Dear Editor and Reviewers,

Thank you so much for giving us the opportunity to revise our manuscript. Replies to all of your suggestions and concerns are presented below in blue. In this revised version, we have 1) updated references and reference list; 2) edited conflicting statements for clarity; 3) included the results of a closure exercise using data from a LISST 100 X instrument. We thank you all again so much for taking the time to review our manuscript – your feedback has undoubtedly contributed to a much improved paper.

Sincerely,

Fernanda – on behalf of co-authors.

Reviewer 1:

*Review of revised manuscript entitled ” Diel variations in the estimated refractive index of bulk oceanic particles” by Henderikx-Freitas et al.*

In general, I find the revisions addressed my comments satisfactorily. I have several additional comments that require further attention and minor revisions.

Dear Dr. Stramski,

Thank you so much for revising our manuscript. Please find below detailed answers to your comments.

1. Line 16 (and elsewhere where relevant): No need to capitalize the word “theory” after “Mie”. In any event, there is internal inconsistency in the manuscript (see Line 406)

Thank you for pointing this out. All references to “Mie theory” were changed to lower case t for consistency.

1. Line 74: You cite 23 in this line but in the list of references this paper has incomplete citation (no journal, volume, page numbers). There are more problems in the list of references as I have indicated at the end of this review. Please note that for your convenience, I provided you with full citation of reference 23 in my first review. BTW, reference 23 demonstrated the photometric immersion refractometry for the suspension of marine bacteria, and not for single cells. The essence of this study was that the beam attenuation coefficient of bacterial suspension was measured in media with varying refractive index.

Thank you. I would like to start by apologizing for the state of our reference list. This was the result of using different versions of the Mendeley citation manager in different computers, while the paper was being edited from different computers. There were a few instances where the list was updated with incorrect formatting from one Mendeley version to another, and I had to keep manually change them continuously –this happened a few times during the review process and I ended up forgetting to check the final version. This is of course not an excuse, but I hope you understand that this in no way reflects lack of respect to the work we cite and how we used them throughout the text.

I have manually gone through all references and updated all missing information.

Regarding the point for Reference 23, we removed the word “single” so that all references can fit appropriately to that sentence. The text now reads: Line 72: “*Microfluidic, photonic, immersion refractometry, and imaging technologies allow for detailed analysis of the index of refraction of cells [1,23]*”.

1. Lines 193-194: Italicize the symbols of variables for consistency.

Thank you. Done.

1. Line 260 figure caption: What do you mean by “…integrated *N*(*Di*)“? The y-axis shows the density function of PSD.

We meant that the values inside each of the eddy were “aggregated” to form one PSD. We removed the word “integrated” for clarity. The y-axis is correct.

1. Lines 415-417: “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].” Although such studies are indeed very rare, the second part of this statement about no evidence for significant variation in *n*’ of detritus is untrue. In my first review I pointed out the results showing the spectra of *n*’ for detrital particles 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. These spectra were derived from single-particle absorption measurements made on multiple detrital particles collected at different sampling depths by Iturriaga and Siegel 1989 (which you cite). The main point is that Fig. 3 in Stramski & Woźniak (2005) demonstrates that *n*’ for detrital particles can vary significantly, so your statement is inaccurate. In the context of your study conducted at 660 nm, it is important to note that while *n*’ of detrital particles can vary significantly, the magnitude of *n*’ at 660 nm remains very low, i.e., on the order of 10-4 or less (as seen in Fig. 3 in Stramski & Woźniak), so the effects of such potential variations in *n*’ on your calculations at 660 nm are expected to be very small. Also, supporting your statement with references [4,25,43] is problematic, especially I find no discussion of variability of *n*’ for detritus in [4] and [25], and while [43] reports on the average *n*’ spectrum based on measurements taken on numerous particles, there is no explicit discussion of variability.

Thank you. The references 4, 25, and 43 were meant to represent the statement “Studies characterizing *n*’ of particles are rare”, so we have moved those to that part of the sentence for clarity. We have also simplified that paragraph to de-emphasize the nuances regarding *n’* (following your previous advice), and it now reads:

Line 407:

*“Particles in this study were assumed to be spherical, homogeneous, to have a constant real refractive index across the entire particle size spectrum, and fixed imaginary component of the index of refraction (n’) across time and size. Studies characterizing n’ of particles are rare [4,25,43]. 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]).”*

1. Lines 444-445: “…not a function of *n*”. This is true but it is only part of the story. You can have a function of *n*, but if the function is not monotonic then you can also have multiple solutions. This is relevant in the context of oscillatory behavior of *Q*c as a function of phase shift parameter. In particular, there can be two solutions in association with the ascending and descending slopes of the first (primary) maximum of *Q*c as a function of phase shift parameter. For “visible” light wavelengths and typical properties of marine particles these two solutions may have physically realistic values of *n*.

We acknowledged in the revised manuscript that multiple solutions could be a theoretical problem, and we provided one example of how multiple solutions could theoretically arise when we wrote “*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*." Furthermore, in Line 225 we note “*Calculation of all possible iterations through a look up table ensured that a single global minimum was obtained*.”

So, although we agree that there are other examples where there could theoretically be multiple solutions such as non-monotonic functions and probably others, for simplicity we will leave the explanation as-is since there is no contradiction between the above example and what we wrote in the text.

(7)Line 495: It is surprising to me that the authors did not make any changes in Acknowledgements in the revised manuscript. I must admit that I have been hesitant and rather uncomfortable to provide this comment because it is not really relevant to reviewer’s job. It is more like a mentorship-related comment and here it is not at all my intention to serve in this role but, in any event, I decided to share this comment as it might inspire the authors to think about their approach to acknowledgements in publications as they further pursue their professional career.

This was a big misunderstanding – I (Fernanda) assumed it was presumptuous to edit the acknowledgements section before finding out if the paper was accepted by the reviewers. It was absolutely our intent to acknowledge both reviewer’s work since they were absolutely critical to improving the manuscript and we are extremely grateful for that. However, I can imagine now a case where the reviewers never go back to see the published manuscript and therefore never get to see that the authors did appreciate their work and feedback, and for that I apologize. I will start editing the acknowledgements section during the review process from now on. Thank you very much for bringing this up to my attention.

(8)References: need more work and more attention to detail.

The correctness of references is a responsibility of authors and it is as important as thecorrectness of any other section of scientific publication. On a related note, if reviewersor readers see the lack of sufficient care in preparation of references, then it can (andlikely will) reduce their confidence that you conducted your scientific work withadequate level of care, rigor, and attention to detail. At the end, while some mistakes areunavoidable, the extent of problems in your list of references is way too large. To me it isquite surprising to see given that this is already a revised manuscript and multiple co-authors are involved in this manuscript. Here are examples that I noticed right away bylooking briefly over the list of references:

Ref 1: appears to be an incomplete citation

Ref 4: incomplete citation, page numbers 13295-13307 are missing

Ref 17: incorrect and incomplete

Ref 19: why “April”? Delete

Ref 23: correct the font of the title (do not italicize). The journal, volume, page numberare all missing

Ref 28: journal should be abbreviated

Ref 27: even your own article is cited incorrectly, check the page numbers and thevolume is missing

Ref 30: incorrect journal abbreviation

Ref 37: incorrect citation, should be “…J. Geophys. Res.109, C01014 (2004)”

**BTW you should check all original papers that you cite from J. Geophys Res. andcite them properly. Over different periods this journal either used or did not use“Oceans” as part of journal’s name and it either used page numbers or used thearticle ID number instead of page numbers.**

Ref 58: citation incomplete

Ref 62: seems incomplete

Ref 63: as above

Ref 65: incorrect citation, should be ”…J Geophys Res 115, C08024 (2010)”

Ref 67: as above, should be “…J. Geophys Res Oceans 126, e2021JC017946 (2021)”

Ref 69: seems incomplete

Ref 71: incorrect citation: the last name of the first author is Koike. Isao is the first name(if there was an error in the journal it does not mean it should be repeated in citations,especially that it was corrected).

Ref 72: as far as I know this is an incorrect citation of IOCCG Protocol Series

Ref 75: seems incomplete

Ref 78: same as above

As mentioned above, sincere apologies for the many problems with the reference list. The list has been carefully edited.

Reviewer 2:  
  
Dear authors,  
I am impressed by your thorough answers to my comments.  
However, there is one comment that you have not fully addressed. The one about closure. You have wrote in your answer to me that you have backscattering and near-forward data collected with a LISST taken at the same time (the LISST does not measure PSDs). Thus you could perform closure by using the parameters of your inverse model to obtain bb and near-forward scattering (what the LISST actually measures). Performing such closure will provide the reader the confidence that your inverse model provide consistent solutions with other measured parameters.  
Unfortunately the reviewing system does not allow me to choose a revision option. Only accept or reject. I thus chose reject even though I do think that your manuscript is very good but lacking in the critical area of demonstrating closure.  
I am uploading a few minor typos/comments I had on the manuscript.  
All the best,  
<http://prism.optica.org/Media/ViewMediaFile?mediaId=6058802&token=e51ad8f9f7818b9089323bc0b41b4c33a947c43c>

Dear Dr. Boss,

Thank you for your suggestions. We have thought a lot about this (as you know!) and you are right – if we are able to show that we can get similar results using different optical data, that would certainly be beneficial to the paper – even if we are still left with all the issues and limitations related to assuming particle sphericity, no change in index of refraction with size, extrapolation of the PSD to small particle size using the IFCB data, and assumption of time-invariant changes in the PSD for that extrapolated portion.

We have decided on presenting a short closure exercise where we show that LISST data can be used to estimate indices of refraction that turn out to be very consistent with the estimates we present in the manuscript. We have added an Appendix section where this analysis is presented. We do not think we have what we need to do a full non-linear minimization of the LISST “VSFs” vs theoretical VSFs at this time because we 1) do not have any calibration data; 2) do not have information on weighting factors for each LISST ring; 3) do not have any information on the noise model required to appropriately conduct a minimization of the theoretical VSF vs the LISST VSFs; 4) our LISST data are very noisy at several angles (even when averaging over one hour!), and because we do not have calibration data we cannot appropriately assess what we can or cannot trust (at least not easily). For those reasons, we have decided on working with a single LISST angle, since that would only require us to come up with only one time-invariant pre-factor to convert *pscat* to VSF, and we would not have to worry about changes in the shape of the LISST VSF that could be due to noise or different sensitivities per angle, etc. This is a brief, proof-of-concept exercise that we hope addresses the desire for some form of closure. Frankly, we were surprised that it worked as well as it did. So thank you for pushing us to perform the exercise!

This analysis is now first mentioned in the Results section, Line 291:

*“Importantly, the diel cycles in estimated n were reproduced by using volume scattering function data collected from a Laser In Situ Scattering and Transmissometry sensor (LISST) during the same cruise (see* ***Appendix A****), providing added confidence that the inverse model described here produces consistent solutions with other measured optical parameters. “*

The Appendix consists of text and 2 figures:

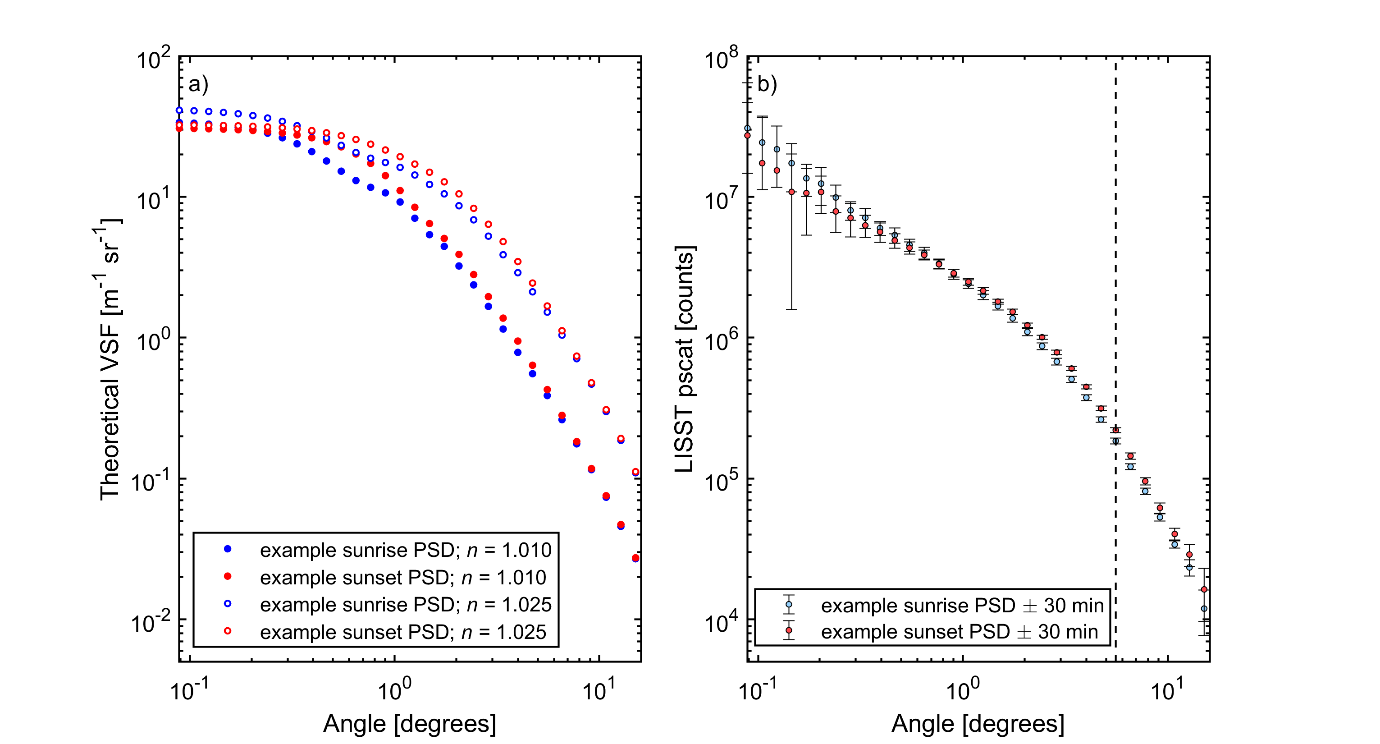
“Appendix A: Estimates of *n* from a LISST instrument – a closure exercise

We performed an exercise to estimate *n* using a LISST-100X Type-B (Sequoia Scientific) instrument instead of a beam transmissometer to show that the inversion approach yields similar results with different optical measurements. A LISST sensor was deployed in underway mode during KM1709 (see [21] for details on data collection). The sensor measured the volume scattering function (VSF) at 670 nm at 32 discrete near-forward angles covering ~15 degrees. Indices of refraction were estimated using an approach analogous to **Fig 2**, where the beam transmissometer data and Mie model were replaced with a LISST-derived VSF dataset from in situ measurements and a theoretical, Mie-VSF model.

*Computation of theoretical VSF*: Theoretical VSFs were computed assuming the same PSD used in the main manuscript (i.e. IFCB-based measurements + extrapolation down to 0.2 µm assuming = 4.57 and *N0* = 104.7252). The M11 Mueller matrix was calculated for each time step and for a range of indices of refraction using a modified version of *fastmie.m* (all data and code are available in our Github repository), at 1461 angles spanning 0 to 180 degrees. Angle-averaged theoretical VSF values matching the angle ranges of the LISST instrument were calculated to allow direct comparisons between the Mie-derived and LISST-derived signals [79].

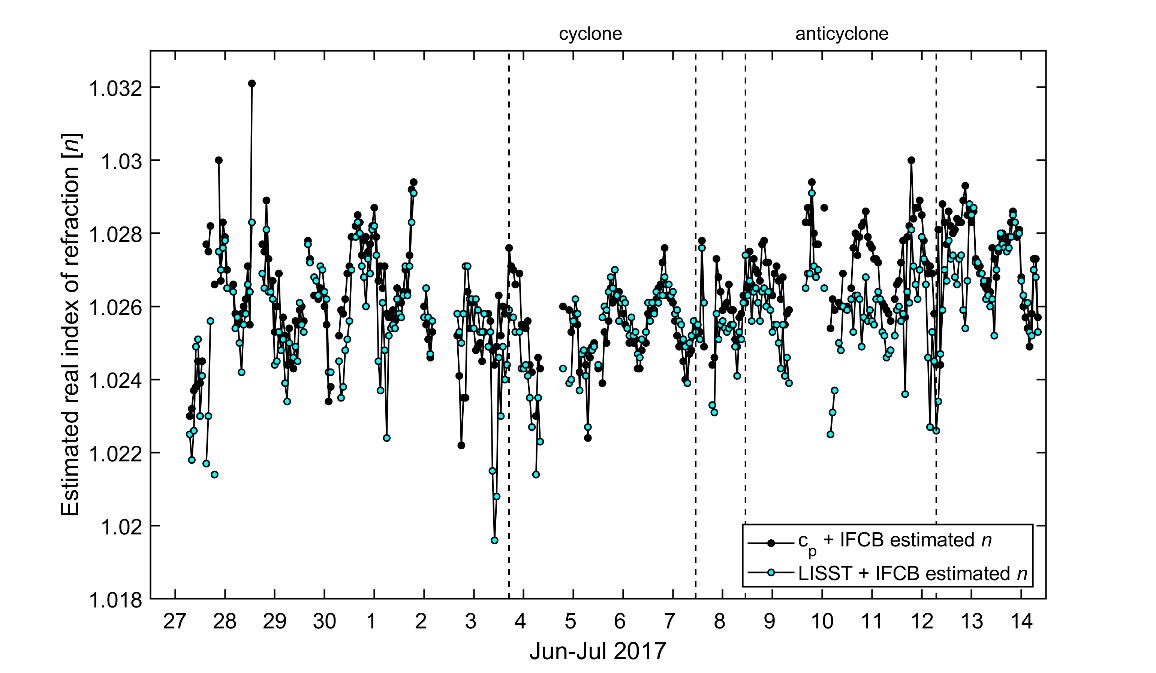
*Computation of LISST VSF:* Raw LISST data were corrected for dissolved background signals similarly to the procedure done for the beam transmissometer [21]. LISST-derived uncalibrated scattered power in each LISST ring corresponding to a scattering angle (*pscat*), which can be theoretically calibrated to VSF units, was calculated from raw LISST data following [79]. Comparisons of LISST *pscat* and theoretical VSF data for the near-forward angles matching the LISST instrument are shown in **Fig. A1**. Typically, conversion of LISST *pscat* data to VSF relies on coefficients obtained from bead calibrations across ring angles [79]. We did not perform any bead calibrations and thus for this exercise we arbitrarily chose the coefficient to be a fixed time-invariant value of 9×10-6 m-1 sr-1 count-1 (i.e. a number that allows retrieved LISST *pscat* to generally match magnitude of theoretical VSFs, see **Fig. A1**).

*Minimization procedure to estimate n:* Given that we do not have knowledge of how the *pscat* to VSF coefficient varies per angle, and we do not have information regarding weighting functions and noise models that would be useful in more accurately performing non-linear minimizations of the multi-angle LISST and theoretical VSFs, we chose to focus on a single LISST angle and how its amplitude varied over time (note also the noise in the LISST *pscat* data at both low and high angles in **Fig. A1**, which impose practical limitations to how much data can be extracted from LISST-100X measurements in clear oceanic waters). We chose to evaluate LISST angle #26 (5.13 degrees) because it showed consistent separation of *pscat* values between day and night pairs of data (see **Fig.** **A1b**), thus maximizing the chance of capturing diel oscillations in parameters (similar results were obtained when the analysis was performed on any of angles 20 through 26 – not shown). More than 85% of the signal measured by angle 26 comes from particles ~ 2 - 14 μm in diameter for our assumed particle size distributions (assuming spherical, homogeneous particles). Indices of refraction for each time point were estimated by minimizing the single angle LISST VSF against theoretical VSF for the same angle. Estimated *n* are presented in **Fig. A2** (cyan markers).



**Fig. A1.** a) Theoretical VSF representing sunrise and sunset PSDs for July 6, 2017 (using a combination of IFCB + extrapolated PSDs), and calculated using two different indices of refraction as examples. b) LISST *pscat* data for the same example sunrise and sunset times of panel a). Errorbars represent ± 30 minutes around sunrise or sunset. Vertical dashed line marks LISST angle 26 (5.13 degrees) used in the minimization procedure.

*Comparing n from two different instruments:* LISST-derived *n* and *cp* – derived *n* showed good agreement (r2 = 0.44), and comparable diel oscillations in *n* variations (see **Fig. A2**). Importantly, all assumptions and limitations discussed in **Sections 3.3** and **3.4,** in addition to limitations and assumptions inherent to the LISST instrument itself, are still applicable to these estimates, and thus this comparison does not serve as a true closure of the result itself. Furthermore, the absolute magnitude of *n* estimates are very sensitive to the choice of calibration constant, which we arbitrarily selected. Thus, we do not place much emphasis on the absolute values of *n* derived from this exercise. Nonetheless, the favorable comparisons in **Fig. A2** rule out diel oscillations in *n* as being caused by a measurement artefact of the beam transmissometer, and add confidence that our inverse model can provide consistent estimates for *n* independent of the optical instrument used.”



**Fig. A2.** Comparison of *n* estimated from *cp* measurements (black, same as Fig. 5a), and *n* estimated from LISST VSF at a single angle (cyan).

Response to PDF comments:

1. Regarding your comment about how *n*’ is absorption (Line 51), we changed the sentence to read:

Line 50*: “**Whereas variations in n’ have minor effect on bulk scattering properties at wavelengths* ***in the red portion of the light spectrum*** *[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. Regarding your comment to Line 70, we edited the sentence to read:

“…*this is certainly not feasible for global, bulk datasets and does not account for potential diel oscillations in n that could affect estimated size.”*

1. Line 78: changed “prohibited” to “prohibitive”.
2. Regarding comment to Line 104 (drift in the C-star): yes, we did see a drift in the raw C-star data throughout the ~3-week long cruise. The drift was accounted for using the filtered measurements.
3. Comment on Line 351 regarding how particle shape may be the largest source of uncertainty. We agree that particle shape is an important source of uncertainty (we mention it in Section 3.4), but we disagree that it is the largest source of uncertainty compared to the extrapolation to 0.2 micrometers. We do not know what the truth is though, and it is OK if people disagree. We touch on this point in Section 3.4 when we talk about how previous work has shown that the beam attenuation coefficient is less sensitive to changes in internal structure than the backscattering coefficient, for instance, which was an added reason why we chose not to deal with bbp in this paper as its use would only compound more and more serious limitations and assumptions on a dataset that is already full of limitations.

Overall, thank you very much for your comments. In particular, pushing us to use the LISST as a source of closure caused a lot of internal debate on our side, but we were pleasantly surprised to see remarkable agreement between the LISST-derived *n* and transmissometer-derived *n*. We would not have undertaken that exercise if it was not for your insistence, and the manuscript is stronger as a result, so thank you!