

饱和激发下叶绿素荧光非线性变化及影响分析

李晓龙¹, 贺岩², 陈卫标², 姜静波¹, 刘庆奎¹, 陈永华^{1*}

1. 中国科学院海洋研究所, 山东 青岛 266071

2. 中国科学院上海光学精密机械研究所空间激光信息传输与探测技术重点实验室, 上海 201800

摘要 叶绿素浓度是海洋初级生产力的重要指标之一, 激光诱导荧光技术可以实现海水叶绿素浓度的快速测量。测量叶绿素浓度的传统激光诱导荧光原理, 是利用叶绿素荧光与水体 Raman 散射的强度比值(I_F/R)进行反演, 即叶绿素浓度 $n_{chl} = CI_F/R$, 其中 C 为系统常量。这是依据叶绿素荧光 685 nm、水体 Raman 散射强度都与激发光强呈线性关系。然而, 该理论并没有考虑诱导荧光饱和现象的存在。当诱导激光强度达到一定程度后, 685 nm 荧光强度随激发光强非线性变化。另外, 值得注意的是, 水体 Raman 散射并不存在信号饱和现象。为了探讨饱和激发造成荧光非线性变化的影响, 在激光诱导荧光技术测量叶绿素浓度的实验中, 设计两种测量方案, 即: 不同激光功率诱导单一浓度样本的荧光测量, 和固定激光功率时不同浓度样本的荧光测量。实验中利用 Nd:YAG 三倍频激光 355 nm 激发获得叶绿素溶液的 404 nm 处 Raman 散射和 685 nm 荧光。实验结果分为 2 部分进行讨论: (1) 为了分析饱和激发造成荧光变化的非线性特性, 通过调节激发光功率测量溶液的受激发射光谱, 发现水体 Raman 散射强度与激发光强呈线性关系, 而 685 nm 荧光强度出现饱和激发下的非线性变化。而且, 随叶绿素浓度的增加, 685 nm 荧光的非线性趋势更为明显, Raman 散射强度与激发光强的线性关系中斜率变小。数据分析表明, 685 nm 荧光数据拟合的 4 阶多项式和 Raman 散射效率值, 可以定性地表征 685 nm 荧光的饱和程度。(2) 考虑实际海洋激光雷达探测叶绿素浓度应用中存在饱和激发荧光非线性现象, 为了分析荧光非线性对传统叶绿素浓度反演理论适用性的影响, 在固定激发光强情况下对不同浓度叶绿素溶液的发射光谱进行测量。将激发光功率调节至 52.00, 80.70, 132.10 和 197.30 $mW \cdot cm^{-2}$, 获取相应激发光强下 685 nm 荧光与水体 Raman 散射的强度比值和叶绿素浓度之间的关系。实验表明, 激发光强不变的情况下, 685 nm 荧光与水体 Raman 散射的强度比值, 与叶绿素浓度仍满足线性关系。但是, 在较高光强激发时, 饱和激发造成的叶绿素荧光非线性变化, 导致利用传统激光诱导荧光理论反演的叶绿素浓度值偏小。因此, 需要对饱和激发下荧光非线性的影响进行修正, 其关系为 $I_F/R = n_{chl}/C + C_F$, 修正值 C_F 不可忽略。另外, 值得一提的是, 修正关系中系统常量 C 随激发光强增加而增大。研究表明, 饱和激发造成的荧光非线性, 会对激光诱导荧光技术测量叶绿素浓度产生影响, 但由于造成荧光非线性因素的复杂性, 仅通过荧光数据拟合获得的多项式, 无法定量说明其影响权重。然而, 当激发光强不变时, 可以实验测量获得基于激光诱导荧光原理的修正关系, 从而准确反演叶绿素浓度。

关键词 激光诱导荧光; 叶绿素浓度; 饱和激发; 非线性荧光; Raman 散射

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引言

叶绿素浓度是海洋初级生产力调查中不可或缺的部分^[1]。激光诱导荧光技术(LIF)已被广泛应用到海洋物质探测领域^[2-4], 采用海洋激光雷达方式, 可高效地获取表层海

水中叶绿素荧光信息, 以反演叶绿素浓度的空间分布^[5]。

饱和激发情况下荧光的非线性特征, 是激光探测物质荧光的重要特性。1988年 Ivanov 和 Fadeev^[6]对激光探测浮游植物时存在的荧光饱和效应进行了初步分析。2013年李晓龙等^[7]基于激光能量参量变化的三维油荧光光谱, 对“异类同谱”的燃料油和原油进行分析, 辅助油种鉴别。2017年 Li

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作者简介: 李晓龙, 1985年生, 中国科学院海洋研究所高级工程师 e-mail: lixiaolong@qdio.ac.cn

* 通讯联系人 e-mail: chen Yonghua@qdio.ac.cn

等^[8]初步分析了叶绿素荧光的非线性变化,其非线性程度随叶绿素浓度增加而变强。本文研究在此基础上,进一步分析叶绿素浓度和激光功率密度与荧光非线性的关系,目的探讨荧光饱和和非线性效应影响下 LIF 技术探测叶绿素浓度的修正方法。

1 饱和和激发荧光原理

当激发光能量密度达到一定程度后,受激发物质的荧光强度与激发光强呈现非线性变化趋势,这就是物质荧光的饱和和激发非线性现象^[9]。

非饱和时荧光强度 I_F^0 与激发光强 I_{laser} 成线性关系

$$I_F^0 = \eta I_{laser} \tag{1}$$

其中, η 为物质的荧光转换效率。

随着激发光强增加,受激荧光出现饱和现象

$$I_F = \beta \eta I_C \tag{2}$$

其中, β 为非线性因子,它取决于物质本身的物理特性, I_C 为诱导荧光饱和的 I_{laser} 。

传统理论中激光雷达反演海洋表层叶绿素浓度^[10]

$$n_{chl} = C \left(\frac{I_F}{R} \right) \tag{3}$$

式中, I_F 为叶绿素荧光强度, R 为海水 Raman 散射强度, C 为系统常量。方程(3)是以 I_F 和 R 都与 I_{laser} 呈线性关系为前提,而并未考虑饱和和激发情况。

2 实验部分

叶绿素荧光测量系统结构如图 1,主要包括激光器、样本室、光谱仪、数据采集、及光强控制系统五个部分。激发源 3 倍频 Nd:YAG 激光脉宽 7 ns,样本室出射窗采用长波通滤光片,截止波长 360 nm。荧光经由耦合透镜、多模光纤进入 Ocean Optics 公司 S2000 光谱仪。激光出射窗前,使用 355 nm 部分反射镜将固定比例的激光能量反射并耦合进入多模光纤。多模光纤采用分叉结构,同时接收荧光和反射的激光信号。

样本采用叶绿素 a 试剂的纯水溶液,放入石英比色皿中进行荧光测量,并考虑各类自然水体中叶绿素 a 浓度保持在 $\mu\text{g} \cdot \text{L}^{-1}$ 量级上,最终配成的样本浓度为 20.25, 40.50, 81.00, 162.00 和 324.00 $\mu\text{g} \cdot \text{L}^{-1}$ 。实验采用两种测量方式,即单一样本在不同激光功率诱导下的荧光测量,和固定激光功率时不同浓度样本的荧光测量。

纯水本身不产生荧光,数据分析时提取激光、Raman 和 685 nm 处峰值,并舍去 Raman 散射或叶绿素荧光峰值波长明显变化的数据。

3 结果与讨论

为了分析荧光饱和的非线性特点,首先分析样本 Raman 散射和荧光的变化,进一步针对 LIF 技术测量叶绿素浓度的应用,分析强激光探测的影响。

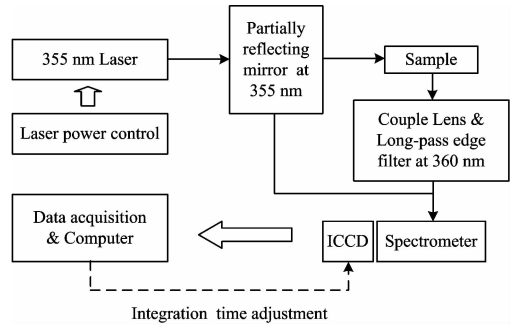


图 1 饱和激发情况下叶绿素荧光测量的实验系统示意图
Fig. 1 The sketch of experiment system for measuring Chl-a concentration under laser induced saturation of fluorescence

3.1 Raman 散射与叶绿素荧光的变化

考虑荧光饱和效应和荧光猝灭间存在联系^[9],受叶绿素浓度的影响。实验对不同浓度的样本进行测量,图 2 和图 3 为改变激光功率获得的 Raman 散射和 685 nm 荧光强度与激光强度的关系,对应叶绿素浓度分别为 162.00 $\mu\text{g} \cdot \text{L}^{-1}$ 和

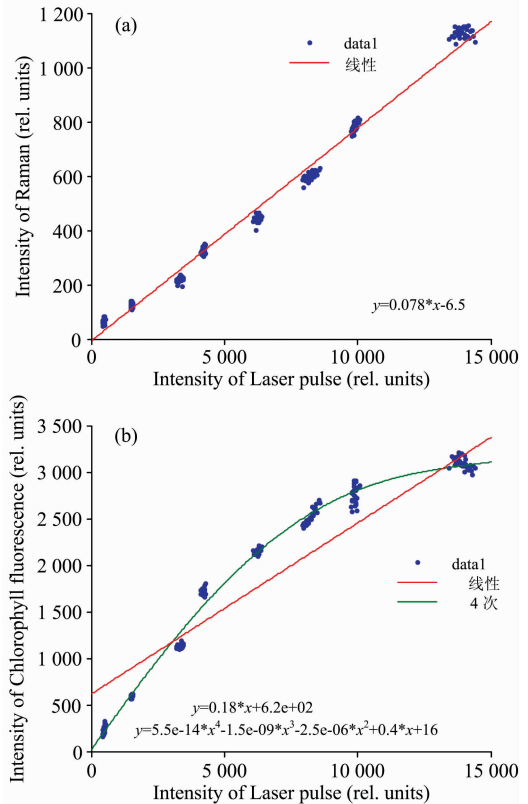


图 2 叶绿素浓度 162.0 $\mu\text{g} \cdot \text{L}^{-1}$ 时水体 Raman 散射和叶绿素荧光强度随激光强度的变化

(a): 水体 Raman 散射与激光强度呈线性变化;

(b): 叶绿素荧光与激光强度呈非线性变化

Fig. 2 The intensity characteristics of Raman and fluorescence of a solution with Chl-a concentration of 162.0 $\mu\text{g} \cdot \text{L}^{-1}$

(a): A linear relationship between Raman intensity and laser intensity; (b): Chlorophyll fluorescence intensity is nonlinear with the emitted laser intensity

81.00 $\mu\text{g} \cdot \text{L}^{-1}$ 。激光光强和 Raman 散射强度呈良好的线性关系，而荧光强度呈非线性变化。

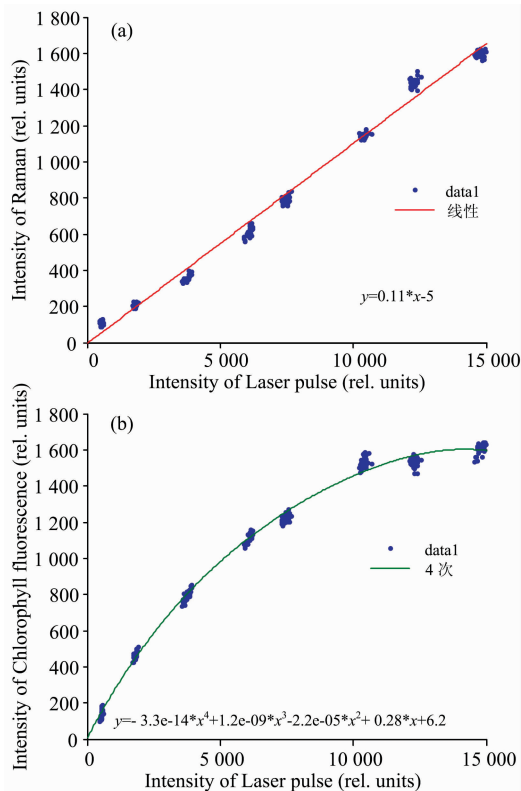


图 3 叶绿素浓度 81.0 $\mu\text{g} \cdot \text{L}^{-1}$ 时水体 Raman 散射和叶绿素荧光强度随激光强度的变化

(a): 水体 Raman 散射与激光强度呈线性变化;

(b): 叶绿素荧光与激光强度呈非线性变化

Fig. 3 The intensity characteristics of Raman and fluorescence of a solution with Chl-a concentration of 81.0 $\mu\text{g} \cdot \text{L}^{-1}$

(a): A linear equation containing water Raman scattering intensity and laser intensity; (b): A nonlinear regression from chlorophyll fluorescence intensity and laser intensity

将浓度 162.00 $\mu\text{g} \cdot \text{L}^{-1}$ 的样本荧光数据分别进行线性和四次多项式拟合，如图 2(b)，线性拟合造成较大方差，而四次多项式拟合基本满足荧光强度分布，与原始数据的相关系数为 99.70%，99.58%，99.75%，决定系数接近于 1。浓度为 324.00，162.00 和 81.00 $\mu\text{g} \cdot \text{L}^{-1}$ 时，荧光与激光强度关系分别拟合为

$$y = -1.3 \times 10^{-13}x^4 + 4.5 \times 10^{-9}x^3 - 7 \times 10^{-5}x^2 + 0.79x + 150$$

$$y = 5.5 \times 10^{-14}x^4 - 1.5 \times 10^{-9}x^3 - 2.5 \times 10^{-6}x^2 + 0.4x + 16$$

$$y = -3.3 \times 10^{-14}x^4 + 1.2 \times 10^{-9}x^3 - 2.2 \times 10^{-5}x^2 + 0.28x + 6.2$$

其中，自变量 x 为 I_{laser} ， x^2 ， x^3 和 x^4 可理解为在单重态湮灭、再吸收及荧光猝灭等作用产生的分量。结合式(2)， $I_{\text{F}} = \beta\eta I_{\text{laser}} + C_0$ ，那么 β 仍与 I_{laser} 存在非线性关系。仅根据拟合多项式，能够描述样本 685 nm 荧光的非线性变化趋势，但无

法确定 β 的具体形式。

由图 2(a)和图 3(a)，可假设 $R = \eta_{\text{R}} I_{\text{laser}} + C_{\text{R}}$ ，其中线性系数 η_{R} 为 Raman 散射转换效率， C_{R} 为常数，理想情况下 $C_{\text{R}} = 0$ 。浓度为 324.00，162.00 和 81.00 $\mu\text{g} \cdot \text{L}^{-1}$ 时，Raman 散射随激光强度的变化均满足线性关系

$$y = 0.058x + 7.9$$

$$y = 0.078x - 6.5$$

$$y = 0.11x - 5$$

由此可见， η_{R} 值随叶绿素浓度增大而减小，拟合出现的偏差 C_{R} 分别为 7.9，-6.5 或 -5，相对于 Raman 散射的强度变化，该值可忽略。

探测光源不变，水中荧光颗粒物增多时，荧光散射截面总和增大，Raman 散射截面总和减小，同时荧光猝灭、再吸收的概率也相应增大^[11]。Raman 散射的转换效率、及 685 nm 荧光的非线性变化，都与样本浓度存在联系。因此，可以将 η_{R} 作为 685 nm 荧光非线性变化程度的参照量。

3.2 激发光强对 n_{chl} 测量的影响

上述实验表明，饱和激发下叶绿素荧光强度不再与激光强度呈线性变化关系，而水体 Raman 强度仍与激光强度呈线性变化，这造成比值 I_{F}/R 与 I_{laser} 呈非线性，如图 5。而根据传统理论(3)， $I_{\text{laser}} \neq 0$ 情况下， I_{F}/R 与 I_{laser} 无关，形成平行于 x 轴的一条直线。

为了探究式(3)的适用性，在激光功率密度为 52.00，80.70，132.10 和 197.30 $\text{mW} \cdot \text{cm}^{-2}$ 时测得 I_{F}/R 与 n_{chl} 的变化，分别对应图 5 中(a)，(b)，(c)和(d)。

由式(3)得： $\frac{I_{\text{F}}}{R} = \frac{1}{C} n_{\text{chl}}$ ，而测量结果满足 $\frac{I_{\text{F}}}{R} = \frac{1}{C} n_{\text{chl}} + C_{\text{F}}$ ，其中 C_{F} 为拟合偏移量。功率密度为 80.70，132.10 和 197.30 $\text{mW} \cdot \text{cm}^{-2}$ 时不可忽略，且随功率密度增大而增大。

另外，值得注意的是，当激发光强增大时， $\frac{1}{C}$ 降低，即

$\frac{I_{\text{F}}}{R}$ 值随 n_{chl} 变化的增幅减小。结合图 4 可知：

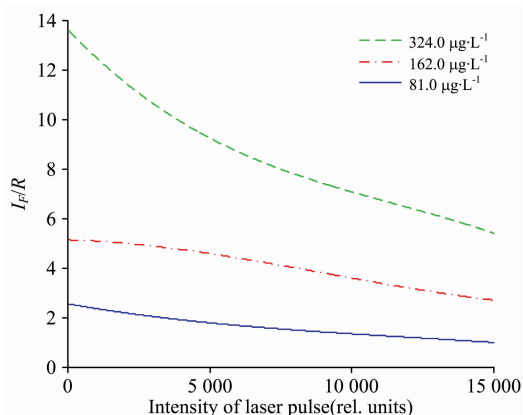


图 4 饱和激发造成的比值 I_{F}/R 非线性变化
Fig. 4 I_{F}/R with Chl-a fluorescence saturation

(1) 饱和激发下 I_{F} 非线性变化，造成反演的 n_{chl} 值随 I_{laser} 增大而减小。

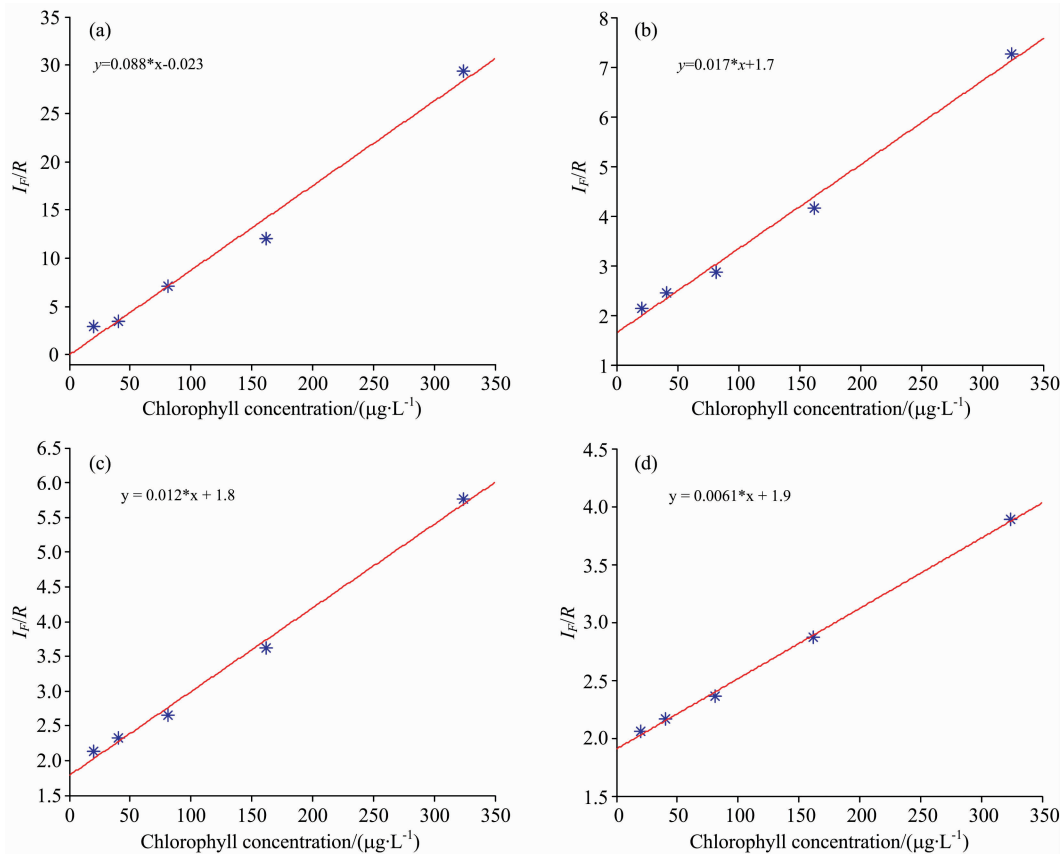


图 5 不同激光光强时 I_F/R 与叶绿素浓度的关系

(a), (b), (c)和(d)对应的激光功率密度分别为 52.0, 80.7, 132.1 和 197.3 $\text{mW} \cdot \text{cm}^{-2}$

Fig. 5 The relationships of I_F/R and Chl-a concentrations under different laser intensities

(a): 52.0 $\text{mW} \cdot \text{cm}^{-2}$; (b): 80.7 $\text{mW} \cdot \text{cm}^{-2}$; (c): 132.1 $\text{mW} \cdot \text{cm}^{-2}$; (d): 197.3 $\text{mW} \cdot \text{cm}^{-2}$

(2)若 I_{laser} 不变, n_{chl} 与 $\frac{I_F}{R}$ 满足线性关系: $\frac{I_F}{R} = \frac{1}{C}n_{\text{chl}} + C_F$, 且 C_F 不可忽略, 系统常量 C 值和 C_F 均随 I_{laser} 增加而增大。

4 结 论

饱和和激发荧光的非线性效应, 是影响 LIF 技术反演叶绿

素浓度的重要因素。由于荧光饱和过程的复杂性, 采用四阶多项式拟合荧光数据, 仅能定性描述其非线性趋势, 结合 n_R 值可间接地说明样品的荧光饱和程度。利用 LIF 技术测量叶绿素浓度, 须对荧光饱和效应的影响进行修正, 反演关系满足 $\frac{I_F}{R} = \frac{1}{C}n_{\text{chl}} + C_F$, 且 C_F 不可忽略。

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Analysis of Nonlinear Variation of Chlorophyll Fluorescence with Saturated Excitation and Its Influence on Chlorophyll Concentration Chlorophyll Concentration Measurement by LIF

LI Xiao-long¹, HE Yan², CHEN Wei-biao², JIANG Jing-bo¹, LIU Qing-kui¹, CHEN Yong-hua^{1*}

1. Institute of Oceanology, Chinese Academy of Sciences, Qingdao 266071, China

2. Key Laboratory of Spatial Laser Information Transmission and Detection Technology, Shanghai Institute of Optics and Fine Mechanics, Chinese Academy of Sciences, Shanghai 201800, China

Abstract As one of the most important indicators for studying marine primary productivity, chlorophyll concentration in seawater can be quickly measured by laser-induced fluorescence (LIF) technology. In the traditional theory for obtaining chlorophyll concentration by LIF, the chlorophyll concentration $n_{\text{chl}} = CI_{\text{F}}/R$, where I_{F} and R are fluorescence intensity of chlorophyll a (Chl-a) at 685 nm and Raman scattering intensity of water respectively, and C is a system constant. Without considering induced fluorescence saturation, this theory is based on an assumption that both the fluorescence intensity at 685 nm and water Raman intensity are linear with the intensity of incident laser. However, experiments confirmed the existence of non-linear relationships between the induced fluorescence energy at 685 nm and laser energy. While the linear relationships between water Raman intensity and pulse intensity have always existed without saturation excitation. In order to explore the effect of non-linear fluorescence change under saturation excitation, two series of measurement were done in the experiments. Fluorescence of the solution with constant Chl-a concentration was measured by varied laser powers, and a constant laser power was used to obtain the solution fluorescence of varied Chl-a concentrations. The third harmonic of Nd:YAG laser at 355 nm was the excitation source. Thus, Raman scattering at 404 nm and fluorescence at 685 nm of Chl-a solutions were the key part of emission spectra. The experiment results were discussed in section 3. In the first part, the emission spectra of Chl-a solutions were measured by LIF with excitation light intensity variation. It shows a linear relationship between Raman scattering and excitation intensity, while fluorescence intensity at 685 nm appeared nonlinear change under saturated excitation. Moreover, fluorescence intensity of Chl-a solution with higher concentration increased to plateau earlier, and the ratio of Raman scattering intensity to excitation intensity in the linear relationship decreased with Chl-a concentration. The data analysis shows that a polynomial of degree 4 fitting the changes of fluorescence intensity and the value of the Raman scattering efficiency can qualitatively characterize the saturation of fluorescence at 685 nm. Secondly, for the purpose of analyzing the effect of fluorescence nonlinearity on the applicability of traditional theory in chlorophyll concentration inversion, with considering the phenomenon of fluorescence saturation existing in the application of ocean Lidar for detecting chlorophyll concentration, the emission spectra of samples with different Chl-a concentrations were measured with a constant excitation intensity. The relationship between I_{F}/R and Chl-a concentration was obtained under the excitation power at 52.00, 80.70, 132.10 and 197.30 $\text{mW} \cdot \text{cm}^{-2}$. Experiments show that I_{F}/R is still in linear relationship with Chl-a concentration under the condition that the exciting radiation is not changed. But, the concentration from the traditional inversion theory by LIF is less than the real Chl-a concentration measured by a high excitation intensity which leads to fluorescence saturation effect. Therefore, the inversion module is necessary to be corrected with C_{F} which is related to fluorescence nonlinearity under saturation excitation. A more accurate inversion is based on $I_{\text{F}}/R = n_{\text{chl}}/C + C_{\text{F}}$. And, it is worth mentioning that the system constant C in this correction module increases with the exciting intensity. Consequently, saturation excitation causes fluorescence nonlinearity and affects the measurement of Chl-a concentration by LIF technology. It is regrettable that the polynomial obtained by the fluorescence data fitting cannot quantify the impact of fluorescence saturation effect, due to the complexity of the nonlinear factors. However, when the excitation power is constant, a corrected inversion can be experimentally obtained and used to measure Chl-a concentration by LIF in field surveys.

Keywords Laser induced fluorescence (LIF); Chlorophyll concentration; Saturated excitation; Nonlinear fluorescence; Raman scattering

* Corresponding author

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