

## An Underwater Holography System for the Study of Plankton

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**Abstract.** A system for the *in-situ* holographic recording and subsequent analysis of plankton and their spatial distribution is described.

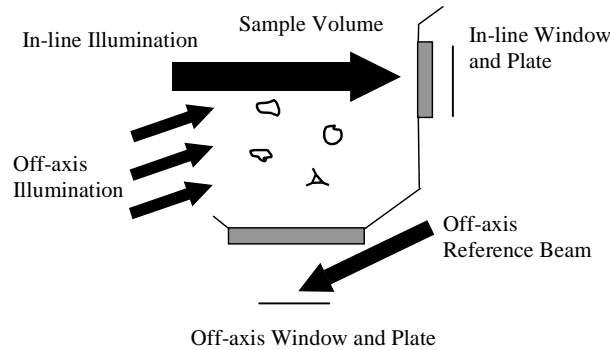
### INTRODUCTION

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 laser 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. A submersible holocamera to record plankton distributions *in situ* was built by Stewart *et al.* [1], and an in-line holography system recently deployed by Katz *et al.* [2] was able to image organisms down to 10 $\mu$ m in size over a sample volume 63mm diameter and up to 900 mm in length, although the holograms required time-consuming manual analysis. The Holomar collaboration is currently building a complete holographic system designed for studies of plankton *in situ*. The system comprises three parts: an underwater holocamera, a hologram replay system, and particle best-focus location and organism classification software.

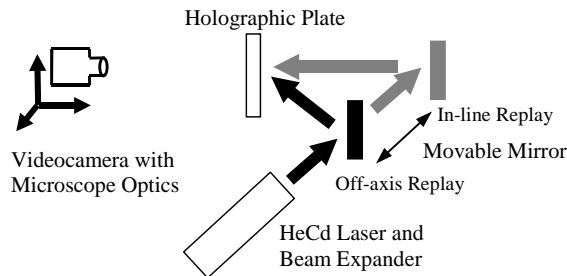
### HOLOCAMERA

The underwater holocamera, 'HoloCam' [3], is 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  $\mu$ m range at concentrations up to several thousand cm<sup>-3</sup> while off-axis holography is better for organisms bigger than 100 $\mu$ m and at much higher concentrations. The use of both geometries with overlapping sample volumes (figure 1) should therefore allow recording of a wider range of organisms under a greater variety of conditions than current alternatives.

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**Figure 1: Schematic of Hologram Sample Volume**



**Figure 2: Schematic of Replay Machine**

## REPLAY SYSTEM AND OBJECT EXTRACTION

The developed holograms are replayed on a dedicated scanning machine ‘HoloScan’ (figure 2). Three computer-controlled stages move a CCD camera fitted with a microscope objective through the real image projected from the holographic plate by a He-Cd (442nm) laser. The laser wavelengths, beam angles and window thickness on HoloCam and HoloScan have been matched, so as to to minimise replay aberrations [4]. The replay geometry is switched between in-line and off-axis simply by moving a mirror between two fixed positions. Some sample images are shown in figure 3.

As the camera is moved depth-wise through the reconstructed sample volume, the images are digitised, cleaned and enhanced. A set of image processing routines has been developed to track objects in the camera’s field of view over successive frames, identifying the plane of best focus for each object. The best focus image is then binarised for automated classification.

## ORGANISM CLASSIFICATION

Automatic classification of plankton images faces two major challenges: first, plankton are living individuals that may cluster together or deform (e.g. their motions associated with swimming); and secondly we have only 2-d image slices of 3-d objects that may be in any orientation with respect to the camera.

The classification system has been implemented as a three-layer neural network (seven input neurons, seven hidden neurons and five output neurons). The inputs are the size of the object and a simple measure of its elongation (the ratio of its diameters along the primary and secondary inertia axes), and some scaling- and rotation-invariant descriptors of shape (the first five Hu moments). The outputs depend on the image set used to train the network: e.g. for in-line holograms the network currently differentiates between Zooplankton; Floc (inorganic or dead matter) and three types of phytoplankton (*Asterionella formosa*, *Ceratium tripos* and *Thalassiosira sp.*) of which holograms of cultured samples were available. The viability of this approach was confirmed in tests, in which zooplankton and floc were identified 100% of the time and *Asterionella*, *Ceratium* and *Thalassiosira* were correctly classified 95%, 94 % and 85% of the time, respectively. The performance of the network (and possibly the range of output classes) will be improved when more images become available once the holocamera has been deployed.



**Figure 3: Reconstructed in-line holograms: *Ceratium Tripos* (left and centre) and *Thalassiosira sp.* (right). All same scale - image height approx 310 $\mu$ m.**

## CONCLUSIONS

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. This is an important advance, as even a single hologram can contain the equivalent of many terabytes of raw data and require weeks of manual analysis (with the attendant risk of data degradation due to operator fatigue). The ability to automatically extract the huge volumes of data is crucial to the use of holography, both in plankton studies and in other fields (e.g. insect swarms). The off-axis hologram recording capability of the holocamera may also be useful in other applications, such as underwater inspection or archaeology.

## REFERENCES

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