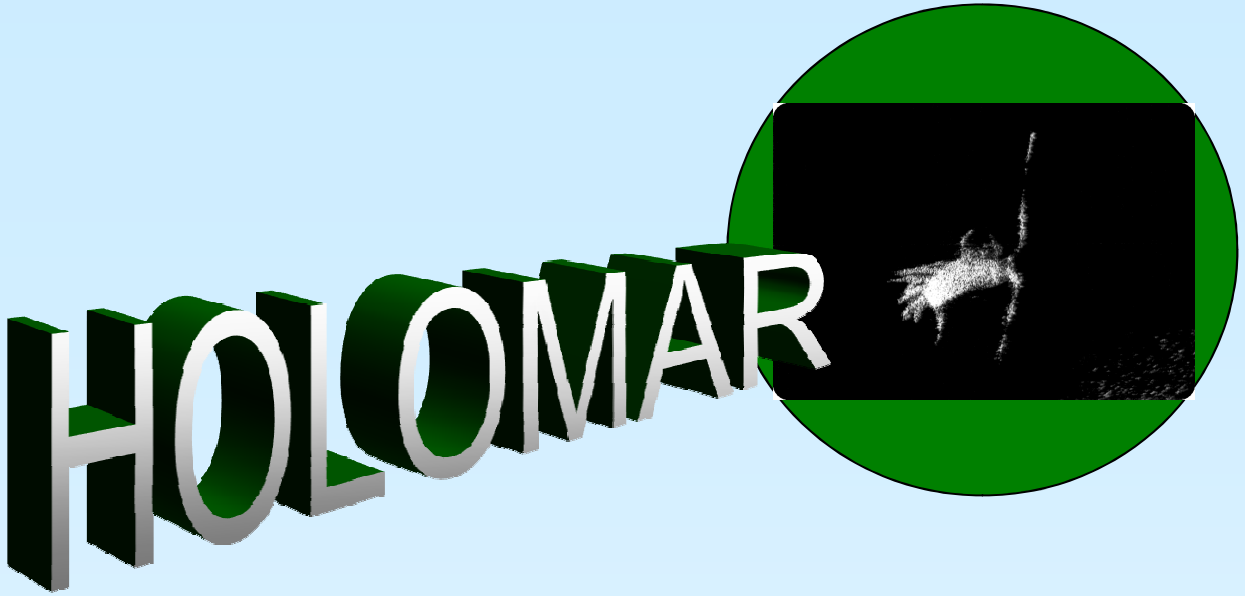
# Development of a holographic system for *in situ* visual recording of plankton


Presented by **Henry Nebrensky** (Brunel University) on behalf of

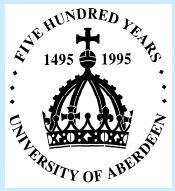
## The HoloMar Collaboration

at *Characterisation of Three-Dimensional Optical Images and Systems*,
National Physical Laboratory, Teddington, Middlesex, UK, 8th July 1999




# High resolution *in situ* HOLOgraphic recording and analysis of MARine organisms and particles

**EC MAST-III Programme**
**MAS-CT97-0079**






**The Partners wish to thank the European Commission (MAST III Programme) for funding the HOLOMAR Project**

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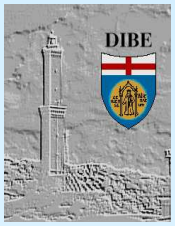



- *Aberdeen University*, SCOTLAND
- *Holo 3*, FRANCE
- *Quantel*, FRANCE
- *University of Udine*, ITALY
- *Brunel University*, ENGLAND
- *Southampton Oceanography Centre*, ENGLAND
- *University of Genova*, ITALY
- *Nemko*, NORWAY


HOLOMAR web site: **http://www.brunel.ac.uk/~phstprh/ holopub/sld001.htm**




# Objectives of HoloMar

- **Develop, construct & evaluate a fully-functioning prototype underwater holographic camera**
  - Holographically record large volumes of the upper water column containing marine 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 marine organisms without operator intervention

# The need to study marine organisms

- **Behaviour of marine biological communities**
  - plays important role in understanding global environment
- **Modelling of chemical cycles assisted by**
  - study of aggregates of biotic or abiotic particles
  - knowledge of distribution & dynamics
  - their inter-relationship with each other
    - local interactions between meso-zooplankton and micro-zooplankton, phyto-plankton and seston.
- **Accuracy of measurements limited by**
  - absence of good measurement techniques
    - typically work on organisms uses counters or photography
  - small data base
  - frailty & wide size range & complexity of aggregates
    - particles vary in size from sub-micrometres to several millimetres

# The benefits of holography in marine 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 to bring 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

# Holography : *a short guide*

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

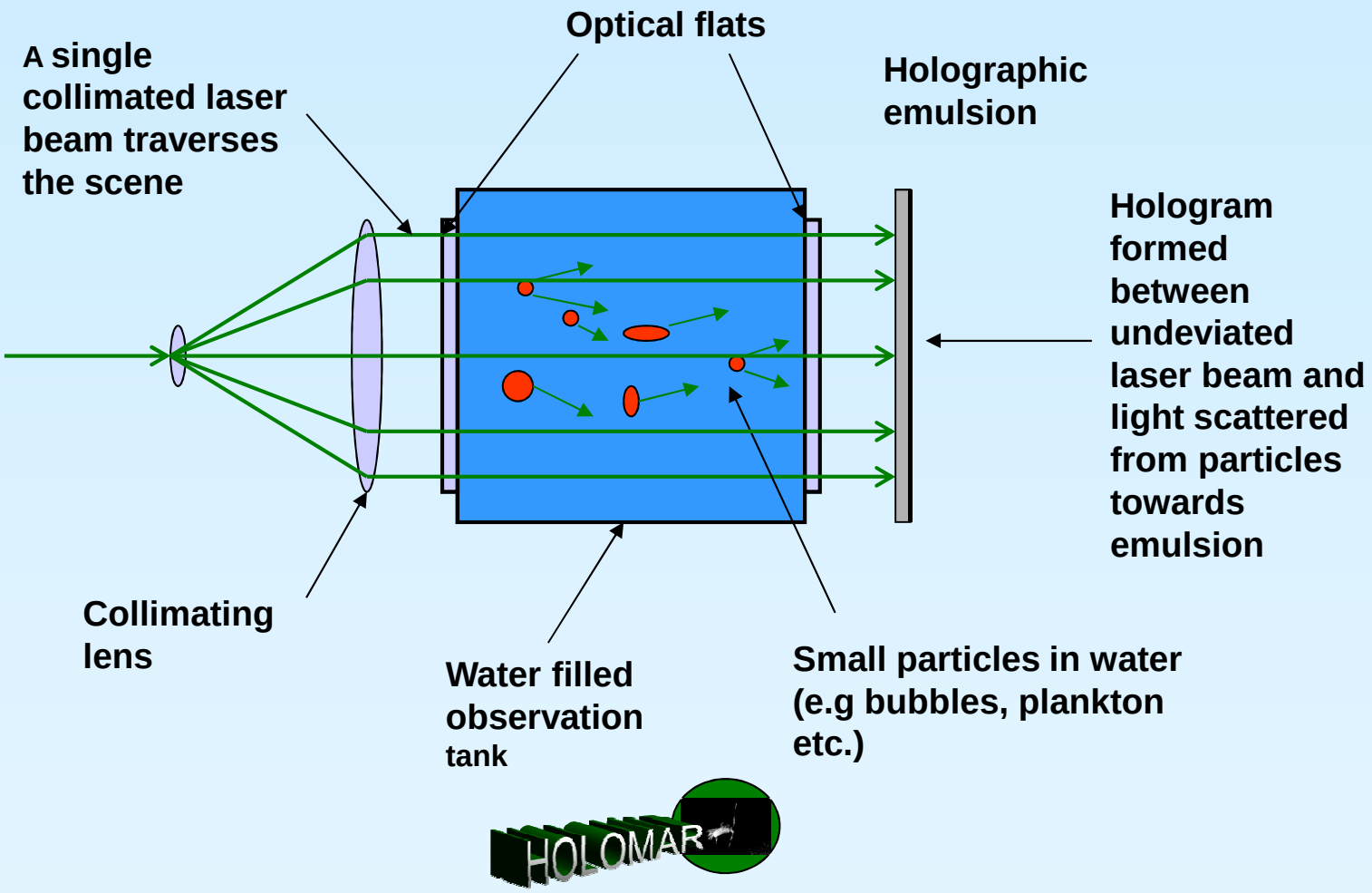
**(*An Unknown Holographer*)**

- *Holography* is a unique form of optical imaging whereby light is reflected and/or scattered from a scene & directly recorded on photographic emulsions
- A hologram records the amplitude AND phase of the incident light
  - A photograph records only the irradiance of the incident light
- A hologram is optically *indistinguishable* from the original
- A hologram provides a true 3-d image of a scene which retains the parallax and perspective of the original scene in sharp focus

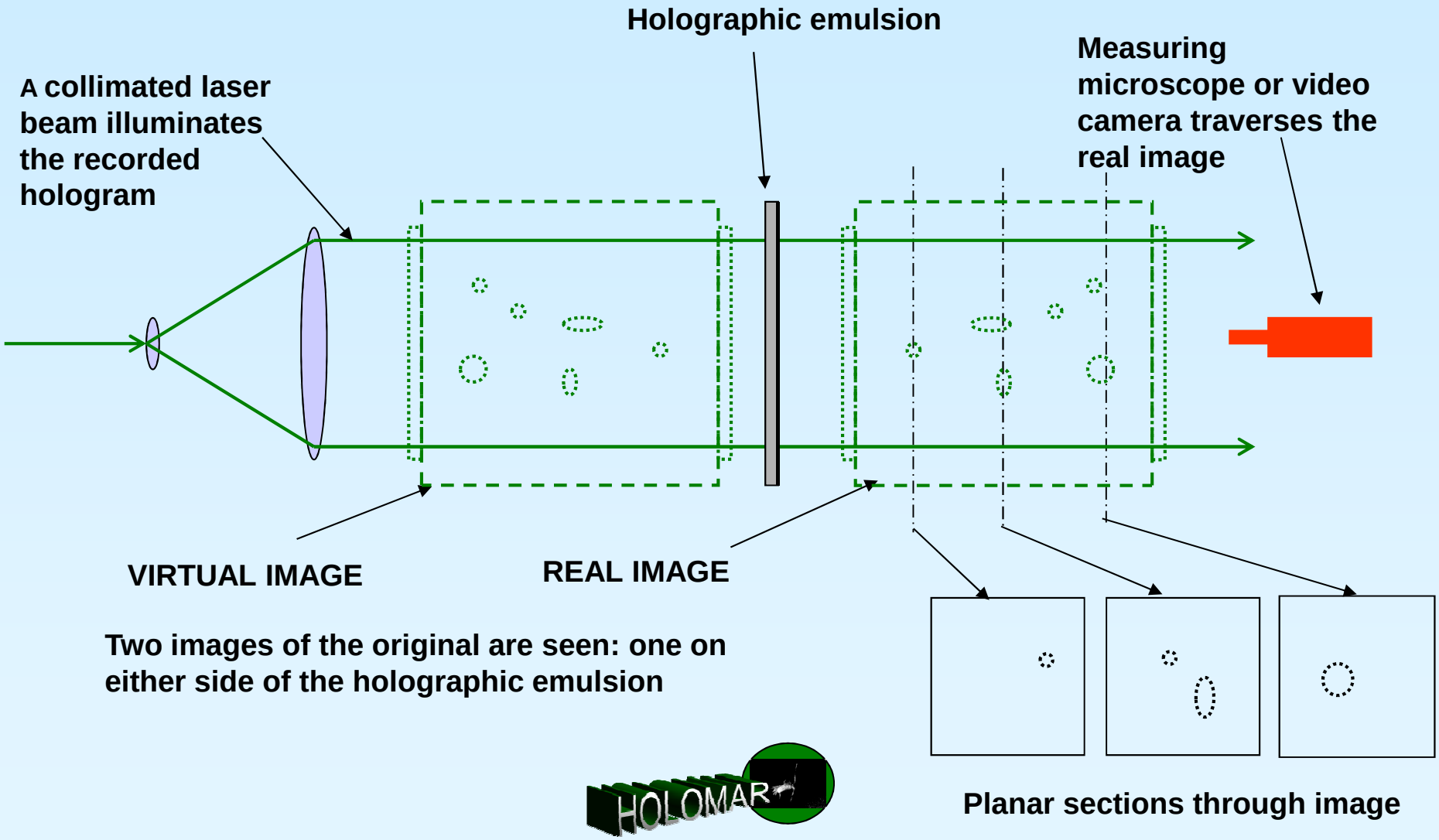
# Concepts of holography: Procedures

- **Holography is a two-step process**
  - recording of the interference pattern
  - replay of hologram produces image
  - a laser is used in recording & (usually) replay
- **Only two holographic techniques are applicable to mensuration**
  - **in-line (Fraunhofer) holography**
    - single beam recording
    - real & virtual images are on same optical axis
    - scene must be essentially transparent
  - **off-axis (Fresnel) holography**
    - two beams used in recording
    - real & virtual images are spatially separate
    - scene may be opaque
- **Two images are produced in replay**
  - virtual image
    - image located behind holographic plate (like viewing through a window)
  - real image
    - image located in space between observer & hologram
    - image is “pseudoscopic” (i.e. back-to-front & reversed left to right)

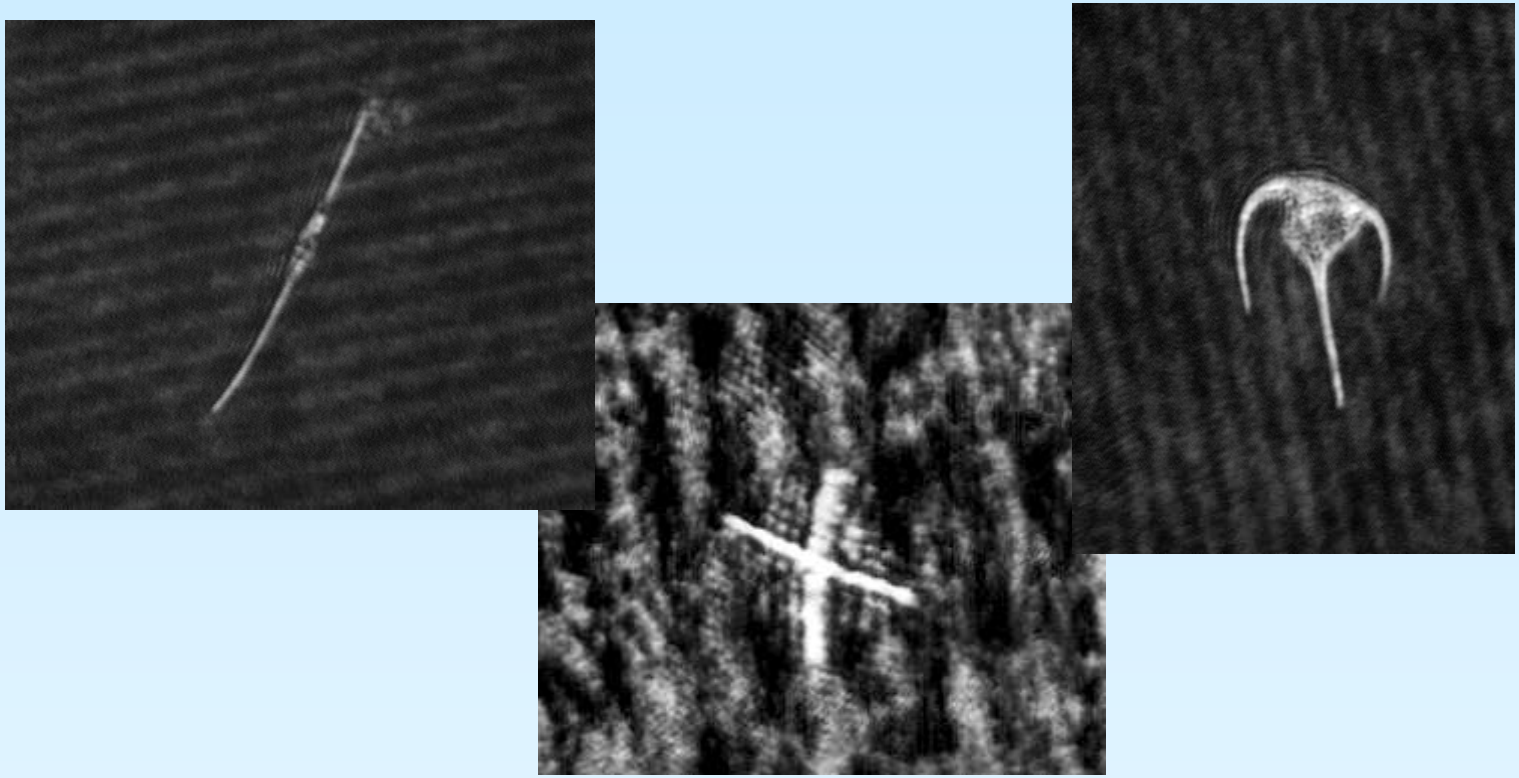
# Recording an In-line hologram



# Replaying an In-line hologram

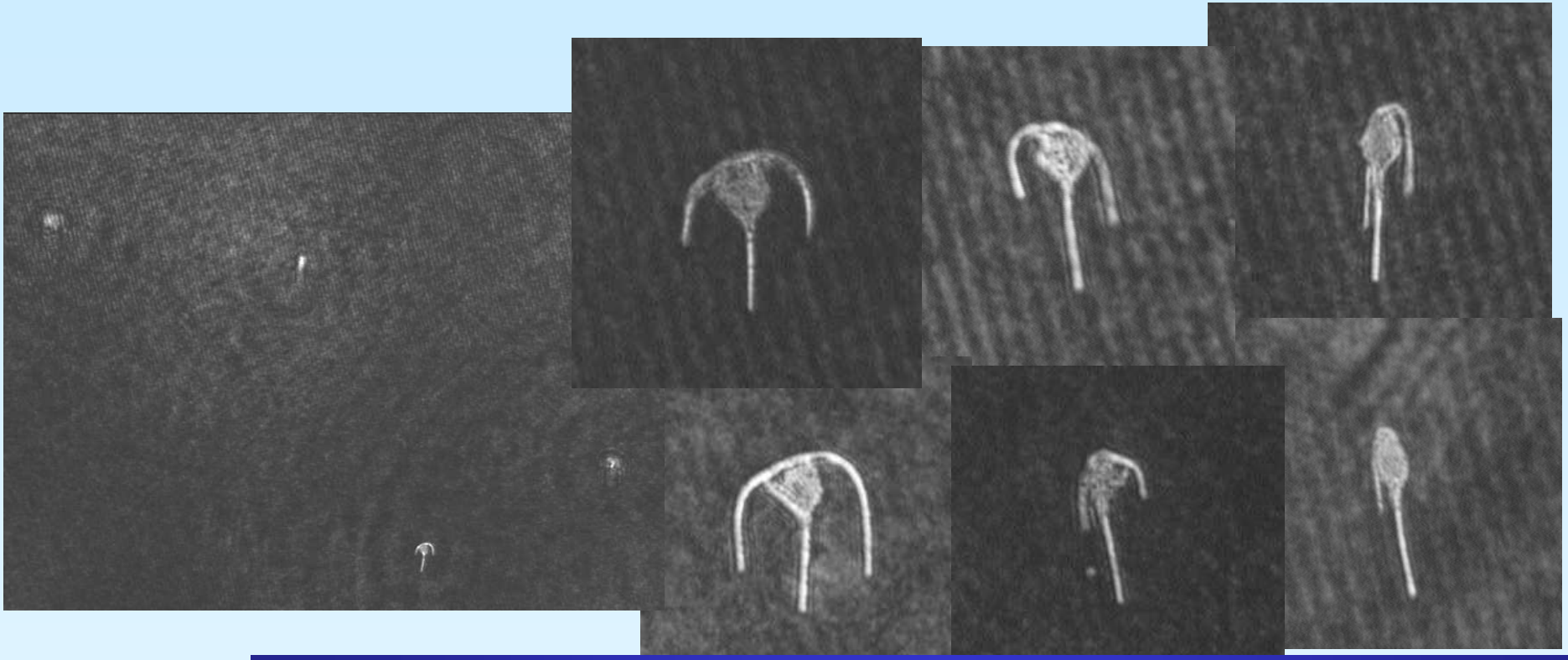


# Some marine organisms



These organisms were all recorded by in-line holography. Shown from left-to-right are a dinoflagellate (150 μm), *Asterionella formosa* (40 μm) and *Ceratium longipes* (200 μm).

# A selection of *Ceratium* images from a single in-line hologram

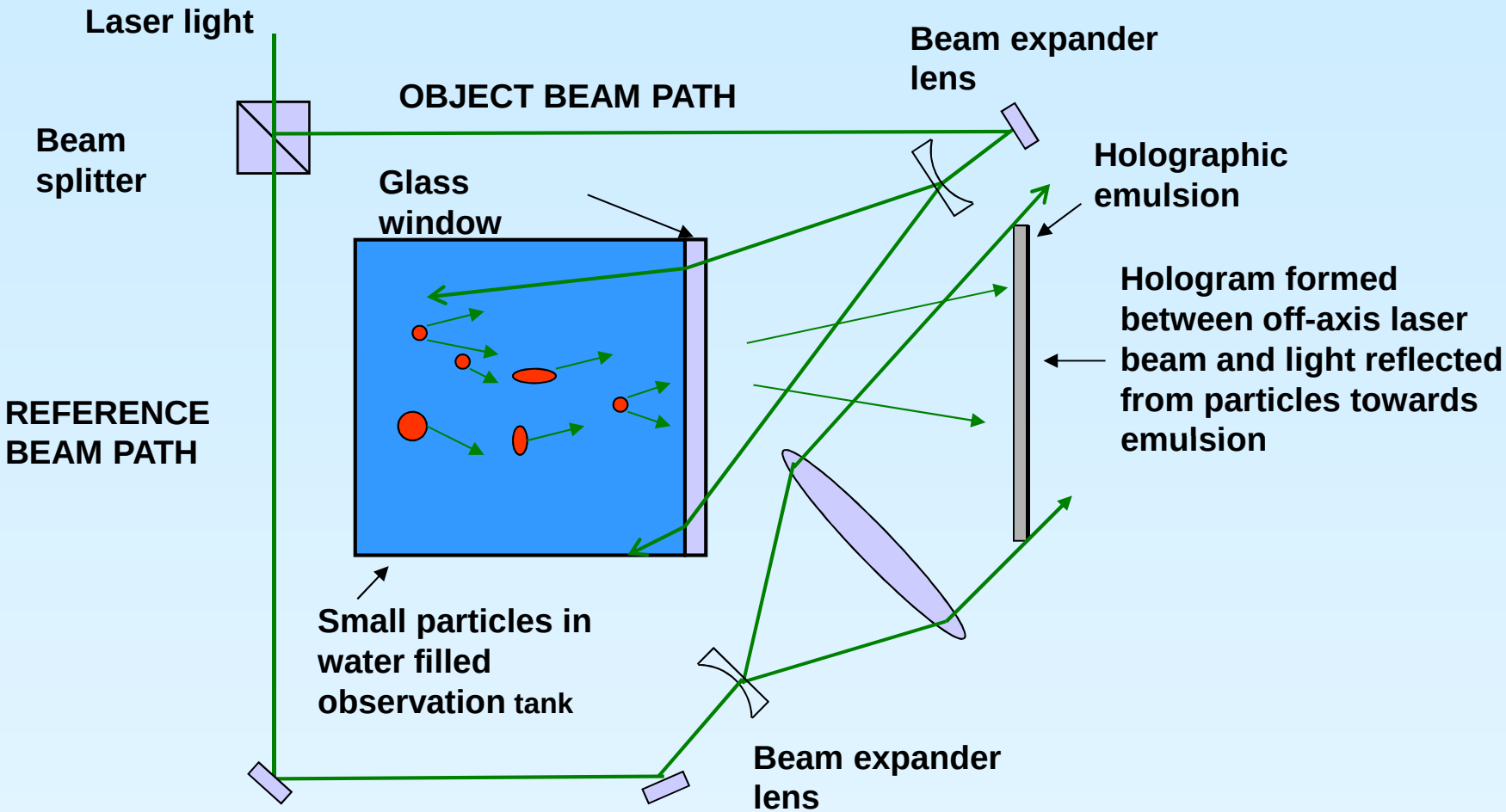


The image on the left is a 5.3 mm by 4 mm plane of an in-line hologram at low magnification. The other images, at higher magnification, show a selection of *Ceratium* organisms (approximately 200 mm long) from the same hologram. They were all recorded by in-line holography in a single exposure of a 2.5 litre volume of water. Each organism was at a different location.

# 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**
- **Recording must satisfy far-field condition**
  - $z > d^2 / \lambda$
    - where z is object-to-film distance, d is max dimension of object to be recorded, $\lambda$ 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 250 μm**
  - at concentrations up to few thousand per cubic centimetre at the smallest sizes
  - down to a few particles per $cm^3$ for larger particles

# Off-axis holography: recording

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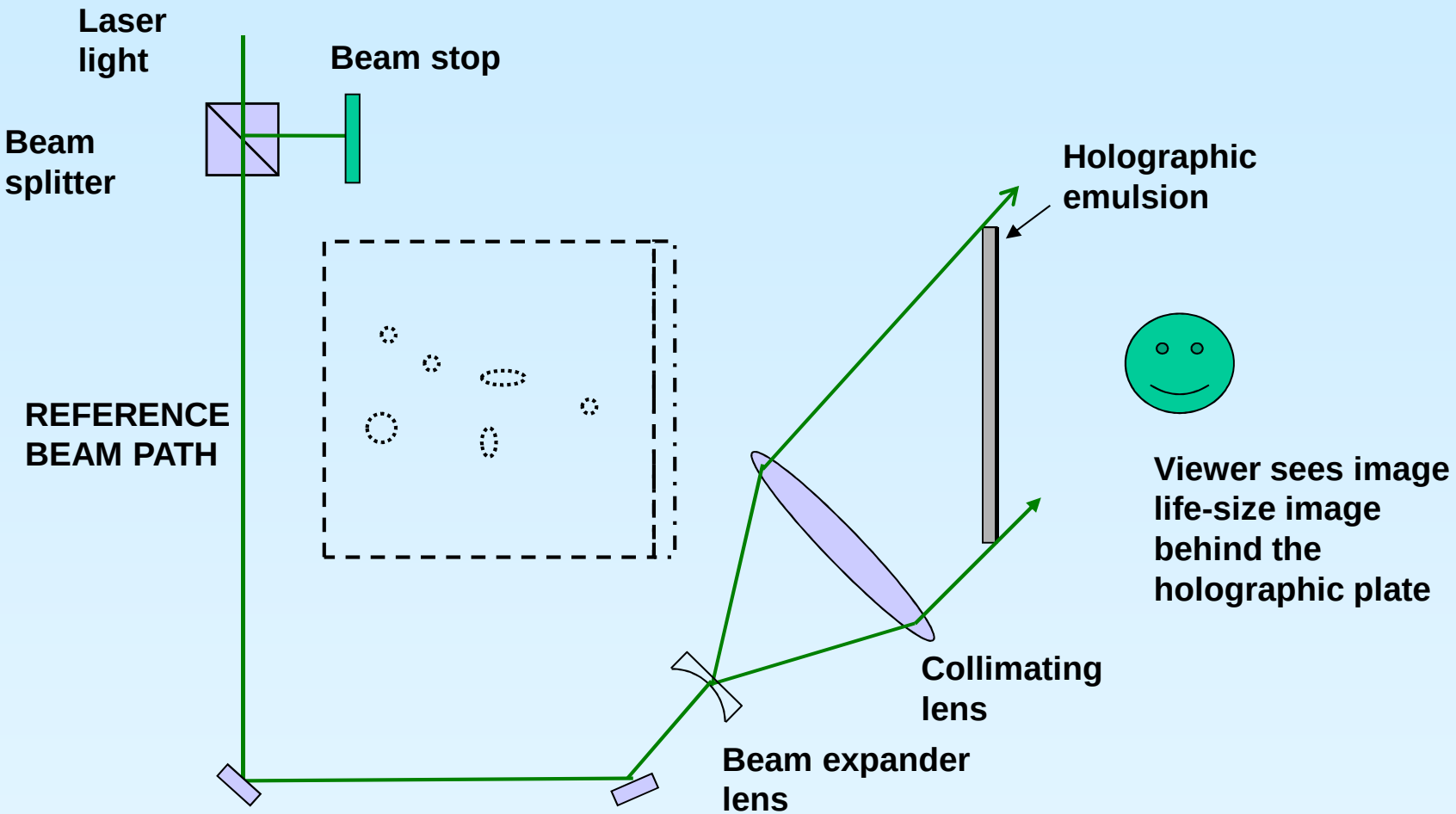
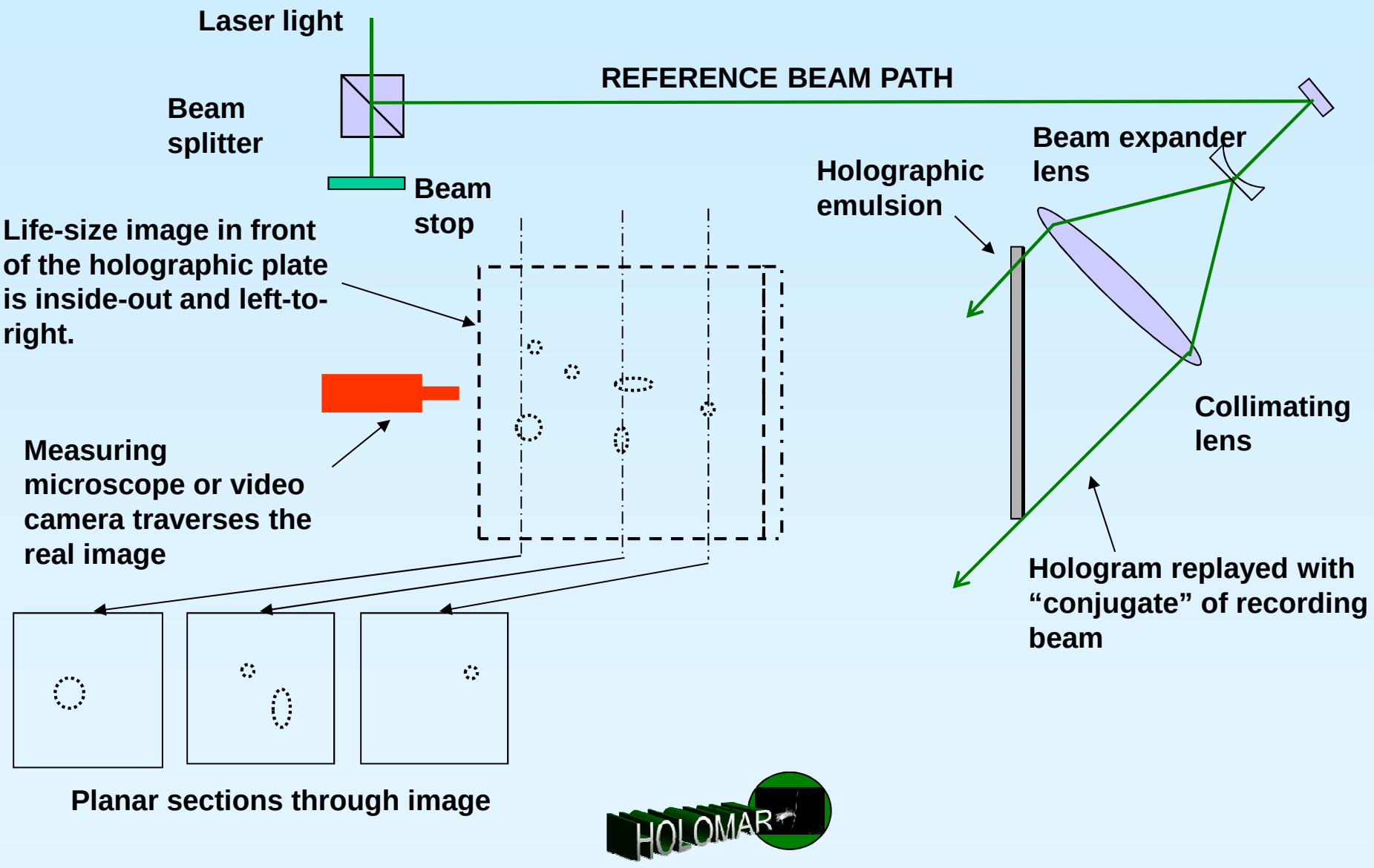
# Off-axis holography: replay of virtual image

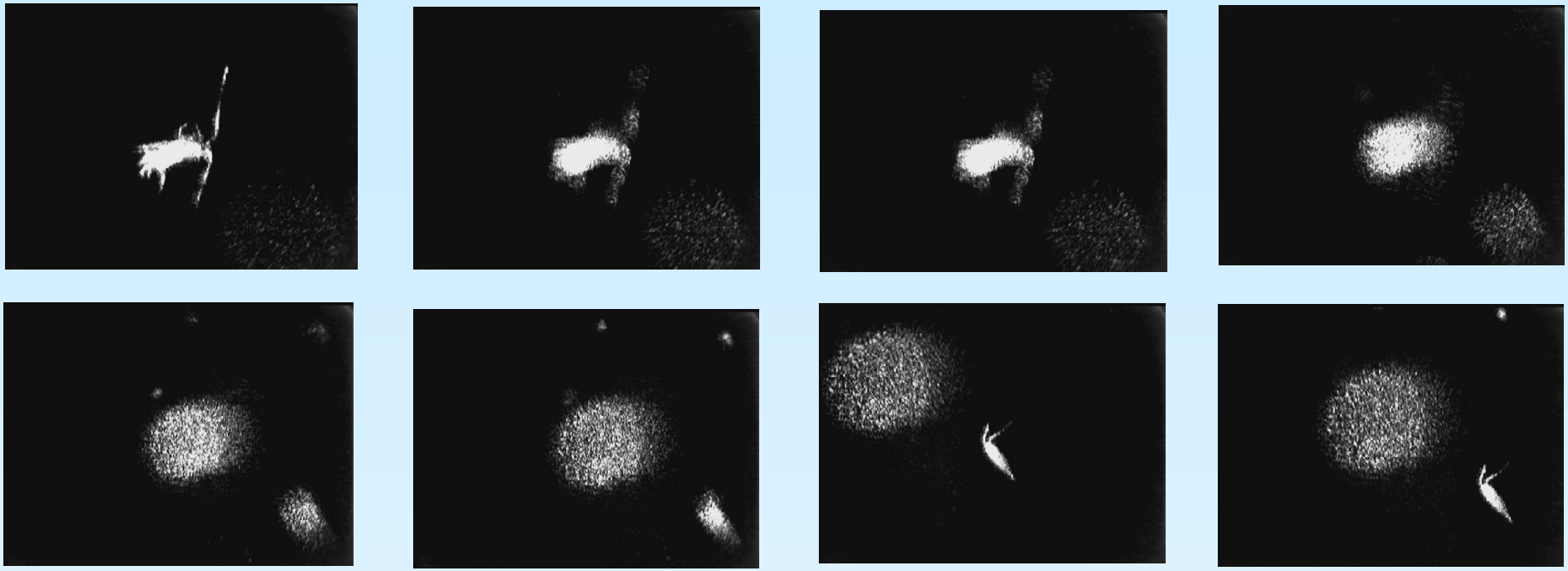



# Off-axis holography: replay of real (projected) image



# 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 organism is about 2 mm long.

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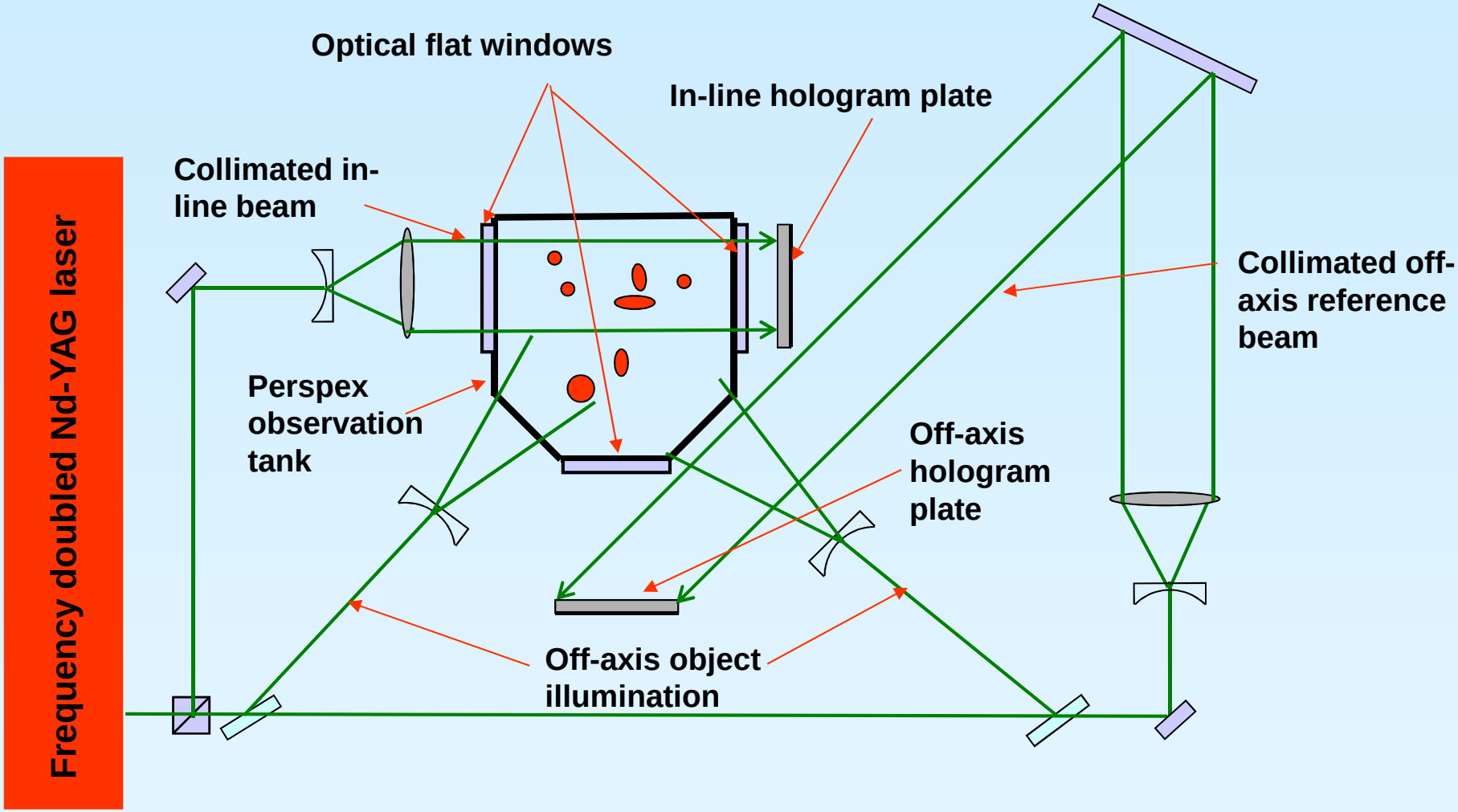
# Off-axis holography: Summary

- **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 upawrds
- **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

# In-line vs. Off-axis

| | In-line | Off-axis |
|---|---|---|
| **Hologram replay** | Fraunhofer real image | Fresnel real images |
| **Subject dimensions** | 1 μm to 1 mm | > 100 μm |
| **Subject transparency** | 95% unobscured field needed | Opaque subjects |
| **Illumination** | Single beam back lit | Multiple beam, front-lit |
| **Particle concentration** | Low concentration | Med/high concentration |
| **Optical density** | Contributes to background noise | |
| **Refractive index mismatch** | Not critical | Significant for finite field angles |

# HoloCam: laboratory layout

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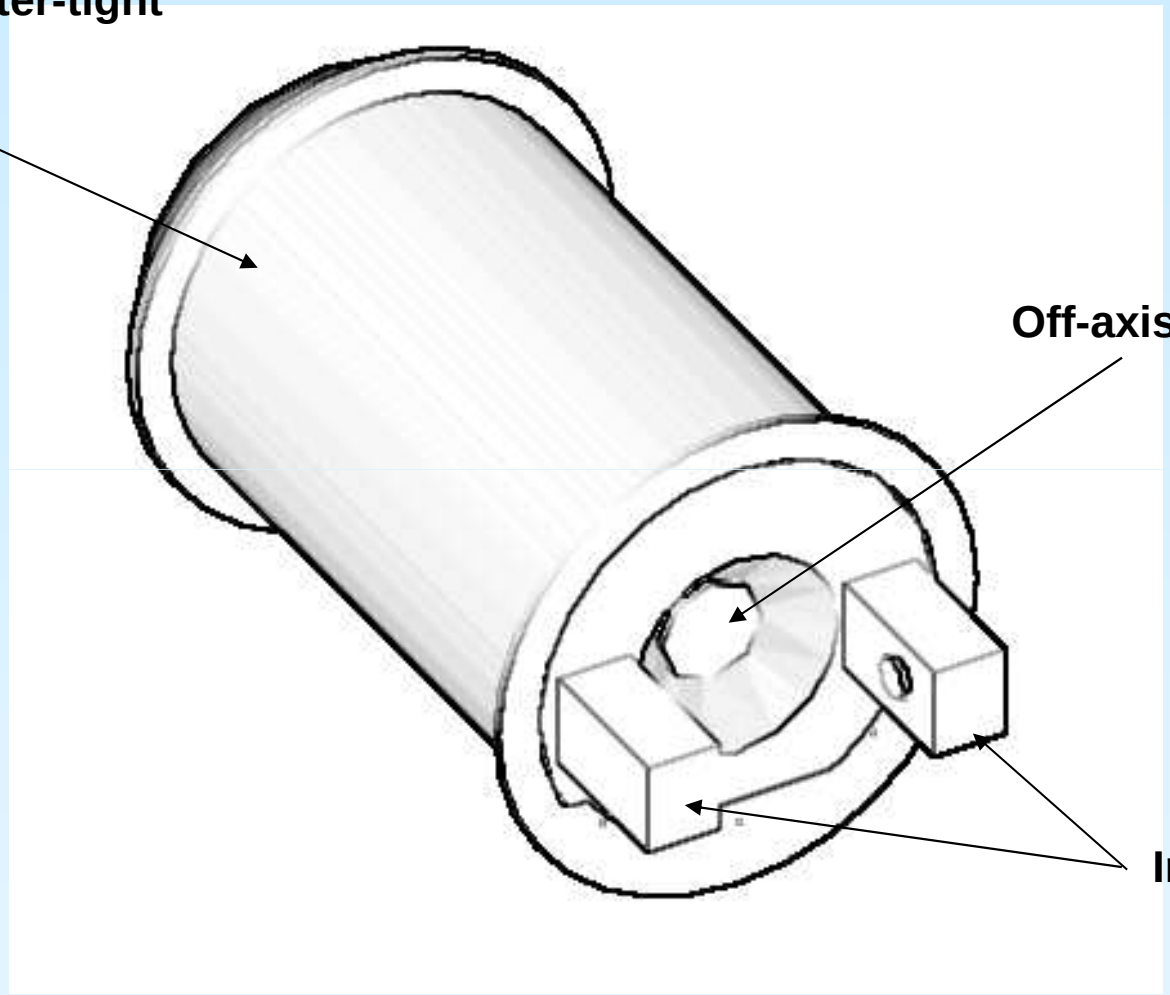
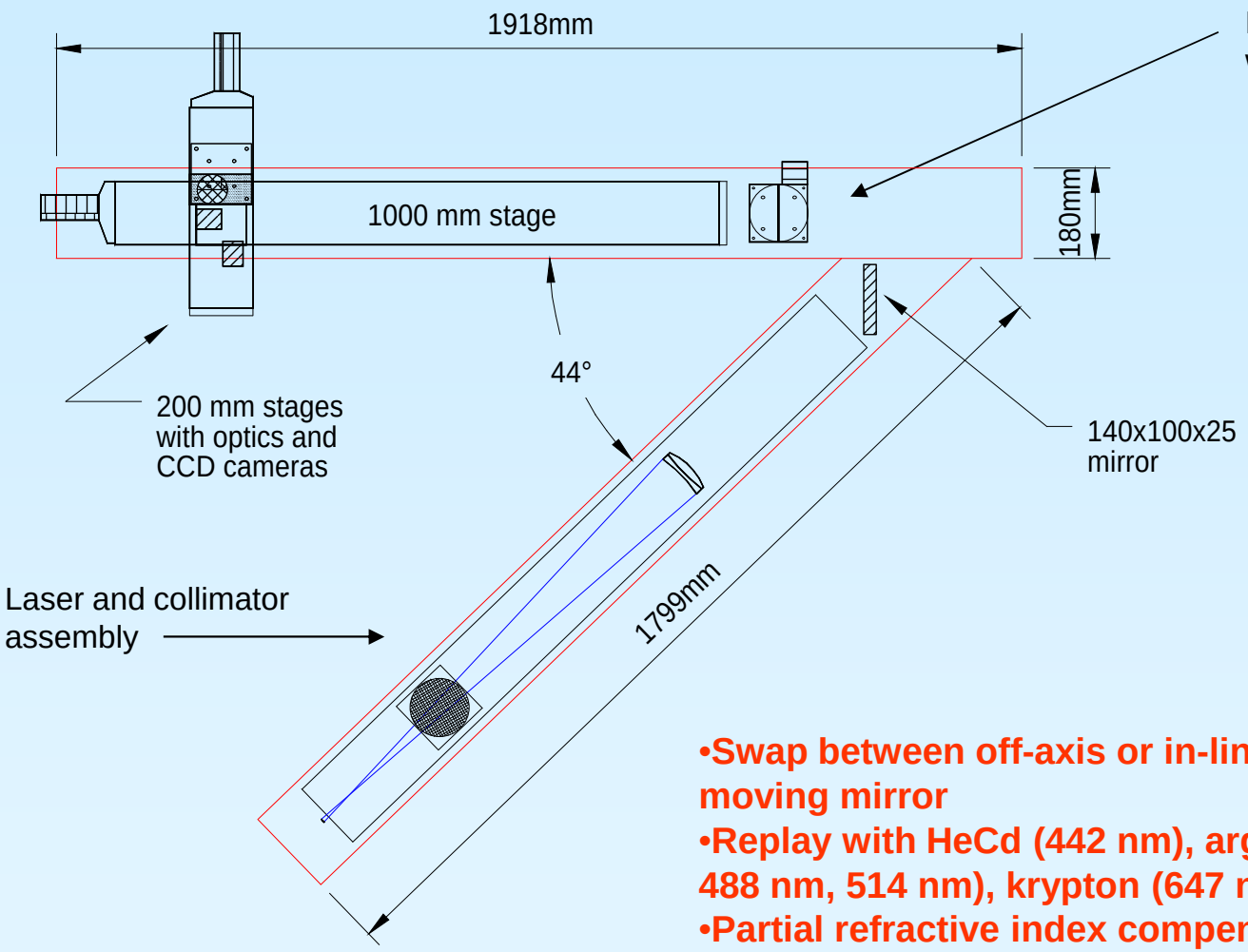
# HoloCam: Outline (3d)



# HoloCam parameters

- **Pulsed frequency doubled Nd-YAG laser**
  - pulse duration 8 ns; pulse energy  650 mJ; 532 nm wavelength (green)
- **Recording volumes up to 100 x $10^3$ $cm^3$**
- **Simultaneous recording of in-line & (front-illuminated) off-axis holograms**
  - recording of partially overlapping volumes of water
- **Operation to a depth of 100 m**
  - Ship or fixed buoy deployment
- **Up to 50 holograms will be recorded at 10 - 60 s intervals**
  - Holograms will recorded on plates
- **Microprocessor control of all holocamera functions**
- **Tilt, laser energy & other parameters monitored**
- **Water temp, pressure & salinity sensors included**

# HoloScan: Replay facility




- **Swap between off-axis or in-line holograms by moving mirror**
- **Replay with HeCd (442 nm), argon (457 nm, 488 nm, 514 nm), krypton (647 nm, 676 nm)**
- **Partial refractive index compensation for off-axis holograms**

# HoloScan Replay & Scanning parameters

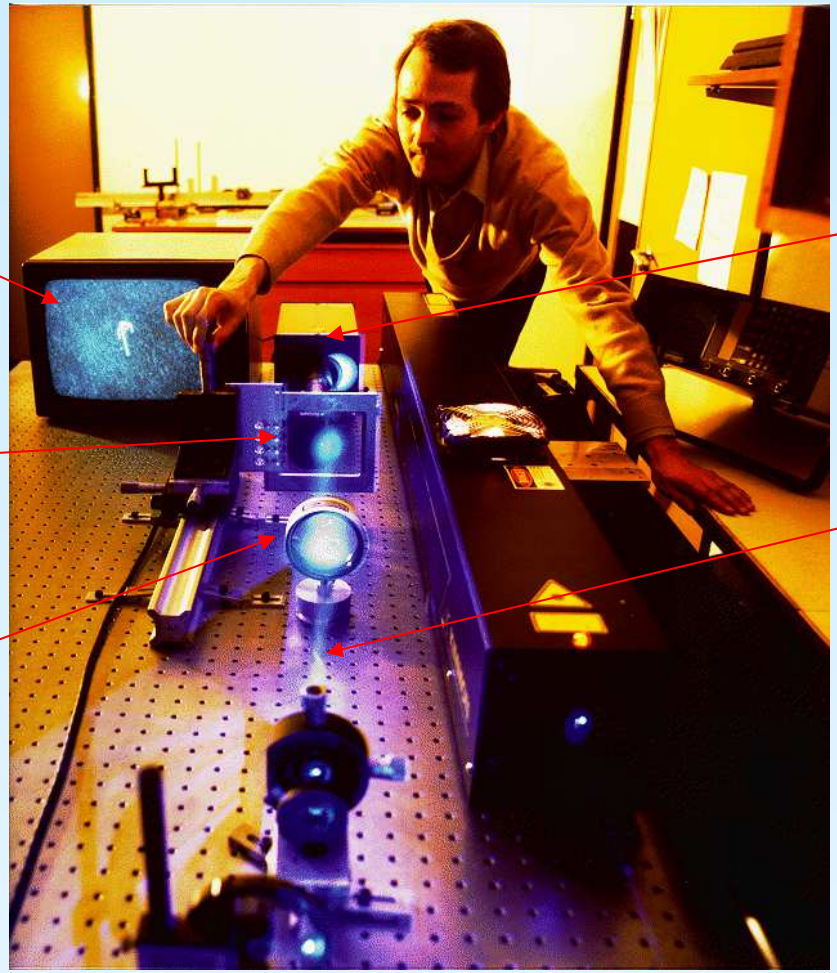
- **Replay by HeCd laser (emitting at 442 nm)**
- **Partial refractive index compensation for off-axis holograms**
- **In-line and off-axis real image replay**
  - allows either type of hologram to be interrogated in same system
- **Video camera mounted on translation stages operated by PC**
  - 2 μm steps in xyz axes
  - precise dimensional measurement of relative location of the organisms
- **Images digitised by framegrabber in control PC**
- **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
- **Large 1000×200×200 $mm^3$ scanning volume**
  - variable magnification views incorporated

# Laboratory replay of in-line hologram

Monitor showing plankton image

Multi-axis plate holder

Collimating lens



Camera mounted on xyz stage views real image

Reconstruction laser beam (HeCd 442 nm)

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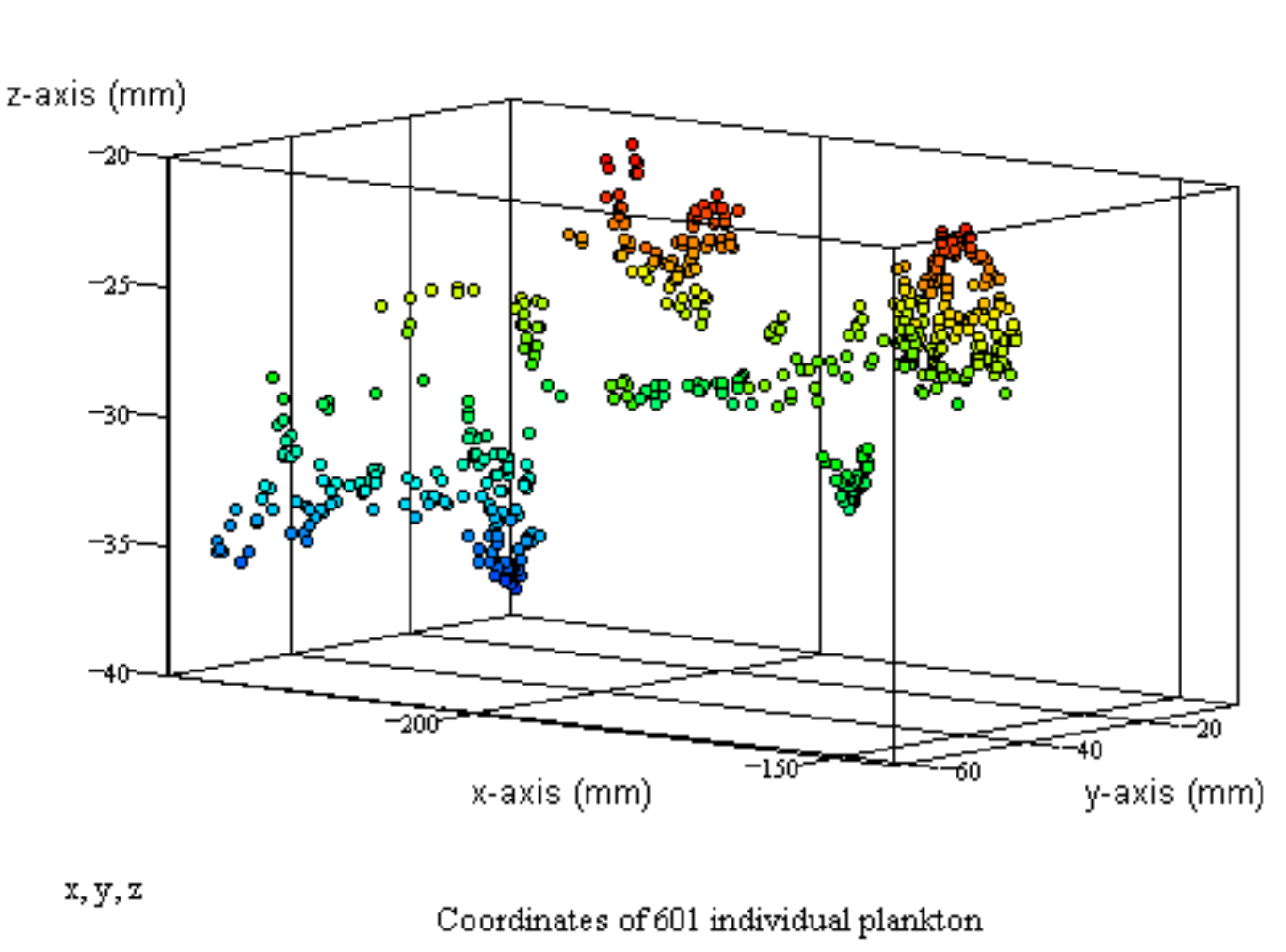
# Plankton co-ordinates: *Asterionella formosa* (live)
## (freshwater species)

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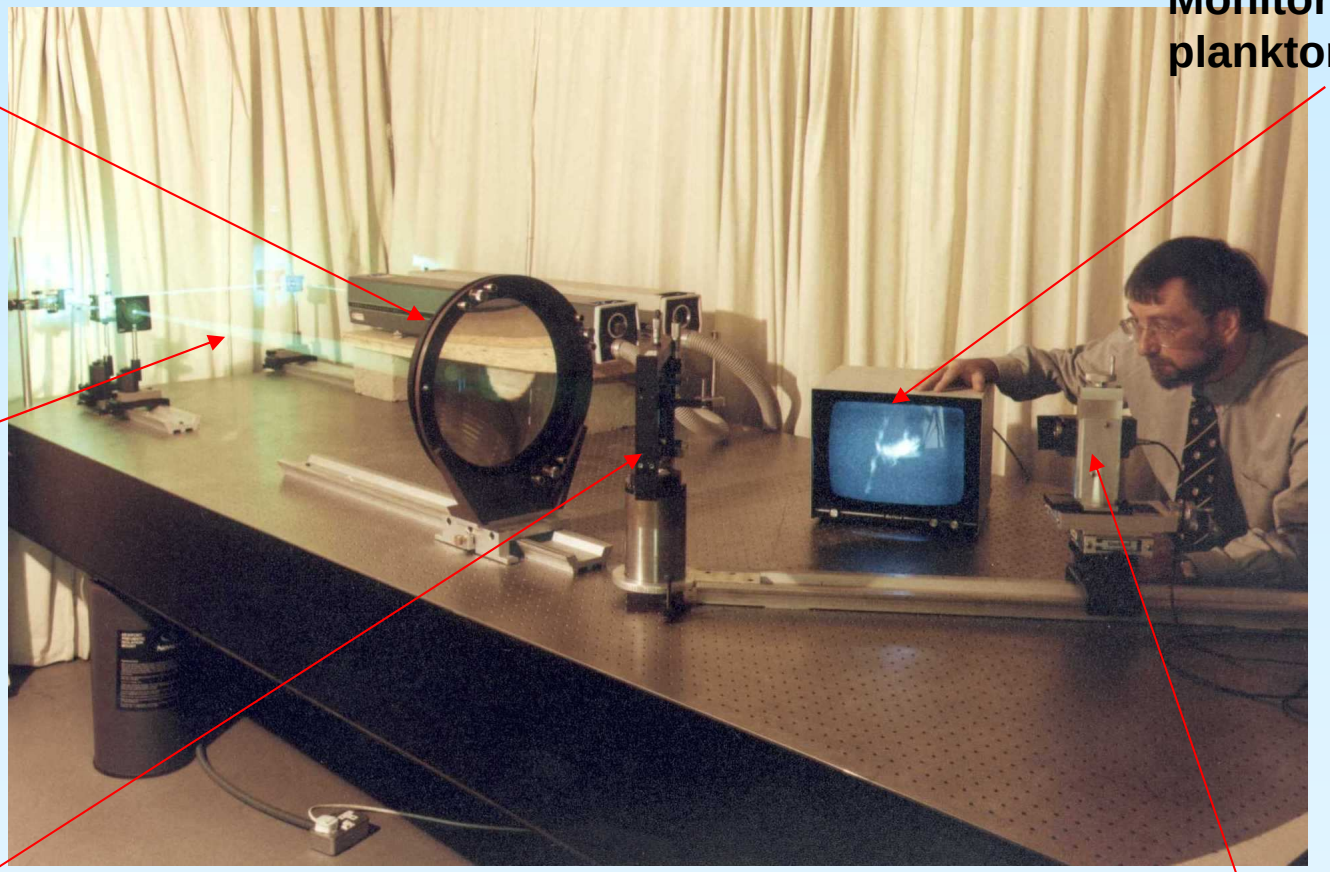
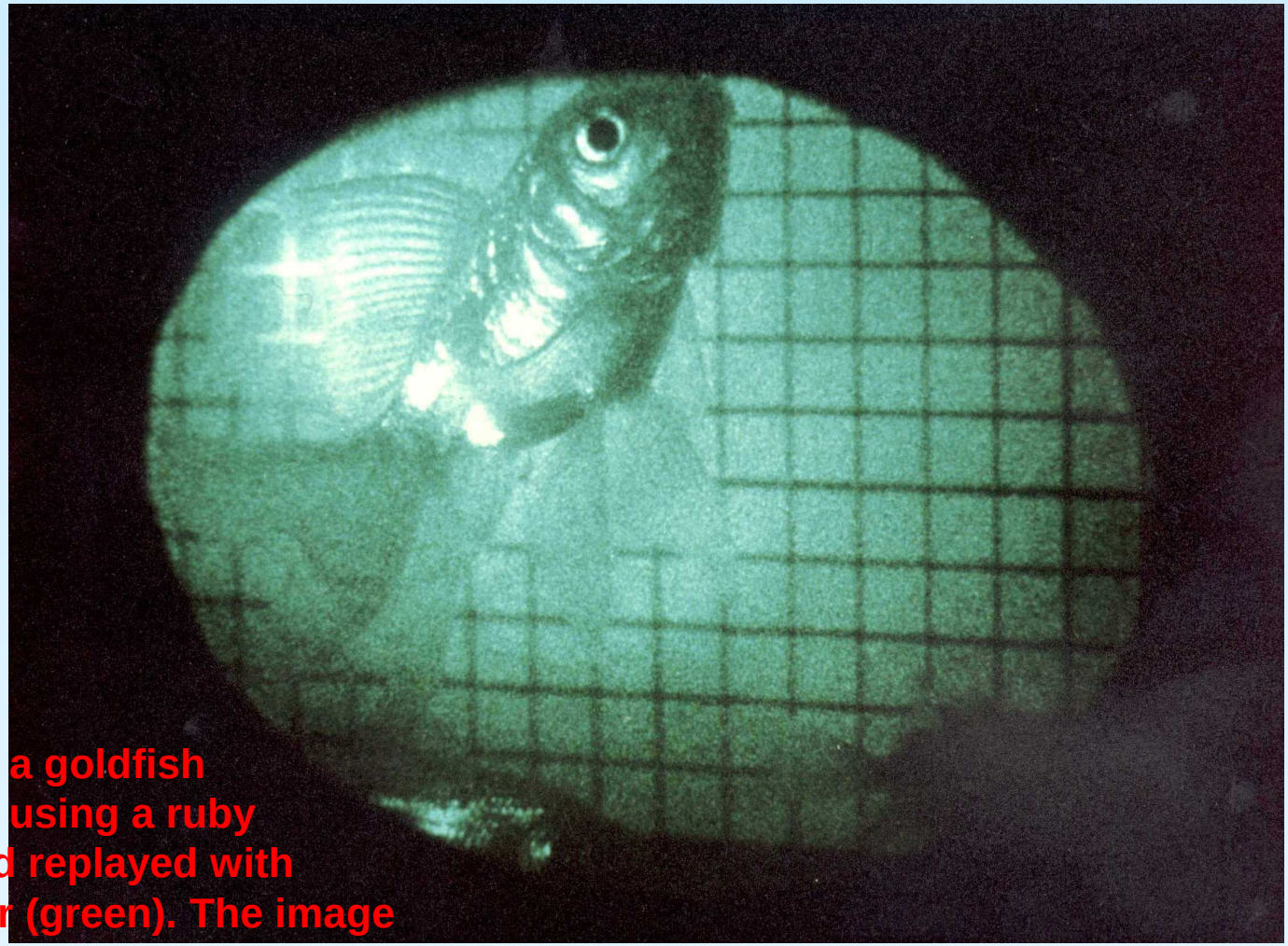
# Laboratory replay of off-axis hologram



# A green goldfish!



**This image of a goldfish was recorded using a ruby laser (red) and replayed with an argon laser (green). The image shows minimal aberrations.**

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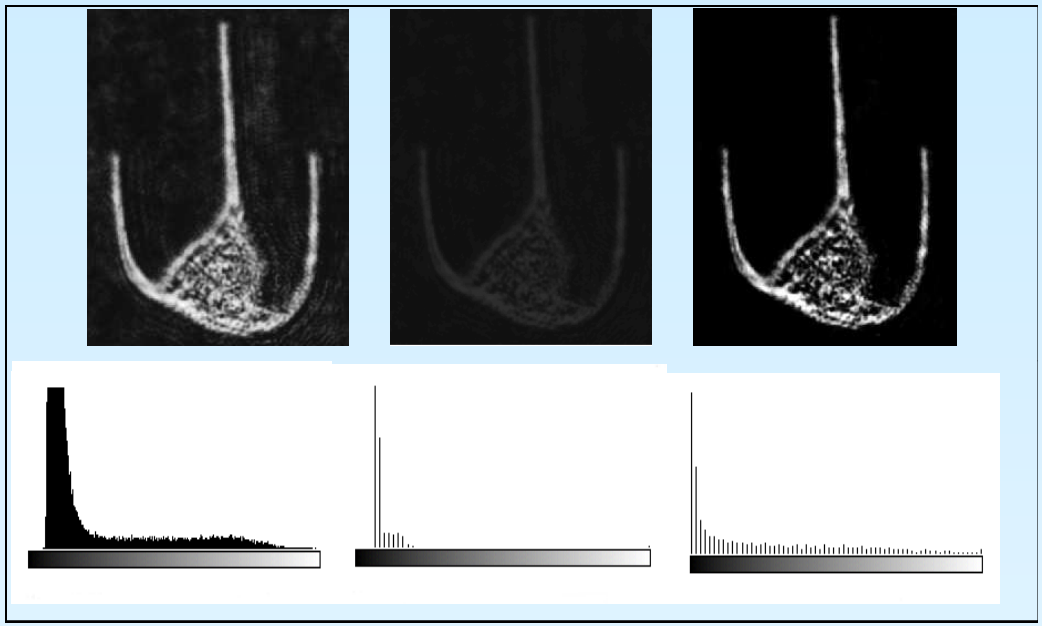
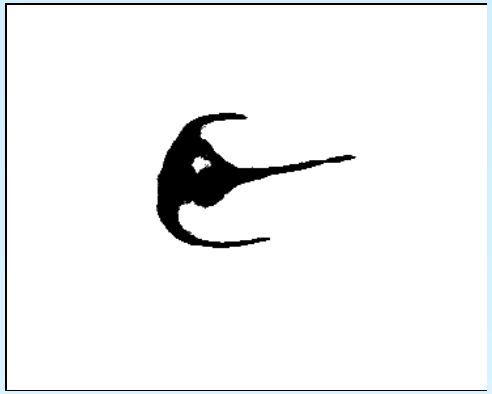
# Data acquisition & image processing

- **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**

# Image enhancement & species identification



Original, cropped & stretched images with relative frequency distributions



Filtered, segmented & pre-processed image prior to identification by neural net techniques

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# Applications of hologrammetry

- **High-resolution imaging & measurement in hazardous or inaccessible environments  environments**
- **Offshore inspection**
  - archiving, corrosion pitting, damage, dimensional measurement
- **Nuclear fuel inspection**
  - inspection of fuel assemblies in cooling ponds & PIE caves
- **Bubble chamber diagnostics**
  - analysis of nuclear particle tracks
- **Combustion processes & liquid atomisation**
  - water droplets, clouds, aerosols
- **Marine life, organisms, bubble fields**
  - meaurement & identification of plankton
- **3d micrography of human eye**
  - analysis of defects in eyes

# The Holographic Data Problem

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

(*An Unknown Holographer)*

- Our in-line sample volume is 100 mm diameter and 1 m long
- The *low*-magnification camera has a field of view about 9 by 6 mm
- That's around 200 images for each slice
- Even assuming a slice every 0.1 mm (bigger than many objects)
  - **2 million images in each holographic plate**

# The Holographic Data Problem

**Manual or semi-automatic analysis generally takes about a person-week for each holographic exposure**

- Brown (ice crystals in clouds) - “a few hours” for a 150 $cm^3$ sample volume
- Borrmann & Jaenicke (snow / fog) - 32 hours / hologram: 8 $cm^3$ and 1000 objects
- Vössing *et al*. (snow / fog) - up to 70 hours for 1 hologram of a 500*l* volume
- Katz *et al*. (plankton) - two weeks for each hologram of 300 to 2000 $cm^3$

**The main issue is operator fatigue leading to measurement errors**

## Automated analysis is needed ...

# The Holographic Data Problem

**... but still a challenge**

At *high* magnification (a 1 mm by 0.7 mm view), one plate can generate **58 *Tera*Bytes** of raw data

- **Need to extract information, not data**
- **How does one characterise the 3-d, projected real image ?**
  - **e.g. brightness and contrast: how to find the brightest and darkest voxels in that 58 Tb?**
  - **Real image properties both fixed in plate and depend on replay laser and viewing camera**