Real-time observation of individual focal adhesion dynamics and cytoskeletons via iSCAT microscopy

Jin-Sung Park,[1] Il-Buem Lee,[1] Hyeon-Min Moon,[1] Jin-Sun Ryu,[2] Sun-Young Kong,[3] Seok-Cheol Hong*,[1,4] & Minhaeng Cho*,[1,5]

[1]*Center for Molecular Spectroscopy and Dynamics, Institute for Basic Science, Seoul 02841, Korea*
[2]*Division of Translational Science, National Cancer Center, Goyang, 10408, Korea*
[3]*Center for Breast Cancer, National Cancer Center, Goyang, 10408, Korea* [4]
Department of Physics, Korea University, Seoul 02841, Korea [5]*Department of Chemistry, Korea University, Seoul 02841, Korea*

* Email : mcho@korea.ac.kr, hongsc@korea.ac.kr

Abstract

Focal adhesion complexes (FAs) are large, dynamic protein systems that form mechanical links between c ytoskeletal actin fibers and extracellular matrix. To date, our current understanding in their structures and dynamic processes has been mostly obtained via fluorescence microscopy such as total internal reflection and super-resolution microscopy. Despite superb specificity, both techniques inevitably bear technical limi tations inherent in fluorescence imaging such as photobleaching of fluorescent probes. Nonetheless, it still remains a challenging issue to observe molecular dynamics of FAs without labelling target proteins. Here we demonstrate a label-free, long-term imaging method to uncover a whole cellular process of individ ual FAs from its initiation, maturation and to its decay using interferometric light scattering (iSCAT) micros copy. To compensate for the lack of chemical selectivity in iSCAT imaging, fluorescently-labeled zyxin prot eins were simultaneously tracked as a major marker for FAs. The iSCAT-based imaging was shown to be se nsitive enough to detect individual binding events of FA proteins onto the glass substrate and their disassem bly in real time. Long-term tracking, key strength of iSCAT, enabled us to monitor a whole passage of FA m etabolism. Mature, zyxin-labeled FAs, imaged as elongated patches, were continuously interpolated into zyx in-free actin fibers. Besides, non-labeled endogenous vesicles traveling along actin fibers were clearly visua lized.

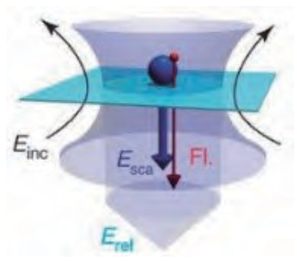
Introduction

◎ Interferometric scattering (iSCAT) microscopy
- New ultrafast and ultrasensitive optical microscopy
- Label-free detection for a very weak signal scattered from nano-objects
- No fluorescent labelling required unlimited measurement time

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

(E_i : Incident electric field, E_r : Reflected electric field, E_s : Scattered electric field, ϕ : Phase difference)

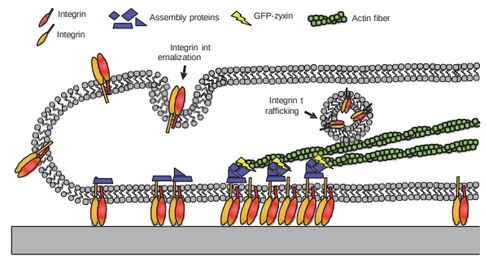
Operating principle of iSCAT detection



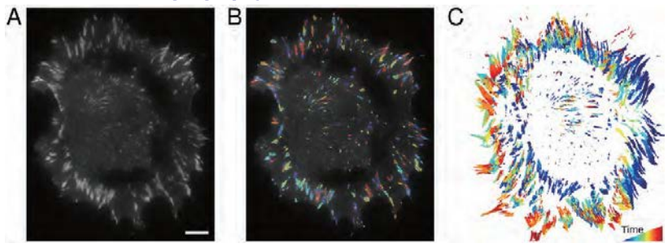
P. Kukura, H. Ewers, C. Muller, A. Renn, A. Helenius & V. Sandoghdar, Nat. Methods 6, 923 (2009)

◎ Focal adhesion (FA) and zyxin adaptor protein
- Dynamic multimetric 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 adhesion formation, 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

Schematic organization of FAs & their adaptor proteins



TIRF imaging of dynamic FAs in a NIH 3T3 cell

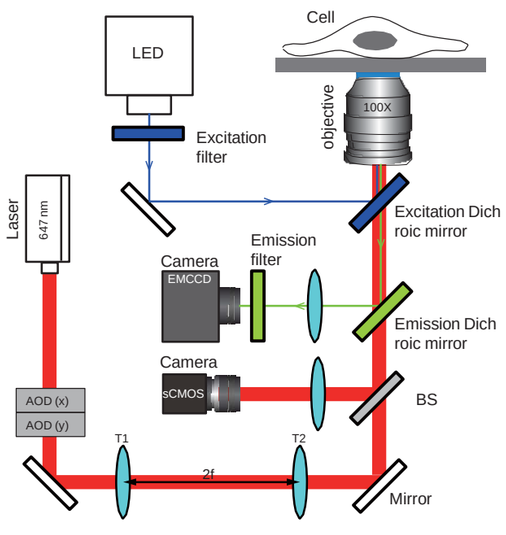


GFP labelling: Paxillin, Total recording time: 200 min

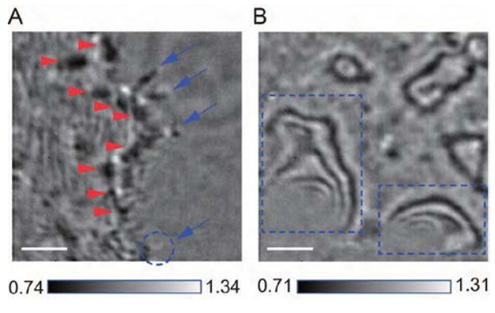
M. E. Berginski et al., PLOS ONE 6(7), e22025 (2011)

Experimental setup

◎ iSCAT microscopy combined with fluorescence detection



AOD: acousto-optic deflector, BS: beam splitters, T1-2, telecentric lens (f = 500 mm), Excitation/em ission wavelength (482/520 nm)

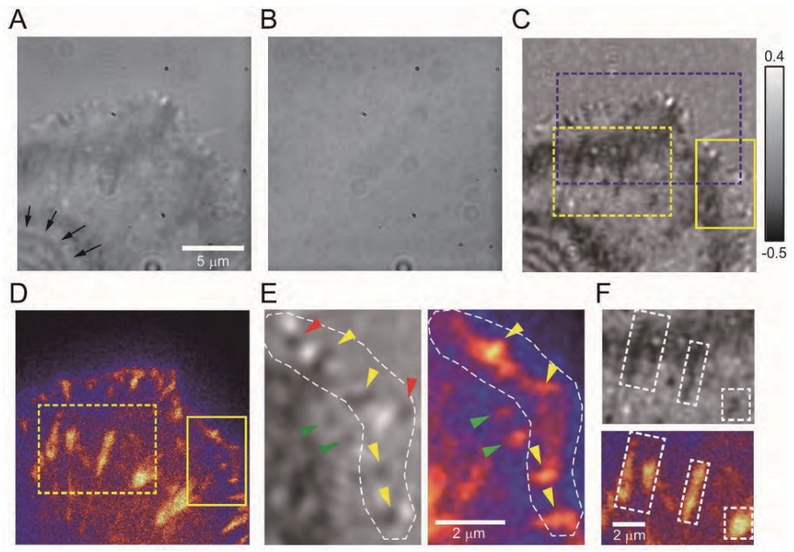


- Cell: COS-7 cell line
- Viewfield: 10 X 10 μm^2
- Pixel size ~ 18.6 nm

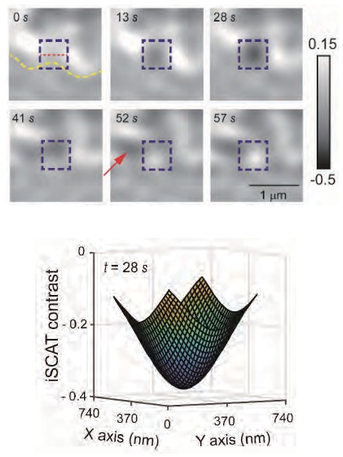
(A) Ultrafine structure of thin filopodia (blue arrows) and focal adhesions (red arrowheads) revealed by iSCAT. (B) Interference fringes caused by the upper cell membrane , conveying 3D topography of the membrane.
Scale bars: 2 μm [J. Park et al., Chem. Sci., 9, 2690 (2018)]

Results

◎ iSCAT and fluorescence image of a GFP-zyxin labelled U2OS cell

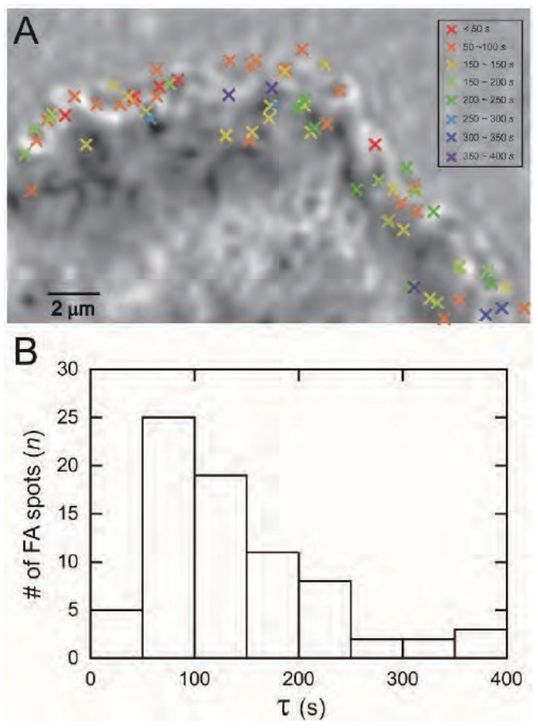


Direct visualization of assembly and disassembly of a single FA



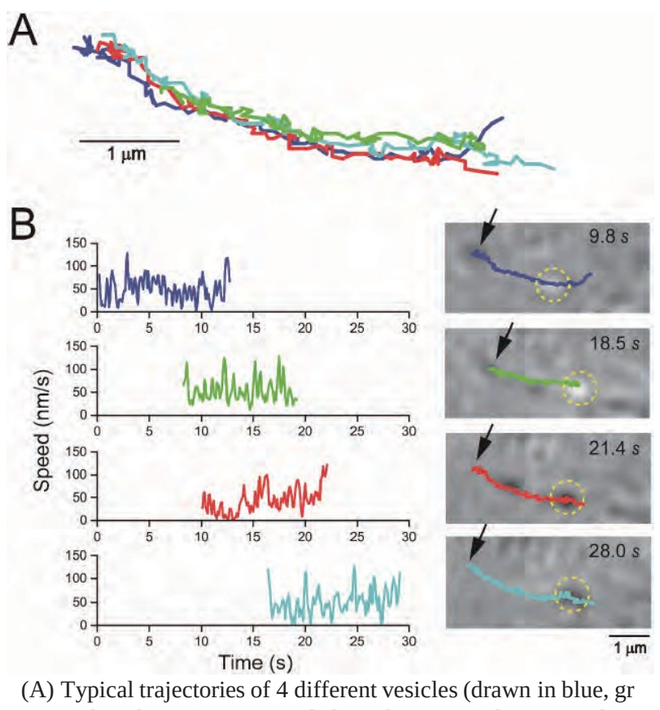
(A) Raw iSCAT image, (B) mean background image of 1,000 consecutive images from a cell-free area, (C) ratiometric i SCAT image obtained from the ratio of (A) to (B), (D) complementary fluorescent image showing zyxin-rich regions, (E) magnified iSCAT image (yellow line-boxed area in (C)) showing the existence of adhesions in the cell boundary (left) a nd its complementary fluorescent image (right), and (F) magnified iSCAT image (yellow dotted-boxed area in (C)) showing the structure of mature FAs (upper) and corresponding fluorescence image (lower).

◎ iSCAT lifetime (τ) measurements of FAs spontaneously formed in a lamellipodium



(A)A total of 75 adhesions were observed for 45 minutes and nucleation sites are indicated by cross symbols in diff erent colors, according to lifetime (τ).
(B)Histogram of τ estimated for the 75 adhesions in (A) . Estimated average value of τ is about 136 ± 80.3 s.

◎ Vesicle transportation along a fibrous structure revealed by label-free iSCAT imaging



(A) Typical trajectories of 4 different vesicles (drawn in blue, gr een, red, and cyan) transported along the same 1-dimensional tra ck. (B) Instantaneous speed of each vesicle (left) and its trajector y redrawn from A is overlaid in iSCAT image (right). Each colo r in graphs represents data from the same vesicle. The arrows in iSCAT images indicate the starting points of each trajectory.

Conclusions

◎ Fluorescence-combined iSCAT microscopy has revealed fine structures of focal adhesions i n a lamellipodium of U2OS cells with GFP-expressed zyxin proteins.

◎ The iSCAT-based imaging was shown to be sensitive enough to detect individual formation of focal adhesions onto the glass substrate and their disassembly in real time. According to our dir ect τ measurement of adhesions in a lamellipodium, we found the mean τ of FA to be 136 ± 80.3 s, well consistent with the previous experimental results based on fluorescent detection (C. K. Choi et al., Nat. Cell Biol., 10(9), 1039 (2008)).

◎ In addition, non-labelled endogenous vesicles travelling in a stop-and-run manner along the actin fibers were simultaneously observed by iSCAT microscopy.

◎ These experimental results show that iSCAT imaging is ideally suited for tracking real-time dy namics of endogenous and exogenous protein complexes in living cells, permitting both fast as well as long-term imaging.

Acknowledgement

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