

SUBMERSIBLE FLOW CYTOMETER FOR MARINE PARTICLE ANALYSIS: DESIGN AND INITIAL TRIALS

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ABSTRACT

We describe the development of a submersible flow cytometer capable of *in situ* single particle analysis. The instrument can be deployed from a ship's winch to a depth of 200 m in profiling mode, or mounted on a submersible research platform. Novel features of the design include blockage-resistant fluidics, solid state optics and recirculating sheath fluid. The optical parameters measured include chlorophyll fluorescence and forward and ninety degree light scattering. Full optical pulse profiles are digitized, which makes it possible to determine the longest dimension of particles to around 1 μ m resolution and to discriminate between the fluorescent and non-fluorescent morphological elements of phytoplankton cells and colonies.

INTRODUCTION

When flow cytometers were first introduced to the marine sciences, instruments were taken to sea in dedicated container laboratories with separate industrial cooling plants (Tarran and Burkill 1992). However the introduction of air cooled argon lasers soon led to compact benchtop designs, and it was anticipated that flow cytometry would make a major contribution to our understanding of the nature and dynamics of particle suspensions in the sea (Demers 1991). The potential value of the technique was demonstrated by the detection of ubiquitous and previously unknown populations of marine prochlorophytes (Chisolm et al. 1988). Early studies of single-cell phytoplankton optics included estimates of absorption cross sections (Perry and Porter 1989), refractive indices (Spinrad and Brown 1986), forward scattering patterns (Cunningham and Buonaccorsi 1992), and anomalous scattering from cyanobacteria (Dubelaar et al. 1987). However from the point of view of marine optics, much of the research momentum seems to have been lost in recent years. The few research groups currently carrying out flow cytometry at sea are concentrating on problems in microbiology (Sieracki et al. 1995), picoplankton physiology (Boelen et al. 2000) and grazing by microheterotrophs (Zubkof et al. 2000). Since modern benchtop flow cytometers are no more expensive than other instruments routinely used aboard ship, the underlying reason can no longer be the cost and difficulty of deployment. Moreover, recent attempts to draw up a database for the optical properties of marine particles (Stramski and Mobley 1997) show that there is an urgent need for information on natural particle suspensions. It is possible however that the general adoption of flow cytometry in marine optics is limited by the fact that commercially available instruments are designed for biomedical work and are

inappropriately configured for general particle analysis. There are several aspects to this problem. Flow cytometers designed for mammalian cells tend to have easily blocked flow systems and to generate flow profiles which disrupt phytoplankton chains and colonies. Laser wavelengths which have been chosen to excite histochemical stains rarely coincide with the main photopigment absorption bands. Most flow cytometers do not precisely control the volume of sample which they analyze, and it is often difficult to translate the data which they generate into accurate numerical concentrations. Furthermore the necessity to bring water samples on board ship restricts the rate and precision with which the distribution of particles in the water column can be measured. In an attempt to alleviate these restrictions, we have designed a new flow cytometer specifically aimed at marine particle analysis. The instrument is based on technology developed under the EU MAST Cytobuoy program (Dubelaar et al., 1999, 2000) and the design goal is a completely autonomous, fully submersible flow cytometer suitable for in situ deployments.

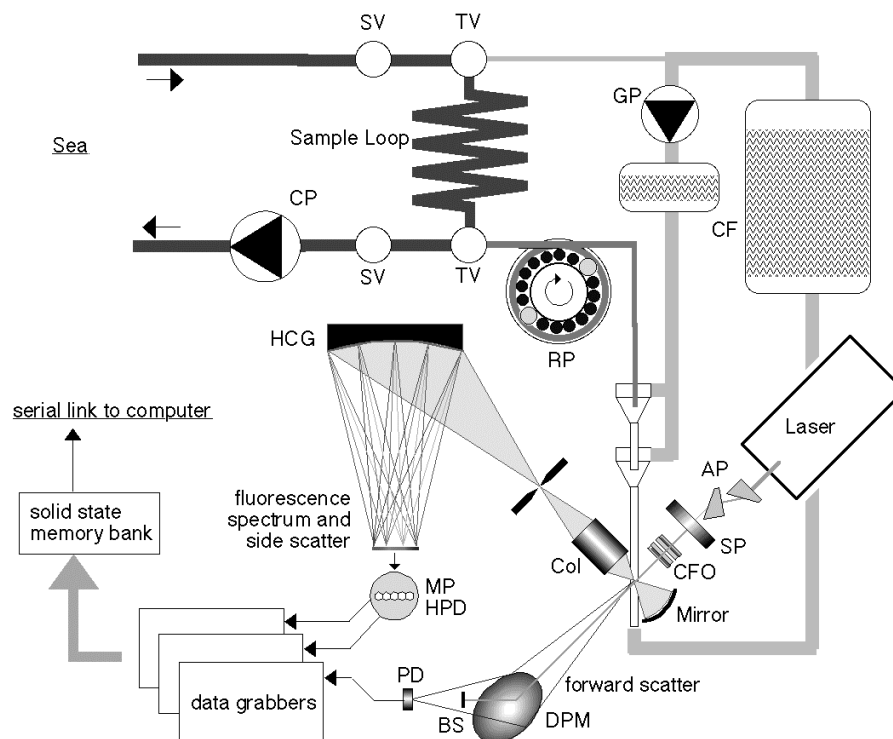


FIGURE 1: Functional design of instrument. SV-safety (isolating) valve; TV-three-way valve; CP-seawater circulation pump; GP-sheath fluid gear pump; RP-roller pump (sample); CF-cartridge filter; AP-anamorphic prism; SP-Savart plate; CFO-cylindrical focusing objective; DPM-double parabolic mirror; BS-beam stop; PD-photodiode; Col-collecting lenses; HCG-holographic concave grating; MPHCD-multipixel hybrid photodiode.

INSTRUMENT DESIGN

The complete flow cytometer (Figure 1) is held at a pressure of one atmosphere in a cylindrical aluminium housing with external dimensions of 415x530 mm, consisting of a tube of 12 mm wall thickness with 38 mm thick end plates. Sea water is pumped into the housing through a 2 mm bore stainless steel tube at a rate of 25 cm s^{-1} at ambient pressure and isolated in a sample loop by means of electrically activated ball valves. The circulation pump is a custom built magnetically coupled unit placed on the exhaust side of the sample loop. The housing and sample intake system has been tank-tested to 250 meters equivalent pressure. The sample loop is then connected into a second hydraulic circuit at one atmosphere, so that it acts as a pressure reduction device for operating at depth. The sample volume is precisely determined by the loop, and it is transferred by a specially designed non-pulsating tube pump into a square section quartz flow cell with a 1 mm wide channel. Hydrodynamic focusing is achieved by coaxial injection into particle-free sheath fluid in a two-stage process designed to minimize lateral displacement of the sample core in the flow cuvette, which might otherwise be generated by movement of the cytometer during deployment. The entry profile of the flow chamber is designed to minimise longitudinal acceleration in order to avoid the disruption of cell colonies and filaments, and the end result is a line of particles oriented in single file with their long axis parallel to the flow direction, entrained in a core of about $70 \text{ }\mu\text{m}$ diameter. These particles pass through the waist of the illuminating laser beam (Figure 2) travelling at 2 m s^{-1} . In order to reduce the internal volume of the instrument, the sheath fluid has a total volume of around 1 litre: it is pumped through two $0.2 \text{ }\mu\text{m}$ cartridge filters (which also serve as reservoirs) and continuously recirculated.

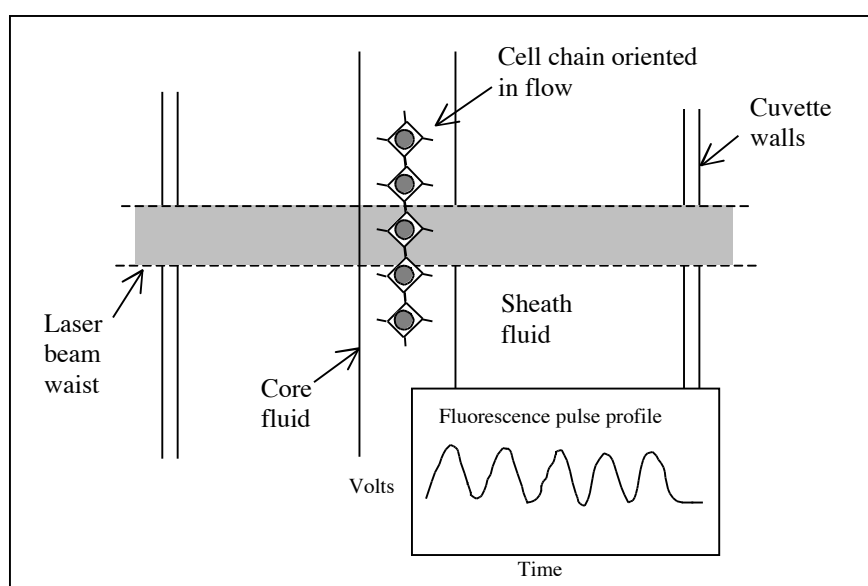


FIGURE 2: Interaction of a chain of cells with the laser beam waist (schematic).

Illumination is provided by a 20 mW solid state laser operating at 675 nm (Circulaser, Blue Sky Research) which coincides with the chlorophyll red absorption peak. In the horizontal plane, a set of anamorphic prisms reduces the beam width to about $320\text{ }\mu\text{m}$ ($1/e^2$ points) and a Savart Plate then redistributes the light intensity to obtain a non-gaussian intensity profile. In the vertical plane, a set of cylindrical lenses brings the laser beam to a focused height of approximately $5\text{ }\mu\text{m}$ in the center of the flow cuvette. The object of this elaborate beam shaping is to produce a relatively wide central flat section of maximum illumination ($\pm 3\%$ over the central $200\text{ }\mu\text{m}$) to cope with possible movements of the sample-bearing core. Forward scatter is collected over a solid angle of 1 to 22 degrees by a photodiode (BPX61) and orthogonal scatter and fluorescence (in the range 690-730 nm) in a solid angle of 42° by an aspherical condenser lens followed by a Steinheil triplet. A custom 96 mm focal length, f . 2.0 holographic concave diffraction grating (American Holographic Inc., Fitchburg, MA) images the fluorescence spectrum of each passing particle onto a multi-pixel hybrid photodiode (DEP, Roden, NL), a detector based on a vacuum photocathode which combines photomultiplier-like performance with solid state technology. Each pixel covers about 20 nm of the fluorescence spectrum, with a maximum of 10 pixels per detector: in the prototype instrument, only one pixel is used for fluorescence detection and a second for wide angle scattering. The interaction of a chain of phytoplankton cells, such as diatoms or cyanobacteria, with the laser beam waist is shown schematically in Figure 2. The waveform from each detector is continuously digitized at 4 MHz and stored in solid state memory (54 kB per optical channel). In the current state of development of the instrument, this data is downloaded via a serial link and post-processing is carried out on a host personal computer: future plans include the installation of real time signal processing. Sampling is initiated by a logic signal and terminated when the memory allocated for a given run has been filled. The instrument can be suspended from a ship's winch with a connecting cable carrying power and control signals, so that particle analyses can be carried out at depths selected from hydrographic or optical profiles. Alternatively, it can be mounted as a completely autonomous unit on an underwater platform. A key objective of this project is deployment on *Autosub*, the UK autonomous underwater vehicle, when it will be possible to pre-program depths and other conditions for sampling at the start of the mission.

PRELIMINARY RESULTS

At the time of writing, the prototype instrument is still undergoing bench tests in the laboratory. Figure 3. shows pulse profiles obtained from a chain of diatom cells (species unknown) , and serves to illustrate a number of features. The overall duration of the forward and side scatter signals defines the length of the chain (around $250\text{ }\mu\text{m}$ in this example), while the periodic nature of the fluorescence signal is due to the presence of discrete chloroplasts distributed along the chain. The influence of these chloroplasts and associated cell structures in modulating the forward scattering signal can also be seen. Figure 4. Shows data from a ship test of the prototype equipment in the Celtic Sea, displayed as a scatterplot of fluorescence (integrated over the pulse profile) against forward scatter with histogram distributions projected on the two axes. This prototype was equipped with a green excitation source and dual fluorescence detectors. The mean

height of the fluorescence signal is indicated as the diameter of the circles, and pulse profiles characteristic of large diatom chains fall within the polygon drawn on the graph.

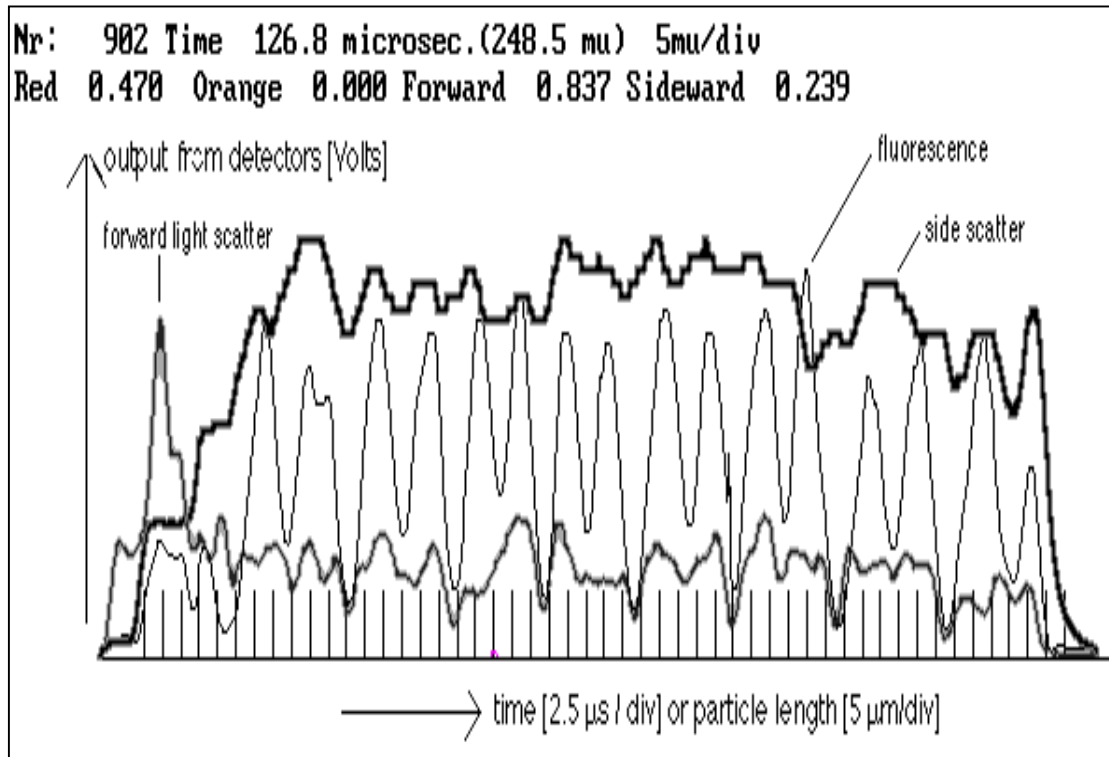


Figure 3. Typical pulse profiles for a diatom chain.

DISCUSSION

The importance of establishing a link between measurements of single particle optics and bulk inherent optical properties was first discussed by Morel (1991), but remarkably little progress has been made since. We hope that the availability of a submersible flow cytometer will open up new possibilities for studying populations of marine particles *in situ*, and there are a number of identifiable topics of current ecological interest which would benefit from the technique. These include detecting thin layers of heterotrophic activity which are believed to exist close to major pycnoclines, monitoring the physiological status of sinking algal blooms, investigating variations in diatom chain length under different physical conditions (e.g. turbulence regimes), and counting phytoplankton cells in waters which bear a heavy burden of other suspended particles. However the main potential contribution to ocean optics is the possibility of accurately characterizing mixed particle assemblages in terms of the numerical proportions of different classes of the materials and of the distribution of sizes within each class. This information should provide new insight into variations in volume scattering functions, particularly in coastal waters. Wider application of flow cytometry to the determination of single particle optical characteristics may help to advance our understanding of the

characteristics of mixed particle suspensions and hence, in an interesting contrast of physical scales, aid in the interpretation of satellite measurements of remote sensing reflectance.

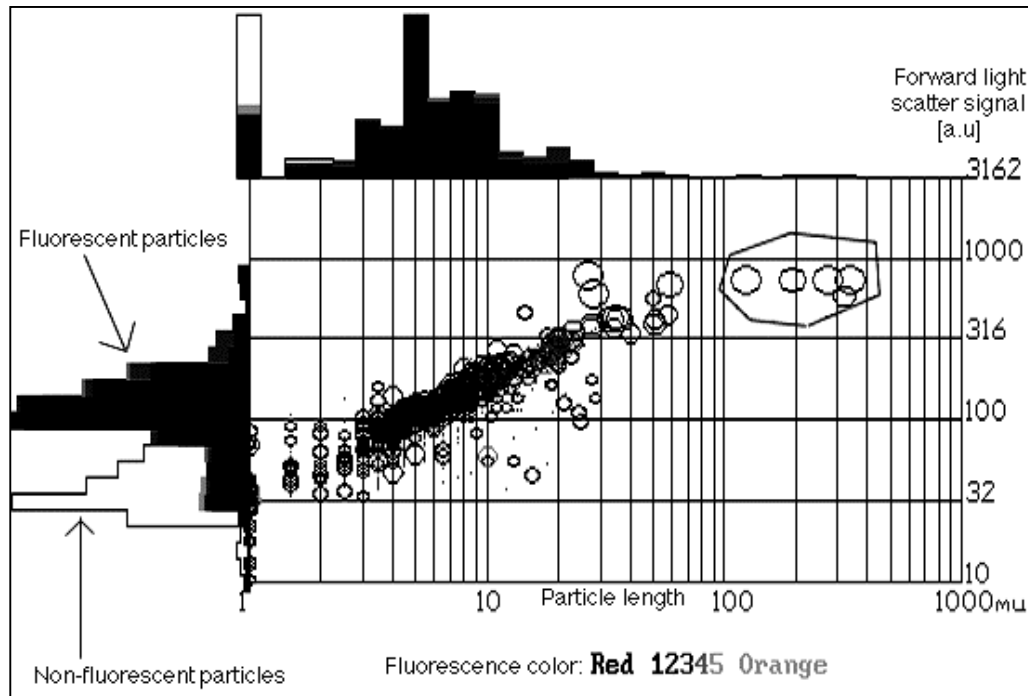


FIGURE 4. Scatter plot of data obtained during shipboard trials of the prototype.

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