Multiple - Wavelength Remote Sensing of Phytoplankton

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ABSTRACT

The results of field remote sensing of brackish - water phytoplankton by tunable lidar on board of a research vessel are presented. The authors have developed study approach based on spectral signature method and the method of selective excitation of phytoplankton pigments to diagnose the variability of pigment composition of phytoplankton community *in vivo*.

1. INTRODUCTION

Phytoplankton is very sensitive indicator of ecological status of water ecosystems. It has been shown by numerous studies that organic pollution or enhanced nutrient loading of natural waters may cause the changes of phytoplankton taxonomic composition and its exceptional blooms [1,2].

The excitation and fluorescence spectra of various micro algae differ by their characteristic features connected first of all with the pigment composition of the cells [3,4]. This allows one to use phytoplankton as a natural fluoroindicator of the state of sea environment.

A promising one among the fluorescent methods is the registration and analysis of spectral signatures of phytoplankton as two - dimensional matrix of fluorescence intensity in the co-ordinates of excitation and emission wavelengths. Such a signature includes the excitation spectra of phytoplankton as well as fluorescence spectra.

The analysis of spectral features of different taxa made it possible to systematize the phytoplankton species according to their pigment composition and to arrange them into groups within some catalogue. A characteristic fluorescent indicator corresponds to each pigment group as a main feature. Such a catalogue compiled includes the spectral signatures of 53 phytoplankton cultures covering the major marine taxa.

At the first stage of catalogue forming the cultures where divided into four groups according to their main accessory pigments: group I - Phycocyanins (Cyanobacteria); group II- Phycoerythrins (Cyanobacteria and red algae); group III - Chl b (green and Prasinophyte algae); group IV - Carotenoids and Chl c (Diatoms, Dinophytes and other algae).

The set of lidar experiments has been carried out in 1991 - 92 in the mouth of the Gulf of Finland to carry out the remote fluorescent diagnostics of phytoplankton by selective sensing of the single group of photosynthetic pigments. The aim of this work was to study the possibility of laser selective excitation of phytoplankton pigments in qualitative and quantitative diagnostics of mixed phytoplankton community.

2. METHOD OF SELECTIVE EXCITATION OF PHYTOPLANKTON PIGMENTS

By analysing of two - dimensional signatures (Fig.1) the most characteristic spectral features of each pigment are easily revealed.

The spectral structure of group I with maxima at 620 nm of excitation and 640 nm of emission corresponds to the fluorescence of Phycocyanin. The fluorescence of Phycoerythrin has typical spectral structure with maximum at 550/580 nm (Fig.1) and corresponds to the phytoplankton group II.

The accessory pigments which have not fluorescence *in vivo* are known to possess the ability to pass stored light energy on to Chl a with it's following fluorescence[5]. These pigments are revealed in the spectral signature as a set of local maxima at emission 680 nm typical for Chl a. In the excitation spectra of Chl a the maximum of Chl b and Chl c are located rather near 480 and 460 nm respectively. There is an additional diagnostic feature of Chl c -containing algae - a wide excitation band of Chl a in the region of 500 - 550 nm, caused by the Fucoxantin absorption and not typical for Chl b -containing algae[6].

The spectral features of different phytoplankton groups are listed in Tab. 1.

Table 1: Phytoplankton groups and their spectral features.

No	Phytoplankton group	Major accessory pigments	Excitation/ Emission, nm
1.	Blue - Green	Phycocyanins	620 / 640
2.	Blue - Green	Phycoerythrins	560 / 580
3.	Green	Chl b	480 / 680
4.	Golden - Brown	Carotenoids and Chl c	460, 520 / 680

To produce a quantitative analysis of pigment composition one needs to calibrate the corresponding intensity of the spectral signature by the pigment concentration, taking into consideration all processes of energy transport from accessory pigment to Chl a.

By taking an approach of non-saturated processes of energy transfer in a cell, the kinetics of Chl a excitation through accessory pigment may be described by the following equations:

$$dN^{(i)*}/dt = (N^{(i)} - N^{(i)*})\,\sigma^{(i)}\,\boldsymbol{F} - (p^{(i)} + p^{f(i)})\,N^{(i)*} - \boldsymbol{K}_{ia}\,N^{(i)*}$$

$$N_a\,dN^{(i)}\,a^*/dt = -(p_a + p_a^f)\,N_a^* + \boldsymbol{K}_{ia}\,N^{(i)} * N_a\,, \qquad (1)$$

where index **a** corresponds to Chl a, $N^{(i)}$ is the number of pigment molecules per cell in group ***i*** at non excited level; symbol * corresponds to the excited level of molecules; $\sigma^{(i)}$ is the absorption cross section of accessory pigment; $p^{(i)}$ and $p^{f(i)}$ are relaxation velocities of excited level by deactivation and fluorescence; $\boldsymbol{K}_{ia}$ is velocity constant of energy transfer from accessory pigment to Chl a; $\boldsymbol{F}$ - intensity of monochromatic light per sec.

When solving the system (1) in approximation of a stationary mode of excitation one can write the number of Chl a molecules excited through accessory pigment $N^{(i)}{}_a^*$ as follows:

$$N_a^{(i)^*} = \frac{n_a\,\boldsymbol{K}_{ia}\,n^{(i)}\,\sigma^{(i)}\,\boldsymbol{F}}{(p^{(i)} + p^{f\,(i)})\,(p_a + p_a^f)} \qquad (2)$$

where $n^{(i)}$ is the total number of pigment molecules per cell of group ***i***. The same approach gives the number of fluorescence photons produced by Chl a at direct excitation by light N_a^* as following:

$$N_a* = \frac{n_a\,\sigma_a\,\boldsymbol{F}}{(p_a + p_a^f)} \qquad (3)$$

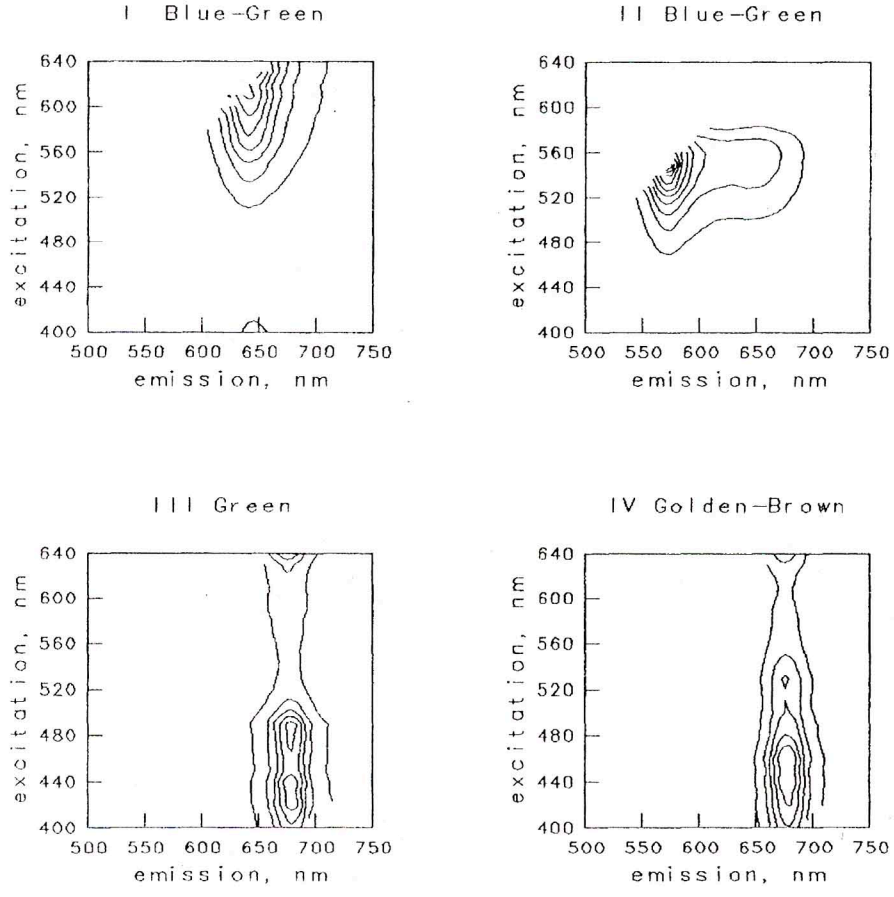

Fig. 1 -Some typical examples from different phytoplankton groups: I - Synechococcus bacillaris SYN; II - Synechococcus sp.; III - Platimonas viridis; IV - Isochrysis galbana.

The corresponding numbers of fluorescence photons $M^{(i)}_a$ produced by Chl a at excitation through accessory pigment ***i*** and at direct excitation M_a are equal to:

$$dM^{(i)}_a = p^f_a N_a^{(i)*} m_i \, dV \, dt , \quad dM_a = p^f_a N_a^{*} m_i \, dV \, dt \quad (4)$$

where m_i is the number of cells of group ***i*** per single volume. The ratio of fluorescence photons emitted by Chl a at different excitation is proportional to:

$$dM^{(i)}_a / dM_a = n^{(i)} \gamma^{(i)} \sigma^{(i)} / \sigma_a \quad (5)$$

In the expression (5) the value $^{(i)} = \boldsymbol{K}_{ia} / (p^{(i)} + p^{f(i)})$ characterises the efficiency of energy transfer from accessory pigment to Chl a. Thus the ratio (5) may be used for quantitative analysis of pigment concentration in the cell if the values of s_a, $\sigma^{(i)}$, and $\gamma^{(i)}$ were measured.

In lidar measurements the fluorescence is expressed by fluorescent factor $\Phi(\lambda_{ex}/\lambda_{em})$ which is determined as the ratio of the integral intensity of fluorescence band with maximum at the wavelength λ_{em} to the Raman backscatter signal of water molecules. By taking into account that in mixed phytoplankton community the excitation in a band of Chl a (direct excitation) will cause the fluorescence of Chl a in all groups of phytoplankton one can rewrite (5) for the fluorescent factors as following:

$$\frac{\Phi(\lambda^{(i)}_{ex}/685)}{\Phi(445/685)} = \frac{\xi^{(i)} \gamma^{(i)} \sigma^{(i)} n^{(i)}_a n^{(i)} m_i}{\sigma_a \sum_{i=1}^{4} n^{(i)}_a m_i} \quad (6)$$

where $\Phi(\lambda^{(i)}_{ex}/685)$ is the fluorescence factor of Chl a excited through accessory pigment ***i*** at the wavelength $\lambda^{(i)}_{ex}$; $n^{(i)}_a$ is a concentration of Chl a per cell in group ***i***; $\xi^{(i)}$ - lidar calibration constants, ***i=1,...,4.*** .

When one of phytoplankton group is dominative, the sum $\Sigma\, n^{(i)}_a m_i$ will be equal to the amount of Chl a in this group. In such a case the ratio $\Phi(\lambda^{(i)}_{ex}/685)/\Phi(445/685)$ is directly proportional to the concentration of accessory pigment $n^{(i)}$. In general case the ratio (6) may be used to diagnose the changes of phytoplankton composition over the sensed area. To provide such a measurements one has to use at least two wavelengths of excitation. The first one must be located in the main absorption band of Chl a (445 nm) and aimed to excite Chl a directly. The second one is selected to be placed into absorption band of accessory pigment ($\lambda^{(i)}_{ex}$).

3. DISCUSSION OF RESULTS

The measurements were performed in August 1991 and July 1992 in the Pojoviken Bay, south-west coast of Finland. The study area is characterized by highly changeable species composition of phytoplankton on small spatial scale caused by complicated hydrophysical picture of the mixing of fresh and salty waters[7]. The remote sensing was carried out on board of small research vessel SADURIA by tunable lidar KLS-10 based on the dye - laser pumped by excimer laser. Sensing was made along the tracks of 15 km with spatial resolution 50 m over the surface. The dye - laser was scanning from pulse to pulse to use different sensing wavelengths. During the underway measurements the fluorescence spectra of phytoplankton integral over the depth of 15 m were registered by gated linear CCD - detector. All measuring tracks were started in the fresh water zone (distances 0 -3 km on the tracks, surface salinity <2.5‰) and gone through mixed waters (3 - 8 km, salinity 4.5‰) to sea water zone (8 - 10 km, salinity 5.7‰)[8]. The water transparency measured by Secchi disk was changed from 2.6 m in fresh water zone to 4.9 m in sea water area. The time of measuring the track did not exceed 3 hours what allowed one to consider roughly the irradiation conditions and the state of water environment stable during the sensing cycle. The water samples were collected at some stations situated along the tracks to provide the pigment's extraction and counting of phytoplankton. At the same stations the remote spectral signatures of phytoplankton were measured.

The major pigments of groups I and II are easily detectable by using the sensing wavelength 515 nm to excite Phycocyanin and 525 nm for Phycoerythrin. Wavelengths actually selected are shorter than the values most effective for the excitation of these pigments. But such shift allows one to avoid overlapping of fluorescent bands with Raman scattering signal in the spectral response. The relative concentrations of Phycocyanin and Phycoerythrin are expressed by fluorescent factors $\Phi(515/640)$ and $\Phi(525/580)$ correspondently. Fig. 2. shows an example of Phycoerythrin and Phycocyanin distributions in the study area. The relative high concentration of both pigments in the fresh - water and sea water zones is caused by presence of Cyanophyceae. The phytoplankton distribution in the mixed water area is changeable and depends on the water streams entering the Gulf. The relative distribution of Phycocyanin and Phycoerythrin gives the information about the ratio of phytoplankton corresponding to I and II groups. Actually the concentration of Phycocyanin in the fresh - waters is greter than in sea water zone. This result is correlated with the data of phytoplankton counting. It was revealed the high amount of Oscillatoria containing Phycocyanin in the beginning of the track and increasing of picoplanktonic Synechococcus sp. numbers when moving to the open sea.

The situation in which one has to distinguish between groups III and IV is more complicated, as their accessory pigments (Chl b, Chl c, Carotenoids) do not fluoresce *in vivo*. To diagnose Chl b and Fucoxantin by the method

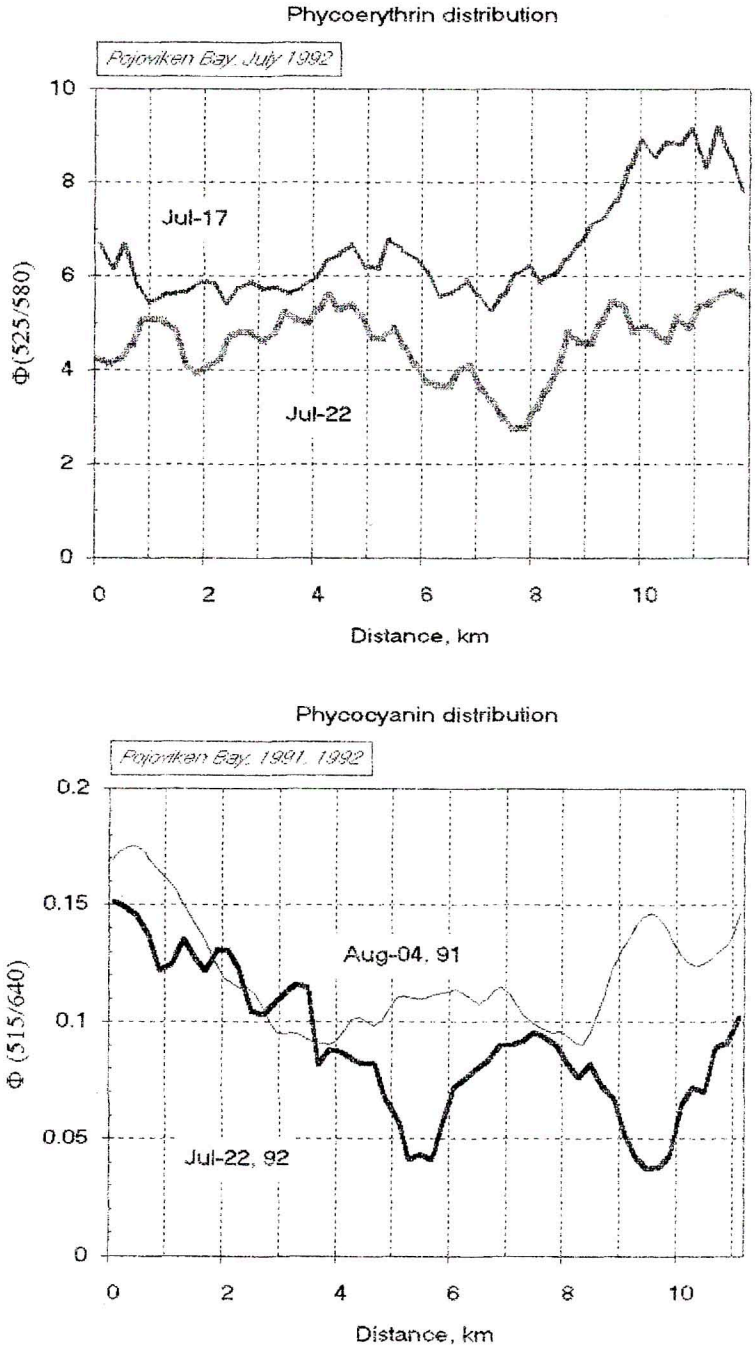

Fig. 2 - Spatial distribution of Phycoerythrin (a) and Phycocyanin (b).

described above the wavelengths 480 nm and 515 were used additionally to direct excitation of Chl a at 445 nm. So far as the intensity of fluorescence of Chl a excited through accessory pigment is proportional to both concentrations of accessory pigment and Chl a (6) the distribution of $\Phi(\lambda^{(i)}_{ex}/685)$ does not correspond simply to the accessory pigment distribution over the water area. At the same time the distribution of selected group of phytoplankton can be revealed by comparison of Chl a fluorescence at direct excitation and excitation through accessory pigment.

Fig. 3 shows one of the examples of remote sensing of Chl b and Carotenoids containing phytoplankton by two wavelengths of excitation.

In the mean part of the track the value of Φ(515/685) for Fucoxantin is increasing when Φ(445/685) has no expressed maximum (Fig.3a). The expressed peak of the ratio Φ(515/685)/Φ(445/685) in the mixed area corresponds to the high concentration of Dinophyceae (*Glenodinium sp.*) and Prymnesiophyceae (*Chrysochromulona sp.*) counted in water samples and containing fucoxantin and its derivatives.

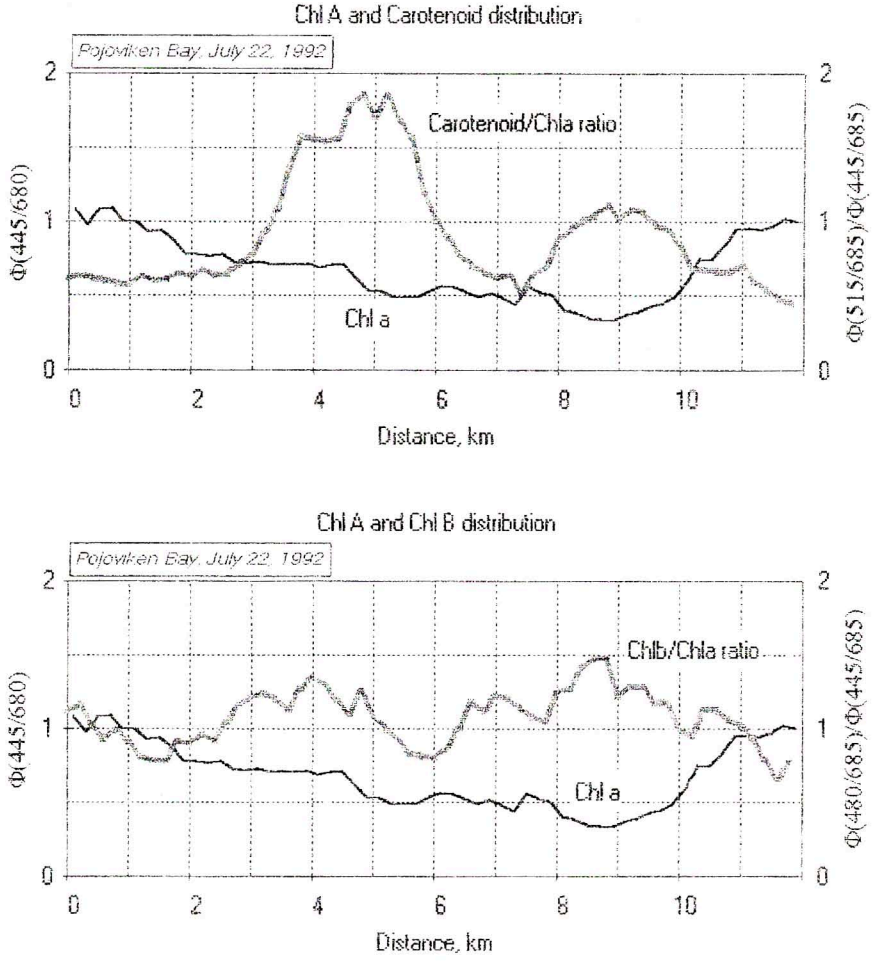

Fig. 3 - Spatial distribution of Carotenoids (a) and Chl b (b).

Relative high concentration of Chl b in the beginning of track is caused by presence of Chlorophyceae in fresh water zone (Fig.3b). Smooth distribution of Chl b (III group) is caused by mixed Prasinophyceae in the study area up to sea waters.

The two - dimensional spectral signatures measured by lidar at the stations produce more detail information about pigment's ratio in a fixed point of aquatoria (Fig. 4) and may be used to identify the dominant group of phytoplankton.

CONCLUSION

The first natural experiments in remote sensing of brackish - water phytoplankton by method of Selective Excitation of Phytoplankton Pigments revealed the possibilities of rapid remote diagnostics of pigment composition in mixed phytoplankton community. To realize such an approach the tunable lidar system is necessary. The sensing wavelengths must be situated in the absorption bands of accessory pigments and Chl a. By comparison of spatial distribution of Chl a fluorescence excited directly and trough accessory pigment it is possible to detect the changes of taxonomic groups of phytoplankton. The remote data must be calibrated separately for different groups of phytoplankton.

The combination of underway lidar measurements and descrete measurements of spectral signatures in characteristic points of aquatoria enables quick evaluation of the water quality, including the registration of annual succession and anomalous nuisance blooms of phytoplankton.

We are very grateful to the staff of Tvärminne Zoological Station, which made it possible to curry out the study. We thank also the specialists of Laser Diagnostic Instruments Ltd. for technical assistance in remote measurements and Dr. Aleksey Shalapjonok (Institute of Biology of South Sea's) for useful discussions.

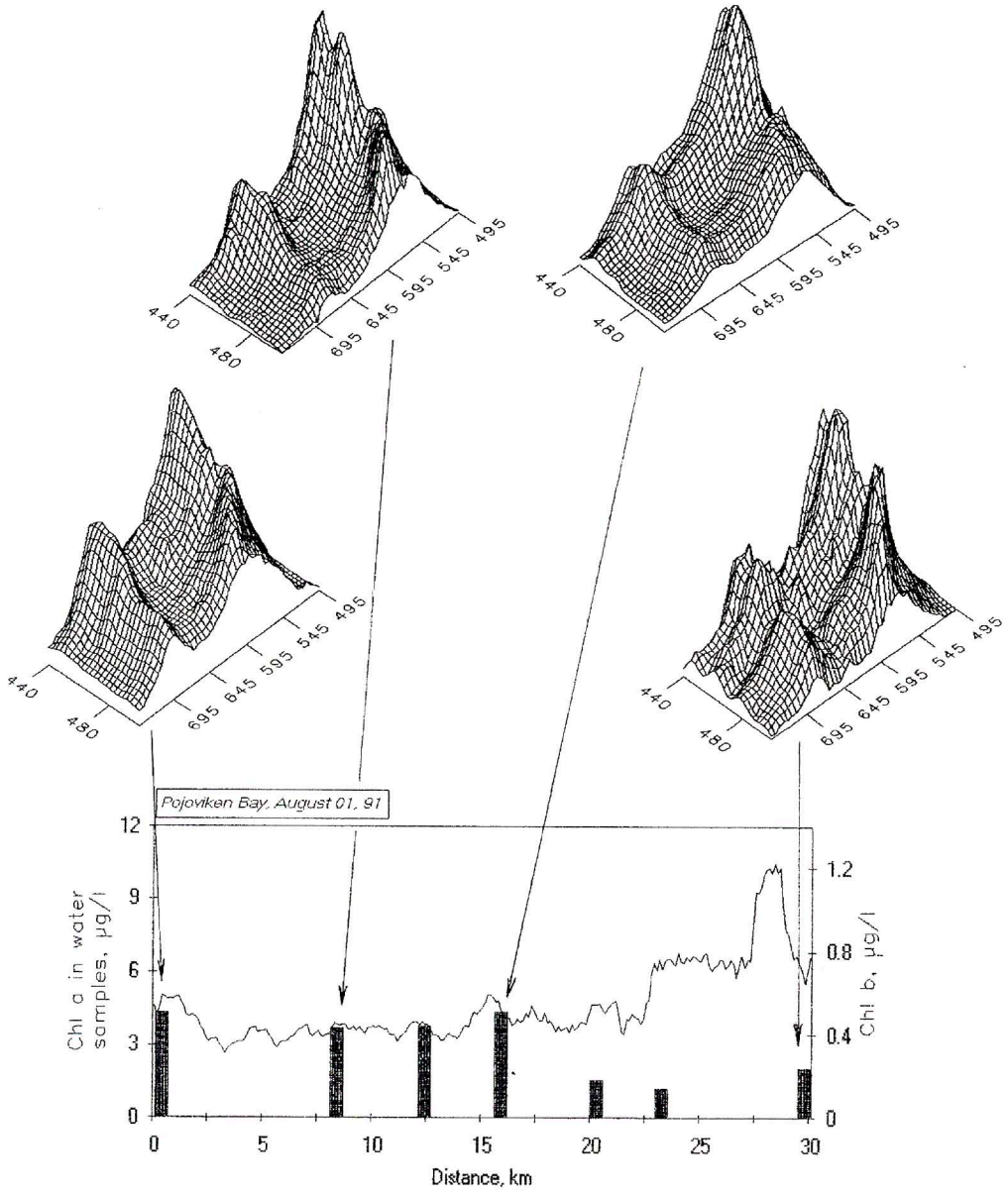

Fig. 4 - Spectral signatures of phytoplankton measured by lidar at the stations along the track. The concentration of Chl a in water samples is represented by bars.

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