Reconstruction of microalgal suspension absorption spectra from reflectance spectra of the cells deposited on GF/F filters

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ABSTRACT

In this communication we present an approach for reconstruction of suspension absorption spectra of microalgae as measured in standard 1-cm spectrophotometric cuvettes from reciprocal reflectance, $R(\lambda)^{-1}$, spectra of cells deposited on glass-fiber filters. Reciprocal reflectance exhibited the highest dynamic range (eight times wider in comparison with the functions $-\log(T)$ and $-\log(R)$) and showed almost linear relationships with suspension absorbance and chlorophyll content from 0.7 to 9.9 $\mu$g/ml. The relationships between suspension absorbance and the $-\log(T)$ and $\log[(1-R)/T]$ functions were non-linear and were fitted by second-order polynomials with high contribution of the quadratic term. The use of $R(\lambda)^{-1}$ allowed the reconstruction of suspension absorbance of six species from key groups of planktonic microalgae in the range from 0.001 to 0.250, with RMSE < 0.02. The advantages of the suggested approach over traditional techniques employing $-\log(T(\lambda))$ of the filters are discussed, including the suitability for plankton studies in the field and for the monitoring of cultivated microalgae.

Keywords: Microalgae optics, absorbance spectra reconstruction, reflectance, transmittance, quantitative filter technique

INTRODUCTION

Spectral information obtained in aquatic environments may be successfully used for the estimation of phytoplankton concentration, its spatial distribution and taxonomic composition (Mayo et al., 1995; Yacobi et al., 1995; Schalles et al., 1998; Morel and Maritorena, 2001). A special challenge is posed when the density of microalgae in water is extremely low (<1 mg m$^{-3}$ of total chlorophyll, Chl), and requires the concentration of relatively large water samples, mostly on glass-fiber filters, followed by spectrophotometry and reconstruction of the absorbance spectra of the suspended particles (Mitchell et al., 2003). The measurements of light absorption by filters carrying microalgal samples (known as quantitative filter technique, QFT) are routinely used for laboratory measurements (Mitchell et al., 2003; Tassan and Ferrari, 2002). Measurement of spectra of cells collected onto filters may be handy in studies of lab and (outdoor) mass cultures of microalgae, especially for characterization of microalgal species possessing cells that tend to clot or rapidly fall on the cuvette floor during

Abbreviations: Chl—(total) chlorophyll(s), CLSM—confocal laser scanning microscope, QFT—quantitative filter technique

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spectrum scanning (Solovchenko et al., 2010, 2011). The reconstruction of absorbance spectra of microalgal cell suspensions from spectra of the cells deposited on filters requires an understanding of the relationships between optical properties of the microalgal cells as measured in standard spectrophotometric cuvettes and on filters. Differing values have been reported for the correlation between absorption by a microalgal suspension sample and attenuation of light passing through the sample collected onto a filter. These studies revealed a wide variation of bio-optical properties of phytoplankton (Dall’Olmo and Gitelson, 2005). This variation is caused by changes in taxonomic composition of the phytoplankton and the environmental conditions where the phytoplankton thrives (Bricaud et al., 2004). However, it should be remembered that, in addition to the mentioned factors, bio-optical variation is also contributed by factors inherent to the spectrophotometry of algal samples deposited on glass-fiber filters, such as strong light scattering. According to Mitchell (1990, 2003), the optical density of microalgae in cuvette and on Whatman glass-fiber filters exhibits non-linear relationships, which could be empirically described by a second order polynomial. A “transmittance–reflectance” method was suggested to account for light losses due to backscattering (Tassan and Ferrari, 1995, 2002). However, this method, though more precise, appears to be more laborious since it requires at least one additional (reflectance) spectrum scan.

Reflectance-based algorithms are widely used in remote sensing of phytoplankton in water bodies (Gitelson et al., 1993; Mayo et al., 1995; Schalles et al., 1998). Gitelson and colleagues successfully applied a reflectance-based technique for remote estimation of cell mass and Chl content in Spirulina (Gitelson et al., 1995) and ultra-high Nannochloropsis culture (Gitelson et al., 2000). Reciprocal reflectance, $R(\lambda)^{-1}$, spectra were successfully employed for non-destructive assay of pigment content in whole higher plant leaves and fruits (Merzlyak et al., 2003; Merzlyak et al., 2005; Gitelson et al., 2009). That experience inspired the attempt to reconstruct the suspension absorption spectra of microalgae from their reflectance spectra measured after deposition on a filter, which is the subject of the current report. The hypothesis that pigment content, absorbance, and reflectance of microalgal cells loaded onto a filter maintain close relationships is seemingly in line with the theory of diffuse reflectance developed in previous years (Atherton, 1955; Aldeeson et al., 1961). To the best of our knowledge, no attempts have been made so far to obtain the spectral characteristic of microalgal samples solely via reciprocal reflectance spectra measurements. Therefore, we investigated the relationships between absorbance of microalgal cell suspensions measured in a standard spectrophotometric cuvette and optical properties (reflectance and absorbance) of the same cells deposited on glass-fiber filters. Finally, we compared the efficiency of different spectral functions in reconstruction of the microalgal suspension absorbance spectra.

**METHODS**

**Microalgal cultures**

Cultures of microalgae representing key groups of phytoplankton were used in this work: the cyanobacteria *Anabaena variabilis* Kütz. ATCC 29413 and *Anacystis nidulans* (Richt.) Drouet & Daily (Synechococcus elongatus Nag.) IPPAS B-267 (Cyanobacteria) cultivated on BG-11 medium (Stanier et al., 1971), the chlorophytes *Chlorella pyrenoidosa* Chick and *Scenedesmus quadricauda* (Turp.) (Chlorophyta) cultivated on Tamiya medium (Tamiya, 1966), and the diatoms *Thalassiosira weissflogii* (Grunow) G. Fryxell & Hasle and *Skeletonema costatum* (Grev.) cultivated on f/2 medium (Guillard and Ryther, 1962). The cyanobacterial cultures were obtained from the collection of the Department of Bioengineering of M.V. Lomonosov MSU, the cultures of the chlorophytes and the diatoms were kindly supplied by Profs. D.N. Matorin and S.I. Pogosyan (Department of Biophysics, Biological Faculty of M.V. Lomonosov MSU) who originally isolated the strains. The cultures were maintained at 25 °C under the irradiance of 100 μmol PAR photons m$^{-2}$ s$^{-1}$. The irradiance was measured with LI-850 quantometer (LiCor, USA)

**Sample preparation**

A suspension aliquot was pelleted by centrifugation and re-suspended in fresh medium to obtain stock suspension of ca. 0.15–0.45 OD at 678 nm. Then, seven dilutions were made stepping 1/8 (from 7/8 to 1/8 of the stock suspension) using corresponding cultivation medium for each species. Absorbance spectra of the stock and each of the diluted samples were recorded. Then aliquots of 0.75 mL were taken from each sample. The aliquots were made up to 15 mL with corresponding cultivation medium and applied on glass-fiber GF/F (Whatman) filters, 21 mm diameter, using a Millipore # xx10 025 03 filter holder with a glass funnel and a vacuum aspirator. All measurements we performed in five replicates.

**Total chlorophyll assay**

Cells were pelleted by centrifugation, transferred to a glass–glass homogenizer with a chloroform–methanol (10 mL, 2:1, v/v) mixture and extracted to remove all...
pigment. The lipid fraction including Chl was separated according to Folch et al. (1957). The chloroform phase was used for further pigment analysis. Total Chl was quantified using absorption coefficients for chloroform (Wellburn, 1994).

**Spectral measurements**

The spectra of the suspensions were recorded with Hitachi 150–20 spectrophotometer equipped with 150 mm integrating sphere fitted with a cuvette compartment (Fig. 1). In 1 cm glass cuvette, the spectra of cell suspensions were taken at two distances: as close as possible to, $D(\lambda;\gamma_0)$ (Fig. 1a), and 1 cm apart from the detector entry window, $D(\lambda;\gamma_1)$ (Fig. 1b), for additional detail see (Merzlyak et al., 2008; Merzlyak and Naqvi, 2000). In all cases, a cuvette with corresponding cultivation medium was used as a reference. The scattering-corrected optical density spectra, $D'(\lambda)$, were then calculated as

$$D'(\lambda) = D(\lambda;\gamma_1) - \frac{D(\lambda;\gamma_1)}{D(\lambda;\gamma_0)} \times [D(\lambda;\gamma_1) - D(\lambda;\gamma_0)]$$

where $D(\lambda;\gamma_0)$ and $D(\lambda;\gamma_1)$ — light attenuation at 800 nm where pigments do not possess detectable absorption, measured as close as possible and 1 cm apart from the detector window, respectively; for further details, see Merzlyak et al., 2008, and Merzlyak and Naqvi, 2000.

Routinely, the transmittance and reflectance spectra of the same samples were recorded after deposition on a glass-fiber filter (GF/F, 25 mm diameter, Whatman) as described above against an empty filter soaked in the corresponding medium (Fig. 1c,d). This approach allowed us to obtain a flat baseline in the spectral range studied (350–800 nm, see the spectra in Figs. 7–12).

In order to characterize optical properties of the filters, in certain experiments transmittance and reflectance spectra were recorded against a BaSO$_4$ reflectance standard (Hitachi, Japan). In this case measured sample spectra were offset by subtracting the spectrum of a blank filter from the measured spectra (see, e.g., Fig. 4). Spectra calculated using both approaches were nearly identical.

For transmittance measurements, the wet filters were mounted with the deposited cells facing the interior of the IS) on the entry ports of the integrating sphere of the spectrophotometer (Fig. 1c) or, for reflectance measure-
ments, at the exit ports of the IS (Fig. 1d). Optical density of the filters, $D' (\lambda)$, was expressed as $-\log T$, where $T$ is transmission. Reciprocal reflectance was calculated as $R' (\lambda)^{-1}$, where $R'$ is reflectance of the filter.

In a separate series of experiments, wet filters with the cells deposited onto them were dried over silica gel under vacuum at room temperature and then saturated with Immersol TM 518N immersion oil (Carl Zeiss, Germany) after transmittance and reflectance measurements, and then the measurements were repeated.

**Optical microscopy**

The integrity of the cells of the studied algal species upon deposition on GF/F filters was controlled visually on the microphotographs made using an Axioskop 2 light photomicroscope fitted with an Axiocam MRc digital camera (Carl Zeiss, Germany).

**Analysis of the algal cell distribution within GF/F filters**

The distribution of cells within GF/F filters was investigated using confocal laser scanning microscope (CLSM) LSM 510 Meta (Carl Zeiss). Fluorescence of Chl present in the algal cells and used as an intrinsic fluorescent probe was excited with the laser line at 633 nm and its emission in the band of 650–710 nm was registered. The recorded z-stacks were processed using the bundled software (Carl Zeiss) to obtain color-coded depth maps representing the distribution of algal cells within the GF/F filter.

**RESULTS**

Our light microscopy examination showed that the cells of the studied algal species suffered no damage upon deposition onto the GF/F glass-fiber filter and were more or less evenly distributed in the plane of the filter surface (Fig. 2a,b). At the same time, the CLSM analysis showed that cell size and the irregular landscape of the filter exerted a considerable impact on the pattern of the cell distribution within the filter depth (Fig. 2b). As apparent from Fig. 2c, virtually all cells were captured within the first 100 μm of GF/F filter depth, while the entire filter is ca. 400 μm thick.

Absorbance spectra of the stepwise diluted microalgal cell suspension samples were recorded in standard spectrophotometric cuvettes and compensated for scattering, as described above in “Methods”, yielding scattering-corrected spectra $D' (\lambda)$ similar to those shown in Fig. 3. For all of the microalgal species studied, the amplitude of the main absorption bands in the red and Soret band of the scattering-compensated suspension absorbance spectra was linearly related with sample cell density and Chl content in the whole range studied ($r^2 > 0.99$). The $D' (\lambda)$ spectra did not show a detectable absorption in the NIR region, and after normalization to the red maximum coincided (not shown), suggesting that distortion of the shape of spectra due to scattering-related effects and photometric inaccuracy did not occur.

The same suspension samples were deposited on GF/F filters and subsequently transmittance, $T' (\lambda)$ (Fig. 4A), and reflectance, $R' (\lambda)$ (Fig. 4B), spectra of the filters were measured. The $T' (\lambda)$ of a wet filter in the NIR was ca. 37% monotonously decreasing to ca. 25% at 350 nm; $R' (\lambda)$ increased from 66% in the NIR to 81% at 350 nm (see curves labeled “0” in Fig. 4).

The measured $T' (\lambda)$ and $R' (\lambda)$ spectra were then used for calculation of absorbance $D' (\lambda)$ (Fig. 4A), and reciprocal reflectance, $R' (\lambda)^{-1}$, spectra (Fig. 4B). The $D' (\lambda)$ of the wet blank filters displayed featureless absorption increasing towards longer wavelengths; the $R' (\lambda)$ amplitude of the blank filters decreased and that of accordingly increased towards longer wavelengths (not shown). After subtraction of blank filter spectrum, the $T' (\lambda)$, $D' (\lambda)$, and $R' (\lambda)$ spectra became almost flat in the NIR (Fig. 4).

The spectra of the microalgal cells deposited on the GF/F filters possessed essentially the same spectral features as the $D' (\lambda)$ spectra and retained the positions of main maxima in the blue and red regions (Figs. 3, 4). In all cases the difference between normalized $D' (\lambda)$ and $D' (\lambda)$ or $R' (\lambda)$ spectra did not exceed 2% with the exception of the cyanobacterium _A. variabilis_ in which the difference reached 6% in the Soret band (not shown). The amplitude of the bands in $D' (\lambda)$ spectra values was, on an average, three times higher (Figs. 3, 4) and the amplitude of $R' (\lambda)^{-1}$ maxima was 25 times higher than that of $D' (\lambda)$ (Figs. 3, 4B). Accordingly, the variation in the magnitude $[R' (\lambda)^{-1}]$ spectra of diluted suspension samples deposited on GF/F filters was more than one order of magnitude higher than that in the corresponding $D' (\lambda)$ spectra.

Saturation of GF/F filters, both blank and loaded with cells, with immersion oil brought about a dramatic reduction in their scattering, e.g., the attenuation in NIR decreased from 0.4 (Fig. 5A) to ca. 0.01 (Fig. 5). Visually the oil-saturated filters became completely transparent resembling a solid piece of glass. As a result, the $D' (\lambda)$ spectra of oil-saturated filters became very similar to $D' (\lambda)$ of the same samples (Fig. 5).

The optical properties of suspended cells, $D' (\lambda)$, and after deposition of the cells onto GF/F filters ($D' (\lambda)$, $-\log [R' (\lambda)]$, and $R' (\lambda)^{-1}$) were compared in the whole spectral range used in this study (Fig. 6). In all cases these relationships could be fitted by a second-order polynomial...
where $x = D_c(\lambda)$, $-\log[R'(\lambda)]$, or $R'(\lambda)^{-1}$.

The comparison revealed that the non-linearity, as it appeared from the contribution of the quadratic term (coefficient $b$) in the polynomial fit equation was considerably higher in the cases of the relationships "$D'_c(\lambda)$ vs. $D'_c(\lambda)$" (Fig. 6A) and "$D'_c(\lambda)$ vs. $-\log[R'(\lambda)]$" (Fig. 6B) than for "$D'_c(\lambda)$ vs. $R'(\lambda)^{-1}$" (Fig. 6C). It should be noted that the latter relationship was characterized by relatively low contribution of the quadratic term ($b$, see Fig. 6) in a wide range of cell densities and regardless of the species studied. As could be seen from Fig. 6C, these relationships departed from linearity at $R'(\lambda)^{-1}$ values higher than 2–2.5.

The empirical equations (eq 2) obtained as a result

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**Fig. 2.** Microalgal cells (the diatom *Thalassiosira weissflogii*) deposited on GF/F glass-fiber under optical microscope (a) and pattern of their distribution as revealed by chlorophyll fluorescence registered with a confocal laser scanning microscope (b, c). In (b), a typical depth map is shown for *T. weissflogii* (the cell depth is color-coded; see the scale at the bottom). In (c), quantitative data on chlorophyll distribution within the volume the filter are presented for the four microalgal species studied.
Fig. 3. Typical $D(\lambda)$ spectra of dilution series (the corresponding dilution factor is indicated below each curve). Total chlorophyll concentration is shown for each sample.

Fig. 4. Representative spectra of GF/F filters carrying different amounts of *C. pyrenoidosa* cells (the same series as presented in Fig. 3). (a) $T(\lambda)$ spectra (black curves) and calculated on their basis $D(\lambda)$ spectra (magenta curves). (b) $R(\lambda)$ spectra (black curves) and calculated on their basis $R(\lambda)^{-1}$ spectra (magenta curves). Dilutions are indicated in each panel in corresponding color; ‘0’—the spectra of blank (carrying no algal cells) GF/F filters.
where \( D_c(\lambda) \) and \( D'_c(\lambda) \) are the measured and the corresponding reconstructed \( D'(\lambda) \) spectra. Results of the reconstruction for all investigated species (excepting the deviating datasets for Chlorella and Anabaena in the cases of \( D'(\lambda) \) and \( R'(\lambda) \), see Fig. 6) are shown in Figs. 7–12. All three spectral functions provided a reasonable precision of \( D'(\lambda) \) reconstruction over whole cell density and spectral range studied (Figs. 7–12). It should be noted, however, that including the whole dataset brought about a decline in the precision of \( D'(\lambda) \) reconstruction via \( D_f(\lambda) \) values in comparison that achieved using \( R'(\lambda) \) values (not shown). Higher deviation of the reconstructed \( D'_c(\lambda) \) from the measured \( D_c(\lambda) \) spectra was observed at higher cell densities during reconstruction with the use of \( D'(\lambda) \). Reconstructed \( D'_c(\lambda) \) spectra retained a tight linear \((r^2 > 0.98)\) relationship with cell density and Chl content of the microalgal suspension sample studied characteristic of the measured \( D'(\lambda) \) spectra regardless of the reconstruction algorithm employed.

**DISCUSSION**

As it could be seen from Fig. 2, the deposition of microalgal cells on glass-fiber filter results in formation of an optically complex system comprised by small pigmented particles (Chl-containing cells) distributed...
within strongly scattering medium (Fig. 4A). Though the spectra of the cells deposited on filters contained the same maxima as could be found in the spectra of suspended cells, the amplitude of the former is considerably higher than that of the latter (Figs. 3, 4), obviously due to effects related with amplification of light path due to multiple scattering in the filter (Roesler, 1998; Lohrenz, 2000). A number of approaches have been proposed for the correction of the microalgal spectra measured using QFT most of which were designed for...

Fig. 7. Reconstruction of *A. variabilis* $D_c(\lambda)$ spectra using (a) $D'(\lambda)$, $-\log[R'(\lambda)]$, and (b) $R'(\lambda)^{-1}$ spectra. Thick solid lines represent $D'(\lambda)$, lines with symbols—$D'_c(\lambda)$; corresponding spectra of RMSE of $D'_c(\lambda)$ is shown below.

Fig. 8. Reconstruction of *A. nidulans* $D_c(\lambda)$ spectra using (a) $D'(\lambda)$, $-\log[R'(\lambda)]$, and (b) $R'(\lambda)^{-1}$ spectra. For designation of the curves see Fig. 7.
transmittance-based measurements (Mitchell, 1990; Roesler, 1998) or transmittance measurements using reflectance measurements for a backscattering correction (Tassan and Ferrari, 1995).

It should be noted, however, that the amount of light reflected by the filter was two to three times higher than the amount of light transmitted (cf. Figs. 3A,B). The balance of light transmitted and reflected by the filter, taking into account the inaccuracy of the measurements, shows that losses of light due to attenuation by the filter per se

Fig. 9. Reconstruction of *C. pyrenoidosa* $D' (\lambda)$ spectra using (a) $D' (\lambda)$, $-\log[R' (\lambda)]$, and (b) $R' (\lambda)^{-1}$ spectra. For designation of the curves see Fig. 7.

Fig. 10. Reconstruction of *S. obliquus* $D' (\lambda)$ spectra using (a) $D' (\lambda)$, $-\log[R' (\lambda)]$, and (b) $R' (\lambda)^{-1}$ spectra. For designation of the curves see Fig. 7.
were relatively small. It appears that the increase in light attenuation observed in samples deposited on filters apparently results from strong backscattering (reflection) of light. This is in line with high reflectance values (ca. 80% in the NIR), a monotonous decrease of transmittance, and an increase in reflectance towards blue-violet which was observed during the filter measurements (curves 0 in Fig. 4). These phenomena could, at least in part, be ascribed to the increase of scattering coefficient with the decrease of wavelength (Butler and Norris, 1960).

Fig. 11. Reconstruction of *T. weissflogii* $D' (\lambda)$ spectra using (a) $D' (\lambda)$, $-\log [R' (\lambda)]$, and (b) $R' (\lambda)^{-1}$ spectra. For designation of the curves see Fig. 7.

Fig. 12. Reconstruction of *Sk. costatum* $D' (\lambda)$ spectra using (a) $D' (\lambda)$, $-\log [R' (\lambda)]$, and (b) $R' (\lambda)^{-1}$ spectra. For designation of the curves see Fig. 7.
A considerable increase in absorption of light by microalgal cells deposited on filters in comparison with suspended cells (Figs. 2, 3) stems from amplification of the effective optical path due to multiple scattering, it could be illustrated by the reduction of amplitude of $D'$ ($\lambda$) to the level characteristic of $D$ ($\lambda$) by saturating the filter loaded with cells in the immersion oil (possessing a refraction index close to that of glass of which the filters are made) thereby removing the glass–air interfaces (Fig. 5).

In this work we tried to employ light reflected by microalgal cells deposited on filters since it appears to constitute a more ample signal in comparison with transmitted light (Fig. 4) for reconstruction of the spectra of corresponding cell suspensions. The relationships between $D'$ ($\lambda$) and spectral function obtained from on-filter measurements appeared to be essentially non-linear (Figs. 6A,B). In line with previous findings for Dunaliella tertiolecta, Chlorella sp., Nannochloropsis sp., and Thalassiosira fluviatilis (Mitchell, 1990; Mitchell et al., 2003) as well as a large number of samples from natural water bodies (Lohrenz, 2000; Roesler, 1998; Tassan and Ferrari, 1998), these relationships were successfully fitted with second-order polynomials with significant contribution of quadratic term: the ratio $\beta/\alpha$ (see eq 3) was equal to 1.63 and 0.99 for $D'$ ($\lambda$) and $-\log[R' (\lambda)]$, respectively (eq 3, Figs. 6A,B). By contrast, the relationships “$D'$ ($\lambda$) vs. $R' (\lambda)$” were considerably more linear ($\beta/\alpha = 0.06$; Fig. 6C). These findings are in accordance with the theory of diffuse reflectance developed by Atherton (1955) widely employed for the description of absorption of dyes applied to textiles or paper. According to this theory, the following is true for the filter loaded with cells in the immersion oil (pos.):

$$R(\lambda)^{-1} = c_1 \varepsilon_1(\lambda) + c_2 \varepsilon_2(\lambda) + ... + c_n \varepsilon_n(\lambda),$$

where $R(\lambda)^{-1}$—reflectance of the medium (blank GF/F filter in this case), $c_i$—concentration and $\varepsilon_i$—absorption coefficients at wavelength $\lambda$ for the pigments present in the system (Atherton, 1955; Aldeeson et al., 1961). Indeed, blank corrected $R' (\lambda)$ was linearly related to Chl content in the range studied ($r^2 = 0.99$). The departure from linearity was observed at higher $R' (\lambda)$ values, probably due to general non-linearity of the relationships between pigment content and reflectance signal in strongly scattering media at high content of the pigment.

An essential point is that the relationships “$D'$ ($\lambda$) vs. $R' (\lambda)$” obeyed a single law for all six species studied (Fig. 6C). This fact suggests that reciprocal reflectance-based approach could be applied for analysis of natural samples with diverse taxonomic composition without compromising the precision of reconstruction. Our pilot experiments with samples containing different proportions of cyanobacteria, green microalgae and diatoms confirmed this suggestion. Furthermore, the reciprocal reflectance-based technique is particularly suited for studying plankton in the field since it could be easily implemented with portable fiber-optic spectrometers and reflectance probes. Another series of pilot experiments demonstrated the feasibility of reliable $R' (\lambda)^{-1}$ measurements even without the use of a conventional integrating sphere.

Collectively, the results obtained in the present work show that the reconstruction of $D'$ ($\lambda$) solely via reflectance measurements is feasible and is at least as efficient as that performed using transmittance measurements. Furthermore, due to the wider dynamic range, the higher linearity of “$R'(\lambda)^{-1}$ vs. $D'(\lambda)$” the sensitivity of $D'(\lambda)$ reconstruction via $R'(\lambda)^{-1}$ increases. As a result, the use of reflectance measurements of cells deposited on filters, and subsequent reciprocating the measured values is advantageous for quantitative characterization of plankton in natural ecosystems and (mass) cultivated microalgae.

**NOMENCLATURE**

$D'$ ($\lambda$)—scattering-corrected OD spectrum of algal cell suspension recorded in standard spectrophotometric cuvette.

$D'$ ($\lambda$)—reconstructed $D'$ ($\lambda$) spectrum.

$D'$ ($\lambda$)—OD spectrum of algal cells deposited onto GF/F filter.

$R'$ ($\lambda$)—reflectance spectrum of algal cells deposited onto GF/F filter.

$R'(\lambda)^{-1}$—reciprocal reflectance spectrum of algal cells deposited onto GF/F filter.

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