Single-particle Tracking with Interferometric Scattering Microscopy (iSCAT)

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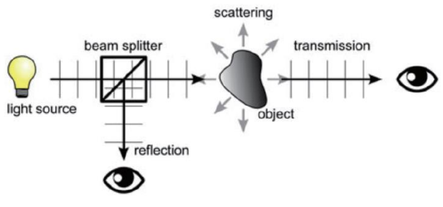
Abstract

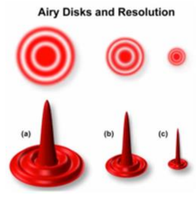
We can observe microscopic world with optical microscope. As techniques for imaging are developed, we can get images of nano-sized particles and molecules with high spatial resolution. Unfortunately, super-resolution imaging techniques using fluorescent molecules suffer photobleaching or photoblinking because lifetime of fluorophore is limited. However, detecting nanoparticles using interference of background reflection and scattered light by themselves, we can directly observe dynamics of nano-materials with high time resolution as well as overcome time limit of fluorescence imaging. In this work, we constructed an optical system of interferometric scattering microscopy (iSCAT). Using iSCAT, we got images of gold nanoparticles with 40 nm diameter and analyzed time trace of their movement.

Background

● Optical Microscope & Diffraction Limit

- Optical Microscope, so called because it uses visible light, is composed of lenses to magnify images of microscopic objectives.
- Due to diffraction, any optical system has a fundamental maximum of the resolution. An optical system with resolution as good as the theoretical limit is called diffraction limited.

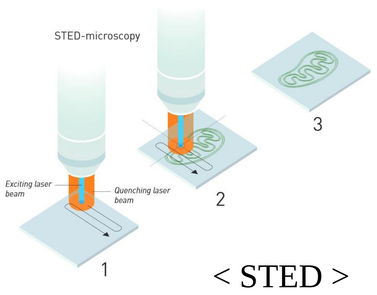


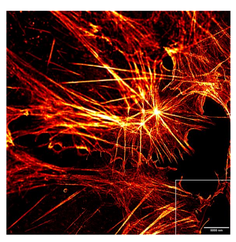


$$d = \frac{\lambda}{2n \sin\theta}$$

● Super-resolution Microscopy

- To overcome diffraction limit, many kinds of super-resolution microscopy technique were developed such as near field microscopy (e.g. NSOM) and photo-active localization microscopy. (e.g. PALM)





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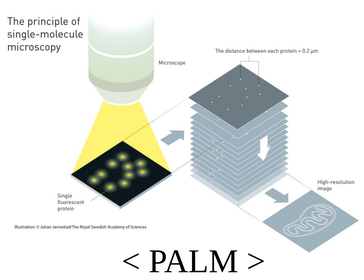
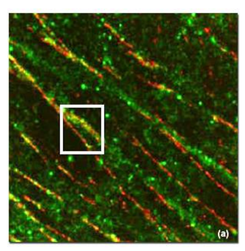
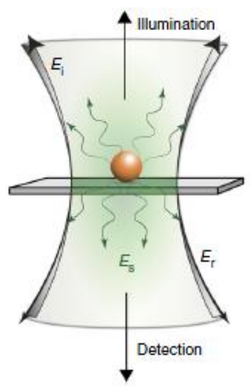
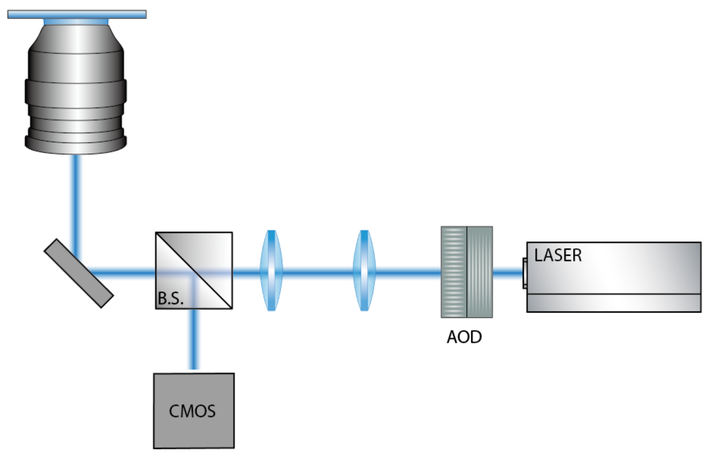




< PALM >

Non-fluorescent Imaging : iSCAT





< Experimental Scheme >

● Interferometric Scattering Microscopy

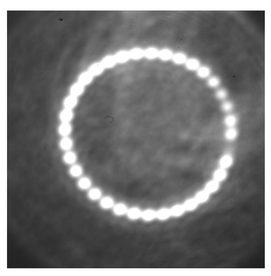
- Interference between reflection field and scattering field by objective particles in sample chamber

$$I_{det} = |E_r + E_s|^2 = |E_i|^2\{r^2 + |s|^2 - 2r|s|\sin\phi\} \cong |E_i|^2\{r^2 - 2r|s|\sin\phi\}$$

- High temporal resolution and localization precision: (~ ms, ~ nm)
- Free from photo-bleaching and photo-blinking : long time observation and label-free imaging

Interferometric Signal Enhancement

● Raster scanning using Acousto-Optic Deflector



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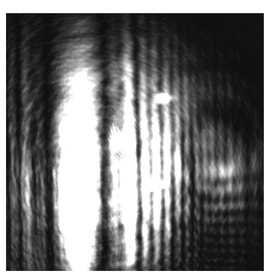

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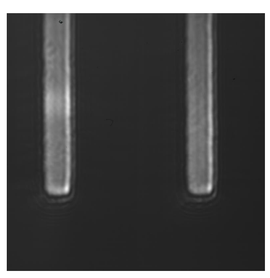

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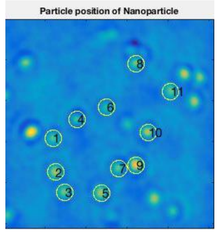
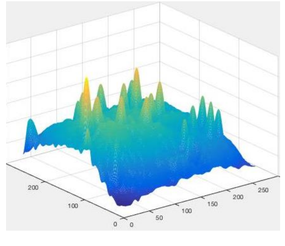
- Illuminating sample region to the imaging camera evenly
- Reducing the effect of laser intensity noise

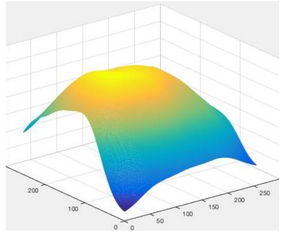
● Image processing for enhanced SNR and particle tracking



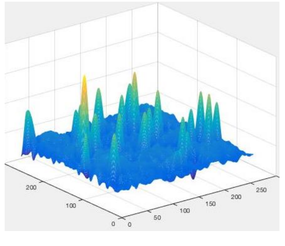
Single particle detection



Raw image



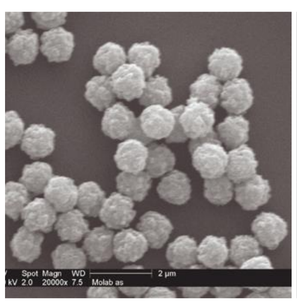
Background image



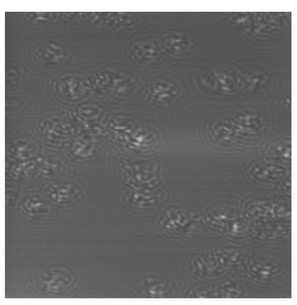
Enhanced image

Results

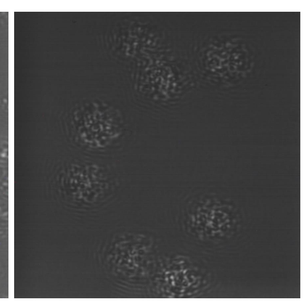
● Images of various micro- and nano-sized particles



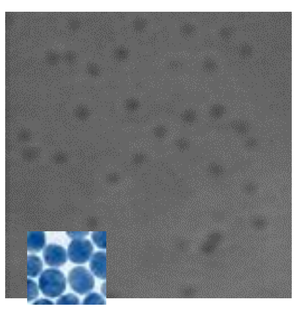
Magnetic bead (SEM image)



Diameter = 1 um (Myone T1, Dynabead)

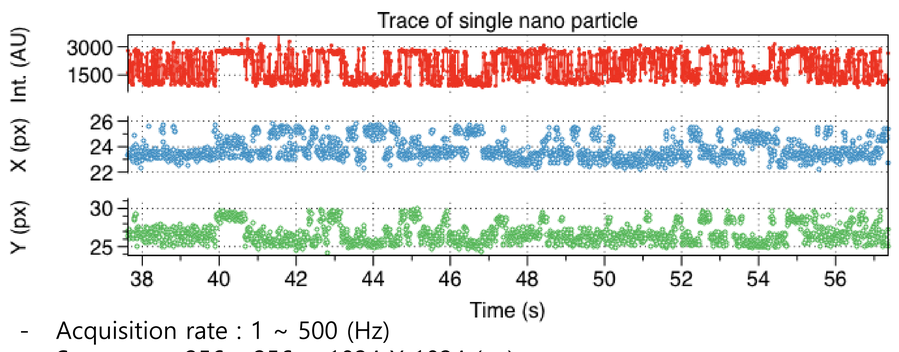


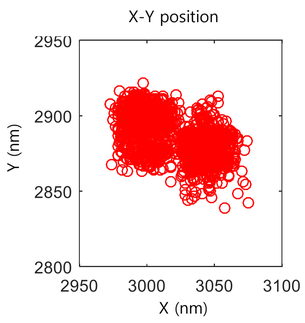
Diameter = 2.8 um (M280, Dynabead)



40nm Gold Nanoparticle

● Single-particle tracking





- Acquisition rate : 1 ~ 500 (Hz)
- Scan area : 256 x 256 ~ 1024 X 1024 (px)
- Pixel resolution : 25 nm / px

References

How the optical microscope became a nanoscope. Nobel Prize 1–7 (2014).
Weisenburger, S., & Sandoghdar, V. (2015). Light microscopy: an ongoing contemporary revolution. *Contemporary Physics*, *56*(2), 123–143.
Ortega-Arroyo, J., & Kukura, P. (2012). Interferometric scattering microscopy (iSCAT): new frontiers in ultrafast and ultrasensitive optical microscopy. *Physical Chemistry Chemical Physics*, *14*(45), 15625.
Arroyo, J. O., Cole, D., & Kukura, P. (2016). Interferometric scattering microscopy and its combination with single-molecule fluorescence imaging. *Nature Protocols*, *11*(4), 617–633.

Acknowledgement

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