

STRUCTURED ILLUMINATION MICROSCOPY (SIM) IMAGING COMPARISON WITH CONFOCAL



Tomomi Nemoto, Ph.D.

Laboratory of Molecular and Cellular Biophysics, Research Institute for Electronic Science, Hokkaido University, Japan

Nikon Imaging center in Hokkaido University, Japan

Kazuaki Sawada

Laboratory of Molecular and Cellular Biophysics, Research Institute for Electronic Science, Hokkaido University, Japan

Ryosuke Kawakami, Ph.D.

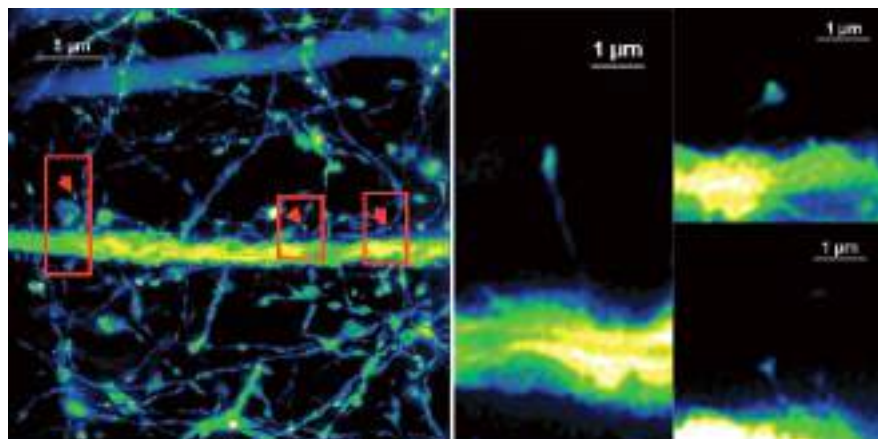
Molecular Medicine for Pathogenesis, Graduate School of Medicine, Ehime University, Japan

The super-resolution microscopy technique structured illumination microscopy (SIM) imaging of dendritic spines along the dendrite has not been previously performed in fixed tissues, mainly due to deterioration of the stripe pattern of the excitation laser induced by light scattering and optical aberrations. To address this issue and solve these optical problems, we applied a novel clearing reagent, LUCID, to fixed brains. In SIM imaging, the penetration to depth of 60 μm with high spatial resolution is improved in LUCID-treated slices. Furthermore, the structured illumination microscopy shows a better method than confocal microscopy for revealing spine morphologies in single dendrites shown as below. Thus, super-resolution SIM imaging represents a promising high resolution imaging method for neuroscience.

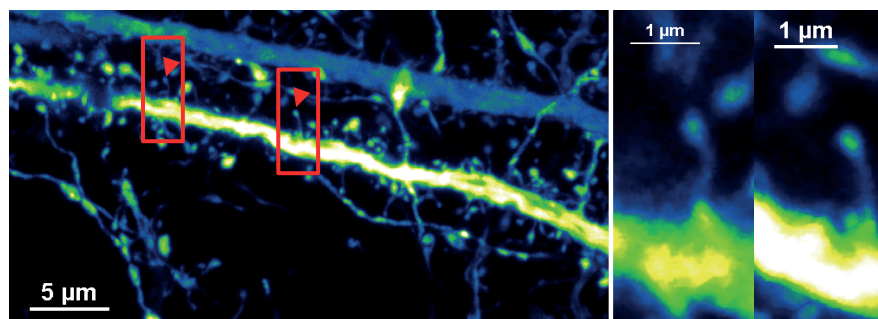
Reference

European Journal of Neuroscience, Vol. 47, pp. 1033–1042, 2018

Structured Illumination Microscopy



Confocal Microscopy





Dr. Jun Kawamoto

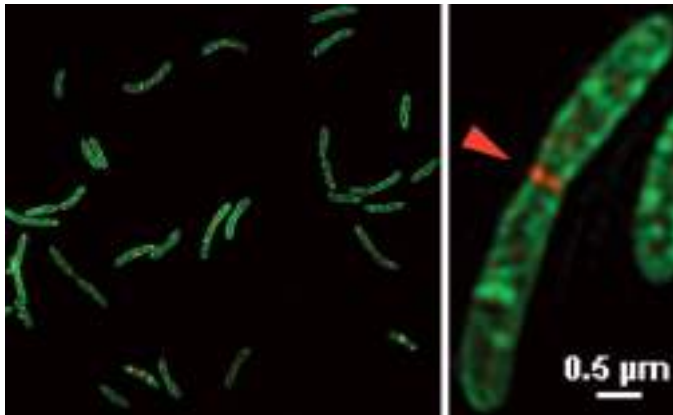
*Assistant Professor of Institute for Chemical Research,
Kyoto University*

The SIM method is an accepted technology in the field of bioscience imaging. We think that it has good point to capture the biological sample image as below.

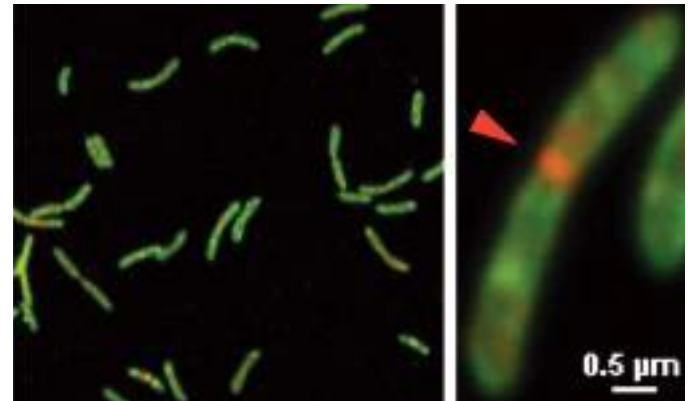
- 1) N-SIM is suitable for observing the distribution of bacterial membrane phospholipids and cell division proteins.
- 2) N-SIM image clearly show the fine structure of a cell division protein which is usually observed as blurred structure in confocal microscopy image.
- 3) N-SIM can also show lateral heterogeneity of bacterial membrane lipid molecules.

Thus we believe the high quality and resolution imaging with SIM will be frequently used, and well utilized in bacteriological science research field from now on.

Structured Illumination Microscopy



Confocal Microscopy



Sample: Bacteria.

The lipid molecule is labeled with Alexa 488 and the cell division protein is labeled with Alexa 555. The encapsulant is ProLong Gold. Use SR Apo TIRF x100.



Dr. Norio Kitagawa

Department of Morphological Biology, Fukuoka Dental College

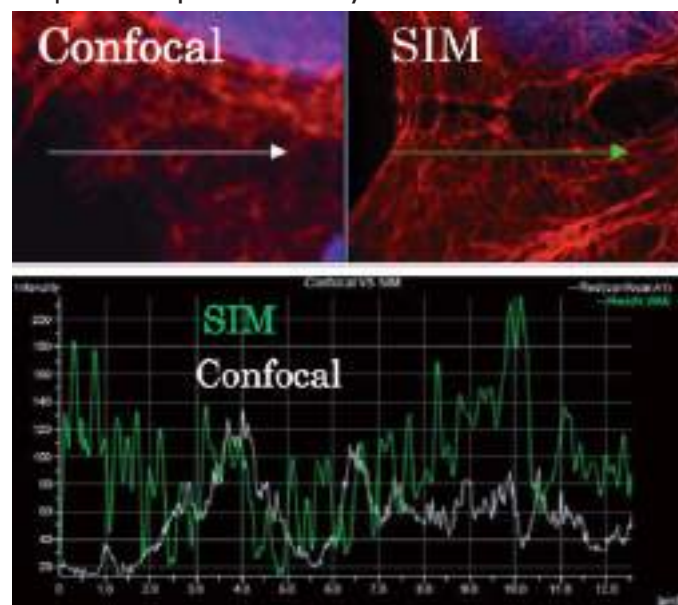
Laboratory URL: <http://www.fdcnet.ac.jp/col/info/teacher/kouza/kouzou.html>

The SIM method is an accepted technology in the field of bioscience imaging. We think that it has good point to capture the biological sample image as below.

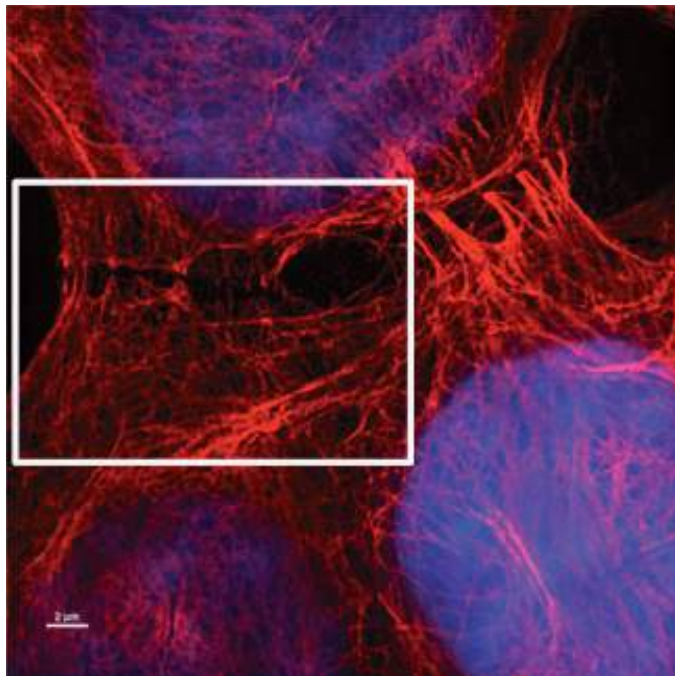
- 1) N-SIM is suitable for observing the distribution change of proteins constituting the cytoskeleton.
- 2) N-SIM image clearly show the structure of cell margin and fine cytoskeleton which is usually observed as blurred structure in confocal microscopy image. An image in three dimensions is also clear, which tends to emphasize blurred portions in confocal image.
- 3) N-SIM can also be expected to exert its ability in images of vesicles and protrusions of cells.

Thus we believe the high quality and resolution imaging with SIM will be frequently used, and well utilized in cell biology research field from now on.

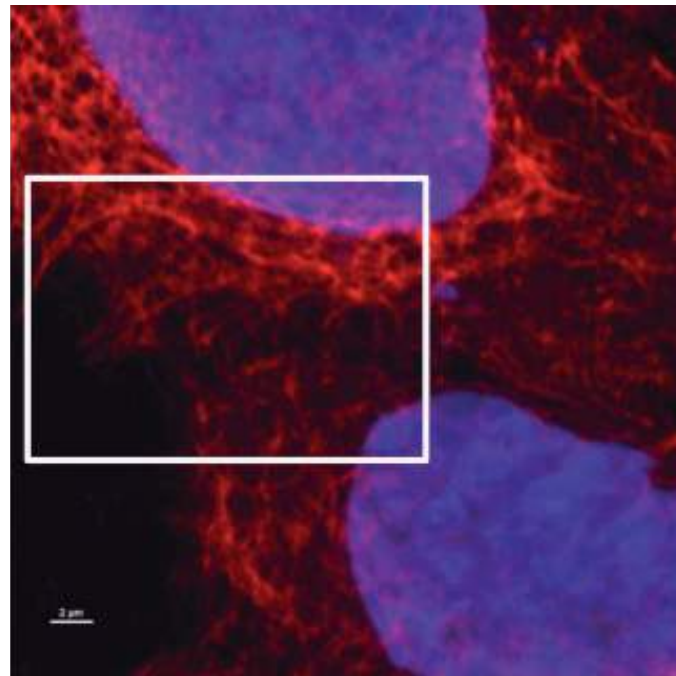
Sample: Human epidermal keratinocytes



Structured Illumination Microscopy



Confocal Microscopy



Primary antibody: rabbit anti-keratin 17 polyclonal antibody, Secondary antibody: anti-rabbit IgG conjugated with Alexa 568. Nuclear stained with DAPI. The encapsulant is a vector shield. Getting Z stack of 5-6 µm thick sample with SR Apo TIRF100x.



Dr. Yoshihiro Inoue

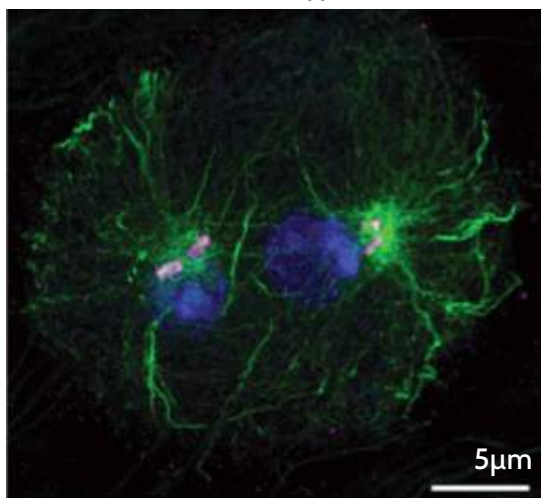
Tsuyoshi Shoda and Yoshihiro Inoue, Department of Insect Biomedical Research, Kyoto Institute of Technology

Laboratory URL: <https://www.kit.ac.jp/en/research/department-of-insect-biomedical-research/>

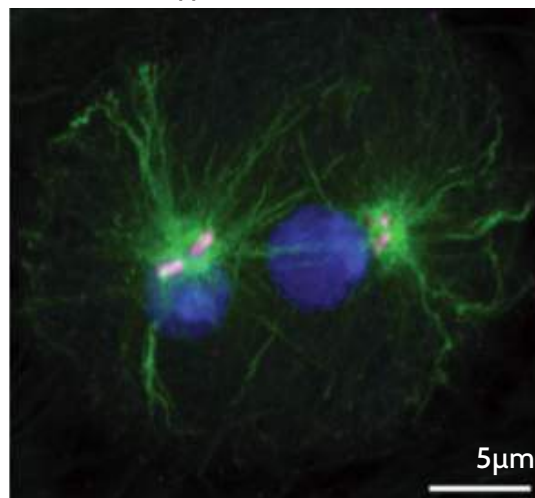
The SIM method is an accepted technology in the field of bioscience imaging. We think that it has good point to capture the biological sample image as below.

- 1) N-SIM allows us to observe intracellular fine structures such as centrioles which are less than 1 μm in length clearly.
- 2) N-SIM can acquire clearer images of microtubule organization centers, compared to blurred images obtained by a confocal microscope. Even in three-dimensional stack images, we can recognize each of microtubules or their bundles using N-SIM.
- 3) N-SIM can also be expected to examine dynamic alterations of cristae structure in mitochondrial inner membrane in both physiological and pathological conditions.

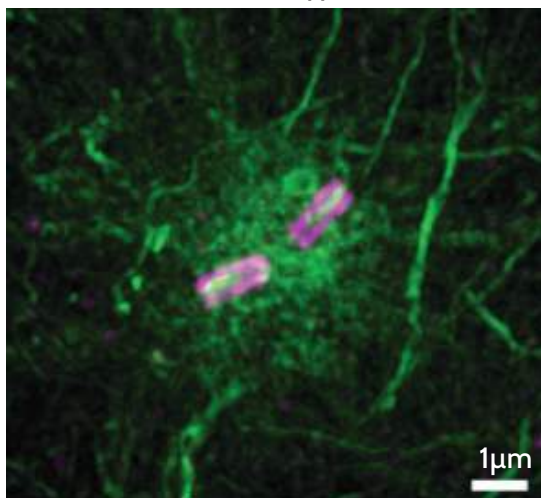
Structured Illumination Microscopy



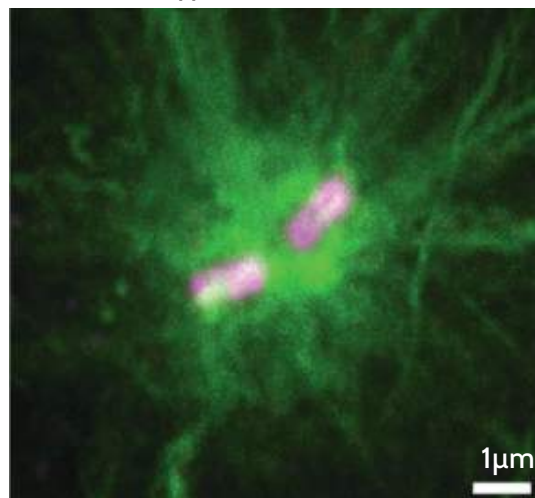
Confocal Microscopy



Structured Illumination Microscopy



Confocal Microscopy



Z-stack imaging of cell division of drosophila primary spermatocyte

The sample is labeled with DAPI, tubulin-GFP and centrosome-Asterless antibody. The mounting medium is Prolong Diamond[†]. The objective lens is Nikon SR Apo TIRF 100x.

[†]1: Thermo Fisher Scientific Inc. Copy Right Reserved.

