Modeling of biological movements with light generated and lightoperated microrobots

Ph.D. dissertation theses

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Introduction

The movement of microscopic biological systems is very different from what is observable in our macro world. This issue is extremely interesting and important, both theoretically and practically with relevance to numerous subjects. For example, it is a very important practical goal to prevent and overcome bacterial infections. This requires a precise characterization of the infection process. A better understanding of the movement of bacteria (single or a large number of individuals together) clearly brings us closer to treat the problem. The movement of bacteria is a peculiar physical problem. It is known that in systems with a small Reynolds number the swimming mechanism is very different from that of the macroscopic world, the detailed characterization of possible realizations is far from complete. There have been observed many interesting effects in this world, for example, the hydrodynamic interaction, the collective motion, the synchronization of certain movements, and so on - it would be necessary to characterize and quantitatively describe these phenomena. The motion of objects of typically micrometer size characterised by small Reynolds numbers have two distinctive features. On one hand, inertia is negligible compared to viscous forces. In addition, due to the micron sizes Brownian fluctuation is relatively large, it is always present during motion. As a result, the mechanism of motion and propulsion is completely different from that in the macro world. Certain swimming modes in the macro world are completely ineffective in the microworld, and vice versa, surprising microscopic mechanisms have emerged.

A most widespread phenomenon of biological movements is collective motion, which can be observed on both microscopic and macroscopic scale. The main characteristics of collective movements is that the behavior of the individual is determined by the effect of the "majority" - the individual shows a completely different behavior as if it were "in itself". For this phenomenon, it is necessary for a large number of individuals to stay close together and interact with each other. In this case an interesting process takes place on the whole system, in which the individuals change their behavior at the same time, and a pattern develops across the entire system.

Another phenomenon of biological movements which has been the object of intensive research in recent years is synchronization. During synchronization, the interaction between two or more cyclic systems (operating at a given frequency) will change the frequency of each system and begin to operate at the same frequency. In the micro-world, the emergence of synchronization is often vital to the locomotion of cells, bacteria, microscopic organisms or the functioning of its organs, for example the synchronized operation of the bacterial flagella or the joint movement of the cilia and the formation of metachronal waves.

There are many interactions that can produce synchronization. According to numerous theoretical works, hydrodynamic interactions may also play an important role in microbiological systems, but the "full picture" of the process of synchronizing in the low Reynold number range is still unclear.

The experimental examination of processes play a key role in the above-mentioned phenomena. On the one hand, it serves as a basis for the generation of theories, on the other hand, control of the theories can be carried out with appropriate experiments. In principle, experiments for the full description are best performed on natural systems. However, experiments in natural systems have an essential limitation that they are usually very complicated, and many of their parameters are not known with sufficient precision. In addition, in order to control the physical models, it is necessary to change the parameters - in natural systems this is usually very limited. Due to these problems quantitative modeling is difficult. One of the obvious solution of this problem is the use of artificial experimental model systems, robots. In the detailed characterization of the moving systems such artificial models would be very helpful that represent the natural function as faithfully as possible from as many aspects as possible, but its parameters are accurately known and can be changed so that the theoretical models can be easily verified.

Objective

In my dissertation, my goal was to design microscopic robot systems with the help of which two problems can be examined, namely microscopic motion and hydrodynamic synchronization.

Light-driven, autonomously moving microscopic objects

In the field of microscopic motion, I wanted to develop moving bodies that obtain the energy for motion from light, but move independently, in the sense that the direction of their movement is not determined by the light. This requirement deviates significantly from the cases of light-driven movements discussed previously. In the simplest case of light-driven motion, the body interacting with light moves in the direction of light travel. Moving the body with a laser tweezer can be easily solved, but then the position of the laser trap is moved and the trapped body moves accordingly. The requirement for the robots to be developed here is that the direction of motion is determined by the robots themselves (their shape, position, etc.). Another requirement is that their movements are regular and directional, so that the persistence lengths that can be assigned to the trajectory of motion are much larger than the size of the body.

I designed two types of microrobots: one type is wedge-shaped and moves by sliding on a surface while the other is roller-shaped and moves by rolling. I planned to build the bodies with two-photon photopolymerization, and I aimed at demonstrating their operation and detailed characterization of their movement.

Hydrodynamic synchronization

In the investigation of the synchronization, the primary aim was to demonstrate hydrodynamic synchronization in the microscopic regime using artificial objects under controlled conditions. To achieve this, I planned to develop and apply a rotor system that was generated and manipulated by light. I designed micron-sized rotors that exhibit a rotational motion around a well-defined axis when trapped by optical tweezers. The system must be capable of simultaneously controlling two microrotors, to fine-tune their relative speed and position, and to precisely characterize their motion. In addition to the demonstration of hydrodynamic synchronization, I also aimed the detailed analysis of the phenomenon and the establishment of its mathematical model.

Methods

Production of structures

All the microstructures used in my experiments were made with two-photon polymerization and during the production of different structures, the same steps had to be taken. The microstructures were polymerized on a 150 μm thick glass cover plate. On the surface of the purified glasses I applied a 16-30 μm thick SU8 photopolymer layer using spin-coat technique. For two-photon polymerization, a 780nm wavelength femtosecond laser was used, with a pulse length of 100fs and a pulse repetition frequency of 100MHz. The average power of the beam was 5mW in front of the rear-facing aperture of the focusing lens. The beam was introduced into an inverted microscope via the epifluorescence port, and focused by a high numerical aperture oil-immersion objective (NA = 1.25). During the polymerization, the sample was moved by a 3-dimensional piezo positioner Photopolymerization was controlled by a computer using a Labview software. After exposure, each sample was developed.

Types of microchannels

I studied the structures in microchannels. During my work I used two different types of microchannels. As a microchannel *type I* microscope cover plate was used. The sides of the channel were made of two parallel cover glasses fixed to the substrate with a light-curing adhesive and finally it was covered by another coverglass from the top. I filled the channel with water containing tween20 detergent. In the case of the *type II* microchannel the bottom of the sample compartment was formed by the free surface of a hanging water droplet. As a carrier of *type II* channel, I used a concavity slide. A drop of water was introduced into the concavity, and the microstructures were introduced in this drop. During the experiment, the substrate was turned downwards, forming a hanging droplet, and then gravity caused the structures to accumulate at the bottom of the droplet – at the water and air interface.

Preparation and driving of wedges and rollers

To make the surfaces of the wedges and rollers reflective, their surface was coated by gold. To perform my experiments, *type I* and *type II* microchannels were used for the wedges, while only *type I* microchannels were used for the experiments with rollers.

Examination of the movement of wedges and rollers was carried out using an Axio Observer A1 inverted microscope. To illuminate both microstructures, I used a CW Ytterbium fiber laser with a maximum power of 10W at 1070nm operating wavelength. Two different layouts were used to illuminate wedges and rollers. The most important difference between them was that the wedges were only illuminated from one direction from above through the condenser lens (upper branch), while the rollers were illuminated from two directions: from above and below. One was the same as used to illuminate the wedges, and the other came through the objective lens also used for observation (lower branch).

Preparation and propulsion of micro-rotors

Following the polymerization, the micro-rotors were placed in a *type I* microchannel. I used optical traps generated by holographic optical tweezers for holding the rotors with the ability to fine tune their position and rotation speed. The rotational speed of each rotor was determined by the intensity of the trapping beams. The intensity of each beam was set holographically. The system was capable of controlling the position and power of the optical trap with arbitrary accuracy. The average frequency of the rotors was approx. 6-8Hz. In order to observe the synchronization it was critical to set nearly identical rotational frequencies. I set 40-50 different speed ratios during a measurement so that the relative speed of the rotor changed monotonically. The relative velocities were changed such that the rotor that was faster at the beginning of the measurement became slower in the end and the speed passed through the equal speed range. Typically, a given rate ratio was kept for 10-40 seconds. The measurements were performed at 3 different rotor spacings from the minimum distance of 6μm to 7μm with 0.5 micron steps.

Evaluating the films

The films about the movements of wedges and rollers as well in ofthe rtos were evaluated with my own motion and orientation analysis programs,

Results

1. I created a light-driven microrobot system. In the system, each particle moves independently, in the sense that the energy for their movement is obtained from the light, but the light has no effect on the direction of their movement. The particles are wedges made by photopolymerization and coated with gold. I studied the movement of these particles under the influence of illumination.

I demonstrated that the microrobots can move due to illumination at both water/glass and water/air interfaces. The energy required for their movement was obtained from the illumination light. The speed of their movement, which is typically 10 μm/s, is proportional to the local intensity of the illumination, while the direction of the movement is determined by the orientation of the structures. I determined the persistence length from the trajectory of motions using the worm-like-chain and the persistent-random-walk model, which ranged from 100 to 1000 μm. The persistence length of the motion of wedges was 1-2 orders of magnitude higher than their size. [**T1**]

2. I prepared a microscopic, autonomously moving roller with a shape that facilitates rolling in the direction determined by its orientation, upon illumination from a direction perpendicular to its axis of rotation.

I investigated the movement of 3-dimensional rollers produced by photopolymerization and coated with a gold layer. I used an optical arrangement to illuminate the rollers where illumination came both from the top and bottom, where the role of the illumination from below was primarily to reduce friction. Experiments have shown that rollers of appropriate shape are capable of continuous light-driven rotation and movement in the expected direction. [**T2**]

3. I gave theoretical evidence that prismatic structures are unable to rotate continuously upon homogeneous illumination perpendicular to the axis of rotation. In the proof, it is fundamental that i) the radiance is a remaining quantity in a lossless (linear) optical system, ii) homogeneously illuminating the structure from one side and rotating it is equivalent to the situation when the structure is placed in isotropic illumination. The proof was performed for both fully reflective and transparent structures.

As a summary of the observations and demonstrations I have formulated the following general statement: A collimated beam can not generate continuous rotation if the light scattering due to the interaction of the radiation and the object is two-dimensional and the interaction is lossless. For the light-driven rotation it is necessary, but not sufficient that the light scattering is 3-dimensional. [**T2**]

4. I demonstrated that the hydrodynamic interaction is capable of synchronizing the rotation of two micro-motors immersed in water. In the experimental demonstration I analyzed the dependence of synchronization on the ratio of the rotation speed and the distance between the two rotors.

I have developed an analytical model for modeling synchronization. The dynamics of the phase difference of the rotors was modeled by the stochastic Adler equation that describes the movement of a Brownian particle in a tilted potential. The distribution of the phase differences of the two rotor was obtained by solving the Fokker-Planck equation. [**T3**]

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