A new device for *in situ* video and fluorescence analysis of marine particles. Applications to phytoplankton studies.

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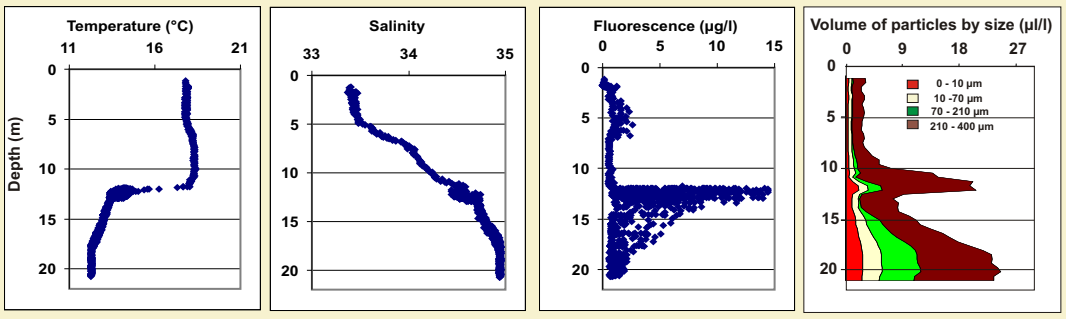
Abstract

A new video-microscope has been developed for in situ investigation on marine particles. A field of view is produced by a laser beam at 473 nm. Individual cells or particles that entered this field of view appear as individual diffraction-limited spots of light, which are resolved from the dark background. A mobile high-pass optical filter (580 nm) can be controlled and displaced in front of the CCD camera, allowing simultaneously imaging and discrimination between fluorescent and non-fluorescent particles. The system allows visualisation of particles ranging from 10 µm to several millimetres, depending on the zoom magnification. With the 580 nm high pass filter, the light detected by the CCD is mainly due to chlorophyll fluorescence (phytoplankton). In coastal ecosystem studies, this new device is well adapted to the description of phytoplankton populations variability.

Objectives : Ecological studies of coastal areas

Understanding the spatial and temporal variabilities of suspended particle populations is essential to many fields of oceanographic research including sediment transport and ecology. Many studies have shown that the vertical density gradient often governs the vertical distribution of particle populations in the water column. For instance, phytoplankton and zooplankton species have often been observed in very thin layers where biological and physical conditions allow optimal growth and accumulation of their populations. Understanding relationships between particle distribution and environmental parameters appears to be necessary in order to better understand the structure and functioning of a given ecosystem.

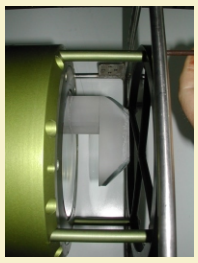


Vertical profiles of Temperature, Salinity, Fluorescence and Particles volume by size (Loire estuary, June 2003).Thanks to the sensors, a thin layer of phytoplankton biomass was identified at 12 m, around the thermocline. High amount of large size particles was detected above and below the thermocline due to the presence of organic agregates. Particle volume also increased in the bottom layer in relation to re-suspension from the bed by current and tides.

In situ Instrumentation

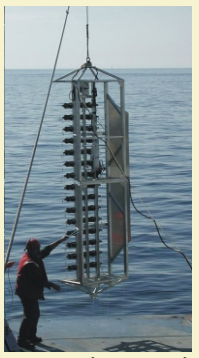
Vertical distributions of particles concentrations and of their characteristics can be obtained by in situ techniques such as diffraction analysis or fluorescence. However a precise identification of their origin can only be achieved through sampling techniques and microscopic observations in laboratory.

Field observations require the deployment of a well-adapted instrumentation during the oceanographic cruises.



Particle Size Analyzer (Cilas-Ifremer)
Diffraction analysis of a laser beam. Measurement of the total amount of particles and of the particle size distribution (30 classes between 0.7 and 400 µm).

Fluorometer
Measurement of the total phytoplankton biomass

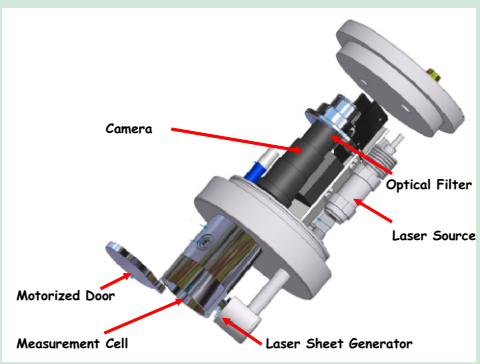


Fine Scale Sampling within stratified water column.

In situ spectral imagery appears as an innovative method to observe and discriminate between particle populations.

Videomicroscopy

Principle



3D view of the basic setup

The system principle is based on the generation of a light sheet which provides a thin illumination field through which particles are observed by a CCD detector. The size resolution and the sensitivity of the camera (EXAVISION , Nîmes, F) were optimised in order to resolve particles ranging from 10µm to several millimetres, depending on the zoom settings. A light source (blue laser at 473 nm, OXXIUS, Lannion, F) has been developed to stimulate organic population and image the emitted fluorescence (pigment) of phytoplankton cells. Equipped with a remote high pass filter (580 nm.), it allows video-microscopy to be considered as a fluorescence imaging system, enabling direct in situ discrimination of suspended particles.

Description

The videomicroscope with its measurement cell and the motorized door. Tests and calibrations on suspended particles can be performed in laboratory by using standard culture cuves.

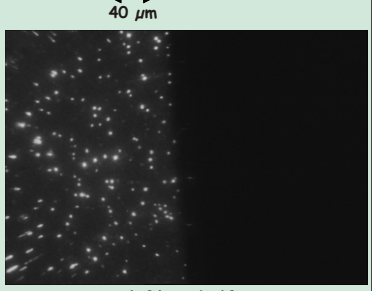
Caméra :
- 752*582 pixels
- Sensibility < 3mlux
- Resolution : 4 µm
- Intensified tube GEN2+
- Photocathode S25
- Maxi Gain 10000
- Zoom *14
- Focus distance : 168 mm in water
- Mini Field : 9 * 6 mm

Laser :
- 473 mm
- 0-15 mW
- Width of the sheet : 3 mm

Mobile optical filter
- Cut off at 580 nm

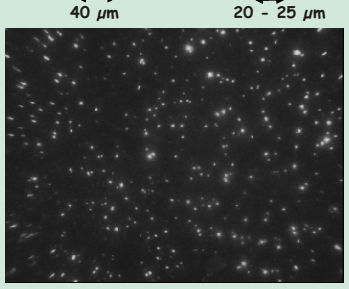
Real time Spectro-Video Visualization

Non fluorescent Polystyrene beads — 40 µm



Optical filter half set in front of the CCD. The non fluorescent beads are not visible in the right part of the image.

Non fluorescent Polystyrene beads — 40 µm + Phytoplankton cells Karenia mikimotoi — 20 - 25 µm



Optical filter completely in front of the CCD. Only the phytoplankton cells are visible.

Computed data

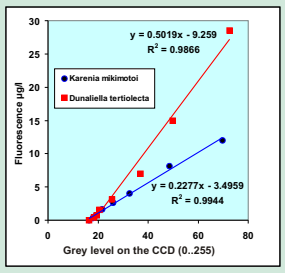
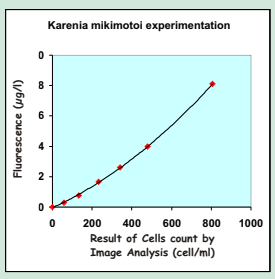


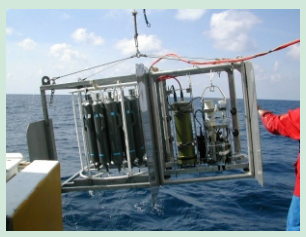
Image analyses techniques allow to extract quantitative results from the video observations. With the filter set in front of the CCD, the received light is related to the total fluorescence emitted by the phytoplankton cells. Calibrations were performed in laboratory with phytoplankton cells using the pigment chlorophyll-a for photosynthesis activity (K. mikimotoi, D. tertiolecta). It was shown that the fluorescence intensities measured by a standard fluorometer were well related to the light energy received by the CCD. Discrepancies appeared when comparing the relationship slopes for each species. This could be due to their specific morphometric properties.

After thresholding of the digitized images, fluorescent particles appear as individual bright spots of light which can be processed by image analysis techniques. Image processing extract informations on the particle characteristics such as size, shape, number or movement trajectories. Through wavelength filtering, more specific data are acquired on organic populations. In situ quantification and observation of the individual fluorescent particles will allow the description of the vertical changes in the pattern of the phytoplankton abundance and composition.



Applications

- In situ characterization and discrimination of particle populations in coastal areas,
- Studies of the phytoplankton variability and relationship with hydrological and physical conditions,
- Analysis of the behaviour pattern of living organisms and of the settling rate of sedimentary particles,
- Studies in laboratory on phytoplankton cultures.



The pelagic profiler on which will be integrated the new video-microscope

Conclusion

Spectro-Videomicroscopy is an alternative to traditional methods for studying distribution and abundance of particles by diffraction analysis. Video allows rapid, objective analysis of light scattering and fluorescence of individual particles. The results indicate that fluorescence vs. video-microscopy techniques can be used on board ship for automated in situ analyses of plankton and particles. Integration of this new device on standard profiler is very helpful for detection and characterisation of vertical layering of phytoplankton species. This instrument is a first step towards in situ spectral imaging techniques to be used as a complement to conventional quantification of phytoplankton groups.

References

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