Phytoplankton Monitoring by Laser Induced Fluorescence

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ABSTRACT

This paper describes some laboratory experiment carried out on different microalgae species, which were irradiated at different visible wavelengths, in order to optimize the excitation wavelength for fluorescence lidar operation in field. As excitation sources, an Ar^+ cw and a He-Ne cw lasers were used. The fluorescence spectra were detected with a resolution of $\approx$ 1 nm. The different algae species showed different spectral signatures, allowing their identification.
Finally, some results on fluorescence behaviour of a light-stressed degrading sample are presented and discussed.

INTRODUCTION

Lidar monitoring of sea and natural waters is mainly carried out with the fluorescence detection of occurring substances, excited at a proper laser wavelength.
A good description of the involved ecological parameters is achieved by performing a spectral analysis of the fluorescence signal along the visible (VIS) band; this kind of detection allows the discrimination of oil pollution, yellow substances, phytoplankton, and chlorophyll content (MEASURES, 1983).
During last years phytoplankton monitoring has been assuming an increasing importance, either in the exploitation for *e.g.* fisheries, or in the evaluation of sea pollution, especially in the monitoring of algae blooming.
Even if many studies have been already performed on phytoplankton surveillance (see e.g. KIM 1973, MUMOLA *et al.* 1973), a considerable effort is still in progress for what concerns the monitoring of the photosynthetic process, both constituting the main goals of this work.

This paper presents and discusses some laboratory experiments performed on phytoplankton fluorescence spectroscopy and introduces preliminary results carried out with a lidar system in a controlled environment.

1. LABORATORY EXPERIMENTS

In general the different pigments contained in phytoplankton have a strong fluorescence yield and show different spectral signatures. Moreover, since chlorophyll fluorescence spectrum, taken *in vivo* on vegetation, varies with photosinthesys (EDNER *et al.* 1991, CECCHI and PANTANI, 1991), it is reasonable to expect that the photosynthetic process can be also monitorable in phytoplankton.

The involved experiments consist of the following main-steps:
- phytoplankton species identification by means of their fluorescence spectrum,
- excitation wavelength optimization,
- monitoring of the photosynthetic process.

Laboratory experiment started on fluorescence spectroscopy of different phytoplankton samples, belonging to four species as shown in Table 1.
The fluorescence was induced at seven different laser wavelengths and detected with an Optical Multichannel Analyzer (EG&G PARC OMA III system) in the band ranging from 550 nm to 800 nm in order to match chlorophyll and other pigments emission.
The experimental set-up is shown in Fig. 1: the sample, contained in a beaker and kept at a constant temperature of 28 °C, was irradiated with the different lines of an Ar^+ and a He-Ne laser. A little monochromator (Jobin-Yvon, H 10) was used to select the Ar^+ lines, while two folding

mirrors were settled to handle the excitation beam and the fluorescence signal, focused with a lens on the input slit of a spectrometer (Jarrel-Ash Mod. 1320), part of the above-mentioned detection system. The slit width was settled at 25 μm. The detector employed is a 512 channel gated and intensified linear diode array (EG&G PARC Mod. 1420). The whole spectral resolution is about 1 nm. Fluorescence spectra of the nine phytoplankton samples, excited with the 514 nm and 633 nm laser lines and corrected for the spectral response of the collection and detection systems, are reported on Figures 2 and 3, respectively.

PHYTOPLANKTON SAMPLES		
CLASS	**TYPE**	**MAIN PIGMENTS**
Bacillariophyceae	Achnanthes	chl *a,c*
Chlorophyceae	Chlorella	chl *a,b*
	Scenedesmus	chl *a,b*
	Tetraselmis	chl *a,b*
Cyanobacteria	Nodularia	chl *a*, phc
	Spirulina Maxima	chl *a*, phc
	Spirulina Subsalsa	chl *a*, phc, phe
	Synechocistis	chl *a*, phc, phe
Xanthophyceae	Heterococcus	chl *a,c*

Table 1: phytoplankton samples used for laboratory experiments. Chl a,b: chlorophyll a,b; phc: phycocyanin; phe: phycoerithrin.

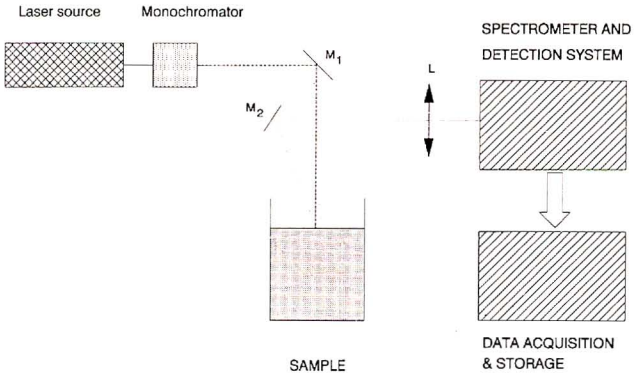

Fig. 1 - Block diagram of the experimental set-up, used in laboratory. M1, M2 are beam-handling mirrors, L is a focusing lens.

The fluorescence spectra show the typical signature of chlorophyll, with the characteristic peak at 685 nm, but also the fluorescence signatures of accessory pigments. In particular, some samples reported in Fig. 2 display fluorescence bands at about 580 nm, due to phycoeritrin, and at about 660 nm, due to phycocyanin, which is partly superimposed to the 685 nm chlorophyll peak. Even if the

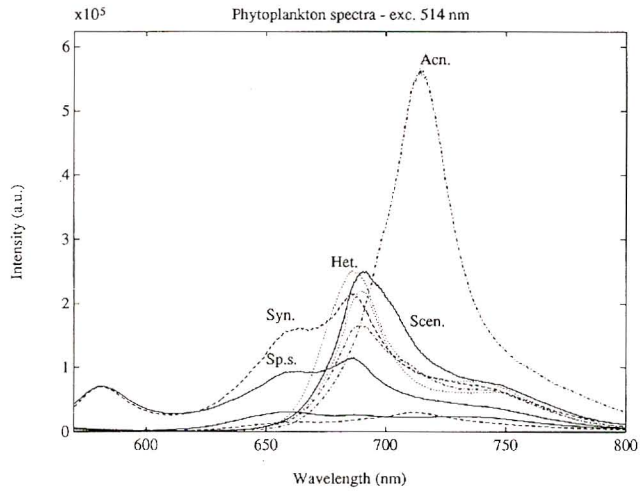

Fig. 2 - Fluorescence spectra of the nine phytoplankton samples. Excitation wavelength: 514 nm.

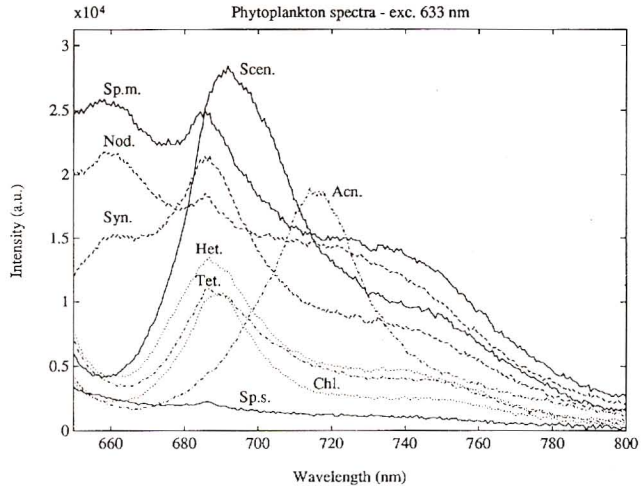

Fig. 3 - Fluorescence spectra of the nine phytoplankton samples. Excitation wavelength: 633 nm.

633 nm line is quite efficient to excite chlorophyll fluorescence, no fluorescence excitation of the other pigments can be of course carried out.

In both cases is quite easy to distinguish *Bacillariophyceae* (Achnantes) and *Cyanobacteria* from the other samples: Achnantes shows a particular peak at 720 nm, while *Cyanobacteria* show the phycocyanin peak, at 660 nm.

Figures 4 and 5 show the same kind of fluorescence spectra excited with the 488 nm Ar^+ laser line. *Bacillariophyceae* show a lowered fluorescence intensity with respect to the previous pictures, but it is still well distinguished from the others. *Chlorophyceae* show a higher intensity, but there are still some problems to identify *Xanthophyceae*.

On the other hand *Cyanobacteria* have a quite low fluorescence intensity but still appreciable.
The other excitation wavelengths do not show an appreciable difference from the 488 nm excitation.

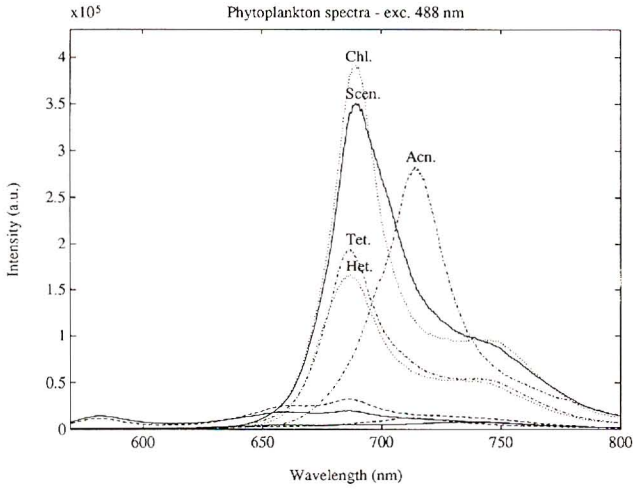

Fig. 4 - Fluorescence spectra of the nine phytoplankton samples. Excitation wavelength: 488 nm.

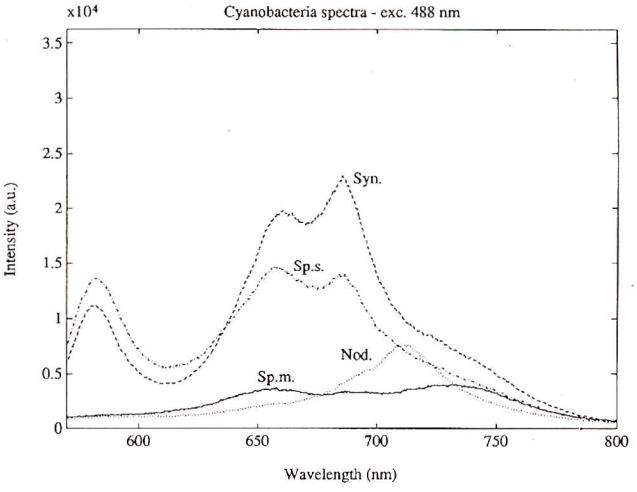

Fig. 5 - Fluorescence spectra of the Cyanobacteria samples. Excitation wavelength: 488 nm.

In order to have a proper identification of the samples, a deconvolution of the different spectra was carried out. This deconvolution is made with Gaussian curves and Fig. 6 gives an example; the error of the fitting is lower than 3%.
Figure 7 reports the deconvolution for the same algae sample, excited with the different wavelengths. The fitting is good, and shows a little variation of the central wavelength of Gaussian curves, which is within ≈ 3 nm for the 685 nm peak, ≈ 7 nm for the 710 nm one, and ≈ 2 nm for the 735 nm one. This test was carried out also to exploit the opportunity of better discriminating the different pigments contained in phytoplankton.

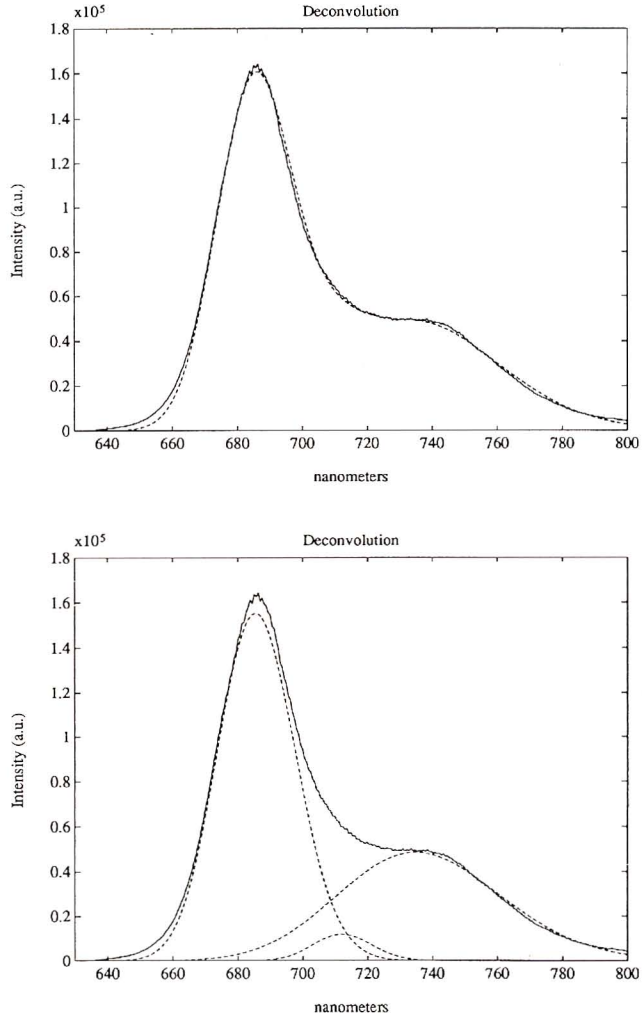

Fig. 6 - Fluorescence spectrum deconvolution with Gaussian curves (Heterococcus excited at 488 nm). Up: detected spectrum (solid line) and its fitting (dashed line). Down: detected spectrum (solid line) and its deconvolving Gaussian curves (dashed line).

The last part of the experiments deals with the fluorescence behaviour during five days of a degrading phytoplankton sample. The sample was Spirulina Maxima on which a light stress was induced.

The algae sample is kept in a dark chamber and has a controlled lamp-irradiation. During the day, the cycle is 16 hours of light and 8 hours of dark. Fluorescence monitoring was carried out with the FLIDAR-3 described by

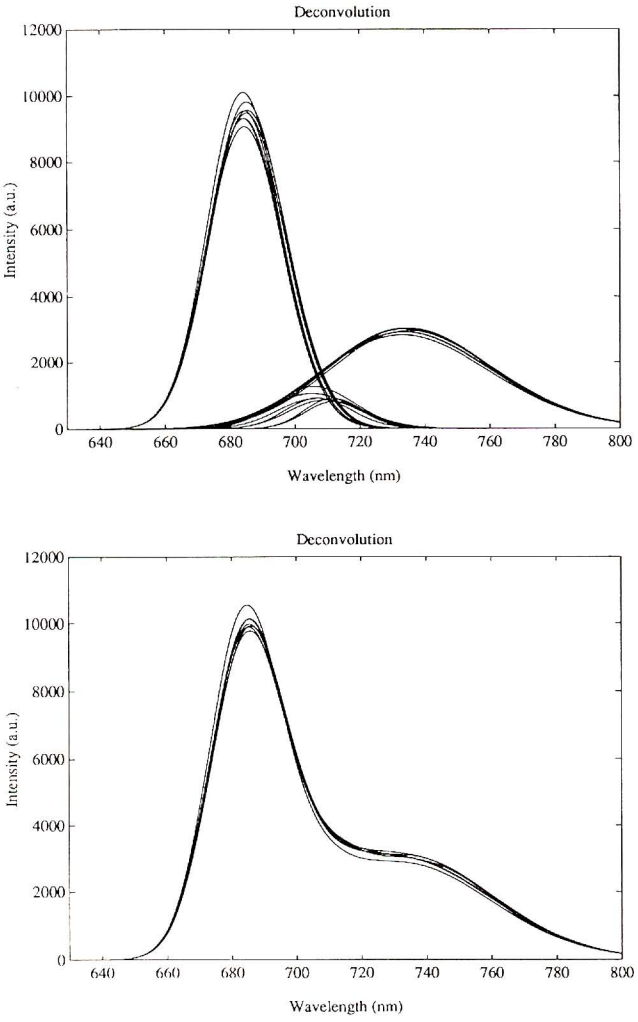

Fig. 7 - Fluorescence spectra deconvolution of the same algae sample (Heterococccus), excited with the different wavelengths. The spectra are area-normalized.

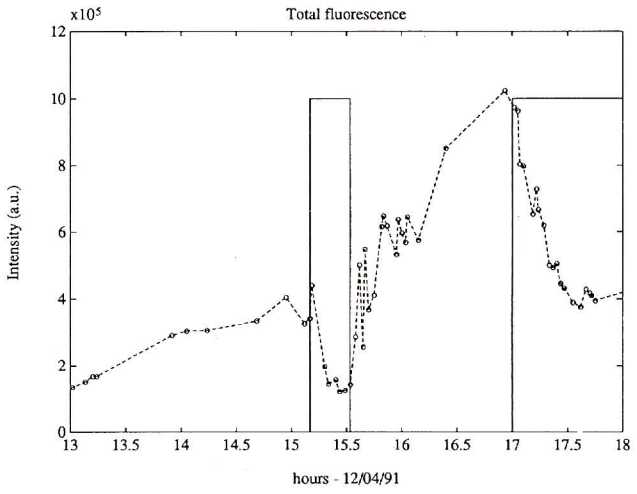

Fig. 8 - Time behaviour of the total fluorescence intensity. The solid line is 1 when lamp is on, 0 when lamp is off.

CECCHI *et al.*, 1991 and the excitation wavelength was the UV one at 308 nm; actually it turned out that Spirulina Maxima shows a higher fluorescence efficiency with the UV excitation with respect to the VIS one. Figure 8 shows the fluorescence intensity, integrated from 550 nm to 800 nm, as a function of time. As soon as the light is switched on, the integrated fluorescence intensity decreases, reaching very low levels, indicating maybe the beginning of photosynthesis on witch a subsequent photoinhibition is superinposed. On the other hand the dark period allows a recover and the fluorescence intensity increases, reaching the same level at the end of each dark period.

After some days of this treatment photooxidation took place and the sample died. Two fluorescence spectra, taken at two analogous times during the dark period, but referring at the initial (health) and final (death) conditions are reported in Fig. 9. The inversion between the two peaks is clearly observable. In vegetation such a behaviour would monitor a light - dark condition, respectively. In phytoplankton this is not true any more: the dashed line of Fig. 9 monitors a light stress: the algae sample does not react to the light and this can be considered as photooxidation index. To monitor this effect, the time-evolution of the ratio 675 nm/740 nm was considered (Fig. 10). This ratio is calculated on a band 40 nm wide, centred at the reported wavelengths.

The ratio shows an increasing trend versus time and reports "quick-jumps" corresponding to two short light switchs-on. The last light period produced a ratio value higher than 1, meaning the reversal between the two peak-intensities (Fig. 9), and referring to the death of the sample.

The rising trend of the ratio could be attributed to the light stress (photoinhibition), while photooxidation is monitored with a value over 1. This is in accordance with the level of Oxygen produced which was also measured during the experiment.

So the ratio could be proposed as a "health-index": the higher value, the higher stress condition is produced, on the contrary a low value should indicate a health state.

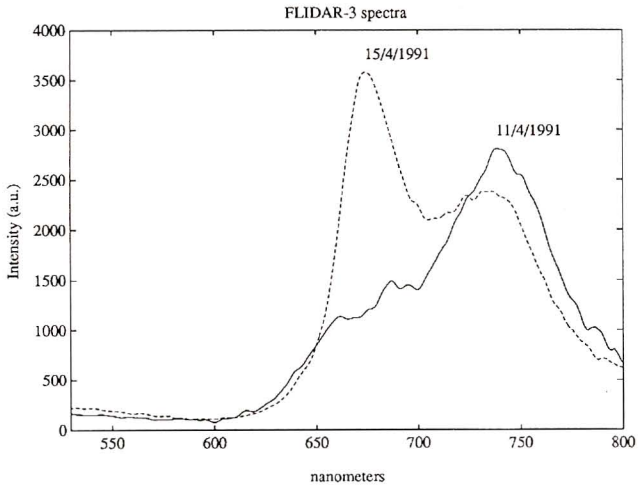

Fig. 9 - Fluorescence spectra of the same phytoplankton sample (Spirulina Maxima), taken at the beginning (solid line) and at the end of the experiment (dashed line).

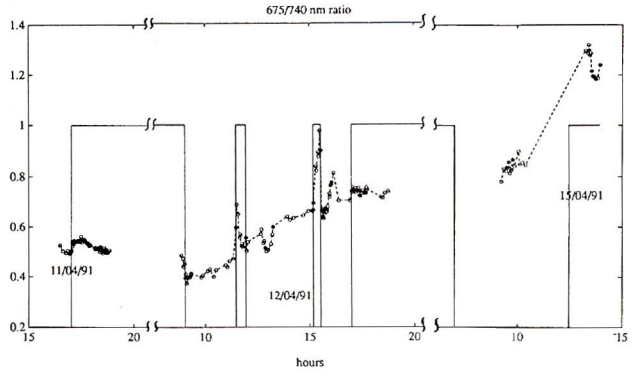

Fig. 10 - Time-evolution of the ratio 675 nm/740 nm. The solid line is 1 when lamp is on, 0 when lamp is off.

CONCLUSION

As a conclusion, we point out the following steps:

- lidar individuation of phytoplankton species is possible, at least for the considered classes;
- there are still some problems in the estimation of a common optimum excitation wavelength: actually *Cyanobacteria* show a higher fluorescence efficiency in the UV than in the VIS, so that a proper analysis at the spectrophotometer for all the samples is required. Such an analysis is quite difficult to carry out, considering the characteristic of algae samples: they precipitate quickly, so a continuous shaking in short-path cuvettes is needed, moreover they require an operation at a constant temperature of 28°C. This spectrophotometer measurements will be performed in the near future;
- a first approach of lidar monitoring to the degradation of a phytoplankton species was carried out, giving interesting indications, and constituting a base for future work.

By the end of 1991, some measurement campaigns, both on sea and on rivers, will be carried out.

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