Multicolor high-speed imaging of single biomolecules with silver, gold, and silver-gold alloy nanoparticles

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Gold nanoparticles (AuNPs) strongly scatter light, whose wavelength matches with its plasmon resonance wavelength. It has been widely used as optical probes to observe various biomolecules, such as lipids and proteins. The motions of labeled-biomolecules were investigated by tracking the bright spot of the optical image of AuNPs. Stepping motion of motor proteins, and diffusional motion of lipids in bio-membranes have been revealed at both sub-millisecond time resolution and nanometer scale localization precision. To investigate the behaviors of multiple biomolecules at high spatio-temporal resolution, increase of the color channels is necessary.

Here, we used silver and silver-gold alloy nanoparticles (AgNPs and AgAuNPs) in addition to AuNPs to achieve multicolor and high-speed tracking of multiple biomolecules [1]. Peak position of plasmon resonance wavelength of AgNPs locates more than 100 nm shorter than that of AuNPs. Furthermore, that of AgAuNPs appears between AgNPs and AuNPs, and its peak position can be tuned depending on their composition ratio. To separately observe each metal NPs at the same time, we constructed multicolor total internal reflection dark-field microscope. We used multiple lasers at 404, 473, and 561 nm as illumination light sources that match with plasmon resonance wavelength of AgNPs, AgAuNPs, and AuNPs, respectively. To obtain scattering image at each wavelength on the single two-dimensional detector, we used spectrophotometer in the imaging optics. With this system, diffusional motions of phospholipids in supported membrane, and stepwise movement of linear motor proteins kinesin-1 along microtubules were observed with time resolution at 100 μs and localization precision at a few nanometers.

Reference:

[1] J. Ando *et al*., *ACS Photonics* **6**, 2870-2883 (2019).