A stereoscopic approach for three dimensional tracking of marine biofouling microorganisms

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ABSTRACT

The extraction and analysis of three dimensional tracking data relating to surface exploration of sessile marine organisms is of great importance for understanding the mechanism of surface colonization (biofouling). The knowledge of behavior can thus be used to develop tools for controlling and influencing undesirable impacts that arise as a consequence of the adhered organisms. This paper describes a stereoscopic system currently in development for tracking barnacle cyprids and allows extraction of 3D swimming patterns for a common marine biofouling organism - *Semibalanus balanoides*. The details of the hardware setup and the calibration object are presented and discussed. In addition we describe the algorithm for the camera calibration, object matching and stereo triangulation. As practical result, several trajectories of living cyprids are presented and analyzed with respect to statistical swimming parameters.

INTRODUCTION

Figure 1: Biomass accumulation on marine vessels and living systems due to biofouling [2].

Biofouling:

- undesired accumulation of biomass on surfaces submerged underwater
- higher total weight and friction for vessels
- higher fuel consumption => higher cost and CO_2 emissions
- cleaning expensive and toxic

Barnacle Lifecycle:

A: *Juvenile / adult barnacle*
within a few months when sexually mature release nauplius
B: Nauplius
feeding up to 7 days growing through 6 moult stages
C: *Cyprid*
D: *Surface exploration*
E: *Cyprid permanently attached*
A: *Juvenile / adult barnacle*

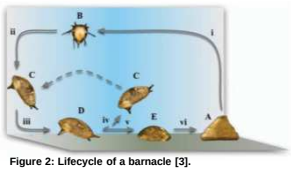


Figure 2: Lifecycle of a barnacle [3].

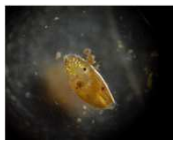


Figure 3: Cyprid of a ***Semibalanus balanoides*** barnacle [4].

Cyprids:

- Last larval stage of the barnacle
- Typical sizes: 500µm - 1000 µm

MATERIALS AND METHODS

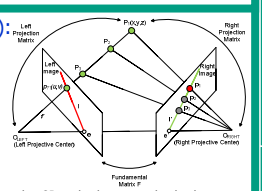
Project overview

1. Calibration
2. Particle detection
3. Graph Matching
4. 3D particle position calculation (Triangulation)
5. Tracking and Statistics

Camera model (projection matrix):

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

K: internal camera parameters
M: external camera parameters (rotation–translation)



Left / Right object matching:

1. The calibration points of each camera are reconstructed to 3D projection rays using back-projection and the projection matrixes
2. The 3D lines in *Pluecker coordinates [6]*: $L = (n, m)$ with n: direction and m: moment
3. The intersection of two lines $L_1 = (n_1, m_1)$ und $L_2 = (n_2, m_2)$:

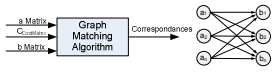
$$X = L_1 \cap L_2 = \left(\frac{n_1 \cdot (n_2 \times m_2)}{\|n_1 \times n_2\|^2} - \frac{(n_1 \cdot n_2)(n_1 \cdot (n_2 \times m_1))}{\|n_1 \times n_2\|^2}\right) n_1 + (n_1 \times m_1)$$

4. The distances between the cross sections and the 3D rays are used as a cost matrix:

$$C(i,j) = \|X \times n_i - m_i\| + \|X \times n_j - m_j\|$$

3D Position determination:

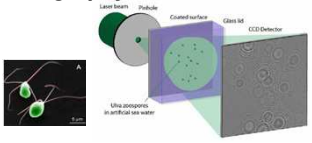
The intersection point (P) of the rays $O_{LEFT}P$ and $O_{RIGHT}P$ corresponds to the real life 3D coordinate of the point $p_{??}$ (left) respectively p_1 (right)



Hardware Setup

STATE OF THE ART

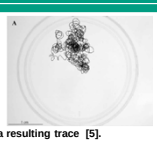
Holography



- Perfect for organisms not larger than 20 µm
- Cyprids – 500 µm

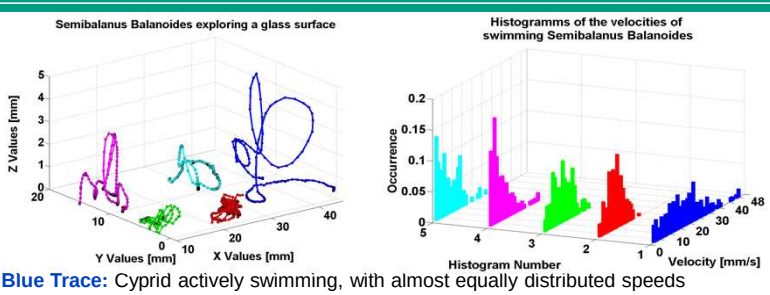
2D Tracking

Figure 4: Hardware setup of a 2D tracking system with a resulting trace [5].



- Use of EthoVision 3.0
- Compare cyprid behaviour between samples
- Proven ability to distinguish between chemistries that prevent settlement
- Can not directly distinguish between searching and swimming cyprids

EXPERIMENTAL RESULTS



Blue Trace: Cyprid actively swimming, with almost equally distributed speeds (0-35 mm/s)
Red Trace, Green Trace: Cyprids probably extensively exploring the surface with speeds lower than 15-20 mm/s.
Cyan Trace, Magenta Trace: Cyprids have a lot of „stop-and-goes“ (high amount of speeds under 5 mm/s), but also a lot of swimming away from the surface, which could indicate a first exploratory stage.

CONCLUSION

We present a **stereoscopic setup** suited for **tracking** of **sub-millimeter sized microorganisms** and the conducted experiments allow to accurately extract trajectories. The resolution is limited only by the resolution of the camera equipment used and the angle in which the two cameras are aligned. One big advantage of the presented algorithm is that it allows a **label-free** tracking of moving particles with conventional cameras. In addition the algorithm is capable of tracking **multiple moving objects** of interest **simultaneously**.

NEXT STEPS

Biological

- Further experiments with different organisms, surfaces and conditions in order to collect statistically relevant data

Algorithmic

- Accuracy improvement (Correction of physical factors, e.g. Refractive Index)
- Automatic classification based on Hidden-Markov Models [1]

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