FOREWORD

Almost exactly 10 years ago, Clarice Yentsch and Paul Horan co-edited a special issue on "Cytometry in Aquatic Sciences" (*Cytometry* 10, 1989), which soon became something like a bible for users of aquatic flow cytometry all over the world. As the past 10 years have seen revolutionary developments in computer- and fluorescence technology, it seemed worthwhile to look at what has been achieved in the aquatic sciences with the help of flow cytometry and what can be expected in the near future. So the title of the workshop held at the Research- and Technology Centre of Kiel University (FTZ) in Büsum, Germany in October 1998 was "Aquatic Flow Cytometry: Achievements and Prospects", the proceedings of which you are holding in your hands. Most of the papers have a reviewing character, but some authors's recent findings are also included. Although 10 years may not seem like a long period, in a field of rapidly developing technology, it is a life time.

The exploration of planktonic communities has been promoted by flow cytometry considerably during the past decade, notably the full recognition of the role of picoautotrophs in oceanic environments. The development of fluorescence technology and specific immunological and molecular labelling methods has probably had the strongest impact on the use of flow cytometry in the field of aquatic science (besides computer technology). More generally, a diversification of research interests into two "schools" has evolved over time: the quest for the oceanic picophytoplankton on the one side, and the measurement, identification and quantification of "larger" in-shore phytoplankton on the other. While for the former group, commercial instruments are generally suitable without major modifications, the latter group realistically requires a special design, including absolute volume counts, a large volume through-put, and the ability to handle filamentous and even colonial species. Naturally, almost all research has been carried out on commercial instruments, only few attempts have been made to construct specialized instruments. So far, such instruments have not been commercially available, and thus have been realized in special projects, such as for the Optical Plankton Analyser (already presented in the Cytometry 10 volume, 1989), its successor, the EurOPA with its various modules, and in subsequent projects (as the Cytobuoy, see p. 255), financed by the Marine Science and Technology Program of the EU.

The papers in this volume cover a wide range, from phytoplankton and bacterial ecology, physiology and taxonomy, to technical aspects like the construction of self-sufficient deployable floating cytometers and imaging modules. A general overview over flow cytometry in phytoplankton research is given by Veldhuis and Kraay (p. 121), who also introduce some interesting novel applications like the assay of automortality of cells (well known as *apoptosis* in the non-aquatic flow world). The paper by Dubelaar and Jonker (p. 135) has a similar scope, but the authors supplement their review with a flow cytometry "poll" among various european marine science institutes, yielding an interesting state-of-the-art snapshot of flow-based aquatic research in Europe. A typical application of flow cytometry in marine microbial ecology, namely the characterization of the ubiquitous coccoid cyanobacteria of the genus *Synechococcus*, is presented by Denis *et al*. (p. 157).

The use of fluorescent antibodies to account for specific cell types is certainly the key application of flow cytometry in the medical field – in aquatic ecology, it is still far from routine, as is demonstrated in detail by Peperzak *et al*. (p. 165). Generally, fluorescence technology has evolved dramatically during the past decade. Most instruments are still equipped with 488nm excitation lines, which represents a reasonable compromise for the excitation of both Chlorophyll a and Phycoerythrine, the two main autofluorescent pigments accessible by flow cytometry. However, DNA stains until recently were only available for UV excitation (DAPI, HOECHST), and those that were available for the 488nm line emitted in the deep red (Propidium Iodide, Ethidium Bromide), considerably overlapping with Chl. a emission. Green-emitting DNA specific probes and physiological probes have much facilitated work on growth rates

(i.e. cell cycle analysis) and other physiological parameters. However, the use of fluorescent probes for the determination of physiological parameters, such as enzyme activities, has more or less just begun (Jochem, p. 183).

Due to their small size, relative uniformity, and lacking autofluorescence, heterotrophic bacteria have not been so easily analyzable by flow cytometry as the phytoplankton; however, the technological progress in staining and labelling procedures has considerably improved the situation, as Gasol and del Giorgio show (p. 197). Especially fluorescently labelled rRNA probes have opened up a new dimension in the identification and specification of aquatic microorganisms, especially for the morphologically indistinguishable picoplankton (e.g. the AIMS project, Jonker *et al*., p. 225).

Several years ago when I (MR) was studying Biological Oceanography at Kiel, we indulged in fancies about a deployable instrument which was able to measure *every* parameter of interest, from the simple things like temperature and salinity, to nutrients and plankton biomass and composition. We called it the "holo-probe". Instruments like the Hardy Continuous Plankton Recorder which were able to collect plankton samples on slowly rotating gauze tissue, were ingenious at the time, but seemed a little awkward to us (as the punch cards and tapes in the early days of computer technology). The ability of many flow cytometers to physically separate individual cells from a mixture or community has long been an outstanding feature of flow cytometry (Reckermann, p. 235), and presents another approach to the recognition and visualization of the unknown organisms. Recent developments like instruments equipped with image-in-flow modules (Kachel and Wieczorrek, p. 247) and especially Dubelaar´s Cytobuoy (Dubelaar and Gerritzen, p. 255) show that our notion of the "holo-probe" was closer to reality than we thought. Indeed, a deployable, freely navigable, remotely controlled instrument, combining the Cytobuoy module with automated nutrient, salinity and temperature analyses seems technologically feasible, provided enough money and effort are spent. A ship-bound version of what might be called a "holo-probe" is currently being planned in the EU "Ferry-Box" project, using "ships of opportunity" like ferries and freight carriers.

So, in conclusion, the use of flow cytometry has become well established in the study of aquatic ecosystems. The analysis of live phytoplankton samples has been much facilitated. Although a species identification can only be achieved in special cases, major taxonomic and/or physiological groups can be easily detected. Thus, flow cytometry has become an integral part of our eco-physiological toolkit, and can be expected to play an increasingly important role in the future, as new technological opportunities evolve. Still – one thing should still be borne in mind when rejoicing about the new technological possibilities – it is all nothing without the human brain and its imagination.

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The Editors