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A new automated flow cytometer for high frequency *in situ* characterisation of heterotrophic microorganisms and their dynamics in aquatic ecosystems

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Abstract –
In aquatic environments, one can distinguish autotrophic phytoplankton on one side and heterotrophic microorganisms on the other side, both compartments known to play a major role in aquatic ecosystem functioning. Phytoplankton communities are the major responsible for primary production. They respond very quickly to environmental changes, even at the hour scale [1], and the automated high frequency sampling and analysis was evidenced as a crucial need to address their dynamics. The development of the first automated flow cytometers about 20 years ago, such as the Cytosense instruments (Cytobuoy, b.v..) allowed investigation of phytoplankton dynamics based on a single cell approach in almost real time while addressing their short-term variability that was previously out of reach [2][3][4][5][6]. Heterotrophic microorganisms such as prokaryotes (bacteria) represent the main consumers and mineralizers of the organic matter in the ocean as well as in inland waters. Small predators such as nanoflagellates and small ciliates are also known to be very important in controlling both phytoplankton and bacteria abundances. These microorganisms are in strong interaction with phytoplankton and may also quickly react to environmental changes. In contrast with phytoplankton, they do not naturally emit an autofluorescence recordable by flow cytometry, which makes their analysis more complex. Their dynamics at short time and spatial scales are poorly documented. To fill this gap and extend the high frequency observation capacity of flow cytometry (FC) to heterotrophic microorganisms, an automated staining module (SM) was jointly designed by MIO and Cytobuoy company in order to be implemented on a modified version of a Cytosense flow cytometer (Cytopro) optimised for small particles. The SM features were combined with those of a Cytosense to match requirements for the sampling, staining with a fluorescent dye, and incubation, mandatory to observe hetrotrophic prokaryotes (HP) by flow cytometry. In this study we present the first results from two experiments conducted with the SM coupled to the new Cytopro version, and thus demonstrate its suitability for the high frequency automated analysis of HP abundance in aquatic environments. This achievement makes a real breakthrough in aquatic microbiology since there is no other available instrument than the Cytopro with the capacity to analyse heterotrophic microorganisms at high frequency and at the single cell level. The reported Cytopro experiment addressed the dynamics of a natural microbial community and was conducted during 7 consecutive days in a microcosm. The automated HP abundance analysis revealed a shift in their abundance between 1 and 3 days, while at the same time we observed the increase of another heterotrophic group, identified as small predators (nanoflagellates) by microscopy. The innovation brought by this study resides in the successful automated *in situ* HP analysis performed at high frequency (every hour) and remotely controlled during extended time periods (days-to-weeks). Subsequently, the Cytopro and its SM were placed on a scientific research vessel for the PEACETIME (ProcEss studies at the Air-sEa Interface after dust deposition in the MEditerranean sea) cruise in the Mediterranean Sea. The seawater was continuously pumped from subsurface along the ship track. Both Cytopro and SM were remotely controlled and operated from land by a MIO scientist thanks to satellite connection to perform both phytoplankton and bacteria analyses at high resolution in the Mediterranean Sea. The reported results demonstrate that Cytopro is suitable for investigating dynamics of both phytoplankton and heterotrophs in aquatic

ecosystems at high temporal resolution over several weeks. The staining module combined with the Cytopro presents a real breakthrough in aquatic microbiology as, in addition to providing a complete picture of the microbial compartment dynamics in real time, it also makes possible to use viability or physiological dyes [7][8] and thus generating a more complete picture of the microbial compartment structure and functioning.

I. REFERENCES

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