Interferometric Scattering Microscopy to Characterize Nanometric Objects and Subcellular Structures: Towards Fast 3D Imaging at Nanoscale.

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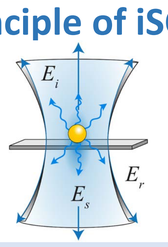
Abstract

It is of great interest to visualize nano-scale objects in bioscience. Fluorescence microscopy, most powerful to date, bears limitations in measurement time, time resolution, and sample preparation. To circumvent such problems, a new scattering-based method named iSCAT (interferometric scattering microscopy) has been recently introduced. Since this technique relies on scattering signal from a target particle such as gold nanoparticle (AuNP) and detects the interference of the signal with a constant reference, it is not subject to the aforementioned limitations: theoretically unlimited SNR to improve time resolution, practically unlimited measurement time for localized scatterers, and lack of photo-blinking and photo-bleaching. Here, we report our recent achievements: First, we utilized polarized scattering from an anisotropic scatterer to characterize the position and orientation of nanorods. From this new scheme (psiSCAT), we capture the orientation of gold nanorods (AuNR) without sacrificing the bright-field image of the entire view-field, in contrast to dark-field imaging [1]. Second, we successfully track the position of nanoparticles along the axial dimension and image the sample at different depths by adopting the remote-focusing (RF) technique. Our RF-iSCAT approach significantly expands the strength and utility of iSCAT [2]. Lastly, we show our iSCAT imaging of biological cells and multi-protein focal adhesion complexes. The iSCAT technique enables us to visualize subcellular structures with remarkable spatial, temporal details and contrast even without labeling [3]. The iSCAT technique would be an indispensable tool in visualizing the nanoscopic biological world.

Principle of iSCAT detection



Scattering cross-section (σ)

$$\sigma = |s|^2 \propto \varepsilon_m^2 \pi \frac{D^6}{4\lambda^4} \left| \frac{\varepsilon_p - \varepsilon_m}{\varepsilon_p + 2\varepsilon_m} \right|^2$$

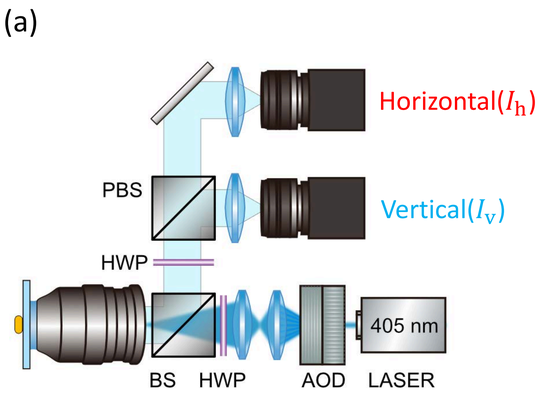
$$I_{\text{detection}} = |E_i|^2 \{ r^2 + s^2 + \boxed{2rs\cos(\Delta\phi)} \} \text{ where } \Delta\phi = \phi_r - \phi_s$$

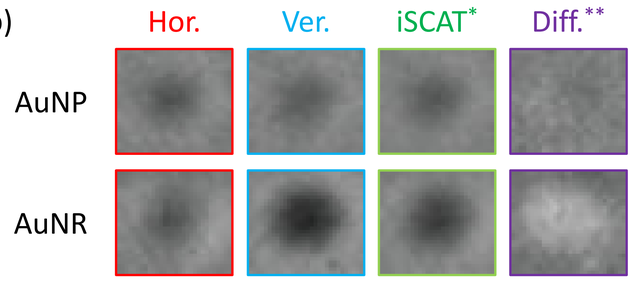
$$I_{\text{ratio}} = \frac{I_{\text{detection}} - I_{\text{background}}}{I_{\text{background}}} = \frac{s^2}{r^2} + \boxed{2\frac{s}{r}\cos(\Delta\phi)}$$

Scattering only | Interferometric

The iSCAT signal varies with D^3 while the dark-field signal (pure scattering) with D^6: the weaker size dependence of iSCAT permits a wider dynamic range of detectable size.

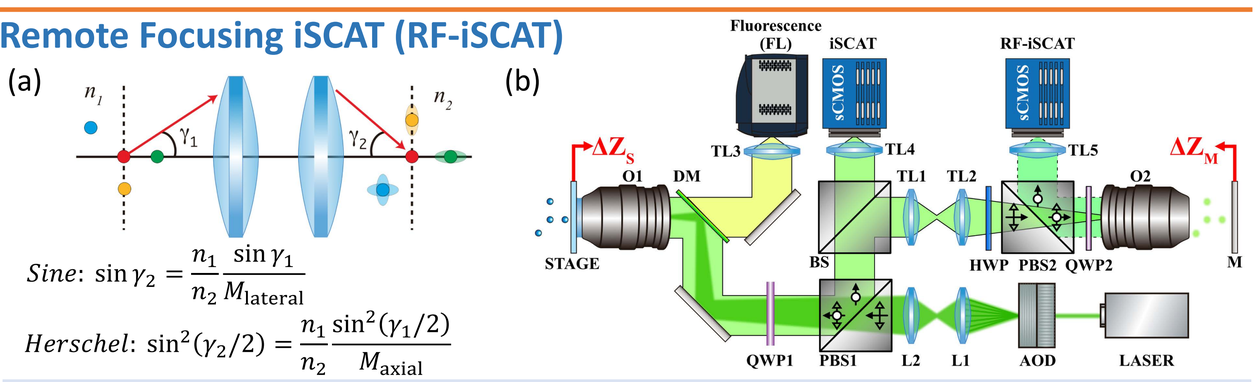
polarization-selective iSCAT (psiSCAT)





(a) Schematic setup of psiSCAT
(b) psiSCAT images (horizontal, vertical, average, differential) of (top) AuNP (40 nm) & (bottom) AuNR (40-110 nm).
*: iSCAT($\frac{I_h+I_v}{2}$), **: Differential($\frac{I_h-I_v}{I_h+I_v}$)

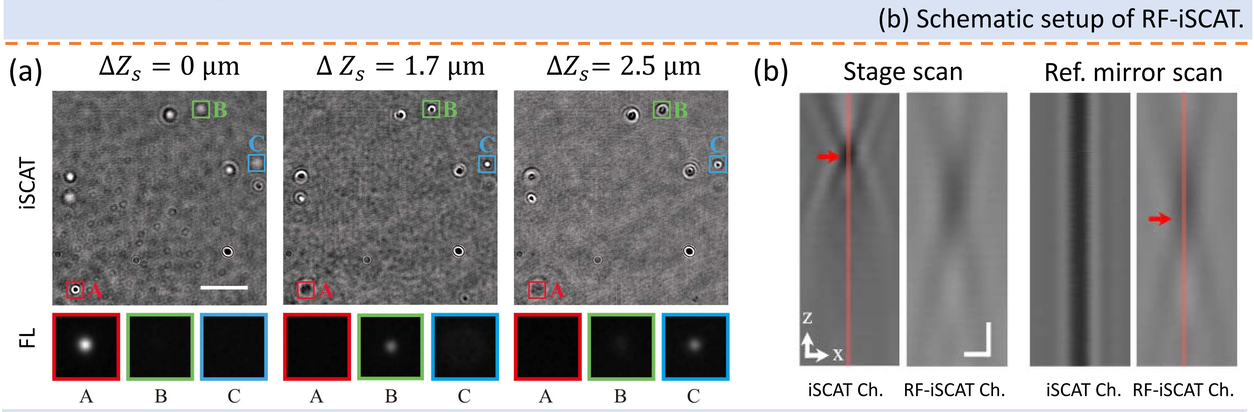
Remote Focusing iSCAT (RF-iSCAT)



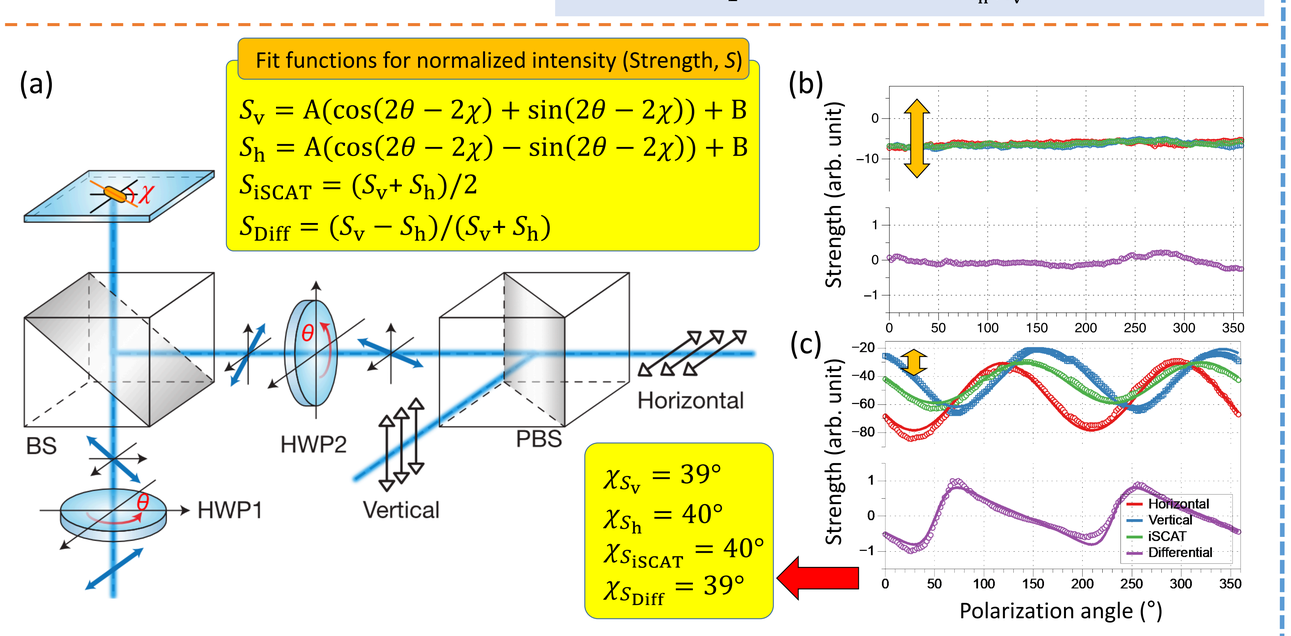
$$Sine\text{: } \sin\gamma_2 = \frac{n_1}{n_2}\frac{\sin\gamma_1}{M_{\text{lateral}}}$$

$$Herschel\text{: } \sin^2(\gamma_2/2) = \frac{n_1}{n_2}\frac{\sin^2(\gamma_1/2)}{M_{\text{axial}}}$$

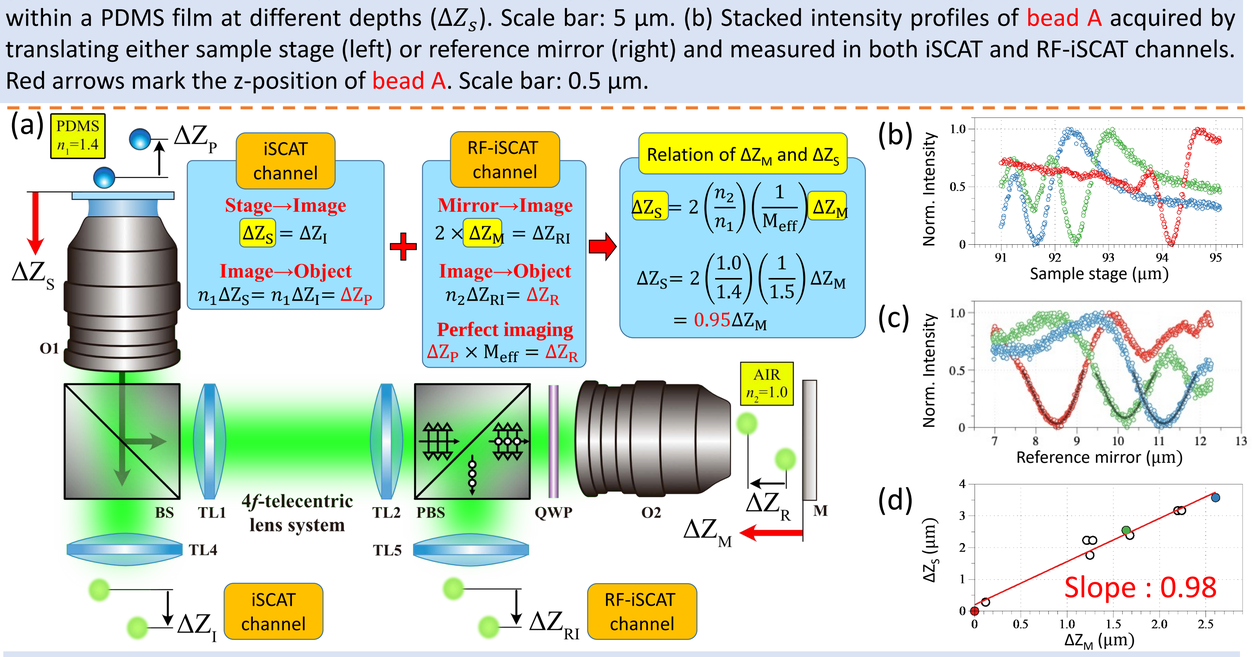
(a) Perfect imaging and optical aberrations: conditions for lateral (Sine) and axial (Herschel) perfect imaging.
(b) Schematic setup of RF-iSCAT.



(a) Representative iSCAT (top) and fluorescence images (bottom) of fluorescent PS beads (A, B, & C; Dia. = 0.2 μm) within a PDMS film at different depths (ΔZ_s). Scale bar: 5 μm. (b) Stacked intensity profiles of bead A acquired by translating either sample stage (left) or reference mirror (right) and measured in both iSCAT and RF-iSCAT channels. Red arrows mark the z-position of bead A. Scale bar: 0.5 μm.

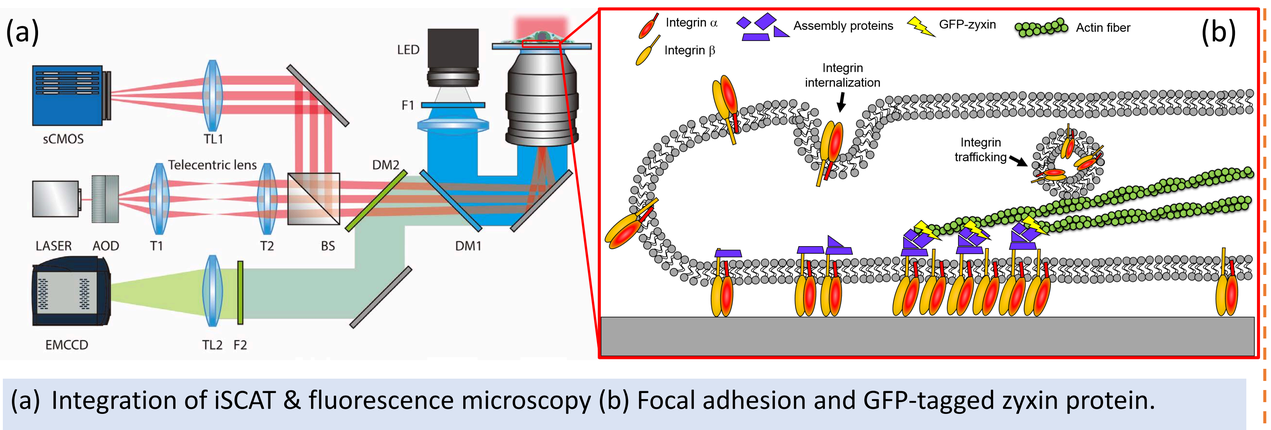


(a) Schematic view of psiSCAT detection. Input polarization (θ) is rotated by HWP1 and output polarization is adjusted by θ by HWP2. Polarization selective detection by PBS provides the orientation of nanorod (χ). (Inset: angle-dependence of various modes of strength) (b, c) Polarization-dependence of strengths from AuNP (b) and AuNR (c). Orange arrows indicate the same scale (ΔStrength = 20) in (b) and (c).



(a) Schematic diagram of key optics for RF-iSCAT and definitions of and relations between various z displacements. (b, c) Normalized intensity variations of the center of bead (A: red, B: green, C: blue) with respect to the position of sample stage (b) or reference mirror (c). (d) Linearity and near identity between the stage height and the mirror position for focusing of beads (each point represents a different bead).

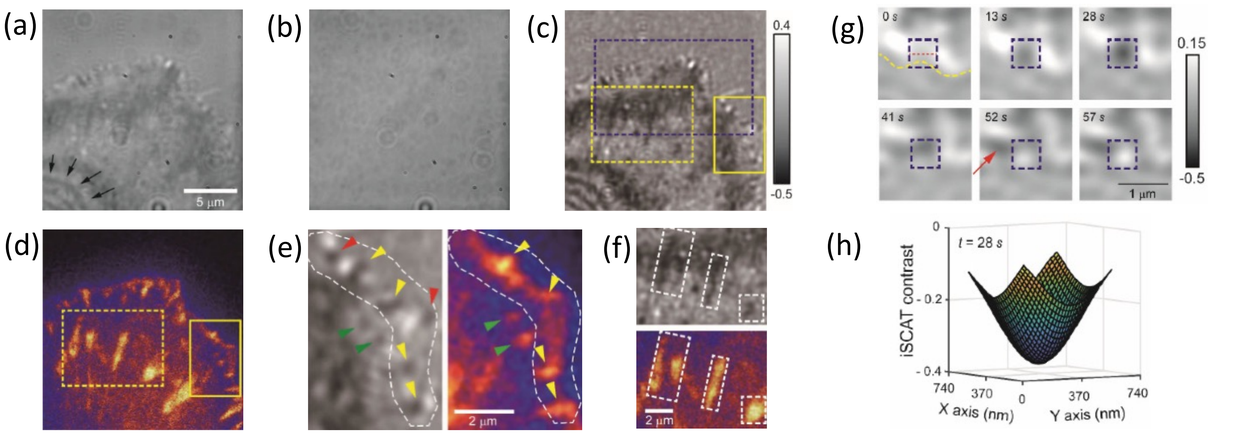
iSCAT + fluorescence microscopy for live cell imaging: focal adhesion study



(a) Integration of iSCAT & fluorescence microscopy (b) Focal adhesion and GFP-tagged zyxin protein.

Focal adhesion (FA): dynamic multi-protein systems, mediating cell adhesion onto extracellular matrix.
- Assembly and disassembly of FAs play a critical role in various key biological functions such as cell migration, proliferation, differentiation, and signal transduction.
- A small portion of focal complexes (FXs), early structure of focal adhesion, grow to form large protein complexes of mature FAs by recruiting many adaptor proteins.
- Among adaptor proteins, zyxin, a zinc-binding phosphoprotein, is localized in the upper region of FA and near actin filament termini.

Fast label-free tracking of FA & specific probing of GFP-labeled FA in U2OS



(a) Raw iSCAT image, (b) mean background image of 1,000 consecutive frames from a cell-free area, (c) ratiometric iSCAT image obtained from the ratio of (a) to (b), (d) complementary fluorescent image showing zyxin-rich regions, (e) magnified iSCAT image (area boxed by yellow solid line in (c)) showing the existence of adhesions in the cell boundary (left) and its complementary fluorescent image (right), (f) magnified iSCAT image (area boxed by yellow dotted line in (c)) showing the structure of mature FAs (upper) and corresponding fluorescence image (lower). (g) Sequential images showing a whole life span of a single nascent adhesion, (h) Gaussian fit to define the center of a nascent adhesion.

Conclusions

- We developed several novel techniques in iSCAT microscopy:
  - The psiSCAT technique can determine the orientation as well as the position of nanorods.
  - The RF-iSCAT technique permits fast, vibration-free, 3D tracking of nano-objects.
- We succeeded in visualizing live cells with iSCAT combined with fluorescence microscopy:
  - The FM-combined iSCAT can image organelles & multi-protein complexes selectively with great temporal and spatial details. It can track them for unlimited measurement time.

References

[1] I.-B. Lee, H.-M. Moon, J.-H. Joo, K.-H. Kim, S.-C. Hong, and M. Cho, ACS Photonics, **5**, 797 (2017).
[2] I.-B. Lee, H.-M. Moon, J.-S. Park, K. Zambochova, S.-C. Hong, and M. Cho, *Optics Letters, under revision*.
[3] J.-S. Park, I.-B. Lee, H.-M. Moon, J.-H. Joo, K.-H. Kim, S.-C. Hong, and M. Cho, Chem. Sci., **9**, 2690 (2018).
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