

Quantitative assessment of picoeucaryotes using taxon specific oligonucleotide probes in association with TSA-FISH and flow cytometry

Isabelle C. Biegala,

F. Not, K. Romari, D. Marie, D. Vaultot, N. Simon

Station Biologique of Roscoff : PICODIV & PICOManche

TSA system compared to monolabeled system

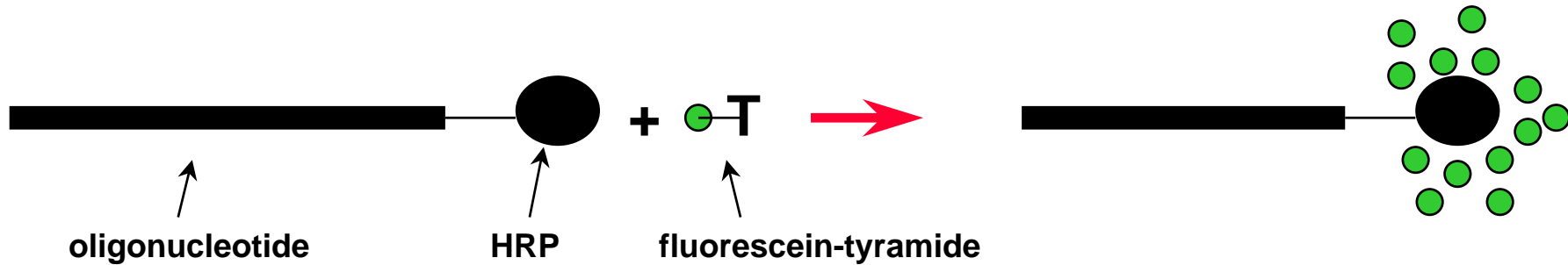
1- Fixation, dehydration, permeabilization

2- FISH:

- Monolabeled probes



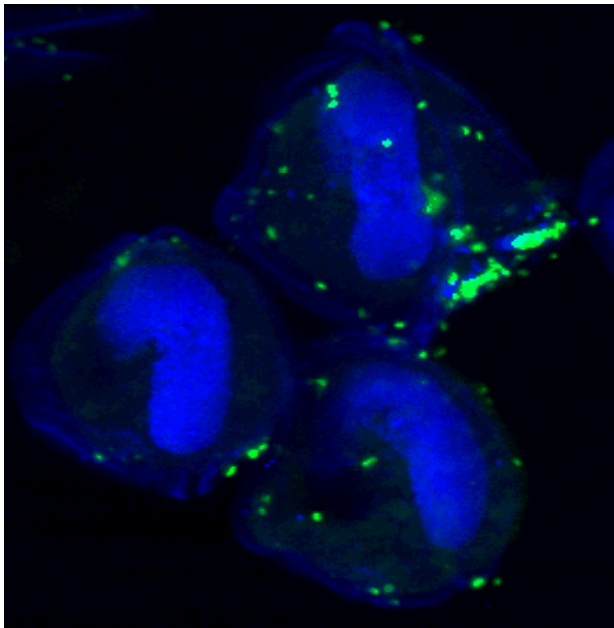
- Tyramide Signal amplification



Application of TSA-FISH

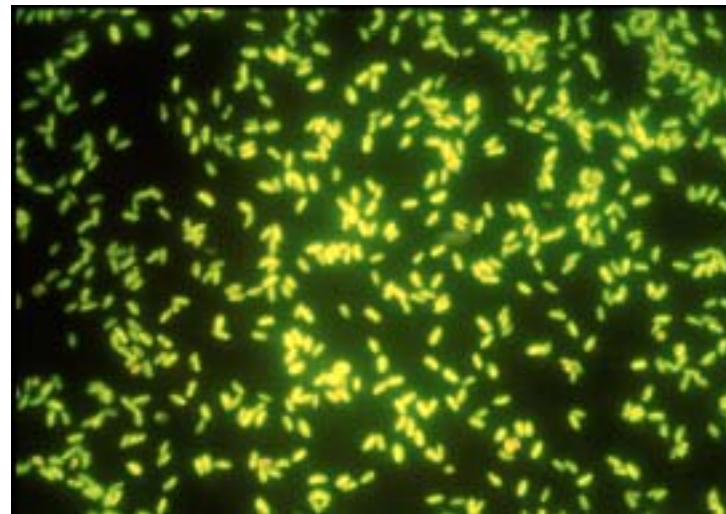
Confocal microscopy

Biegala et al. 2002



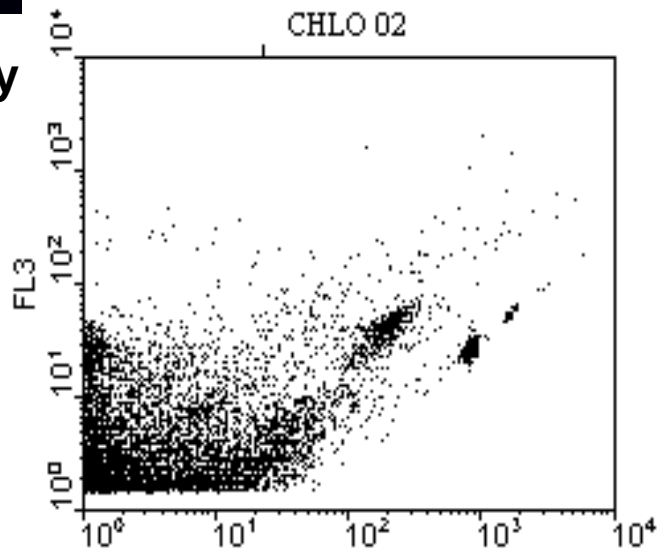
Epifluorescent microscopy

Not et al. 2002



Flow cytometry

This study



TSA-FISH and Flow cytometry

Prokaryotes

Lebaron et al. 1997
heterotrophic bacteria
Failed with FCM

Schönuber et al. 1997
heterotrophic bacteria
+/- = 20 - 40

Eukaryotes

None with TSA-FISH

Simon et al. 1995 : monolabeled probe
Nonaeukaryotes: Log & stationary phase
+/- = 3.5 - 80
Picoplankton: log
+ ≠ -

Rice et al. 1997 : monolabeled probe
Nanoplankton: log & stationary
+/- = 2 - 3

Problems

- Signal / background ratio too low
- Cell loss
- Cell clump



Quantification on natural samples not possible

Protocol for TSA-FISH & FCM

1- Cell collection, fixation and dehydration

- Collection by centrifugation (surfactant)
- Fixation PFA
- Dehydration ETOH

2- FISH

-Sonication

-Hybridization

-Wash

-Equilibration

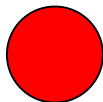
-Sonication

-TSA (FITC)

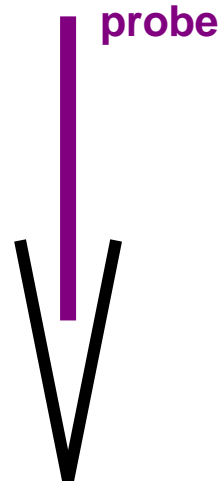
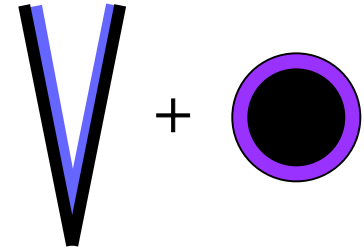
-Wash

-Sonication

-PI



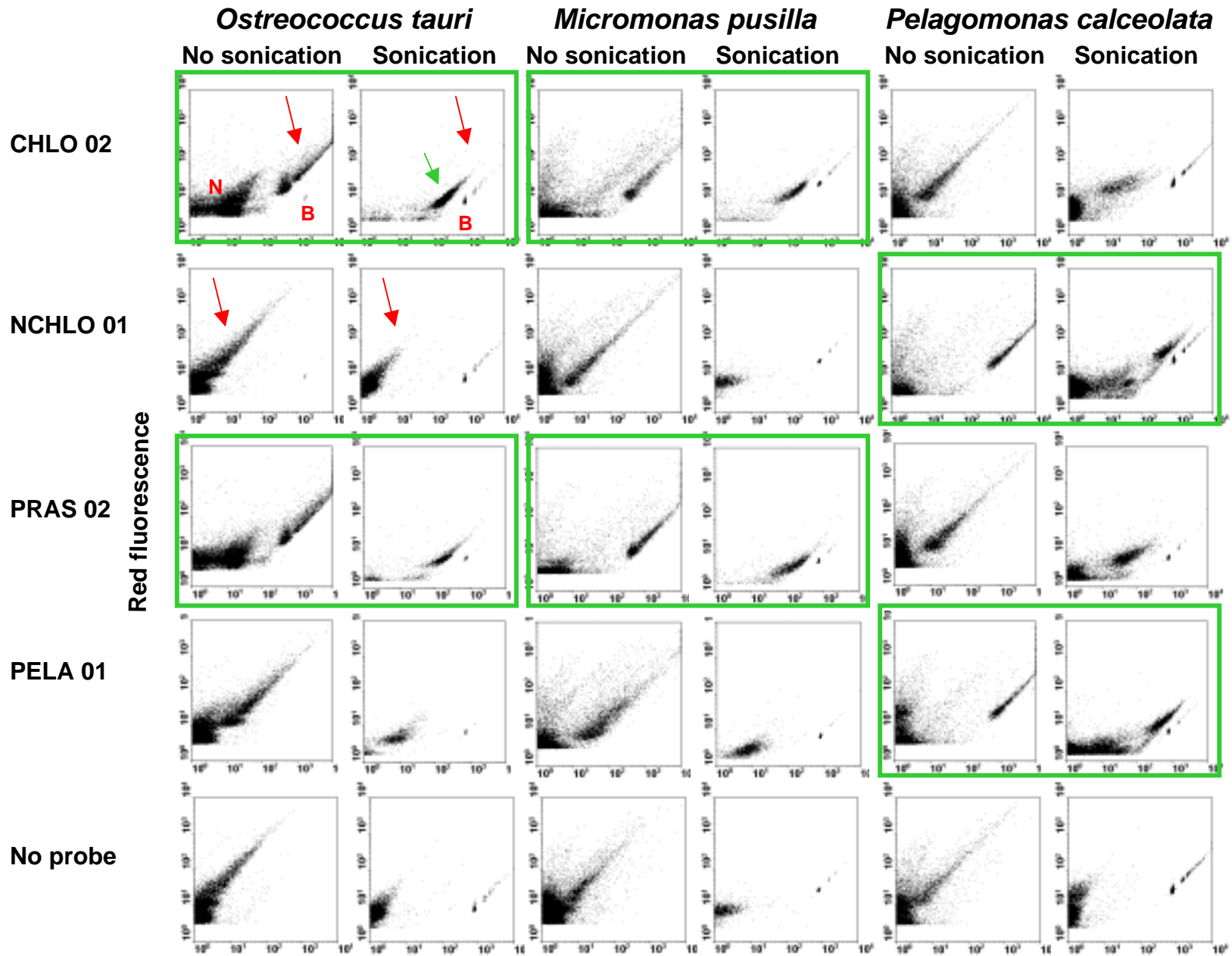
Silicone and pluronic



3- Flow cytometry

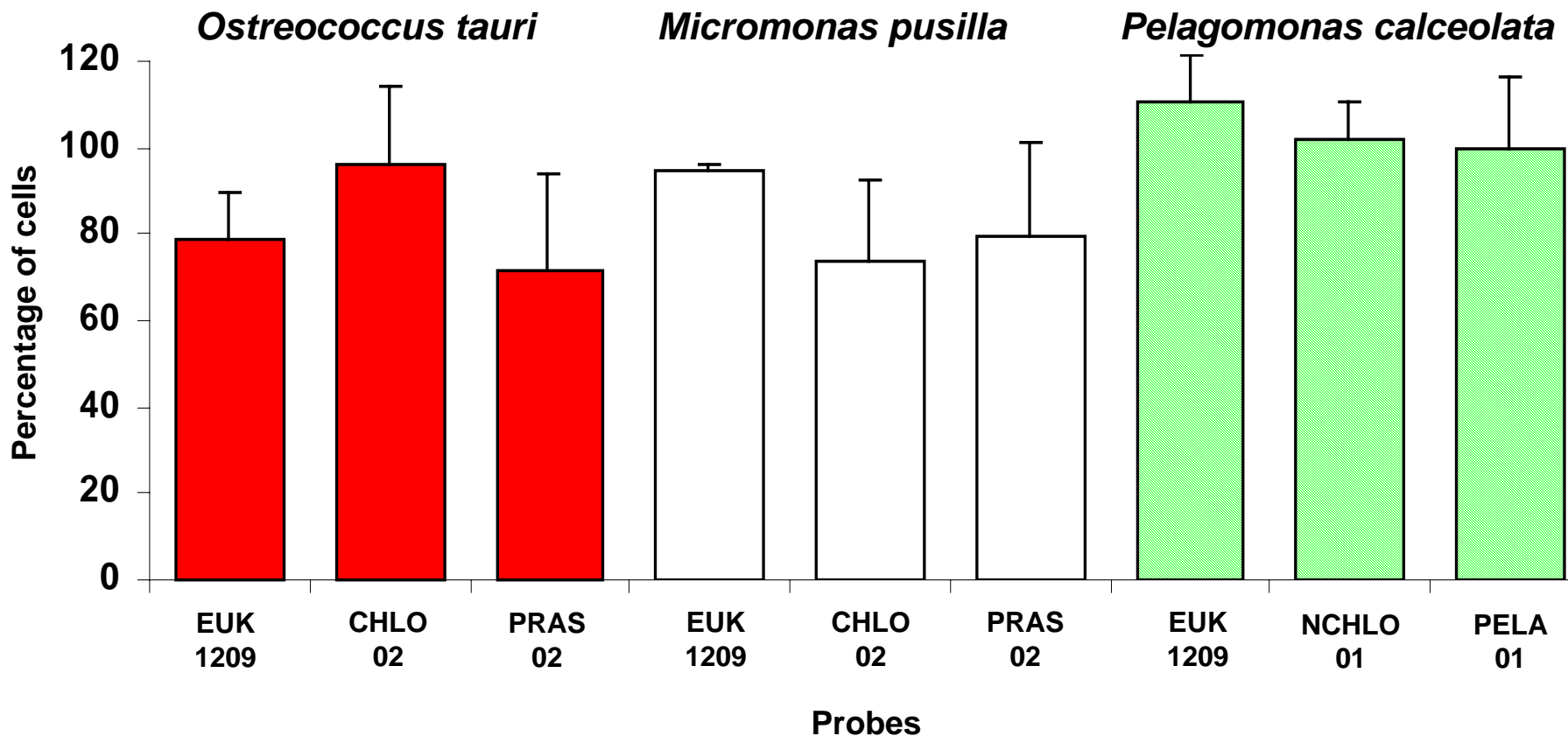
single laser 488 nm

Probes specificity and use of sonication to remove cell clumps



Red fluorescence

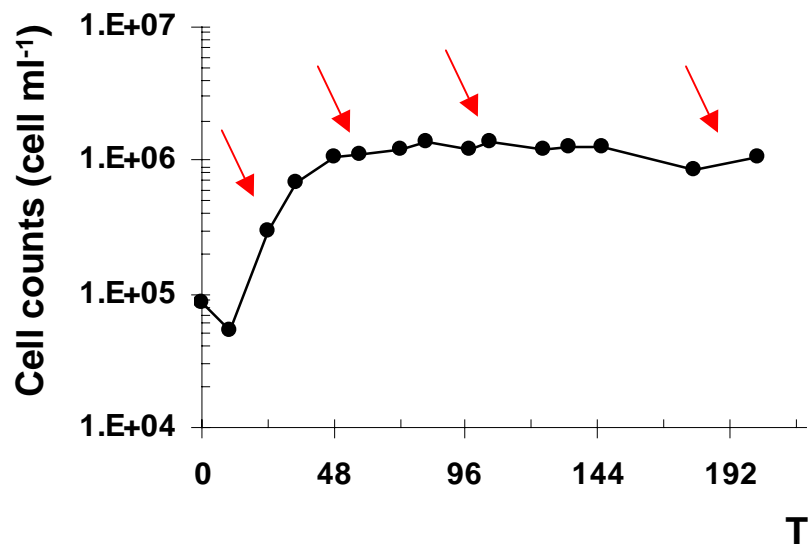
Use of surfactants and sonication to avoid cell loss



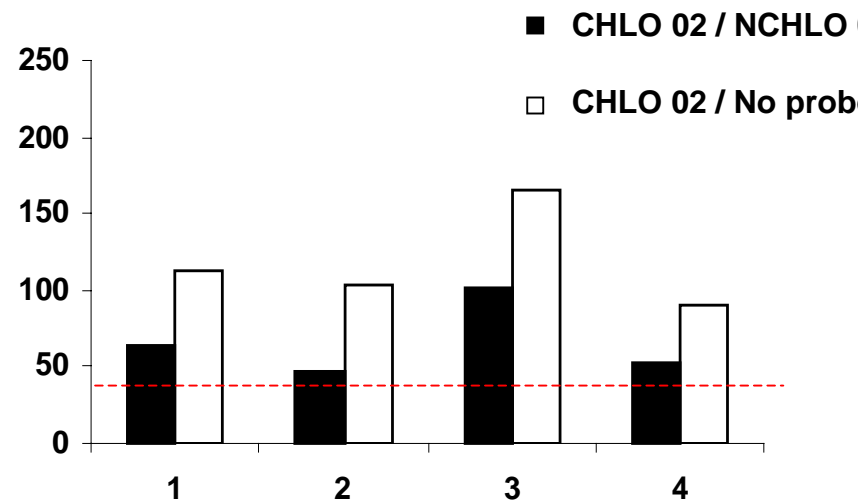
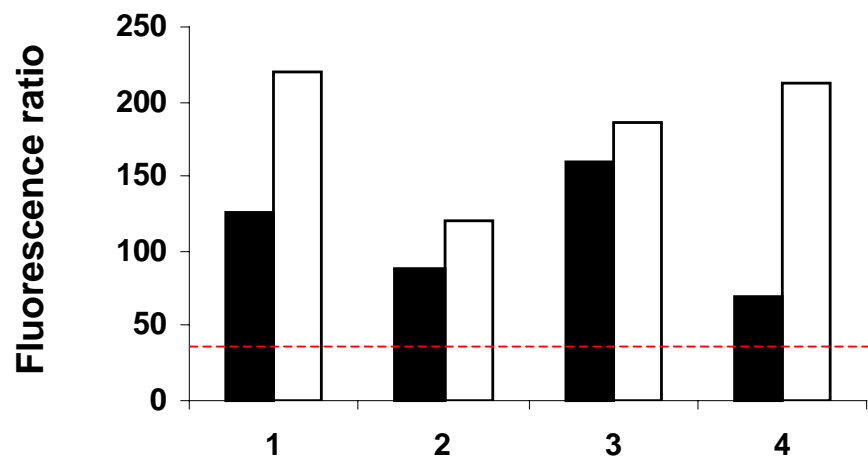
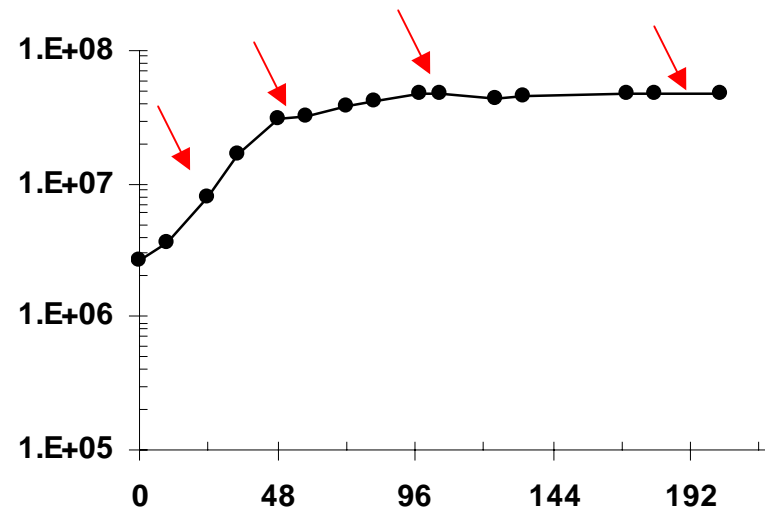
N=19 moyenne= 90% ±18

Fluorescence stability over different growth stages

Chlamydomonas concordia



Ostrococcus tauri

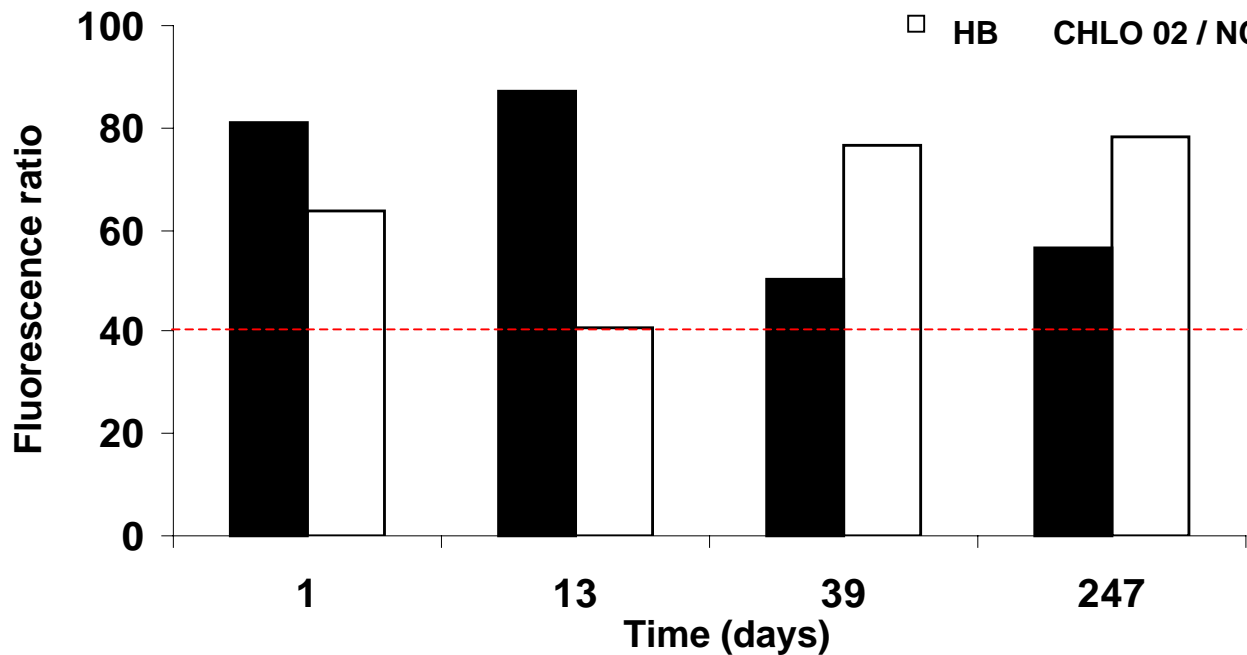


Fluorescence stability over time

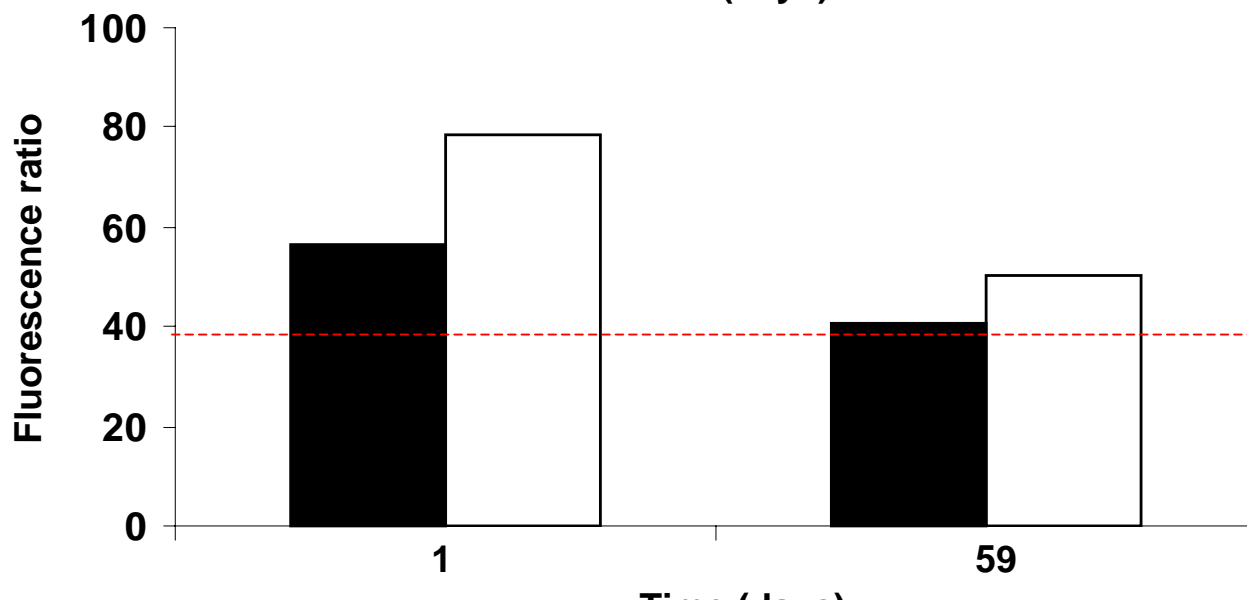
■ ETOH CHLO 02 / NCHLO 01

□ HB CHLO 02 / NCHLO 01

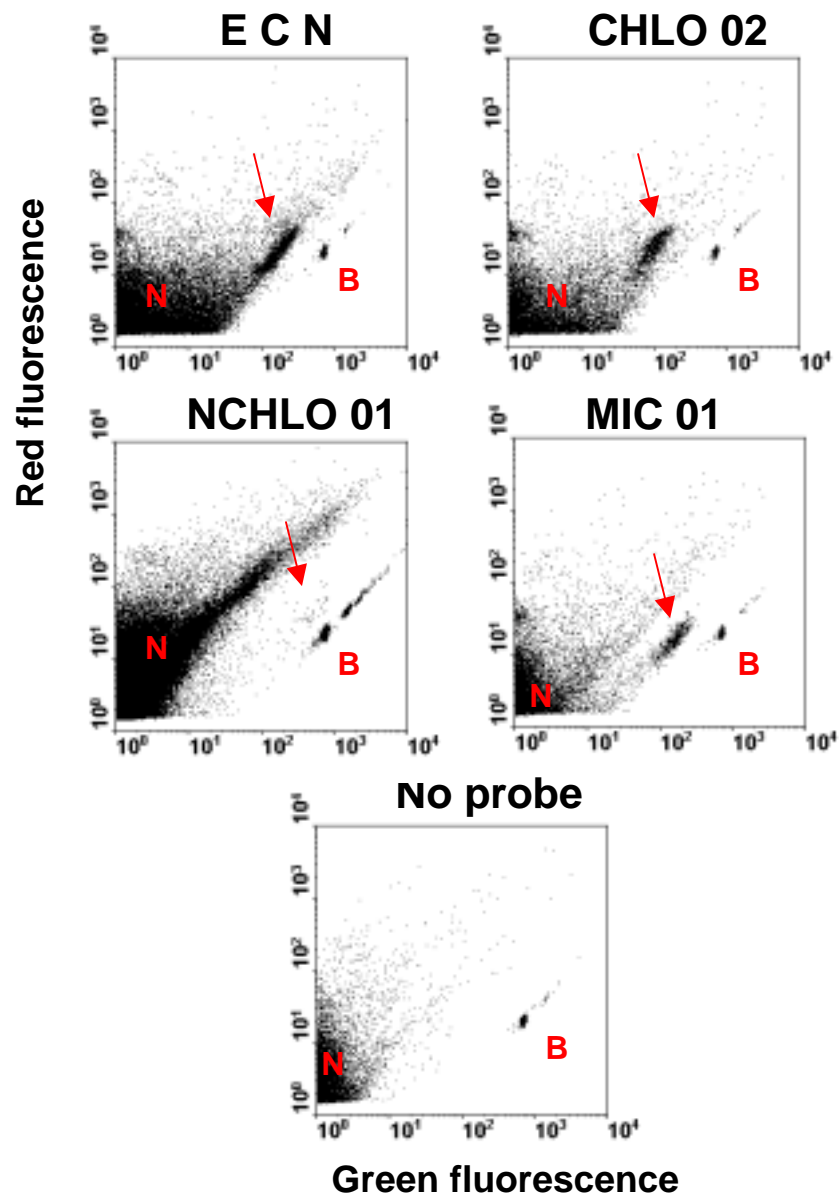
Before
Hybridization



After
Hybridization



Natural environment: estuarine station Dourduff 24/10/01

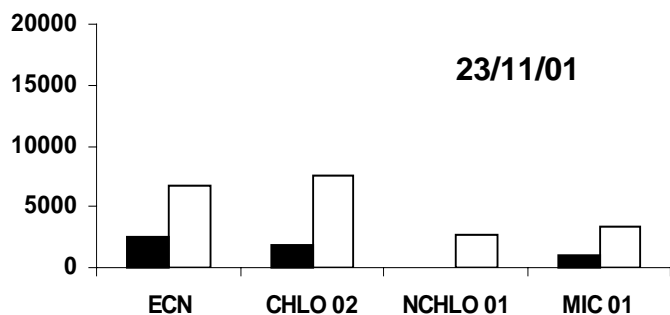
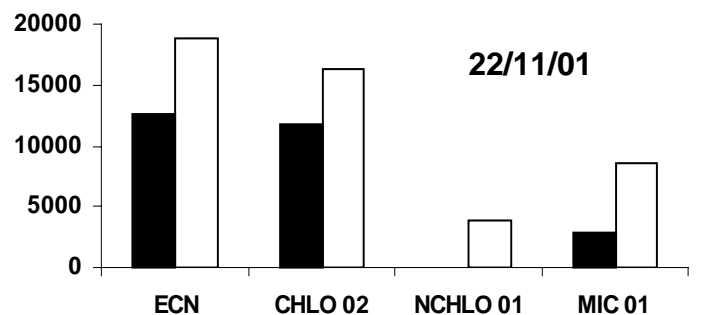
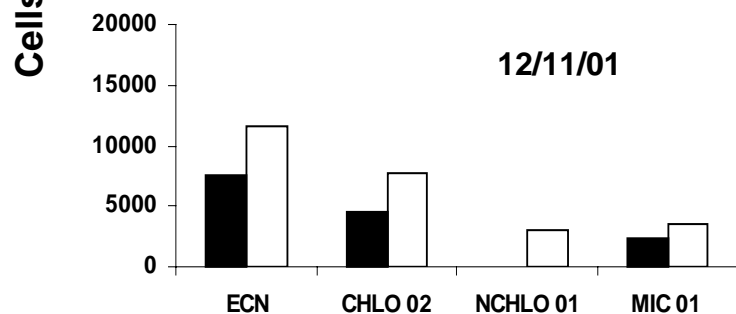
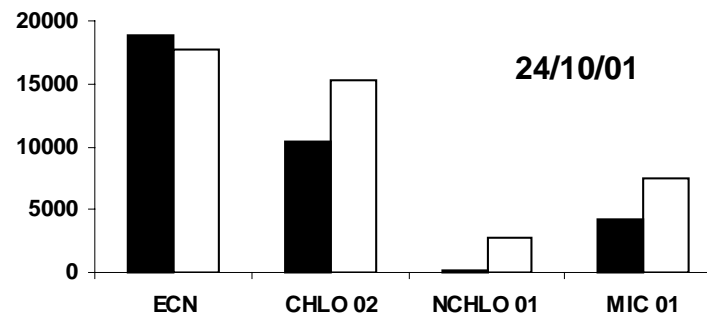
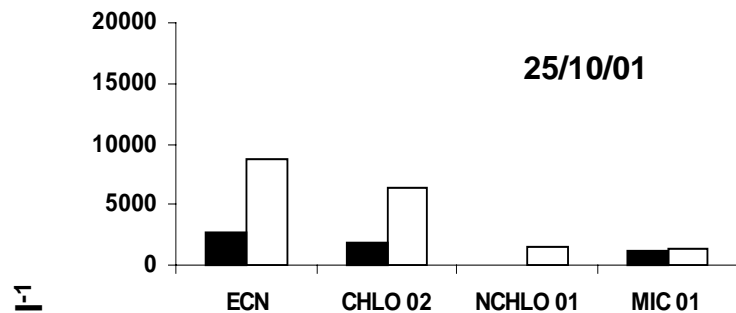


Comparison of counts FISH+cytometry / FISH+microscopy

■ Cytometry
□ Microscopy

Astan

Dourduff



Probes

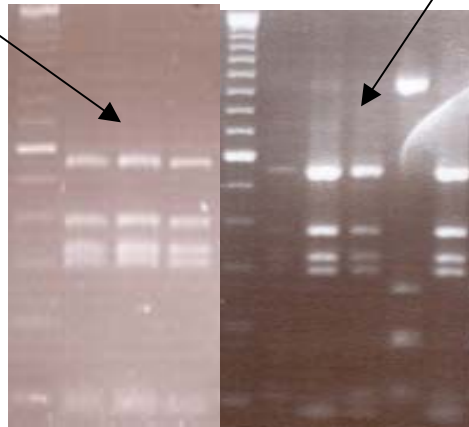
Cells ml⁻¹

Cloning hybridized cells after cytometry sorting

O. tauri

Clones from non-hybridized culture

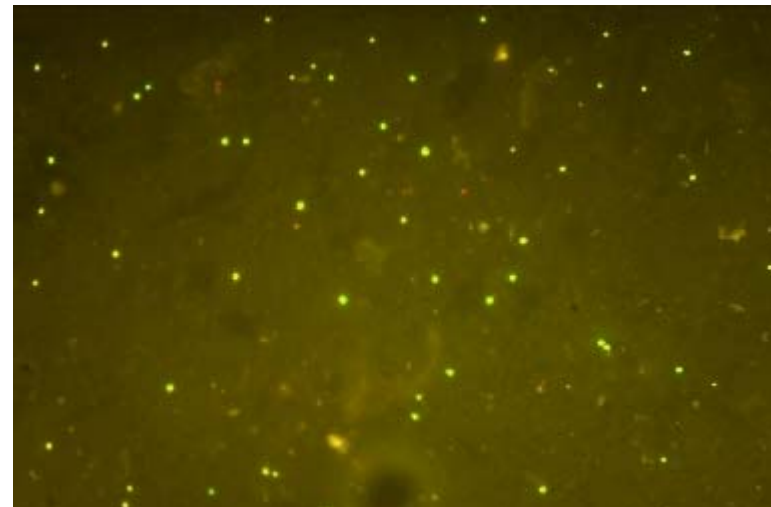
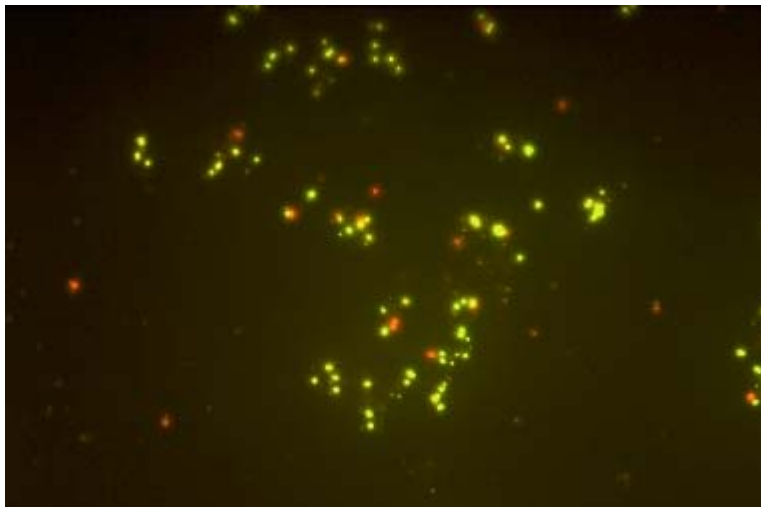
Clones from hybridized (CHLO 02) culture



O. tauri + *P. calceolata* (CHLO 02 + IP)

Prior sorting

After sorting



Conclusions and work in progress

1- Cultures

TSA-FISH + FCM is fully quantitative

Tests on some heterotrophic cultures of picoeukaryotes in progress

2- Natural environment

First time quantitative assessment with FISH + FCM

As little as 35 ml to be collected for one measurement

Improve cell collection process → oceanographic cruises : in progress

3- Sorting and further analysis

On cultures : in progress

**Future : to be tested on natural samples to identify organisms from groups
we have no sequences from actual clone libraries**