An Underwater Holography System for *in-situ* Plankton Studies

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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 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. The Holomar collaboration has built 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.

The underwater holocamera, *HoloCam* [1], is self-contained within a pressure housing designed for operation down to 100m. Up to 45 holograms may be recorded on glass plates using a purpose-built pulsed Nd:YAG (532nm) laser. Uniquely, the camera incorporates 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 thus allows recording of a wider range of organisms under a greater variety of conditions than previous alternatives. *HoloCam* is currently undergoing sea trials in inshore waters around Oban, Scotland.

The developed holograms are replayed on a dedicated scanning machine *HoloScan*. 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 minimise replay aberrations [2]. The replay geometry is switched between in-line and off-axis simply by moving a mirror between two fixed positions.

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 tracks 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 (figure 1).

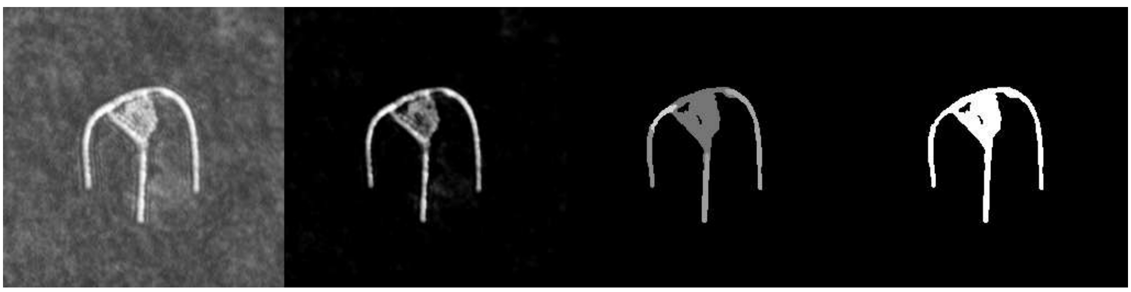

Figure 1: *Ceratium Tripos*, about 250µm long. From left to right: raw reconstructed image, cleaned and stretched image, image after region growing, and binarised image to be passed to neural net

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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 neurones, seven hidden neurones and five output neurones). 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 after the current sea trials.

The major hurdle faced by holographic techniques to date has been the challenge of data extraction, 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). This project has explicitly incorporated the needs of the hologram replay and image processing right through back to the holocamera, and 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. 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 *HoloCam* may also be useful in other applications, such as underwater inspection or archaeology.

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