Alkaline phosphatase activity under climate change: a single cell measurement approach using microfluidic technology

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Introduction: A global context

From 1998 to 2006 → **+6.600.000 Km²** of low concentration of phytoplankton

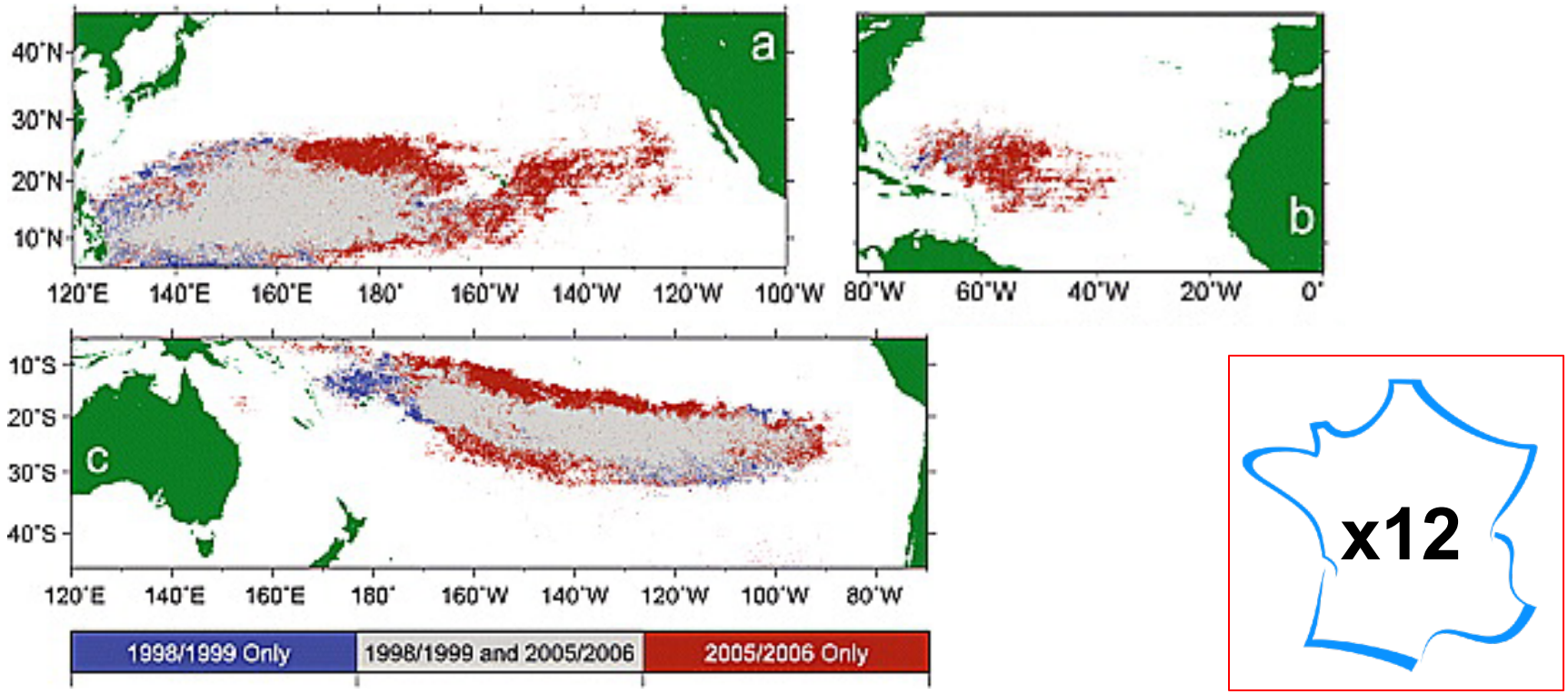


Fig. 1: Areas in North Pacific (a), North Atlantic (b) and South Pacific (c) with surface chlorophyll less than or equal to 0.07 mg.chl/m3 depending on time (Polovina *et al*., 2008).

Introduction: Alkaline phosphatase an indicator of nutrient starvation?

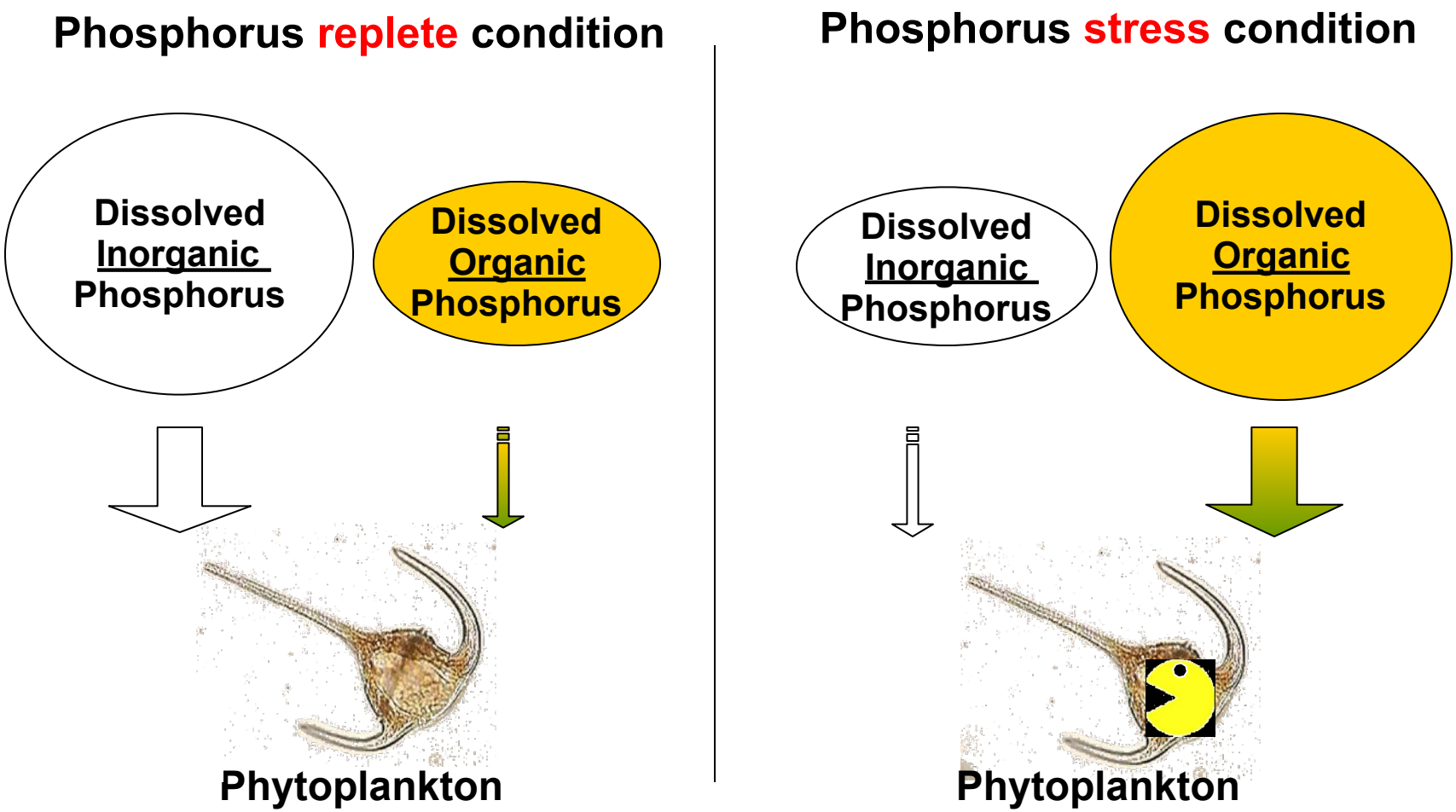


Fig. 2: Replete versus stress phosphorus conditions.

Technical limitations using the classical methods

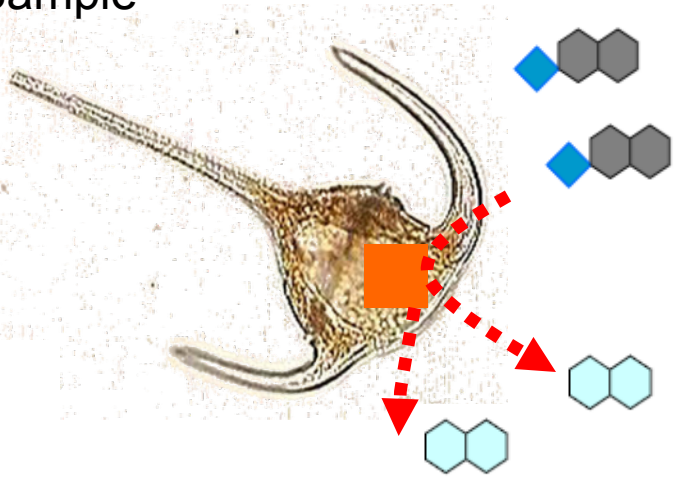
- Bulk measurement

MUF phosphate (MUFP or DiFMUP)

water soluble fluorescent product (MUF or DiFMU)

extracellular phosphatase

Sample



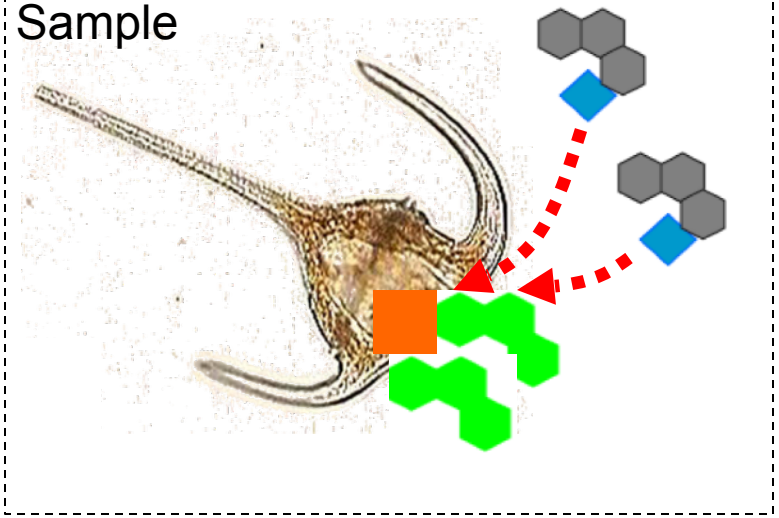
- Presence/absence of APA

ELF97 phosphate (ELFP)

water insoluble fluorescent product (ELFA)

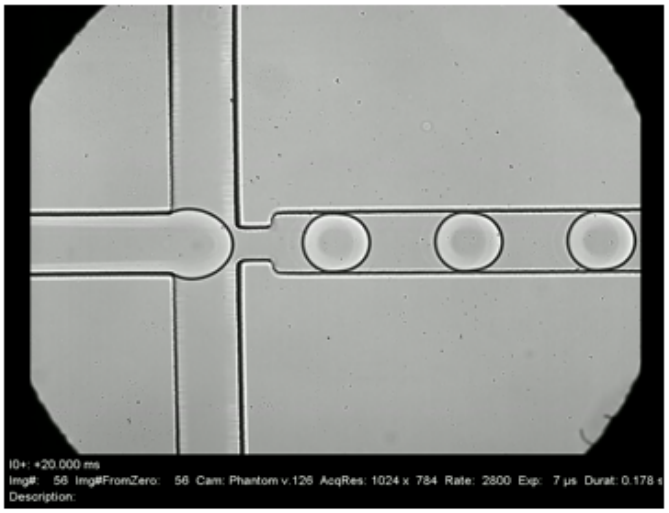
extracellular phosphatase

Sample



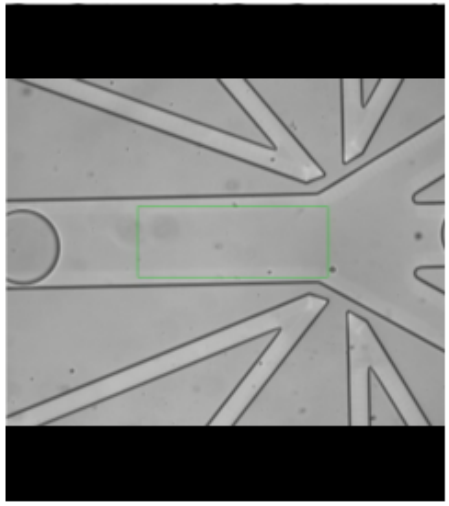
Which planktons activate the alkaline phophatase and in which extents?

Microfluidic: a suitable tool for working at a single cell level

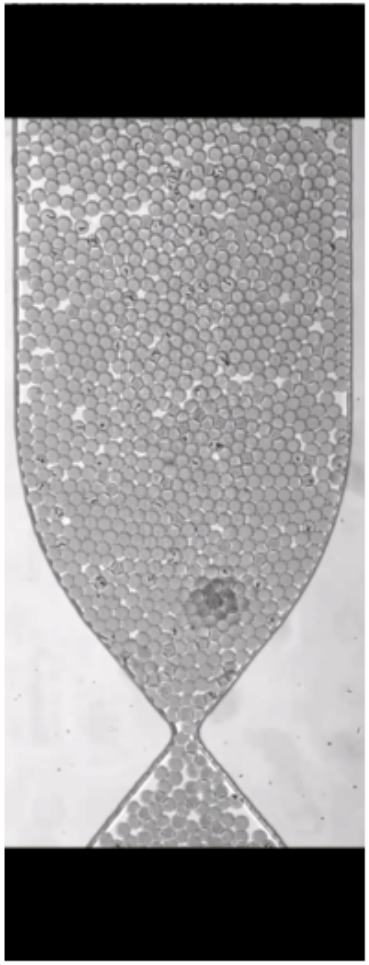


Droplet generation

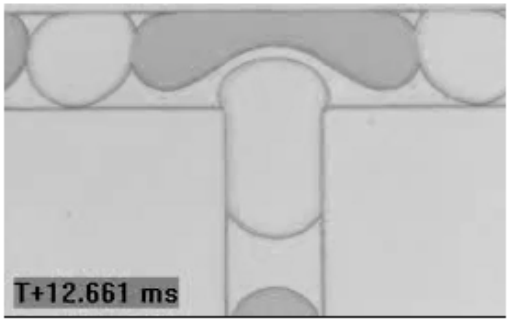
Injection



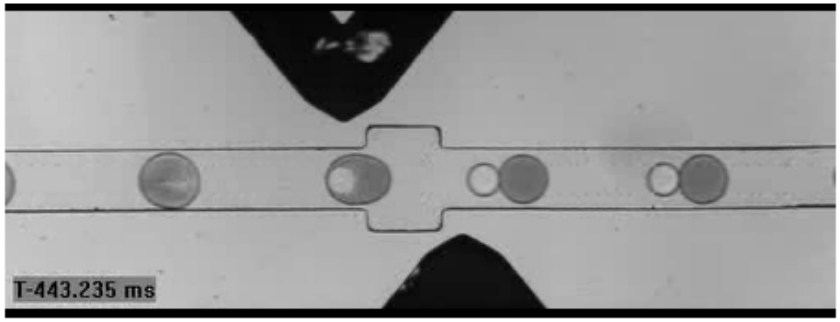
Sort



Incubation

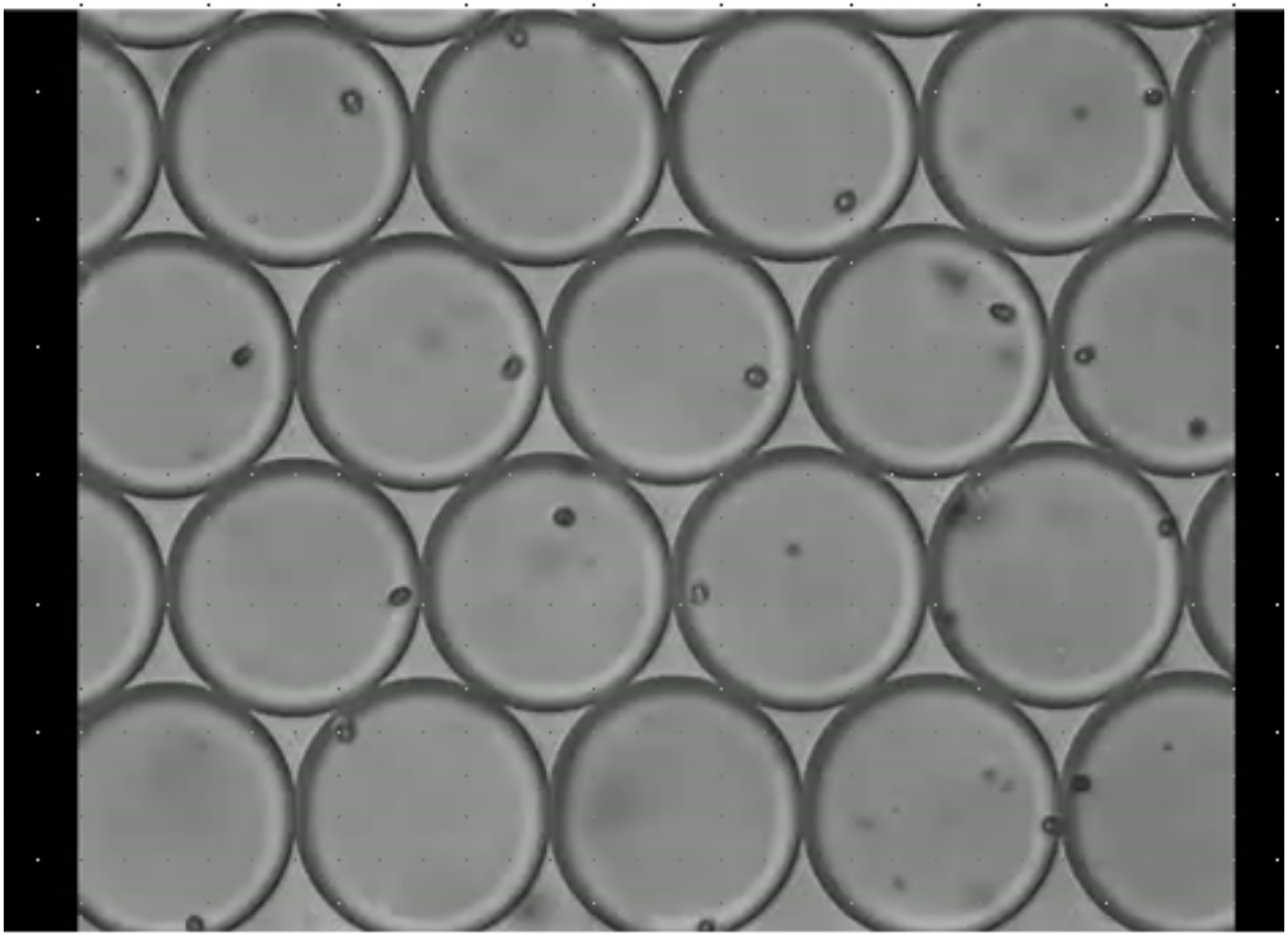


Split



Fusion

Incubation of single cells in the droplets



Video: Example of living planktons encapsulated in droplets.

Methods: experimental setting

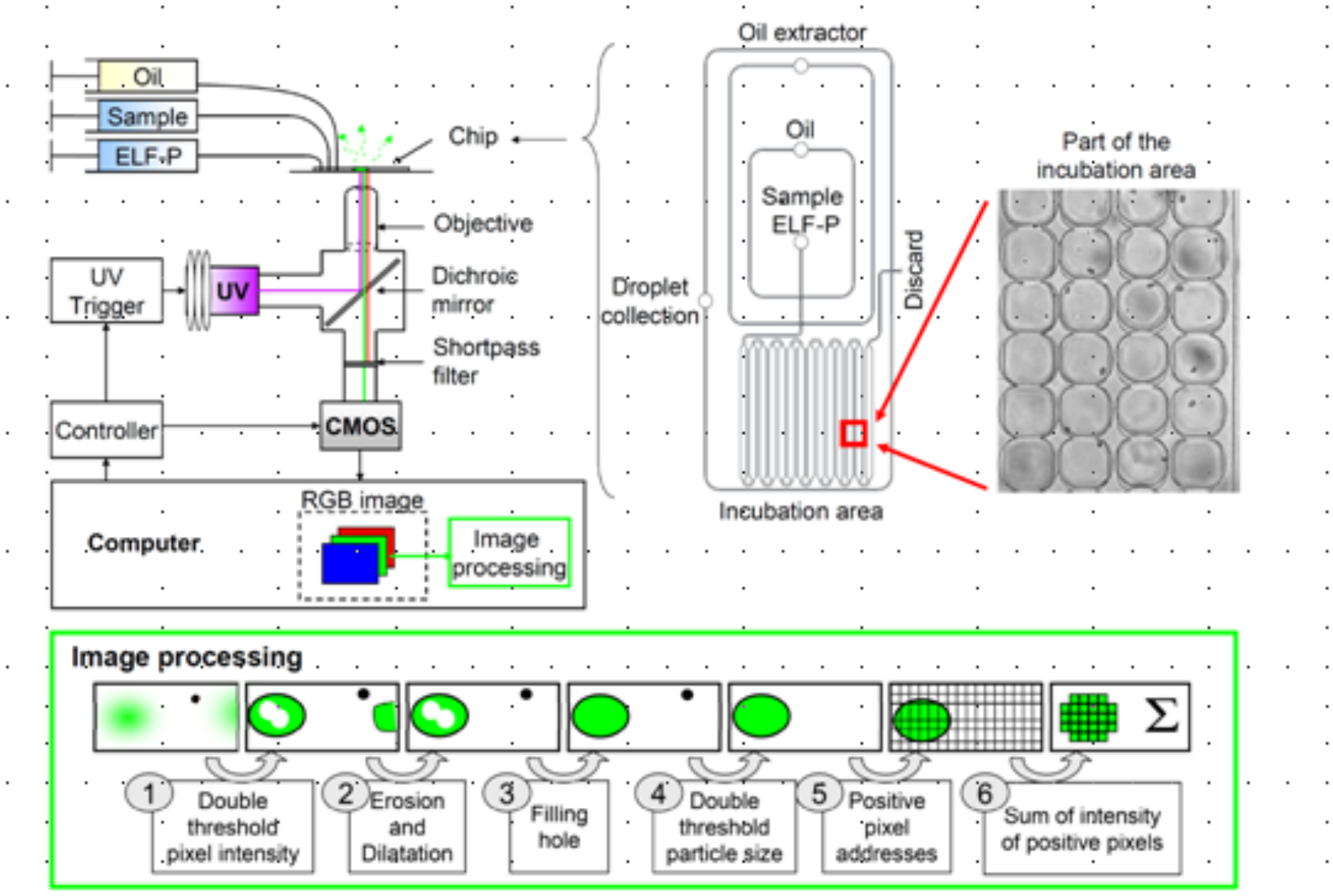


Fig. 3: Details of the experimental setting and chip.

Results: Alkaline phosphatase activity of plankton

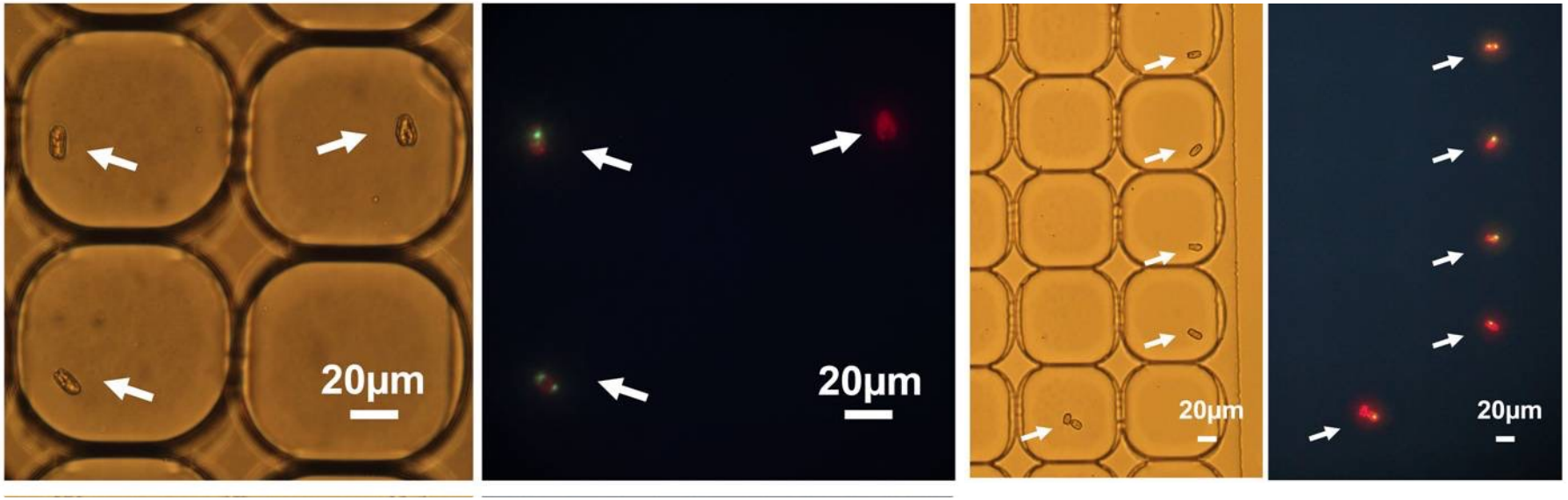


Fig. 4: Photomicrographs of cells labelled and encapsulated in droplets. Left and right panels show the images of cells encapsulated in the droplets in bright-field and fluorescence microscopy, respectively.

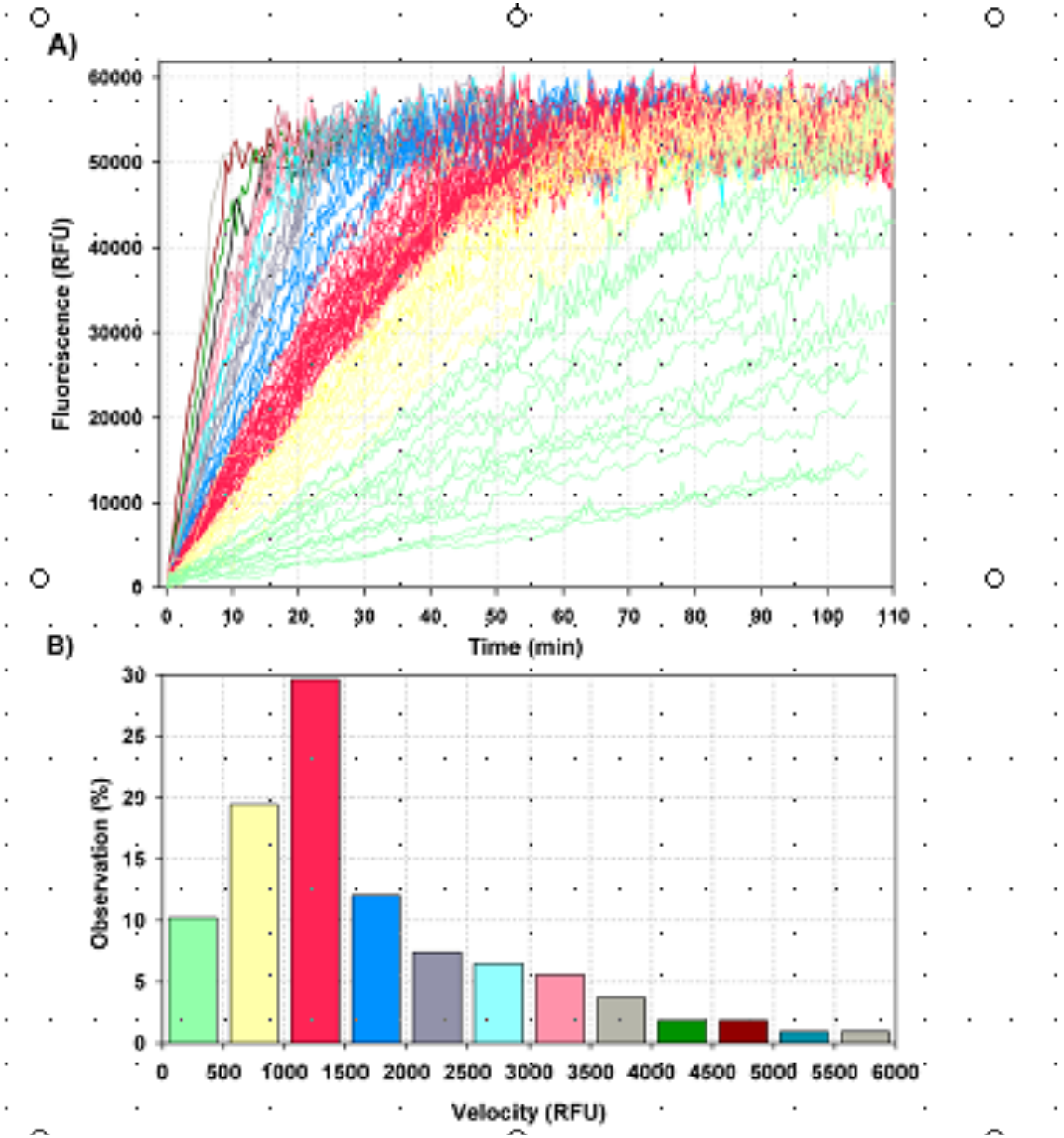


Fig. 5: Variability of the labelling kinetic of *Tetraselmis* sp. under the same environmental conditions.

Conclusions…

1. We developed a microfluidic platform suitable to measure alkaline phosphatase kinetic at a single cell level.
2. Both quantitative and qualitative information of the alkaline phosphatase activity can be obtained in real-time.
3. Phosphorus stress can be measured at a single cell level.

… and perspectives.

1. Measure the phosphatase alkaline released in the dissolved fraction of the sample.
2. Extend the method to others activities (e.g. study the effects of drugs on cells, toxins…)

Colleagues and partners working in this project

Yolanda Del Amo

Déniz Pekin

Thomas Beneyton

Jean-Christophe Baret

Céline Charbonnier

Sabrina Bichon

Thank you for your attention