OPTICAL PARTICLE CHARACTERIZATION

P. H. Burkill and C. P. Gallienne,
Plymouth Marine Laboratory, West Hoe,
Plymouth, UK

doi:10.1006/rwos.2001.0322

Particles and Their Properties

Particles are ubiquitous in ocean waters, where they are intimately involved in defining the optical properties, productivity, and biogeochemistry of our seas. Marine particles exist in a wide range of sizes and concentrations, and exhibit an inverse relationship between size and concentration, and a positive relationship between concentration and ambient nutrient concentration (the 'trophic status') in surface waters of the ocean.

Particles range in size from the largest marine organisms (blue whales, *c.* 70 m length) down to the size that arbitrarily divides particles from dissolved materials. In biological oceanography, this is defined operationally as 0.2 μm. But here we will focus on particles that fall within the size range of the plankton. Plankton organisms range from viruses (*c.* 0.05 μm) up to larger zooplankton such as euphausiids (*c.* 2 cm). However, even within this size range, many particles are not living but instead contribute to the large pools of detritus that often predominate over living particles in the ocean.

Particle Characterization

A wide array of techniques is available for the characterization of marine particles. Although most are based on optical properties, nonoptical techniques, such as the acoustic doppler current profiler (ADCP) and the multifrequency echosounder, can also be used to quantify and characterize particles such as large zooplankton and fish in sea water. Optical characterization techniques vary considerably in their resolution. At one extreme, satellite-based remote sensing can be used to quantify and characterize the marine phytoplankton across whole ocean basins (*see* **Ocean Color from Satellites**). At the other extreme, microscope-based techniques and analytical flow cytometry resolve single particles. For the biologist, microscopy is the benchmark procedure for identification of plankton. This is true whether the particles of interest are viruses, which are typically analyzed by electron microscopy, or bacteria, protozoa, or larger zooplankton, which are analyzed by light microscopy. As an adjunct to light microscopy, fluorescence-based techniques are used increasingly to characterize, and sometimes quantify, the chemical properties of cells. Such approaches can be extremely powerful, particularly when used in conjunction with fluorescently labeled molecular probes. Such probes can be tailored to target specific taxonomic groups. Although microscopy remains the benchmark, for the simple reason that 'what you see, you believe,' it is time consuming and costly. A wide range of techniques offer rapid analysis of particles. However, these tend to be 'black box' techniques and should always be used with appropriate controls. No single technique provides a panacea in particle analysis, and it is often useful to combine two or more complementry techniques.

Rapid optical techniques for analyzing plankton-sized particles may be based on scattered, fluorescent or transmitted light. Scattering and fluorescence methods are applicable to smaller particles (< 500 μm equivalent spherical diameter (ESD)), where as larger particles, such as zooplankton, are usually analyzed by transmission techniques. Two techniques that have been developed rapidly in the last decade, are analytical flow cytometry (AFC) and optical plankton counting (OPC). AFC and OPC are particularly suitable for the analysis of smaller particles (viruses to protozoa) and larger particles (metazoa), respectively.

Analytical Flow Cytometry

Technique Analytical flow cytometry (AFC) is a generic technique based on the multiparametric analysis of single particles at high speed. Originally developed for medical hematology and oncology, AFC is used increasingly in biological oceanography. Its strengths are derived from its quantitative capability, versatility, sensitivity, speed, statistical precision, and ability to identify and, in many instruments, sort particle subsets from heterogeneous populations. Its drawbacks are its cost and, for commercial instruments, the small volume of sample (*c.* 0.5 cm^3) analyzed. Particle characterization and quantification in flow cytometry relies on cellular fluorescence and light scatter, and the power of the technique derives from the ability to make multiple measurements simultaneously on each cell at high speed. Typically, up to 5000 cells can be analyzed

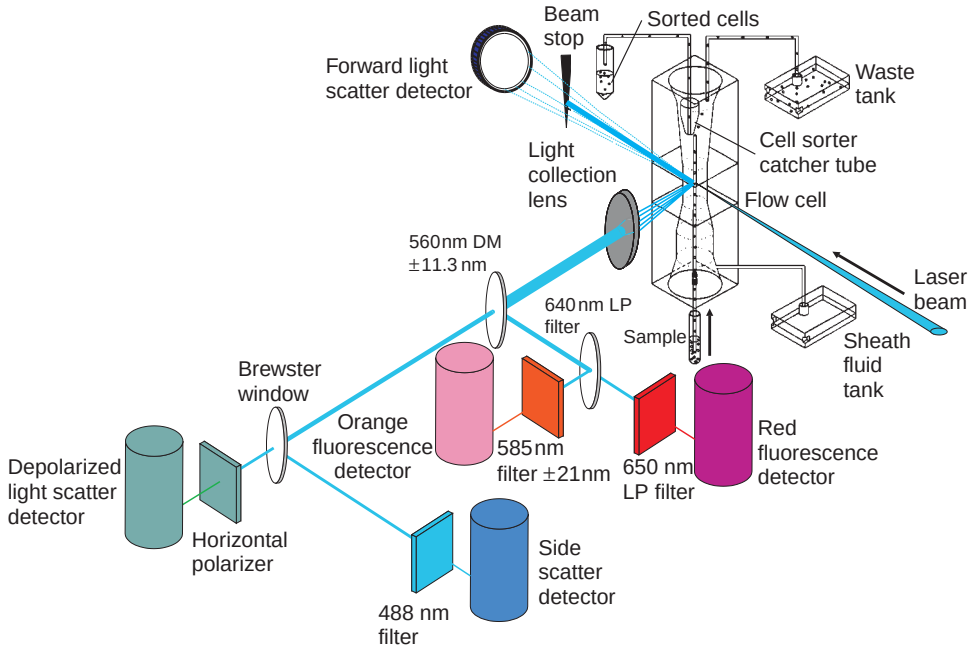

Figure 1 Operating principles of AFC in which samples containing the particles of interest are passed singly across a laser beam. Each particle scatters light and this is collected by forward and side light scatter detectors. Birefringent particles will tend to depolarize the vertically polarized laser light and this is measured at the appropriate detector. Fluorescence from each particle is collected and spectrally filtered so the wavelength of interest is detected by photomultiplier tubes. Output from each sensor is digitized and the data are transferred to a computer. Particle size and refractive index are determined by the light scatter and the chemical properties are determined by fluorescence. Particles exhibiting appropriate properties can be collected by sorting, whereas other particles pass to waste. (Figure produced by Glen Tarran, Plymouth Marine Laboratory.)

per second and sorting rates of $> 10\,000\,s^{-1}$ with $> 98\%$ purity, can be achieved.

The principles of AFC (**Figure 1**) are based on hydrodynamically focusing a suspension that is streamed coaxially through a flow chamber so that individual particles pass singly through the focus of a high intensity light source. Suitable light sources include coherent wave lasers since these provide a very stable light beam that can easily be focused to small dimensions. Light flux from the highly focused light source generates enough fluorescence from individual cells for this to be measured in the few microseconds taken to traverse the light beam. As the particles traverse the beam, they scatter light and may also produce fluorescence. Cellular fluorescence arises from autofluorescent cells or cells stained with a fluorochrome, and this is collected by a high numerical aperture lens located orthogonally to the irradiation source and the sample stream. The light collected is spectrally filtered sequentially by dichroic mirrors which reflect specific wavelengths into photomultiplier tubes (PMT). The PMTs are optically screened by band-pass filters. The quantity of light incident upon each PMT, responding within a given color band, is then proportionally converted into an electrical signal. The signal is amplified, digitized, and stored transiently in computer memory. The data are then displayed on a computer screen and stored onto disk as 'list mode' data. The list mode data can be considered to be analogous to a spreadsheet in which each row represents a particle, each column represents a different AFC sensor with values that represent quantitative optical signatures of each particle. The advantage of list mode data, as with data in any spreadsheet, is that it can be replayed, reanalyzed, and redisplayed.

Commercial cytometers are usually equipped with light-scatter detectors that are situated in the narrow forward and an orthogonal angle, as well as two or three fluorescence PMTs. Although a single laser is normal, AFC instruments can be equipped with two or more lasers to increase the number of fluorochrome excitation wavelengths. Some cytometers may use arc-lamp excitation particularly if UV irradiation is required. There is also a move to the use of diode lasers for applications that demand low power use. Flow chambers may vary in their hydrodynamic, optical, mechanical, and electrical characteristics to achieve high sensitivity and good stream stability. AFC instruments often have quartz cuvette sensing zones to improve sensitivity and to allow the application of UV irradiation. Specialized cytometers can also measure particle volumes based on the Coulter principle of electrical impedance

alteration as particles flow through a restricted orifice. Other specialized instruments may generate images of particles in the sensing zone. Data processing and display procedures have been developed which handle fully crossed-correlated multidimensional data and this is achieved by microcomputers. Considerable developments have taken place in the last few years to apply sophisticated procedures such as multiparametric statistics or neural net generation, to identify and characterize particles from within heterogeneous mixtures.

Many AFC instruments are able to sort cells and this is invaluable for identification, manipulation or as a gateway to other analysis procedures. High speed 'sorting-in-air' is based on developments in ink-jet printing. Two populations may be sorted from the sample stream that undergoes oscillation, driven by a piezo-electric crystal that is mechanically coupled to the flow chamber. The crystal, driven at 30–40 kHz, produces uniform liquid droplets of which a small percentage contain single cells. The 'sort logic' circuitry compares processed signals from the sensors with pre-set, operator-defined ranges. When the amplitude falls within the pre-set range, an electronic time delay of a few microseconds is activated. This triggers an electrical droplet-charging pulse at the moment the cell arrives at the droplet formation break-off point. The droplet-charging pulse causes a group of droplets to be charged, and subsequently, deflected by a static electric field into a collection vessel. Cells failing the pre-set sort criteria do not trigger droplet-charging, and so pass undetected into the waste collector. Other sorting procedures include one in which a collecting arm moves into the sample stream to pick up particles that meet the programmed sort criteria. Sorting is an essential adjunct to AFC and is crucial to verifying both satisfactory instrument and analytical protocol operation.

Applications Oceanographic applications of AFC are now diverse and continue to expand rapidly. Although the fundamental principle of AFC remain constant, recent developments in optical sensitivity and design of AFC instruments have aided new applications. Fluorochrome chemistry and molecular biology are both richly endowed fields and developments in fluorescent assays of biochemical constituents, coupled with the ability to target individual taxa have proved invaluable for AFC applications in marine biology. Detection limits are adequate to measure cellular attributes of many planktonic cells.

Cellular fluorescence may be derived from two basic categories:

1. autofluorescence in which the fluorescent molecule of interest occurs naturally in the cell;
2. applied fluorescence in which the fluorescent dye is applied, or otherwise generated, and fluorescence is accumulated within the cell.

Phytoplankton Analysis of phytoplankton by AFC is based on the presence of chlorophyll, a highly autofluorescent compound that is found in all viable plants. Chlorophyll is the phytoplankton's principal light-harvesting pigment, and absorbs light strongly in the blue and red regions of the visible spectrum. Blue light cellular absorption coincides with the emission of the argon ion laser at 488 nm that is commonly used in flow cytometers. Chlorophyll fluorescence is emitted in the far red ($\lambda_{em} = 680$ nm), thereby offering a useful Stokes' shift of some 200 nm. This window means that phytoplankton can be readily characterized and quantified by flow cytometers equipped with an argon laser (or other blue light source) and suitable spectral filtration (such as a 650 nm longpass filter) of fluorescent light emitted by cells onto a sensitive photomultiplier tube.

As well as chlorophyll, some phycobiliproteins are also autofluorescent. One of these is phycoerythrin which is found in cyanobacteria and cryptophytes. Although phycoerythrin absorbs light in the green–blue end of the spectrum, the 488 nm emission of the argon is sufficiently close to excite this compound. In studies of phytoplankton, fluorescence from phycoerythrin is measured by a separate photomultiplier tube that is spectrally filtered to collect emissions at 585 nm. Based on this differentiation and coupled with light scatter measurements (the magnitude of which is roughly proportional to cell size), AFC can readily differentiate and quantify the phytoplankton groups shown in **Table 1**.

In recent years, the application of powerful multivariate statistical and neural net procedures have been applied to further characterize algal taxa from within the complex mixtures that are typical of sea water. Multivariate statistics that have been used

Table 1 Routine AFC analysis of phytoplankton based on optical characteristics

Differentiation	*AFC criteria*
Phytoplankton	Chlorophyll autofluorescence
Prochlorococcus	Low chlorophyll and light scatter
Synechococcus	Low phycoerythrin and light scatter
Cryptophytes	Phycoerythrin and light scatter
Coccolithophores	High orthogonal light scatter and laser depolarization

include quadratic discriminant analysis and canonical variate analysis. The latter is a useful graphical technique for analyzing and displaying data, whereas quadratic discriminant analysis can discriminate over two-thirds of mixtures of 22 algal taxa, with classification rates $>70\%$. Such approaches are more than two orders of magnitude faster than conventional flow cytometric analyses for discriminating and enumerating phytoplankton species.

Artificial neural nets (ANN) have proved to be extremely powerful in increasing AFC capability for differentiating algal taxa. This approach is based on training an ANN to recognize the optical characteristics of individual taxa. This is achieved by presenting the net with AFC data derived from unialgal cultures. The unknown samples are then analyzed by the AFC under the same conditions and the data passed through the trained ANN. The net outputs identification probabilities for each cell analyzed. Several types of ANN have been used and it is now possible for nets to differentiate and recognize >70 taxa with high accuracy. Considerable developments are anticipated in this field in the coming years.

As well as providing procedures for differentiation of phytoplankton from other particles, there are AFC protocols for quantifying cellular attributes of phytoplankton. These include the cellular concentrations of chlorophyll, phycoerythrin, protein and DNA as well as enzymes such as ribulose-1,5-bisphosphate carboxylase. AFC instruments are capable of great sensitivity and are able to quantify concentrations of cellular chlorophyll in phytoplankton in the range of about 1–2000 fg cell^{-1}.

In practice, marine phytoplankton are typically analyzed using a fresh sample of sea water without pretreatment. The sample is analyzed at a constant rate so sample volume can be determined from analysis time. Chlorophyll-containing phytoplankton and those containing phycoerythrin are registered by the red and orange fluorescence emitted from single cells as they traverse the laser beam. Typical sample analysis time is generally 4–5 min. Examples of data generated by AFC protocols for the analyses of natural waters are shown in **Figure 2**.

Bacteria Bacteria, traditionally quantified by epifluorescence microscopy, can now be differentiated from other particles and analyzed by AFC (**Figure 2**). Both approaches are based on the intercalation of a fluorochrome with the cell's nucleic acid. However, such intercalation is universal and often does not differentiate between autotrophic and heterotrophic bacteria. A range of fluorochromes have been used including 488 nm absorbing YOYO-1, YO-PRO-1, PicoGreen and SYBR Green as well as the more traditional UV excited bis-benzimide Hoechst 33342 or 4′,6-diamidino-2-phenylindole (DAPI). Of these, SYBR Green offers the practical advantage that autotrophic and heterotrophic bacteria can be differentiated readily.

It is now possible to quantify the cellular protein and DNA content of bacteria in natural waters.

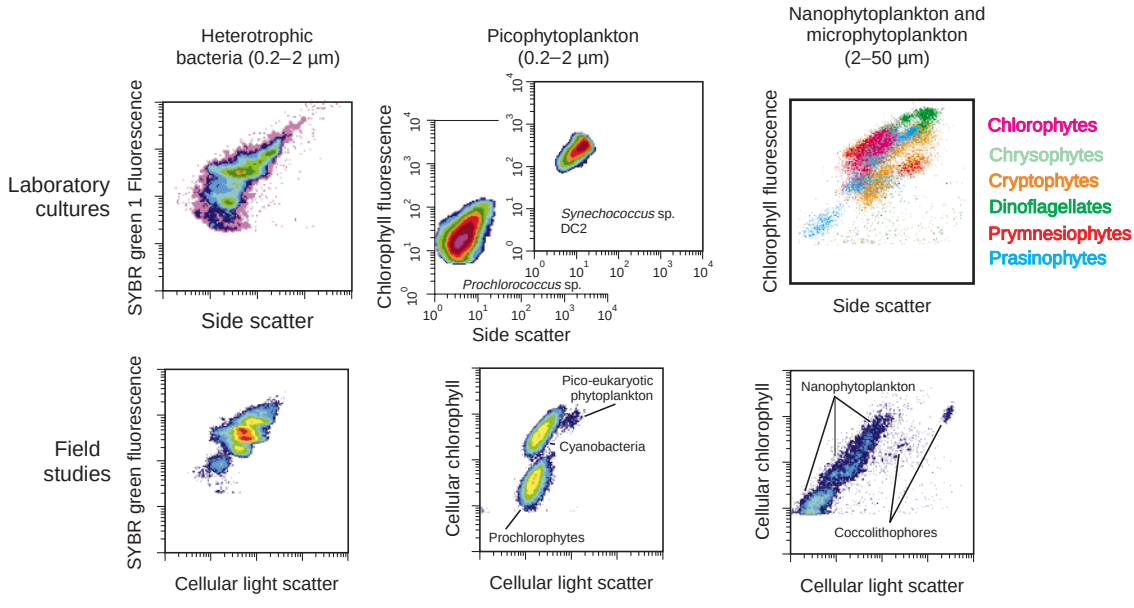

Figure 2 AFC characterization and differentiation of bacteria and phytoplankton in lab culture and in natural communities carried out at the Plymouth Marine Laboratory. Bacteria are measured using SYBR Green fluorescence and the different phytoplankton are measured by chlorophyll autofluorescence. Analysis can be verified by microscopic analysis of flow-sorted particles. (Figure produced by Glen Tarran, Plymouth Marine Laboratory.)

Such AFC techniques use the intensity of SYPRO-protein or DAPI–DNA fluorescence of individual marine bacteria. Cultures of various marine bacteria have been measured in the range 60–330 fg protein $cell^{-1}$, but the amount of natural bacterioplankton from the North Sea in August 1998 was shown to be only 24 fg protein $cell^{-1}$. The total DNA of natural bacteria has been estimated to be about 3 fg $cell^{-1}$ by AFC techniques.

Changes in bacterioplankton community composition have also been assessed by molecular biological AFC techniques. The combination of AFC analysis and sorting combined with denaturing gradient gel electrophoresis of polymerase chain reaction (PCR)-amplified 18S rDNA fragments and fluorescence *in situ* hybridization has been shown to be a rapid method of analyzing the taxonomic composition of bacterioplankton. Experimental manipulation of natural water samples resulted in a bacterial succession from members of a *Cytophaga flavobacterium* cluster, through gamma-proteobacteria and finally alpha-proteobacteria.

Protozoa *AFC*-based techniques for the analysis of protozoa have, so far, been based on molecular probes. Ribosomal RNA species-specific probes to various members of the common heterotrophic flagellate genus, *Paraphysomonas*, have been developed (**Figure 3**). However, they have been restricted to laboratory applications since naturally occurring organisms exhibit cellular fluorescence levels that are often too low to distinguish from background. This observation may either be a reflection of poor probing efficiency or it may be due to the organisms low growth rates *in situ*.

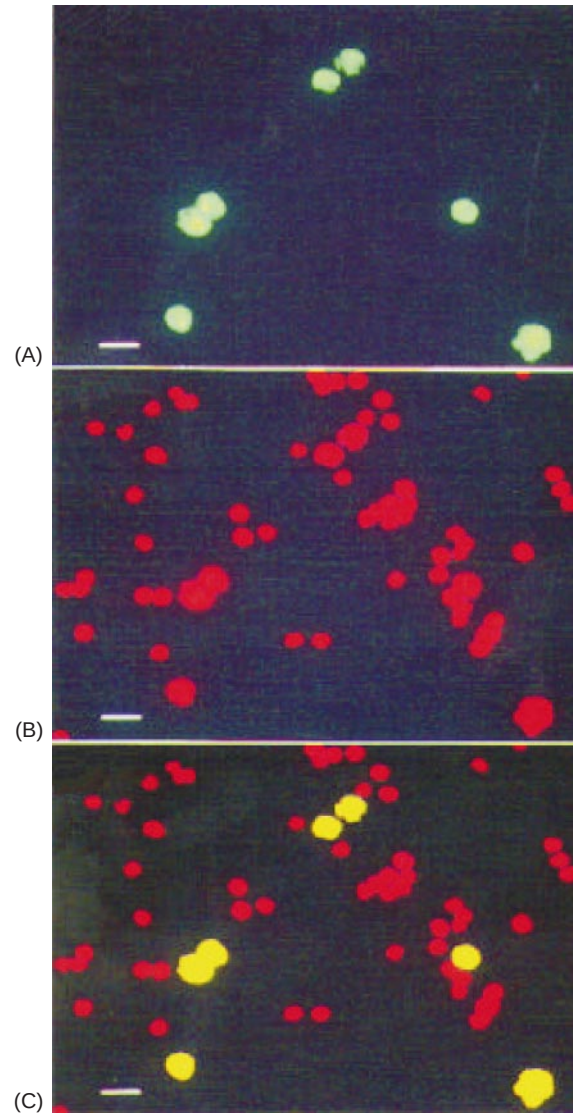

Figure 3 Photomicrographs showing a mixture of four *Paraphysomonas* species after hybridization with a mixture of PV1 and EUK probes tagged with fluorescein and rhodamine, respectively. PV1 is specific for *P. vestita* whereas EUK labels all eukaryotes. The mixture was irradiated at (A) 488 nm to show *P. vestita* labeled with PV1, (B) 568 nm to show organisms labeled with EUK, and (C) both 488 and 568 nm to reveal both probes. Scale bar is 10 μm. (Reproduced from Rice *et al.* (1997) with permission from the Society for General Microbiology.)

Viruses Viruses are now thought to be one of the most abundant types of particles in the ocean. AFC protocols are now available for enumerating natural marine viruses based on staining with the nucleic acid-specific dye SYBR Green-I. Interestingly AFC-based counts are often higher than those obtained by microscopy, suggesting that further development work is needed. However, this AFC protocol reveals two, and sometimes three, virus populations in natural samples, whereas microscopy would only differentiate one pool of viruses. Cultures of several different marine virus families (Baculoviridae, Herpesviridae, Myoviridae, Phycodnaviridae, Picornaviridae, Podoviridae, Retroviridae, and Siphoviridae) have also been stained with a variety of highly fluorescent nucleic acid-specific dyes. Highest fluorescence is achieved using SYBR Green I, allowing DNA viruses with genome sizes between 48.5 and 300 kb (kilobases) to be detected. Small genome-sized RNA viruses (7.4–14.5 kb) are at the current limit of detection by AFC.

Zooplankton and larval fish Although commercially available AFC instruments are directly applicable for the analysis of microbial cells, it is also possible to adapt the generic AFC concept for the analysis of metazoan organisms. This involves a scaling up of flow chambers and the associated fluidics system. The Macro Flow Planktometer built within the EU

MAROPT project has been applied to organisms as large as larval fish. An inherent property of this system is that it incorporates 'imaging in flow' as part of the analysis. Images may be stored either photographically or electronically and then be available for subsequent image analysis.

In-situ AFC As we move towards operational oceanography, there is an increasing need for autonomous, *in situ* instrumentation. An important step towards this has been made recently with the development of CytoBuoy, an AFC instrument housed in a moored buoy and capable of wireless data transfer. CytoBuoy is one of the very few AFC instruments to have been designed and built purely for aquatic use. Its characteristics include enhanced optics and electronics designed to obtain maximal information on particle characteristics. Whereas standard cytometers reduce these to single peak or area 'list-mode' numbers, time resolved signals are preserved fully and transferred to the computer as raw data. Pulse shape signals aid identification considerably and allow, for the first time, a true measurement of particle length. The CytoBuoy concept has also been taken a step further and has been redesigned as a functional module of the UK Autosub autonomous underwater vehicle.

Future trends The generic capability of AFC lends itself to a variety of applications. The thrust in recent years has been towards greater taxonomic resolution and this has been aided by the application of molecular techniques particularly involving oligonucleotide probes. These have many advantages including the ability to be tailored to target particular groups of organisms. Here the level of taxonomic targeting may range from general (e.g. differentiation of classes of phytoplankton) down to individual species. Molecular probes may also be coupled with chemotaxonomic capability that can analyze cellular function such as specific enzyme production.

Flow cytometry has opened up our ability to characterize marine particles with a greater degree of taxonomic resolution and further development of techniques such as ANN will increase this capability considerably. This should allow characterization of natural populations objectively in close to real-time. The quest for greater analytical resolution will undoubtedly continue. However, it is also possible that a taxonomic identification watershed may soon be reached. So far, the focus of AFC protocols has generally conformed to traditional taxonomic criteria. But there may be another route that remains to be explored. This involves an approach to classifying particles based directly on flow cytometry variables of light scatter, fluorescence, time of flight etc. Such an approach might prove worthwhile because of its direct simplicity. How such an approach would compare to traditional taxonomic identification remains to be addressed, and that remains an exciting challenge for the future.

Optical Plankton Counting

The Optical Plankton Counter (OPC) has been designed to analyze those zooplankton called the mesozooplankton whose size range conventionally spans 0.2–20 mm.

Technique The OPC uses a collimated beam of light through an enclosed volume, received by a photosensor. When a particle interrupts this beam of light the sensor produces an electronic response proportional to the cross-sectional area of the particle (**Figure 4**). This response is digitized and this digital size is converted into ESD using a semi-empirical formula. This is the diameter of a sphere having the same cross-sectional area as the particle being measured, and can be simply converted into volume. The OPC comes in two versions: a towed instrument for *in situ* use and a benchtop laboratory version. The towed version has a sampling tunnel of 22 cm × 2 cm. The laboratory version has a glass flow cell 2 cm square.

Applications The OPC is capable of large-scale, rapid and continuous sampling of zooplankton, providing a reliable measure of size distributed abundance and biovolume, between 0.25 and 20 mm ESD,

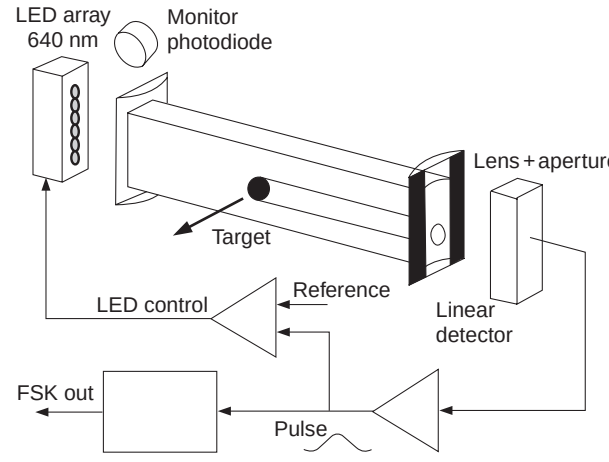

Figure 4 Schematic of the operating principle of the optical plankton counter. LED, light-emitting diode; FSK; frequency shift keying.

at data rates up to $200\,s^{-1}$. The use of the *in situ* OPC on various towed platforms is now well established. The laboratory version is intended for characterizing preserved samples. It has also been deployed at sea in pump-through mode producing continuous real-time data on surface zooplankton abundance and size distribution, permitting near continuous sampling of epipelagic zooplankton across ocean basin scales. **Figure 5** shows data from the OPC in this mode on the Atlantic Meridional Transect. The data in **Figure 5** show one of 11 transects completed to date, each comprising a near-continuous 13 000 km transect of size distributed biovolume in the North and South Atlantic, illustrating the power of this kind of instrument. Data at this level of detail and spatial resolution acquired autonomously and continuously could not have been gathered over such large spatial scales by any other means.

Operational considerations Bias and coincidence require calibration of the instrument against some other sampling device. Initial calibration uses spherical glass beads of known size. Several researchers have noted nonlinearity in this calibration at the extremes of the size range, and have suggested that the operational size range should be reduced. Sensor response time and coincidence limit the densities at which the OPC can operate. Coincidence occurs when more than one particle is in the beam at the same time. They register as one larger particle and abundance will, therefore, be underestimated, and biomass overestimated. Coincidence in the towed OPC can be reduced in areas of high abundance by inserting into the sampling tunnel a transparent plate, reducing the sampling volume to one-fifth. There is a finite probability of coincidence occurring at all concentrations, increasing with abundance and flow rate through the instrument. It has been determined experimentally that at a count rate of $30\,s^{-1}$, more than 90% of particles will be counted. Smaller particles far outnumber larger ones, so coincidence will result in a loss of these smaller particles, and biovolume is underestimated to a lesser degree than abundance. Towed at $4\,m\,s^{-1}$, the standard OPC will pass $17.6\,l\,s^{-1}$ through the sampling tunnel. Coincidence will therefore begin to have a significant effect on estimated abundance above concentrations of $1700\,m^{-3}$, or $8500\,m^{-3}$ with the flow insert.

Object orientation can also present problems in this type of counter. Elongated organisms present a very different cross-sectional area depending upon whether they are side-on or end-on. Biovolume may be considerably underestimated in the latter case. It has been shown that on average the cross-sectional area measured for a randomly oriented object will be greater than 70% of the true value. Biovolume may also be overestimated by the spherical model assumed in the OPC calibration – most zooplankton have a shape closer to that of an oblate spheroid. We use an ellipsoidal model based on cross-sectional area of the particle. From cross-sectional area the length and width of the ellipsoid can be calculated (assuming a length to width ratio typical for copepods).

Several cases have been reported of OPCs producing counts many times higher than those from concurrent net samples. Comparison between OPC and a Longhurst Hardy Plankton Recorder (LHPR) showed that abundance and biovolume recorded by the OPC were consistently four times higher than for the LHPR (200 μm mesh). Using a 53 μm mesh showed that there had been significant undersampling of zooplankton $< 350\,\mu m$ ESD by the LHPR. Studies like these show that care is required when

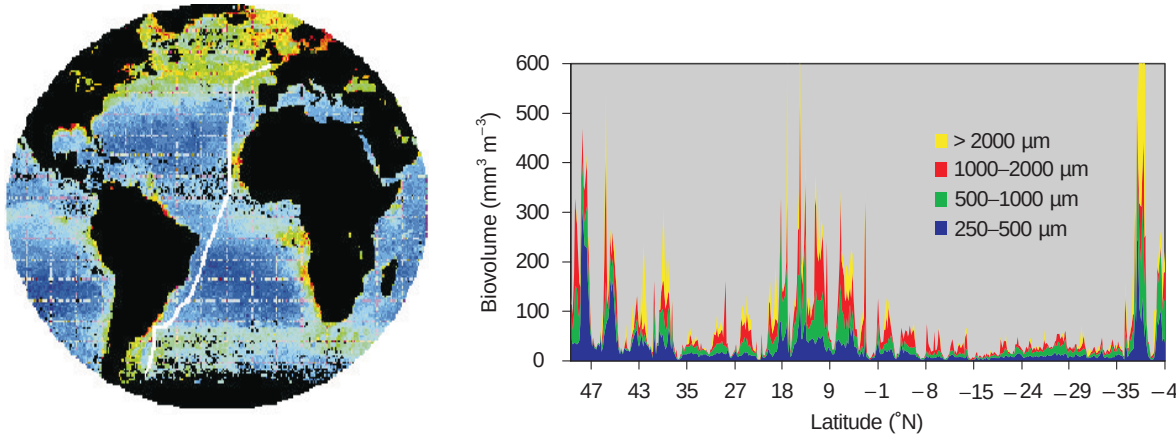

Figure 5 A 13 000 km transect of size-distributed epipelagic zooplankton biovolume in the North and South Atlantic from the OPC in continuous surface sampling mode on the Atlantic Meridional Transect.

comparing any two sampling methods. When comparing the OPC to net systems, careful selection of mesh size or OPC lower size threshold is required. Our own investigations indicate that the most suitable mesh size for a net used in comparison with the standard OPC is 125 μm (250 μm ESD, 3:1 ellipsoidal model, mesh size 75% of width of smallest animal to be quantitatively sampled).

It must be emphasized that OPC cannot discriminate living from nonliving particles. All particles within its operating range will be detected, including detritus, marine aggregates, air bubbles, etc. A recent study found average abundance in the Faroe–Shetland Channel and north–western North Sea to be 2.5 times higher for an OPC than for net samples and up to 40 times higher at extremely low concentrations. This was put down to detrital particles/marine aggregates, which may in some cases be the most abundant particle type in the water, and are often too fragile to be sampled by nets. Detrital particles may be considered part of the plankton, being significant in marine food webs, but their presence in large numbers will bias comparisons between OPC and net derived estimates of abundance.

Particle Imaging Instruments

Automated particle counters (electronic, acoustic, and optical) can give reliable data on zooplankton abundance and size distribution, but tell us little or nothing about the species present, except where dominant species are already known and well separated in size. Larger autotrophic particles may be counted as zooplankton, and in areas where high concentrations of detritus or marine aggregates are present, they are not discriminated from zooplankton. To make these discriminations, information on shape as well as size is needed, requiring the use of imaging devices.

Photographic methods High-speed silhouette photography has been undertaken at sea without the need for sample preservation. Samples in a shallow tray on top of 8×10-inch (20×25 cm) film were exposed by a xenon strobe. Samples could then be counted and identified from the film, to provide a permanent record. A towed version of the system using concentrating nets ahead of a camera system was subsequently developed. Although this system avoided some of the problems associated with the deployment and use of net systems, and preservation of samples, a human investigator still carried out counting and identification of the samples. Automated image processing of digitized photographic images could alleviate this problem, but duration and spatial resolution is limited by the amount of film that can be carried.

Video methods The most advanced video method for producing abundance and size distribution indicators of good temporal and spatial resolution, together with near-real-time classification to taxonomic groups, is the video plankton recorder. This consists of a towed frame 3 m long with four cameras each having concentric fields of view (5–100 mm) covering the size range of interest (0.5–20 mm ESD). The imaged volume is defined by the oblique intersection of the cone defining the camera field of view, and that produced by a collimated, strobed 80-watt xenon light beam 1 μs pulse duration, producing dark field illumination. Sample volume varies from 1 ml to 1 liter. Fiber-optic telemetry is used to send video data to the surface, where information is recorded to tape. Subsequent upgrading of this system is expected to permit preprocessing to be done in real time, and near real time production of size and species distributions. Concurrent calibration of the device is provided by an integrated LHPR system. The device can differentiate detrital material and zooplankton, unlike optical and acoustic systems. The video plankton recorder has been used extensively around George's Bank in the north-west Atlantic.

Video cameras used in scientific imaging typically produce 10–100 million bytes s^{-1}, and image-processing algorithms are highly computer intensive. For real-time acquisition systems to be used at sea in continuous sampling mode, assuming $1\,m^{-3}\,min^{-1}$ as the required sampling volume, and at typical oceanic zooplankton abundance of 50–5000 m^{-3}, we need to be able to process 3000–300 000 animals per hour. Although currently available technology can resolve this problem to some degree, the solution is not yet likely to be economical in size or cost.

ROV devices Remotely operated vehicles (ROVs) carrying video cameras have been used for *in situ* studies of zooplankton abundance and behavior. Gelatinous organisms are notoriously difficult to sample using traditional methods, and an ROV carrying a video camera has been successfully deployed for their study. ROVs are usually restricted to small-scale, observational studies, but may be useful for the location of zooplankton patches. Small imaging volume may preclude the study of rarer taxa, and poor image quality may preclude the study of smaller taxa. Zooplankton also exhibit

attraction/avoidance responses to the presence of ROV systems.

See also

Acoustic Scattering by Marine Organisms. Autonomous Underwater Vehicles (AUVs). Carbon Cycle. Fish Larvae. Fluorometry for Biological Sensing. Inherent Optical Properties and Irradiance. Krill. Microbial Loops. Ocean Color from Satellites. Plankton. Plankton Viruses. Population Genetics of Marine Organisms. Remotely Operated Vehicles (ROVs).

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ORBITALLY TUNED TIME SCALES

T. D. Herbert, Brown University, Providence, RI, USA

doi:10.1006/rwos.2001.0245

Introduction

Geologists rely on a variety of 'clocks' built into sediments to place paleo-environmental events into a time frame. These include radiometric decay systems, annual banding in trees, corals, and some marine and lake sediments, and, increasingly, the correlation of isotopic, geochemical, and paleontological variations to pacing supplied by changes in the Earth's orbit. Variations in three parameters of the orbital system – eccentricity, obliquity, and precession – cause solar insolation to vary over the Earth as a function of latitude, season, and time, and hence cause global changes in climate. Because the timing of orbital changes can be calculated very precisely over the past 30 million years, and because their general character can be deduced for much longer intervals of geological time, orbital variations provide a template by which paleoceanographers can fix paleoclimatic variations to geological time. Paleoceanographers now commonly assign either numerical ages or elapsed time to sediment records by optimizing the fit of variations in sediment composition, fossil context, or isotopic ratios to a model