Electronic Supplementary Information (ESI)

for

HSI colour-coded analysis of scattered light of single plasmonic nanoparticles

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1. Experimental section

1.1 Apparatus

The size and morphology of the AuNPs were imaged through scanning electron microscopy (SEM) (S-4800, Hitachi, Tokyo, Japan). The scattering and UV-vis absorption spectra of AuNPs colloid were performed with F-4500 Fluorescence Spectrophotometer (Shimazdu, Japan) and UV-3600 spectrophotometer (Hitachi, Tokyo, Japan), respectively. Dark-field imaging was carried out through a BX51 optical microscope (Olympus, Japan) equipped with a high numerical dark-field condenser (U-DCW, 1.2–1.4). White light from a 100 W tungsten lamp passed through dark-field condenser and interacted with AuNPs to scatter colour light. The scattered light was collected by a 100× objective lens and the images were taken by a DP72 single chip true colour CCD camera (Olympus, Japan). The originally acquired images were all 24-bit truecolour TIFF/BMP picture files.

1.2. Reagents

The 50 nm AuNPs were purchased from Pinano Technology Co. Ltd. (Beijing, China). The used probe DNA sequence (5’-SH- TTT TTg CTA TTT gAT ggC-3’) and target DNA sequence (5’-ATT AAAgCT CgC CAT CAAATAgCAA-3’) was synthesized by Sangon Biotech Co., Ltd. (Shanghai, China). Ethanol, 1-butanol, glycol, dimethylsulfoxide (DMSO) were analytical reagent grade and were used without further treatment. Oil used as the solvent for dark-field imaging of AuNPs was from Olympus special for confocal fluorescence microscope. Milli-Q purified water (18.2 MΩ at 25 ℃) was used throughout.

1.3. Dark-field microscopic imaging of AuNPs immersed in different solvents

To observe the scattering light of AuNPs, a homemade device made of a slide glass and cover glass was used to produce a simulated flow cell. Different solvents (water, ethanol, 1-butanol, glycol and DMSO) were pipetted into the cell in turn to observe the variations in the scattering light of AuNPs immersed in different dielectric

environments. Dark-field images of AuNPs deposited at the bottom of the cell in each condition were taken respectively and a characteristic region containing AuNPs was selected for analysis.

1.4. HSI analysis of the attachment of thiolated DNA

To qualitatively analyze the attachment of thiolated DNA to AuNPs, bare AuNPs with a size of 50 nm were deposited on a glass slide firstly, and then 150 μL 500 nM probe DNA solution was added in the cell to start the reaction of thiolated DNA binding to AuNPs through Au-S bond at room temperature. The binding reaction was essentially time-consuming in this condition, but here it completed quickly because the thiolated DNA in the solution using pH 3.0 buffer, immensely promoting the reaction. Dark-field images of AuNPs in the same area before and after reaction were taken and converted into HSI colour space of image through computer programming, after which the hue values in HSI of scattered light colour spots were calculated with software.

To gain quantitative understangding, bare AuNPs with a size of 50 nm were deposited on a glass slide firstly, and then 150 μL different concentration of probe DNA sequence solution (0.1, 1, 10, 50, 100, 200, 500, 1000 nM) was added respectively in the cell for 5 min using pH 3.0 buffer at room temperature. After reaction, each solution dark-field images were taken and hue shift was calculated using computer programming. Then we use origin 8.0 software to draw curve about relationship of hue shift and different probe DNA concentration.

To analyze the DNA hybridization on the surface of AuNPs, bare AuNPs with a size of 50 nm were deposited on a glass slide firstly, and then 150 μL 100 nM probe DNA solution was added in the cell for 5 min using pH 3.0 buffer at room temperature. Then, different concentration of target DNA sequence solution (1, 10, 50, 100, 200, 500, 1000 nM) was added respectively using Tris HC1 pH 7.4 buffer (Containing Na^+ 1.4 M, K^+ 0.2 M, Mg^{2+} 0.1 M) for 120 min at room temperature. After reaction, each solution dark-field images were taken and hue shift was calculated using computer programming. Then we use origin 8.0 software to draw curve about relationship of hue

shift and different target DNA concentration.

1.5. Data analysis

When collect the original dark-field images by dark-field microscopy (DFM), we use HSI colour model analysis for scattered light of AuNPs, and through hue values in HSI colour model to reflect scattered light change. So we need to calculate the hue values of each particle in the dark-field images. The digital image contains a fixed number of rows and columns of pixels, and pixels are the smallest individual element in an image, holding quantized values that represent the colour at any specific point (Fig. S3, ESI). If calculate hue value of particle, we need to calculate hue values of all pixels corresponding this particle and calculate the mean of these hue values. Herein we need to analyze dark-field images of AuNPs at pixel level.

In this study, the digital hue values of each particle need to be calculated. In order to realize it, the algorithm of our proposed include three steps. Firstly, we do image segmentation in dark-field images, and we can get the particles without any background information from the images (Fig. S1). The hue values can be calculated from these particles. Image segmentation is a process of pixel classification, here we aim to segment colour spot in dark-field images from the background. Because the colour of particles is quite distinct from that of background and the background is same colour, it will be clear to apply thresholding for image segmentation. In this step, we need to convert colour image to greyscale image. The greyscale image $f(x,y)$ is two dimensional and each pixel has single value (such as intensity). By this way, the particles and background pixels have grey levels group into two dominant modes. We can use thresholding to separate these modes. The thresholding as following equation:

$$g(x,y)=\begin{cases}1 & if\ \ f(x,y)\geq T\\ 0 & if\ \ f(x,y)<T\end{cases}$$

We just need to set threshold 'T', then any point(x,y) for which $f(x,y)<T$ is a background point, otherwise, the point is an object point.

However, there is a problem that we don't known the hue values of pixels belong to which particle, and we can't get the hue value of selected particle. To solve these problems, we give the each particle in dark-field image a label using image tags in the second step of our algorithm. Here, we use a matrix which has same column and row as the dark-field image, and label a concrete number in pixel corresponding particle, these pixels belong a same particle have a same number tags (Fig. S1). We scan each row from up to down of the dark-field image at pixel level, and calculate values of each pixel, such as black colour value of the pixel is (0,0,0) if we use RGB image. Then use this value compare background value (we can set all pixels of background values are the same value when complete image segmentation), we can judge this pixel belong to particles if the value not equal to background value. We write down this pixel position and use binary tree traversal algorithm found all the pixels which adjacent together. These pixels are belong same particle, so we label the same number tags in matrix at the same position. Then, we back the original position and continue scan the pixels, we will use binary tree traversal algorithm found all the pixels which adjacent together if detect the value of pixel not equal to background value and not label in tags matrix, and do the same process until scan all pixel in the dark-field image. Finally, we can find all the pixels belong to each particle after image tags step through scan the matrix.

Thirdly, the hue values of each particle can be calculated. In this experiment, the dark-field images which we obtained are RGB image, the hue value of a pixel can be calculate from RGB information of same pixel using the equation (1). When get the hue value of each pixels, we can sum the hue value of all pixels which belong to same particle using the image tags, and get the mean hue value of each particle.

2. Additional figures

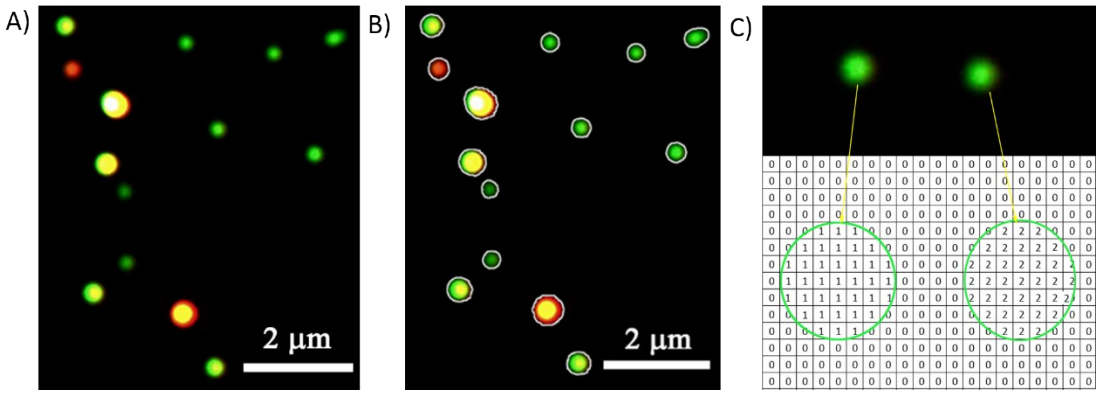

Fig. S1 The schematic illustration of the computer image processing. When get the DFM image (A), we can use computer programming to calculate the hue values of particles in DFM image. First, separate each particles from background in DFM image using image segmentation (B). Second, label each particles in pixel with image tags (C), and upper in C are two particles in DFM image, lower in C tag each pixel (the same number 1 or 2 represent these pixels belong one particle, 0 represent background) . Then, the hue values of single AuNPs in DFM image can be calculated according to tags in pixel.

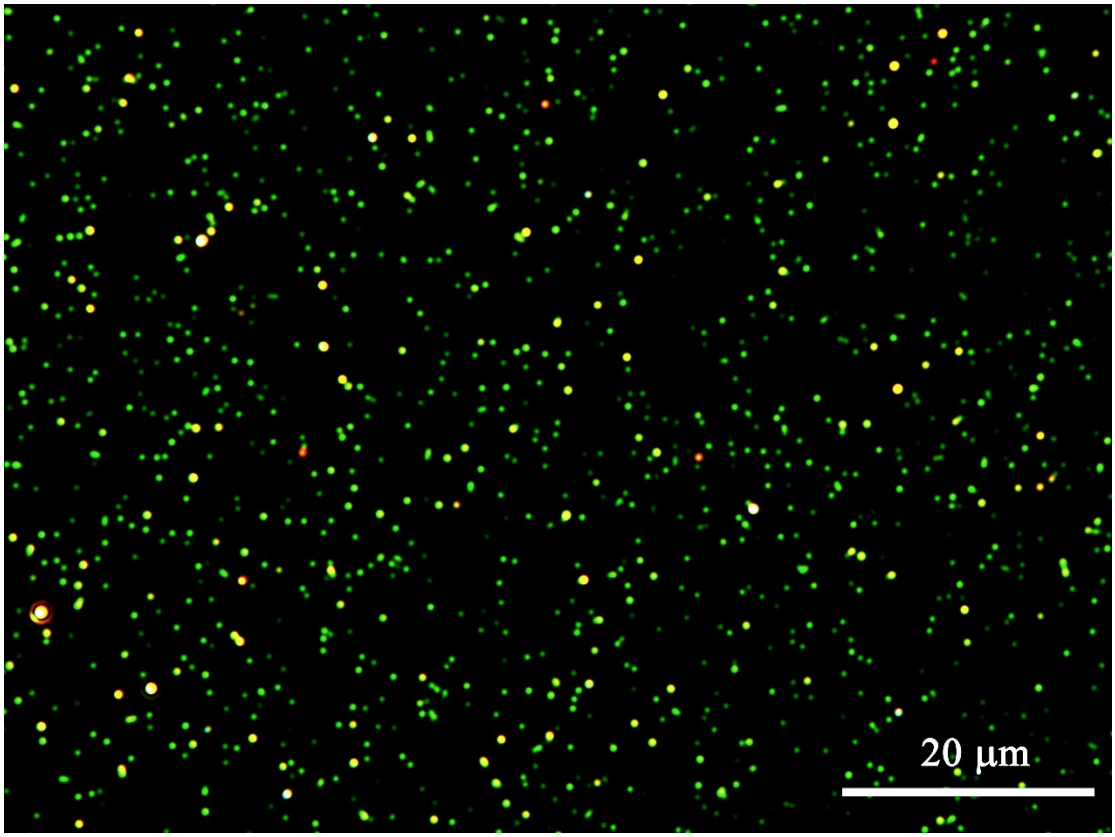

Fig. S2 Scattered light iDFMs of AuNPs with an average diameter of about 50 nm，and most of them are green. The image scale bar corresponds to 20 μm.

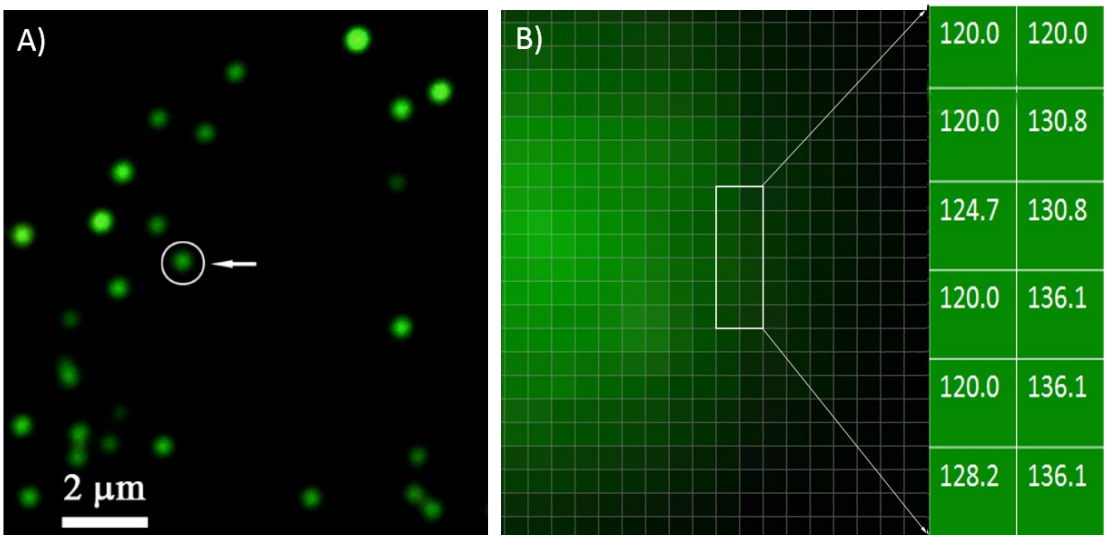

Fig. S3 Digitization of DFM images of AuNPs using HSI colour system. (A) Light scattered dark-field microscopic images of AuNPs. (B) Magnified image of the indicated AuNPs in (A) at pixel level, and the scattered light of the selected pixels in (B) are digitized and expressed as digital information of hue values as listed on the right.

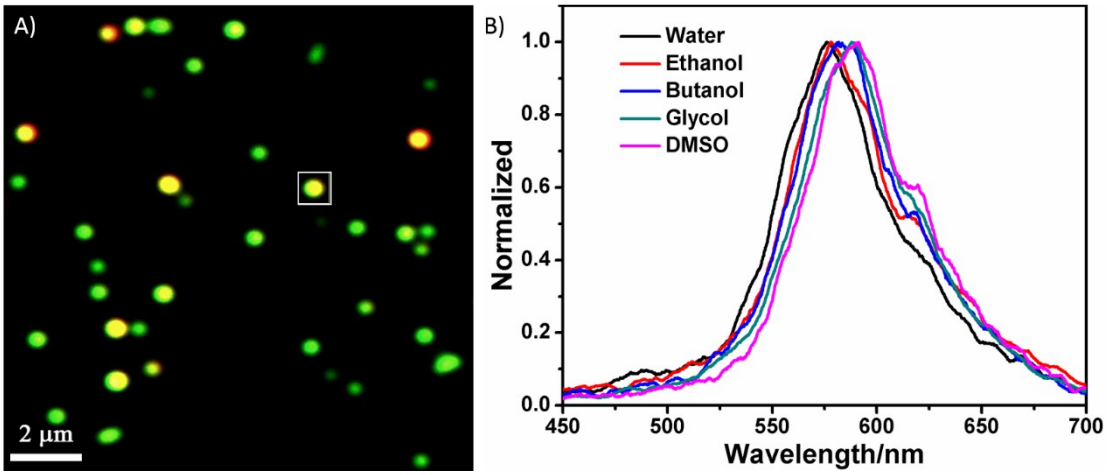

Fig. S4 The DFM image and scattering spectrum of the chosen AuNPs bathed in different solvents. (A) DFM image of AuNPs bathed in DMSO. (B) The spectrum of the selected AuNPs bathed in water, ethanol, 1-butanol, ethylene glycol (EG) and DMSO.

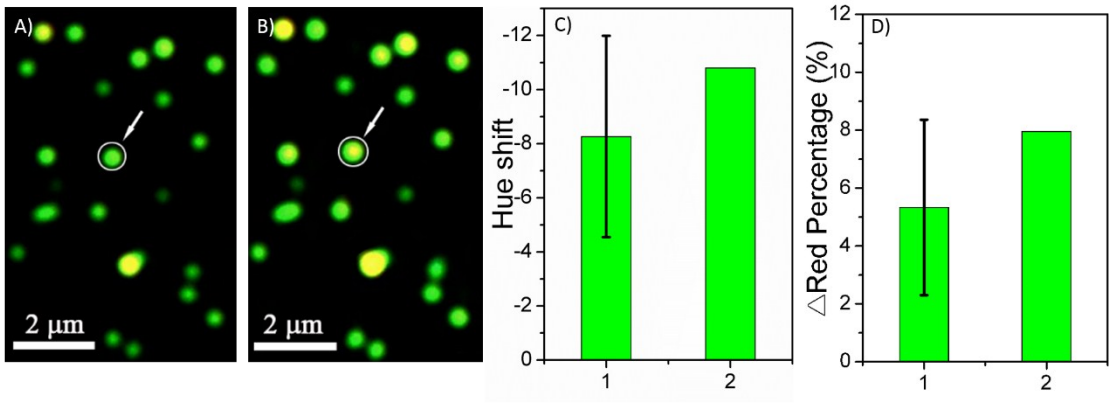

Fig. S5 DFM image of the AuNPs before (A) and after (B) the attachment of thiolated DNA to the corresponding AuNPs in A. The shift of hue values of the Dark-field scattering light color in A and B (C) and 1 represent mean hue values of all AuNPs in A and B, and 2 represent the hue value of the selected particle. Red percentage shift values of scattering light color in A and B using RGB analysis (D).

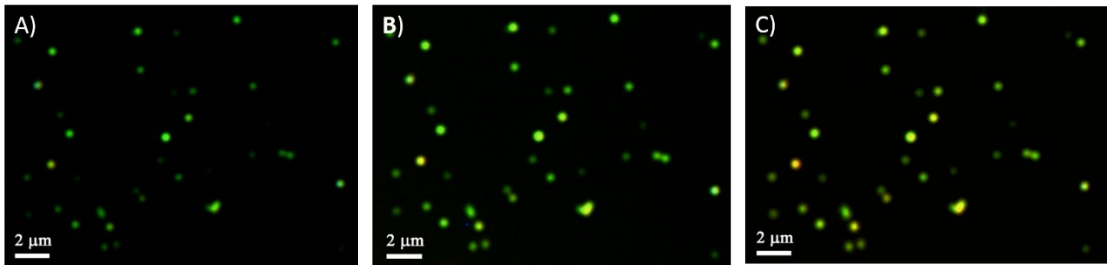

Fig. S6 Typical DFM images of DNA hybridization on the surface of AuNPs. (A) DFM image of AuNPs bathed in water on clean glass slide. (B) DFM image of the attachment of thiolated DNA to AuNPs. (C) DFM image of DNA hybridization on the surface of AuNPs after attaching thiolated DNA on AuNPs. The scattered light colour of particles in DFM image change from green (A) to yellow-green (B) after attaching thiolated DNA to AuNPs, and the colour of particles change from yellow-green (B) to yellow (C) after DNA hybridization on the surface of AuNPs.