1. Introduction

About sixty years ago, Erwin Schrödinger raised in his book 'What is Life?'[1] the question whether physics and biology are compatible and whether living organisms can be explained by the laws of physics. In his book, Schrödinger discussed questions about genetic stability and mutations in terms of basic physical principles by assuming that genes have a molecular basis. He did this at a time when the word 'gene' was still used as a conceptional term. Exactly in the publication year of 'What is Life?' it was revealed that genes consist of DNA (deoxyribonucleic acid) molecules. An eighteen year old student was so impressed by Schrödinger's book that he desired to research the gene. A few years later, he revealed - together with Francis Crick - the double-helix structure of DNA.[2]

At the time of Watson's and Crick's discovery, living cells were described as 'chemical reactors', small vessels which contain a complex composition of substances undergoing chemical reactions through diffusion and random collision.[3] Since then, the structural knowledge about the molecular cell components increased strongly by techniques like X-ray crystallography, nuclear magnetic resonance spectroscopy and electron microscopy. Simultaneously, more and more attention was drawn to the question of the structural basis of biomolecular interactions.

Cells were no longer considered as homogeneous reaction vessels but rather as small 'factories' containing molecular 'machines' to carry out highly specialized processes like DNA transcription, RNA translation, organelle transport, chromosomal segregation, cell motility or protein folding. On the scale of individual molecules, many of these processes are mechanical processes and can be described by basic physical concepts like force, torque, energy or power.[3]

The recent advent of experimental tools for the direct mechanical manipulation of single cells or even single molecules enables the investigation of the mechanical cellular machinery. One of the tools that are in particular suitable for this purpose are optical tweezers. Optical tweezers consist of a tightly focussed laser beam which allows the three-dimensional trapping of small objects like beads. A small bead, attached to a single molecule or a cell can be trapped by optical tweezers and used as a handle to measure mechanical properties such as forces, torques, energies or powers.

In the work presented here, optical tweezers with an interferometric fluctuation tracking system were applied to study cellular mechanics during the process of phagocytosis. Phagocytosis is a central cellular mechanism in the innate mammalian immune system. Although many cell types can undergo this process under some conditions, it is a major function of macrophages, which are cells specialised on phagocytosis. When an invading bacterium binds to the membrane of a macrophage, the cell membrane starts to wrap around the invader and internalizes the bacterium. Thereby, the bacterium is enclosed into an intracellular membrane-organelle, the phagosome.

The process of phagocytosis is highly interesting from a biomedical point of view as well as from a biophysical one. Biomedically, phagocytosis is very important because macrophages are able to kill most of the potential infectious agents fast and with only little inflammation. However several pathogens, are able to undermine this process. One of these pathogens is *Mycobacterium tuberculosis*, the causative agent of the disease tuberculosis, which causes about two million deaths per year. The mechanisms used by *M. tuberculosis* to survive inside macrophages are still poorly understood and a deeper knowledge of the phagocytosis process might help in fighting this disease. So far, phagocytosis was mainly investigated by conventional light and electron microscopies, which provide primarily kinematic and structural information (kinematic and structural information at a resolution on the order of hundreds of nanometers in the case of conventional light microscopies and structural information at a resolution in the range of Ångström in the case of electron microscopies). However, the mechanical properties of the molecular machinery driving this process, like the physical forces and energies involved were barely known up to now.

Biophysically, phagocytosis involves various interesting aspects: The first step of phagocytosis is the binding of the bacterium to the macrophage plasma membrane. This process involves the (sequential) bacterial binding to receptors in the cell membrane. The dynamics of this binding process should depend on e.g. the receptor density within the membrane or the strength of the receptor-ligand bond. The next phagocytosis step is then the wrapping of the membrane around the bacterium. This process is thought to be caused by a remodeling of the actin-cortex which is located on the cytosolic side of the plasma membrane. After the bacterium is taken up by the cell, the newly formed phagosome is transported inside the cell by molecular motor proteins along cytoskeletal tracks. Neither the dynamics of the binding process nor the mechanical properties of the uptake and the subsequent three-dimensional intracellular transport were investigated so far on the molecular level of nanometers and picoNewtons.

In order to tackle these tasks, optical trapping and tracking tools were developed and their optical properties were compared to theoretical predictions based on Fourier-optics and extended Mie theory (Chapter 2). These tools were then applied to investigate the mechanical properties of phagocytic binding, uptake

and intracellular transport in single living cells (Chapter 3). Finally, a Brownian dynamics simulation was developed to validate the experimental data and to increase to amount of information that can be gained experimentally about mechanical cellular processes (Chapter 4).

2. Optical trapping and tracking

Perhaps in some future time the human mind may succeed in utilising processes and forces that will open new ways to overcome the limitations of resolution, which now seem insurmountable to us ... But I think that the tools which some day will aid our senses ... much more effectively than the contemporary microscopes, will probably have little in common with these microscopes other than the name.

— Ernst Abbe, (1840 - 1905)

2.1 Introduction

2.1.1 Optical tweezers

In 1986 Arthur Ashkin[4] was able to show, that micrometer-sized dielectric particles immersed in water can be stably trapped in three dimensions with a highly focused laser beam. Such an optical trap - also called optical tweezers - was used already one year later to trap viruses and bacteria and to move them in a controlled way.[5]

The usage of optical tweezers allows to apply forces in the picoNewton range to trapped particles and to track them simultaneously with nanometer or even sub-nanometer[6] precision. Due to these possibilities, optical tweezers have become a routinely used tool in physics and biology.[7–10]

They were used in colloidal physics[11] to investigate e.g. excitations in lipid membranes.[12] Other studies explored the entropic interactions in binary colloids[13] or the hydrodynamic coupling of particles to surfaces.[14]

In the field of biopolymer research optical tweezers were applied to study e.g. the mechanical properties of DNA[15–17] or RNA[18] in stretching and unfolding experiments.

As the forces and step sizes of molecular motor proteins are on the level of picoNewtons and nanometers, optical tweezers are in particular well suited for the investigation of these proteins. The mechanochemical properties of the

microtubule-based motor kinesin[19,20] as well as the actin-based motors myosin-Va[21] and -VI[22] were studied *in vitro*. Other motors, which were also studied at the single-molecule level were viral DNA-packaging motors[23,24] or RNA polymerase.[6]

Simultaneous to the broad application of optical tweezers, experimental and theoretical studies were performed on basic aspects of optical trapping.[7,25–28]

2.1.2 Atomic force microscopy

Also in 1986, atomic force microscopy (AFM) was developed by Binning, Gerber and Quate.[29] In AFM, a fine tip attached to a mechanical cantilever is used as a local probe to scan surfaces with nanometer resolution. During the scanning process, the cantilever is bending due to atomic interactions between the tip and the sample. This bending can be measured by detecting a laser beam that is deflected at the back of the tip. The detection of the laser is usually done by using a quadrant photo diode (QPD).

Besides surface topography measurements in air and vacuum, which are widely used in the field of solid state physics, AFM can be also used to study biomolecular structures in liquids. By chemical functionalization of the AFM tip, it is possible to measure specific biomolecular interactions.[30] That way, adhesion forces between individual ligand receptor pairs were measured.[31]

Because of the possibility to work in liquid solutions with a resolution in the nanometer range, the AFM is in principle an instrument to study surface structures of living cells. However there are some restrictions, which hamper its application to cell biological and biophysical questions. Due to the mechanical cantilever it is only feasible to analyze the side of a membrane that is facing towards the AFM tip. To perform measurements inside a living cell, it is inevitable to destroy the plasma membrane of the cell. Furthermore, the forces, the AFM usually exerts on the sample, are sufficiently high to push aside the lipids in the plasma membrane, so that only the more stable cytoskeletal structure is measured.[32]

2.1.3 Photonic force microscopy

The basic idea of the photonic force microscope (PFM)[33–35] is to replace the mechanical cantilever and the small tip of an AFM by an optical tweezers and a therein captured small bead with a diameter of several hundreds of nanometers (Figure 2.1).

In the PFM a trapped particle acts as a probe, which driven by extended Brownian motion scans its local environment. Light scattered by the particle and un-

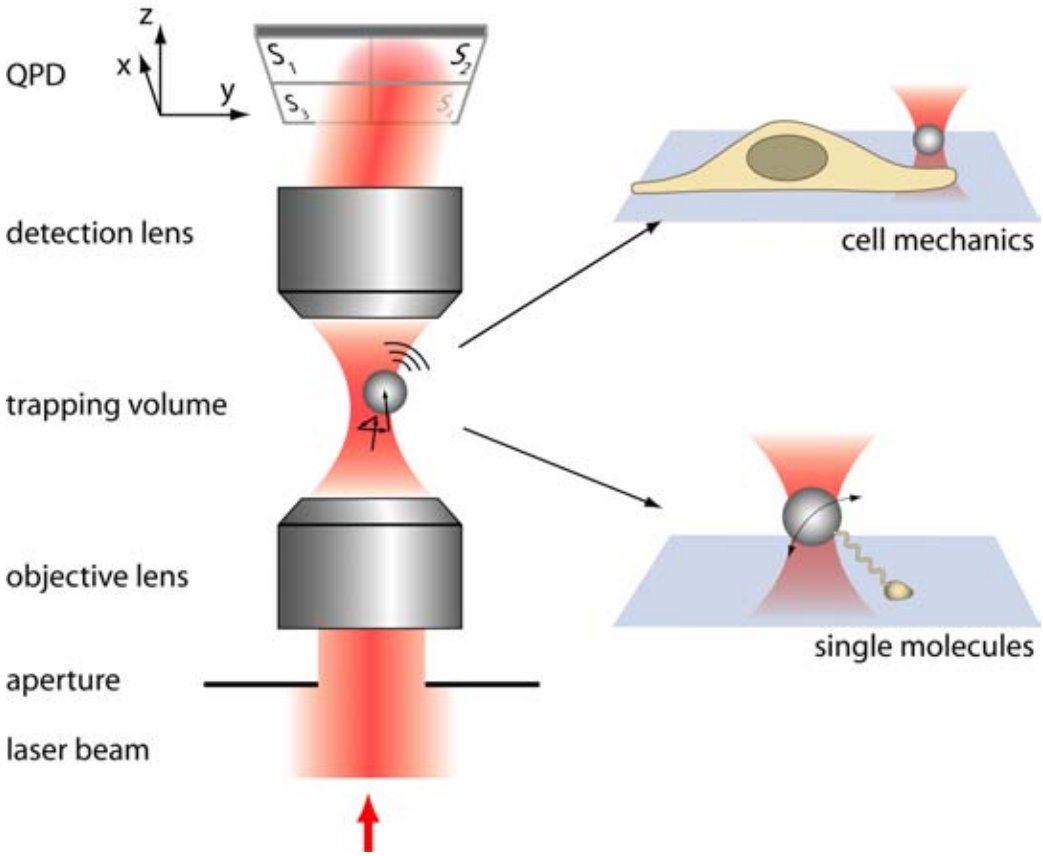

Figure 2.1 Principle of the photonic force microscope. A laser beam is focused by an objective lens with a high numerical aperture. A particle in the focal region interacts with the focused light and the resulting optical forces generate a 3D optical trap. Driven by Brownian motion, the particle fluctuates in the trapping region and thereby scans its local environment. The 3D position of the particle is measured by a quadrant photodiode (QPD) with nanometer precision at up to one MHz sampling rate. This technique is applied in cell mechanical or single molecule experiments.

scattered light generate an interference pattern that is detected by the QPD (Figure 2.1). The signals from the QPD are used to determine the three-dimensional position of the particle with nanometer precision at a rate of up to one Megahertz. Two dimensional position detection of a trapped particle was done first by Denk and Webb[36] and then later by Ghislain and Webb.[37] They were able to measure the position fluctuations of the particle in lateral direction, i.e. perpendicular to the optical axis. Position detection in all three spatial dimensions was first achieved by Friese et al.[38] and Pralle et al.[33] For particles smaller then the trapping laser wavelength, a theoretical description of optical trapping and 3D position detection was done by Rohrbach et al.[27,28,39–41]

The thermal position fluctuations of a trapped particle are altered by its environment due to external forces acting on the fluctuating probe. The PFM is therefore a tool for measuring weak interactions between the trapped probe and its environment by recording the probe's 3D position fluctuations. The instrument is used to investigate diffusion in cell membranes,[42] to image 3D cavity structures,[43] to study the mechanical properties of single molecules,[44,45] as well as surface forces, molecular binding forces, and small variations in local diffusion and viscosity. Other studies used optical tweezers in combination with an interferometric quadrant photo diode (QPD) detection system to track the motion of single molecular motors,[46] and to investigate diffusion within living cells by microrheology.[47]

If an optically trapped fluctuating bead is used as a local probe to image 3D interaction potentials, it is usually displaced from the trap center during the measurement. If - for example - the binding of a bead to a cell membrane is studied to investigated cell mechanics (Figure 2.1 and 3.8), the bead is displaced from the trap center on the one hand due to the binding process itself. On the other hand it is additionally displaced if the membrane moves the bound bead actively. However, the interferometric 3D position detection with the PFM is only unique and linear in limited regions around the trap center.[39,40,48] The unique detection region is the region where the position signals can be inverted in order to provide the unambiguous 3D bead position. In order to know whether the measured QPD signals reproduce the bead movement, it is important to know the exact size of the unique and the linear region in three dimensions. The introduction of a force clamp feedback control system[10,49,50] enables trapping and tracking over a significantly extended range. There, the time-averaged displacement of the bead is compensated by a deflection of either the optical trap or the sample stage.

In summary, the essential components of the PFM[34,51] are the following ones:

- the optical forces of the optical tweezers,
- the 3D detection of the particle's position with a precision of a few nanometers at a sampling rate of up to Megahertz,

- the utilization of the particle's thermal motion to scan its local environment.

In particular, the last item is the extension of the PFM compared to regular optical tweezers systems.

2.1.4 Outline of this chapter

This chapter describes the main components of the PFM setup used in this work. In this context a sample heating device was developed, which keeps (biological) probes at 310 K (37° C) while minimizing thermal drift and thermal equilibration time.

If strong external forces are acting on a trapped particle, it might get displaced from the trap center so far that it leaves the linear or unique 3D tracking region. In order to reposition the particle in such a case, a piezo stage feedback system was set up. The linear and unique 3D tracking regions were measured for various trapping lens numerical apertures. The measured detection signals were compared to theoretical predictions based on extended Mie-theory.

Furthermore, optical force profiles were measured in lateral and axial direction to identify the linear force range. In addition a brief introduction into the theory of optical forces is given together with a comparison of theoretical and experimental optical force constants and force profiles.

Finally a theoretical investigation is done about the usability of cylindrical PFM probes for surface scanning or single-molecule experiments.

2.2 Photonic force microscope setup

2.2.1 Overview

Figure 2.2 shows a picture and a schematic of the main PFM setup parts. An infrared (IR) laser beam is tightly focused by the objective lens (OL) to optically trap small particles. The interference of the unscattered light and the light scattered by the particle is projected by the detection lens (DL) onto a quadrant photodiode (QPD), which enables 3D particle tracking. A halogen lamp is used for DIC and a Hg-lamp for fluorescence imaging.

2.2.2 Trapping and detection optics

An ultra-low noise (< 0.03% between 10 Hz and 10 MHz) diode-pumped Nd:YAG-laser (CrystaLaser) is used for optical trapping and position detection.[34] Its wave-