Summary

This thesis consists of two main parts. The first part concerns the technique of optical trapping and optical trap calibration. In the second part optical traps are used to study the cooperative overstretching of DNA in the presence of a bis-intercalating dye, and collective effects in molecular motors are reviewed.

If light interacts with a particle and the direction of the light changes, momentum is transferred from the light to the particle. Near the focus of a laser the forces associated with these momentum changes can be high enough to capture silica or polystyrene beads, called optical trapping. The beads can be used as handles, which can be conveniently seen in the microscope, to hold and manipulate biomolecules, by attaching DNA, molecular motors, or other biomolecules to them. The force and displacement ranges of optical traps (picoNewtons and nanometers) make them a powerful tool to study mechanical properties and interactions on a single-molecule level. Information on bead displacements and external forces on the bead is contained in the light that is transmitted through the sample. However, in order to extract that information, the trap has to be calibrated. In Chapter 2 the general design of the setups and some of the calibration methods that we have used are described. When the bead is displaced from the center of the trap, a shift in the intensity distribution of the light in the back focal plane of a collimating lens, which depends linearly on the distance moved, is measured using a quadrant photodiode. Chapter 2 describes ways to determine the distance calibration factor, that relates the detector signal in Volts to actual distances, from the Brownian motion of the bead in the trap. If the distance of the bead from the center of the trap is known, the restoring force of the trap on the bead can be calculated using the trap stiffness, which can also be found from the Brownian motion of the bead.

When an oil-immersion objective is used to create the optical trap, the refractive index mismatch between the coverslip and the watery fluid in the sample chamber causes smearing of the focal spot which depends on the distance to the cover slip surface. Especially the high-angle rays, which contribute considerably to the trapping force, stay close to the surface. This causes the trap stiffness to decrease with increasing distance to the surface. In Chapter 3 we measured the dependence of the trap stiffness on the distance to the surface in the presence (oil-immersion objective) and
absence (water-immersion objective) of spherical aberrations, in the lateral and axial direction, for two different bead materials. The experimental data could be explained qualitatively with a model based on the intensity in the focus, irrespective of the angle of incidence. When a water-immersion objective is used to create the optical trap there are two counteracting refractive index mismatches. Therefore the trap stiffness does not depend on the distance to the surface in this case.

Spherical aberrations also influence the distance calibration factor. In Chapter 4 the depth dependence of the distance calibration factor is studied using a novel, active calibration method that consists of rapidly scanning the laser over the bead using Acousto-Optic Deflectors (AODs). This method is particularly useful when some parameters that are needed in the calibration method based on the Brownian motion of the bead are not known. For instance, in dense visco-elastic media, like actin or microtubule networks, the viscous drag coefficient is not known. The active calibration method using AODs has been compared to the passive power spectrum method based on the Brownian motion of the bead. Both yielded the same results. With the new calibration we found that when using a water-immersion objective the detector sensitivity is independent of the distance to the surface, and when using an oil-immersion objective it decreases with increasing distance to the surface.

In Chapter 5 optical traps were used to study the overstretching transition of DNA with the fluorescent dye Atto-Dino 2. Upon intercalation of the dye, which proceeds slowly, the length of the DNA increases, and the overstretching plateau becomes less flat. This is explained as decreased cooperativity due to the prevention by the intercalated dye molecules of large-scale formation of single-stranded (ss) DNA-regions. The binding constant and the size of the binding spot of the dye were found from a fit of the total length increase as a function of dye concentration to the McGhee and Von Hippel binding isotherm. The optical-tweezers setup has been used to measure fluorescence-detected linear dichroism to study the force-dependent orientation of the chromophores with respect to the DNA axis. Starting from zero-force the tension on the DNA is gradually increased, aligning the DNA as well as the chromophores that are bound to it. The effective angle of these chromophores peaks around 73° when the DNA is stretched to its contour length. When the tension is increased into the overstretching force regime the angle decreases slightly, as does the fluorescence intensity. This suggests that the motion of the chromophores in between the basepairs increases.

These experiments are to our knowledge the first tension-dependent linear dichroism experiments to study the overstretching transition of DNA. In future studies of the overstretching regime other dyes could be used, which would improve the signal to noise ratio. By measuring up to higher forces the end of the overstretching regime could be studied. It would also be interesting to follow the association of dye in the sample chamber in real-time, at different tensions. This would make it possible to study the distribution of ss-DNA regions in the overstretching regime, which would show up as dark regions since the dye does not bind to ss-DNA. Fluorescence
anisotropy measurements could provide insight on the degree of movement of the dye while it is intercalated.

Chapter 6 of this thesis is a literature study on different levels of collective effects in molecular motors. Sometimes different motor domains in the same molecular motor need to interact strongly (like in the case of kinesin 1) in order to stay attached to the track for more than one cycle, and different models for processive movement are discussed. In other cases the motors work in large clusters (like in the case of the muscle protein myosin II) that generate their own collective effects, as each motor is mechanically coupled to the others. Theoretical models for collections of motors are discussed, showing how mechanical coupling can lead to spontaneous motion and instabilities, or oscillations when an external load is added. A third example of collective behavior is thin filament regulation by proteins such as the troponin-tropomyosin complex. Upon binding of Ca\textsuperscript{2+} to troponin C conformational changes through the complex open up seven myosin binding spots on the actin that were not accessible at first.

In recent years the focus in optical trapping experiments has been on single-molecule experiments. However, this study shows that optical trapping experiments with multiple motors, different combinations of motors, or regulated tracks could provide novel insights into collective effects in larger, multi-molecular motor structures.