Absorption, luminescence and scattering of single nano-objects

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CHAPTER 1

Introduction

In this thesis, we study radiative (luminescence, scattering) and nonradiative (absorption) properties of single nano-objects, in particular single molecules, organic dye nanoparticles, and gold nanoparticles. We gain insight into the effect of physical structure and composition on the optical properties of a single nano-object by means of correlated absorption, luminescence, and scattering signals. In this introduction, we give a brief overview of single nano-objects, we describe their optical properties, and provide a short description of the methods for optical detection of individual nano-objects. We also discuss the advantages of studying single particles and point out the specific requirements and advantages of each detection method. At the end, we provide the contents of the following chapters of the thesis.

1.1 Single nano-objects

Nano-objects, with dimensions between atomic scale and bulk, include isolated molecules, semiconductor nanocrystals, organic dye nanoparticles, metal colloids, etc. They exhibit novel material properties that differ from bulk properties, e.g., the optical properties of nano-objects depend on their chemical structure, composition, size, shape, and local environment. A variety of nano-objects with tunable optical properties can be synthesized. In order to use nano-objects for their applications, it is of fundamental interest to understand how the optical properties change as functions of physical properties and local environmental conditions . After the optical properties

are characterized, nano-objects find novel scientific and technological applications. For example, organic nanoparticles have become building blocks of organic light-emitting diodes, thin-film transistors and photovoltaic solar energy collectors.[1,2] Semiconductor nanocrystals and single molecules serve as microscopic single-photon sources[3–5] and they pave the way for quantum information processing.[6–8] Gold nanoparticles provide very stable optical signals and they serve as contrast agents in microscopy.[9–12] They are also convenient tools for optical sensing[13–16], because their optical properties are very sensitive to changes in their local environment. Depending on the experimental conditions, they can be nontoxic and be used for biomedical applications.[17] Their potential use as novel agents for cancer therapy was reported.[18–21] Gold nanoparticles also have potential for applications in data storage media, since they can increase the capacity of standard DVDs by several thousand times.[22] Additionally, nano-objects have found applications for technological advances in our daily lives. For instance, harmful effects of sun's ultraviolet radiation are diminished by coating glasses with nanoparticles which reflect ultraviolet rays. Antibacterial protections in refrigerators and dishwashers are made by covering the inner walls with metal nanoparticles. The number of examples of these applications is increasing everyday. Scientific and technological developments continue to benefit from fundamental insights into materials' intrinsic properties at the nanoscale. This thesis contributes to understanding the intrinsic properties of single nano-objects and further extends the potential of nano-objects for intriguing applications.

1.2 Advantages of single nano-object studies

Investigations of material properties of nano-objects have mostly relied on measurements from ensembles. Even with the best synthesis method, however, it is almost impossible to obtain nano-objects with the same size and shape. The distribution of material properties causes an inhomogeneous broadening of observed signals. The details are usually not accessible and the observations suffer from averaging. Furthermore, signals which are obtained from ensembles of nano-objects are liable to error because of heterogeneity. For instance, scattering signals quickly decay for metal nanoparticles with sizes smaller than 30 nm. In a suspension of nanoparticles, aggregated

nanoparticles may be present and they have much stronger scattering signals than single particles. Thereby, the ensemble average diameter, which is measured by scattering methods, can be biased towards larger sizes. Despite their known drawbacks, ensemble methods remain irreplaceable because the obtained data from optical signals still carry a wealth of information. Moreover, the experiments can be performed in a relatively simple way (both from equipment and sample preparation points of view).

Although experimental studies on single nano-objects are more challenging, they have significant advantages over measurements on ensembles:

1. It is inevitable to have a distribution of sizes and shapes even with the best synthesis methods. Distribution of variables in a sample is only accessible by measuring properties at the single-particle level. In addition, several optical properties of the same nano-object can be correlated. Fundamental understanding and novel insight into the effect of size and shape on the optical properties become accessible by combining optical and electron microscopy on the same particles.[23]

2. Single nano-objects can be used to probe their nano-environment and they can serve as sensitive optical sensors. Optical signals may vary due to spectral or orientational changes of nano-objects. Fluctuations of the optical signal from a single nano-object can be directly observed. They can be followed to study nanoscale environmental changes, such as refractive index, viscosity, etc.[24] The sensitivity of optical properties to local changes is also enhanced since the inhomogeneous broadening is eliminated. For instance, the red-shift in the plasmon resonance of a gold nanorod related to an increase in the effective refractive index of the medium is sensitive enough to detect the binding of single non-absorbing molecules.[25]

3. Optical signals from single nano-objects can be localized and used to determine positions of nano-objects. Single-molecule trajectories can be determined by precise determination of a molecule's position as a function of time. Their quantitative analysis can be applied to learn about intracellular transport.[26,27] It is also possible to obtain 2-dimensional[28] and 3-dimensional[29,30] trajectories of single gold nanoparticles, which are diffusing in living cells to obtain information about biological events.

4. With single-particle measurements one can select and study rare cases of objects which would be very difficult to extract from ensembles, e.g., pairs of gold nanoparticles.[31,32]

5. Single nano-objects can be harnessed as nano-sized tools. For example, they can be used as local heat sources for cancer therapy.[21]

1.3 Optical properties of single nano-objects

The first far-field optical detection of single molecules at low temperature[33] opened the way for new experiments which could only be performed in ensembles previously. Later, far-field optical methods became more advanced with modern optics and detectors[34–37] and in parallel single molecule fluorescence studies have been extended to detection and spectroscopy of different nano-emitters and nano-absorbers.[38–44] Nano-objects of different sizes, shapes and compositions exhibit a large variety of optical properties.

Single molecules are small (a few nanometers) quantum systems. A molecule can be excited with a few μWs of an excitation light within its absorption band. Fluorescent molecules release the absorbed energy mostly by emitting photons at longer wavelengths than their excitation. The fluorescence signal is then easily separated from the excitation light. Fluorescent molecules are used as valuable tools for a variety of applications ranging from biophysics to soft-matter studies.[24,45–48] They can serve as labels to tag non-fluorescent molecules (protein, DNA, etc.). For instance, kinesin, a molecular motor, was tagged with a fluorescent Cy3 molecule to understand its movement along microtubules. This way, new insight into cellular functions such as cellular cargo transport was gained.[49] Another example is dynamics of polymers near the glass transition temperature. Orientational and translational diffusion of a molecule which was embedded in a polymer matrix were studied to understand the heterogeneity of polymer above and close to glass transition temperatures.[50] For fluorescence detection of single molecules, it is more advantageous to use a molecule which has short fluorescence life-time, high quantum yield, strong one-photon absorption and high photostability. However, these characteristic properties are difficult to obtain and a large number of synthesized molecules do not meet requirements for fluorescence detection. Blinking[51,52] and photobleaching[53] of sin-

gle molecules usually limit the overall experimental process by reducing the time over which the sample can be observed. Moreover, there are many naturally created important molecules (such as DNA) which do not emit photons efficiently. For these reasons, absorption-based detection methods can be complementary to fluorescence-based methods for investigation of molecules which efficiently absorb light but are not necessarily fluorescent (see Chapter 2). These methods would open up new perspectives for a wide range of molecules.

Organic dye nanoparticles are solid particles that are composed of organic compounds. Reprecipitation has been widely employed for preparation of organic nanoparticles due to its simplicity and its ability to control sizes.[54,55] Sizes of organic dye nanoparticles range from tens of nm to micrometer and their morphology is different from inorganic particles such as semiconductor and metal nanoparticles.[54]. Organic dye nanoparticles consist of many molecules, because the molecules are usually compactly packed in the nanoparticle.[55,56] Van der Waals intermolecular interactions and coverage of nanoparticles surfaces with molecules can, in principle, complicate their investigation.[57,58] For instance, fluorescence quenching can occur due to formation of dark $\pi - \pi$ stacked complexes. Fortunately, quenching is usually precluded by introducing bulky or twisted groups to the dye such that these groups restrict intramolecular aggregation of dyes in the nanoparticle.[55,57,59] Moreover, molecules with weakly overlapping absorption and luminescence spectra are usually selected for the formation of dye nanoparticle to minimize the reabsorption of the emitted light within the nanoparticle.[55,57] Thus, organic nanoparticles can produce high fluorescence signals for their detection. In addition to their luminescence, absorption signals can also be used for the detection of dye nanoparticles, which exhibit significant radiationless deactivations through efficient vibrational processes (see Chapter 3).[56] These particles have found diverse applications over the past decades in bioimaging[55,60] and biotechnology studies.[61] For instance, the use of organic nanoparticles as drug delivery systems was recently reported.[61]

In addition, organic nanoparticles can form dewetting-induced short (several microns) or long range (several millimeters) ordered patterns on their thin films.[56,62–64] These thin films could serve as building blocks of organic transistors, organic light emitting diodes, etc.[2,65] However, revealing single morphological domains among patterns with complex 3D structures

can be difficult. For instance, near-field scanning optical microscopy (NSOM) is in principle very useful for morphological and optical correlations.[66] However, the depth of field of NSOM can be limited for studying patterns with different heights compared to that of conventional optical methods. Recent investigations of single organic nanoparticles and their patterns due to the aforementioned difficulties were performed by single-particle spectroscopy. These studies have led to novel discoveries about the photophysical properties of organic dye nanoparticles and of their patterns.[43,56]

Gold nanoparticles are complex many-electron systems. Electromagnetic resonances due to collective oscillations of conduction electrons, called surface plasmons, give fascinating properties to gold nanoparticles. The resonant nature of the surface plasmon makes gold nanoparticles very efficient absorbers and scatterers of light, and their detection by optical methods turns out to be relatively easy. The photoluminescence quantum yield of individual gold nanoparticles can be as high as 10^{-5}.[67] Although the quantum yield of gold nanoparticles is much smaller than the quantum yield of single molecules, gold nanoparticles are still easily imaged by their photoluminescence since they absorb many more photons than single molecules (see Chapter 4 and 5). Unless nanoparticles exhibit any high temperature mediated shape transformation[68,69], their optical signals are perfectly photostable making them suitable candidates for numerous applications where high photostability and brightness are required.[11,70,71] Thus, gold nanoparticles provide advantages over common optical probes such as single fluorophores and quantum dots, which usually suffer from intermittency or loss of fluorescence signal.[51]

The optical properties of gold nanoparticles depend on their size, shape and local environment. The amount of absorbed and scattered light by gold nanospheres at a given wavelength in a homogeneous medium can be calculated analytically from Mie theory.[72] For gold nanospheres, the resonance occurs at a frequency which corresponds to green light. For this reason, a suspension of gold nanospheres appears red in transmission and greenish in scattering.

In the case of gold nanorods, there are two resonant modes, which are due to collective oscillation of conduction electrons along the long and the short axis of the nanorod. These modes are called longitudinal and transverse plasmon resonances, respectively. It is interesting that the longitudinal plasmon

resonance can be tuned from visible ($\sim$ 600 nm) to near-infrared (850 nm) by changing the aspect ratio of the nanorod from 2 to 4.[73] In addition, optical signals of gold nanorods depend on the relative orientation of the nanorod with respect to the excitation polarization.[74–76] The tunable and polarization-dependent optical properties of a gold nanorod can find applications as local sensors at the nanoscale.[71,77]

The surface plasmon resonance of gold nanorods are also sensitive to the changes in their local dielectric environment. For instance, the plasmon resonance wavelength shifts to longer wavelengths when the refractive index of the medium is increased. The sensitivity to refractive index changes is higher for nanorods with a larger aspect-ratio.[78] This sensitivity is exploited in a wide range of applications, such as gas-sensing or molecular binding.[15,79–81]

1.4 Optical detection of single nano-objects

Investigating optical and physical properties of nano-objects on an individual basis requires sensitive methods. Technological developments combined with increasing interest in scientific and technological applications of single nano-objects have led to recent advances in methods to study properties of nano-objects at the single-particle level. Compared to scanning probe microscopy methods, optical techniques are much less invasive and relatively easy to adapt. Optical methods can access individual nano-objects in very complex environments (i.e. cell) or in thick specimens (i.e. polymer film).

Single-particle spectroscopy is applied to nano-objects by studying only one nano-object per laser spot. Nano-objects are separated by distances larger than the size of a laser spot (a few hundred nanometers) in order to inhibit interactions between individual nano-objects ensuring the investigation of at most one particle at a time.

Fluorescence microscopy is a commonly used technique and is capable of detecting luminescent single nano-objects with high sensitivity. When luminescence is too weak to be detected, scattering or absorption methods can be used to detect single nano-objects. The dark-field scattering signal can be an option for plasmonic particles with sizes larger than 30 nm in diameter. For nano-objects that are smaller in size and very weakly luminescent, absorption detection methods can be employed since they are sensitive to very small sizes[38] (of the order of a molecule's size[82]). Moreover, absorption methods

can be applied to any nano-object that absorbs light efficiently.[38,39,82,83] If it is possible to access both radiative (fluorescence, scattering) and nonradiative (absorption) signals, it is also interesting to correlate different signals of the same nano-object. In the following section, we describe the above-mentioned complementary techniques that we have used for the investigation of single nano-objects.

Fluorescence spectroscopy

Fluorescence microscopy has been extensively utilized for detection of single fluorescent nano-objects since the first single-molecule fluorescence detection by Orrit and Bernard in 1990.[33] It provides a broad range of methods for sensitive fluorescence detection of organic or inorganic substances.[84] The luminescence signal is obtained after the nano-object is excited with light in its absorption band. The signal can easily be isolated from background scattering of excitation light using proper filters. Thus, a very high signal-to-noise ratio can be achieved.

Wide-field as well as confocal techniques can be utilized for fluorescence detection of single nano-objects. In wide-field luminescence microscopy, a collimated beam (a few tens of micrometers in diameter) is used to illuminate the sample making it possible to simultaneously detect and follow all luminescent nano-objects distributed over the illuminated area using a CCD or an image-intensifier. In this technique a background signal that originates from emissions away from the focal plane reduces the image contrast. Alternatively, a total internal reflection configuration can be used to largely reduce the out-of-focus signals. In this configuration, an excitation beam is incident on a sample at an angle larger than the critical angle. An evanescent wave is formed on the surface at the light-reflection interface, and the intensity of this wave decays exponentially away from the surface. Only a thin layer ($\sim$100 nm) above the sample is illuminated, therefore the background signal is largely reduced.

In the confocal method, an area ($\sim 10^5$ nm^2) of the size of a diffraction limited spot on the sample is excited. The fluorescence signal is imaged onto a photodetector through a pinhole which is placed in the imaging plane of the detection path. Images with better contrast can be obtained since the luminescence from out-of focus planes of embedding medium is rejected sup-

pressing the fluorescence background. It is also possible to record 3D images with this method by moving the sample or the pinhole in 3D.

Although fluorescence microscopy is very sensitive and has a variety of applications, it can only be applied to luminescent nano-objects. In addition, blinking or bleaching of single molecules or semiconductor nanocrystals decreases their observation time. In order to detect nonluminescent nano-objects, alternative methods are required.

Scattering spectroscopy

Light that is scattered off nano-objects can be used as an alternative signal to fluorescence. The suitability of scattering methods for detection of tiny particles, with sizes below the wavelength of light, was first demonstrated around 1900.[85] The technique has become useful for characterization of spectral and polarization properties of single nano-objects (e.g. silver nanoparticles, gold nanoparticles, quantum dots).[9,77,86–88] In order to detect single nano-objects with this technique, which is mostly applied for detection of plasmonic nanoparticles, either the scattered light alone or the interference of the scattered wave with a reference wave can be used. In dark-field microscopy, light that is directly scattered from a particle is detected on a dark background.[9,89] The advantage of dark-field methods is that it is relatively easy to implement and requires only a standard microscope equipped with low-cost objectives. Wide-field images are recorded in which several hundreds of nanoparticles are visualized simultaneously. Gold nanoparticles as small as 30 nm can be detected using this technique. However, the directly-scattered light intensity varies with the sixth power of the radius of particles and decreases steeply for particles with diameters smaller than 30 nm. The background signal from other scattering objects dominates and nanoparticles can no longer be distinguished.[71]

Nanoparticles with diameters smaller than 30 nm can be detected through bright-field illumination, exploiting the interference of the scattered field ($E_s = sE_i$) with a reference field ($E_r = rE_i$). The detected intensity is the square modulus of the sum of the reference field and the scattered field $I_d = |E_r + E_s|^2 = E_i^2 \left(r^2 + |s|^2 - 2r\,|s| \cos\theta \right)$, where E_i is the incident wave and θ is the phase difference between the two fields. In bright-field experiments, direct scattering signal $|s|^2$ is negligible for small nanoparticles. The

signal has to be detected against a background signal $|r|^2$ and scales with the interference term $-2r\,|s|\cos\theta$. The interference signal scales with the amplitude of the scattered field. Therefore, the interference signal varies with the third power of the radius of the nanoparticle and is more sensitive to small nanoparticles than the dark-field signal. However, this method lacks specificity for the scattering signals which can also be generated from other scatterers such as impurities, roughness, or local refractive index inhomogeneities. There is a background signal against which the interference signal should be distinguished. Therefore, the scattering signals derived from the interference are usually sensitive to particles down to 10 nm in diameter.[86,87] For detection of individual nanoparticles smaller than 10 nm, absorption based methods, which can create an image contrast only for absorbing objects and suppress background signal from other impurities or interfaces, can be advantageous.[90]

Photothermal (absorption) microscopy

Only a few existing methods have been proved able to directly detect absorption of individual molecules and other nano-objects at room temperature.[42,82,88,91–94]. Photothermal (absorption) microscopy is one of these.[38,39,82,83,90,95–98] In this method, a heating beam is chosen in the absorption band of nano-objects and its intensity is modulated. After a nano-object is excited by the heating beam, the absorbed energy is released as heat. As a consequence, an inhomogeneous refractive index profile builds up around the nano-object, i.e., a time-dependent thermal lens. The thermal lens scatters a probe beam, which is often tuned in a transparency region of the nano-object. The incident wave itself or the reflection of the incident wave from glass-medium interface can be used as the reference field.[95] Interference of the scattered field with the reference field creates small modulated changes in the detected intensity of the probe light. The photothermal signal is obtained by isolating the small modulated component from the detected probe intensity using a lock-in amplifier. In this method, the probe intensity can be very high and the photon noise on the probe beam is considerably reduced. Moreover, the signal is proportional to the nano-object's absorption cross section which scales as the volume of nanoparticles and the background signal from impurities or interfaces is suppressed. Thus, nano-objects with very

small sizes can be detected with this technique.[38] For instance, the photothermal technique is capable of detecting gold nanoparticles with sizes down to 1.4 nm[38] and its sensitivity is not limited to gold nanoparticles.[39,83,96,99]. Moreover, the recent advancements in absorption methods have led to push the sensitivity of absorption-based detection methods to the single-molecule level.

Detection of a single molecule by its absorption used to be a challenging task which could be achieved only by low-temperature experiments until very recently.[100–103] These experiments were favored by the fact that the peak absorption cross section of a molecule is increased by a factor of about 10^6 compared to room temperature values. At ambient conditions, the absorption cross section of a single molecule is very small compared to the diffraction-limited area of a focused laser light. Only one photon in ten millions is absorbed by a molecule which has absorption cross section of ~ 1 Å^2 and is excited with a diffraction-limited laser spot. In addition, rejection of background is not as easy as in fluorescence microscopy methods in which the background is largely removed by proper interference filters.

In 2010, three different groups have independently applied three different methods that allowed the detection of single molecules by their absorption at room temperature. Recently, we have improved the sensitivity of photothermal detection method enabling us to detect single organic molecules by their absorption with a signal-to-noise ratio of 10 in an integration time of 300 ms at room temperature (see Chapter 2).[82] These methods open up new perspectives for utilization of a wide range of molecules, which efficiently absorb light but do not necessarily fluoresce.

After our work, two papers reported single-molecule sensitivity in absorption. Sunney Xie's group has used a ground state depletion microscopy which employs two lasers on resonance with the absorption of a molecule. The first (pump) beam was modulated at 1.75 MHz. It depleted the ground state of the molecule giving rise to a modulation of the absorption of the second (probe) laser at a nearby frequency. The absorption signal was obtained by separating the high frequency fluctuations of the probe laser.[91] This technique is similar to photothermal microscopy in that it detects cross-talk between two beams at different frequencies, but relies on an electronic nonlinearity instead of a thermal one. Repeated line scans are followed to detect absorption of a single molecule and relatively high excitation powers are re-

quired for repeated saturation of the molecule. It is still a challenge to detect dyes with lifetimes of the order of picoseconds or shorter with this method.

Sandoghdar's group utilized a direct extinction method in which the attenuation of the light beam was observed in transmission by direct absorption of a molecule. The sensitivity of this conventional technique was improved by using a balanced photodetector to compensate for the laser noise and by an index matched sample geometry to suppress background scattering.[92,104] Inhomogeneities, e.g. topographic or refractive-index variations, in the sample give rise to background fluctuations which reduce the image contrast. This technique requires averaging of several tens of images to create a contrast for attenuation of light through single-molecule absorption which limits its time resolution. This technique also requires index-matching conditions and minimizing surface roughnesses. Measurements in heterogeneous samples are challenging with this approach.

1.4.1 Correlation of absorption, luminescence and scattering

Simultaneous insight into absorption and luminescence properties further opens new perspectives on the photophysics of labels. Combining different signals of the same label can also expand the observation time and tracking capabilities if labels blink or bleach.

We correlate absorption, luminescence, and scattering signals of the same individual nano-objects. This allows us to

1. specifically determine the quantum yields of single nano-objects (see Chapter 3, 4, 5).
2. estimate the number of molecules per nanoparticle if the nanoparticle is formed of many dyes (see Chapter 3).
3. provide a better description and understanding of photoluminescence of single nano-objects (see Chapter 4, 5).

1.5 Plasmon resonance of a single gold nanorod near a dielectric interface

The influence of surface roughness on intermolecular and surface forces between stationary and sliding solid-solid interfaces is of fundamental interest

for scientific and technological applications.[105–108] Most surfaces in nature are rough[109]. When two solid surfaces are brought together, the contact area between two surfaces can be much smaller than the apparent contact area.[109,110] The real contact area depends on the roughness of the two surfaces and on the normal force between the two surfaces.[111,112] For this reason, sensitive techniques are utilized to study the complex heterogeneity at the contact interfaces. Mostly scanning probe methods such as atomic force microscopy have been utilized. However, the area probed using these methods is usually limited by the size of the tip and the topography images can be affected by the tip shape.[113] Moreover, the deformations under and around the tip can be very different from deformations at distant locations. Optical microscopy techniques can be utilized to extend the observation area and to perform sensitive topography measurements. With the large field of view of optical techniques, the coupling between asperity points over large areas can be directly viewed.

The plasmon resonance of a gold nanoparticle is very sensitive to the changes of local refractive index. Moreover, it was found that the sensitivity of the plasmon resonance is highly distance-dependent because the amplitude of the EM field decays rapidly away from the surface of the gold nanoparticles.[114–117] The effect of the distance between two nanorods, and their relative orientation on their plasmon resonance were studied.[31] In coupling studies of gold nanoparticles, a red-shift in the plasmon resonance was observed while two nanoparticles were brought closer to each other.[118] The sensitivity of plasmons to the coupling distance was used in some of applications.[79,119] For example, DNA hybridization was visualized by the observed blue-shift in the plasmon resonance of the dimers as nanoparticles were brought apart during hybridization.[120] Although coupling of gold nanoparticles with different sizes, shapes, and orientation have been extensively studied,[31,114–117] the effect of the distance between a glass substrate and a gold nanorod on the plasmon resonance of the nanorod has not been measured yet.

For gold nanorods that are immobilized at a glass-medium interface, there is no analytical solution to describe the effect of the substrate on optical properties. Usually, the average of the glass and medium refractive indices is taken as an effective refractive index of the medium. This approximation, however, does not work well in different geometrical configurations of a

nanoparticle in a medium, i.e. different embedding ratios of nanotriangles in PVA.[121] Thus, it is interesting to study the plasmon resonance of a gold nanorod at varying nanorod - dielectric interface distances. In chapter 6 we study plasmon resonances of gold nanorods with respect to an approaching glass substrate. Studies about contact and friction can benefit from the distance-dependent plasmon resonance of gold nanorods.

1.6 Outline of this thesis

In this thesis, we will explore the optical properties of single nano-objects that are immobilized on a glass substrate by using single-particle detection tools. A common theme of this thesis is the absorption measurement and its correlation with luminescence and scattering measurements. We will get insight into the optical properties of nano-objects for their further utilization. In the following, we provide a short overview of the contents of each chapter mainly discussing the important aspects and results of each study.

Chapter 2 We explore different ways (e.g. index matching, using a liquid with high photothermal strength, careful compensation of chromatic aberrations, etc.) to optimize the sensitivity of photothermal imaging and demonstrate that this method can detect a few nW of dissipated power (in an integration time of 10 ms), which corresponds to the power dissipated by a molecule. The achieved improvement has allowed us to detect a single organic dye molecule by its absorption at room temperature with a signal to noise ratio as high as 10. This remained a challenge for two decades.

Chapter 3 We perform simultaneous measurements of absorption and luminescence from the same individual organic dye nanoparticles to characterize their optical properties and sizes. These nanoparticles are prepared by reprecipitation of a dye solution in acetone in water. They show excellent properties for simultaneous measurement of both absorption and luminescence signals. Particularly, the nanoparticles' emission is highly red-shifted from their absorption band which suppresses re-absorption. Moreover, efficient vibrational energy relaxation processes allow the detection of dye nanoparticles in absorption measurements. We quantified the number of molecules in individual dye

nanoparticles. We used 3 independent methods which were absorption microscopy, luminescence microscopy and AFM. These methods revealed consistent results illustrating the potential of correlated absorption and luminescence studies for characterization of complex systems.

Chapter 4 We study the absorption and luminescence properties of single gold nanospheres. Most of the energy absorbed by gold nanoparticles is released as heat and a very small part of it is released by emitting photons. Simultaneous detection of absorption and luminescence allows us to estimate the luminescence quantum yield of gold nanoparticles directly at the single-particle level, which was not possible before. Although the observed luminescence quantum yield is very low, the ability of gold nanoparticles to efficiently absorb light makes them detectable in luminescence measurements. We also found that the luminescence quantum yield of single gold nanospheres does not depend on their size due to the combination of two effects, which are the radiative coupling and the screening factor. Additionally, we describe a laser induced mechanism that can be employed to enhance the luminescence of 20 nm diameter by a factor of 3 to 4.

Chapter 5 We investigate luminescence properties of single gold nanorods to further reveal the effect of the particle shape on the observed luminescence and to investigate the mechanism of luminescence from gold nanoparticles. We studied absorption and luminescence properties of single gold nanorods with aspect ratios ranging from 1 (sphere) to 3.5 by correlating absorption, luminescence and scattering signals at the single-particle level. We found that a quantum yield as high as 10^{-5} can be obtained from nanorods that have a plasmon resonance at about 650 nm. Furthermore, we confirmed the plasmonic influence on the luminescence and observed a weak component (around 500 nm), which contributes to this luminescence. We analyze these spectral components by correlating luminescence and scattering spectra of individual nanorods with different plasmon resonances and performing polarization sensitive measurements on a single gold nanorod.

Chapter 6 It is interesting to investigate the sensitivity of plasmon reso-

nances of individual gold nanorods to the proximity of a dielectric surface. Gold nanorods are very sensitive to the changes in their dielectric environment and can be useful in the study of contact mechanics. We experimentally study the influence of a nearby dielectric interface on the longitudinal plasmon resonance of a gold nanorod. We recorded the luminescence spectrum of a single gold nanorod that is localized on a glass substrate at varying distances. For distances shorter than 400 nm between the nanorod and the glass substrate, we observed significant changes in the plasmon resonance of a gold nanorod and the observed changes are reversible. The obtained results, although preliminary, are promising for application of single gold nanorods as a plasmonic distance sensor.