Digital In-line Holographic Microscopy of marine microorganisms in multi-media environment

G.H. Sendra[1*], S. Weiße[1], M. Heydt[1], M.E. Callow[2], J.A. Callow[2], M. Grunze[1,3], A. Rosenhahn[1,3]

1 Applied Physical Chemistry, Ruprecht-Karls-University Heidelberg, Im Neuenheimer Feld 253, D-69120 Heidelberg, Germany
2 School of Biosciences, University of Birmingham, B15 2TT Birmingham, United Kingdom
3 Institute of Functional Interfaces, Karlsruhe Institute of Technology, PO Box 3640, D-76021 Karlsruhe, Germany

*E-mail: sendra@uni-heidelberg.de

Digital In-line Holographic Microscopy (DIHM) has shown to be a suitable technique for tracking motile marine microorganisms. It is a transmission technique that uses a spherical coherent source to illuminate the sample and the optical field intensity is registered by a camera without any lens (free propagation). When light has to go across media with different refractive indices, the wavefront is distorted and the light that reaches the detector does not correspond to the original spherical wave. The impact of media before and after the sample was analyzed and a correction method was developed for the reconstruction process. This new approach was then tested not only in controlled experiments but also in the analysis of the exploratory behavior of motile spores of the marine fouling alga, *Ulva linza*, on surfaces with different chemical termination.

Digital In-line Holographic Microscopy

A NEW MICROSCOPIC PRINCIPLE [1]

By Dr. D. GABOR
Research Laboratory, British Thomson-Houston Co., Ltd., Rugby

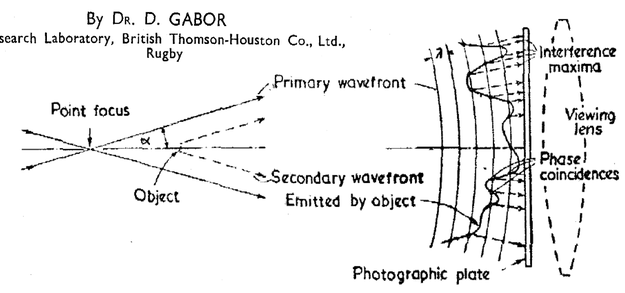
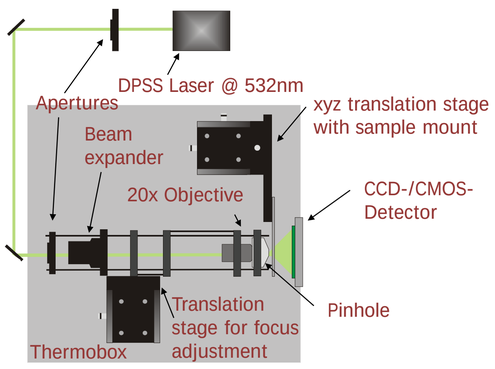


Fig. 1. INTERFERENCE BETWEEN HOMOCENTRIC ILLUMINATING WAVE AND THE SECONDARY WAVE EMITTED BY A SMALL OBJECT

Schematic drawing of the setup:



Intensity distribution [2]:

$$I(\vec{r}) = |\psi_0(\vec{r})|^2 + \psi_0^*(\vec{r})\psi_S(\vec{r}) + \psi_0(\vec{r})\psi_S^*(\vec{r}) + |\psi_S(\vec{r})|^2$$

Source — Hologram — Twin image — Self interference

Reconstruction [2]:

$$K(\vec{r}) = \iint_S I(\vec{\xi}) \exp\left(i\frac{k\vec{\xi}\vec{r}}{\xi}\right) d^2\xi$$

Phase information preserved

Resolution [2]:

$$\delta_{lateral} = \frac{0.61\lambda}{NA} \qquad \delta_{depth} = \frac{\lambda}{NA^2} \qquad NA \approx \frac{a}{2L}$$

Angular spectra propagation [4]

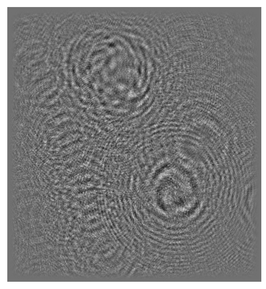
$$\psi_z(x,y) \approx F^{-1}\{H \cdot F\{I - I_0\}\} \qquad H(f_x, f_y) = e^{i2\pi\frac{\bar{z}}{\lambda}\sqrt{1-(\lambda f_x)^2-(\lambda f_y)^2}}$$

Reconstruction implemented by Repetto et al [5]

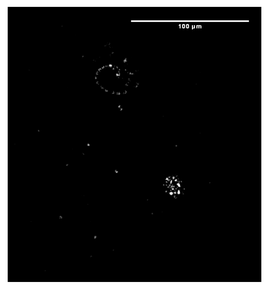
$$\sqrt{f_x^2 + f_y^2} < \lambda^{-1}$$

Series of recorded holograms and reconstructed images [3]:

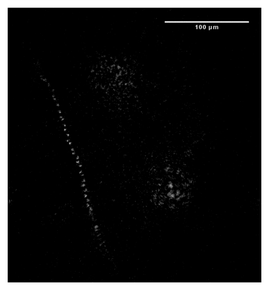
a) Accumulated hologram consisting of 50 single frames



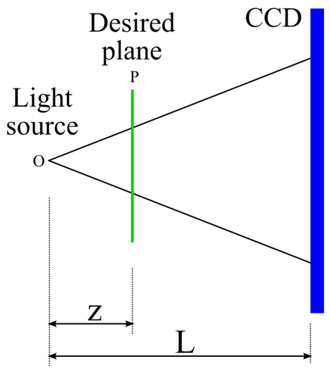
b) Reconstruction of the hologram (a), 1300 µm above the pinhole



c) Reconstruction of the hologram (a), 1830 µm above the pinhole



Reconstruction in multi-media environment

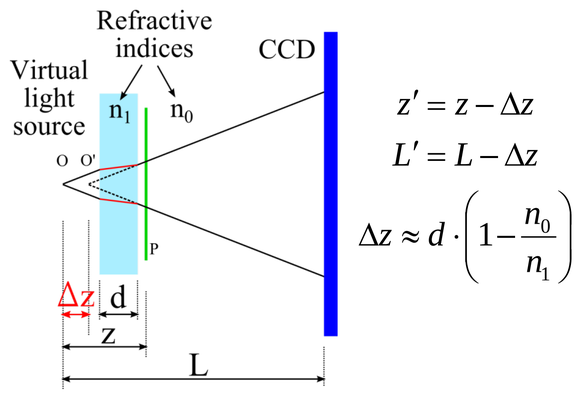


Reconstruction distance

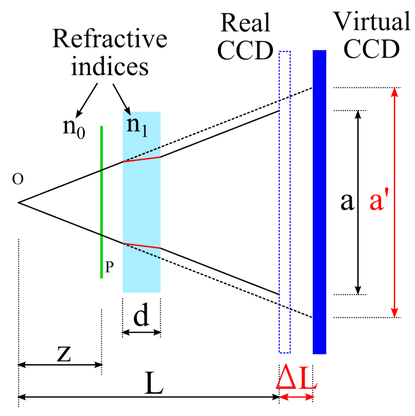
$$\bar{z} = \frac{L}{z}(L - z)$$

Lateral magnification:

$$M = \frac{L}{z}$$



$$z' = z - \Delta z$$
$$L' = L - \Delta z$$
$$\Delta z \approx d \cdot \left(1 - \frac{n_0}{n_1}\right)$$



$$L' = L + \Delta L$$
$$\rightarrow \quad \Delta L \approx d \cdot \left(\frac{n_1}{n_0} - 1\right)$$
$$a' = K \cdot a$$
$$\rightarrow \quad K \approx \frac{L + \Delta L}{L - d\left(1 - \frac{n_0}{n_1}\right)}$$

Calibration

Samples for the calibration measurements were standard glass slides of 20 x 60 x 1 mm (Carl Roth GmbH + Co. KG, Karlsruhe, Germany) and ibidi Luer I 0.8 plastic cuvettes (Ibidi Biodiagnostics, Martinsried, Germany), sputtered with 20 nm thick Chromium lines on their upper and lower surfaces respectively. Masks of two different dimensions were used, namely 10 x 3000 µm and 25 x 3000 µm. The lines were sputtered in a relative orientation of 90°between them, forming a cross in the axial projection, in order to have distinguishable front and back marks.

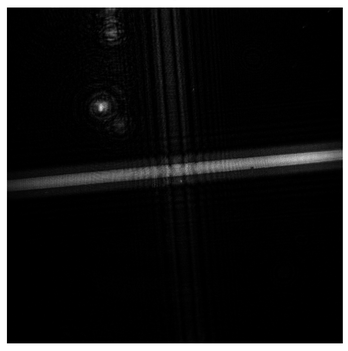
Evaluation of sample displacement

Sample displacement (µm)	Δz_1 (µm)	Δd (µm)
-250±10	20	10
+250±10	5	5
+500±10	5	20
+750±10	20	10

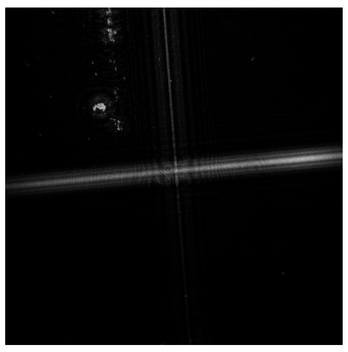
Evaluation of variation in the refractive index

Sample refractive index	z_1 (µm)	d (µm)
1.33	475±5	1970±5
1.47	475±5	1975±5

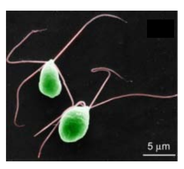
First plane



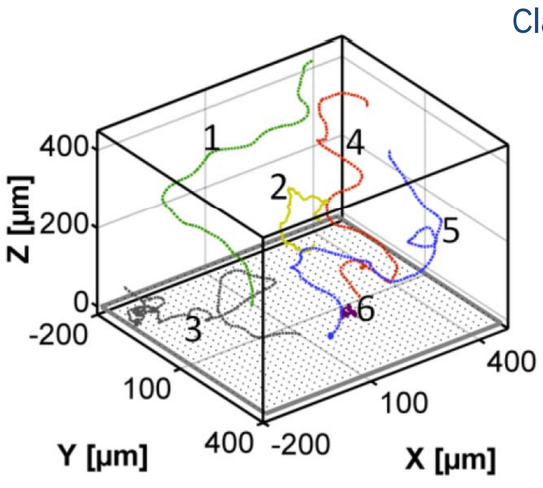
Second plane

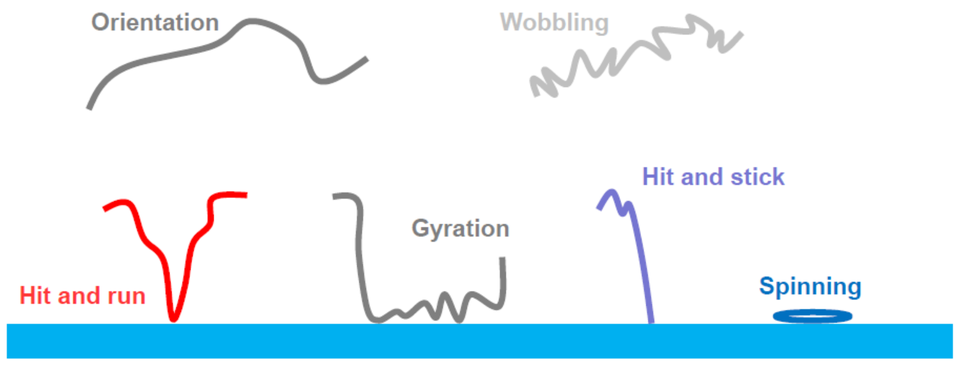


Application to marine microorganisms: *Ulva linza*



Classification of trajectories [3]





Motion patterns within 1 Minute after injection

Further analysis are being done to these and other surfaces such as EG_1OH, EG_6OH, including studies on the effect of preconditioning and preincubation of the surfaces, changes in velocities and evolution in time

Literature

[1] Gabor, D. (1948). "A new microscopic principle." Nature **161**: 2.
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[3] Heydt, M., A. Rosenhahn, et al. (2007). "Digital in-line holography as a three-dimensional tool to study motile marine organisms during their exploration of surfaces." Journal of Adhesion **83**(5)**:** 417-430.
[4] Goodman, J.W. (1968) "Introduction to Fourier Optics" (McGraw-Hill, New York, 1968), Chap. 8.
[5] Repetto, L., E. Piano & C. Pontiggia (2004). "Lenless digital holographic microscope with light-emitting diode illumination." Optics Letters **29**(10): 1132-1134 .