Investigation of motile marine organisms with Digital In-line Holographic Microscopy in situ and under lab conditions

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Introduction

Biofouling caused by different micro- and macro-organisms such as algae, diatoms or invertebrates is a major problem in marine industries [1]. The investigation of the settlement behavior of these organisms on different surfaces or coatings allows to reveal selection strategies.

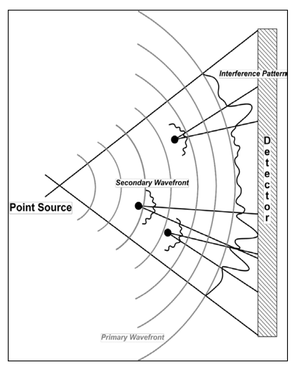
In previous work swimming and settlement behavior of *Ulva linza* zoospores under lab conditions was observed in the vicinity of different chemistries with a digital in-line holographic Microscope [2].

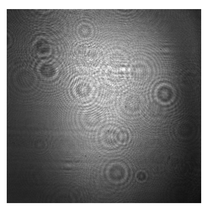
For the investigation of marine organisms in their native environment experiments on a test facility situated at the Indian River Lagoon in Melbourne Beach Florida were performed.

Holography

$$I(\vec{r}) = \left|\Psi_{in}(\vec{r})\right|^2 + \Psi_{in}^{*}(\vec{r})\Psi_{sc}(\vec{r}) + \Psi_{in}(\vec{r})\Psi_{sc}^{*}(\vec{r}) + \left|\Psi_{sc}(\vec{r})\right|^2$$

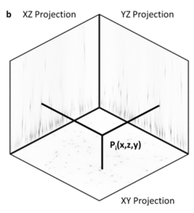
Source — Hologram — Twin image — Self interference





Hologram

Reconstruction



3D-Projection

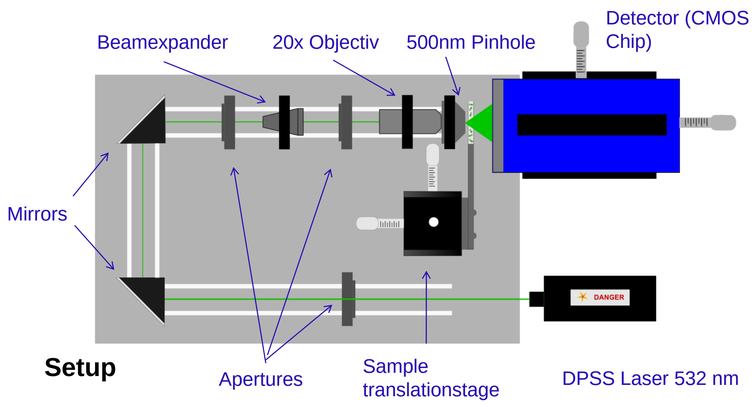
$$K(\vec{r}) = \int_S I(\vec{\xi}) \exp\left(\frac{ik\vec{\xi}\vec{r}}{\xi}\right) d^2\xi$$

Kirschhoff-Helmholtz Transformation

Resolution $\delta_{lateral} = \frac{0{,}61\lambda}{NA}$ $\delta_{depth} = \frac{\lambda}{NA^2}$ $NA = \frac{D}{2L}$

Field experiments

- Transportable holographic Setup in in-line geometry, first proposed by Gabor in 1948 [3]
- Experiments performed in a mobile laboratory (working in a Van)
- independent from electrical power supply; working with car batteries
- Seawater taken out of seawater tank and filtered with 30µm netfilters
- Record of Hologram series with up to 18000 frames (10 frames per second)



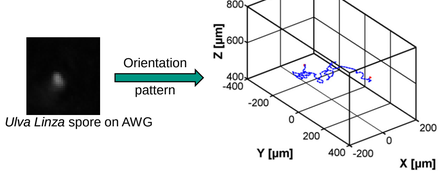
Tracking Marine Biofoulers

Comparison Lab versus Field

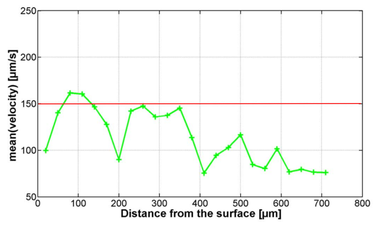
The motile zoospores of the green algae *Ulva linza* are established model organisms to investigate exploration behaviour and settlement dynamics related to different chemical cues. Settlement is a critical stage in *Ulva* life cycle. The selection of a suitable surface is important for their survival and reproduction. However *Ulva linza* has to be collected from a seashore prior to experiments. The zoospores can be released and measured in the laboratory at University of Birmingham.

The step from a model organism to a conglomeration of marine biofoulers under native conditions is a big challenge but necessary to correlate between lab and field.

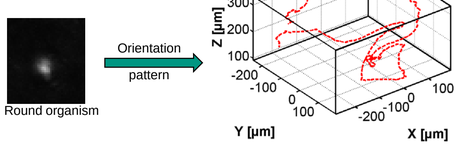
Lab [4]



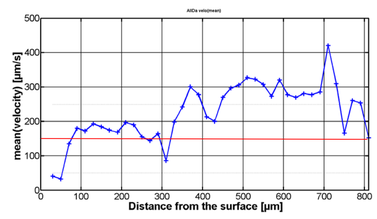
Ulva Linza spore on AWG



Field

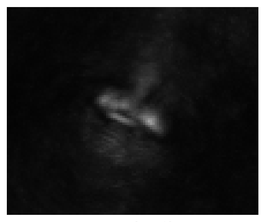


Round organism

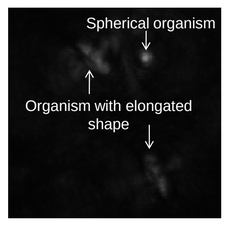


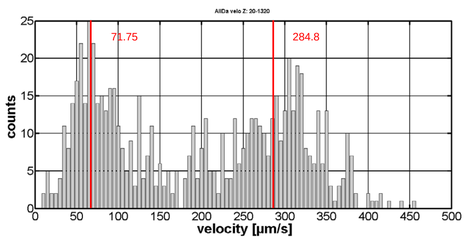
Mean velocity of tracked organisms

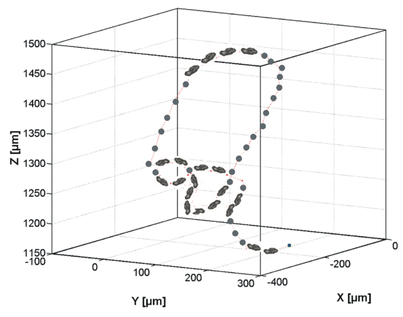
Observation of active swimming organisms with elongated shape



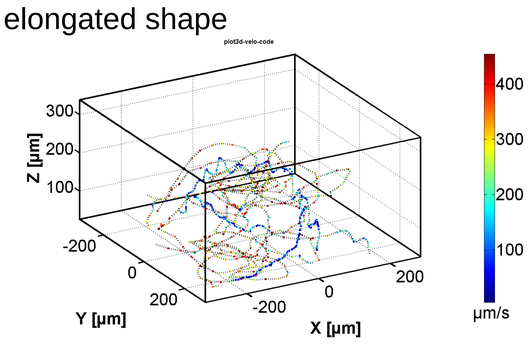
xy-projection







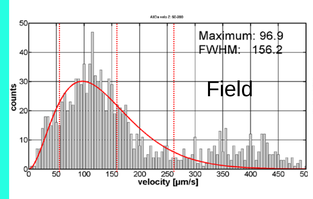
3D trajectory with shape of organisms observed in xy-projections



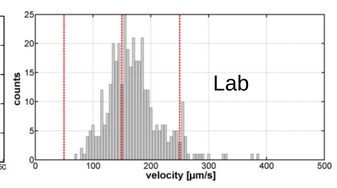
Velocity coded 3D-trajectories

- Velocity of *Ulva linza* spores under lab conditions shows a split in a slow and a fast swimming fraction after longer incubation time in the observation chamber
- Organisms in field experiment show a broader velocity distribution without spliting even after a very long incubation time
- Velocity distribution of organisms with elongated shape splits in two fractions
  fast swimmers (mean velocity: 284.8 µm/s) and slow swimmers (mean velocity:71.75 µm/s)
- Elongated organisms swim along thier longitudinal axis and have a round shape relating to their transversal axis

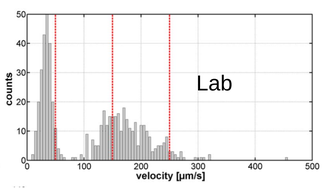
Velocity distributions of *Ulva linza* on AWG and spherical undefined organisms measured in the field



Marine organisms after 3 h in observation chamber



Ulva spores after 35 s in observation chamber



Ulva spores after 11:57 min in observation chamber

Outlook

- Reproduction and further investigations of settlement behaviour of *Ulva linza* spores on surfaces with different surface properties
- Differentiation and possibly identification of marine species from field experiments on the basis of their shape and velocity
- Further correlation of field work with lab experiments
- Correlation of holographic data with other data obtained in the field (settlement kinetics, Microfluidics, Conditioning film formation, ect.)

Acknowledgment

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Literature:

[1] Rosenhahn, A., Ederth, T. & Pettit, M. E. Advanced Nanostructures for the control of biofouling: The FP6 EU Integrated Project AMBIO. *Biointerphases* **3**, IR1-IR5 (2008)
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