3D- Tracking of biofouling Microorganisms with digital in-line holographic Microscopy

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Digital in-line holographic microscopy, based on Gabor´s initial idea of a lensless microscope[1], is an imaging technique which allows to track microorganisms in three dimensions. A so-called “source wave” interferes with the wave scattered off the swimming objects and forms an interference pattern (Hologram) on the detector which contains three-dimensional information of the objects investigated. To obtain real space information from the Hologram, a reconstruction algorithm is applied[2]. The reconstructed data provides 3D trajectories of single spores with a 10 Hz time resolution and thus allows a qualitative and quantitative analysis of swimming behavior and settlement kinetics of microorganisms such as marine biofoulers or pathogen bacteria down to a lenght of 1-2 µm.

In our recent work the swimming and settlement behavior of *Ulva linza* zoospores as a common motile biofouling organism was investigated in the vicinity of surfaces with different chemistry[3-5]. We analyzed the effect of fast and abnormal settlement of *Ulva* spores on a charged Arginin containing oligopeptide surface[6] by digital holography to study the exploration behavior and kinetics of the colonization of the surfaces. As step towards application of holography at ocean test sites in the field, we constructed a compact holographic setup and tested it at the FIT test site and studied the swimming behavior of small marine organisms in their native environment.

We also applied holography to study motile biofilm forming bacteria. Using a large CMOS sensor, we were able to resolve and track rod shaped bacteria with a length of 2 µm, namely the pathogen *Pseudomonas aeruginosa*. We will show the first three-dimensional trajectories for a free swimming *Pseudomonas aeruginosa*.

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