A first attempt at flow cytometry off the coasts of Tanzania and Comoros Island

Keshnee Pillay[1] Edward Senkondo[2], Masumbuko Semba[3], Jerry Geofrey Mang'ena[4], Fadhili Mustafa Malesa[4], Ceiça Alfredo Chioze[5], Samir Ali Said[6], Fatma Hassan Manyenze[7], Riaan Cedras[8], Seshnee Maduray[1], Kuhle Hlati[1], Marco Worship[1]

[1]*Department of Environmental Affairs: Oceans and Coasts, P.O. Box 52126, V&A Waterfront, Cape Town, South Africa, 8000*
[2]*Tanzania Fisheries Research institute, P. O. Box 9750, Dar es Salaam, Tanzania*
[3]*Nelson Mandela African Institution of Science and Technology, Arusha, Tanzania*
[4]*Insitute of Marine Science, University of Dar es Salaam, P.O.Box 668, Mizingani Road, Zanzibar*
[5]*National Institute of Fisheries Research, Av. Mao Tsé Tung 389, Maputo, Mozambique, 4603*
[6]*The National Agency of Maritime Affairs, BP 97 Moroni, Union of Comoros*
[7]*Pwani University, P. O. Box 195, 80108 Kilifi, Kenya.*
[8] *University of Western Cape. Private Bag x17, Bellville, 7535*

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Background

Flow cytometry is a technology that measures optical attributes of a single particle or organism flowing through a fluid medium. The instrument is made up of three systems: fluidics, optics and electrics. The fluidics system transports the particles to the light beam, where the optics system illuminates the particles and returns the light to the appropriate detectors and the electronics system converts the detected light signals into electronic signals that can be recognized by a computer and analysed using special software. The application of flow cytometry is vast with applicability in many different fields of science. Of interest is using this technology for counting and determination of phytoplankton taxa based on the signature of pigments found in different plankton.

Methodology

Water samples were collected during the International Indian Ocean Expedition 2 onboard the *SA Agulhas II* during the July 2018 cruise around the coasts of Tanzania and Comoros Islands. 15ml of water was taken from the surface and F_{max} on every station. These samples were preserved with 1% formalin and stored at -20°C until further analysis. These samples were fully defrosted and 1ml of sample was analysed in triplicate using a BD Accuri™ C6 Cytometer. This particular cytometer has the ability to conduct absolute counting by calculating volume analysed thus making calculating a biomass a very simple task. Using the standard model "3 blue 1 red", 480nm emissions are read on the FL1, FL2 and FL3 lasers (blue) and 640nm emissions are read on the FL4 laser (red).

Results

The forward and side scatter detector yielded information on the relative size and complexity of the cells analysed when compared to each other. This analysis gave results of nano- and pico-phytoplankton (0.2µ - 20µm). Chlorophyll (detected by red laser, wavelengths >640nm) is present in all photosynthetic organisms therefore on its own cannot be used to distinguish

between taxa. But this can be used to determine the biomass of the photosynthetic organisms i.e. phytoplankton that is alive and the base of production. Using the accessory pigments found in an organism, taxa can be distinguished. Using the most abundant of these, phycobilins (wavelengths between 450 – 650nm) and carotenoids (wavelengths 450 – 490nm) broad taxa identification was accomplished. Species level determination of marine free-living procholorophytes was of interest. This group was discovered in 1988 with the use of an early flow cytometer and are too small for detection through traditional microscopy. The pigment composition of marine *Synechococcus* and *Prochlorococcus* produces a very characteristic signature. Both taxa have a similar level of both forward light scatter and side scatter, although *Synechococcus* tends to give a slightly higher signal. However, *Synechococcus* cells are easily recognized by the large orange fluorescence due to their phycobilins, compared to prochlorophytes, which lack this. To the authors knowledge this is the first flow cytometry results for the open ocean in the region of Tanzania and Comoros.

Conclusion

Flow cytometry allows fast, accurate and reliable results without the intensive training of other techniques. Traditional microscopy does not allow the same or even comparable sample turnover rate that flow cytometry offers. This means flow cytometry is less time-consuming and over time more affordable that traditional microscopy. With respect to very small picoplankton and viruses flow cytometry comes into its own as it is fast becoming the go-to method for analysis of these groups.

Keywords: Flow cytometer; Comoros; Tanzania; picoplankton; *Synechococcus*; *Prochlorococcus*