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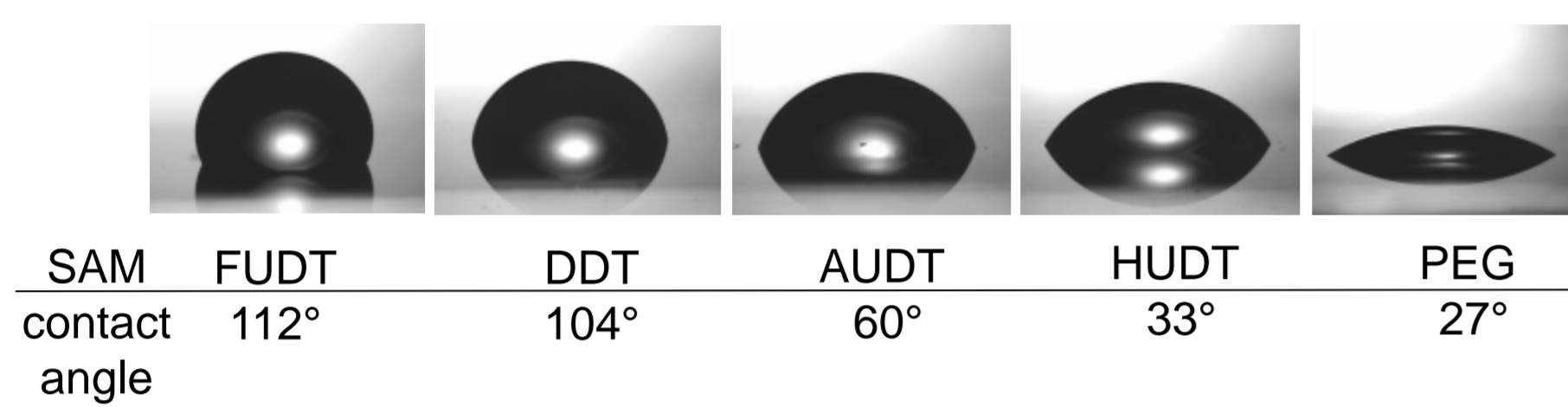
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## Colonization of SAMs with different wetting properties and hydration

- Self assembled monolayers allow to tune the physicochemical properties of a surface like wettability and hydration which are important factors for biofouling [1]
- To study the influence of surface chemistry on the colonization of biofouling organisms under real conditions SAMs with different wettability and a series of EG-containing SAMs with different EG-chain length were submerged for different duration in seawater at the FIT testfacility



## Field experiments at the FIT testsite

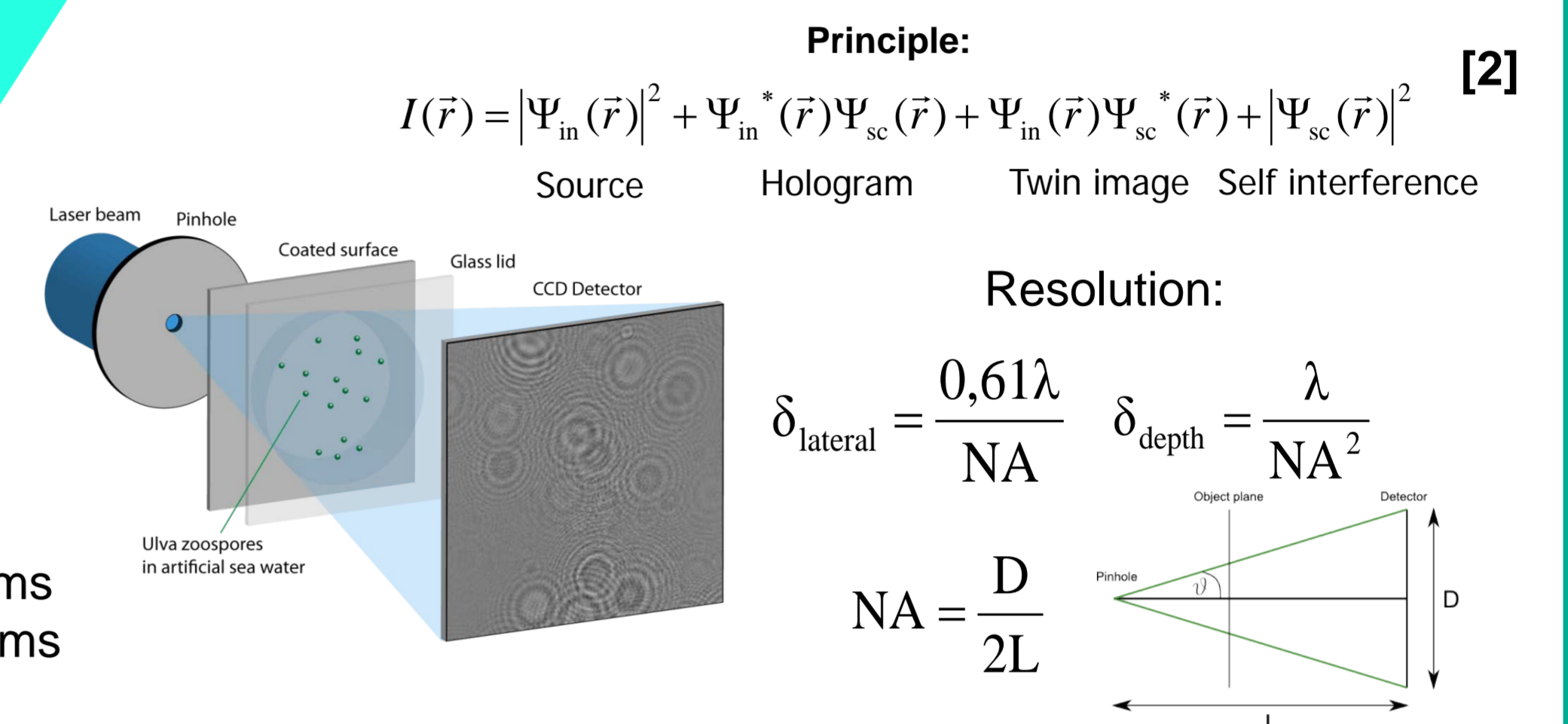


## Lab experiments

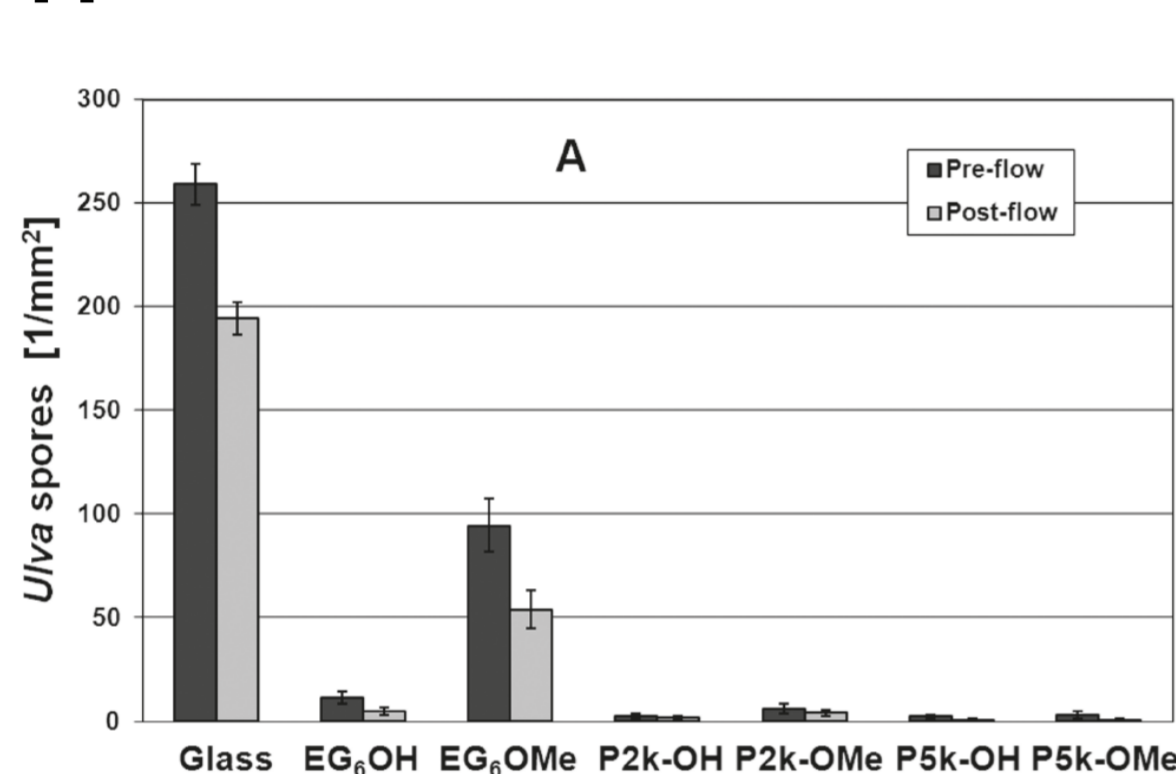
Comparison of the biofouling performance of model organisms measured under lab conditions and the behaviour of organisms measured under native conditions in the field

## 3D tracking with digital in-line Holography

- Digital in-line holographic microscopy allows to track marine organisms in three dimensions which provides a qualitative and quantitative analysis method for biofouling dynamics
- For field experiments the holographic setup was built at the testfacility in a mobile lab

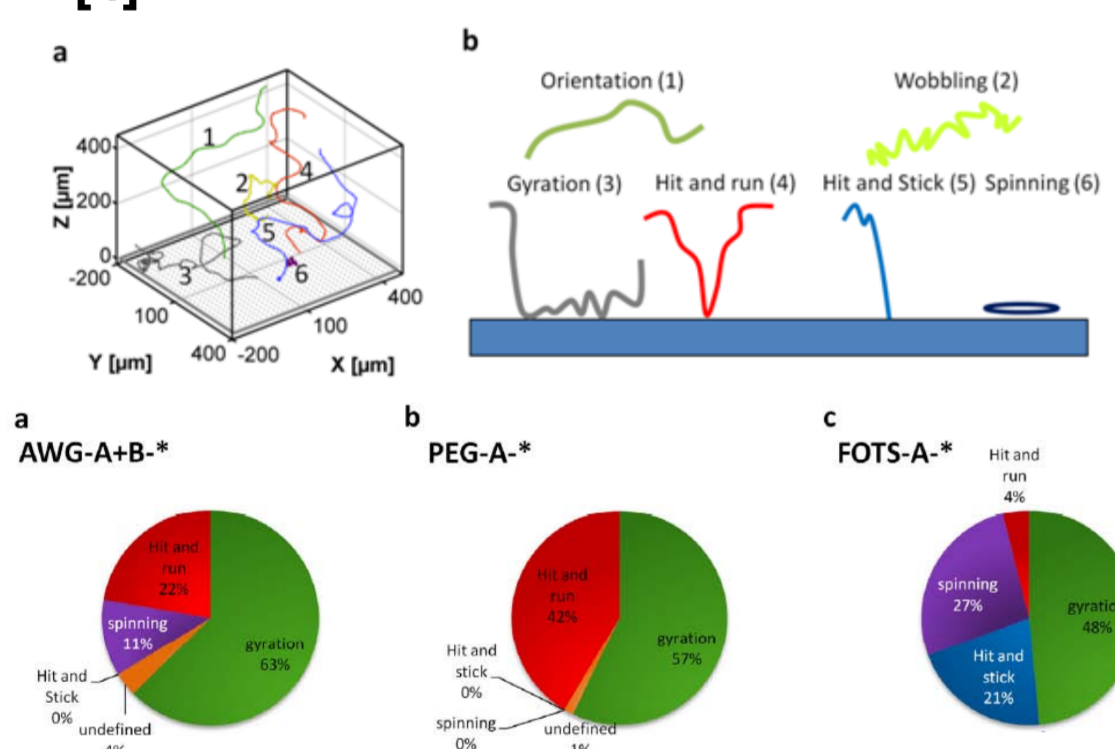


## Ulva linza zoospores as model organisms for soft macrofoulers Settlement assays on SAMs



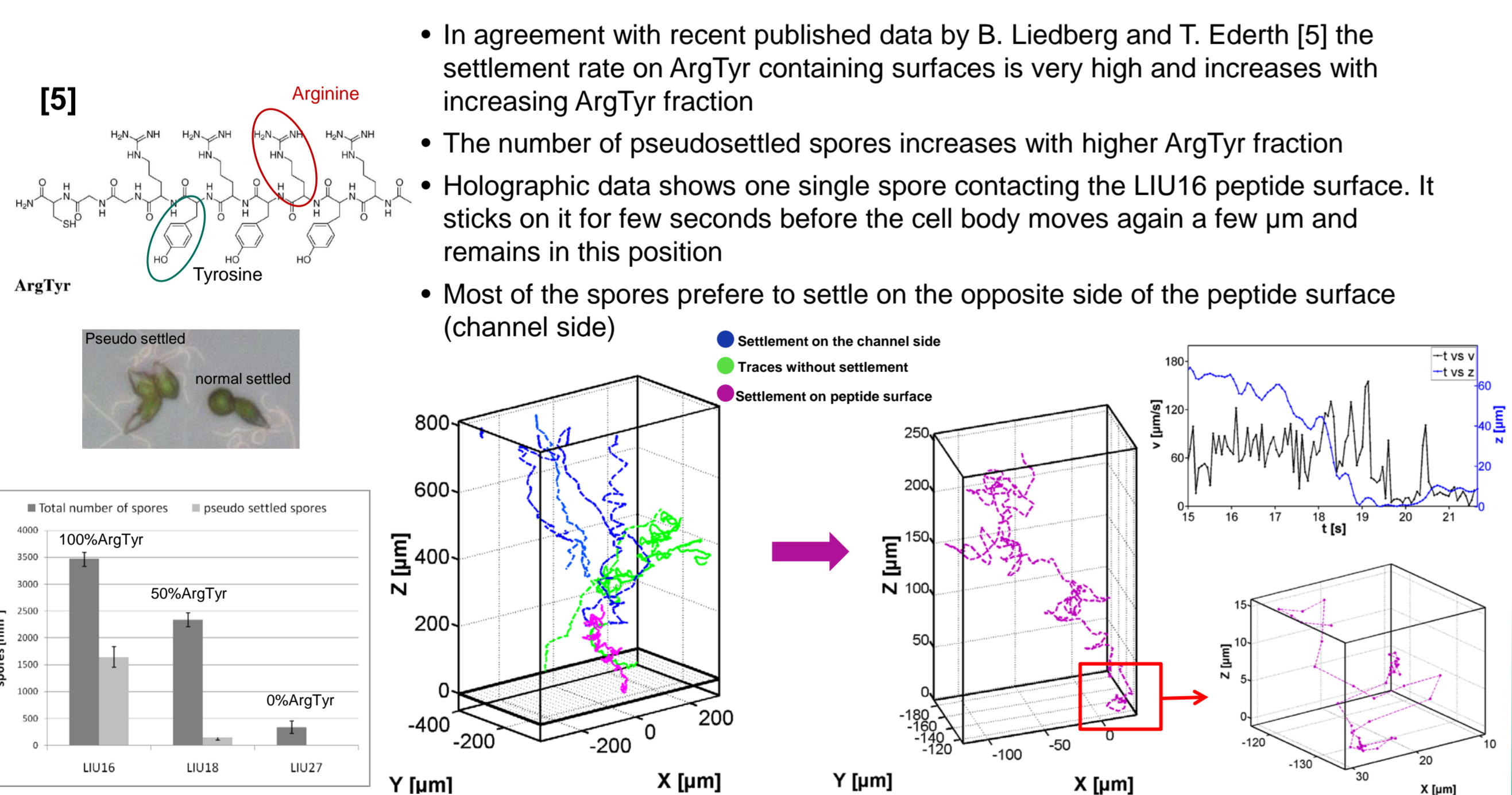
- Surfaces with hydroxyl end-group termination show a low number of attached spores
- Highest settlement could be observed for oligomeric EG<sub>6</sub> with methyl-termination
- Very low attachment on all PEG surfaces
- Number of attached cells increases with contact angle
- Results for the attachment of *Navicula perminuta* are similar to results for *Ulva* spores

## Tracking of Ulva spores with in-line holography in vicinity of different surfaces – measurements under lab conditions



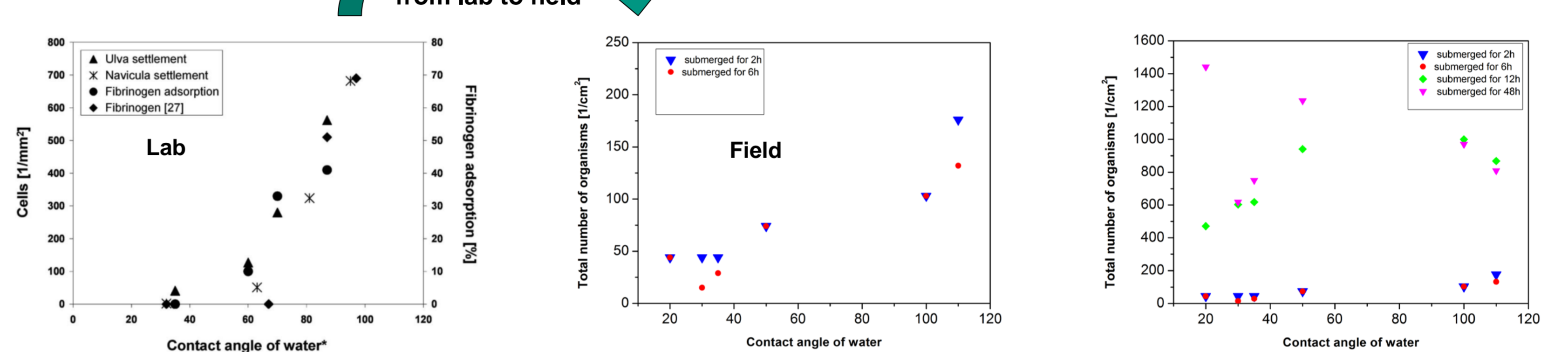
- Traces of *Ulva linza* spores can be classified into 6 different motion pattern
- The occurrence of these motion pattern is chemistry dependent
- The „hit and run“ pattern indicates a not suitable surface for *Ulva* spores, which is a dominant pattern at the PEG surface

## Settlement behaviour of Ulva spores on charged ArgTyr- oligopeptide surfaces

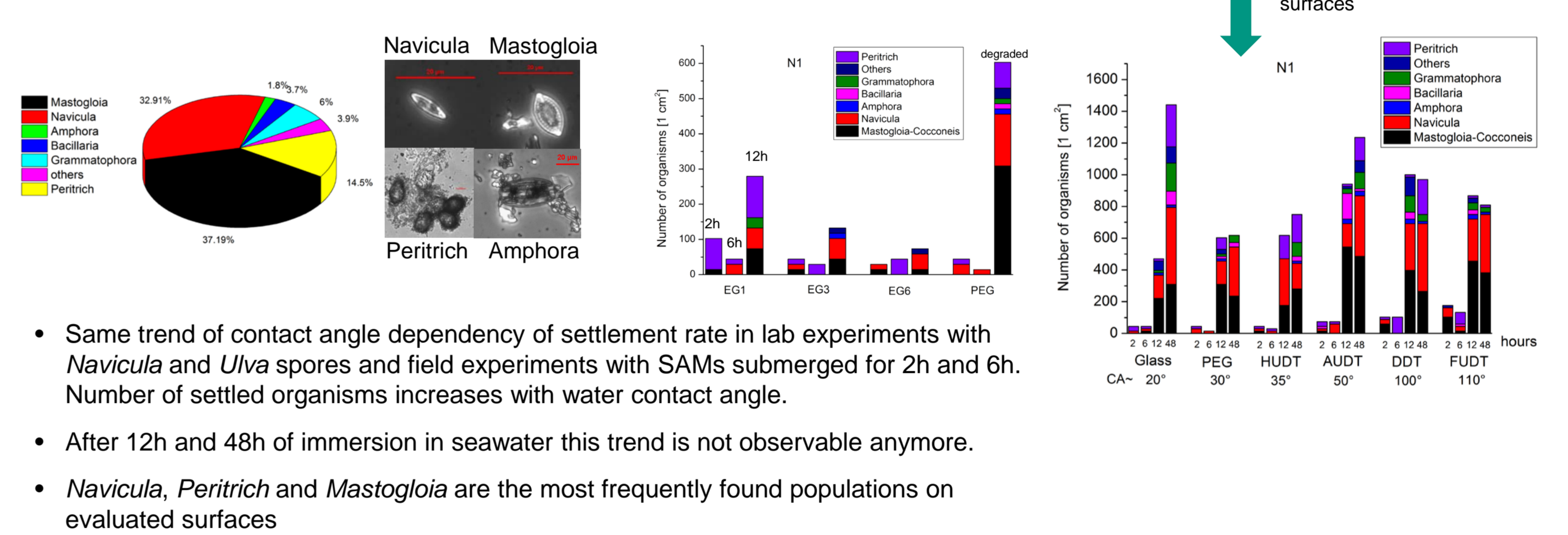


- In agreement with recent published data by B. Liedberg and T. Ederth [5] the settlement rate on ArgTyr containing surfaces is very high and increases with increasing ArgTyr fraction
- The number of pseudosettled spores increases with higher ArgTyr fraction
- Holographic data shows one single spore contacting the LIU16 peptide surface. It sticks on it for few seconds before the cell body moves again a few µm and remains in this position
- Most of the spores prefer to settle on the opposite side of the peptide surface (channel side)

## In situ surface colonization of SAMs with different wettability at the FIT testsite

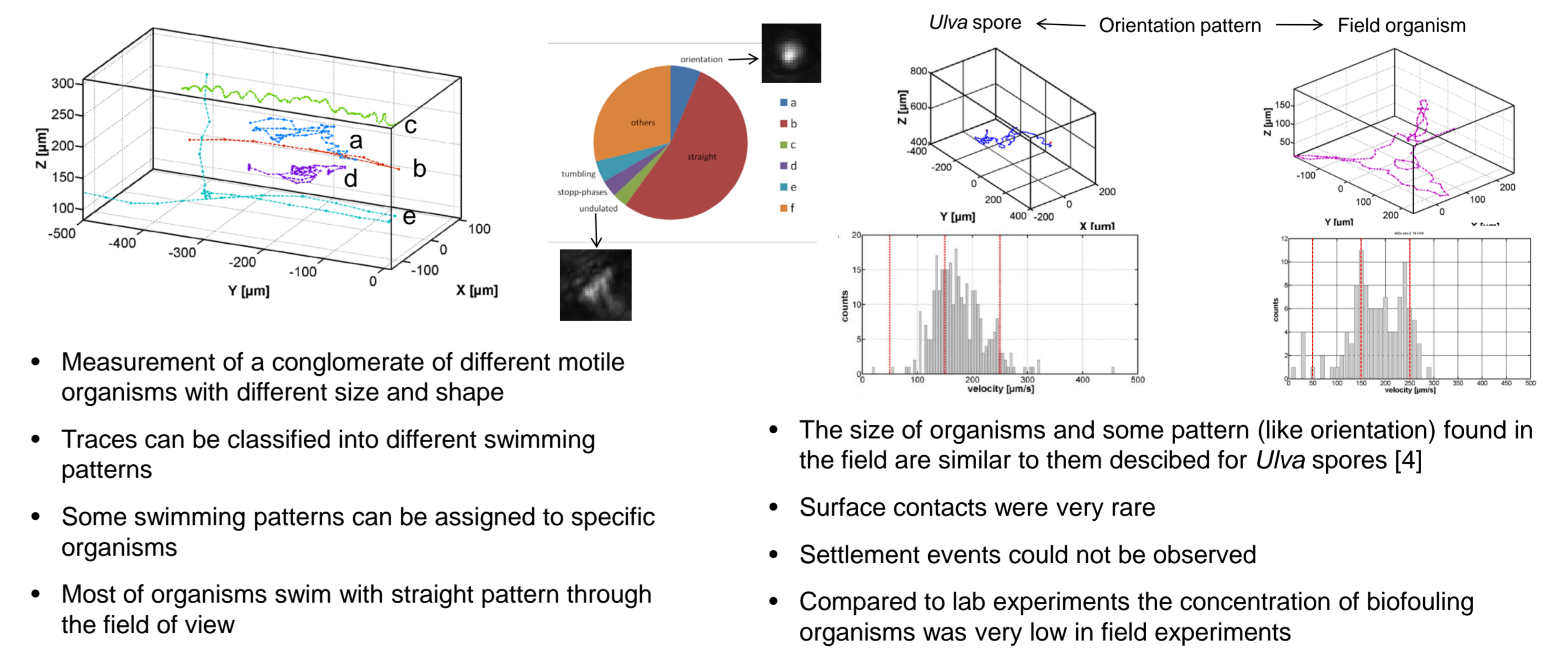


## Microscopic population-analysis of attached organisms



- Same trend of contact angle dependency of settlement rate in lab experiments with *Navicula* and *Ulva* spores and field experiments with SAMs submerged for 2h and 6h. Number of settled organisms increases with water contact angle.
- After 12h and 48h of immersion in seawater this trend is not observable anymore.
- Navicula*, *Peritrich* and *Mastogloia* are the most frequently found populations on evaluated surfaces

## Tracking of motile marine microorganisms measured under field conditions



- Measurement of a conglomerate of different motile organisms with different size and shape
- Traces can be classified into different swimming patterns
- Some swimming patterns can be assigned to specific organisms
- Most of organisms swim with straight pattern through the field of view
- The size of organisms and some pattern (like orientation) found in the field are similar to them described for *Ulva* spores [4]
- Surface contacts were very rare
- Settlement events could not be observed
- Compared to lab experiments the concentration of biofouling organisms was very low in field experiments

- Short time colonization of surfaces submerged for 2h and 6h shows an increased number of attached organisms with increasing contact angle similar to experiments performed in the lab
- With increasing incubation time in seawater (12 h and 48 h) this effect is not distinct anymore. The most frequently observed organisms are *Mastogloia*, *Navicula* and *Peritrich*
- With Holography different swimming patterns of motile marine organisms could be classified in the field
- Patterns similar to them described for *Ulva* spores could be found. Most of recorded organisms have a size between 4 and 6 µm
- Within a field of view of 600 µm no settlement event could be observed. The biofouling performance in this short time observation was very low because concentration of biofouling organisms was very low

- To verify the results for the colonization of SAMs with different chemistries further experiments would be reasonable to include other factors like weather or seasonable differences in organism occurrence
  - To observe more fouling events holography should be repeated in a season with increased occurrence of biofouling organisms and generally higher fouling pressure
- Further experiments in June or July

## Acknowledgment

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## Literature:

- Rosenhahn, A., Schilp, S., Kreuzer, H. J. & Grunze, M. The role of "inert" surface chemistry in marine biofouling prevention. *Phys. Chem. Chem. Phys.* **12**, 4275-4286, (2010).
- Kreuzer, H. J., Jericho, M. J., Meinertzhagen, I. X., W. B. Digital in-line holography with photons and electrons. *Journal of Physics-Condensed Matter* **13**, 10729-10741 (2001).
- Schilp, S. et al. *Physicochemical Properties of EG-Containing Self-Assembled Monolayers Relevant for Protein and Algal Cell Resistance*. *Langmuir* **25**, 10077-10082, (2009).
- M. Heydt. *How do spores select where to settle? A holographic motility analysis of Ulva zoospores on different surfaces* Dissertation thesis, Heidelberg, (2009).
- Ederth, T. et al. *Anomalous settlement behavior of Ulva linza zoospores on cationic oligopeptide surfaces*. *Biofouling* **24**, 303-312, (2008).