HoloCam: A subsea holographic camera for recording marine organisms and particles

G Craig[a],
S Alexander[a], S Anderson[a], DC Hendry[a], PR Hobson[b], RS Lampitt[c], B Lucas-Leclin[d], H Nareid[a], JJ Nebrensky[b], MA Player[a], K Saw[c], K Tipping[c], J Watson[a],

[a]Dept of Engineering, Aberdeen University, Aberdeen AB24 3UE, Scotland
[b]Dept of Electronic and Computer Engineering, Brunel University, Uxbridge, Middlesex UB8 3PH
[c]Southampton Oceanography Centre, Empress Dock, Southampton SO14 3ZH, England
[d]Quantel, 17 Avenue de l'Atlantique, BP23, 91941 Les Ulis, France

ABSTRACT

The HoloCam system is a major component of a multi-national multi-discipline project known as HoloMar (funded by the European Commission under the MAST III initiative). The project is concerned with the development of pulsed laser holography to analyse and monitor the populations of living organisms and inanimate particles within the world's oceans. We describe here the development, construction and evaluation of a prototype underwater camera, the purpose of which is to record marine organisms and particles, *in-situ*. Recording using holography provides several advantages over conventional sampling methods in that it allows non-intrusive, non-destructive, high-resolution imaging of large volumes (up to 10^5 cm^3) in three dimensions. The camera incorporates both in-line and off-axis holographic techniques, which allows particles from a few micrometres to tens of centimetres to be captured. In tandem with development of the HoloCam, a dedicated holographic replay system and an automated data extraction and image processing facility are being developed. These will allow, optimisation of the images recorded by the camera, identification of species and particle concentration plotting.

Keywords: Holography, Underwater, Plankton

1. INTRODUCTION

It has been known for some time that the earth's climate is intimately connected to the chemical and physical processes occurring within our oceans. It is also known that the proportions and species of plankton within the upper water column are a real-time indicator of the oceans condition. Therefore it can easily be concluded that monitoring these minute organisms will yield invaluable information concerning our ever changing environment[1,2,3].

Present methods of sampling these creatures are not well suited to observing precise spatial relationships or preserving some of the more delicate organisms. For example, electronic counting techniques tend to destroy the plankton and flocs and consequently obviate any visual identification. Another common technique, high-resolution photography, can only resolve a narrow depth of field per exposure. Hence, sampling a large volume takes a considerable time, during which the particle distribution may change.

Holographic recording and imaging offers biologists an alternative to conventional imaging techniques for the analysis of marine systems[4-10]. The advantage of holography over other visual inspection techniques is that it permits non-intrusive and non-destructive analysis of the organisms and particles in their natural environment. It provides this by producing a high resolution, 3-dimensional image that retains both parallax and perspective information. Since a pulsed laser with a relatively short pulse length is used for the recording, the object scene is effectively frozen at the recording instant allowing even fast moving particles to be recorded.

Through replay of the hologram, the spatial distribution and relative location of the particles can be analysed as well as the individual particles themselves. Using a combination of holographic techniques detail of around 10μm can be resolved and volumes up to $10^5 cm^3$ can be recorded. Since the holograms themselves are recorded on emulsion covered glass plates the method also provides a permanent record for archiving.

2. RECORDING AND REPLAY OF UNDERWATER HOLOGRAMS

Although recording underwater introduces some additional complications[11,12] the general principles of holography remain unchanged. From the several different holographic techniques available[13] two have been found particularly suitable for field application as high resolution imaging tools, namely in-line and off-axis.

2.1 In-line recording

In in-line holography a single beam of coherent light is directed through the subject volume onto a holographic plate (figure 1). At the plate the light diffracted by the objects in the beam path interferes with the undiffracted portion to form the hologram.

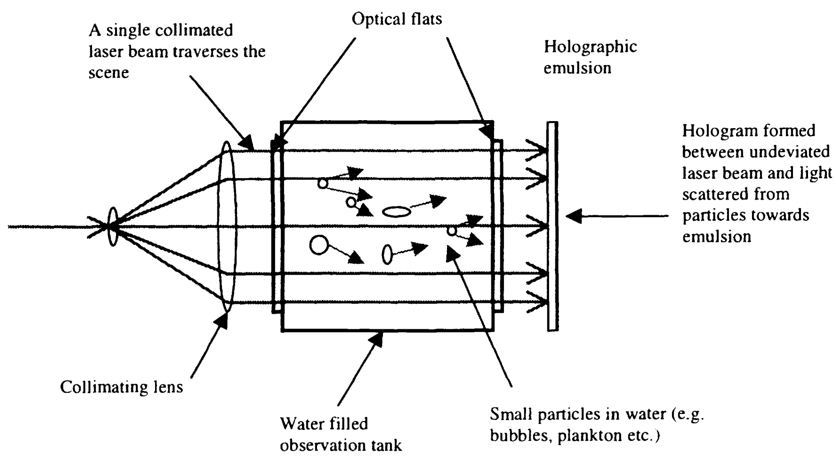

Figure 1: Recording an in-line hologram

This process relies on transmission of the undiffracted wavefront and can therefore only be used with a relatively highly transparent subject (approximately 90%). The upper particle size that can be recorded using in-line is set by the Fraunhofer[13] condition, which states:

$$Z > d^2/\lambda$$

Where Z is the object to plate distance, d is the maximum dimension of the particle to be recorded and λ is the wavelength of the recording light. This limits the maximum recordable particle size to around 250 μm. However, the main advantage of in-line holography is that it allows particles down to around 10 μm or lower to be resolved.

2.2 Off-axis recording

In comparison, the off-axis technique utilises two separate beams to record the hologram. The first, the reference beam, is directed onto the plate at an angle (60° in this case) without transversing the subject volume (figure 2). The subject is simultaneously illuminated with diffuse laser light and a portion of this is reflected and interferes with the reference beam at the plate to form the hologram. Since this process relies on light reflected from the subject much larger objects may be recorded. Maximum dimensions are only limited by the field of view of the camera and the coherence length and available power of the laser. However, the lower limit of resolution is restricted, primarily by speckle and optical system constraints, to around 30-40 μm[13] (depending on geometry and conditions). This technique is more versatile than the in-line and is suited to recording larger particles in higher concentrations and larger volumes.

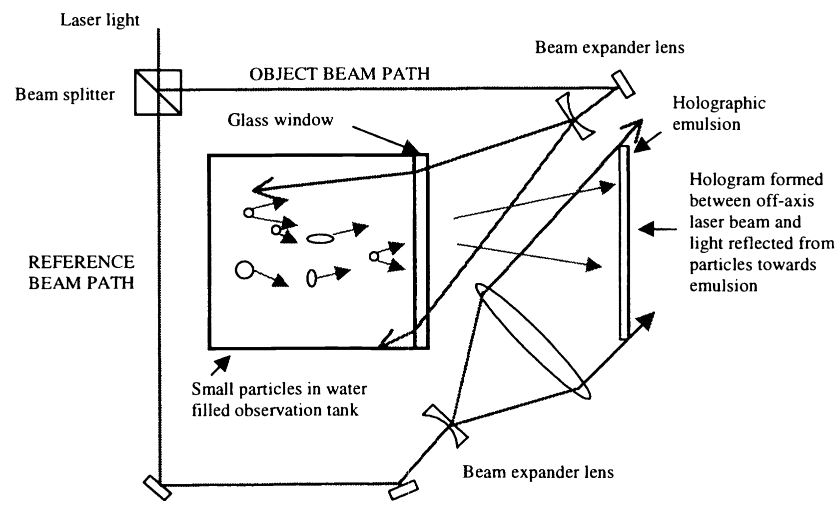

Figure 2: Recording an off-axis hologram

2.3 Replay

Although this paper is primary concerned with the development and construction of the HoloCam, the full potential of the system can not be realised without considering the associated replay and data analysis systems, HoloScan. After the holograms have been recorded they are developed by a wet chemical process similar to that used in monochrome photography and dried. To replay the hologram the plate is placed in the phase conjugate of the recording reference beam. To simplify matters the holograms are recorded with a collimated beam, which means that for optimum results they are replayed with an identical collimated beam.

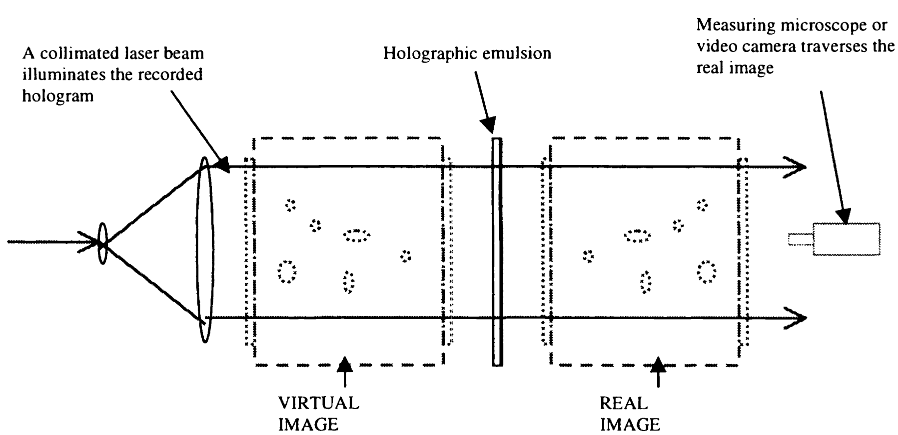

Figure 3: Replay of an in-line hologram

Replay of the hologram allows the recorded scene to be projected into free space where detailed analysis can be carried out on the image. In both off-axis and in-line replay two images are formed, the real and the virtual. The virtual image is located behind the plate and the real image is projected into free space infront of the plate. Replay of the in-line hologram is shown above in figure 3. The image is viewed with a video camera or microscope mounted on computer controlled micropositioning stages which travel through the projected volume allowing individual particles to be brought into focus. Within the project, software is being developed to automate this laborious task. Once the interesting particles have been located a through focus sequence of images is sent to image enhancing software then onto a neural network which attempts to classify the subject.

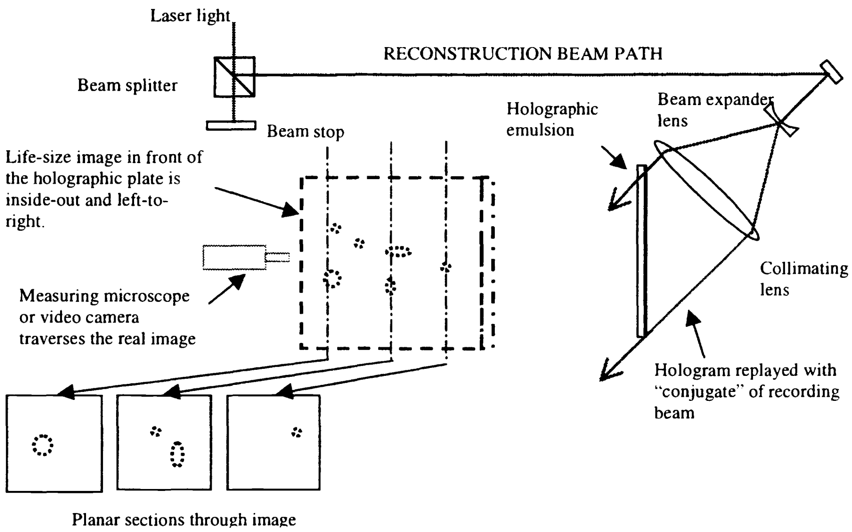

Figure 4: Replay of an off-axis hologram (real image)

The complete reconstruction system will consist of a dedicated hologram replay and data analysis facility. Specially developed image processing and neural network algorithms will allow identification of species at the family or genus level together with the ability to automatically extract information regarding shape, dimensions, relative position and measurement of local concentration and distribution of a variety of marine organisms. However, the replay and data extraction facility will not be discussed further in this paper.

2.4 Subsea Holography

Since our interest is in the recording of marine organisms, we expect the water itself to influence the quality of the images produced. An increase in the overall turbidity of the water will adversely affect both techniques and would be expected to create background noise that will reduce image fidelity. Holograms of particles recorded directly in water will also be affected by the refractive index change when replayed in air, which will increase the optical aberrations in the reconstructed image. With in-line holography, only a small amount of spherical aberration is introduced since both reference and object beam angles are normal to the recording plane[14]. However, in the off-axis case, the resulting refractive index mismatch does produce significant aberrations, most noticeably astigmatism and coma, which increase with the field angle of the subject in the reconstructed image. Previous work[12] on the origins of image aberrations resulting from refractive index mismatch suggests that image degradation can be reduced by replaying in air at the effective wavelength of the beam in water (i.e. a replay wavelength equal to the construction wavelength divided by the refractive index).

In our work, a green (532 nm) laser is used, and so the ideal compensation is given by 532 nm/1.33, i.e. a replay wavelength of 400 nm. However, no suitable laser lines exist in this region of the spectrum. Furthermore, complete correction assumes that the entire holographic system be located in water. Since this is both impractical and undesirable, the holograms are recorded with the holographic plate in air behind a planar, glass window. Analysis of the aberration balancing which can be accomplished shows that good compensation can still be achieved if the hologram to window separation in the recording geometry is about a fifth of the window thickness. A typical window thickness of 30 mm suggests an optimum air gap of around 6 mm for object points that lie directly on the optical axis of the system. Such a small air gap, though, constrains the angle of reference beam which can be accommodated. Since an angle of about 60° is required to allow high resolution recording, a larger air gap is required. Fortunately, our analysis also shows that using a larger air gap shifts the optimum replay wavelength to a longer value[15]. For an air gap of 120 mm, a blue replay wavelength of 442 nm achieves a good performance over a full field angle of about 40°. It should be noted that refractive index compensation is a particular requirement of the off-axis holograms but is not so vital for in-line holograms.

3. HOLOCAM

The holographic camera consists of the following components; a watertight pressurised housing with support rig (figure 5), a laser with associated power supply, 2 plate holders, holographic optics and the control system. The overall dimensions of the camera will be approximately 2 m long by 1 m in diameter. The initial prototype system will be capable of either ship deployment or attachment to a fixed buoy and will allow recording down to a depth of 100m. To eliminate the possibility of condensation occuring on the windows the housing will be purged with nitrogen before deployment

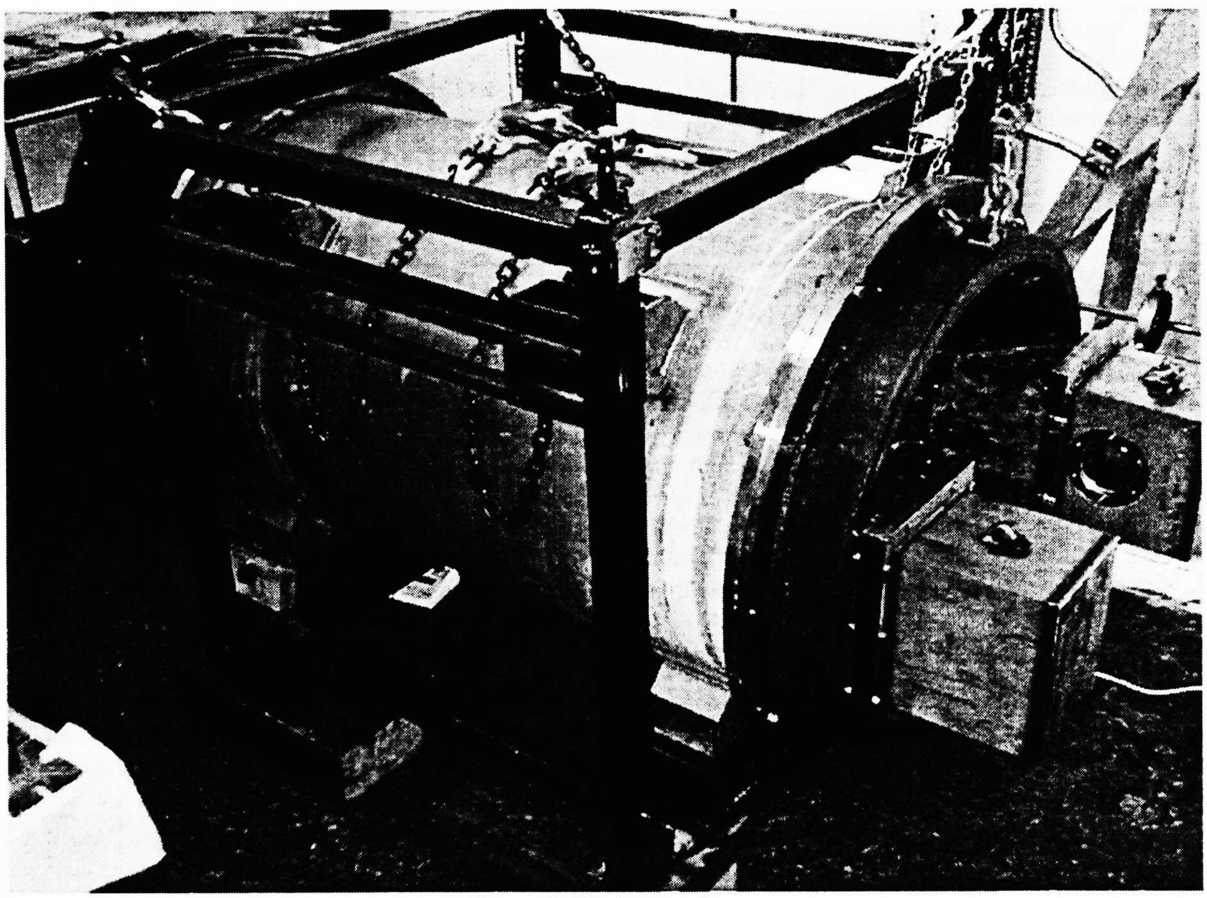

Figure 5: Main housing

The optical window for the off-axis mode is recessed into the housing (figure 6) and allows return of the reflected/scattered light from the object volume. The distance between the plate-holder and the window is set at 120 mm (to allow access for the reference beam, as discussed above). The in-line hologram is recorded between the two arms protruding from the front of the camera. As can be seen the off-axis mode records a larger volume than the in-line but a significant degree of overlap between the two is provided. Compared with the in-line mode, the off-axis hologram records almost the entire volume delineated by the front window to the end of the arms.

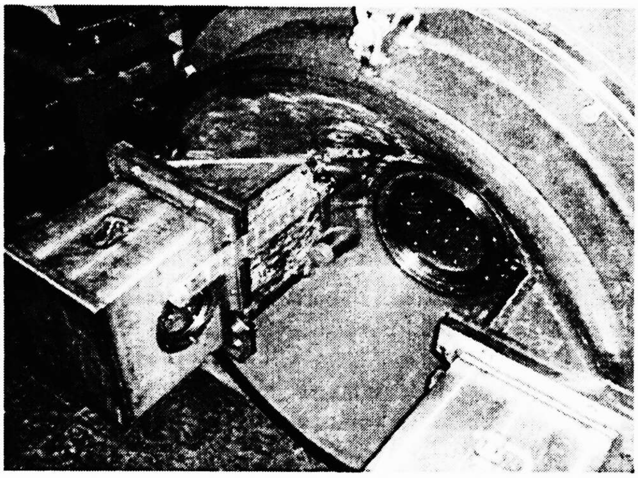

Figure 6: Camera front

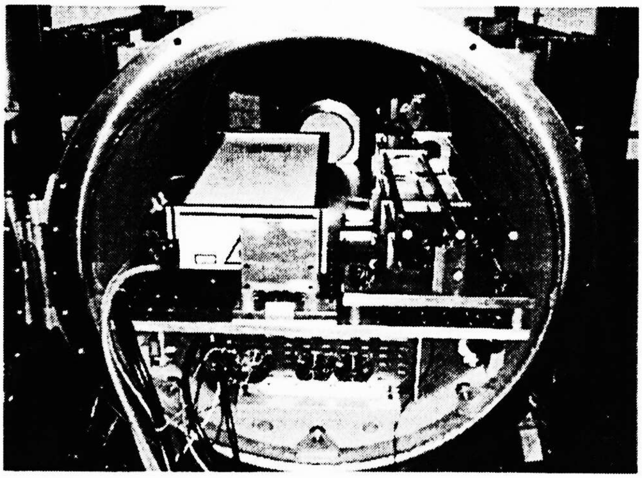

Figure 7:Camera rear

A CTD (salinity-temperature-depth) profiler will also be mounted on the frame, which will allow external conditions such as seawater temperature, salinity and depth to be monitored and recorded. A conventional video system will also be provided to allow observation of the scene prior to recording.

3.1 The laser

The laser is a Q-switched, frequency-doubled Nd-YAG with pulse duration of less than 10 ns and output energy of 700 mJ. The laser emits light at a wavelength of 532 nm, in a single longitudinal mode and has a coherence length in excess of 2 m. The wavelength was chosen to coincide roughly with the peak transmission window of seawater. The laser has been specifically developed by Quantel France, for our application. The optical head assembly consists two gain-medium/flashlamp assemblies (oscillator and amplifier), passive Q-switch, lithium triborate (LBO) frequency doubler crystal and ancillary optical elements. The laser base plate and the optical base plate are manufactured from the same specification of aluminium alloy to eliminate any problems that may occur due to thermal expansion. The power supplies and cooling system for the laser are located beneath the main optical baseplate on a secondary wooden baseplate (figure 7). Output from the laser is linearly polarised, in a horizontal plane but transport through the beam steering mirrors effectively rotates this, so that holographic recording occurs with vertically polarised light.

3.2 The optics

The arrangement of in-line and off-axis holographic geometries is designed to permit simultaneous recording of partially overlapping volumes of water, to enable some element of cross-correlation between holograms to be carried out. The in-line path records a water column of 500 mm long by 100 mm diameter. The centre-line of the in-line optics is approximately 400 mm from the front face of the housing.

Figure 8 shows a plan of the main optical baseplate, which supports the laser head, the holographic optical assembly and the plate holders. The general beam paths for the in-line and off-axis geometries are shown together with the essential optical components. As with the laser baseplate the optical mounts are manufactured from the same aluminium alloy. The laser produces two output beams: one contains an energy of 100 mJ and the other 600 mJ. The 100 mJ beam is split into two roughly 50:50 beams at the beam splitter (BS). The straight through path forms the illuminating beam for the in-line mode and the reflected path forms the reference beam for the off-axis mode. Both beams are expanded and collimated using a Galilean-type beam expansion system to a diameter of 100 mm with a wavefront flatness of $\lambda/8$ over the whole aperture

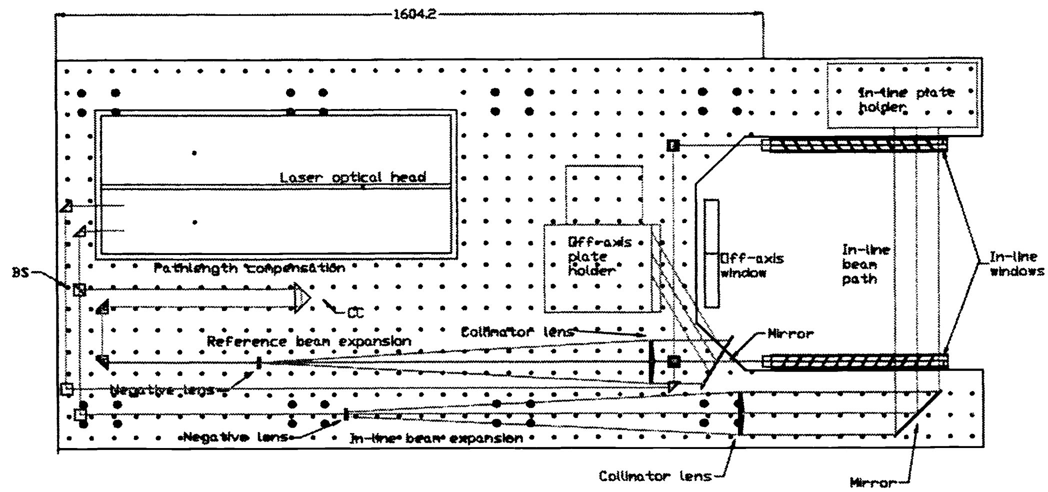

Figure 8: Plan of main internal baseplate

The in-line beam passes into the starboard arm before being directed through a window into the water, through another window and onto the hologram plate, which is held in the other arm. These windows are high quality optical flats ($\lambda/10$) as is the off-axis front window.

The off-axis beam passes through beam steering and path-length compensation assemblies, before being collimated to form the reference beam. Path length compensation takes the form of a series of mirrors and a roof prism, which fold the beam path. The prisms position is adjusted so that the paths followed by reference and illuminating beams are nominally the same in length. This is an important point in off-axis holography and is necessary to ensure that the "fringes" which are recorded in the hologram have optimum contrast and that full advantage is taken of the laser's 2 m coherence length. The off-axis reference beam is then folded at a mirror and is incident on the plate at an angle of 60° to the normal.

3.3 Off-axis illumination

For illumination of the off-axis volume, experiments have shown that side illumination provides the best images when recording plankton[16]. A novel system has been devised to provide roughly even side illumination to the off-axis volume, using a custom-designed "lightrod". This encompasses a hollow pipe with 10 glass plates distributed evenly along the length, positioned at 45° to the main axis. Each surface acts as a partial reflector, deflecting about 4% of the beam sideways into the object volume. The last plate is a totally reflecting mirror, which deflects the remaining portion of the light into the water. The glass reflectors are sealed in a Perspex tube to protect against water penetration. The curvature of the tube acts as a cylindrical lens, which helps to spread the light in a vertical plane (with respect to the paper). The outer surface of the tube (facing the water volume) is lightly roughened to provide an element of diffusion of the light and provide evenly distributed illumination throughout the recording volume. Three of the lightrods are mounted on the outside of the camera (two starboard, one port), with the 600 mJ laser output being split evenly between them. A number of alternative designs were evaluated, before this solution was adopted on grounds of robustness and simplicity but these will be discussed in detail elsewhere[16].

3.4 The plate holders

Through evaluation of available recording mediums it was determined that optimum holographic images were produced when recording using Agfa-Gevaert Millimask plates. Separate plate holders were developed for the in-line and off-axis configurations, each containing 25, 100 mm square plates. The off-axis holder is positioned at the front of the housing and the in-line is located within one of the arms. The glass plates are located in specially developed plastic plate holders; these are in turn stacked in a cassette, which is detachable from the main body of the plate holder mechanism. The plastic holders protect the fragile glass plates and provide a mechanical interface for the plate extraction and movement mechanisms. The cassettes ensure no exposure to stray light, which allows the plates to be easily transported to and from the darkroom processing facility.

When a holographic recording is required a grabbing mechanism removes the end plate from the front of the cassette and moves it to the exposure position. After exposure the same mechanism returns the plate to the back of the stack and prepares for the next recording. The plate stack is moved forward within the cassette by a spring-loaded pressure plate at the rear of the cassette housing, there are no other moving parts within the cassette. The grabbing mechanism, in the main body of the plate holder, consists of two translators driven by DC motors via leadscrews. One translator is responsible for extraction and insertion of the plates, while the second moves between extraction, exposure, and insertion positions. Position detection is provided by microswitches and motor control by FET switches, including mark-space ratio modulation for speed control. The control sequencing is through software, running on the micro-controller system described below.

This design was selected for robustness and compactness. Unlike a linear "slide-projector" mechanism, the cassette itself does not move, and so requires little more space than the minimum occupied by the stack of plates alone. The main cost in this approach is the time taken to translate each plate the whole length of the cassette between extraction and insertion. However, the maximum target speed for the camera of one exposure every 10 seconds is achievable.

3.5 The electronic control

The Topside Control Console (PC) runs the main control program (a Windows based program written in Visual C++) providing a user interface as well as displaying system information and storing data for each recording. A network of 3 micro-controller boards (Siemens C167CR) has been chosen as the backbone of the in-camera control system. Communication between topside and the camera modules will be via a main umbilical cable using the CAN bus protocol[17]. The CAN protocol has a high noise tolerance providing reliable data transfer for lengths greater than 150 m and will easily allow several more micro-controller units to be added if necessary.

The first micro-controller board controls the laser (through a RS232 link) as well as thermally regulating the internal volume. The thermal control comprises a network of resistive heaters and temperature sensors in order to maintain the camera baseplate temperature at 20 °C (±1 °C). Thermal breaks are provided between the baseplate and the main housing. This is required to control thermal expansion of the components and ensure optimal laser operation. The second board relays data from the CTD environmental sensors to the topside console. It also controls the motors for the in-line plate movement. The final controller operates the off-axis plate movement and monitors the humidity and tilt sensors.

4. SYSTEM TRIALS AND PERFORMANCE

At the time of writing the camera is still in construction. However, the laser and optical components have been installed (figure 7) and are operating reliably. A large water tank is currently being fabricated which will encompass the front of the camera and allow completion of final laboratory testing. Unforeseen delays have meant that the in-line plate holder and control system will not be completed until June 2000. Once these have been installed and tested thoroughly the complete system will be deployed in Southampton docks for initial field trials. These if successful will ultimately lead to ship deployment sometime in the autumn.

A series of photographs of reconstructed holographic images may be see in figure 9. These were recorded in the laboratory in a Perspex tank using the camera's laser and optical assembly. The images were taken using the off-axis configuration of a sample of preserved marine plankton.

Figure 9: Plankton images from off-axis hologram

ACKNOWLEDGEMENTS

The authors wish to thank *The European Commission* for support of this work under the MAST-III initiative (MAS3-CT97-0079) and also the support of our partners at the Universities of Udine and Genoa (Italy) and at Holo3 (France).

REFERENCES

1. P.S. Amy, B.A. Caldwell, A.H. Soelder, R.Y. Morita & L.J. Albright, "Microbial activity and ultrastructure of mineral based marine snow from Howe Sound, British Columbia", *Can. J. Fish. Aquat. Sci.* 44, 1135-1142,1987.
2. R. Lampitt, W.R. Hillier & P.G. Challenor, "Seasonal and diel variation in the open ocean concentration of marine snow aggregates", *Nature* 362, 737-739, 1993.
3. A. Rogerson, & J. Laybourn-Parry, "Aggregate dwelling protozooplankton communities in estuaries", *Arch. Hydrobiol.* 4, 411-422, 1992.
4. J. Watson, "Hologrammetry and its applications", *J. Imaging Tech.* 15, 38-46, 1989.
5. P.R. Hobson, "Precision coordinate measurements using holographic recording", *J.Phys.E, Sci. Inst.* 21, 138-145, 1988.
6. C. Knox, ("Holographic microscopy as a technique for recording dynamic microscopic subjects", *Science* 153, 989-90, 1966.
7. K.L. Carder, *et al.*, (1982) "In situ holographic measurements of the sizes and settling of oceanic particulates", *J. Geophys. Res.* 87, 5681-5685, 1982.
8. L.O. Heflinger, G.L. Stewart & C.R. Booth, "Holographic motion pictures of microscopic plankton", *Applied Optics* 17, 951-954, 1978.
9. J. Katz, T.J. O'Hern & A.J. Acosta, "An underwater holographic camera system for detection of microparticles", *Proc. ASME Cavitation and Multiphase Flow Forum*, New Orleans, 1984.
10. P.R. Hobson, & J. Watson, "Accurate three-dimensional metrology of underwater objects using replayed real images from in-line and off-axis holograms" *Meas. Sci. Technol.* 10: 1153-1161, 1999.
11. J. Watson, P.W. Britton & A.C.S. Cran, "Resolution of Holographic Images of Underwater Objects", Optics and Laser Technology 9, 97-101, 1987.
12. J.M. Kilpatrick, "An Evaluation of Optical Aberrations in Underwater Hologrammetry", Phd Thesis: University of Aberdeen, 1991.
13. P. Hariharan, *Optical Holography*, Cambridge University Press, 1984.
14. X. Fang, & P.R. Hobson, "Effect of spherical aberration on real-image fidelity from replayed in-line holograms of underwater objects" *Appl Opt*, 37: 3206-3214, 1998.
15. E. Krantz, "An evaluation of optical holography applied to imaging in situ plankton" PhD Thesis, University of Aberdeen, 1999.
16. H. Nareid, J. Watson, G. Craig, M.A. Player & K. Tipping, "A novel illumination system for off-axis holography of small particles" submitted to International Symposium on Applied Photonics, Glasgow, May 2000.
17. R. Bosch, *Controller Area Network Specification*, GmbH, 1991.