Orientation-Sensitive Imaging of Anisotropic Particle with Interferometric Scattering Microscopy-type Method

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Abstract

Single molecule detection techniques have allowed one to observe biological systems. It is difficult to observe nanoscopic phenomena optically because of diffraction limit of optical detection. To overcome this limit, various super-resolution microscopy, such as fluorescence microscopy, have been developed. Although there are numerous advantages of fluorescence microscopy techniques, they usually suffer saturation of signal, photobleaching and photoblinking of fluorophores. These problems disrupt stable and long-term tracking of targets of interest. Here, we developed new experimental scheme based on interferometric scattering microscopy (iSCAT), which collects the interference between the scattering field from target and the reference field from interface of chamber. We constructed orientation sensitive optical system with linear polarized light and dual camera system. With signal difference between two images of which the linear polarized light is perpendicular to each other, orientational information of nanorod were delivered and also, we observed Brownian rotational motion of a single anisotropic gold nanorod.

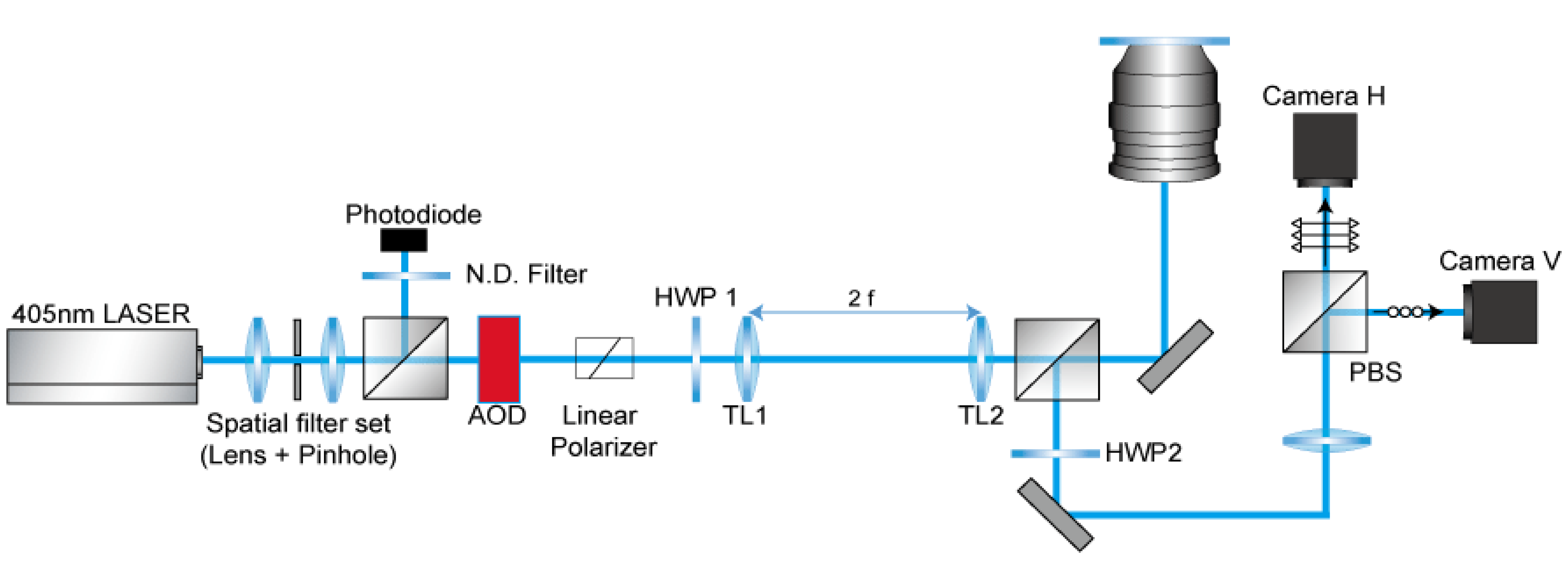
iSCAT-type orientation-sensitive Imaging

● Interferometric Scattering Microscopy

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

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

● Orientation-sensitive Imaging Experiment Scheme



< Experimental Scheme >

- Illuminate targets with linearly polarized light and collect the signal of two polarization states ,which is perpendicular to each other by using a polarizing beam splitter, with dual camera system (h-pol and v-pol).

$$I_h\approx\frac{1}{2}|E_i|^2\left\{r^2-2r|s_h|\cos\phi_h\right\}$$

$$I_v\approx\frac{1}{2}|E_i|^2\left\{r^2-2r|s_v|\cos\phi_v\right\}$$

- Generate a differential image using the h-pol image and the v-pol image, which has anisotropy information, with numerical operation.

$$\delta I\equiv\frac{I_h-I_v}{I_h+I_v}=\frac{|s_h|\cos\phi_h-|s_v|\cos\phi_v}{\left\{r-\left(|s_h|\cos\phi_h+|s_v|\cos\phi_v\right)\right\}}$$

- In addition to advantages of iSCAT, it delivers orientational information of anisotropic particles: free from photo-bleaching and photo-blinking, high temporal resolution and localization precision: (~ ms, ~ nm)

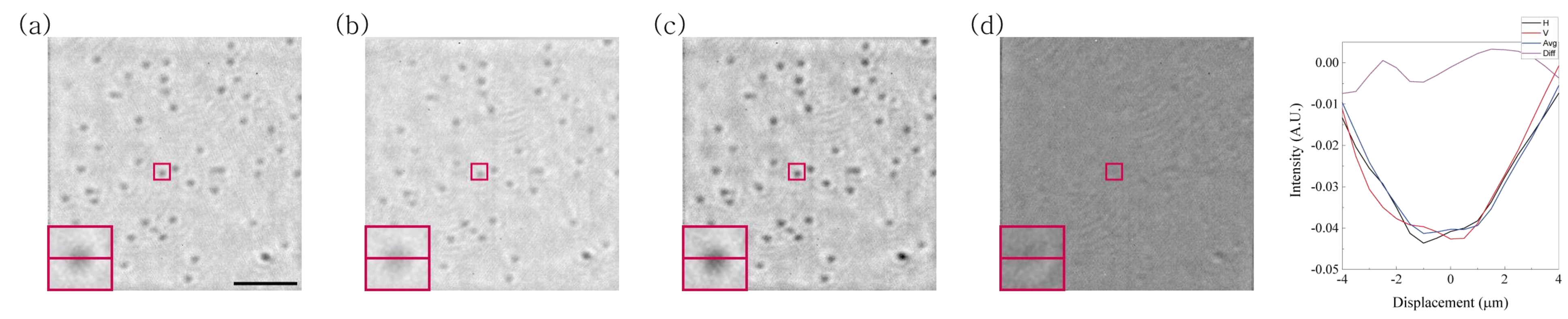
- The data can be fitted with the functions below. These functions contain the effects of relative orientation of AuNR and polarization-sensitive detection by H and V camera.

$$I_h''=I_0''\left[1+\beta\left\{\cos(2\theta-2\chi)-\sin(2\theta-2\chi)\right\}\right]\quad I_{sum}''=2I_0''\left(1-\beta\sin(2\theta-2\chi)\right)$$

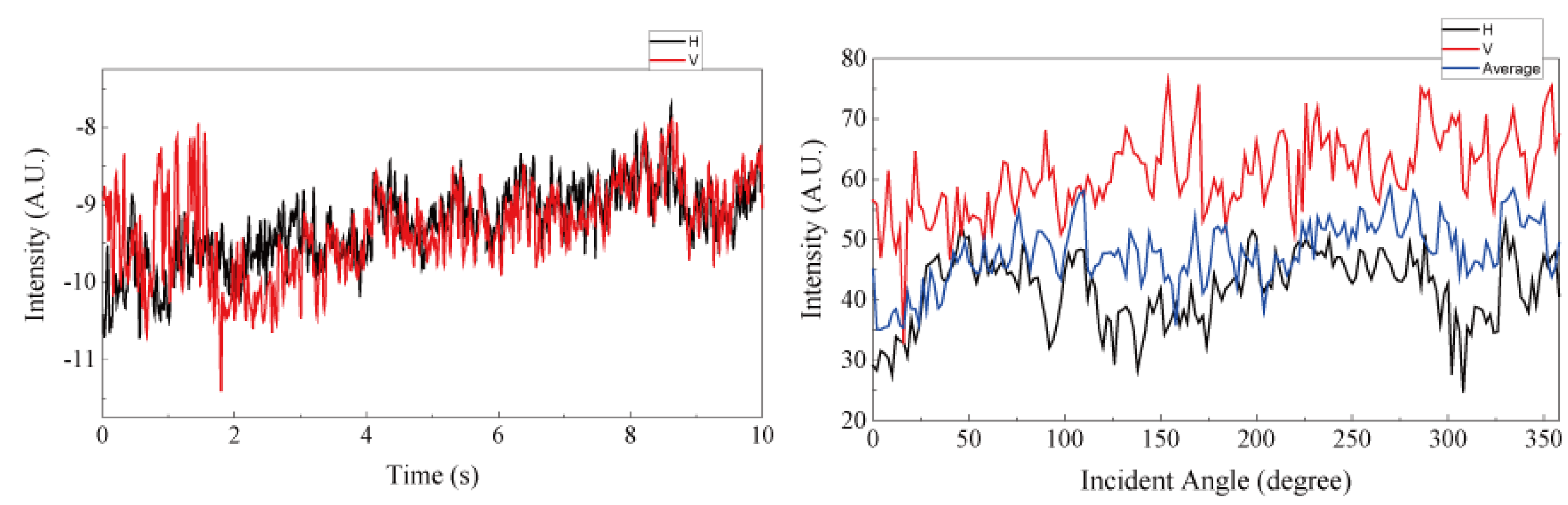
$$I_v''=I_0''\left[1-\beta\left\{\cos(2\theta-2\chi)+\sin(2\theta-2\chi)\right\}\right]\quad \delta I''\equiv\frac{I_h''-I_v''}{I_h''+I_v''}=\frac{\beta\cos(2\theta-2\chi)}{1-\beta\sin(2\theta-2\chi)}$$

Results

● 40 nm Isotropic Gold Nanoparticle (AuNP) Imaging

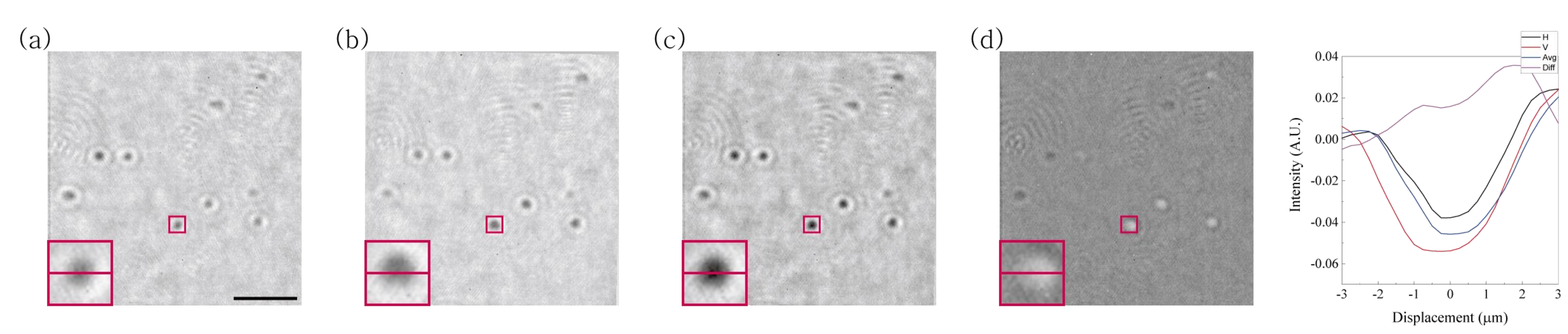


< Images of AuNPs and cross-sectional profile of the signal spot in a box. (a) h-pol image of AuNPs. (b) v-pol image of AuNPs. (c) average image of AuNPs. (d) differential image of AuNPs. >

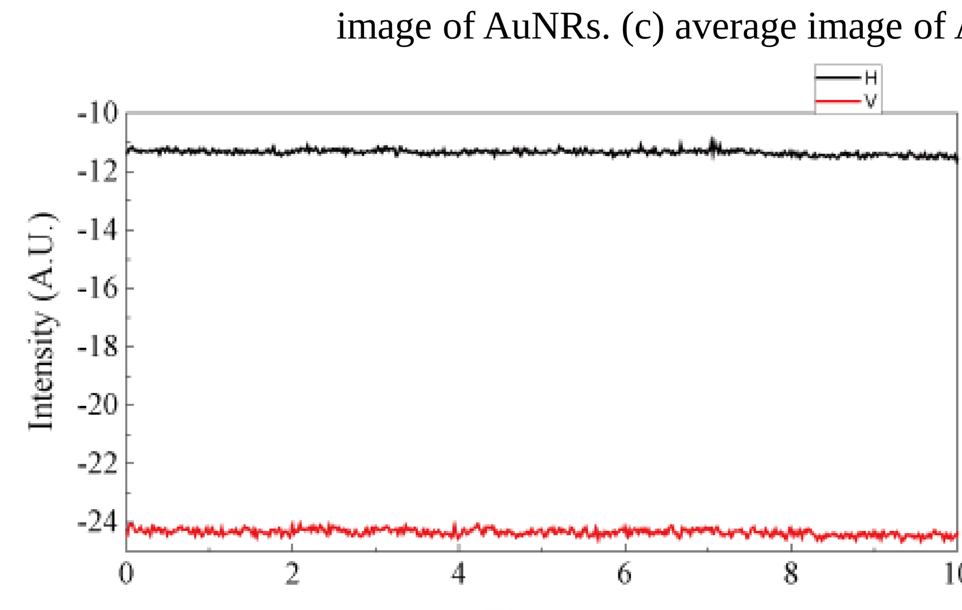


<. Time-trace of a AuNP signal intensity and signals of a single AuNP as a function of incident polarization. >

● 30-100nm Anisotropic Gold Nanorod (AuNR) Imaging

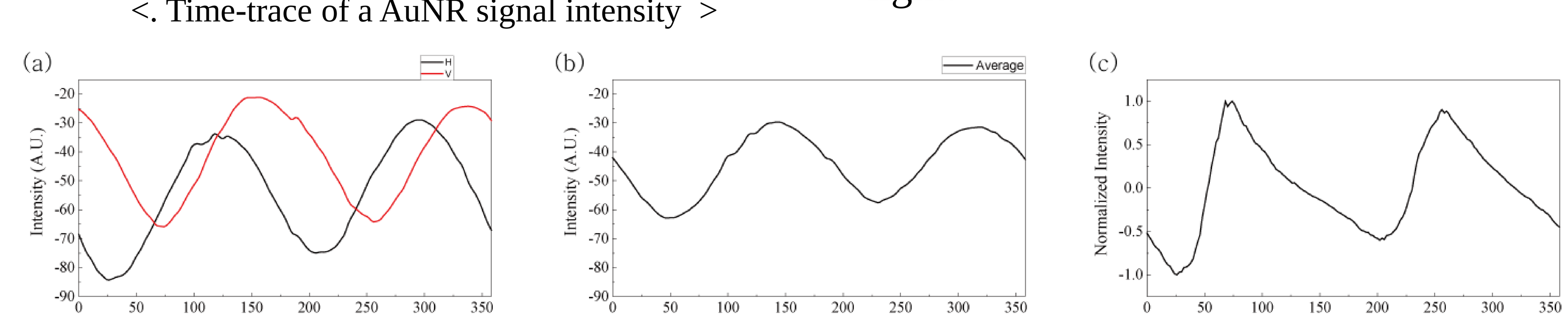


< Images of AuNRs and cross-sectional profile of the signal spot in a box. (a) h-pol image of AuNRs. (b) v-pol image of AuNRs. (c) average image of AuNRs. (d) differential image of AuNRs. >



<. Time-trace of a AuNR signal intensity >

- Although elongated shape of AuNR did not appeared, but AuNR has intensity level difference between the h-pol image and the v-pol image.
- This difference comes from anisotropy of AuNR and the relative orientation between AuNR and the incident polarization of light which affects the ratio of polarization component of scattered light.



< Signals of a single AuNR as a function of incident polarization. The orientation of the AuNR obtained from (a)-(c) are in good agreement. ((a) χ=51.4˚ for H and 49.7˚, (b) χ=50.6˚, (c) χ=50.8˚ >

References

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