Gold nanorod photoluminescence : applications to imaging and temperature sensing

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INTRODUCTION

Gold nanorods are ideal candidates for complementing fluorophores in labelling applications. The presence of the surface plasmon resonance generates large absorption and scattering cross sections, thus making the detection of single nanoparticles possible under a light microscope. In this introduction we will review the current status of light microscopy, particularly of fluorescence microscopes. We will introduce some properties of gold nanoparticles including the plasmon resonance and we will focus into the luminescence emission. Finally we will briefly introduce the experimental chapters of this thesis, that correspond to applications of the luminescence ranging from imaging to temperature sensing.

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1.1. LIGHT MICROSCOPY

MICROSCOPES have become indispensable tools in material science and biology. The first microscopes developed by Antoni Van Leeuwenhoek in the XVII century were aimed at studying fabrics; it didn't take long however to discover that nature was hiding amazing details beyond what the bare human eye could see. The first microscope builders focused into developing better lenses in order to obtain sharper images and therefore being able to observe even smaller structures.

With the development of the wave theory of light a fundamental limitation for optical microscopes appeared: the diffraction limit. Abbe realized that no matter how good a lens is, there will always be a limit to how much it is possible to focus light. This limit is determined mainly by the wavelength of the employed light beam and by the maximum acceptance angle of the lens. Shortening the wavelength of the sources is therefore a possible strategy to increase the resolution of a microscope.

Particles with wavelengths shorter than optical wavelengths, such as electrons, opened the possibility to investigate much smaller structures[1]. Notably in the field of virology the electron microscope provided the evidence that researchers were long looking for: the existence of particles smaller than what optical microscopes were able to resolve. Electron microscopes are a very powerful tool but they require special sample preparations that don't allow the study of biological processes *in vivo*.

With a similar strategy, the resolution of optical microscopes can be increased with the use of ultraviolet sources. Some biological samples show emission of light at longer wavelengths when irradiated with UV light, nowadays simply referred to as autofluorescence. In 1911 German physicist Oskar Heimstädt used the the emission from bacteria to build the first fluorescence microscope[2]. The difficulties to focus enough UV light into the sample and the low efficiency in collecting the emission made him skeptical of the success of his design. He ended his work stating[3],

> If and to what degree fluorescence microscopy will widen the possibilities of microscopic imaging only the future will show.

Fluorescence however was not a new phenomenon. It was first observed and characterized during the second half of the 19th century. The pioneering works of Stokes showed that some materials when irradiated with short wavelengths emit light at longer ones. Only in the 1930s did the first applications of fluorescent materials start to emerge for biological applications. Fluorophores were employed to stain biological samples, allowing to easily detect tissue components or bacteria. Oskar Heimstädt could rest assured that his invention was starting to revolutionize biology.

The following decades witnessed a phenomenal increase in technical developments, including the advancement of epi-illimuniation, the confocal microscope and the improvement of filters and light sources. The dichromatic mirror, the final key element of a fluorescence microscope, was introduced in 1967[4]. The wealth of information that could be retrieved thanks to fluorescence and its simple implementation were crucial for the success of the fluorescence microscope and its establishment as a standard tool in almost any biological or material science laboratory. Fluorescence microscopes however do not overcome the diffraction limit.

At the end of the XX century a major breakthrough occurred in the field of optics: the detection of a single-molecule fluorescence in 1990[5]. Single molecules opened the door to determine material properties that would have been hidden by ensemble averaging. The first studies were performed at low temperature (few Kelvins) and gave access to properties not only of the fluorescence of molecules themselves but also of the hosting matrices, mainly polymers and crystals. Single molecules are the bridge between the diffraction limit of far field optics and the atomic scale properties of materials.

Single-molecule microscopy also changed the way imaging can be performed. If two fluorophores are separated further away than the diffraction limit, their centers can be determined with a precision that scales as $\approx 1/\sqrt{N}$, with N the number of recorded photons. The possibility of localizing single molecules beyond Abbe's limit shows how useful single-molecule detection could be. For example, tracking of single molecules[6] could be used to study diffusion with unprecedented spatial and temporal resolutions.

In relevant biological samples, however, the density of fluorophores is such that they are not further apart than the diffraction limit. In the late 1990s it was found that the fluorescence of molecules can be switched on and off by irradiating them with specific wavelengths[7]. This led to the development of a wide variety of techniques that rely on switching on and off molecules[8], and therefore they can be individually localized[9]. Post processing the information of each fluorescent molecule allows to reconstruct images with a spatial resolution an order of magnitude higher than the diffraction limit[10].

Molecules, however, show two phenomena known as blinking[11] and bleaching[12]. At room temperature it is impossible to prevent fluorophores from going to dark states, meaning that their fluorescence signal will disappear. Blinking characterizes the process by which fluorescence disappears for a comparatively short period of time. If the molecule undergoes an irreversible transition to a dark state, the process is called bleaching. Blinking and bleaching put a hard limit to the experiments that can be performed with single molecules, since they cannot be observed for extended periods of time. Tracking is limited to few seconds, and imaging is limited to few frames.

As single-molecule detection allowed to bridge the length mismatch between visible light and biologically relevant scales, new agents that can fill the gap between biologically relevant time scales and fluorophores' observation times are of utmost importance. In this direction different approaches were taken, including the use of scattering instead of fluorescence[13], the use of semiconductor quantum dots[14] and of metallic nanoparticles[15]. The latter are the focus of this thesis and of the next few sections.

1.2. GOLD NANOPARTICLES

Metallic nanoparticles have been utilized for a very long time. In a fortuitous way Romans dispersed gold salts into oxide mixtures that then they melted to obtain red-coloured glass; the beautiful Lycurgus cup[16] is the only surviving complete example of an artifact made out of such glasses. Nanoparticles in the glass have preserved their optical properties for centuries and can still be admired today. In medieval times the technique was rediscovered and became common in the fabrication of red-coloured glasses for churches throughout Europe.

The explanation of the phenomenon however only came one century ago. Gustav Mie in 1908 calculated the scattering of a plane wave incident on spherical particles[17] by

fully solving Maxwell's equations. This solution is known today as Mie scattering. The original paper compared measured and calculated scattering spectra of gold nanospheres; both experiments and theory show a peak at around 550 nm. The weaker interaction with light of longer wavelengths explains the reddish color of colloidal gold nanoparticle suspensions.

The peak observed in the spectra is related to a resonance of the oscillating conduction electrons on the surface of the metal and is known as plasmon resonance. For particles much smaller than the incident wavelength, a simplification of the Mie formalism can be made by considering only the first order electrostatic approximation. In this case the polarizability of a nanosphere of volume V is given by[18]

$$\alpha_{\text{sphere}} = 3\epsilon_0 V \frac{\epsilon(\omega) - \epsilon_m}{\epsilon(\omega) + 2\epsilon_m} \tag{1.1}$$

where ϵ_0 is the permittivity of vacuum, $\epsilon(\omega)$ is the permittivity of the metal as a function of the incoming excitation frequency ω and ϵ_m is the permittivity of the surrounding medium. The absorption cross section can thus be calculated as $\sigma_{\text{abs}} = k\text{Im}(\alpha)$ and the scattering as $\sigma_{\text{scatt}} = k^4|\alpha|^2/(6\pi)$.

From equation 1.1 it is possible to see that a resonance will appear when $\text{Re}(\epsilon(\omega)) = -2\epsilon_{\text{m}}$. It is important to note that the energy at which this resonance appears is therefore dependent not only on the particle's material properties but also on the surrounding medium's optical constants. In the case of elongated nanoparticles, some correction factors can be introduced to the polarizability. Several computer packages[19–21] exist to calculate with a great precision absorption and extinction cross sections of arbitrary geometries and therefore it is not worth entering into the specifics of the calculations[1].

It is important to point out, however, that while nanospheres have resonances that slightly change with their radius, elongated particles such as nanorods present a longitudinal resonance that strongly depends on their aspect ratio. The more elongated particles will have resonances with lower energies. Moreover particles with resonances to the near infrared region show a narrower resonance[22], making them interesting candidates for sensing applications. In biological conditions, nanoparticles with resonances towards longer wavelengths are particularly relevant because cells are typically transparent to near-infrared wavelengths.

A standard procedure to obtain gold nanoparticles is synthesis through wet chemical methods[23]. Even in the best of cases there will be a dispersion of shapes in the sample. The differences between nanoparticles can be observed on electron micrographs, but also optically. Slightly different particles will show different resonances[24] and this becomes more significant for elongated particles. Minute changes in shape will lead to different plasmonic resonances, that can be observed under the microscope. In a sample of such particles it is unlikely that two of them will have the exact same resonance. Dimers and clusters will therefore have a characteristic signature since more than one resonance will be observed in general.

The distribution of resonance energies has another important advantage: studying properties that depend on the resonance does not require a new synthesis nor changing

[1] A complete description of how to calculate the plasmon resonance with the ADDA package can be found at: https://www.aquicarattino.com/science/plasmon-resonance/

the sample. Once the nanoparticles are immobilized on a substrate it is possible to characterize them individually, select the ones with particular resonances and perform the rest of the experiments on them. All the chapters in this thesis show results that were gathered through the study of different particles in the same sample and under the same exact conditions: from chemical reactions in chapter 2 to electron phonon coupling in chapter 5 and anti-Stokes luminescence in chapters 3 and 4.

Besides the geometrical factors, nanoparticles also show a broad distribution of their quantifiable properties. For example, the quantum yields of nanoparticles that are apparently equivalent (similar in size, same resonance energy) can differ from each other by almost an order of magnitude[25]. In every chapter of this thesis variations from particle to particle can be observed. Chapter 2 shows that the etching rates can vary from particle to particle, in chapter 5 we have observed that the broadening rates when increasing temperature can differ from particle to particle. It means that particles differ from each other more than what an optical or scanning electron microscopes can resolve. The only way of validating the observed results is therefore through accumulating significative statistics on single-particle measurements.

1.3. LUMINESCENCE FROM GOLD NANOPARTICLES

Light emission from gold and copper was first observed by Mooradian[26] in 1969. In his work, electrons and holes in the metal were excited with visible light and the emission was observed at longer wavelengths. Strikingly, the emission quantum yield (i.e. the number of emitted photons per absorbed photon) that he estimated was in the order of 10^{-10}. In subsequent years several studies showed that this low number could be increased with the presence of sharp edges[27] or tips[28], but still it would be much lower than the typical fluorescence yield of organic dyes, on the order of few percent at least.

When transitioning from bulk gold to nanoparticles, the interaction of light with metals will be highly influenced by the presence of the plasmon resonance[29]. On one hand nanoparticles will have large absorption cross sections in specific wavelength regions, as explained in the previous section. On the other hand the emission spectrum will also be enhanced for frequencies around the plasmon resonance. Previous work has already shown a big overlap between the scattering and the emission spectra of gold nanoparticles[25].

The emission quantum yield of single gold nanoparticles is several orders of magnitude higher than bulk values partly due to the presence of sharp structures such as edges and tips. Typical quantum yield values are in the order of 10^{-6}[25], several orders of magnitude lower than those of organic dye molecules, but the absorption cross section can be in the order of $10^{-2}\,\mu\text{m}^2$, 6 orders of magnitude higher than those of dye molecules. The combination of both factors makes it possible to use luminescence to detect single gold nanoparticles in a standard fluorescence microscope.

The photoluminescence of gold nanoparticles can be excited mainly through two different approaches. It is possible to use a short wavelength laser, e.g. 532 nm, to excite interband transitions in gold[30], as well as the plasmon resonance of spheres or the transverse plasmon resonance of rods. The emission from particles can be collected after placing a notch or long pass filter in the detection path to prevent the excitation light to reach the detectors. Illuminating with a short wavelength allows one to collect the entire

plasmonic emission, but doesn't fully exploit the advantage of the larger absorption cross section that the resonance provides.

The other approach to observe luminescence from nanoparticles is to excite them close to their resonances. In this way it is possible to benefit from the higher absorption cross section leading to lower excitation powers. Recent studies have shown that the emission quantum yield does not change significantly between exciting at the resonance and exciting with a shorter wavelength around 532 nm[31]. However, the photoluminescence itself is also coupled to the plasmon; if excited in resonance, the emission will be concentrated around the excitation wavelength[32]. Detection filters may therefore block an important part of the emission spectrum.

Throughout this thesis luminescence will refer to all the emission from a nanoparticle at different wavelengths than the excitation wavelength and that scales linearly with the excitation power. Luminescence makes it is possible to observe single gold nanoparticles under a conventional fluorescence microscope. A notch or long pass filter in the detection path efficiently blocks the excitation light while allowing the luminescence emission to go through. Moreover it is possible to select nanoparticles with resonances at wavelengths at which no other sources absorb or emit, effectively lowering the background.

For dye molecules the Stokes shifted fluorescence can be understood as a consequence of energy conservation: a photon of a given energy excites the electrons of a material that subsequently relax back by emitting another photon, by transferring energy in the form of heat to the medium or a combination of both. It is expected therefore that the emitted photon has a lower energy than the incident one. This is always valid unless the excited electrons can somehow gain energy from the medium before relaxing back radiatively. If this happens, the process is called anti-Stokes and the emitted photons possess a higher energy than the excitation.

When excited at resonance, gold nanoparticles exhibit anti-Stokes luminescence with intensities that can be compared to the Stokes shifted emission. In brief, the mechanism proposed for the emission of photons with higher energies is the interaction of electrons and holes with phonons in the gold lattice before they recombine radiatively. Note that in this process the interaction with other thermal charge carriers is also possible. Assuming that the anti-Stokes emission from gold nanoparticles depends only on the population of phonons, the general shape of the emission is expected to be

$$\bar{I} \approx \left(\exp \frac{\hbar\omega}{k_B T} - 1 \right)^{-1}. \tag{1.2}$$

where ω is the frequency of the emitted photon, k_B is Boltzmann constant and T is the temperature.

Notably in equation 1.2 the only adjustable parameter is temperature. If properly characterized, the anti-Stokes emission spectrum should provide a way to estimate the absolute temperature of the particles without any previous calibration.

1.4. APPLICATIONS OF GOLD NANOPARTICLES

1.4.1. TUNING THE RESONANCE OF GOLD NANOPARTICLES

The previous two sections highlighted different strategies for detecting single gold nanoparticles such as nanospheres or nanorods. The principal characteristic of the particles is their localized surface plasmon resonance. The resonance wavelength (or energy) will be given by the geometry of the particle and by the surrounding medium's properties, such as its refractive index. The geometry of the particles is determined during the synthesis procedure, where the average length and width can be tuned. However, when the particles are deposited on a substrate, their resonance is already determined.

Chapter 2 focuses into tuning the plasmon resonance *in-situ*, once they are immobilized on a substrate and optically characterized. Currently two approaches exist for tuning the plasmon resonance after synthesis: (1) it is possible to tune the refractive index of the medium using an electric or magnetic field[33]. (2) It is possible to induce shape modifications of the nanoparticles either through chemical[34–38] or physical means[25, 39, 40]. In the majority of the reports a blue shift of the plasmon resonance has been observed.

In the case of chemical etching of the nanoparticles, almost all works have focused on bulk measurements in suspension. The tips of the particles tend to be more reactive because they are less protected by the surfactants that prevent aggregation of particles. This leads to an anisotropic reaction that slowly transforms elongated particles into spheres and that softens sharp edges or tips, yielding an overall blue-shift of the resonance.

Chapter 2 shows that through well known chemistry between gold and cyanide ions it is possible to induce a red-shift of the plasmon. This is modelled through an isotropic etching of the particles, and a good agreement between calculations and experiments is obtained. The main difference with previous work is the absence of a capping agent on the particles' surface. Controllably changing the shape of nanoparticles is of great importance for experiments where a specific resonance is needed.

1.4.2. IMAGING THROUGH DETECTION OF ANTI-STOKES EMISSION

Gold nanoparticles are ideal candidates for labelling biological samples because they prove to be innocuous to the cell[41] but also because they can be observed for extended periods of time[28, 42]. One of the drawbacks of gold nanoparticles is their low quantum yield. Since the absorption cross section of the particles scales as their volume, detecting smaller particles in presence of background requires a specific approach.

To overcome these difficulties, several techniques have been developed for imaging gold nanoparticles, including two-photon excited luminescence[43], photothermal heterodyne detection[44] and interferometric detection[45]. Each of these methods is useful but their operation requires dedicated setups and a high level of expertise.

Chapter 3 of this thesis shows that it is possible to image gold nanorods in biologically relevant conditions through detection of their anti-Stokes emission. By placing a short-pass filter in the detection path the background level is reduced significantly, while the luminescence signal from the particles remains high. This is valid even for cells stained with ATTO 647N, a dye with high quantum yield that absorbs light of the same wavelengths as the rods. In these conditions it is not possible to observe any single

nanoparticle through conventional Stokes-shifted emission while the anti-Stokes scheme presents a signal-to-background ratio higher than 10.

The technique presented in chapter 3 can be readily implemented in any conventional microscope by the addition of the appropriate filters. It does not require any special operation nor infrastructure. Moreover any data analysis tool for tracking, imaging, centroid extraction, etc. of single labels can readily be implemented without further modifications.

1.4.3. GOLD NANOPARTICLES AS NANO-THERMOMETERS

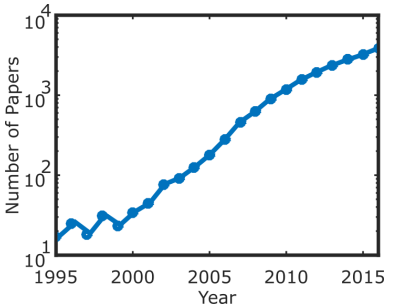

Figure 1.1: Number of papers published containing the terms Plasmonic Photo Thermal Therapy since 1995. Note the logarithmic scale in the y-axis.

During the past two decades there has been an increasing interest in gold nanoparticles as possible agents for medical treatments[46–48]. The strong interaction between particles and light makes them ideal candidates not only for labelling but also for releasing heat into very localized environments [46, 47, 49, 50]. This simple approach can be used for instance to induce death of cancer cells and is normally referred to as Plasmonic Photo Thermal Therapy (PPTT or PTT depending on the author). Figure 1.1 shows the number of papers published in this field since 1995. The increase in the number of publications is more than exponential, and depicts the relevance this technique is gaining over time.

After decades of research there is however almost no information regarding the temperatures that need to be reached by the nanoparticles to induce cell death. Much less is available at a single-particle/single-cell level. Moreover the field of thermometry at the nanoscale is subject to a heated debate[51, 52] since some experimental findings[51] contradict expected values from thermodynamic considerations[53].

Chapter 4 of this thesis focuses into the characterization of the mechanisms that give rise to anti-Stokes luminescence. Discarding multi-photon processes, photons with higher energies than the excitation energy require interactions with thermal baths. In a nanoparticle electrons and holes can interact with phonons before recombining radiatively, as discussed in section 1.3.

By carefully fitting the luminescence spectra of single gold nanorods and nanospheres with a function similar to equation 1.2 it is possible to extract the surface temperature of the particles. The method presented in chapter 4 does not depend on any ad-hoc calibration and can be performed in any confocal microscope with a coupled spectrometer. The chapter shows the increase in temperature with increasing laser powers and also

shows the changes that the luminescence spectra undergo when increasing the medium's temperature.

The calibration-free procedure is a major improvement over previous techniques in the field of nano-thermometry. The results from the chapter can have a significant impact on an emerging community that addresses one of the most pressing health issues of our time.

1.4.4. PLASMON DAMPING AS A FUNCTION OF TEMPERATURE

Luminescence is not the only method for detecting gold nanorods with an optical microscope. Gold nanoparticles have a large scattering cross section coinciding with the plasmon resonance. Exciting nanoparticles with white light allows one to record the scattering spectra in any confocal microscope coupled to a spectrometer. Since the resonance itself is affected by the surrounding conditions[54, 55], it is possible for example to use it for studying changes in the refractive index of the medium. However the plasmon resonance energy is not the only interesting property of the nanoparticles; the plasmon damping rate can also be used to detect changes in the surrounding conditions.

In principle there are four main mechanisms responsible for damping of the plasmons[22, 56, 57]: electron-phonon coupling, electron-surface interactions, electron-electron collisions and radiative damping. Out of those only the coupling with phonons shows an appreciable dependence on temperature[54, 55]. Therefore studying the dependence of the plasmon width with temperature could lead to an alternative approach to measure temperature changes.

Chapter 5 focuses on the characterization of the plasmon resonance of single gold nanorods at various temperatures. In the range of temperatures studied (between 293 K and 350 K), the plasmon width increases linearly with temperature. The broadening is assigned to an increase in the electron-phonon damping rate, and the observations can be understood through the Debye model of phonons. Measuring the broadening of the resonance can then be related to changes in temperature of the surrounding medium.

The scattering of gold nanorods is much more efficient than their luminescence, not only because of their large scattering cross section but also because of the low quantum yield of their emission. Therefore the powers needed for recording scattering spectra are much lower than the ones employed when exciting the luminescence of the particles. These lower powers enable studies of the plasmon resonance without inducing a significative increase of temperature. However the broad distribution of widths and broadening rates found in the studies of chapter 5 does not allow to perform an absolute temperature measurement but only to measure a relative change. This is similar to other experiments performed with quantum dots[51] and therefore expands the toolbox of available techniques for thermometry at the nanoscale.

1.5. ONE PROGRAM TO RULE THEM ALL

All modern laboratories rely on computer equipment to perform measurements, ranging from integrated micro controllers to powerful computers. A micro controller, for example, is responsible for maintaining a stable temperature of a heating plate; a fast computer on the other hand can analyze data online in high throughput experiments and take decisions

on the fly. Experiments performed at CERN and in other particle physics accelerators heavily rely on computers to discard millions of non-interesting events and save the relevant ones. However, for the average experimentalist there is a big gap between what is needed and what is available.

Flexible, open source programs to control experiments are hard to find in the Internet if they exist at all. The absence of a solution generates a double negative effect: researchers find themselves reinventing the wheel more often than desired and experiments are based on what can be done and not on what is desired to be done. For example, a simple home-built confocal microscope requires a dedicated computer program to run, which can take months to develop. Commercial software normally lacks the flexibility that new science needs, limiting the creativity of researchers while planning experiments.

All the chapters of this thesis relied on a flexible computer program that allowed to plan complex experiments building on software rather than being limited by it. The program has been made open source and can be found on Github[2]. It started to be developed for simplifying repetitive tasks such as refocusing on a particle or triggering a spectrometer. Later it evolved into a fully functional graphical user interface (GUI) for performing and visualizing 2D and 3D scans, acquiring fast timetraces, monitoring an optical tweezer and communicating with serial devices as well as over the network. The latest developments of the software open the possibility to define an application programming interface (API) for easy integration with applications on smartphones or to control several independent setups through the local network or also through the Internet.

Chapter 2 shows results where several particles were analyzed while being etched with potassium cyanide. Studying several nanoparticles under the same conditions is crucial for characterizing the dependence with the plasmon resonance and to discard any systematic error. Refocusing on the particles by hand is too slow for processes that happen as fast as the ones shown in the chapter and therefore the experiments wouldn't have been possible without a specialized computer program.

Chapter 3 shows the scanning capabilities of the software for imaging purposes. Moreover the specific program for acquiring the power dependence plots can be written in about 20 lines of code. When varying the temperature of the sample as done in chapters 4 and 5, being able to refocus on a reference particle to compensate for the drift of the setup was of utmost importance.

The software even if developed with an optical microscope in mind, can be easily extended to other configurations. Choosing Python as the programming language provides platform independence; it can run without inconvenience on several Windows versions, Mac OS and Linux. The main objective of the program is to provide a lower level layer on which to build creative solutions to complex problems.

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