High resolution analysis of a North Sea phytoplankton community structure based on *in situ* flow cytometry observations and potential implication for remote sensing.

We thanks the reviewers for their substantial comments that enable to improve the paper and the message we want to deliver. We would like to express our surprise concerning the fact that the majority of the questions is about PHYSAT while the main part of our work is about cytometry measurements analysis (at high frequency). PHYSAT maps are shown as a first preliminary test to show the high potential of coupling such innovative in situ data with remote sensed observations.

We do not claim that we propose a new PHYSAT version in this paper (even if we hope it will be the case in the future based on this first work). We just show that two distinct situations detected with the flow cytometry approach can be linked with two different anomalies from remote sensing. We suppose that the title was not clear enough so that we have changed it to clarify this point. We have added some sentences in the text in order to decrease reader's expectations about this aspect.

We hope the paper fulfills the reviewer's expectations now we have clarified some points and we are waiting for a positive decision for publication in Biogeosciences.

Reply to reviewer 1:

The aim of this paper is to develop specific approaches to characterize the phytoplankton community structure and its high frequency variation in time and space. For this purpose, the authors combine plankton community structure using automated flow cytometry and remote sensing algorithm such as PHYSAT. The manuscript is well written and the topic is very interesting and relevant for environmental and ocean colour researches. I do recommend it for publication, however the authors should improve the paper (see comments above).

General comments:
The authors are interested in a regional area of the North Sea. They have used ocean colour data within 4 km of spatial resolution. Maybe, the study could improve using satellite images with 1.1 km of spatial resolution.

Reply:
At the time the matchups were selected, the remote sensing dataset available for PHYSAT was the 4km resolution. We would like to insist on the fact that the aim of the paper was not to improve PHYSAT. PHYSAT maps are just shown as a potential application so that the question of resolution seems not essential at this stage. However, we agree with the reviewer, future application and PHYSAT development will need the best resolution.

Once PHYSAT has been applied, the frequency of occurrence of the two distinct anomalies (N1 and N2) were very low. Can you explain why these percentages are very low? Could authors explain the phytoplankton community that include N1 and N2?

Reply:

Again, PHYSAT maps are shown as a potential application. The two anomalies found during the 5 days cruise can't be representative of all the variability over the area. A lot of other kind of anomalies exist (in different areas and seasons) and will potentially be identified using cytometry in the future. This explains why the frequencies of the two anomalies found in this first test are not high everywhere.
Our aim is to show that it's possible to find different anomalies with different composition based on cytometry. This shows for the first time that PHYSAT is not limited to dominant cases as it was before with HPLC (pigments) data. Areas where the frequencies of the two anomalies are low correspond to other sets of anomalies not found within our 2 days matchups between the in situ measurements and the satellite pixels. At this scale of anomalies selection, the variability is high and this variability is used in the paper of BenMustapha et al., 2014.
Text has been added to better explain that our aim is not to improve PHYSAT and to furnish a new method adapted to the area. We only want to show (for the first time) that cytometry analysis at high frequency can be potentially used to label PHYSAT anomalies in the future as two distinct types of anomalies have been associated to two different in situ compositions. This work encourages people to develop in situ cytometry measurement and coupling with remote sensed data.

« This paper shows for the first time that SFC datasets can be used for labeling PHYSAT anomalies at the daily scale. The SCF is a powerful automated system aimed to be implemented in several vessels of opportunity and monitoring programs for future PHYSAT anomalies identification at the daily scale and at the community structure level."

Minor comments:
Page 15625, Lines 14-15. You can include more studies perform with PHYSAT in regional scales such as Mediterranean Sea.

Reply: Thank you for this reminding, we have added the Navarro et al. 2014 reference in the list.

Page 15630, Lines 25. Please, give more information about the turbid mask using in this study.

Reply: The turbid mask used in this paper is the one described in the paper of Vantrepotte. As our paper is not focused on the improvement of PHYSAT, we decided not to explain again this method here. We would like to invite the reviewer to read the Vantrepotte et al. paper for more information as we have apply the method without any changes.

Page 15631, Line 5. Although PHYSAT is a well know method established in the scientific literature, I think that the authors should give more information about PHYSAT method using in this study.

Reply: We thank the reviewer for its interest in PHYSAT. However, as our paper is not focused on the development of a new regional exhaustive, we decided not to explain in detail this method here. Papers referenced in the manuscript about PHYSAT methodology are easily accessible on the internet for more detail.

Page 15635, Line 11. Which LUT has been used to calculate Ra?

Reply: Once again PHYSAT is not the main aim of our paper. However the LUT is based on the relationship used in Alvain et al. 2005 and 2008. This relationship has been applied to regional dataset used in this study. A sentence about that has been added in the text:

"PHYSAT radiance anomalies (Ra) were calculated based on the 2005 method (Alvain et al., 2005) and the average signal was recalculated to fit the sampling area. "

Page 15635 , Line 15. Explain why authors did not use the Ra 555 nm.

Reply: This wavelength was not implemented in the PHYSAT calculation for MODIS at the time it was processed for the paper. We think it's not crucial as we only want to show that the two specific phytoplankton compositions detected by cytometry can be associated with two specific anomalies at different wavelengths.

Figure 9. Please, include in fig 9c and 9d the threshold from Table 3 to compare with the individuals spectra.

Reply: The thresholds were highlighted on the figure 9.

Reply to reviewer 2 :

This study attempts to address issues inherent to the spatial and temporal variability of phytoplankton community structures in marine ecosystems. The authors present an interesting novel method to help improve estimates of phytoplankton community structure derived from satellite imagery using calibration from high-resolution flow cytometry data. The authors conducted a 4 days-survey of the phytoplankton communities in the North Sea using a scanning flow cytometer and compared their results with estimates derived from PHYSAT algorithm, a model that estimates the dominant phytoplankton groups based on anomalies in satellite-based ocean color. While the authors used a interesting method a significant and collected a significant amount of data, I think the authors failed to present and discuss their results in a meaningful way. The authors did not address a specific question with that method and no substantial conclusion was reached. In my opinion, the manuscript is lengthy, there are too many figures (5-8 can be supplemental), and the discussion section does not discuss the findings in a broader context. The manuscript focuses too much on the scanning flow cytometer and not enough on the coupling between flow cytometry data and PHYSAT model output. The author's conclusions are 1) Abundances of phytoplankton vary along the transect (line 14-15), 2) the sum of the red fluorescence of each individual phytoplankton cells correlated with bulk chlorophyll estimates (line 15-18), 3) the high-frequency Cytobuoy enable 2-3 more matchups with satellite data than traditional, low-frequency water sampling. As is, these three main results feel short of my expectations considering the amount of data collected in this study. I was disappointed after reading the manuscript due to the high expectations built in the title, which are not met by the current version of the manuscript. I recommend the authors to rewrite the discussion and resubmit a more concise version of the manuscript.

Reply: We thank the reviewer for his interest in the PHYSAT method and its expectation of a more detailed description of the importance in getting synoptical basin scale phytoplankton community distribution. However, our main aim is not to furnish a new PHYSAT method with an improve and exhaustive labeling adapted to the North Sea in this paper. We only want to show that cytometry observations can potentially be used in association with remote sensing data. It's the first time that specific composition (not only dominance based on pigments analysis) can be associated with specific

anomalies. We don't have the objective to give a new PHYSAT method or a regional PHYSAT observation at this stage.

To clarify this, the title was modified in order to decrease the level of expectation although the importance of getting high resolution phytoplankton community structure at the basin scale either for ecological or biogeochemical purposes is clearly mentioned in the discussion.

The paper describes the distribution of phytoplankton community structure, size and contribution to chlorophyll from high frequency in situ analysis, and then the data set enables a first attempt to label two different types (not all of them…) of PHYSAT anomalies with in situ automated flow cytometry. We think that presenting the in situ phytoplankton datasets (distribution, size and contribution to fluorescence) is still an important descriptive part useful for any ecological survey in the area or comparison with other datasets.

Furthermore, at this stage of research and with such little sampling days, the use of the data set is not representative enough for any broad conclusion on phytoplankton distribution in the North sea. It is not possible with this little level of time/seasonal resolution to get substantial and mean full conclusion. It would overtake the capacity afforded by the present dataset. However, we really think that our work is interesting enough by showing, for the first time, that two sampling days of cytometry can furnish matchups for a first remote sensed anomalies labeling, with a description of the phytoplankton community instead of the phytoplankton dominant group only as previously described.

Reply to reviewer 3:

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 (Ra in the text, nLw* in Fig9) would complete the manuscript. The additional material does not need to be long but should help in 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 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 (~10-30 µm, 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 findings 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
l.8: Dodge et al. (1977) or Dodge (1977)?

Reply : done

l.11: "spatial"
l.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
l.1: "The combination of SFC
...
"
l.2: "associated with"

Reply= done

l.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.

l.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.

l.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
l.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

Fig.7: "Small black scares": "diamonds"?

Reply: Indeed

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

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

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 N2

...

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Reply: The distribution of frequencies corresponding to the two anomalies is not widespread. The reason is because this work if a first test based on 7 days mapping used to process these maps. The idea is not to extrapolate the phytoplankton community structure over en entire season because we expect serious changes from one week to the other. We would like to remind the reviewer that our main aims 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 futures 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.