

PROTIST NEWS

Meeting Report: EU Workshop “Analysis of Single Cells in the Marine Phytoplankton” (ASCMAP), Alfred Wegener Institute for Polar and Marine Research, Bremerhaven, Germany, April 15–21, 2002

From 15–21 April 2002, the EU workshop ASCMAP (Analysis of Single Cells in Marine Phytoplankton) took place at the Alfred Wegener Institute for Polar and Marine Research in Bremerhaven, Germany. A substantial part of the workshop was based on results obtained during two EU projects, AIMS (Automated Identification of Microbial Populations; MAS3-CT97-0080) and PICODIV (Monitoring the diversity of photosynthetic picoplankton in marine waters; EVK3-CT-1999-00021). The aim of the workshop was to combine three important techniques in modern phytoplankton analysis – flow cytometry, artificial neural networks and molecular probes – which were presented by talks, posters and practical hands-on demonstrations to the participants. The interest in this combination was obviously huge and around 90 participants and speakers from more than two dozen countries attended the workshop. In total, over 40 talks and the same number of posters were presented at the workshop in addition to half a dozen different practical demonstrations; hence only a fraction of them can be mentioned here.

Flow cytometry, a method originally developed for the field of medicine, has already found its place in the analysis of aquatic organisms. The principle is based on the analysis of single cells in a flow stream which pass through a narrow light beam (laser or lamp) at high speed. The light that is scattered from the cells or emitted in the form of fluorescence, is detected and can be used to identify and characterize the cell. Depending on the type of flow cytometer used, up to a dozen parameters can be measured per cell at a time.

The basics of this technique, the instrumentations and their possible applications in marine biology were presented by leading experts in the field: Peter Burkill (Southampton, UK), Glen Tarran (Plymouth, UK), Bill Li (Dartmouth, Canada), Alex Cunningham

(Glasgow, UK), Marcus Reckermann (Büsum, Germany) and Marcel Veldhuis (Texel, The Netherlands).

After an introductory talk of Peter Burkill which focused on the development as well as the recent and future applications of flow cytometry in the analysis of aquatic single cells, George Dubelaar (Bodegraven, The Netherlands) explained the special technical requirements necessary for flow cytometric applications in the marine environment. His work led to the development of the CytoBuoy and Cyto-Sub flow cytometers that can be used for autonomous sampling and monitoring on board ship. These special instruments were also practically demonstrated during the workshop. A similar topic was addressed by Glen Tarran, who showed data from the use of flow cytometers on board ship collected during different cruises. His impressive results demonstrated clearly the power of this approach for investigating phytoplankton directly during a cruise. After the data are collected by flow cytometry, they must be processed and analysed, which is not a trivial task as was shown by Richard Jonker (Amsterdam, The Netherlands), who emphasized the importance of controls and objective criteria. An additional feature of some flow cytometers is the possibility not only to characterize cells but also to sort them out of mixed assemblages for later analysis or culturing. Markus Reckermann explained this approach and its advantages using the high-speed sorter FACSVantage (Becton Dickinson) and his investigations of the phytoplankton community at the German Wadden Sea. The problems that arise when one tries to derive optical properties of cells from flow cytometric data were addressed in the talks of Alex Cunningham and Marcel Veldhuis. Phytoplankton with its wide variety of cell shapes and pigments is quite different compared to the cell types that are analysed by flow cytometry in the

medical field. The different cell characteristics provide unique opportunities for research approaches, but can also make them especially difficult to work with. These special features must therefore have an impact on the design of experiments, the development of new methods and also of future flow cytometers that are to be used in the aquatic environment. In the last of the tutorial talks about this topic, Bill Li applied flow cytometry in his investigation of phytoplankton communities in the Northern Atlantic with the aim of finding macroecological patterns with an approach to “sacrifice” detail information in order to see the big picture of variation and fluctuation in the ocean. For this kind of research, flow cytometry is especially well suited as it leads to large amounts of data in a relatively short time.

Practical demonstrations were an integral and important part of the workshop. Participants could not only see how the methods they had heard about in the talks work in the laboratory, but they could also actually try the methods by themselves. For the flow cytometry part that meant the analysis of a large variety of phytoplankton lab cultures with three different machines (FACSCalibur, Becton Dickinson; Epics, Coulter; CytoBuoy, DRIE) for which the participants could learn how to handle samples, adjust the cytometers, measure of the cells and even do cell sorting. During the “advanced” course, the participants then analysed unknown cultures and mixtures of phytoplankton species and tried to identify them.

The second part of the workshop dealt with Artificial Neural Networks (ANNs), a tool which shows great potential for automatic identification of biological data and which is especially well suited for the analysis of flow cytometric data. In general, ANNs are computers or computer programs that try to simulate the function of neurons in the brain and should be able to solve a wide range of problems that are either difficult or impossible to be solved by conventional computer programs.

The main talks about this topic, which is not easily accessible to most phycologists because of its strong mathematical background, were presented by Lynne Boddy (Cardiff, UK), Colin Morris and Arnaud Autret (both Treforest, UK). All three explained the basics of ANNs, different algorithms and approaches, like Back Propagation, Radial Basis Function Networks or Kohonen Self-Organizing Maps, in an illustrative way that showed the possibilities of ANNs in analysing single cells as well as the possible drawbacks. Special emphasis was put on the computer software AIMSNet that simulates a neural net on Windows-based computers and which was developed by Malcolm Wilkins from the group of Lynne Boddy during the EU project AIMS. Also, during the

demo session the participants had the chance to become familiar with a neural network by using this program for the analysis of flow cytometric data. After the introductory part, Jo Brenner (Basel, Switzerland) and Patrick Quinn (Zurich, Switzerland) gave examples of how ANNs could be integrated into marine science. Jo Brenner showed the use of ANNs and their good correlation to “classical” methods in analysing data from a cruise to the Orkney Islands, Scotland. Additionally, he combined this with the use of flow cytometry and the application of molecular probes, thus illustrating the possibility of combining the three techniques of the workshop. A quite different application was demonstrated by Patrick Quinn who used the specially developed computer program COGNIS for the identification of fossil coccolithophores by image analysis. This program does not only identify the cells by a backpropagation network, but is also able to control the microscope, which makes it into a powerful automatic tool for the identification of these nanofossils.

When “normal” flow cytometric or microscopic parameters fail in identifying phytoplankton cells, specific molecular probes can be useful for this purpose. Here, molecular probes are short oligonucleotides, developed based on unique sequences of the rRNA and labelled with fluorochromes for the detection in whole-cell hybridization experiments. Under the right experimental conditions, these probes bind only to the species or algal group in question and can therefore identify them in mixed assemblies.

The introductory talk about this technique was given by Rudolf Amann (Bremen, Germany), who spoke in detail about the general considerations that are necessary to think about before developing and applying probes to cells, in this case bacteria. His talk clearly showed how important a reasoned probe design is for successful application, which includes many details like the position of base mismatches in relation to non-target organisms or the position of the probe site on the rRNA molecule. Recent developments in the methodology to boost up the probe’s signal strength complemented the lecture. The development and application of probes in general for the identification of phytoplankton and an overview of available probes and those under development for higher groups, classes, clades, genera, species and strains was presented by René Groben (Bremerhaven, Germany). A variety of these probes were also used by the participants during the practical demonstrations to identify different species in a mixture of algal cells using *in situ* hybridization and fluorescence microscopy. The application of some of these probes for DNA microarrays was successfully demonstrated by Katja Kerkmann (Bremer-

haven, Germany. This novel and fast method allows the analysis of samples with a large number of probes at the same time. In addition, she presented a handheld device that can be used for the identification of *Alexandrium* species in the field. Special emphasis on probes for toxic species was given in a talk by Don Anderson (Woods Hole, USA) who used probes for *Alexandrium tamarense* and *A. ostenfeldii* on a field research in the Gulf of Maine. The comparison of two different methods for probe application, *in situ* hybridisation and sandwich hybridization, showed some discrepancies that will need further investigation, but nevertheless gave valuable results for the analysis of toxic phytoplankton in field samples. Dave Scanlan (Warwick, U.K.) presented results about molecular investigations of two important genera of marine picoplankton, *Synechococcus* and *Prochlorococcus*, that led to the development of specific probes for different clades of these organisms. These probes have already been successfully used for determining the occurrence and dynamics of these groups in different waters, e.g., the gulf of Aqaba. The special problems that arise when analysing picoplankton were addressed by Isabelle Biegela (Roscoff, France) in the context of the development of new probes for prasinophytes. The small size of the cells and as a consequence the low signal strength can be overcome by the use of the TSA (tyramide signal amplification) system, which was also practically demonstrated by her. This talk presented results from the EU project PICODIV as well as those later given by Daniel Vaultot, Fabrice Not (both Roscoff, France) and Carlos Pedrós-Alió (Barcelona, Spain). This very successful project investigates the occurrence and abundance of picophytoplankton in different marine areas and makes heavy use of flow cytometry and molecular methods, like probes, sequence analysis and fingerprinting techniques. Erika Magaletti's (Rome, Italy) talk about cell recognition by antibodies and cell cycle proteins and their use in physiological experiments complemented this part of the workshop.

As mentioned, the talks given by the tutorial speakers were supplemented by a number of contributed talks and poster presentations that showed various examples of the usefulness of the techniques presented here. Among other applications, flow cytometry was used widely for the determination of phytoplankton abundance in different areas of the world, e.g., the Mediterranean Sea (Gérald Grégori, Marseille, France; Nenad Jasprica, Dubrovnik, Croatia) and the Red Sea (Noga Stambler, Ramat-Gan, Israel), or for investigating marine viruses (e.g., Aud Larsen, Bergen, Norway; Dominique Marie, Roscoff, France; Martin Mühlhng,

Coventry, UK; Stéphan Jaquet, Bergen, Norway). Molecular biological analysis of different phytoplankton groups, e.g. *Pseudo-nitzschia* (Caroline Cusack, Galway, Ireland) or *Dinophysis* (Bente Edvardsen, Oslo, Norway) was presented, including molecular probes and PCR techniques (e.g., Silke Kröger, Lowestoft, UK; Rebecca Gast, Woods Hole, USA; Antonella Penna, Pesaro, Italy), as well as innovative technical methods for phytoplankton monitoring, especially of toxic species (e.g., Cheryl Rafuse, Halifax, Canada; Martin Beutler, Plön, Germany; Heidi Sosik, Woods Hole, USA; Marco Berzano, Camerino, Italy; Annick Wilmotte, Liège, Belgium). Again, this was only a part of all the different research topics presented by the participants.

One day of the workshop was dedicated to a practical session where the participants had the possibility to apply the techniques they had learned before to field samples that they had brought with them. The analysis by flow cytometry and the use of probes showed impressively the possibilities of these methods in analysing field samples, but also some limitations that still need to be overcome.

Overall, it was a very successful workshop that was fuelled by the enthusiasm of the participants and their willingness to learn new methods that they can now apply to their own field of research. The combination of the three different techniques of flow cytometry, artificial neural networks and molecular probes proved to be a powerful approach for answering questions about the occurrence and ecology of single cell organisms in the marine environment. It is also highly evident that they will be of even wider use in the future. Hence, the seven day workshop was very intense but widely appreciated by all who attended.

Readers who are interested in more information about the workshop are welcome to visit the homepage <http://www.sb-roscoff.fr/Phyto/PICODIV/index.html> and follow the link to ASCMAP 2002. The workshop was funded by the EU (HPCF-2001-00390) and the City of Bremerhaven and would not have been possible in this form without the help of a number of companies, who are all gratefully acknowledged by the organizers. The authors want to thank Klaus Valentin, Gundula Eller and Katja Kerkmann for their help in preparing this manuscript.

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