

Transdisciplinary Research at NanoLSI





Kanazawa University established the Nano Life Science Institute (NanoLSI) in 2017 to fulfill its selection for the World Premier International Research Center Initiative (WPI), an effort launched by the Ministry of Education, Culture, Sports, Science and Technology to develop research centers that have a salient presence in the global scientific community for their world-class research environments and superlative standards of research. NanoLSI is now the staging ground of bold explorations for pioneering the uncharted nano realm of the life sciences.

— *Elucidating the truths of biological phenomena on the nanoscale*

Over the centuries, humankind has developed various microscopes to peer into microcosms invisible to the unaided eye. Those observations have taught us about all sorts of physical properties and the origins of diverse phenomena, and in so doing have helped science to evolve. However, there's a world we are still unable to observe directly—the nanoscale structures and dynamics of atoms and molecules. This is the uncharted nano realm, and we greatly need to develop the technologies that will allow us to unlock its mysteries.

The surface and interior of the cells that make up our bodies are populated with countless molecules that perform many functions in diverse ways, giving rise to biological phenomena. For this reason, our observation of their behavior and interactions would be a key to understanding and controlling the mechanisms of biological phenomena. However, science and technology have yet to advance to the level necessary for fully bringing that dream to reality. NanoLSI thus seeks to chart the uncharted nano realm of the life sciences in order to endow humankind with a fundamental understanding of the many different phenomena of life.

**Takeshi
Fukuma**

Director of
WPI-NanoLSI



— *Bringing innovation to the life sciences through transdisciplinary research*

NanoLSI is a community of scientists who for many years have produced world-class research achievements spanning four domains: Nanometrology, Life Science, Supramolecular Chemistry, and Computational Science.

Leveraging cutting-edge technologies in scanning probe microscopy (SPM)* and integrating/expanding our four research domains, we strive to develop nanoendoscopic (nanoprobe) technologies that will enable us to directly observe the relationships and action of molecules that perform critical roles inside and outside cells, and thus gain the insights needed to analyze and manipulate them. By witnessing biological phenomena unseen before, we will be able to bring forth tremendous advances to the life sciences and establish a new domain of science, "Nanoprobe Life Science."

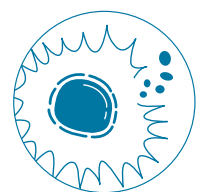
*Scanning probe microscopy (SPM): Microscopy that measures a specimen's surface form and properties via an ultrafine-tipped probe tracked near the specimen's surface.

— *A one-of-a-kind research center*

Since the beginning, NanoLSI scientists have regularly shared insights, perspectives, and methodologies with their colleagues in the institute's other research domains. By engaging in such transdisciplinary dialogue, they have developed a deep mutual understanding that helps them to advance world-leading research in their respective fields and to make effective use of the shared body of knowledge and research expertise. The result is a close-knit community of diverse scientists, and it is our goal to nurture and evolve the ideas that only such a community can produce. As a one-of-a-kind research center, we are firmly committed to generating a steady stream of new discoveries and concepts, and translating them into advances in science and technology, in a constant cycle of creation and evolution.

The Tale of an Adventure to the Secrets of Life

Life is made possible because the molecules inside and outside the cells of our bodies carry out certain duties. But, what are their shapes? How do they work? At NanoLSI, we endeavor to unravel the secrets of life by answering such questions.

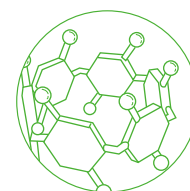
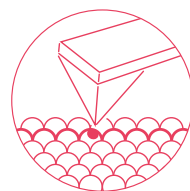


Life Science

I already know the basic functions of most molecules. But, since I haven't seen what actually goes on in the cell, such as the actual shape and movements of the molecules, I still don't know their physiological functions.

Nanometrology

I have cutting-edge technologies in microscopy that allow me to make video observations of the shape and behavior of molecules. With those technologies, I can develop even more amazing microscopes that can see molecules within the world of cells. However, there are still some challenges that need to be overcome in viewing the molecules inside cells.

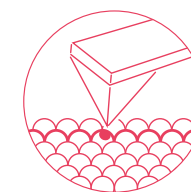


Supramolecular Chemistry

I can make special molecules that are able to distinguish and move molecules I'm interested in. Eventually, I'll become able to manipulate molecules inside cells, and then I can help develop new types of microscopes!

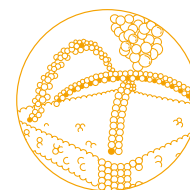
Nanometrology

Wow! That means we'll be able to directly view molecules both inside and outside cells! Now, since we're talking about viewing the behavior of tiny molecules with technologies that haven't been realized before, the next step is to prove that our understanding of them is right.



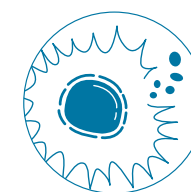
Computational Science

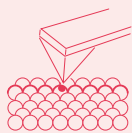
I have tons of data I've collected, so I can use simulations to back up our understanding of the tiny molecules' behavior. Let's pool together our expertise and test which patterns are able to explain the molecular behavior!



Life Science

Thanks to our exploration of the world of cells, we've gotten closer to uncovering the truth behind biological phenomena, like the mechanisms of how life starts and how diseases develop. Our research will open the door to a future where we can aid the development of new ways to diagnose and treat people!

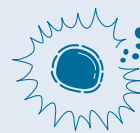




Nanometrology

Developing nanoprobe technologies

The Nanometrology group brings together many scientists who have pursued world-leading research utilizing two forms of SPM: atomic force microscopy (AFM), which offers high-speed, high-resolution, and three-dimensional observation, thanks to the application of unique developmental technologies, and scanning ion-conductance microscopy (SICM), which enables observation of the soft surfaces of cells because the probe does not come into contact with the specimen's surface. While continuing to evolve both AFM and SICM, this group seeks to establish nanoprobe technologies that, through fusion with supramolecular chemistry, realize direct observation, analysis, and manipulation of biomolecular dynamics.

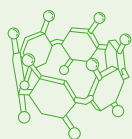


Life Science

Elucidating the basic principles of fundamental cellular functions

By examining molecular dynamics through comparison of normal cells and cancer cells, we can learn the molecules' physiological functions and gain understanding of the mechanisms by which their abnormalities lead to cancer. Using newly developed nanoprobe technologies such as SPM and molecular sensors that react only to specific molecules, the Life Science group observes "as is" the molecules essential to biological phenomena, with the aim of elucidating the basic principles of fundamental cellular functions, including cell proliferation, differentiation, death, movement, and so on. In the future, this group will utilize its discoveries to develop new methods of diagnosing and treating cancer and many other diseases.

A fusion of four sciences Nanoprobe Life Science



Supramolecular Chemistry

Manipulating molecule with supramolecular forces

This group freely designs molecules through the ingenious use of diverse molecular structures. It seeks to develop supramolecules as sensors that bind only to a specific metabolite or protein through molecular recognition—whereby the size and shape of a molecule are identified based on its interactions with other molecules—and thus can be used to reveal the intracellular distribution of the metabolite or protein. In addition, the group plans use molecular machines—whose structures change in response to external stimuli such as light and ions—to realize endoscopic manipulation techniques for manipulating the shape and position of specific molecules in a cell during observation with a nanoprobe microscope.



Computational Science

Bridging the gap between imaging and reality

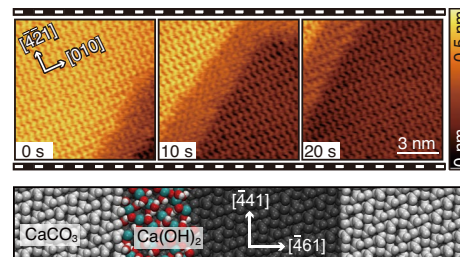
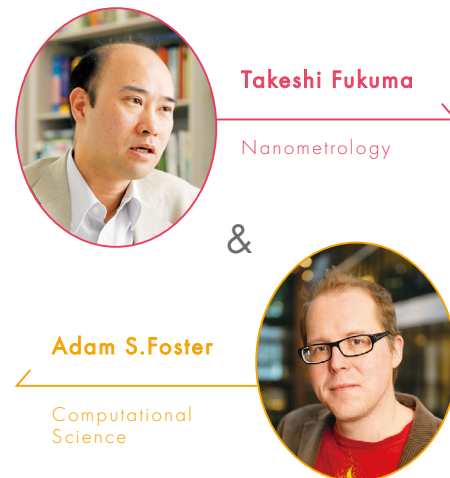
It is not easy to identify new principles of dynamics only through observation with novel measurement technologies, notably SPM, which visualizes the nano-level structure and properties of the target molecule through its interactions with the probe. Sophisticated simulation technologies are also necessary. The Computational Science group endeavors to understand the reality of biological phenomena by elucidating the principles of dynamics. To do this, they reproduce the patterns of molecular dynamics inferred from the images obtained from imaging techniques and integrate the knowledge and experiences of various disciplines.

Scientists in NanoLSI's four research groups—Nanometrology, Life Science, Supramolecular Chemistry, and Computational Science—are pursuing diverse transdisciplinary research projects driven by nanometrology.

New insights from the merger of original AFM technology and sophisticated simulation

> Shedding light on the unknown origins and principles of natural phenomena

Prof. Takeshi Fukuma has developed technologies for frequency modulation atomic force microscopy (FM-AFM), which enables high-speed, high-resolution observation at the atomic level, and three-dimensional AFM (3D-AFM), which can be used to observe the three-dimensional spatial distribution of water molecules and molecular chains, among others. Seeking to uncover new insights in well-known phenomena, he has turned his attention to calcite, a mineral that, as the world's largest carbon pool, poses a critical impact on the global carbon cycle. Using high-speed FM-AFM, he became the first in the world to succeed in atomic-resolution observation of the calcite dissolution process, and he has elucidated the dissolution mechanism at the atomic level by combining his technique with Prof. Adam Foster's high-precision simulation analyses.



The dissolution process of calcite surface as observed with high-speed FM-AFM (top) and a simulation model demonstrating the molecular-level changes (bottom).

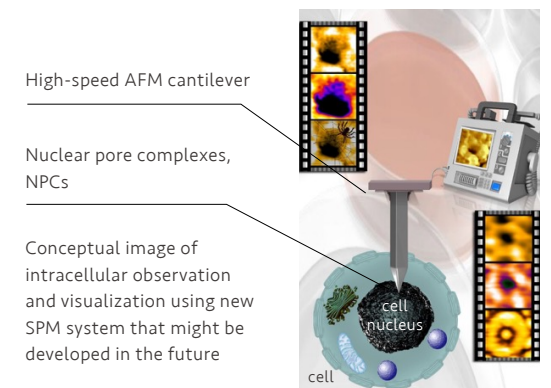
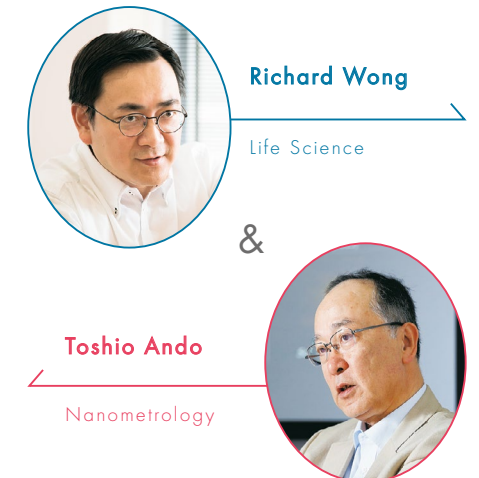
> The next challenge: Elucidating mechanisms of biological phenomena

The structure of chromatin—largely made up of DNA, the carrier of genetic information—plays a major role in gene expression and control, which provide the foundation for life activities. With the aim of elucidating the genetic abnormalities behind various diseases through investigation of the three-dimensional structure and dynamics of chromatin, the two professors are working to improve the AFM and simulation technologies necessary for analyzing the three-dimensional structure of biomolecules.

Nuclear pore complexes: Exploring molecular nanogates at the nano level

> Uncovering the action of NPCs, molecular nanogates

Nuclear pore complexes, NPCs, are the sole passageway for the transport of materials between a cell's nucleus and cytoplasm. They are protein complexes that monitor the vast quantity of information used in biological phenomena and selectively control the exchange of genetic information. They are indispensable to biological phenomena, as attested by their control of the proliferation- and metastasis-related signaling in cancer cells. Prof. Richard Wong has isolated the nuclear membranes of human colorectal cancer cells and observed them using the high-speed AFM system developed by Prof. Toshio Ando. Through this research, he has shed light on NPC structure and dynamics, and uncovered the characteristic deformation of NPCs when the cancer cells die from the administration of cancer drugs.



> Future development of artificial NPCs could pave way for nanotherapies

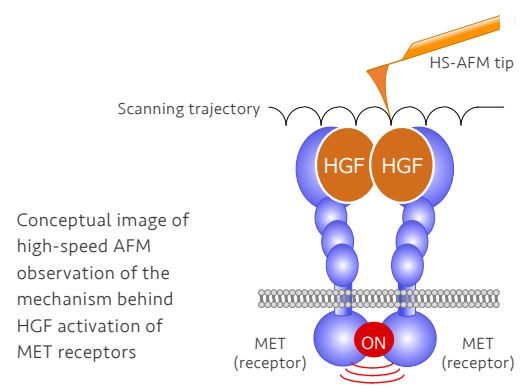
In its current state, the technology used by Prof. Wong can observe NPCs only in isolated nuclear membranes. However, if an SPM system capable of observing the NPCs inside living cells were to be innovated, it may be possible to reveal NPC functions and dynamics in molecular interactions inside and outside the cell. By merging cellular biology's insights on biological functions, high-speed AFM's revelations of structures, and clinical understanding of pathology, scientists may find ways to create therapeutic technologies for nano-level control of abnormalities in various biological phenomena, such as by developing artificial NPCs.



Dynamic structural biology for activating and controlling signaling molecule HGF

> An effort to unravel the true mechanism behind HGF-MET receptor activation

Hepatocyte growth factor (HGF) is a protein that promotes cell proliferation and survival when it binds with its receptor, MET. Since HGF plays a key role in tissue regeneration/repair and is involved in cancer metastasis as well, it is used in regenerative medicine and cancer diagnosis/treatment. Prof. Kunio Matsumoto, in a quest to find the true mechanism of how HGF activates MET receptors, has teamed up with Assoc. Prof. Mikihiro Shibata, who has used high-speed AFM to capture the actual behavior of biomolecules. To date, Shibata and his colleagues have visualized the behavior of DNA/RNA and proteins related to the editing of genomic information, the blueprint of life. In this research project, they have started to shed light on the unknown realm of the dynamic structure of MET activation.



> Innovative discoveries may open door to new drugs and other therapies

Elucidation of the dynamics of the interactions that take place between the miniscule proteins in the body would not only provide substantive understanding of functions and structural changes, but also open the door to the creation of new drugs and other forms of therapy. The ability to use high-speed AFM for direct video observation in a near-in vivo environment offers the potential for new discoveries that shatter established thinking and thus contribute to the advancement of the life sciences. As a potential precedent for such evolution, this project will continue to engage in transdisciplinary research founded on the elucidation of HGF-MET dynamics.

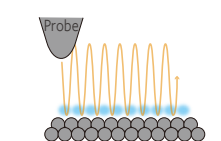
Designing an original probe for 3D spatial distribution imaging of target molecules

> Developing technologies for 3D spatial distribution imaging of target molecules

3D-AFM, which was developed by Assoc. Prof. Hitoshi Asakawa and colleagues, enables three-dimensional spatial imaging of molecular structures, based on the interactions between the probe and the atoms/molecules. Meanwhile, pillararenes, molecules first discovered by Prof. Tomoki Ogoshi, are simple rings of symmetric pillar-shaped structures. By designing a pillararene with a suitable ring size or recognition sites, the pillararene can selectively incorporate into its ring a target molecule with subtle differences in size or shape, like a key and keyhole relationship. This project is exploring the possibilities for attaching molecule-recognizing pillararenes on probes to detect the distinctive forces acting between the pillararenes and the target molecule in three-dimensional space, and thus enable visualization of the spatial distribution of tiny target molecules in real space.

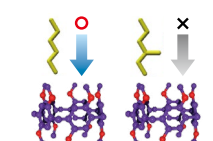


Three-dimensional observation by 3D-AFM



Acquiring 3D data by moving the probe in both horizontal and vertical directions

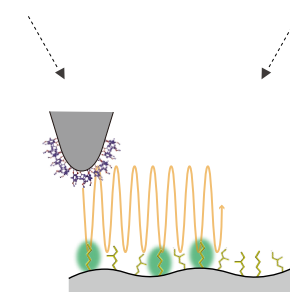
Molecular recognition with pillararenes



Molecules with even just small differences in size or shape are not incorporated

> Striving toward fusion with computational science and life science

Going forward, scientists will not only need to use simulations to develop better understanding of measurement data, but also design supramolecules that can recognize specific proteins or metabolites essential to functional analysis for understanding biological phenomena. NanoLSI is stepping up research for developing technologies in both areas, aiming to bring about a fusion of both areas with computational science and life science.



Visualization of a target molecule's distribution by using an originally designed probe that reacts only to molecules taken in by pillararenes