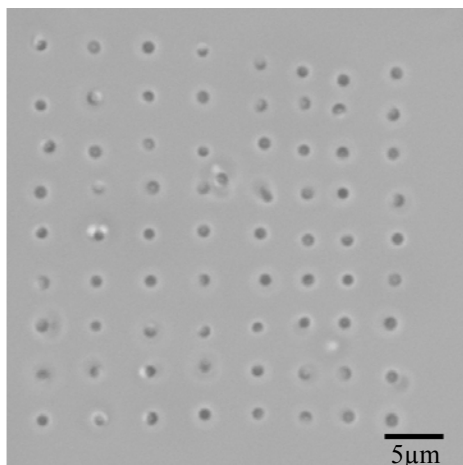


P19 – Holographic Optical Tweezers Aided Investigations on *Bacillus subtilis*

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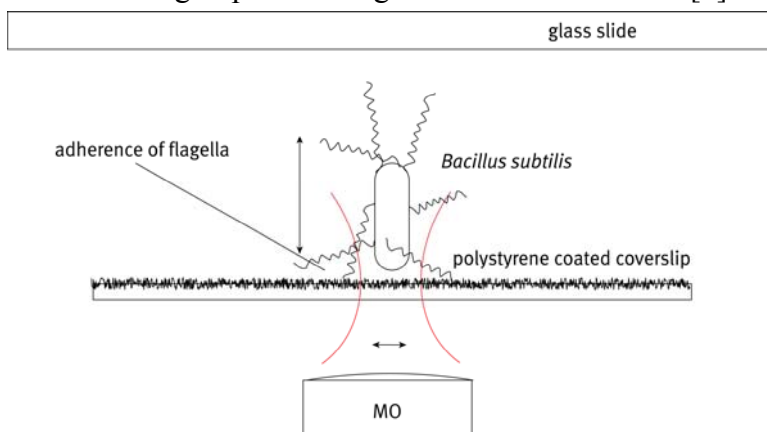


Fluid flows on microscopic scales are important for cell-cell interactions and could also be of future interest for mixing processes on small ranges. For a general study on these flows we chose *Bacillus subtilis* because of its well understood flagella motor as a model system to get a better understanding of hydrodynamic interactions between several bacteria.

Flow fields created on the one hand by a single bacterium [1] and on the other hand by large colonies of bacteria [2] which show cooperative behaviour have been investigated recently. Our system enables us to study the interesting region in between to get a better understanding when and how this behaviour appears and most importantly to study flows created by well defined

arrangements of bacteria. For this purpose we implemented a Holographic Optical Tweezers system [3] that traps up to about 100 bacteria simultaneously in all 3 dimensions at a wavelength of $\lambda = 1064$ nm. At this wavelength photodamage to *Bacillus subtilis* [4] is minimal and fluid flows can be studied as unaffected and natural as possible.

We use our tweezers system as a tool to adhere multiple bacteria at the same time to polystyrene coated glass surfaces. This robust technique provides us a good basis for further studies on hydrodynamic interactions between bacteria and their influences on fluid mixing.



- [1] L. H. Cisneros et al., "Unexpected Bipolar Flagella Arrangements and Long-Range Flows Driven by Bacteria near Solid Boundaries" *Phys. Rev. Lett.* **101** (2008), 168102-1
- [2] L.H. Cisneros et al., "Fluid dynamics of self-propelled microorganisms, from individuals to concentrated populations" *Exp. Fluids* **43** (2007), 737
- [3] E. R. Dufresne and D. G. Grier, "Optical tweezer arrays and optical substrates created with diffractive optical elements" *Rev. Sci. Instr.* **69** (1998), 1974
- [4] K. C. Neuman et al., "Characterization of Photodamage to Escherichia coli in Optical Traps" *Biophys. J.* **77** (1999), 2856



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Holographic Optical Tweezers Aided Investigation on *Bacillus subtilis*

Fluid flows on microscopic scales are important for cell-cell interactions and could also be of future interest for mixing processes on small ranges. For a general study on these flows we chose *Bacillus subtilis* because of its well understood flagella motors as a model system to get a better understanding of hydrodynamic interactions between several bacteria. We use our tweezers system as a tool to adhere multiple bacteria at the same time to polystyrene coated glass surfaces. This robust technique provides us a good basis for further studies on hydrodynamic interactions between bacteria and their influences on fluid mixing.

Optical Trapping



Figure 1: Ray optics approach: Refraction of rays of different intensity leads to F_1, F_2 and thus to a net force F_{pos} towards the focus.

- For a transparent particle of diameter d different approaches (for $d \gg \lambda$, $d = \lambda$, $d \ll \lambda$) all yield a force, dependent on the intensity gradient of the light [1]:

$$F = \alpha \nabla E^2$$

- Weakly focused beams are able to trap particles in lateral directions
- Strongly focused beams yield 3d control of the particle position (gradient force outweighs scattering force)

Holographic Optical Tweezers Setup

- 1064 nm or 532 nm laser coupled into an inverted fluorescence microscope

- SLM generates arbitrary phase holograms and thus any desired intensity distribution in the sample plane

- High resolution flow field analysis with a high-speed camera (469 Hz @ full resolution; 3500 Hz with ROI)

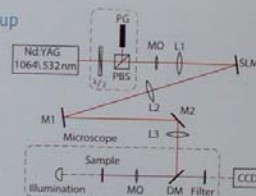


Figure 2: Holographic optical tweezers (HOT) setup. The laser illuminates the SLM completely and is then scaled to slightly overfill the back aperture of the oil immersion objective.

Preliminary Work on Particle Trapping



Figure 3: 6 μm polystyrene spheres trapped in 3 dimensions and moved by a sequence of precalculated holograms

- The HOT system is a useful tool to control suspended microparticles such as

- Polystyrene spheres (<200 nm up to several μm in diameter)
- Zeolite L (porous crystalline material)
- Bacillus subtilis* (nonhazardous bacteria; is used as a biophysical model system)

- SLM displays a sequence of holograms to create periodic movement (fig. 3) and can even be used for interactive particle control

- Distance dependent particle-particle interactions can be investigated



Figure 4: 3x3 array of 3d trapped *Bacillus subtilis* bacteria

- Approx. 1 mW in the focal plane is sufficient to trap microparticles
- Our 2 W laser system is able to trap several dozens of particles
- Simultaneous control over up to 72 (fig. 4) particles in all 3 dimensions

Structuring *Bacillus subtilis* on Surfaces

- Bacillus subtilis* has very low absorption at 1064 nm [2] → use of ND:YAG laser to
- yield a good trapping efficiency
- avoid photodamage
- Flagella can be found all over the bacteria's body (fig. 5)
- Flagella are able to adhere to polystyrene → enables us to investigate their rotational behavior

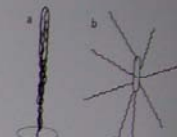


Figure 5: a) flagella rotate in the same direction and bundle together for a directed movement b) some of all flagella reverse rotation direction → bacterium tumbles

Laser Guided Bacterial Adherence

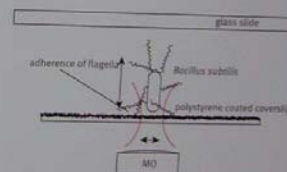


Figure 6: The *Bacillus subtilis* bacterium was optically trapped and moved to the designated position near a polystyrene coated surface.

- A small array of *Bacillus subtilis* is positioned with HOT close to a polystyrene coated surface (fig. 6)
- Soon most bacteria stick to the surface and can not be removed by the laser
- The array can be enlarged easily by repeating this procedure (fig. 7)
- Controlled arrangement of rotating bacteria provides a well defined basis for flow field analysis [3]

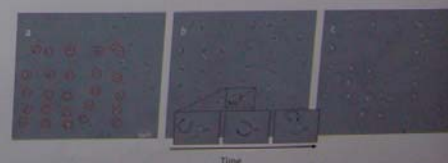


Figure 7: We used HOT to trap and to position *Bacillus subtilis* in a 3x3 array as described above. a) b) moving the microscope objective relative to the sample leads to a movement of the bacteria in contrast to laser-trapped bacteria. c) defocusing; insets: example of a living and rotating bacterium

- Flow field analysis via particle image velocimetry (PIV) of fluorescent tracer particles
- Interactions between rotating bacteria can be analyzed without any laser influences

References

- [1] A. Ashkin, *Phys. Rev. Lett.* **44** (1970), 154-158
- [2] K. C. Neuman et al., *Bioophys. J.* **77** (1999), 2856-2863
- [3] Chiriac et al., *Phys. Rev. Lett.* **101** (2008), 168102-1-168102-4