

Laser induced saturation of DOM fluorescence in natural water

S. Patsayeva, R. Barbini⁺, F. Colao⁺, R. Fantoni⁺, A. Palucci⁺ and R. Reuter⁺⁺

Moscow State University, Physics Department, Moscow, 119899 Russia

email: svetlana@lidar.phys.msu.su

⁺ ENEA, Dip. Innovazione, Divisione Fisica Applicata

CR Frascati, C.P.65, 00044 Frascati, Italy

email: Barbini@frascati.enea.it

⁺⁺ Carl von Ossietzky Universität Oldenburg, FB Physik, D-26111 Oldenburg, Germany

email: r.reuter@las.physik.uni-oldenburg.de

ABSTRACT

In remote sensing of natural waters using luminescence techniques the remotely detected data need to be carefully calibrated and adjusted to laboratory conditions. One of the phenomena that can complicate the calibration procedure is the effect of fluorescence saturation. Saturation becomes important if the rate of chromophore excitation exceeds the rate of their deactivation. Especially when measuring fluorescence with laser pulse excitation this effect appears as a non-linearity of the detected signal versus the intensity of excitation pulses. This is noticeable if the photon flux exceeds about 10^{24} photons per second and cm^2 , which corresponds to laser pulses of about 1 MW and is typical in many laboratory experiments.

In this paper we report on the theory of the effect and on experimental results found with natural water samples. On the basis of the non-linear balance equations for photon absorption and spontaneous emission a kinetic theory is developed. Solutions for quasi-stationary and non-stationary conditions are given which include singlet-singlet annihilation as an additional non-radiative deactivation channel. It has been observed in experiments that the normalised fluorescence of marine water samples measured at low excitation intensity can be two times higher than excited with 10 mJ pulse energy. The data are interpreted in view of the theory, and possible explanations are given for the observed fluorescence saturation of complex mixtures of organic molecules such as dissolved organic matter in water.

1. INTRODUCTION

Significant analyses of the aquatic environment require sensitive and accurate techniques for remote monitoring of dissolved organic matter (DOM) and phytoplankton chlorophyll in natural water. Dissolved organic matter is an important part of natural water systems and is related to their ecological state. Currently, fluorescence techniques using water Raman scattering

for fluorescence standardisation are widely applied for measurements of these organic compounds.¹⁻⁴ These techniques are based on the hypothesis that a linear relation exists between normalised fluorescence intensities and organic matter concentrations.

However, a phenomenon that often complicates the calibration procedure is the effect of fluorescence saturation. This is caused by the limited life-time of molecules in their excited state and depends on the intensity of light exciting the sample.⁵⁻⁷ It becomes noticeable at irradiation levels of 10^{24} photons per second and cm^2 . These are typical with most laser-based laboratory fluorimeters having a laser peak power in the order of 1 MW and higher.

This situation has to be taken into account in calibration exercises for remote sensing, that is when remotely measured fluorescence data shall be adjusted to *in situ* data ("ground truth"). In airborne or shipboard remote sensing experiments the power of a laser beam is several orders of magnitude lower at the water surface than in laboratory experiments with the same type of laser source. Then, an increase of remotely sensed fluorescence signals from organic substances, normalised to water Raman scattering, can result if such data are compared with measurements in the laboratory for the given type of water.

This effect leads to an overestimation of the content of organic substances in natural waters. Its value depends on characteristics of the laser source such as its wavelength, pulse duration and power, spectral properties of the fluorophores such as fluorescence life-time and absorption cross-section, and the presence of luminescence quenchers in the water is relevant as well.

Several publications^{5,6,8,9} report on fluorescence saturation of organic dyes in water. This technique, called spectroscopy of saturated fluorescence, makes use of strong pulse lasers for fluorescence excitation. In several publications the effect of fluorescence saturation was studied for dissolved organic matter of natural origin^{10,11} and chlorophyll a in phytoplankton.^{12,13}

In this paper, we report on a solution of the kinetic balance equations for photon absorption and fluorescence emission with quasi-stationary and non-stationary excitation. An analytical solution is given that includes singlet-singlet annihilation as a further non-radiative deactivation process, not reported previously. Laser spectroscopic experiments were done with the goal to identify the relevance of these effects in fluorometric studies of dissolved organic matter in seawater.

2. THEORY

A. Terminology

The exciting photon flux density ρ denotes the number of photons at the excitation wavelength per (cm² s) illuminating a water sample. In this section we consider a simplified model of an organic molecule with two electronic energy levels (that is, ground and first excited singlet state) and vibrational sub-levels. B_{12} , A_{21} , and B_{21} are the Einstein coefficients of absorption, spontaneous and stimulated emission for transitions between the ground state and the first excited singlet state. A_{21}^* is the rate of non-radiative deactivation processes.

Because electronic levels of organic compounds in solutions are broad, a molecule is transferred by photon absorption to a vibrational sub-level of the excited electronic singlet state. Photon emission starts from the lowest vibrational sub-level of the excited singlet electronic level due to very fast intra-level deactivation. Hence the energy of the absorbed photon is not the same as the emitted one, and the Einstein coefficient for stimulated emission becomes $B_{21}=0$. In the following we set $B_{12} = B$ for brevity.

The Einstein coefficient for absorption B has also the meaning of an absorption cross-section in the Bouguer-Lambert-Beer absorption law by an optically thin layer, $I = I_0 \exp(-Bnl)$, where I_0 and I denote the intensities of the initial and attenuated light beams, with l being the length of the optical path, and n the number of absorbing molecules in the illuminated volume.

This does no longer hold in the presence of high photon numbers, for example due to intense laser irradiation. Then the rate of molecular excitation is higher than the rate of deactivation (radiative and non-radiative). The medium becomes more transparent at the wavelength of irradiation. This effect is called saturation of population in the excited state, and the fluorescence measured under such conditions is called saturated fluorescence. Hence, fluorescence saturation results in a deviation from a linear dependence of the fluorescence intensity versus the intensity of the exciting light.

B. Fluorescence saturation

In a first approach we consider idealised exciting pulses with a square spatial and temporal distribution. The sample is homogeneously irradiated. The excitation photon flux density $\rho(t)$ is assumed to be constant during the laser pulse $0 < t \leq \tau_p$, and it is $\rho(t)=0$ at $t < 0$ and $t > \tau_p$. Singlet-triplet transfer of organic molecules, and the interaction of excited molecules causing fluorescence quenching (singlet-singlet annihilation) are neglected.

The population n_2 of the excited level changes due to photon absorption by molecules in the ground state. The number of excited molecules increases according to

$$dn_2^+ = n_1 \rho B dt,$$

where n_1 is the population density in the ground state. A reduction of the excited state population is caused by spontaneous emission and non-radiative deactivation including quenching by other molecules:

$$dn_2^- = n_2 A dt,$$

where we set $A = A_{21} + A_{21}^*$. The kinetic equation for the number of excited molecules becomes:

$$dn_2 = (n_1 \rho B - n_2 A) dt.$$

The total number of absorbing molecules $n_0 = n_1 + n_2$ is constant during the observation time, if singlet-triplet conversion and photochemical processes are not taken into account. To find the molecules n_2 in the excited state we use a system of two balance equations:

$$\begin{cases} \frac{dn_2}{dt} = n_0 B \rho(t) - n_2 (A + B \rho(t)) \\ n_0 = n_1 + n_2 \end{cases}$$

The square laser pulse as a boundary condition leads to

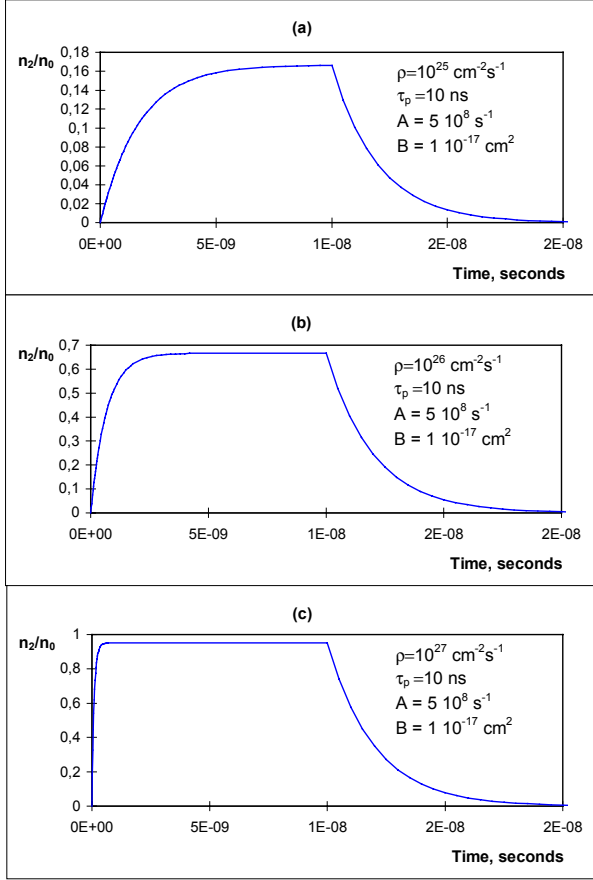
$$\begin{cases} \frac{dn_2}{dt} = n_0 B \rho - n_2 (A + B \rho) & 0 < t \leq \tau_p \\ \frac{dn_2}{dt} = -A n_2 & t > \tau_p \end{cases}.$$

The solution for the excited state population $n_2(t)$ is then:

$$n_2 = \frac{n_0 B \rho \{1 - \exp[-(A + B \rho)t]\}}{A + B \rho} \quad 0 < t \leq \tau_p$$

$$n_2 = \frac{n_0 B \rho}{A + B \rho} \frac{[\exp(A \tau_p) - \exp(-B \rho \tau_p)] \exp(-At)}{\exp(A \tau_p) - \exp(-B \rho \tau_p)} \quad t > \tau_p$$

The temporal behaviour of the relative excited state population n_2/n_0 is illustrated in Figs. 1a-c with different values of the excitation photon flux density.



During illumination with the laser pulse ($0 < t \leq \tau_p$) the density of excited molecules grows according to

Fig. 1a-c: Laser-induced saturation of the excited state, assuming a „square“ excitation pulse.

$\{1 - \exp[-(A + B\rho)t]\}$ up to the maximum value at the end of a pulse. As it is seen from formula the stationary density of excited molecules $n_{stat} = \lim_{t \rightarrow \infty} n_2(t)$ is $n_0 B\rho / (A + B\rho)$. This value can be reached during the pulse if $(A + B\rho)\tau_p \gg 1$. That means “quasi-stationary” excitation by the prolonged pulses of duration $\tau_p \gg \tau = 1/A$. In case of pulses when $\tau_p \sim \tau = 1/A$ the stationary density of excited molecules $n_0 B\rho / (A + B\rho)$ can be achieved within the pulse duration if the excitation rate

$B\rho$ is higher than the deactivation rate A : $B\rho \gg A = 1/\tau \sim 1/\tau_p$.

Following the pulse the density of excited molecules decays exponentially as $\exp(-At)$. The number of fluorescence photons excited by a single laser pulse is then

$$N_{fl} = \int_0^{\tau_p} A_{21} n_2(t) dt + \int_{\tau_p}^{\infty} A_{21} n_2(t) dt.$$

The number of water Raman scattered photons N_{Ram} emitted during single laser pulse is proportional to the total number of photons illuminating the sample during the pulse. Since the cross-section of water Raman scattering for a given excitation wavelength, the experimental geometry, and the number of water molecules in the cuvette are constant, we set the factor of proportionality to 1. Then the term $N_{Ram} = \rho \tau_p$ is a measure of laser power.

Fluorescence normalised to the water Raman scatter intensity, the so-called fluorescence parameter,^{5,6} is defined as $\Phi = N_{fl} / N_{Ram}$. It follows:

$$\Phi = \frac{N_{fl}}{N_{Ram}} = \frac{1}{\rho \tau_p} \left\{ \int_0^{\tau_p} A_{21} n_2(t) dt + \int_{\tau_p}^{\infty} A_{21} n_2(t) dt \right\}$$

$$= \Phi_1 + \Phi_2$$

where

$$\Phi_1 = \frac{n_0 A_{21} B}{(A + B\rho)^2 \tau_p} \{ (A + B\rho)\tau_p - 1 + \exp(-(A + B\rho)\tau_p) \}$$

and

$$\Phi_2 = \frac{n_0 A_{21} B}{(A + B\rho) A \tau_p} \{ (1 - \exp(-(A + B\rho)\tau_p)) \}$$

In conventional fluorescence spectroscopy both N_{fl} and N_{ram} are proportional to the laser power, and their ratio is independent of ρ . With high power excitation, that is at high ρ values, this does not hold any longer.

The main results of our study on laser induced saturation of fluorescence are reported in Fig. 2. Fig. 2a shows laser induced saturation of fluorescence when the fluorescence signal is not proportional to the excitation intensity. The non-linearity is noticeable in conditions where the excitation photon flux is higher than 10^{24} photons per second and cm^2 , that is in laboratory experiments with pulse laser sources. As a consequence the fluorescence normalised to the laser power (or: to the water Raman scatter intensity) is not invariant with alterations of the excitation intensity (Fig 2c).

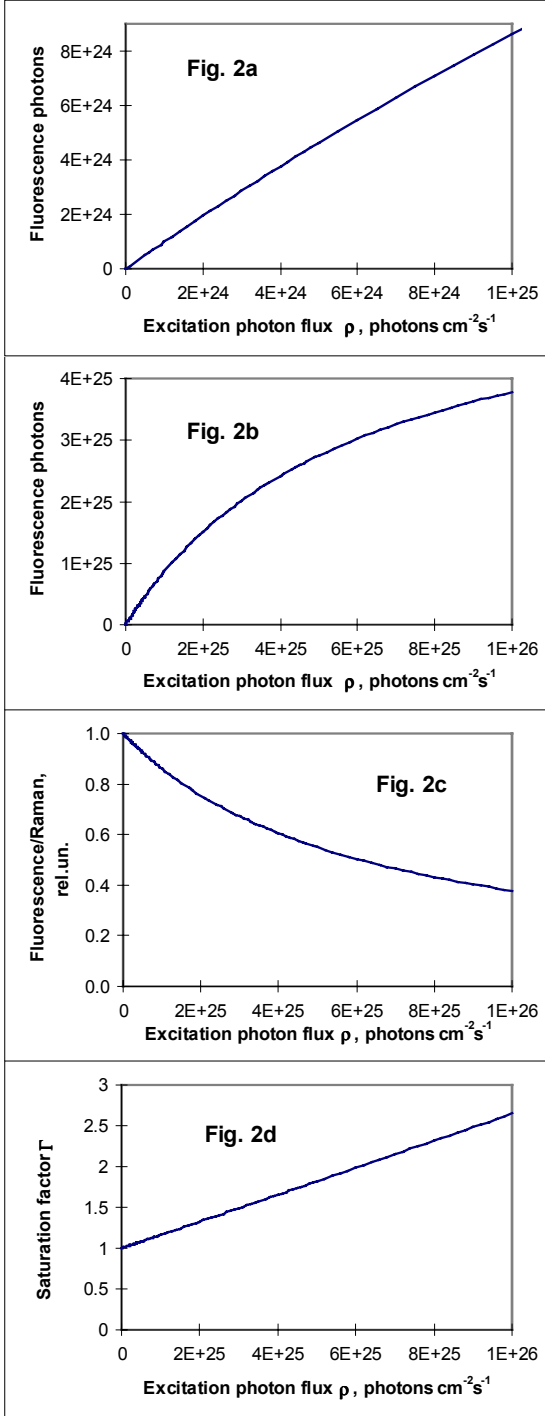


Fig. 2a-d: Laser-induced saturation of fluorescence. $A=5 \cdot 10^8 \text{ s}^{-1}$, $B=1 \cdot 10^{-17} \text{ cm}^2$, $\tau_p=10 \text{ ns}$.

To examine the dependence on the laser pulse energy in more detail we consider two extreme cases. First, when $\rho \rightarrow 0$, the fluorescence parameter Φ (the normalised fluorescence signal) corresponds to the “non-saturated” value $\Phi_0 = n_0 B(A_{21}/A)$ that is independent of ρ .

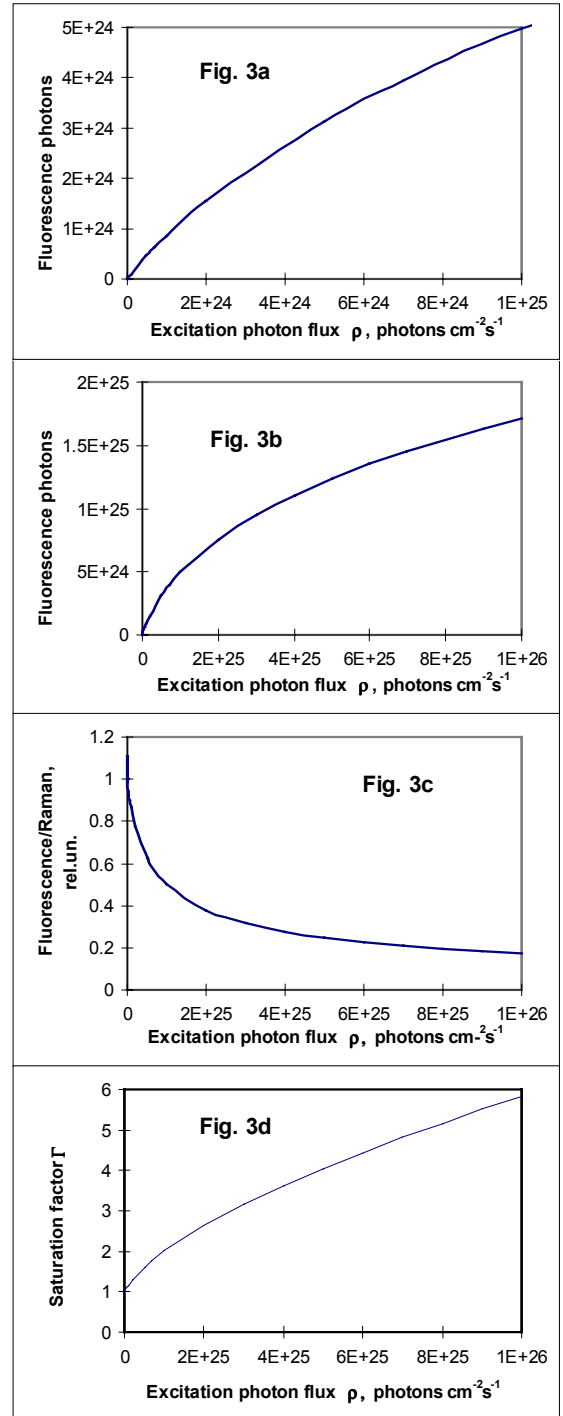


Fig. 3a-d: Laser-induced saturation of fluorescence with singlet-singlet annihilation ($Gn_0=10^{10} \text{ s}^{-1}$). $A=5 \cdot 10^8 \text{ s}^{-1}$, $B=1 \cdot 10^{-17} \text{ cm}^2$, $\tau_p=10 \text{ ns}$.

This holds when exciting fluorescence with CW laser or lamp sources. Otherwise, when $\rho \rightarrow \infty$ at high power pulsed laser excitation the fluorescence signal becomes independent of the excitation intensity. This is the case with strong saturation, and the fluorescence parameter (or normalised fluorescence signal)

$\Phi \rightarrow \frac{n_0 B A_{21}}{(A+B\rho)} \frac{A\tau_p + 1}{A\tau_p}$ becomes inversely propor-

tional to the laser pulse energy. This deviation of the fluorescence from its non-saturated value can be described with the "saturation factor"^{5,6}

$$\Gamma(\rho) = \frac{\Phi_0}{\Phi(\rho)},$$

where $\Phi_0 = \lim_{\rho \rightarrow 0} \Phi(\rho)$. By definition $\Gamma(0) = 1$ when

there is no saturation. When $\rho \rightarrow \infty$ (strong saturation) the saturation factor is linear with the excitation photon

flux ρ , i.e. $\Gamma(\rho) \rightarrow (1 + \frac{B}{A}\rho) \frac{A\tau_p}{A\tau_p + 1}$. Fig.2c shows

this linear dependence of the saturation factor $\Gamma(\rho)$ for an excitation photon flux of up to 10^{26} photons per second per square centimeter.

Under quasi-stationary conditions where the laser pulse duration τ_p exceeds the lifetime of excited molecules $\tau = 1/A$, it becomes $A\tau_p \gg 1$, and the saturation

factor $\Gamma(\rho) \rightarrow (1 + \frac{B}{A}\rho)$ is linear. Then the slope B/A

of the regression line $\Gamma(\rho)$ can be used as an estimation of the molecular absorption cross-section or fluorescence life-time.^{5,6}

In case of non-stationary conditions, when $\tau_p \ll \tau = 1/A$ and $A\tau_p \ll 1$, the function $\Gamma(\rho \rightarrow \infty)$ is approximated by $\tau_p(1 + \frac{B}{A}\rho)$, and the coefficients of

the regression line can again be used for an estimation of molecular spectroscopic characteristics. The difference of non-stationary excitation from the quasi-stationary case is that $\Gamma(\rho \rightarrow \infty)$ depends on the value of laser pulse duration. So this technique can be suggested for estimations of the laser pulse duration τ_p using saturation of fluorescence for dye molecules with known spectroscopic characteristics (absorption cross-section and fluorescence lifetime). It is necessary that the fluorescence lifetime of organic compound under investigation should exceed the laser pulse duration.

C. Singlet-singlet annihilation

At high concentrations the interaction of excited molecules becomes important, and an annihilation of excited singlet states S^* can take place: $S^* + S^* \rightarrow S + S^{**} \rightarrow S + S^*$. This so-called singlet-singlet annihilation (SSA) is an additional channel of molecular deactivation, leading to an increase of the total rate of molecular deactivation. The SSA rate $Gn_2^2/2$ is proportional to the square of the number of excited molecules and the SSA constant G . The factor 1/2 takes into account that only

half of the molecules return to the ground state as a result of this effect.

The temporal behaviour of the excited state population in case of singlet-singlet annihilation for "square" laser pulses is described by the equation:

$$\begin{cases} \frac{dn_2}{dt} = n_0 B \rho - n_2 (A + B\rho) - \frac{G}{2} n_2^2 & 0 < t \leq \tau_p \\ \frac{dn_2}{dt} = -A n_2 - \frac{G}{2} n_2^2 & t > \tau_p \end{cases}$$

The solution is

$$n_2(t) = \begin{cases} \frac{2n_0 B \rho}{(A + B\rho) + Q \operatorname{cth}(tQ/2)} & 0 < t \leq \tau_p \\ \frac{n_2(\tau_p) \{1 - \operatorname{th}(A(t - \tau_p)/2)\}}{1 + \left\{1 + \frac{G}{A} n_2(\tau_p)\right\} \operatorname{th}(A(t - \tau_p)/2)} & t > \tau_p \end{cases}$$

with

$$n_2(\tau_p) = \frac{2n_0 B \rho}{A + B\rho + Q \operatorname{cth}\left(\frac{Q}{2} \tau_p\right)}$$

and

$$Q = \sqrt{(A + B\rho)^2 + 2Gn_0 B \rho}.$$

The fluorescence parameter is then

$$\Phi = \frac{A_{21}}{\rho \tau_p} (I_1 + I_2)$$

with

$$I_1 = \int_0^{\tau_p} n_2(t) dt = \frac{2n_0 B_{12} \rho}{A + B\rho + Q} + \frac{2}{G} \ln \frac{A + B\rho + Q + (Q - (A + B\rho))e^{-Q\tau_p}}{2Q}$$

$$I_2 = \int_{\tau_p}^{\infty} n_2(t) dt = \frac{2}{G} \ln \left(1 + \frac{G}{2A} n_2(\tau_p)\right)$$

It is interesting to compare the fluorescence saturation in two cases, i.e. with and without SSA. It can be shown that an increasing molecular deactivation rate through SSA also influences the effect of fluorescence saturation.

The same approximation for $\Phi(\rho \rightarrow 0)$ holds in both cases, i.e. $\Phi_0 = n_0 B(A_{21}/A)$. However, $\Phi(\rho)$ and $\Gamma(\rho)$ behave very differently. It is very important that in case of SSA the dependencies $\Phi^1(\rho)$ and $\Gamma(\rho)$ are essentially nonlinear at high ρ values in contrast to the case without SSA. Under the same excitation conditions the effect of fluorescence saturation is more noticeable in the presence of SSA, and the saturation factor is higher than with negligible interaction of excited molecules. Fig.3 illustrates the influence of SSA on the effect of fluorescence saturation, with the same molecular parameters and pulse duration as in Fig. 2. It is seen in Figs. 2a and 3a that the nonlinearity of the fluorescence response versus laser pulse power becomes noticeable at lower ρ values. The dependencies of $\Phi^1(\rho)$ and $\Gamma(\rho)$ are apparently nonlinear, and the saturation factor Γ at $\rho=10^{26} \text{ cm}^{-2} \text{ s}^{-1}$ is about two times higher in case of SSA (Figs. 2c,d and 3c,d).

2. EXPERIMENTAL RESULTS

A. Description of the experiment

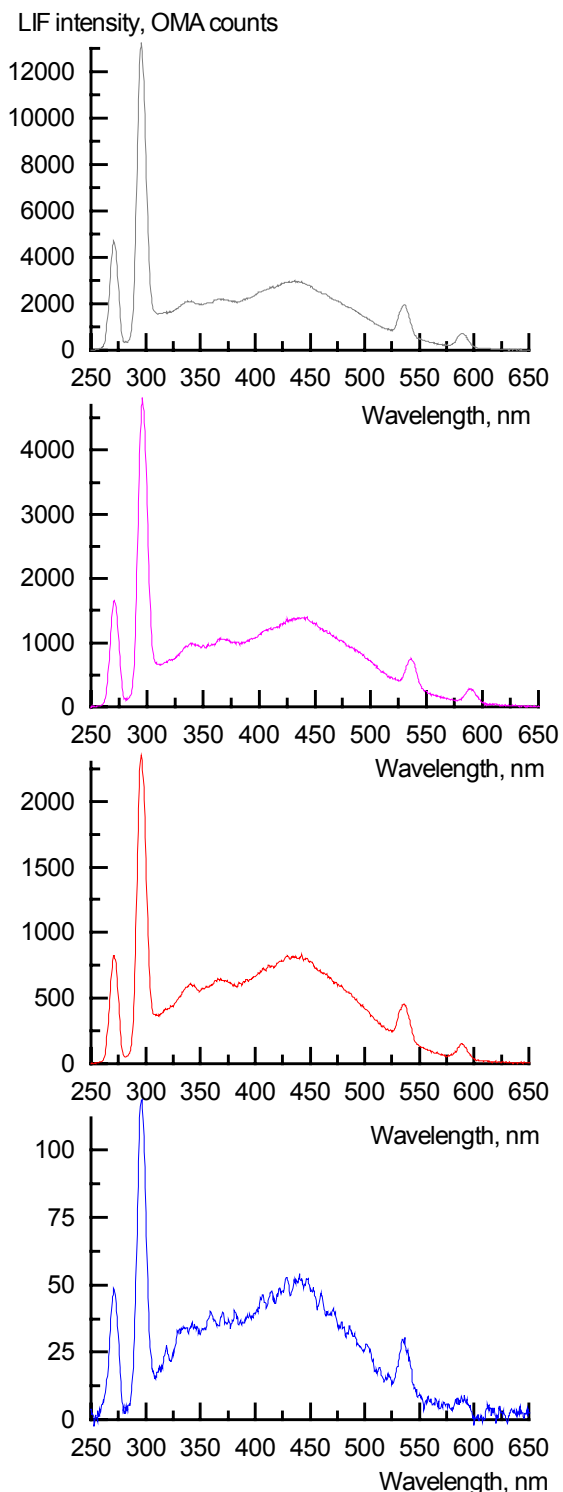
Laboratory measurements of DOM fluorescence emission were made at the lidar laboratory, ENEA, Frascati (Italy). Seawater samples, freshly collected at the coast of the Tyrrhenian Sea near Anzio harbour, were analysed in a quartz cuvette (2.2 cm x 1.0 cm x 4.1 cm), either after a static filling or during a fast continuous flow without turbulence (flow speed one litre/minute).

The laser fluorometer built at ENEA Frascati³ consists of four main subsystems: UV laser source, sample holder and collection optics, fluorescence detector with high spectral resolution, electronics for instrument control and data acquisition. Two laser types were used as UV excitation sources. A short pulse Q-switched Nd:YAG laser, equipped with second, third and fourth harmonic crystals for emission at 355 or 266 nm wavelength, with 10 mJ pulse energy and about 300 ps pulse length. Secondly, a XeCl excimer laser with 308 nm emission wavelength, 10 mJ pulse energy and 20 ns pulse length. Both lasers, designed for remote sensing applications, were emitting a low divergence nearly Gaussian square-shaped spot on the sample cuvette with a size of 0.12 cm² for the YAG laser and 1.6 cm² for the excimer laser.

A flow-through quartz cuvette with three optically polished surfaces was used. The cuvette was equipped with inlet and outlet lines for constant refilling by fresh samples.

A collection optics was mounted at a 90° position nearby the cuvette. It included an appropriate low pass filter for blocking scattered light at excitation wavelength, a 10 cm lens and a quartz fibre with a length of 10 m.

Fig. 4. Fluorescence spectra of sea water sample excited at 266 nm with different de-

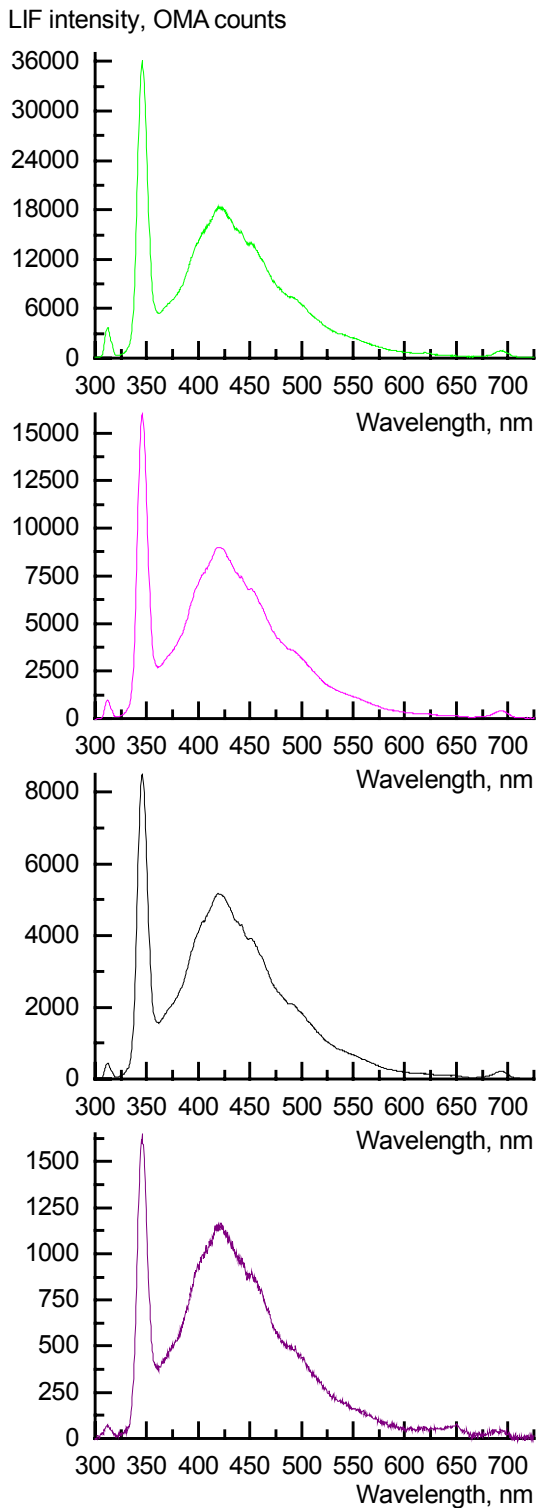


gree of laser pulse attenuation.

A gateable 1024 channel Optical Multichannel Analyser EG&G Model OMA III served as the signal detector. It was mounted on a small monochromator with 32 cm focal length, equipped with a low resolution grating having 147 grooves per millimetre. A proper

optical matching between the quartz fibre

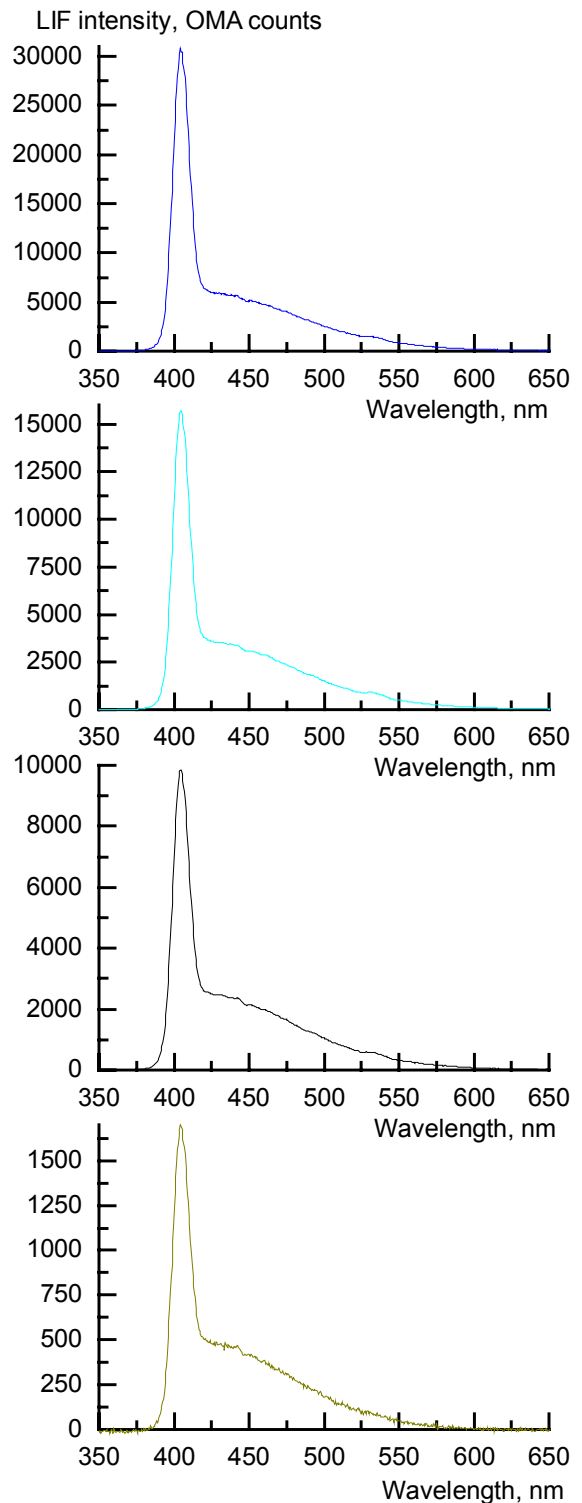
Fig. 5. Same as in Fig. 4, but with 308 nm



excitation wavelength.

and the monochromator was achieved with a small lens refocusing the signal on the 0.5 mm wide entrance slit of the monochromator.

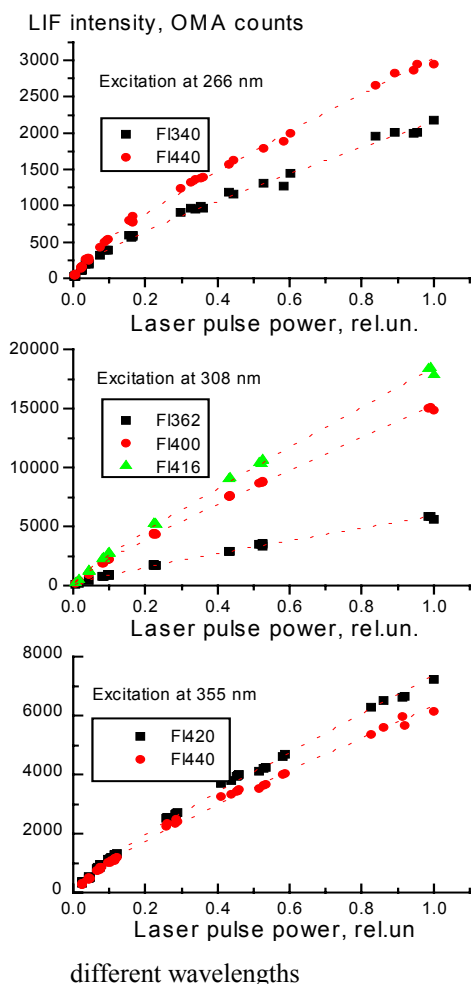
Fig. 6. Same as in Fig. 4, but with 355 nm excitation wavelength.



Single shot spectra were measured over a spectral range of about 500 nm from the UV to the red portion of the spectrum. The laser fluorometer was operated at 10 Hz

repetition rate. The spectra were averaged *off line* over 50 or 100 individual measurements.

Fig. 7. Fluorescence intensity versus laser pulse power for seawater samples excited at



B. Spectral data processing

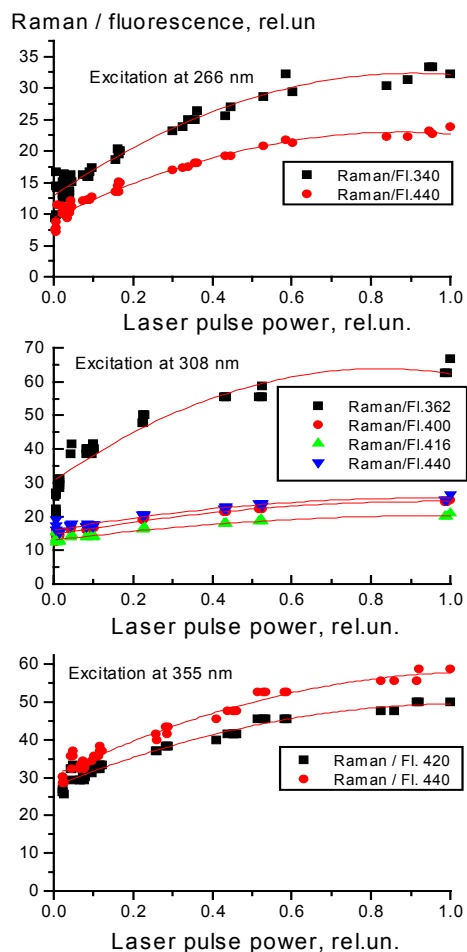
First, the apparatus baseline of the monochromator and multichannel analyser was determined for all spectra using a linear approximation between the signals measured at the lower and upper end of the spectrum. The fluorescence spectra are shown in Figs. 4-6, with the fluorescence intensity scaled in OMA counts.

Then the integral of the water Raman scatter band was evaluated. The fluorescence background in the region of the Raman band was again determined in a linear approximation at positions nearby the leading and trailing edge. Fluorescence signals at selected emission wavelengths were averaged over a 5 nm interval and divided by the wavelength integrated water Raman peak, yielding normalised fluorescence spectra given in Raman units.¹⁴

We suppose that the integral water Raman scatter signal is proportional to the laser pulse intensity illuminat-

ing the sample. Absorption in the sample cuvette can be neglected because of the low content of absorbing and scattering matter in the analysed water samples. Therefore, the water Raman peak area is also a measure of the laser pulse energy in relative units.

Fig. 8. Ratio of water Raman scattering to fluorescence intensity versus laser pulse



power for seawater samples.

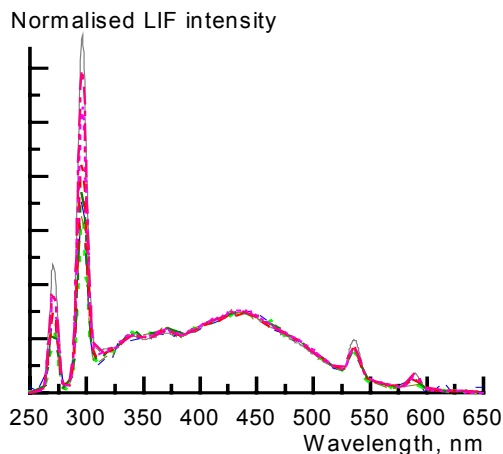
C. Photobleaching with high laser irradiation

As a first step of the experiment, the stability of the fluorescence emission of the water samples with respect to the laser pulse energy was analysed. For this purpose, spectra were taken without water flow through the cuvette, with 266 and 355 nm excitation and at different laser power levels.

Fluorescence spectra of natural water excited at 266 nm usually consist of two overlapping bands when excited at wavelengths below about 300 nm.¹⁴ The band with a maximum in the 240 to 360 nm region is from the aromatic amino acid tryptophan bound into proteins and mostly called protein- or tryptophan-like fluorescence. The visible fluorescence with maxima at about 420 to

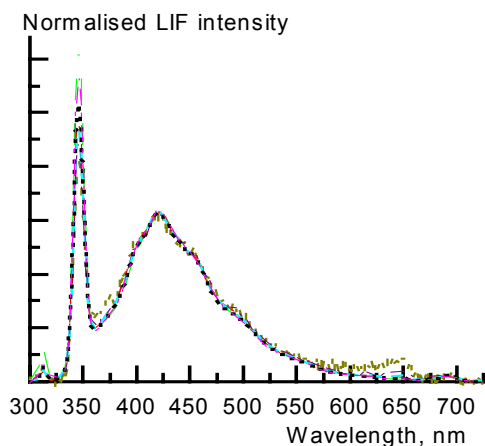
450 nm is caused by dissolved coloured humic substances (gelbstoff, yellow substances).

Fig. 9. Spectral response of seawater with 266 nm excitation at different degrees of laser pulse attenuation. Spectra are normalised



to fluorescence maximum

Fig. 10. Same as Fig. 9, but with 308 nm excitation.



Visible DOM fluorescence from humic substances in water is affected by both wavelengths of irradiation, with higher efficiency at 266 nm. At this wavelength both emission bands undergo significant photodegradation, but the efficiency of the process is larger for the tryptophan-like band than for the humic substance band. After prolonged irradiation (about 500 laser shots with pulse energy of 10 mJ) the humic substance fluorescence prevails in the spectrum.

Attenuated laser pulses with a 100 times lower energy gave rise to the same continuous process of photodegradation from spectrum to spectrum. The need of using samples that have not been irradiated with laser light before being investigated for fluorescence saturation effects was obvious from these findings. Therefore,

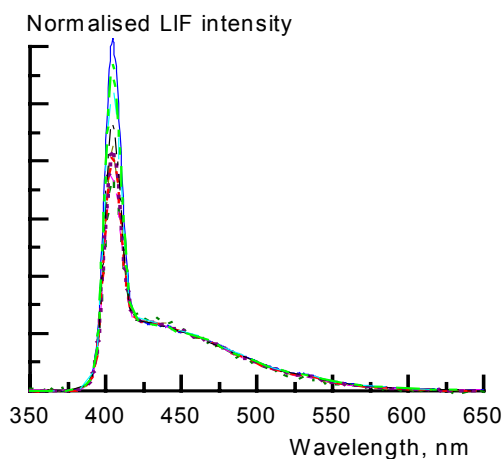
these measurements were done with a flow-through cuvette such that a fresh water sample was in the illuminated volume prior to each laser shot.

D. Fluorescence saturation

Measurements with continuously flowing seawater samples were made with 266, 308, and 355 nm excitation. A pulse energy of about 10 mJ was used as a starting value for an initial spectrum with every excitation wavelength. However, the excimer and the Nd:YAG laser beams differed in their laser spot size in the sample cuvette, and in their pulse length and temporal distribution.

Fig. 11. Same as Fig. 9, but with 355 nm excitation..

Fig. 4 presents the experimental results obtained with



266 nm excitation. Two fluorescence emission bands are apparently seen in the spectrum. The lines at 266 and 290 nm correspond to the laser excitation wavelength and to the water Raman stretching band. Consequently, the peaks at 532 nm and 580 nm are the second order of Raleigh and water Raman scattering.

Figs. 5 and 6 show the effects with 308 and 355 nm excitation. Each spectrum includes the broad fluorescence emission of humic substances, and the lines of elastic and water Raman scatter.

As seen in Figs. 4-6, the intensity fluorescence of organic substances in seawater including UV and visible spectral bands correlates with the laser pulse power. Attenuation of the laser power reduces both the DOM fluorescence and water Raman scattering. But this correlation is non-linear, which is further illustrated in Figs. 7-8. The fluorescence intensity in detector counts is given in Fig. 7 as a function of the laser pulse energy derived from the water Raman peak area. This nonlinearity is due to fluorescence saturation, and the deviation from linearity is increasing with higher pulse energy.

Because the dependence of fluorescence measured in OMA counts versus laser power is non-linear, their ratio or normalised fluorescence is not a constant value.

With 266 nm excitation the normalised fluorescence increases about three times if the laser power is attenuated by a factor of 100. The increase is 1.5 times with 308 nm excitation and attenuation by a factor of 18, and almost a factor of two with 355 nm excitation and attenuation by a factor of 38.

Fig. 8 demonstrates the dependence of normalised fluorescence versus laser pulse power. The ordinate axis shows the ratio of the water Raman scatter signal to the fluorescence signal (reverse value of fluorescence parameter). It is clearly seen from the figure that this ratio is decreasing while reducing the laser pulse power, and rising while increasing the laser pulse power.

Fluorescence saturation has been observed with samples of seawater excited at 266 nm on both the characteristic UV and visible fluorescence bands. The normalised fluorescence intensities for both emission bands showed strong dependence on the laser pulse energy (see Figs. 7 and 8, the plot in the centre).

E. Fluorescence bandshape

To compare the spectral shape of the fluorescence emission the data in Figs. 9-11 are normalised to maximum of the humic substance fluorescence at 440 nm. The shape of the fluorescence spectra excited at a given wavelength remains constant during the measurements.

This holds also for the spectra with 266 nm excitation. The ratio of the tryptophan-like to the humic substance fluorescence also remains constant. These results measured with the flow-through sample differ from the data obtained under static sample conditions with high laser radiation levels.

The results on seawater photobleaching received in the present paper and earlier investigations¹⁵ further support the different chemical nature of chromophores responsible for UV and visible emission bands in the fluorescence spectrum. But as it is clearly seen from experimental data both fluorescence bands for two types of chromophores are saturated by strong laser pulses in the same extent. The ratio of two fluorescence intensities keeps remarkably constant while the laser pulses are attenuated by a factor of 100.

To explain that the spectral shape for gelbstoff fluorescence and the ratio of UV to visible fluorescence keep constant we made the model calculations of saturation curves for organic matter in water. We used the model that the fluorescence band in natural water consists of at least two components which correspond to different types of fluorescent centres. We varied the molecular parameters (absorption cross-section and fluorescence life-time for two fluorophores) trying to achieve the re-

sulting spectral shape independent of the laser pulse intensity.

Parameters of laser source	Lifetime of fluorophore [ns]		Absorption cross-section [10^{16} cm ²]		$\frac{N f_1}{N f_2}$
	1st	2nd	1st	2nd	
Nd:YAG laser: $\tau_p = 300$ ps, $\rho = 4 \cdot 10^{26}$ / (s cm ²)	0.2	0.2	1	1.1	1.08
	0.5	0.5	1	1.1	1.09
	1	1	1	1.1	1.09
	5	5	1	1.1	1.10
	10	10	1	1.1	1.10
	5	10	1	1	1.02
XeCl laser: $\tau_p = 20$ ns, $\rho = 1 \cdot 10^{24}$ / (s cm ²) ²	2	10	1	1	1.10
	1	1	1	1.1	1.01
	5	5	1	1.1	1.03
	10	10	1	1.1	1.04
	5	6	1	1	1.04
	5	10	1	1	1.16
	2	10	1	1	1.36

Table 1. Results of the theoretical calculation of the intensity ratio $N f_1 / N f_2$ for two fluorescence bands with different values of fluorescence lifetime and absorption cross-section.

Table 1 presents the results of model calculations for two types of laser sources we have used in the work. As it is seen from the table for the excitation by the YAG laser the minor variations (10%) in absorption value cause noticeable changes in the fluorescence ratio. And a similar deviation in the fluorescence ratio is achieved if the fluorescence lifetime differs in 5 times (400%). The experimentally found constancy of spectral shape means that the absorption should not vary in more than 10% for different types of fluorophores (including both protein-like material and gelbstoff). And this conclusion is also valid if we vary the value of absorption for first fluorophore (10^{-17} , $5 \cdot 10^{-17}$, 10^{-16} , $5 \cdot 10^{-16}$ cm²).

It is not possible to find the real value of absorption cross-section for substances in natural water from our experimental data, because many other characteristics including concentration and quantum yield are unknown. But the noted spectral behaviour gives us the opportunity to limit the dispersion of absorption to 5-10%. This information is important for the interpretation of natural water fluorescence, because it means that the same type of molecules absorbs light and then transfers the energy of photo-excitation to different fluorescent centres. Certainly there are many sub-

stances in water absorbing light without fluorescence emission, but in our work we consider only absorbing acts which further give fluorescence emission. We can use the expression “excitation cross-section” instead of absorption cross-section.

The calculations made for the excimer laser showed that both factors (absorption and fluorescence lifetime) for two possible fluorophores are important to keep the spectral shape constant. The excitation cross-section could not vary in more than 30% and fluorescence lifetime could not vary in more than 1.5 times to keep the spectral shape practically constant. This conclusion on absorption is weaker than the previous one made from the consideration of YAG-laser as an excitation source. But from the present consideration of spectra excited at 308 nm we have the following limitation for dispersion of fluorescence lifetime for all possible chromophores contributing to gelbstoff fluorescence. Its upper limit is given by the value of $\pm 50\%$.

On the basis of experimental material using two excitation sources we conclude that at certain excitation wavelengths there is one possible type of chromophore (“absorber”) in water that mainly contributes to photo-excitation of fluorescence. Various groups emitting at different wavelengths (including protein-like fluorescence and gelbstoff fluorescence) are built in a matrix containing “absorbers” in the way that excitation cross-section for such macromolecules is almost the same (with an accuracy of 5-10%), and fluorescence lifetime excited at 308 nm is not varied in more than 50% for different fluorophores.

F. Discussion of experimental results

The most important experimental results on fluorescence saturation in non-filtered samples of natural seawater can be formulated as following:

1.) The fluorescence response of natural water (including tryptophan-like and gelbstoff spectral bands) under excitation by a pulsed laser source is non-linear versus excitation intensity. And consequently the fluorescence intensity normalised by water Raman scattering is strongly dependent on the laser pulse power. Normalised fluorescence intensities increase almost three times with 266 nm excitation (attenuation 100 times), 1.5 times for excitation at 308 nm (attenuation 18 times), and almost twice and for excitation at 355 nm (attenuation 38 times).

The decrease of normalised fluorescence observed in the work with rising laser pulse power is caused by the effect of fluorescence saturation. The value of the effect is a function of laser parameters such as pulse duration and excitation photon flux.

The effect of fluorescence saturation is negligible under conditions of remote sensing because of insufficient value of excitation photon flux. But the effect of fluo-

rescence saturation becomes important when remotely detected data are adjusted to fluorescence data measured under laboratory conditions under laser pulsed excitation. Then operating at high values of laser pulse power we may underestimate the concentration of fluorescent species in water (protein-like substances or DOM in our case) in laboratory conditions, and hence to overestimate the remotely measured concentration.

2.) The shape of fluorescence spectra keeps constant for all three excitation wavelengths. The intensity ratio of protein-like fluorescence to gelbstoff fluorescence excited at 266 nm also keeps constant with an accuracy of 3% at all used values of laser power. This is valid only for spectra measured for the sample in flow. The need of the sample flowing during spectrum excitation was proved by the experiments. A model could explain this invariance in the fluorescence band shape providing that the same absorber is held responsible for the excitation of all the macromolecules involved.

3) As a sequence of fluorescence saturation the ratio of water Raman scattering to fluorescence intensity (reverse Raman units) is a function of laser pulse power. The dependence observed in the work is apparently non-linear: the slope of the regressive curve is higher at attenuated laser power.

The relevant plot made in the theoretical part of the paper (see Fig. 2, curve $\Gamma(\rho)$ in the down plot) is linear. The theoretical plot is calculated considering the effect of fluorescence saturation without interaction between excited molecules. The deviation of the experimental plot from linear illustrates the complexity of the photo-excitation process in organic matter of natural origin. It could be caused by intermolecular interaction such as the considered singlet-singlet annihilation.

3. CONCLUSIONS

1. The effect of fluorescence saturation for natural seawater observed in the work is very remarkable for all three wavelengths of excitation. We can resume that a laboratory calibration of remotely operated laser fluorosensors requires to take into account the much higher laser pulse power of such instruments. Otherwise the adjustment of remotely detected data to laboratory conditions would not be correct, especially when data measured with a laser fluorosensor are compared with spectra measured using a convenient lamp instrument.

2. In experiments of fluorescence saturation the shape of fluorescence spectra keeps constant for all three excitation wavelengths. The intensity ratio of protein-like fluorescence to gelbstoff fluorescence excited at 266 nm also keeps constant at all used values of laser power. This is valid only for spectra measured for the sample in flow. The need of the sample flowing during spectrum excitation was proved by the experiments.

On the basis of experimental material we conclude that at certain excitation wavelengths there is one possible type of chromophore ("absorber") in water that mainly contributes to photo-excitation of fluorescence. Various groups emitting at different wavelengths (including protein-like fluorescence and gelbstoff fluorescence) are built in a matrix containing "absorbers" in the way that excitation cross-section for such macromolecules is almost the same, and fluorescence lifetime excited at 308 nm is not varied in more than 50% for different fluorophores.

3. The deviation of the experimental saturation curve from linear illustrates the complexity of the photo-excitation process in organic matter of natural origin. It could be caused by intermolecular interaction (singlet-singlet annihilation, for instance).

4. ACKNOWLEDGEMENTS

The work was done within the frame of a project supported by INTAS (grant number 94-748), Brussels.

5. REFERENCES

1. R.M. Measures, „Laser Remote Sensing. Fundamentals and Applications”, John Wiley & Sons, New York 1984.
2. R. Reuter, D. Diebel, and T. Hengstermann, „Oceanographic laser remote sensing; measurements of hydrographic fronts in the German Bight and in the northern Adriatic Sea”, *International Journal of Remote Sensing* 14, pp. 823-848, 1993.
3. R. Barbini, F. Colao, R. Fantoni, A. Palucci, and S. Ribezzo, “Remote sea water quality monitoring by means of a lidar fluorosensor”. *Proceedings SPIE Int. Opt. Soc.* 2586 Global process monitoring and remote sensing of the Ocean and sea ice, Eds. D.W.Deering and P.Gudmandsen, pp. 46-55, 1995.
4. R. Reuter, H. Wang, R. Willkomm, K. Loquay, T. Hengstermann and A. Braun, “A laser fluorosensor for maritime surveillance: experimental results”, *EARSel Advances in Remote Sensing*, Vol. 3, No 3, pp. 152, 1995.
5. A. Chekalyuk, V. Fadeev, G.Georgiev, Zh.Nickolov, “Application of laser induced saturation of molecular fluorescence for lifetime measurements”, *Opt. Comm.*, vol. 38, No 3, pp. 177-181, 1981.
6. A. Chekalyuk, V. Fadeev, G.Georgiev, T.Kalkanjiev, Zh.Nickolov, “Determination of fluorescence quantum yields using a spontaneous Raman scattering line of solvent as internal standard”, *Spectr. Lett.*, vol.15, N 5, pp. 355-365, 1982.
7. E. Baulin, V. Fadeev, “The effect of fluorescence saturation and remote sounding of water media”, *Atmospheric and oceanic Physics. (USSR)*. V.21, No 1, pp. 105-107, 1985.
8. S. Dzhasim, N. Serov, V. Fadeev, A. Chekalyuk, “Determination of spectral-luminescent characteristics of complex organic compounds by the fluorescence of saturation method”, *Journal of Applied Physics (USSR)*. V.56, No 2, pp. 252-258, 1991.
9. S. Dzhasim, N. Serov, V. Fadeev, A. Chekalyuk, “Determination of spectral-luminescent characteristics of complex organic compounds by the fluorescence of saturation method”, *Journal of Applied Physics (USSR)*. V.56, No 2, pp. 252-258, 1991.
10. S. Patsayeva, V. Fadeev, E. Filippova, V. Chubarov, V. Yuzhakov, “The saturation effect of dissolved organic matter fluorescence”, *Moscow Univ. Physics Bull.* Vol. 33, No 5, pp. 38-42, 1992.
11. S. Patsayeva, “New methodological aspects of the old problem: laser diagnostics of dissolved organic matter”, *EARSel Advances in Remote Sensing*, Vol.3, No 3, pp. 66-70, 1995.
12. M.Yu. Gorbunov, V.V. Fadeev, A.M. Chekalyuk, “The use of laser saturation fluorometry for the study of mechanisms of chlorophyll a fluorescence build-up in phytoplankton under condition of mineral nutrition shortage”, *Moscow University Physics Bulletin*, v. 47, No 4, pp. 47-53, 1992.
13. M.Yu. Gorbunov, A.M. Chekalyuk, “Diagnostics of primary photosynthesis processes by laser saturation fluorometry”, In "Laser Study of Macroscopic Biosystems", J.E.I. Korppi-Tommola, ed., *Proc. SPIE* 1992, pp. 57-62, 1992.
14. S. Determann, R.Reuter, P. Wagner P., and R. Willkomm “Fluorescent matter in the eastern Atlantic Ocean. 1: method of measurement and near-surface distribution”, *Deep-Sea Research* 41, 659-675, 1994.
15. S. Patsayeva, V. Fadeev, E. Filippova, V. Chubarov, V. Yuzhakov, “Temperature and ultraviolet radiation influence on luminescence spectra of dissolved organic matter”, *Moscow Univ. Physics Bull.*, 32, 6, 71-75, 1991.