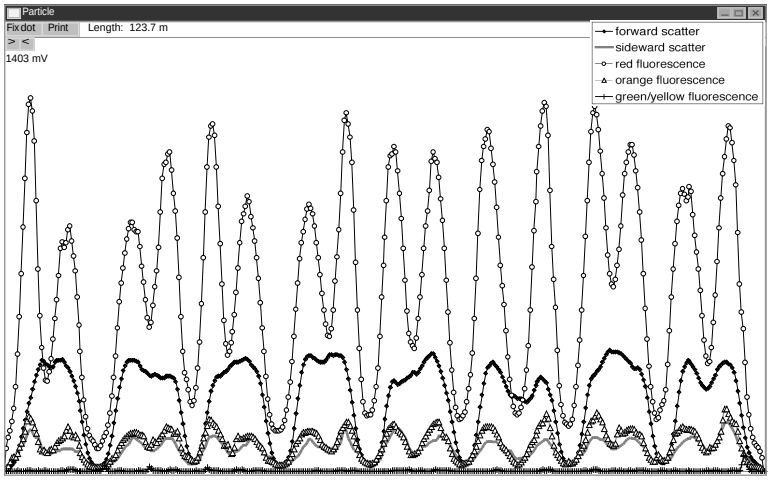
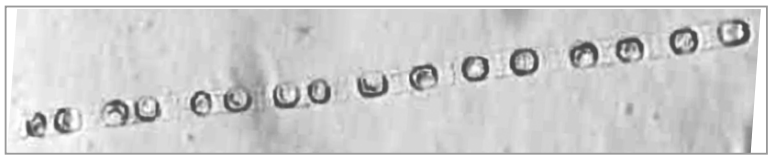
Screening of ballast water with scanning flow cytometry

George B. J. Dubelaar
CytoBuoy b.v., Nieuwerbrug, The Netherlands

Various methods for ballast water treatment are under development nowadays. Both the development and the operational control of ballast water treatment installations are best served by quantitative, fast, and if possible autonomous analysis techniques to measure the treatment efficiency. One of the 'difficult' control entities are the small suspended particles, consisting of bacteria, phytoplankton and other microorganisms as well as organic debris and inorganic sediment particles.

The primary goal of the treatment of ballast water is to prevent the dispersion of non indigenous marine and freshwater organisms. Selective removal of specific organisms, especially microorganisms, from large water flows is practically impossible. Therefore BWT systems tend to combine two or more general treatments, such as general particle removal by cycloning or filtering, killing organisms by UV or chemicals. Next to high capacity treatment during ballasting, low capacity in-tank methods may also be effective such as blocking of cell replication by ultrasound. Each of these methods has its challenges. Cyclones are less effective with low density particles such as phytoplankton, filtration is getting increasingly difficult with smaller particles, UV radiation or chemicals are less effective with certain species and/or cysts. Similarly, no single analysis technology is capable of direct detecting, counting and analysing the complete range of target particles and concentrations to control and monitor these processes: the development of BWT installations therefore requires a comprehensive and complementary range of analysis technologies. Flow cytometry is a versatile analysis technology for a wide range of particles and concentrations that may well serve as the cornerstone for such a system, and constitutes a good candidate technology for applications requiring a single realtime technology such as a inline control of BWT systems performance or control of discharged ballast water by authorities.

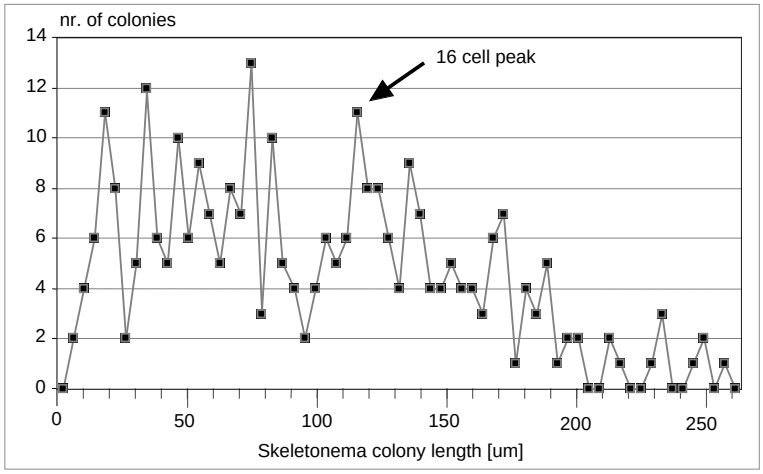




Scanning flow cytometry (SFC) yields 1-dimensional low resolution profiles obtained from particles flowing through a laser beam, which limits the data load as compared to collecting images and allows fast sample processing. As in standard flow cytometers, the particle suspension is injected into a particle free carrying fluid that narrows down the suspension into a very thin line of fluid in which the particles are gently stretched out into a single file and aligned along the path of flow. This 'line of particles' is moving at a fixed high speed exactly through the middle of a sharply focussed laser beam. From each passing particle the scattered laser light is measured at two angles as well as several colors of laser induced fluorescence emitted by chlorophyll and/or other fluorescent pigments in the particle or microorganism. This provides direct morphological information and accurate particle length spectra, even for colony formers such as Pseudonitschia sp. As an example a scan is shown of a single Skeletonema colony together with a photo of such a particle. The length of particles (=124 um for this particular colony), the number of "humps or cells" they contain (=16 for this colony) and the mean "hump or cell" size (=7.5 um for this colony) are automatically generated.

We designed a compact and robust series of such scanning flow cytometers for live, untreated samples - sampling discrete or online in the lab, ship or in-situ platforms. Whereas standard flow cytometers analyze only small single cells, these CytoBuoy systems analyze particles from submicron in size up to 600 µm in diameter and lengths of several millimeters. This eliminates the need for size fractioning and the danger of instrument clogging. A much higher flow rate as compared to standard flow cytometers allows sufficient individuals of low abundant species to be 'catched'.

Sediment particles are easily identified and their concentration (number of particles per analyzed volume of sea water) and total scatter (mass indication) can be generated for many size classes to obtain an indication of sediment removal efficiency. Sometimes even different types of sediment can be distinguished. Photosynthetic microorganisms such as algae and cyanobacteria are easily recognized by their strong red autofluorescence. We could typically distinguish between 30 and 50 groups in natural samples of fresh and marine waters. These may include also chlorophyll lacking organisms such as heterotrophs basing on their distinct morphology. By comparing the raw measured data to previously determined templates, a fast overview of specific particle types present and their abundance can be obtained. Other properties, such as cellular fluorescence or scatter, and length can also be generated for the whole sample



or for any sub-group distinguished. This is shown in Fig. 2 which represents the length distribution of Skeletonema particles as found in a sea water sample. The diagram clearly shows the discrete distribution of the chain lengths as governed by the cell division process.

The fast and quantitative diagnostic capabilities of the CytoBuoy type of flow cytometers may be of great help for the fast screening of ballast water by generating countings and accurate size spectra for sediment particles, phytoplankton and other groups of particles. This can be used to monitor the efficiency of organism targeted treatments, or even serve as a feed back mechanism to actively control treatment performance.

More information can be found on www.cytobuoy.com.