SUMMARY

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. The plasmon of gold nanorods depends on the ratio between their width and length and covers the range between 540 nm for spheres and even above 800 nm for elongated particles, thus almost the entire visible and near-infrared spectrum. The surface plasmon presents great opportunities in (bio-)sensing, enhanced spectroscopies, photothermal therapy and for concentrating light below the diffraction limit.

Chapter 1 of this thesis is a brief overview on fluorescence microscopy and on the basic properties of gold nanoparticles. Microscopy and specifically fluorescence microscopy is the result of a long process of technical improvements in optics and light sources, but also of the labels used to prepare the samples. From simpler molecules to genetically encoded proteins, the wealth of resources available nowadays is remarkable. In this context, gold nanoparticles can find their way because of their stability over time.

The resonance wavelength (or energy) of metallic nanoparticles will be given by their geometry 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. Once the particles are deposited on a substrate, their resonance is already determined. It is possible, however, to induce shape modification to the particles through chemical means.

Previous works have focused on bulk measurements in suspension. In this case, 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.

When particles are excited by a monochromatic light source, such as a laser, they will emit light at different wavelengths than the excitation wavelength. This emission is generally referred to as luminescence and is commonly used for imaging and tracking nanoparticles under confocal microscopes. When the excitation wavelength coincides with the plasmon resonance, the emission will happen not only at longer wavelengths, i.e. lower energies, but also at shorter wavelengths. This emission is called anti-Stokes emission and possesses intriguing properties.

Chapter 3 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 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. The results of chapter 3 can have a major impact in the way nanoparticles are imaged and detected in biological conditions.

During the past two decades there has been an increasing interest in gold nanoparticles as possible agents for medical treatments. The strong interaction between particles and light makes them ideal candidates not only for labelling but also for releasing heat into very localized environments. This simple approach can be used, for instance, to induce death of cancer cells and is normally referred to as plasmonic photo thermal therapy.

Chapter 4 focuses on 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. The distribution of phonons in gold follows Bose-Einstein statistics, where the only free parameter is the temperature. We therefore propose in the chapter that anti-Stokes emission can be used for sensing temperature at the nanoscale.

By carefully fitting the luminescence spectra of single gold nanorods and nanospheres 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 temperature. The calibration-free procedure is a major improvement over previous techniques in the field of nano-thermometry.

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 plasmon damping rate is affected by the surrounding conditions it can also be used to detect changes in temperature. From the mechanisms involved to explain the plasmon damping rate, only electron-phonon coupling is dependent on temperature.

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. Measuring the broadening

of the resonance can then be related to changes in temperature of the surrounding medium. The powers needed for recording scattering spectra are much lower than the ones employed when exciting the luminescence of the particles. 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.