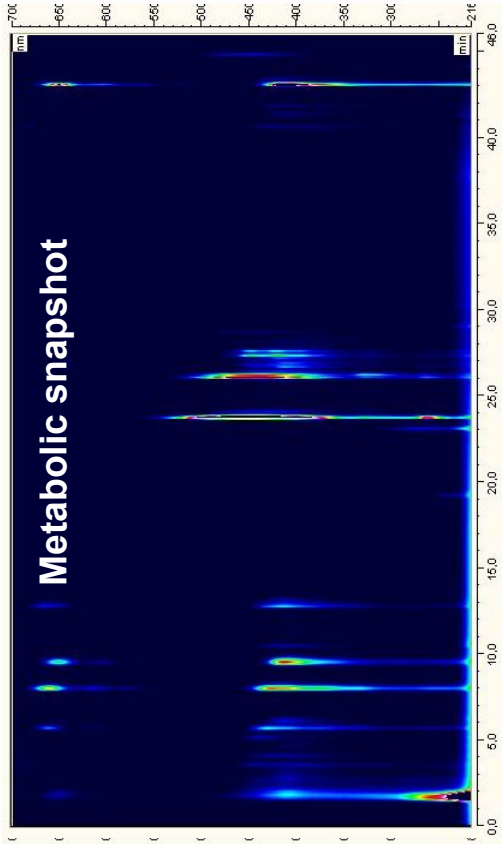


Methodology in the work flow

Efficient metabolite extraction → **Total extract** → **Microalgae chemodiversity** → **OMICs studies**



Serive et al, 2012.
***Bioresource Technology* 124**

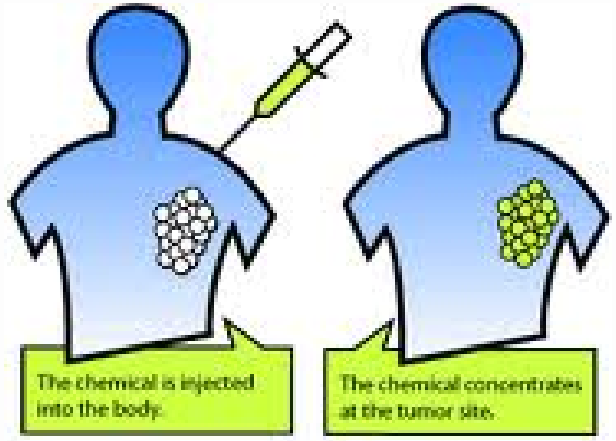


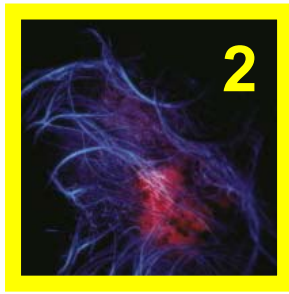
- **Genomic**
- **Transcriptomic**
- **Proteomic**
- **Metabolomic**
- **Pigmentomic**

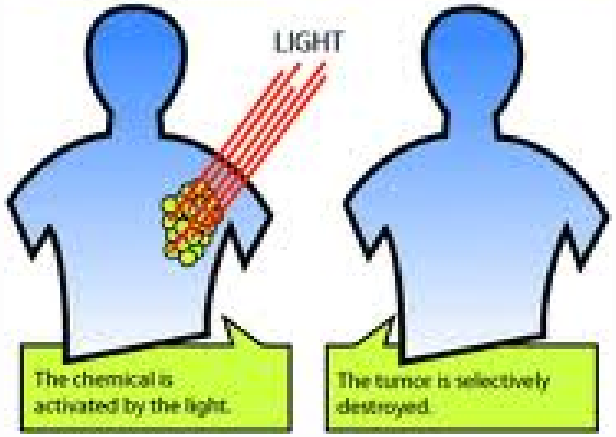
Photomer project

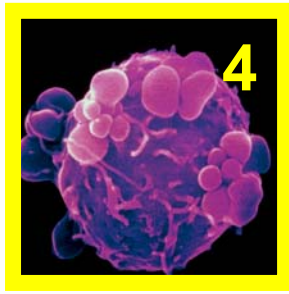
PHOTOMER

Identify new photosensitizers from microalgae for PhotoDynamic Therapy









Pigment analysis and spectral screening

45 microalgae strains composition

→ Spectral screening of interest in pharmacognosy.

→ High interest for oceanography applications.

Laboratoire de Physiologie et Biotechnologie des Algues
IFREMER / RBE / BRM / LABORATOIRE PBA

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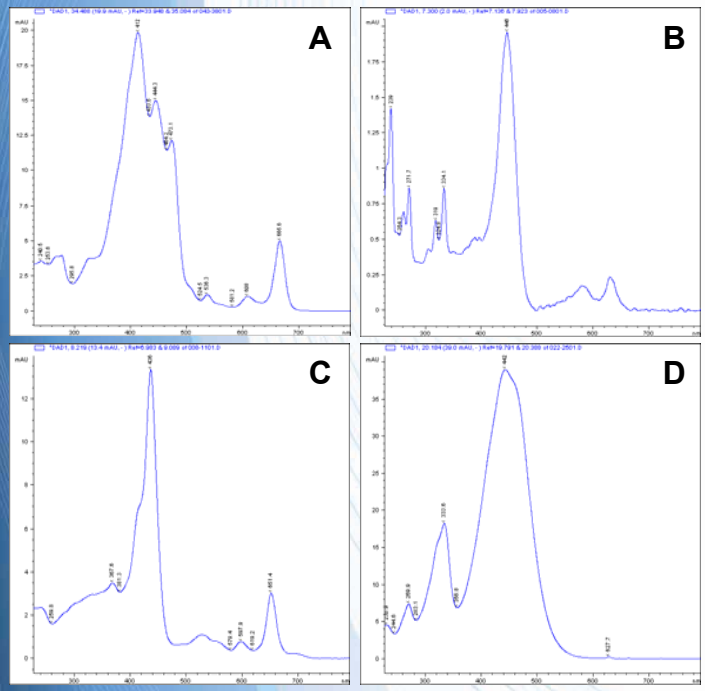
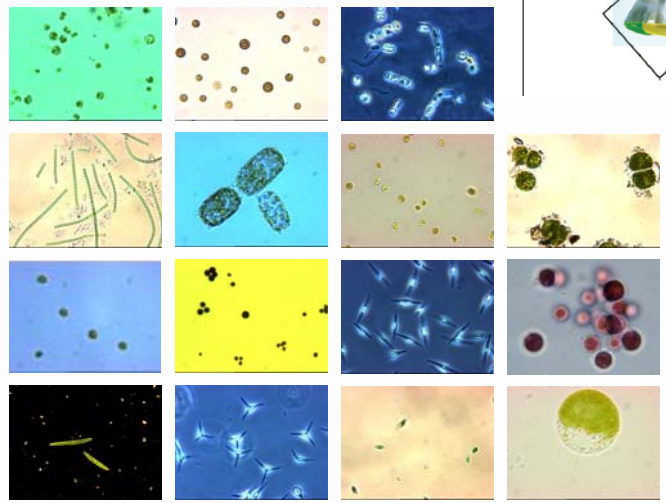
Benoît SERIVE
Mars 2012

Composition pigmentaire de 45 souches de micro-organismes photosynthétiques

PBA





Examples of original spectra from the following strains:

A- ***Alexandrium minutum*** **B-** ***Alexandrium tamarense***

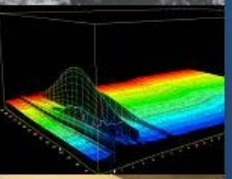
C- ***Chlorarachnion reptans*** **D-** ***Phaeodactylum tricornutum***

OCEAN CHemodiversity Against Cell cycle Targets

- Research of new marine inhibitors targeted against desease-relevant proteins kinases

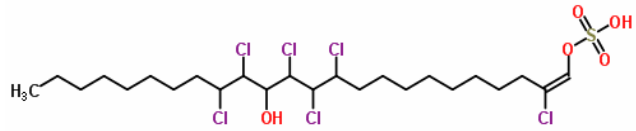


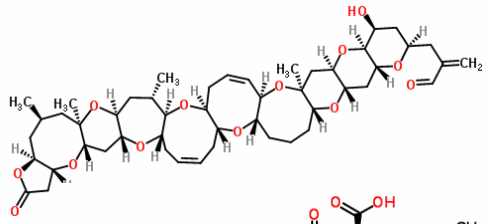


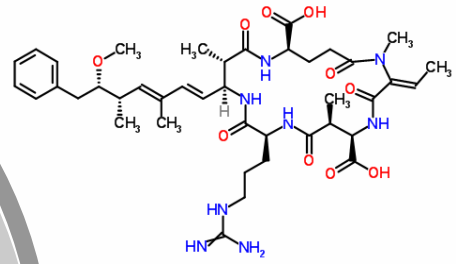


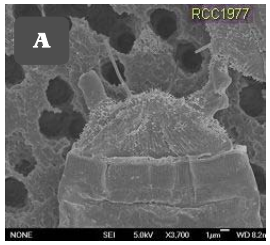


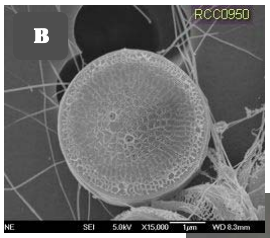
J-B Charcot











SEVENTH FRAMEWORK PROGRAMME

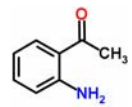
EUROPEAN COMMISSION

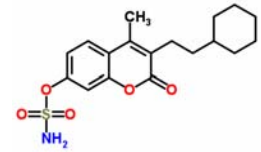
cnrs

CNRS UPMC Station Biologique Roscoff

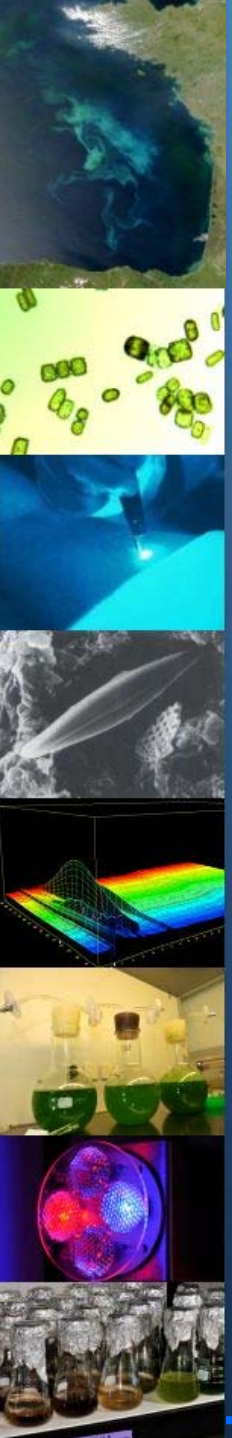
Griffith UNIVERSITY Eskitis Institute

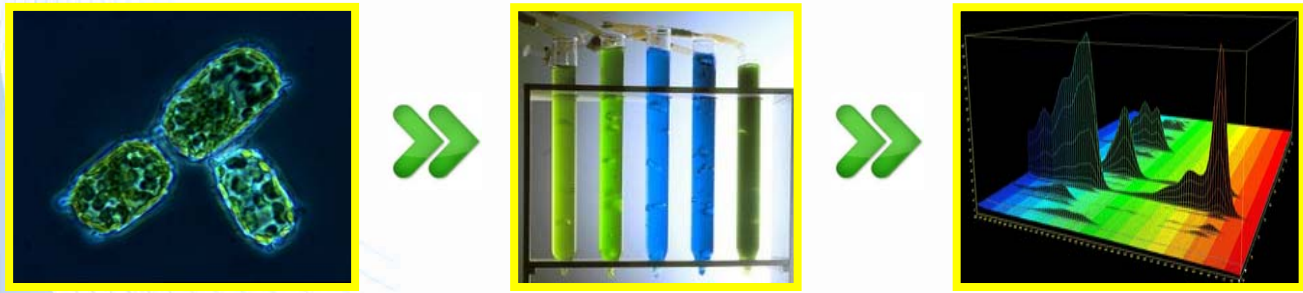
Roscoff RCC Culture Collection





Bruker SolariX 12.0 Tesla FTMS





Thank you for your attention

Contact: Benoit.Serive@live.fr