<Over view of each Bio-SPM technology>

Atomic resolution/3D-AFM (FM-AFM)

FM-AFM（Frequency-modulation Atomic Force Microscope）can visualize subnanometer-scale surface structures of biomolecules in solution. Combined with 3D scanning technique, it can also visualize 3D distribution of hydration and flexible surface structures at solid-liquid interfaces. The imaging rate of FM-AFM and 3D-AFM is typically 1 min/frame. The optimal spatial resolution of the instrument is 0.3 nm in the lateral direction and 0.01 nm in the vertical direction. In the case of biomolecular imaging, the practical resolution is mostly determined by the fluctuation of the surface structures rather than the instruments. For more details, see the following articles:

1. H. Asakawa, S. Yoshioka, K. Nishimura, T. Fukuma, “Spatial Distribution of Lipid Headgroups and Water Molecules at Membrane/Water Interfaces Visualized by Three-Dimensional Scanning Force Microscopy”, ***ACS Nano*** 6, 9013-9020 (2012).
2. H. Asakawa, K. Ikegami, M. Setou, N. Watanabe, M. Tsukada, T. Fukuma, “Submolecular-Scale Imaging of α-Helices and C-Terminal Domains of Tubulins by Frequency Modulation Atomic Force Microscopy in Liquid”, ***Biophys. J.*** 101, 1270-1276 (2011).
3. T. Fukuma, “Water distribution at solid/liquid interfaces visualized by frequency modulation atomic force microscopy”, ***Sci. Technol. Adv. Mater.*** 11, 033003 (18 pages) (2010).

High-speed AFM (HS-AFM)

HS-AFM (High-speed Atomic Force Microscope) can visualize moving objects in solution. Its temporal resolution is typically 100 ms/frame, while the spatial resolution is 2-3 nm in the lateral direction and 0.15 nm in the vertical direction. When it is applied to protein molecules in action, the acquired HS-AFM images can provide a significant insight into how the molecules function. For more details, see the following review articles:

1. T. Ando, T. Uchihashi, S. Scheuring, "Filming biomolecular processes by high-speed atomic force microscopy", ***Chem. Rev.*** 114, 3120-3188 (2014).
2. T. Ando, T. Uchihashi, N. Kodera, "High-speed AFM and applications to biomolecular systems", ***Annu. Rev. Biophys.*** 42, 393-414 (2013).
3. T. Uchihashi, N. Kodera, T. Ando, "Guide to video recording of structure dynamics and dynamic processes of proteins by high-speed atomic force microscopy", ***Nature Protocols*** 7, 1193-1206 (2012).

Scanning Ion Conductance Microscopy (SICM)

SICM has a unique measurement principle and provides unprecedented opportunity that enables submicroscale functional imaging of single live cells by a combination of nanoscale local stimulation and noncontact topography imaging. The imaging rate of SICM is 30-300 s/frame. Spatial resolution of the instrument is 10 nm in the lateral direction and 5 nm in the vertical direction. For more details, see the following articles:

1. P. Novak, C. Li, A. I. Shevchuk, R. Stepanyan, M. Caldwell, S. Hughes, T. G. Smart, J. Gorelik, V. P. Ostanin, M. J. Lab, G. W. J. Moss, G. I. Frolenkov, D. Klenerman, and Y. E. Korchev, “Nanoscale live-cell imaging using hopping probe ion conductance microscopy”, ***Nat. Methods*** 6, 279-281 (2009).
2. V. O. Nikolaev, A. Moshkov, A. R. Lyon, M. Miragoli, P. Novak, H. Paur, M. J. Lohse, Y. E. Korchev, S. E. Harding, and J. Gorelik, “beta(2)-Adrenergic Receptor Redistribution in Heart Failure Changes cAMP Compartmentation”, ***Science*** 327, 1653-1657 (2010).
3. Zhou, M. Saito, T. Miyamoto, P. Novak, A. Shevchuk, Y. Korchev, T. Fukuma, Y. Takahashi, “Nanoscale Imaging of Primary Cilia with Scanning Ion Conductance Microscopy,” ***Anal. Chem.*** 90, 2891-2895 (2018).

AFM for Cell Measurement

Based on high-speed AFM or 3D-AFM, NanoLSI is developing AFM technologies for measuring the structure, dynamics or mechanical properties of the surface or inside of cells at a nano scale. High-speed AFM successfully

visualized the surface structure of bacteria at a molecular scale and nano-motion of the terminal portion of nerve cells. Based on 3D-AFM, we developed a nanoendoscopy-AFM technique. Using this technique, we succeeded in three-dimensional observation of cell nucleus or actin fibers inside live cells, the measurement of two-dimensional nanodynamics of inner scaffold of plasma membrane, and the measurement of the surface stiffness of cell nucleus. For more details, see the following articles:

1. H. Yamashita, A. Taoka; T. Uchihashi, T. Asano, T. Ando, Y. Fukumori, “Single-molecule imaging on living bacterial cell surface by high-speed AFM”, ***J. Mol. Biol.*** 422 (2), 300-9 (2012).
2. M. Shibata, T. Uchihashi, T. Ando, R. Yasuda, “Long-tip high-speed atomic force microscopy for nanometer-scale imaging in live cells”, ***Sci. Rep.***, 5, 8724 (2015).
3. M. Penedo, K. Miyazawa, N. Okano, Furusho, H. Ichikawa, T, S. Alam Mohammad, K. Miyata, C. Nakamura, T. Fukuma, “Visualizing intracellular nanostructures of living cells by nanoendoscopy-AFM”, ***Sci. Adv.*** 7 (52), eabj4990 (2021).
4. K. Kobayashi, N. Kodera, T. Kasai, YO. Tahara, T. Toyonaga, M. Mizutani, I. Fujiwara, T. Ando, M. Miyata. “Movements of Mycoplasma mobile gliding machinery detected by high-speed atomic force microscopy”, ***mBio*** 12: e00040-21 (2021).