Electronic Supplementary Information

High-speed imaging and tracking of very small single nanoparticles by contrast enhanced microscopy

Ching-Ya Cheng, Yi-Hung Liao, and Chia-Lung Hsieh* Institute of Atomic and Molecular Sciences (IAMS), Academia Sinica, Taiwan Email: <u>clh@gate.sinica.edu.tw</u>

Figure S1 COBRI images of 15 nm AuNP as bright spots and dark spots

Figure S2 Performance of background correction

- Figure S3 Precision of measuring COBRI contrast of 10, 15, and 20 nm AuNPs.
- Figure S4 COBRI imaging of single nanoparticles: 10 nm and 20 nm AgNPs, 50 nm and 80 nm SiO2-NPs, and 20 nm Fe3O4-NP

Figure S5 TEM images of eight nanoparticle samples

Figure S6 Size distribution of eight nanoparticle samples estimated from TEM images

Complex refractive indexes of materials used in calculating the scattering cross section of nanoparticles



Figure S1. COBRI images of 15 nm AuNP as bright spots (a) and dark spots (b). The inversion of contrast occurs by adjusting the particle position relative to the microscope objective.



Figure S2. Performance of background correction. The 10 nm AuNPs are barely seen in the raw image because their contrast is smaller than the heterogeneous background due to inhomogeneous illumination and scattering of supporting coverglass. When the background is properly estimated and removed (see Experimental Section), single 10 nm AuNPs are clearly visualized in background-corrected image. Note that the color maps of the raw and background-corrected images are different. Scale bars: 1 µm.



Figure S3. Precision of measuring COBRI contrast of 10, 15, and 20 nm AuNPs. More than 500 particles are analyzed for each sample. The contrast of every nanoparticle under analysis is measured multiple times (at least 50 times) by recording a video of the particle at 1,000 fps. From each recorded frame, one contrast is estimated for one particle appearing in the given frame by 2D Gaussian fitting. The precision of COBRI contrast of a given particle is defined as the standard deviation of all estimated contrast values of the particle. In Figure S3, we display the histograms of the precision of contrast estimation for 10, 15, and 20 nm AuNPs, whose average values are 4.6×10^{-3} , 3.4×10^{-3} , and 4.3×10^{-3} , respectively.



Figure S4. COBRI imaging of single nanoparticles, including 10 nm and 20 nm AgNPs, 50 nm and 80 nm SiO2-NPs, and 20 nm Fe3O4-NP. The contrast of each particle sample is measured at single-particle level and the histogram of measured contrast is plotted. Scale bars: $1 \mu m$.







80 nm SiO2-NP

200 nm

50 nm SiO2-NP

50 nm



20 nm Fe3O4-NP



Figure S5. TEM images of eight nanoparticle samples used in this work.



Figure S6. Size distribution of eight nanoparticle samples used in this work, estimated from the TEM images.

Complex refractive indexes of materials used in calculating the scattering cross section of nanoparticles

Material	Refractive index
Gold (Au)	0.5439 + 2.2309i
Silver (Ag)	0.0540 + 3.4290i
Silica (SiO2)	1.4607
Iron oxide (Fe3O4)	2.1687 + 1.0809i