HOLOMAR

Introduction to Holography and its Application in Aquatic Biology

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If a photograph is worth a thousand words, then a hologram is worth a million photographs!

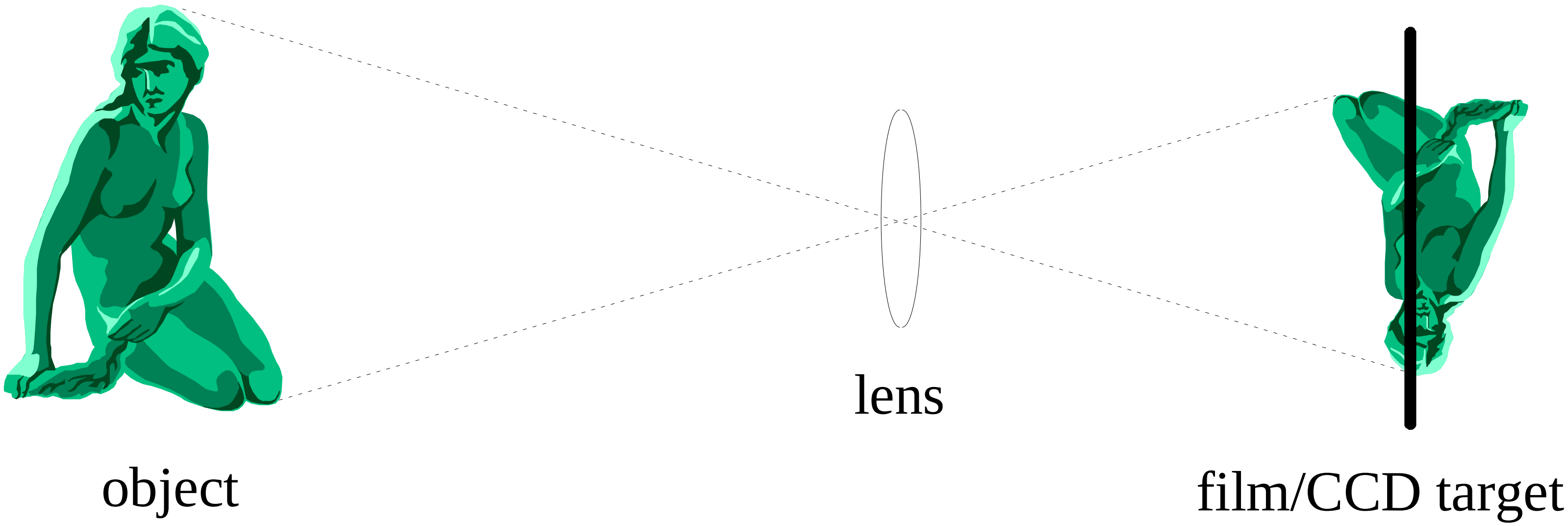
(*Any Holographer will tell you that***)**

You don't understand holography, you just get used to it!

(attributed to *Albert Einstein*)

Holography vs. Photography

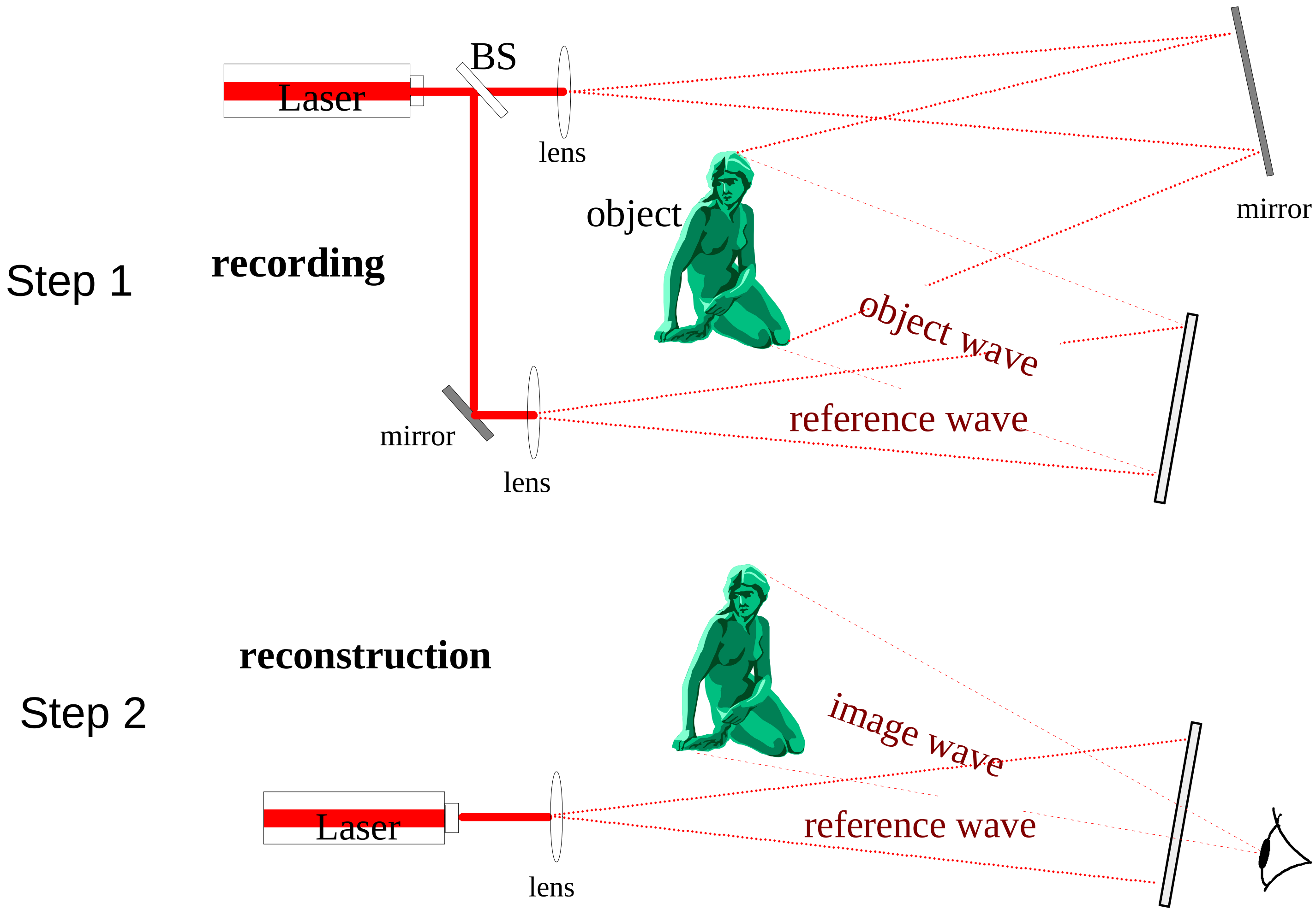
Photography is a 1-step imaging method



- image in sharp focus only in film/CCD target plane
- records intensity only (square of amplitude)
- all phase information lost
- no depth information
- suffers from parallax & perspective distortion
- small depth-of-field

Holographic Recording

Holography is a 2-step imaging method



Properties of a hologram

- ***Holography*** **is a unique form of optical imaging**
  - light reflected or scattered from scene recorded on photosensitive sensor
  - interferometrically combined with a known reference wave
- **A hologram is fully three-dimensional**
- **A hologram preserves parallax information**
  - allows the viewer to look around objects which are in the foreground
  - a stereo-photograph is 3-D but does not preserve parallax
- **A hologram maintains the true perspective of objects**
  - objects in the foreground are in proportion to those in the background
- **Images offer potentially diffraction-limited resolution**
- **A hologram is in focus from the film to the furthest reaches of the object**
  - depth of field is hundreds of times that of a microscope of the same resolving power
- **A hologram records both the phase and amplitude of light**
  - it is phase which provides direction of light and so gives three-dimensionality
  - a photograph contains only irradiance (square of amplitude) and no phase
- **A hologram (off-axis only) contains the entire image**
  - can be broken into many small pieces & still contains the entire image
- A classical hologram is optically *indistinguishable* from the original

The benefits of holography in aquatic biology

- **Records live species in natural environment**
  - non-intrusive, non-destructive, *in situ* interrogation
  - can record large volumes of water column in one short exposure
- **True three-dimensional imaging of organisms**
  - retention of parallax & perspective information
  - high image resolution over large depth-of-field
  - wide recording dynamic range
- **Ability to isolate individual planes of the image**
  - move viewing plane through image volume brings individual species into focus
- **Aids study of marine biological communities**
  - measurement of distribution of organisms & interrelationships
  - measurement of size & relative position of organisms
  - species identification & classification at genus level

Objectives of HOLOMAR

- **Develop, construct & evaluate a fully-functioning prototype underwater holographic camera**
  - Holographically record large volumes of the upper water column containing aquatic plankton & seston
- **Design, develop & construct a fully-functioning hologram replay facility**
  - Replay holograms in the real image mode for high resolution inspection & measurement
- **Record, analyse & interpret holograms using specially developed image processing algorithms**
  - Identification of species, size, relative location & distribution of aquatic organisms

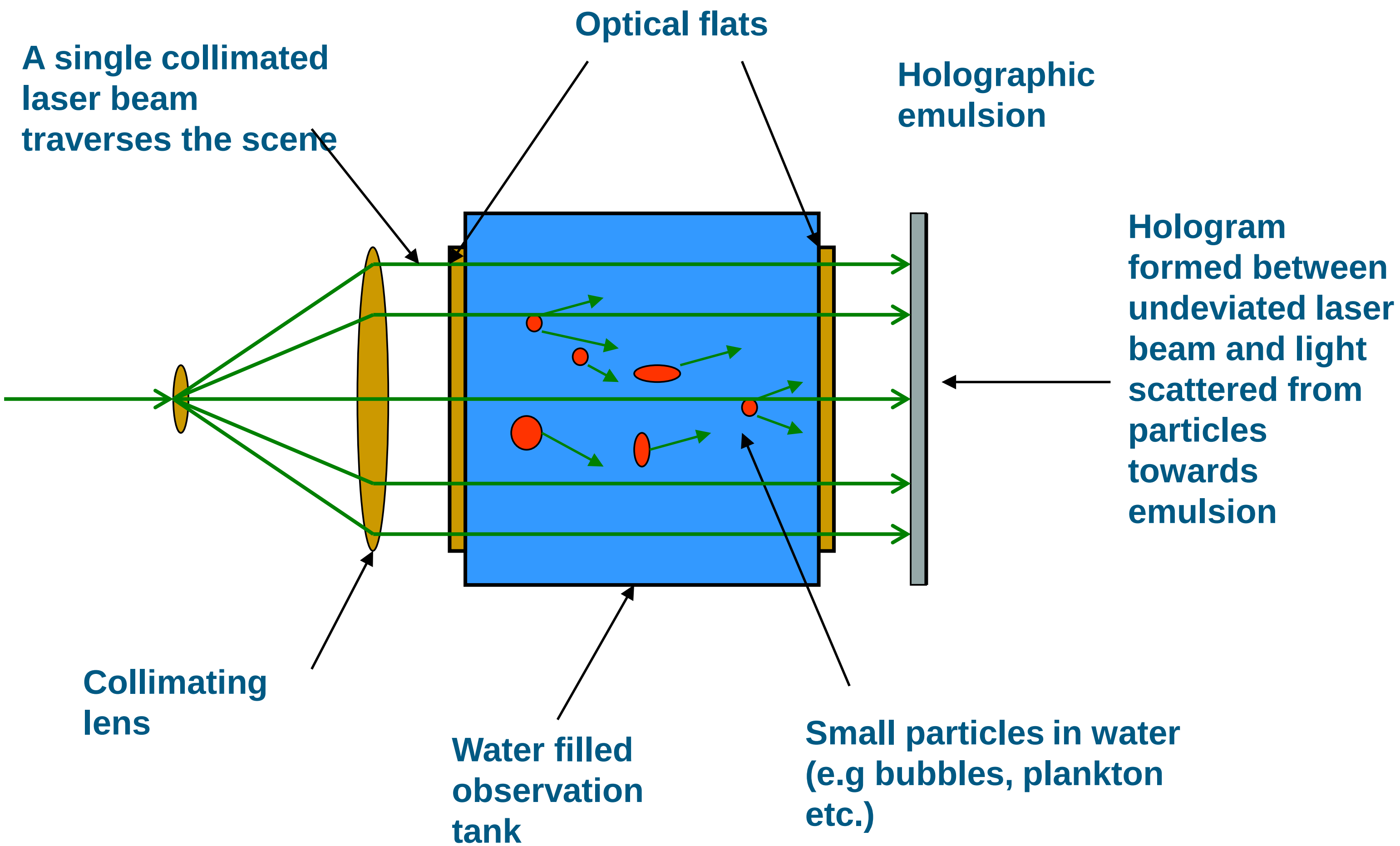
Two Underwater Holographic Cameras

- *HoloMar* Camera
  - Classical recording on photographic plates
    - Simultaneous recording in in-line and off-axis modes
    - Laboratory replay for inspection & measurement
    - Identification of species, size, location & distribution
- *eHoloCam*
  - Digital recording on CMOS sensor
    - Numerical reconstruction in PC
    - Identification of species, size, location & distribution
    - Holographic video

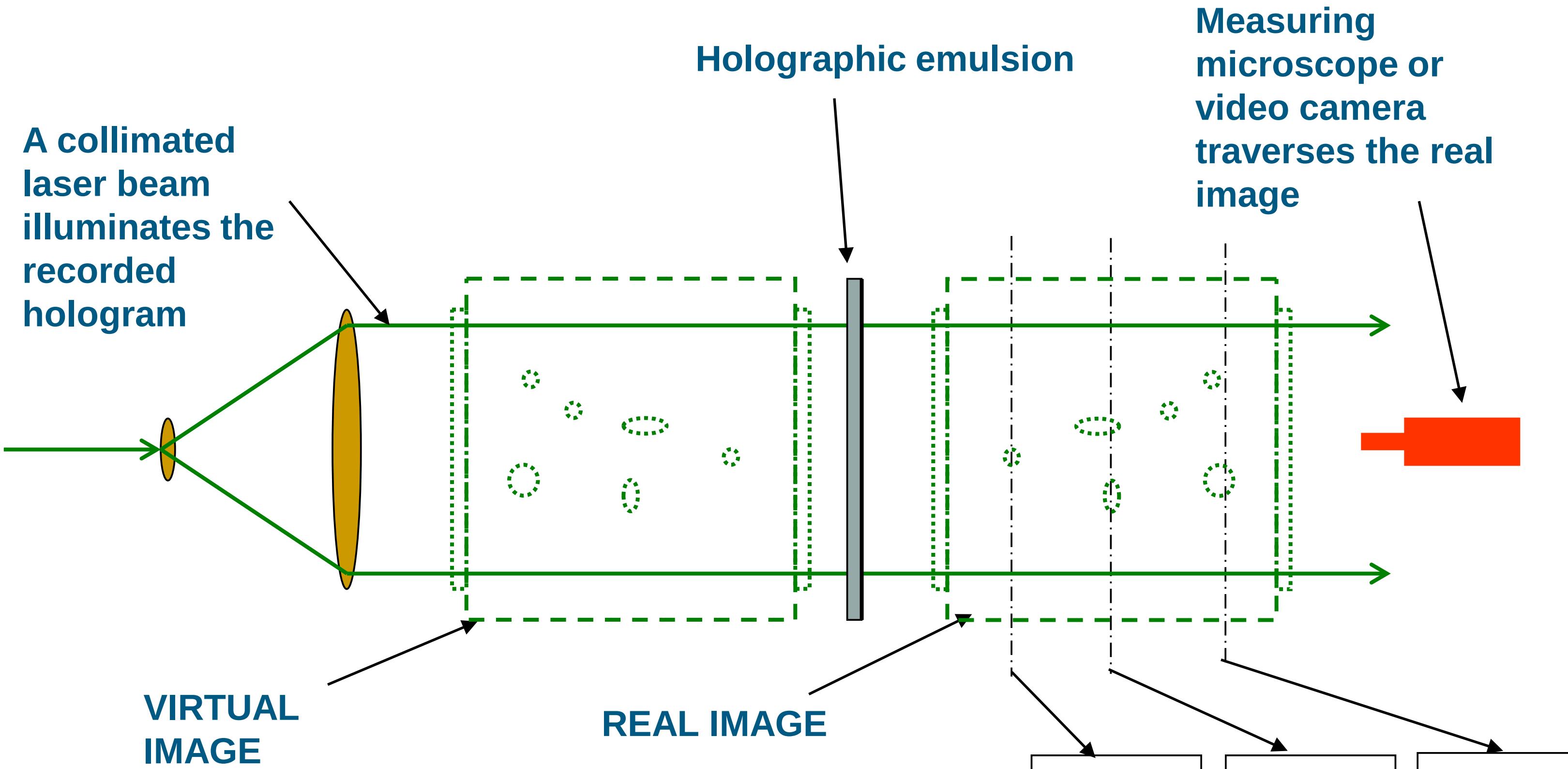
Brief History of Underwater Holography

- 1966 Knox (IL, living plankton, plate immersed in water)
- 1970 Stewart, Beers and Knox (field - *RV Ellen Scripps)*
- 1978 Heflinger *et al* (OA holograms, replay into water)
- 1984 Katz, O'Hern and Acosta (in-line)
- 1985 Watson (off-axis, offshore industry)
- 1988 O'Hern, d'Agostino and Acosta (IL, cavitation)
- 1989 Costello *et al* (in-line, sedimentation trap, orthogonal)
- 1991 Kilpatrick and Watson (aberration correction)
- 1995 Hobson, Lampitt, Watson *et al* (IL and OA tank trials)
- 1999 Katz *et al* (ROV, in-line technique, real data extracted)
- 2000 Owen and Zozulya (in-line, digital holography)
- 2001 Watson et al - HOLOMAR (IL/OA, large volume)
- 2001 Jericho – Digital holography of biological samples
- 2003 Katz – Digital holography
- 2004 Watson et al - eHoloCam

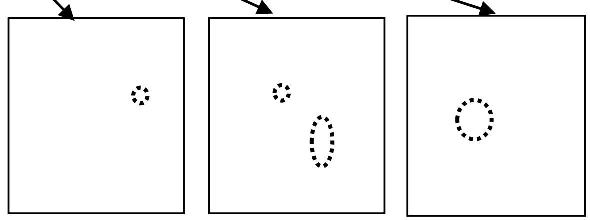
Recording an In-line hologram



Replaying an In-line hologram



Two images of the original are seen: one on either side of the holographic emulsion



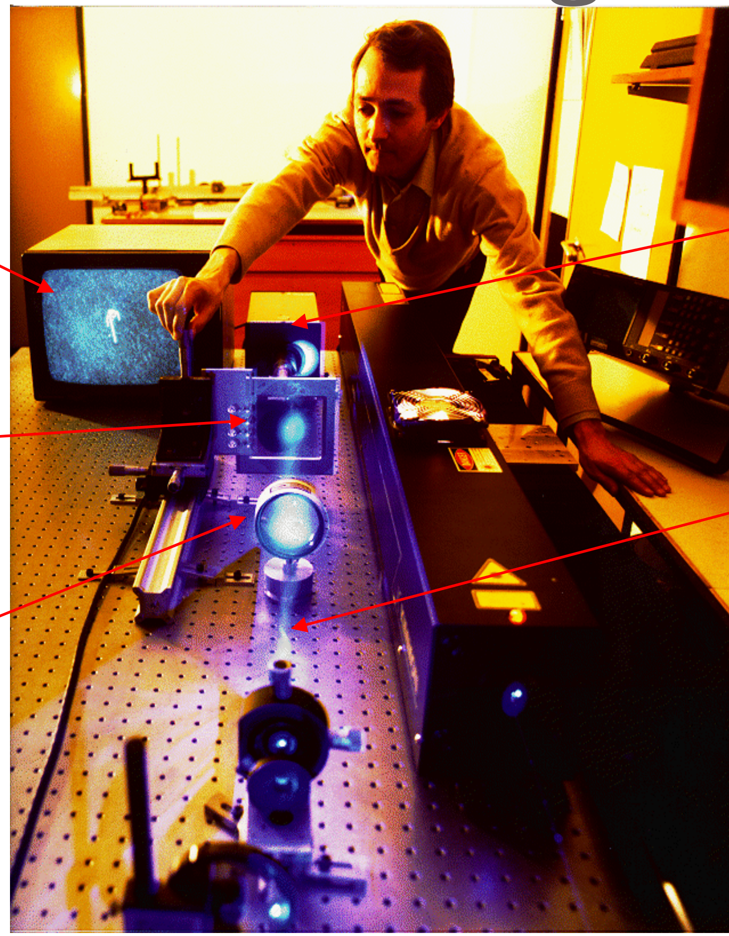
Planar sections through image

Replay of in-line hologram

Monitor showing plankton image

Multi-axis plate holder

Collimatin g lens



Camera mounted on xyz stage views real image

Reconstruction laser beam (HeCd 442 nm)

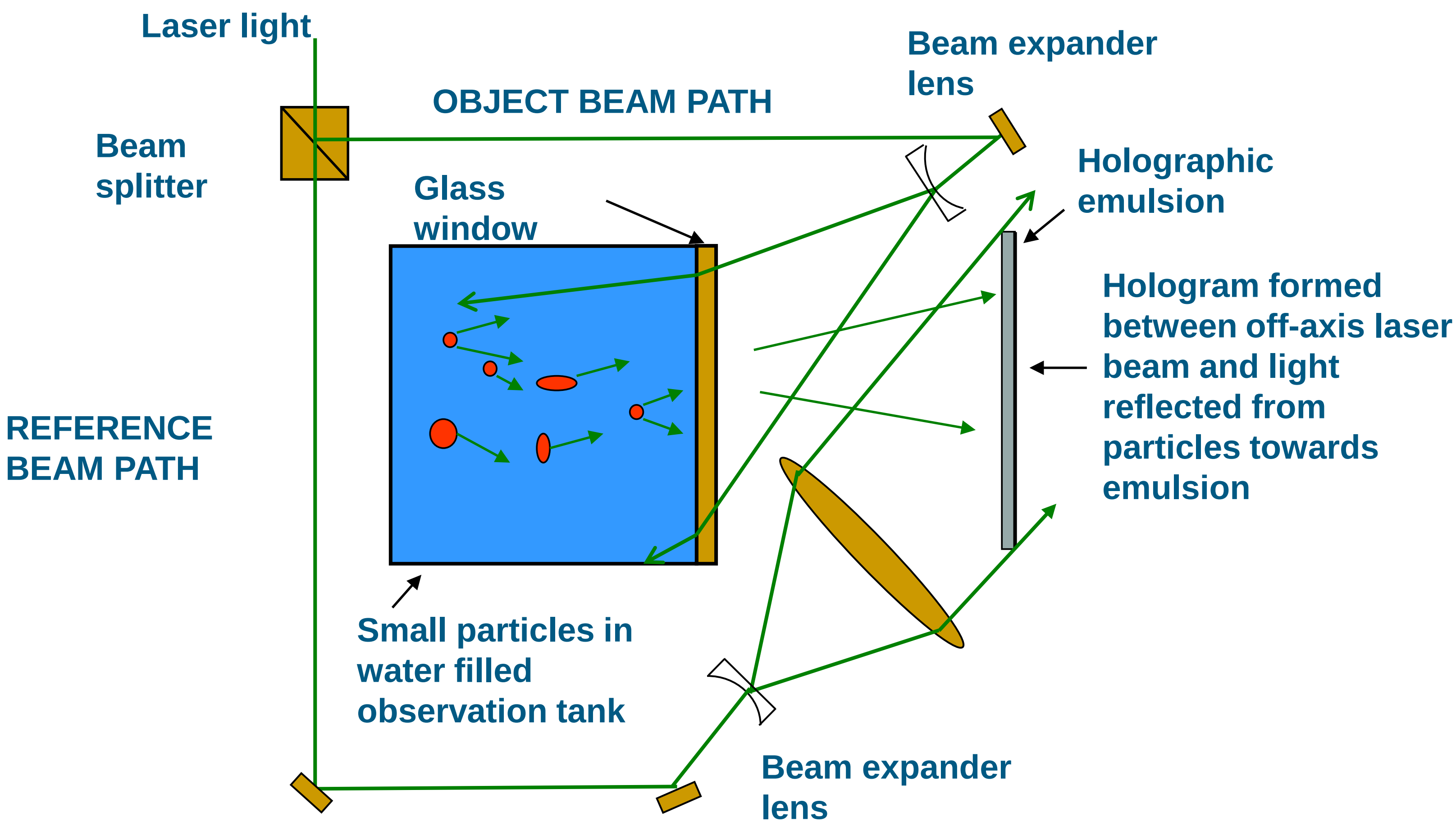
Change of focus through an in-line hologram

The camera shifts away from the best focus in increments of 0.50 mm. These hologram images were recorded with a ruby laser (694 nm) and replayed with Ar-ion (514 nm). The object is about 200 μm long. The change of image with defocus is very nearly symmetrical on either side of the "best focus”.

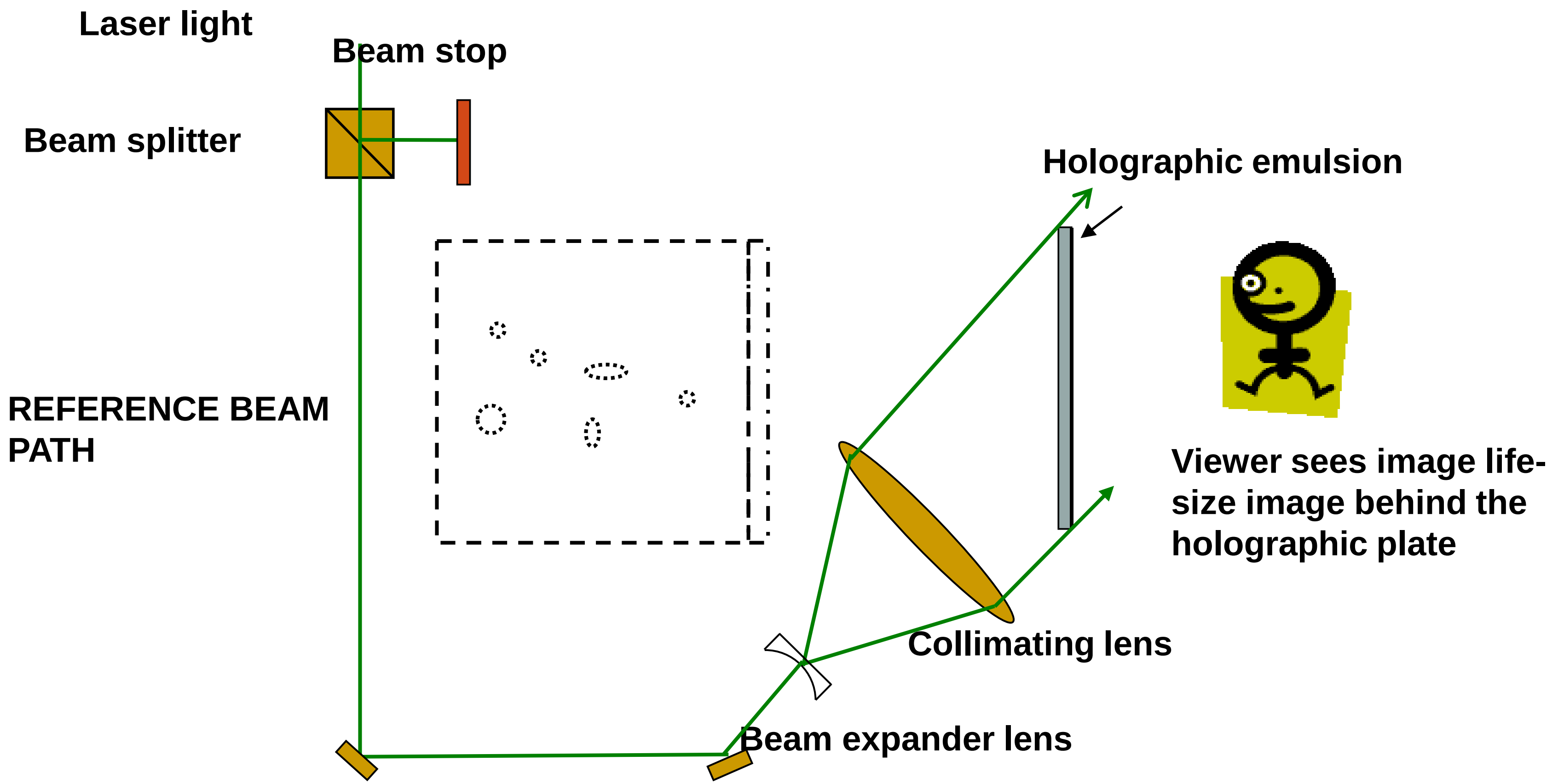
In-line holography: Summary

- **A single parallel beam of light traverses the medium**
  - the scene is back-lit
  - the medium must be essentially transparent
    - about 80-90% transparency needed over entire field for good images
- **Replay produces both virtual & real images**
  - both images are on the same optic axis and can obscure each other
- **A parallel beam of light is used in replay**
- **Far-field recording condition may limit object size**
  - $z > d^2 / \lambda$
    - z is object-to-film distance, d is max dimension of object to be recorded, λ is object beam wavelength
  - sets upper limit on size of particles which can successfully be recorded
- **Can resolve particles in the range 5 μm to a few millimetres**
  - concentrations to few thousand per cubic centimetre at the smallest sizes
  - a few particles per cm^3 for larger particles

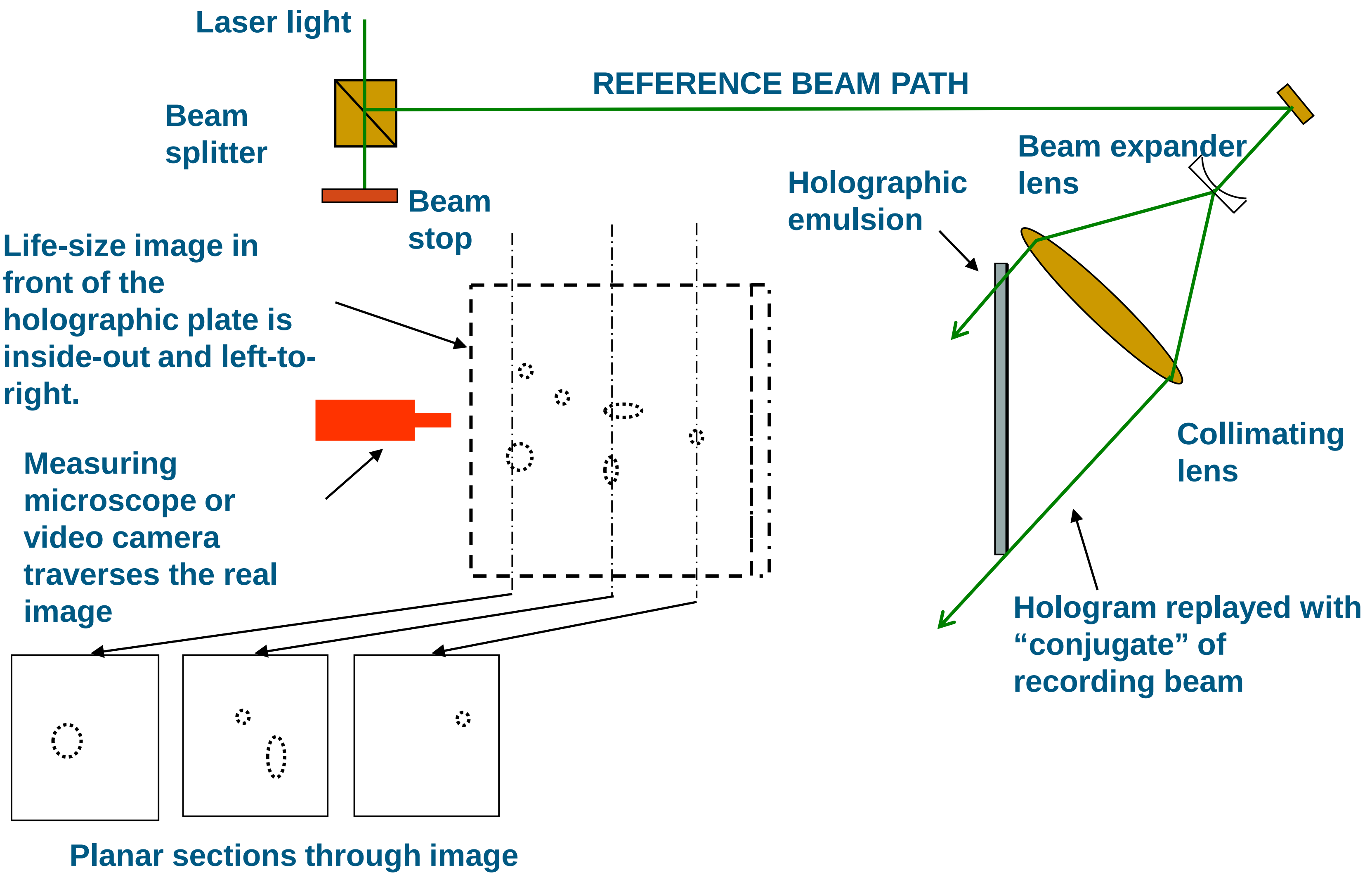
Off-axis holography: recording



Off-axis holography: replay of virtual image



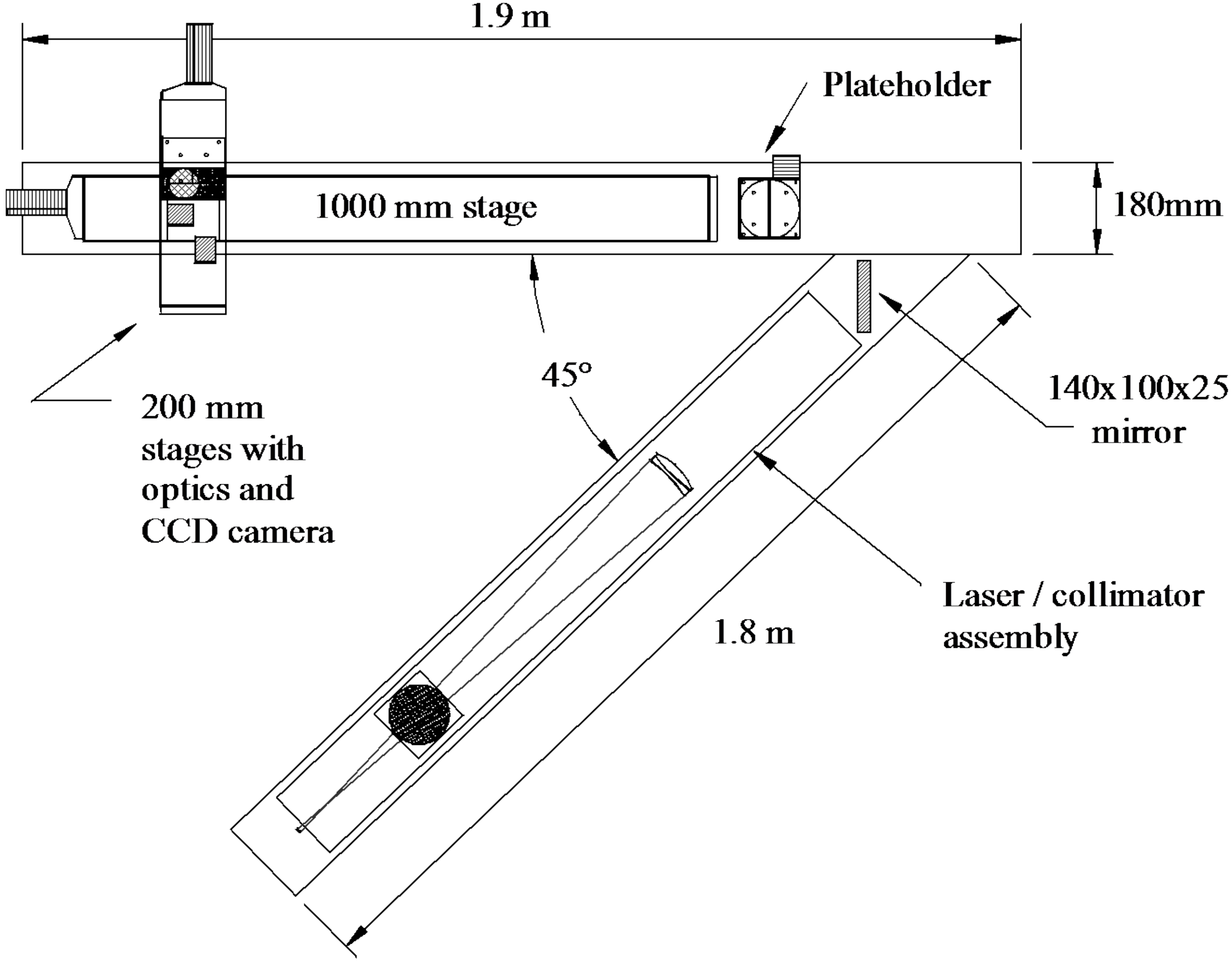
Off-axis holography: replay of real (projected) image



HoloMAR-SCAN Replay & Scanning parameters

- **Replay by HeCd laser (emitting at 442 nm)**
- **In-line and off-axis real image replay**
  - allows either type of hologram to be interrogated in same system
- **Partial refractive index compensation for off-axis holograms**
- **Video camera mounted on motorised translation stages**
  - 2 μm steps in xyz axes
  - precise dimensional measurement of relative location of the organisms
- **Holographic plates are mounted in precision holder**
  - motorised translation and rotation in all six degrees of freedom
  - allows optimisation of the resolution and brightness of the hologram
    - $< 0.5°$ permitted in each rotational axis
  - orientated at the exact recording angle (up to 60°) for the off-axis mode or normal to the reconstruction beam for the in-line case
- **Large $1000\times200\times200$ mm^3 scanning volume**
  - variable magnification views incorporated

HoloMAR-SCAN: Replay Facility



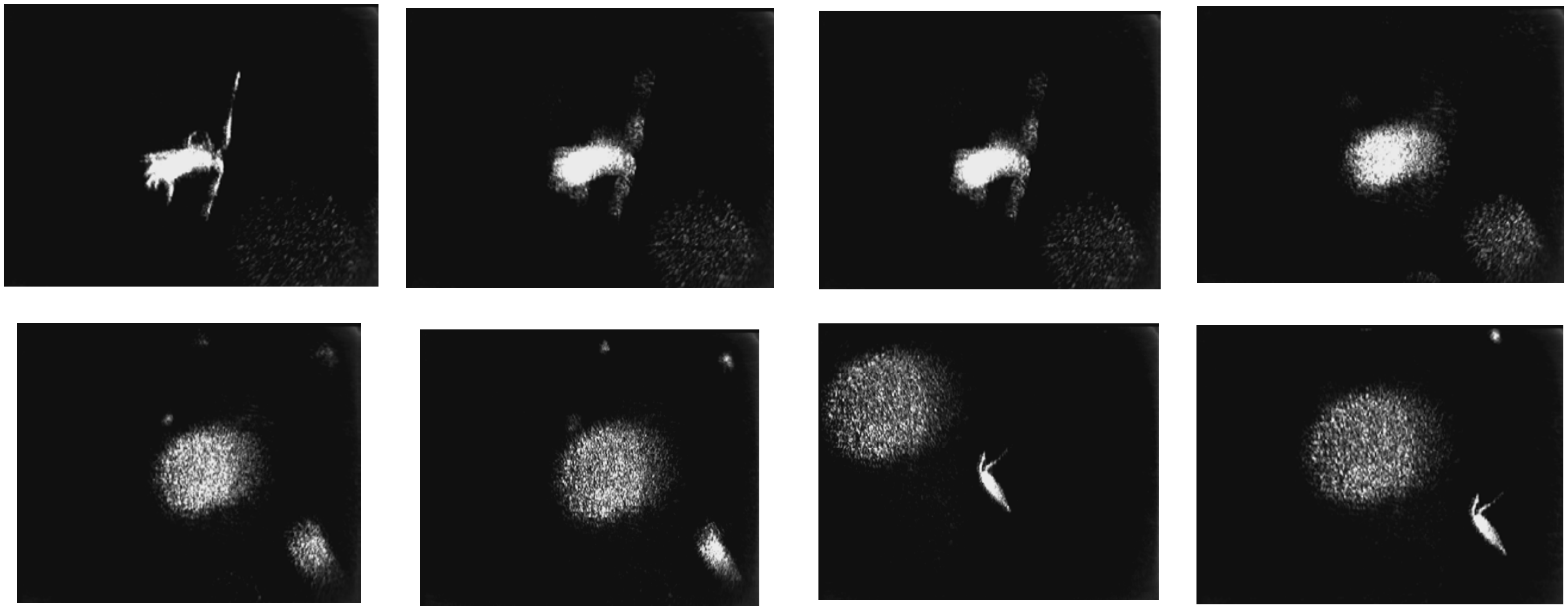
Through-focus video of off-axis hologram

Clip shows a th
section of an o
hologram. The
17.5 mm by 13
represents a tr
30 mm through the image
volume. Copepod is about 4
mm long.

l. Water
n of
hologram.

Creatures from the Deep!

Change of image plane through an off-axis hologram



A sequence of images from a single off-axis hologram showing a translation of the video camera from one focused organism to another. The two organisms (copepods) are 49.0 mm apart, vertically separated by 3.5 mm and horizontally separated by 1.5 mm. The holograms images recorded with a ruby laser (694 nm) and replayed with Kr-ion (647 nm). The organisms are about 2 mm long.

Off-axis holography: Summary

- **Two beams of light are used**
  - one is expanded to a diverging beam & illuminates the scene
  - the other is expanded (& often collimated) & directly illuminates the emulsion
- **Real & virtual images are spatially separated**
  - replay optimised for either virtual or real images
- **Multiple beam illumination (front and/or side lit) is possible**
- **Off-axis method is better for larger organisms & opaque scenes**
  - from about 100 μm upwards at much higher concentration levels
  - there is a lower practical recording limit of about 100 μm for off-axis holograms
  - particle concentrations from a few hundred per cubic centimetre upwards
- **Recording and replay conditions must be carefully matched**
  - suffers potentially from refractive index mismatch at finite field angles
  - characteristics of original preserved
  - longitudinal, lateral & angular magnifications all unity under right conditions
- **Because of recording in water & replay in air**
  - image can suffer from optical aberrations at finite field angles
  - the aberrations can be minimised by replaying under specific conditions

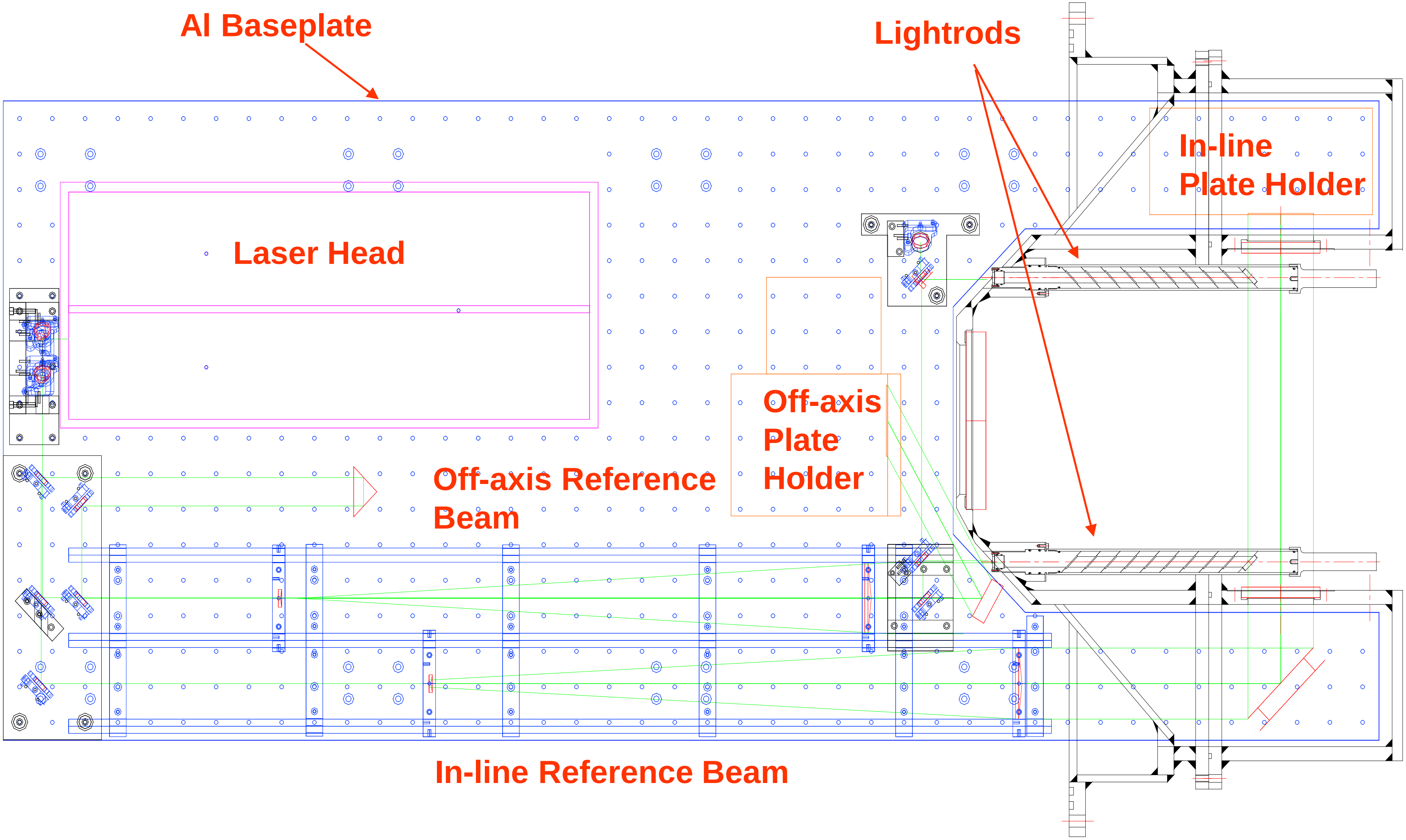
HOLOMAR parameters

- Pulsed frequency-doubled Nd-YAG laser
  - 8 ns duration; 650 mJ energy; 532 nm wavelength
- Simultaneous recording of IL & OA holograms
- In-line: Resolution < 10 µm over 2.5 litres
- Off-axis: Resolution < 100 µm over ~ 12 litres
  - but nearly 100 litres are imaged
- Operation to a depth of 100 m
  - Up to 45 holograms per dive
  - PC control of camera functions from surface
- 2.3 m long x 1.1 m diam; 2.3 tonne!!!

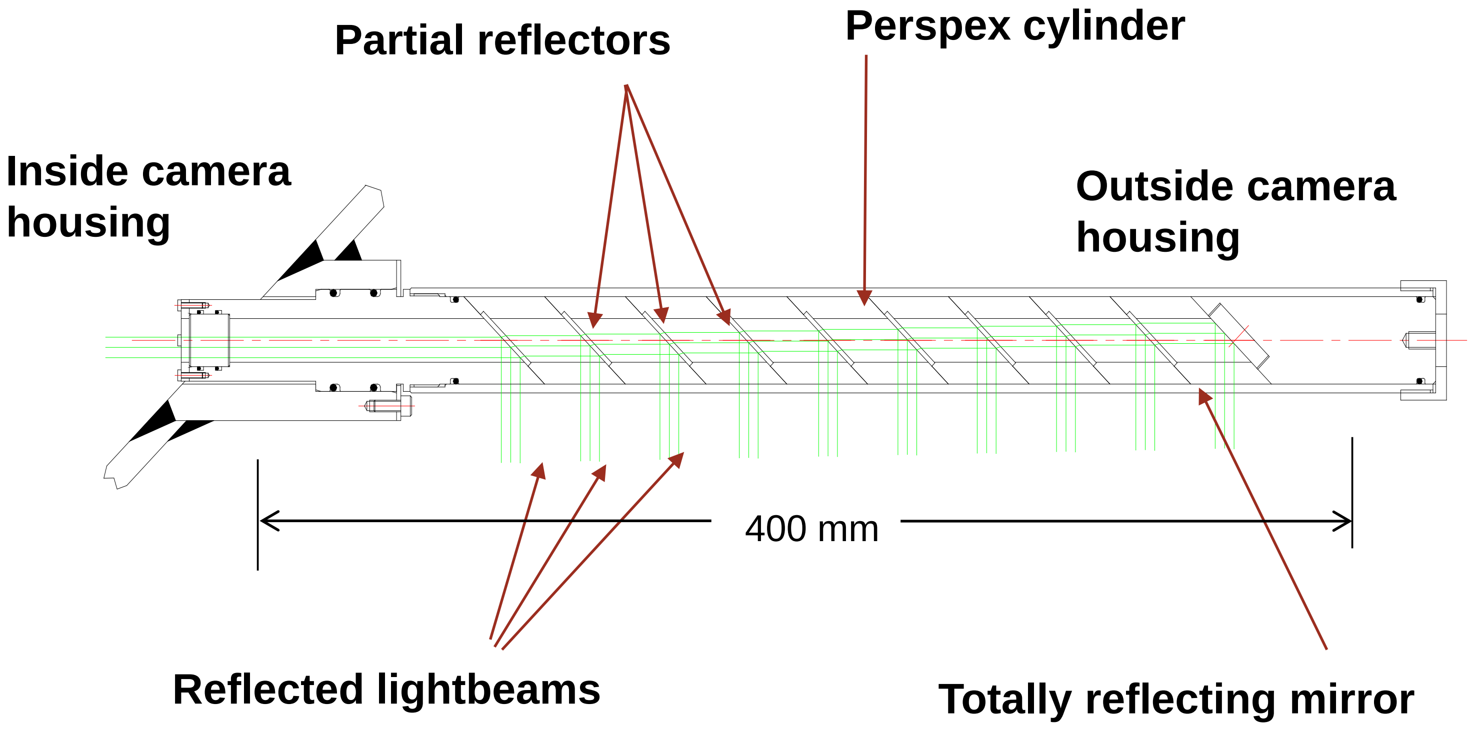
HoloMAR-CAM parameters

- Pulsed frequency-doubled Nd-YAG laser
  - pulse duration 8 ns; pulse energy 650 mJ; 532 nm wavelength
- Simultaneous recording of in-line & off-axis holograms
  - recording of partially overlapping volumes of water
- In-line Resolution ~ 10 µm over 2.5 litres
- Off-axis Resolution ~ 80 µm over ~ 12 litres
  - but nearly 100 litres are imaged
- Operation to a depth of 100 m
  - Up to 45 holograms per dive at a maximum rate of 6 per minute
  - Microprocessor control of all camera functions from surface PC
- Water temp, pressure & salinity sensors included

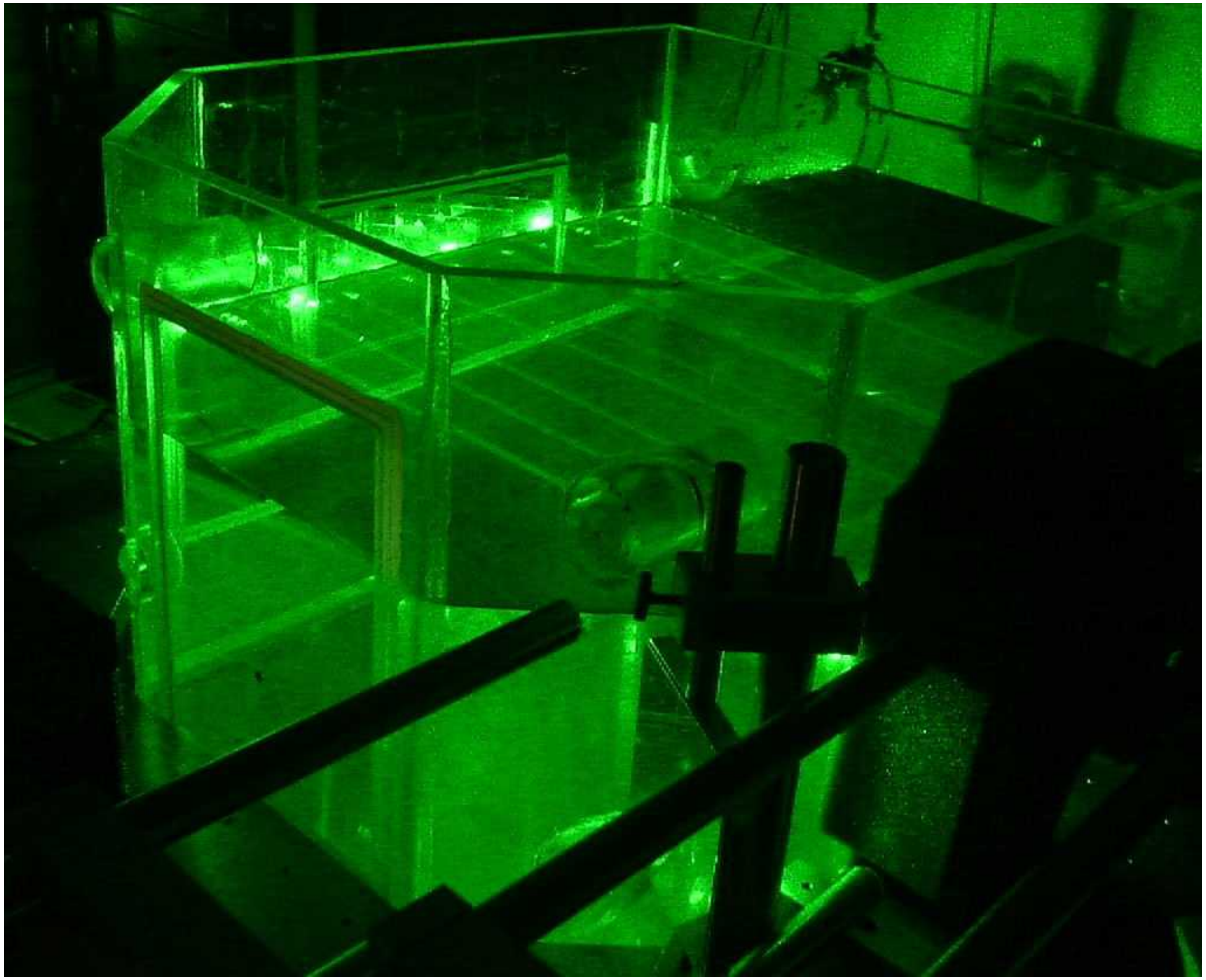
HoloMAR Layout



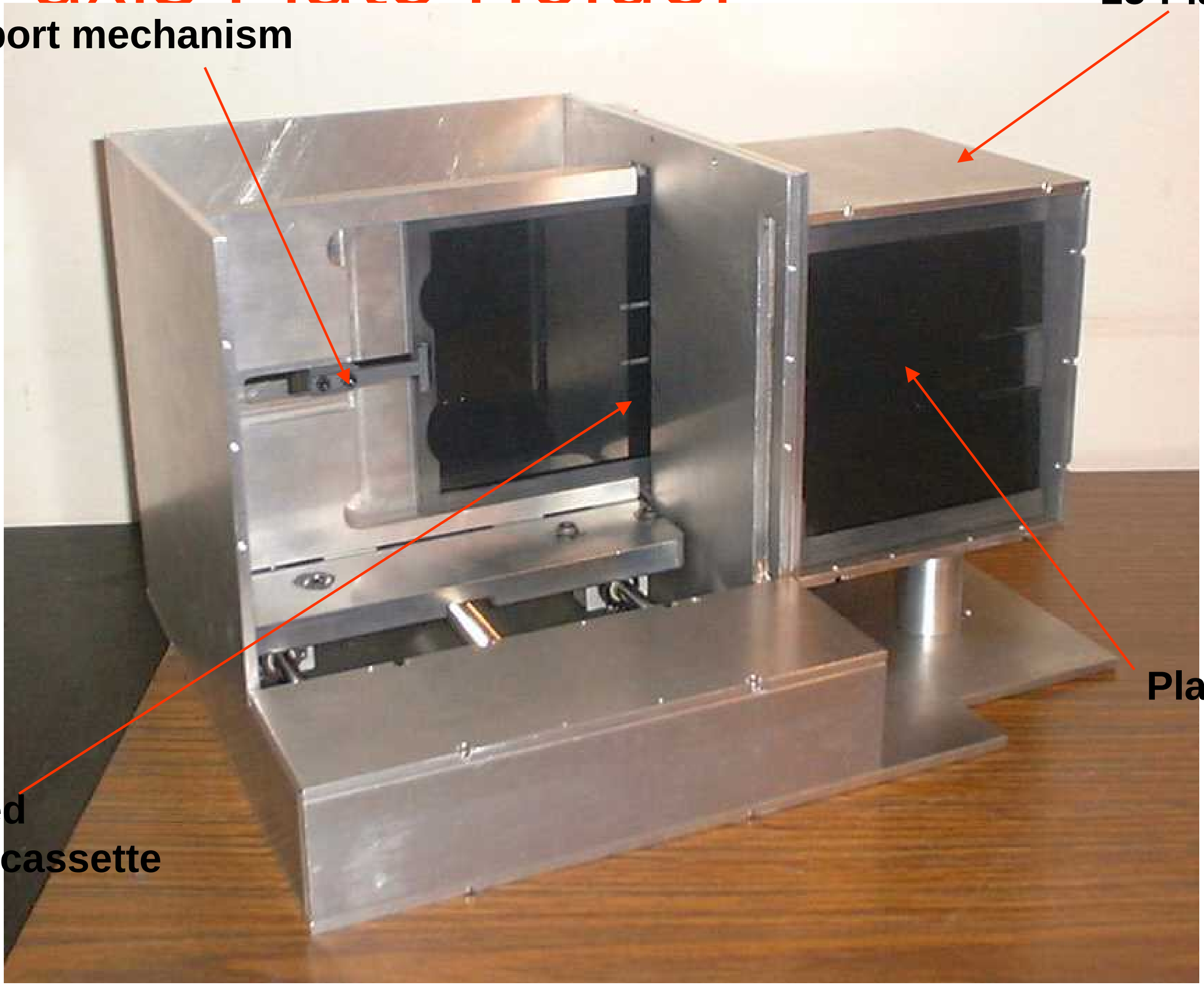
Illumination Lightrod



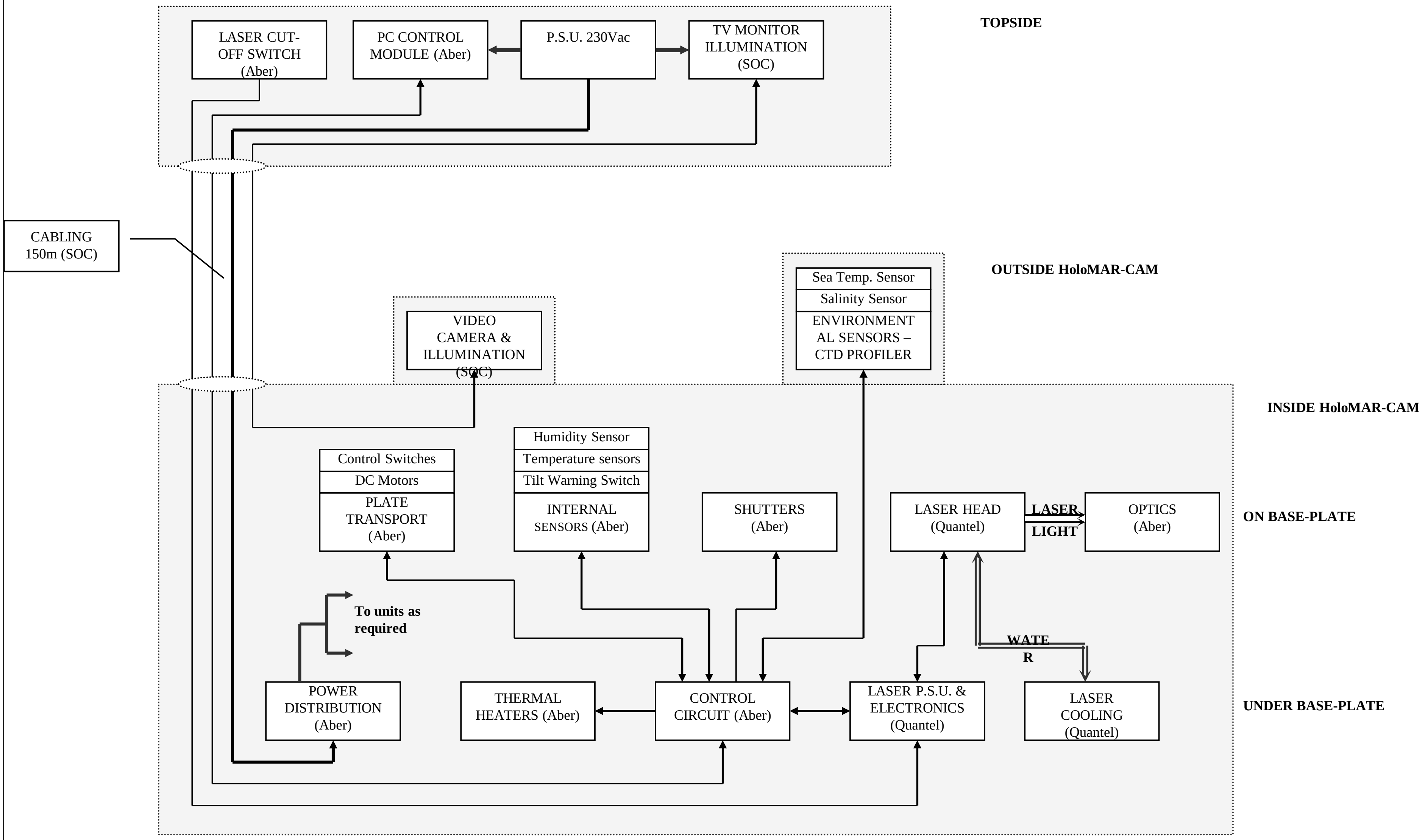
Illumination Pattern



Off-axis Plate Holder



The HoloMAR-CAM System Diagram



Successes of HoloMar

- A fully operational underwater holographic system designed, built and sea trialed.
  - Two cruises produced > 300 holograms
  - Data of biological relevance now being acquired.
- BUT……
  - Bulky, heavy & difficult deployment & manoeuvrability
  - Wet chemical processing of holograms
    - Limited availability of holographic emulsions
  - Complex image processing
  - Not easily mounted on ROV's, AUV's
  - Limited in operational depth

HOLODATA: Data acquisition & image processing

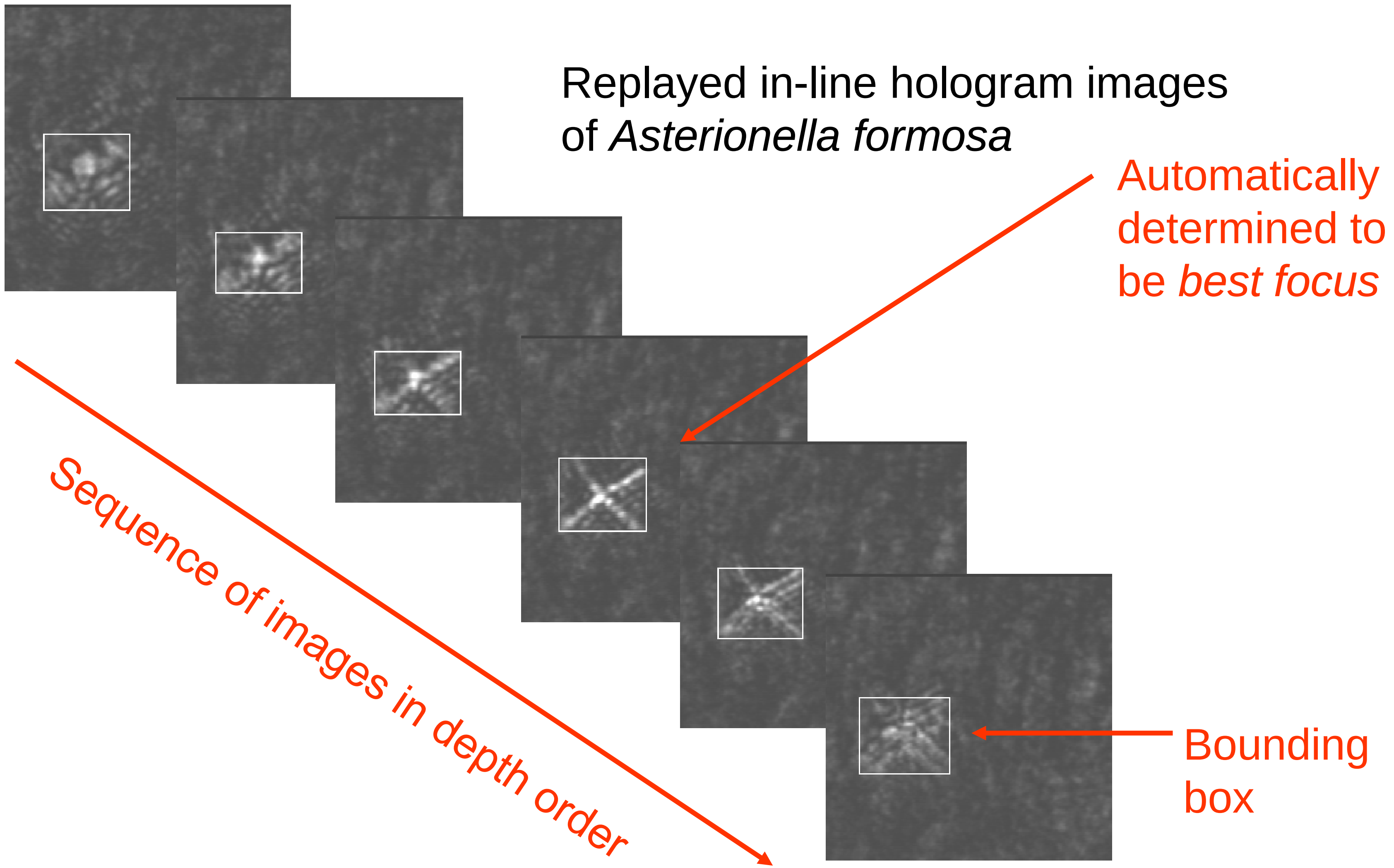
Images received from multi-frame averaging frame grabber in HoloMAR-SCAN

- **Tracking**
  - camera explores image volume and selects and localises organisms
  - based on a best-focus algorithm and on repeatability along z-axis
- **Noise cleaning and image enhancement**
  - stop band filtering removes unwanted components
  - image histogram manipulated to exploit all possible 256 grey levels
  - a median filter is employed to reduce speckle noise
- **Image segmentation through region growing approach**
  - produces grey level image based on threshold levels
  - separates significant regions from background and removes spurious information
- **Binarisation and Hu moments implementation**
  - objects are binarised and Hu moments implemented
  - fed to the neural network system
  - identification and classification according to family or genus
- **Measurement of local concentration & distribution**

Data acquisition & image processing

- **Global adjustment of hologram for brightest and sharpest image**
  - orientation of plate holder and angle of reference beam
    - this may be manual/visual or computer-controlled
- **Global search of hologram on low magnification for macroscopic feature recognition**
- **Digital processing for image enhancement**
  - specially developed image processing algorithms
  - facilitate enhancement of images prior to identification
    - edge enhancement
    - grey level filtering
    - noise removal
    - speckle removal
    - best focus of image
- **Species identification**
  - specially developed image processing algorithms
  - based on neural networks recognition
    - enable identification of individual organisms at family or genus level
    - identification regardless of orientation & scaling of organism
    - size measurement & relative position
- **Measurement of local concentration and distribution**

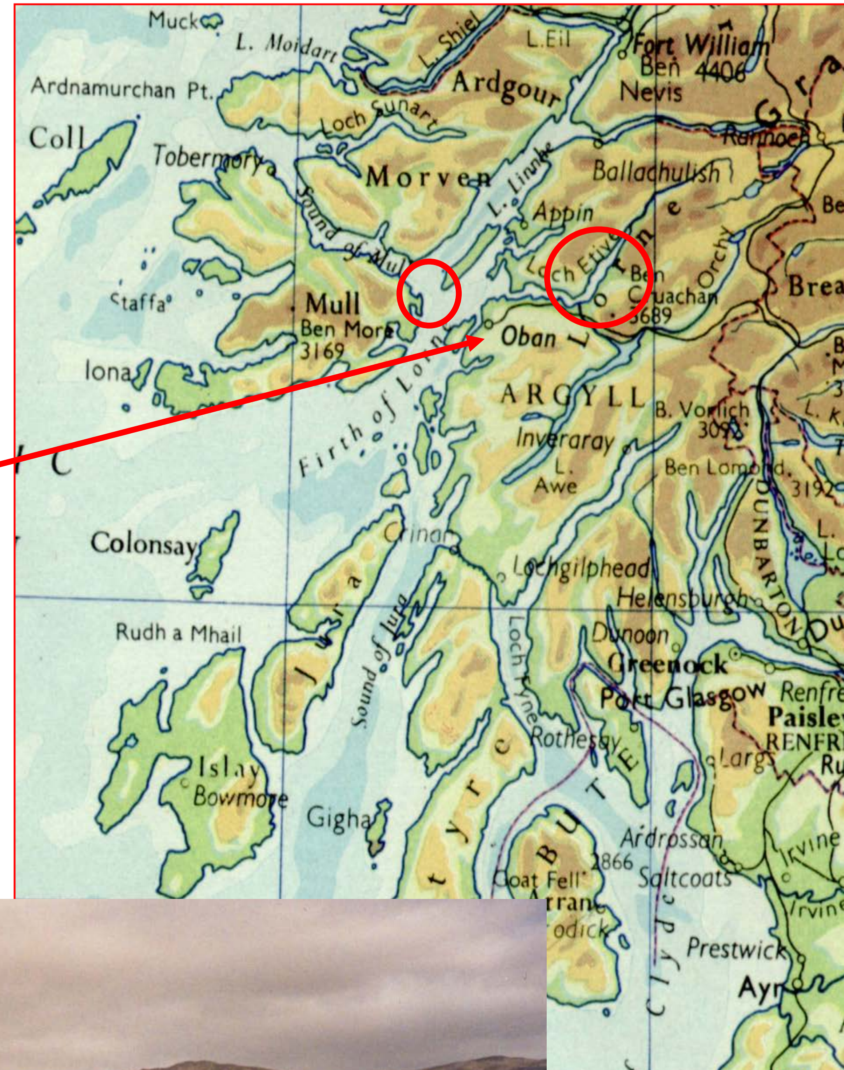
Tracking and focal plane finder



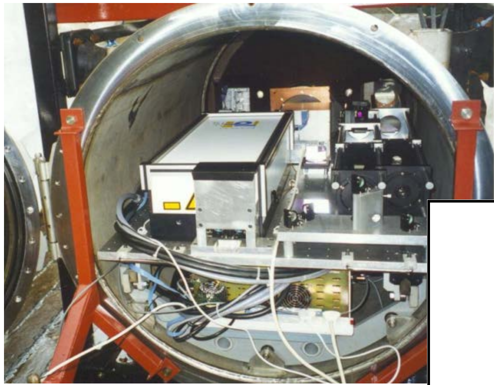
Loch Etive - Ship Trials 2000 & 2001

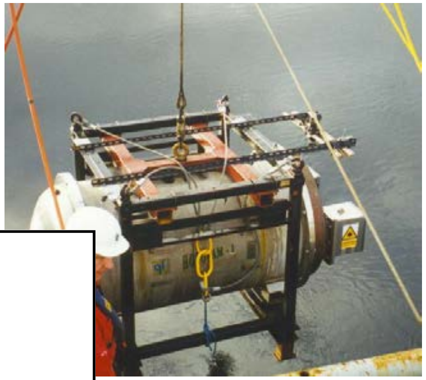
- **Two cruise trials of HoloMAR-CAM in Loch Etive & Lismore, SCOTLAND**
  - Cruise 1: 30 October - 1 November 2000 - Bonawe Deep, Loch Etive
  - Cruise 2: 16 September - 21 September 2001 - South of Lismore & Bonawe Deep
- **Three dives with complete HoloMAR-CAM to 100 m (Cruise 1)**
  - 18 pairs (1 in-line, 1 off-axis) of holograms recorded per dive
  - Recorded at 5 - 10 m intervals down to 100 m
  - HoloMAR-CAM held at each depth for 3 - 5 mins before recording
- **Six dives with complete HoloMAR-CAM to 100 m (Cruise 2)**
  - Two dives off Lismore (12 pairs + 18 pairs)
  - Four dives in Loch Etive (18 pairs per dive)
  - Recorded at 5 - 10 m intervals down to 100 m
- **Two holograms lost due to plate jams**
- **One batch of off-axis/in-line lost due to base plate slippage.**

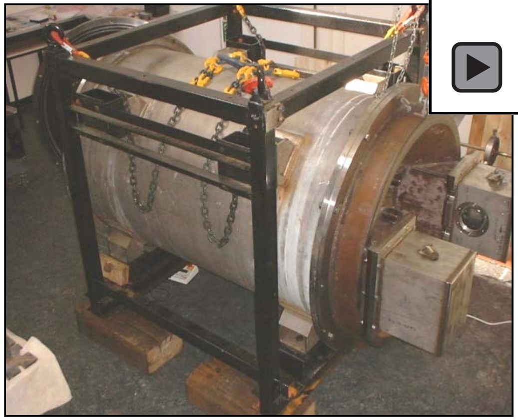
Loch Etive Ship Trials

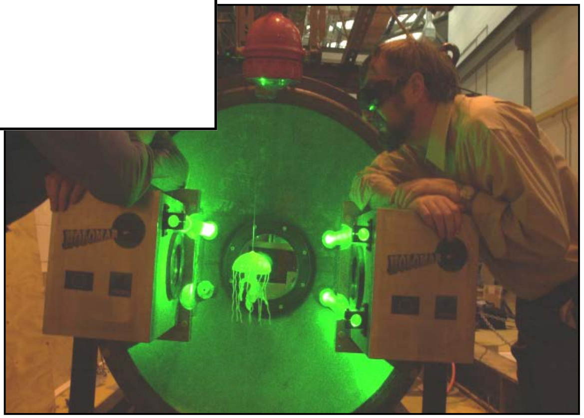


HoloMar Recording

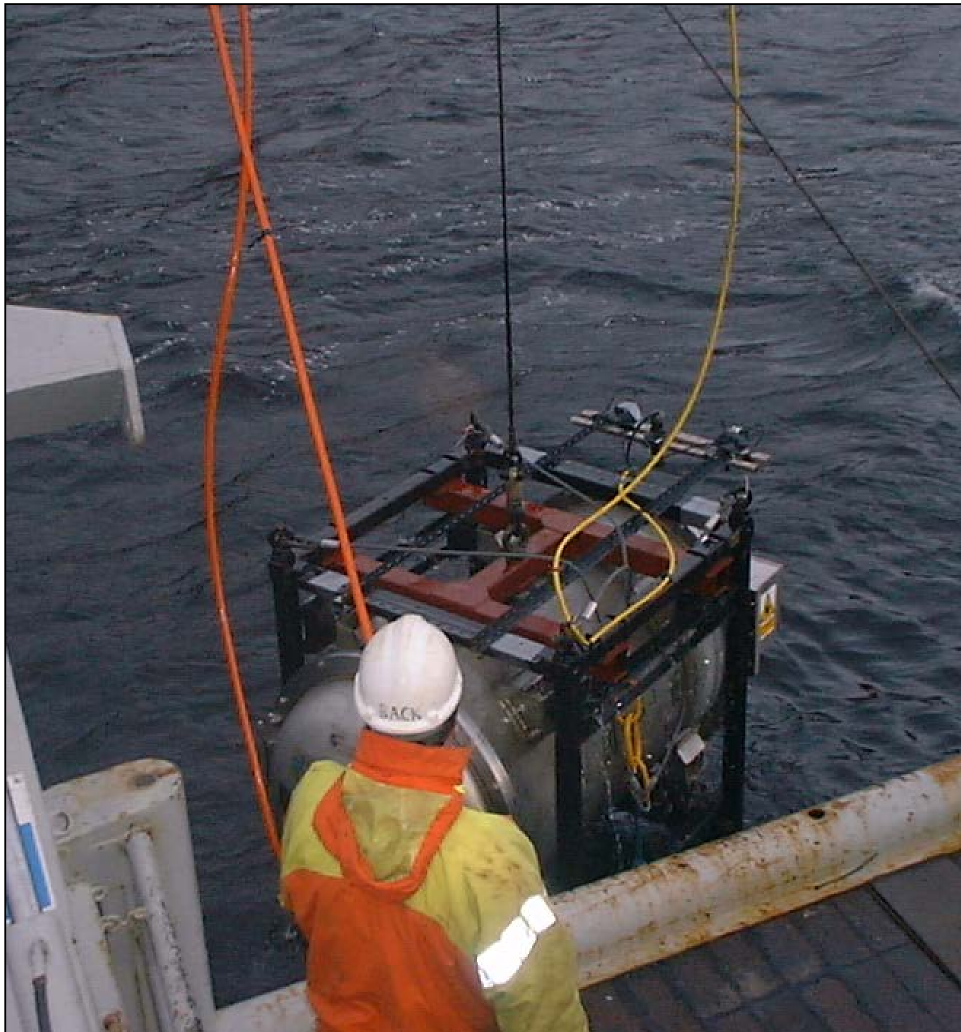


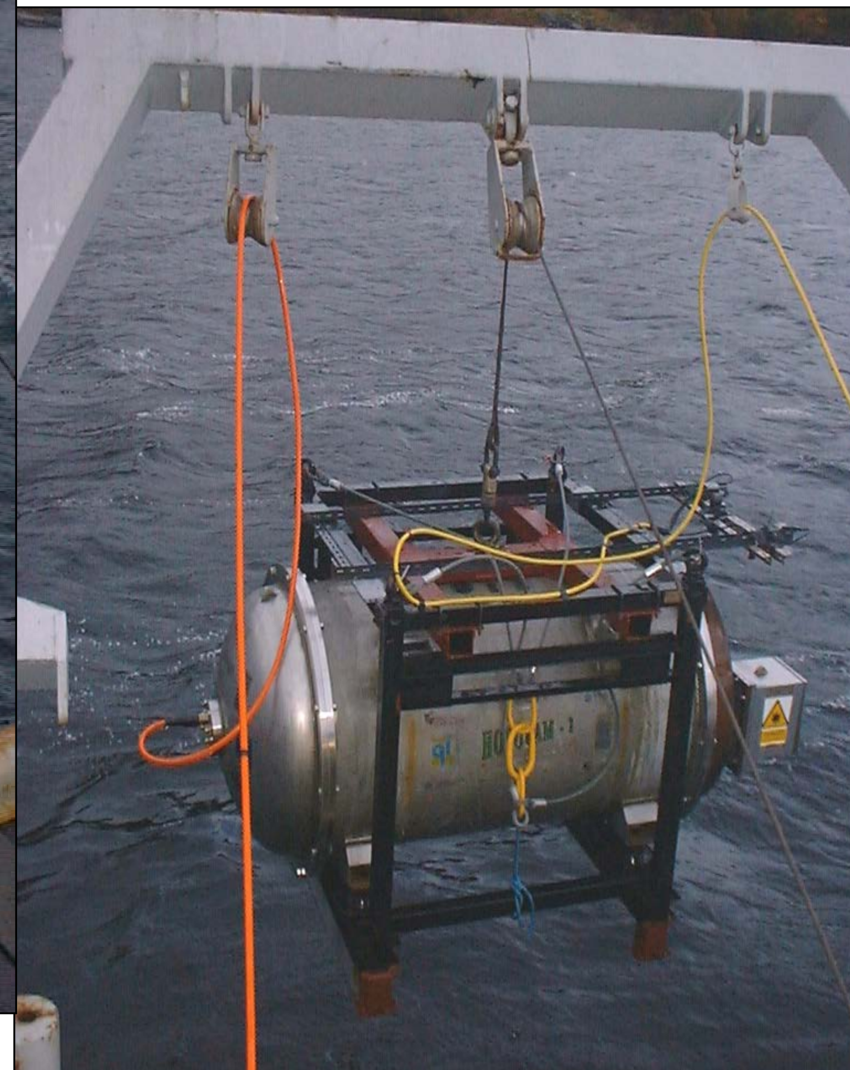




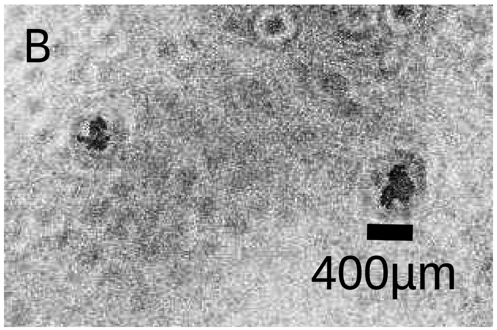


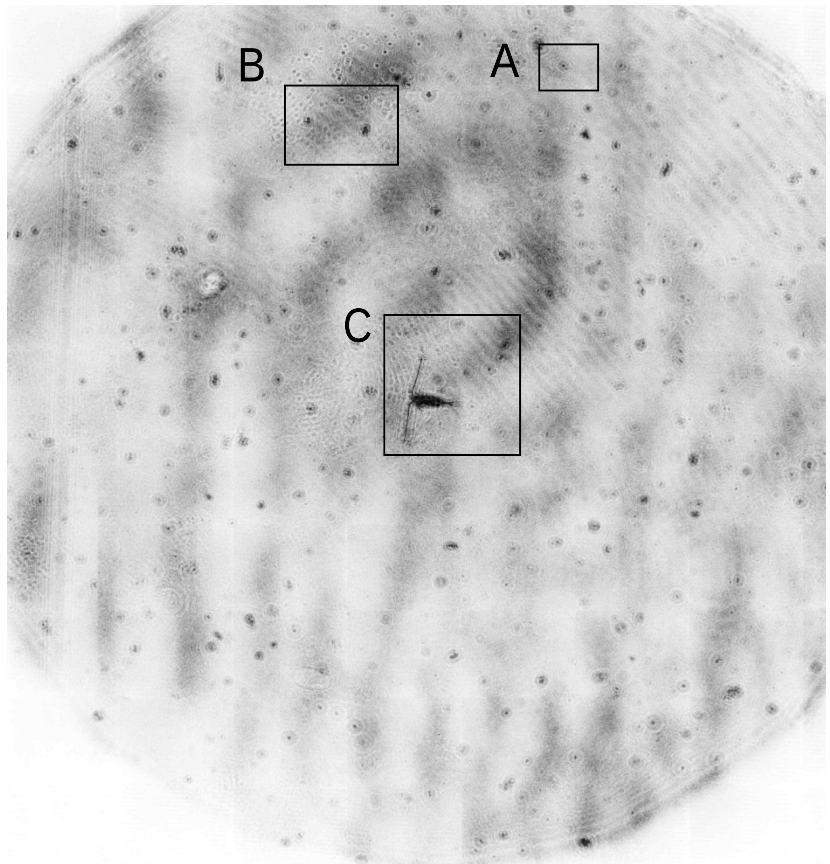
Launching HoloMAR-CAM in Loch Etive



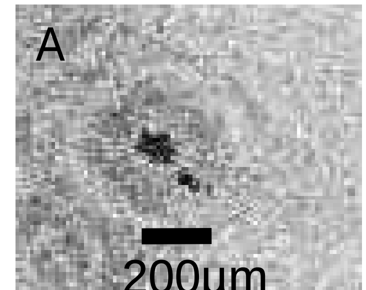


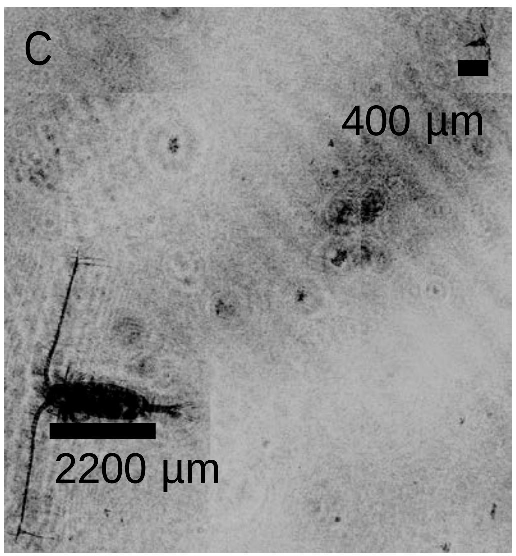
Resulting Automatic detection of small particles from composite image





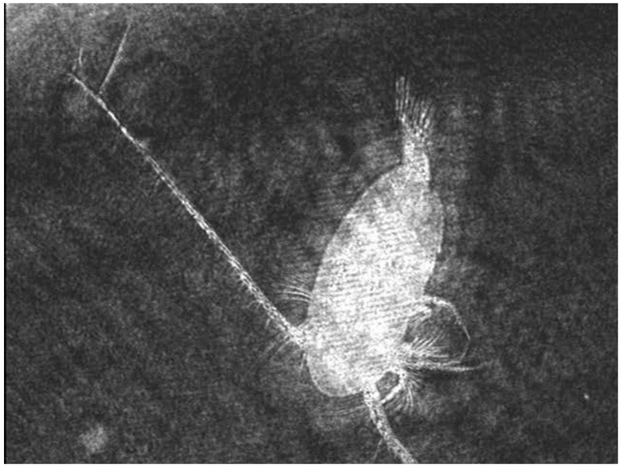
Sub-sampled reconstruction, from raster scan with 20 x 20 segmented array



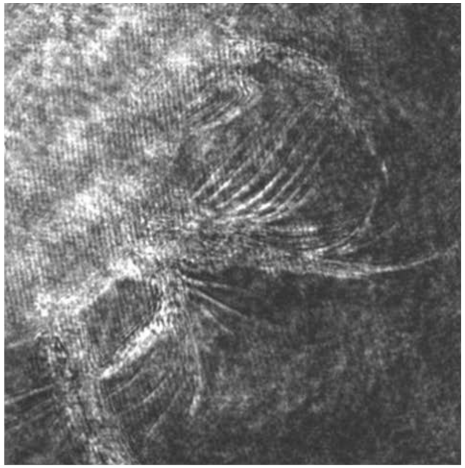


Loch Etive (2000) Holograms - in-line

Images of calanoid copepods in stage V development.

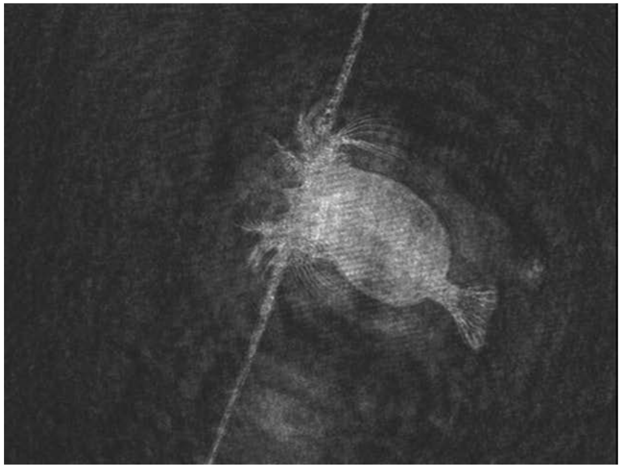


(a)

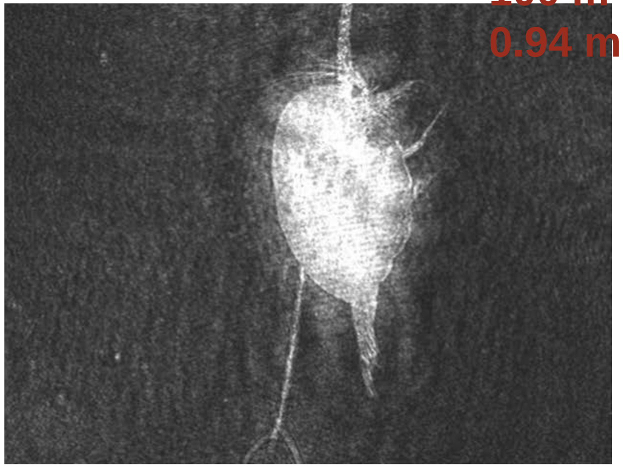


(b)

(a) recorded at 70 m (2.15 by 0.72 mm). (b) is an enlarged detail of (a).
(c) recorded at 80 m (1.9 by 0.75 mm).
(d) recorded at 100 m (2.06 by 0.94 mm).

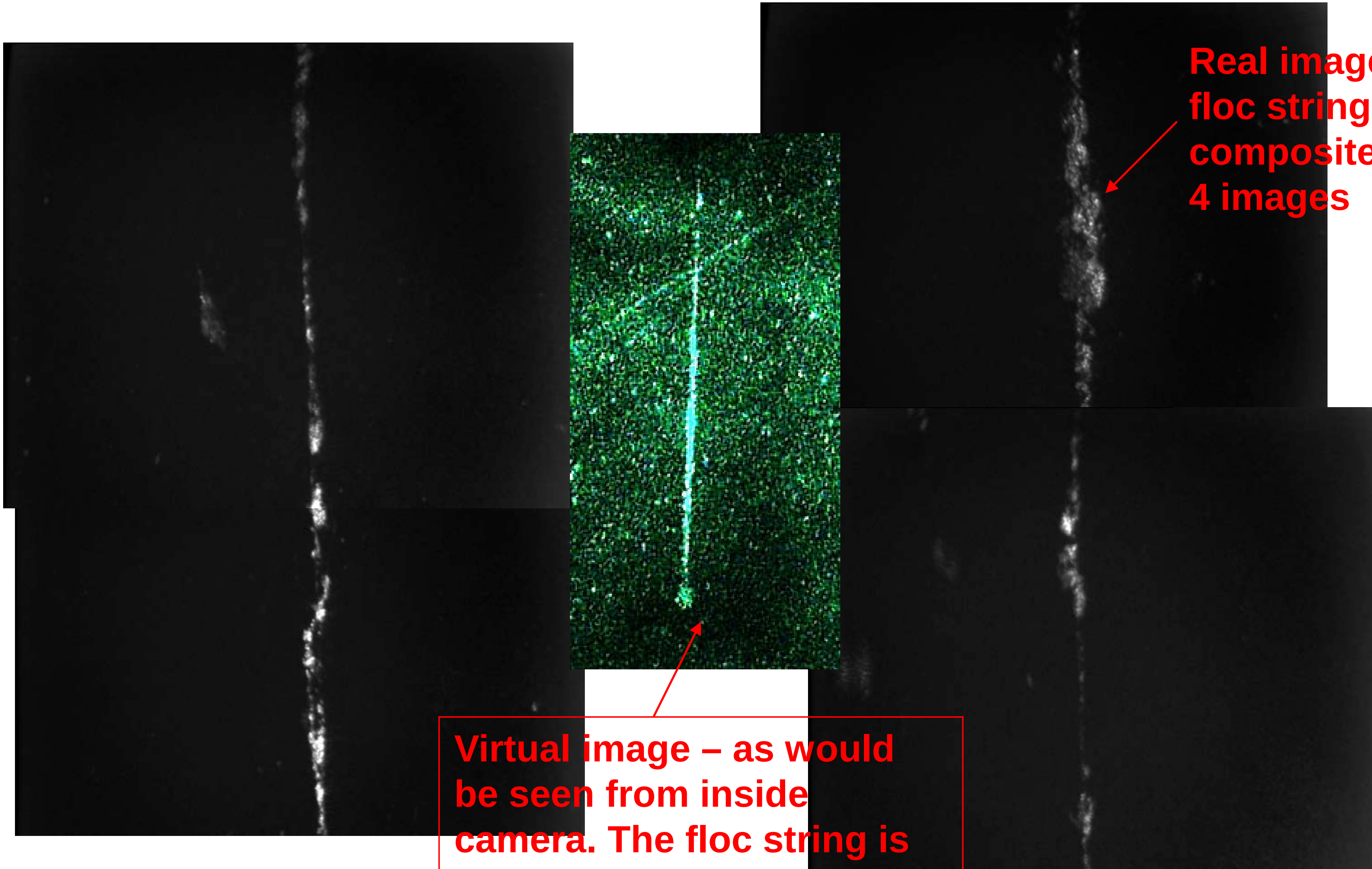


(c)

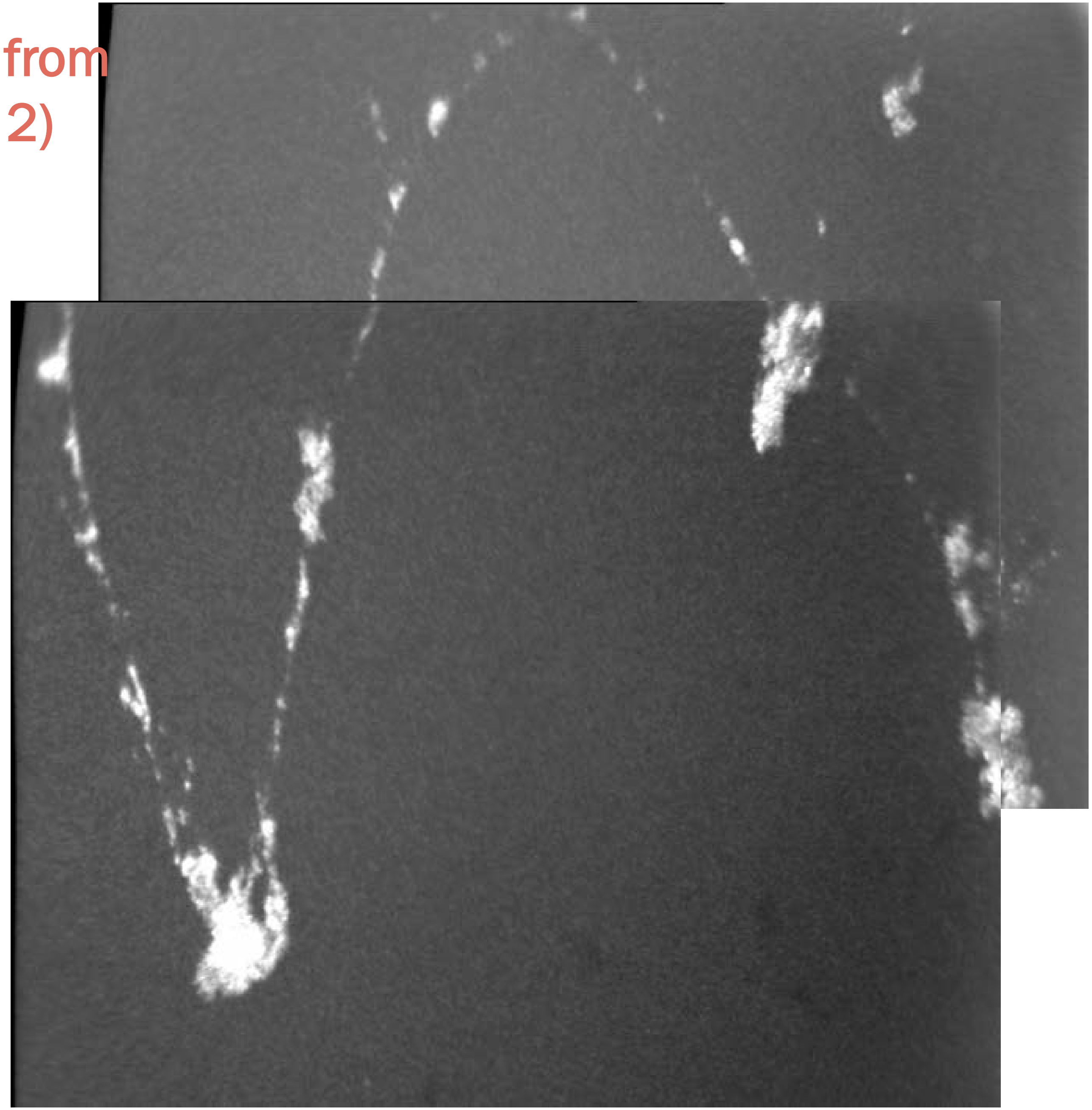


(d)

Long floc string in Loch Etive (2001)

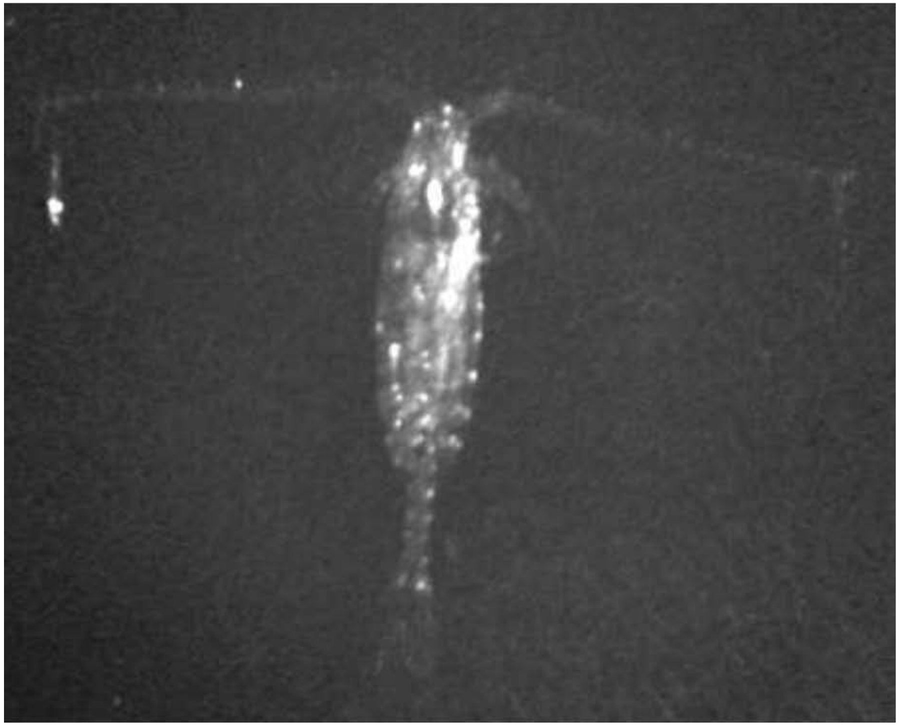


Off-axis Hologram from Loch Etive (Cruise 2)

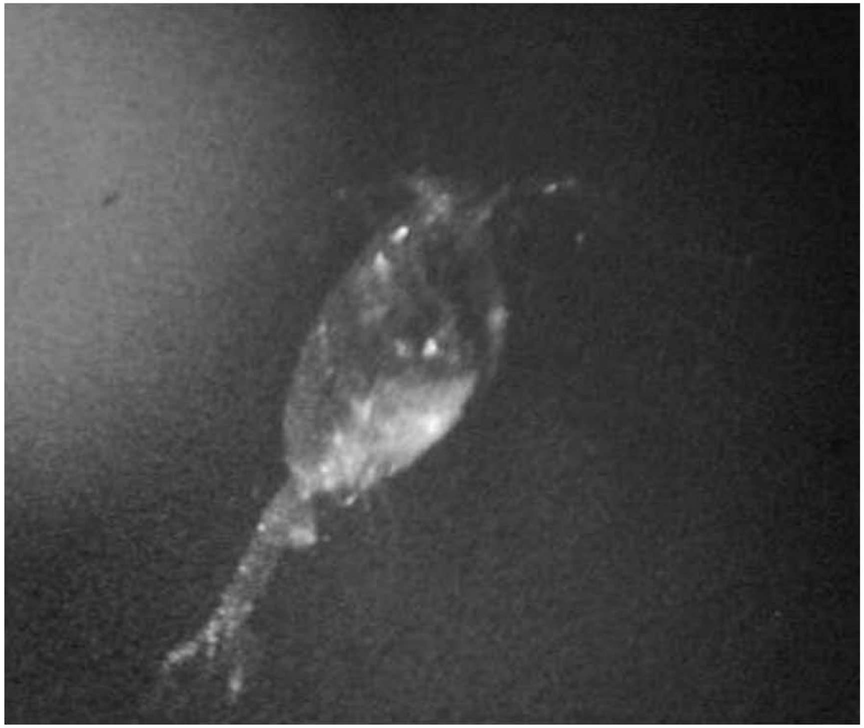


This string of floc was recorded at 100 m depth. Each panel of the composite photograph corresponds to a sensor area of 9 mm x 5.5 mm. The filaments between the floc are about 15 - 40 μm wide.

Loch Etive Holograms - off-axis (Cruise 1)



(a)



(b)

These two holograms were recorded at 60 m depth and are taken from the same hologram. They are adult calanoid copepods, about 5 to 7 mm long.

Through focus video of off-axis hologram

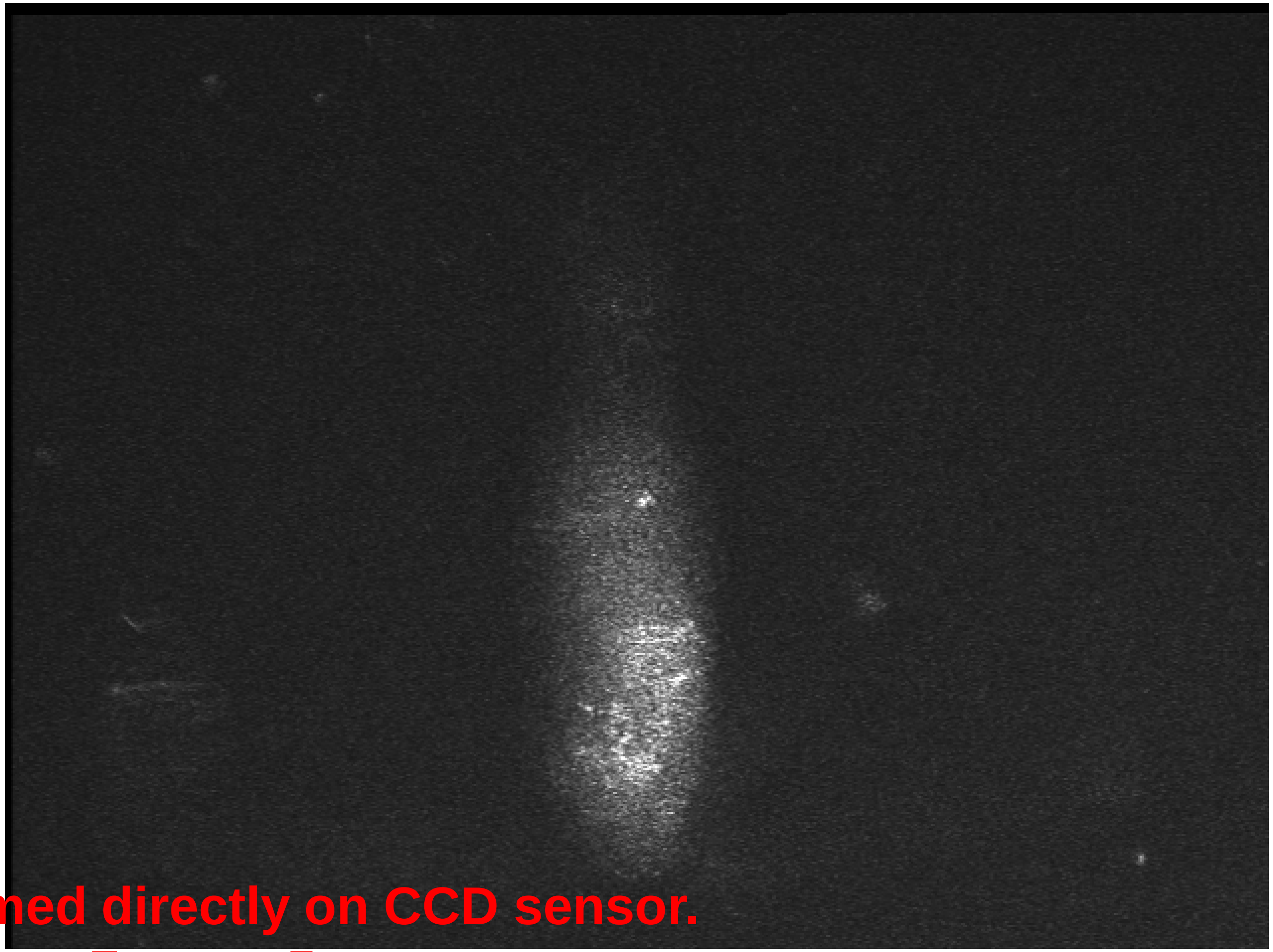
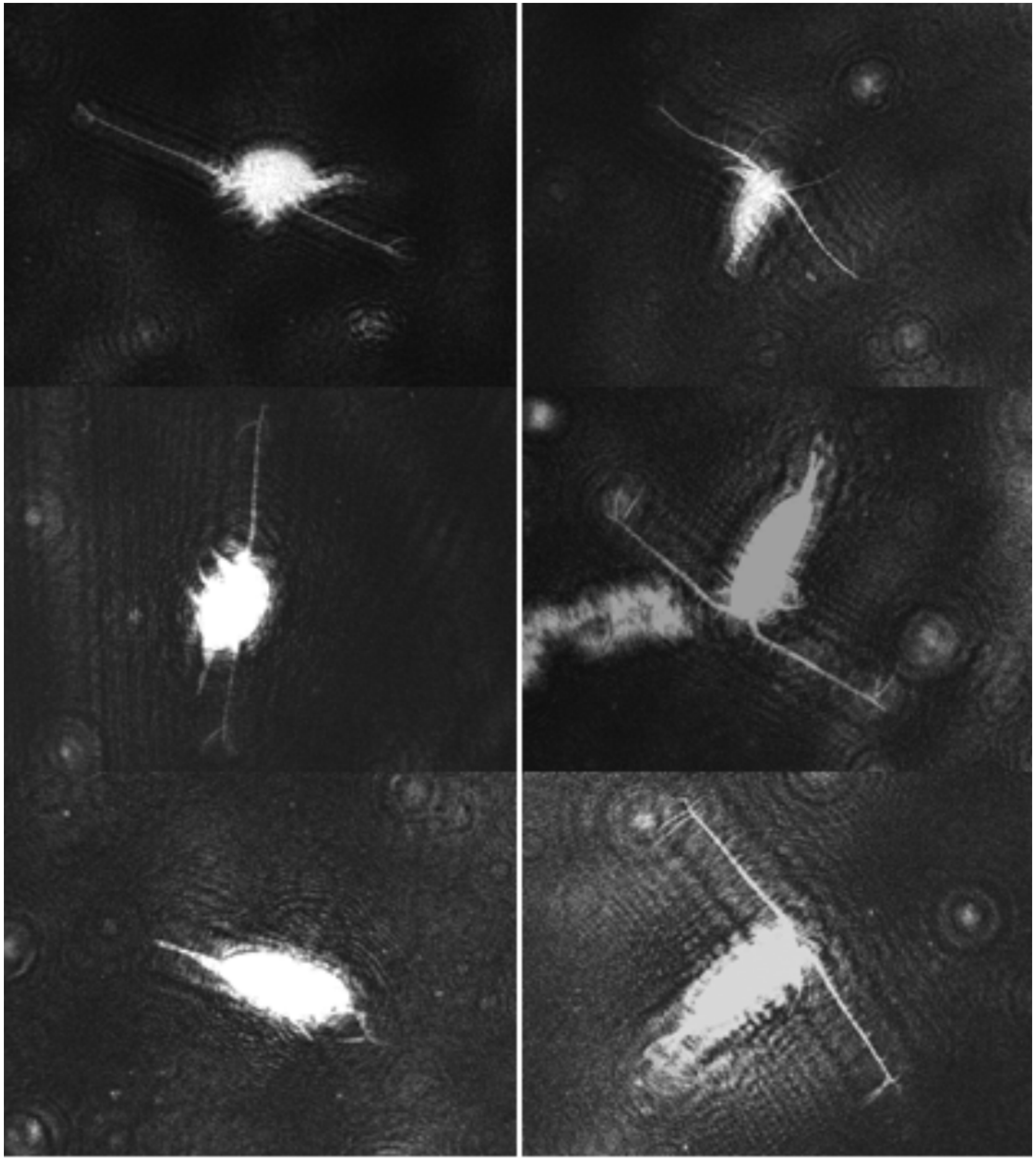


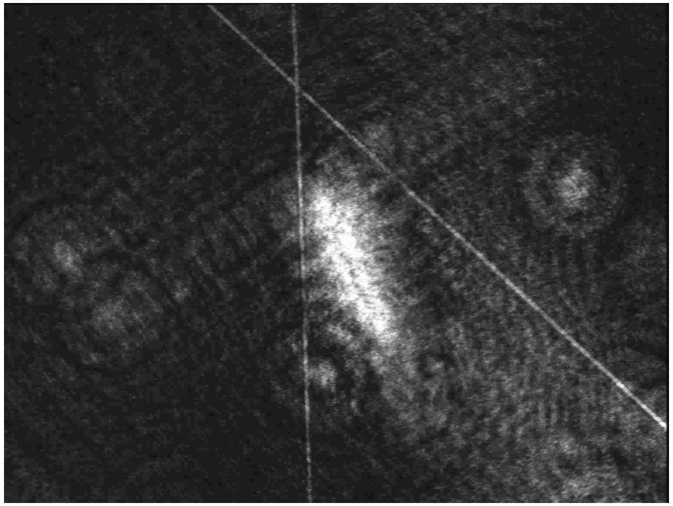
Image formed directly on CCD sensor.
Area is about 7 mm x 5 mm.

Loch Etive Holograms - in-line

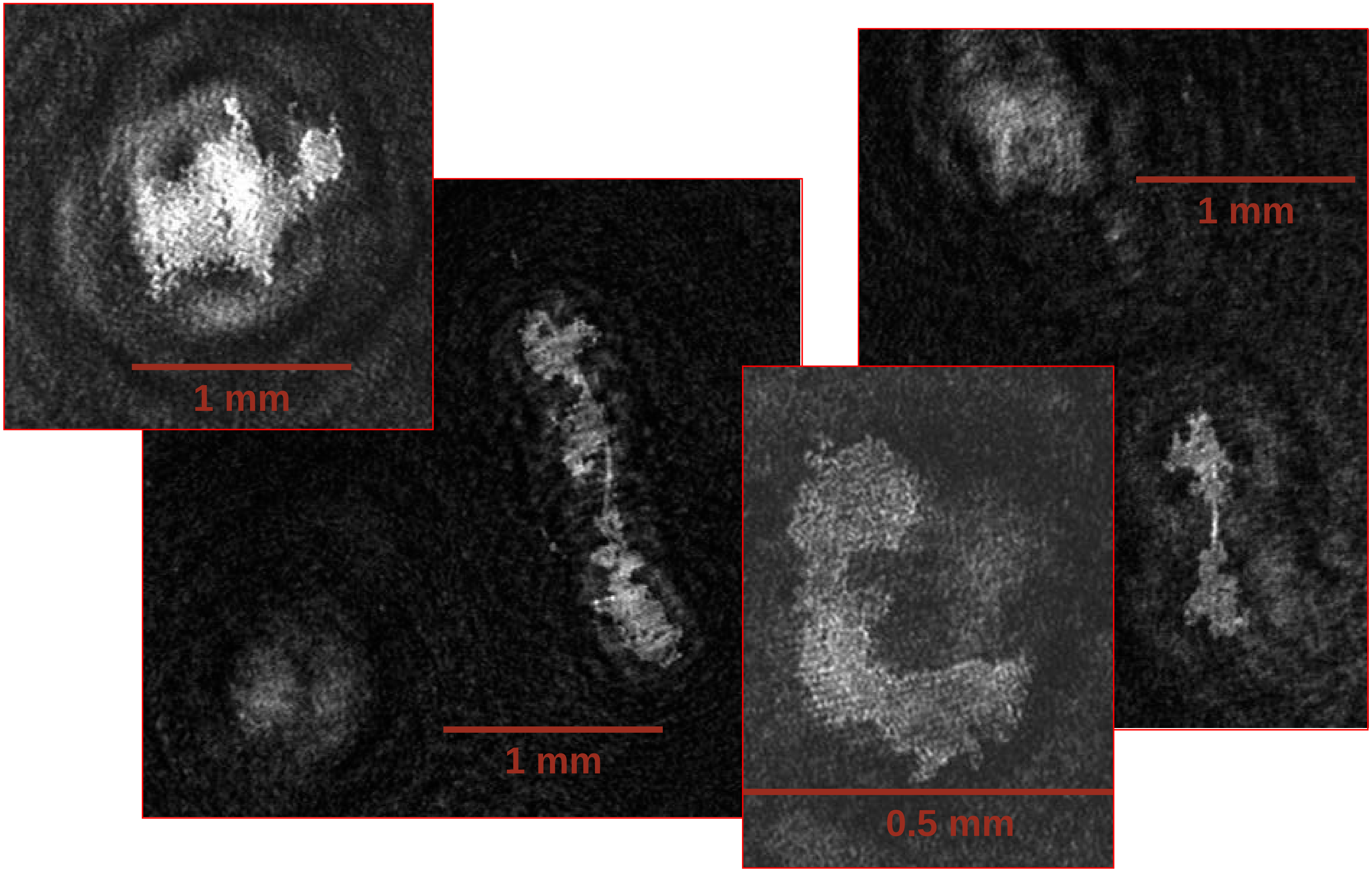


A mosaic of six different calenoid copepods replayed from in-line holograms from cruise 2. In each frame the area displayed is 8.9 mm × 6.3 mm and the surface of the loch is at the top. The out-of-focus patches seen in many of the frames are typically from floc.

The image below shows two 50 μm fiducial wires with an out-of-focus copepod behind them.



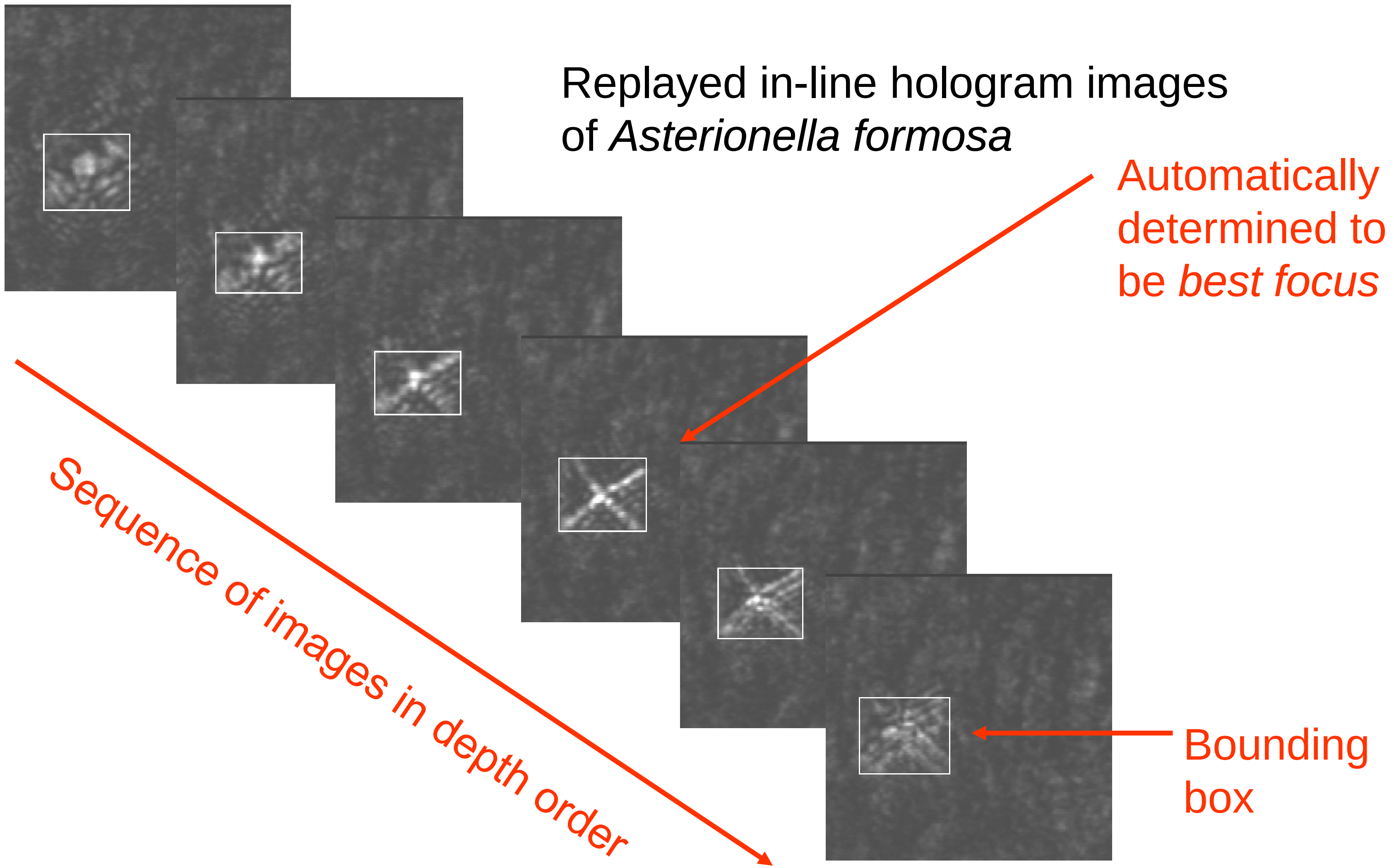
In-line Holograms of Floc (Cruise 1)



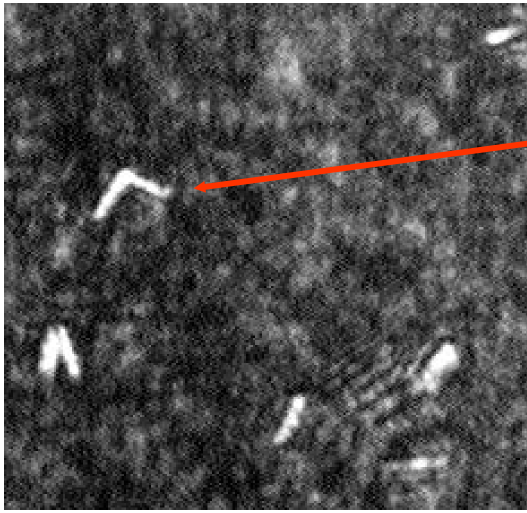
Summary

- A fully operational underwater holographic facility has been designed, built and commissioned and sea tested.
  - HoloMAR-CAM the submersible camera
  - HoloMAR-SCAN the dedicated replay machine
  - HoloMAR-PROC the suite of image processing and classification code
- The first and second cruises produced 300 holograms
- Verification and optimisation of the image processing and classification software is underway.
- Data of biological relevance now being acquired.
- The multidisciplinary (and multi-national) aspect of the HoloMar project was a key to its success.

Tracking and focal plane finder

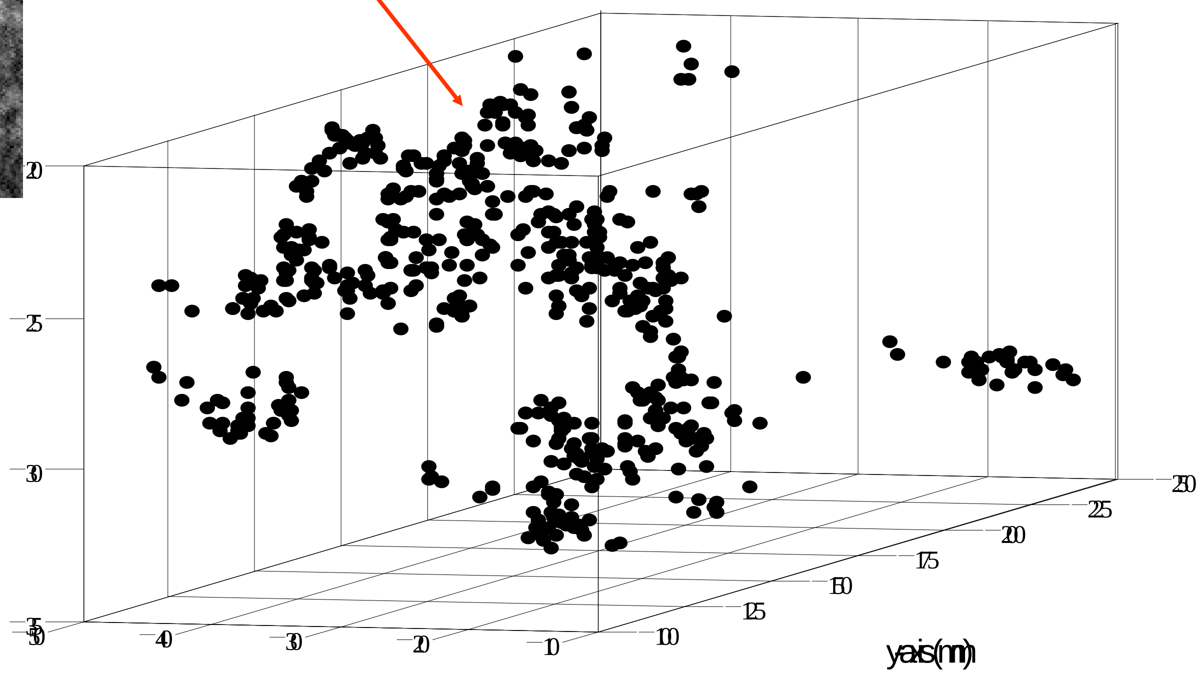


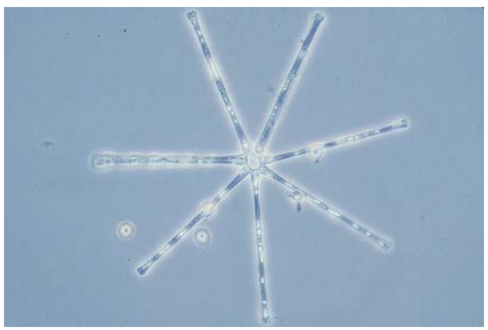
Coordinates - In-line holograms (tank tests)



Every organism in 98 cm^3 located in 3D

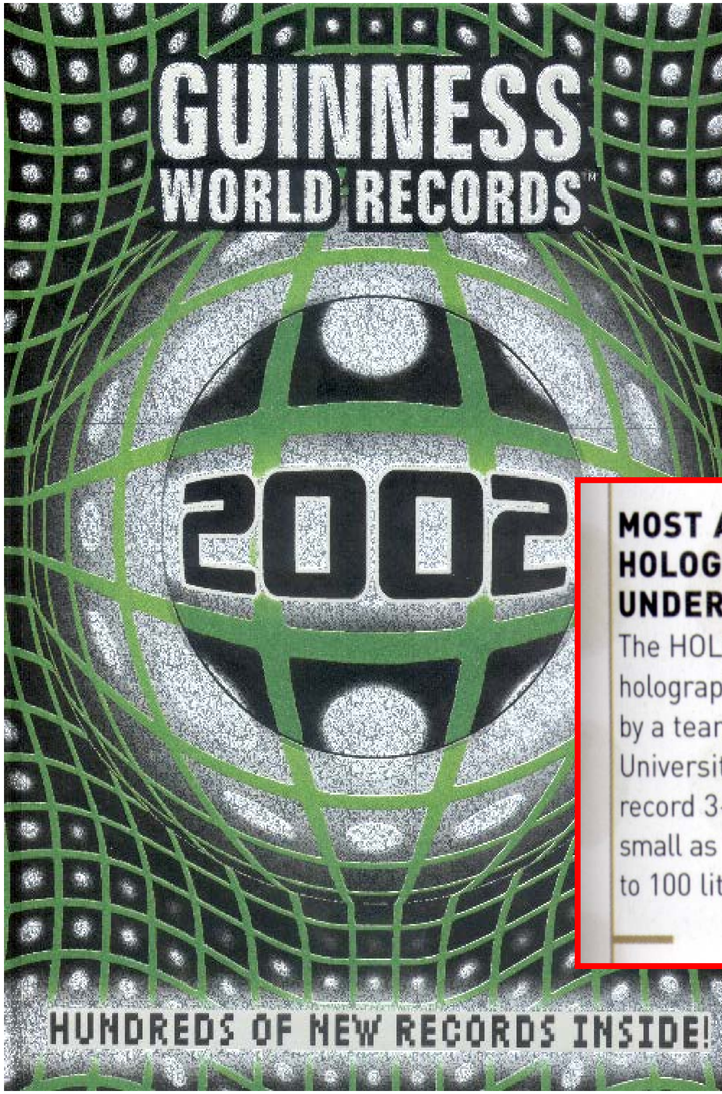
Asterionella cloud and coordinates





x, y , z

Guinness World Records 2002



MOST ADVANCED HOLOGRAPHIC UNDERWATER CAMERA

The HOLOMAR underwater holographic camera was developed by a team led by Aberdeen University, Scotland (UK). It can record 3-D images of objects as small as 100 microns across in up to 100 litres (26 gal) of water.

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