

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 several years ago already, 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 (see for example work on zeolite nanocrystals in the group of J. Hofkens in Leuven, and 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 quantum 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 resonances because coupling to plasmons enhances the radiative rate by antenna effects. Therefore, photoluminescence can be used as an alternative contrast signal to dark-field scattering. This method is convenient to follow particles with a fluorescence detection setup, although the intensities required for luminescence detection are much higher than for scattering. Stokes photoluminescence is emitted at lower energies than the excitation laser. Because the emission background from electron-hole recombination is spectrally broad, plasmon resonances appear as enhancement of this background, and provide similar information as plasmon-enhanced scattering. Anti-Stokes photoluminescence is emitted at wavelengths shorter than the excitation laser's, and convey information about the actual temperature of the excited particle, through the ratio of anti-Stokes to Stokes emission (see Carattino et al., NanoLett. 2018). This may be interesting in photothermal therapy, particularly.

Luminescence can be excited by the absorption of two (or more) photons from short excitation pulses. The advantage of multiphoton absorption is that long wavelengths are less prone to photodamaging cells or sensitive media, and are less efficiently 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 interesting for the generation of nonlinear optical effects, whose intensity scale as higher powers of the excitation intensity. 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 combined frequencies such as $2\omega_1 - \omega_2$, which can be spectrally separated with high selectivity from the exciting pulses. Photothermal detection is a special case of four-wave mixing, where the temperature change produced by absorption of frequency ω_1 is detected through the scattering of the other frequency, ω_2 , producing a signal at $\omega_1 - \omega_1 + \omega_2$.

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 the same particles in both types of images. Moreover, electron microscopy often damages the particles, so that all optical measurements should be done first, before ascertaining that the spots arise from single nanoparticles.

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 probability that two nanorods at the same position have the same aspect ratio (within experimental error) is usually negligible.

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, and 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. An arbitrary cutoff of background and noise in experimental histograms may well suppress the weaker signals from isolated particles and smaller clusters.

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, the appearance or disappearance of the signal in a single step corresponding to adsorption or desorption of the particle or cluster from an interface with a liquid, a strong polarization response 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 considerably by a plasmonic resonance. As a consequence, both the excitation and the emission by a molecule placed in the vicinity of a nano-antenna may 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 of the combined particle-emitter system. This new dipole results from that of the molecule and from charges and currents induced by the molecule in the antenna.

Of course, the excitation and emission enhancements are in general different, and depend on the spectral positions 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 most efficient enhancement process is usually the excitation. Enhancing the radiative rate usually does not enhance the global fluorescence intensity, as every absorbed photon leads to emission. At resonance, the total enhancement can reach 1000 for a single molecule in the near field close to the tip of a gold nanorod. Much higher values are obtained in small gaps between the particles (for example in particles-on-a-mirror geometries, see work by Baumberg's lab), and in higher-order nonlinear processes.

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.

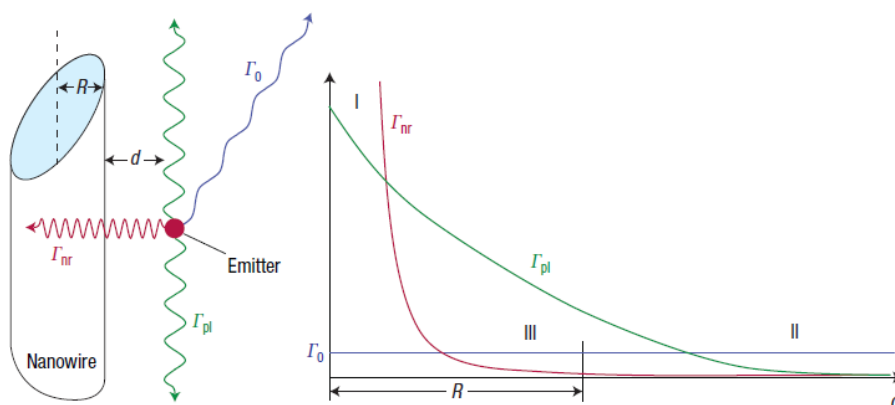


Figure 15.4 : Behavior of the radiative enhancement (green solid line) and of the non-radiative quenching rate (red solid line) in the vicinity of a metal nanostructure (here, a silver nanowire) as functions of the distance d between the point dipole and the metal. Quenching dominates at short distances, 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} cm². 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 Raman enhancement 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 nanoparticles 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 $10^{13} - 10^{14}$. 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 about 50 years now, there is still some debate as to what are the exact mechanisms 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 have begun to change this situation. Several groups have started TERS (tip-enhanced Raman scattering) studies in this hope and obtained spectacular images of single molecules with different areas involved in different molecular vibrations. Presently, there is a general agreement on the dominance of the electromagnetic enhancement. The other enhancement mechanisms mentioned hereafter have not been unambiguously demonstrated.

i) The by-far most important enhancement mechanism 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 photon wavelength. The size of a molecular scatterer is clearly not well adapted to efficient absorption and emission at optical wavelengths. The process can thus be vastly enhanced by antennas. Metal structures or colloids are such antennas. Moreover, 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 in Raman scattering 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 at the plasmon resonance can concentrate the field even more (lightning-rod effect), up to factors of 10^2 - 10^3 for the field. 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 suggest 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 do not seem unreasonable.

iii) Finally, recent theoretical work and TERS experiments suggest 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} cm² per molecule.

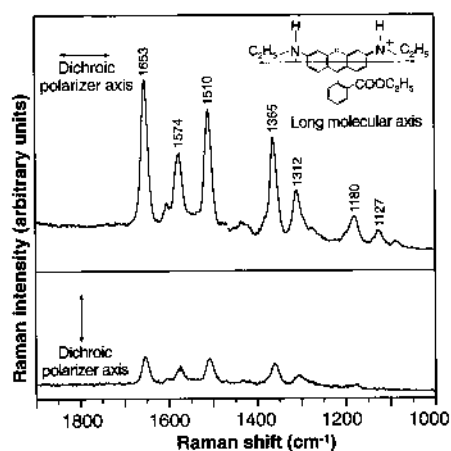


Figure 15.5 : Example of the SERS spectrum of a single Ag colloid, presumably arising from a single rhodamine 6G molecule (Reprinted with permission from S. Nie and S. R. Emory, *Science* 275 (1997) 1102 ; Copyright 1997 AAAS).

Measuring the Raman spectra of a single molecule, not only under a STM tip, but in a real sample such as a biological cell 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 very small volumes, down to 1 nm^3 . 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 a 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 the close environment of a 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 other 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's 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. Their 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, and they give rise to very specific signals such as up-conversion (emission of shorter waves upon moderate excitation). However, they present rather weak optical transitions, so that only nanoparticles with many rare-earth ions are detectable. Rare-earth-doped nanoparticles are often used as labels in biology. Because their transitions and their interactions are weak, larger concentrations of rare-earth ions can be achieved than with molecules. The problems of the large size of such labels 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 stable and bright biological labels as well as sources of single photons. Because of the large index of refraction of diamond, of its chemical inertness and biocompatibility, diamond nanocrystals can be used as non-fluorescent, scattering probes of biological systems.

Exercise 15.1: In the Drude model of a metal, the dielectric permittivity is given by:

$$\varepsilon(\omega) = \varepsilon_2 - \frac{\omega_p^2}{\omega \left(\omega + \frac{i}{\tau} \right)}$$

- i) *Express the Drude time τ with the conductivity σ of the metal, assuming the latter to be independent of the frequency. Explain why this probably is a good approximation. What is the physical interpretation of the constant permittivity ε_2 ?*
- ii) *Inject this expression into the polarizability $\alpha_0(\omega)$ of a sphere in the electrostatic approximation (see text) and show that the plasmon resonance in this approximation is Lorentzian. What is the associated half-maximum width FWHM?*

Emission of radiation by the sphere is not included in the above electrostatic model. To account for it, we correct the polarizability according to the following expression:

$$\alpha(\omega) = \frac{\alpha_0(\omega)}{1 - i \frac{k^3}{6\pi\varepsilon_0} \alpha_0(\omega)}$$

iii) Interpret physically this expression by taking the example of a Lorentzian resonance, $\alpha_0(\omega) = A + \frac{B}{\omega - \omega_0 + i\gamma}$, and show that our radiative correction adds a

radiative damping term. Give its expression and explain why it scales as the cube of the emitted wavevector and as the volume of the particle.

iv) For very small particles, interactions of the conduction electrons with the surface cannot be neglected any more. It turns out that these additional electron scattering events increase the Drude rate (inverse of the Drude time) by an amount v_F / D proportional to the Fermi velocity and to the inverse particle diameter. Prove that the plasmon resonance width presents a minimum as the particle diameter is varied, and provide an estimate of the particle's diameter giving rise to the narrowest resonance.

Exercise 15.2: Photothermal signal due to the plasmonic response.

i) Use the above model to estimate the sensitivity of the plasmon resonance to the index of refraction of the medium surrounding the sphere.

ii) Assuming the dielectric around the sphere to have thermal conductivity κ , and the metal's conductivity to be infinite, calculate the temperature profile in steady state around the sphere for a heating power P_h .

iii) Using the real and imaginary parts of the polarizability discussed above, and neglecting radiative corrections, calculate the photothermal response of the sphere when it is heated by a heating beam with a given intensity.

iv) Another contribution of the scattered field arises from the thermal lens, which is the inhomogeneity of the index of refraction of the medium around the heated sphere.

Under which conditions do these two field components interfere constructively? Discuss under which conditions the sphere's contribution dominates the thermal lens's contribution to scattering.