15.4. Luminescence

Very small noble metal *clusters* of a few to a few tens of atoms have been shown to luminesce. Claims of single cluster detection have been published in recent years, but no consensus has been reached yet. If it is indeed possible to detect such clusters individually via their emission, and if they are photostable enough, they would be very attractive labels once conjugated to biomolecules, provided they are sufficiently stable chemically and photochemically. Such small clusters can also be used as dopants of other nanoparticles, for example zeolite nanocrystals (work in the group of J. Hofkens in Leuven, work on silver clusters in DNA hairpins in the group of D. Bouwmeester in Leiden).

Larger gold or silver nanoparticles do also luminesce, albeit with a very low efficiency. These much larger particles are completely photostable at moderate intensities (provided their temperature does not exceed the melting temperature for spheres or the reshaping temperature for rods and other non-spherical particles). Simultaneous measurements of the luminescence intensity and of the photothermal signal enable determination of the quantum yield particle by particle. It turns out that the quantum yield is nearly independent of the particle size and is rather low, typically about 10^{-6}-10^{-7} for nanospheres, and about 10 times higher for nanorods. Even such small yields, however, suffice for detection of particles above 20 nm, because of their large absorption cross-section.

The mechanism of luminescence is a subject of discussion in the literature. One of the possible scenarios runs as follows. As briefly mentioned in the pump-probe section above, excitation of a plasmon quickly gives rise to a hot electron-hole pair, which can radiatively recombine, albeit with a very low probability. This recombination background is spectrally very broad, as electron and holes can occupy a broad continuum of levels in the conduction and valence bands. The broad emission can then be enhanced in narrow plasmon bands by the particle's plasmons, which explains why the emission spectrum usually reflects the plasmon resonances of the particle. The absence of a dependence of the quantum yield on size (for particles less than 60 nm in size) is surprising. The following explanation has been proposed by Feldmann's group (Dulkeith et al., PRB 2004). Larger particles are expected to give rise to a larger antenna effect than smaller particles, and the radiative rate should scale as the volume of the particle. On the other hand, the screening of the field from electron-hole pairs by surrounding electrons also increases with particle size. A scaling of this

effect with volume therefore would account for a constant luminescence yield when the particle size varies.

The luminescence thus presents pronounced plasmonic structures. Therefore, it can be used as an alternative contrast signal to dark-field scattering, with similar characteristics. This is convenient to follow particles with a fluorescence detection setup, although the intensities required for luminescence detection are much higher than for scattering. Luminescence can be excited by the absorption of one, two, or more photons. The advantage of multiphoton absorption is that long wavelengths are less prone to photodamage cells and are less scattered by tissues. Two-photon excited luminescence is particularly interesting for tracking individual particles in scattering environments such as live cells.

15.5. Nonlinear optics

The field enhancement in metal nanoparticles and in other plasmonic structures is particularly important for nonlinear optical effects, which vary as higher powers of the electric field. Single metal nanoparticles give rise to detectable signals by second- and third-harmonic generation. Four-wave mixing is a non-degenerate frequency mixing process in which two pulses at frequencies ω_1 and ω_2 give rise to a signal at frequency $2\omega_1 - \omega_2$, which can be spectrally separated with high selectivity from the exciting pulses.

15.6 Validation of single-nanoparticle signals

In contrast to single fluorescent molecules and semiconductor nanocrystals, single gold nanoparticles yield very stable signals, that do not bleach or blink. Therefore, it often is difficult to make sure that the optical signals one measures stem from single nanoparticles, and not from aggregates or from more complex objects. Here is a list of possible criteria helping to identify single-particle signals.

i) correlation of the optical image with electron microscopy, of with sufficiently resolved AFM (STM) images. This is the best proof, but it requires a lengthy and difficult analysis, in particular fiducial markers to recognize particles in both types of images.

ii) lineshape in a scattering or photoluminescence spectrum. This works particularly well for nanorods, thanks to their intense and narrow longitudinal plasmon resonance,

spread by inhomogeneity in aspect ratios. The chance that two nanorods at the same position have the same aspect ratio (within experimental error) is negligibly small.

iii) statistical properties of a distribution of signals. This method is very useful for samples with low dispersity, contrary to ii), which just requires dispersity. Usual distributions of signal strengths are Poisson-like, in the most favorable case single particles dominate pairs, who themselves dominate triplets, etc. To be convincing, the histograms must show the characteristic 'desert' between the noise floor and the first peak corresponding to isolated single particles.

iv) More specific checks. Those include the scaling of the signal with particle size (again a statistical check), the order of magnitude of the signal expected from theory, when known, appearance or disappearance of the signal in a single step corresponding to adsorption or desorption from an interface with a liquid, a strong polarization for anisotropic objects such as rods, complementary spectroscopic analysis such as the acoustic vibration of the particle (two particles will present two different vibration frequencies), etc.

15.7 Fluorescence enhancement

We have seen that the electric field of an incoming wave may be strongly enhanced in the vicinity of a metallic nanostructure (or nano-antenna). This enhancement arises from a lightning rod effect for a quasi-static field, but can be further enhanced by a plasmonic resonance. As a consequence, both the excitation and the emission by a molecule placed in the vicinity of a nano-antenna will be enhanced. The first effect just follows from the stronger local near field, while the second effect, named after Purcell, can be described as an enhanced density of photon states in the Fermi golden rule, or, alternatively, by an enhanced dipole moment. This new dipole results from that of the molecule and from the currents induced by the molecule in the antenna. Of course, the two enhancements are in general different, and depend on the frequencies of the excitation and of the fluorescence with respect to the plasmon resonance. The fluorescence enhancement is particularly strong for emitters with very weak quantum yields. For good emitters, the limiting step is usually the excitation, so that enhancing the radiative rate does not enhance the global fluorescence intensity. At resonance, the total enhancement can reach 1000 for a single nanorod.

Part of the Purcell enhancement of the 'emission rate' is caused by the direct energy transfer (Förster) from the molecule to the metal, resulting in quenching of the fluorescence ('emission' doesn't distinguish between radiative emission or energy transfer to the metal followed by non-radiative dissipation). This effect, which dominates at short distances (less than 10 nm) must be distinguished from the excitation of the dipole mode of the particle or structure, which is dominant at distances comparable to the size of the particle (larger than about 20 nm). The dependence of these two channels is sketched schematically in Fig. 15.4.
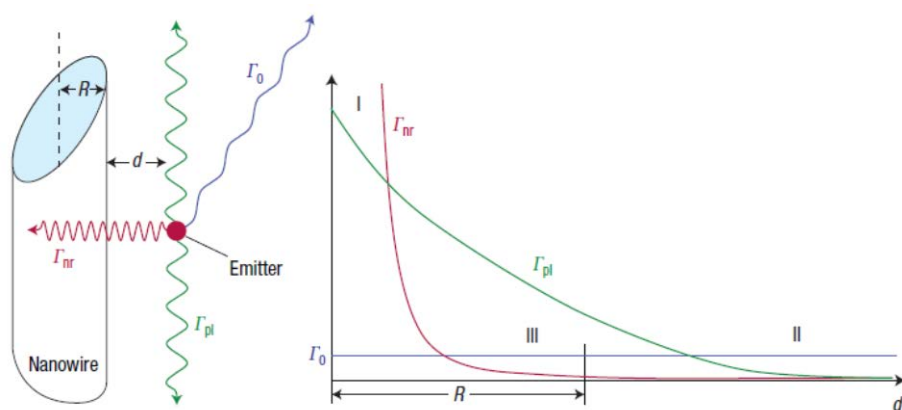

Figure 15.4 : Behavior of the radiative enhancement (green solid line) and of the non-radiative quenching rate (red solid line) in the vicinity of a metal nanostructure (here, a silver nanowire) as functions of the distance d between the point dipole and the metal. Quenching dominates at short distances, whereas radiative emission through currents in the antenna dominate at distances comparable to the nanostructure size.

15.8. Surface-Enhanced Raman Scattering

15.8.1. Early observations on surfaces, colloids

Raman scattering is normally a very weak effect, with a scattering cross-section of about 10^{-28} cm^2. In usual experiments, the only way to observe it is to use intense laser light, a large number of molecules (at least several millions in a monolayer at the focus of an optical microscope, which requires 100 s of accumulation for an acceptable signal), and a sensitive multichannel detector such as a CCD. Yet, it is known since the 1970's that *rough* metal surfaces can enhance the Raman scattering by many orders of magnitude. The roughness plays a crucial part in the enhancement. The factors estimated at that time were of the order of millions or billions, but have

kept increasing since. This effect is known as SERS. Similar giant enhancements were later observed with evaporated metal islands on dielectric substrates, or with colloids and clusters of metallic nanoparticles. These are usually prepared by reduction of a noble metal salt in a solution, leading to particle sizes in the range 10-100 nm. The colloidal solution can then be 'doped' with Raman-active molecules (in practice dyes and other conjugated molecules) at a very low concentration. In 1996, two groups (those of K. Kneipp and S. Nie in the USA) announced the observation of single molecules by SERS, with enhancement factors as large as $10^{14} - 10^{15}$. Such enhancement factors cause the Raman cross-section of a single molecule to exceed the fluorescence cross-section of a good laser dye (typically a few 10^{-16} cm^2).

15.8.2. Possible origins of the colossal enhancement

Although SERS is known for more than 30 years now, there is still debate as to what are the exact causes of the enhancement. This is because surface roughness, colloid sizes and shapes, cluster configurations, and the sites molecules occupy on the surfaces are still poorly known. Single-molecule investigations and tip-enhanced spectroscopy may change this situation in the next few years. Several groups have started SERS studies in this hope. Presently, there is a general agreement on the necessity of at least two or three independent enhancement mechanisms.

i) The first one, by far most important, is the electromagnetic (or plasmon) enhancement. In normal Raman experiments, one relies on the scattering of a photon into another photon by a molecule, a very small object relative to the wavelength. The size of the scatterer is clearly not adapted to the wavelength, and the efficiency of the process can be vastly enhanced by antennas. Metal structures or colloids are such antennas. It must be realized that an antenna works both ways: first by concentrating the incident field on the object, second by better coupling the currents in the object to the radiated field. A field enhancement factor is thus squared for the ratio of the scattered and incident fields, but brought to the power of four for the ratio of intensities! For a spherical particle, the enhancement of the laser field at frequency ω_L is :

$$\frac{\varepsilon(\omega_L) - \varepsilon_1}{\varepsilon(\omega_L) + 2\varepsilon_1} \left(\frac{r}{r+d} \right)^3$$

where r is the radius of the particle and d the distance of the molecule to the surface. Taking now the enhancement of the radiated field at frequency ω_R into account, the electromagnetic Raman enhancement becomes :

$$G = \left| \frac{\varepsilon(\omega_L) - \varepsilon_1}{\varepsilon(\omega_L) + 2\varepsilon_1} \right|^2 \left| \frac{\varepsilon(\omega_R) - \varepsilon_1}{\varepsilon(\omega_R) + 2\varepsilon_1} \right|^2 \left(\frac{r}{r+d} \right)^{12} .$$

For spherical particles, this factor can reach 10^7, but it decreases extremely rapidly with distance. Ellipsoids can concentrate the field even more (lightning-rod effect), up to factors of 10^{11}. Similar or even higher factors are reached in the gap between two colloids, and even larger ones can be found locally in colloid clusters.

ii) Some observations show that the electromagnetic factor does not act alone. A moderate enhancement of the Raman effect (by a factor 10-100) is found already on flat metal surfaces. The SERS effect is found to depend on the potential applied to an electrode. SERS does not apply to any chemical compound, but has been found only with conjugated molecules (so far). This could arise from resonance of the Raman signal with the molecule's excited state(s), but might also indicate some degree of coupling between the molecular orbitals and the electron states in the metal is involved. The theory of these effects is difficult, but enhancement factors of 100-1000 seem quite reasonable.

iii) Finally, recent theoretical work suggests that some part of the enhancement could arise from opto-mechanical coupling of the Raman vibration to the plasmon resonance, and from large optical forces in the huge field gradient of the hot spot.

15.8.3. Single-molecule observations, potential advantages
The enhancement factors are difficult to estimate from experiments, because the number of molecules in active sites is a priori unknown. The fraction of 'hot' molecules has kept decreasing since the first experiments, so that the most reliable estimate now comes from single-molecule experiments. An independent argument for the strength of the enhancement effect was provided by K. Kneipp and co-workers [K. Kneipp et al., Phys. Rev. Lett. 78 (1997) 1667], and rests on a nonlinear effect. When illuminating Ag colloids with adsorbed molecules by means of a pulsed laser, these authors found that the intensity of the anti-Stokes scattering grows quadratically with intensity. They attributed this to a very efficient pumping of the vibration by SERS, so that the vibrational population is larger than the thermal population given by the

Boltzmann factor. Estimating the vibrational lifetime to 10 ps, they concluded that the Raman cross-section must reach $10^{-16} \, cm^2$ per molecule.
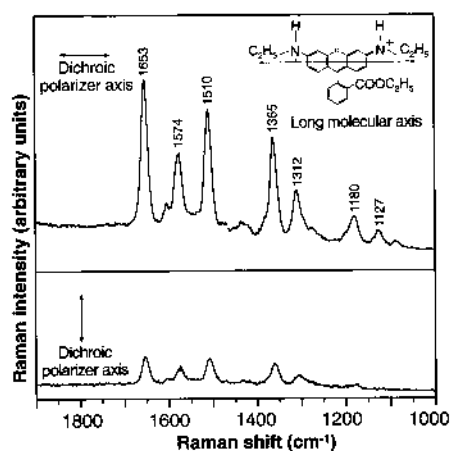

Figure 15.5 : Example of the SERS spectrum of a single Ag colloid, presumably arising from a single rhodamine 6G molecule (Reprinted with permission from S. Nie and S. R. Emory, Science 275 (1997) 1102 ; Copyright 1997 AAAS).

Measuring the Raman spectra of a single molecule would open many fascinating possibilities. It would provide the vibrational fingerprint of the molecule (frequencies, widths, coupling strengths, of several molecular vibration modes ; see an example in Fig. 15.5), which is itself a much more detailed indicator of the chemical environment of the molecule than the broad fluorescence spectrum. Infrared and Raman spectroscopies are current tools in chemistry to characterize molecules and their interactions. Two further advantages with respect to fluorescence are:
i) that the saturation intensities for vibrations are much higher than for excited states (because of the short vibrational lifetime), which means that more intense signals and shorter timescales would be available ;
ii) because only vibrational states of the molecule are populated, but not its excited state, photobleaching is much less likely, and long accumulation times are possible.

However, a big disadvantage of SERS is that it is still poorly understood, and that the accurate position of the molecule with respect to a metal structure is very difficult to control and to maintain. It would be very difficult, for example, to measure the SERS of a single protein molecule in a cell. Furthermore, obtaining quantitative data for

molecules in the focal volume has proved impossible so far, although tip-enhanced Raman scattering coupled to an STM is a big progress in this direction.

15.9. Applications of optical experiments on single metal NPs

15.9.1. Labeling and tracking:
Gold nanoparticles are attractive as labels because of the strength and stability of their optical signals (no blinking, no bleaching). Moreover, in dielectric samples, they present a large contrast with non-metallic surroundings, which facilitates their detection.

15.9.2 Antennas:
Plasmonic structures are used to concentrate electromagnetic energy into small volumes. A simple example is the gap between two metal nanospheres, discussed in the introduction. Just as for radiowave or microwave frequencies, (nano-) antennas help match the large optical wavelength to microscopic emitters (molecules, nanocrystals, etc.). These structures can also channel emission into confined modes, for example for propagation along a nanowire, or into a desired direction with Yagi-Uda antenna.

15.9.3. Sensing:
The plasmon resonance of a particle is sensitive to the index of refraction in its immediate environment. This effect makes it possible to detect slight modifications of refractive index in close environment of particle, for example binding of organic molecules to the surface of the particle, or a change of solvent around the particle.

15.9.4. Trapping and manipulation:
Because of their collective electronic response, metal nanoparticles are much more polarizable than dielectric particles. This feature makes it possible to trap individual gold nanoparticles down to diameters as small as 20 nm for gold nanospheres, or 10 nm for gold nanorods. Such efficient trapping opens the way to manipulation of objects with sizes comparable to those of organelles in cells, which would be impossible with dielectric particles.

15.10 Other small particles

Hereafter, we briefly mention and discuss the advantages and disadvantages of some nano-sized labels.

15.10.1. Polymer beads

Small latex beads (a few tens of nm in diameter) can be doped with fluorophores, to improve the label lifetime before photobleaching. However, to avoid self-quenching (i.e. the complete loss of fluorescence due to the formation of dimers and to FRET to those dimers), the concentration cannot be higher than 10^{-3} M. A 100-nm diameter bead thus contains at most a few hundred dye molecules. A further advantage of such a label is that the molecules are better protected from small reactive molecules such as oxygen and water. They main drawback is that they are big, which leads to two problems for single molecule microscopy :

i) they are physically too big to enter crowded structures, and may modify interactions between biomolecules ;

ii) attaching them to a single biomolecule is difficult, because there are in general several active sites on the surface, which can bind two or more molecules.

Single conjugated polymer molecules or small clusters can also be used as luminescent probes.

15.10.2. Rare-earth doped nanoparticles

Rare-earth ions are often luminescent, but present weak optical transitions. A possible way to use them as labels is to include a large number of them in a nano-particle. Because their transitions are weak, larger concentrations can be achieved than with molecules. The problems of the size of such labels would still persist, though.

15.10.3. Single N-V vacancies in diamond nanocrystals

Diamond presents a number of colored centers which can fluoresce. One of them has been studied in great detail, the nitrogen-vacancy center (NV for short). In a NV-center, one of the carbons has been replaced by a nitrogen (which happens naturally in diamonds), while one of the neighbor carbons is missing (vacancy). This system

carries an additional electron. It has triplet ground and excited states, and fluoresces strongly at room temperature. J. Wrachtrup and colleagues have detected single NV centers in 1997 [A. Gruber et al., Science 276 (1997) 2012]. In diamond nanocrystals usually either 0 or 1 NV center is present. The main advantage of this system is its high resistance to bleaching, related to the strength and high melting point of the diamond lattice. Single N-V centers in diamond nanocrystals have been proposed as sources of single photons.