Techniques for 3D tracking of marine microorganisms during surface exploration

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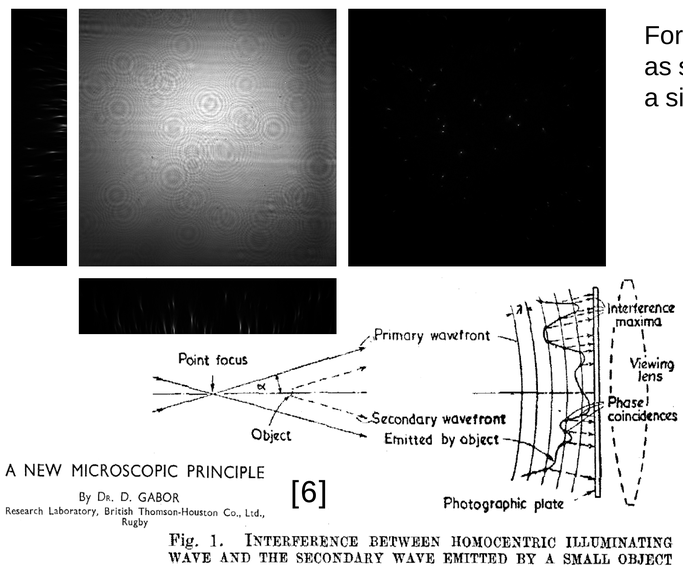
When microorganisms approach a surface, they change their exploratory behavior depending on the chemical, physical, biological, and topographical properties encountered. The analysis and understanding of the exploration movements allows to determine the immediate response of the microorganisms and thus directly impact the design of new fouling resistant surfaces. Because of the dynamic and three-dimensional nature of the settlement motions of microorganisms, it is difficult to use conventional light microscopy for their investigation. The application of three dimensional measurement methods produces data with high resolution in the temporal and spatial direction. In the presented work we show a comparison and evaluation of two different techniques for tracking of microorganisms depending on their size: digital in-line holographic microscopy (DIHM) [1] and stereoscopic imaging (SI) [2]. The holographic approach has proven to be stable and robust for microorganism sizes smaller than 20 µm, while the stereoscopic method is suitable for microorganisms larger than 200 µm. The corresponding setups are described and compared with respect to their: applicability under real life conditions, minimal and maximal resolution, complexity of reconstruction algorithms, resistance to noise, and accuracy of the measurement results. Emphasis will be on the calibration procedure, as the presence of water as a medium with different refractive index than air needs to be considered. The image processing algorithms for obtaining the position in 3D coordinates are also evaluated [3, 4]. After obtaining the cell positions, an automatic tracking algorithm is applied to obtain the trajectories [5]. From such traces, the changes in the exploratory behavior of the microorganisms depending on the chemistry of the surface are quantitatively analyzed. Both techniques were tested with different marine fouling microorganisms: spores of the green algae for digital in-line microscopy and barnacle cyprids for stereoscopic imaging.

Digital In-line Holographic Microscopy

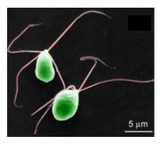


A NEW MICROSCOPIC PRINCIPLE
By Dr. D. GABOR
Research Laboratory, British Thomson-Houston Co., Ltd., Rugby

[6]

Fig. 1. Interference between homocentric illuminating wave and the secondary wave emitted by a small object

For tracking microorganisms such as spores of *Ulva linza*, that have a size of approximately 5 µm



Intensity distribution [7]:

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

Source | Hologram | Twin image | Self interference

Reconstruction [7]:

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

Optical resolution [7]:

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

Angular spectra propagation [8]

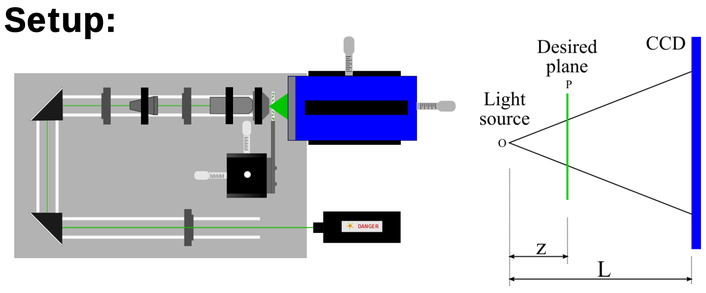
$$\psi_z(x,y) \approx F^{-1}\{H \cdot F\{I - I_0\}\}$$

$$H(f_x, f_y) = e^{i2\pi\frac{\bar{z}}{\lambda}\sqrt{1-(\lambda f_x)^2-(\lambda f_y)^2}} \qquad \sqrt{f_x^2 + f_y^2} < \lambda^{-1}$$

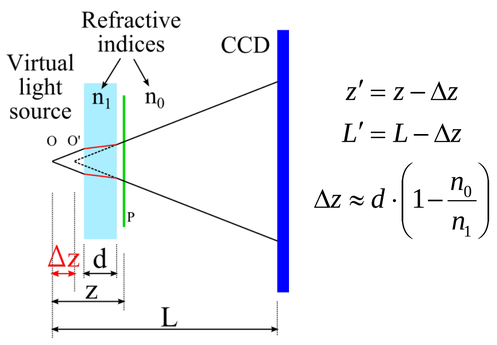
Position and magnification [3]

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

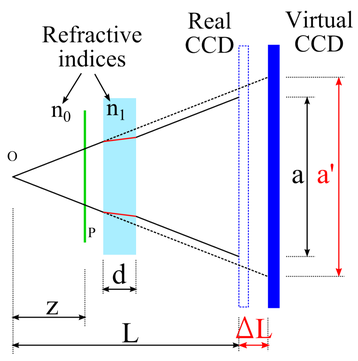
Setup:



The multi-media environment:



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

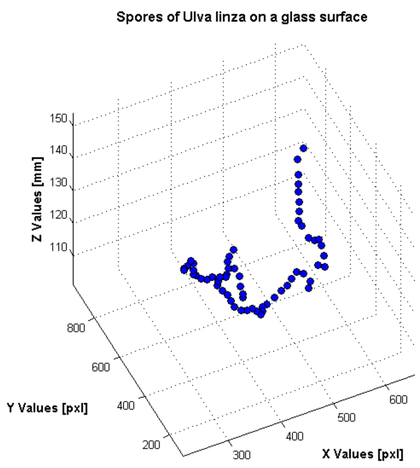


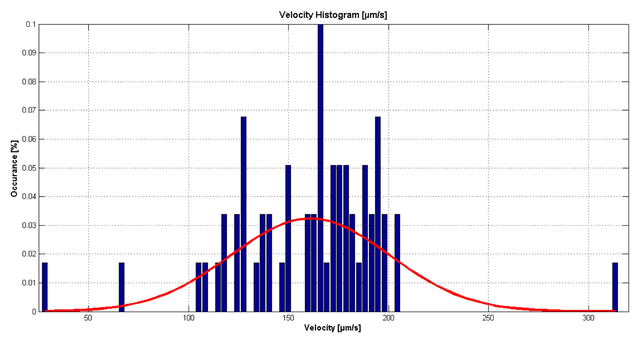
$$L' = L + \Delta L$$
$$\rightarrow \quad \Delta L \approx d \cdot \left(\frac{n_1}{n_0} - 1\right)$$
$$a' = K \cdot a$$
$$\rightarrow \quad K \approx \frac{L + \Delta L}{L - d\left(1 - \frac{n_0}{n_1}\right)}$$

Calibration procedure:

1. Determination of the pinhole-camera distance *L*.
   - A sample with two known calibration marks is used, or alternative marks or stuck particles/microorganisms in the experiment chamber. It is an iterative process to find the correct distance between the two marks.
2. Determination of one reference plane in the experiment, to establish the correct particle position *z*.
   - Marks or stuck particles in the chamber can be employed.

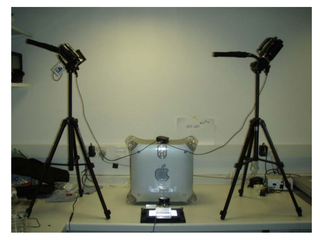
Tracking example: a spore of *Ulva linza* over a glass surface





Stereoscopy imaging

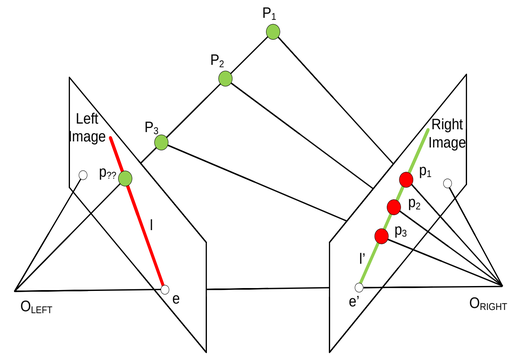
For tracking microorganisms such as barnacle cyprids (*Balanus amphitrite* or *Semibalanus balanoide*), that have a size between 200 and 1000 µm



Camera model:

$$\begin{pmatrix} U \\ V \\ S \end{pmatrix} = \underbrace{\begin{pmatrix} k_u f & 0 & u_0 \\ 0 & k_v f & v_0 \\ 0 & 0 & 1 \end{pmatrix}}_{K} \begin{pmatrix} 1 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 \\ 0 & 0 & 1 & 0 \end{pmatrix} \underbrace{\begin{pmatrix} r_{11} & r_{12} & r_{13} & t_1 \\ r_{21} & r_{22} & r_{23} & t_2 \\ r_{31} & r_{32} & r_{33} & t_3 \\ 0 & 0 & 0 & 1 \end{pmatrix}}_{M} \begin{pmatrix} x \\ y \\ z \\ 1 \end{pmatrix}$$

K: internal camera parameters
f: camera projective center distance
(k_u, k_v): image pixel coordinates.
M: external camera parameters (rotation–translation)
(*U*/*S*, *V*/*S*): perspective position in the camera image
(x, y, z): real position



Fundamental matrix (F):

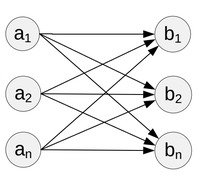
$$\mathbf{x'}^T\mathbf{F}\mathbf{x} = 0 \rightarrow \begin{bmatrix} x_1'x_1 & x_1y_1 & x_1' & y_1x_1' & y_1'y_1 & y_1' & x_1 & y_1 & 1 \\ . & . & . & . & . & . & . & . & . \\ x_n'x_n & x_ny_n & x_n' & y_nx_n' & y_n'y_n & y_n' & x_n & y_n & 1 \end{bmatrix} \begin{bmatrix} f_1 \\ . \\ f_9 \end{bmatrix} = \mathbf{o}$$

x and x' are calibration points (corresponding points in both images)

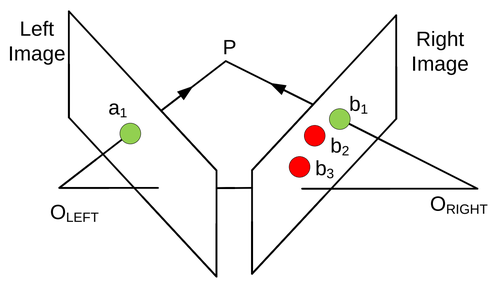
Left / Right object matching:

$$\begin{bmatrix} x_1 \\ . \\ x_8 \end{bmatrix} \begin{bmatrix} f_1 & f_2 & f_3 \\ f_4 & f_5 & f_6 \\ f_7 & f_8 & f_9 \end{bmatrix} [x'_1 \quad . \quad x'_8] \rightarrow \begin{bmatrix} 0 & big & big \\ big & 0 & big \\ big & big & 0 \end{bmatrix} = C_{CostMatrix}$$

a Matrix, $C_{CostMatrix}$, b Matrix → Graph Matching Algorithm → Correspondances

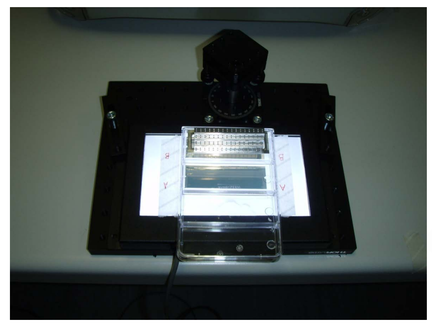


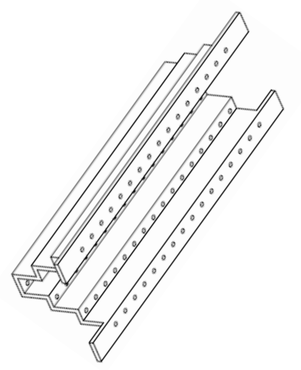
3D Position determination:



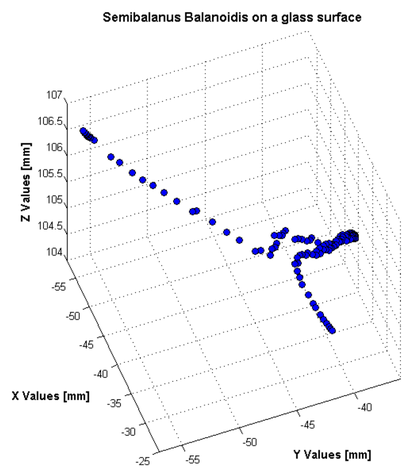
The intersection point (P) of the rays $O_{LEFT}P$ and $O_{RIGHT}P$ corresponds to the real life 3D coordinate of the point a_1 respectively b_1

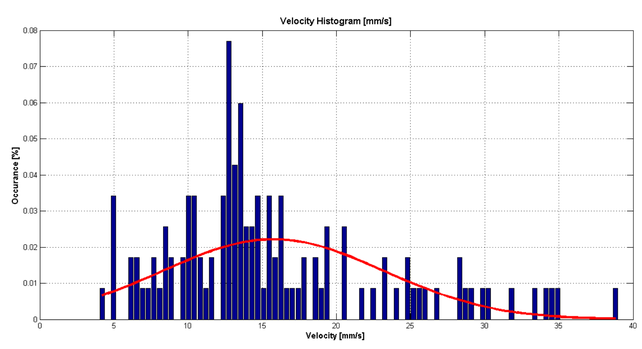
Calibration:





Tracking example: a cyprid *S. balanoide* over a glass surface





Conclusions

Both techniques showed to be useful for tracking microorganisms. For sizes between 1 and 20 µm DIHM is the appropriate technique, while for cyprids whose sizes are between 200 and 1000 µm stereoscopy imaging has exhibited good preliminary results and appears to be a promising technique. They offer advantages such as label free tracking of living organisms, determination of 3D positions, simultaneous tracking of multiple organisms, measurement on surfaces with low transparency.
The exploration behaviour of algae (*Ulva linza*) and cyprids (*Balanus Amphitrite*) is being analyzed over the following surfaces: EG_1OH, EG_6OH, PEG2000OH, $C_{12}COOH$, $C_{12}NMe_3$ and Fluorinated (FOTS) surfaces. In the last case, the effect of preincubation in artificial seawater or water already used by spores is also considered for *Ulva linza*.

Acknowledgments

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