1. INTRODUCTION

The magnitude of sun-induced chlorophyll fluorescence is now routinely retrieved by satellite observations in the open ocean using Fluorescence Line Height (FLH) algorithms, Letelier (1996). This retrieval is much more complicated in the coastal waters where the fluorescence component overlaps with NIR elastic scattering component. The overlap occurs because the minimum of the total algal chlorophyll absorption exists in the same spectral region as fluorescence, Gitelson et al (2002), Schalles (2006). For a comprehensive picture of these overlapping effects, it should be noted that total absorption spectra, including algal re-absorption can also affect the apparent fluorescence spectral shape observed, and this impact, as well as the impact of spectral dependence of algal scattering on total reflected spectra requires further study. We report here the results of radiative transfer simulations and a series of field and laboratory measurements which examine these impacts. The laboratory measurements include fluorescence measurements obtained from laser and white light illumination and comparisons of fluorescence retrievals using our recently developed polarization discrimination technique for the separation of fluorescence from elastic reflectance, Ahmed et al (2004), Gilerson et al (2006). The results provide more comprehensive insights into the relationships between fluorescence magnitude and chlorophyll a concentration [Chl] for various coastal water environments. These relationships were used to provide synthetic reflectance spectra for testing the performance of fluorescence retrieval algorithms for a variety of water compositions typical for coastal waters as a function of [Chl] and to estimate errors for different fluorescence retrieval techniques for these conditions.

2. LABORATORY MEASUREMENTS AND OBSERVED CHANGES IN CHLOROPHYLL FLUORESCENCE SPECTRAL SHAPE DUE TO REABSORPTION IN ALGAE

The spectral features of the chlorophyll a fluorescence signal is well known, Babin et al (1996) with a peak centered at 685nm and a generally Gaussian with FWHM 25 nm, Gower et al (1999), independent of the excitation wavelength. This description is commonly included in simulation models Gower et al (1999) and in algorithms for fluorescence retrieval, Letelier (1996). At the same time it is also known Babin et al (1996) that fluorescence overlaps with the strong changes in the chlorophyll absorption. As a consequence, fluorescence can be partially reabsorbed during propagation through the algae and the spectral shape and peak of the water leaving fluorescence modified (shifted) potentially affecting FLH measurements. To assess the quantitative effects of reabsorption mechanisms on the observed water leaving signal, a series of controlled laboratory experiments using cultured algae Isochrysis sp. at various chlorophyll concentrations were undertaken.

To examine the impact of reabsorption as a function of [Chl] total path absorption, experiments were first carried out using the arrangement shown in Fig. 1a. A horizontal ion laser light beam at 488 nm was used to illuminate algae, in the glass cuvette C, at various depths with the fluorescence signal detected above water level by a fiber optic probe connected to an Ocean Optics spectrometer.
In all of our lab experiments, [Chl] was estimated using WET Labs ACS absorption measurements of algae samples and making use of the expression, Boss et al (2004):

\[ [\text{Chl}] = (a(676) - a(650)) / 0.014. \] (1)

Maximum sample concentration determined by this process before dilution was 794 mg/m$^3$.

Fig. 2 shows fluorescence spectra recorded above the surface level with the horizontal laser illumination of Fig 1a at different depths. The position of the fluorescence peak as the function of excitation depth for the same algae is shown in Fig. 3. As can be seen there is an essentially linear shift in the fluorescence peak position for both concentrations. Experimental results are compared in Fig. 3 (solid line) with a theoretically estimated shift of the water leaving fluorescence spectra given by

\[ F_{\text{surf}}(\lambda) = F_{\text{0}}(\lambda) \exp(-a(\lambda)h), \] (2)

where $F_{\text{surf}}(\lambda)$ is a fluorescence spectrum detected just above the surface for an excitation depth $h$, $F_{\text{0}}(\lambda)$ is the original fluorescence spectrum excited by the laser at any depth which we take to be well approximated by the fluorescence detected just above the surface for near surface excitation with $h \approx 5$ mm. The absorption $a(\lambda) = a_{ph}(\lambda) + a_w(\lambda)$ is the total absorption spectrum consisting of algae absorption $a_{ph}(\lambda)$ measured by WET Labs ACS instrument and water absorption $a_w(\lambda)$, Pope and Fry (1997). Experimentally recorded shifts are much higher than theoretical estimates; this may be the result of multiple scattering effects which are known to significantly enhance the effective path length.

While the above results are of interest theoretically, they do not correspond to natural conditions. To better approximate natural illumination conditions, the arrangement of Fig. 1b was used. In this, laser light delivered through a fiber optic light guide and illuminated algae from above the water in a cuvette with walls blackened to minimize reflections. With this illumination geometry, no large shifts were observed in the position of the fluorescence peak.
This result is consistent with calculations which were performed by integrating the fluorescence signal through all vertical layers taking into account the [Chl] absorption relevant for each layer. Maximal shifts for overhead illumination were typically not more than 2-3 nm even for [Chl] = 250 mg/m$^3$. It should be pointed out however, that these shifts can be magnified if fluorescence yield is non-uniform with higher values occurring significantly below the surface. In these situations, larger fluorescence contributions from deeper layers will reach the surface with larger reabsorption shifts. As an example, in Fig. 5 we compare unaltered fluorescence spectra with the normalized fluorescence shape expected at the surface simulated for vertical sun illumination and fluorescence efficiency changing linearly from 0 to a maximum value of 0.02 at the depth of 2 m. The peak is shifted to 689 nm, and the FWHM is 27 nm instead of the unperturbed 25 nm.

An arrangement similar to the one of Fig. 1b was also used to test the accuracy of the fluorescence discrimination technique previously described, Gilerson et al (2006), for fluorescence retrievals from samples illuminated by white light. A polarizer placed in front of the detector enabled detection of reflectance spectra at different polarizations and retrieval of
fluorescence as described before, Gilerson et al. (2006). The retrieved fluorescence was then compared to retrievals obtained by modifying the white light illumination of the algae through different filters allowing us to directly separate the reflectance and fluorescence spectra. In the first experiment, a green band pass, 400-600 nm filter was used which allowed us to measure the fluorescence directly without any overlapping elastic backscatter. In the second experiment, a long pass red filter transmitting beyond 650 nm was used. In this case, the fluorescence is obtained by subtracting the signal detected for filtered illumination which is comprised only of elastic scattering from that for the unfiltered illumination, which includes fluorescence. The fluorescence retrieved with the polarization discrimination technique, as seen in Fig. 6, was a good match to that retrieved from filtered measurements. All measurements take into account the filter transmission efficiency and the algae’s chlorophyll absorption as measured by the WET Labs ACS instrument.

3. FIELD MEASUREMENTS AND FLUORESCENCE CONTRIBUTIONS TO NIR REFLECTANCE AND IMPACTS ON PERFORMANCE OF FLH ALGORITHMS

In-situ field measurements were carried out at 42 stations in the Chesapeake Bay area in July 2005 using a ship-deployed profiling package assembled by WET Labs (Philomath, Oregon). This package consists of three instruments: an ACS recording absorption a and attenuation c at 82 wavelengths between 400 nm and 750 nm, an ECO-scattering meter measuring the volume scattering function at 117° at 7 wavelengths, fluorescence signals of chlorophyll and CDOM and a CTD to obtain temperature, salinity and pressure data. A data logger was used to power all the instruments, acquire data, coordinate different timing schemes and transmit the data up through a single cable to the computer. The depth of the profiler was raised and lowered to sample the depth profile. Absorption and attenuation channels of the ACS instrument were calibrated with deionized water as recommended. Data were corrected for temperature, salinity and scattering. The upwelling radiance $L_u(\lambda)$ from the water was collected and delivered by fiber bundles to a GER spectroradiometer. Downwelling irradiance $E_d(\lambda)$ was determined by calibration observations of a Lambertian Spectralon plate with a 99% reflectance factor. The fiber probe for upwelling measurements was placed just beneath the surface, and the remote sensing reflectance $R_s(\lambda)$ was obtained by normalizing the upwelling radiance to the downwelling irradiance:

$$R_s(\lambda) = \frac{L_u(\lambda)}{E_d(\lambda)}$$

For polarization measurements, a polarizer was added to the end of the fiber collector probe. Consecutive measurements of $L_u(\lambda)$ were then made with the fiber probe in the principal scattering plane and the polarizer transmission axis in horizontal and vertical orientations. The detector was calibrated to take into account the polarizer transmission. Chlorophyll concentrations were determined from the ACS absorption spectra using expression (1). These data generally correlated well with near surface data from water sample analysis, except under algal bloom conditions.

Fluorescence retrieved using the polarization discrimination technique at a typical station in the Chesapeake Bay, Fig. 7, showed that its magnitude constitutes 30-40% of the total reflectance peak in the NIR. Although the impact of fluorescence on the shape and magnitude of the NIR peak is relatively small even for high [Chl], the actual fluorescence magnitude of

![Fig. 7. Retrieval of fluorescence component from reflectance in Chesapeake Bay using polarization discrimination, [Chl] = 41 mg/m³](image-url)
fluorescence is significant and is a useful parameter for the analysis of photosynthetic activity, [Chl] and algae blooms. Unfortunately, the position of fluorescence on top of the NIR elastic scattering peak creates significant difficulties for disentanglement and for its accurate retrieval especially from satellite sensors with a limited number of spectral bands. This coupling is more difficult since the relative efficiency connecting FLH to [Chl] concentration is not fixed. To examine the coupling relationship between [Chl] and FLH in coastal waters, we retrieved the fluorescence signal at more than 30 stations as part of the Chesapeake Bay campaign. The retrieval algorithm is based on a LSQ fit of the modeled irradiance reflectance spectra into measured ones, with the 2 parameter model given as

\[ R_{\text{mod}}(\lambda) = \frac{0.37 \cdot b_m(\lambda)}{a_m(\lambda) + b_m(\lambda)} + FL \cdot \Phi(\lambda) \]  

(4)

where \(a_m(\lambda)\) is an absorption spectrum, and \(b_m(\lambda) = c_m(\lambda) - a_m(\lambda)\) is the scattering spectrum measured by the ACS instrument in the WET Labs package for wavelengths from 400 to 750 nm. The backscatter spectrum can then be connected to the total scattering as

\[ b_b(\lambda) = \tilde{b}^* b_m(\lambda), \]  

where \(\tilde{b}\) is to be used as a fitting parameter and considered to be spectrally flat. The second parameter is the magnitude of fluorescence \(FL\) which multiplies the standard Gaussian shape profile \(\Phi(\lambda)\) centered at 685 nm with a 25 nm FWHM. Results of fluorescence height retrieval as a function of [Chl] concentration are shown in Fig. 8. These results are combined with the fluorescence determined using MODIS fluorescence line height (FLH) algorithm utilizing 667, 678 and 745 nm bands. For comparison we show (solid line) a plot of the expression for fluorescence given by Gower et al (2004)

\[ FL = 0.0015 \frac{[\text{Chl}]}{1 + 0.2 \cdot [\text{Chl}]}, \]  

(5)

where the factor 0.0015 was adjusted to reflect irradiance values underwater and a plot (dashed line) of this expression modified with fluorescence proportional to [Chl] where the coefficient determined by 0.0015/(1+0.2[Chl]) from formula (5) for [Chl] = 25 mg/m³

\[ FL = 0.00025 \cdot [\text{Chl}]. \]  

(6)

All three data sets show rough agreement in terms of orders of magnitude. While the relationship between [Chl] and fluorescence is close to linear for [Chl] < 30 mg/m³, we see evidence of strong saturation for higher [Chl]. The reason for this saturation is not obvious. Previous laboratory tests, Gilerson et al. (2006), using our polarization discrimination technique showed near linearity even for significantly higher [Chl]. We confirmed these results using ion laser light excitation at 488 nm of cultured algae, in the experimental setup of Fig 1b. Small saturation effects can be observed only for [Chl] concentrations higher than 200 mg/m³ in Fig. 9. To explain the apparent saturation observed in the field tests, we note that for the data in Fig. 8, [Chl] was determined from water samples near the surface. These data correlate well for concentrations below 50 mg/m³ with \(a(\lambda)\) ACS measurements about 1 m below the surface, in accordance with expression (2), which was used as another proxy for [Chl]. However, for the algal bloom station with [Chl] = 236 mg/m³, the ACS value was significantly lower ~ 60 mg/m³, which probably indicates the existence of a strong negative vertical gradient of [Chl] which might also explain the poor fit of modeled irradiance reflectance of the WET Labs data in the NIR into the measured irradiance reflectance for those stations. Similar results were achieved...
with Hydrolight simulations in which the WET Labs ACS absorption $a(\lambda)$ and attenuation $c(\lambda)$ data were used as input. For these simulations, the backscattering ratio was initially assumed to be 1% and then adjusted to match measured reflectance at 560 nm. Fluorescence was determined as the difference between measured and calculated reflectance. For many stations, reflectances simulated by Hydrolight are a better match than those obtained using approximation (3), except for the algae bloom case. This again points to possible significant depth changes in [Chl] and the need for examination of [Chl] depth profiles for bloom conditions.

Finally, to examine the performance of satellite sensor FLH algorithms for coastal water conditions, we used an IOCCG data set of irradiance reflectances modeled using Hydrolight [http://www.ioccg.org/groups/OCAG_data.html](http://www.ioccg.org/groups/OCAG_data.html) for various compositions with $1 < \text{[Chl]} < 30 \text{ mg/m}^3$. The fluorescence was superimposed on top of these reflectances as a Gaussian shape with the center at 685 nm and FWHM 25 nm and the fluorescence magnitude was modeled according to formula (5) to take into account the coupling observed. Results for MODIS and MERIS FLH algorithms and formula (5) are shown in Fig. 10 and 11. The MODIS algorithm shows a spread of at least twofold in fluorescence magnitude. This does not improve by shifting the on-line central band to 685, 690 nm or higher. In comparison, the MERIS algorithm shows even worse performance, but preliminary results show that it can be improved by the change of the central band.

It is important to emphasize that these results are based on the IOCCG reflectance set which has: a) 10 nm resolution, b) several assumptions which do not accurately depict IOP in the NIR zone, c) mineral concentrations generally assumed to co-vary with [Chl], which is not necessarily true for coastal waters. More accurate and diversified synthesized and field data sets are needed for more thorough performance evaluation.
4. CONCLUSIONS

Substantial spectral shifts due to reabsorption were found to occur in the fluorescence spectral shape observed near the surface for algae illuminated by the laser light at different depths. These shifts were qualitatively consistent with a simple Beer’s Law attenuation whose effective path length is modified by multiple scattering. However, for illumination conditions closer to those in nature, i.e. from above the water, the changes in the fluorescence spectra were relatively small, supporting the use of the unaltered fluorescence spectra in simulation and fitting procedures. While quantitative analysis shows that the fluorescence overlap does not significantly detract from the validity of NIR ratio for [Chl] retrieval algorithms for typical coastal water conditions, the fluorescence contribution can typically represent 30 % of the peak reflectance in the NIR for many coastal waters, and the retrieval of fluorescence independent of [Chl] can have a significant impact on assessing correlations between these two parameters. Preliminary retrievals of [Chl] and FLH based on our field measurements, show that a weak linear dependence does occur between these two parameters. Furthermore, it is observed, mainly in algal bloom conditions, that FLH saturates with high [Chl] values. Laboratory measurements, however, show that these saturations should only occur at higher [Chl] levels. This discrepancy suggests that a significant variability exists in the depth profiles of fluorescence efficiency and [Chl] concentration. Clearly, additional simulations and experiments are needed to estimate proportionality between fluorescence reflectance component and [Chl] in the conditions of high [Chl] and algae blooms, taking into account possibility of the strong non-uniform depth [Chl] distribution.

Using a model connecting FLH to [Chl] concentration, we were able to modify the IOCCG reflectance data set to account for fluorescence and assess different satellite algorithms. Estimation of MODIS and MERIS FLH algorithm performance showed that the MODIS algorithm performs better, but that twofold errors in FLH can be easily expected, especially for [Chl] greater than 20 mg/m³. These results need to be confirmed with more detailed synthesized and field data sets with higher resolution and more accurate values in the NIR.

Finally, to ensure that our fluorescence measurements are correct, the polarization discrimination method used to retrieve fluorescence was compared in white light experiments where the separation of fluorescence was obtained both through polarization discrimination and illumination through a variety of chromatic filters. Unlike previous laser induced validation experiments where only the shape of the spectral feature was validated, the filter based method provides quantitative agreement in both the shape and magnitude of the retrieved fluorescence signal under the same white light excitation.

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REFERENCES


