Moscow University Physics Bulletin
Vol. 46, No. 6, pp. 59–65, 1991

Vestnik Moskovskogo Universiteta. Fizika
UDC 535.372.2

METHOD OF REMOTE LASER MONITORING OF PHOTOSYNTHESIS EFFICIENCY IN PHYTOPLANKTON

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The method and equipment are described which allow remote measurements of the horizontal distributions of the phytoplankton photosynthetic activity to be carried out *in situ* aboard a moving vehicle (ship, plane, or helicopter). In the course of measurements the relative yield of the variable fluorescence of chlorophyll *a* is determined, this quantity characterizing the efficiency of light utilization in the primary reactions of photosynthesis. It is shown that for the measurements to be correct, the photon flux density of the activating pulse in the volume probed must be no smaller than 5×10^{22} cm^{-2} s^{-1}, that of the probing pulse must not exceed 10^{22} cm^{-2} s^{-1}, and a time interval of 40-70 μs between these pulses must be ensured. The results of laser determinations of the photosynthetic activity of phytoplankton over extensive water areas of the North-West Atlantic are discussed.

1. INTRODUCTION

Because of its unique ability to photosynthesize, the phytoplankton plays the key role in the functioning of ecosystems in seas, oceans, and inland water basins. It is the primary link of the trophic chain, supplies oxygen supply to the water and atmosphere and controls the carbon dioxide content there. In view of the urgency the problems of ecological control of these processes have recently acquired, there is a need to develop up-to-date rapid methods of assessing the photosynthetic activity whose potentialities would be adequate to the problems faced.

To assess the photosynthesis efficiency various characteristics of the process can be measured: the rate of carbon dioxide fixation, oxygen release, relative yield of the variable fluorescence of chlorophyll, the delayed luminescence, and others [1, 2]. We shall not dwell here on a comparative characteristic of these methods, which have shown themselves to advantage under laboratory conditions; we would only like to indicate a feature that is common to all of these methods: their contact character, i.e., the necessity of working with samples, which makes difficult the application of these methods to ecological monitoring problems.

In the present communication we propose a remote laser method of assessing the efficiency of phytoplankton photosynthesis which enables rapid measurements to be carried out *in situ* with a high spatial resolution aboard a moving ship, helicopter, or plane. The method was developed on the basis of the studies on remote laser monitoring of the phytoplankton fluorescence intensity carried out in our laboratory [3-5], and also investigations into the mechanisms of fluorescent response of phytoplankton to powerful pulsed photoexcitation [6, 7].

2. METHOD OF DETERMINING THE RELATIVE YIELD OF THE CHLOROPHYLL VARIABLE FLUORESCENCE

The photosynthesis efficiency in algae and higher plants in many respects depends on the state of the reaction centers of photosystem II (PSII) [2]. Under favorable external conditions the majority of the PSII reaction centers are in the open state, and ensure a maximum efficiency of utilization of the energy of absorbed light in the primary photosynthesis reactions; a deterioration of the environmental conditions (shortage of mineral nutrients, presence of toxicants, etc.) brings about an increase in the proportion of "closed" (or inactive) PSII reaction centers, and the efficiency of utilization of the absorbed light energy diminishes.

For a quantitative assessment of the photosynthesis efficiency wide use is made of the method of measuring the relative yield of the variable fluorescence of chlorophyll *a* [2]. This method is based on the fact that transition of the PSII reaction centers to the "closed" state with a reduced primary quinone acceptor Q_A (for instance, on addition of diuron or under the effect of a saturating flash of light) causes an increase in the intensity of chlorophyll fluorescence from the initial level I_{fl}^0 to the maximum level I_{fl}^{max} due to the addition of variable fluorescence [1, 2].

The difference $I_{fl}^{max} - I_{fl}^0$, normalized on I_{fl}^{max}, characterizes the efficiency of utilization of the absorbed energy of light in the primary reactions of photosynthesis [8, 9] (in the subsequent exposition we shall use the term "photosynthesis efficiency" for the sake of brevity). For the majority of algae species in good functional state, the value of $\eta = (I_{fl}^{max} - I_{fl}^0)/I_{fl}^{max}$ ranges within 0.6-0.7 and comes down to 0.1-0.3 under the effect of various unfavorable factors [2, 9].

3. METHOD OF LASER DETERMINATION OF THE RELATIVE YIELD OF THE CHLOROPHYLL *a* VARIABLE FLUORESCENCE

The proposed method of remote determination of the photosynthesis efficiency in phytoplankton is in fact a laser modification of the contact method of measuring the relative yield of the variable fluorescence η with the use of saturating and probing flashlight pulses [9, 10]. Remote operation of the method and the possibility to determine η *in situ* aboard a moving vessel are provided by the use of two locked-in pulsed lasers (an activating laser and a probing laser) to excite the fluorescence of algae in the near-surface layer of water.

To determine the initial level of chlorophyll *a* fluorescence (I_{fl}^0) single probing pulses are used, as in the case of remote monitoring of the phytoplankton fluorescence intensity [3-5]. When the maximum fluorescence level (I_{fl}^{max}) is measured, excitation is effected with double pulses of the activating and probing lasers, directed to one and the same volume of water with a time lag. The pulses of the first laser are used as the activating ones for bringing the PSII reaction centers over to the closed state. The fluorescence intensity I_{fl}^{max}, corresponding to the closed PSII reaction centers, is determined from the spectrum of an echo-signal recorded in response to the probing pulse.

In the course of remote laser monitoring [3-5], optical spectra are recorded that contain bands of the chlorophyll *a* fluorescence of phytoplankton and bands of spontaneous Raman scattering of water. As a quantitative measure of the fluorescence intensity, use is made of the fluorescence parameter $\Phi = I_{fl}/I_R$, where I_{fl} and I_R are the intensities of the phytoplankton fluorescence and of the Raman scattering of water in the spectrum of the echo-signal (the "internal reference" method [3]). This parameter is practically independent of the distance to the water surface and of the state of the surface (foam, waves) [3, 5], this being of special importance in remote measuring from a moving vessel. It is apparent that with the internal reference method the relative yield of the variable fluorescence is determined by the formula $\eta = (\Phi^{max} - \Phi^0)/\Phi^{max}$. The values of Φ^{max} and Φ^0 are calculated from the spectra obtained upon excitation with an activating pulse and without preliminary activation.

4. OPTIMIZATION OF LASER EXCITATION MODES

For correct determination of the phytoplankton photosynthesis efficiency by the method of remote laser probing, the modes of algae fluorescence excitation must meet certain requirements.

First, the photon flux of the activating pulse onto the volume probed must be sufficiently powerful for the pulse-induced processes to bring about the transition of the majority of the initially open PSII reaction centers to the closed state.

Second, the time lag between the activating and the probing pulses must be chosen so that it be sufficient for the fluorescence quenchers induced by the activating pulse to become deactivated, yet insufficient for the reduced primary quinone acceptor Q_A to become oxidized by the secondary acceptor Q_B [1, 2].

Third, when measuring the initial Φ^0 and the maximal Φ^{max} of the fluorescence parameter, one must take into account the specific character of powerful pulsed laser photoexcitation, in particular, the effect of fluorescence saturation [6, 7, 11].

Development of a laser procedure for determining the relative yield of variable fluorescence with consideration for the above requirements was carried out under laboratory conditions on pure cultures of algae

and also under field conditions aboard the *Vitiaz* research ship in the North-West Atlantic in the spring of 1990. Measurements were carried out with a laser spectrofluorimeter-lidar [5], incorporating an additional YAG:Nd^{3+} laser for generating activating pulses. The main results of the investigations are presented below.

1. Figure 1 shows the results of measuring the dependence of the intensity of fluorescence Φ^p excited by the probing pulse on the photon flux density F_A of the preceding activating pulse for the green algae *Chlorella vulgaris*. As F_A increases, the number of the PSII reaction centers closed by the pulse grows and, as a consequence, the intensity of the fluorescence Φ^p excited by the subsequent probing pulse grows as well.

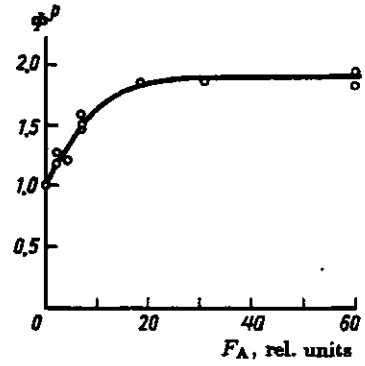

Fig. 1

Dependence of the intensity of chlorophyll *a* fluorescence excited by the probing pulse on the photon flux density F_A of the preceding activating pulse for green algae *Chlorella vulgaris*. The time lag between the pulses $\tau = 50$ μs; maximum photon flux density $F \cong 2 \times 10^{23}$ cm^{-2} s^{-1}.

An analysis of the Φ^p versus F_A curves for a number of marine and fresh-water algae has shown that in probing on the wavelength of 532 nm with 10 ns pulses the photon flux density F_A in the probed volume must be higher than 5×10^{22} cm^{-2} s^{-1} for the value of Φ^p to differ from its maximum value $(\Phi^p)^{max}$ by no more than 10%.

2. With a view to optimizing the time lag τ between the activating and the probing laser pulses for different cultures of algae, the relationship between the intensity of the fluorescence Φ^p excited by the probing pulse and the time lag τ was measured under laboratory conditions.

A typical experimental curve is presented in Fig. 2. The initial portion of the Φ^p rise in Fig. 2 is defined by the process of deactivation of the fluorescence quenchers induced by the activating pulse and by the transfer of the PSII reaction centers to the closed state by *P*680, Pheo Q_A^- [9, 10] (*P*680, Pheo are the primary donor and the intermediate acceptor of the PSII reaction centers, respectively). From 40 to 70 μs after having reached the maximum Φ^{max}, Φ^p begins to diminish as a result of oxidation of Q_A^- by the secondary acceptor Q_B. The characteristic time of this process is 200-800 μs [1, 2]. Our measurements showed that Φ^{max} is only 10-15% lower than the fluorescence level recorded in the presence of diuron.

Thus, as in the case of excitation with flashlight pulses [9, 10], a time lag of 40-70 μs between the activating and the probing pulses proved to be optimal for determining the relative yield of the variable fluorescence.

3. In using laser pulses for the excitation of algae fluorescence, one should take into account the fluorescence saturation effect, which manifests itself in the appearance of a nonlinear dependence of the fluorescence intensity on the intensity of the probing laser radiation [6, 11].

Figure 3 shows the curves of the initial (Φ^0) and the maximum (Φ^{max}) fluorescence intensity level versus the photon flux density F of the probing pulse. The activating laser pulse with $F \cong 10^{23}$ cm^{-2} s^{-1} used to measure Φ^{max} was 50 μs ahead of the probing pulse. The saturation effect in this case causes a diminution of the value of Φ as F grows, since the intensity of the normalizing signal of the Raman scattering of water is proportional to F.

The differences in the behavior of these curves can be explained by different mechanisms of saturation of the constant and the variable components of the fluorescence [6] and, accordingly, by a more rapid saturation

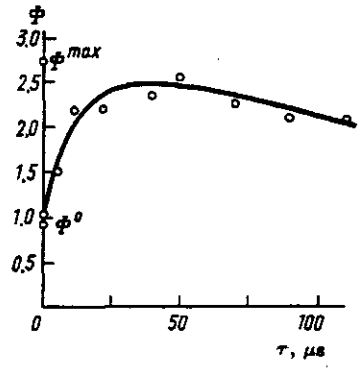

Fig. 2

Intensity of chlorophyll *a* fluorescence excited by the measuring pulse versus the time lag τ between the pulses. The photon flux density of the activating pulse $F_A \cong 10^{23}$ cm^{-2} s^{-1}; Φ^0 and Φ^{max} are the initial and the maximum (in the presence of diuron) values of the fluorescence parameter.

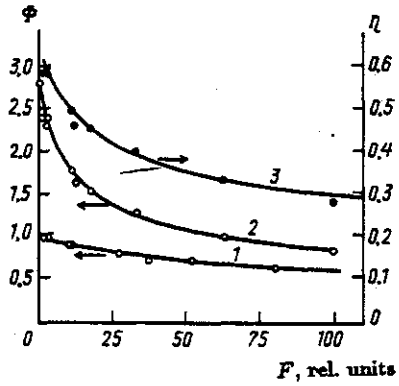

Fig. 3

Saturation curves of chlorophyll *a* fluorescence (algae *Chlorella vulgaris*): dependence of the fluorescence intensity (fluorescence parameter) Φ on the photon flux density F of the probing laser radiation for the initial level of the fluorescence intensity Φ^0 (1) and for the maximum level Φ^p (2); the relative yield of the variable fluorescence η as a function of F (3). The maximum photon flux density $F \cong 5 \times 10^{23}$ cm^{-2} s^{-1}.

of the latter component with increasing F. The result is that the value of the relative yield of the variable fluorescence, $\eta = (\Phi^{max} - \Phi^0)/\Phi^{max}$, thus determined depends on the photon flux density F of the probing pulse and diminishes as this density increases (curve 3 in Fig. 3).

A proper consideration for the fluorescence saturation effect is not an easy problem, even if measurements are carried out under laboratory conditions [11]. When η is determined remotely, from a moving vessel, the difficulties increase, because the effective photon flux density of the probing pulse in the water near-surface layer constantly changes as a result of waves, foaming, changes in the probing geometry, and other factors that are difficult to control. Therefore, when a sufficiently powerful probing radiation is used, the values of η will change in an uncontrollable manner in the course of measurements because of variations in the fluorescence saturation.

A way out of this situation may lie in limiting the photon flux density in the probing pulse to F_{max}, since at this value the effect of chlorophyll *a* fluorescence saturation in algae may be neglected with a known accuracy. In such a case the measured values of η will be close to the value of the unsaturated parameter

$\eta_0 = \lim_{F \to 0} \eta(F)$, which is independent of F and coincides with the value of this parameter measured by the two-pulse flashlight procedure [9, 10]. The estimates obtained by us as a result of measuring the saturation parameters of the fluorescence of various algae cultures have shown that for the values of the relative yield of the variable fluorescence η, as measured by the laser technique, not to differ from η_0 by more than 10%, F_{max} must not exceed 10^{22} cm^{-2} s^{-1}.

Thus, in remote laser assessments of the relative yield of the variable fluorescence, an optimization of the excitation regime requires, as follows from Sections 1-3, that the following conditions should be met.

(i) The photon flux density of the activating pulse in the water volume probed must not be lower than 5×10^{22} cm^{-2} s^{-1}.

(ii) The time lag between the probing and the activating pulse must be 40-70 μs.

(iii) The photon flux density of the probing pulse must not exceed 10^{22} cm^{-2} s^{-1}.

We have taken these requirements into consideration when developing the equipment and procedure for remote measurements of the photosynthesis efficiency; these requirements constituted the basis for making a number of design and methodological decisions.

5. EQUIPMENT AND PROCEDURE FOR MONITORING THE PHOTOSYNTHESIS EFFICIENCY

The set of equipment was built around a ship lidar, which has shown itself to advantage under marine conditions and which was employed for remote monitoring *in situ* of the phytoplankton fluorescence

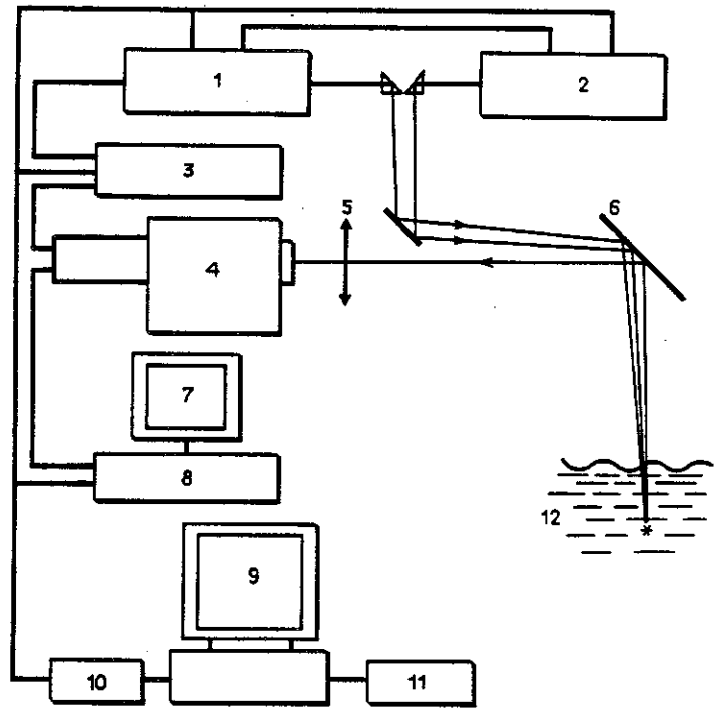

Fig. 4

Schematic diagram of a lidar: activating and probing lasers (1, 2); generator of high-voltage pulses that enable the receiver of the optical multichannel analyzer (OMA) (3); OMA polychromator and detector (4); objective lens (5); turnable mirror (6); OMA monitor (7); OMA bracket (8); microcomputer (9); interface (10); graph plotter (11); outboard water (12).

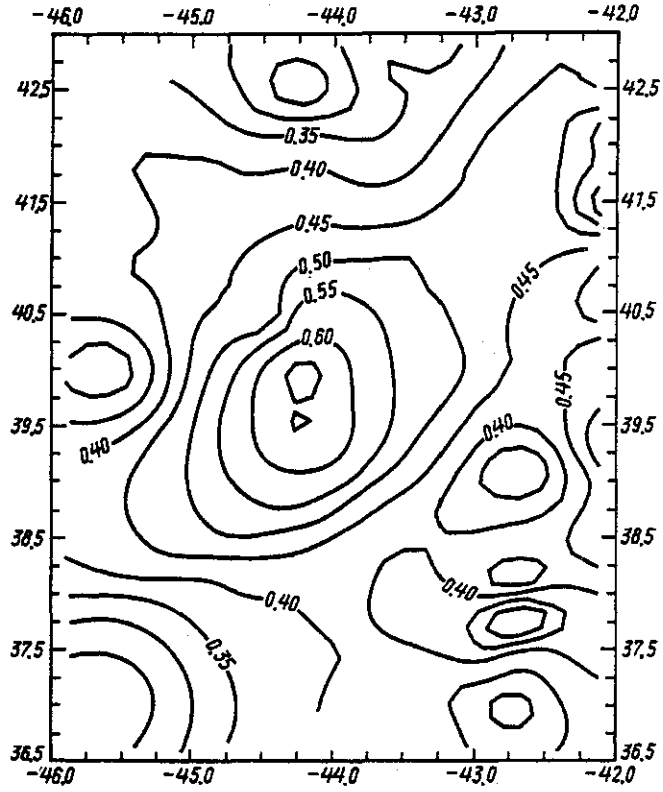

Fig. 5

Diagram illustrating the distributions of the relative yield of the chlorophyll variable fluorescence in the near-surface phytoplankton. The diagram is based on the results of measurements made in the North-West Atlantic in May 1990. The X-axis shows degrees of west longitude and the Y-axis those of north latitude.

intensity [4, 5]. We had to introduce an additional laser emitter into the equipment set, because it is technologically difficult to provide generation of two pulses that would meet the above-cited requirements with the use of one laser emitter. The optical system of the lidar and the software were modified accordingly.

The schematic diagram of the set is presented in Fig. 4. The set comprises the following units and systems: two pulsed YAG:Nd^{3+} frequency-doubled lasers, operating in synchronism with a variable 2-150 μs delay; an adjusting optical system; an optical multichannel analyzer (OMA); and a microcomputer interfaced with the above devices. The first and second lasers emit activating and probing pulses with pulse powers of 3 and 0.6 MW and repetition frequencies of 5 and 10 Hz, respectively. The emission wavelength is 532 nm, the divergence is 5×10^{-3} rad. These characteristics of the lasers ensure the required conditions of exciting the phytoplankton fluorescence at a distance to the water surface of 10-15 m in a layer 3-6 m thick.

The optical system of the lidar ensures a coaxial entry of the activating and probing pulses into the near-surface water layer and the delivery of the echo-signal to the entrance slit of the OMA, which carries out spectral analysis of this signal. Gating of the OMA detector with 1 μs enabling pulses synchronously with the probing pulses of the laser allows selective recording of the echo-signal that originates in response to the probing pulses. The microcomputer controls the devices of the set and processes the spectral information coming from the OMA.

In the course of measurements the near-surface layer is excited alternately with single probing pulses (for the determination of Φ^0) and with probing pulses doubled with the activating ones (for the determination of Φ^{max}). For the signal/noise ratio to be satisfactory, additive accumulation of the laser pulse spectra corresponding to "single" and "doubled" excitation, is carried out for several tens of pulses in different cubes

of the computer memory. Then the results of measurements are averaged over the portion of the route traveled by the vessel during the period in question. Depending on the state of the water surface, the length of the averaging section of the route may vary from 10 to 100 m with the lidar located aboard the ship.

The minimal distance between the points in which successive measurements are carried out is determined by the product of the time required for recording the spectrum by the receiving system and for data processing and the speed of the moving vessel. In our case this distance amounts to 200-300 m, and this figure determines the spatial resolution of the method.

We tried out the method of laser assessment of the photosynthesis efficiency in the spring of 1990 in the North-West Atlantic. Systematic measurements in the region of mixing of the warm Gulf Stream current with the cold Labrador current in the period of spring efflorescence gave valuable data on the character and scope of horizontal variability of the phytoplankton photosynthesis efficiency.

The diagram presented in Fig. 5 and illustrating the distribution of the relative yield of the variable fluorescence η, is of special interest. This diagram was plotted on the basis of the results of measurements performed in the testing area of 350 by 500 km in size, located between 37° and 44° north latitude and between 42° and 46° west longitude. Without going into a detailed analysis of the distribution particulars, we would like to point to a considerable variability of the parameter η not only on the synoptic scale, but on the mesoscale as well. Within the testing area three zones of high photosynthesis efficiency ($\eta \cong 0.5$-0.7) and three regions of very low photosynthesis efficiency ($\eta \cong 0.2$-0.3) were registered. The zones of high photosynthesis efficiency coincided with the anticyclonic vortex formations found in the process of hydrological survey.

The most effective is analysis of laser monitoring results by comparing the fluorescence intensity distributions and the relative yield of the variable fluorescence. As the first parameter carries information on the concentration of chlorophyll *a* [5, 12] and the second on the photosynthesis efficiency, using this approach one can not only obtain rapid information on the specific features of the horizontal distributions of the phytoplankton and on its photosynthetic activity within vast water areas, but also predict the situation development.

In conclusion the authors express their gratitude to L. V. Ilyash and V. A. Polynov from the Biological Department for providing pure algae cultures.

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1 March 1991 Department of Quantum Radiophysics