Dear Reviewers,

First of all, we feel so honored that the two of you took the time to read this manuscript and provide such thorough thoughts and suggestions. We could not have picked two better or more appropriate people to review a paper on a topic such as the index of refraction, so thank you very much for all the feedback (and thank you Editors for inviting them!).

Because of your suggestions, we have considerably modified the manuscript, and we believe the paper has been much improved.

On the data side, we now 1) have a more transparent processing scheme, with direct information on how particle diameters were obtained; 2) have a less biased estimate of mean particle diameters per bin; 3) have added constraints to the power-law fit which resulted in even more conservative error bound estimates of *n* and their diel oscillations; 4) have included results of additional sensitivity analysis that helped test the impact of large particles in the overall retrievals; 5) have added two new figures (Figure 1b-c) that highlight how large particles do not contribute significantly to cp in our dataset; 6) all figures have been updated to reflect updated analysis and/or correct typos.

On the data interpretation / presentation side, we have 1) added appropriate references to the text, following suggestions; 2) addressed the implications of changes in *n* not affecting Qc and Qc\* for large particles, as well as addressed the fact that large particles do not contribute significantly to cp in our dataset; 3) de-emphasized the Justification of method assumptions section and changed the tone of Section 3.4 (and previous 3.5) to focus more on the thorough characterization of the limitations of the methodology; 4) merged the Outlook and Conclusions section to focus primarily on the need for future studies to consider retrieving *n* variability from single particle instruments; 5) changed the title of the manuscript to reflect the main result of the paper; 6) more clearly emphasized the main result of the paper (diel oscillations in n) in the Abstract, Justifications, and Conclusions sections.

All of our responses to the specific comments are denoted in blue below, and line numbers refer to the clean version of the manuscript.

Thank you very much again,

Sincerely,

Fernanda Henderikx-Freitas, on behalf of co-authors

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Review of the manuscript entitled ”In situ variability in the index of refraction of oceanic particles” by Henderikx-Freitas et al.

First of all, thank you so much Dr. Stramski for your careful and thorough review of our manuscript. As mentioned above, we could not have chosen more appropriate scientists to look at this manuscript, and we feel deeply honored that you took the time to share so many insights.

**General comments:**

1. The manuscript describes the estimation of a single value of (bulk) refractive index that is “assigned” to the entire assemblage of suspended particles in seawater from measurements of the bulk optical beam attenuation coefficient and incomplete particle size distribution using a Mie scattering theory that assumes the sphericity and homogeneity of all particles. Despite major limitations of both the approach itself and physical interpretation of such estimates of bulk refractive index for highly complex, polydisperse and heterogeneous assemblages of natural particle populations, an important aspect of this study is demonstration of diel variability in the estimated bulk refractive index which, I believe, has scientific value that can support the publication of this study after improvements of the manuscript. I also think that my suggestions for revisions of text and addition of a few calculations to the sensitivity analysis can be addressed, so I will be looking forward to improved manuscript and its publication.

Thank you – we largely share many of your concerns and understand the hesitation. We have made modifications to the manuscript to increase the transparency of the limitations and potential issues with our method thanks to your suggestions. We are excited that you see the value in the observations of the diel oscillations in *n* that we estimate, and this aspect of the paper is further emphasized now (see more below).

The authors made significant effort to provide a fairly comprehensive background of the problem at hand and a description of limitations including the results of sensitivity analysis. These are important strengths of the manuscript, especially that in reality the quantitative characterization of the actual refractive index (or rather the distribution of actual values of refractive index) for natural assemblages of marine particles is not possible with any existing approach. Also, the authors attempt to support the various statements in the manuscript with multiple references, often times with references published some decades ago which are highly relevant or pioneering in the context of the topic of this study. In recent years I have seen that this particular aspect of publications is often very weak or largely ignored, so the authors of this manuscript are to be commended for their effort to describe the background and bring to the attention of readers the relevant studies from the past. Still, I suggest some improvements in references. Finally, I found the manuscript well-written with good logical flow.

Thank you – we happily adjusted references as needed (see details below).

1. Given the actual complexity of natural assemblages of marine particles in terms of their physical, chemical and, hence, optical properties, in general I am not in favor of the application of *inverse modeling applied to ensemble/bulk measurements* (which unavoidably is based on multiple highly oversimplifying and speculative assumptions) with a purpose to predict the characteristics of natural particle assemblages, which is done in this study by combining the bulk measurements of beam attenuation, incomplete size distribution, and Mie theory. The way such inverse models are set (including this study) represents an ill-posed problem for natural assemblages of marine particles. My view is that this type of oversimplifying inverse modeling is justifiable mainly as a research tool which can aid in identifying and/or understanding some patterns that are potentially present in real marine environments and also as a guiding tool to design/develop better and more appropriate instrumentation and experimental approaches which will be needed in the future to address the characteristics of actual particle assemblages in a more rigorous fashion. My view is based largely on my research experience in optics of marine particles, which also includes studies in which particle refractive index was estimated for specific populations/types of particles (with relatively narrow size distributions), but not for highly complex polydisperse assemblages consisting of various types of particles spanning several orders of magnitude in particle size.

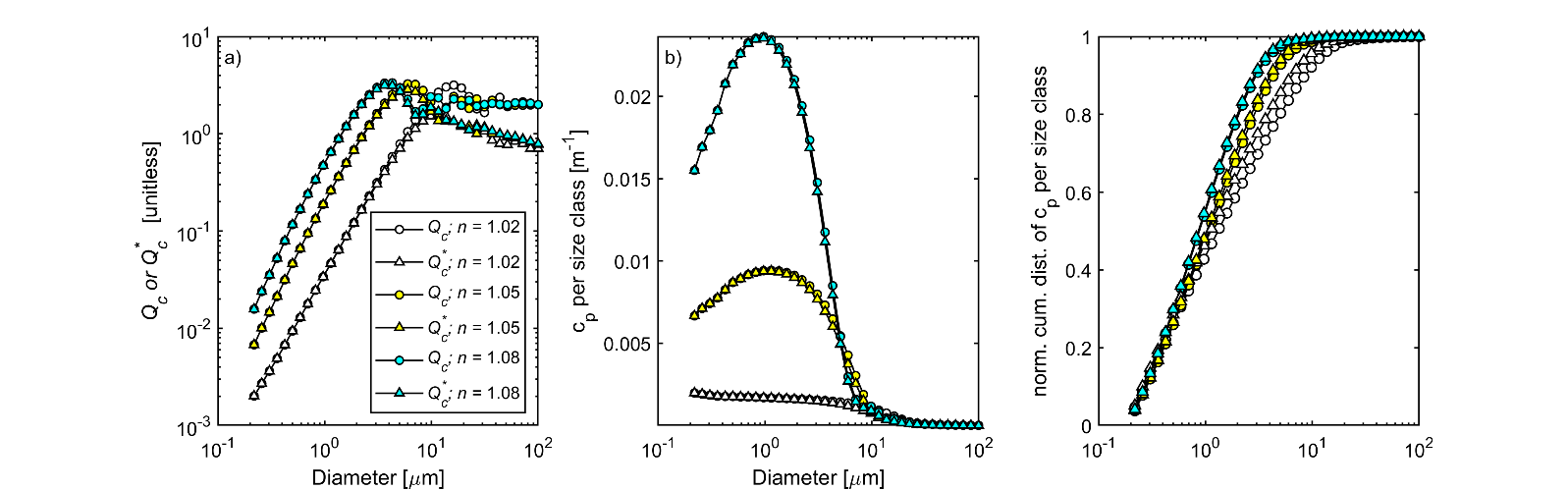
Because of the extent and implications of unavoidable assumptions in such inverse optical models, I would not undertake a study to estimate a single bulk refractive index of the entire population of marine particles based on bulk measurement of beam attenuation, incomplete size distribution and Mie theory with a purpose to characterize the actual particle assemblages, say in some concrete marine environment such as NPSG in this study. Nevertheless, I am generally supportive of the publication of this study, primarily because the presented diel variability in the estimated bulk refractive index (regardless of the weaknesses associated with limitations of the approach, physical interpretation of such bulk refractive index, and the estimated magnitude of this index) most likely reflects the presence of diel variations in actual refractive index of various phytoplankton cells in the ocean. This expectation derives from basic laboratory research with phytoplankton cultures which was made in the past and, importantly, the diel pattern with a minimum near sunrise and maximum near the end of daytime presented in this manuscript is qualitatively consistent with those previous basic studies. Thus, in my view the main value of this study is that it potentially provides evidence (despite the weaknesses of the approach) that the diel patterns observed previously in laboratory cultures of single phytoplankton species do exist in real oceanic environment where many phytoplankton species co-exist with many other types of microorganisms and non-living particles.

We largely agree, and believe that the inversion technique we describe and its application to the North Pacific Subtropical Gyre represents an initial exploration tool that could motivate future studies, such as single-particle-resolved refractive index variability. We have taken care to re-emphasize each of these assumptions we have made throughout the manuscript, and we have altered the tone to highlight the impacts of many of these assumptions in the sensitivity analysis portion of this manuscript. We now emphasize the diel oscillation in *n* aspect of the paper as well as connections with previous laboratory experiments and speculations.

1. As mentioned above, I generally like the extent of description of background and limitations of the approach used in this study. However, I think that the manuscript requires further improvements in terms of presentation of background and limitations, and these improvements seem especially important in view of the fact that the application of the proposed approach in the context of real polydisperse assemblages of marine particles could be discredited on the basis that it involves too extensive and speculative assumptions and does not meet the adequate level of scientific rigor. Apart from the problem that the presented approach is essentially unsuitable to highly polydisperse heterogenous particulate assemblages, another important limitation which is not addressed in the manuscript is the fact that the attenuation efficiency factor *Qc* (at a given wavelength) is insensitive to changes in refractive index of particles within the domain of optically large particles. In other words, in this domain the Mie theory predicts that *Qc* reaches an asymptotic (constant) value of 2 (in physical optics literature it is often referred to as “extinction paradox”). In this domain the changes in particle size or refractive index have no effect on *Qc*. For wavelengths from the visible spectrum (such as 660 nm in this study) and for typical range of refractive index of marine particles, this asymptotic regime is expected to be achieved already at a particle size of about 10 µm and then the nearly constant value of *Qc* = 2 extends beyond this particle size. The point is that the authors apply the inversion model to highly polydisperse system of particles which includes the domain of optically large particles (i.e., insensitivity of *Qc* to refractive index) which is one of the reasons why this approach is ill-posed and why the estimates of bulk refractive index obtained by matching the measurement of bulk beam attenuation with Mie theory predictions have weak foundation for physics-based interpretation of these refractive-index estimates. The limitation associated with potential multiple solutions from the inverse method should also be addressed.

We largely share and understand your point of view, and we have thoroughly modified the manuscript following several of the suggestions. Additionally, while we do think this method is unsuitable at distinguishing individual refractive index variation of sub-populations, the method itself (provided more data were available in the 0.2-5 um range for instance) is theoretically suitable for measuring the average refractive index of particles < 10 um, especially given that not many alternatives exist. We have added Figures 1b-c, which highlight the particle ranges that most influence beam attenuation for typical particle size distributions in our samples, and also which diameter classes are most sensitive to changes in refractive index.

New Fig 1:



***Fig. 1. a)*** *Mie Theory calculations of the**attenuation efficiency of particles (Qc, circles) and Qc corrected for the acceptance angle of 1.2° of the C-Star instrument (Qc\*, triangles) for three different n values (blue, yellow, white) and wavelength of light of 660 nm. n’ was fixed at 0.0003.* ***b)*** *contribution of size classes ranging from 0.2-100 μm to theoretical cp using Eq. 2, where N(Di) was modeled as a hypothetical size distribution with a power law slope of -4 (see Section 2.2.2).* ***c)*** *normalized**cumulative distribution of cp data presented in plot b).*

The new Figs. 1b-c is first introduced in Line 151:

*“**Note that Qc is the only term in the definition of cp that contains a dependence on m (Eq. 1). However, in the domain of optically large particles (i.e. > ~ 10 μm for the range of indices of refraction shown in Fig. 1a), changes in the refractive index have no effect on Qc. This essentially applies an upper limit of ~ 10 μm to the range of relevant particle diameters for which n is estimated using Eq. 2 (see further discussion in Section 3.3). Nonetheless, note that particles > 10 μm typically make up only a small portion of the cp signal in open ocean environments (< 1-5% for a range of indices of refraction and assuming a hypothetical size distribution with a power law slope of 4; see Section 2.2.2; Fig. 1b-c).”*

We return to this point in Section 3.3 (Sensitivity analysis):

Line 389 – *“As shown in Fig. 1, our application of RINV is mostly sensitive to particle dynamics in particle sizes < 10 μm, both because Qc (and Qc\*) do not change significantly with changes in n in that size range, and because the contribution of particles > 10 μm to cp is < 1-5% (or < 0.5% if the data in Fig. 4a where a of 4.57 was considered). This point can be further demonstrated when Eq. 4 is executed for particle size distributions truncated at 10 μm, 20 μm, and 50 μm. Assuming a of 4.57, average n retrievals for each of the three iterations are essentially identical to each other and to the original result in Fig. 4a: 1.027 ± 0.001, 1.027 ± 0.001, and 1.026 ± 0.001, respectively, with similar diel oscillations in [n-1] of 11.0 ± 4.0, 10.9 ± 4.3, and 11.3 ± 3.7, respectively.”*

Regarding the multiple solutions comment, we agree that, theoretically, inversion is potentially prone to multiple solutions. However, we have clarified the manuscript to explain that in the water we measure, where beam attenuation is dominated by particles < 10 micron, overall beam attenuation increases with refractive index, and thus we can converge on one and only one solution to minimize the error between actual and measured beam attenuation. We make this point more clear in Section 2.2.3:

Line 221: *“**Implementation of the RINV method included building a lookup table of theoretical cp\* using the constructed N(Di) and n ranging from 1.0010 to 1.2000 at 0.0001 intervals (Eq. 4). Then, for every datapoint in time, the squared difference between modeled cp\* and measured cp (via the C-Star transmissometer, 660 nm) was minimized to yield the estimated n of that bulk sample. Calculation of all possible iterations through a look up table ensured that a single global minimum was obtained. Variations in (3.91 - 5.23) and N0 (104.3076 - 106.5477), equivalent to one standard deviation from the fit, were used to evaluate the sensitivity of n to changes in both the slope and magnitude of the size distribution of the 0.20 – 5.54 μm size range (Section 3.3). Mean particle sizes for bins in the 0.20 – 5.54 μm size range were appropriately recalculated whenever different N0 and values were used.”*

And we revisit this topic as well in Section 3.4

Line 443: “*Mathematical inversions can theoretically yield multiple solutions. This would be the case for example if the entirety of the water sample analyzed contained particles for which Qc\* is not a function of n. The signal in our measured samples was dominated by particles < 10 μm (see* ***Fig. 1****). In this size regime, Qc\* increases with n (****Fig. 1b****) and therefore RINV was able to converge to a single estimate of n at each time point.*”

Below I provide specific suggestions for improvements in the manuscript. My comments are largely based on my experience in research area relevant to this manuscript and involve some of my previous work, so I have identified myself as a reviewer. If the authors have questions or are interested in more discussion during the revision, please feel free to contact me.

**Specific comments:**

(1) Title of the manuscript

I think the title should be changed to better reflect the actual content and approach of your study, for example something along those lines:

“Estimation of diel variations in the refractive index of oceanic particles using beam attenuation and particle size distribution”.

Note that in this suggestion “estimation” is the key word from the standpoint of your inverse approach, “diel variations” are the key words from the standpoint of your most important results and, finally, specifying the region is also essential given that your data are restricted to one oceanic region. All these key words are missing in your title which is too general.

Thank you for the suggestion, we agree. We have changed the title to “Diel variations in the estimated refractive index of bulk oceanic particles”.

1. Lines 34-36 and references therein:

To the best of my knowledge, the paper Stramski, D., and A. Morel. 1990. Optical properties of photosynthetic picoplankton in different physiological states as affected by growth irradiance. Deep-Sea Research, 37, 245–266, is the first study that reported on the relationship between the real part of refractive index and intracellular carbon concentration based on measurements of phytoplankton cultures. In addition, the paper Stramski, D. 1999. Refractive index of planktonic cells as a measure of cellular carbon and chlorophyll *a* content, Deep-Sea Res. I, 46, 335-351, was specifically devoted in its entirety to the relationships between the real and imaginary parts of refractive index of phytoplankton cells and the intracellular carbon and chlorophyll-a concentrations, respectively (FYI, Equation 9 in this paper has a typographical error, it should include “+” instead of “– “). My intent is not to specifically advocate citation of my studies but these two papers represent an important contribution to literature in the context of your study and specific text in lines 34-36. I think it makes sense to add these references, especially that you generally made good effort with regard to selection of various references, so I do not know why you omitted these two references given their significance to this subject area.

Thank you for the very appropriate suggestions, the references have been added.

1. Line 41: Add “scattering (*bp*)” after “(*cp*)”

Done.

1. References [4, 9-11]: Add reference 3 which is, to the best of my knowledge, the first study devoted to experimental demonstration of diel variations in phytoplankton spectral absorption and scattering properties, cell size, and complex refractive index.

Done.

1. Line 49: “…measure the optical scattering cross-sections”: This is not true. The scattering cross-section is a single-particle optical property extremely difficult to measure directly. Even single-particle techniques such as flow cytometry have not generally been developed and suitably calibrated for the purpose of quantitative measurement of scattering cross-sections of individual particles (which has units of m2). The instruments that measure bulk scattering properties (like laser diffraction instruments) belong to the category of ensemble scattering techniques, so they do not provide a measurement of scattering cross-sections of individual particles. Obviously, the satellite instruments do not measure the scattering cross-sections of individual particles either.

Thank you for pointing this out – the original sentence did not convey what we meant to say. We have edited that sentence to read:

Line 44: “*Ignoring the effects of m variability can significantly affect use and interpretation of data from many flow cytometers, diffraction instruments, and satellite detectors. Data from these instruments are commonly used in combination with an optical model to estimate particle size, often the parameter of choice for biogeochemistry applications*.”

1. Line 54: “variations in *n*’ have minor effect on bulk scattering….”. This is true only at wavelengths where absorption is negligible or weak, so for phytoplankton this can be approximately true only outside the main absorption bands of phytoplankton pigments. Within the absorption bands, the effect of *n*’ on scattering can be strong, which has been demonstrated in multiple papers where spectra of both absorption and scattering coefficients were measured on phytoplankton cells. Therefore, please correct the sentence to clarify this point.

We clarified the sentence and now it reads:

Line 50: “*Whereas variations in n’ have minor effect on bulk scattering properties at wavelengths where particulate absorption is negligible or weak [3,4], variations in the choice of n within reasonable ranges can lead to large changes in the estimated particle sizes, volumes, areal distributions, and numerical abundances of a population of particles [3,4]*”.

1. Line 57: Reference [4]: Again, I think that adding the earlier reference [3] which represents a study similar to [4] but with the diatom rather than cyanobacterium, is relevant in this context.

Done (see excerpt above).

1. Line 63: You cite just [17] (which is a recent paper from 2018) in the context of determinations of *n* for Prochlorococcus. Please cite the paper Morel, A., Y.-H. Ahn, F. Partensky, D. Vaulot, and H. Claustre. 1993. Prochlorococcus and Synechococcus: A comparative study of their optical properties in relation to their size and pigmentation, J. Mar. Res. 51, 617-649. To my knowledge, this is the first study where the optical properties including determinations of refractive index of Prochlorococcus were made. See also Table 1 in reference [36] where the values of refractive index for Prochlorococcus and a number of other plankton species are compiled from different studies.

Thank you for this reference! We now cite the Morel et al 1993 paper.

1. Lines 65-70: The major problems and errors associated with a commonly used approach to convert cell volume to carbon content using some fixed empirical relationships (like the relationships from reference [19] that you cite) are discussed and demonstrated (even if a single species is considered) in Stramski (1999) (see my comment #2 regarding this paper). As discussed in Stramski (1999), the relationship between the real part of refractive index and intracellular carbon concentration is founded on stronger mechanistic basis and provides a proper avenue to make scientific advancements by essentially abandoning the use of highly uncertain conversion from cell volume to cell carbon. Also, as described in Stramski (1999), the approach involving the refractive index requires an appropriate combination of light scattering (and possibly also absorption) measurements, particle size measurements, and inversion method to estimate the refractive index. Importantly, note that for potential application to natural assemblages of marine particles Stramski (1999) proposed to advance this methodology based on single-particle optical and size measurements, and not bulk optical measurements on entire populations of marine particles with application of inverse models to these bulk measurements like in your study. This is because the inverse problem is generally an ill-posed problem due to shortcomings of the ensemble methodology and complexity of particle assemblages. Although technological capabilities have existed for many years to develop or refine the single-particle instrumentation to include more rigorous particle optical and size measurements compared, for example, with typical flow cytometers, unfortunately the required technological development has not been realized until now. I bring this information in case you were not aware of this important historical background when you decided to embark on a study with the use a combination of bulk optical measurement of beam attenuation and inversion model applied to these bulk attenuation measurements. I alluded earlier to the point that I would not conduct a study based on such bulk/inverse approach for natural seawater samples (because it is essentially an ill-posed problem) but, on the other hand, it must be admitted that no appropriate instrumentation is as yet available which would allow to conduct single-particle measurements from which reasonable determinations of refractive index could be made on a particle-by-particle basis.

Thank you for your feedback. This comment in particular has helped us with the restructuring of the scope of the manuscript to highlight that this is a bulk particle estimation mostly sensitive to particles in a particular size range. It is our hope that, in the spirit of incremental scientific progress, the methods described here would serve as a foundation for more detailed analysis to be done as technological advances are made to link bulk optics with the single-particle analysis.

1. Lines 75-78: Regarding approaches for detailed or direct determinations of refractive index please consult and include reference to paper by Jonasz, M., G. Fournier, and D. Stramski. 1997. Photometric immersion refractometry: A method for determining the refractive index of marine microbial particles from beam attenuation. Appl. Opt., 36, 4214–4225. This paper describes and demonstrates the application of an experimental method to determine the refractive index of marine heterotrophic bacteria. This immersion refractometry method is well-founded on physical principles and the paper by Jonasz et al. (1997) is a unique contribution to literature in this subject area, especially in the context of application to marine particles. Although that study demonstrated the application to marine bacteria, the underlying principles are applicable to other types of marine particles, and more generally even to polydisperse systems of many types of particles although additional restrictions would be involved in the interpretation of results for polydisperse heterogenous samples. The photometric immersion refractometry method is, however, labor intensive and therefore not suited for routine applications to analysis of marine particles, which is one reason why it was not further applied to such analyses. Nevertheless, it certainly deserves to be mentioned in your background about refractive index determinations.

Thank you, we have added this important paper to the references.

1. Lines 84-87: I think that this statement does not characterize accurately enough these specific methods for estimating *n* of different plankton microorganisms. Importantly, the work by Bricaud & Morel (which you omitted) as well as my work (including references 3 and 4, but also Stramski, D., and D.A. Kiefer. 1990. Optical properties of marine bacteria, p. 250-268. In: Ocean Optics X, R. W. Spinrad [ed.], Proceedings, Society of Photo-optical Instrumentation Engineers, Vol. 1302, SPIE, Bellingham) utilized a combination of measurements of spectral beam attenuation, spectral absorption coefficient and particle size distribution as well as optical theory (either van de Hulst approximation or Mie theory) to solve for *n* and *n*’. Your statement indicates that these methods were based on measurements of cross-sectional area and number distributions which is inaccurate and misleading because, in addition to particle size distribution, the key components were the measured spectra of beam attenuation and absorption coefficients. I suggest adding a reference Bricaud, A., and A. Morel. 1986. Light attenuation and scattering by phytoplanktonic cells: a theoretical modeling. Appl. Opt. 25, 571-580. This study used van de Hulst approximation (rather than Mie theory) but, in my opinion, it is a very important contribution to literature regarding determinations of refractive index of phytoplankton. The study of Stramski and Kiefer (1990) (mentioned above) has also significance in this field because it advanced the approach of Bricaud & Morel by employing the Mie theory and it was later used by us in [3] and [4]. You may also find of interest to look at Fig.1 in Stramski & Kiefer (1990) showing a flowchart of our inverse model and see the differences in comparison to your inverse model in shown in your Fig. 2.

References have been appropriately added, thank you. We have also added more complete information regarding the datasets included in those referenced analysis. The sentence now reads:

Line 81: *“Estimates of n based on measurements of cross-sectional areas, spectral absorption and attenuation coefficients, and number distributions of specific plankton have been described [3,4,12,30–3]*”.

Thanks for pointing us in the Stramski and Kiefer 1990 direction – it was great to see the flowchart.

You also indicated in line 86 that these earlier studies were species-specific which you wrote it in a way that it may suggest it was a limitation. So, I’d like to clarify this point. The inverse methodology to estimate refractive index in the work conducted by Bricaud, Morel, myself and my co-workers, was applied by purpose and scientific rationale only to specific types of particles (i.e., plankton species with a relatively narrow size distribution). We did not extend this application to polydisperse assemblages of marine particles with broad size distributions because such extension has major scientific problems and limitations. Both Morel’s team and my team have developed the inverse methodology long time ago and we have had data available of optical properties (including beam attenuation and spectral data) and particle size distributions (the broader size range than in your study) measured on natural assemblages of marine particles, and yet for purely scientific reasons we have not conducted a study similar to yours on entire assemblages of marine particles. In addition, in the background paragraph on refractive index determinations, I also suggest adding a reference Agagliate, J., R. Röttgers, K. Heymann, and D. McKee. 2018. Estimation of suspended matter, organic carbon, and chlorophyll-a concentrations from particle size and refractive index distributions, Appl. Sci., 8, 2676. If you are unfamiliar with this paper, I think you’ll find it interesting and relevant to your work.

Our intent was not to minimize those contributions at all, but rather highlight that species-specific studies have shown variable conclusions regarding how *n* varies over time, and set the stage for the next paragraph which brings to attention that bulk estimates of *n* are rare (understandably for all the reasons you have mentioned throughout the review). We nonetheless modified the sentence to read:

Line 83*: “Estimates of n based on measurements of cross-sectional areas, spectral absorption and attenuation coefficients, and number distributions of specific plankton have been described [3,4,12,30–35], but variable conclusions have been gained regarding how n changes as particles grow*.”

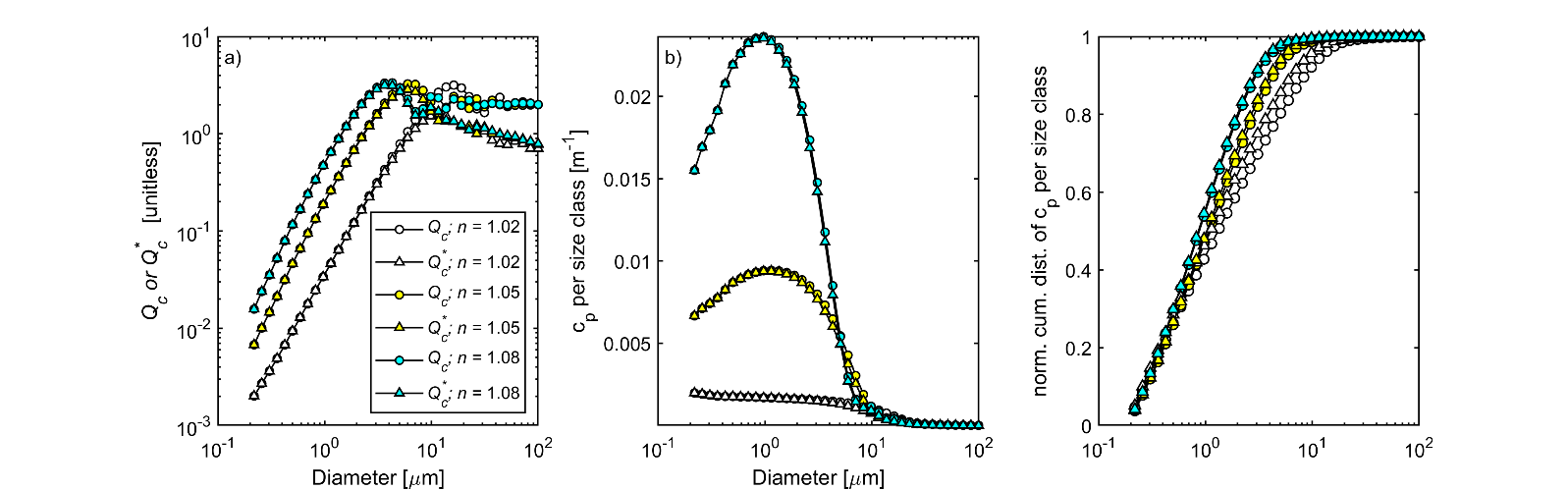
1. Line 130: Add “for homogeneous spherical particles” after “Mie theory model”

Done, thank you.

1. Line 131-132: The statement about the dependence of *Qc* on *m* is quite critical. As indicated in my general comment (3), *Qc* does not depend on *m* within the domain of optically large particles and the bulk beam attenuation measurements are affected to some degree by particles from this domain. This is an important issue which should be addressed in section 2.2.1 and also later in the manuscript in sections 3.3 and 3.4. With regards to Fig. 1, I am puzzled by the results for *Qc*\* which decreases with increasing particle size to values lower than 1 for a diameter of 100 µm. In other words, it suggests that if *Qc* is underestimated due to inclusion of light scattered at very small angles <1.2o, the asymptotic regime where *Qc* is about 2 does not exist anymore. I would not expect such strong effect associated with such small range of near-forward angles, but if it is indeed the case, I think it deserves more emphasis in the manuscript. This is because the fact that the beam attenuation meter is imperfect and collects light scattered at angles <1.2o can provide some advantage to your inverse method in a sense that *Qc*\* exhibits some sensitivity to *n* even in the domain of optically large particles, which is not the case for true *Qc*. Another issue that should be addressed in the description of inverse method is the possibility of obtaining multiple solutions of *n*, which naturally occurs because *Qc* at a given wavelength is not a monotonic function of particle size or refractive index. Also, please include information in the caption of Fig. 1 about light wavelength for which these calculations were made (reminding readers that all your results are for 660 nm is important).

Thank you for these suggestions. We have added Figures 1b-c to clarify how beam attenuation depends on size class, as well as to clarify how sensitive RINV solutions for refractive are for size classes given how Qc changes with *n* assumptions.

New Fig. 1:



***Fig. 1. a)*** *Mie Theory calculations of the**attenuation efficiency of particles (Qc, circles) and Qc corrected for the acceptance angle of 1.2° of the C-Star instrument (Qc\*, triangles) for three different n values (blue, yellow, white) and wavelength of light of 660 nm. n’ was fixed at 0.0003.* ***b)*** *contribution of size classes ranging from 0.2-100 μm to theoretical cp using Eq. 2, where N(Di) was modeled as a hypothetical size distribution with a power law slope of -4 (see Section 2.2.2).* ***c)*** *normalized**cumulative distribution of cp data presented in plot b).*

Line 151: *“Note that Qc is the only term in the definition of cp that contains a dependence on m (Eq. 1). However, in the domain of optically large particles (i.e. > ~ 10 μm for the range of indices of refraction shown in Fig. 1a), changes in the refractive index have no effect on Qc. This essentially applies an upper limit of ~ 10 μm to the range of relevant particle diameters for which n is estimated using Eq. 2 (see further discussion in Section 3.3). Nonetheless, note that particles > 10 μm typically make up only a small portion of the cp signal in open ocean environments (< 1-5% for a range of indices of refraction and assuming a hypothetical size distribution with a power law slope of 4; see Section 2.2.2; Fig. 1b-c).”*

Line 389: *“As shown in Fig. 1, our application of RINV is mostly sensitive to particle dynamics in particle sizes < 10 μm, both because Qc (and Qc\*) do not change significantly with changes in n in that size range, and because the contribution of particles > 10 μm to cp is < 1-5% (or < 0.5% if the data in Fig. 4a where a of 4.57 was considered). This point can be further demonstrated when Eq. 4 is executed for particle size distributions truncated at 10 μm, 20 μm, and 50 μm. Assuming a of 4.57, average n retrievals for each of the three iterations are essentially identical to each other and to the original result in Fig. 4a: 1.027 ± 0.001, 1.027 ± 0.001, and 1.026 ± 0.001, respectively, with similar diel oscillations in [n-1] of 11.0 ± 4.0, 10.9 ± 4.3, and 11.3 ± 3.7, respectively.”*

We more directly address the possibility of multiple solutions in Section 3.4:

Line 443: “*Mathematical inversions can theoretically yield multiple solutions. This would be the case for example if the entirety of the water sample analyzed contained particles for which Qc\* is not a function of n. The signal in our measured samples was dominated by particles < 10 μm (see* ***Fig. 1****). In this size regime, Qc\* increases with n (****Fig. 1b****) and therefore RINV was able to converge to a single estimate of n at each time point. Despite the limitations of the method, the strength of our analysis is in the fact that results showed the presence of robust diel cycles in n which qualitatively agree with laboratory studies of diel variations estimated for phytoplankton cultures [3,4], potentially providing evidence that (as speculated by many of these early studies), a diel cycle in n should be observed in real oceanic environments where various living and non-living organisms co-exist*.”

1. Fig. 2: I think the second box from the top should read “0.2 – 4 µm” instead of 2 – 4 µm”

Thank you. The typo was corrected and updated to reflect the updated size ranges

1. Lines 168-170: You make a general statement about approximating the particle size distribution (PSD) with a single-slope power function and in this general context you cite two recent papers from 2015 [37,38]. In such general context, it is appropriate to cite more fundamental and/or pioneering work about the use of power function for approximating marine PSDs, such as a paper by Bader, H. 1970. The hyperbolic distribution of particle sizes. J. Geophys. Res., 75, 2822–2830, and a comprehensive book by Jonasz, M., and G. Fournier. 2007. Light Scattering by Particles in Water: Theoretical and Experimental Foundations. San Diego, CA: Academic Press.

Great suggestions, thank you. This important historical context has been added.

1. Lines 193-194: FYI, the spectra of imaginary part of refractive index of detrital particles are presented in Fig. 3 in Stramski, D., and S. B. Woźniak. 2005. On the role of colloidal particles in light scattering in the ocean. Limnol. Oceanogr., 50, 1581–1591. In this paper you will also find data of particle size distribution in the submicron range (from earlier studies of Wells and Goldberg 1994 and Yamasaki et al. 1998), which show large variations in both the concentration and shape of PSD in the submicron range. Note that these data are not supportive of the use of a single-slope power function for approximating or extrapolating PSDs into the range of small-sized particles.

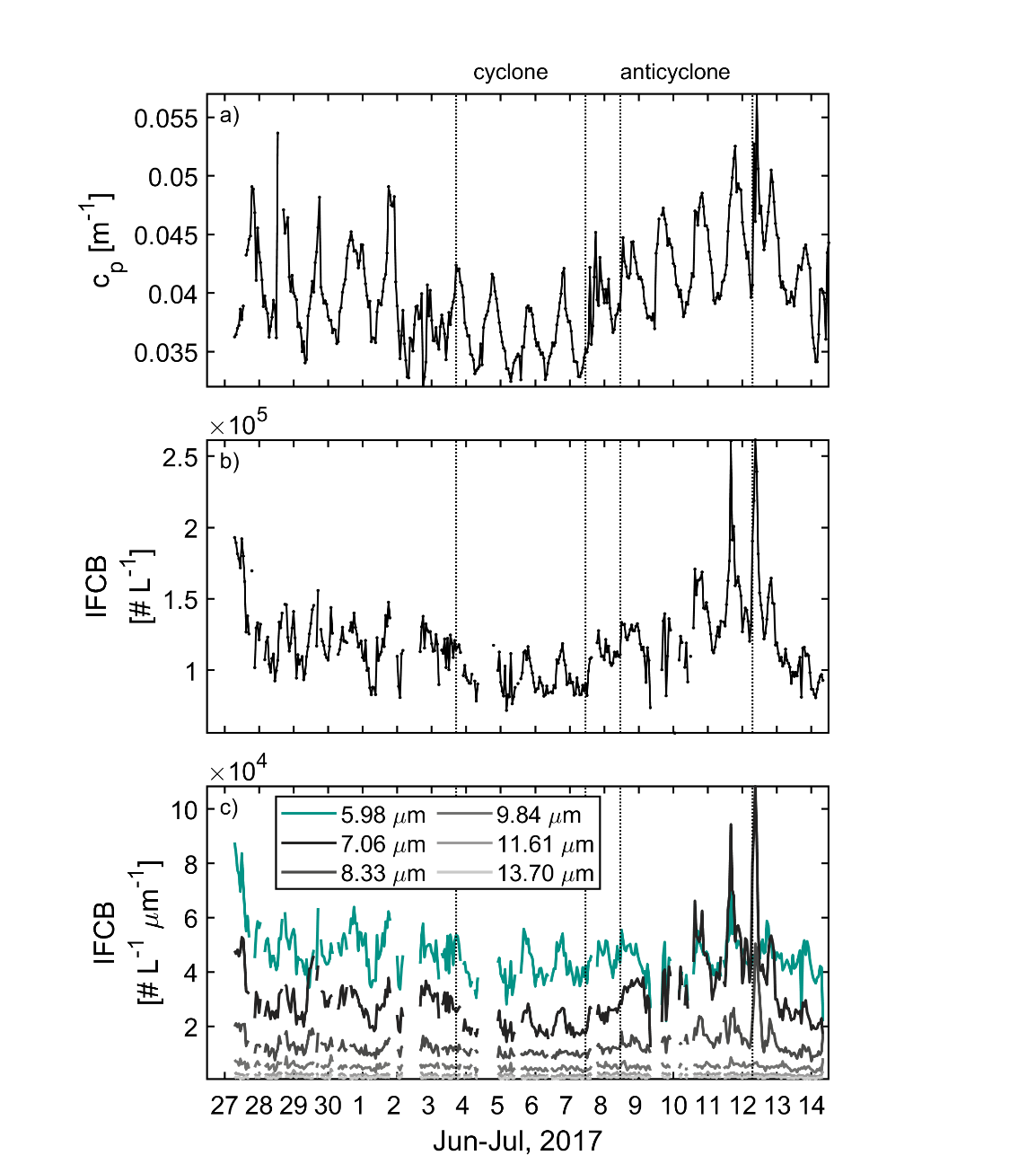
Thank you for this! We’ve taken care to point this out in Section 3.4:

Line 399: *“The RINV method described here depends on assumptions and limitations that must be recognized. We assume a power-law particle size distribution for particles smaller than those directly measured by the IFCB. Although the magnitude of the N(Di) spectrum in Fig. 4 itself agrees with previously published size distributions in the ~ 1 - 100 μm size range for the NPSG [44,45], in situ PSDs are notoriously complex and often deviate from the classic power law distribution [46,65–67].”*

1. Fig. 3d: I think the curves for particle concentration are not directly comparable because of differences in the width of size bins. Why don’t you show the curves of particle size distribution normalized by the bin width (i.e., the density function of PSD)?

Done. We have updated Figure 3 to showcase a) beam attenuation at 660nm, b) Total IFCB particle abundance, and C) the normalized particle abundances for select particle sizes.

Updated Fig. 3:



***Fig. 3.******a)*** *particulate beam attenuation coefficient at 660 nm, cp.* ***b)*** *total particle abundance measured by the IFCB in the 5.54-109.26 μm size range.* ***c)*** *particle abundance normalized by binwidth per size class measured by the IFCB. Only diameters < 14 μm are plotted to aid visualization. Size class 5.98 μm, used as a reference diameter, is highlighted in teal. Dashed vertical lines denote the Lagrangian sampling periods inside cyclonic (Jul 4-7) and anticyclonic (Jul 8-12) eddies.*

1. Lines 243-244: You provide the percentage change for refractive index increment, *n*1, but then in parenthesis you give the values of *n* rather than *n*–1. This can be confusing. For clarity you may want to add the vales of *n*–1 for which the percentage change was calculated. I think it would still be OK to keep the values of *n*; it is better to have some redundancy than potential confusion. Similar situation in lines 268-269.

For clarity, we have modified this sentence to read:

Line 266 “*Average values of n inside the anticyclonic eddy (1.0271 ± 0.0010) were ~ 7% higher than values in the cyclonic eddy (1.0254 ± 0.0009) if calculated from n-1 refractive increments (i.e. 0.0271 vs 0.0254), but not significantly different*.”

1. Lines 269-271: The qualitative consistency of diel patterns with previous laboratory based studies provides the key support of your results about the potential existence of similar diel patterns in real marine environments and this support is essential in view of significant limitations of the inverse approach. So, I suggest also including reference [3] in line 270.

Done.

1. Lines 294-295: The addition of reference Stramski (1999) would be a good fit in this context.

Done.

1. Lines 302-303: Change the order to “…the diatom *Thalassiosira* *pseudonana* and cyanobacterium *Synechococcus*” for consistency with the order of references [3,4].

Done, thank you.

1. Section 3.3 Sensitivity Analysis: I suggest conducting additional calculations to add results of sensitivity analysis with particle size distribution truncated at a few particle diameters from the range of optically large particles but smaller than 100 µm. This can be important in the context of behavior of *Qc* in the range of relatively large particle diameters (see also comment #13). For example, it might be useful to examine and demonstrate the sensitivity of results to particle size distribution extending only to 10, 20 and 30 µm, or perhaps 10, 30, and 50 µm.

We performed this analysis and discuss it in Section 3.4. We also make the point that cp in our dataset is already weakly sensitive to this size range as well, so large particles both do not contribute significantly to cp, nor do they contribute with n estimated via the inversion.

Line 389: *“As shown in Fig. 1, our application of RINV is mostly sensitive to particle dynamics in particle sizes < 10 μm, both because Qc (and Qc\*) do not change significantly with changes in n in that size range, and because the contribution of particles > 10 μm to cp is < 1-5% (or < 0.5% if the data in Fig. 4a where a of 4.57 was considered). This point can be further demonstrated when Eq. 4 is executed for particle size distributions truncated at 10 μm, 20 μm, and 50 μm. Assuming a of 4.57, average n retrievals for each of the three iterations are essentially identical to each other and to the original result in Fig. 4a: 1.027 ± 0.001, 1.027 ± 0.001, and 1.026 ± 0.001, respectively, with similar diel oscillations in [n-1] of 11.0 ± 4.0, 10.9 ± 4.3, and 11.3 ± 3.7, respectively.”*

1. Line 361: Is the percentage difference for *n* – 1?

Yes. This is now clarified.

1. Lines 365-366: See comment #18.

We now make sure to specify *[n-1]* whenever that value is used in the calculation, including in Figure captions.

1. Section 3.4. Justification of method assumptions: While it is very good to have such section devoted to the various assumptions and limitations of the method, I suggest changing the emphasis in some statements that appear to defend some assumptions too much although these assumptions are not really defendable. I believe it is better to acknowledge that at this stage of this kind of inverse modeling the use of multiple assumptions that can deviate significantly from reality is unavoidable but the results of sensitivity analysis show the presence of diel pattern that is qualitatively consistent regardless of parameters used in the sensitivity analysis and, very importantly, also consistent with earlier findings from laboratory studies of diel variations in phytoplankton cultures. In my view, this is the key message that can more efficiently defend your study in view of significant assumptions and limitations of the method. I think you can use this message to revise text in section 3.4, and it can also be helpful for some edits of text in the Outlook and Conclusions sections.

One example assumption that is not defendable is the use of single-slope power function over an extended particle size range (lines 377-379). There is ample evidence presented in many publications that such assumption is generally unjustifiable given the experimental data of PSD. This is true not only in the context of using a single-slope function to extrapolate the PSD to particles smaller than 4 µm but significant deviations from a single-slope approximations, for example distinct peaks, shoulders, valleys, and changes in slope across different size ranges, are often observed in the ocean. Only under specific environmental situations and/or within the limited size ranges, the single-slope power law may provide reasonably good approximation, and I am not convinced that your specific PSD data are sufficient to support such approximation. The deviations from a single-slope approximation have naturally implications to proportions of concentrations of differently-sized particle, and hence to calculated optical properties such as beam attenuation. It is also important to acknowledge that the variations in the shape of PSD across the particle size range are best observed with techniques that allow high size resolution measurements (such as Coulter Counter technique). The PSDs measured in your study have relatively low size resolution which may not be good enough to clearly reveal the features in the PSD shape. Often times such features in PSD are associated with specific populations of plankton and your study emphasizes the significant role of Crocospharea, so it is possible that the associated feature can be dampened in low resolution size measurements.

We have considerably changed the tone and message of Section 3.4 and 3.5 following your suggestions. In Section 3.4, we have de-emphasized our attempts to justify our assumptions based on what we agree is very incomplete data. We explicitly cite some of the references in the list provided below when we mention how in situ PSDs are complex and often deviate from a power-law, i.e.: Line 401: “*Although the magnitude of the N(Di) spectrum in* ***Fig. 4*** *itself agrees with previously published size distributions in the ~ 1 - 100 μm size range for the NPSG [44,45], in situ PSDs are notoriously complex and often deviate from the classic power law distribution [46,67–69]*.” We have removed our attempts to justify an invariant shape of the PSD in the submicron range. We end Section 3.4 with a few sentences highlighting the strength of the manuscript, with a more explicit mention and connection to the early laboratory studies, as suggested.

New Section 3.4, Line 399:

*“The RINV method described here depends on assumptions and limitations that must be recognized. We assume a power-law particle size distribution for particles smaller than those directly measured by the IFCB.* *Although the magnitude of the N(Di) spectrum in* ***Fig. 4*** *itself agrees with previously published size distributions in the ~ 1 - 100 μm size range for the NPSG [44,45], in situ PSDs are notoriously complex and often deviate from the classic power law distribution [46,65–67].*

*Particles in this study were assumed to be spherical, homogeneous, and to have a constant refractive index across the entire particle size spectrum. The modification of Mie theory to assume heterogeneous structures where cell membrane and internal organelles have different optical properties has been shown to help better characterize bulk optical properties such as the particulate backscattering coefficient [68,69], while modifications for more complex particle shapes has shown to be crucial for characterizing the optical properties of aerosols [70]. However, oceanic cp is generally deemed less sensitive to these assumptions [69]. It is clear that individual particles in a sample will present a range in indices of refraction; no parameterization relating indices of refraction to size is available (and there is no reason to believe a simple one should exist; [4]). Additionally, the imaginary component of the index of refraction was assumed to be constant and fixed at 0.0003. Studies characterizing n’ of particles are rare, but there is no evidence for the n’ of detritus (which makes up the bulk PC in the NPSG) to vary significantly [4,25,43].*

*Clearly, application of an inverse model requires making multiple assumptions that are unavoidable in the absence of a complete dataset. Certain observations served as general guidelines to support choices made throughout the analysis, and should be mentioned. In the case of the magnitude of the size distribution extrapolation to small sizes, data from a flow cytometer showed that Prochlorococcus counts averaged ~ 1.8 × 108 L-1 during KM1709 [21], or 3.6 × 108 L-1 μm-1 if a bin width of 0.5 μm is assumed, which is less than the extrapolated value of ~2.1 × 109 L-1 μm-1 at 0.6 μm estimated here (****Fig. 4a****). This is expected given that Prochlorococcus are just a fraction of the particles in that size range, and suggesting the raw magnitude of the extrapolated particle size distribution is at least probable. During this cruise, bulk optical signals of cp (660 nm) correlated well with the IFCB abundances and volume concentrations measured (****Fig. 3;*** *[21]), indicating that particles outside the range of the IFCB (< 5 μm) either co-varied with the larger-sized particles (> 5 μm); made up a stable background signal; or a combination of both. This also suggest that the bulk particle dynamics was captured by the IFCB despite the limited size range of the instrument. Apart from size classes 7 - 8 μm, day-to-day variability in IFCB size classes was relatively stable throughout the cruise (****Fig. 3c****). The form of the size distribution below 1 μm is poorly known [42].* *Submicron particles are believed to be dominated by non-living materials, whose abundance are not expected to oscillate significantly over the diel cycle [25]. Moreover, their relative abundance compared to living particles is expected to increase with decreasing size ranges [71], and thus any diel cycles may be further muted as particle sizes decrease. Finally, the shape of the binned 5.54 – 29.06 μm portion of particle size distribution does not appear to shift significantly over the diel cycle (****Fig. 4b****), with samples collected at 7:00 vs 20:00 h (local time) yielding the same average power law slope fit ( = 4.57 ± 0.43 vs 4.57 ± 0.40), and end-of-day samples generally showing more elevated particle abundances, as expected from* ***Fig. 3*** *(i.e. fit N0 = 104.71 ± 0.03 at 07:00 h vs 104.76 ± 0.04 at 20:00 h).*

*Mathematical inversions can theoretically yield multiple solutions. This would be the case for example if the entirety of the water sample analyzed contained particles for which Qc\* is not a function of n. The signal in our measured samples was dominated by particles < 10 μm (see* ***Fig. 1c****). In this size regime, Qc\* increases with n (****Fig. 1b****) and therefore RINV was able to converge to a single estimate of n at each time point. Despite the limitations of the method, the strength of our analysis is in the fact that results showed the presence of robust diel cycles in n which qualitatively agree with laboratory studies of diel variations estimated for phytoplankton cultures [3,4], potentially providing evidence that (as speculated by many of these early studies), a diel cycle in n should be observed in real oceanic environments where various living and non-living organisms co-exist.”*

Section 3.5 is now merged with Conclusions, and mentions the more appropriate datasets that could/should be used in order to tackle potential changes in n over short time scales.

Revitalized Conclusion section, Line 454:

*“**The index of refraction of particles (n) is a key parameter to determine how light scattering signals can be converted into particle size, often the parameter needed for biogeochemical applications. Yet, little is known about how n varies over time and space, and in situ measurements in the ocean – to our knowledge, do not exist. We describe a method to estimate the index of refraction of bulk particles < 10 μm, in situ, based on high frequency measurements of the particulate beam attenuation coefficient (cp) and particle size distribution collected from an Imaging FlowCytobot (IFCB) in the surface waters of the North Pacific Subtropical Gyre. We describe daily amplitudes in n from sunrise to sunset that were relatively robust even when evaluating the assumptions inherent to our methodology (ranging from 11.3 ± 4.3% to 16.9 ± 2.9%). Importantly, our estimates are qualitatively consistent with laboratory-based estimates that showed that n of specific phytoplankton species varies significantly over the diel cycle, with potential implications for the interpretation of optically-derived data. Such variability estimates could be crucial for more accurate estimates of biomass and growth rates from time-varying changes in abundance and particle size inferred from scattering-based in situ instruments, and improvement of satellite-based algorithms of the particle size and production, as very few global algorithms have been distributed with uncertainty estimates [72].*

*The accurate characterization of refractive index variability has implications for both remote sensing and in situ measurements, both of which largely rely on optical scattering by plankton and detritus, and which are used to understand the ecological functioning of the ocean. Accurate estimations of the index of refraction have proven integral in modeling the size, shape, polarization properties, and distribution of aerosols and their impacts on climate models [70,73]. Thus, better characterization of oceanic n in situ could also prove useful for the modeling of polarimetric qualities of phytoplankton, similar to what has been done for aerosols, and which would be highly relevant for the increased capabilities of measurements and algorithm development from the upcoming Plankton, Aerosol, Cloud, Ocean Ecosystem (PACE) mission [74]. Widespread estimates of the refractive index also have the potential to influence retrievals of the chlorophyll-to-carbon parameter through modeling of the backscattering coefficient and the density of carbon per cell, which has implications for our understanding of critical ecological information from spaceborne sensors [75]. Future studies to validate and explore variation in n over time and space will improve understanding of ocean biogeochemistry and improve bio-optical models aiming at retrieving particle size and production. Development of capabilities to determine the refractive index on a particle-by-particle basis, over high temporal and spatial resolutions, potentially using a combination of multi-angle flow-cytometry, optical diffraction tomography, and cell counters [6,26,76–78] could circumvent many of the limitations of the inverse method described here. Together with measurements of cell composition, high spatial and temporal resolution measurements of particle refractive index could provide a means to revolutionize our understanding of carbon cycling, particle diversity, and energy transfer in the ocean.”*

With regards to relevant publications on marine PSDs, in comment #16 I mentioned a few papers on submicron size range. You can also see large changes in slope of PSD

(including submicron range) measured in the South Pacific Subtropical Gyre in the paper:

Loisel, H., J.-M. Nicolas, A. Sciandra, D. Stramski, and A. Poteau. 2006. Spectral dependency of optical backscattering by marine particles from satellite remote sensing of the global ocean. Journal of Geophysical Research, 111, C09024.

You may find it useful to look over our recent paper which demonstrates the inadequacy of single-slope approximation for PSD data collected in different oceanic environments:

Reynolds, R. A., and D. Stramski. 2021. Variability in oceanic particle size distributions and estimation of size class contributions using a non-parametric approach. J. Geophys. Res., Oceans, 126, e2021JC017946. In fact, there are many publications demonstrating significant deviations of measured PSDs from a single-slope approximation. I happen to have such (non-exhaustive) list which I can share with you:

Bader, H. (1970). The hyperbolic distribution of particle sizes. J. Geophys. Res., 75, 2822–2830.

Gordon, H. R., & Brown, O. B. (1972). A theoretical model of light scattering by Sargasso Sea particulates. Limnol. Oceanogr., 17, 826–832.

Jonasz, M. (1983). Particle-size distributions in the Baltic. Tellus B Chem. Phys. Meteorol., 35, 346–358. https://doi.org/10.3402/tellusb.v35i5.14624

Risović, D. (1993). Two-component model of sea particle size distribution. Deep Sea Res. Part I, 40, 1459– 1473.

Wells, M. L., & Goldberg, E. D. (1994). The distribution of colloids in the North Atlantic and Southern Oceans. Limnol. Oceanogr., 39, 286-302.

Jonasz, M., & Fournier, G. (1996). Approximation of the size distribution of marine particles by a sum of log-normal functions. Limnol. Oceanogr., 41, 744–754.

Yamasaki, A., Fukuda, H., Fukuda, R., Miyajima, T., Nagata, T., Ogawa, H., & Koike, I. (1998). Submicrometer particles in northwest Pacific coastal environments: Abundance, size distribution, and biological origins. Limnol. Oceanogr., 43, 536-542.

Reynolds, R. A., Stramski, D., & Mitchell, B. G. (2001). A chlorophyll-dependent semianalytical reflectance model derived from field measurements of absorption and backscattering coefficients within the Southern Ocean. J. Geophys. Res., 106(C4), 7125–7138.

Stavn, R. H. (2004). Suspended minerogenic particle distributions in high-energy coastal environments: Optical implications. J. Geophys. Res., 109, 201–219.

Loisel, H., Nicolas, J.-M., Sciandra, A., Stramski, D., & Poteau A. (2006). Spectral dependency of optical backscattering by marine particles from satellite remote sensing of the global ocean. Journal of Geophysical Research, 111, C09024.

Jonasz, M., & Fournier, G. (2007). Light scattering by particles in water: Theoretical and experimental foundations. San Diego, CA: Academic Press.

Reynolds, R. A., Stramski, D., Wright, V. M., & Woźniak, S. B. (2010). Measurements and characterization of particle size distributions in coastal waters. J. Geophys. Res., 115, C08024.

Woźniak, S. B., Stramski, D., Stramska, M., Reynolds, R. A., Wright, V. M., Miksic, et al. (2010). Optical variability of seawater in relation to particle concentration, composition, and size distribution in the nearshore marine environment at Imperial Beach, California. J. Geophys. Res., 115, C08027.

White, A. E., Letelier, R. M., Whitmire, A. L., Barone, B., Bidigare, R. R., Church, M. J., et al. (2015). Phenology of particle size distributions and primary productivity in the North Pacific subtropical gyre (Station ALOHA). J. Geophys. Res. Oceans 120, 7381–7399.

Bochdansky, A. B., Clouse, M. A., & Herndl, G. J. (2016). Dragon kings of the deep sea: marine particles deviate markedly from the common number-size spectrum. Sci. Rep., 6, 22633.

Reynolds, R. A., Stramski, D., & Neukermans, G. (2016). Optical backscattering of particles in Arctic seawater and relationships to particle mass concentration, size distribution, and bulk composition. Limnol. Oceanogr., 61, 1869–1890.

Organelli, E., Dall’Olmo, G., Brewin, R. J. W., Nencioli, F., & Tarran, G. A. (2020). Drivers of spectral optical scattering by particles in the upper 500 m of the Atlantic Ocean. Opt. Expr., 28, 34147–34166.

Cael, B. B., & White, A. E. (2020). Sinking versus suspended particle size distributions in the North Pacific Subtropical Gyre. Geophys. Res. Lett., 47, e2020GL087825.

Runyan, H., Reynolds, R. A., & Stramski, D. (2020). Evaluation of particle size distribution metrics to estimate the relative contributions of different size fractions based on measurements in Arctic waters. J. Geophys. Res. Oceans, 125, e2020JC016218.

Reynolds, R. A., & Stramski, D. (2021). Variability in oceanic particle size distributions and estimation of size class contributions using a non-parametric approach. J. Geophys. Res.: Oceans, 126, e2021JC017946.

Yamada, Y., Fukuda, H, Umezawa, Y., & Nagata, T. (2021). Geographic variation of particle size distribution in the Kuroshio region: Possible causes in the upper water column. Front. Mar. Sci., 8, 768766.

Woźniak, S. B, Meler, J., & Ston-Egiert, J. (2022). Inherent optical properties of suspended particulate matter in the southern Baltic Sea in relation to the concentration, composition and characteristics of the particle size distribution; new forms of multicomponent parameterizations of optical properties. J. Mar. Syst., 229, 103720.

1. Line 393: Consider adding a reference to support higher concentration of non-living particles compared with microorganisms (including Prochlorococcus) in the submicron range, for example Koike, I., S. Hara, K. Terauchi, and K. Kogure. 1990. Role of submicrometre particles in the ocean. Nature 345, 242-244.

This is a great addition, thank you for this suggestion!

Sentence now reads:

Line 433*: “Submicron particles are believed to be dominated by non-living materials, whose abundance are not expected to oscillate significantly over the diel cycle [25]. Moreover, their relative abundance compared to living particles is expected to increase with decreasing size ranges [71], and thus any diel cycles may be further muted as particle sizes decrease.”*

1. Lines 398-400: I think there is no sufficient evidence about stable background associated with submicron particles. Although data on submicron particles are scarce, there are a few studies which showed large variations in abundance of submicron particles which, in turn, must have implications to optical properties. I think you do not need to use this argument to defend your extrapolation to submicron range as described in my comment #25.

We followed your suggestion and now this aspect of the text is de-emphasized. See previous comment.

1. Lines 412-413: Indeed, one of the major problems of the inverse method applied to bulk (ensemble) measurements on all particles is that variations in refractive index among different types of particles across the entire range of particle sizes is ignored. I believe that for this reason alone this ensemble/inverse methodology is not suited to study natural assemblages of marine particles and, personally, I am not in favor in investing a lot of effort and resources to pursue this ensemble/inverse methodology in the future. However, I also think that developing a novel capability to quantitatively characterize the refractive index of marine particles in a more rigorous way is very important to advancing science. Specifically I believe the proper course for future work is to focus efforts and resources on development of a capability to determine the refractive index on a particle-by-particle basis. Such development was advocated in Stramski (1999) which postulated that both conceptually and technologically such development is possible. I have touched upon this topic in comment #9. I suggest that in section 3.5 Outlook you add a discussion about such avenue for future advancements towards determinations of refractive index on a particle-by-particle basis. In Outlook I would also downplay the further potential of methodology based on inverse method applied to bulk measurements. Please note that the idea to develop the single-particle refractive index technique for populations of natural particles which I advocated in the 1999 paper, has some analogy to determinations of particle sizes. The particle sizing methods that rely on ensemble measurements of all particles in suspension with subsequent inversion to particle size distribution have major limitations and uncertainties. In contrast, sizing particles based on single-particle analysis represent a much more robust and generally more accurate methodology (albeit uncertainties and challenges exist like in any experimental methodology).

We agree with the reviewer – at the same time, we also believe that the most science will done by the product of robust techniques and ready availability! We have modified the Conclusions to focus on the future and need for better capabilities to more accurately estimate *n*.

Line 445: Replace “measure” with “determine”. You did not measure the refractive index.

Done. Thank you.

1. Line 469: To my knowledge measurements of *n* were never made in the ocean. The refractive index was determined in some way, but not measured.

Agreed. We replaced all incorrect mentions of “measured” *n* by estimated *n*, particularly in the Conclusions section (see sentences/words in bold):

Lie 454: *“The index of refraction of particles (n) is a key parameter to determine how light scattering signals can be converted into particle size, often the parameter needed for biogeochemical applications. Yet, little is known about how n varies over time and space,* ***and in situ measurements in the ocean – to our knowledge, do not exist****. We describe a method to estimate the index of refraction of bulk particles < 10 μm, in situ, based on high frequency measurements of the particulate beam attenuation coefficient (cp) and particle size distribution collected from an Imaging FlowCytobot (IFCB) in the surface waters of the North Pacific Subtropical Gyre. We describe daily amplitudes in n from sunrise to sunset that were relatively robust even when evaluating the assumptions inherent to our methodology (ranging from 11.3 ± 4.3% to 16.9 ± 2.9%). Importantly, our estimates are qualitatively consistent* ***with laboratory-based estimates*** *that showed that n of specific phytoplankton species varies significantly over the diel cycle, with potential implications for the interpretation of optically-derived data. Such variability estimates could be crucial for more accurate estimates of biomass and growth rates from time-varying changes in abundance and particle size inferred from scattering-based in situ instruments, and improvement of satellite-based algorithms of the particle size and production, as very few global algorithms have been distributed with uncertainty estimates [72].*

***The accurate characterization of refractive index variability*** *has implications for both remote sensing and in situ measurements, both of which largely rely on optical scattering by plankton and detritus, and which are used to understand the ecological functioning of the ocean.* ***Accurate estimations of the index of refraction*** *have proven integral in modeling the size, shape, polarization properties, and distribution of aerosols and their impacts on climate models [70,73].* ***Thus, better characterization of oceanic n in situ*** *could also prove useful for the modeling of polarimetric qualities of phytoplankton, similar to what has been done for aerosols, and which would be highly relevant for the increased capabilities of measurements and algorithm development from the upcoming Plankton, Aerosol, Cloud, Ocean Ecosystem (PACE) mission [74].* ***Widespread estimates of the refractive index also*** *have the potential to influence retrievals of the chlorophyll-to-carbon parameter through modeling of the backscattering coefficient and the density of carbon per cell, which has implications for our understanding of critical ecological information from spaceborne sensors [75]. Future studies to validate and explore variation in n over time and space will improve understanding of ocean biogeochemistry and improve bio-optical models aiming at retrieving particle size and production. Development of capabilities to determine the refractive index on a particle-by-particle basis, over high temporal and spatial resolutions, potentially using a combination of multi-angle flow-cytometry, optical diffraction tomography, and cell counters [6,26,76–78] could circumvent many of the limitations of the inverse method described here****. Together with measurements of cell composition, high spatial and temporal resolution estimations of particle refractive index*** *could provide a means to revolutionize our understanding of carbon cycling, particle diversity, and energy transfer in the ocean.”*

1. Line 472: I do not agree that this is “a **straightforward** way to obtain *n*”

This is a fair criticism. We have removed that sentence.

1. Regarding the sections 3.5 Outlook and Conclusions I think they can be merged. The considerations regarding the outlook can likely be reasonably embedded in Conclusions. As described in my earlier comments, I suggest several revisions of text in these sections.

Sections have been merged and simplified.

Dariusz Stramski

Review of “In situ variability of the index of refraction of oceanic particles”. Reviewer: Emmanuel Boss, University of Maine.

This paper is an important paper addressing a long term enigma regarding what causes died cycles in beam attenuation? Changes in particle size? Changes in particle composition? or both? Using a set of data from a novel automated microscope (IFCB) the author set an inverse model to assess the likely value of the bulk index of refraction and its diet variability. While the value of the bulk index of refraction is found to not be robust to model assumptions, its diel variability does seem to be robust.

The paper is clear, well written and of interest to JGR.

I have some major comments that I believe, if addressed, could significantly improve this paper.

Dear Dr. Boss, thank you so much. As mentioned above, we could not have imagined better reviewers to check out this paper, so thank you so much for taking the time and for all the suggestions.

* 1. There is no attempt for any closure between the measurements of this paper and other measurements you have done on the cruise. Closure it the cornerstone of our science. How do we know that the IFCB indeed captured the bulk of the particles it images given that its trigger is tunable (in order not to trigger on noise)? For an example assessing these issues see Haentjens et al., 2022, L&O.

We now more explicitly mention previous comparisons with instruments such as the LISST, as well as a rationale for choosing the minimum viable IFCB diameter (see below).

Section 3.4, Line 426: *“…During this cruise, bulk optical signals of cp (660 nm) correlated well with the IFCB abundances and volume concentrations measured (****Fig. 3;*** *[21]), indicating that particles outside the range of the IFCB (< 5 μm) either co-varied with the larger-sized particles (> 5 μm); made up a stable background signal; or a combination of both. This also suggest that the bulk particle dynamics was captured by the IFCB despite the limited size range of the instrument*.”

Section 2.1, Line 120: “*The lowest particle size determined to be accurately detected by the IFCB was selected as the size bin with peak cell abundance using data aggregated over the entire cruise*”.

* 1. On the same vain you should analyze the IFCB PSDs to see where the threshold setting for image triggering stops affecting the PSD on the small size. You need to justify the 4um Dmin based on your data (see Haentjens et al., 2022 for methodology).

A series of important developments have occurred once we started revising the paper. One of our goals with this paper was to have all of our data and code readily available to allow clear, transparent, and prompt reproducibility. We wanted the data processing and analysis to be as simple as possible, and as transparent as possible, especially given the many assumptions and limitations involved when dealing with inversion algorithms with very incomplete data. While reprocessing the IFCB data in order to address some of the issues you brought up below (e.g. using cross-sectional areas instead of biovolumes, etc), we were puzzled that our reprocessed diameters (either from cross-sectional areas or biovolume) were larger than the original values used in the manuscript (and used in the Dugenne et al (2020) and Henderikx-Freitas et al (2020) papers), such that Dmin shifted from ~ 4.3 um to ~5.98 um (mean bin diameters). We were reminded that an important modification to the Moberg and Sosik (2012) algorithm was made in the Dugenne et al (2020) paper during calculation of their biovolumes and therefore estimated spherical diameters (ESD). This modification claims to have originated from a careful analysis where the first author was convinced that the Moberg and Sosik 2012 biovolume overestimated “true” ESDs when visually looking at pixel sizes of spherical particles. However, the modification is clearly cruise-specific and hard to verify and reapply by new data users. Thus, we made the difficult decision to re-calculate diameters from biovolume as in Moberg and Sosik (2012) using the most up-to-date Matlab script, since that at least guarantees that our method is transparent, reproducible, and consistent with the community standards. As seen below, however, this implies that our PSDs are further shifted to the right compared to the previous version of the manuscript.

Whereas in the original manuscript we simply cited Dugenne et al (2020) to refer the reader to the IFCB processing, we now explicitly mention:

Section 2.1, Line 113: “*Estimated spherical diameters (ESD) were computed directly from biovolume of individual particles following [39], with most recent MATAB code extracted from Dr. Heidi Sosik’s Github repository (*[*https://github.com/hsosik/ifcb-analysis/tree/master/feature\_extraction/biovolume*](https://github.com/hsosik/ifcb-analysis/tree/master/feature_extraction/biovolume)*).”*

As a result, we have revised all of our IFCB PSD data, and our Dmin is now set to 5.98 um (average diameter). That is the bin with peak particle abundances, as also characterized by the Haentjens et al., 2022 paper. Thanks for pointing this paper out, we had not seen it yet!

The text now reads: Section 2.1, Line 117: “*The lowest particle size determined to be accurately detected by the IFCB was selected as the size bin with peak cell abundance using data aggregated over the entire cruise*”. We also acknowledge that detection efficiency decreases at either limit of the IFCB range: “*An Imaging FlowCytobot (IFCB; [38]) was used in underway mode to measure particle size distribution in the ~ 3 - 100 μm size range from surface waters throughout the cruise, although detection efficiency is known to decrease at either limit of that rang*e”.

* 1. Cp of particles larger than 4um is mostly dependent on their cross-section. Why not look at the slope of Cp vs. cross-section (e.g. appendix of Berenfeld and Boss, 2006) to get a sense of the likely amount of relevant particles the IFCB captured?

Given that the IFCB already misses a huge portion of the PSD (i.e. < 5.54 um according to our new thresholds), the slope of cp vs IFCB-measured cross-section per m^3 of that limited size range would not be as informative as an “efficiency factor” as the data in the 2006 paper is (where the cross-section data was obtained from a Coulter Counter). We have been able to show on a previous paper that this ratio was indeed reasonable for LISST data during this cruise, and LISST data and IFCB data correlated very well in the size region where sizes overlapped (but of course, the LISST data has inherent problems with their black box inversion which already assumes n values). We do make a point to reference our previous Applied Optics 2020 paper where we emphasize how the temporal oscillations in several optical properties (cp, bbp, IFCB counts, IFCB volume concentrations in the nano-plankton size range), all agreed well with each other, suggesting that at least the dynamics of the bulk particle load was captured by the IFCB. Our previous analysis also suggested that the IFCB underestimated particle concentrations in the nano-plankton size range by ~ 30% compared to the LISST (although the LISST inversion clearly doesn’t provide the most accurate estimate either, and no other ground-truthing data were available to assess the bias). Fortunately, changing IFCB particle abundances by a factor of 3 would not significantly affect our main results (as evidenced by the sensitivity analysis where slope and No are varied within a wide range).

* 1. The imaginary part of the index of refraction, n’, is dependent for ‘soft’ particles (those with index of refraction close to one) *only* on the absorption coefficient (Ven de Hulst and papers by Morel and Bricaud). Hence you can get a sense of its variability from your particulate absorption measurements. This is in contrast to what you wrote in the ‘Outlook’ section.

Unfortunately, we do not have particulate absorption measurements for this cruise. Our Outlook section is now merged with Conclusions, and both were edited to focus on the need for single particle measurements of the index of refraction in the future, as well as to focus on the strengths of the analysis as pointed out by both reviewers: the somewhat robust estimates of diel changes in [n-1].

* 1. You ignore a major factor: particle shape. The IFCB software computes volume but you can also get average cross-sectional area from which non-spherically can be gained. Large particle are highly non-spherical and Cp of particles > 4um is mostly dependent on their cross-section to the beam. If you assume random orientation, you would likely be able improve your model by using this cross-section rather than using that based on a sphere with the same volume.

On one hand, we disagree that we “ignore” the issue of particle shape – we state that particle sphericity is one of our assumptions. On the other hand, we completely see your point that different measurements of particle size are more appropriate or accurate than others. Ideally, one would use the cross-sectional areas directly in a different form of Equation 4 (the cp equation), since cross-sectional area is already the parameter of interest. However, we are using Mie Theory which assumes spherical particles, and Qc specifically requires the diameter as an input, thus any calculated cross-sectional area would need to be converted to diameter in the application of Equation 4. We have gone back and forth on what to do here. We performed the analysis using cross-sectional areas and saw that the VAST majority of particles only changed diameter by about 0.3 um compared to the estimated spherical diameter obtained from biovolume. We also checked that the bulk of the particles imaged by the IFCB in this dataset can be considered spheres (eccentricity for instance is nearly always bigger than 0.75). In line with our argument in Point 2, we then chose to work with the more standard biovolume estimates (and ESDs derived from it) than deriving ESDs from cross-sectional areas.

* 1. What about diatom vacuole dynamics?

This is super interesting! This issue might be slightly outside the scope of this paper (given that diatoms made up < 1% of imaged particles from the IFCB), but we did add vacuole dynamics as a means to generally explain potential changes in *n*, in Section 3.2. The sentence now reads:

Line 311: *“Whereas daytime increases in cp, cell abundances, and volume concentrations (****Fig. 3a-c****) imply both bulk cell division and particle growth processes occurred during daylight hours during this cruise (see also [21,56]), it is not clear how n of bulk particles should change over the diel cycle. Changes in species composition, carbon per cell, water content, vacuole dynamics, or rearrangements in internal structures can all lead to changes in n [4,9,25,63].”.* We added the Stramski 1999 and Behrenfeld et al 2021 citations.

* 1. The mid-point is a poor description of the mean-size in a bin for a power law distribution. See discussions in Haentjens et al., 2022 and <https://www.oceanopticsbook.info/view/opticalconstituents-of-the-ocean/level-3/creating-particle-size-distributions-data>.

Thank you for this suggestion – we have changed our average diameters according to the non-linear weighting in the ocean optics web book when appropriate in the manuscript, and now all mean diameters are slightly shifted to the left compared to our previous estimates. We would like to point out though that although we originally used the word “mid-point” to characterize the mean size, the calculation was done using the geometric mean and not arithmetic mean. Apologies for the confusion.

In line with these changes and in the spirit of aiming to obtain estimates as accurate as possible with the data available, we decided to weight our bins by the number of particles in each bin during the power-law fit procedure. This allowed estimating the slope and No values to be used in the extrapolation to smaller sizes. As a result, slopes retrieved now represent an even more conservative range (3.91 to 5.23 instead of the previous 4.3 to 5.1). As showcased in Section 3, diel oscillations are more variable than initially presented given that the range in assumptions was now expanded, but comparisons are still robust (i.e. errorbars overlap).

The calculation of mean bin size and new fit procedure are described in Line 179:

*“We used a power-law model to extrapolate particle abundances to 0.20 – 5.54 μm using* ***Eq. 3****, where N0 is the particle abundance at reference diameter D0, and is the particle size distribution slope [44–47]. (…) where N0 and were estimated from a weighted linear regression model on log-transformed cruise-integrated IFCB-based N(Di) data normalized by binwidth, and log-transformed diameter in the 5.54 – 29.05 μm size range (bins 21 - 30), with data weighted by the number of particles in each bin using the MATLAB function fitlm.m. The upper value of ~ 29 μm was chosen as it represents the largest bin to systematically contain at least 10 particles L-1 μm-1. For the purpose of applying the fit to the IFCB data alone, a temporary mean particle size per bin was determined as the average diameter of all particles falling within each pre-determined bin. Once N0 and were obtained, the mean particle size per bin for each of the 38 bins was recalculated following [48,49], yielding mean particle sizes ranging between 0.21 μm (Dmin) and 99.88 μm (Dmax).*

We clarify that mean diameters per bin in the 0.2-5 um size range were recalculated whenever different slopes and No were considered (as in the Sensitivity Analysis section):

*Line 229: “Mean particle sizes for bins in the 0.20 – 5.54 μm size range were appropriately recalculated whenever different N0 and values were used.”*

* 1. L.115: IFCBs typically take > 23min to cycle a sample.

Our samples took between 18-21 minutes to complete.

* 1. I would replace ‘macromolecules’ with materials throughout.

Done, thank you.

* 1. You do not have any direct measurements of the index of refraction. Hence, maybe in the title and text you should use ‘proxies of the index of refraction’.

We removed all mentions of “measured index of refraction” and similar to “estimated index of refraction”. We do not think our estimates are “proxies” though because they are direct outputs (with a whole lot of assumptions!) and are presented in the correct units. What we agree is that our estimates are not validated and were not directly measured, so we have changed all text accordingly.

We have changed the title to “Diel variations in the estimated refractive index of bulk oceanic particles”.

Minor comments:

The text has [n-1] throughout but it is not in the figures or defined in the text where you talk about n and not n-1.

Done. Thank you and sorry for the confusion.

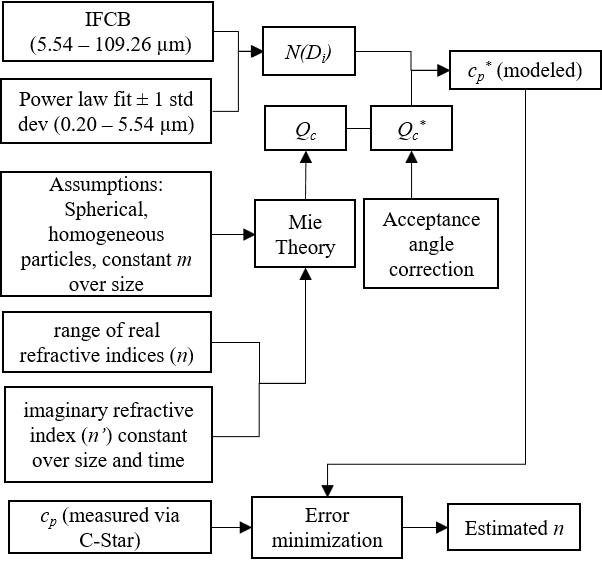
L.222- I would replace ‘net growth’ with ‘accumulation’.

Done. Thanks!

Fig. 2: add the wavelength you use and replace 2 with 0.2.

Done and done.

Updated Fig 2:



Dear authors: I am often wrong. If you feel my review is off the mark feel free to contact me and if convinced I will be more than happy to change it.