

STEREOSCOPIC METHODS FOR THREE-DIMENSIONAL TRACKING OF EXPLORATORY BEHAVIOR OF BARNACLE CYPRIDS

S. Maleschlijski¹, G. H. Sendra², A. Di Fino³, L. Leal-Taixe⁴, N. Aldred³, A. S. Clare³,
B. Rosenhahn⁴, A. Rosenhahn^{1,2}

¹Institute of Functional Interfaces, KIT, PO Box 3640, 76021 Karlsruhe, Germany

²Applied Physical Chemistry, University of Heidelberg, INF 253, 69120 Heidelberg, Germany

³School of Marine Science and Technology, Newcastle University, Newcastle NE1 7RU, UK

⁴Leibniz University Hannover, Appelstr. 9A, Hannover, Germany

An important step before colonization of surfaces by sessile marine biofouling organisms is surface exploration. During this step, the microorganisms evaluate different surface properties as well as different environmental factors in order to find an appropriate site for permanent settlement. This exploration is a fast process involving three-dimensional swimming motions and its quantitative analysis requires respectively the recording of temporally resolved three-dimensional swimming trajectories. Two dimensional tracking [1] has been proven to be suitable for identification of attractive and repelling surfaces, in terms of biofouling, and provided a new insight into selection strategies [2]. However, a clear separation between active (exploration, swimming) and passive (floating) phases of the swimming trajectories proves to be complicated. Another imaging method (Digital Inline Holographic Microscopy, DIHM) provides the needed features, but works optimally only with objects at size of around 20 μm [3], which is not sufficient in the case of cyprids (depending on the species, size varies in the range of 100 μm – 1000 μm). We present a transportable, submersible stereoscopic system which can be applied to record three dimensional video data and extract swimming trajectories of multiple, label-free objects. The hardware setup is presented and discussed. Introducing the mathematical basics of stereoscopy, we describe the resolution and perform empirical error analysis in order to obtain the error values of the system. First trajectories of barnacle cyprids on chemically different surfaces (PEG2000-OH, $\text{C}_{11}\text{NMe}_3^+\text{Cl}^-$, acid washed glass (AWG)) are extracted and evaluated in respect to known patterns (swimming velocity, swimming angle, etc.).

The authors gratefully acknowledge the funding from the following projects: DFG Ro 2524/2-2, Ro 2497/7-2, ONR N00014-08-1-1/16 and Seacoat (Marie Curie ITN).

- [1] J. P. Marechal, C. Hellio, M. Sebire, and A.S. Clare, “Settlement behaviour of marine invertebrate larvae measured by EthoVision 3.0”, *Biofouling*, **2004**, 20, (4-5), 211–217.
- [2] Nick Aldred, Guozhu Li, Ye Gao, Anthony S. Clare and Shaoyi Jiang, “Modulation of barnacle (*Balanus amphitrite* Darwin) cyprid settlement behavior by sulfobetaine and carboxybetaine methacrylate polymer coatings”, *Biofouling*, **2010**, 26, (6), 673-683.
- [3] M. Heydt, A. Rosenhahn, M. Grunze, M. Pettitt, M.E. Callow and J.A. Callow, “Digital in-line holography as a three-dimensional tool to study motile marine organisms during their exploration of surfaces”, *Journal of Adhesion*, **2007**, 83, (5), 417–430.