February 2022

Summary of Research Center Project

*Briefly describe the general plan of your project (Compile in English within 2 pages.)

Center name: Nano Life Science Institute (NanoLSI)（Within 15 words）

Host institution: Kanazawa University

Head of host institution: Koetsu Yamazaki, President of Kanazawa University（Name, Affiliation）

Center director: Takeshi Fukuma, Professor of Kanazawa University（Name, Affiliation）

Administrative director: Yoshihiro Fukumori, Professor of Kanazawa University（Name, Affiliation）

1) Overall Image of Your Center

NanoLSI is well-known for its world-class bio-scanning probe microscopy (SPM) technologies such as high-speed atomic force microscopy (HS-AFM) developed by Ando, in-liquid frequency-modulation AFM and 3D-AFM developed by Fukuma, and functional scanning ion conductance microscopy (SICM) developed by Korchev. In addition, we have been developing novel nanoprobe technologies for imaging the structures, dynamics, and chemical and mechanical property distributions at the surfaces and/or inside living cells. Attracted by these technologies, many life scientists have visited our institute from different countries to observe various nanoscale biological phenomena and understand their fundamental mechanisms. Thus, NanoLSI is well recognized as one of the world's most influential centers of Bio-SPM collaborations. In addition to the Bio-SPM researchers, we have internationally top-level researchers in the life sciences, supramolecular chemistry, and computational science. The interdisciplinary collaborations among these four disciplines are another distinctive feature of our institute. Our life scientists have different expertise in cell biology (NPCs, exosomes, among other things), pharmaceutical science (drug metabolism), and cancer development and progression, allowing us to pioneer novel applications in various life science fields. Meanwhile, we use our knowledge on supramolecular chemistry for producing molecular sensors, integrating them into a nanoprobe, and visualizing distributions of target molecules around a living cell. Furthermore, we also use our expertise in computational science for understanding mechanisms of biological phenomena from the SPM data obtained. Through these activities, we are pioneering new research discipline "nanoprobe life science."

2) Research Activities

1. Development of novel nanoprobe technologies

Compared with major bio-imaging techniques such as fluorescence microscopy and electron microscopy, Bio-SPM has distinctive advantages of molecular-scale or sub-10-nm-scale imaging and quantitative nanomechanical measurements. However, the former is possible only for "*in vitro*" systems constructed on a solid surface and the latter is impossible for the intracellular components. Thus, the majority of the nanodynamics at the surface and/or inside of the living cells have remained inaccessible. In this project, we aim to overcome this limitation by developing novel nanoprobe technologies for visualizing nanoscale structures, dynamics, chemical and mechanical property distributions at the surfaces and/or inside of the living cells. Major projects include the development of (1) 2D/3D nanoendoscopy-AFM for intracellular imaging, (2) HS-SICM/AFM for molecular-scale cell-surface imaging, (3) SICM for chemical mapping inside and around living cells, (4) molecular sensors and nano-devices for sensing and manipulation, and (5) modeling, simulation, and AI-based analysis techniques for understanding the physics behind the Bio-SPM data. Through these efforts, we aim to create a paradigm shift from "*in vitro*" to "*in vivo*" Bio-SPM technology. Meanwhile, we also keep updating our world-class Bio-SPM technologies such as HS-AFM, 3D-AFM, and SICM. For example, we aim to enhance the imaging rate of HS-AFM from 10 fps to 100 fps for visualizing faster protein dynamics. In this way, we will continue to create novel nanoprobe technologies for visualizing as yet unseen nanoscale biological phenomena.

2. Understanding of basic cellular functions and their cancer-specific abnormalities

So far, Bio-SPM applications have been mostly limited to biophysics research. In this project, we aim to expand this application range to molecular cell biology and medical science for the development of the "nanoprobe life science" field. To this end, we aim to present impactful applications of bio-SPM techniques in different life science disciplines. In the past five years, PIs at NanoLSI from different life science fields explored possible applications of newly developed live-cell imaging techniques and determined to work on the focused subjects related to (1) intracellular transport through nuclear pore complexes and its correlation with the infection mechanisms. (2) cell-cell communication via exosomes to induce cancer cell specific response and clarify the nanoscale mechanism. (3) cell dynamics altered by anti-cancer drug using the originally designed aptamer, (4) dynamic changes in the cell surface structures and mechanics altered by

cancer progression, (5) visualizing distribution of one of the metabolites around a living cell for understanding its correlation with cancer-specific metabolism, (6) machine learning classification for single cell-level cancer diagnosis, and (7) growth factor receptor dynamics at the living cell surfaces for understanding its correlation with cancer progression.

3. Establishment of "Nanoprobe Life Science" field

We aim to create a world-leading center for international collaborations on nanoprobe life science. So far, we have established three programs for promoting Bio-SPM collaborations such as the Bio-SPM summer school, Bio-SPM collaborative research program, and NanoLSI visiting fellows program. Through these programs, we will perform many collaborative studies with international visitors and clarify nanoscale mechanisms of various biological phenomena. In this way, we will play a central role in the establishment of the "nanoprobe life science" field.

3) Interdisciplinary Research

We combine the world-class Bio-SPM and supramolecular chemistry techniques to develop innovative nanoprobe methods, including nanoendoscopy. With our unique nanoprobe technologies and computational analysis techniques, we aim to understand fundamental nanoscale mechanisms of basic cell functions and their cancer-specific abnormalities. In this way, we combine our expertise in nanometrology, supramolecular chemistry, computational sciences, and life sciences to establish a novel research field, "nanoprobe life science." To this end, we will continue to take various measures such as the NanoLSI T (Transdisciplinary) meeting, Luncheon meeting (webinar), and NanoLSI Colloquium. The proposals stimulated by the top-down and bottom-up measures are financially supported by Transdisciplinary Research Promotion Grants.

4) International Research Environment

We will retain five or more foreign PIs and two international satellites and solicit more robust activities by the foreign PIs at NanoLSI by strengthening collaborative research with domestic PIs and other NanoLSI researchers. We will place emphasis on the employment of overseas postdoctoral researchers as well as outreach programs. We will retain the deployment of 60 or more Bio-SPMs and 6 EMs together with an open facility policy for international visiting researchers. The deployment of experienced and multi-lingual technicians will also be retained. We will provide multi-layered financial support for young overseas researchers such as a startup budget, annual basic research budget and transdisciplinary research promotion grant. URAs' support will be provided for overseas researchers to obtain KAKENHI and other external research funds. Wide-ranging administrative and livelihood support will also be provided for overseas researchers in English, including detailed explanation of the terms of the employment contract and Japanese tax system, accompanying during resident registration, accommodation search and interpreting during signing of the contract with a real estate agent, etc. In addition, support is provided for the families of overseas researchers. We will hold several international research meetings per year, including the NanoLSI international symposium and the NanoLSI-iCeMS joint international symposium.

5) Center Management

NanoLSI has been officially positioned in the statutes of Kanazawa University as an independent institute of the university. This revision of the statutes has endorsed the Center Director's discretionary power in terms of the personnel arrangements, budget execution, and representation of NanoLSI. The Center Director will take the initiative in developing interdisciplinary research in particular. The Administrative Director will work in cooperation to realize the Center Director's vision of NanoLSI management. We will continuously apply for a research evaluation-dependent annual salary scheme in order to maintain an internationally competitive salary. The research-focused scheme for competent researchers, namely the Research Professorship, will be continuously applied for full-time NanoLSI researchers to exempt them from non-NanoLSI duties and allow them to focus on their own research with maximal effort. We will make further efforts to hire female scientists at both the PI level and assistant or associate professor levels. For fostering future generation researchers of NanoLSI, all 27 full-time researchers of NanoLSI who have an educational assignment will be engaged as educators in the Graduate School of Frontier Science Initiative, Division of Nano Life Science. In addition, most of the PIs or Jr. PIs will give lectures to other graduate or undergraduate program students in various departments, helping us to recruit them to the Graduate School. We will provide a basic scholarship for the graduate students of the Graduate School from Kanazawa University's own fund. In addition, the governmental scholarships given to Kanazawa University will place a priority on the graduate students in the Graduate School. Kanazawa University will conform to its commitment and will proactively reassess its existing organization and develop system reforms. The promised deployment of 22 tenured or tenure-track researchers to NanoLSI has been completed and will be sustained.