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Measurement of light absorption by aquatic particles: improvement of the quantitative filter technique by use of an integrating sphere approach

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Abstract

Determination of particulate absorption in natural waters is often made by measuring the transmittance of samples on glass-fiber filters with the so-called quantitative filter technique (QFT). The accuracy of this technique is limited due to variations in the optical properties of the sample/filter composite, and due to uncertainties in the path-length amplification induced by multiple scattering inside the filter. Some variations in the optical properties of the sample/filter composite can be compensated by additional measurements of the filter's reflectance (Transmittance-Reflectance method, T-R,[1]). We propose a different, rarely used approach, namely to measure the filter's absorptance in the center of a large integrating sphere, to avoid problems with light losses due to scattering. A comparison with other QFTs includes a sensitivity study for different error sources and determination of path-length amplification factors for each measurement technique. Filter to filter variability induced much lower errors in absorptance compared to a measured transmittance. This reduced error permits more accurate determination of the usually low absorption coefficient in the near infrared spectral region. The error of the T-R method was lower than that of the transmittance measurement but slightly higher than that of an absorptance measurement. The mean path-length amplification was much higher for the absorptance measurement compared to the T-R method (4.50 vs. 2.45) but was found to be largely independent of wavelength and optical density. With natural samples the path-length amplification was less variable for the absorptance measurement, reducing the overall error for absorption to less than ±14%.

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Measurement of light absorption by aquatic particles: improvement of the quantitative filter technique by use of an integrating sphere approach.

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1 Introduction

Since the first approaches to determine the light absorption by phytoplanktonic particles in water [2, 3, 4], the absolute quantification of particulate absorption in natural waters has remained difficult. The determination of particulate absorption at low concentrations in most natural waters requires sensitive methods, but the ability of particles to scatter light adversely affects these measurements at all concentrations. Only recently has the use of integrating cavity absorption meters (ICAM), insensitive to scattering errors, provided more accurate determination in oceanic waters [5, 6]. However, filter pad techniques have been routinely and widely used for many decades and it is desirable to further improve these methods and to validate their performance with the more accurate ICAM methods. Most of the lab techniques used to determine particulate absorption are simple and can be conducted using a dual-beam spectrophotometer (for summary see [7]). One approach to determine phytoplankton absorption is to measure the attenuation of an algal suspension in a photometric cuvette [3, 8, 9], when the cuvette is positioned in front of the detector, in front of a diffusive plate placed between cuvette and detector, or in front of an integrating sphere, such that all forward-scattered light can be measured by the detector. However, the back- and sidewards-scattered light is lost and, thus, is a source for significant errors.

Different methods have been developed to correct for these losses [10, 11] but typically the attenuation determined at >750 nm (assuming negligible absorption by particles in this spectral region and a wavelength-independent scattering) is subtracted from the attenuation values of all other wavelengths and the result taken as absorption (null point correction). To avoid errors by back-scattered light, the cuvette can be positioned in the center of an integrating sphere [12, 8, 13, 14, 15, 16, 17]. These techniques are applicable for samples with relatively high particle concentrations; on the other hand they are limited to low, optically thin, concentrations of particles to avoid errors by multiple scattering in the cuvette. Particles in natural aquatic samples have to be concentrated to obtain a sufficient optical density. This is done by retaining particles on a filter. The attenuation of this filter is measured in a photometer by determining the transmittance of the filter when placed directly in front of the detector window or in front of an integrating sphere [4, 18, 19]. A special method of this quantitative filter technique (QFT, [20]) is the "Transmittance-Reflectance" (T-R) technique of Tassan and Ferrari [1, 22], where the attenuation by particles on a filter is derived from light, which is transmitted through and reflected by the filter. This method allows the correction of errors induced by back-scattering by the filter and the particles itself. Furthermore it is assumed that this method is more accurate compared to simple transmission methods in waters with high concentrations of mineral particles [22, 17] that adversely change the scattering properties of the sample/filter composite. However, the method needs additional measurements with the same filter in the "reflectance" mode and some assumptions for its calculation, (for details see [1, 22]. Glass-fiber filters, which are used for the QFT, have a high scattering coefficient. Thus, a collimated light beam is scattered inside the filter and transformed into a diffuse light field. As a result, these filters strongly amplify the optical path-length because of multiple internal scattering and the fact that the particles are distributed in the depth of the filter not just on its surface. Normally, the amplification factor (β), which is the ratio of the real optical to the geometric path-length [21], is determined empirically. These determinations have been done e.g. by comparison with measurements in a cuvette using algal cultures [23, 24, 20, 25, 1, 26, 27]. Errors in the determination of β occur because the cuvette measurements and/or the QFT could be susceptible to significant wavelength-dependent scattering errors [28]. Furthermore β might be variable and dependent on the particle distribution in the filter depth. Additional errors are introduced by sample filtration, freezing and storage procedures

[29, 30, 31, 32]. Some errors of the QFT discussed above are related to (multiple) light scattering and thereby to incomplete capture of scattered light when the filter is measured in front of an integrating sphere or in front of the detector window. These errors can be compensated to some extent by measuring backscatter losses as in the T-R technique [1, 22] but could be completely avoided by placing the filter in the center of an integrating sphere (e.g. [33]) to directly determine its absorptance. The required special integrating spheres were custom-made instruments until a few years ago and, consequently, this kind of measurement has been made in well equipped optical laboratories only. Since a few years they have been commercially available as accessory equipment for many common spectrophotometers and are already used in a number of laboratories, e.g. to measure absorption in a cuvette placed in its center (see above), as well as to measure particulate absorption on filters [34]. To the best of our knowledge there has been no methodological work done to standardize the procedure, to perform an error and sensitivity analysis, and to analyze the necessary amplification corrections for conducting measurements with filters inside an integrating sphere. Some measurements of particulate absorption of dust particles in suspension [15, 16] showed significant particulate absorption in the near infrared region of 700 - 900 nm (NIR). Significant absorption in the NIR spectral region would preclude using these wavelengths for null point correction. Tassan and Ferrari (2003)[17] showed significant absorption up to 750 nm in natural samples measured by the T-R method, but the regular "transmittance" method is highly susceptible to filter to filter variations (e.g. due to filter wetness) making exact determination of the reference background difficult. This is also a problem for the "reflectance" measurement, but the T-R technique can partly correct for these filter to filter differences. We will show the advantages of filter measurement in the center of an integrating sphere, i.e. that the overall precision and accuracy of the QFT is strongly improved, and describe the necessary correction for path-length amplification, which is as well strongly changed compared to a transmittance measurement. As the scattering error for the absorption determination inside an integrating sphere is insignificantly small and, hence, no scattering offset (null-point) correction is required, this setup can be used to measure the low particulate absorption in the NIR spectral region.

2 Materials and Methods

2.1 General procedure

Measurements were performed with two types of glass fiber filters, GF/F (Whatman) and GF-5 (Macherey and Nagel), in a dual-beam UV/VIS spectrophotometer (Lambda 800, Perkin Elmer) that was equipped with a 150 mm integrating sphere (Labsphere Inc. U.S.A.). The sphere was made from Spectralon® (Labsphere Inc. U.S.A.) and allowed placement of a sample in its center with the help of special center-mount sample holders, as well as placement of a sample in front of (transmission port) and behind (reflection port) the integrating sphere. For all measurements inside the sphere we used a custom-made, clip-style, center-mount filter holder that allowed arranging different angles between the sample and the incident light beam. We performed measurements in three different modes (in front, behind, and at the center of the integrating sphere). These three modes can be considered as measurements of the optical properties transmittance, (T), reflectance, (R), and absorptance, (A). Theoretically we should find that $T + R + A = 1$. Transmittance is measured by placing the filter in front of the entrance of the integrating sphere. Absorptance is measured by placing the filter at the center of the sphere. In both cases the reflectance ports are closed with a white Spectralon® reflectance standard. Reflectance is measured by placing the filter behind the integrating sphere without placing a white reflectance plate behind the filter, so the black cover behind the port acts as a light trap. In all cases the reference reflectance port is covered by the reflectance standard. The filters used had a diameter of at least 47 mm (47 or 55 mm). The transmittance and reflectance measurements were performed with complete filters. For absorptance measurements each filter was cut in up to four rectangular pieces of about 1 x 2 cm. During measurements the filters were never completely soaked with water to avoid any water droplets that might fall down inside the sphere and damage the detectors at the bottom of the sphere. The general procedure was to soak the filter with water for at least 1 h and then briefly put it on a tissue to remove free water. We will show later that filter wetness has a minor effect on the overall attenuation of the filter when placing a filter inside an integrating sphere. The filter was placed at the center of the integrating sphere by the use of a clip-style filter holder (Labsphere Inc. U.S.A.). Filters were placed perpendicular to the light beam and the wavelength scan with the spectrophotometer was started promptly.

Spectral measurements were performed from 300 to 900 nm (occasionally only 350 to 750 nm) with a resolution of 2 nm (slit width: 2 - 4 nm, scan speed: 60 - 200 nm/min). We checked the effects of slit width and scan speed on the overall optical density (OD) spectrum and the signal to noise ratio. In the range of 1 to 4 nm and 100 to 500 nm/min these effects were insignificant for the spectral range of 350 to 750 nm. At shorter and longer wavelengths the signal to noise ratio decreased with decreasing slit width and increasing scan speed. Our software permits use of different settings for scan speed and slit width for different spectral regions, thus, the whole scan was optimized for all wavelengths for good signal to noise level and minimal total scanning time to avoid drying of wet filters during the scan. For the spectral region between 830 and 900 nm we used a slit width of 4 nm and a slow scan speed of 60 nm/min, for 300 to 830 nm we used 2 nm and 200 nm/min. Before each set of measurements the instrument was allowed to warm up for at least one hour. The positions of the two light beams were checked after the optical compartment and the sphere was cleaned from dust using ultraclean compressed air. This ensured that the sample light beam passed through the filter for both situations, when the filter was in front of or in the center of the sphere, and that the reference beam illuminated exactly the center of the respective reflectance standards that cover the reflectance ports, and that the sample beam crosses the filter patch in ca. 5-10 mm distance from the clip holder. The baseline of the spectrophotometer is adjusted by performing an "autozero" run either without any filter or with a dry filter in the center of the integrating sphere. For this run the entrance ports were open, and the reflectance ports were covered with Spectralon® reflectance standards. Between measurements the baseline was checked by either performing a scan with the same setup as during the "autozero" scan, or by verifying the value at 750 nm.

2.2 Filter preparation

We observed specific spectral characteristics of some Whatman GF/F filter batches (Fig. 1, upper panel) that indicated contamination of the filters with an unknown chemical compound originating from the blue plastic tray that contains the filter. The contamination was observed on filters from new filter packs, was highly variable between filters from any given pack and was observable in different packs from the same batch of filters. The contaminant was soluble in water as well as in organic solvents and, hence,

could influence all kinds of measurements. If detectable in the absorption it was e.g. detected by HPLC analysis techniques, which is used for measuring phytoplankton pigments, or as dissolved matter in the CDOM (chromophoric dissolved organic matter) fraction (pers. observations). It should be noted that the peak was not as obvious when the filters were measured in front of the integrating sphere. In this case the attenuation of the filter is high, so the fractional contribution from the contamination is significantly less. A much lower peak was also seen in original GF-5 filters (Fig. 1, lower panel). Combustion of the filters at 450°C for 4 hours removed this contamination, and was, hence, used for filter preparation. This combustion did not influence the optical properties of uncontaminated filters significantly (Fig. 1, lower panel). In the following, all measurements were made with combusted GF-5 filters. Saldanha et al. (2004)[35] showed that this filter type has the same particle retention characteristics as the commonly used GF/F filter type.

2.3 Cultures and samples

Cultures of several microalgal species were provided by the Alfred Wegener Institute for Polar and Marine Research (AWI), Bremerhaven, or isolated from the North Sea. These cultures include different diatom species isolated from the North Sea, as well as cultures of *Prymnesium parvum*, *Isochrysis galbana*, *Trichodesmium erythreum*, and *Nannochloropsis* sp. A culture of *Prochlorococcus marinus* was provided by the University of Freiburg, Germany. The cultures were cultivated as batch cultures in 1 liter glass bottles (Duran, Schott) at 20° C, with an illumination of ca. 20 μmol photons m^{-2} s^{-1} with 24h of light. Samples from natural water were collected on a variety of cruises from the River Elbe, the North Sea, the Baltic Sea and the Atlantic Ocean.

3 Assessment

The absorption coefficient, a [m-1], of a particle suspension retained on a filter is calculated from the OD of a sample and a reference filter, OD_s and OD_r, respectively, as

$$a = 2.303(OD_s - OD_r)F/V * 1/\beta \ [m^{-1}], \tag{1}$$

where F [m^2] is the filter clearance area, V [m^3] the filtered sample volumen, β the path length amplification, and the factor 2.303 converts $\log_{10}$ to the natural logarithm, as $OD = -log_{10}T$ (T is transmittance). We consider two main error sources that influence the overall accuracy and precision of the spectrophotometric measurements of particles retained on filters. One is the variation in optical density of the filter used, including variations over a single filter and between different filters induced by differences in filter structure, thickness and in filter wetness, all influencing the optical properties of empty filters used as reference/blank filters. Similar differences can be assumed for the sample/filter composite. These variations can be wavelength-dependent or -independent as they might be induced by different scattering properties of the filter and the sampled particles. Furthermore they determine differences in attenuation between a sample filter and a reference filter that are not related to the particulate absorption of the sample. The other main error source is related to the optical path-length amplification inside the filter. It may vary for several reasons such as the overall optical properties of the filter itself, the optical properties of the sample/filter composite or the size and kind of particles collected, as well as the particle distribution in the filter depth. The influence of this amplification might also vary for different optical setups for measuring the absorption of particles (i.e. T, R or A). To determine variations in OD due to the first error source, we performed tests to compare absorptance with transmittance and reflectance measurements, as well as with measurements following the T-R method of Tassan and Ferrari[1, 22]. These measurements included tests to determine: 1) the influence of the water content of the filter, 2) the optical variability of filters from a single batch, and 3) the dependence on structural differences of the two filter sides. Lastly we determined the overall error of the whole procedure including sample filtration and measurement. The second error source was investigated by determining 1) the effects of path-length amplification for each setup (T, R, A, T-R), 2) its dependence on the filter load and 3) its sample to sample variability. Additional sources for a systematic error are the determination of V and F, but these are not related to the optical measurements itself and are relatively small. The precision of the determination of V is 1-2 %, that for F with a caliper rule (precision: $\pm$0.05 mm) ca. 2 % (filter patch diameter: ca. 41 mm).

3.1 Filter to filter variations

In a first set of experiments the OD of empty, dry filters was measured using the different setups (T, A and R) followed by measurements of empty, wet filters that were soaked in purified water and subsequently were measured over a period of 40 min to follow changes in OD when the filters were drying. The transmittance of an empty, dry filter was very low (<6 %), the corresponding OD for this measuring mode, OD_f^T, was, hence, >1.2 (Fig. 2, upper panel). Accordingly, the reflectance of this filter was high and between 94 and ∼100 %, the corresponding OD, OD_f^R, between 0.029 and 0.003 (Fig. 2, upper panel). The absorptance of an empty, dry filter was very low (<1 % and partly negative), the corresponding OD, OD_f^A, was between -0.01 and 0.003 (Fig. 2, lower panel), the artificial negative values were observed mainly at shorter wavelength. The OD_f^T of an empty filter that was soaked in purified water was between 0.36 and 0.60 (Fig 2, upper panel) and the OD_f^R between 0.14 and 0.24 (Fig. 2, upper panel). This corresponds to a transmittance of 24 to 41 % and a reflectance of 72 to 58 %, respectively. Thus, soaking the filter with water increases the transmittance of the filter with a corresponding decrease in reflectance. Hence, the T/R ratio of a filter changes when the filter is soaked in water. OD_f^A of such a wet filter was only slightly different from that of a dry filter and was between 0.001 and 0.02, and mainly below 0.006 (Fig. 2, lower panel). The corresponding absorptance was <5 %, and mainly <1.5 %. It has previously been observed that transmittance decreased slightly over time when filters were soaked in water but eventually stabilized when the filter was soaked for more then one hour (see [22]). These changes for transmittance and reflectance were observed here as well (data not shown). Reference filters for T, R and T-R measurements should be soaked in water for more than 1 hour as proposed by Tassan and Ferrari 2002 [22]. However, when filters were measured inside the integrating sphere, no significant differences in absorptance were observed with time after soaking for the spectral region measured (data not shown). In addition, differences in OD_f^A between the two structurally different filter sides were tested and found to be insignificant for dry and for wet filters (data not shown). Changes in optical density between dry and wet filters were examined in more detail by conducting repetitive measurements with 5 - 7 filters that were first measured shortly after filtering 100 mL purified water through them, and then again every 10 min for 40 min in total, with the filters left to dry in air on an open petri slide between measurements. The

OD_f^T of a soaked filter increased with time at all wavelengths (Fig. 2a) approaching the values of a dry filter, whereas OD_f^A of the same filter changed only slightly, with pronounced changes at wavelengths <350 nm only (see Fig. 2b). Next we re-examined the variability in OD_f of different filters, and how OD_f varies with the water content of the filter, as these variations determine the quality of empty filters when used as reference blanks. This has been examined earlier for T and T-R measurements [28, 1, 22] (see Table 2). Repetitive measurements with different filters (n = 5 - 14) were used to determine standard deviations (σ) for measurements of empty, dry filters and empty, wet filters (in this case measured immediately after soaking with purified water). Figure 3a shows σ_{OD} for dry filters that were used to determine the variability of filters from one filter batch. The mean σ_{OD} in the spectral range of 300 - 890 nm was similar when measured as transmittance and reflectance with a value of ∼0.0051 (range: 0.004 - 0.012). That when measured as absorptance was 0.0003, about one order of magnitude lower (range: 0.0001 - 0.0010, Fig. 3a), a value just 2 - 3 times the noise level of the spectrophotometer for the settings used. The mean σ_{OD} for the T-R method (0.0041, range: 0.003 - 0.010) was slightly lower than that for transmittance and reflectance alone. The highest values were observed in all cases at shorter, ultraviolet and at longer, infrared wavelengths, and were mainly a function of the spectrophotometric precision. For wet filters (Fig. 3b) the mean σ_{OD} for transmittance was 0.0022 (range: 0.0015 - 0.005) whereas that for absorptance was again lower (mean: 0.0004, range: 0.0002 - 0.0010), and only slightly higher than the respective σ_{OD} for dry filters. For the T-R measurements the mean σ_{OD} was 0.0006 (range: 0.0004 - 0.0009), and at some wavelengths still higher than that for absorptance but significantly lower than that for transmittance alone and lower than the respective σ_{OD} for dry filters. A second set of experiments was conducted with filters prepared from different algal cultures to examine the variability in sample filters. The observed variability includes errors related to the filtration procedure (like filter homogeneity, filtered volume precision and filter wetness), and to any filter to filter variability of the path-length amplification inside the filter. We measured 7 - 10 different filters through which the same volume of an algal culture was filtered. The regular procedure for the T-R technique is followed in such a way that we prepared filters with OD_f^T between 0.1 and 0.3. For absorptance measurements we prepared filter with OD_f^A between 0.01 and 0.1 (see below). The mean σ_{OD} for absorptance measurements of a particle sample was 0.0009 (range: 0.0003 - 0.0020, n = 10) and was OD-dependent

(Fig. 3c). The mean relative error was 3.0 % (range: 1.6 - 5 %) and less OD-dependent (note that the OD was >0.01 at all wavelengths used for this calculation). The mean σ_{OD} for OD_f^T was 0.0051 (range: 0.0025 - 0.0076, n = 7), and that for OD_f^{TR} was 0.0023 (range: 0.0005 - 0.0057, n = 7). In the NIR spectral region, for which no significant absorption is expected for an algal culture, the observed error for a sample filter is nearly the same as that for an empty, wet filter. In summary, in all cases (dry and wet, empty and sample-loaded filters) the highest variability and thereby the largest error in determining OD is observed for the transmittance and reflectance measurement, the lowest for the absorptance measurement, with the error of the T-R method being lower than that for the transmittance measurement alone and slightly higher than that for the absorptance measurement. The error of the T-R method is notably higher than that of the absorptance measurement in the NIR spectral region where it is particularly important to distinguish between absorption and scattering for baseline correction purposes. A smaller error would make the absorption measurement more accurate, potentially to the point where a null point correction could be avoided. The relative errors of the OD_f^A and OD_f^{TR} were similar as higher filter loads were used for the T-R measurements. We will show later that lower filter loads are preferable to decrease influence of particle density on the path length amplification.

3.2 NIR absorption

The results described above make it obvious that simple transmittance measurements will generally only give correct absorption results if filter to filter variations are null-point corrected, using the OD at a wavelength at which negligible absorption can be assumed. The validity of this assumption can be assessed from measurements we performed during this exercise. We show results of OD_f^A for an algal culture and a sample from the North Sea together with that of a reference filter (Fig. 4). Two different sample volumes were used for each sample: 1) to obtain a maximal OD below 0.1 (Fig. 4a), and 2) to obtain OD $\gg$ 0.1 (Fig. 4b) to increase the sensitivity in spectral regions with low absorption (i.e. NIR). The original measurements are shown without any corrections. It is evident that the algal culture does not posses any significant absorption at wavelengths >730 nm, as the OD at those wavelengths is not different from that of the reference filter even when high sample volumes are used as in Fig. 4b. In contrast the natural sample showed significant absorption in the NIR spectral region and in this case us-

ing higher sample volumes gave OD up to 0.1 in the NIR region. A negligible NIR-absorption can only by shown for pure algal cultures. Natural samples always contain higher amounts of detrital material that seems to absorb in the NIR region strongly. A scatter error correction (null point correction) would, hence, always result in an underestimation of absorption. Considering the extent of the scattering error and the significant absorption in the NIR, simple transmission measurements of the particulate absorption can hardly be correct. At longer wavelengths the relative error of this method can be quite larger.

3.3 Path length amplification

To determine effects of path-length amplification by multiple scattering inside the filter we measured the absorption of different algal cultures and of a large set of samples taken during three cruises: one on the River Elbe down to the inner German Bight, one covering open ocean and coastal areas of the Baltic and the North Sea, and one crossing the Atlantic Ocean. Sample filters were prepared with different filtration volumes to obtain a maximum OD_f between 0.02 and 0.4. Samples from algal cultures were measured immediately after filtration. Natural samples were shock-frozen in liquid nitrogen, stored at <-20 °C and measured about 2 weeks later. The theoretical absorption coefficient ($a^{\beta=1}$) is determined using the specific sample volume and filter clearance area but ignoring path-length amplification. To determine the individual amplification factor, β, for each filter we measured the presumably real particulate absorption coefficient, a [m^{-1}], with a point-source integrating-cavity absorption meter (PSICAM, spectral range 400 - 720 nm) [6, 36]. Particulate absorption with the PSICAM was determined by measuring the total absorption of all water constituents and subtracting the absorption of the specific sample filtrate (glass fiber filter, particle size $<\sim$0.7 μm). The filtration was done carefully under low vacuum to avoid cell breakage. The absorption of the filtrate was relatively low compared to the particulate absorption, errors in particulate absorption determination by cell breakage are considered to be negligible, and the overall error is in the range of a few percent only (see [6, 36]). Figure 5 shows the absorption coefficient of a culture and the theoretical absorption, $a^{\beta=1}$, observed on a filter measured as absorptance, transmittance, and reflectance, together with that for the T-R calculation (using the tau formulation of [22]). The highest values for $a^{\beta=1}$ were obtained when the filter was measured in reflectance mode R,

the lowest for the T-R method. Measurements in transmittance mode were higher than that of the T-R method, whereas the absorption determined as absorptance was lower than that when measured as reflectance. The absorption obtained by the T-R method was more than twice the real absorption, while the absorption determined as absorptance was about 5 times the real absorption. These differences in $a^{\beta=1}$ are the result of differences in path-length amplification for transmitted and reflected light, the effect for reflected light being about double that for transmitted light. Next we show the results of a first experiment with different filter loads, i.e. different volumes of the same algal culture filtered onto separate filters. There is general agreement in the literature that the OD for a sample filter (compared to that of a reference filter) should not be too high (e.g. <0.4, [20]). On the other hand, Tassan and Ferrari (2002)[22] found indications of a nonlinear relationship between OD and the path-length amplification factor when OD >0.4. A set of filters was prepared with the maximal OD_f^T between 0.04 and 0.3 (i.e. OD_f^A: 0.06 - 0.46) and for each filter the transmittance and absorptance was measured (Fig. 6a). The theoretical absorption coefficients calculated for each of these samples was not constant but decreased with increasing sample volume in both cases; it was between 2 to 3 times higher than the real absorption coefficient when measured as transmittance, and 3 to 5 times higher when measured as absorptance (Fig. 6b). However, the relative difference between filters with different sample volumes was the same for both kind of measurements, as the two theoretical absorption coefficients, $a_T^{\beta=1}$ and $a_A^{\beta=1}$, are linearly correlated (Fig. 6c, for this comparison the transmittance measurements had been null point corrected). The amplification for absorptance measurements was again about 1.5 times higher than for transmittance independently of the filter load and, hence, absolute OD_f. However, when these theoretical absorption coefficients were plotted against the real absorption coefficient, only the filter with the lowest filter load showed a good linear correlation (Fig. 6d) with an amplification of 4.59 ($r^2 = 0.9987$). With increasing filter load the relationship deviated increasingly from linearity and showed a dependence on the absolute OD (not on the wavelength) probably induced by densely packed particles. This OD-dependent amplification has been regularly observed in other studies and is normally compensated by an OD-dependent amplification correction factor, $b = 1/\beta$, using e.g. a second order polynomial function (e.g. [20, 25, 37]). In the experiment presented here changes in path length amplification with OD can already be observed in the range of 0.1 to 0.3 (OD_f^T), but below 0.1 the deviation from linearity

is insignificant. Thus, another set of filters was prepared with lower filter loads and the OD of these filters was then measured as transmittance, absorptance, and reflectance (Fig. 7). The maximal value of OD_f^A was now between 0.02 and 0.11 (Fig. 7a). The corresponding theoretical absorption at each wavelength, $a_A^{\beta=1}$, for these samples was rather constant with only the highest filter load showing a 10 % lower value; it was about 5 times higher than the real absorption (Fig. 7b). The absorption coefficient was also calculated using the T-R method (small deviations at >750 nm for T, R and T-R measurement were null point corrected). The T, R and T-R theoretical absorption values exhibited strong linear correlations with A measurements (Fig. 7c), with different slopes for each set of measurements and some artifacts for $a_T^{\beta=1}$ and $a_R^{\beta=1}$ that were a consequence of the much lower OD obtained with these methods and, hence, lower signal to noise ratio. The T-R method showed a much better correlation to A measurements than T and R measurements alone as a result of compensation for filter to filter differences. Plotting theoretical absorption coefficients against the real absorption coefficient showed good linear correlations for each measurement mode (Fig. 7d). The highest correlation coefficients were obtained for absorptance measurements and for T-R, as those techniques respectively avoid or compensate for filter to filter variability. The resulting amplification factor was again highest for the absorptance measurement (it was higher for reflectance measurements, but these were not used here to obtain absorption) with a factor of 5, and lowest for the T-R method, 2.3. Fig. 7d shows that there was still some systematic effect of increasing filter load (i.e. OD) on path-length amplification even for these reduced sample loads. However, if the maximal OD_f^A of the sample filter was below 0.1, the variability in amplification was below 5 %. Filter load experiments with natural samples from the Elbe River that had high amounts of detrital material showed similar results and a constant amplification for the same sample when different sample volumes were used and OD_f^A was kept below 0.1 (data not shown). The above experiments demonstrated clearly that OD needs to be kept very low in order to have a constant path-length amplification effect over all wavelengths and over all OD. Standard errors for simple transmittance measurements at low ODs are too large to use the results without a null-point correction which we have seen may not be justifiable for natural samples. Precise measurements can only be provided by A and T-R methods, the latter still being more susceptible to filter to filter variation in OD. The following comparison was, thus, made for the A and T-R method only. A large set of sample filters was used

to examine the variability of the path-length amplification in filters prepared from algal cultures and from natural water samples. The real absorption coefficient was determined again using a PSICAM. Ten different algal cultures were used for absorptance measurements, but only six cultures for the T-R measurements. Algal species were chosen which did not show any absorption artifacts induced by the filtration procedure. For example, species that contain water-soluble phycobilin proteins lose some of these pigments during filtration and the corresponding loss in absorption induces strong artifacts in the correlation plot, sometimes observed as a hysteresis (e.g. [24, 26]). We were able to observe this in detail by comparing filter absorption spectra with PSICAM spectra (data not shown). Filters were prepared in replicates with the same sample volume and averaged. In addition, different sample volumes were used to prepare several sets of filters from the same culture. In all cases the correlation between the calculated theoretical absorption and the real absorption was highly linear (r^2 = 0.987 - 0.999, linear regression forced through the origin). However, the slopes of these linear regressions (i.e. β) were highly variable and between 3.5 and 5.4, mean 4.50 (n = 23) for the absorptance measurements and between 2.2 and 2.8, mean 2.39 (n = 15) for samples measured using the T-R method (Fig. 8a). We did not examine species-specific differences in detail, but in both cases lowest values were observed for diatoms (Bacillariophyceae) and highest values for non-diatom species without any correlation to cell size. As the variability in the amplification factor for algal culture samples was rather large, we examined the variability from filters of natural samples in more detail. Samples from natural waters were taken from the River Elbe with high amounts of detrital material, from the Baltic Sea, the North Sea and the Atlantic Ocean. The comparison of the T-R method was only done with samples taken in the Atlantic Ocean to minimize the influence by absorption of detrital material, thereby avoiding problems with null-point correction. Figure 8b shows the result of all measurements in absorptance mode (n = 31) and with the T-R method (n = 34). In general the linearity of the correlation for each sample was as good as for culture samples. The amplification for the T-R method varied between 2.0 and 3.2 (mean 2.50), while for the absorptance measurements it varied between 3.8 and 5.1 (mean 4.47). Compared to the amplification factors from algal cultures, the variability was higher for the T-R method, but lower for the absorptance measurements. However, the mean value was the same for absorptance measurements of algal cultures (4.47 vs. 4.50) but somewhat higher for the T-R method (2.50 vs. 2.39).

On a relative basis the amplification for the T-R method ranged between 80 and 128 % of the mean, for the absorptance measurements it ranged between 86 and 113 %. The reasons for these variations have not been examined in detail but are currently considered to be natural variability induced by the physical and optical properties of the particles collected on the filters. But we were e.g. aware that lab measurements with cultures were done by measuring the OD of the filters directly after filtration on a dry filter, whereas for natural samples the filters were measured after the filter were stored and the filter could be considered to be soaked in water for a much longer time. With a single culture it was tested whether a longer soaking of empty filter in water has an influence on the path-length amplification by measuring samples on filters that were not soaked in water before filtration and filters that were soaked before for >1h. The OD for the same amount of culture measured on previously soaked filters was about 10 % higher than that measured on filters that were not previously soaked (data not shown), indicating that path-length amplification is depending on the optical changes in filters induced by soaking glass-fiber filters in water.

4 Discussion

Some optical properties of glass fiber filters have been described elsewhere (e.g. [28, 22]). A first striking difference between transmittance, reflectance and absorptance measurements is the absolute optical density of a dry empty filter (Fig. 2a). Whereas OD_f^T was very high (>1.2), values for the reflectance and absorptance methods were about two to three orders of magnitude lower. Dry glass-fiber filters possess strong backscattering that leads to low light transmission through the filter and high reflectance. The low absorptance of dry filters is, thus, a first indication that the setup proposed here is not sensitive to scattering by the filter. When calculating the sum of transmittance, reflectance and absorptance at each wavelength values between 100 and 101 % were obtained, supporting the validity of the measuring concept used. The low, but significant negative values observed in the absorptance spectrum (Fig. 2b) can be explained by higher reflectivity of the filter material compared to that of the reflectance standard closing the reflectance port at these wavelengths. The significant positive values can be explained by either absorption of the glass material or changes in the overall light field when a filter is placed in the center of the integrating sphere that leads e.g. to an

increase in absorption by construction parts of the filter holder. (Note that any effect on light that leaves and re-enters the filter after being reflected at the cavity wall is compensated by use of the reference beam.) Theoretically these artifacts could be reduced by placing a dry empty filter before the reflectance standards in each reflectance port or by placing it in the center when performing a baseline. But lastly, when the filters are fully moistened the observed attenuation measured by absorptance does not vary significantly and does not lead to a large error (Fig. 2b). When filters were soaked in water and measured wet, the transmittance decreased and the reflectance increased, whereas the absorptance changed only slightly (as a result of the change in filter reflectance relative to the reflectance standard). From this we can deduce that the overall T/R ratio of a filter is different for dry and wet filters, and as a result transmittance and reflectance measurements are highly dependent on the wetness of the filter. For T and R measurements filters need to be measured fully soaked with water to have constant optical conditions; any drying of the filter needs to be avoided. Roesler (1998) observed an increase in T with a drying filter after about 4 min and could regain the initial T by adding water to the filter[28]. In addition, soaking a dry filter changes its T/R ratio constantly for the first 60 minutes after placing the filter in water before it reaches a constant value. This is known and soaking for about one hour is recommended for the T-R method [22]. In contrast to the above, the absorptance of a filter does not change significantly with time when soaking in water and is not much different from that of a dry filter. The absorptance shows a low absorption signal from water inside the filter at wavelengths >700 nm (clearly seen at wavelengths >900 nm pers. obs.). The influence of drying is significant only at shorter wavelengths and is most probably a result of changes in filter reflectance with water content at these wavelengths. The overall error for sample measurements was examined for the different optical setups (A, T and T-R). This was done with an algal culture that did not possess significant absorption in the NIR spectral region. The largest error was found for the transmittance measurement, the lowest for the absorptance measurement. As different filter loads were used for each setup, it is more appropriate to look at the relative error for those wavelengths for which significant absorption can be assumed (OD >0.01). This relative error was again largest for the transmittance measurement (mean 4.5 %) but equally low for the absorptance and T-R measurements (mean: 3.0 and 2.8 %, respectively). However, the error in the NIR spectral region, where no absorption by the algal sample was observed, was lowest for the

absorptance measurement, and for each method very similar to the error for empty filters (Fig. 3). The overall error for the T-R method reported by Tassan and Ferrari 2002 (see Table 2) was very similar to our measurements (σ_{OD}: 0.0020 vs. 0.0023), but the variability in OD_f of empty filters was higher (σ_{OD}: 0.0015 vs. 0.0006). The errors for T and R measurements were also higher (e.g. $\sigma_{OD,T}$: 0.0050 vs. 0.0022). This could be explained by either the use of larger filters or the use of a larger integrating sphere in the present study, as these normally perform better in collecting all scattered light. Compared to the transmittance measurement in front of an integrating sphere, changes in attenuation induced by the water content of the filter are much lower when the filter is measured inside the sphere. Less care has to be taken with respect to the water content and soaking of a reference filter. However, differences in the water content might change multiple scattering inside the filter and with it the path-length amplification. Because of this strong dependence on the water content of the filter and generally higher sensitivity to filter to filter differences, variability of OD_f in wet filters is relatively high for transmittance measurements, making the application of a null point correction often necessary. The T-R method showed much lower variability in calculated OD_f because the additional reflectance measurement is used to compensate for filter to filter variability in transmittance and it compensates for changes in backscattering by the sample particles like minerogenic particles (for which it was originally developed[37]). When the filters are measured inside an integrating sphere this variability is reduced even further, the null point correction is obsolete and OD_f^A >0.0008 can be considered as significant absorptance. As the small methodological error for measurements in the center of an integrating sphere also applies for the NIR (700 - 890 nm) spectral region, NIR absorption can be determined quantitatively, something that could not be achieved with this accuracy using other methods yet (e.g. [13, 15, 16]). However, at longer wavelengths (>900 nm) strong absorption by water itself will lead to significant errors. With respect to the error in the NIR, transmittance measurements will often need a null point correction, and the T-R method will not fully compensate for variability at NIR wavelengths, whereas the absorptance measurement will not need any null point correction, and should therefore be more accurate in the NIR spectral region. When NIR absorption is high, the T-R method can be used for measurements of absorption in the NIR region as well (see [17]). In terms of measurement effort, the T-R technique needs an additional measurement (the reflectance of the filter) and a complex calculation scheme with

additional empirically measured factors (tau) and additional assumptions regarding the equality in overall transmission of sample and reference filter (see [22] for details). Furthermore, T-R measurements, even when conducted very carefully, often exhibit exceptionally strong differences in OD for particular reference filters. These filters were not used for the earlier calculations of σ_{OD} (see [22]). Differences in OD_f are wavelength-independent and could therefore be compensated by null point correction. However, that would exclude the possibility of performing measurements of absorption in the NIR spectral region. Including results from these "bad" filters in the error calculation would increase σ_{OD} only slightly for the T-R measurement. Therefore we would not recommend any null point correction for the T-R method, but when performing measurements with low NIR absorption, multiple reference and sample filters should be measured to avoid potential influence from a single "bad" filter. The OD_f of these "bad" filters when measured as absorptance was not different from other reference filters and, hence, did not represent an additional error source for this technique.

4.1 Path length amplification

The remaining and major uncertainty for these measurements is related to the optical path-length amplification inside the filter. The amplification factor, β, has been determined several times by determining the real absorption, mainly by using algal cultures, in a photometric cuvette (e.g. [23, 24, 20, 25, 1, 26, 27]) or using an AC-9 instrument [28]. In most cases β was observed to dependent on the OD, and the obtained relationships were described using e.g. a second order polynomial. Roesler (1998) [28] proposed a constant factor of 2 for all ODs and wavelengths arguing that any deviation from linearity in the relationship of β vs. OD or wavelength was artificial due to measurement errors related to scattering losses when determining either the real or the filter pad absorption. Several filter load experiments showed that β was dependent on OD_f [33, 38]. This dependence is observed in our experiments as well when OD_f is high. However, when OD_f is low the variation in amplification with OD_f is insignificant. When OD_f is high, the probability for particle shelf shading is high. This self shading can only be avoided by measuring sample filters with low OD_f. The methodology used here to determine the true absorption (PSICAM) does not have a significant scattering error due to the integrating cavity design and is as well not affected by particle self-shading under the low particle concentrations used.

The same low scattering error can be assumed for the same reason for the absorptance measurements inside the spectrophotometer's integrating sphere. The linear correlation of the transmittance and absorptance measurement for a single filter (see Fig. 6c, r^2=0.9991) shows that transmittance and T-R measurements are subject to very low wavelength-dependent scattering error. Decrease of β with increasing OD is therefore not an artifact induced by scatter losses but is mainly induced by self-shading of the particles inside the filter when the filter load is too high. When filter load and, thus OD, is low, OD_f is a rather linear function of absorption (Fig. 7d) and there is only a small range in β for each filter absorption measurement mode. However, β is much higher when the filter is measured inside the sphere than when measured using the T-R method. Path-length amplification also has a different effect on OD_f for transmittance and reflectance measurements. Reflectance measurements showed, rather exactly, two times the amplification of transmittance measurements. In transmittance mode only light that leaves the filter in the forward direction is collected, so the absorption is determined from this signal only, as light totally reflected by the filter is not collected by the detector whether is gets absorbed after being reflected or not. When measuring in reflectance mode the situation is the opposite. The optical situation inside a filter is complex as the parallel light beam entering the filter becomes increasingly diffuse, so, the relative amount of diffuse light collected is higher for reflected light. The path-length amplification for reflectance measurements could be higher because the particles are not homogeneously distributed inside the filter, but are concentrated on one side of the filter. From the assumption that the filter acts as a good light diffuser, Roesler (1998) proposed that amplification for the transmittance measurement should be about 2. Following this approach, the amplification for the filter measured in absorptance mode should theoretically be double, i.e. 4. Our measurements showed that amplification for absorptance measurements was not double compared to the transmittance mode, but was instead higher by a factor of ca. 1.5 (see Fig. 6c). Despite the differences in amplification between measurement modes, our experimental data showed that any of the modes tested provided absorption data without significant scattering errors and that the only error sources were the amplification factor and the filter to filter variability. Thus, for algal cultures (for which it is safe to assume zero NIR absorption) the transmittance measurement provides a good proxy for absorption measurements without wavelength-dependent artifacts by scattering when filter to filter variations are compensated by a null point correction

and the individual amplification effect is known. Proper knowledge of the amplification factor makes data from any of these measurement modes valuable. However, we showed that the variability of the amplification factor was rather large for algal cultures. For the T-R method it ranged from 2.0 to 3.2 and for the absorptance measurement it ranged from 3.5 to 5.4. For natural samples the variability in β for the absorptance measurements was lower, with β ranging from 3.8 to 5.1 only. At least for natural samples the amplification effect for absorptance measurements is about double that for T-R measurements but on a relative basis is less variable. Only a small part of the observed variation can be explained by errors made in either the PSICAM or the QFT measurements. Under lab conditions the PSICAM is very accurate. (However, in the field, calibration of the PSICAM can lead to errors as large as 5 %). In this case we observed large variability in amplification factors for natural samples and cultures when PSICAM measurements were very accurate. Describing the light field inside a sample/filter composite is rather complicated and influenced by 1) the parallel light beam that becomes increasingly diffuse as it penetrates through the filter, 2) the spatial allocation of particles inside or on the filter, and 3) the individual scattering properties of the particles. As 2) and 3) influence 1), a constant amplification factor is unrealistic. It was not the intention of the work presented here to examine whether the amplification depends directly on specific optical properties of the particles sampled. Nevertheless we observed some specific details in the measurements of algal cultures. For example, the amplification effect was always lowest for diatoms species and higher for non-diatom species. The high variability in amplification observed for algal cultures could, therefore, result from the use of cultures from quite different algal groups. A second source for this larger variability could be the use of filters that were not previously soaked in water, as this pretreatment influences path-length amplification as well. Considering the possible errors, an amplification factor of 4.5 can be used for the measurements inside an integrating sphere, resulting in a maximal relative error from sample to sample variability in amplification of ± 14 % for natural samples. For the T-R method a mean amplification factor of 2.45 would have a maximal relative error of ca. ± 25 %. The natural samples came from quite different environments, like coastal and offshore waters from the North and the Baltic Sea, from a river and from the Atlantic Ocean. Nevertheless, when optical properties of the particles collected play a role for this variability the overall error by this variability could be larger in other environments. The exact determination of the path-length amplification factor

remains the major error source in the quantitative filter technique, as most other measurement errors can be reduced by the use of absorptance measurements inside an integrating sphere. Of course there are other, previously documented problems with the QFT that do further increase its uncertainty (see [29, 30, 31, 32]). However, the exact path-length amplification can be determined with a PSICAM measurement for each individual sample. In coastal waters with high particle concentrations the PSICAM would actually give better results, but in oligotrophic waters its sensitivity is limited. However, even in these waters the absorption at ∼442 nm is high enough to be measured with good precision with the PSICAM, and this measurement could be used to determine the individual amplification factor across the spectrum. All our results support the fact that the amplification factor is constant over wavelengths for a single filter (if OD_f <0.1). Despite variability of the amplification factor, the accuracy of the whole procedure can be elucidated from measurements of an algal culture with negligible absorption in the NIR spectral region, and a natural sample with significant NIR absorption. In the case of the algal culture, the OD in the NIR region does not differ from that of the reference filter even when the maximal OD is ten times higher than recommended. For the natural sample the NIR absorption is much higher than the proposed sensitivity limit of 0.0008. We therefore propose that this method can be used to determine the particulate absorption in the NIR spectral region with high accuracy especially considering that the path-length amplification is wavelength-independent (for low OD) and is therefore the same for the VIS, UV and NIR spectral region.

5 Conclusions

The commonly used technique to measure the absorption of a filter in transmittance mode performs much worse in terms of sample to sample (and filter to filter) variability than a measurement in the absorptance mode inside an integrating sphere. The transmittance mode can hardly be used for filters with low OD_f without a null point correction of the spectrum. Unfortunately it is precisely under the condition of low OD_f that the influence of path-length amplification is wavelength- and OD- independent. However, we did not observe any wavelength-dependent artifacts induced by scattering (see measurement of an algal culture) but a rather wavelength-independent shift that would allow a null point correction for algal cultures that do not

possess absorption in the NIR. On the other hand, we showed that the T-R method compensates for a variability that is related to differences in the T/R ratio of different filters, and of sample and reference filter. It can be used (for the UV to NIR spectral range) without a null point correction in cases where NIR absorption is high. However, T-R measurements were found to be susceptible to the effect of "bad" filters that are occasionally encountered, and appropriate precautions are required. The proposed measurement of particles retained on glass-fiber filters inside an integrating sphere has some advantages compared to the other two techniques: 1) the overall precision is high, as many variations in the OD measurements that are related to errors due to back scattering and to varying filter scattering properties (moisture, thickness, structure etc.) are avoided, 2) no null point correction is needed, hence, 3) the higher precision and lower absolute error allow more precise measurements in the NIR region, where absorption is sometimes relatively low, 4) the variation of the path-length amplification and, hence, the error due to this variation is reduced to max. ± 14 % for natural samples; we showed that with low filter loads β is rather constant for a single filter, however if OD are higher a non-linear OD_f to a relationship of β can be used but will increase the overall error, 5) as the path-length amplification effect is a factor of two greater than for the T-R method, less material is needed to obtain the recommended OD_f, 6) the workload is reduced to a single scan compared to the T-R method and less care has to be taken for preparing the reference and sample filters. Critically for all QFT methods is the variability and non-linearity of the path length amplification in the filter at higher filter loads. The sources for this variability have to be examined in more detail. As spectrophotometers with large integrating spheres are commercially available and becoming more commonplace, we expect that with the described method the overall accuracy of the determination of the particulate absorption will strongly increase and that e.g. remote sensing data can be improved in the future as the algorithms require highly precise measurements of the inherent optical properties, like particulate absorption. The combination with PSICAM measurements allows determination of amplification factors individually for single filters and cross-validation of both methods. It combines a method with theoretically unlimited sensitivity (QFT) with one that does not need additional sample treatment like filtration, freezing, or storage (PSICAM). The overall error for determination of particulate absorption in natural waters could most probably be reduced to values below 5 %.

Appendix A. Proposed measurement procedure

Filter preparation

New 25 or 47 mm GF/F (Whatman) or GF-5 (Macherey & Nagel) filters are combusted at 450°C for 4-5 hours. Before use the filters are soaked in purified water for more than one hour.

Filtration

The required amount of sample suspension (to achieve 0.02 <ODmax <0.1) is filtered onto a filter using a clean glass filter holder and a clean glass filtration unit. To achieve precise absorption measurements for different parts of the spectrum, different volumes of the same sample are filtered, which means more than one triplicate of filters per sample. A higher filter load will normally prevent determination at shorter wavelengths, but will improve the determination at longer wavelengths, especially in the NIR region. Very small volumes (e.g. required for highly turbid waters) are homogeneously distributed over the filter by filling the filter funnel with filtered water of the sample and dispensing the small sample volume into it before starting the filtration. The glass filter holder is checked beforehand with a larger volume, such that any inhomogeneity on the filter - due to a partly clogged holder - can be recognized and the holder replaced. The diameter of the clearance area of the filter is measured with a caliper rule (precision: ±0.05 mm).

Spectrophotometric measurement

The filter can be measured directly in a spectrophotometer equipped with an integrating sphere that allows placing the filter inside the sphere. Frozen and stored filter should be soaked in a few drops of water with the same salinity as the sample water for a few minutes. Then the filter is put briefly on a clean tissue to remove free water from the filter. The filter is placed inside the sphere on a filter holder in such a way that the sample light beam is perpendicular to the filter. To place the filter inside the sphere it might be necessary to cut it into a piece that fits on the specific center-mount filter holder. Reference filters are prepared from the same filter pack as sample filters and measured in the same way after being soaked in filtered seawater

for at least 1 hour. Before measurements commence the spectrophotometer should be turned on to warm up, the sample compartment and integrating sphere cleaned, and the baseline recorded with a dry, empty filter, open sample and reference entrance ports, and reflectance ports closed with highly reflective Spectralon standards. The position of the sample and reference light beam should be checked by eye. Scans are performed to yield the best signal to noise ratio in a reasonable scanning time. The baseline should be regularly checked by a measurement of the same dry, empty filter.

Absorption calculation

The attenuation of a reference filter (OD_r) is subtracted from that of the sample filter (OD_s) and, assuming no losses by scattering, the absorption coefficient a [m^{-1}] is calculated using Eq. (1), with individual filtered sample volume, V, and filter patch area, F. For the recommended values of (OD_s-OD_r) <0.1 a general path length amplification factor of $\beta = 4.5$ can be used. In case (OD_s-OD_r) >0.1 (valid range 0.1 - 0.5) a polynomial function (based on data from Fig. 6) can be used to calculate β as

$$\beta = 6.475(OD_s - OD_r)^2 - 6.474(OD_s - OD_r) + 4.765. \quad (2)$$

References

[1] S. Tassan and G. M. Ferrari, "An alternative approach to absorption measurements of aquatic particles retained on filters," Limnol. Oceanogr. **40**, 1358-1368 (1995).

[2] K. Shibata, A. A. Benson, and M. Calvin, "The absorption spectra of suspension of living micro-organisms," Biochim. Biophys. Acta **15**, 461-471 (1954).

[3] K. Shibata, "Spectrophotometry of intact biological materials. Absolute and relative measurements of their transmission, reflection, and absorption spectra," J. Biochem. **45**, 599-623 (1958).

[4] C. S. Yentsch, "A non-extractive method for the quantitative estimation of chlorophyll in algal cultures," Nature **179**, 1302-1304 (1957).

[5] R. M. Pope, A. D. Weidemann, and E. S. Fry, "Integrating cavity absorption meter measurements of dissolved substances and suspended particles in ocean water," Dyn. Atmos. Oceans **31**, 307-320 (2000).

[6] R. Röttgers, C. Häse, and R. Doerffer, "Determination of particulate absorption of microalgae using a Point Source Integrating Cavity Absorption Meter," Limnol. Oceanogr. Methods **5**, 1-12 (2007).

[7] B. G. Mitchell, A. Bricaud, and others, "Determination of spectral absorption coefficients of particles, dissolved material and phytoplankton for discrete water samples," in *Ocean Optics Protocols for Satellite Ocean Color Sensor Validation, Revision 2*, G. S. Fargion, and J.L. Mueller, eds. NASA/TM-2000-209966, NASA Goddard Space Flight Center, Greenbelt, MD. Chapter 12, pp. 125-153 (2000).

[8] N. B. Nelson and B.B. Prézelin. "Calibration of an integrating sphere for determining the absorption coefficient of scattering suspension," Appl. Opt. **32**, 6710-6717 (1993).

[9] J. T. O. Kirk, *Light and photosynthesis in aquatic ecosystems, 2nd ed.* (Cambridge Univ. Press 1994).

[10] J. Ducha and S. Kubin, "Measurements of in vivo absorption spectra of microscopic algae using bleached cells as a reference sample," Arch. Hydrobiol. Suppl. **49**, 199-213 (1976).

[11] D. Stramski and J. Piskozub, "Estimation of scattering error in spectrophotometric measurements of light absorption by aquatic particles from three-dimensional radiative transfer simulations," Appl. Opt. **42**, 3634-3646 (2003).

[12] H. Haardt and H. Maske, "Specific in vivo absorption coefficient of chlorophyll a at 675 nm," Limnol. Oceanogr. **32**, 608-619 (1987).

[13] M. Babin and D. Stramski, "Light absorption by aquatic particles in the near-infrared spectral region," Limnol. Oceanogr. **47**, 911-915 (2002).

[14] M. Babin and D. Stramski, "Variations in the mass-specific absorption coefficient of mineral particles suspended in water," Limnol. Oceanogr. **49**, 756-767 (2004).

[15] D. Stramski, S. B. Wozniak, and P. J. Flatau, "Optical properties of Asian mineral dust suspended in seawater," Limnol. Oceanogr. **49**, 749-755 (2004).

[16] D. Stramski, M. Babin, and S. Wozniak, "Variations in the optical properties of terrigeneous mineral-rich particulate matter suspended in seawater," Limnol. Oceanogr. **52**, 2418-2433 (2007).

[17] S. Tassan and G. M. Ferrari, "Variability of light absorption by aquatic particle in the near-infrared spectral region," Appl. Opt. **42**, 4802-4810 (2003).

[18] C. S. Yentsch, "Measurements of visible light absorption by particulate matter in the ocean," Limnol. Oceanogr. **7**, 207-217 (1962).

[19] H. G. Trüper and C. S. Yentsch, "Use of glass fiber filters for the rapid preparation of in vivo absorption spectra of photosynthetic bacteria," J. Bact. **94**, 1255-1256 (1967).

[20] B. G. Mitchell, "Algorithms for determining the absorption coefficient for aquatic particulates using the quantitative filter technique," in *Ocean Optics 10. Proc SPIE 1302*, pp. 137-148 (1990).

[21] W. L.Butler, "Absorption of light by turbid samples," L. Opt. Soc. Am. **52**, 292-299 (1962).

[22] S. Tassan and G. M. Ferrari, "A sensitivity analysis of the 'transmittance-reflectance' method for measuring light absorption by aquatic particles," J. Plankton Res. **24**, 757-774 (2002).

[23] B. G. Mitchell and D. A. Kiefer, "Chlorophyll a specific absorption and fluorescence excitation spectra for light-limited phytoplankton," Deep-Sea Res. **35**, 639-663 (1988).

[24] A. Bricaud and D. Stramski, "Spectral absorption coefficients of living phytoplankton and nonalgal biogenous matter: a comparison between the Peru upwelling area and the Sargasso Sea," Limnol. Oceanogr. **35**, 562-582 (1990).

[25] C. S. Cleveland and A. D. Weidemann, "Quantifying absorption by aquatic particles: a multiple scattering correction for glass-fiber filters," Limnol. Oceanogr. **38**, 1321-1327 (1993).

[26] B. Arbones, F. G. Figueiras, and M. Zapata, "Determination of phytoplankton absorption coefficient in natural seawater samples: evidence of a unique equation to correct the path-length amplification on glass-fiber filters," Mar. Ecol. Prog. Ser. **137**, 293-304 (1996).

[27] Z. V. Finkel and A. J. Irwin, "Light absorption by phytoplankton and the filter amplification correction: cell size and species effects," J. Exp. Mar. Biol. Ecol. **259**, 51-61 (2001).

[28] C. S. Roesler, "Theoretical and experimental approaches to improve the accuracy of particulate absorption coefficients derived from the quantitative filter technique," Limnol. Oceanogr. **43**, 1649-1660 (1998).

[29] J. C. Goldman and M. R. Dennet, "Susceptibility of some marine phytoplankton species to cell breakage during filtration and post-filtration rinsing," J. Exp. Mar. Biol. Ecol. **86**, 47-58 (1985).

[30] D. Stramski, "Artifacts in measuring absorption spectra of phytoplankton collected on a filter," Limnol. Oceanogr. **35**, 1804-1809 (1990).

[31] H. M. Sosik, "Storage of marine particulate samples for light-absorption measurements," Limnol. Oceanogr. **44**, 1139-1141 (1999).

[32] I. Laurion, F. Blouin, and S. Roy, "The quantitative filter technique for measuring phytoplankton absorption: interference by MAAS in the UV waveband," Limnol. Oceanogr. Methods **1**, 1-9 (2003).

[33] H. Maske and H. Haardt, "Quantitative in vivo absorption spectra of phytoplankton: detrital absorption and comparison with fluorescence excitation spectra," Limnol. Oceanogr. **32**, 620-633 (1987).

[34] S. G. H. Simis, S. W. M. Peters, and H. J. Gons, "Remote sensing of the cyanobacterial pigment phycocyanin in turbid inland water," Limnol. Oceanogr. **50**, 237-245 (2005).

[35] F. M. P. Saldanha-Correa, S. M. F. Gianesella, and J. J. Barrera-Alba, "A comparison of the retention capability among three different glass-fiber filters used for chlorophyll-a determinations," Brazilian J. Oceanogr. **52**, 243-247 (2004).

[36] R. Röttgers and R. Doerffer, "Measurements of optical absorption by chromophoric dissolved organic matter using a point-source integrating-cavity absorption meter, " Limnol. Oceanogr. Methods **5**, 126-135 (2007).

[37] S. Tassan and G. M. Ferrari, "Measurement of light absorption by aquatic particles retained on filters: determination of the optical path-length amplification by the 'transmittance-reflectance' method," J. Plankton Res. **20**, 1699-1709 (1998).

[38] S. E. Lohrenz, "A novel theoretical approach to correct for path-length amplification and variable sampling loading in measurements of particulate spectral absorption by the quantitative filter technique," J. Plank. Res. **22**, 639-657 (2000).

Table 1: Table of notations

A	absorbance, i.e fraction of light that is absorbed, dimensionless
T	transmittance, i.e. fraction of light that is transmitted, dimensionless
R	reflectance, i.e. fraction of light that is reflected, dimensionless
T-R	transmittance-reflectance method of Tassan and Ferrari [1]
OD	optical density as $-log()$ of transmission, dimensionless
OD_s	OD of a sample filter, dimensionless
OD_f	OD of an empty filter, dimensionless
$OD_f^{A,T,R,T-R}$	OD of a filter measured as A, T, R, of by T-R, dimensionless
λ	wavelength, [nm]
a	absorption coefficient, [m^{-1}]
$a^{\beta=1}$	a for a filter sample w/o correction for path-length amplification, [m^{-1}]
$a^{\beta=1}_{A,T,R,T-R}$	$a^{\beta=1}$ determined for a measurement in mode A, T, R, or by T-R, [m^{-1}]
β	path-length amplification factor, dimensionless

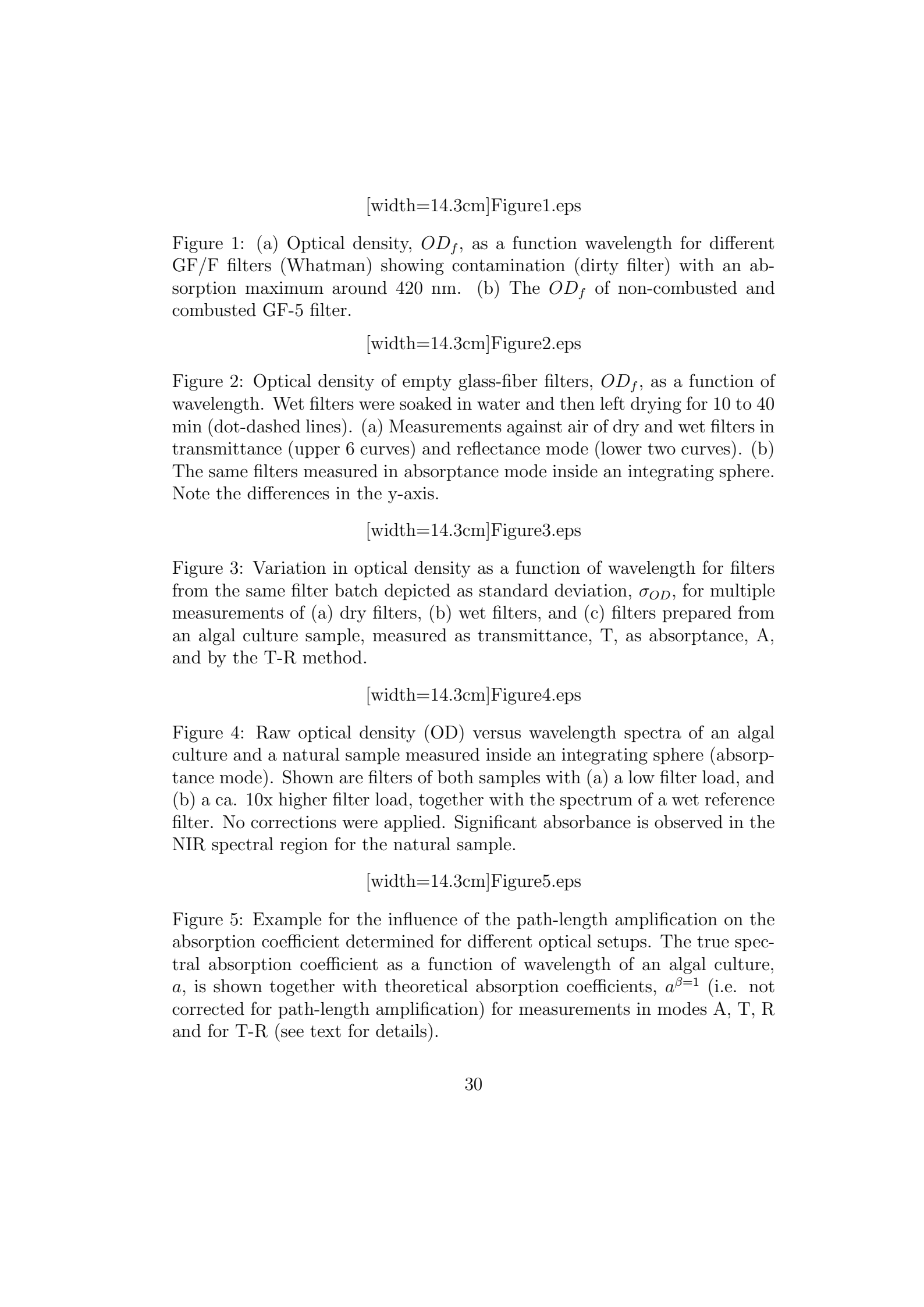

Figure 1: (a) Optical density, OD_f, as a function wavelength for different GF/F filters (Whatman) showing contamination (dirty filter) with an absorption maximum around 420 nm. (b) The OD_f of non-combusted and combusted GF-5 filter.

Figure 2: Optical density of empty glass-fiber filters, OD_f, as a function of wavelength. Wet filters were soaked in water and then left drying for 10 to 40 min (dot-dashed lines). (a) Measurements against air of dry and wet filters in transmittance (upper 6 curves) and reflectance mode (lower two curves). (b) The same filters measured in absorptance mode inside an integrating sphere. Note the differences in the y-axis.

Figure 3: Variation in optical density as a function of wavelength for filters from the same filter batch depicted as standard deviation, σ_{OD}, for multiple measurements of (a) dry filters, (b) wet filters, and (c) filters prepared from an algal culture sample, measured as transmittance, T, as absorptance, A, and by the T-R method.

Figure 4: Raw optical density (OD) versus wavelength spectra of an algal culture and a natural sample measured inside an integrating sphere (absorptance mode). Shown are filters of both samples with (a) a low filter load, and (b) a ca. 10x higher filter load, together with the spectrum of a wet reference filter. No corrections were applied. Significant absorbance is observed in the NIR spectral region for the natural sample.

Figure 5: Example for the influence of the path-length amplification on the absorption coefficient determined for different optical setups. The true spectral absorption coefficient as a function of wavelength of an algal culture, a, is shown together with theoretical absorption coefficients, $a^{\beta=1}$ (i.e. not corrected for path-length amplification) for measurements in modes A, T, R and for T-R (see text for details).

Table 2: Standard deviations of the optical density, σ_{OD}, for various error sources given for the full wavelength range and the mean over all wavelengths.[a]

	Transmittance mode	T-R	Absorptance mode
Wetting the filter to saturation	0.0015 - 0.0050 **⌀0.0022** (0.005) *0.005*	0.0004 - 0.0009 **⌀0.0006** *0.0015*	0.0002 - 0.0010 **⌀0.0004**
Measuring the filter 2 to 10 min after wetting	**⌀0.04** (0.01)	n.d.	**⌀0.0008**
Changing the filter in the same batch (dry filters)	0.0040 - 0.012 **⌀0.0051** (0.006)	0.0030 - 0.010 **⌀0.0041**	0.0001 - 0.0010 **⌀0.0003**
Changing the filter in the same batch (wet filters)	0.0015 - 0.005 **⌀0.0022**	0.0004 - 0.0009 **⌀0.0006**	0.0002 - 0.0010 **⌀0.0004**
Overall error of repetitive measurements of samples	0.0025 - 0.0076 **⌀0.0051** (0.003 - 0.008)	0.0005 - 0.0057 **⌀0.0023** *0.002*	0.0003 - 0.0020 **⌀0.0009**
Percentage error	3 - 6% **⌀4.5%** $OD_{max} = 0.3$ (3 - 17%, ⌀6.5%)	1.7 - 5% **⌀2.8%** $OD_{max} = 0.3$	1.6 - 5% **⌀3.0%** $OD_{max} = 0.1$

[a] Shown in brackets are values of Tassan and Ferrari (1995)[1] and in italics values of Tassan and Ferrari (2002)[22]
n.d., not determined

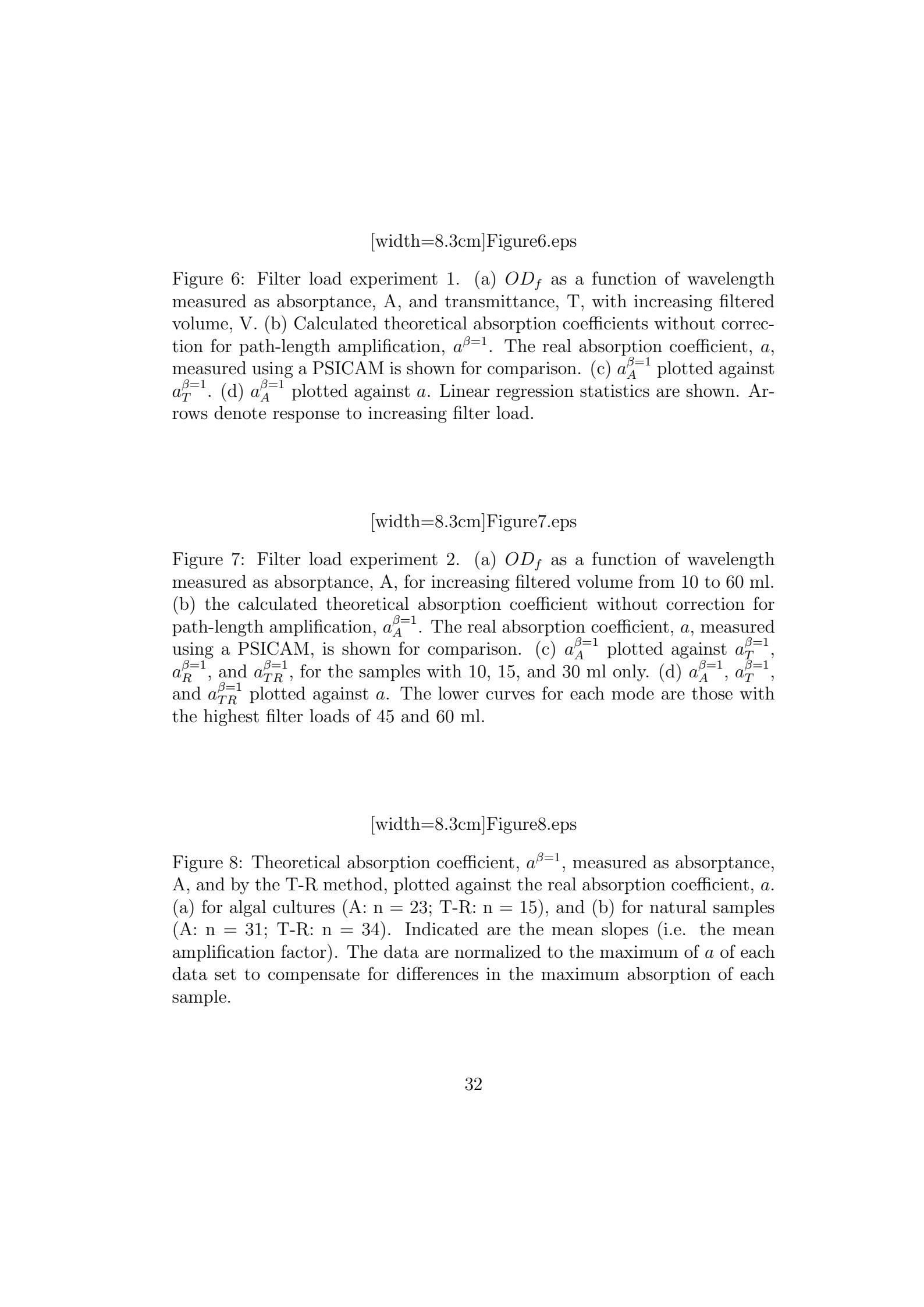

Figure 6: Filter load experiment 1. (a) OD_f as a function of wavelength measured as absorptance, A, and transmittance, T, with increasing filtered volume, V. (b) Calculated theoretical absorption coefficients without correction for path-length amplification, $a^{\beta=1}$. The real absorption coefficient, a, measured using a PSICAM is shown for comparison. (c) $a_A^{\beta=1}$ plotted against $a_T^{\beta=1}$. (d) $a_A^{\beta=1}$ plotted against a. Linear regression statistics are shown. Arrows denote response to increasing filter load.

Figure 7: Filter load experiment 2. (a) OD_f as a function of wavelength measured as absorptance, A, for increasing filtered volume from 10 to 60 ml. (b) the calculated theoretical absorption coefficient without correction for path-length amplification, $a_A^{\beta=1}$. The real absorption coefficient, a, measured using a PSICAM, is shown for comparison. (c) $a_A^{\beta=1}$ plotted against $a_T^{\beta=1}$, $a_R^{\beta=1}$, and $a_{TR}^{\beta=1}$, for the samples with 10, 15, and 30 ml only. (d) $a_A^{\beta=1}$, $a_T^{\beta=1}$, and $a_{TR}^{\beta=1}$ plotted against a. The lower curves for each mode are those with the highest filter loads of 45 and 60 ml.

Figure 8: Theoretical absorption coefficient, $a^{\beta=1}$, measured as absorptance, A, and by the T-R method, plotted against the real absorption coefficient, a. (a) for algal cultures (A: n = 23; T-R: n = 15), and (b) for natural samples (A: n = 31; T-R: n = 34). Indicated are the mean slopes (i.e. the mean amplification factor). The data are normalized to the maximum of a of each data set to compensate for differences in the maximum absorption of each sample.