Retrieval of phytoplankton size from bio-optical measurements: theory and applications

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The absorption coefficient of a substance distributed as discrete particles in suspension is less than that of the same material dissolved uniformly in a medium—a phenomenon commonly referred to as the flattening effect. The decrease in the absorption coefficient owing to flattening effect depends on the concentration of the absorbing pigment inside the particle, the specific absorption coefficient of the pigment within the particle, and on the diameter of the particle, if the particles are assumed to be spherical. For phytoplankton cells in the ocean, with diameters ranging from less than 1 μm to more than 100 μm , the flattening effect is variable, and sometimes pronounced, as has been well documented in the literature. Here, we demonstrate how the *in vivo* absorption coefficient of phytoplankton cells per unit concentration of its major pigment, chlorophyll *a*, can be used to determine the average cell size of the phytoplankton population. Sensitivity analyses are carried out to evaluate the errors in the estimated diameter owing to potential errors in the model assumptions. Cell sizes computed for field samples using the model are compared qualitatively with indirect estimates of size classes derived from high performance liquid chromatography data. Also, the results are compared quantitatively against measurements of cell size in laboratory cultures. The method developed is easy-to-apply as an operational tool for *in situ* observations, and has the potential for application to remote sensing of ocean colour data.

Keywords: phytoplankton size; average diameter; bio-optical measurements; specific absorption; package effect; diagnostic pigments

1. INTRODUCTION

Phytoplankton communities in aquatic ecosystems are size-structured. Taxonomically diverse species of phytoplankton are distributed in a continuum of cell sizes ranging from less than 1 μm to more than 100 μm , but the size structure is known to be variable. Size regulates a number of important bio-geochemical processes and functions of an aquatic ecosystem. For example, small cell size favours faster nutrient uptake (because of the higher surface-area-to-volume ratio) and large cells sink faster than small ones. Cell size structure of phytoplankton in the ocean varies with variations in nutrient availability [1], mixing and turbulence [2], and selective grazing by higher trophic organisms [3]. Large and small cells of phytoplankton have distinct distributions along resource gradients in the ocean: the large cells dominate in nutrient-rich eutrophic waters, whereas the small cells dominate in nutrient-poor oligotrophic waters [1,4]. Metabolic rates of marine organisms are dependent on their size [5]. But, despite its undoubted importance in ecosystem functioning, it is difficult to obtain accurate measurements of phytoplankton cell size.

As pointed out in a recent review by Nair *et al.* [6], direct measurements of phytoplankton size by microscope is extremely time-consuming, and results may be affected by the sample preservation methods used. Automated methods such as flow cytometry rely on indirect calibration methods for determining size; standard instruments have a limited size range (often an upper limit of less than 20 μm); the instruments are very expensive and require specialist operators. Size analyses by indirect methods such as the use of diagnostic pigments from high-performance liquid chromatography (HPLC) provide at best a partition into three size classes—picoplankton (less than 2 μm), nanoplankton (2–20 μm) and microplankton (more than 20 μm)—and the method is admittedly approximate [7]. For example, if pigments diagnostic of diatoms are found in the sample, they are automatically classed as microplankton, even though smaller diatoms are known to occur in nature. Furthermore, the concentrations of diagnostic pigments in a phytoplankton species may vary according to growth conditions, adding further uncertainties to the interpretation. Phytoplankton absorption characteristics have been used to infer the fraction of small and large cells in a sample [8,9], but the methods available currently do not provide quantitative estimates of cell size.

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Table 1. Notations used in the text.

notation	description	unit
d	diameter of phytoplankton cell	m
λ	wavelength of incident light	nm
C	concentration of chlorophyll a in a sample	mg Chl- a m $^{-3}$
p_i	concentration of absorbing pigment per unit cell volume	mg Pigment- i m $^{-3}$
c_i	concentration of chlorophyll a per unit cell volume	mg Chl- a m $^{-3}$
ρ	optical thickness of intact phytoplankton cell	dimensionless
c_0	proportionality constant in size-scaled intra-cellular concentration	mg Chl- a m $^{-2.94}$
m	size-scaling exponent of intra-cellular concentration	dimensionless
a_{cm}	absorption coefficient of the cell material	m $^{-1}$
a_{ci}	equivalent to a_{cm} when chlorophyll a is the only absorbing pigment	m $^{-1}$
a_p	total phytoplankton absorption coefficient	m $^{-1}$
a_c	total absorption coefficient by chlorophyll a only	m $^{-1}$
a_u	total absorption coefficient by pigments other than chlorophyll a	m $^{-1}$
a_p^*	absorption coefficient of phytoplankton normalized to chlorophyll a	m 2 (mg Chl- a) $^{-1}$
a_c^*	<i>in vivo</i> specific absorption coefficient of chlorophyll a	m 2 (mg Chl- a) $^{-1}$
a^m	maximum value of absorption coefficient normalized to chlorophyll a	m 2 (mg Chl- a) $^{-1}$
a_{cm}^*	specific-absorption coefficient of absorbing pigment inside a cell	m 2 (mg Pigment) $^{-1}$
a_{ci}^*	specific-absorption coefficient of chlorophyll a inside a cell	m 2 (mg Chl- a) $^{-1}$

In this article, we develop a new method based on absorption characteristics of phytoplankton to estimate the mean cell size of phytoplankton populations. Light absorbed by a cell at any wavelength, per unit incident light, is a nonlinear function of the intra-cellular pigment concentration, the absorption properties of the pigments and cell size [10,11]. These properties of the cell are also known to modulate the shape of phytoplankton spectra in the visible domain, an effect commonly known as the flattening effect (or sometimes as packaging effect or particle effect) [12–14]. We exploit the flattening effect to develop a model for estimating the average diameter of a phytoplankton population. Sensitivity analyses are carried out to evaluate the potential errors in the results arising from assumptions in the model. The results for an extensive *in situ* dataset are tested qualitatively against results from HPLC analysis. The outputs are also compared quantitatively against measurements of size on phytoplankton cultures grown in the laboratory [14]. The proposed method has the advantage of ease of implementation as a routine technique for use at sea, and also has the potential to be applied to remotely sensed data.

2. MATERIAL AND METHODS

2.1. Data

The *in situ* dataset used consists of 1932 samples from 50 cruises collected over a period of 13 years (1994–2006) mostly by scientists from the Bedford Institute of Oceanography (Canada). The data come predominantly from the northwest Atlantic, but were also collected on cruises in the Arabian Sea, off Vancouver Island, in the Gulf of Mexico and off the coast of Chile. Chlorophyll a concentration and accessory pigments were measured using reverse-phase, HPLC according to Stuart & Head [15]. Chlorophyll concentration was also estimated using Turner Fluorometer.

Particulate absorption was measured using the filter technique, as detailed in Bouman *et al.* [4].

Further, we have used here a subset of previously published data of Sathyendranath *et al.* [14] that contain data on absorption, pigments, cell-number densities and cell sizes of some 20 individual species of phytoplankton belonging to *Platymonas*, *Dunaliella*, *Tetraselmis*, *Hymenomonas* and *Chaetoceros* genera grown in the laboratory. Measurement details of these data are provided in Sathyendranath *et al.* [14].

2.2. Theoretical background

Phytoplankton cells are treated here as homogeneous spheres, as in [11,13,14]. All the optical properties discussed are functions of wavelength λ . But, for simplicity the wavelength dependence is not stated explicitly henceforth, except where it is essential to do so. The notations used in the following sections are listed in table 1. For a ray of light passing through the centre of the spherical cell, the dimensionless optical thickness ρ of the cell can be expressed as a function of the cell diameter d (say, in m) and the absorption coefficient of the cell material a_{cm} [13,14]:

$$\rho = d \times a_{cm}. \quad (2.1)$$

The quantity a_{cm} is the product of p_i (the concentration of absorbing pigment per unit volume of the cell) and a_{cm}^* (the specific absorption coefficient of the absorbing pigment inside the cell), so that $a_{cm}^* = a_{cm}/p_i$.

Now, if all the absorbing material contained in N cells were dissolved in unit volume of a medium, say water, the absorption coefficient of the hypothetical solution would be given by [14]

$$a_{sol} = \frac{\pi}{6} d^3 N a_{cm}. \quad (2.2)$$

Also, the dimensionless absorption efficiency Q_a of a cell defined as the ratio of the light absorbed by the cell

to the light incident on it, can be written as a function of ρ , according to Duysens [12] and Van de Hulst [16]:

$$Q_a(\rho) = 1 + \frac{2 \exp(-\rho)}{\rho} + 2 \frac{\exp(-\rho) - 1}{\rho^2}, \quad (2.3)$$

and the absorption coefficient a_p of a suspension of N particles in the medium is then given by

$$a_p = \frac{\pi}{4} d^2 N Q_a(\rho), \quad (2.4)$$

where $\pi d^2/4$ is the cross-sectional area of a particle. Comparison of equations (2.2) and (2.4) shows that the absorption coefficient of a substance, dissolved uniformly in a medium (a_{sol}), would differ from the absorption coefficient of the same material in the form of discrete particles suspended in the medium (a_p). The ratio of these two properties defines the flattening effect, F (dimensionless), which can be written as

$$F(\rho) = \frac{a_p}{a_{\text{sol}}} = \frac{3 Q_a(\rho)}{2 \rho}, \quad (2.5)$$

so that the specific absorption coefficient a_p^* of intact cells of phytoplankton in suspension in water can be written as

$$a_p^*(\rho) = a_{\text{cm}}^* \times F(\rho) = \frac{3 a_{\text{cm}}^* Q_a(\rho)}{2 \rho}. \quad (2.6)$$

For many practical purposes, chlorophyll a is considered to be the main pigment in phytoplankton, and the absorption properties of phytoplankton are reported relative to the concentration of chlorophyll a . The absorption coefficient of cell material, when chlorophyll a is the only absorbing pigment inside the cell, can be written as a_{ci} ; and is defined as the product of c_i (in $\text{m}^{-3} \text{mg Chl-}a$), the concentration of chlorophyll a per unit volume of the cell, and a_{ci}^* (in $\text{m}^2 (\text{mg Chl-}a)^{-1}$), the specific absorption coefficient of chlorophyll a inside the cell, such that $a_{ci}^* = a_{ci}/c_i$, and optical thickness $\rho_c = d \times a_{ci}$. In this case, the specific absorption coefficient of chlorophyll a a_c^* (in $\text{m}^2 (\text{mg Chl-}a)^{-1}$) of intact cells of phytoplankton in suspension in water can be written as

$$a_c^*(\rho) = a_{ci}^* \times F(\rho_c) = \frac{3 a_{ci}^* Q_a(\rho_c)}{2 \rho_c}. \quad (2.7)$$

3. MODEL IMPLEMENTATION AND SENSITIVITY ANALYSIS

Equation (2.7) shows that the *in vivo* specific absorption coefficient of phytoplankton cells carries implicit information on the cell size, since it is a monotonic function of ρ_c , which in turn is a function of cell diameter d . So, given the *in vivo* chlorophyll-specific absorption coefficient a_c^* , the cell diameter of phytoplankton can be calculated from equation (2.7). But three problems have to be surmounted before equation (2.7) can be used to retrieve d from a_c^* : first, the intracellular chlorophyll a concentration c_i is unknown and has to be parameterized as a function of diameter; and second, a_{ci}^* is a wavelength-dependent unknown

quantity, which has to be determined; and finally, since phytoplankton contain other pigments in addition to chlorophyll a , to obtain a_c^* we have to account for the contribution of materials other than chlorophyll a to phytoplankton absorption. We now consider how these three issues may be addressed.

3.1. Intracellular chlorophyll a concentration c_i

Intracellular chlorophyll a concentration (c_i) is known to be highly variable among phytoplankton species [13,14,17]. Recently, Marañón *et al.* [18] studied concentration of chlorophyll a per cell as a function of cell size using size-fractionated phytoplankton. Their data were collected from contrasting marine environments with different light and nutrient conditions, over different periods of the year, covering temperate, subtropical and equatorial regions; and the measurements included surface, subsurface and deep-water (up to 140 m) samples (detailed in [18], table 1). Although it is well understood that the intracellular concentration of chlorophyll changes with the light environment [19,20], the parameterization of Marañón *et al.* [18] does not capture such effects: instead, it represents the general dependency of intracellular chlorophyll concentration on cell size. They calculated chlorophyll a content per cell by dividing the chlorophyll a concentration of each size fraction by the number of cells within that size fraction, and showed that cell content of chlorophyll a varied as a function of cell diameter [18].

Further, they reported that the isometric scaling between cell volume and chlorophyll a per cell contrasts with previous observations of phytoplankton in laboratory cultures, and they derived a size-scaling exponent 0.98, much higher than that reported previously [18, fig. 3]. From their results, we can infer that the intracellular concentration (c_i) in general varies with cell size according to:

$$c_i = c_0 d^{-m}, \quad (3.1)$$

where m is the size-scaling exponent and c_0 the proportionality constant. The parameters m and c_0 of this relationship can be calculated from the numerical relationship reported in ([18], fig. 3). The relationship between the cell volume and the concentration of chlorophyll a per cell given by Marañón *et al.* [18] is $[\text{chlorophyll } a][\text{cell}]^{-1}$ (in $\text{pg unit} = 10^{-2.42} \times [\text{cell volume in } \mu\text{m}^3]^{0.98}$). If we divide both sides of this relationship by $[\text{cell volume}] = (\pi/6)[\text{cell diameter}]^3$, then the equation expresses the intra-cellular concentration of chlorophyll a (c_i) as a function of cell diameter. If c_i is expressed in $(\text{mg Chl-}a \text{ m}^{-3})$ and d in (m), the magnitudes of the parameters of equation (3.1) can be estimated as $c_0 = 3.9 \times 10^6$ ($\text{mg Chl-}a \text{ m}^{-2.94}$) and $m = 0.06$ (dimensionless). Based on equation (3.1), we can express ρ_c and a_c^* as

$$\rho_c = a_{ci}^* \times c_0 d^{-m} \times d = a_{ci}^* c_0 d^{1-m} \quad (3.2)$$

and

$$a_c^*(\rho_c) = a_{ci}^* \times F(\rho_c) = \frac{3 a_{ci}^* Q_a(\rho_c)}{2 \rho_c}. \quad (3.3)$$

Figure 1. Bio-optical basis of combining cell diameter to specific absorption. (a) Variation of intracellular chlorophyll *a* concentration with cell diameter (red line, derived from Marañón *et al.* [18]); (b) phytoplankton absorption efficiency under assumptions of constant (black) and variable (red) intracellular pigment concentrations; (c) specific absorption of chlorophyll a_c^* against diameter at $\lambda = 676$ nm under assumptions of constant (black) and variable (red) intracellular pigment concentration; the blue dots represent the chlorophyll-normalized absorption a_p^* data of laboratory cultures taken from Sathyendranath *et al.* [14]. Possible error (percentage) in estimated diameter across the cell size owing to error in assigning (d) the exponent m ; (e) the parameter c_0 and (f) the specific absorption of unpackaged chlorophyll inside the cell a_{ci}^* ; different thickness of lines correspond to 10% (light grey line), 20% (dark grey line) and 30% (solid black line) errors in the respective quantities.

The intracellular chlorophyll *a* concentration (c_i) is plotted as a function of the diameter d of the phytoplankton cell in figure 1*a* using the above parameterizations. Compared with the case under the assumption of a constant intracellular pigment concentration, the absorption efficiency $Q_a(\rho_c)$ increases more sharply as a function of diameter (figure 1*b*), when c_i is allowed to vary with cell size according to equation (3.1).

3.2. Choice of a_{ci}^*

Although phytoplankton absorption models are generally parameterized as a function of chlorophyll *a* concentration, many pigments other than chlorophyll *a* contribute to light absorption by phytoplankton cells [14,21,22]. Variability in abundance and composition of these auxiliary pigments and the structure of the pigment–protein complexes within the cell [22] could be a source of variation in the absorption properties of phytoplankton pigments. Therefore, the specific absorption coefficient of unpackaged chlorophyll *a* of a cell (i.e. a_{ci}^*) has been difficult to measure in practice. To minimize the effect of auxiliary pigments, it is desirable to choose

a wavelength at which the contribution of pigments other than chlorophyll *a* to phytoplankton absorption is minimum. We know that chlorophyll *a* is typically responsible for most of the absorption at the red peak in phytoplankton absorption at around $\lambda \sim 676$ nm. Therefore, we assume that, in laboratory cultures maintained at high concentrations, the background contributions to the absorption at 676 nm from substances other than chlorophyll *a* may be negligible, such that $a_{cm}^* = a_{ci}^*$. In laboratory cultures, the magnitude of a_{cm}^* at the red peak near $\lambda \sim 676$ nm has been measured in the range $[0.025, 0.028] \text{ m}^2 (\text{mg Chl-a})^{-1}$ [13,21,23–26]. We note that, those reports have been based on laboratory cultures of both large (e.g. *Coscinodiscus* sp. by [26]), and small phytoplankton (e.g. *Prochlorococcus* and *Synechococcus* by [24]). Since by definition, the specific absorption coefficient of chlorophyll *a* (a_c^*) should be maximum when it is unpackaged, we choose the magnitude of a_{ci}^* to be the maximum of the reported values. In other words, we choose $\lambda = 676$ nm for the implementation of the model, and fix the magnitude of $a_{ci}^*(676)$ at $0.028 \text{ m}^2 (\text{mg Chl-a})^{-1}$.

For a given cell size d of phytoplankton, the specific absorption coefficient of chlorophyll a a_c^* at $\lambda = 676$ nm can be determined directly from equation (3.3). We note that when the equation is implemented with a variable c_i , the change in a_c^* (676) with cell diameter d is steeper than that expected under assumption of a constant c_i (figure 1c). The result for the variable c_i is consistent with the data for specific absorption (figure 1c) from laboratory cultures reported previously by Sathyendranath *et al.* [14]. Now, according to figure 1c, given the magnitude of a_c^* at 676 nm of a phytoplankton sample, the diameter of the cells can be calculated from equation (3.3).

3.3. Specific absorption coefficient of chlorophyll a a_c^*

In field samples [9,27], especially at low chlorophyll concentrations, $a_p^*(\lambda)$ sometimes appears to be greater than $0.028 \text{ m}^2 (\text{mg Chl-a})^{-1}$, the maximum value for $a_c^*(\lambda)$ reported from laboratory cultures, suggesting that the absorption by pigments other than chlorophyll a may not always be negligible at 676 nm.

Under those circumstances, the total phytoplankton absorption $a_p(\lambda)$ at any wavelength λ can be expressed as

$$a_p(\lambda) = a_c(\lambda) + a_u(\lambda), \quad (3.4)$$

where $a_c(\lambda)$ refers to absorption by chlorophyll a only, and $a_u(\lambda)$ refers to that by other pigments. Dividing both sides of equation (3.4) by the concentration of chlorophyll a C , we get the coefficients of light absorption normalized to chlorophyll a . Assuming that the concentration of chlorophyll a and that of other pigments are expressed in common units, we can write,

$$a_p^*(\lambda) = \frac{a_p(\lambda)}{C} = \frac{a_c(\lambda)}{C} + \frac{a_u(\lambda)}{C} = a_c^*(\lambda) + \frac{a_u(\lambda)}{C}. \quad (3.5)$$

Here, $a_c^*(\lambda)$ is the specific absorption of chlorophyll a , and the left-hand side is the absorption of phytoplankton normalized to chlorophyll a . For many practical purposes the second term on the right-hand side of equation (3.5) is often ignored; i.e. it is assumed that $a_c^*(\lambda) \approx a_p^*(\lambda)$ in the red absorption peak of chlorophyll a . Equation (3.5) suggests that if $a_u(\lambda)$ does not decrease with chlorophyll a , then in the limiting case, the assumption of $a_c^*(\lambda) \approx a_p^*(\lambda)$ may overestimate the magnitude of $a_c^*(\lambda)$ significantly. But, since total phytoplankton absorption $a_p(\lambda)$, which is a sum of $a_c(\lambda)$ and $a_u(\lambda)$, is known to decrease with chlorophyll a , it is obvious that both $a_c(\lambda)$ and $a_u(\lambda)$ would decrease independently with chlorophyll a . It means that the chlorophyll a normalized phytoplankton absorption $a_p^*(\lambda)$ cannot increase beyond a certain ceiling. From equation (3.5) it is now clear that, in the limiting case the magnitude of $a_p^*(\lambda)$ has a maximum upper bound, and at that point $a_p^*(\lambda) \gg a_c^*(\lambda)$.

Based on these considerations, we make two assumptions: (i) at any wavelength λ , as the total phytoplankton absorption decreases, $a_p^*(\lambda)$ approaches a maximum value, say, $a^m(\lambda)$; and (ii) the specific absorption coefficient of chlorophyll a , $a_c^*(\lambda)$, has an asymptotic maximum value $a_{ci}^*(\lambda)$, which is the

specific-absorption at wavelength λ of unpackaged chlorophyll a *in vivo*.

Clearly, owing to the package effect, the magnitude of chlorophyll a specific phytoplankton absorption $a_c^*(\lambda)$ is always less than that of $a_{ci}^*(\lambda)$. However, as mentioned in §2.2, the package effect decreases for small cells, and for cells with smallest diameter the package effect is negligible, i.e. as $d \rightarrow 0$, $a_c^*(\lambda) \rightarrow a_{ci}^*(\lambda)$. But when the cell diameter d is very small, the total amount of chlorophyll a held by the cell is also negligible, i.e. $C \rightarrow 0$ when $d \rightarrow 0$. Thus, as $C \rightarrow 0$, the maximum value of chlorophyll a specific phytoplankton absorption $a_p^*(\lambda)$ (at any wavelength λ) should represent the specific absorption of unpackaged chlorophyll a $a_{ci}^*(\lambda)$ (at that wavelength). On the other hand, in this limiting case, owing to our assumption (i), the chlorophyll-normalized phytoplankton absorption $a_p^*(\lambda)$ approaches to the maximum value $a^m(\lambda)$. Thus, we have the following two limiting conditions:

- A1. As $a_p^*(\lambda) \rightarrow a^m(\lambda)$, we have $a_c^*(\lambda) \rightarrow a_{ci}^*(\lambda)$; and
- A2. As $a_p^*(\lambda) \rightarrow 0$, we have $a_c^*(\lambda) \rightarrow 0$.

These trends can be captured in a continuous function as follows:

$$a_c^*(\lambda) = \frac{a_p^*(\lambda)}{1 + \sigma_c(\lambda)a_p^*(\lambda)}. \quad (3.6)$$

In the above equation, at any given wavelength (say, $\lambda = 676$ nm) chlorophyll a specific absorption (a_c^*) gradually increases with phytoplankton absorption normalized to chlorophyll a (a_p^*) to a saturation level (figure 2a), and the parameter $\sigma_c(\lambda)$ determines how slowly the saturation level is reached. Using (A1) and (A2), we find the magnitude of the parameter $\sigma_c(\lambda)$ as:

$$\sigma_c(\lambda) = \left(\frac{1}{a_{ci}^*(\lambda)} - \frac{1}{a^m(\lambda)} \right). \quad (3.7)$$

In particular, for $\lambda = 676$ nm the maximum numerical value of $a_{ci}^*(676)$ reported for laboratory cultures is $0.028 \text{ m}^2 (\text{mg Chl-a})^{-1}$ [13,26], as noted in the previous section.

The magnitude of $a^m(676)$ can be determined from phytoplankton absorption spectra based on *in situ* measurements. To obtain a possible numerical value of $a^m(676)$ we perform quantile analysis for a_p^* (676) values against chlorophyll a concentrations. We consider the *in situ* dataset consisting of 1932 samples collected from 50 cruises during 1994–2006 (see §2.1 for details). For these samples the 95 per cent quantile value of $a_p^*(676)$ turns out to be $0.0412 \text{ m}^2 (\text{mg Chl-a})^{-1}$. Therefore we take $a^m(676) = 0.0412 (\text{mg Chl-a})\text{m}^{-2}$, and we note that this saturating value of chlorophyll-normalized absorption is consistent with the *in situ* data reported by Bricaud *et al.* [28] and Barocio-Leon *et al.* [27].

For this estimated value of $a^m(676) \text{ m}^2 (\text{mg Chl-a})^{-1}$, we find from equation (3.7) the magnitude of $\sigma_c(676) \approx 11.44 (\text{mg Chl-a})\text{m}^{-2}$. It is noteworthy that, at any wavelength λ ($= 676$ nm, say) chlorophyll-normalized phytoplankton absorption (a_p^*) and the specific absorption of chlorophyll a (a_c^*) follow similar trends against

Figure 2. Computation and sensitivity of chlorophyll *a*-specific phytoplankton absorption: (a) *in vivo* specific absorption of chlorophyll *a* a_c^* (676) as a function of chlorophyll *a*-normalized absorption a_p^* (676) using equation (3.6) given in text; (b) error % in a_c^* (676) estimation as a function of a_c^* (676) values owing to error in parameter a^m —different thickness of black lines correspond to 10% (light grey line), 20% (dark grey line) and 30% (solid black line) errors in a^m ; and (c) observed phytoplankton absorption normalized to chlorophyll *a* a_p^* (red circle) and specific absorption of chlorophyll *a* a_c^* (blue plus) at $\lambda = 676$ nm as functions of observed chlorophyll *a* concentrations.

chlorophyll *a* (figure 2c). However, owing to the systematic elimination of light absorption by pigments other than chlorophyll *a* (using equation 3.6), the magnitude of a_c^* is marginally lower than a_p^* across the chlorophyll *a* range (figure 2c).

3.4. Sensitivity analysis

The magnitude of the diameter d calculated in the method described in previous sections will be sensitive to four model parameters, c_0 , m , a_{ci}^* (676) and a^m (676). We assess, through a sensitivity analysis, the potential errors in estimated diameter owing to uncertainties in assigning each of these parameters. We assume that the relative errors associated with the magnitudes of any two parameters are independent of each other (i.e. the error covariance terms are taken to be zero). Keeping all other parameters unchanged, the error in diameter ($\Delta d/d$) arising from an error ($\Delta m/m$) in the parameter m is calculated from

equations (3.1)–(3.2):

$$\frac{\Delta d}{d} = \left[\frac{m(-6 \log_e 10 + \log_e d)}{1 - m} \right] \times \frac{\Delta m}{m}. \quad (3.8)$$

This equation suggests that a positive error in m may lead to an underestimation, and a negative error to an overestimation, of the cell diameter, and that the magnitude of the relative error in diameter is maximum for small cells and decreases gradually towards large cells (figure 1d). Moreover, within a reasonable size range for phytoplankton, the relative error in estimated diameter never exceeds the relative error associated with the parameter m itself (figure 1d).

Similarly, keeping other parameters unchanged the error in diameter ($\Delta d/d$) arising from an error in c_0 (i.e. $\Delta c_0/c_0$) is calculated from equations (3.1)–(3.2):

$$\frac{\Delta d}{d} = -\frac{1}{1 - m} \times \frac{\Delta c_0}{c_0}. \quad (3.9)$$

The error in this case is flat across the size range; a positive error in c_0 results in a negative error in estimated diameter, and the relative error in diameter is almost equal to that in the parameter c_0 itself (figure 1e).

Further, keeping all other parameters unchanged, the error in estimated diameter corresponding to an error in a_{ci}^* (i.e. $\Delta a_{ci}^*/a_{ci}^*$) is calculated from equations (3.2)–(3.3):

$$\frac{\Delta d}{d} = \left[\frac{1}{(1-m)(1-(\rho_c/Q_a(\rho_c))(\partial Q_a(\rho_c)/\partial \rho_c))} - (1-m) \right] \times \frac{\Delta a_{ci}^*}{a_{ci}^*}. \quad (3.10)$$

This expression suggests that a positive error in a_{ci}^* may lead to a positive error in cell diameter. Although the relative error is high for very small cells (especially for picoplankton and nanoplankton with diameter less than 10 μm), it decreases sharply for large cells, and for large phytoplankton the relative error is likely to be much less than the relative error in a_{ci}^* (figure 1f).

Finally, the uncertainty in determining chlorophyll a specific absorption (i.e., $\Delta a_c^*(\lambda)/a_c^*(\lambda)$) arising from uncertainty in the saturation parameter $a^m(\lambda)$, when other parameters are unchanged, is calculated from equations (3.6)–(3.7):

$$\frac{\Delta a_c^*(\lambda)}{a_c^*(\lambda)} = - \left[\frac{a_c^*(\lambda)}{a^m(\lambda)} \right] \times \frac{\Delta a^m(\lambda)}{a^m(\lambda)}. \quad (3.11)$$

Thus, a positive error $a^m(\lambda)$ may lead to a negative error in $a_c^*(\lambda)$. The magnitude of the possible error reduces for low $a_c^*(\lambda)$; and the relative error is always less than that in the parameter $a^m(\lambda)$ (figure 2b).

We note that the accuracy of cell size determined from equations (3.2)–(3.3) depends on the correct choice of parameters c_0 , m and a_{ci}^* (676). However, the analysis above suggests that a small uncertainty in these parameters is not likely to lead to magnified errors in size estimation.

3.5. Implementation of the method

The method for determining cell diameter from specific absorption coefficient outlined above was applied to over 1800 measurements of a_p^* (676). We assigned the model parameters as discussed above, and the cell diameters were computed from equations (3.2)–(3.3) numerically, using the Newton–Raphson scheme [29]. Sensitivities of the model to our assumptions were computed numerically using equations (3.8)–(3.11). Scripts written in MATLAB were used for all computations.

4. RESULTS AND DISCUSSION

4.1. Computed diameters compared with size classes

Using diagnostic pigments Vidussi *et al.* [7] and Uitz *et al.* [30] have estimated fractions of total chlorophyll associated with picoplankton, nanoplankton and microplankton. In spite of its limitations (as noted in [7]) the

method gives a rough estimation of the relative proportions of the three size classes in a sample. We have used the pigment method to calculate the fractions of three size groups in our samples.

For comparison with the estimated cell size, we categorize a sample as dominated by picoplankton, nanoplankton or microplankton if the fraction of that size class is higher than the fractions of either of the other two size classes. The samples dominated by pico-, nano- and micro-plankton appear sequentially, with some overlapping, from low to high chlorophyll concentration. The samples with high chlorophyll are generally dominated by cells of large diameter and those with low chlorophyll by cells of small diameter (figure 3a). Further, the microplankton-dominated and picoplankton-dominated samples classified by diagnostic pigments appear in separate clusters consistent with the estimated diameters (figure 3a).

The results from the new method proposed here are thus consistent with information on the gross community and size structure of the phytoplankton inferred from the diagnostic pigments.

4.2. Size variability among taxonomic groups

Based on major pigments identified by HPLC, phytoplankton samples dominated by certain taxonomic groups can be distinguished [31]. Using this method samples dominated by diatoms, prymnesiophytes, *Prochlorococcus*, other cyanobacteria and green algae were identified. When a phytoplankton population is dominated by a particular phytoplankton group, the cell diameter calculated from absorption is consistent with the nominal size of that particular taxonomic group. Samples identified as being diatom-dominated tend towards large diameters (figure 3b); and samples dominated by *Prochlorococcus* have the smallest diameters, with other phytoplankton groups occupying intermediate size ranges (figure 3b and table 2).

The derived mean sizes of samples dominated by diatoms, prymnesiophytes, other cyanobacteria and green algae are also consistent, respectively, with their typical cell sizes. However, we note that the derived size of *Prochlorococcus* dominated samples, the smallest among five groups, is around 3 μm , which appears to be a significant overestimation, because these organisms are known to have a diameter of 1 μm or even less [32,33]. Some of this discrepancy may arise from differences in the methods compared. Although pigment analysis used here determines the dominant phytoplankton type, this method does not imply that a population is necessarily composed of only the dominant type. When a population is dominated by the smallest cells, the presence of even a small portion of large cells will increase the average size, which could explain cell sizes of more than 1 μm for samples which were identified through the pigment method as dominated by *Prochlorococcus* (table 2).

Another source of error may lie in the optical method as implemented here. According to figure 1f, errors in estimated diameters arising from errors in the parameter a_{ci}^* could be highest for the smallest diameters. Further, apart from divinyl chlorophyll *a*, the cells of

Figure 3. Comparison of the estimated diameter with *in situ* size class: (a) estimated diameter of phytoplankton population as a function of chlorophyll *a* concentration. Green, blue and red dots represent samples dominated, respectively, by picoplankton, nanoplankton and micro-plankton classified by diagnostic pigments; (b) estimated diameter as a function of chlorophyll *a* for samples dominated by diatoms (yellow dots), prymnesiophytes (red dots), *Prochlorococcus* (black dots), other cyanobacteria (blue dots) and green algae (green dots) as identified by pigments.

Prochlorococcus contain appreciable levels of divinyl chlorophyll *b* that absorbs light at 676 nm. The errors in measurement of diameter associated with *Prochlorococcus* may therefore also be owing to the presence of different ecotypes that vary in their intracellular concentration of divinyl chlorophyll *b* [34].

4.3. Comparison with laboratory data

The comparison with HPLC data presented above can be only qualitative at best, since the pigment data are only indicative of size classes, and deviations from these common trends are known to occur [7]. As a more explicit test, we compared diameters estimated from absorption data against diameters measured in laboratory culture, using data published by Sathyendranath *et al.* [14]. This study reported the

phytoplankton absorption values normalized to chlorophyll $a_p^*(676)$ and the average cell diameters of cultures of *Platymonas*, *Dunaliella*, *Tetraselmis*, *Hymenomonas* and *Chaetoceros* grown in the laboratory.

Now, using the reported values of $a_p^*(676)$, the chlorophyll-specific phytoplankton absorption $a_c^*(676)$ can be obtained from equation (3.6); and using the reported diameters, the $a_c^*(676)$ values can be calculated from equations (3.2)–(3.3). The $a_c^*(676)$ values obtained by these two methods (figure 4a) were significantly correlated, correlation coefficient $r = 0.74$ ($p < 0.005$). When the $a_c^*(676)$ values obtained from equations (3.2)–(3.3) were regressed against those from equation (3.6), the slope of the regression line turned out to be 1.07 with a 95% CI of [0.99 1.14], suggesting that the two sets of values fell along the 1 : 1 line, and that the $a_c^*(676)$ values of the laboratory cultures obtained independently from two different sets of equations used in our method were consistent with each other (figure 4a).

Similarly, the cell diameters computed from $a_c^*(676)$, obtained from equation (3.6), and the diameters measured in culture were significantly correlated with correlation coefficient $r = 0.69$, $p < 0.005$ (figure 4b). Further, when the estimated diameters were regressed against measured diameters, the slope of the regression line was 1.07 with a 95% CI of [0.94 1.21], suggesting that the estimated and measured diameters fell along the 1 : 1 line, and hence that those were also consistent with each other (figure 4b).

5. CONCLUDING REMARKS

Bio-optical properties of phytoplankton population have been used in this study to obtain estimates of the average cell size of the population. The method developed here requires prior knowledge of only a few biological properties that can be obtained easily in the field. We have performed sensitivity analyses to assess possible errors in measuring the cell size from absorption coefficient, and have demonstrated that the uncertainties are not high, especially when estimating the size of large-cell-dominated populations. The method has been tested on a large dataset collected during several cruises, and we have demonstrated that the outcome of the method is consistent with the products of another method, based on pigments, that is often used to assess phytoplankton size classes. Moreover, the method was able to retrieve cell sizes of phytoplankton cultures grown in the laboratory.

Using our algorithm, we have estimated the cell diameters of those samples that were initially identified, based on composition of diagnostic pigments, as diatoms, prymnesiophytes, other cyanobacteria or green algae. The estimates are consistent with the size ranges of those phytoplankton types reported in the literature. The pigment-based method, when used to identify taxonomic groups (such as diatoms), can only be indicative of a broad size range associated with the groups. The optical method proposed here is able to estimate the average diameter of a phytoplankton sample, and is hence able to provide further information

Figure 4. Comparisons of the estimated diameters with laboratory culture data taken from Sathyendranath *et al.* [14]: (a) chlorophyll *a* specific absorption $a_c^*(676)$ values calculated from equation (3.2)–(3.3) using the diameters measured in laboratory culture against those calculated from equation (3.6) using $a_p^*(676)$ values measured in laboratory culture; (b) phytoplankton cell diameters computed by the current method against those measured in laboratory culture.

Table 2. Statistics of the estimated diameters for the mixed-population and for samples dominated by individual taxonomic groups.

dominating phytoplankton group	number of samples	diameter mean $\pm$ std (μm)	diameter minimum (μm)	diameter maximum (μm)	diameter median (μm)
diatoms	64	23.7 $\pm$ 8.8	6.6	48.9	23
Prymnesiophytes	4	6.8 $\pm$ 2.6	4.9	10.6	5.8
<i>Prochlorococcus</i>	5	3.3 $\pm$ 0.3	2.9	3.7	3.2
other cyanobacteria	6	7.2 $\pm$ 5.1	2.5	15.1	5.5
green algae	7	4.5 $\pm$ 2.6	1.7	9.0	4.3

on the variability in cell size within a taxonomic group or even within samples of a given size class such as pico-, nano- or micro-phytoplankton.

The method used here relies on measurements of phytoplankton absorption coefficient at 676 nm and chlorophyll *a* concentration to estimate the mean cell size of the phytoplankton population. The concentration of chlorophyll *a* is generally measured either by HPLC or by Turner fluorometer. We have applied our algorithm to estimate phytoplankton diameter both from HPLC and Turner fluorometer. We found that (data not shown) both the estimates were very similar. Thus, the chlorophyll values estimated by either method can be used for diameter estimation. Furthermore, chlorophyll *a* concentration is a standard product from remote sensing of ocean colour. Methods have also emerged [6,35] to estimate phytoplankton absorption coefficient from ocean-colour data. These two products taken together raises the possibility of estimating phytoplankton cell size by remote sensing.

Since our algorithm produces estimates of average cell size of phytoplankton samples, these estimates can be related quantitatively to the major size classes of phytoplankton. For example, given that the fractions of biomass of different size classes (picoplankton, nanoplankton or microplankton) in plankton samples

can be estimated from other methods (e.g. the diagnostic pigments), one can derive relationships between the biomass fractions and the average cell sizes obtained by our method. These relationships will be useful for application to ecosystem models.

The sensitivity studies point to directions where further work is needed to achieve improvements in the proposed method. Clearly, the results are very sensitive to errors in the assumed values of the *in vivo* intracellular absorption characteristic of the pigments, especially for small cell sizes. The other source of error is related to assumptions regarding the intracellular concentration of chlorophyll *a*, which is in principle susceptible to variation, in response to changes in the light environment. Typically, phytoplankton populations in the natural environment occupy a broad size range, and the effect of the size structure on the optical properties (see [36]) is not explored in the model used here, which assumes a single size for the population.

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