Summary

DNA contains the genetic instructions specifying the biological development of all cellular forms of life. DNA is transcribed to produce messengerRNA, which in turn is translated by ribosomes into proteins that are the "the machinery of life". These proteins perform a crucial role in many processes within the living cell. Understanding interactions between these molecules is enhanced by single molecule force spectroscopy and imaging techniques that yield new information such as the structure of these molecules, the stoichiometry in which they form complexes, the forces that hold them together and the mechanical and dynamic behavior of individual molecules and complexes thereof. With conventional techniques these properties are masked in the ensemble average.

A single molecule force spectroscopy technique that allows mechanically stretching of a single DNA molecule while simultaneously measuring the forces acting on the molecule is optical tweezers (OT). This technique is capable of holding small micron-sized spherical particles in the focus of a strongly convergent light beam, as demonstrated in the late eighties by Ashkin *et al.* (1986). A displacement of these spherical beads within the focus can be related to the force required to initiate this positional shift. The beads form a perfect handle for fixing the ends of a single DNA molecule, allowing the molecule to be stretched, from which the mechanical properties such as the Young's modulus and the persistence length of the molecule can be deduced.

Nowadays the technique is mostly applied to study DNA-protein interactions in which structural changes and mechanical properties are altered or displacements of the molecule are generated. Important information that is not obtained by OT is the positional information about the DNA-binding proteins. The main goal of the work described in this thesis is to construct a microscope in which OT and scanning probe microscopy (SPM) are combined, to be able to localize proteins while simultaneously controlling the tension within the DNA molecule. This apparatus enables the study of the effect of tension in the molecule on the functional properties of DNA-binding proteins. An advantage of the 'Scanning Probe Optical Tweezers', i.e. SPOT-microscope, is that the DNA-protein interactions are not obstructed by the presence of a supporting surface as is the case for imaging using conventional SPM techniques.

The SPOT-microscope consists of a reflection-based OT apparatus in combination

with a MWNT probe fixed to a glass μ-pipette. This probe was injected through an entry-hole into a flow cell in which a single DNA molecule was suspended between two 2.6 μm polystyrene beads, one of them immobilized on a μ-pipette integrated in the flow cell and one held by the optical trap. To allow the probe to get close to the optical trap and the DNA molecule, the backscattered trapping laser light from the trapped bead was used for force detection as opposed to the transmitted light as used in most optical tweezers instruments. In the development of the reflection-based OT apparatus we found that the use of a position sensitive detector for measuring the deflection of the backscattered laser light resulted in a linear detection range of 0.57 μm for 2.5 μm beads, a factor of 2 higher than obtained with a quadrant detector.

Furthermore the frequency response of the detector plays a crucial role in the trap stiffness calibration, which is essential in force spectroscopy. It is obtained by fitting a Lorentzian function to the power spectral density of the deflection signal. Due to the transparency of silicon for near-infrared light (>850 nm) the detection bandwidth was limited. Several detectors were characterized as a function of the wavelength, applied bias voltage and total light power, using a two-LED wobbler system. In AFM and OT applications the low-pass effect of the commonly used silicon detectors leads to serious errors in the force constant determination of the probe. We have shown that this low-pass effect can be compensated for using the frequency response of the detector as determined with the LED-wobbler.

Scanning back and forth with a pipette with an ending diameter of 2 μm and a MWNT (40-90 nm) as probe, we were able to localize single 450 nm beads coupled to the middle of the DNA molecule. The main differences found for these two probes was the magnitude of the interaction forces, 68 pN for the pipette and 40-50 pN for the MWNT that resulted in a different interaction range, 1.5 μm for the pipette and 0.5 μm for the MWNT.

We also localized DIG-sites with an α-DIG coated pipette (2 μm diameter), using the strong binding affinity of this antibody-antigen pair. This strong affinity resulted in a high interaction force of the probe with the DIG molecules ($\sim$13) that were located over a range of 90 nm approximately in the middle of the DNA molecule. Due to the convolution of this range and the size of the μ-pipette the onset of the force in different scans was spread over a range of approximately 1 μm.

We have shown that the SPOT-microscope is capable of 'feeling' individual beads and small molecules on a single DNA molecule. The next step is to really localize individual proteins, where the interaction force between the probe and the proteins will play a crucial role. Instrumental improvements such as further development of the scanning probe and the detection scheme are possible ways to achieve the full potential of the SPOT-microscope.