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# Optical Detection of Very Small Nonfluorescent Nanoparticles

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*Abstract:* We discuss an interferometric method for the optical detection of very small nonfluorescent nanoparticles. In particular, we show that single gold nanoparticles with a diameter as small as 5 nm can be detected. We discuss the potential of such tiny particles as optical labels for biological studies. Furthermore, we show that our interferometric method can be also used for the detection and tracking of unlabelled biological nano-entities such as viruses or microtubuli.

**Keywords:** Interferometric detection · Label-free · Nanoparticle · Optical microscopy · Particle tracking

## Introduction

Detection, identification, and tracking of single molecules and very small nanoparticles is an ultimate goal in analytical chemistry. The main challenge in each specific case boils down to a struggle with the signal-to-noise ratio (SNR). In general, one can improve the SNR either by increasing the signal or reducing the noise. In optical studies, the former can be achieved by increasing the collection efficiency, the detector efficiency, the excitation efficiency, and the interaction cross section. Two typical contributors to the noise are the detector noise and the laser intensity or frequency noise in optical experiments. However, the most serious source of trouble is usually the background. Although the average value of the background can be often identified and subtracted from the measured signal, any fluctuations in the background translate into a noise on the signal that could be hard to eliminate. It is, therefore, very important to reduce the background as far as possible.

Over the years, many clever techniques have been invented and applied to achieve a sufficiently high SNR for the detection of very small quantities of matter. The most impressive and significant breakthrough was the detection of single *fluorescent* molecules in late 1980s and early 1990s. In a pioneering experiment, Moerner and Kador used a double lock-in scheme to detect a single dye molecule embedded in an organic crystal at cryogenic temperature of $T < 2$ K *via* high-resolution spectroscopy in transmission [1]. At such low temperatures, the homogeneous linewidths of the individual molecules can become so narrow that they no longer overlap for a dilute sample. After this demonstration, Orrit and Bernard showed that a single molecule can be detected more effciently under the same conditions if one detected the red (Stokes) shifted fluorescence instead of examining the transmitted light at the excitation wavelength [2]. This fluorescence excitation spectroscopy method has become a workhorse of single molecule spectroscopy ever since.

Somewhat in parallel to the cryogenic single molecule detection in the early 1990s, scientists from several fields attempted to reach single molecule detection sensitivity at room temperature. At that time, it was believed that the signal from a single molecule would be too low compared with the noise of the background fluorescence at room temperature because the homogeneous linewidth is many orders of magnitude larger and therefore the absorption cross section is much lower. Betzig and Chichester employed scanning near-field optical microscopy (SNOM) to reduce the size of the illumination to a subwavelength spot, therefore reducing background fluorescence [3]. They obtained the first spatial images of single molecules. Shortly after that Nie *et al.* showed that room-temperature single-molecule detection would be even easier using a confocal arrangement [4]. These developments were rapidly extended to single molecule detection in a wide array of arrangements, including wide-field illumination and total internal reflection. Furthermore, other single emitters such as semiconductor nanocrystal [5] and color centers [6] could be detected using the same setups.

Fluorescent emitters have played a central role as labels for optical microscopy and for biophysical investigations for more than half a century. However, their limited photostability has turned out to be a severe limitation for studies at the single emitter level. The most prominent limiting property of fluorescence is photobleaching, which typically results in a total emission of about $10^6$–$10^9$ photons before a photochemical process eliminates the fluorescence signal. Another troublesome feature of single emitters has been blinking due to random intermittent transitions to dark states. Bleaching and blinking clearly prevent long time and continuous monitoring of the system under study. Furthermore, integration times longer than several milliseconds are typically necessary in the detection of single fluorescent emitters, limiting the time resolution in many experiments. It is, therefore, of high interest for biological studies to develop optical methods not based on fluorescence. One possibility discussed in this special issue is Raman spectroscopy. In this article,

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we present another alternative based on the interferometric detection of light scattering from nano-objects [7][8].

## Scattering from Nano-objects

For a spherical particle with a diameter $D$ that is much smaller than the wavelength of light λ, the scattering cross section SCS reads [9]

$$SCS \propto k^4 |\alpha(\lambda)|^2 \quad (1)$$

where α denotes the electrostatic polarizability of the particle given by

$$\alpha(\lambda) = \frac{\pi D^3}{2} \frac{\varepsilon_p(\lambda) - \varepsilon_m(\lambda)}{\varepsilon_p(\lambda) + 2\varepsilon_m(\lambda)} \quad (2)$$

Here $\varepsilon_p(\lambda)$ and $\varepsilon_m(\lambda)$ signify the dielectric constants of the particle and the medium, respectively [9]. An important feature of these equations is that the intensity of the light scattered from a nanoparticle scales as $D^6$. Thus, the scattering signal of a 10 nm particle is 1,000,000 times weaker than that of a 100 nm particle.

The detection of individual colloidal particles made of glass or polymer is indeed possible in a conventional dark-field microscope (where the illumination is mostly spatially filtered out of the detection path), as long as $D$ is larger than about 100 nanometers. Detection of smaller particles becomes very challenging in the condensed phase because one requires to distinguish the signal of a single particle from stray background scattering caused by any slight corrugation or fluctuation of the refractive index in the sample. This is a fundamentally different situation than that of detecting the fluorescence of single molecules, which can be separated from the background very efficiently by using spectral filters.

## Gold Nanoparticles

Gold nanoparticles (gnp) are inert and have been used in biological electron microscopy applications for a long time [10]. These particles can be fabricated in a large variety of sizes (see ref. [11]), are chemically stable and are biocompatible. Radiation at optical frequencies causes a collective oscillation of the electrons in a gnp, called plasmons. If the gnp is much smaller than the wavelength of light, it is a Rayleigh scatterer and its scattering cross section can be approximated by the same expression as in Eqn. (1). It turns out, however, that the complex dielectric constants of gold conspire to give rise to a plasmon resonance in the dipolar polarizability of a gnp. This resonance is determined by several factors such as the size and shape of the gnp as well as the dielectric constant of its surroundings. For spherical gnps of diameter less than 50 nm, the plasmon resonance wavelength is around 530–540 nm in water and is fairly insensitive to the particle size. The optical properties of gold nanoparticles have been the subject of many excellent review articles and textbooks [12][13].

For a given diameter $D$, metallic nanoparticles typically have larger polarizabilities than common dielectric particles. Thus, single gold nanoparticles can be detected in the same dark-field configuration as discussed earlier, even when they have diameters as small as D ≅ 30–40 nm [14][15]. Nanoparticles of this size have been used for particle tracking applications in biophysics [16], but detecting and following smaller particles becomes a great challenge. To this end, gold particles have been at a disadvantage compared to fluorescent molecules, which are typically about one nanometer in size or luminescent semiconductor nanocrystals, which range up to 20 nm, depending on whether and how they have been made biocompatible [17].

## Interferometric Detection of the Scattering Signal

Recently, efforts have been undertaken to detect individual gold nanoparticles as small as 5 nm. An effective strategy has been to make use of the particle absorption, which scales only as $D^3$ rather than $D^6$ [9]. One can either directly record the absorption [18] or measure the refractive index change in the heated vicinity of an absorbing particle [19][20]. Detection of very small particles, down to 5 nm in the former case and 1.4 nm in the latter, has been achieved.

In our laboratory, we have developed an alternative method for direct detection of gold nanoparticles *via* elastic scattering, exploiting the interference between the background reflection as a reference beam and the scattered field [7]. Let us consider $E_i$ to be the incident electric field at the location of the sample, $E_r$ the electric field of the light reflected from the sample and $E_s$ the electric field of the light scattered from the particle on the detector. The measured intensity $I_m$ can be then written as

$$I_m = |E_r + E_s|^2 = |E_i|^2 (r^2 + s^2 - 2|r||s| \sin\varphi) \quad (3)$$

where $r$ is the field reflectivity, and $s = |s| e^{i\varphi}$ is the complex scattering amplitude with phase φ. The first term on the right denotes the background intensity. The second term is the scattered intensity, which becomes smaller than the noise of the first term for very small particles. The third term, however, is proportional to $D^3$ and overwhelms the purely scattered light for very small particles. The signal is amplified by a reference beam, which in this case is simply the background reflection. The interferometric detection of very small signals has been discussed in various contexts in the recent years [21–23].

The experimental arrangement is sketched in Fig. 1a). The samples were prepared by spin coating a dilute solution of gold nanoparticles onto a microscope cover glass. A droplet of water or immersion oil was placed on the sample and contained by a small Teflon cell. For illumination we used an intensity-stabilized laser with a wavelength λ = 532 nm near the peak of the plasmon resonance of a gnp. The experiments were performed on a commercial inverted microscope, which had been modified to house a piezoceramic motion stage to scan the sample in the focus of the objective. The optical signal was collected in reflection, passed through a 50% beam splitter and focused by the microscope tube lens onto a variable diameter confocal pinhole. A photomultiplier served to detect the signal.

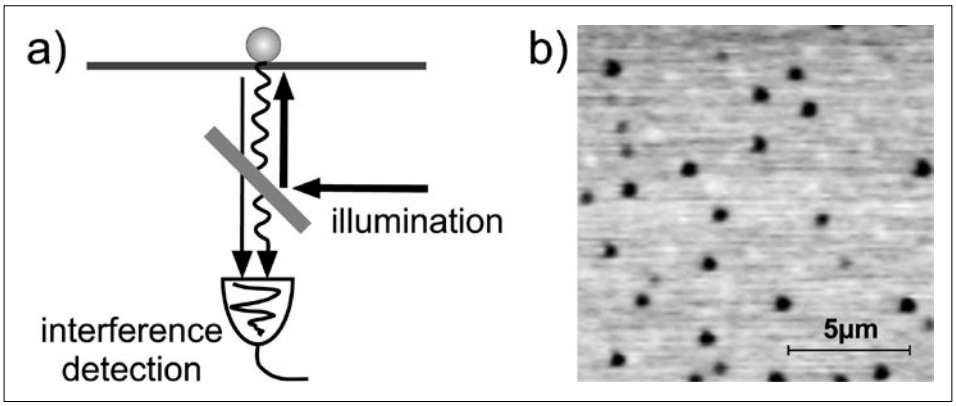


Fig. 1. a) Schematic drawing of the measurement arrangement. The illumination laser beam is focused onto the sample. The reflected beam and the light scattered from the particle interfere on the detector to produce the recorded signal. b) An example of the image obtained in this experiment. Gold nanoparticles of diameter 5 nm at the oil–glass substrate appear dark.

Fig. 1b) shows an example of a scanning confocal microscope image recorded for gnps of 5 nm diameter at the oil–glass interface whereas Fig. 2a) displays an image of 5 nm particles at the water–glass interface – a configuration of great importance for biological studies. Fig. 2b) shows the normalized intensity σ defined as:

$$\sigma = \frac{I_m - I_r}{I_r} \quad (4)$$

for a cross-section of the image in Fig. 2a). Here $I_m$ is the intensity measured from a particle at the center of the focus, and $I_r$ is the intensity of the reflected light without the particle, as determined from the average background in the same scan. For very small particles, the pure scattering term in Eqn. (3) can be neglected so that σ becomes proportional to the scattering amplitude *s*. Particles appear dark against the background due to the destructive interference between the scattered and reflected fields caused by the Gouy phase in the reflection of the focused incident beam.

The method presented here also works for a wide-field arrangement [8] although care has to be taken to avoid wavefront abberations and interferences that would result in unwanted spatial modulation within the camera image. We also point out that since the scattering signal does not saturate, one can substantially reduce the integration time by increasing the illumination power. In this manner, one could improve the time resolution of particle tracking experiments down to the microsecond level [8].

When studying single nanoparticles, it is important to verify that one does not detect aggregates. Since the resolution of the optical microscope is limited to about 200 nm in our case, we have examined the size of the optical signal instead of its spatial distribution. Fig. 2c) shows a histogram of the signal σ for a large number of 5 nm particles. The width of the histogram is less than twice the value of the peak signal, showing that we indeed detect single particles. There are, however, also some particles that deliver a considerably larger signal at the tail of the histogram distribution. Furthermore, we remark that according to Eqn. (2), an uncertainty of only 1–2 nm in the particle diameter leads to a variation in *s* of a factor of 2.

## Direct Detection of Small Unlabelled Entities

The background of the image in Fig. 2a) shows slow variations spread over many scan lines. These fluctuations are caused by scattering from very small irregularities in the glass coverslip. Repeated scans of the same sample produce exactly the same features, proving that the variations are not due to the measurement noise. This observation reveals the extremely high sensitivity of our method to small scattering objects. Indeed, we have shown that unlabelled microtubuli can be detected with a very high SNR ratio in this configuration [8]. Fig. 3a) illustrates the topology of a microtubule in a schematic manner while Fig. 3b) shows an image of a few microtubuli moving on a kinesin surface prepared as in ref. [24]. Recently the interferometric method discussed in this work was also used to detect viruses in a microfluidic channel [25].

While the sensitivity to small scattering objects can be exploited for studying unlabelled entities, it could also cause unwanted background signals when detecting gold nanoparticle labels. However, the scattering of non-metallic objects is wavelength independent while gold nanoparticles possess plasmon resonances [9][13]. We have shown that by simultaneously recording the signal at two different wavelengths, this spectral signature can be used to distinguish the gold nanoparticles against the background scattering [8].

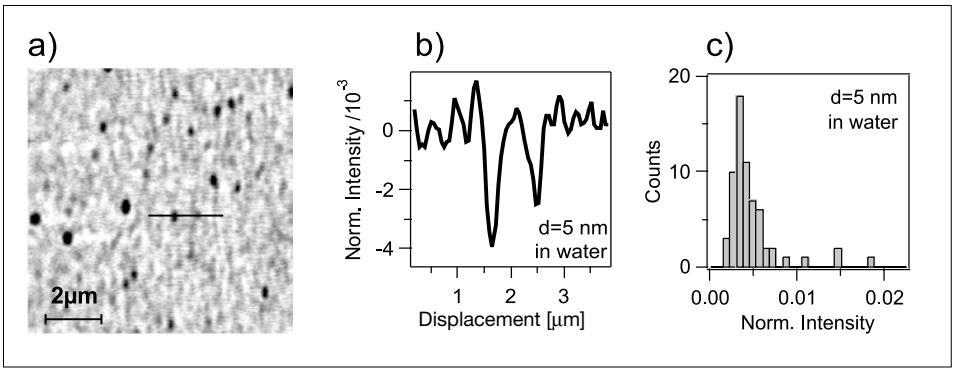


Fig. 2. a) A confocal scan image of 5 nm gold particles at the water–glass interface. b) A cross section of the image in a). c) A histogram of the signal visibility recorded from 5 nm particles. The narrow distribution is a clear proof that we have detected single particles. Fig. adapted from ref. [8]

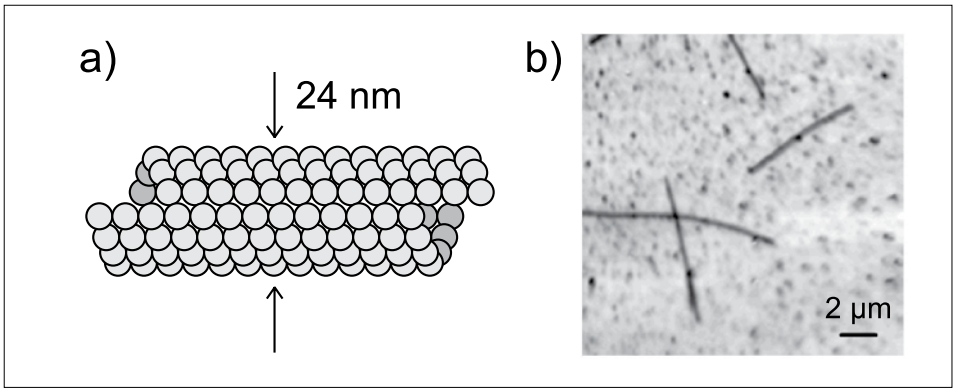


Fig. 3: a) Schematic drawing of a microtubule. b) An interferometric confocal image of unlabelled microtubuli moving on a kinesin surface which are prepared as described in ref. [24]. The microtubules cause a normalized intensity of σ = 0.02.

## Conclusion

We have demonstrated that the optical detection scheme described by Lindfors *et al.* [7], which was initially demonstrated in immersion oil covered samples also allows the detection of particles as small as 5 nm even at a water–glass interface, with a normalized intensity change of around 0.3%. To detect this small signal, a correspondingly low noise illumination and detection scheme is required. The detection sensitivity is high enough that also dielectric nano-objects such as biological macromolecules can be detected in controlled environments. The detection can be done not only in a scanning confocal arrangement but also in a wide-field microscope, enabling simultaneous observation of many particles in large systems. Moreover, the interferometric detection of the scattered light allows the observation of single gold nanoparticles in very short times of the order of a microsecond. Finally, the wavelength dependence of the plasmon resonance allows us to distinguish between gold nanoparticle labels and background scatterers. We believe the method presented here will be a powerful addition to the analytical tool box of nanometer scale studies.

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[1] W.E. Moerner, L. Kador, *Phys. Rev. Lett.* **1989**, *62*, 2535.
[2] M. Orrit, J. Bernard, *Phys. Rev. Lett*. **1990**, *65*, 2716.
[3] E. Betzig, R.J. Chichester, *Science* **1993**, *262*, 1422.
[4] S. Nie, D.T. Chiu, R.N. Zare, *Science* **1994**, *266*, 1018.
[5] S.A. Empedocles, D.J. Norris, M.G. Bawendi, *Phys. Rev. Lett*. **1996**, *77*, 3873.
[6] A. Gruber, A. Dräbenstedt, C. Tietz, L. Fleury, J. Wrachtrup, C. von Borczyskowkin, *Science* **1997**, *276*, 2012.
[7] K. Lindfors, T. Kalkbrenner, P. Stoller, V. Sandoghdar, *Phys. Rev. Lett*. **2004**, *93*, 037401.
[8] V. Jacobsen, P. Stoller, C. Brunner, V. Vogel, V. Sandoghdar, *Opt. Express* **2006**, *14*, 405.
[9] C.F. Bohren, D.R. Huffman, 'Absorption and Scattering of Light by Small Particles', John Wiley and Sons, **1983**.
[10] M. Horisberger, J. Rosset, *J. Histochem. Cytochem.* **1977**, *25*, 295.
[11] U. Kreibig, H. Bönnemann, J. Hormes, in 'Handbook of Surfaces and Interfaces of Materials', Ed. H. Nalwa, Academic Press, San Diego, **2001**.
[12] S. Eustis, M. El-Sayed, *Chem. Soc. Rev.* **2005,** *35*, 209.
[13] U. Kreibig, M. Vollmer, 'Optical Properties of Metal Clusters', Springer Berlin, **1995**.
[14] S. Schultz, D.R. Smith, J.J. Mock, D.A. Schultz, *PNAS* **2000**, *97*, 996.
[15] C. Sönnichsen, S. Geier, N.E. Hecker, G. von Plessen, J. Feldmann, H. Ditlbacher, B. Lamprecht, J.R. Krenn, F.R. Aussenegg, V.Z.-H. Chan, J.P. Spatz, M. Müller, *Appl. Phys. Lett*. **2000**, *77*, 2949.
[16] T. Fujiwara, K. Ritchie, H. Murakoshi, K. Jacobson, A. Kusumi, *J. Cell Biol.* **2002**, *157*, 1071.
[17] X. Michalet, F.F. Pinaud, L. Bentolila, J. Tsay, S. Doose, J. Li, G. Sundaresan, A.M. Wu, S.S. Gambhir, S. Weiss, *Science* **2005**, *307*, 538.
[18] A. Arbouet, D. Christofilos, N.D. Fatti, F. Vallée, J.R. Huntzinger, L. Arnaud, P. Billaud, M. Broyer, *Phys. Rev. Lett.* **2004**, *93*, 127401.
[19] D. Boyer, P. Tamarat, A. Maali, B. Lounis, M. Orrit, *Science* **2002**, *297*, 1160.
[20] S. Berciaud, L. Cognet, G.A. Blab, B. Lounis, *Phys. Rev. Lett*. **2004**, *93*, 257402.
[21] J.S. Batchelder, M.A. Taubenblatt, *Appl. Phys. Lett.* **1989**, *55*, 215.
[22] T. Plakhotnik, V. Palm, *Phys. Rev. Lett.* **2001**, *87*, 183602.
[23] A.A. Mikhailovsky, M.A. Petruska, M.I. Stockman, V.I. Klimov, *Opt. Lett.* **2003**, *28*, 1686.
[24] H. Hess, G.D. Bachand, V. Vogel, *Chem. Eur. J.* **2004**, 10, 2110.
[25] F.V. Ignatovich, L. Novotny, *Phys. Rev. Lett*. **2006,** *96*, 013901.