A new device for \textit{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 \textit{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 presents the vertical distances 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.

In situ Instrumentation

Vertical distributions of particles concentrations and of their characteristics can be obtained by \textit{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.

**Videomicroscopy**

**Principle**

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 populations and image the emitted fluorescence (pigment) of phytoplankton cells. Equipped with a remote high pass filter (580 nm), it allows videomicroscopy to be considered as a fluorescence imaging system, enabling direct in situ discrimination of suspended particles.

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

**Real time Spectro-Video Visualization**

**Computed data**

Image analysis 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. Observations 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.

**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 obtained \textit{in situ} video analysis are currently being validated on board ship. For \textit{in situ} analyses of plankton and particles. Integration of this new device on standard profiling is very helpful for detection and characterisation of vertical layering of phytoplankton species. This instrument is a first step towards \textit{in situ} spectral imaging techniques to be used as a complement to conventional phytoplankton group analyses.

**References**