Flow Cytometric sorting of phytoplankton : Possible problems and a recently developed application

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Using Flow Cytometry of phytoplankton provides us an unique possibility to examin sub-populations. Especially in natural environments we encounter a great variety of species, growth stages and morphology, even in one species. However, it can be important to obtain physiological parameters of pure sub-populations.
Two sources of error and a recently developed application are described .
The first described problem (I) is the sorting of filamentous algae. The trichome length of Cyanobacteria such as Oscillatoria and Prochlorothrix often exceed the droplet width.
To avoid a bias in population length, it is better to sort 3 drops, using as middle drop the one after the one calibrated.
The second problem (II) is the discrimination of Chlorophytes and Diatoms, maintaining the ability to discriminate the Cyanobacteria.
This can be achieved by using a flow cytometer in which the particle passes 3 lasers. Discriminating Cyanobacteria from Eukariotes can be done with a blue laser (488 nm), while the discriminating within the Eukariotes can be done with the combination of a red laser (647 nm) and a green one (530 nm).
As a example of the use of sorted phytoplankton, a description is given of determining growth speed of phytoplankton groups, using isotope ratio mass spectrometry (III).

I. Sorting of filamentous algae

Sorting filamentous phytoplankton gives us the opportunity to learn more about different stages (long/short i.e. old/young filaments), preferential grazing etc., provided your sorting is right. The problem is, that filaments are often so long, that they are present in 2 drops. When you align the sorting with beads, you find the right drop (the **0**-drop (Fig. 1)). However, the length distribution, compared with the unsorted distribution in this drop indicates (Fig. 2) that you pick the wrong drop. Good results are achieved sorting 3 drops at the same time (**0**, **+1** and **+2**).

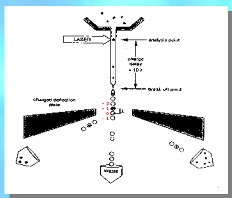

Fig. 1. Flow sorting principle

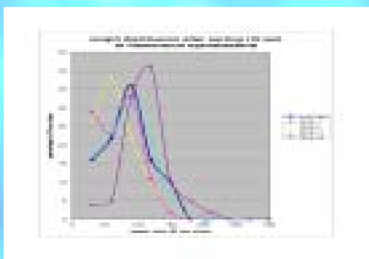

Fig. 2. Length distribution of sorted filaments

II. Discrimination of Chlorophytes and Diatoms

Using a blue laser (488 nm) it is possible to discriminate the real Cyanobacteria from the ukariotes (Fig. 3). However, the Chlorophytes and Diatoms are not separable. If we, however, apply a combination of red laserlight (647 nm) and green laserlight (530 nm) we can learly separate these algae, but the Cyanobacteria are now undistinguishable from the Chlorophytes. For that reason we must apply 3 laser successively.

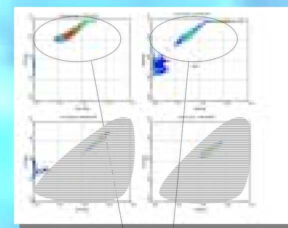

Fig. 3. Orange(640 nm) vs. red(675 nm) fluorescensce , induced by a 488 nm laser (gray: Cyanobacterial aerea)

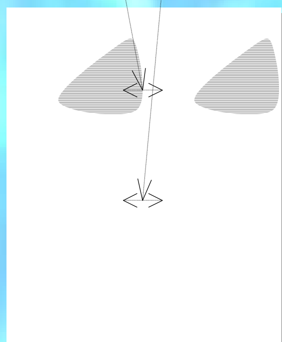

Fig. 4.
Left panels : Red fluorescence (from green laser) vs. red fluorescence (from red laser).
Right panels : red fluorescence (from green laser) vs. orange fluorescence (from green laser).
Algae : Chlorophytes (top row), gray=Diatoms aerea, Diatoms (middle row) and Cyanobacteria (bottom row).

III. Pyrolysis-IRMS ^{13}C-analysis of FCM-sorted cells (for method see separate folder)

Assessment of *in situ* growth rates of phototrophs by ^{13}C-CO_2 labelling and combined FCM/py-IRMS analysis

- unfiltered Lake Loosdrecht water was confined in a small laboratory scale enclosure enabling close controle over the degree of ^{13}C enrichment of the inorganic carbonate of the sample.

- target populations for FCM-sorting (see also Fig. 1 on principle handout):
  - *Oscillatoria*-like filaments (50 to 250 µm)
  - *Prochlorotrix*-like filaments (50 to 250 µm)
  - Eukaryotic algae (diatoms and green algae; Ø 4 to 10 µm)

- monitoring of target FA-profiles and δ^{13}C signature by py-IRMS (3 x 24 hours; 2 to 3 sortings per day from enclosure). Calculation of population-specific growth rates from the rate of ^{13}C enrichment observed in membrane-derived FAs.

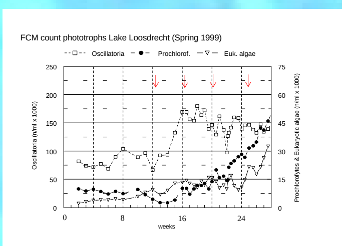

Fig. 5 Algal contribution in Lake Loosdrecht, 1999. Red arrows indicate sample data for growth rate determination (table 1)

Table 1. Growth rates (d^{-1}) of predominant phototrophs in Lake Loosdrecht using ^{13}C-CO_2 enclosure technique (spring 1999; dates indicated with red arrows in **Fig. 5.**)

	March 25	April 20	May 18	June 22
Cyanobact.	0.033	0.031	0.05	0.08
Prochlorof.	0.014	0.021	0.023	0.046
Euk. algae	0.14	0.14	0.2	0.43

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