

# ***Interactive comment on “Phytoplankton community structure in the North Sea: coupling between remote sensing and automated in situ analysis at the single cell level” by M. Thyssen et al.***

**M. Thyssen et al.**

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Some methodological aspects are described too succinctly, particularly for non-experts. This is the case with Section 2.1 that would deserve some general explanatory sentences in several paragraphs to make it clearer. This is also the case for the optical classification used to select the match-ups. How was it derived and applied? Same comment for the PHYSAT approach. It has been well described in the literature but at least the equation defining the anomaly ( $R_a$  in the text,  $nLw^*$  in Fig9) would complete the manuscript. The additional material does not need to be long but should help in

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making the overall text clearer and more fluid without the necessity to consult the cited literature. It would also ease the discussion. See also more detailed comments below

Reply: We thank the reviewer for its interest in the PHYSAT method and for the willing in describing the method more in details. However, the PHYSAT part is only a first attempt to use the cytometry dataset (analysed in detail in this paper) for potential labeling. We do not think the different methods should be described again in this paper. Please note that it has been done in the methodology description papers (Alvain et al., 2005; 2013). The description of the use of the flow cytometer in the material and method chapter is much more complete than any paper using flow cytometry in order to evidence the difference between this specific instrument and conventional flow cytometers. The discussion (Section 4) is interesting but should be completed. For instance for a non-expert, it might not be clear to what degree the associations between clusters and phytoplankton types (p.15638-15639) are solid or informed speculation. What about Micro1 in that respect (it is not mentioned)? Something should also be said in terms of optical properties: do the types preferably found associated with anomalies N1 and N2 have specific optical properties, and are these consistent with the anomalies? See also more detailed comments below

Reply: The description of the phytoplankton clusters in terms of genus or functional types are based on a combination between the knowledge of the North Sea phytoplankton ecosystem (pheaocystis blooming, diatoms and dinoflagellates) and the specific combination between pigment content and size, as for orange fluorescing cells (synechococcus and cryptophytes). The associations are thus based on speculations. A sentence was added in the discussion to make it clearer. "Thus cluster identification at the species level is speculative and, as any cytometric optical signature, it needs a sorting and genetic or microscopic analysis analysis to be resolved at the taxonomical level. This deep level of phytoplankton diversity resolution requirement is although not needed in biogeochemical processes studies in which functionality is preferred to taxonomy (LeQuéré et al., 2005). In this context, most of the optical clusters could be

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described at the plankton functional type level because of some singular similarities combining abundance, size, pigments and structure proxies obtained from optical SFC variables (Chisholm et al. 1988; Veldhuis and Kraay 2000; Rutten et al. 2005; Zubkov and Burkill 2006).” Micro1 cluster was left behind and additional description of it was added in the discussion. “The Micro1 cluster could correspond to small nanoplanktonic diatom cells ( $\sim 10\text{--}30\ \mu\text{m}$ , Fig. 6G). Regarding the size range, this cluster could represent several species. They were mainly found within the Humber area.” We did not try to explain the Ra with the optical properties resulting from the phytoplankton community. Indeed, IOP are not measured for this case study and once again, this paper doesn’t propose a new or revised PHYSAT method. So we don’t think appropriate to study this aspect at this time. Sentence to clarify the main objective of this paper have been added (PHYSAT application is just a first very preliminary test in order to shown the potential of cytometry data, the crux of the study).

Another point of discussion is about the optical classification. A working hypothesis is that the selected match-ups are characterized by relatively clear waters not affected by sediments. Still the considered area is known to often present significant amounts of sediments and/or CDOM, and the data used in Vantrepotte et al (2012) are mostly from coastal waters. Overall, could the anomalies in nLw be explained by subtle changes in sediment and/or CDOM concentrations?

Reply: Yes they could, this is the reason why the use of the Vantrepotte et al reflectance signature classification makes the anomalies processing based on similar optical classes as robust as possible. However, as we don’t propose a new PHYSAT version but only use 5 days of high frequency data of cytometry to show future potential use of such in situ dataset, it’s really too early to answer this question (we agree with the reviewer, this question has to be addressed in the future PHYSAT development in order to take into account more waters types).

It would be nice to also illustrate the nLw spectra associated with the match-ups, and not only the anomalies (Fig .9). This is important to understand how specific the find-

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ings are: are they likely to change completely from one cruise to the next, or are there elements to suggest that they can be the first blocks to actually build a bridge between in-situ determinations of community structure and remote sensing?

Reply: As discussed by the reviewer, the data is expected to change from one cruise to the other since we are looking at a daily/weekly scale community structure from radiance anomalies. It is expected that the increase of in situ datasets will afford large combination between anomalies and phytoplankton community structure in the North Sea. This would result in theoretical validations of anomalies and a quantitative description of the anomaly. But this is not the aim of the present paper. As explained to Reviewer 1, this paper is a first trial between high potential instruments but on a very short time scale. This paper show the high power of implementing in situ automated single cell analysis systems such as flow cytometry with remote sensing (by showing two distinct types of anomalies associated with two different cytometry compositions). We do not pretend to propose an adapted PHYSAT method at this stage. Currently several projects in the North Sea and in the Mediterranean sea aims to implement this type of flow cytometers on ships of opportunity. So that, based on this first attempt that shows the potential, more PHYSAT anomalies will be potentially linked to phytoplankton functional community changes in the future.

p.15639 I.8: Dodge et al. (1977) or Dodge (1977)?

Reply : done

I.11: “spatial” I.17-20: not clear what is meant here about the PHYSAT algorithm.

Reply: sentence was simplified: “The PHYSAT method was built on an empirical relationship between dominant phytoplankton functional types from in situ HPLC analysis and Ra. The method was thus limited to dominance cases only as HPLC analysis can’t give us more information.”

p.15640 I.1: “The combination of SFC ... ” I.2: “associated with”

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Reply= done

I.6: “Spatial succession ... ”: it should be made more explicit why and how this applies to the presented results in terms of species. How is it relevant to the identified clusters?

Reply: The possible identification of the clusters based on several referenced works, geography, sizes, abundances and images when available are described before in the discussion. We let the readers make their own conclusion about them but we prefer to keep the cluster’s name as it is described in the Results section. The idea is to keep precocious about the identification power of cytometry.

I.17: “Myrionecta”: please specify what this species refers to, zooplankton? (isn’t “rubra”?)

Reply: yes indeed, it is Myrionecta rubra or Mesodinium rubrum and not rumbra. It refers to microzooplankton (ciliate) which keep the pastids of their main food source, a cryptophyte cell, to perform photosynthesis as a supplement to hetrotrophic nutrition, and they are visible with the image in flow device as they pass through the tubing.

I.21-27: how does this paragraph relate to the rest of the discussion?

Reply: This sentence was replaced at a proper place in the discussion, i.e. before describing the possible species belonging of the clusters.

p.15644 I.11: “phytoplankton” , “growth” Fig.2: “Presented data are”

Reply= done

Fig.3: are the colors consistent across the different panels? (do they always refer to the same cluster?)

Reply= yes

Fig.3b: “Maximum” (y-axis) does not appear fully on my copy.

Reply= corrected

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11, C9218–C9224, 2015

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Fig.7: “Small black scares”: “diamonds”?

Reply: Indeed

Fig.9: is there a unit for  $nLw^*$ ? Is it the same thing as  $R_a$  referred to in the text?

Reply: There was an error in the legend, it corresponds to the  $R_a$ .

Fig.10: “Wilcox”? C7230 Fig.11: “Wilcox”?

Reply: Indeed, it is the `Wilcox.test()` used for the Wilcoxon test.

Fig12: it is not easy to distinguish colors in the blue-green range, so it is hard to see the distribution of frequencies. They seem low, particularly for  $N_2$  ... .

Reply: The distribution of frequencies corresponding to the two anomalies is not widespread. The reason is because this work is a first test based on 7 days mapping used to process these maps. The idea is not to extrapolate the phytoplankton community structure over an entire season because we expect serious changes from one week to the other. We would like to remind the reviewer that our main aim is not to furnish a new PHYSAT method at this stage, but to show, for the first time, that high frequency phytoplankton analysis based on cytometry can contribute to the labeling of remote sensed anomalies. It would be much too presumptuous regarding the small data sets collected and its complexity in terms of community, although highly profitable on such a little period of sampling. Of course, based on our results future studies will be pursued in order to improve PHYSAT and to give more detailed and representative maps. But this is too early at this stage.

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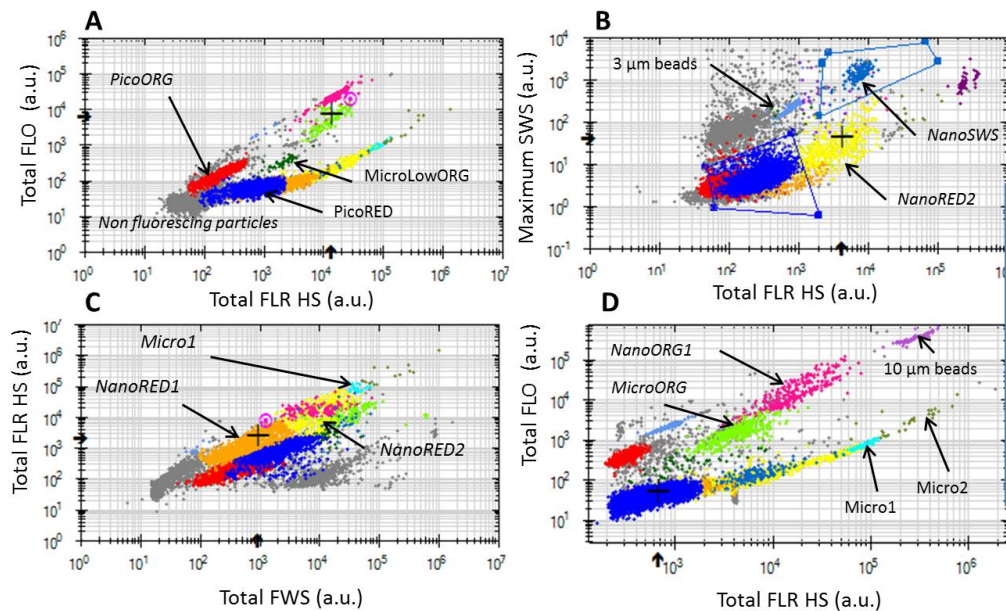


Fig. 1. Figure 3

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