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# Scattering measurement of single particle for highly sensitive homogeneous detection of DNA in serum

Liang Zhu[a,b], Guohua Li[a,b], Yonghong He[a,b,*], Hui Tan[c], Shuqing Sun[a,b]

[a] Institute of Optical Imaging and Sensing, Shenzhen Key Laboratory for Minimal Invasive Medical Technologies, Graduate School at Shenzhen, Tsinghua University, Shenzhen 518055, People's Republic of China

[b] Department of Physics, Tsinghua University, Beijing 100084, People's Republic of China

[c] The First Affiliated Hospital of Shenzhen University, Shenzhen Key Laboratory of Neurosurgery, Shenzhen 518035, People's Rebublic of China

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ABSTRACT

A highly sensitive homogeneous method for DNA detection has been developed. The system relies on two kinds of gold nanorod (AuNR) probes with complementary DNA sequences to the target DNA. In the presence of the target DNA, two kinds of AuNR probes are assembling into dimers or small aggregates. The target-induced AuNR aggregate has higher scattering intensity than that of a single AuNR because of the plasmonic coupling effect. Dark field microscopy was utilized to image the single particle and measure its scattering intensity. We wrote our own Matlab code and used it to extract the scattering signal of all particles. Difference in distribution of scattering intensity between the single AuNR and its aggregate provides a quantitative basis for the detection of target DNA. A linear dynamic range spanning from 0.1 pM to 1 nM and a detection limit of ~ 30 fM were achieved for the detection of DNA in serum sample.

## 1. Introduction

Highly sensitive and convenient DNA assays are of great importance in the practice of diagnostic medicine and biological research [1,2]. For example, highly infective hepatitis virus is an enormous threat to life and difficult to afford ideal treatment. Efficacious methods for the diagnosis and therapy of this deadly virus would be particularly important. Conventional DNA assay requires standard procedures containing capture probe immobilization, specific target binding, recognition of target with high-intensity tags, multiple steps of washing cycles and signal readout [3–6]. Due to its high complexity, time-consuming experimental process, labor-intensive manual operations and well-trained experts are commonly required to perform it for reliable results. To overcome these difficulties, rapid one-step homogeneous DNA assay has attracted a lot of attention in recent years. Most of these methods can generally be divided into three categories including fluorescence quenching [7–9], Förster resonance energy transfer (FRET) [10–13] and target-induced gold nanoparticle (GNP) aggregation [14]. However, the first two types of methods are always criticized for the instability of the fluorescent reagents containing unmanageable fluorescence quenching and photoblinking.

In contrast, methods based on target-induced GNP aggregation are generally considered to be a superior one as the excellent optical and chemical properties of GNPs. The basic idea of this method was first proposed by Mirkin et.al., who found that AuNPs modified with DNA capture probes can self-assemble into larger aggregates by the addition of the complementary oligonucleotides [15], leading to a change in plasmon resonance absorption that can be observed by UV–Vis extinction spectrum. In turn, we can utilize this change to quantify the concentration of the target. This is a typical homogeneous colorimetric measurement and has been generalized into various applications by using different ligand-receptor pairs [16–19]. But the major drawback to limit its broad practical applications lies in the low detection sensitivity.

Up to date, attempts to develop methods based GNP aggregation for higher detection sensitivity are focused on the quantitative measurement of the degree of GNP aggregation. These methods can be divided into two categories: ensemble measurement and single particle detection. Ensemble methods are on the basis of scattering measurement techniques such as nonlinear light scattering [20], linear light scattering [21] and dynamic light scattering (DLS) [22,23]. As compared with the ensemble measurement, single particle detection method is thought to have great potential in detection sensitivity because it is capable of detecting an individual biomolecule [24]. Typical methods include inductively coupled plasma mass spectrometry (SP-ICP-MS) [25], high-sensitivity flow cytometer [26], methods based on Brownian

* Corresponding author at: Institute of Optical Imaging and Sensing, Shenzhen Key Laboratory for Minimal Invasive Medical Technologies, Graduate School at Shenzhen, Tsinghua University, Shenzhen 518055, People's Republic of China.

*E-mail addresses:* heyh@sz.tsinghua.edu.cn (Y. He), tanhui@iccas.ac.cn (H. Tan), sun.shuqing@sz.tsinghua.edu.cn (S. Sun).

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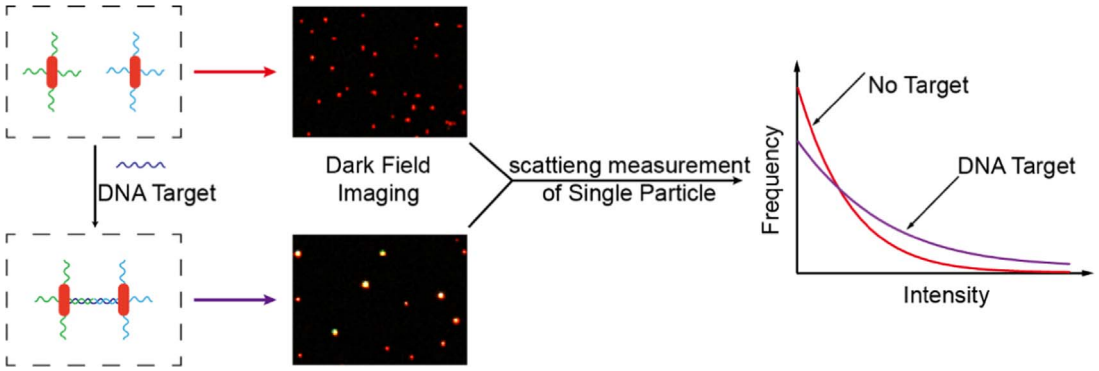


**Fig. 1.** Schematic illustration of the detection principle.

motion [27–29], darkfield microscopy [30] and optical anisotropy imaging [31].

Gold nanorods (AuNR) are a class of special plasmonic nanoparticles. Its plasmon extinction splits into two distinct bands corresponding to the light absorption along the longitudinal and transverse axis. They can be synthesized in a simple manner and are highly stable and dispersible in solutions. As compared with AuNPs, a single AuNR is much easier to be distinguished from the substrate under the dark field image due to the much larger scattering cross section in their extinction bands [32–36]. However, the excellent ability of single particle identification has not been applied in the promising detection methods based on target-induced nanoparticles aggregation.

In this work, we reported the development of a sensitive homogenous DNA assay based on target-induced AuNRs aggregation and scattering measurement of single particle. As illustrated in Fig. 1, AuNR dimer or small aggregate is formed by the addition of the target DNA with the aid of complementary DNA sequences on AuNR surfaces. As compared with the single AuNR, the scattering intensity of the AuNR aggregate is much larger than that because of the strong plasmonic coupling effect. The scattering intensity of particles is measured by dark field microscopy and extracted by a home-made single particle analysis program. The higher concentration of the target DNA indicates that there is a much higher proportion of particles with high intensity scattering, providing us a quantitative basis to detect the target DNA.

## 2. Materials and methods

### 2.1. Materials and chemicals

Ultrapure water was used throughout the whole work. Cetyltrimethyl Ammonium Bromide (CTAB), hydrogen tetrachloroaurate (III) hydrate ($HAuCl_4$), 2-(N-Morpholino) ethanesulfonic acid (MES), sodium borohydride ($NaBH_4$), 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), Tris-(2-carboxyethyl)-phosphine (TCEP), 1,4-dithiolthreitol (DTT), silver nitrate ($AgNO_3$), polyvinylpyrrolidone (PVP, M.W. 8000), Sodium Dodecyl Sulfonate (SDS), ethanolamine were purchased from Sigma-Aldrich. (3-aminopropyl) triethoxysilane (APTES) was purchased from Nanjing Chen Gong organic silicon material co., LTD (China). Phosphate Buffered Saline (PBS) was prepared before use. DNA sequences used in this work are listed as follows: DNA1: 5′-GTG TGG ATA ATA GAG AAT-$A_{10}$-$(CH2)_6$-SH-3′; DNA2: 5′-SH-$(CH2)_6$-$A_{10}$-AGT GCT TAT GCA GCA AAT-3′; perfectly matched target DNA: 5′-ATT CTC TAT TAT CCA CAC CTA TTT GCT GCA TAA GCA CT-3′; single base-pair mismatched target DNA 1: 5′-ATT CTC TAG TAT CCA CAC CTA TTT GCT GCA TAA GCA CT-3′; single base-pair mismatched target DNA 2: 5′-ATT CTC TAT TAT CCT CAC CTA TTT GCT GCA TAA GCA CT-3′; single base-pair mismatched target DNA 3: 5′-ATT CTC TAT TAT CCA CAC CTA TTT GCT GCA GAA GCA CT-3′. All these DNA sequences were purchased from Beijing Genomics Institution (China).

### 2.2. Details for characterization

Extinction spectrum was recorded by a U-4100 UV–Visible spectrophotometer (Japan). The spectrum range was set from 400 to 800 nm. Step size was set at 0.5 nm and the measurement speed was set as 600 nm/min. In measurement of the extinction spectrum of AuNR solution, 2 mL sample was added into a clean cuvette with size 1 × 1 × 4 $cm^3$ (4 cm was the height). The cuvette was placed into the sample channel and then close the cover on it. Run the program and record the raw data. High resolution transmission electron microscopy (HRTEM) was conducted to observe the morphology of the AuNRs. In order to prepare the sample for observation with HRTEM, 1 mL of freshly prepared AuNR solution was centrifuged at 8500 rpm for 10 min at first. Then the supernatant was removed and the resulting pellet was shaken well. About 8 μL of the pellet was dropped into a clean copper wire mesh. The copper wire mesh was placed into a clean vacuum drying oven to remove the moisture. After thoroughly drying the sample, the copper wire mesh can be used for observation with HRTEM.

### 2.3. Modification of the glass slides

Glass slides were immersed in the piranha solution (98% $H_2SO_4$/30% $H_2O_2$ = 7:3) for at least 6 h to decompose the impurity particles on their surface, and subsequently rinsed in ultrapure water for sonication for 5 min at least 5 times to thoroughly remove all the potential impurities. Extreme care should be taken to use piranha solution as it is very dangerous. The clean slides were immersed in the 20% v/v APTES ethanol solution for at least 6 h. As a result, the slide was functionalized by APTES to form high intensity amino groups-grafted hydrophobic surface. The obtained APTES-modified glass slide was rinsed in ethanol with sonication for 5 min at least 5 times followed by stored in a 60 °C clean vacuum drying oven before use.

### 2.4. Synthesis of CTAB-stabilized AuNRs

AuNRs with an average length of 51.7 nm and width of 24.3 nm were synthesized according to a reported seed-mediated method with some modifications [37]. In brief, 240 μL of 100 mM $NaBH_4$ solution, 400 μL of 24 mM $HAuCl_4$ and 40 mL of 100 mM CTAB solution were added into a flask (100 mL) with vigorously mixing. The mixture turned yellow brown suggesting the formation of the seed solution for AuNR growth. Subsequently, the growth solution was prepared by mixing 40 mL of 200 mM CTAB, 400 μL of 24 mM $HAuCl_4$ and 300 μL of 5 M HCl into a flask. Two hundred microliters of 40 mM of $AgNO_3$ and 320 μL of 100 mM ascorbic acid were further added. At last, 56 μL of the seed solution was added. The flask was covered with aluminum foil and kept standing for about 8 h at room temperature. The color of the mixture turned purple indicated the formation of the AuNRs. These obtained AuNRs were centrifuged and stored at 10 mL of 0.01 M CTAB solution in the dark prior to use.