An Underwater Holography System for the Study of Plankton

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In order to understand the biology of organisms such as plankton, it is necessary to know about not only single organisms and their numbers, but also about the interactions between individual organisms, and indeed between species. Unfortunately conventional sampling techniques such as bottles and nets often destroy valuable information about the spatial relationships between the various individuals. Pulsed holography is a powerful technique for the study of particle fields as it allows instantaneous, non-invasive high-resolution recording, and the later replay of real images from which one can obtain the size, shape, three-dimensional position and - if multiple exposures are made - velocity of every object in the sample volume. The Holomar collaboration is currently working on a complete holographic system designed for studies of plankton *in situ*. The system comprises three parts:

- an underwater holocamera, self-contained within a pressure housing designed for operation down to 100m. Up to 40 holograms may be recorded on glass plates using a purpose-built pulsed Nd:YAG (532nm) laser. Uniquely, the camera will incorporate both the 'in-line' and 'off-axis' holographic geometries: in-line holography can record organisms in the 5 to 250 µm range at concentrations up to several thousand cm^{-3} while off-axis holography is better for organisms bigger than 100µm and at much higher concentrations. The use of both geometries with overlapping sample volumes (of tens of litres) should therefore allow recording of a wider range of organisms under a greater variety of conditions than current alternatives.

- a dedicated scanning machine that automatically locates the plankton images within the reconstructed sample volume. Three computer-controlled stages scan a CCD camera fitted with a microscope objective through the real image projected from the holographic plate by a He-Cd (442nm) laser, to minimise replay aberrations.

- image analysis and identification software. A set of image processing and analysis routines has been developed that can locate the plane of best focus of an object within a sequence of images and generate a suitable binarised representation for input to a neural net trained to differentiate between several forms of phyto- and zoo-plankton. By integrating the software and replay machine it will be possible to generate the identity and location of every organism within the recorded sample volume without operator intervention.

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