White Paper | Cytek® Aurora | Cytek® Northern Lights™

CYTEK®
TRANSCEND THE CONVENTIONAL

Characterizing Marine Phytoplankton with Full Spectrum Profiling™ Technology

Introduction

The marine phytoplankton community, composed mostly of unicellular photosynthetic microorganisms ranging in size from less than one micron to several hundred microns, is responsible for almost half of the atmospheric oxygen produced each year by photosynthesis[1,2]. In addition, Sean Nee et al. described life on Earth as mostly microscopic and unicellular, with organisms between 0.2 and 100 µm in size contributing to 50% of the biomass on Earth[3]. Consequently, gathering information about the structure and dynamics of these unicellular organisms, and notably the phytoplankton community, is critical to our understanding of how our global ecosystem functions and how it reacts to changes in climate.

Flow cytometry (FCM) has been widely used to study marine planktonic microorganisms since the late 1970s. Since then our understanding of the primordial role of unicellular microorganisms in the global ecosystem and the biogeochemical cycles of elements on the Earth has been increasing[4,5]. For marine microbial ecologists, FCM makes it possible to study the major compartments of the microbial ecosystem such as virioplankton, bacterioplankton, small phytoplankton, and small predators at a single cell level from a multiparametric perspective[6]. FCM allows for improved statistical strength with its ability to study thousands of phytoplankton cells per second, far surpassing throughput using microscopic interrogation.

With the Full Spectrum Profiling™ (FSP™) technology now onboard the Cytek® Northern Lights™, Aurora and Aurora CS systems, we are collecting the full spectrum of emitted light from these cells. Compared to conventional FCM, which captures only sections of the emitted light, as determined by the cytometer's filters and mirrors, FSP technology measures the entire emission spectra produced by each cell; this ultimately increases the optical resolution of the various phytoplankton groups identified in natural seawater samples. Samples were collected in the frame of the French Coastal Observation Service SOMLIT (National Observation Service of the CNRS-INSU). SOMLIT is a monitoring network deployed on 12 ecosystems of the French coast. In this paper, we will show you how phytoplankton samples were analyzed using FSP technology and discuss the benefits of this technology for marine phytoplankton studies.

Materials and Methods

Phytoplankton samples were collected on the surface of French coastal sea waters (Dinard, Arcachon, Banyuls-sur-Mer, Marseille, Villefranche sur-Mer; France). Cryotubes were used to collect 2 mL of seawater from each location. Samples were fixed with a mixture of glutaraldehyde (0.25% final dilution) and pluronic (0.01% final dilution)[7] for 20 minutes at room temperature and then stored at -80°C after liquid nitrogen fast freezing. They were kept frozen until analysis in the laboratory. After thawing, samples were analysed using FSP technology, without the need for prior staining, to examine the intrinsic autofluorescence given off by their photosynthetic pigments.

Samples were analysed using a four-laser (355, 405, 488, 640 nm) Cytek® Aurora system. Photosynthetic organisms such as phytoplankton contain chlorophyll which emits red fluorescence when excited by the 488 nm laser. The red fluorescence was used to define photosynthetic organisms using the B8 detector. A threshold has been set on B8 detector from the blue laser which corresponds to the pic emission wavelength of the chlorophyll. The flow rate was set at 100 µL/min and approximately 500 µL of sample was acquired, enough to detect less abundant populations like nanophytoplankton. The default CytekAssaySettings were modified to maximize the separation of the different clusters of the cyanobacteria Synechococcus sp. (UV9 gain value was increased to 2014 by 500%) (results not shown). Violet SSC was used rather than blue SSC to improve the optical resolution of the smallest populations such as

Cytek Biosciences, Inc. 47215 Lakeview Blvd, Fremont, CA 94538 1-877-922-9835 sales@cytekbio.com

the cyanobacteria Prochlorococcus.

Analyzing Phytoplankton

To examine phytoplankton cells, acquisition focuses on the autofluorescence of the photosynthetic pigments. This way, it is possible to ignore other nonfluorescent particles such as bacteria or debris, present in large numbers in marine water. The composition of the seawater samples depends on many environmental variables such as depth, the location where the sample was collected, or the type of water, coastal vs. open ocean, even if some populations are ubiquitous. A lot of nonbiological particles lead to significant background noise in the water and make it difficult to extract the signal of biological populations from it, particularly for the small or dim fluorescent cells such as the minute cyanobacteria Prochlorococcus. Phytoplankton populations can be differentiated by their optical scattering properties (Figure 1). Because of the small size of the cells, the "Height" of the optical pulses is the most informative metric. Indeed, the "Width" and the "Area" of the pulses are less efficient (except for cell cycle analysis), particularly for the small particles/cells whose size is smaller than the width of the laser beams (typically around 5 µm). The most abundant populations in these marine water samples are the smallest, such as viruses which are <1 µm in diameter with a concentration up to 107-8 events/cm3 and challenge the optical detection limit of FCM instruments.

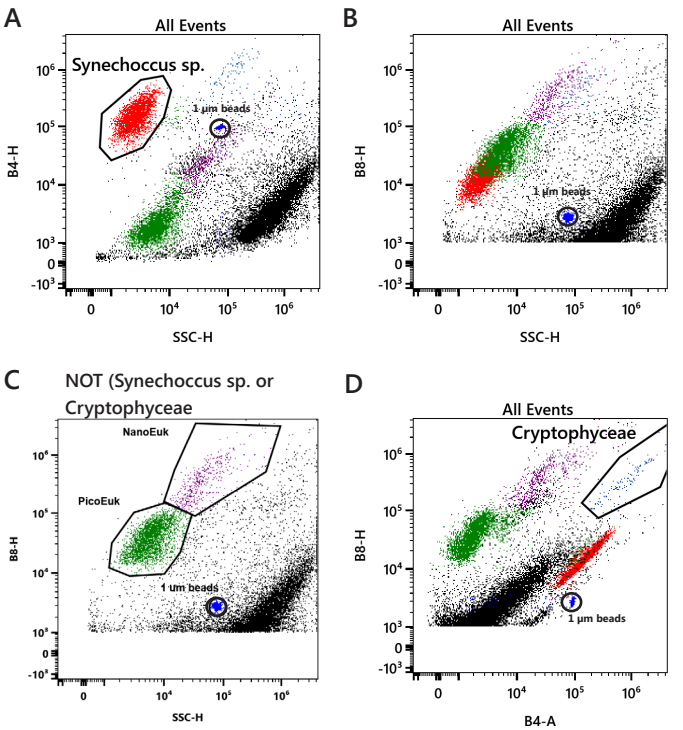

Figure 1: Dot plots produced from the analysis of a seawater sample by the Cytek Aurora system and used to gate the various phytoplankton populations.

The particles displayed in Figure 1 are not homogeneously distributed on the dot plots, but tend to group into several clusters based on their fluorescence and scatter intensities. To better resolve the various clusters, it is mandatory to take into consideration the multiparametric dimension of the flow cytometry analysis and perform a complex gating strategy based on the combination of several dot plots. In Figure 1A, side scatter versus fluorescence channel B4, which corresponds to the peak emission channel of phycoerythrin (PE), visually differentiates phycoerythrin-containing cells corresponding to the cyanobacteria Synechococcus sp. Another group of cells, the Cryptophyceae group, is usually mixed with eukaryotic nanophytoplankton groups but distinguished by the presence of PE. One can gate this specific class by using its high fluorescence in channels B8 and B4 (Figure 1D).

Finally, after applying an "OR GATE" between Synechococcus sp. and Cryptophyceae group, then a "NOT GATE" to the "OR GATE", it is possible to distinguish and gate the eukaryotic pico- and nanophytoplankton populations (PicoEuk and NanoEuk, respectively, Figure 1C). Picophytoplankton refer to cells with a size less than 2-3 µm, and nano-eukaryotic phytoplankton refer to cells between 2-3 and 20 µm[8]. The limit between them is not always obvious but the use of fluorescent microspheres may help[8].

Using FCM, most microbial phytoplankton species can be monitored to understand the structure and dynamics of this autotrophic compartment in the ocean in relation with the hydrology and trophic interactions.

Application Advantages with FSP Technology

The Cytek® Northern Lights™, Aurora and Aurora CS systems are differentiated from other flow cytometers by their Full-Spectrum Profiling (FSP) technology. With FSP technology, you can record the complete fluorescence spectrum of every single cell and capture information about each cell's autofluorescence. The blue (488 nm) laser beam excites different accessory pigments within phytoplankton. These accessory pigments act as energy donors to chlorophyll-a which in turn emits red fluorescence. The higher the amount of pigment, the higher the intensity of fluorescence emission. Since the geographical location (latitude) and the depth that the marine samples were collected from influence both the quality and the quantity of irradiation available for photosynthesis, this leads to different pigment compositions in the various phytoplankton species, and

Cytek Biosciences, Inc. 47215 Lakeview Blvd, Fremont, CA 94538 1-877-922-9835 sales@cytekbio.com

consequently, different fluorescence spectra. Cytek's full spectrum flow cytometry captures the overall fluorescence signal of these pigment combinations at once using FSP technology. Of course, the more lasers available on the instrument, the more possibility of exciting the various pigments: phycoerythrin is optimally excited by a yellow-green laser (561 nm) but can also be excited by a blue laser (488 nm); chlorophyll-a can be directly excited by violet (405 nm) and far red (>630 nm) lasers, and is indirectly excited by a blue laser; the accessory pigments are excited by a blue laser and transfer the energy to chlorophyll-a.

Figure 2A displays the gating strategy used to define Cryptophyceae, NanoEuk and PicoEuk populations. The various fluorescence spectra produced by the pigments contained in the major flow cytometry groups after the excitation by the UV, violet, blue and red lasers onboard the Cytek® Aurora system are shown in Figure 2B. Each spectrum has some variance as different populations of phytoplankton adapt to the intensity and quality of light, water depth, and particle load in their environment.

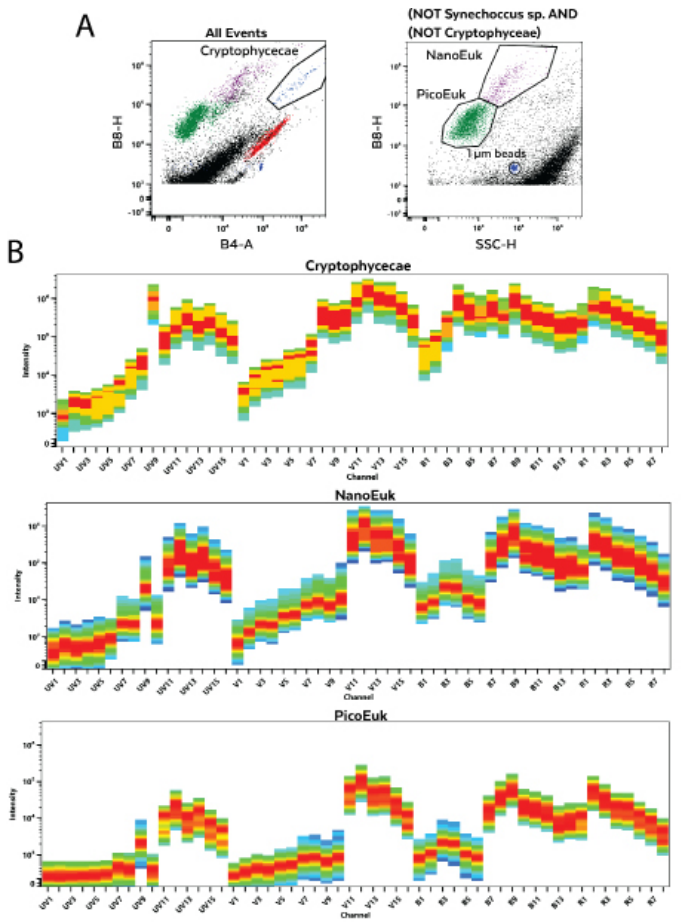

Figure 2: A) the major groups of phytoplankton are displayed in a commonly used SSC vs Red fluorescence channel dot plot. B) the corresponding full spectrum signature recorded for each phytoplankton group is shown in a spectrum plot obtained from a four-laser Cytek Aurora system (355, 405, 488, and 638 nm). The color in each plot is used as a scale to highlight the density of the spectra within each detector channel, e.g. the lowest density of cells in blue to the highest in red.

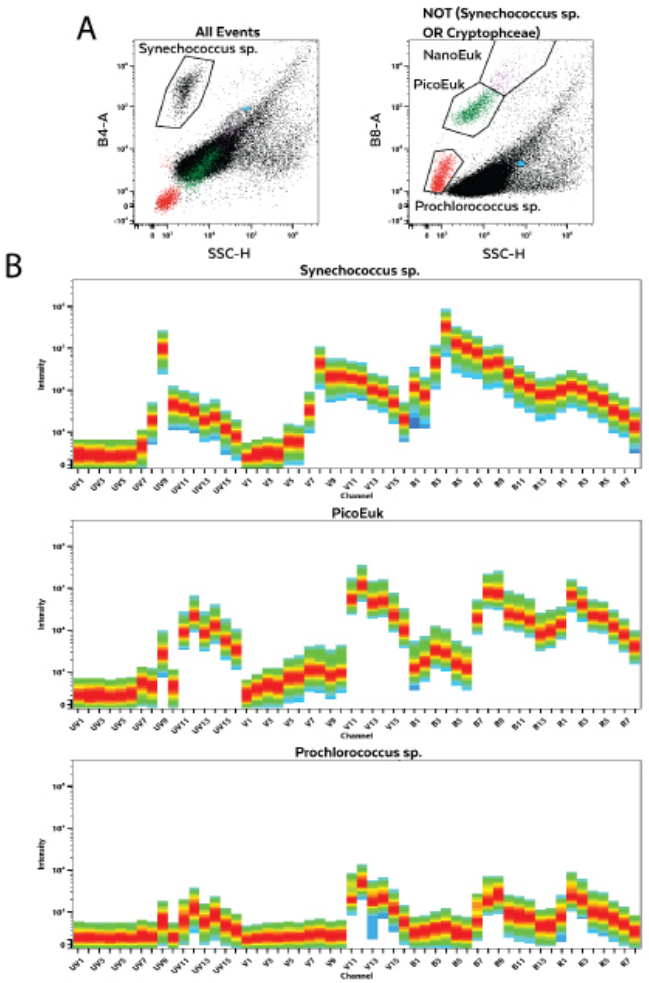

Figure 3: A) Dot plots and B) full spectrum signatures recorded for the smallest cells of the phytoplankton community, among which the Prochlorococcus (cyanobacteria).

Even the very dim and very small Prochlorococcus (Figure 3A) can be resolved with the Cytek® Aurora system which is sensitive enough to collect the dim red fluorescence of these cyanobacteria (Figure 3B). All of Cytek's full spectrum systems are sensitive enough to collect the fluorescence spectra of the Prochlorococcus cells and are able to discriminate their signal from background noise.

Figure 4 shows the spectra of Synechococcus sp.-like populations sampled across five French coastal marine stations at the same time. The green arrow indicates the maximum fluorescence of these populations caused by

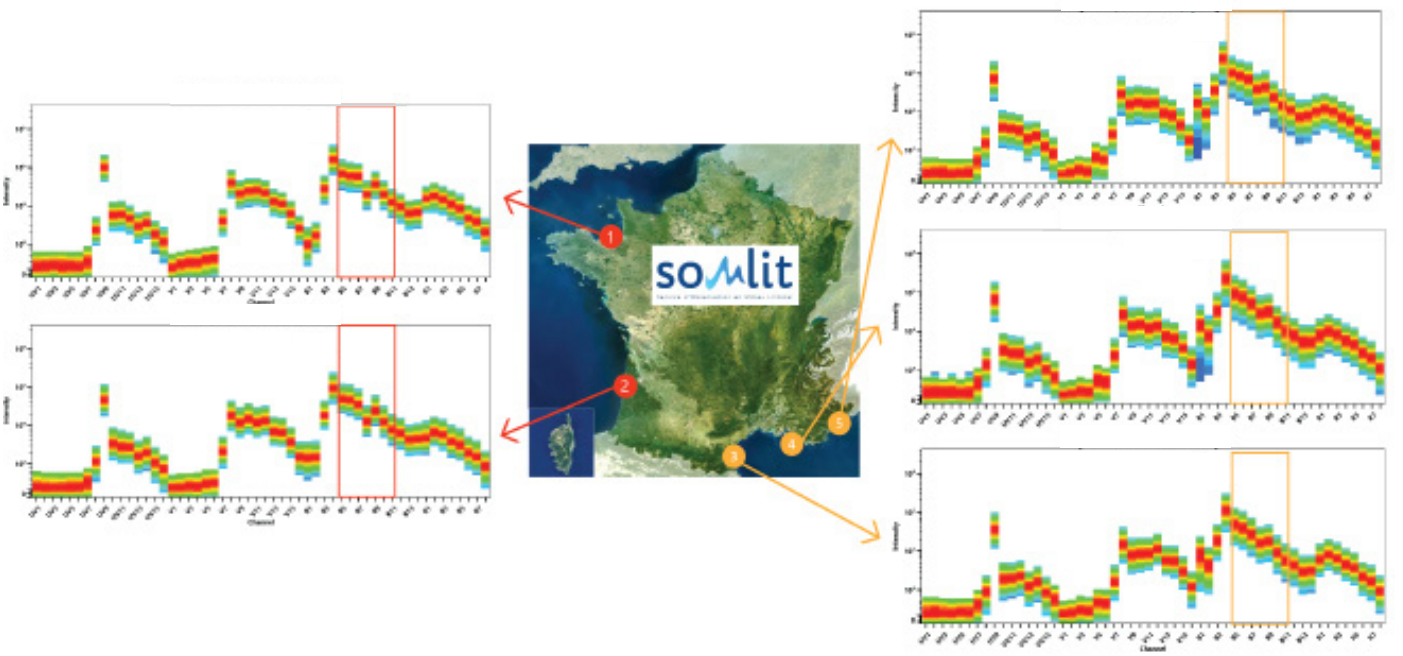

Figure 4: Full spectrum signatures recorded for the Synechococcus cluster at 5 different stations of the SOMLIT network. The peak intensity is highlighted by the green arrow. There are small differences in the shape of the signatures between the samples from the Atlantic ocean (red rectangles) and the Mediterranean Sea (orange rectangles).

the phycoerythrin pigment. The maximum fluorescence (green arrow), caused by the phycoerythrin pigment, is similar between samples collected at the five different marine stations. However, there are slight spectral differences between the samples collected in the Atlantic ocean (red rectangles) and the Mediterranean Sea (orange rectangles) which could not be detected using conventional FCM. As displayed in Figure 4, samples collected from the Atlantic Ocean (1-2) share the same spectra as indicated by the red rectangle. Similarly,

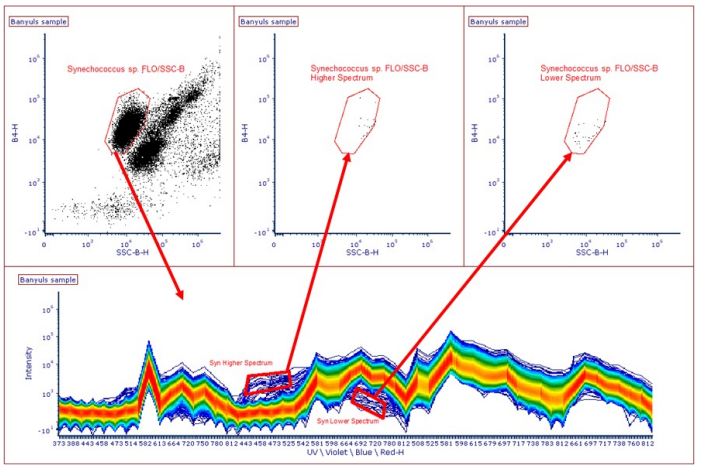

Figure 5: Evidence of the heterogeneity of the Synechococcus cluster from variation in its full spectrum signature.

samples collected in the Mediterranean Sea (3-5) share the same spectra as each other (orange rectangle) but their spectra is distinct from the spectra observed in samples 1-2 obtained from the Atlantic ocean.

Another interesting feature when working with spectrum plots, rather than a discrete measurement of a single narrow band of fluorescence emission, is the improved ability to discriminate the phytoplankton groups and the background noise of the seawater. Background noise is mostly generated by non-fluorescent particles, such as heterotrophic prokaryotes, small predators like nanoflagellates, and small debris in seawater. In Figure 5, the full spectrum of the Synechococcus cluster was explored using FCS Express™ Version 7 Software (De Novo Software). When gating on two different regions of the spectrum plot, labeled “Syn Higher Spectrum” and “Syn Lower Spectrum”, there is evidence that within this phytoplankton group, a small number of particles show slightly different spectra compared to the rest of the group. The first set of particles from the “Syn Higher Spectrum” gate show higher blue-green fluorescence while the second set of particles from the “Syn Lower Spectrum” gate show lower red fluorescence. Both of these sets emit in response to violet excitation and show a different pattern than the bulk of the Synechococcus sp. cells. Sorting out these populations with a spectral cell sorter, such as the recently released Aurora CS system, would allow further exploration of these populations and

would allow us to characterize these cells and determine whether these populations represent a subspecies of Synechococcus sp., or are Synechococcus sp. in a different physiological state, or are a different species altogether.

Summary

The analysis of full-spectrum signatures of phytoplankton using FSP technology onboard with the Cytek Aurora system allows a better characterization of the various microbial phytoplankton communities compared to conventional FCM. Additionally, FSP technology helps to better separate background noise in heterogeneous marine water samples from the phytoplankton signals. As briefly demonstrated here, it is also possible to explore spectral heterogeneity within population clusters, heterogeneity that is not discernible with conventional FCM. FSP technology paves the way for visualization of marine microbial ecosystems. Recent advances in cell sorting utilizing FSP technology will allow further interrogation of marine water samples and will help bring new insights into the functioning of marine photosynthetic life.

Authorship

David Pecqueur[1], Gerald Gregori[2] and Juliette Desfrancois[3]

1. Sorbonne University, CNRS, BioPIC Platform, FR3724, Oceanological Observatory of Banyuls-Sur-Mer, Banyuls-sur-mer, France.
2. Aix Marseille University, University of Toulon, CNRS, IRD, Mediterranean Institute of Oceanography (M.I.O), Marseille, France
3. Cytek Biosciences, Inc.

David Pecqueur and Gerald Gregori conceived the study and prepared the manuscript with technical application support from Juliette DesFrancois.

Acknowledgements

The authors would like to thank the SOMLIT National Observatory services and its team for providing the samples that were used in this study.

References

1. Azam, F., Fenchel, T., Field, J. G., Meyer-Reil, R. A. and Thingstad, F. (1983) The ecological role of water column microbes in the sea. Mar Ecol-Prog Ser 10:257–263.
2. Field, C. B., Behrenfeld, M. J., Randerson, J. T., and Falwoski, P. G. (1998). Primary production of the biosphere: integrating terrestrial and oceanic components. Science 281, 237–240. doi: 10.1126/science.281.5374.237
3. Gasol J.M., Morán X.A.G. (2015) Flow Cytometric Determination of Microbial Abundances and Its Use to Obtain Indices of Community Structure and Relative Activity. In: McGenity T.J., Timmis K.N., Nogales B. (eds) Hydrocarbon and Lipid Microbiology Protocols. Springer Protocols Handbooks. Springer, Berlin, Heidelberg. https://doi.org/10.1007/8623_2015_139
4. Marie D., Brussaard C., Partensky F. and Vaulot D. Flow cytometric analysis of phytoplankton, bacteria and viruses. 1999. In: Current Protocols in Cytometry. John Wiley & Sons, Inc. 11.11.1-11.11.15.
5. Marie, D., Rigaut-Jalabert, F. and Vaulot, D. (2014), An improved protocol for flow cytometry analysis of phytoplankton cultures and natural samples. Cytometry, 85: 962–968. doi: 10.1002/cyto.a.22517.
6. Nee, S. More than meets the eye. Nature 429, 804–805 (2004). https://doi.org/10.1038/429804a
7. Pomeroy, L. R., 1974. The ocean‟s food web: a changing paradigm. BioScience 24: 499–504.
8. Sieburth J.M., Smetacek V., Lenz J (1978). Pelagic ecosystem structure : heterotrophic compartments of the plankton and their relationship

Find out more at www.cytekbio.com.

Please contact sales@cytekbio.com for any questions

CYTEK®
TRANSCEND THE CONVENTIONAL

N9-20079 Rev. A