

## Electronic Supplementary Information (ESI)

# Making Gold Nanoparticles Fluorescent for Simultaneous Absorption and Fluorescence Detection on the Single Particle Level

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## Experimental setup

Two configurations of the experimental setup (depicted below) were used to conduct the experiments of simultaneous photothermal and fluorescence detection

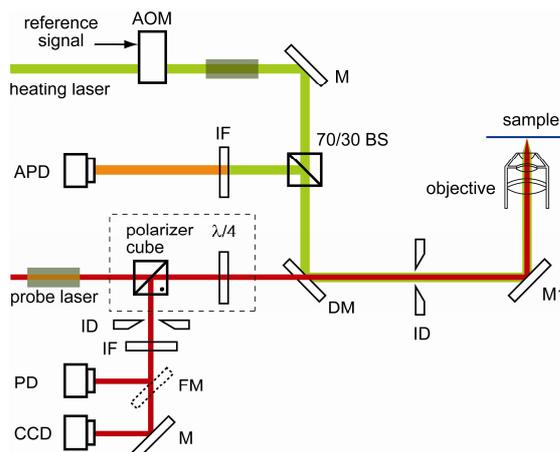


Figure S1. Scheme of the experimental setup for simultaneous photothermal and fluorescence detection. The heating beam is provided by a laser diode at 532 nm (Shanghai Lasers, power 150 mW). AOM – acousto-optical modulator, M – mirror, FM – flip mirror, BS – beam splitter, DM – dichroic mirror, ID – iris diaphragm,  $\lambda/4$  – quarterwaveplate, IF – interference filters, APD – avalanche photodetector, PD – photodiode, CCD – video camera. Polarizer, spatial filters are not shown in this scheme. Grey boxes schematically indicate the positions of the telescopes and beam expander. A dashed line indicates the position of the “cat’s eye” parts (a polarizer beam splitter and a waveplate) that can be replaced by a beamsplitter, 50/50 BS. The probe beam at 790 nm is provided by a Ti:sapphire laser (Mira, Coherent, pumped with Coherent Verdi V10). Details of the setup are described elsewhere [A. Gaiduk, P.V. Ruijgrok, M. Yorulmaz, M. Orrit, *Chem. Sci.*, 2010, 1, 343]

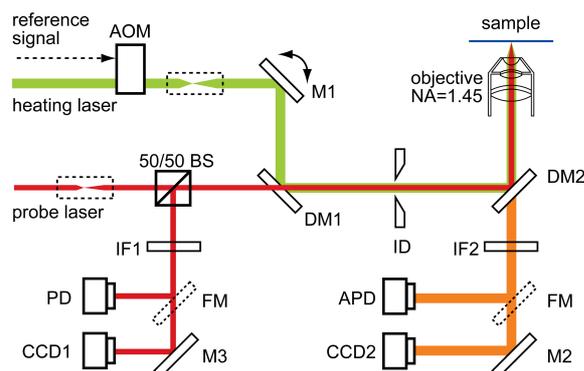


Figure S2. Scheme of the experimental setup for simultaneous photothermal and fluorescence detection. The heating beam is provided by an Ar-Ion laser (Coherent Innova 300) or by a laser diode at 532 nm (Shanghai Lasers). The probe beam at 800 nm is produced by a Ti:sapphire laser (S3900s, Spectra Physics) pumped with the Ar-Ion laser. AOM – acousto-optical modulator, M – mirrors, FM – flip mirrors, BS – beam splitter, DM – dichroic mirrors, ID – iris diaphragm, IF – interference filters, APD – avalanche photodetector (optionally spectrograph), PD – photodiode, CCD – video cameras. Positions of beam-expanding telescopes are indicated with dashed boxes. Spatial filters are not shown in the schematic representation.

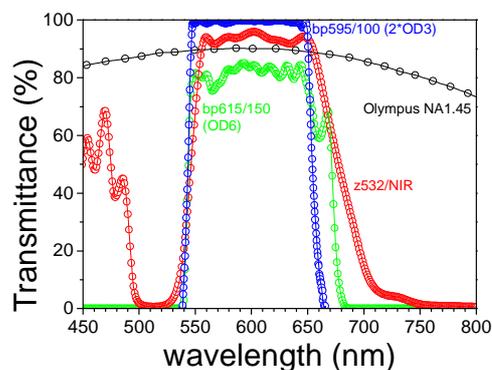


Figure S3. Transmittance of the microscope objective, dichroic mirror and optical filters in the experimental setup (Fig.S2) for simultaneous photothermal and fluorescence detection.

## Absorption and fluorescence spectra of 20 nm gold NPs solution

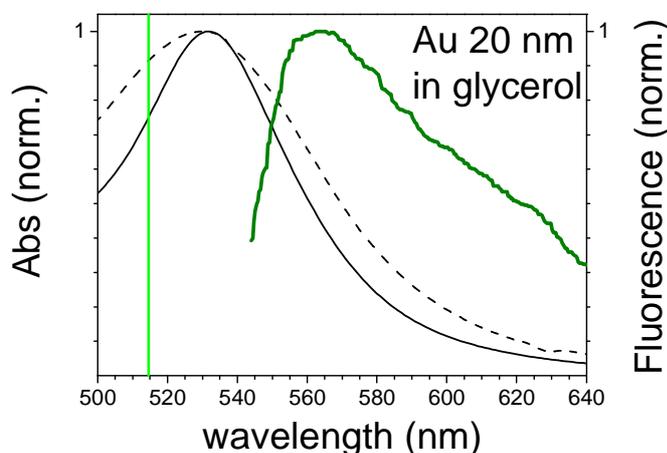


Figure S4. Normalized absorption and fluorescence spectra of 20 nm diameter gold NPs.

Solid black line: Calculated absorption spectrum in glycerol according to Mie theory.

Dashed black line: Measured ensemble absorption spectrum in a glycerol:water mixture (3:1). The broader shape of the spectra arises from the distribution of NP sizes and shapes.

Solid green line: Fluorescence spectrum from a single NP made fluorescent after moderate heating on the surface of glass in glycerol. The steep decrease of the fluorescence spectrum in the short-wavelength region is due to the optical transmission of dichroic mirrors and interference filters (Fig.S3).

A solution of gold NPs in glycerol:water mixture (3:1) showed no measurable fluorescence, and the spectrum did not change after illumination with 514 nm laser light for >2 hours with the laser intensity about 100 W/cm<sup>2</sup>.

The vertical solid green line indicates the fluorescence excitation at 514 nm.

## SEM, AFM and photothermal imaging of 20 nm gold NPs

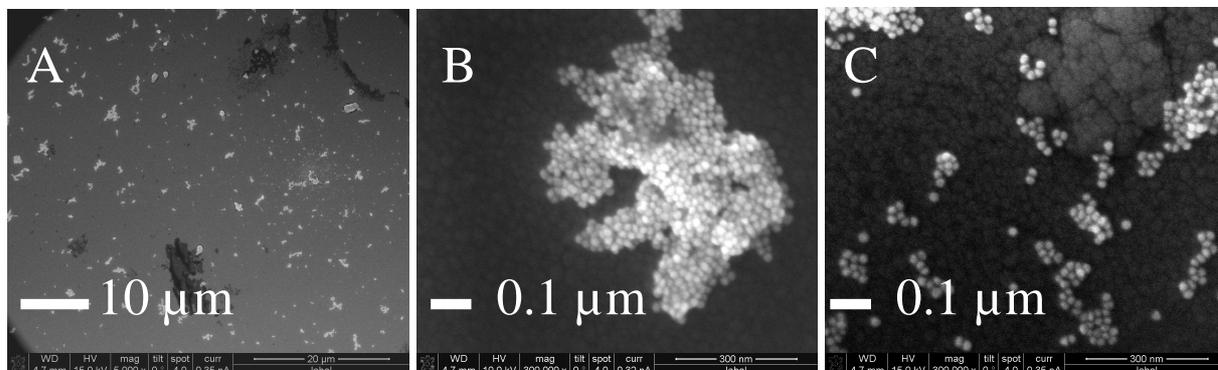


Figure S5. SEM images of 20 nm gold particles prepared by drop casting of 50  $\mu\text{L}$  of undiluted NPs solution on the surface of ITO-glass substrate. Images are taken at different magnifications. (A) shows a large area of the sample and illustrates different aggregates of particles in this area. (B) is the magnified image of a big NPs cluster. (C) is the zoom into an area containing smaller clusters and also where individual NPs can be resolved. SEM images show that this preparation method does not result in the homogeneous sample containing individual NPs separated from each at distances required to resolve them with optical microscopy.

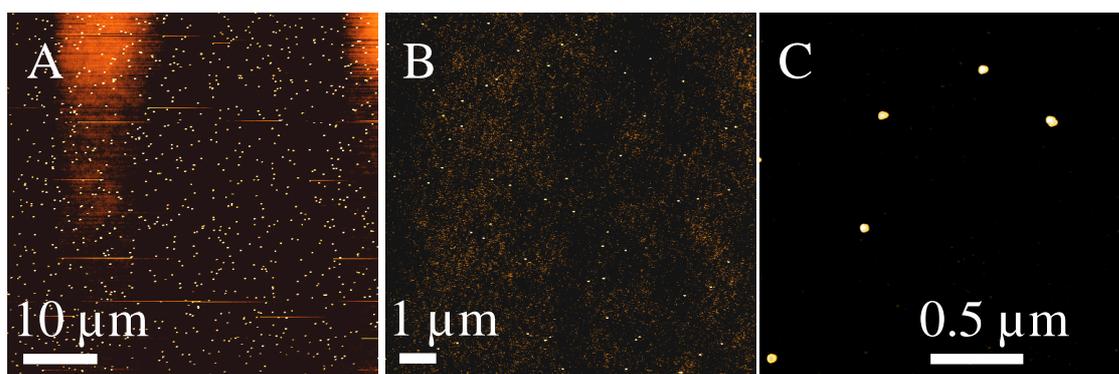


Figure S6. AFM topography images of 20 nm gold NPs prepared by spin coating on cleaned glass surface, as described in the main text. Images of  $50 \times 50 \mu\text{m}^2$  (A),  $10 \times 10 \mu\text{m}^2$  (B) and  $2 \times 2 \mu\text{m}^2$  (C) show no aggregates. Most of gold NPs are separated from each other at distances greater than 300 nm, large enough to resolve single particles with optical imaging.

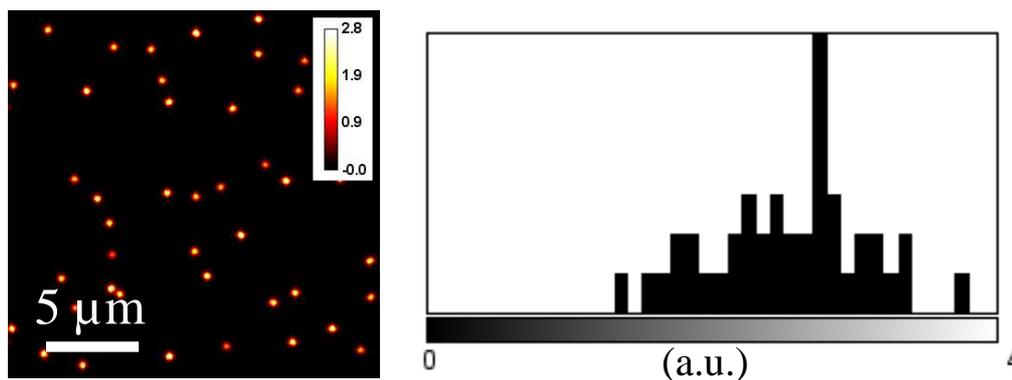


Figure S7. Photothermal microscopy image of 20 nm gold NPs (42 NPs) prepared by spin coating on cleaned glass surface, as described in the main text. The image shows homogeneously distributed NPs with no aggregates. Most of individual gold NPs are separated from each other at a distances greater than 300 nm, large enough to be resolved with optical imaging. The histogram shows a unimodal distribution of photothermal signals, well offset from the background, with a mean value of  $2.53 \pm 0.53$  (a.u.). The variation of photothermal signal of about 21% is in a good agreement with expected variation value is 24% (based on manufacturer specifications of the NPs diameters of 19.9 nm with less than 8% variation coefficient).

## Calculations of the fluorescence quantum yield

Fluorescence quantum yield ( $\eta_{fluor}$ ) is defined as the ratio of the number of emitted photons ( $N_{em}$ ) to the number of absorbed photons ( $N_{abs}$ ):

$$\eta_{fluor} = \frac{N_{em}}{N_{abs}} \quad (\text{Eq.S1})$$

The number of emitted photons depends on the number of detected photons ( $N_{det}$ ) and the detection efficiency ( $Det.Eff.$ ) of the setup as follows:

$$N_{em} = \frac{N_{signal}}{Det.Eff.}, \quad (\text{Eq.S2})$$

where  $N_{signal}$  is either the background-corrected fluorescence, or background signal.

The number of the photons absorbed by a NP is given as follows:

$$N_{abs} = \frac{\sigma_{NP} \cdot I_{heat}}{h\nu_{heat}}, \quad (\text{Eq.S3})$$

where  $\sigma_{NP}$  is the absorption cross section of the NP at the heating wavelengths in the media,  $I_{heat} = P_{heat} / A$  is the irradiance (power density) of the heating light with the power  $P_{heat}$  focused into a diffraction limited spot of area  $A$ ,  $h\nu_{heat}$  is the energy of a single photon.

To estimate the lowest detectable quantum yield in a particular experiment, the number of the photons absorbed by background (in the absence of NPs) is calculated as follows:

$$N_{abs} = \frac{A \cdot I_{heat}}{h\nu_{heat}} = \frac{P_{heat}}{h\nu_{heat}}, \quad (\text{Eq.S4})$$

## Photothermal and fluorescence imaging of 20 nm gold NPs after solvent exchange

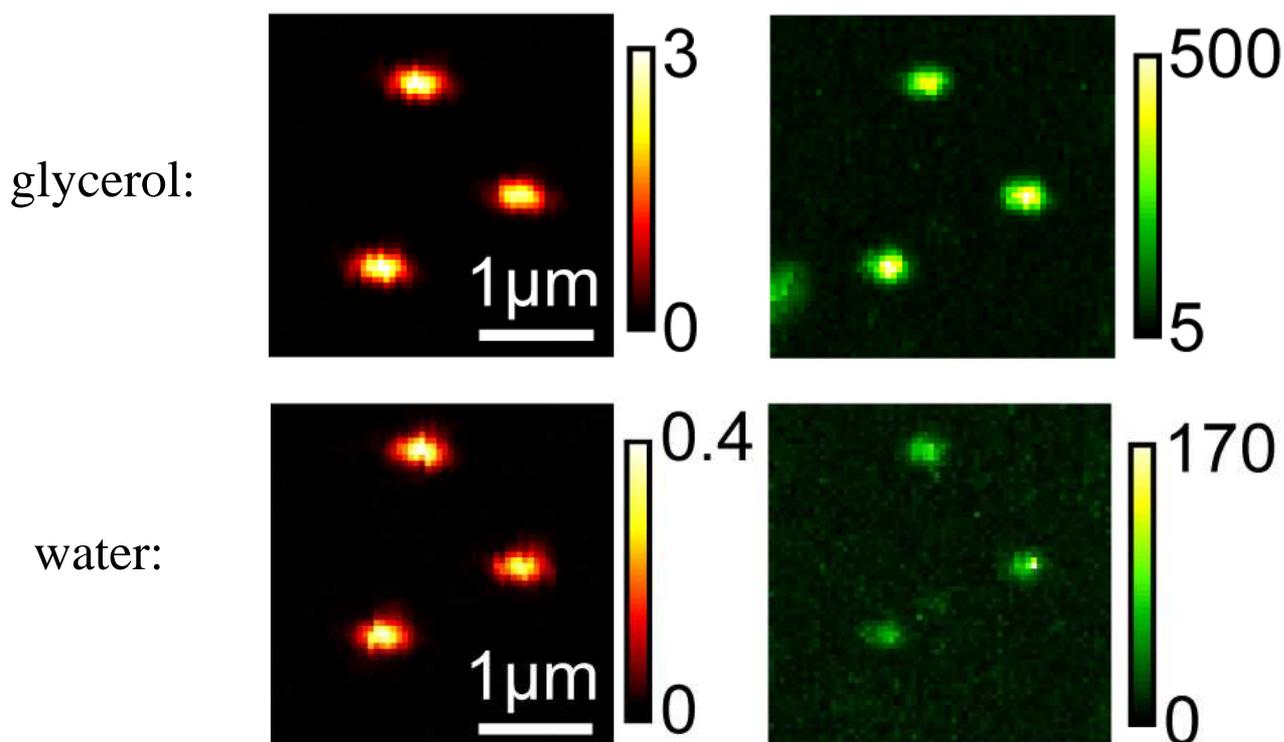


Figure S8. Simultaneously obtained photothermal (left, in a.u.) and fluorescence (right, in counts/10ms) images of the same 20 nm gold NPs in different media.

Top row: Images in glycerol. Particles were made fluorescent according to the procedure described in the main text. The fluorescence quantum yield is about  $7.6 \cdot 10^{-7}$ .

Bottom row: Glycerol is exchanged with water in several gentle washing steps. The same particles are imaged in water. As expected, the photothermal signal decreases in water [A. Gaiduk, P.V. Ruijgrok, M. Yorulmaz, M. Orrit, *Chem. Sci.*, 2010, 1, 343]. The fluorescence signal is detectable after solvent exchange. The fluorescence quantum yield is about  $2.6 \cdot 10^{-7}$  and the fluorescence background is reduced.