Dear Melilotus Thyssen,
Thanks for your opinion, here follow the answers of your questions:

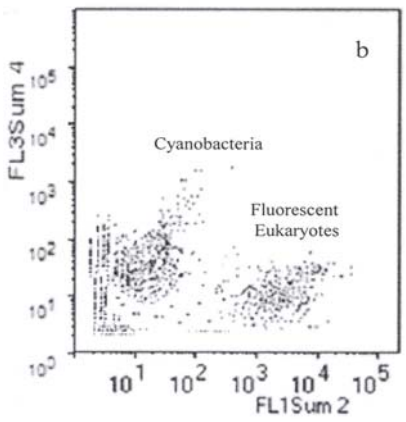
P6247 I19: I agree when you say that flow cytometers do not analyze the total suspended particles but particles present in the analyzed volume passing through the flow cell however, beyond a peristaltic pump that takes the sample to the flow chamber, the CytoSence (CytoBuoy) technology uses a volumetric injection pump system that allow all particles in a given sample (user predefined volume) be enumerated. Anyway, the word Total will be changed as you indicate.

P6248 I3: Sorry, I was thinking only in how fast the sample runs in the system, but it is really more appropriated to use the term mm3.s-1. It will be changed. In relation to trigger levels it was 0 (without any) in Figure 2a since our objective is to detect all possible particles in the sample (of course we have huge amount of noise) but in the case of Figure 2b it was applied a level of 30 (beyond this it was gated out since it is a normal procedure in cytometry).

P6248 I4-5-6: Again I agree with you and these procedures were made in the lab before field operations. However, in the sea it is a very complicated operation. In fact, we did not transport the buoy inside the motorboat, we hauled it. The option to connect a tube with beads solution was not considered in that time but in this case we should have used one tube something like 3 meters long. In our case, the logistic support comes from the Brazilian Navy and their boats are not so small like an inflatable that could be better.

P6248 I16: You are correct about the possibility of detecting and measuring stained cyanobacteria in this way, but I have considered two things: i – If we have an in-situ measurement it is always more real than enumerations from small and fixed samples. ii in natural samples we do not know what sort of organisms are present and their sensibility in relation to fixation and transport. Of course this argument can be also used to heterotrophs and anyone can ask about damages provoked by the hydrodynamic forces inside the tubes and the system or high speed in flow rates. We never know!

P6249 I3: Yes I can, it will be provided.
OBS. Noise detection is one of our ongoing research objectives and not easy to find. In my experience it is not limited to the established threshold, it can be found over it. We have tested the imaging in flow technology coupled to the CytoBuoy and CytoSub and found many empty images. There are numeric values in their matrixes but anything was really photographed in the range of 10 to 300 micrometers (looking for zooplankton). We have been talking with the manufacture that saw the same. Noise or something wrong? In my view, in monitoring operations and systems all data must be classified by pattern recognition models and one class should be noise. I mean, models must recognize noise. We have to keep in mind that the ultimate goal is to work in Real Time or very near it.



P6**249 L4:** As I explained above, the conditions to work with buoys in the sea are not always good, sometimes it can be hard. Connect tubes was not really thought. In this way, we have preferred to order to the manufactures the development of a new and redesigned system to allow us to use beads, fluorochromes or specific fluorescent probes on board like the CytoBot developed by Olson and Sosik in USA. In relation to shifts, of course large changes can be related to instrumental errors, but in my experience using the MoFlow sorter instrument, I have seen large changes in the clusters since they are not made of one species, but can have many ones according their similarities in optical conditions. Not only cell division but nutrients, salinity, temperature etc can influence the optical signature of each particle (organism) making many overlaps. So, such changes in cytometric patterns are usual and have to be viewed with care.

P6249 L4: I agree with you and it will be changed as suggested.

P 6249 L4: No, I am not referring to size; maybe it should be more appropriated to use fluorescent eukaryotes.

P6249 L10: Sorry, it was my fault; all measurements were converted to milliliters and will be corrected.

P6249 L13: definition of suspended particles is, all particles present in the sample. Unfortunately I can not provide an estimation of cell-size based in numerical values of TOF and FWS length since I used the old CytoWave software (.cpf) and this version do not allow us to export this information however I can access a histogram as the following. Probably I can include this one as Figure 2c.