Michel Denis, J Oceanogr Mar Res 2018, Volume 6
DOI: 10.4172/2572-3103-C1-004

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Recent advances in the *in situ* automated high-frequency observation of marine microorganisms at the single cell level to address their spatial-temporal dynamics

Marine microorganisms play a major role in oceanic biogeochemical processes, both regarding organic matter production, the feeding source of the entire marine food chain and its mineralization. Their large diversity and short cell cycles make them very sensitive to changes in their environment. Their size classes cover 4 orders of magnitude and their diversity encompasses several thousand species. To overcome these difficulties and account correctly for their dynamics in the natural environment in the frame of global change and carbon cycle, it is critical to observe them *in situ* at an hour timescale and at the single cell level. Similarly, to better apprehend their spatial distribution dynamics, high-frequency observation has to be carried out at submesoscale. Building on the first commercialized automated *in situ* (Cytosub, Cytobuoy.com, NL), flow cytometer dedicated to phytoplankton, we addressed its dynamics at the hour time scale and its spatial distribution at sub-mesoscale. Our 12-year experience leads to several improvements of the instrument features. For practical reasons, we preferentially used the non-submersible Cytosense on pumped seawater and demonstrated its efficiency when analyzing pumped seawater from a coastal laboratory, a buoy or a ship where the instrument could be remotely controlled providing internet access. *In situ*, high-frequency observation of phytoplankton was also critical to validate the algorithm (PHYSAT) developed to identify the dominant phytoplankton groups from remote sensing data. To extend this approach to non-photosynthetic microorganisms, we designed and developed (in close collaboration with Cytobuoy), a new instrument (Cytopro) equipped with an automated staining module that is now validated and tested on a cruise. The Cytopro is more focused on analyzing small cells, particularly heterotrophic prokaryotes (at best, every half hour). This advanced technology opens the access to new fields of research requiring *in situ* high-frequency observation of marine microorganisms.

Biography

Michel Denis was recruited at the CNRS in 1969 to develop physics approaches in biology: A study of electron transfer in the mitochondrial respiratory system with the help of various optical, potentiometric and magnetic techniques. He joined the Center of Oceanology of Marseille (COM) in 1985 to apply these approaches to marine microorganisms whose respiratory systems were largely unknown. His activity evolved towards the individual study of marine microorganisms using flow cytometry, an emerging technique in the marine environment at the end of the 1980's. He thus introduced this approach to COM and trained students in this discipline.

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