

Electronic Supplementary Information (ESI)

for

Precision improvement in dark-field microscopic imaging by
using gold nanoparticles as internal reference: a combined
theoretical and experimental study†

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1 Effect of oil dropped on the dark-field condenser on the scattered light of AgNPs

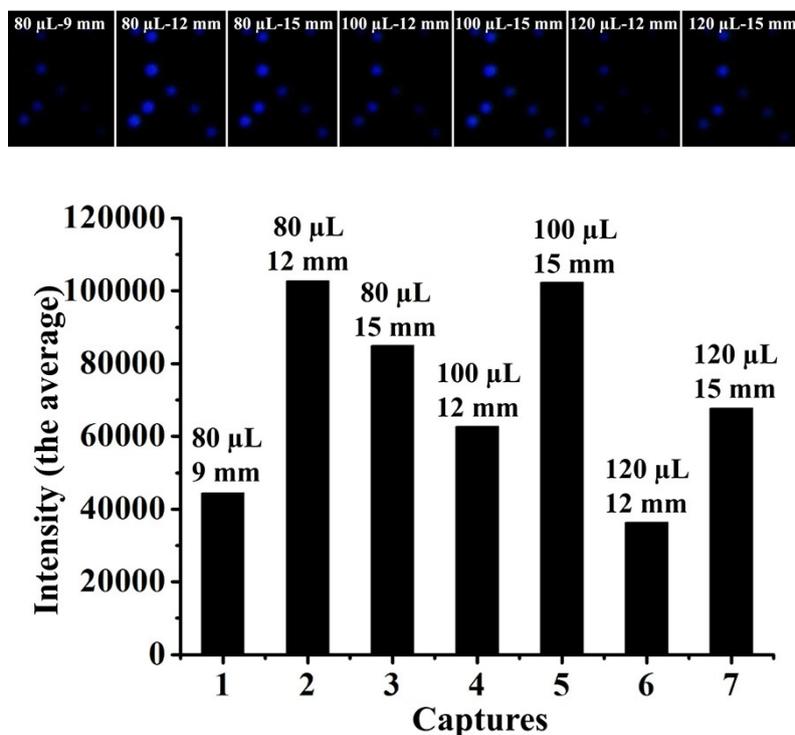


Fig. S1 iDFMs of the same AgNPs from the same region under different capturing conditions (top) and the average light intensities for them after expressed as digital information (bottom). Different amount of oil (about 80 μL , 100 μL , 120 μL) was dropped onto the dark-field condenser and oil circles in different size (about 9 mm, 12mm, 15 mm in diameter) were used.

We investigated the effect of the oil, which is necessary between the dark-field condenser and the slide, on the scattered light of the same AgNPs from the same region. To evaluate the effect of the oil, different amount of oil (about 80 μL , 100 μL , 120 μL , the oil was taken by pipettor) was dropped onto the dark-field condenser, after which the condenser was adjusted to touch the glass slide to form oil circles with different sizes (about 9 mm, 12mm, 15 mm in diameter), and iDFMs were captured under different conditions (each capture was corresponding to each condition) which are shown in Fig. S1 (top). Since the scattered light of AgNPs under condition (about 100 μL , about 9 mm) and condition (about 120 μL , about 9 mm) was too weak to observe, we did not show it here. After expressed as digital information, the average light intensities of AgNPs in different captures are shown in Fig. S1 (bottom).

The intensity of the same AgNPs varied severely, and the intensity changed from less than 36000 to more than 100000. Thus it can be concluded that the amount and the disperse of the oil on the condenser have significant influences on the acquired data. In practical applications, the exact amount of oil dropped on the condenser is almost impossible to be precisely controlled even when pipettor is used considering the severe residual of the oil which is unavoidable. On the other hand, the disperse of the oil is also impossible to be precisely controlled considering the mechanical drift of the glass slide from the stage (or adjustments of the relative position between sample and the dark-field condenser).

2 Characterization of AuNPs and AgNPs

The AuNPs and AgNPs were successfully synthesized according to modified literature procedures as described in the previous section. Fig. S2-1, 2-2 and 2-3 display SEM images of the as-prepared AuNPs and AgNPs. AuNPs with an average diameter of 13 nm (Fig. S2-1, S2-7) and 54 nm (Fig. S2-2, S2-7) had localized surface plasmon resonance (LSPR) peaks located at 516 nm and 526 nm (Fig. S2-4), respectively. AgNPs had an average diameter of 27 nm (Fig. S2-3, S2-7), and they were uniform quasi-spheres, which was consistent with the narrow LSPR peak located at 406 nm (Fig. S2-4). The iDFM of AuNPs-54 nm is shown in Fig. S2-5, in which the scattered light colors are dominated by green, which originates from the spherical nanoparticles. Due to its uniform morphology, the scattered light colors of AgNPs (Fig. S2-6) are dominated by blue (up to 98 %). Thus, we could identify the nanoparticles scattering green as AuNPs and those scattering blue as AgNPs in the iDFMs of mixtures containing AuNPs and AgNPs.

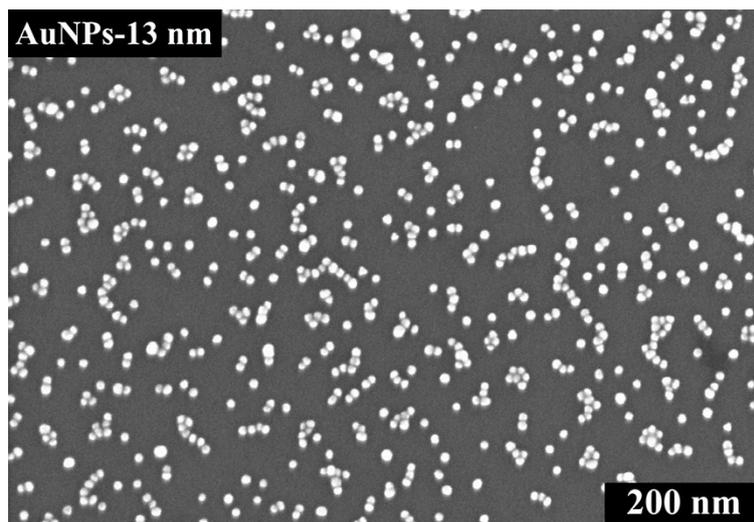


Fig. S2-1 SEM image of AuNPs-13 nm.

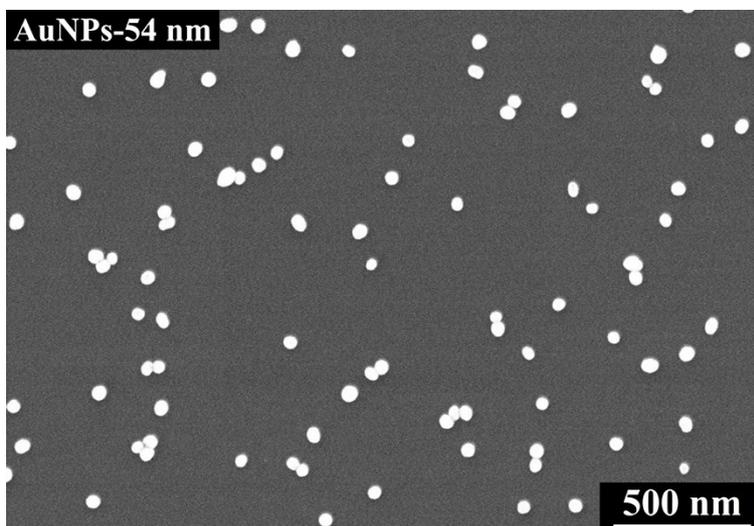


Fig. S2-2 SEM image of AuNPs-54 nm.

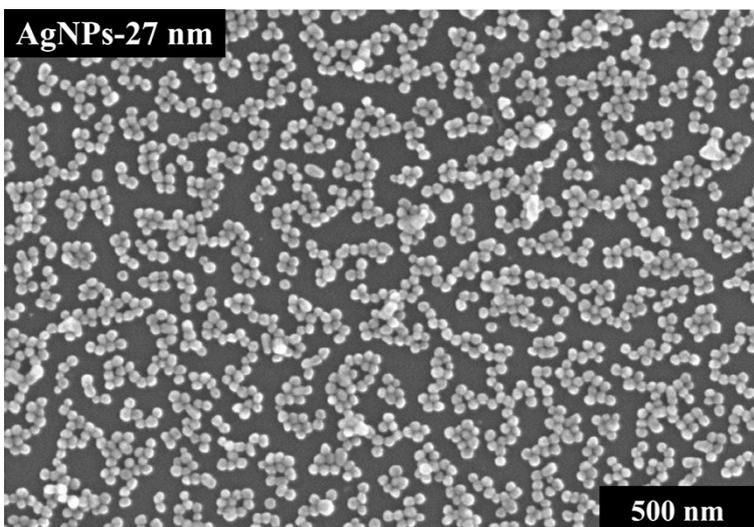


Fig. S2-3 SEM image of AgNPs-27 nm.

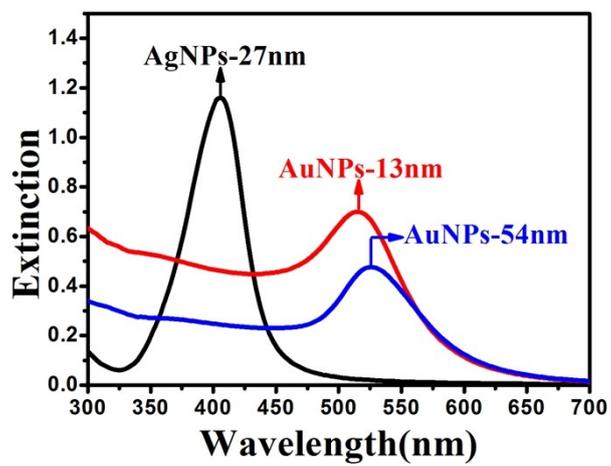


Fig. S2-4 Extinction spectrum for AuNPs and AgNPs.

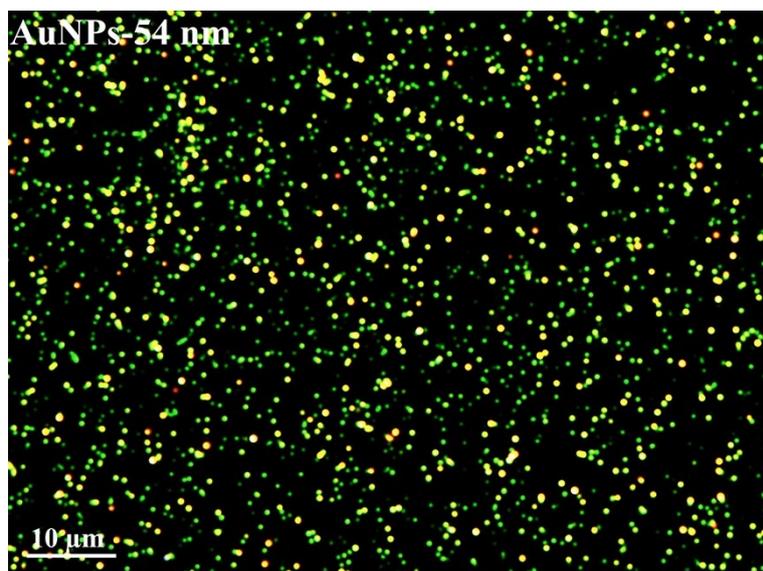


Fig. S2-5 The full dark-field microscopic image for AuNPs-54nm.

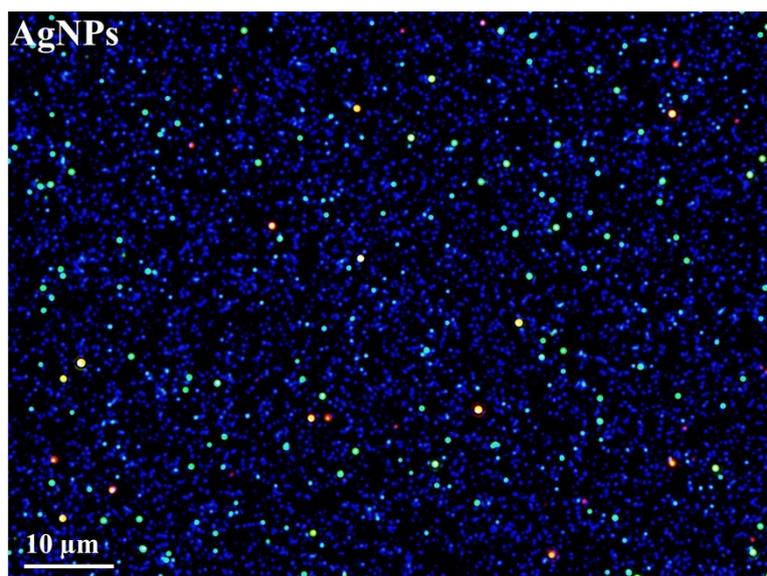


Fig. S2-6 The full dark-field microscopic image for AgNPs.

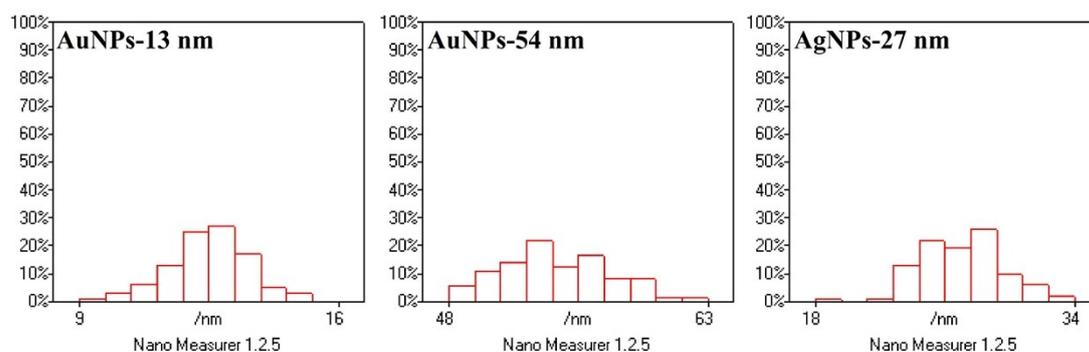


Fig. S2-7 The size distribution in SEM images for AuNPs-13 nm, AuNPs-54 nm, and AgNPs-27 nm, respectively.

3 Typical iDFM for the mixture nanoparticles

The AuNPs used as the IR did not interfere with the dynamics of the reaction between AgNPs and O_2 or H_2O_2 in our work. In all of our investigations, the AuNPs and AgNPs were first mixed in solution, and then fixed to the glass slides by the electrostatic adsorption between the positively charged slide and the negatively charged nanoparticles. The distance between two single nanoparticles was large enough (more than $0.5 \mu m$, more than 10 times of the diameter of these nanoparticles, see Fig. S3-1). After that, the slides loaded with these nanoparticles were exposed to O_2 or H_2O_2 or glucose. Therefore, the mutual interference between single nanoparticles can be neglected.

The quality and usefulness of analytical data depend critically on the validity of the sample. In order to achieve reliable results, the sample should be an unbiased representative of the whole. Therefore, these typical nanoparticles which were used in this study should be randomly selected and they were evenly distributed in the whole image (see Fig. S3-1).

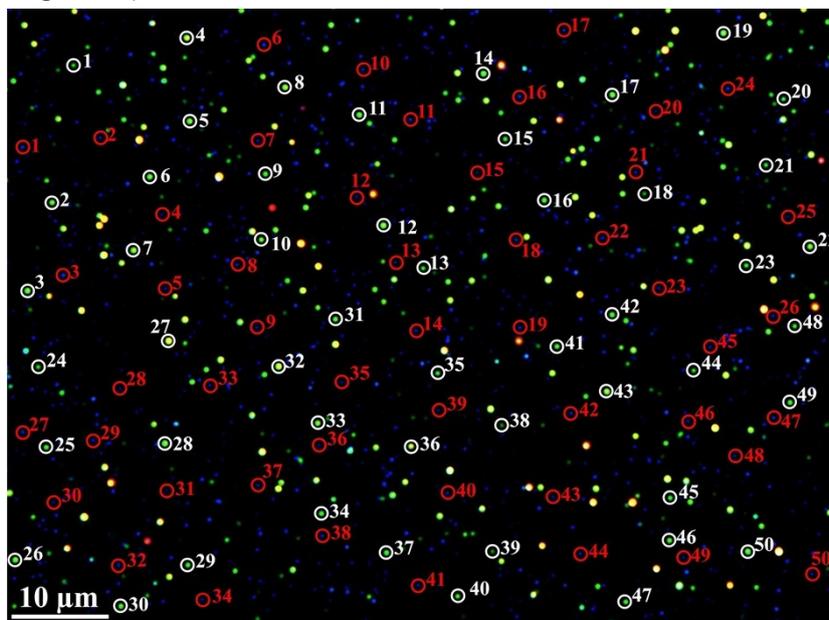


Fig. S3-1 Illustration of the way for choosing typical light spots to represent all blue and green spots, respectively.

In order to investigate the influence of sampling positions on the scattering light intensity, the oxidation process of AgNPs in air at room temperature was studied. iDFMs of the same nanoparticles (composed of a mixture of AuNPs and AgNPs) in the same region were captured before and after exposing these nanoparticles to air (as described in the experimental section). The initial light intensities of AgNPs (I) and the intensity changes of AgNPs (ΔI) measured at different positions might be different. Thus these two parameters could reflect the fluctuation of the data obtained in different positions. In order to investigate the influence of sampling positions in a simplified way, these images were separated into four equal parts in the same way to represent four different positions in these images (as shown in Fig. S3-2A, parts a-d). Then four series of light intensities of AgNPs (corresponding to the average intensities of points 1-9, 10-15, 16-23, 24-30 in different exposure time, respectively.

These points were randomly selected and evenly distributed in each position to represent all AgNPs in this position.) were collected from these four parts, respectively. $\Delta I/I$ was introduced to represent the relative change in light intensity of AgNPs and each series of $\Delta I/I$ is shown in Fig. S3-2B. The data series showed similar tendencies, and in general, $\Delta I/I$ was found to decrease with increasing exposure time in air. However, the $\Delta I/I$ for the same time in these four series was different. Thus, in order to ensure the validity of the acquired data, these typical nanoparticles chosen to represent all nanoparticles should be evenly distributed in the whole image.

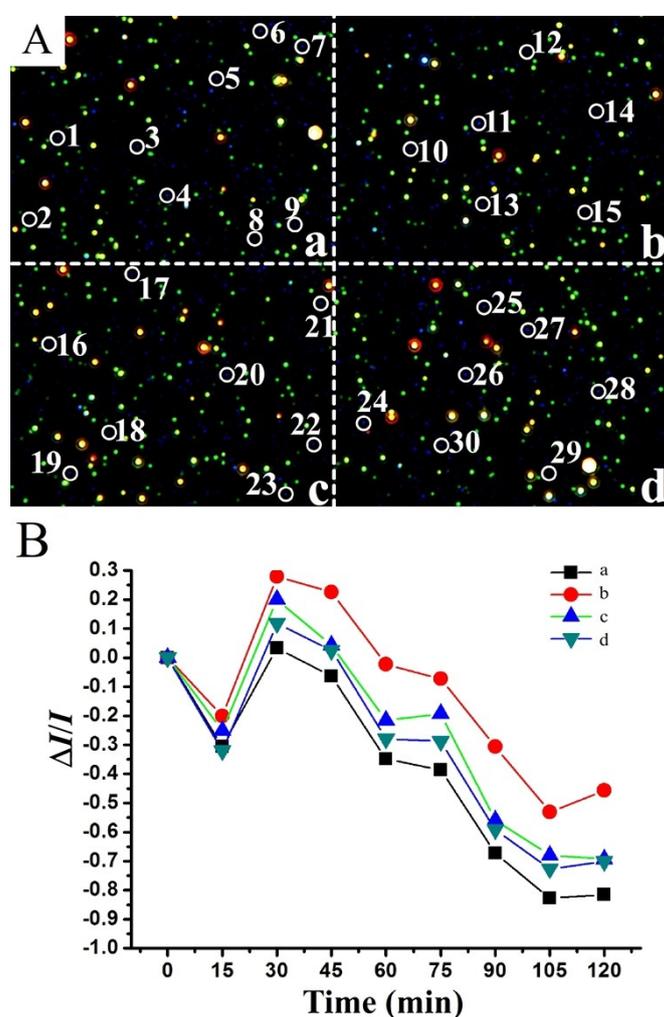


Fig. S3-2 The influence of sampling positions on detection results. (A) iDFM of AgNPs and AuNPs mixture, showing how these images were separated and positions of these chosen AgNPs. (B) Four series (a-d) of $\Delta I/I$ corresponding to the average intensities of points 1-9, 10-15, 16-23, 24-30 in Fig. S3-2A (a-d), respectively. I is the initial light intensity of AgNPs and ΔI is the

change in the light intensity of AgNPs. The graph illustrates the change of $\Delta I / I$ in these four series with increasing exposure time in air.

4 The derivation process for introducing the calibration factor α

Since $\bar{I}_{\text{blue}} / \bar{I}_{\text{green}}$ would remain unchanged without further chemical reactions or physical changes, this rule could also be suitable for the nanoparticles which had finished their reactions (they would not be subjected to further chemical reactions or physical changes).

$$\frac{I_{\text{blue}}'}{\bar{I}_{\text{green}}'} = \frac{I_{\text{blue}}^{\chi}}{\bar{I}_{\text{green}}^{\chi}}$$

(1)

Thus,

$$I_{\text{blue}}^{\chi} = \frac{\bar{I}_{\text{green}}}{\bar{I}_{\text{green}}'} I_{\text{blue}}' = \alpha I_{\text{blue}}$$

(2)

Where, \bar{I}_{green} and \bar{I}_{green}' represent the average values of the intensities of the green scattered light from AuNPs before and after chemical reactions or physical changes, respectively. I_{blue}' and I_{blue}^{χ} represent the observed intensities of the blue light from AgNPs after chemical reactions or physical changes before and after regulation, respectively.

5 Monitoring the interaction between AgNPs and oxygen

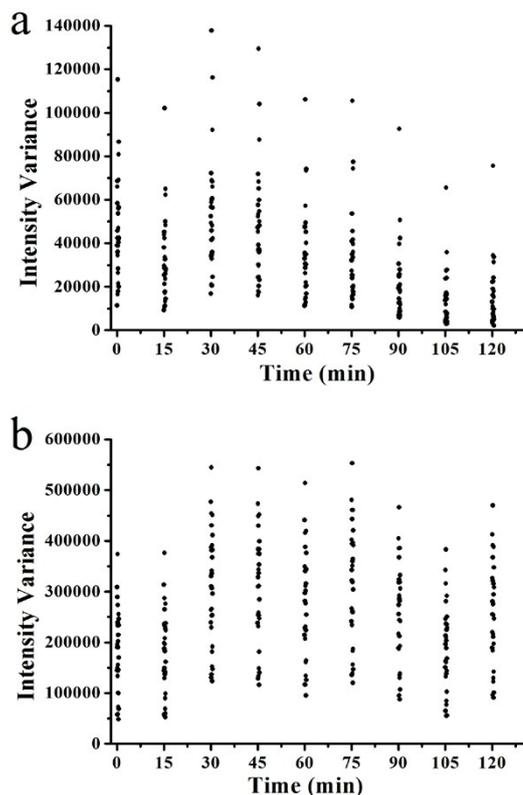


Fig. S4-1 The light intensities of the same 50 AgNPs (a) and AuNPs (b) before and after their direct exposure to air, respectively. The mixture of AgNPs and AuNPs were absorbed on a region by an electrostatic attraction. The 50 AgNPs and AuNPs were randomly selected from the mixture nanoparticles to represent all AgNPs and AuNPs in this region, respectively.

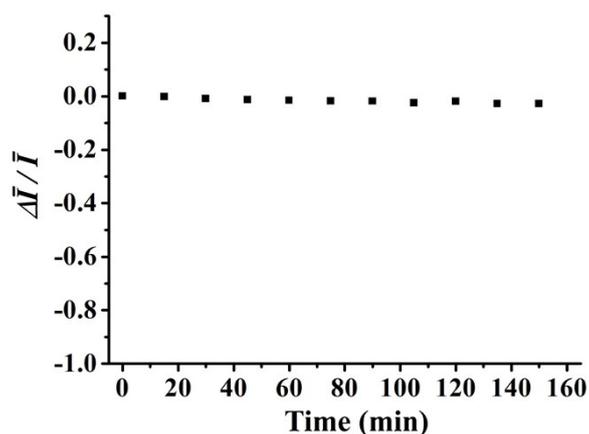


Fig. S4-2 Monitoring of the oxidation of AgNPs in oxygen which is naturally solved in water at room temperature. iDFMs of the same region before and after the oxidation of AgNPs were captured and used. The change of $\Delta \bar{I} / \bar{I}$ with increasing the exposure time of AgNPs in oxygen solved in water. \bar{I} is the average value of the light intensities of AgNPs from the whole region at the beginning and $\Delta \bar{I}$ represents the change in the average value of the intensities of AgNPs from the whole region after their exposure in oxygen solved in water.

The influence of the oxygen naturally solved in water was also investigated. As seen in Fig. S4-2, the oxygen naturally solved in water had little influence on the intensity of the scattered light of AgNPs. The aqueous medium was used between the cover glass and the glass slide, thus, all nanoparticles were not directly exposed to air in all of our investigations except in the process of monitoring the oxidation of nanoparticles in air. Thus the aqueous medium can be considered as one way to protect AgNPs from being oxidized in air.

6 Co-locating the same nanoparticles with DFM image and SEM image

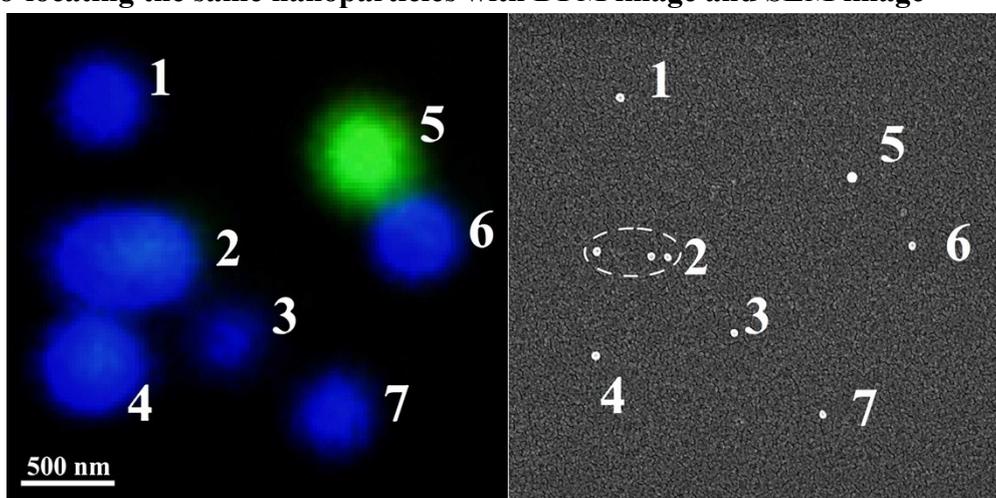


Fig. S5 DFM (left) and SEM (right) image of the same nanoparticles in the same region.

7 SEM images of AgNPs before and after their reaction with H_2O_2

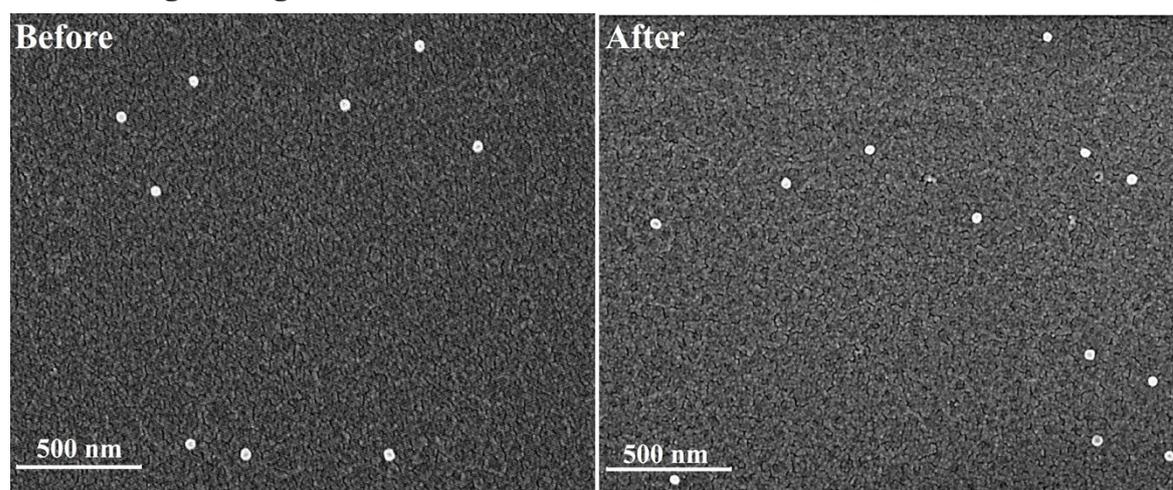


Fig. S6 SEM images of AgNPs before and after their reaction with H_2O_2 .

8 Comparison of the scattered light from the mixture nanoparticles before and

after their exposure to glucose

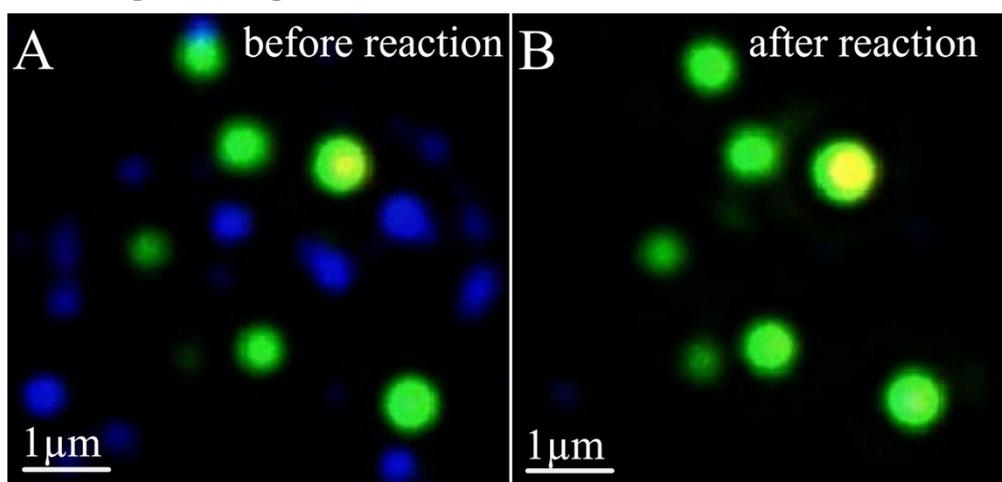


Fig. S7 iDFMs of the mixture nanoparticles from the same region before (A) and after (B) their exposure to glucose. The green spots are AuNPs while the blue spots are AgNPs.

9 The extinction spectrum for the bare AuNPs and AuNPs-TAMRA

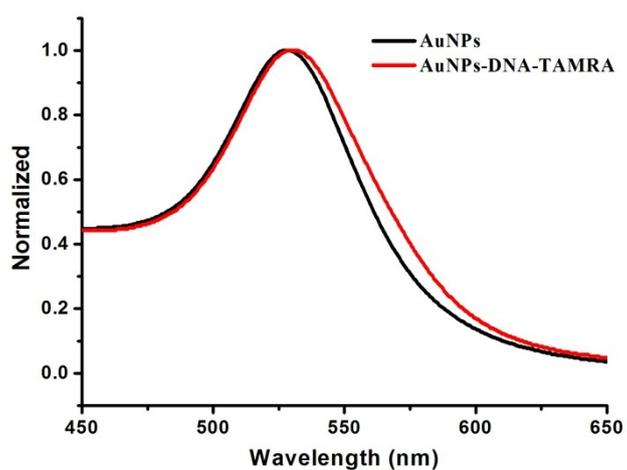


Fig. S8 The extinction spectrum for the bare AuNPs and AuNPs-TAMRA.