Live-cell Imaging with Interferometric Scattering Microscopy (iSCAT)

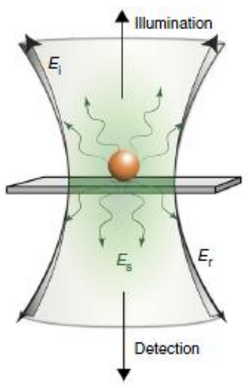
JongHyeon Joo[1,2], JinSung Park[1], Il-buem Lee[1,3], HyeonMin Moon[1,3], SeokCheol Hong †[,1,3], MinHaeng Cho †[,1,2]

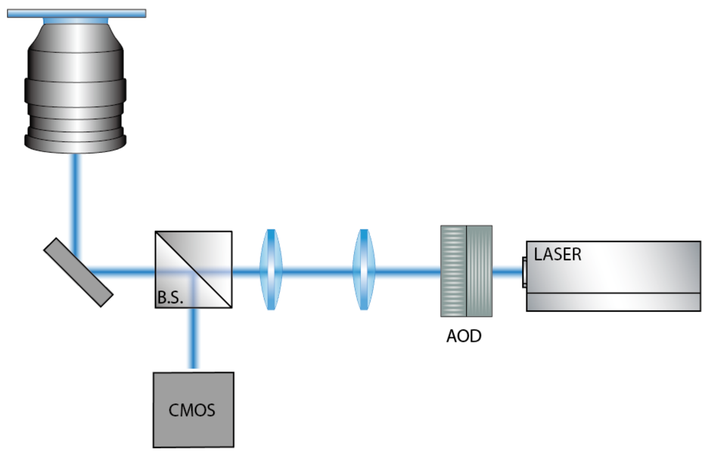
[1]Center for Molecular Spectroscopy and Dynamics, Institute for Basic Science (IBS), Korea University, Seoul 02841, Republic of Korea
[2]Department of Chemistry, Korea University, Seoul 02841, Republic of Korea
[3]Department of Physics, Korea University, Seoul 02841, Republic of Korea

Abstract

Optical detection of single living-cell has been studied using fluorescent labeling as the basis of single-molecule sensitivity. Despite the many advantage of using fluorescence, the intensity and duration impose fundamental limits on the imaging speed and precision for real-time tracking studies. By using interferometric scattering microscopy (iSCAT) which detects interferometric signal from objectives, we can observe targets with high time resolution as well as overcome time limit of fluorescence imaging. In this work, we observed complex cell structures (e.g. nucleus) and constructed overall living-cell mosaic using iSCAT.

Non-fluorescent Imaging : iSCAT





< Experimental Scheme >

● Interferometric scattering detection

- The interferometric signal can be observed at the detector because of the interference between the reflection field and the scattering field.

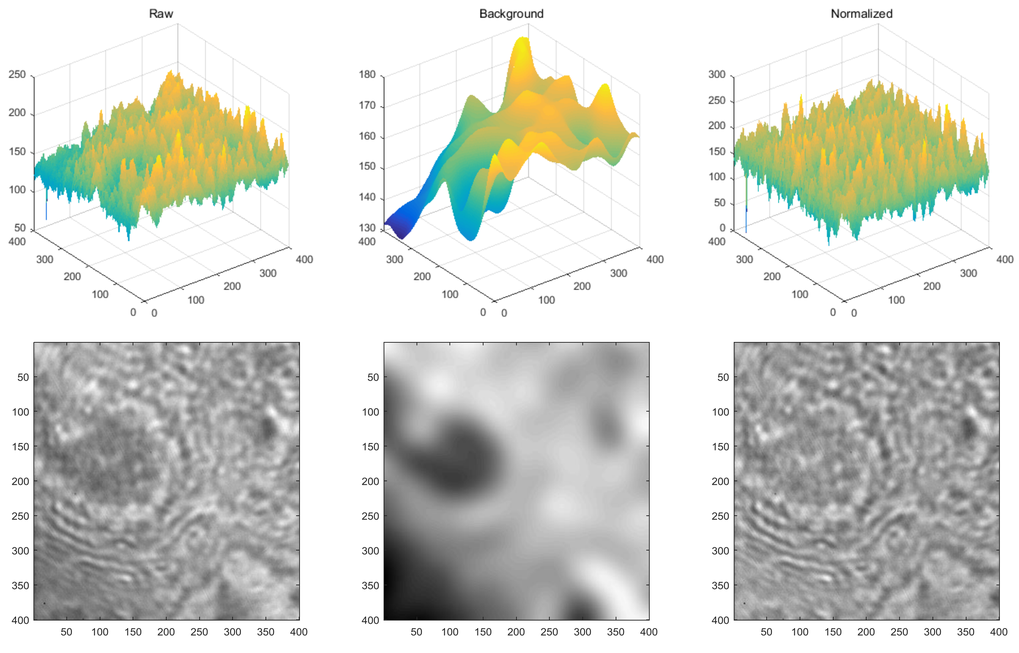
$$I_{det} = |E_r + E_s|^2 = |E_i|^2\{r^2 + |s|^2 - 2r|s|\sin\phi\} \cong |E_i|^2\{r^2 - 2r|s|\sin\phi\}$$

- Fluorescence detection exhibits unique photophysical and chemical effects, such as photoblinking and bleaching, which limit the total achievable photon yield
- Sensitive and precise localization of nanostructures is possible.
- Indefinite observation times

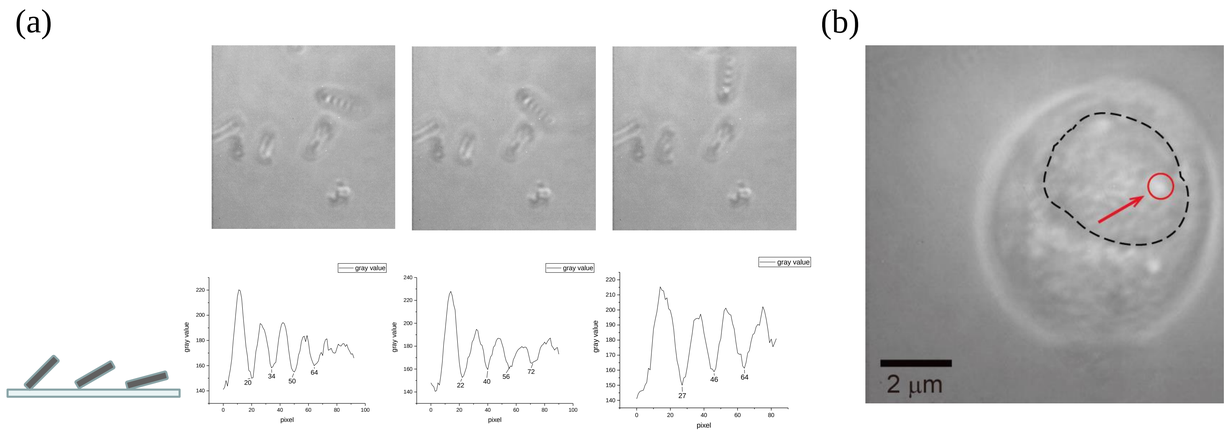
● Removing background intensity

- To remove uneven background noise, raw image is divided by background field image.
- This process enhances signal-to-noise ratio of collected images.

$$I_{red} = \frac{I_{det}}{I_{background}} = \left\{1 - 2\frac{|s|}{r}\sin\phi\right\}$$



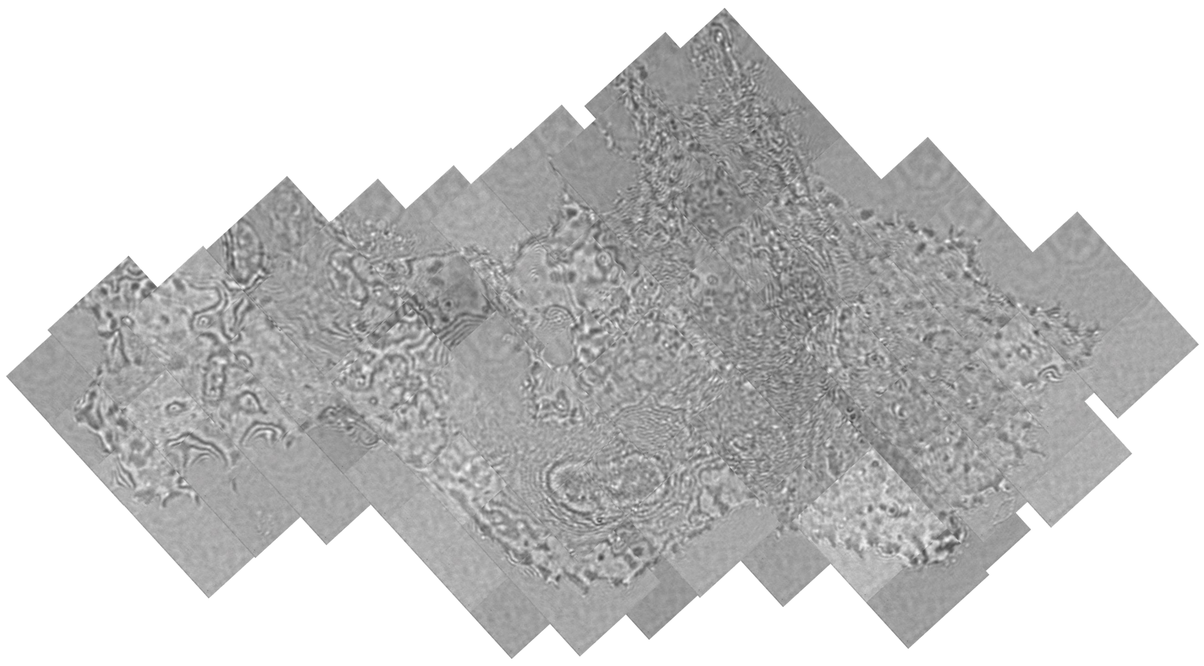
Interferometric Signal of Microorganisms



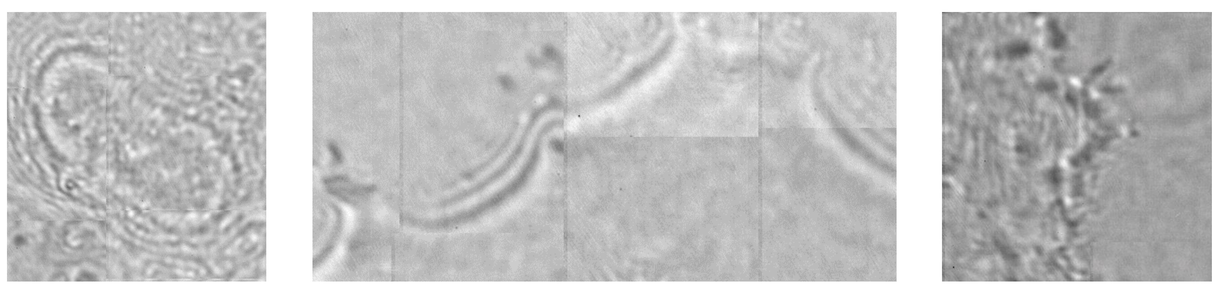
● iSCAT Imaging of (a) *Escherichia coli* (*E. coli*) OP50 and (b) yeast

- With analyzing interferometric signal, standing angle of *E. coli* can be assumed.
- Intracellular structures of yeast was observed and amorphous mass inside can be tracked.

iSCAT Imaging of COS-7 cells



< A mosaic of live COS-7 cell >



(a) Nucleus (b) Cell lamellipodium (c) Cell protrusion

- Acquisition rate : 25 (Hz)
- Scan area : 400 X 400 (px)
- Pixel resolution : 25 nm / px

References

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.

Limozin, L. & Sengupta, K. Quantitative reflection interference contrast microscopy (RICM) in soft matter and cell adhesion. *Chem. Phys. Chem. 10*, 2752-2768 (2009).

Acknowledgement

This work was supported by the Institute for Basic Science (IBS) R023-D1.