NEW NUCLEIC DYES FOR PICO- AND NANOPLANKTON CYTOMETRIC ANALYSIS

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Introduction. Flow cytometry (FCM) is a promising tool in the field of aquatic phytoplankton ecology because it allows for multi-parameter assessment of the physiological state of individual cells in an algal population. It can help to elucidate major questions such as phytoplankton taxa identification, the evaluation of cell quantity and viability, and the measuring of phytoplankton and general microbial metabolic activities. Traditionally, microalgal characterization is performed by microscopic analysis using UV-excited nuclear dyes (e.g. Hoechst and DAPI) or dyes that are excited in the blue-green part of the spectrum such as propidium iodide and eosin. The development of multi-laser cytometric systems has widened the possibilities for multi-parametric analysis and cell sorting of phytoplankton populations. Notwithstanding, significant algae autofluorescence originating from different types of chlorophyll and accessory pigments may overlap with propidium iodide and/or eosin staining and affect the resolution of algae clusters and cell sorting.

Materials and methods. We applied different nucleic dyes from the SYTOX family (SYTOX Orange, SYTOX Green, SYTOX Blue; Life Technologies, Carlsbad, USA) to characterize and sort phytoplankton populations using the 4-laser FACSAria cell sorter (BD Biosciences, San Jose, USA). SYTOX Blue dye (excited by 405 nm violet laser) has insignificant spectral overlap with algae autofluorescence, and therefore provides a strong signal and makes it possible to differentiate small algal clusters in phytoplankton samples.

Results. Combining SYTOX Blue staining with CountBright absolute counting beads (Life Technologies, Carlsbad, USA) and autofluorescence assessment allowed us to develop a new method of analysis of algal growth kinetics and viability (validated with *Emiliania huxleyi* pure algal cultures).

Conclusions. We conclude that using fluorescent dyes such as SYTOX Blue excited by violet laser can be a helpful approach for a number of analytical and algae cell sorting applications such as FACS sorting of non-cultivated phytoplankton clusters for whole genome amplification (WGA) and the assessment of growth and viability of mixed algal cultures.

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