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Optical imaging and localization of prospective scattering labels smaller than a single protein

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HIGHLIGHTS

- The smallest scattering labels show potential for single particle tracking.
- Scattering objects as small as 2 nm are optically imaged.
- Position estimate is better than 10 nm.
- Extremely small scattering labels are ultimately photostable and biocompatible.

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ABSTRACT

Labeling of biological matter is a key technique enabling visualization and real-time tracking of dynamic processes beyond the diffraction limit. While the precision of fluorescence-based localization microscopy is limited by signal saturation, a new generation of small scattering labels (such as gold nanoparticles) have brought the localization precision below the size of a single protein. However, the size of scattering labels is also the most obvious handicap, as they are usually significantly larger than the resulting localization precision. We address this inconsistency and demonstrate detection and precise localization of gold nanoparticles of only 2 nm in diameter using interferometric detection of scattering (iSCAT). This size is considerably smaller than the size of a small protein and in combination with the possibility of tracking the nanoparticle position, we foresee a new concept of detailed studies of biomolecular systems in the future.

1. Introduction

Modern optical microscopy offers an unprecedented insight into the structure and dynamics of biological matter. Imaging resolution and sensitivity is constantly being pushed to new levels of detail [1]. Due to the non-destructive nature of the interaction of light with biological matter, it is possible to visualize various dynamic biophysical processes in their native state down to the level of individual biomolecules. Molecular labels are usually incorporated to discriminate processes under study from the complex background in a biological sample.

The vast majority of recent progress made in single-molecule optical microscopy has been based on the detection of fluorescence signals. In these techniques, fluorophores are added into a system under study by labeling or direct protein mutation. Time series of diffraction-limited images are recorded and centroid positions of fluorophore images are fitted with the point-spread function of the microscope [2]. The fluorescence saturation sets a fundamental limit on the detection speed as well as localization precision because it limits the number of photons that can be emitted by the fluorophore and collected by the detector within a given time interval [3]. Furthermore, the photostability of fluorophores limits the total number of emitted photons, and therefore observation time, before they photobleach. As a result, the localization precision of state-of-the-art fluorescence-based microscopy has been reported to be in the range of 10–20 nm, with temporal resolutions in the order of 1 ms [4].

Conversely, elastic scattering of light does not suffer from any of these limitations. The detection of scattered light by any particle including single molecules is limited only by the sensitivity of the optical detection system and the level of background scattering. To distinguish an object of interest from the background signal, a dielectric or metallic particle is typically used as a scattering label. A seminal report on the topic involved the use of differential interference contrast (DIC) microscopy, where the position of a 190-nm latex bead attached to a kinesin was tracked with a 1–2 nm localization precision [5]. There have

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recently been a number of works published based on dark-field microscopy to track smaller gold nanoparticles (GNPs) down to 40 nm in diameter. In these works, previously unseen behavior of biophysical systems has been revealed, including stepping details of kinesin motors [6] and long-term study of chaperon dynamics [7].

A common objection to using scattering labels is their large size and mass, both of which are often much larger than the analyte molecule. A number of factors must be accounted for, including the label diffusivity, drag force, the length of label anchor, maximum speed of investigated motion, and the accessibility of label binding site [8]. Indeed, a majority of these factors are linked to the size of the scattering label and the challenge of using smaller nanoparticles as scattering labels is an ongoing effort of modern microscopy.

Imaging of GNPs as small as 10 nm was reported using dark-field microscopy [9]. Among more indirect methods, photothermal detection exploits the absorbing nature of gold nanoparticles to differentiate them against a non-absorbing background, enabling to track GNPs as small as 5 nm, and specifically image even smaller gold clusters [10,11].

Interferometric detection of scattering, coined as iSCAT, has been a particularly successful technique for detecting extremely small scattering signals. Recent improvements in the sensitivity of single protein detection reported for the first time by Piliarik et al. in 2014 [12] has led to increasing attention in single-molecule bioanalytics [13–16]. In principle, iSCAT is a simple homodyne interferometer overlapping the weak scattered beam with a reference light beam, where both share a common optical path [17]. In combination with scattering labels, iSCAT has allowed for tracking GNPs as small as 5 nm [18], observed motion of motor proteins [19], protein diffusion in lipid membranes and live cells [20–22], and increased the temporal resolution of localization trajectories up to 1 MHz [23].

Here, we demonstrate that gold nanoparticles as small as 2 nm in diameter can be imaged and localized using iSCAT. The contrast from GNPs with different sizes is directly optically detected as an instant local fluctuations in the light scattered at a glass-water interface due to the particle immobilization. We present images of the 2-nm GNPs in an environment with minimized background scattering, i.e. on an atomically flat surface, and validate the possibility of tracking the GNP by direct optical imaging with a localization precision of only a few nanometers.

2. Methods

2.1. Experimental setup

A simplified scheme of the iSCAT imaging optical layout is depicted in Fig. 1. The output of a continuous wave laser emitting at 561 nm (Laser Quantum, United Kingdom) was circularly polarized, modulated with an acousto-optic modulator (AA OPTO-ELECTRONIC, France), synchronized with the camera readout, and spatially filtered into a speckle-free beam. The illuminating beam was then transmitted through a 70:30 beam splitter and propagated through the focus (at the back focal plane) of a high-NA microscope objective (α Plan-APOCHROMAT 100× 1.46NA, Carl Zeiss AG, Germany) to illuminate area of $4.5 \times 4.5\ \mu m^2$ on the functionalized coverslip with a cuvette containing aqueous sample. The total power density incident on the sample was between $80\ \mu W\ \mu m^{-2}$ for 60-nm GNPs and $520\ \mu W\ \mu m^{-2}$ for 2-nm GNPs. Light reflected on the glass-water interface and light scattered by nanoparticles in the vicinity of the glass surface were collected by the same microscope objective and redirected at the 70:30 beam splitter to be imaged on a CMOS-based camera (Photonfocus AG, Switzerland).

The coverslip with a cuvette containing liquid sample was mounted on top of a 3-axis piezo stage (Physik Instrumente GmbH & Co. KG, Germany), which enabled positioning of the sample with sub-nanometer precision.

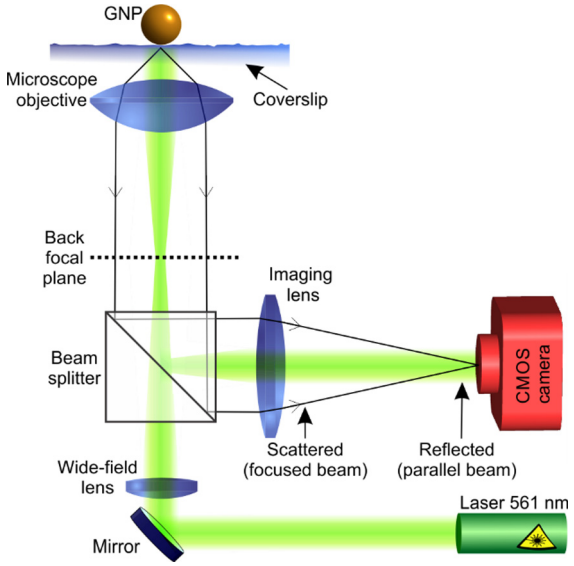

Fig. 1. Optical layout of iSCAT imaging.

2.2. Image acquisition and data processing

The exposure time of the camera was 10 μs and a region of 128 × 128 pixels was acquired using a custom LabView program at 7000 frames per second. Temporal averaging of 100 frames was used to image GNPs larger than 5 nm, whereas up to 1000-frames averaging was used to image the smallest nanoparticles. To suppress the effect of wavefront aberrations and background scattering from objects that are not connected with the sample, the sample was periodically moved in one of the lateral axes with a displacement amplitude of 300 nm using the piezoelectric scanner via a lock-in principle [12].

2.3. Detection of GNPs on the glass surface

Glass coverslips (Marienfeld, Germany) were rinsed with acetone, ethanol and deionized water and dried with nitrogen after each rinsing step. Then, they were treated with O_2 plasma for 90 s at 30% power in a plasma cleaner (Diener, Germany). Cleaned coverslips were drop-casted with 0.01% poly-L-ornithine solution (Sigma-Aldrich) for one minute and rinsed with deionized water.

Colloidal solutions of citrate-capped GNPs with mean diameters of 2, 5, 10, 15, 20, 30, 40 and 60 nm were purchased from BBI solutions (UK). In order to obtain a statistically relevant values of the interferometric contrast of the particles, a series of individual GNPs were observed in real time as they were arriving on poly-L-ornithine-functionalized glass surface from aqueous colloidal solution.

The functionalized coverslip was placed in the focus of the microscope objective and an acrylic glass cuvette was mounted on top of the coverslip. The cuvette was filled with 200 μL of deionized water. After establishing a stable image of the coverslip surface, 10 μL of GNP solution (100-times dilution of a stock solution) was injected into the cuvette. By analyzing fluctuations in the time series of consecutive images, we extracted the iSCAT signal of a statistically significant number of nanoparticles (i.e. tens to hundreds), which were bound to the glass surface within the field of view.

2.4. Immobilization of GNPs on mica

The highest quality muscovite mica (V1 grade from Electron Microscopy Sciences, USA) was cleaved and attached to the cover glass slide using an optical glue (NOA 61 from Norland Inc., USA). The thickness of the glue was reduced by spinning the sample for 30 s at 12,000 RPM. The glue was then cross-linked by the UV light, mica was

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