

## Theoretical and experimental approaches to improve the accuracy of particulate absorption coefficients derived from the quantitative filter technique

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### Abstract

The quantitative filter technique (QFT), whereby particles are concentrated onto glass-fiber filters and analyzed in a spectrophotometer, is used extensively to estimate the absorption coefficients of aquatic particles. A number of empirically derived correction factors ( $\beta$ ) have been developed to account for the amplified optical path length associated with the highly scattering glass-fiber filters. Published results are inconsistent, and  $\beta$  remains the largest source of uncertainty in estimated absorption coefficients. In this study, path-length amplification was estimated from the average cosine of diffusely traveling photons in the filter pad using a theoretical approach. This amplification factor, combined with variability in blank filter pad optical density, explains many of the confounding observations in the literature. Absorption coefficients for phytoplankton cultures and field samples were estimated from a modified QFT using the new model for path-length amplification and tested against absorption coefficients measured with a nine-wavelength absorption and attenuation meter (ac9, WETLabs). A linear regression between the modeled and measured particulate absorption coefficients was highly significant ( $r^2 > 0.99$ ,  $n = 99$ ), with estimated slope and intercept not significantly different from 1 and 0, respectively ( $P < 0.001$ ). The model outperforms published, empirically derived correction factors over a broad range of absorption coefficients and particulate compositions. Results indicate that the modified QFT combined with the new model for path-length amplification yields accurate estimates of spectral particulate absorption coefficients regardless of the concentration or composition of the particulate material.

The color and clarity of light emanating from the ocean provide useful indices of the composition and concentration, respectively, of the dissolved and particulate material that lies within. Clear blue waters are relatively bereft of material, whereas turbid green waters are rich in light-absorbing and light-scattering materials. Similarly, the color of oceanic particulate material concentrated on a filter pad provides evidence of the composition and concentration of that particulate material, owing largely to the phytoplankton-derived pigments.

Although technical advances in spectrophotometry have enabled precise characterization of pigment composition and concentration in solvent extracts, absolute quantification of the particulate absorption coefficient has remained elusive because suspended particles scatter light. The ocean is a relatively dilute medium; therefore, concentration of particles onto a filter is beneficial for standard spectrophotometric analysis of discrete water samples (Yentsch 1962; Trüper and Yentsch 1967; Yentsch and Phinney 1989). This method, named the quantitative filter technique (QFT) by Mitchell (1990), is also useful for separating the total absorption coefficient into contributions by the dissolved and particulate

fractions. The particulate fraction can be further separated into absorption by phytoplankton (essentially, *in vivo* pigment absorption) and tripton components (Kishino et al. 1985). However, scattering by both the particles and the filter-pad fibers amplifies the mean path length that the photons travel through the filter/particle system, artificially enhancing the derived absorption coefficients (Butler 1962; Bohren 1987).

Most filter-pad correction schemes have used the fact that phytoplankton cultures can be grown to an optical thickness sufficient for spectrophotometric measurements in 1-cm cuvettes. Absorption coefficients calculated from these optical-density measurements are used to develop empirical relationships to estimate the particulate absorption coefficients of the same material concentrated on a filter pad over a range of particle loads (Kiefer and SooHoo 1982; Mitchell and Kiefer 1984, 1988; Maske and Haardt 1987; Bricaud and Stramski 1990; Mitchell 1990; Roesler 1992; Cleveland and Weidemann 1993; Moore et al. 1995; Arbones et al. 1996). The essence of this correction factor is that the filter-pad measurement is scaled to match the suspension measurement of like material over a range in optical density (particle loading). The derived correction factor ( $\beta$ ) is then applied to the optical density of field samples concentrated on filters. However, although some independent investigators report comparable results (e.g., Mitchell 1990; Cleveland and Weidemann 1993), most do not; the  $\beta$  correction remains the largest source of uncertainty in estimating particulate absorption coefficients. There are a number of unresolved observations related to  $\beta$ , such as nonunique solutions (a hysteresis when  $\beta$  is expressed as a function of optical density, leading to an apparent wavelength dependence; Bricaud and Stramski 1990; Roesler 1992; Arbones et al. 1996) and vari-

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ations in the asymptotic value of  $\beta$  at high optical densities (ranging from approximately 1 to 3).

Theoretical derivations of scattering corrections for particles in suspension are numerous (Shibata et al. 1954; Duyens 1956; Amesz et al. 1961; Bricaud et al. 1983; Kirk 1992; Roesler and Zaneveld 1994; Zaneveld et al. 1994; Bricaud et al. 1995), but those for particles concentrated on a filter pad are rare (Clarke 1982; Clarke et al. 1987; for soot particles on Nucleopore filter). This paper presents a theoretical approach to deriving a correction factor to account for path-length amplification due to scattering by glass fibers, with the goal of increasing the accuracy of estimating particulate absorption coefficients using the QFT.

## Background

The optical density measured spectrophotometrically in a cuvette is given by

$$D = \log_{10} \frac{I_0}{I}, \quad (1)$$

where  $D$  is the dimensionless optical density,  $I_0$  is the intensity of the incident beam, and  $I$  is the intensity of the transmitted beam. In a dual-beam spectrophotometer,  $I_0$  is a measure of the intensity of the beam transmitted through a reference material, and  $I$  is a measure of the beam transmitted through the sample material where the reference and sample materials are in the same geometric configuration. In practice, spectrophotometers contain inherent signals associated with fluctuating beam intensities, nonuniformity of the detector, and geometric variations between sample and reference compartments. A baseline scan, with reference material in both the sample and reference holders, is used to correct for these instrumental phenomena. The baseline-corrected optical density of a sample,  $D'$ , is given by the difference between a sample and baseline optical density measurements

$$D' = D_S - D_B = \log_{10} \frac{I_R}{I_S} - \log_{10} \frac{I_{RB}}{I_{SB}} = \log_{10} \frac{I_{SB}}{I_S}, \quad (2)$$

where the subscripts  $R$ ,  $S$ , and  $B$  indicate reference, sample, and baseline, respectively, and  $I_R = I_{RB}$ . For a homogeneously dispersed sample, the transmission of the incident beam can be described by the natural logarithm of the change in the emergent beam through the sample:

$$K = \frac{1}{l} \log_e \frac{I_{SB}}{I_S}, \quad (3)$$

where  $K$  is the diffuse attenuation coefficient ( $\text{m}^{-1}$ ) and  $l$  is the geometric path length of the sample (m). The attenuation coefficient and the optical density are related by the equation

$$K = \frac{1}{\log_{10} e} \frac{D'}{l}. \quad (4)$$

The suspended particles and the medium are understood to both absorb and scatter the light in the incident beam. If, however, scattering losses are minimized by maintaining optically dilute samples and configuring the acceptance region of the detector is configured to maximize the collection of

## Notation

$a$	Absorption coefficient, where subscripts $c$ and $f$ indicate an estimate derived from a cuvette or filter-pad optical density measurement in a spectrophotometer, $m$ and $ac9$ measured with an ac9 before and after scattering correction, $\text{m}^{-1}$ .
$b$	Scattering coefficient derived from the difference between the attenuation and absorption coefficients measured in an ac9, $\text{m}^{-1}$ .
$\beta$	Correction factor for pathlength amplification of absorption due to scattering. Subscripts $c$ and $f$ indicate amplification associated with the cuvette and filter-pad measurements, respectively, dimensionless.
$c$	Attenuation coefficient measured in an ac9, $\text{m}^{-1}$ .
$D$	Optical density determined spectrophotometrically where ' indicates baseline correction, subscript $s$ and $B$ indicate sample and baseline scans, and $f$ and $c$ indicate measurement made on a filter pad or in a cuvette, respectively. The subscript GFF indicates the optical density of a blank filter pad, and 750 indicates the optical density at the null wavelength; dimensionless.
$\Delta D'_{\text{GFF}}$	The difference in optical density between two baseline-corrected blank filter pad scans, dimensionless.
$I$	Relative intensity of a spectrophotometric beam, where subscript 0 indicates the incident beam, $R$ and $S$ indicate the transmitted beam through the reference and sample compartments, and $B$ indicates a baseline scan; dimensionless.
$K$	Diffuse attenuation coefficient of the net transmitted irradiance, $\text{m}^{-1}$ .
$l$	Geometric pathlength for a sample in a spectrophotometer, where subscripts $c$ and $f$ indicate cuvette and filter-pad configurations, respectively; m.
$\lambda$	Wavelength, nm.
$r$	Optical pathlength of the filter pad, mm.
$\sigma_{\text{GFF}}$	The sample standard deviation of optical density between three or more baseline-corrected blank filter-pad scans, dimensionless.
$\sigma_a$	The uncertainty in particulate absorption coefficients derived from $\sigma_{\text{GFF}}$ , $\text{m}^{-1}$ .
$\tau$	Geometric pathlength of the filter pad, equal to pad thickness, mm.

forward scattered light, the absorption coefficient can be approximated by the measured attenuation:

$$a \approx \frac{1}{\log_{10} e} \frac{D'}{l}. \quad (5)$$

The accuracy of the absorption coefficient derived from Eq. 5 depends on the relative magnitudes of absorption and scattering by the particles and the medium, the absolute magnitude and angular distribution of the scattering coefficient, and the detector's collection efficiency for scattered light. The scattering problem is twofold. First, scattered photons that are not captured by the detector are attributed to loss by absorption. Second, with each scattering event, the path length of the photons is increased relative to that of unscattered photons, thereby increasing the probability of absorption. Both processes result in overestimation of the absorption coefficient.

A number of configuration and measurement schemes have been developed to minimize scattering by the suspend-

ed particles, to maximize the detection of scattered radiance, or both. The scattering by the particles in the sample cuvette and the reference cuvette can be matched by using the same particles bleached of their absorbing components (Ducha and Kubin 1976). Scattering can be minimized by placing the particles in a medium with a similar index of refraction (Barer 1955; Mitchell and Kiefer 1984; Allali et al. 1995). Scattered light collection can be increased by using an integrating sphere (Nelson and Prézelin 1993), by placing a quartz or opal glass diffuser between the sample and the detector (Shibata 1958; Bannister 1988), or by placing the sample immediately adjacent to the detector (Bricaud et al. 1983). In all cases, all of the backward scattered light and some portion of the side- and forward-scattered light is not captured by the detector. These effects have been quantified for long-path-length-reflecting tube absorption meters using Monte Carlo simulations (Kirk 1992). It should be noted, however, that the optical configurations used in that study were based upon early versions of WETLabs absorption meters, which were optically superior to standard spectrophotometers with regard to beam collimation, detector sensitivity, and detection range. Thus, the results of those simulations are too optimistic for commercial spectrophotometers and especially optimistic for 1-cm cuvette applications.

Typically, the loss of scattered photons is indicated by an offset in the red wavelength region (usually at the value of 750 nm or an average about the value of 750 nm), where there is negligible absorption by particulate material. Any optical density signature in this wavelength region is assumed to be due to scattering losses. The most common approach is to subtract this offset from the entire spectrum (the null wavelength correction,  $D'_{750}$ ), the assumption being that the scattering is spectrally flat. More sophisticated correction schemes involve estimating the spectral shape of the scattering coefficient from the particle size distribution and Mie theory (Bricaud et al. 1983).

Regardless of what portion of the scattered light is detected or corrected for, the path-length amplification that results from any scattering event necessitates a correction to obtain accurate absorption coefficients. The path-length correction factor,  $\beta_c$  (the subscript *c* indicates the cuvette), is defined as the ratio of the optical path length to the geometric path length in the sample (Butler 1962). In the spectrophotometer, the optical path length is affected by scattering from both medium and particles, the relative magnitudes of which depend upon the particle concentration. Particles that are large relative to the wavelength of light will exhibit diffraction-dominated scattering, and the angular distribution of scattered light will be primarily forward, minimally enhancing the optical path length. As the magnitude of scattering increases, the influence of side- and back-scattering on optical density becomes more important, as does the magnitude of the optical path length. For particles whose size approaches the wavelength of the light (in the size range for Rayleigh or Rayleigh-Gans theory), the angular distribution of scattered light will be enhanced in the side and backward directions, thereby increasing the optical path length more than larger particles do. Thus, Eq. 5 is amended as follows to include both scattering losses by the particles (scattering

losses due to the medium will be accounted for by the medium in the reference cuvette, assuming no multiple scattering) and the path-length amplification due to scattering by the medium and particles:

$$a \approx \frac{1}{\log_{10} e} \frac{D'_c - D'_{750}}{\beta_c l}, \quad (6)$$

where  $D'_c$  is the baseline-corrected optical density of the sample measured in a cuvette,  $D'_{750}$  is the null wavelength correction attributed to scattering losses by the particles in suspension, and  $\beta_c$  is the path-length amplification in the cuvette due to scattering by the particles and the medium.

The absorption coefficient of natural samples has rarely been determined from suspension measurements in cuvettes because the concentration of the material is not sufficient, because the scattering by the natural particles is larger than that by phytoplankton cultures, or both. The QFT results in concentrated particulate material on the filter, increasing the effective geometric path length of the particles in the absence of the medium. Glass-fiber filter pads are inherently strong scatterers. Visual inspection of the filter pads in the path of a collimated beam indicates that the beam is highly diffused, implying a significantly enhanced optical path length. The absorption coefficient of the particulate material on the filter pad is determined from

$$a = \frac{1}{\log_{10} e} \frac{D'_f - D'_{750}}{\beta_f l}, \quad (7)$$

where  $D'_f$  is the measured optical density of the particles on the filter,  $D'_{750}$  is the null wavelength correction associated with scattering losses on the filter pad (cf. Iturriaga et al. 1991),  $\beta_f$  is the correction factor for the path-length amplification by the filter, and  $l$  is the geometric path length equal to the ratio of the sample volume to the filter's effective area.

Path-length amplification by the filter pad,  $\beta_f$ , has not been measured directly, nor have there been theoretical predictions. The value is estimated from cuvette and filter-pad measurements of optical density of phytoplankton cultures. The absorption derived from the cuvette measurement is assumed to be free of path-length amplification (i.e.,  $\beta_c = 1$ , although in most cases the authors report subtraction of the red offset, which implies scattering and therefore path-length amplification). Equating Eq. 5 and Eq. 7 yields

$$\beta_f = \frac{D'_f - D'_{750} l_c}{D'_c - D'_{750} l_f}. \quad (8)$$

Calculated this way,  $\beta_f$  exhibits a decreasing exponential dependence on the magnitude of the optical density measured on the filter pad (i.e., the loading of the particulate material; see Fig. 1a). At low filter loadings,  $\beta_f$  is highly variable and can exceed 10, while at high filter loadings the variability decreases and  $\beta_f$  converges to approximately 2 (Mitchell and Kiefer 1988; Bricaud and Stramski 1990; Roesler 1992; Hoepffner and Sathyendranath 1993; Arbones et al. 1996). Rarely have other asymptotic values been observed, although notable values of 1 (J. Cullen pers. comm.) and 4 (Moore et al. 1995, for *Prochlorococcus*) have been reported.

Not only do the value and variability of  $\beta_f$  increase with

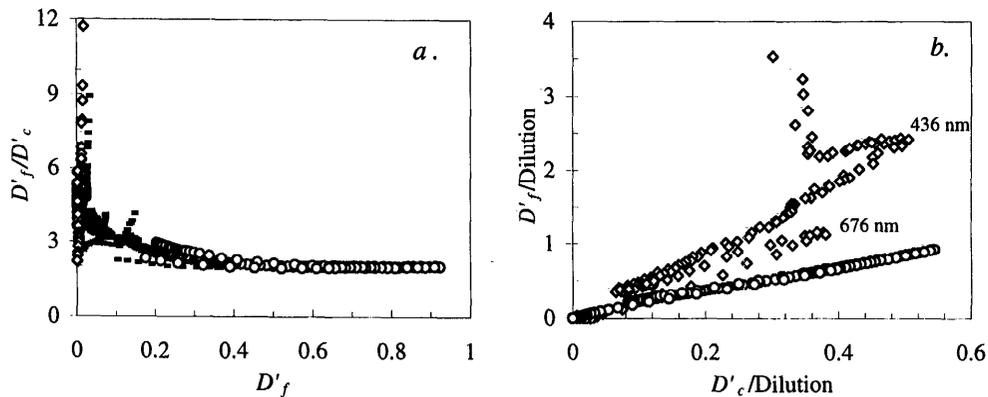


Fig. 1. (a) The ratio of optical density measured on a filter pad to that measured in a 1-cm cuvette ( $\beta_f$ ) as a function of the filter pad's optical density. The points are for a set of six samples of mixed species phytoplankton cultures with a >200-fold range in dilution (chlorophyll concentrations ranging from approximately 42.5 to 8,500  $\mu\text{g liter}^{-1}$ ). The geometric path length was 1 cm for both cuvette and filter-pad measurements (i.e., the volume filtered was equivalent to a 1-cm height of sample over the effective filter area). Circle and diamond symbols represent most and least concentrated samples, respectively. (b) The optical density measured on a filter pad versus that measured in a cuvette for the most concentrated (circles) and least concentrated (diamonds) samples in (a). The optical density has been scaled to the dilution factor for each sample. The red (676 nm) and blue (436 nm) absorption peaks are identified for the least concentrated sample.

decreasing  $D'_f$ , but multiple values of  $\beta_f$  are observed for a single value of  $D'_f$  within a single spectrum (Bricaud and Stramski 1990; Roesler 1992; Arbones et al. 1996). This hysteresis is most clearly observed by expressing  $D'_f$  as a function of  $D'_c$  (corrected for null wavelength scattering) for two different filter loadings (Fig. 1b). At high filter loadings, the relationship between  $D'_f$  and  $D'_c$  is linear with a slope of 2. At low filter loadings, there is a nonlinear relationship because of enhanced values of  $D'_f$  that increase toward the blue end of the spectrum.

## Model Development

Developing a correction factor for path-length amplification based on filtered and cuvetted samples requires that corrections be determined for scattering losses and path-length amplification in the cuvette. The scattering corrections and path-length amplification will vary from sample to sample depending upon the particles' concentration, size distribution, and scattering efficiency. In view of these complicating factors, it is preferable to derive a correction factor independently of suspension optical density measurements.

The optical regime of the filter-pad measurement is quite different from that of the cuvette. Particles on the filter may do little to change the optical path length of the filter pad alone. Although the nominal pore size of glass-fiber filters is 0.7  $\mu\text{m}$ , the spacing between the fibers is much larger, and the pore size is determined by the tortuous path of the particles through the filter. Stramski (1990) observed that even large phytoplankton were not completely retained by glass-fiber filters and suggested that samples be passed through more than once to increase retention. The result is that even large particles are imbedded within the filter matrix. Scattering by these particles will be insignificant rela-

tive to scattering by the fibers because the diffused light field cannot become more diffused by additional scattering.

Diffused light can, however, become less diffused because of absorption. The angular distribution of the light field in a suspension can be significantly decreased by preferential absorption of photons traveling at large angles. This is not likely to be an important process on the filter pad, because the thickness of the pad (<1 mm) determines both the thickness of the absorbing layer and the difference in geometric path lengths of photons traveling directly through the filter compared with those traveling at larger angles. In a 1-cm cuvette, the difference in geometric path length, and hence the probability of absorption of diffusely traveling photons is not negligible.

The differences in the cuvette and filter pad optical regimes may actually simplify the path-length amplification problem for the filter pad. The amplification in the cuvette must be uniquely determined for each sample because scattering by the suspended particles in the sample and the medium itself may cause the amplification. Conversely, for filtered samples, scattering by the filter pad, not the particles, dominates the path-length amplification. (The media is removed by the filtration process.) Thus, if the light field is approximately uniform within different filters because of the strong scattering coefficients, then a single path-length amplification for filter pads could be parameterized and that amplification would apply to all samples, independent of the specific particles.

The QFT simplifies the optical environment compared to cuvettes by (1) removing the effects of an absorbing and scattering medium (i.e., water), (2) significantly reducing the geometric path length through the sample, and (3) creating a diffuse light field within the filter pad.

The filter pad is a good analog of a plane, parallel, ho-

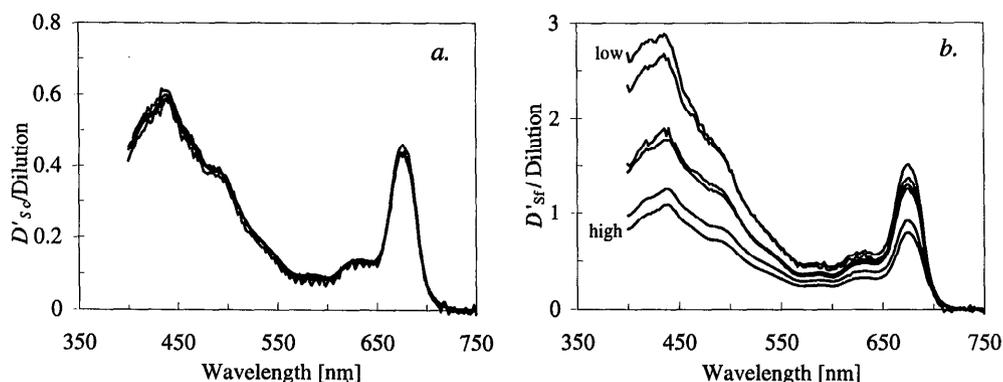


Fig. 2. The optical density spectra of the six samples in Fig. 1 measured in a 1-cm cuvette (a) and on a glass-fiber filter pad (b). The geometric path length was 1 cm for both cuvette and filter-pad measurements; all spectra have been scaled to the sample dilution. The most concentrated sample (high filter pad loading) in (b) has the lowest scaled optical density; the least concentrated sample (low filter pad loading) has the highest scaled optical density. Measurements were made with an SLM-Aminco DW-2C dual-beam spectrophotometer configured with a quartz diffusing plate so that large-angle forward-scattered light was collected by the end-on PMT (Amesz et al. 1961). A beam scrambler was placed between the diffusing plate and the detector to reduce errors associated with the nonuniformity of the PMT surface by further diffusing the photon field. Filtered media was used as reference material in the cuvette, and a blank wetted filter was used as the reference for the filter-pad measurements (data from Roesler 1992).

mogeneous medium of infinite extent relative to the incident collimated beam. The path-length amplification factor of a photon traveling through the filter is the ratio of the actual path length,  $r$ , to the geometric path length or thickness of the filter,  $\tau$ . Although it is not possible to measure the exact path length of each photon, it is possible to express the actual path length as a function of the cosine of the angle of the transmitted photon,  $\cos \theta$ :

$$r = \frac{\tau}{\cos \theta}. \quad (9)$$

If multiple scattering occurs and there is redirection of the photon, the fractional path length is expressed as a function of the fractional thickness scaled to the cosine of the angle (i.e., equivalent to breaking up the path into segments). For the suite of photons composing the light field in the filter pad, Eq. 9 can be simplified to express the average amplification by the average cosine of the light field. For a diffuse light field,

$$\frac{r}{\cos \theta} = \frac{\int_{2\pi} \cos \theta \, d\omega}{\int_{2\pi} d\omega} = 0.5, \quad (10)$$

where  $d\omega = \sin \theta \, d\theta \, d\phi$ , the incremental solid angle. Thus, the average optical path length in the filter is estimated to be twice the geometric path length, which translates into a value of 2 for path-length amplification, a result born out by observations under high filter-pad loading.

If this were an appropriate model for path-length amplification,  $\beta_f$  would be a constant for all filter pads, not a function of filter-pad loading or a function of wavelength. That there are apparent variations in  $\beta_f$  under low filter-pad

loading suggests that other processes may influence the optical regime and hence the empirical estimation of  $\beta_f$ .

The cuvette optical density spectra used to derive the empirical values of path-length amplification, as shown in Fig. 1, appeared to be relatively free of scattering losses and path-length amplification. Although the optical thickness of the suspensions ranged from 0.002 to 0.5 in optical density units, (OD), the scattering losses as estimated by the offset in the red wavelengths were less than 2% of the value at 436 nm. Scaling the optical density to the concentration of the suspension yielded no change in the magnitude or spectral shape of the optical density spectra, which would have suggested multiple scattering and path-length amplification (Fig. 2a). Such observations are not typical for most particle suspensions. Verification of significant scattering losses and path-length amplification would have further confounded the values of the derived  $\beta_f$ .

Associated filter-pad measurements indicated that the blue end of the spectrum was enhanced compared with the red end (Fig. 2b) and that this enhancement decreased as the loading on the filter increased. All of these measurements incorporated a baseline subtraction with a blank filter pad. If, however, there were a small difference in optical density between the blank baseline and the sample filter pads, that small difference could have significantly affected the sample measurement under reduced particle loads, becoming less significant as the contribution by the particles increased—a classic signal-to-noise problem.

Therefore, the path-length amplification correction factor that is derived empirically contains biases due to processes other than path-length amplification. My supposition is that path-length amplification within the filter pad is relatively constant and equal to 2. Apparent variations from this value arise when  $\beta_f$  is empirically derived from filter-pad and cu-

vette measurements that contain other signatures associated with processes such as (1) path-length amplification and scattering losses in the suspension measurements, (2) variability in the optical density between the baseline and sample filter pads, or (3) change in the optical density of the blank reference filter pad during measurements.

My goal was to test whether the theoretical approach to path-length amplification yields more accurate absorption coefficients than one based upon empirical derivations that include other sources of variability. Path-length amplification was distinguished from other processes associated with the filter-pad methodology. Refinements of the QFT methodology were used to identify and minimize the source of optical variability in glass-fiber filter pads, and the magnitude of remaining variability was quantified. The remaining variability determined the uncertainty in the estimated absorption coefficients and was included in the model for obtaining absorption coefficients from QFT:

$$a(\lambda) \pm \sigma_a(\lambda) = 2.303 \frac{D'_f(\lambda) - D'_{750} \pm \sigma_{\text{GF/F}}(\lambda)}{2l}, \quad (11)$$

where  $(\lambda)$  indicates spectral dependence, 2.303 is the coefficient representing the transformation from  $\log_{10}$  to  $\log_e$ ,  $D'_{750}$  is the null wavelength correction accounting for differences in the magnitude of optical density between sample and baseline filters, 2 is the path-length amplification factor for filter pads, and  $l$  is the ratio of the volume filtered to the effective area of the filter pad. The uncertainty in the absorption coefficient is given by  $\sigma_a$ ; the calculated spectral standard deviation in the absorption coefficient is determined from the standard deviation associated with spectral variations in optical density between filter pads,  $\sigma_{\text{GF/F}}$ . Note that the value of the uncertainty varies inversely to the geometric path length of the sample (so it is preferable to filter larger volumes of sample). More important is that the relative magnitude of the uncertainty in absorption depends on the relative magnitudes of  $D'_f$  and  $\sigma_{\text{GF/F}}$ . That is, high particle loadings lead to larger values of  $D'_f$  and smaller uncertainties.

## Methods

Optical density (D) spectra for samples collected on Whatman GF/F glass-fiber filters were measured with an empty reference compartment or one containing a neutral-density filter ( $D = 1.0$ , Esco) for both the baseline and sample scans. The basis for choosing a neutral-density filter is twofold. First, the intensity of the sample and reference beams are more closely matched; this increases the resolution of the measurements and reduces errors associated with the detection of signals with very different magnitudes by a single photomultiplier tube (PMT) over millisecond time scales. Second, the optical properties of a neutral-density filter remain constant over time, in contrast to a wetted filter pad. One drawback to a glass neutral-density filter is the strong absorption by the glass in the ultraviolet region of the spectrum. A quartz neutral-density filter or an empty reference compartment enables the wavelength range to be extended into the ultraviolet.

The optical density of blank filter pads and pads with par-

ticulate samples used for model testing were measured with a Cary 3E dual-beam spectrophotometer over the wavelength range of 200–900 nm with a 2-nm half-maximum bandpass. This instrument exhibits no instrumental drift over periods of 6–10 h and has a resolution of 0.0001 optical density units.

*Optical density of blank filter pads*—The optical density spectra of blank Whatman GF/F glass-fiber filters (corrected with a baseline scan using empty sample and reference compartments),  $D'_{\text{GF/F}}$ , were measured to quantify the variability between filters and variability associated with filter orientation, moisture content, and filtered volume. Interfilter variability was examined for filters from the same lot (five filters) and from different lots (five filters from each of four lots). Filter orientation was assessed for individual filters rotated 90°, 180°, 270°, and 360° from the position of the initial scan, because variations over the filter have been suggested to be a source of variability (Stramski 1990).

The effect of filter-pad water content on  $D'_{\text{GF/F}}$  was examined for individual filters with an empty reference compartment and a correction for baseline with empty sample and reference compartments. The wavelength was held constant at 400 nm and  $D'_{\text{GF/F}}$  was monitored for 15 min at 1-s intervals while the filter dried. Subsequent readings were taken after incremental additions of 10  $\mu\text{l}$  MilliQ to the filter pad. The influence of filtration volume on  $D'_{\text{GF/F}}$  was assessed by varying the volume of MilliQ water filtered through a blank baseline and blank “sample” filter pads from 10 to 850 ml.

*Model testing*—Field samples were collected from the continental shelf south of Nantucket during the Office of Naval Research (ONR)-sponsored Coastal Mixing and Optics Experiment in August 1996. These samples and seven monospecific phytoplankton cultures representing diverse taxa and cell sizes (1–20  $\mu\text{m}$  diameters) were used to test the path-length amplification model. The absorption coefficients estimated from  $D'_f$  using Eq. 11 were tested against absorption coefficients measured with a WETLabs ac9. The discrete field samples and phytoplankton cultures were filtered onto Whatman GF/F glass-fiber filters and immediately run in the Cary 3E spectrophotometer using a neutral-density filter in the reference compartment for baseline and sample scans. A blank filter pad for the baseline scan was prepared by filtering through it a volume of reference water equivalent to the volume filtered for samples. Five additional blank filter pads from the same box were similarly prepared and analyzed to evaluate the variability in optical density for that particular lot of filters. All filters were kept moist in a covered petri dish on a bed of wet Kim Wipes. The baseline scan was collected with one of the blank filter pads with a neutral-density filter in the reference compartment to maintain approximately the same intensity of sample and reference beams incident on the PMT. Immediately after the baseline scan, another scan was collected on the same filter without moving it. After it was baseline-corrected, this “zero” scan was examined to ensure that the shape of the spectrum was flat with a value of zero and only small fluctuations of order 0.0001 (the level of instrumental noise). The remaining blank filter pads were scanned and baseline-

corrected. The sample standard deviation of these five blank scans was used to calculate  $\sigma_{\text{GFF}}$  and the uncertainty of the spectral absorption coefficients (Eq. 11).

The discrete phytoplankton culture samples were fed through the dual path of the ac9 via gravity flow. A blank of the GF/F-filtrate media was subtracted from the optical coefficients to quantify the contribution due solely to particulates. The absorption and attenuation coefficients for the particulate fraction of the field samples were measured in situ, during water sample collection, using two ac9s. A 0.2- $\mu\text{m}$  filter was placed on the intake port of one of the instruments. Subtraction of the filtered instrument signal from the unfiltered instrument signal (after temperature and scattering corrections were applied; *see below*) yielded the spectral absorption and scattering coefficients of the particulate fraction in situ. Spectrophotometric measurements of the filtrate for 0.2- $\mu\text{m}$  and GF/F filters yielded no measurable differences in the visible wavelength range for this particular data set.

The temperature dependence of absorption and attenuation at each of the nine wavelengths was quantified over 5–30°C in both instruments, using filtered MilliQ water in a temperature-controlled circulator bath. Temperature dependence in absorption and attenuation was observed only in the 715-nm channel; the dependence was linear over the temperature range. Absorption and attenuation at 715 nm were corrected for variations between the in situ or discrete water samples and the pure water calibration temperature (Pegau and Zaneveld 1993; Pegau et al. 1997).

After the temperature correction was applied, a nonzero absorption signal was observed at 715 nm. Particulate and dissolved materials exhibited negligible absorption in this region; thus, the signal was attributed to scattering losses out of the reflecting tube (Kirk 1992). Scattering coefficients are generally not spectrally flat, and subtraction of the signal at 715 nm from the entire measured absorption spectrum will not accurately correct the absorption coefficient for scattering losses (the approach used with cuvettes in conventional spectrophotometers). A spectral correction was calculated as a fraction of the spectral scattering coefficient. A first-order scattering coefficient was calculated from the difference between the measured attenuation and absorption spectra:

$$b'(\lambda) = c(\lambda) - a'(\lambda). \quad (12)$$

Note that the absolute magnitude of the scattering spectrum was slightly underestimated because the absorption spectrum was overestimated by the fraction of included scattering. In most natural waters, the single-scattering albedo (ratio of scattering to attenuation) exceeds 0.8 and the magnitude of the scattering error in the absorption measurement is less than 20% of the absorption coefficient; therefore, the scattering coefficient in Eq. 12 is underestimated by approximately 5%. This underestimation minimally affected the estimation of the spectral shape of the scattering coefficient. The error in the absorption meter due to scattering out of the acceptance region of the detector was removed from the entire spectrum, accounting for the spectral variations in the scattering coefficient:

$$a(\lambda) = a'(\lambda) - a'(715) \frac{b'(\lambda)}{b'(715)}, \quad (13)$$

where  $a'(715)$  is the estimate of scattering loss and the ratio is the spectral scattering estimate scaled to its 715-nm value.

The presence of scattering by suspended particles and the medium in the absorption tube indicates that there may be path-length amplification. A primary concern is to minimize the probability of multiple scattering by maintaining an optically dilute scattering environment ( $D < 0.1$ , van de Hulst 1981). Under single-scattering optical regimes, the maximal amplification of the path can be determined from the geometry of the collimated beam, detector, and the reflecting tube. Monte Carlo simulations of reflecting tube absorption meters indicate that path elongation due to scattering is “fortuitously” balanced by path shortening from photons trapped in the cuvette wall, due to multiple reflection (Kirk 1992). Again, these results are applicable to reflecting tube absorption meters with configurations akin to WETLabs instrumentation only; they may not be applicable to 10-cm cuvette measurements and certainly not to 1-cm cuvette measurements in conventional spectrophotometers.

## Results

*The optical properties of blank filter pads*—The  $D'_{\text{GFF}}$  was approximately 4.5 at 200 nm, with a steep decrease to a value of 2.5 at 400 nm, followed by a more gradual decrease to approximately 2.3 at 700 nm (Fig. 3a). The strong ultraviolet signal was likely due to the absorption coefficient of the glass fibers, whereas scattering by the filters dominated the signal in the visible range.

The variation in  $D'_{\text{GFF}}$  was defined as the difference in optical density between two scans,  $\Delta D'_{\text{GFF}}$ , or as the standard deviation of optical density among three or more scans,  $\sigma_{\text{GFF}}$ . The moisture content of the blank pad influenced the optical density. When left to dry in the sample holder, a saturated blank filter pad maintained a constant  $D'_{\text{GFF}}$  over approximately 4 min, after which  $D'_{\text{GFF}}$  increased by 0.0014 in the next 10 min. Resaturation via incremental water additions resulted in a subsequent decrease in  $D'_{\text{GFF}}$ , which ultimately returned to the initial value.

A baseline spectrum, collected before sample scans, was subtracted from sample scans. The preparation of the baseline filter pad, in particular the unequal filter volumes for baseline and sample filters, was found to significantly influence  $\Delta D'_{\text{GFF}}$  (Fig. 3b). When the filter volumes were equivalent, the  $\Delta D'_{\text{GFF}}$  was less than 0.001. However, as the sample volume increased relative to the baseline volume, the value of  $\Delta D'_{\text{GFF}}$ , although quite variable, grew as large as 0.007. No negative values of  $\Delta D'_{\text{GFF}}$  were observed, suggesting that the filter fibers were modified during filtration and that this modification always increased the filter pad's optical density.

Filter orientation resulted in  $\Delta D'_{\text{GFF}}$  values from 0.001 to 0.011, depending upon the uniformity of the filter; typical values were on the order of 0.005. The  $\sigma_{\text{GFF}}$  was approximately 0.006 within a filter lot and 0.008 among the four lots. For comparison, the instrument noise and drift for the instrument was approximately 0.0001 over periods of >10 h. All the variations in optical density were observed to be largest in the blue region of the spectrum, decreasing in the red as did the absolute magnitude (Fig. 3a).

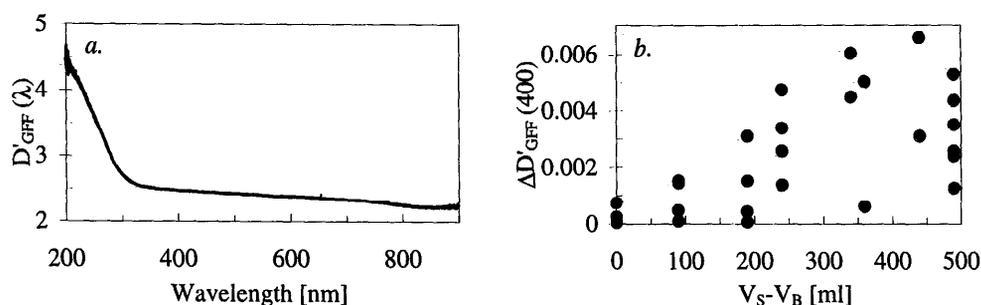


Fig. 3. (a) The average value of the baseline-corrected optical density spectra,  $D'_{\text{GFF}}$ , for 20 blank filter pads from four different lots. Sample and reference compartments were empty during the baseline scan. The reference compartment was empty during the sample scans. (b) The  $\Delta D'_{\text{GFF}}$  at 400 nm as a function of the difference in the volume of MilliQ water filtered for the baseline ( $V_B$ ) and sample ( $V_S$ ) filter pads. The volumes ranged from 10 to 850 ml.

The variability in blank filter pads directly affects the measurements of sample optical density. This was demonstrated by the change in spectral shape of the phytoplankton optical density with particle loading on the filter pad (Fig. 2a). At low particle loads, the deviation in the optical density between the sample filter pad and the baseline filter pad made a significant contribution to the sample optical density. Because  $\Delta D'_{\text{GFF}}$  appeared to be amplified in the blue relative to the red, the blue-to-red peak ratio of the sample optical density became artificially enhanced (a value of 2.11). The filter pad's contribution to total optical density decreased as the concentration of cells on the pad increased, and the blue-to-red peak ratio decreased to the value observed in suspension (1.37).

The  $D'_f$  was approximately 0.012 for the least concentrated sample at 440 nm. The average value of  $\Delta D'_{\text{GFF}} = 0.005$  represented almost 50% of the measured sample optical density, essentially doubling the estimated absorption. Under higher particle loads, the influence of blank-pad variations were negligible except in spectral regions for which optical density was low (i.e., the green region, where minimal pigment absorption occurs). Under moderate particle loading but in a region of minimal absorption (600 nm), the  $D'_f = 0.05$ . The  $\Delta D'_{\text{GFF}} = 0.005$  resulted in an overestimation of approximately 10%, causing the appearance of higher absorption in regions of minimal pigment absorption. These errors were not due to path-length amplification but merely to an inaccurate blank-filter measurement.

The inconsistent features observed previously in  $\beta_f$  can be explained by these results. (1) The trend for  $\beta_f$  to increase as  $D'_f$  decreases has two likely sources. First, the contribution of  $\sigma_{\text{GFF}}$  to  $D'_f$  becomes larger as the loading on the filter pad decreases. Secondly, the volume of water filtered through the baseline reference filter pad rarely approaches the volume of water filtered through the sample filter pad, which leads to a  $\Delta D'_{\text{GFF}}$  value of  $>0$  (Fig. 3b). (2) The fact that  $\sigma_{\text{GFF}}$  can be positive or negative causes the observed variability in  $\beta_f$  that had been observed at small  $D'_f$  values (i.e.,  $\beta_f$  ranging from 1.6 to 5, Bricaud and Stramski 1990; and 1.5 to 12, Roesler 1992). (3) Since  $\sigma_{\text{GFF}}$  tends to be larger in the blue than in the red region of the spectrum, there is an apparent spectral enhancement in the blue at low particle loading; this is the previously observed "hysteresis" seen in the relationship be-

tween the optical density of a sample measured on the filter to that in suspension (Fig. 1). (4) Finally, path-length amplification often occurs in the cuvette measurements of optical density used to derive  $\beta_f$ , which causes either variability in the value at low  $D'_f$  or causes the asymptotic value to be less than the predicted value of 2. The observed asymptotic value of 1 indicates comparable path-length amplification in the suspension and on the filter pad, hence a highly scattering suspension. The combination of all of these factors has likely contributed to the inconsistencies between reported values of  $\beta_f$  and its dependence on optical density. The empirical models have incorporated both  $\beta_c$  and  $\sigma_{\text{GFF}}$  into  $\beta_f$ .

*Testing the model on independent data*—The difficulty in testing the path-length amplification model is acquiring data for which the absorption coefficient can be measured accurately, independent of the filter pad. Although the ac9 is not free of scattering errors and path-length amplification, its geometrical configuration, sensitivity, and direct measurement of scattering yield absorption measurements with minimal errors (Kirk 1992; Roesler and Zaneveld 1994; Zaneveld et al. 1994; Bricaud et al. 1995).

The absorption coefficients of six monospecific phytoplankton cultures, one mixed culture, and four field samples were measured with the ac9 and modeled from  $D'_f$  using Eq. 11. All of the methodological precautions explored in the previous section were taken to minimize filter pad variability. The absorption coefficients for each sample were well predicted by the model (Fig. 4) over the range in chlorophyll concentrations ( $0.05$ – $66 \mu\text{g liter}^{-1}$ ) and particle absorption coefficients ( $0.005$ – $1.6 \text{ m}^{-1}$ ) ( $r^2 > 0.99$ ; Table 1). The slope and intercept between modeled and measured absorption coefficients are 1.005 and 0.023, not statistically different from 1.0 and 0.0, respectively ( $P < 0.001$ ). The error bars (shown only at the ac9 wavelengths for clarity) represent the uncertainty associated with the estimated absorption due to variations in the optical density of blank filter pads.

For comparison, the absorption coefficients for the 11 samples were also estimated from  $D'_f$  using three published models of  $\beta_f$ . The linear approximation for  $\beta_f$  derived by Mitchell and Kiefer (1984; their Eq. 6), modified for a single GF/F filter (Mitchell and Kiefer 1988), is parameterized as

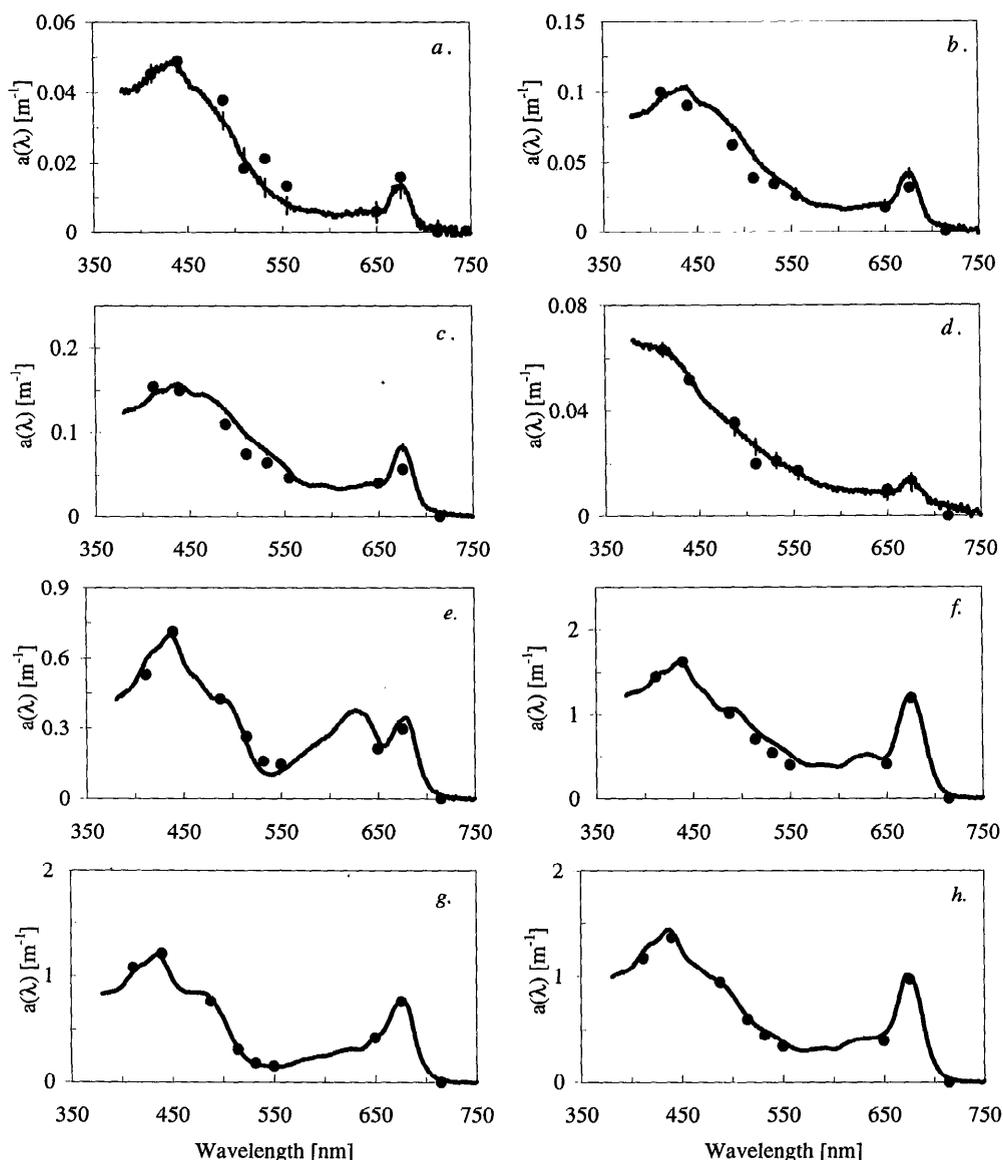


Fig. 4. Particulate absorption coefficients measured with an ac9 (●) and estimated from  $D'_f$  for discrete samples using Eq. 11 (bold line; error bars are shown at ac9 wavelengths). (a-d) Field samples and in situ measurements collected from the continental shelf south of Nantucket in August 1996 at approximately 10, 20, 30, and 62 m, respectively. (e-h) Cultured phytoplankton samples, *Synechococcus* spp., *Thalassiosira weissflogii*, *Dunaliella salina*, and a mixed culture of the previous three species plus *Chaetoceros gracilis*, *Nitzschia closterium*, and *Isochrysis galbana*.

Table 1. Results of linear regression between modeled and measured absorption coefficients (Fig. 5).

Model	Slope	Intercept	$r^2$
This study	1.005*	0.023**	0.99
Linear $\beta$	1.138	-0.048**	0.97
Power $\beta$	1.100	-0.024**	0.99
Quadratic $\beta$	1.566	-0.070**	0.98

\* Slope not significantly different from 1 ( $P < 0.001$ ).

\*\* Intercept not significantly different from 0 ( $P < 0.001$ ).

$$\beta_f = [1.3(2.26 - 1.5D'_f)]. \quad (14a)$$

A power function was fit by Bricaud and Stramski (1990) to the same data set (Mitchell and Kiefer 1984, their figure 3) to extend the range of the correction factor to lower optical densities than the linear model could accurately predict:

$$\beta_f = 1.63 \times D'_f{}^{-0.22}. \quad (14b)$$

The absorption coefficients were then calculated using Eq. 7. More recently, a correction factor was derived as the best-fit quadratic equation to the relationship between  $D'_c$  and  $D'_f$  (Mitchell 1990), yielding a corrected sample optical density

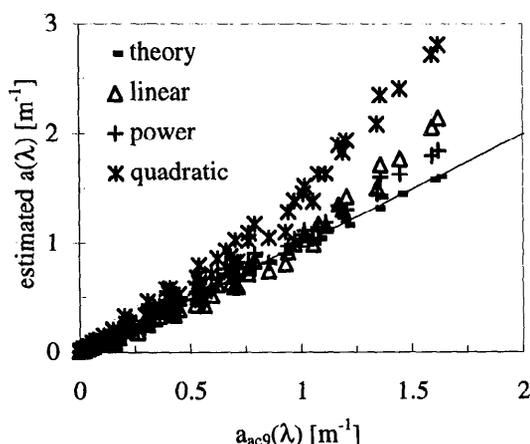


Fig. 5. Relationship between the absorption measured with the ac9 and that estimated from filter-pad optical densities using three previously published models for  $\beta$ : quadratic (asterisks), linear ( $\Delta$ ), power (+), and the theoretical model developed in this paper (dashes). The line represents the one-to-one relationship. Samples include the four field samples and seven phytoplankton cultures measured at nine wavelengths ( $n = 99$ ). The statistical results are presented in Table 1.

$$\text{corr } D'_f = 0.392D'_f + 0.655D'^2_f, \quad (14c)$$

which is then used in Eq. 7 without  $\beta_f$ . Since the publication of this last model, a number of authors have examined it under wide conditions (Cleveland and Weidemann 1993; Moore et al. 1995; Arbones et al. 1996). The coefficients on the first and second terms vary from 0.29 to 0.48 and 0.05 to 0.75, respectively, depending upon the nature of the particles, the optical thickness of the samples, and the loading on the filter pads. For this test, Mitchell's (1990) values were used because they are not sample-specific and they lie well within the range of the other values.

The empirical  $\beta_f$  models tend to underestimate the absorption coefficients for the field samples (characterized by small  $D'_f$  and small absorption coefficients) and overestimate the absorption coefficients for the cultures (large  $D'_f$  and large absorption coefficients). This is to be expected, as the empirical  $\beta_f$  models include contributions by filter-pad variability at low  $D'_f$  values and may contain  $\beta_c$  errors at high optical densities,  $D'_c$ . The pooled data (11 samples at 9 wavelengths) are shown in Fig. 5. Linear slopes and intercepts were calculated by least-squares linear regression between modeled and measured absorption coefficients (Table 1). The slopes determined for the empirical  $\beta_f$  models ranged from 1.1 to 1.6; all were significantly different from 1.0 ( $P < 0.1$ ). In all cases, the intercepts on the regressions were not significantly different from zero ( $P < 0.001$ ). Both the linear and quadratic  $\beta_f$  models exhibited nonlinearity at high absorption values.

## Discussion

The QFT has been used for more than 30 years to estimate the absorption coefficients of particulate material in the ocean. Concerns over the correction factor for filter pads have led to a reliance on absorption determined from optical

density measured on suspensions in cuvettes. Results from this study, however, suggest that once a correction factor for the filter pad is established, the QFT yields more accurate estimates of absorption coefficients than the cuvette method for particles with a range of absorption to scattering ratios. Although the QFT is generally reserved for estimating particulate absorption coefficients for field samples, where the concentration of particulate material is necessary, I suggest that this method become the standard for analyzing cultures in the laboratory as well, particularly for concentrated suspensions or those containing particles with a high single-scattering albedo (i.e., tripton, sediment, and some phytoplankton).

There are two sources of error in the filter-pad method. The first is methodological and can be minimized. The optical density of blank filter pads can be quite variable, depending upon filter-to-filter uniformity, filter moisture content, and blank filter preparation (i.e., filtered volume). A blank filter must be used in the sample compartment for a baseline scan. Variations in the optical density between the baseline filter pad and the sample filter pad can lead to a significant signal-to-noise problem at low optical densities, where the signal due to the variations between the pads approaches the signal due to the particles on the pad. Using a second blank filter in the reference compartment for the baseline and sample scans confounds the problem even more.

The second source of error for the filter-pad method is scattering-induced path-length amplification caused by the filter-pad fibers. The measured optical density is more related to the diffuse attenuation coefficient than to the absorption coefficient (Yentsch and Phinney 1992). The model presented here is based on the observation that the filter pad is highly scattering and the assumption that it creates a diffuse light environment. The average optical path length of photons through the filter is estimated from the average cosine of a diffuse light field, which results in a path-length amplification of 2.

A number of predictions concerning the path-length amplification correction factor fall out of this result. (1) Because the amplification is due to intense scattering by the filter, adding particles results in a negligible contribution to path-length amplification. (2) The amplification should be constant for a filter regardless of the concentration of absorbing particles on the filter, and thus should be independent of  $D'_f$ . (3) Once a correction factor is defined accurately for filter pads of a given type (i.e., GF/F versus GF/C), the factor will apply to all samples regardless of the composition of the particles on the filter.

These two features of the filter-pad correction explain the apparent disagreement among published correction factors that were empirically derived. The asymptotic value of an empirically derived  $\beta_f$  can be  $< 2$  if the optical path length in the cuvette was amplified by particle scattering. The asymptotic value of an empirically derived  $\beta_f$  may exceed 2 if there is self-shading by strongly absorbing particles in the suspension and  $D'_c$  is artificially too low. Values of an empirical  $\beta_f$  are observed to increase from the asymptotic value as the  $D'_f$  decreases because of low particle loading on the filter pad or low absorption coefficients in a particular spec-

tral band. Under these conditions, the optical density of the particles on the filter becomes comparable to the variable filter-pad optical density,  $\sigma_{\text{GFF}}$  or  $\Delta D'_{\text{GFF}}$ . The variable filter-pad optical density is greatest in the blue region of the spectrum and smaller in the red region, leading to the observed hysteresis effect. Additionally, low particle loading on the filter is typical for oligotrophic water samples because of the paucity of material in the water. Two to five liters of water is necessary to concentrate enough material to measure the optical density. Blank filter pads for the baseline correction are not typically prepared with 2–5 liters of reference water filtered through them, adding to the magnitude of  $\Delta D'_{\text{GFF}}$ , the hysteresis, and ultimately to spectrally biased absorption coefficients.

The correction factor for path-length amplification derived here yielded absorption coefficients highly comparable to those measured with the ac9 on discrete samples and collected in situ, where the samples ranged from pure phytoplankton cultures to field samples dominated by phytoplankton to those dominated by tripton. Other empirically derived  $\beta_f$  models did not perform as well, underestimating the absorption at low optical density and overestimating the absorption coefficient at high optical density, exhibiting a larger scatter about the linear regression, or exhibiting nonlinear behavior. These empirical models may be incorporating the errors associated with filter-pad variability and/or path-length elongation in the cuvette measurement. Errors due to filter-pad variability have been minimized in the  $D'_f$  spectra in the model testing. Thus, I expect that the published models would overestimate absorption at high  $D'_f$  values and underestimate absorption at low  $D'_f$  values, as was observed. The power model for  $\beta_f$  (Bricaud and Stramski 1990) performed well with only 10% overestimation and low scatter about the linear regression. The quadratic expression appears to overestimate the absorption peaks and performs poorly at high sample loading (high  $D'_f$  values). While Mitchell (1990) recommended that his model not be used under these conditions, high sample loading is a preferable situation for measuring  $D'_f$  because the uncertainty in absorption due to the filter pad variability ( $\sigma_a$ , which is only estimated,) can be quite large at small optical densities.

The QFT for estimating particulate absorption spectra of discrete samples is still recommended even if in situ instrumentation is being used. The total particulate material collected on the filter can be partitioned into phytoplankton and nonphytoplankton (tripton) components via extraction with methanol (Kishino et al. 1985) and hot water (Roesler and Perry 1995) to remove the soluble pigments from phytoplankton cells. The uncertainty term will be unique for each sample, as the volume filtered varies (affecting  $l$ ). It will also represent a different proportion of the particulate and tripton absorption spectra, since  $D'_f$  will be different for the two treatments. The uncertainty of each will determine the uncertainty in the calculated phytoplankton absorption coefficient.

The QFT has undergone many modifications and improvements since it was first introduced by Yentsch in the 1960s. However, since that time it has remained the best method for estimating the absorption coefficients of the particulate fraction in seawater. My results suggest that the QFT can be

more accurate than has been anticipated and that the method yields more accurate results than those obtained from suspension measurements under many conditions, provided the necessary steps are taken to minimize filter-pad error and maximize the accuracy of the correction factor.

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