Biogeosciences Discuss., 10, C5168–C5175, 2013
www.biogeosciences-discuss.net/10/C5168/2013/

Biogeosciences
Discussions
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Interactive comment on "Using a two-layered sphere model to investigate the impact of gas vacuoles on the inherent optical properties of *M. aeruginosa*" *by* M. W. Matthews and S. Bernard

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Received and published: 19 September 2013

The authors wish to thank the referee for their insightful comments. The major comments with respect to the real refractive index of the shell layer and the manuscript terminology have been addressed in turn below.

1. size and real refractive index of the shell layer

The referee has commented on both the value determined of the real refractive index (n = 1.12) and volume of the shell layer. The authors provided a defence of this value in the paper through comparison with previously used n values in two-layer modelling efforts and literature values of n for protein cell walls. The authors have omitted an

important reference to values determined for eukaryote chloroplasts as reviewed by Bernard et al. (2009) (table 2). The mean value of n for chloroplasts was 1.14 according to this study. Since the shell layer chromatoplasm in prokaryotic M. aeruginosa contains the photosynthetic lamella (comparable to chloroplasts) concentrated by gas vacuolation, the chromatoplasm is comparable to the chloroplast. Therefore a value of 1.12 does seem reasonable to the authors. It is important to note that the chloroplast/chromatoplasm n = 1.12 is vastly different from that bulk homogeneous cell n, which in this case is 0.97 with Vg = 50%. It is also noteworthy that value of n = 1.12 determined using Rrs produced the best comparison with Zhou et al (2012) and Volten et al's (1998) measurements, in contrast with statements made by the referee.

Added to Line 20 p. 10554. "The mean value for chloroplast in eukaryotes as reviewed by Bernard (2009) was 1.14, which is comparative to the chromatoplasm"

"Therefore, a value of 1.12 for the shell layer might be reasonable considering reported values of two-layer simulations and values reported for eukaryotic chloroplasts."

Regarding the poor comparison with Volten et al.'s (1998) VSF measurements in the backward direction, the following to explanations could be given: 1) unaccounted for light due to backscattering suggested by the referee; and 2) the VSF in the backward direction is much more influenced by non-sphericity (Clavano et al. 2007) - the disagreement could be caused by the assumption of sphericity of the core and shell layers. A brief explanation for the poor agreement has been provided as follows: Added to Line 26, p. 10551. "This is most likely caused by the assumption of sphericity, since the VSF in the backward direction is heavily influenced by non-sphericity (Clavano 2007)."

Considering that Volten et al.'s data are normalised by Petzold's VSF at 90 degrees, there is no way to perform a quantitative check of the scattering/backscattering coefficients. Therefore the comparison is performed for non-quantitative visual purposes.

Regarding the use of Rrs to refine the estimate of n the referee commented that the authors did not take account of all water components in the modelling. However,

the referee appears to be mistaken: the ecolight simulations did include the components CDOM and tripton (see methods sections). The recently published manuscript Matthews, M. W., & Bernard, S. (2013). Characterizing the Absorption Properties for Remote Sensing of Three Small Optically-Diverse South African Reservoirs. Remote Sensing, 5, 4370–4404 provides the background of the specific IOPs used in these simulations. It is also important to note that the M. aeruginosa blooms investigated have extremely high biomass (chl a from 70 – 1500 mg m-3). Therefore these blooms may be effectively treated as "cultures" with the result that the overwhelming influence on Rrs is cyanobacteria (see Matthews& Bernard 2013 figs. 18-19 for ternary plots showing relative influence of absorption from components).

The authors would argue that the method for tuning the 1+e value using Rrs is both sound and appropriate given that only the value of the shell-layer n was allowed to vary over a restricted range in the simulations, which is the primary deterministic factor for backscattering, as stated by the referee.

The referee also suggested that the 2-layered model can be used directly in the inverse ADA. Despite the use of the ADA for two layered spheres by Quirantes & Bernard (2004, Journal of Quantitative Spectroscopy and Radiative Transfer) it is now Bernard's opinion that such a model violates the ADA assumptions (soft sphere) by introducing internal structure. Therefore such an application is not performed here.

The referee has suggested that a) it is premature to simulate Rrs using the backscattering terms derived and B) to derive algorithms from the simulated Rrs. While the authors would argue it is not premature to simulate Rrs, the referee is quite right that it is premature to develop algorithms. Section 4.2 was intended only as a sensitivity study regarding validation of existing algorithms, and for further verification of the model through comparison with existing algorithms (e.g. Matthews et al 2012). Therefore, this section has been slightly rewritten to ensure the intention is not misunderstood.

As regards simulating Rrs, there will undoubtedly be uncertainties in both the modelled

bb* and Rrs. The authors were encouraged by the excellent agreement between the magnitude of modelled bb* and the independent measurements of Zhou et al. (2012) (fig. 7F), although the agreement of the spectral shape is poorer. However, even measurements from backscattering meters (HS2, BB9 etc) contain substantial uncertainties caused by the underlying assumptions of Mie theory (sphericity). The Rrs simulations are a first order modelling attempt given the absence of hyperspectral bb* measurements of vacuolate/non-vacuolate M. aeruginosa, and given that one of the primary aims of the paper is to estimate spectral bb. To the author's knowledge, the attempt presented here is state-of-the-art by avoiding the use of empirical relationships for Rrs using ecolight-s, further reducing the expected uncertainty.

2) Loosely defined terms.

The referee has raised concerns regarding loosely defined terms used in the paper. This has been addressed by the addition of a table defining the symbols (as per referee #1 recommendations), and in addition by complying with the detailed comments made by the referee.

Detailed changes:

The referee's detailed comments have been adhered to and are presented below.

1. The relationship between 1+e and n has been more clearly defined. A brief section of text has been added to the beginning of the methods section: Edited at Line 5 p. 10546. "The details of Mie and Aden-Kerker theory of light scattering with small particles may be found in (Morel 1986, Bernard 2009). Briefly, the complex refractive index (m) is composed of real (n) and imaginary parts (n') according to m = n-in'. n is said to vary according to 1+epsilon+Delta n where 1+epsilon is the central value around which n varies and Delta n is the spectral variation as predicted by the Kramers-Kronig or Ketteler-Helmholtz theories."

2. Backscattering probability is the same as the backscattering ratio in percent. This

term has been changed to the latter throughout.

3. The notation for n‘ has been made consistent throughout the paper.

4. n values have been provided relative to water throughout the paper, and its calculation has been defined at the beginning of the methods section for clarity (see 1. above).

Edited Line 5 p. 10536: “The refractive index of the vacuole is close to 0.825 while that of the other cellular material is close to 1.028 relative to the medium (water).” Edited Line 14 p. 10536: “Calculating the homogeneous refractive index of the cell using the values of Fuhs (1969) for the gas vacuole and chromatoplasm and V_g:V_c ranging from 2–90% gives n_m varying over a considerable range between 1.02 and 0.84 relative to water.”

Added Line 6 p. 10539: “n is provided relative to water as n=n/n_w where n_w is 1.334.” Edits Line 6-7, 17, p. 10541.

5. P10549 L507, The authors agree with the referee’s comment.

6. P 10549 L507, As the volume occupied by the vacuole increases > 50%, there is a decrease in Qa from experimental values. There are theories that gas vacuole shield light in cyanobacteria, diverting light away from the photosynthetic lamella, resulting in a reduction in observed absorption. This is supported by absorption measurements mainly made using conventional geometries. This effect can however be explained more easily by the fact that vacuoles backscatter light away from the photoreceptor and is eliminated when using an integrating sphere (Ogawa 1979). The effect of reduced Qa observed in our data is not expected to support light shielding theories. Rather the reduction in Qa is likely be an artefact caused by volume equivalence assumption used to scale n‘ (eq. 1).

7. Applications: Section 4.2 has been slightly re-written to avoid misunderstanding: Edited section 4.2:

Heading: Sensitivity study of existing empirical models for estimating chl-a

“While semi-analytical algorithms based on bio-optical models and solved using a variety of optimisation procedures are often used for deriving water constituents, forward modelled R_rs from bio-optical models also allow empirical relationships for water constituents to be validated (e.g. Matthews 2011, Dekker 2001). In this example, the sensitivity of existing empirical relationships between chl-a and a 710:665 nm band ratio and a baseline subtraction algorithm called the maximum peak height (MPH) algorithm (Matthews 2012), were tested using simulated R_rs for a population of vacuolate M. aeruginosa. E-S simulations were run for chl-a between 20 and 1000 mg m-3 for vacuolate cells (see section 4.1). b_bphi* was extended to 900 nm in order to facilitate computation of the MPH variable using the value at 750 nm. Noise was introduced by randomly varying the concentration of TR and a_g(440) in the ranges 1 to 50 g m-3 and 0.5 to 5 m-1, respectively, inside the natural variability expected for Hartbeespoort (Matthews 2013). This tested the sensitivity of the empirical relationships to variations in background constituent concentrations.

Fig. 10 shows the R_rs spectra and the resulting empirical relationships derived for the 710:665 ratio and the MPH variables. The best fit determined for the 710:665 ratio was:

$$chla = 7.294 \times exp^{1.2739\times(R_{rs}(710)/R_{rs}(665))}, \quad R^2 = 0.99$$

while that for the MPH variable was:

$$chla=222173mph^2 + 5231.9mph + 14.625, \quad R^2 = 1.0 $$

The high correlation coefficients demonstrate the robustness of empirical-type algorithms for providing chl-a estimates in hypertrophic waters, as confirmed by the simulations. This confirms that empirical studies such as Schalles (1998) and Matthews (2012) have a strong bio-optical basis, provided that the dominant water constituent is

phytoplankton (in this case the species tested is M. aeruginosa). The approach also demonstrates how empirical relationships derived from red/NIR optical signals might be species-specific since they are based on b_bphi*. Therefore, empirically based algorithms are likely to be sensitive to species variability. The sensitivity study showed that the empirical relationships are stable despite variability in background concentrations of TR and gelbstoff."

Edited at Line 3, p. 10559. "Empirical relationships for estimating chl-a in eutrophic/hypertrophic waters are robust even under variable tripton and gelbstoff concentrations, and are likely to be sensitive to species variability."

Abstract: "A sensitivity analysis of empirical algorithms for estimating chl-a in eutrophic/hypertrophic waters suggests these are robust under variable constituent concentrations and likely to be species sensitive."

Interactive comment on Biogeosciences Discuss., 10, 10531, 2013.

Symbol	Definition	Unit
m	Complex refractive index	
n	Real refractive index	
n'	Imaginary refractive index	
n_w	Real refractive index for water	
n_m	Homogeneous real refractive index	
$1+\epsilon$	The central value of n	
Δn	The variation of n around $1+\epsilon$	
d	Diameter	μm
r_{eff}	Effective radius	μm
V_{eff}	Effective variance	
$F(d)$	Size distribution function	
V_g	Gas vacuole volume	
V_c	Core layer volume	
V_s	Shell layer volume	
c_i	Intracellular chl a concentration	kg m^{-3}
a	Absorption coefficient	m^{-1}
b	Scattering coefficient	m^{-1}
c	Attenuation coefficient	m^{-1}
b_b	Backscattering coefficient	m^{-1}
a_ϕ^*	Chl a specific absorption coefficient	m^2 mg^{-1}
b_{ϕ}^*	Chl a specific scattering coefficient	m^2 mg^{-1}
$b_{b\phi}^*$	Chl a specific backscattering coefficient	m^2 mg^{-1}
a_{tr}^*	Tripton mass specific absorption coefficient	m^2 g^{-1}
b_{tr}^*	Tripton mass specific scattering coefficient	m^2 g^{-1}
b_{btr}^*	Tripton mass specific backscattering coefficient	m^2 g^{-1}
a_g	Gelbstoff absorption coefficient	m^{-1}
a_w	Water absorption coefficient	m^{-1}
b_{bp}	Particulate backscattering coefficient	m^{-1}
b_{bp}^*	Chl a specific particulate backscattering coefficient	m^2 mg^{-1}
$\tilde{b_b}$	Backscattering ratio	
$\tilde{b_f}$	Forward scattering ratio	
Q_a	Optical efficiency factor for absorption	
Q_b	Optical efficiency factor for scattering	
Q_c	Optical efficiency factor for attenuation	
$\bar{Qa}$	The experimental mean absorption efficiency factor	
Q_c^{NAE}	The non-absorbing efficiency factor for attenuation	
R_{rs}	Remote sensing reflectance	sr^{-1}
β	Phase function	m^{-1} sr^{-1}

Fig. 1.