The development and sea trials of a subsea holographic camera for large volume in-situ recording of marine organisms

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ABSTRACT

We describe the development, construction and sea testing of an underwater holographic camera (*HoloCam*) for *in situ* recording of marine organisms and particles in large volumes of sea water. *HoloCam* comprises a laser, power supply, holographic recording optics and plate holders, a water-tight housing and a support frame. Added to this are control electronics such that the entire camera is remotely operable and controllable from ship or dock-side. Uniquely the camera can simultaneously record both in-line and off-axis holograms using a pulsed frequency doubled Nd-YAG laser. In-line holography is capable of producing images of organisms with a resolution of better than 10 μm (at concentrations up to a few thousand per cubic centimetre at the smallest sizes). Off-axis holograms of aquatic systems of up to 50,000 cm^3 volume, have been recorded. Following initial laboratory testing, the holo-camera was evaluated in an observation tank and ultimately was tested in Loch Etive, Scotland. In-line and off-axis holograms were recorded to a depth of 100 m. We will present results on the test dives and evaluation of the camera performance.

1. INTRODUCTION

In recent years holographic recording and imaging of the marine environment has begun to offer biologists an alternative to conventional techniques and provides a number of advantages for the analysis of marine systems. Holography offers non-intrusive, non-destructive recording of living, motile organisms and particles in their natural environment [1, 2, 3]. Its simultaneous large depth-of-field and high resolution, three-dimensionality, and ability to "optically section" the replayed image, allows enumeration and identification of specific organisms together with their size, orientation and three dimensional spatial distribution [4, 5, 6, 7, 8, 9, 10]. Using short pulse lasers allows the *in-situ* recording of living, motile, organisms and inanimate particles in their natural environment and with their accurate spatial distribution accurately "frozen" in time. Compared to a photograph vastly more data can be recorded in a hologram. Totally transparent organisms can be recorded with excellent contrast without using special illumination or interferometric techniques.

Several subsea holographic cameras have been used before to successfully study marine systems, notably those by Carder [11], Heflinger [12] and Katz [13, 14]. More recently an all-digital holographic camera has been developed by Owen and Zozulya [15] and deployed with great success. All these cameras, however, have been based solely on the use of in-line reference beam holography (ILH). Although providing the highest resolution (down to a few micrometres dimension), ILH is limited by the concentrations of particles which can be recorded and the maximum size of object which can be imaged. We have developed a unique holographic camera "HoloCam" [16] for the simultaneously recording of both in-line (ILH) and off-axis holograms (OAH) of overlapping volumes of the water column. We report here the first sea deployments of the camera down to a depth of 100 m in Loch Etive (a sea loch in the West of Scotland), and outline the successful recording of about 300 holograms which are rich in plankton and particles.

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2. HOLOCAM

The prototype HoloCam consists of a watertight stainless steel pressure housing containing the holographic optics, laser and power supplies. The overall dimensions of the housing are approximately 2.4 m long by 1 m in diameter and the camera weighs 2.3 tonne in air when fully loaded and ballasted.

HoloCam is based around a Q-switched, frequency-doubled Nd-YAG laser (Quantel SA). The output wavelength of 532 nm coincides with the blue-green transmission window of seawater and so minimises beam attenuation and scattering. An output energy of some 700 mJ in a single pulse provides sufficient energy to record large subject volumes in off-axis holography. In contrast IL holograms can be recorded with energies of less than 50 mJ. By adopting passive Q-switching with solid saturable absorber a pulse duration of 10 ns is obtained; this also reduces the complexity and size of the laser over the alternative active Pockel's cell Q-switch. The short pulse duration freezes movement of the organisms and particles in the water column and also removes the constraints on vibrational stability required to record good holograms. To ensure that high quality holograms with a large depth can be recorded it is necessary to design the laser to operate in a single longitudinal mode.

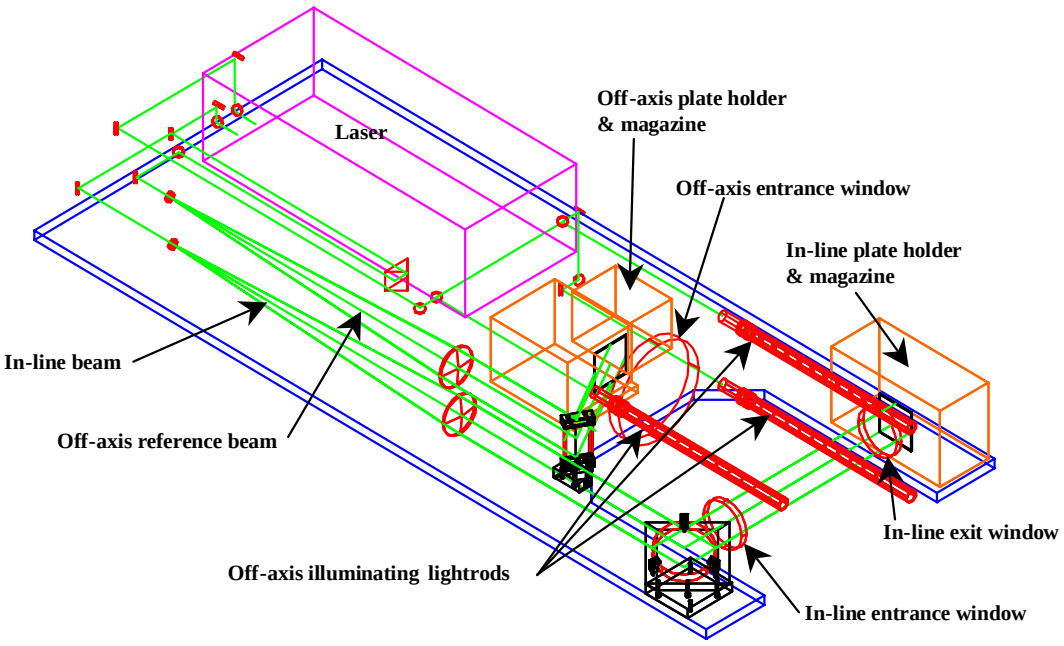

Fig 1: Schematic of HoloCam

The camera utilises the ability to record simultaneous in-line and off-axis holograms. In-line holography can record small particles from a few micrometres dimension up to several millimetres provided that the target concentration is low enough to allow at least 90% of the incident light to pass through the medium. The use of OAH allows us to extend the range of particle sizes which can be resolved up to tens or hundreds of centimetres, but at a lower resolution of about 100 μm. Furthermore, dual deployment of ILH and OAH allows the recording of object concentrations as low as a few particles per cubic metre up to many thousands and on to total opacity. Another benefit of dual deployment is that, for opaque organisms, ILH records essentially an outline of the organism so only its profile is known. By contrast OAH records shape, surface texture and structure of the organism which helps in identification of the larger species. A consequence of adopting off-axis holography, with its corresponding optical complexity and requirement for high laser energy, is the large size and weight of HoloCam.

The laser head assembly consists of flashlamp-pumped oscillator and amplifier assemblies, passive Q-switch, lithium triborate (LBO) frequency doubler crystal and ancillary optical elements. The laser baseplate and the HoloCam optical

baseplate are manufactured from the same specification of aluminium alloy to reduce any problems that may occur due to differential thermal expansion. The power supplies and cooling system for the laser are located beneath the main optical baseplate on a secondary wooden baseplate. 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 reference beams. The output beam is split into two beams using an arrangement of polarisers and half-wave plate. This system allows the adjustment of the energy ratio between reference beams and object beam (notionally 100 mJ and 600 mJ respectively) by simply rotating the first half-wave plate. The single mode repeatability is higher than nine shots out of ten and the spatial beam profile is fairly uniform in intensity. A significant bonus in the design was the uniform spatial output profile of the beam. This allowed us to design the holographic optics without the need for spatial filtering of the beam.

The optical layout of HoloCam is shown in figure 1. The reference beam (100 mJ) is further split into two 50:50 beams to form separate in-line illuminating/reference and off-axis reference beams. Both beams are expanded and collimated, using Galilean expansion systems, to 92 mm clear aperture. The in-line beam is directed through an entrance window ($\lambda/4$ flatness) into the water, through the exit window back into the camera and onto the hologram plate. The in-line geometry records a water volume of 92 mm diameter by 470 mm long (about 3,500 cm^3 volume). The centreline of the path is 450 mm from the front face of the camera. The off-axis reference beam passes through beam steering and path-length compensation assemblies, before being collimated to a diameter of 92 mm. The beam is folded by a mirror and strikes the holographic plate at 60° to the normal. The off-axis entrance window allows return of the reflected/scattered light from the object volume. The 120 mm separation between the plate-holder and the window allows access for the reference beam. Illumination of the off-axis recording volume is provided by the 600 mJ beam after being split into three "lightrods" positioned through the wall of the camera and protruding into the object volume [17, 18]. Each lightrod consists of a PMMA tube containing a series of glass plates distributed evenly along the length at 45° to the main axis. Every glass surface acts as a partial reflector, deflecting about 9% of the beam sideways into the water. The last plate is a totally reflecting mirror, which reflects the remaining portion of the light into the water. The lightrod on the opposite side of the in-line plate holder window contains fewer reflectors and has a reduced active length to avoid any stray illumination striking the in-line plate. The off-axis hologram records almost the entire volume delineated by the front window to the end of the arms (about 50,000 cm^3). The line of sight of the off-axis hologram is at right angles to that of the in-line. A grid of 50 μm diameter wire fiducials is placed on the inside of the in-line entrance window and a grid of 100 μm wires inside the off-axis window.

To achieve high quality images, holograms have to be recorded on high-resolution photographic materials; this is particularly true for OAH where a resolving power of better than 4000 mm^{-1} is needed. The constraints are less stringent for ILH but the use of the same high-resolution emulsion (low grain size) for both ensures that the holograms are low-noise. Agfa *Millimask* has been shown to meet our requirements. To provide the “fringe” stability needed for high quality holograms it is desirable that the holographic emulsion be laid down on a flat glass plate substrate. No plate holders of the specification required by us were available and it was necessary to design and develop two separate motor-driven plateholder and transport mechanisms. Because of space restrictions the IL plateholder takes 20 plates and the off-axis takes 25. The glass holographic plates (102 mm square) are located in plastic frames and stacked in a detachable light tight cassette. The plastic frames protect the glass plates and provide a mechanical interface for the plate extraction and movement mechanisms. 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 it is replaced at the back of the cassette. The cassettes ensure no exposure to stray light and allow the plates to be easily transported to and from the darkroom processing facility. The control sequencing is through software, running on the micro-controller system described above. One plate can be exposed every 10 seconds.

Prior to operation the cassettes are loaded and placed in the plateholders and the plateholders located in position of the baseplate. The baseplate containing laser and optics is then slid on runners into the camera. Pre-alignment of the optics on the baseplate with respect to the lightrods ensures that they receive the appropriate beams. To eliminate the possibility of condensation occurring on the windows the housing is purged with 99% pure nitrogen before deployment.

Power and communication is provided through an umbilical cable from the surface. The Topside Control Console (a standard PC) runs the main control program (a Windows based program written in MS Visual C++) providing a user interface as well as displaying system information and storing data for each recording. A network of four Siemens

C167 micro-controller boards provides the backbone of the in-camera control system. Communication between topside and the camera modules is via the main umbilical cable using the CAN (Controller Area Network) bus protocol [19]. One micro-controller controls the laser (through a RS232 link), another controls the motors for the in-line plate movement, a third operates the off-axis plate movement and the fourth handles temperature, humidity and tilt sensors.

3. RECORDING IN LOCH ETIVE

We chose *The Bonawe Deep* in Loch Etive (a sheltered sea-loch on the west coast of Scotland) for the first sea deployment of HoloCam following advice of marine biologists at *SAMS* (Scottish Association for Marine Science, Dunnstaffnage, Oban). Since *SAMS* also operates a mooring at this location and have conducted several Doppler and CTD (conductivity-temperature-depth) profiles in this area, a wealth of supporting background data is available. Two separate deployments were undertaken in October/November 2000 and September 2001.

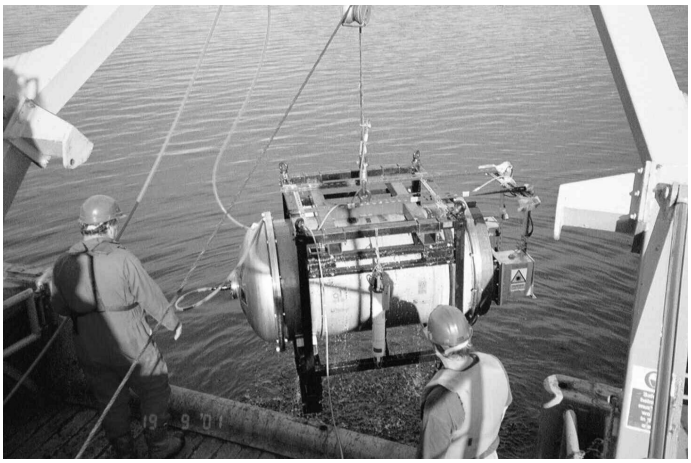

Fig 2: Lowering of HoloCam into Loch Etive

In Deployment 1, the camera was lowered into Loch Etive in a water depth of 141 m from the research vessel *RV Calanus* (figure 2) on three occasions: once on 31 October 2000 and twice on 1 November 2000. The water temperature and salinity were meeasured using a separate CTD (current-temperature-depth) probe. A series of holograms was recorded between the surface and 100 m at 5 or 10 m intervals. At each depth a pair of holograms was recorded simultaneously, one in-line and one off-axis. On dive 1 seventeen in-line holograms (one failure occurred due to a plate holder jam) and eighteen off-axis holograms were recorded. On each of the other dives a full set of 18 pairs was recorded. Video observation (with lights) was used on dive 1 while the camera was lowered to its target depth. The lights were switched off 30 s prior to each exposure in order to reduce the attraction of swimming organisms to the lights. On the other dives no lights or video observation was used. A waiting time of 2 to 3 minutes was allowed at depth before recording to allow the camera and the organisms to settle. The water temperature was measured to be about 8°C at 1 m to 14°C at 100 m and the salinity increased from 3 psu (practical salinity units) at 1m to 28 psu at 30 m. Weather was bright and clear with a calm sea for all dives, although conditions turned to cloud and rain during the third dive.

The second deployment took place about 11 months later. During the downtime between deployments significant modifications were made to *HoloCam* to improve its operation and functionality. These included incorporation of remote steering of beams into the lightrods to improve alignment; improvements to plateholders to improve reliability and reduce liklihood of plate jams. Other improvements were to the method of opening /closing of housing and insertion/removal of optical baseplate.

In deployment 2, the *HoloCam* was used at two different locations: dives 1 and 6 were at a point North of Lismore (off the Isle of Mull) and dives 2 to 5 were back at the *Bonawe Deep* used in deployment 1. On dive 1, 24 holograms were

recorded (12 each), and on all the other dives 36 holograms (18 off each) were recorded. The weather conditions were again very good, with clear dry conditions on all dives. Typical salinities were from 8 psu at the surface rising to 28 psu at about 40 m depth. Water temperatures varied between 12°C at surface and 9°C at about 30 m. On this occasion lights were used on all dives and switched off immediately prior to initiating the hologram record sequence giving a time of about 40 s between lights going off and hologram being recorded.

All the holograms were recorded on Agfa Millimask plates. Wet chemical processing of the holograms was carried out on shore. Each set of holograms corresponding to one dive was processed in one session in batches of twelve. Development was in undiluted Tetenal Neofin Blue for 2 min at 20°C followed by fixing for 2 min.

4. RESULTS OF DIVES

Full quantitative evaluation of the holograms takes place by replaying the holograms in a customised and automated reconstruction and data analysis facility (HoloScan) [20]. This process involves projection of the holographic real image which is then scanned by a video camera mounted on precision xyz micropositioning stages. Specially developed software allows tracking, focusing and location of individual organisms followed by classification and evaluation of population densities. Furthermore, HoloScan also incorporates the facility to alter the replay wavelength and beam angle. This is necessary, particularly for off-axis holograms, in order to minimise optical aberrations in the replayed image by the method of "index compensation" [21, 22, 23, 24]. Initial holograms were viewed in both virtual image and real image modes and scanned and evaluated manually.

Qualitative examination of all recorded holograms showed that they were of a consistent recording quality. Visual inspection of the unmagnified virtual image (off-axis holograms) revealed each hologram to contain large numbers of marine organisms and particles. Qualitatively one can see that the highest concentrations of marine organisms occurred at the greater depths (below about 40 m).

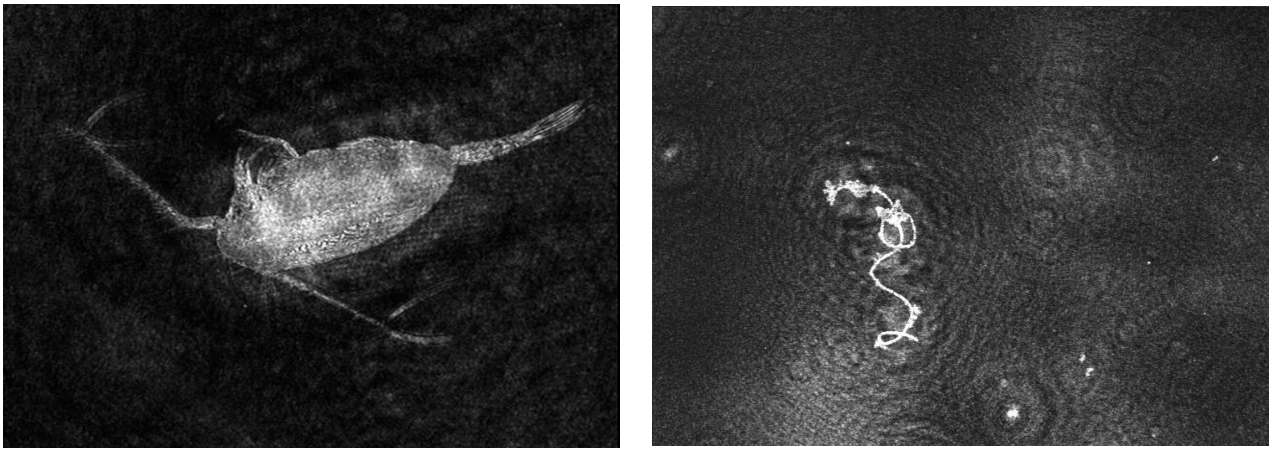

Fig 3: Images from In-line holograms

Figures 3 shows two sample images taken from real image projection of in-line holograms, which were recorded in Loch Etive. The first image is of a calanoid copepod (*Calanus finmarchicus* or *Calanus helgolandicus*) probably in Stage V of development; it is approximately 2 to 3 mm long and was recorded at a depth of 90 m. The second image shows what is possibly a chain forming diatom, about 4 mm long, with floc attached to it (60 m depth). The holograms reveal the presence of large numbers of organisms in the size range from 0.5 mm to a few millimetres. In each of these holograms there were significant amounts of detritus and other particles below 500 μm dimension. The top of each image is towards the surface of the water. There has been no post image processing on any of these images.

Similar observations are apparent in the off-axis holograms shown in figure 4. Two images are shown, both taken from the second deployment and from the same hologram recorded at 100 m depth. The first image is of an adult calenoid

copepod (about 6 mm long) whereas the second image is thought to be another example of a chain forming diatom with floc attached to it.

For the smaller unicellular organisms the in-line holograms have superior resolution and can show internal structure in transparent plankton such as some *Ceratium* species. Larger (> 1mm) multicellular organisms will tend to record as silhouette. Off-axis holograms show the true three-dimensionality and orientation for these larger organisms, although it is not possible to focus on the whole depth of the organism at one time. In general the two techniques are complimentary with the off-axis expected to be the only technique capable of recording the larger multicellular organisms, or of recording high population densities of organisms. For example in fig 4 the structure on the tail can be seen and this would not be seen in an equivalent in-line hologram.

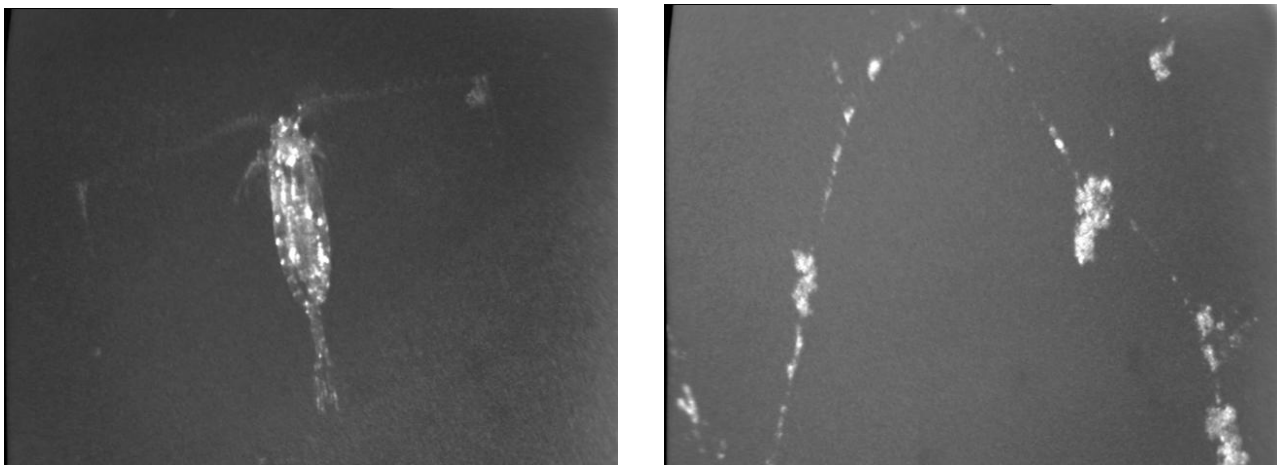

Fig 4: Images from Off-axis holograms

5. CONCLUSIONS

A unique holographic camera (HoloCam) has been designed and developed for subsea recording of simultaneous in-line and off-axis holograms of marine plankton and other particles and organisms. The camera has been deployed from a ship in a Scottish sea loch and 36 pairs of in-line and off-axis holograms recorded at various intervals down to a design depth of 100 m. The holograms reveal themselves to be rich in plankton and other marine particles and many examples of calanoid copepod and marine snow can be seen. The in-line holograms show detail down to about 10 μm resolution. The off-axis holograms are able to reveal more clearly the three-dimensional orientation and surface texture of the organism.

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