Electronic Supplementary Information

Individually color-coded plasmonic nanoparticles for RGB analysis

Yue Liu^a, Jian Ling^b, and Cheng Zhi Huang^{*a,c}

 ^a Education Ministry Key Laboratory on Luminescence and Real-Time Analysis, School of Chemistry and Chemical Engineering, Southwest University, Chongqing 400715, China
^b School of Chemical Science and Technology, Yunnan University, Kunming 650091, China
^c College of Pharmaceutical Science, Southwest University, Chongqing 400716, China
*Corresponding author: chengzhi@swu.edu.cn

Experimental section

1. Apparatus

The absorption and scattering spectra of AuNPs colloid solution were measured with UV-3600 UV-Vis-NIR Spectrophotometer (Shimazdu, Japan) and F-4500 Fluorescence Spectrophotometer (Hitachi, Japan), respectively. S-4800 scanning electron microscope (SEM) (Hitachi, Japan) was employed for imaging the size and shape of AuNPs. Dark-field imaging was carried out through 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 passes through dark-field condenser

and interacts with AuNPs to scatter color light. The scattering lights from AuNPs were collected by a 100× object lens (Adjustable numerical aperture from 0.6 to 1.3) and images were taken by DP72 single chip truecolor CCD camera (Olympus, Japan) controlled by IPE software (a simplified edition of IPP, Media Cybernetics, U.S.A.). The acquired images were all 24-bit truecolor TIFF/BMP picture files.

2. Reagents

Chloroauric acid tetrahyrate (HAuCl₄·4H₂O) was purchased from Sinopharm Chemical Reagent Co. Ltd (Shanghai, China). 11-mercaptoundecanoic acid (MUA) was purchased from Sigma-Aldrich (Missouri, USA). Sodium citrate, 1-propanol and 1-octanol are analytical reagent grade for use without further purification. Oil as a solvent for dark-field imaging of plasmonic nanoparticle was from Olympus special for confocal fluorescence microscope. All water used was purified with an LD-50G-E Ultra-Pure water system (Lidi Modern Waters Equipments Co., Chongqing, China).

3. Synthesis of AuNPs

For synthesis of AuNPs with intense scattering light that could be clearly observed under a dark-field microscope, the procedure was as follows (X. Ji, X. Song, J. Li, Y. Bai, W. Yang, X. Peng, *J. Am. Chem. Soc.*, 2007, **129**, 13939). In a clean cone-shaped bottle, 50 mL of HAuCl₄·4H₂O solution (0.25 m mol/L) was added and brought to boiling. Shortly, 0.3 mL of citrate sodium (50.0 m mol/L) was added under vigorous stirring. The mixture was kept boiling and refluxed for 15 minutes, then heat source was evacuated and the colloid solution was cool to room temperature with continuous stirring.

4. Dark-Field imaging of AuNPs bathed in different solvents

To observe the scattering light of AuNPs bathed in different dielectric environments, a homemade device, which consisted of slide glass and cover glass only to give birth to a simulative flow cell, was employed. Firstly, AuNPs were deposited to the bottom of the cell. Secondly, water, 1-propanol, 1-octanol and oil were pipetted into the cell in turn, and dark-field images were taken.

5. RGB analysis of molecular binding

For qualitative analysis of thiols binding to AuNPs, bare AuNPs were deposited on a glass slide following by adding 70 μ L of 1-butanol containing 1.0 m mol/L MUA into the cell. Then, the reaction of MUA molecules binding to AuNP was taken place through Au-S bond at room temperature for 60 minutes. Before and after the reaction, dark-field images of AuNPs in the same region were taken and the RGB values of scattering light color spots were calculated with software.

6. Data Analysis

The process of dark-field images and the calculation of primary RGB color values were carried out through Image-Pro Plus (IPP) software (Media Cybernetics, U.S.A.). Color scattering light has three independent properties, namely, hue, saturation and lightness. The scattering intensity is determined by lightness, while the scattering light color of a plasmonic nanoparticle is determined by hue. One scattering color spot represents an individual nanoparticle, and one spot consists of many pixels, all of which have three color channels, namely RGB. Thus, for calculating the RGB color values of an individual nanoparticle, all pixels of a color spot are involved.

Additional Figures



Fig. S1 (A) Principle of coordination of tricolor-RGB. By adjusting red (R), green (G) and blue (B) lights, whose wavelengths are 700.0 nm, 546.1 nm and 435.8 nm, respectively, it can make the same light with C in diffusers by standard observer through diaphragm. (B) Three-dimensional coordinate of tricolor with integral color values from 0 to 255 for 24-bit truecolor in computing. So there are 16777216 kinds of colors in theory resulting from the calculation of ($256 \times 256 \times 256$).



Fig. S2 (A) Absorption and scattering spectra of AuNPs. The absorption and scattering peaks of AuNPs are located at 532 nm and 565 nm, respectively. (B) SEM image of AuNPs.



Fig. S3 The chromaticity diagram according to 1931 Commission International de 1'Eclairage (CIE) (http://cvision.ucsd.edu/gallery/CIE1931.gif). *x* axis represents the proportion of R primary color and *y* axis is for G primary color. And the axis representing B primary color can be deduced from the equation of x + y + z = 1. As we find, *x* has a tendency to increase, while *y* decreases from green to red.



Fig. S4 The relationships of P_R and P_G with light wavelength of standard spectral color (A and B) and corrected standard spectral color with gamma value of 0.6 (C and D). Gamma correction is the name of a nonlinear operation used to code and decode luminance or tri-stimulus values in video or still image systems. Commonly, a linear response is given by a gamma value of 1.0 (A and B). However, to get optimal images, the gamma corrections of the truecolor CCD camera or display are always not set as 1.0. Thus, the intensity of the output on TV and computer display devices is not directly proportional to the R, G, and B applied electric signals. For example, if we correct the standard spectral color with gamma value of 0.6, P_R and P_G will be nonlinear with light wavelength (C and D).

A)	B)	C)	D)

Fig. S5 Dark-field scattering images of an AuNP with red scattering light bathed in

water (A), 1-propanol (B), 1-octanol (C), and oil (D).