1.4 Outline of this thesis

This section gives a brief summary of the contents per chapter in this thesis.

Chapter 2. Kinesin walking through the spotlight

In this chapter the confocal approach is described in detail, including the setup and data-analysis. The velocity distribution obtained with this approach is compared to the velocity distribution determined with classical wide-field fluorescence microscopy. We show that our confocal approach can determine the velocity of a single passing motor. Furthermore the photo-bleaching behavior of the attached fluorophores while traversing the confocal spot in combination with the increasing signal to noise ratio at increasing excitation intensities is discussed. The correlation technique for this type of data is introduced and via simulations its power in discriminating submillisecond intensity fluctuations in the fluorescence signal is investigated.

Chapter 3. Kinesin’s step dissected with single-motor FRET

Four different homodimeric constructs of kinesin, each with a specific single-cysteine on each motor domain, are labeled with a donor and an acceptor fluorophore. Both the donor and acceptor fluorescence intensities are measured simultaneously and FRET between the two is observed for two constructs. These experiments show that an intermediate state exists (∼3 ms) when kinesin steps at saturating ATP concentrations (step time is ∼12 ms). This intermediate state has one-motor domain bound to the microtubule while the other motor domain is not microtubule-bound, is rotated and close to the microtubule-bound domain. It is proposed that this state occurs directly after ATP binding and precedes ADP release of the microtubule-unbound motor domain.

Chapter 4. Alternating-site catalysis by Kinesin-1 confirmed with single-motor FRET using fluorescent ATP analogues

The binding and unbinding of single-fluorescently labeled ATP molecules was studied using single-labeled kinesin motors in our confocal setup. We observed kinesin moving processively in the presence of only fluorescently-labeled ATP and determined the Michaelis-Menten parameters. For fluorescently-labeled ATP \( v_{\text{max}} = 247 \pm 99 \text{ nm/s} \) and \( K_M = 32 \pm 22 \mu\text{M} \). For regular ATP \( v_{\text{max}} = 575 \pm 9 \text{ nm/s} \) and \( K_M = 13.4 \pm 0.6 \mu\text{M} \). After analyzing the autocorrelation of many single events in the presence of fluorescent ATP or mixtures of fluorescent ATP and regular ATP we found that the average fit parameters are well described by simulations of models that obey the alternating site mechanism, whereas single-site catalysis models all yielded poor fits to the obtained data.
Chapter 5. Novel ways to determine Kinesin-1’s run length and randomness, using total-internal-reflection fluorescence microscopy

The goal of the research described in this chapter was to develop an experimental approach that can determine important motility parameters of kinesin with a measurement technique that is available to many research groups in the world. We used TIRF microscopy to obtain three key motility parameters of kinesin; the velocity, the number of rate-limiting steps in one single 8-nm step and the run length, all at different ATP concentrations. The average run length of hundreds of motors is obtained in a few minutes by analyzing the intensity profile over a microtubule that contains many labeled kinesins. Although single motors can not be discerned in this approach, the average run length can be obtained by analyzing the shape of the concentration profile. This approach is quick compared to other available techniques and does not require any correction for photo-bleaching due to the low excitation powers. We measure an average run length of 1220 ± 50 nm that is independent of the ATP concentration. The velocity is determined by analyzing the displacement-trajectories of many individually-tracked labeled kinesin motors. Analysis of the variance of the displacement trajectories allows us to determine the number of rate-limiting steps in one single 8-nm step, an analysis technique generally referred to as randomness analysis, and we verified that the randomness at saturating ATP lies between 0.33 and 0.5.