Summary

In the rapidly developing field of nanotechnology where objects of ever smaller sized are fabricated, ever more sensitive methods are needed to detect and analyze these objects in detail. Detection by optical means is often preferred since the optical approach is non-invasive, operates under ambient conditions, is chemically specific and not limited to the surface. Chapter 1 describes some of the challenges that need to be overcome when studying individual nanoparticles. Detection is hampered by the tiny cross-section, as only a small fraction of the incident light will interact with the particle. The unperturbed light will lead to a large background from which the signal of interest has to be recovered. The most commonly used optical methods are based on the detection of non-resonant fluorescence in combination with filters to reject the resonant background light. Indeed, individual fluorescent molecules are being studied in detail and widely used as local reporters in, for instance, life sciences. The reliance on fluorescence comes however at a price. Fluorescence is a slow process, thus limiting the timescales that can be studied. The ultrafast (femto and picosecond) molecular processes that play an essential role in a large number of important systems, such as the energy transfer in light harvesting complexes can therefore not be studied directly on a single molecule level. A second disadvantage of fluorescence based detection is that molecules with sufficient fluorescence efficiency and stability are required, which severely restricts the range of systems that can be directly observed. In this thesis we explore novel routes to address both the ultrafast time scales and the detection of single non-fluorescent nanoparticles.

A novel detection method that enables ultrafast processes to be probed on the single molecule level is presented in the second chapter. While still detecting the fluorescence the intricate balance between stimulated absorption and emission is used to probe the initial energy redistribution of the electronic excited state. In this Single Molecule Pump Probe (SM2P) experiment, two short saturating pulses with variable delay are used to excite a molecule. A short intense laser pulse will, under ambient conditions, leave a complex organic molecule in the excited state at most fifty percent of the time. On

a femto- to picosecond timescale, the molecule will relax from the initially excited vibrational state to the groundstate of the electronic excited state via interaction with other vibrational states. From this lower lying state the molecule will return to the groundstate on a nanosecond timescale by emitting a fluorescent photon. A second pulse arriving at the same time has an equal probability of exciting the molecule from, or stimulating the molecule back, to the electronic groundstate. The total fluorescence probability will therefore not increase by adding a second pulse at zero delay. However, when a second pulse arrives after the molecule has had sometime to redistribute its energy, the second pulse can no longer stimulate the molecule back to the groundstate. Therefore, the total fluorescence probability increases when two pulses are used with some delay between them. By varying the delay between the two pulses and measuring the resulting change in fluorescence, the timescale of the energy redistribution is probed.

In chapter 2 the SM2P setup is described in detail. Simulations are presented showing that redistribution times even shorter than the pulse length can be recovered with confidence. Experiments are presented on a number of common single molecule fluorophores in varying matrixes. It was found that chemically identical molecules in the same sample can have widely varying energy redistribution times. Varying the matrix or the excitation wavelength did not result in different average redistribution times. Chemically different molecules however did show different average redistribution times. This leads us to conclude that the processes responsible for the initial energy redistribution are mainly *intra*molecular rather than *inter*molecular. The large variations from molecule to molecule in the same sample are attributed to conformational variations induced by the nanoenvironment, resulting in differences between the coupling of the electronic and nuclear degrees of freedom. The presented experiments reveal for the first time the ultrafast energy pathways in individual molecules.

Such ultrafast processes are particularly relevant in excitonically coupled systems where the excited state energy is delocalized over two or more chromophores. The extent of this delocalization is dependent on, and reduces, the interaction between the electronic and vibrational modes of the molecule. With the SM2P method we now have for the first time the ability to study these interactions which occur on a femto- to picosecond timescale, on individual coupled molecules. SM2P results on a model trimer system, consisting of three identical subunits arranged in a head-to-tail fashion are presented in chapter 3. The excitonically coupled molecules show a much longer energy distribution time than the monomeric subunits, indicating that indeed the interaction between the electronic and vibrational modes is reduced leaving the

molecule longer in the initially excited state. These SM2P experiments are complemented by single molecule spectral data that also show a decrease in the vibrational coupling and lifetime measurements that allow the excitonic coupling strength to be measured. For the first time, our ultrafast experiments directly show the relation between the electronic-vibrational coupling and the exciton delocalization length on individual molecules.

To extent the range of single nanosystems that can be studied, we have developed a novel contrast mechanism that does not have the drawbacks of fluorescence based detection. In chapter 4 it is shown that different types of nanoparticles can be detected by their modification of the polarization state of the transmitted light. In a heterodyne interference polarization scanning optical microscope (HIPSOM) the incoming laser light is split in two parts, forming a signal and reference branch. The light in the signal branch is focused on the sample and the transmitted light is collected and mixed with the reference branch. By selecting orthogonal polarization states in the reference and signal branch only light that has interacted with the particle and undergone a polarization change will contribute to the interfere signal. The strong reference branch selectively amplifies this signal, resulting in a background free, shot-noise limited detection scheme. A signal-to-noise analysis shows that the detection of individual molecules based on their dipolar absorption is feasible with this setup. Furthermore, both amplitude and phase of the field scattered by nanoparticles are retrieved. In chapter 4 results are presented showing the detection of gold nanoparticles with a diameter of 2 nm and a signal-to-noise better than 10. Owing to this excellent signal-to-noise the position accuracy for 6 nm sized particles is better than 3 nm.

In chapter 5 a systematic study is presented into the image formation mechanism for gold nanoparticles with a diameter between 2 and 100 nm. Following Mie theory it is shown that for particles much smaller than the incident wavelength (<10 nm) the scattered field can be accurately described by that of an oscillating dipole. The orientation of this induced dipole is parallel to the incident polarization. In the far field the radiated dipole field is again parallel to the dipole orientation, such small particles will therefore not lead to a polarization conversion and not result in any signal in the HIPSOM. However, due to strong focusing the light just outside the center of the focus does undergo a polarization conversion. This converted light is scattered by the particle and results in detectable signal when the particle is at four positions just outside of the center of the focus resulting in the observed cloverleaf pattern. The field scattered by larger particles can no longer be accurately described by a dipole field, and higher order terms are needed. This results in depolarization of the incident field even when the

particle is in the center of the focus. For larger particles (>15 nm) the recovered pattern was observed to transform from the cloverleaf pattern to a single gaussian feature. The sharp transition allows particles of different size to be accurately discriminated. The specific particle size where the transition occurs can be varied by changing the numerical aperture of the illumination objective. The ability to measure both the phase and the amplitude of the scattered field makes the HIPSOM a valuable to tool to study individual nanoparticles.

The new methods and results presented in this thesis open the door for new research directions in the ultrafast and ultrasensitive detection of individual nanoparticles. Chapter 6 presents some of the systems that can be addressed with these novel techniques. Finally, ideas are presented to further improve the versatility of both experimental configurations.