World Premier International Research Center Initiative (WPI)
Executive Summary (For Interim Evaluation)

Host Institution	Kanazawa University	Host Institution Head	Koetsu Yamazaki
Research Center	Nano Life Science Institute (NanoLSI)		
Center Director	Takeshi Fukuma	Administrative Director	Yoshihiro Fukumori

Instruction:
Summarize the Self-Evaluation Report for Interim Evaluation (**within 4 pages** including this page).

I. Summary

At the Nano Life Science Institute (NanoLSI), we aim to produce innovative nanoprobe technologies for visualizing unseen nanoscale biological phenomena, thereby elucidating their fundamental mechanisms. During the first half period, we have extended the capabilities of our unique world-leading bio-scanning probe microscopy (Bio-SPM) techniques such as high-speed atomic force microscopy (HS-AFM), three-dimensional AFM (3D-AFM) and scanning ion conductance microscopy (SICM). In addition, we also worked on the development of various novel live-cell Bio-SPM imaging techniques, and made it possible to visualize intra-cellular structures, dynamic changes in the cell surface mechanics and pH mapping, inside and around living cells at the nanoscale.

Meanwhile, we established systems to promote Bio-SPM collaborations such as the Bio-SPM Summer School, Bio-SPM Collaborative Research Program, and NanoLSI Visiting Fellows Program. Through these programs, many researchers from many different countries visited NanoLSI to perform unique nano bio-imaging studies with our Bio-SPM technologies. The results obtained, such as the HS-AFM imaging of proteins and DNAs, and SICM imaging of living cells, have been published in high profile journals. NanoLSI is now well recognized as one of the biggest centers of Bio-SPM collaborative research. In parallel, we actively explored applications of the newly developed live-cell imaging techniques and defined several focused research subjects on various cell functions and their cancer-specific abnormalities. In the next five years, we will make major efforts on these subjects to have strong impacts on various life science disciplines, leading to the establishment of the nanoprobe life science research field.

We established systems and environments for transdisciplinary research among four disciplines: nanometrology, life science, supramolecular chemistry, and computational science. We built a new NanoLSI building for realizing the under-one-roof concept and organized weekly, quarterly and annual meetings. We also introduced an internal grant system to promote collaborations among young researchers. In addition, to establish an international research environment, our overseas researchers' ratio was kept over 30%, two satellite research centers were launched at ICL in London and UBC in Vancouver, annual international symposia were held in Tokyo, London, Vancouver, and Kanazawa(online). Our institute is now well recognized globally, and the internationally co-authored papers' ratio dramatically increased from 17% in 2016 which was the preceding year of the WPI grant period to 50% in 2020. Furthermore, we played central roles in system reforms at Kanazawa University. For example, we introduced a research assistant (RA) support system for master course students and a performance dependent salary system. We also established a new graduate school division tightly connected to NanoLSI. We are therefore making steady progress in all of the four WPI missions: the top world-level research, internationalization, a fusion of disciplines, and system reforms.

II. Items

1. Overall Image of Your Center

NanoLSI is well-known for its world-leading Bio-SPM technologies such as HS-AFM (Ando), in-liquid FM-AFM & 3D-AFM (Fukuma), and functional SICM (Korchev). In addition, we are developing novel nanoprobe technologies for imaging structures, dynamics, and chemical and mechanical property distributions at the surfaces and inside of living cells. Attracted by these unique technologies, many life scientists have visited our institute from different countries to see various nanoscale biological phenomena and understand their fundamental mechanisms. Thus, NanoLSI is well recognized as one of the world's biggest 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 various new possibilities of interdisciplinary research.

2. Center's Research Activities

After the launch of NanoLSI, individual PIs from the four different disciplines continued to work on their ongoing, internationally recognized projects and, at the same time, started work on various new projects. These new projects can be categorized into three parts: development of novel nanoprobe technologies, especially for live-cell measurements, Bio-SPM studies on cell functions and cancer development and progression, and Bio-SPM collaborations with many visiting researchers. Through these activities, we aim to have strong impacts on a wide range of life science research fields, leading to the establishment of a new research field, nanoprobe life science. During the first half period, we published 345 papers (48% internationally co-authored; 84 with an IF > 10; 109 with an IF > 7), gave 199 invited talks at international meetings, and acquired 3,844 million yen external funds (97 grants > 10 million yen). The major achievements are summarized in the following ten categories.

[1] Development of 2D- and 3D-AFM imaging inside living cells

By inserting a long nanoprobe into a living cell, we succeeded in 3D imaging of intra-cellular components (e.g., nucleus, actin fibers, among other things) and 2D imaging of nanodynamics at the inner surface of the cell membranes. This is a great breakthrough to overcome the major limitation of conventional AFM, whose high-resolution applications are limited to in-vitro systems.

[2] Development and application of measuring technologies on the surface of living cells

With high-speed SICM, we succeeded in visualizing dynamic changes in the cell surface structures and elasticity distributions and elucidated their dependence on cancer progression. We also developed a method to perform high-resolution AFM imaging of basal cell membrane surfaces with sub-10 nm resolution and enabled us to compare them directly with STED microscopy images.

[3] Development of nanoprobe-biosensors for chemical mapping inside and outside of living cells

Cellular chemical heterogeneity is a hallmark of cancer. We have recently developed a range of novel nanoprobe-biosensors for oxygen gradients, ROS species, ATP, and pH. Importantly, these nanoprobes can be integrated with our advanced SICM, which enables us to map intracellular and extracellular chemicals of living cancer cells with higher spatial and temporal resolution.

[4] Further improvement of high-speed AFM technologies

By redeveloping all devices in the HS-AFM and by introducing a new scanning mode, "only trace imaging", the scanning performance of HS-AFM was improved ~10 times than was possible before. Furthermore, a concave/convex substrate and a manipulator function were newly added to HS-AFM. These improvements greatly contribute to a deeper understanding of biological phenomena at the nanometer level.

[5] Development and application of AFM data analysis technologies using mathematical and computational sciences

We explored the sensitivity of characteristic electronic structure signatures to the properties of the tip and how the analysis could further be applied to organic systems. To advance the quantitative interpretation of resolution-limited AFM experiments, we have developed the BioAFMviewer software platform which integrates simulated AFM scanning and optimized fitting of atomic protein structures to experimental images.

[6] Promotion of supramolecular chemistry research and its application to life science and SPM nanometrology

We developed a new biosensor for quantitative detection of 1-MNA in crude biological samples and examined enhancement of sensitivity and selectivity of the biosensors through polymer conjugation and detection of 1-methylnicotinamide (1-MNA) by a SICM probe.

[7] Nano-dynamics research on proteins, DNA and organelles by HS-AFM

We demonstrated that HS-AFM is a suitable tool to characterize the structure and dynamics of intrinsically disordered proteins and directly to image genome editing process by CRISPR-Cas9. Furthermore, we successfully revealed the structures involved in droplet-like autophagosome precursor formation by liquid-liquid phase separation

[8] Functional imaging of living cells

We have shown that we can perform nanoscale topography and QNM using a scanning procedure with no detectable effect on living cells, allowing long-term QNM as well as detection of nanomechanical properties under drug-induced alterations of actin filaments and microtubules.

[9] New discoveries in cell biology using Bio-SPM technologies

To obtain more detailed information on the structure of extracellular vesicles (EVs), we performed 3D-AFM force mapping, which unexpectedly revealed a non-homogeneous and bumpy structure of EVs with

distinct local nano-domains that can be associated with the heterogeneous presence of signaling molecules. We found deformation and loss of the nuclear membrane pore barrier during cell death and revealed the conformational dynamics of influenza protein during docking onto the EVs by HS-AFM.

[10] Progress in cancer research and new achievements by applying SPM technology

We discovered critical functions of a microbiota-derived metabolite and a vitamin-related metabolite, for dysfunction of hematopoietic stem cells and drug resistance of malignant cells, respectively. Based on the findings, we promoted transdisciplinary projects concerned with the development of a visualization system for small chemical compounds with SPM technology, leading to deep understanding of pathophysiological roles of metabolites.

- New NanoLSI research building and deployment of Bio-SPM

The host institution, Kanazawa University (hereinafter referred to as KU), completed the new NanoLSI Research Building in September 2020. The new building has a total floor area of 6840 m^2, consisting of a basement and 4 floors above ground. Features of the facility include 65 scanning probe microscopes (SPM) (47 atomic force microscopes, AFM, and 18 scanning ion conduction microscopes, SICM) and 6 electron microscopes (one transmission electron microscope, TEM, and five scanning electron microscopes, SEM). Most of these microscopes are located in the basement.

3. Feeding Research Outcomes Back into Society

- Intellectual property utilization based on patented technology, license status

Distinctive inventions by NanoLSI researchers concern scanning probe microscopes. KU and NanoLSI have patented these inventions and possess a total of 32 patented technologies. Of these, 14 patents have been licensed to four domestic and overseas scanning probe microscope manufacturing companies.

- Outreach programs for external researchers, development of joint research

NanoLSI places emphasis on outreach programs for external researchers. The purpose is to introduce and disseminate NanoLSI's scanning probe microscope (Bio-SPM) technology to external researchers in the life sciences, which will lead to joint research. They include the Bio-AFM Summer School for young researchers for one week, the Bio-SPM collaborative research program for mid-career researchers for about two weeks, and the NanoLSI Visiting Fellows Program for senior researchers and their research groups for one month. Participants were selected on the basis of calls for applications. As a result of these programs from FY2017 to FY2020, a total of 121 researchers participated (73 domestic researchers and 48 overseas researchers from 18 countries). Since FY2017, through these outreach activities for researchers, 19 co-authored papers were published via the Bio-AFM Summer School by NanoLSI researchers and external researchers, and 7 co-authored papers, through the Bio-SPM Technology Joint Research program.

4. Generating Fused Disciplines

Our major goal is to establish the new interdisciplinary research field of Nanoprobe Life Science by combining expertise in nanometrology, life science, supramolecular chemistry, and computational science. For the fusion of supramolecular chemistry and other fields, we will design and synthesize molecular sensors and machines and integrate them into nanoprobes, enabling them to detect a specific target molecule or ion or to provide a local stimulus to a biological system. For the fusion of Bio-SPM and other fields, we will develop novel nanoprobe technologies for imaging, analyzing, and manipulating nanodynamics on the surface of and inside living cells. For the fusion of computational science and other fields, we will elucidate mechanisms of both biological phenomena and their nanoprobe measurements. For the fusion of life science and other fields, we will elucidate nano-level mechanisms of basic cellular functions and their cancer-specific abnormalities.

- NanoLSI transdisciplinary research promotion grants

In FY2018, NanoLSI established a new grant program under which sums of between ¥0.5-2 million are awarded to transdisciplinary research projects pursued by two or more young researchers in different fields. From FY2018 to FY2020, 92 applications were received, and 63 research projects were selected, to which a total of ¥72.8 million was granted. The average amount of this financial support was approximately ¥1.2 million per project. At the end of each fiscal year, the institute's PIs provide grant recipients with feedback on their projects in the form of written evaluations and advice based on reports and short presentations. This grant program was extremely effective in providing young researchers with strong encouragement to give concrete shape to their ideas for transdisciplinary research and to enable them to be implemented.

5. Realizing an International Research Environment

- Attracting top researchers from abroad

Of the 16 PIs at NanoLSI, 5 are from overseas. One of the five is a full-time researcher at NanoLSI, while the other four stay at NanoLSI for one to three months a year while being employed permanently at an overseas research institution. In order to promote joint research with these four overseas PIs, one assistant professor in NanoLSI is assigned to each of them. In addition, one research associate in charge

of joint research is assigned to each of the two laboratories designated as overseas satellites at Imperial College London, UK, and the University of British Columbia, Canada, with the financial support of NanoLSI.

- **Employment of postdoctoral researchers from abroad**

The numbers of postdoctoral researchers hired from abroad have increased from 3 in FY2017 to 24 in FY2020; the proportion of those to the sum of all postdoctoral researchers hired have thus increased from 42.9% to 80.0%.

- **International research meetings**

Since its inauguration in October 2017, NanoLSI has held 22 international research meetings: 6 NanoLSI-hosted symposia, 11 co-hosted symposia, 3 workshops, in which a total of 2,005 researchers including 1,817 domestic and 188 overseas researchers participated. Two training meetings were held, in which 24 overseas and 22 domestic young researchers participated.

6. Making Organizational Reforms

NanoLSI has implemented various system reforms based on the initiative of the Center Director and with the support of the President. The main system reforms include a research-focused system for NanoLSI full-time researchers, evaluation-dependent annual salary system, transdisciplinary research promotion grants for young researchers, junior PI program for talented young researchers, establishment of graduate school "Division of Nano Life Science," the independence of NanoLSI, and top leaders' regular meeting between KU president and the Center Director for quick top-down decision-making.

7. Future Prospects

Based on the fundamental technologies that we developed in the first half period, we aim to further develop novel live-cell nanoprobe technologies for imaging structures, dynamics, and chemical and mechanical property distributions at the surfaces and inside of living cells. With these technologies, we aim to present several impactful examples of nano life science research on the mechanisms of various cell functions and their cancer-specific abnormalities.

Specific targets include intra-cellular transport through nuclear pore complexes, cell-cell communication through extracellular vesicles, cell responses induced by anti-cancer drugs, alterations in cell mechanics induced by cancer progression, pathological roles of a cancer-specific metabolite 1-MNA, and single-cell diagnosis of drug resistant cancer cells. The life science PIs working on these subjects are well known in each field. Hence their presentations on novel Bio-SPM applications in academic meetings or publications should attract much attention. By these means, we intend to expand the Bio-SPM application area from biophysics to molecular cell biology, and medical and pharmaceutical sciences, leading to the establishment of the nanoprobe life science field.

In order to realize the prospected research plan in the second half period and after the completion of the WPI grant, NanoLSI, which is open to the outside, will acquire new external collaborators through collaborative/joint research with world-leading Bio-SPM technologies such as HS-AFM, in-liquid FM-AFM & 3D-AFM, and functional SICM. We aim to produce research outcomes at the highest international level by reinforcing collaborative relationships and to maintain our activities as a globally outstanding research center.

8. Host Institution's Concrete Plan toward Achieving the Center's Independence over the Next 5 Years (from its sixth year)

KU will continue to provide multi-layered and priority support to NanoLSI through its concrete action plan during the second half of the WPI grant period to provide budgets, personnel affairs, and infrastructure development. The support will include 400 million yen per year as personnel costs for NanoLSI researchers, research expenses of 60 million yen per year, the research focus of NanoLSI researchers, and provide internationally competitive salary levels. It also includes expanding enrolment of the graduate school "Division of Nano Life Science", priority support to the maintenance of the new NanoLSI research building, priority employment of able technicians, and administrative staff to NanoLSI. To secure long-term NanoLSI researcher positions beyond the WPI grant period, KU will set up 22 permanent NanoLSI positions.

9. Others

For the long-term survival of NanoLSI, harmonization and synergistic effects between NanoLSI's position as an independent research institute within the University and its involvement in graduate school education will revitalize the research environment by fostering future generations of NanoLSI researchers and the involvement of doctorate students in NanoLSI activities via the graduate school "Division of Nano Life Science", an educational unit paired with NanoLSI. KU and NanoLSI will provide various forms of preferential treatment for the students of the "Division of Nano Life Science" including priority provision of scholarship from KU Foundation, full or half tuition fee and/or entrance fee exemption, and provision of research assistant salary.

World Premier International Research Center Initiative (WPI)

Self-Evaluation Report for Interim Evaluation

Host Institution	Kanazawa University	Host Institution Head	Koetsu Yamazaki
Research Center	Nano Life Science Institute (NanoLSI)		
Center Director	Takeshi Fukuma	Administrative Director	Yoshihiro Fukumori

Common Instructions:
* Unless otherwise specified, prepare this report based on the current (31 March 2021) situation of your WPI center.
* As a rule, keep the length of your report within the specified number of pages. (The attached forms are not included to this page count.)
* Use yen (¥) when writing monetary amounts in the report. If an exchange rate is used to calculate the yen amount, give the rate.

1. Overall Image of Your Center (write within 2 pages including this page)

Describe the Center's current identity and overall image.
- List the Principal Investigators in Appendix 2, and enter the number of center personnel in Appendix 3-1, 3-2, diagram the center's management system in Appendix 3-3, draw a campus map in Appendix 3-4, and enter project funding in Appendix 3-5, 3-6.

The Center's Current Identity

World-Leading Unique Bio-SPM Technologies

At Nano Life Science Institute (NanoLSI), we aim to produce world-leading advanced nanoprobe technologies for visualizing unseen nanoscale biological phenomena and to elucidate their mechanisms. Even before the establishment of NanoLSI, Kanazawa University was well known for its activities on bio-scanning probe microscopy (SPM) studies.

For example, Ando et al. developed high-speed atomic force microscopy (HS-AFM) and made it possible to directly visualize nanoscale dynamic behaviors of various proteins, DNAs, and lipids. Fukuma et al. developed liquid-environment frequency modulation AFM (FM-AFM) and three-dimensional AFM (3D-AFM), making it possible to visualize subnanometer-scale biomolecular surface structures and their 3D hydration shells. In addition, Korchev, who established the fundamentals of the current bio-scanning ion conductance microscopy (SICM) techniques, joined our institute when NanoLSI was launched. Since the establishment of NanoLSI, we have been making efforts to expand the capabilities of these unique Bio-SPM technologies and also to develop novel nanoprobe technologies especially for live cell imaging.

Co-existence of these three groups, who can produce original Bio-SPM technologies, is one of the biggest features of our institute and hence NanoLSI is well recognized in the nanoscience research field.

Center of Nanoprobe Life Science Research

With our unique Bio-SPM technologies, we have so far visualized various biological structures and phenomena for the first time in the world and given major impacts in the nano life science field. Many researchers from around the world visited our institute and performed nanoprobe life science studies using these Bio-SPM techniques, with which important nanodynamics of proteins or cells are directly visualized. Such experiments have provided missing information not accessible using other bio-imaging tools such as fluorescent microscopy or cryo-electron microscopy.

At NanoLSI, there are various systems to promote such research visits, including Bio-SPM Summer School (formerly named as Bio-AFM Summer School), Bio-SPM Collaborative Research, and NanoLSI Visiting Fellows Program. Through these programs, more than 121 researchers have visited our institute and more than 26 papers have been published as an outcome of such collaborations. This is clear evidence demonstrating the high visibility of our institute and the strong need for such unique Bio-SPM technologies in the life science field. In the meantime, we also continue to explore new possibilities for Bio-SPM studies. While Bio-SPM has been widely used for biophysics studies mainly with purified or synthesized biomolecules, its applications in molecular cell biology and medical sciences are still limited.

Here at NanoLSI, we have 7 PIs from different disciplines of life sciences from basic cell biology to medical and pharmaceutical sciences. Among them, four PIs are also affiliated with the Cancer Research Institute at Kanazawa University that has more than 50 years' history and is well recognized in the cancer research field. These life scientists have been actively exploring the use Bio-SPM technologies for their research and some of the achievements have been already published. Through these activities, NanoLSI is becoming well recognized in a wider range of life science research fields.

Fusion of Four Different Research Disciplines

As we aim to use advanced Bio-SPM technologies for life science research, it is natural to combine our expertise in nanometrology and life sciences. In addition, we are exploring possibilities to combine knowledge in supramolecular chemistry and computational sciences, where we have strengths at

Kanazawa University. Co-existence of top world-level researchers from these four research disciplines is unique and the strong advantage of our institute (Fig. 1).

(Computational Science)

To establish a new measurement technology, the principle of the measurement should be supported by theory and verified by simulation. In addition, interpretation of the data obtained often requires new analysis methods. At NanoLSI, we have computational scientists who have strong experience in Bio-SPM modeling and simulation. With their expertise, we have been making efforts to establish a theoretical basis and analysis methods for the newly developed techniques. This is an important and unique role that we are playing in the nanoprobe life science community.

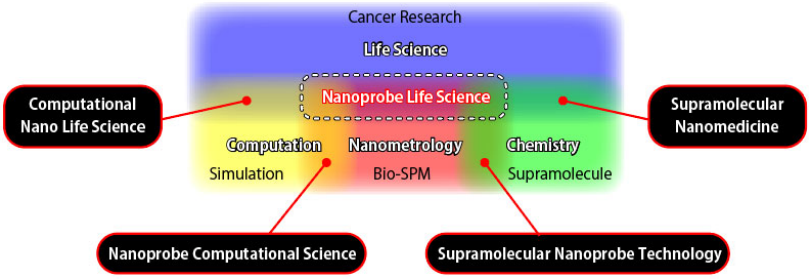

Fig. 1: Relationship between the four major research disciplines.

(Supramolecular Chemistry)

At our institute, there are four PIs from supramolecular chemistry, who can design and synthesize unique molecules and precisely control their inter-molecular interactions. We aim to use this technology to produce new molecular sensors to detect biomolecules and metabolites, and to chemically functionalize the SPM probes or substrates. Although basic knowledge of chemistry has been used for similar purposes, few advanced supramolecular chemistry principles have been applied to the development of Bio-SPM technologies. At NanoLSI, we are actively exploring such possibilities, which is one of the unique features of our institute.

Research environment

With the completion of the new NanoLSI research building and the arrangement of 65 scanning probe microscopes (SPM) and 6 electron microscopes (EM), a diverse and independent research environment has been established under one roof. For the maintenance of 65 Bio-SPMs and 6 EMs deployed in the new building, a technician specializing in AFM and a technician specializing in EM are placed in NanoLSI. Both technicians are able to assist users of the equipment in English. In order to maximize the research focus of overseas researchers, the administrative staff members assigned to the NanoLSI Administrative Office provide support in English.

Outreach programs for external researchers

Based on the scanning probe microscope (Bio-SPM) technologies for life science research that NanoLSI researchers themselves have developed, it is very important that NanoLSI promotes joint research with external researchers resulting in co-authored publications. NanoLSI will continue to develop outreach activities for external researchers as a priority, i.e., the Bio-SPM Summer School, the Bio-SPM Technology Joint Research Program, the NanoLSI Visiting Fellows Program, and to place an emphasis on joint research with external researchers.

Junior PI program

Talented excellent young researchers who are expected to form the next generation at NanoLSI are hired applying the conditions of a tenure track application with the tenured posts being secured in advance. They are treated as independent researchers in the same way as PIs with a start-up budget of 10 million yen and personnel expenses for one subordinate young researcher.

Fostering young researcher

NanoLSI provides multi-layered support for young researchers to initiate and conduct fused research and to obtain external grants. First, when a young researcher is employed at NanoLSI, they are provided with a startup budget of 1 million yen. After spending the startup budget, a basic research budget of half a million yen is provided every year. In addition to this budgetary support, Transdisciplinary Research Promotion Grants of 0.5 to 2 million yen per year are provided after joint review by PIs of young researchers' fused research proposals. In order for young researchers to obtain external research funds, a competent URA is assigned to NanoLSI who supports the preparation of application documents through close consultation.

Graduate school "Division of Nano Life Science"

For the long-term survival of NanoLSI as a remarkable organization, harmonization and synergistic effects between NanoLSI's position as an independent research institute within the University and its involvement in graduate school education will revitalize the research environment by fostering future generations of NanoLSI researchers and the involvement of doctorate students in NanoLSI activities via the Graduate School of Frontier Science Initiative, Division of Nano Life Science, an educational unit paired with NanoLSI; it is now in operation. This Graduate School attracts more excellent students from Japan and overseas than we can accept.

2. Center's Research Activities (within 8 pages)

2-1. Research results to date

Give an overall picture of the Center's research activities. Select 10 representative research results achieved during the period from 2017 through March 2021. Number them [1] to [10] and provide a description of each.

- In Appendix 1-1, list the papers underscoring each research achievement (up to 20 papers) and provide a description of each of their significance. And in Appendix 1-4 list the center's research papers published in 2020.

We have been working on the development of novel nanoprobe technologies and on nano life science research (Fig. 2). While we continued to expand the capabilities of our world-leading Bio-SPM technologies (e.g., HS-AFM, FM-AFM, 3D-AFM, and SICM), we also developed various live cell imaging techniques for visualizing structures, dynamics, and mechanical properties at the surfaces and inside living cells.

We also explored possibilities for using our expertise in supramolecular chemistry to detect biomolecules and metabolites, as well as to functionalize nanoprobes and substrates. Meanwhile, we also utilized our skills in mathematical and computational sciences (e.g., modeling, simulations, and machine learning) for verifying the principles of the newly developed techniques, interpreting the obtained Bio-SPM data, and understanding the mechanisms of life science phenomena.

With the developed technologies and other existing bio-imaging tools, we performed various nanoprobe life science studies. Through collaborations with life scientists worldwide, we continued to present many HS-AFM studies on the nanodynamics of proteins, DNAs, and organelles and SICM studies on cellular dynamics and functions. In the meantime, life scientists at NanoLSI continued to perform their internationally top level life science research on basic cellular functions (e.g., cell-cell communication, intra-cellular transport, metabolism, etc.) and cancer development and malignant progression. In addition, they actively pursued possibilities for using Bio-SPM technologies in their research, and some of the achievements are already published. Through this transdisciplinary research, we are pioneering the new research area "nanoprobe life science".

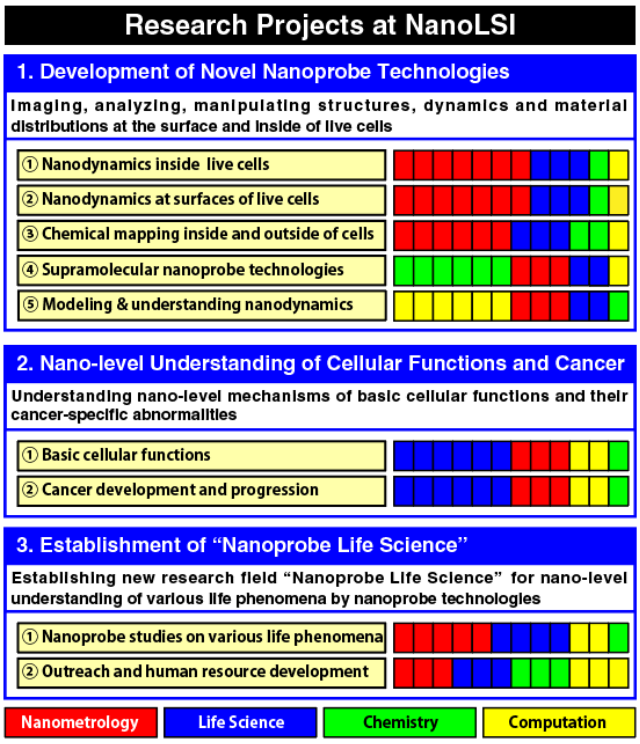

Fig. 2: Research projects at NanoLSI and contributions from the four major disciplines to each project.

A short summary of the achievements from Oct. 2017 till Mar. 2021 are listed below.

- Papers: 345 (48% internationally co-authored; 84 with an IF > 10; 109 with an IF > 7),
- Invited talks at international meetings: 199,
- Funding: 3,844 million yen overall (97 grants >¥ 10 million).

Hereafter, we summarize our research achievements in ten categories.

[1] Development of 2D- and 3D-AFM imaging inside living cells

AFM has been widely used for visualizing the nanodynamics of various biomolecules and their assemblies to understand the mechanisms of their biological functions. However, such high-resolution AFM imaging were mostly performed with biological systems either extracted from a cell or reconstructed on a solid substrate. To overcome this limitation, we have developed an intra-cellular AFM imaging technique referred to as "nanoendoscopy". Just like inserting an endoscopic camera into a human body, we insert a specially designed long nanoprobe into living cells and scan it in 2D or 3D for visualizing intra-cellular structures and dynamics (Fig. 3a).

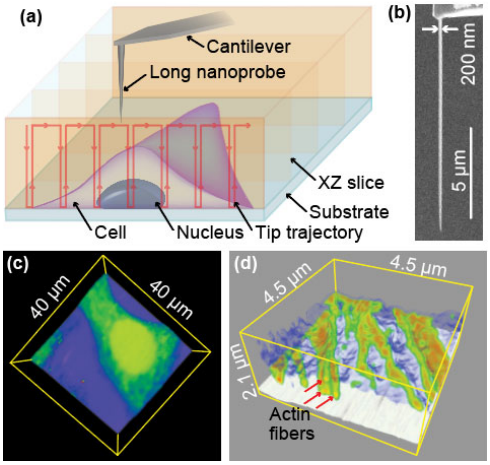

Fig. 3: (a) Principle of Nanoendoscopy. (b) Long nanoprobe. (c) Living HeLa cell. (d) Actin fibers in the live fibroblast cell.

So far, we established a method to fabricate a long nanoprobe (Fig. 3b, ***Sci. Rep. 2021***) and succeeded in imaging the whole cell structure (Fig. 3c), 3D configurations of the actin fibers (Fig. 3d), and 2D nanodynamics of the inner scaffold of the bottom plasma membrane (*Sci. Adv. 2021*) and NPCs at the nuclear surfaces. Importantly, our fluorometric assay

reveals that such imaging does not produce detectable changes in cell viability. Unlike previous AFM techniques using ultrasonic waves or elastic responses, this method allows an AFM probe to directly access the target intra-cellular components so that we can exploit the full range of AFM capabilities such as high-resolution imaging, nanomechanical mapping, and molecular recognition. These features should greatly expand the range of intra-cellular structures and properties observable in a living cell.

[2] Development and application of measuring technologies on the surface of living cells

SICM can image the surface physical properties of specimens, such as topography, surface charge density, and viscoelasticity, in ionic solutions without mechanical probe—sample contact. This unique capability is advantageous for measuring fragile cells, but its possible imaging rate and spatial resolution are far lower than those desired in biological studies.

We have continuously improved the spatiotemporal resolution of SICM. So far, our system performs the highest spatiotemporal resolution in existing SICM systems (***Rev. Sci. Instrum. 2019***). We established a method for measuring the geometry of a SICM probe tip with a sub-nm resolution (Fig. 4a, ***Anal. Chem. 2020***), allowing us to perform the qualitative mechanical mapping of living cells by SICM. These developments enabled simultaneously visualization of the dynamic change in topography and mechanical properties of living cancer cells (Fig. 4b, *Biomaterials.* 2021) and revealed the variation of mechanical properties of cancer cells depending on cancer driver genes (Fig. 4c).

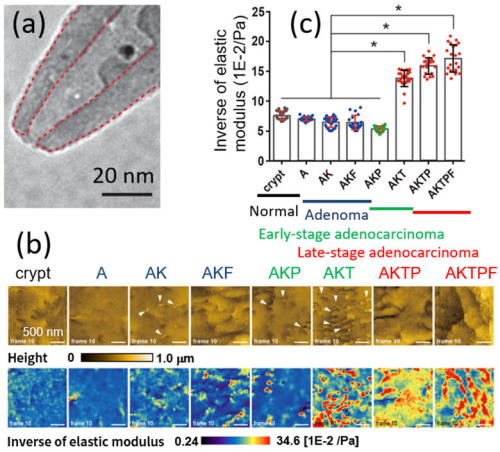

Fig. 4: (a) High-resolution SICM probe. (b) Height and stiffness maps and of cancer cells with different cancer driver genes. (c) Averaged stiffness of measured cells.

AFM enables observation of intact samples with sub-nanometer resolution, but there have been no reports of observations of living animal cell surfaces with a resolution less than hundreds of nanometers. The reason for this would be that the cell surface is very soft and easily fluctuates during AFM observation.

In this study, we developed a novel method of AFM observation using Micro Porous Silicon Nitride Membrane (MPM). As a result, protrusions of less than 10 nm in diameter were successfully identified on the living colon cancer cell surface (unpublished data). Furthermore, to recognize specific molecules in the AFM images, we superimposed the AFM images onto the images using the stimulated emission depletion (STED) microscope and confirmed that the localization of the E-cadherin molecule observed in the STED images corresponded to the protruding structures on the cell surface observed in the AFM images (unpublished data).

[3] Development of nanoprobe-biosensors for chemical mapping inside and outside of living cells

Cellular chemical heterogeneity is a hallmark of cancer. While a number of techniques have been developed and help to understand the role of the chemical heterogeneity in cancer progression and treatment, these sensors are restricted by their chip-like design exhibiting relatively large (several micrometers) electrode dimensions. These have limited their ability to perform highly localized measurements in small volumes, especially in biological samples such as the extracellular and intracellular domains of individual cells.

In the context of our nanotechnology development, we have recently designed a range of novel nanoprobe-biosensors for oxygen gradients, ROS species, ATP, and pH. Importantly these nanoprobes can be integrated with our advanced SICM, which can be applied to map intracellular and extracellular chemicals with higher spatial and temporal resolution. For example, we have recently constructed label-free zwitterionic-like cross-linked glucose oxidase and poly-l-lysine pH-sensitive nanoprobes at the tip of dual-barrel nanopipettes (Fig. 5a-b). These SICM feedback-controlled pH-nanoprobes allow SICM topography and 3D extracellular pH mapping of living breast cancer MCF7 cells with pH sensitivity better than 0.01 units, fast response times down to ~2 ms, and higher spatial resolution of ~50 nm (Fig. 5c). As demonstrated in another example, carbon-filled nanoprobes as small as 2 nm radius can be functionalized

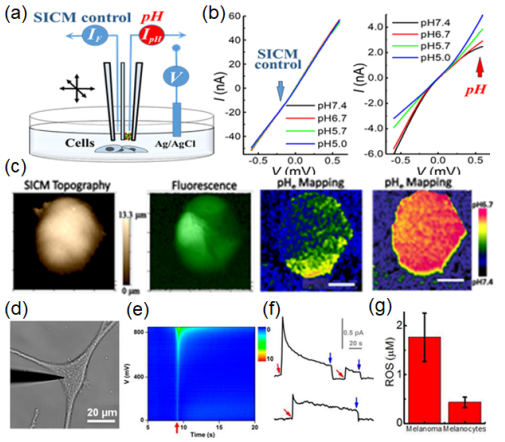

Fig. 5: (a) Schematic demonstrating simultaneously SICM imaging and extracellular pH sensing. (b) Nanoprobe shows pH sensitive in pH-barrel but not SICM-barrel. (c) SICM & fluorescence imaging and pHe mapping of $CD44^{GFP}$ MCF7 cells. (d) Optical image of ROS-nanoprobe intracellular detecting. (e) Voltammograms of ROS-nanoprobe penetrate in melanoma. (f) Current traces of ROS-nanoprobe inside (red arrows) and outside (blue arrows) of melanoma. (g) ROS levels measured with ROS-nanoprobe inside melanoma and melanocytes.

with Pt as ROS-nanoprobe biosensors, which can be applied to detect intracellular ROS of melanoma and melanocytes with high sensitivity (Fig. 5d-g). (***Nat. Commun. 2019,*** IF: 12.121)

[4] Further improvement of high-speed AFM technologies

The power of high-speed AFM (HS-AFM) has been demonstrated by an increasing number of imaging studies on biological molecules. However, the vast majority of biological processes have not been visualized by HS-AFM. In order to apply HS-AFM technologies to a wider range of biological phenomena, further improvement of HS-AFM technologies are thus necessary.

After NanoLSI was established, we improved devices in the HS-AFM (i.e., cantilever (Fig. 6a), optical beam deflection system, amplitude detector and Z-scanner) to improve the scanning performance of HS-AFM. Notably, a trace imaging mode that eliminates the backward scanning in X-axis was only recently introduced (Fig. 6b, ***Rev. Sci. Instrum. 2021***, Patent application number (PAN): 2020-199938), enhancing the speed performance of HS-AFM ~2.5 times. The combination of the developed devices enables us to perform HS-AFM imaging ~10 times faster than before.

Furthermore, we have been developing the following devices to extend the functions of HS-AFM: (1) AFM substrate with controlled concave/convex shapes (Fig. 6c, ***Front. Immunol. 2020***, Issued patent: JP677310), (2) HS-AFM with nano-manipulator enabling us to manipulate a target object during HS-AFM imaging (PAN: 2019-149584). These new HS-AFM technologies will greatly contribute to a deeper understanding of biological phenomena at the nanometer level.

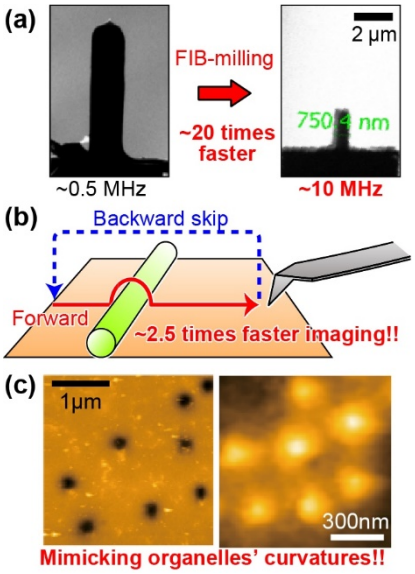

Fig. 6: (a) Ultra-small cantilever milled by FIB. (b) Only trace imaging mode. (c) Concave/convex substrate.

[5] Development and application of AFM data analysis technologies using mathematical and computational sciences

In terms of developing methods for the simulation of high-resolution SPM, we have expanded our machine learning infrastructure to predict electrostatic potentials and hydration structures, while adapting multichannel experimental data as input and taking the first steps in autonomous AI instruments operation. We have also extended our molecular modeling tools to the study of complex two-dimensional materials (***Nature 2020,*** IF = 42.779). In particular, we explored the sensitivity of characteristic electronic structure signatures to the properties of the tip and how the analysis could further be applied to organic systems (***Adv. Funct. Mater. 2021,*** IF: 16.836). To advance the quantitative interpretation of resolution-limited AFM experiments, we have developed the BioAFMviewer software platform, which integrates simulated AFM scanning and optimized fitting of atomic protein structures to experimental images (***PLoS Comput. Biol. 2020***). Mathematical modelling and computer simulations were applied to understand pioneering interactive HS-AFM experiments of ATP-less walking of the myosin V molecular motor and provide an explanation in terms of conformational dynamics and energetics. As a result, the classical model emphasizing the importance of ATP-induced chemo-mechanical motions, has to be reexamined.

Further progress was in the development of an analysis method for HS-AFM movies based on statistical mechanics. The novel analysis method was then applied to HS-AFM movies filming repeating associations and dissociations of scorpion toxin to a K^+ channel (Fig. 7) (***Sci. Adv. 2019,*** IF: 13.117). Combining the results of the analysis with the discrete state Markov model, the binding dynamics of the toxin to the channel were found to occur solely via the induced-fit pathway, and the states of the channel at each moment in the HS-AFM movies were successfully classified as a high- or low-affinity state to the toxin.

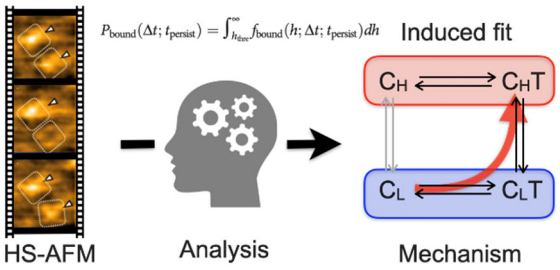

Fig. 7: HS-AFM movie and mechanism deduced from analysis based on statistical mechanics on the movie

[6] Promotion of supramolecular chemistry research and its application to life science and SPM nanometrology

We developed unique stimuli responsive helical polymers (***JACS 2018, 2019, 2020,*** IF: 14.612), a simple and versatile living polymerization system for the synthesis of telechelic helical polymers (***Angew. Chem. 2020x2,*** IF: 12.959, ***JACS 2021***) and various responsive host molecules with new switching functions (***JACS 2019***) as well as a new receptor for carboxylate anions such as lactate. We also developed unique cellulose-based nanomaterials (***Nat. Commun. 2019,*** IF: 12.121, ***Angew. Chem. 2018, 2020x2,*** IF: 12.959, ***Adv. Mat. 2019, 2020x3,*** IF: 27.398), new responsive metal complexes

(***Angew. Chem. 2020***), planar chiral supramolecular assemblies (***Nat. Commun. 2019, JACS 2018, 2019***) and chiral transfer and amplification systems based on pillar[n]arenes (***Chem. Sci. 2021, Angew. Chem. 2020x3***)

Based on our unique building blocks for producing various supramolecular assemblies and systems, we have performed interdisciplinary research with life science and SPM nanometrology scientists (Fig. 8). We developed a new biosensor for quantitative detection of 1-methylnicotinamide (1-MNA), which is produced by the cancer-associated nicotinamide N-methyltransferase (NNMT), even in crude biological samples (***Commun. Chem. 2020***), and examined enhancement of sensitivity and selectivity of the biosensors through polymer conjugation and detection of 1-MNA by a SICM probe. In addition, we have started to develop new sensor molecules that can bind O_2 and lactate or measure the temperature to probe the environment of cancer cells using the nanoprobe technology. We developed supramolecular nanoprobes by collaboration with SPM nanometrology groups. Arrangements of pentagonal and hexagonal molecules on the surface were directly observed by high-resolution AFM measurements (***Commun. Chem. 2018***). Development of supramolecular probes, which can directly monitor the host-guest interactions as 3D images by high-resolution AFM, and direct-visualization of host-guest complexation and decomplexation by HS-AFM have been investigated.

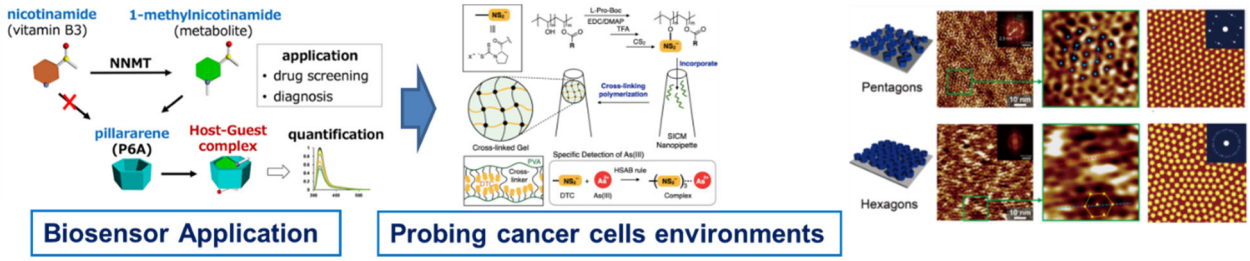

Fig. 8: Interdisciplinary research of supramolecular chemistry group with life science and SPM nanometrology groups.

[7] Nano-dynamics research on proteins, DNA and organelles by HS-AFM

Structural and dynamics analysis of IDPs by HS-AFM

Intrinsically disordered proteins (IDPs) are ubiquitous proteins that are entirely or partly disordered and play important roles in diverse biological phenomena. Their structure dynamically samples a multitude of conformational states, thus making their structural analysis very difficult with conventional methods such as X-ray crystallography, electron microscopy and NMR spectroscopy. In contrast, we demonstrated that HS-AFM is a suitable tool to characterize the structure and dynamics of IDPs (***Nat. Nanotech. 2021***, IF: 31.538). Successive HS-AFM images of an IDP molecule can not only identify constantly folded and constantly disordered regions in the molecule, but can also document disorder-to-order transitions. Moreover, the number of amino acids contained in these disordered regions can be roughly estimated, enabling a semiquantitative, realistic description of the dynamic structure of IDPs.

Real-space and real-time dynamics of CRISPR-Cas9 visualized by HS-AFM

The CRISPR-associated endonuclease Cas9 binds to a guide RNA and cleaves double-stranded DNA with a sequence complementary to the RNA guide. The Cas9–RNA system has been widely used for genome editing. Here, we used HS-AFM to visualize the real-space and real-time dynamics of CRISPR-Cas9 in action. HS-AFM movies visualized that the nuclease domain (HNH) in Cas9 fluctuates upon DNA binding, and subsequently adopts an active conformation, where the active site of HNH is docked at the cleavage site in the target DNA (Fig. 9). Our HS-AFM data provides unprecedented details about the functional dynamics of CRISPR-Cas9, and highlights the potential of HS-AFM to elucidate the action mechanisms of RNA-guided effector nucleases from distinct CRISPR-Cas systems.

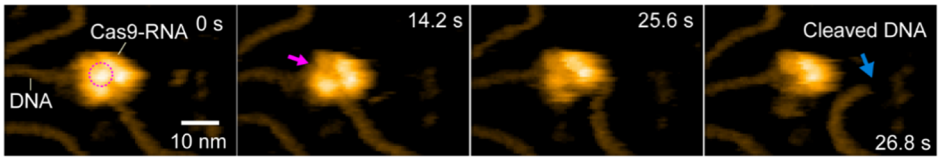

Fig. 9: Successive HS-AFM images of a Cas9–RNA–DNA complex during DNA cleavage.

Oligomeric structure of microbial rhodopsins in the membrane

Oligomeric assembly is a common feature of membrane proteins and often relevant to their physiological functions. Determining the stoichiometry and the oligomeric assembly of membrane proteins in the membrane is generally challenging because of their large size, complexity, and structural alterations under experimental conditions. Here, we directly observed the oligomeric states in the membrane of various microbial rhodopsins by using HS-AFM (***Sci. Rep. 2018, JPCB 2018, Biophys. J. 2020***). In particular, heliorhodopsin (HeR) is a distinct, abundant group of microbial rhodopsins discovered by functional metagenomics. We presented

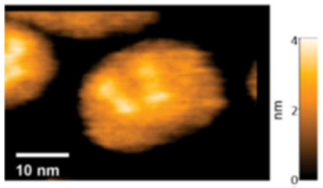

Fig. 10: HS-AFM images showing *Ta*HeR forms dimers in the lipid membrane.

the crystal structure and HS-AFM images of HeR to form a stable dimer in the membrane (Fig. 10) (***Nature 2019***, IF: 42.779).

Various applications of HS-AFM to nano-dynamics research

Our HS-AFM imaging studies on a vast array of purified protein systems successfully revealed their functional mechanisms, structural formation processes, physiological roles, or others: To name a few, droplet-like autophagosome precursor formation by liquid-liquid phase separation (Fig. 11a,b; ***Nature 2020***), interconversion between trimer and dimer complexes of the translocase of the outer mitochondrial membrane complex (***Nature* 2019**), membrane tube constriction and fission by dynamin helical polymers (***e-Life* 2018**) , mechanical role of inner lumen proteins of doublet microtubules in cilia and flagella (Fig. 11f-h; ***Nat. Commun. 2019,*** IF: 12.121), ATPase-driven conformational changes of AAA+ chaperone ClpB (***Nat. Commun. 2018***), and stabilization of cairca-24 h oscillation of clock-protein KaiC phosphorylation by a short-time scale dynamic interaction between KaiC and KacA (***Nat. Commun. 2018***).

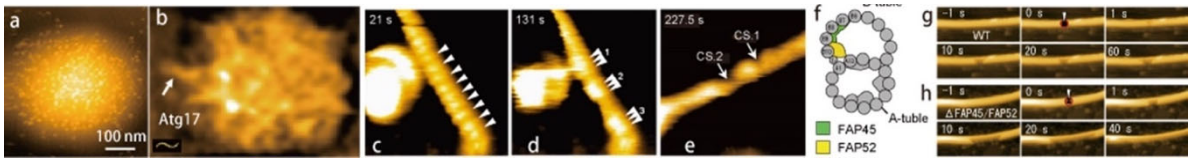

Fig. 11: (a, b) Autophagosome precursor formed by liquid-liquid phase separation, (c–e) helical rings of dynamin surrounding membrane tube (c), dynamin ring clustering upon addition of GTP (d), and appearance of membrane constriction sites at bare regions (e), (f–h) cross-section of doublet microtubule (f), and tip force-induced depolymerization of WT B-tubule (g) and FAP45/ FAP52-deleted B-tubule (h).

[8] Functional imaging of living cells

Mechanical properties of living cells determined by cytoskeletal elements play a crucial role in a wide range of biological functions. However, low-stress mapping of mechanical properties with nanoscale resolution but with a minimal effect on the fragile structure of cells remains difficult. SICM for quantitative nanomechanical mapping (QNM) is based on intrinsic force interactions between nanopipettes and samples and has been previously suggested as a promising alternative to conventional techniques. We have provided an alternative estimation of intrinsic force and stress and demonstrated the possibility to perform qualitative and quantitative analysis of cell nanomechanical properties of a variety of living cells (Fig. 12a). Force estimation on decane droplets revealed that the forces applied using a nanopipette are much smaller than when using atomic force microscopy. We have shown that we can perform nanoscale topography and QNM using a scanning procedure with no detectable effects on living cells, allowing long-term QNM as well as detection of nanomechanical properties under drug-induced alterations of actin filaments and microtubules (***Nanoscale 2021***).

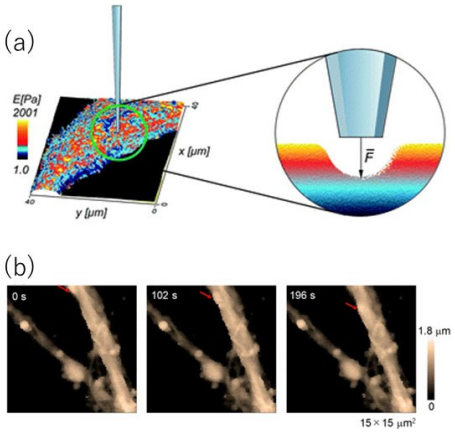

Fig. 12: (a) Schematic illustration of mechanical properties imaging of live cell using SICM. (b) Time-lapse SICM images of cargo transport (red arrows) in the dendrites of a hippocampal neuron. The scan size was 15 × 15 μm^2.

SICM is an effective tool for visualizing the nanoscale topography changes of the cell surface without labeling. Dynamic reassembly of the cell membrane, cytoskeleton, and structural changes represented by virus particle internalization and dendritic spines, cargo transport, and synapse formation. The temporal resolution of SICM is a critical issue for live-cell time-lapse imaging. We developed a new scanning method to reduce the scanning time by half. The time-lapse images provided quantitative information on the dendritic spine and synaptic bouton volume changes and formation process of the neural network that are closely related to memory. Furthermore, we directly measured HIV-like particle assembly and found that they can reach full size in 20 s and release in 0.5 to 3 min. Compared to previous estimates, this is more than 10 times faster. In our opinion this is a highly important discovery that GFP tagging affects virus particle assembly and release (***PNAS 2020,*** IF: 9.412, ***Anal. Chem. 2020***)

[9] New discoveries on cell biology using Bio-SPM technologies

At NanoLSI, various cell biologists try to unravel the basic principles of cellular functions by observing nanostructures and nanodynamics of cells with Bio-SPM.

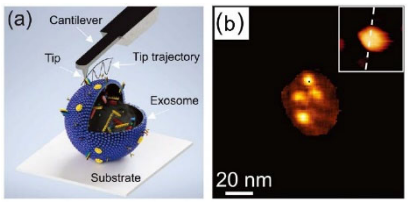

Fig. 13: (a) 3D-AFM force mapping of EV. (b) Presence of distinct local domains bulging out from EV surface.

As an immunologist, the goals are to understand how immune cells communicate with each other through cell adhesion and extracellular vesicles (EVs). We discovered a novel type of immune cell that presents tissue antigens (***J. Exp. Med. 2019,*** IF: 11.743) and are working on how antigen presentation by EVs differs from that of other cells using a novel analytical method combining super-resolution microscopy and SICM. To get more detailed information on the structure of EVs, we performed a 3D-AFM force mapping, which unexpectedly revealed a non-homogeneous and bumpy structure of EVs with distinct local nano-domains that can be associated with the heterogeneous presence of signaling molecules (Fig. 13, ***Nanoscale 2021***). Moreover, the 3D-AFM force mapping also revealed a malignancy-dependent increase in EV stiffness due to changes in the amount of elastic fiber-related proteins. This finding may imply that malignant cancer cells can become more flexible and easier to metastasize through the release of these proteins by EVs.

We have accumulated the know-how for spatiotemporal visualization of native nuclear pore complex (NPC) and chromatinization of DNA (***Biomaterials 2020,*** IF: 10.317) and enabled visualization of the structure and dynamics of the NPC at a nanometer scale. It is shown that deformation and loss of the nuclear membrane pore barrier would be one of the dying codes of cancer cells (***ACS Nano 2017,*** IF: 14.588). In addition, we have conducted joint studies to reveal the conformational dynamics of influenza protein hemagglutinin (HA) from an ellipsoid to a Y-shape during docking onto the exosomes by HS-AFM (***Nano Lett. 2020,*** IF: 11.238). We have been studying how molecular-level biochemical activities lead to macroscale tissue organization and have developed a new method to convert inert proteins into intercellular signaling proteins and program de novo tissue patterns (***Science 2018, Science 2020,*** IF: 41.846). By combining synthetic biology with Bio-SPM technology, we aim to visualize and manipulate the reconstruction of life phenomena at the nano-level to obtain a deeper understanding.

[10] Progress in cancer research and new achievements by applying SPM technology

We have established gastrointestinal cancer mouse and human organoid model systems that recapitulate cancer development and progression. Using this system, we have demonstrated a novel concept of polyclonal metastasis, in which genetically heterogeneous cell clusters develop metastatic foci (***Nat. Commun. 2021,*** IF: 12.121) (Fig. 14). Moreover, using gastric cancer organoids, we confirmed AQP5 as a novel gastric cancer stem cell marker (***Nature 2020***, IF: 42.779). These results contribute to development of a novel clinical strategy against alimentary tract cancer.

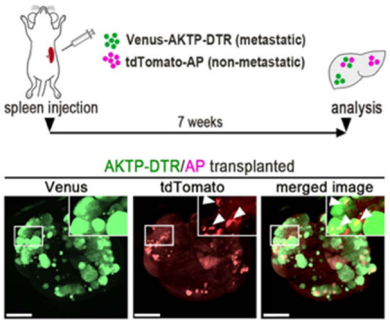

Fig. 14: Experimental proof of a novel concept "polyclonal metastasis".

We have revealed several critical cell fate determinants for normal and malignant hematopoiesis (***Cell Stem Cell 2018,*** IF: 20.860***, Nat. Immunol. 2019,*** IF: 20.479). Among them, we discovered critical functions of a microbiota-derived metabolite and a vitamin-related metabolite, for dysfunction of hematopoietic stem cells and drug resistance of malignant cells (Fig. 15), respectively. Based on these findings, we promoted transdisciplinary projects for the development of a visualization system for small chemical compounds with SPM technology (***Commun. Chem. 2020***), leading to a deep understanding of the pathophysiological roles of metabolites.

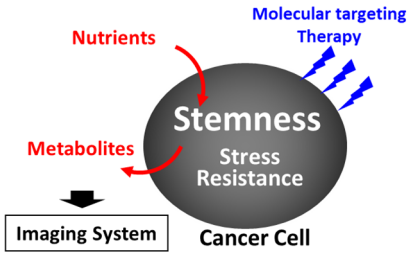

Fig. 15: Critical metabolism for drug resistance of cancer stem cells.

Using RaPID screening, we obtained macrocyclic peptides that specifically bind to HGF or MET (***Nat. Chem. Biol. 2019,*** IF: 12.587, ***Nat. Commun. 2021,*** IF: 12.121). HiP-8 (HGF-inhibitory peptide-8), a cyclic peptide consisting of 12 amino acids, binds to and inhibits HGF (Fig. 16). HiP-8 is an excellent tool for PET molecular imaging for cancer detection. High-speed AFM analysis indicated that HiP-8 restricted the highly flexible molecular dynamics of HGF into static. This study established a novel concept that a small cyclic peptide can inhibit the molecular dynamics of a target protein.

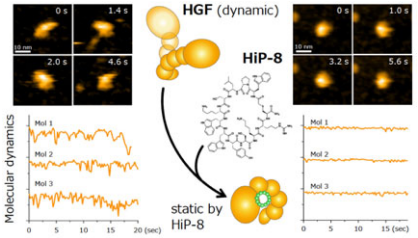

Fig. 16: HiP-8-induced inhibition of molecular dynamics of HGF.

2-2. Research environment including facilities and equipment

Describe the degree to which the Center has prepared a research environment appropriate for a world premier international research center, including facilities, equipment and support systems, and describe the functionality of that environment.

New NanoLSI Research Building, Bio-SPM, Technicians

The host institution, Kanazawa University (hereinafter referred to as KU), completed the new NanoLSI Research Building in November 2020, and NanoLSI researchers started work in the new building in December. The new building has a total floor area of 6840 m^2, consisting of a basement and 4 floors above ground. Researchers in the fields of nanometrology, life science, supramolecular chemistry, and computational science form a research core under one roof. At the same time, the 4th floor is connected to the important collaboration partners, the Cancer Research Institute and the College/Institute of Science and Technology, through an aerial corridor to facilitate collaboration between researchers. The structural features of the new building include the installation of a dry area (empty moat) that protects the entire building from external vibrations propagating on the ground, and the installation of floating floors that prevent vibrations transmitted from inside the building at key points. Due to these features, high-precision anti-vibration measures are now possible that could not be realized at existing facilities. It has been reported that these anti-vibration measures successfully reduced vibration noise and increased the resolution of observations using scanning probe microscopes (SPM) and electron microscopes (EM).

Features of the facility include 65 scanning probe microscopes (SPM) (47 atomic force microscopes, AFM, and 18 scanning ion conduction microscopes, SICM) and 6 electron microscopes (one transmission electron microscope, TEM, and five scanning electron microscopes, SEM). Most of these microscopes are located in the basement, where there is less vibration, forming the research floor that is the hallmark of NanoLSI. The electron microscopes are used for observing samples; SEMs are used for processing AFM probes, and TEM is used for measuring the diameter of nanopipettes for SICMs. In addition to SPMs and EMs, the research environment has been enhanced by an animal room, a treatment room, and three P2-level laboratories. Furthermore, a shared chemistry laboratory has been established with a total of 16 draft chambers with high-speed variable air volume control (Variable Air Volume) with excellent energy-saving performance.

In addition, in order to promote fused research in the above four fields, a shared laboratory without partitions has been established on every floor. All explanations of how to use the experimental equipment and the reservation system are in IT and English.

Regarding the technicians, one expert in AFM speaks three languages (English, Chinese and Japanese), and one expert in EM exclusively for NanoLSI is fluent in English. In addition, one technical staff member from the host institution is involved in the development of the NanoLSI research environment.

2-3. Competitive and other funding

Describe the results of the Center's researchers to date in securing competitive and other research funding.

- In Appendix 3-6, describe the transition in acquiring research project funding.

Total amount of external funds secured (by year), amount secured by PIs, amount secured by other full-time researchers

From FY2017 to FY2020, the external research funds secured by all NanoLSI researchers consisting of PIs, NanoLSI full-time researchers other than PIs, and associated researchers who work at NanoLSI and other departments of KU are shown below. In each year, the amount of external funds secured exceeded the WPI subsidies received.

	Number of NanoLSI researchers	Total amount of external funds	Allocation amount from the host institution
FY2017	50	¥886,045,607	¥573,713,736
FY2018	72	¥866,977,892	¥629,295,011
FY2019	74	¥1,044,068,024	¥701,070,766
FY2020	83	¥1,047,075,206	¥711,349,232

The amounts of external funds secured by PIs and their proportion of the total amount per year are shown below. The amount secured was maintained at a level of 600 million yen or more and a proportion of 60% or more.

	Number of PIs	Amount of external funds secured by PIs	Proportion of the total amount
FY2017	16	¥702,496,072	79%
FY2018	16	¥644,422,801	74%
FY2019	16	¥686,279,039	66%
FY2020	16	¥625,859,132	60%

The amounts of external funds secured by NanoLSI's full-time researchers other than PIs and the proportion to the total amount per year are shown below. Both the amount secured and the proportion have been increasing.

	Number of full-time researchers	Amount of external funds secured by full-time researchers	Proportion of the total amount
FY2017	9	¥110,210,661	12%
FY2018	28	¥140,890,554	16%
FY2019	33	¥217,608,827	21%
FY2020	44	¥262,520,048	25%

Acquisition of major competitive external research funds

The program titles, number of grants and amounts of major competitive external research funds acquired by PIs and other NanoLSI full-time researchers between FY2017 and FY2020 were as follows:

- KAKENHI Grant-in-Aid for Specially Promoted Research: 1 grant (1 Co-PI), 158,600,000 yen
- KAKENHI Grant-in-Aid for Scientific Research (S): 2 grants (1 PI, 1 Co-PI) 161,980,000 yen
- KAKENHI Grant-in-Aid for Scientific Research (A): 14 grants (9 PIs, 5 Co-PIs) 247,104,000 yen
- AMED Practical Research for Innovative Cancer Control: 7 grants (3 PIs, 4 Co-PIs) 370,511,730 yen
- AMED Project for Cancer Research and Therapeutic Evolution: 5 grants (3 PIs, 2 Co-PIs) 264,393,680 yen
- AMED AMED-CREST: 1 grant (1 PI) 50,221,817 yen
- JST Strategic Basic Research Programs (CREST): 5 grants (3 PIs, 2 Co-PIs) 324,624,105 yen
- JST Strategic Basic Research Programs (PRESTO SAKIGAKE): 8 grants (7 PIs, 1 feasibility study) 144,125,384 yen
- JST Development of Advanced Measurement and Analysis Systems: 1 grant (1 PI) 102,317,422 yen
- The Human Frontier Science Program: 1 grant (1 Co-PI) 12,195,000 yen (Exchange rate: ¥108.4 per US dollar)

2-4. State of joint research

Describe the results of joint research conducted with other research organizations both in and outside Japan.

Co-authored research papers that states "WPI" and NanoLSI

From 2018 to March 2021, NanoLSI researchers published a total of 282 papers co-authored with external researchers outside KU. The number of co-authored papers with researchers belonging to overseas research institutes is 161, which is 48% of the co-authored papers.

Among these co-authored papers, 6 papers were published with RIKEN (Center for Biosystems Dynamics Research) and one with the University of Tsukuba (Microbiology Research Center for Sustainability). NanoLSI has concluded cooperation agreements with these institutions. With overseas institutions with which NanoLSI has concluded cooperation agreements, 12 co-authored papers were published with Imperial College London, UK, a satellite of NanoLSI, and 20 co-authored papers with the University of British Columbia, Canada, another satellite of NanoLSI. In addition, of the domestic institutions with which NanoLSI has not concluded any agreements but with which 10 or more co-authored papers were published, 26 co-authored papers were published with Kyoto University, 22 with Japan Science and Technology Agency, 18 with Osaka University, 16 with Nagoya University and 15 with the University of Tokyo. Overseas institutions with which NanoLSI has not concluded any agreements were involved in 28 co-authored papers with Aalto University, Finland, and 5 co-authored papers with the Fritz Haber Institute of the Max Planck Society, Germany. Two of overseas PIs belong to these institutions respectively.

2-5. Appraisal by society and scientific organizations

Describe how society and/or scientific organizations in and outside Japan have recognized the Center's research achievements.

- To substantiate the above evaluation, list the main awards received and invitational/Keynote lectures given by the Center's researchers in Appendix 1-3.

NanoLSI researchers and their research output have been acknowledged in the scientific community and society both domestically and internationally. This is demonstrated by the fact that since the inauguration of NanoLSI in October 2017, NanoLSI researchers, mainly PIs, have been invited to give 199 international invited lectures and have received 37 domestic and international awards. The dissemination of research results by NanoLSI researchers through these international invited lectures and awards has led to an increase in awareness of NanoLSI.

Representative international invited lectures:

- Prof. T. Ando, Plenary talk at the 8th Multifrequency AFM Conference 2020
- Prof. M. Oshima, Lecture at Annual Meeting of the Korean Society of Cancer Prevention 2019
- Prof. T. Fukuma, Keynote speech at International scanning Probe Microscopy Conference 2018

Examples of notable awards:

- Prof. T. Ogoshi, Japan Society for the Promotion of Science Prize FY2020
- Prof. S. Yano, Commendation for Science and Technology by the Minister of MEXT FY2020
- Prof. T. Fukuma, Japan Society for the Promotion of Science Prize FY2018

3. Feeding Research Outcomes Back into Society (within 2 pages)

3-1. Applications of research results

Describe the applications created from research results, their effect in spawning innovation, intellectual properties (IPs) obtained, and joint research activities conducted with corporations, etc.

Intellectual property utilization based on patented technology, and license status

Distinctive inventions by NanoLSI researchers concern scanning probe microscopes. KU and NanoLSI have patented these inventions and possess a total of 32 patented technologies. Of these, 14 patents have been licensed to four domestic and overseas scanning probe microscope manufacturing companies, and the patented technology has thus been implemented.

Portfolio analysis and utilization strategy of patent technologies

For the further social implementation of these patented technologies, the intellectual property department of KU conducted, in collaboration with a private research company, a portfolio analysis of valid patents in Japan related to scanning probe microscopes in FY2020.

After searching for patents and applications that have been registered and published in Japan based on keywords related to scanning probe microscopes and international patent classification, patent search experts read and analyzed a total of 388.

32 out of the 388 (8%) are patents/applications by KU and NanoLSI. These 32 patents have features and relative advantages in increasing the scanning speed of scanning probe microscopes and controlling samples, solutions, and temperatures.

Furthermore, it was found that KU and NanoLSI have exclusive patents in Japan for the six patented technologies related to the scanning ion-conducting microscope (SICM).

Based on the results of the survey of the current state of technological development of the scanning probe microscope and the characteristics of the patented technology, KU and NanoLSI are planning to further develop patented technology licensing and promote social implementation that contributes to improving the performance of scanning probe microscopes.

Status of joint research with private companies

In addition to the social implementation of patented technology, NanoLSI researchers are conducting joint research with corporate researchers based on their own research results. From FY2017 to FY2020, a total of 93 joint research projects were conducted with 33 private companies, and a total of ¥154,506,260 was provided by these companies for joint research. Of these, PIs became the major driving force, by whom a total of 75 projects with 26 private companies with a total contract amount of ¥116,214,560 were carried out.

3-2. Achievements of Center's outreach activities

* Describe what was accomplished in the center's outreach activities during the period from 2017 through March 2021 and how the activities have contributed to enhancing the center's "globally visibility." In Appendix 5, describe the concrete contents of these outreach activities and media reports or coverage of the activities.

Outreach programs for external researchers, and development of joint research

NanoLSI places emphasis on outreach programs for external researchers. The purpose is to introduce and disseminate NanoLSI's scanning probe microscope (Bio-SPM) technology to external researchers in the life sciences, which will lead to joint research.

They include the Bio-SPM Summer School for young researchers for one week, the Bio-SPM Collaborative Research for mid-career researchers for about two weeks, and the NanoLSI Visiting Fellows Program for senior researchers and their research groups for one month. Participants were selected on the basis of calls for applications. As a result of these programs from FY2017 to FY2020, more than 121 researchers participated (73 domestic researchers and 48 overseas researchers from 18 countries).

In FY2020, however, the Bio-SPM Summer School and NanoLSI Visiting Fellows Program were canceled due to COVID-19. The Bio-SPM Collaborative Research was held for domestic researchers in the form of accepting external researchers or conducting remote joint research, while taking into account the infection status of COVID-19.

Since FY2017, through these outreach activities for researchers, 19 co-authored papers were published via the Bio-SPM Summer School by NanoLSI researchers and external researchers, and 7 co-authored papers, through the Bio-SPM Collaborative Research.

A program to introduce and disseminate Bio-SPM technology developed by NanoLSI to external life science researchers is a strategic approach of importance for the development of joint research between NanoLSI researchers and external scientists leading to outstanding results. NanoLSI will continue to develop outreach activities for researchers as a priority, i.e. the Bio-SPM Summer School, the Bio-SPM Collaborative Research, the NanoLSI Visiting Fellows Program.

In addition, in anticipation of independence after the completion of the WPI project, we will approach industry to promote joint research with companies, especially with those companies already collaborating with NanoLSI. More specifically, we will disseminate research results on a human resources exchange platform in which those companies participate.

International research meetings

International research meetings such as the NanoLSI Symposium held each year have been held 20 times, either sponsored by NanoLSI only or co-sponsored by NanoLSI and other research institute(s). These international meetings were attended by a total of 2,005 researchers including 1,817 domestic researchers and 188 overseas participants from FY2017 to FY2020. Due to the influence of COVID-19, most of the international research meetings in FY2020 were held online. Of the four NanoLSI Symposia, that in FY2018 was held in London, England in collaboration with the overseas satellite, Imperial College London. In FY2019, the NanoLSI Symposium was held in Vancouver, Canada in collaboration with the University of British Columbia, another overseas satellite.

Press releases of research results

Press releases on research results were issued 62 times, 26 in English only and 28 in both Japanese and English. The English releases used three distribution services: PR Newswire (CISION), EulekAlert! (AAAS) and Asia Research News. With these distribution services, the English releases were posted on an average of 17 news sites per release in FY2019 and FY2020. The average value of the Altmetric Score (an index showing the degree to which the relevant paper is attracting attention on the Web) related to these distributions is 152.5. It is thus evident that the outreach activities were effective. Furthermore, a Nature Spotlight on Kanazawa featuring NanoLSI was published in Nature Vol.562, No.7726, 11 October 2018.

Outreach for the general public

Regarding outreach for the general public, a total of 25 reports on NanoLSI research appeared in newspapers and television from FY2017 to FY2020. The NanoLSI website was created with content introducing the research and resulting publications. The section "Education" presents the outline, faculty, and financial support for the Graduate School of Frontier Science Initiative, Division of Nano Life Science to help attract excellent graduate students from inside and outside Japan. "Diversity in NanoLSI" presents the research activities of female researchers. Further, we used Facebook, Twitter, and YouTube to take advantage of the immediacy and ease of information dissemination to both the research community and the general public.

In addition, we created "World of Nanoscale; Do Life Scientists See Dancing Protein in Their Dreams?" (Japanese content) in collaboration with WPI headquarters and a publisher, introducing life science research using Bio-SPM involving NanoLSI researchers. Twenty brochures and pamphlets were published such as "Overview of NanoLSI," "Transdisciplinary Research at NanoLSI," "Open Facility Programs at NanoLSI" and "Recruiting Flyer for the Graduate School Course of NanoLSI." In FY2020, the online approach was strengthened since opportunities for direct contact with the target audience were very limited. Specifically, we produced videos that introduced the newly built NanoLSI Research Building.

NanoLSI attaches great importance to outreach for the general public, especially from the perspective of fostering future generations of researchers. In the second half of the WPI grant period, when the foundation of the research activities of NanoLSI has been laid and the establishment of the Graduate School of Frontier Science Initiative, Division of Nano Life Science is completed to foster future generations of NanoLSI researchers, we plan to strengthen our efforts to attract excellent students in Japan and overseas. At the same time, we will try to raise funds by crowdfunding for activities aimed at excellent high school students.

4. Generating Fused Disciplines (within 3 pages)

4-1. State of strategic (or "top-down") undertakings toward creating new interdisciplinary domains

Describe the content of "top-down" measures taken by the Center to advance research by fusing disciplines. For example, measures that facilitate doing joint research by researchers in differing fields.

Our major goal is to establish the new interdisciplinary research field of Nanoprobe Life Science by combining expertise in nanometrology, life sciences, supramolecular chemistry, and computational sciences. As for fusion of Bio-SPM and other fields, we will develop novel nanoprobe technologies for imaging, analyzing, and manipulating nanodynamics on the surface of and inside living cells. As for fusion of supramolecular chemistry and other fields, we will design and synthesize molecular sensors and machines and integrate them into nanoprobes, enabling them to detect a specific target molecule or ion or to provide a local stimulus to a biological system. As for fusion of computational sciences and other fields, we will elucidate mechanisms of both biological phenomena and their nanoprobe measurements. As for fusion of life sciences and other fields, we will elucidate nano-level mechanisms of basic cellular functions and their cancer-specific abnormalities. In order to advance research by fusing disciplines, the following top-down measurements have been taken.

Employment of 6 Jr. PIs

Based on the idea of the Center Director, 6 active young researchers, Jr. PIs have been employed. Regarding the securing of positions for 6 tenure positions have been secured at the President's discretion. Jr. PIs are key players to promote fused research and are treated as independent researchers in the same way as PIs with a start-up budget of 10 million yen and personnel expenses for one subordinate young researcher. These 6 Jr. PIs are promoting fused research into the design and synthesis of chemical sensors, the modeling and simulation of cellular systems, AFM imaging of various living cells, AFM hard/software and FPGA development, the design and synthesis of cellular systems, and the dissection of nuclear events through deep sequencing.

Employment of young researchers for the director's discretionary posts

At the discretion of the Center Director, a budget has been set aside for hiring young researchers; in addition to 6 Jr. PIs, 6 young researchers have been hired performing studies related to fused research. These 6 young scientists are carrying out research on combining AFM with optical microscopy, analyzing dynamics in movies of biomolecules taken by HS-AFM and computational simulations, combining high-speed AFM with optical tweezers to explore biodynamics induced by an external force, development of HS-AFM imaging using tips functionalized with synthetic ligands for molecular recognition and cell manipulation, combining SICM with intracellular sampling, and characterization of physical properties of living cells including morphologies and nanomechanical properties.

NanoLSI T (Transdisciplinary) -meeting

This program was launched at the initiative of the Center Director at the start of FY2018 as a weekly, voluntary forum for researchers to engage in free discussion over tea. It attracted many participants and contributed to the advancement of transdisciplinary research, but with a view to promoting disciplinary fusion more systematically, the approach was altered from the sixth week onwards. First, scheduling was changed to enable individual meetings of two research groups from different fields to be held periodically. Secondly, the format was altered so that a number of young researchers from each discipline could give short presentations. Since FY2018, 62 T-meetings has been held. This format provided an extremely worthwhile opportunity for young researchers and PIs to gain direct knowledge of each other's research. Encounters and intensive discussion at T-meetings have led to joint research projects executed between PIs or between young researchers in the different fields.

NanoLSI Colloquium

Beginning shortly after the WPI was launched in FY2017, four study meetings were held last year, in order for PIs to learn about each other's research and gain basic knowledge in each of the institute's major fields. These were extremely useful in the overall planning of interdisciplinary research proposals. In FY2018, the study meetings were developed into a colloquium where research groups inform one another of the progress of their projects. The colloquia are now held periodically and attended by all members of the institute, including young researchers. They involve PIs reporting on their groups' progress and, where necessary, young researchers reporting on progress in relation to specific topics. In FY2020, this social gathering was converted to a virtual chat online due to COVID-19.

Advisory Board Meetings

We have appointed 12 researchers from the four major research disciplines as advisory board members. The first meeting was held in Kanazawa on 20-21 February 2019, and we asked all the advisors to come and familiarize themselves with our strategy and current status of research and management. The Advisory Board mostly encouraged us to follow the current direction. Throughout FY2019, several prominent scientists were invited to a one-day seminar to discuss the promotion of interdisciplinary research involving supramolecular chemistry, life sciences, and nanometrology, which had been

particularly remarked upon in the FY2019 site visit report. Afterwards, an intensive discussion was held between the Advisor, Prof. Suga (Univ. Tokyo), and researchers from NanoLSI to develop a goal-oriented strategy for the fusion between Bio-SPM and supramolecular chemistry, which was very fruitful for considering the policy direction of NanoLSI.

Luncheon meeting and webinar

We started a luncheon meeting in FY2019. At a luncheon meeting, young researchers introduce themselves and their research for about 30 minutes, followed by a free exchange. Since FY2019, a total of 31 luncheon meetings have been held about once every two weeks have been held. This deepened their understanding of each other's backgrounds and should greatly contribute to matching collaboration among young researchers in the different fields. The luncheon meeting has been converted to a luncheon webinar due to COVID-19 since FT2020.

4-2. State of "bottom-up" undertakings from the center's researchers toward creating new interdisciplinary domains

Describe the content of "bottom-up" measures taken by the Center to advance research by fusing disciplines. For example, measures that facilitate doing joint research by researchers in differing fields.

The following financial support programs have been set up in response to the proposals for fused research by young researchers stimulated by the top-down measures described in 4.1.

NanoLSI Transdisciplinary Research Promotion Grants

In FY2018, NanoLSI established a new grant program under which sums of between ¥0.5-2 million are awarded to transdisciplinary research projects pursued by two or more young researchers in different fields. From FY2018 to FY2020, 92 applications were received, and 63 research projects selected, to which a total of ¥72,760,000 was granted. The average amount of this financial support was approximately ¥1,150,000 per project. At the end of each fiscal year, the institute's PIs provide grant recipients with feedback on their projects in the form of written evaluations and advice based on reports and short presentations. This grant program was extremely effective in providing young researchers with strong encouragement to give concrete shape to their ideas for transdisciplinary research and to enable them to be implemented.

4-3. Results of research in fused research fields

Describe the Center's record and results by interdisciplinary research activities yielded by the measures described in 4-1 and 4-2.

- In Appendix 1-2, list up to 20 of the Center's main papers on interdisciplinary research that substantiate the above record of results, and describe their content.

We have continued to establish a new research field "nanoprobe life science" by integrating knowledge from the four research fields: nanometrology, supramolecular chemistry, computational sciences, and life sciences. In FY2017, we started to work on various transdisciplinary subjects combining measures as described in 4-1 and 4-2. Measures described in 4-1 and 4-2 have produced results of interdisciplinary research in FY 2019 and FY 2020. Specific examples are given below.

NanoLSI T (Transdisciplinary)-meeting contributed to the advancement of joint research between research groups from different fields. This measure led to several publications in FY 2019 and FY2020 including papers 7 and 10 in Appendix 1-2. In paper 10, young researchers from the Fukuma and Hanayama groups are listed as the 1st and 2nd authors, indicating that T-meeting research promotes joint research between young researchers in different fields. The NanoLSI colloquium is a good place for NanoLSI members to get to know the research work of a particular group. The Hirao group presented a talk about 1-methylnicotinamide (1-MNA) which is a metabolite with a low-molecular weight. Ogoshi wondered if pillararene may bind to 1-MNA and they started collaborative research. This led to results combining Supramolecular Chemistry and Life Science (Paper 2 in Appendix 1-2). The NanoLSI Transdisciplinary Research Promotion Grant was very effective in promoting interdisciplinary research among young researchers from different fields. Research results have been produced since FY2019 (Papers 2 and 9 in Appendix 1-2).

Five excellent research results are described below and the NanoLSI members are shown underlined and in italic.

Nanometrology x Life Science

Molecular dynamics suppressed by novel macrocyclic peptide (*Nat. Chem. Biol.*, *2019*, IF: 12.587)

K. Sakai, T. Passioura, *H. Sato*, K. Ito, H. Furuhashi, M. Umitsu, J. Takagi, Y. Kato, H. Mukai, S. Warashina, M. Zouda, Y. Watanabe, *S. Yano*, *M. Shibata*, H. Suga and *K. Matsumoto*

Through a cross-disciplinary approach, they found that HiP-8 (HGF-inhibitory peptide-8), a macrocyclic peptide consisting of 12 amino acids, specifically binds to HGF. Biochemical analysis indicated that HiP-8 binds to HGF through multivalent binding interfaces. High-speed AFM analysis indicated that HiP-8 restricted the dynamic domain movement of HGF into static closed conformations. This study established the novel concept that a small macrocyclic peptide can inhibit the molecular dynamics of a target protein.

Supramolecular Chemistry x Life Science

Pillar [6] arene acts as a biosensor for the quantitative detection of a vitamin metabolite in crude biological samples (*Commun. Chem., 2020*, IF: 4.253)

M. Ueno, T. Tomita, H. Arakawa, *T. Kakuta*, T. Yamagishi, J. Terakawa, T. Daikoku, S. Horike, *S. Si*, K. Kurayoshi, C. Ito, A. Kasahara, *Y. Tadokoro*, *M. Kobayashi*, T. Fukuwatari, I. Tamai, *A. Hirao* and *T. Ogoshi*

This study was supported by a NanoLSI transdisciplinary research promotion grant. Metabolic syndrome is associated with obesity, hypertension, and dyslipidemia, and increased cardiovascular risk. Therefore, quick and accurate measurements of specific metabolites are critical for diagnosis. Through a cross-disciplinary approach, the authors found that water-soluble pillar [5] arene (P5A) forms host–guest complexes with both 1-MNA and nicotinamide, and water-soluble pillar [6] arene (P6A) selectively binds to 1-MNA at the micromolar level. P6A can be used as a "turn-off sensor" by photo-induced electron transfer (detection limit is 4.38×10^{-6} M). Their findings demonstrate that P6A can be used as a biosensor to quantify 1-MNA in crude biological samples.

Nanometrology x Life Science

High-Speed AFM Reveals Molecular Dynamics of Human Influenza A Hemagglutinin and Its Interaction with Exosomes (*Nano lett., 2020*, IF: 11.238)

K. Lim, *N. Kodera*, H. Wang, M. S. Mohamed, *M. Hazawa*, A. Kobayashi, *T. Yoshida*, *R. Hanayama*, *S. Yano*, *T. Ando*, and *R. W. Wong*

Influenza A hemagglutinin (HA) is one of the crucial virulence factors that mediate host tropism and viral infectivity. Presently, the mechanism of the fusogenic transition of HA remains elusive. Through a cross-disciplinary approach, they used high-speed atomic force microscopy (HS-AFM) to decipher the molecular dynamics of HA and its interaction with exosomes and revealed that the native conformation of HA in the neutral buffer is ellipsoidal, and that HA undergoes a conformational change in an acidic buffer. Real-time visualization was obtained of the fusogenic transition by HS-AFM. These results suggest that the mechanism possibly fits to the "uncaging" model, and the HA intermediate appears to be Y-shaped.

Nanometrology x Life Science

High-Speed SICM for the Visualization of Nanoscale Dynamic Structural Changes in Hippocampal Neurons (*Anal. Chem., 2020*, IF: 6.785)

Y. Takahashi, *Y. Zhou*, T. Miyamoto, H. Higashi, N. Nakamichi, Y. Takeda, Y. Kato, *Y. Korchev*, and *T. Fukuma*

This study was a collaboration with Imperial College London. Dynamic reassembly of the cytoskeleton and structural changes represented by dendritic spines, cargo transport, and synapse formation are closely related to memory. However, the visualization of the nanoscale topography is challenging because of the diffraction limit of optical microscopy. The temporal resolution of Scanning ion conductance microscopy (SICM) is a critical issue for live-cell time-lapse imaging. Through a cross-disciplinary approach, the authors have developed automation region of interest (AR)-mode SICM and a fast *Z*-axis scanner for improving the temporal resolution of topography imaging. The newly developed algorithm requires half of the scanning time and allows for the visualization of dynamic cargo transport, changes in the volume of dendritic spines and synaptic boutons, and the growth cones of hippocampal neurons without labeling. Furthermore, translocation of plasmalemmal precursor vesicles (ppvs), for which fluorescent labeling has not been established, were also visualized along with the rearrangement of the cytoskeleton at the growth cone.

Nanometrology x Computational Science

Computed Atomic Force Microscopy Images of Chromosomes by Calculating Forces with Oscillating Probes (*J. Phys. Chem. C, 2020*, IF: 5.344)

T. Sumikama, *A. S. Foster*, and *T. Fukuma*

This study was a collaboration with Aalto University, Finland. Experimentally obtained AFM images have been compared with the simulated ones; however, such conventional images of biomolecules have usually been computed by calculating the equidistance surface from given atomic positions, not by calculating force. Here, we use a polymer model of a chromosome, as a representative biomolecule, and the AFM probe, and computed isoforce surfaces upon the fiber. The oscillation of probes utilized in the dynamic mode of AFM measurements was also implemented in the simulation. The computed isoforce images were clearer than the conventional equidistance ones, and very similar images to those from isoforce measurements were obtained when the diameter of the probe was reduced to approximately 30% in the equidistance images. Thus, the probe was found to approach very close to samples beyond the estimation of the equidistance surface, contributing clear AFM images.

5. Realizing an International Research Environment (within 4 pages)

5-1. International Circulation of Best Brains

5-1-1. Center's record of attracting and retaining top-world researchers from abroad

Describe the participation of top-world researchers as PIs and their stays as joint researchers at the Center.

・ In Appendix 3-2, give the number of overseas researchers among all the Center's researchers, and the yearly transition in their numbers. In Appendix 4-2 give the achievements of overseas researchers staying at the center to substantiate this fact.

Overseas PIs and their achievement

Of the 16 PIs at NanoLSI, 5 are from overseas. One of the five is a full-time researcher at NanoLSI, while the other four stay at NanoLSI for one to three months a year while being employed permanently at an overseas research institution. In order to promote joint research with these four overseas PIs, one assistant professor in NanoLSI is assigned to each of them. In addition, one research associate in charge of joint research is assigned to each of the two laboratories designated as overseas satellites at Imperial College London, UK and University of British Columbia, Canada with the financial support of NanoLSI.

Since the inauguration of NanoLSI in FY2017, Prof. A. Foster, Aalto Univ., Finland has produced eight co-authored papers with other NanoLSI researchers. Prof. Y. Korchev, Imperial College London, UK has produced six co-authored papers, and Prof. A. Mikhailov, Fritz Haber Institute of the Max Planck Society, Germany has produced one co-authored paper. Prof. M. MacLachlan, Univ. Of British Columbia, Canada has submitted one co-authored paper and is preparing two more co-authored papers; these will be published in FY2021.

These four overseas PIs have obtained substantial external research funds such as the Commission of the European Communities, NanoCellSense grant on a nanotechnology-based approach for label-free single-cell analysis of cytoplasmic proteome, the NSERC Discovery grant on new directions in supramolecular macrocycle and coordination chemistry, and the Magnus Erhnrooth Foundation on Graph Neural Network (GNN) molecular prediction in their own counties. Also in Japan, Prof. Y. Korchev has acquired KAKENHI Basic Research (B) and Prof. A. Mikhailov, Basic Research (C).

As for the contribution of overseas PIs to the fostering of young researchers, Prof. A. Foster and Prof. A. Mikhailov gave intensive lectures at the Graduate School of Natural Science and Technology, KU. Once restrictions due to COVID-19 are eliminated and overseas PIs are again able to visit Japan, they plan to give intensive lectures at the graduate school of NanoLSI, the Graduate School of Frontier Science Initiative, Division of Nano Life Science.

In FY2018, Prof. Y. Korchev, Head of the NanoLSI overseas satellite at Imperial College London, acted as an organizer and held the 2nd NanoLSI International Symposium in London, which was attended by 37 foreign researchers including 5 invited speakers and 16 Japanese researchers. In FY2019, Prof. M. MacLachlan, who is also the Head of NanoLSI overseas satellite at University of British Columbia, Canada acted as an organizer and held the 3rd NanoLSI International Symposium in Vancouver, which was attended by 55 foreign researchers including 7 invited speakers and 17 Japanese researchers. In addition, in response to the demand for research dissemination using podcasts and providing audiovisual data files on the Internet worldwide, NanoLSI made a program in which four overseas PIs presented their research.

In this way, the four overseas PIs contribute to the research and educational activities carried out by NanoLSI and play an important role in disseminating the research results worldwide.

NanoLSI visiting fellows

Prof. Anthony Watts, Department of Biochemistry, University of Oxford, who is the Managing Director of the European Biophysics Journal, was invited in FY2018 by the NanoLSI Visiting Fellows Program, which invites eminent researchers from overseas and their research groups. In FY2019, Dr. Lorena Redondo Morata, Researcher, Institut National de la Santé et la Recherche Médicale (INSERM), France was invited. In FY2020, it was planned to invite Prof. Zoya Leonenko, Department of Biology, Waterloo Institute for Nanotechnology, University of Waterloo, Canada but the Visiting Fellows Program was canceled due to COVID-19.

5-1-2. Employment of young researchers at the Center and their job placement after leaving the Center

Describe the Center's employment of young researchers, including postdoctoral researchers, and the positions they acquire after leaving the Center.

・ Enter the following to substantiate the facts provided above:

- In Appendix 4-3, describe the Center's state of international recruitment of postdoctoral researchers, the applications received, and selections made.
- In Appendix 3-2, give the percentage of postdoctoral researchers employed from abroad
- In Appendix 4-4, describe the positions that postdoctoral researchers acquire upon leaving the Center.

When recruiting postdoctoral researchers internationally, the host institution, KU, has a policy of hiring them as specially appointed assistant professors whose three-year term can be renewed. In accordance with this policy, NanoLSI conducts recruitment of postdoctoral researchers through international announcements, and almost all overseas postdoctoral researchers are hired as specially appointed assistant professors. From FY2017 to FY2020, international recruitment of postdoctoral researchers was

conducted for 34 postdoctoral researcher positions. A total of 256 applications were received, of which 192 were applications from overseas. The success rate of applicants for these 34 positions was about 13%. Of these, 27 were hired from overseas, resulting in 80% from international recruitment.

The numbers of postdoctoral researchers hired and the proportion of those from overseas per year, both the number and the proportion having been increasing, are shown below:

	Postdoctoral researchers employed	Overseas postdoctoral researchers	Proportion overseas/total
FY2017	7	3	42.9%
FY2018	28	17	60.7%
FY2019	23	17	73.9%
FY2020	30	24	80.0%

Of the 27 overseas postdoctoral researchers employed by NanoLSI, 5 have already obtained their next research positions and are advancing their academic careers. Their next positions after WPI Center, i.e., NanoLSI are as follows:

- Assistant Professor, Kyoto University, Japan;
- Lecturer, China Pharmaceutical University, China;
- Scientist, Swiss Federal Institute of Technology in Lausanne, Switzerland;
- Postdoctoral Researcher, Rennes Institute of Chemical Science, University of Rennes 1, France; and
- Technical Engineer, Shenzhen HUASUAN Tech. Co., Ltd, China.

5-1-3. Overseas satellites and other cooperative organizations

・ In Appendix 4-1, describe the state of cooperation with overseas satellites and other cooperative organizations. In Appendix 4-5, describe the state of the Center's agreements concluded with these organizations.

Researcher Exchange between NanoLSI and overseas satellites

Following the adoption of NanoLSI as a WPI Center in FY2017, KU and NanoLSI concluded a cooperation agreement with Imperial College London (Imperial), UK and another with The University of British Columbia (UBC), Canada on long-term collaborative research projects concerning WPI Center activities. Five researchers, including 2 PIs, visited the Imperial satellite for one day in conjunction with the NanoLSI Symposium held in London in FY2018. One overseas PI from the Imperial satellite visited NanoLSI for 33 days. In FY2019, one overseas PI from the satellite visited NanoLSI for 32 days, and one other researcher visited for 12 days. In FY2020, one researcher of the Imperial satellite was transferred to NanoLSI in order to strengthen joint research. There were no other researcher exchanges due to COVID-19.

Two doctoral and one postdoctoral researcher visited the UBC satellite for 103 days, 99days, and 34 days respectively in FY2018. One overseas PI from the satellite visited NanoLSI for 38 days. In FY2019, 13 researchers, including 4 PIs, visited the satellite in conjunction with the NanoLSI Symposium held at UBC. In FY2020, there were no researcher exchanges due to COVID-19.

State of agreements between NanoLSI and overseas satellites

The following stipulations are included in the cooperation agreement with Imperial: (1) the PI who is responsible for the research project at Imperial College London, (2) the research conducted by the PI concerned, (3) the financial support provided by KU and NanoLSI, and (4) general rules concerning intellectual property rights arising from the results generated from the research, such as joint patents between the two parties, mutual approval of the use of patents, and profit sharing. Regarding financial support, the salary of the PI subject to KU payment as a joint appointment, the salary of one subordinate researcher under the PI, and the basic cost to be paid to the postdoctoral researcher are stipulated.

The cooperation agreement with UBC is almost the same, but the method of paying salaries to the PI by KU is different. There is a request from UBC related to the joint-appointment system with Japanese research institutes to avoid affecting the tax status of UBC regarding tax incentives from the State of British Columbia. KU accepts this and pays the salary to the PI according to a separate contract between KU and the PI, while the cooperation agreement stipulates that this separate contract is regarded as an integral part of the cooperation agreement.

5-2. Center's record of holding international symposia, workshops, research meetings, training meetings and others

・ In Appendix 4-6, describe the main international research meetings held by the Center.
・ In Appendix 4-6, describe the main international research meetings held by the Center.

Since its inauguration in October 2017, NanoLSI has held 22 international research meetings: 6 NanoLSI-hosted symposia, 11 co-hosted symposia, 3 workshops, and 2 training meetings.

The major international research meetings hosted by NanoLSI were as follows:

- "International Symposium on Atomic Force Microscopy at Solid-Liquid Interfaces" in Kanazawa, Japan on 8-9 November 2017: 11 overseas and 65 domestic participants;

- "The 2nd NanoLSI International Symposium - Towards Establishment of New Research Field: Nanoprobe Life Science - in London, UK on 19 November 2018: 37 overseas and 16 domestic participants;
- "The 3rd NanoLSI International Symposium - Supramolecular Chemistry and Nanoprobes in Life Sciences – in Vancouver, Canada: 55 overseas and 17 domestic participants;
- "MLM2020 "The 1st International Conference on Big Data and Machine Learning in Microscopy" in Kanazawa, Japan on 15-17 January 2020: 25 overseas and 15 domestic participants; and
- "The 4th NanoLSI Symposium - Bio-imaging, Sensing and Manipulation for Medical Science – by online; 21 overseas and 253 domestic participants.

In addition, the training meetings hosted by NanoLSI were as follows:

- "The 7th Bio-AFM Summer School (current name Bio-SPM Summer School)" at NanoLSI on 27 August – 1 September 2018: 12 overseas from 10 countries and 10 domestic participants; and
- "The 8th Bio-AFM Summer School" at NanoLSI on 19-24 August 2019: 12 overseas from 11 countries and 12 domestic participants.

5-3. System for supporting the research activities of overseas researchers

Describe the Center's preparations to provide an environment conducive for overseas researchers to concentrate on their work, including for example living support in various languages or living support for their families.

Support for overseas researchers has been a special focus of NanoLSI since its inauguration in FY2017. The following support has been provided:

One of the features of NanoLSI in terms of equipment is the deployment of 65 Bio-SPMs and 6 EMs. These instruments are not only used by overseas researchers belonging to NanoLSI, but also by many overseas researchers who visit NanoLSI through the various programs described in 5.2 above for shared equipment. In order to support the maintenance of these instruments and to assist overseas researchers who use them under this open facility system, one technician who specializes in AFM and speaks three languages, English, Chinese, and Japanese, and one technician who is fluent in English and specializes in EM are deployed exclusively for NanoLSI.

Regarding research, one URA is assigned, whose main responsibility is to support the acquisition of external research funds by young overseas researchers belonging to NanoLSI. With support by URA such as individual consultation and elaboration/confirmation of the application form of overseas researchers, 13 out of 17 young overseas researchers who have belonged to NanoLSI for more than 6 months have their own KAKENHI as of April 2021. For planning and operating outreach projects for researchers such as the Bio-SPM Summer School, another URA is assigned to be in charge of prior contact with participating overseas researchers and care after visiting NanoLSI.

In order to maximize the research focus of overseas researchers belonging to NanoLSI, the administrative staff members assigned to the NanoLSI Administrative Office provide support in English. The kind of support is wide-ranging, including explanation of the terms of the employment contract, personnel rules and the Japanese tax system, and accompanying the researchers during resident registration, driver's license acquisition or renewal, accommodation search and interpreting during signing of the contract with a real estate agent, interpreting at the time of private car purchase, interpreting when renting a parking lot, interpreting when opening a bank account, applying for a credit card, etc. In addition, support is provided for the families of overseas researchers, including accompanying and interpreting during hospital visits, for finding a nursery school, employment support for spouse, and introduction to community activity circles. In order to spread the experience and know-how regarding support for overseas researchers throughout KU during the personnel rotation of administrative staff, support is provided by KU's own staff rather than by outsourcing to external companies.

5-4. Others

Describe the Center's policy for sending Japanese researchers overseas to gain international experience, and give examples of how the Center is working to create career paths for its researchers within a global environment of researcher mobility.

NanoLSI receives research funding of 60 million yen each year from the host institution KU, of which 2 million yen is for each PI per year and 500,000 yen each for other NanoLSI full-time researchers to promote international research. A total of 23 NanoLSI researchers and doctorate students were supported in FY2018; 33 NanoLSI researchers and doctorate students visited foreign countries in FY2019 for presentations at international conferences and for joint research at overseas institutions. FY2020 results will be limited to one international conference due to COVID-19.

In addition, NanoLSI, in collaboration with the overseas satellite at Imperial College London, held the regular NanoLSI International Symposium in FY2018 in London, England, in which 15 Japanese researchers participated. Similarly, in FY2019, the NanoLSI International Symposium was held in Vancouver in collaboration with the satellite at the University of British Columbia, in which 16 Japanese researchers participated.

When hiring talented young researchers as Junior PIs, who are treated as independent researchers like PIs, experience in research at overseas institutes is taken into account; in fact, 3 out of 6 Junior PIs

employed have such experience. Two were hired directly from overseas research institutes by NanoLSI, which provides a better research environment. One of the three has been hired by NanoLSI from a domestic research institute having previously worked at an overseas institute.

Since the inauguration of NanoLSI in FY2017, three of the young Japanese researchers hired as postdoctoral researchers or specially appointed assistant professors have obtained the following academic positions:

- Research Associate, Weil Cornell Medicine, USA;
- Assistant Professor, Kanazawa University, Japan; and
- Assistant Professor, Kanazawa University, Japan.

6. Making Organizational Reforms (within 3 pages)

6-1. Decision–making system in the center

Describe the strong leadership that the director is giving on the Center's operation and its effect, and the division of roles and authority between the Center and its host institution.
- In Appendix 3-3, draw a concrete diagram of the Center's management system.

During the launch period of NanoLSI, which was inaugurated in October 2017, a Steering Board was established consisting of the Center Director and four PIs, who acted as coordinators of each research field. Meetings were held every other week to share the management policy of the Center Director with the Steering Board. The management direction of the Center was thus unified and important matters were decided by consultation.

After that, the Steering Board was suspended from the latter half of FY2018 when the management policy of the Center Director was well understood and management became stable.

Now, the Administrative Director and administrative staff report the progress of major projects and activity plans to the Center Director almost every week, and the Center is operated by transmitting the instructions of the Center Director to each of the following working groups: Alliance Formation, Open Facilities, Transdisciplinary Research Promotion, Research Outreach, Researcher Development, and New Research Building.

To ensure that all members of NanoLSI are well informed about the Center's policies, a Faculty Board consisting of PIs and professors is held every month and minutes in English and meeting materials are shared with all staff.

From FY2021, the Steering Board has been replaced with the Future Planning Board consisting of the Center Director, the Administrative Director and three PIs, where the progress of NanoLSI research is regularly assessed, medium- to long-term issues are discussed and policies are confirmed.

At the beginning of FY2020, NanoLSI was officially positioned in the statutes of KU as an independent institute of KU. With this revision of the statutes, the personnel rights, budget execution rights, and representation rights of the Center Director of NanoLSI have been established both in real and institutional terms.

Throughout the above process, both the research units and the administrative office of NanoLSI share the management policy of the Center Director, and every member participates in the operation of the Center in a unified manner.

Regarding cooperation and the sharing of roles between the host institution and NanoLSI, a regular meeting with the President, Vice President in charge of general affairs, finance, and facilities, the Center Director, and the Administrative Director is held every month. Here, reports on the activities of NanoLSI are presented, intentions are unified on how to deal with important matters in the operation of the Center, and the sharing of roles between KU and NanoLSI in dealing with these issues is decided.

6-2. Arrangement of administrative support staff and effectiveness of support system

Describe the assignment of the Center's administrative support staff who have English language and other specialized skills, effort made in establishing the support system, and the system's effectiveness.

KU has positioned the success of NanoLSI as one of the most important aspects of university management and has prioritized the placement of competent administrative staff in NanoLSI. Eighteen administrative staff are assigned to the NanoLSI Administrative Office, 14 of whom can perform their duties in English.

In addition to English skills, administrative staff with unique abilities resulting from their work experience and career paths have been appointed. Examples are given below:

- A member with experience of working in international scientific affairs at MEXT and as an administrative manager at a national research institute is currently in charge of external negotiations and management of NanoLSI;
- A member with experience as a freelance writer and of planning and setting up events is currently in charge of the planning and management of projects such as public relations and symposia;
- A member with extensive administrative experience at KU in project promotion and management is currently responsible for budget accounting of NanoLSI and improvement of the research environment including facilities and equipment;
- A member with experience of university management as a secretary to the President is currently in charge of the planning and coordination of general matters at NanoLSI;
- A member with experience at an overseas company and working in the international exchange department of KU is currently responsible for personnel and labor management of NanoLSI overseas researchers.

As shown by those examples, NanoLSI has administrative staff who have the ability not only to engage in routine office work but also to actively participate in NanoLSI's administrative management. They contribute to strengthening NanoLSI and the maximization of the research focus of the Center's scientists.

6-3. System reforms advanced by WPI program and their ripple effects

Concisely itemize the system reforms made to the Center's research operation and administrative organization, and describe their background and results. Describe the ripple effects that these reforms have on the host institution. (If any describe the ripple effects on other institutions.)

NanoLSI has implemented various system reforms based on the initiative of the Center Director and the support of the President. The main examples of system reform are as follows:

Top Leaders' Meeting

A regular meeting consisting of the President, Vice President in charge of general affairs, finance, and facilities, the Center Director, and the Director of the Administrative Department of NanoLSI is held every month to realize quick top-down decision-making and the initiation and execution of business. —introduced in FY2017

Research-Focused System for NanoLSI full-time researchers

KU's unique research focus system, the Research Professorship, was revised to exempt full-time NanoLSI researchers from non-NanoLSI duties. —introduced in FY2017

Evaluation-dependent annual salary system

The Center Director evaluates the annual research progress reports submitted by all NanoLSI researchers, and the evaluation score is multiplied by the NanoLSI researcher allowance (professor: 170,000 yen per month, associate professor: 150,000 yen per month, assistant professor: 110,000 yen per month) to determine the annual salary for the following year. The evaluation results for FY2020 were SS (score 2.1-3.0) for 1 researcher, S (score 1.1-2.0) for 22 researchers, and A for 44 researchers. —introduced in FY2018

Transdisciplinary Research Promotion Grants

As part of the start-up support for young researchers, annual financial support of 0.5-2 million yen is provided for a fused research project in which young researchers of NanoLSI and/or students of the Graduate School of Frontier Science Initiative, Division of Nano Life Science from two or more fields out of nanometrology, life science, supramolecular chemistry, and computational science participate. —introduced in FY2018

Junior PI Program

Six excellent young researchers who are expected to form the next generation at NanoLSI are hired applying the conditions of a tenure track application with the tenured posts being secured in advance. They are treated as independent researchers in the same way as PIs with a start-up budget of 10 million yen and personnel expenses for one subordinate young researcher. —introduced in FY2018

Graduate school "Division of Nano Life Science"

For fostering future generation researchers of NanoLSI, the Graduate School of Frontier Science Initiative, Division of Nano Life Science was established in FY2020 as an education unit paired with NanoLSI; it is now in operation. This Graduate School attracts more excellent students from Japan and overseas than we can accept.

The implementation of the above reforms of NanoLSI had the following ripple effects on the host institution, KU:

Ripple effect 1:

Following the implementation and success of the evaluation-dependent annual salary system by NanoLSI, KU revised their system to apply the personnel salary system, which combines performance evaluation and the annual salary structure, to all faculty members (researchers) hired after FY2019.

Ripple effect 2:

The establishment and early success of NanoLSI in FY2017 helped KU establish the Nanomaterials Research Institute in FY2018 and the Advanced Manufacturing Technology Institute in FY2019. It also helped the reorganization of the existing Cancer Research Institute, leading to the development of a group of institutes featuring fused (transdisciplinary) research, and contributed to the reorganization of KU, which aims at cross-disciplinary education and fused research.

6-4. Support by Host Institution

The following two items concern the support that the host institution provides the Center. Describe the measures that the host institution has taken to sustain and advance the Center's project. That include the item of support that it committed to at the time of the initial project proposal submittal.

6-4-1. Record of host institution support and its effects

・ In Appendix 6-1, describe the concrete measures being taken by the host institution.

In order to support the start and development of NanoLSI from FY2017 to FY2020, the host institution, KU, provided labor costs of 400 million yen per year, research expenses of 60 million per year, research space of 3000 m^2 for researchers belonging to NanoLSI, and essential support such as space charge exemption. Also, the research-focused promotion system of KU's original Research Professorship was primarily applied to NanoLSI full-time researchers. At the same time, office staff with both high English capability and administrative ability were prioritized for NanoLSI.

With the strong leadership of the President, KU negotiated with MEXT to secure a budget of 1.5 billion

yen for the construction of a new NanoLSI research building and KU's own funds of 650 million yen were allotted to give a total budget of 2.15 billion yen. Construction of the NanoLSI Research Building (research space, 6840 m^2) was thus completed in FY2020.

In order to secure excellent young researchers who are expected to form the next generation, NanoLSI has earmarked 6 tenure posts at the President's discretion for hiring these young researchers as tenure-track Jr. PIs. In order to support the leadership of the Center Director, a meeting between the President and the Center Director was held every month, which helped to make quick decisions and implement plans for NanoLSI operations. In order to establish the personnel rights and budget execution rights of the Center Director, the statutes of KU were revised for NanoLSI to be an independent institute.

With the above support of KU, NanoLSI has established the foundation for a world-class research institute.

6-4-2. Position of the Center within the host institution's mid-term plan

- To Appendix 6-2, excerpt the places, in the host institution's "Mid-term objectives" and/or "Mid-term plan" that clearly show the positioning of the WPI center within its organization.

KU has stated in the Third term (FY2016-FY2021) Mid-term objectives that KU will promote advanced and original research and enhance diverse basic research as well as strengthening the research implementation mechanism to establish a research institution with the highest international credentials.

Further, the Mid-term plans stipulate that it will promote nanotechnology research using innovative AFM technology. In addition, the Mid-term plans stipulate that with the aim of obtaining a fundamental understanding of the mechanisms of various life phenomena at the nano level, the "Nano Life Science Institute", adopted by the World Premier International Research Center Initiative (WPI) will be established, and a system will be created and operated to fully demonstrate the independent management of the Institute.

In this way, KU has clearly committed to full support for the establishment and operation of NanoLSI in its Mid-term objectives and plans.

6-5. Others

6-5-1. System for fostering young researcher (e.g. start-up funding)

NanoLSI provides multi-layered support for young researchers to initiate and conduct fused research and to obtain external grants.

First, when a young researcher is employed at NanoLSI, they are provided with a startup budget of 1 million yen. After spending the startup budget, a basic research budget of half a million yen is provided every year. In addition to this budgetary support, Transdisciplinary Research Promotion Grants of 0.5 to 2 million yen per year are provided after joint review by PIs of young researchers' fused research proposals.

In order for young researchers to obtain external research funds, a URA is assigned to NanoLSI who supports the preparation of application documents through close consultation. For the basic research budget of half a million yen per year, the research funds of 60 million yen provided from KU to NanoLSI every year are used as financial resources.

Furthermore, a Junior PI Program peculiar to NanoLSI was established to support particularly talented young researchers who are expected to form the next generation of NanoLSI. This program provides a world-class research environment, such as treatment as an independent researcher in the same manner.

6-5-2. Participation of female researchers

- On the transition in the number of female researchers, enter the figures in Appendices 3-1 and 3-2.

In FY2017 when NanoLSI started the WPI project, there were 6 female researchers out of a total of 49, and with the progress of the WPI project, the number of female researchers increased to 11 out of 83 NanoLSI researchers in FY2020. However, the proportion of female researchers remains around 13%. Since both the FY2020 Site Visit Report and the WPI Follow Up Report recommend improving the participation of female scientists, NanoLSI will implement a gradual correction of gender balance. The specific measures take into account the progress of research each year in line with the strategic plan and roadmaps in the second half of the WPI grant period, where the researchers needed will be appointed from among female scientists. Through these measures, the number of female researchers will be gradually increased to 13 for FY2021, 15 for FY2022, and 17 for FY2023. At the end of FY2023, the female researcher ratio should be over 20%.

In response to the above recommendation, negotiations have already been completed with two female researchers who will participate in NanoLSI from FY2021. In addition, in order to gain the participation of a new female PI, a working group consisting of the Administrative Director and three PIs has been established. This working group will try to recruit a new female PI on campus or from off-campus through joint appointment contracts.

7. Future Prospects (within 2 pages)

7-1. Policy and plan for achieving the Center's research objectives in the future

[Policy] Our policy remains the same as that for the first five years. We will continue to produce novel nanoprobe technologies for visualizing the nanodynamics of various biological phenomena. With these unique Bio-SPM technologies and other life science research tools, we investigate nanoscale mechanisms of protein and cell functions, and diseases such as cancer.

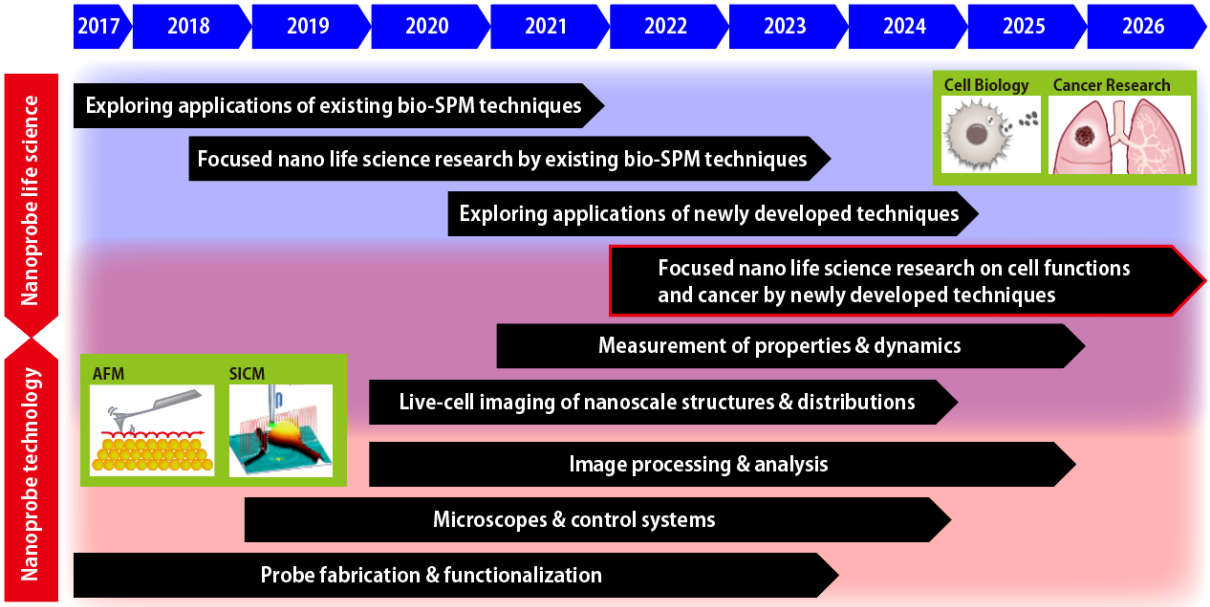

Fig. 17: Research plan for the development of novel nanoprobe technologies and nanoprobe life science

[Plan] In the first half period, we developed fundamental methods for probe fabrication and functionalization, prototypes of microscopes and control systems, and basic image processing and analysis methods. With the developed methods and systems, we started to visualize nanoscale cellular structures, dynamics and properties. In the meantime, cell biologists and cancer researchers actively explored various applications of the existing Bio-SPM technologies in their individual research disciplines and some of them led to a more focused study on a specific biological phenomenon (see Sec. 2-1 for more details). In parallel, they also explored possible applications of newly developed live-cell Bio-SPM technologies and are now moving towards more focused research projects. In the second half period, while we will continue all of these efforts, we aim to present new findings on the nanoscale mechanisms of cell functions and cancer using the developed live-cell nanoprobe technologies. These achievements should have major impacts not only on biophysics but also on cell biology and medical science, leading to significant development of the nanoprobe life science research field.

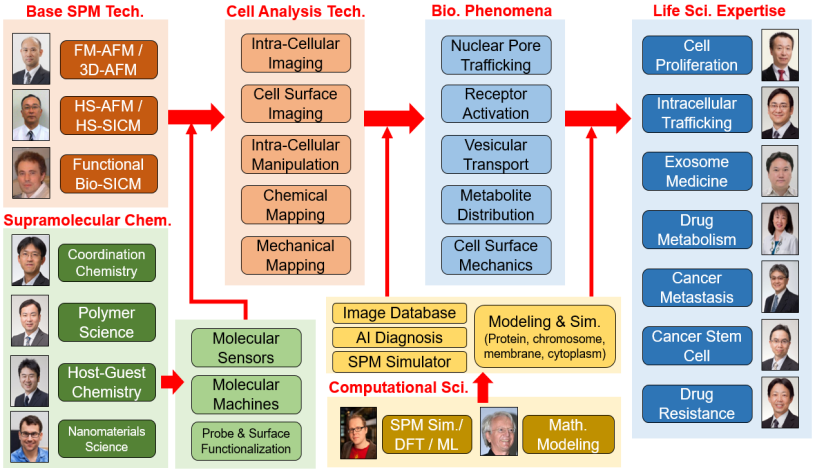

Fig. 18: Strategy for the development of new nanoprobe technologies and their applications to life science research.

[Focused Subjects] At NanoLSI, we do not aim to work on a single life science problem. Instead, individual life scientists having different expertise will work on different life science issues related to cell functions and cancer development and progression. We believe this is the best way to expand the application area of the Bio-SPM technologies and to establish the nanoprobe life science research field. Although we cannot describe all research subjects here, some of the focused applications of newly developed nanoprobe technologies led by the life science PIs are listed below.

(1) We will use the newly built super high resolution of 3D-AFM (nano-endoscopy) and upgraded HS-AFM to directly visualize NPC periphery. Membrane-less NPCs are assembled via liquid-liquid phase separation (LLPS) and are actively involved in bidirectional nucleocytoplasmic transport,

chromatinization, nucleophagy and cell division. Moreover, NPC is often hijacked by various viruses to accomplish their replications as well as to suppress host immune response. Thus, visualization of these scenarios represents an inspiring and challenging development over the next 5 years since many of these vital processes occur only fleeting through feeble intermolecular interactions with NPC.

(2) We will analyze extracellular vesicles (EVs) before secretion in the donor cells and after uptake in the recipient cells, locally at the single particle level using nanopipettes to track molecular and characteristic changes through collaboration with SPM groups. In addition, we will try to understand the mechanisms of immune response via EVs at the nanoscale by immobilizing EVs derived from immune cells or designer EVs expressing immune molecules on nanopipettes to act locally on cells and investigate their responses.

(3) We will develop an aptamer molecule which specifically inhibits RNA editing, because RNA editing is known to be upregulated in various cancer cells and be involved in the progression of cancer. We evaluate the effects of the aptamer on cancer cell viability and invasion ability as well as cell morphology by using SICM.

(4) We will characterize nano-level mechanical properties of cancer cells to understand the mechanisms for genetic alteration-induced malignancy. Established organoid cells have been examined by HS-SICM to identify specific nano-scale surface structures as well as physical properties like stiffness. Preliminary results suggest distinct properties of metastatic cells.

(5) We will develop a novel imaging system for 1-MNA by SICM with a new pillararene sensor developed at NanoLSI, for mapping the extra- or intra-cellular distribution of the metabolite at the nano-scale, leading to a deep understanding of the pathological roles of cancer-specific metabolites.

(6) We will establish cell classification methods using machine learning, which enable the diagnosis of drug resistant tumor cells at a single cell level by AFM. We measured the surface profile, elastic modules, and adsorption of living tumor cells by AFM, and found that tumor cells with an epithelial-to-mesenchymal transition associated with drug resistance had a smoother surface compared with drug sensitive tumor cells.

(7) We will directly observe and verify the dynamic mechanism of membrane MET receptor activation/clustering by HGF and engineered artificial ligands on lipid bilayer and living cells, using high-resolution/speed AFM and Bio-SPM with a peptide-modified cantilever. We will verify MET clustering in MET-amplified cancer cells, and thereby elucidate abnormal activation and drug resistance.

7-2. Center's plan to maintain its posture as a globally visible institute after WPI funding ends

In order to realize the research plan described in 7-1 after the completion of the WPI program and maintain our activities as a globally outstanding research center, we propose the following measures concerning NanoLSI after the WPI program ends, including promotion of close cooperation with the host institution, KU, and collaboration with research institutes inside and outside KU. Specifically, we propose the basic policies of 1) members, 2) human resource development, and 3) international research environment.

Members: 1) PIs will be continuously employed and the level of research corresponding to a WPI Center will be maintained. Personnel expenses will be permanently secured by the KU's own funds. 2) After the completion of the WPI program, 70 or more researchers will be secured to maintain the level and scale of research activities. 3) An overseas researcher ratio of 30% or more will be maintained. 4) As research support staff, 2 URAs, 2 technicians and a sufficient number of administrative staff who can carry out their work in English will be deployed. 5) The research professor system will be applied to all the faculty members (excluding the participating members), and the annual salary system that can be supplemented according to the amount of external funding obtained will be applied to maintain an internationally competitive salary level.

Human Resource Development: 1) The tenure-track system will be maintained for Jr. PIs in order to foster next generation PIs. 2) Excellent doctorate students of the Graduate School "Division of Nano Life Science" will be supported in terms of their career development to become internationally top-level researchers. 3) Administrative staff will be fostered in NanoLSI as a place for OJT for obtaining a better command of English, which will lead to the internationalization of the entire University.

International Research Environment: 1) Cooperative agreements will be concluded with top research institutes both domestically and overseas. 2) A support system will be established for acquisition of international research grants. 3) The existing programs, i.e. "the Bio-SPM Summer School," "the Bio-SPM Collaborative Research," and "the NanoLSI Visiting Fellows Program," will be reinforced in order to acquire new external collaborators through collaborative/joint research with world-leading Bio-SPM technologies such as HS-AFM, in-liquid FM-AFM & 3D-AFM, and functional SICM, aiming to produce research outcomes at the highest international level as a globally outstanding research center.

8. Host Institution's Concrete Action Plan toward Making its center an autonomous research institute in the second half of the grant period (from the 6th year of the center's operation)

Describe the Host Institution's plan for realizing a research system including the allocation of resources (e.g. personnel, infrastructure) that will sustain the Center as a "top world-level research institute" after its WPI funding period ends. To enable this, describe the assets that the Host Institution will provide the Center (e.g. expected acquisition of external funding, allocation of personnel, provision of budgets). Describe actions that the Host Institution has taken toward achieving the Center's independence up to the point of this midterm evaluation.

Kanazawa University's medium- to long-term plan and positioning of NanoLSI

The host institution, KU states in its Third Mid-term objectives/Mid-term plans (FY2016-FY2021) that KU further promotes strong research such as nanotechnology using innovative atomic force microscopy technology, innovative material development using supramolecular technology and research on cancer metastasis and drug resistance mechanisms, in a systematic and intensive manner through the on-campus COE system. At the same time, KU states that the research implementation mechanism will be strengthened with the aim of becoming a research center at the highest international level for interdisciplinary, i.e., fused research.

Based on the Mid-term objectives/Mid-term plans, KU reorganized its existing institute, the Cancer Research Institute, and subsequently established NanoLSI in FY2017, the Nanomaterials Research Institute in FY2018 and the Advanced Manufacturing Technology Institute in FY2019. Thus a group of multi-disciplinary, fused research institutes has been established. In FY2020, KU contributed 650 million yen from its own funds and, together with the budget from MEXT, the new NanoLSI Research Building was completed. While KU aims to provide a research base at the highest international level in each of these four research institutes in their own multi-disciplinary, i.e., fused academic field, NanoLSI is particularly positioned as a pioneering, path-breaking institute. It is clearly stated in the Mid-term plans that "a system will be constructed and operated in which the autonomous operation of the Institute (NanoLSI) will be fully demonstrated."

In order to fulfill the above Mid-term objectives and plans, and to move to the next set of Mid-term objectives and plans to reinforce KU as research base at the highest international level, KU will continue to provide multi-layered and priority support to NanoLSI through its concrete action plan during the second half of the WPI grant period as described below:

Provision of Budgets

With the following budget and financial support, KU as the host institution will provide NanoLSI with more funds than the WPI program subsidy.

KU will contribute 400 million yen per year as personnel costs for researchers belonging to NanoLSI.

KU will continue to support research expenses of 60 million yen per year through the on-campus COE system of KU as in the first half of the grant period.

Ten million yen per year will be provided for the maintenance of the new NanoLSI Research Building.

For students belonging to the Graduate School of Frontier Science Initiative, Division of Nano Life Science, the educational unit that fosters future generations of NanoLSI researchers, ¥50,000 per month for a master's student and ¥100,000 per month for a doctorate student will be provided as a scholarship from KU's own funds. In addition, scholarships for the WISE Program for Nano-Precision Medicine, Science, and Technology, which was applied for by KU and funded by MEXT, will give priority to students in the Graduate School of Frontier Science Initiative, Division of Nano Life Science. Similarly, a scholarship aimed at improving the financial condition of doctorate students, the Fellowship for Fostering Top Scientists in Fused Disciplines, which MEXT will provide to KU, will give priority to students in the Graduate School of Frontier Science Initiative, Division of Nano Life Science.

Regarding the amount of external funding for NanoLSI, after the research staffing was almost completed, over one billion yen was secured both in FY2019 and FY2020. In addition to 16 PIs, 6 young researchers have been employed as Jr. PIs who will lead the next generation, and an excellent research team is in place. It is expected that external funds of around 1 billion yen can be secured each year also in the near future.

Personnel Affairs

By preferentially applying the personnel measures described below to NanoLSI, KU will maintain the research focus of NanoLSI researchers and provide internationally competitive salary levels. In addition, KU will secure NanoLSI researcher positions for a long period beyond that of the WPI grant.

KU's Research Professor system will be continuingly applied to full-time researchers belonging to NanoLSI and exempting them from non-NanoLSI duties, KU will secure the research focus of NanoLSI full-time researchers.

KU will maintain an internationally competitive salary level by applying a salary structure according to the performance of individual researchers, i.e., combining rigorous performance evaluation and evaluation-dependent salaries with NanoLSI's unique research allowance.

Regarding the enrollment capacity of students (currently 6 students per year) of the Graduate School of Frontier Science Initiative, Division of Nano Life Science, enrollment will be increased from 6 to 10 in both the master's and doctorate programs by consolidating the overall graduate school enrollment capacity of the KU.

To secure long-term NanoLSI researcher positions beyond the WPI grant period, KU will apply the following personnel measures to NanoLSI:

The President's discretionary positions (8 positions) currently assigned to NanoLSI will be secured as permanent and sovereign positions of NanoLSI. Currently, the positions (14 positions) originating from other Institutes or Colleges, and assigned to NanoLSI, will be secured as permanent positions of NanoLSI by which researchers belong to NanoLSI while involved in graduate school education in the originating research institute/college. For young researchers, a considerable number of positions will be secured by using NanoLSI's abundant external funding.

Infrastructure development

By maintaining and developing the research infrastructure, KU will secure a world-class research environment for NanoLSI.

NanoLSI is already officially positioned in the statutes of KU as an independent institute of KU. At the same time, the independence of NanoLSI will be confirmed by defining the independent operational structure of NanoLSI in the next Mid-term objectives/Mid-term plans, and positioned as a permanent organization backed by KU's medium- to long-term strategy.

A monthly leaders' meeting, which is a top-down decision-making body, attended by the President and Vice President in charge of general affairs, finance, and facilities (KU side) as well as the Center Director and the Administrative Director (NanoLSI side) was held during the first half of the WPI grant period. This will continue during the second half of the WPI grant. These meetings ensure strong and flexible decision-making by the Center Director and close cooperation between KU headquarters and NanoLSI.

The Facilities Department of KU will give priority to supporting the maintenance of the new NanoLSI research building. For the maintenance of 65 Bio-SPMs and 6 EMs deployed in the new building, a technician specializing in AFM and a technician specializing in EM will continue to be placed in NanoLSI. Both technicians are able to assist users of the equipment in English.

As support for young and overseas researchers, a URA who supports acquisition of research funds by overseas researchers, and a URA who plans and operates outreach projects for researchers, such as the Bio-SPM Summer School, will continue to be assigned.

The administrative system will be maintained in which all NanoLSI operations, such as research work, daily life, administrative procedures, and various communications from the office, are carried out in English. Instead of fixing the administrative staff assigned to NanoLSI, turnover will be assured by personnel transfer, positioning the NanoLSI Administrative Office as a place for OJT to carry out duties in an English environment.

9. Others (within 1 page)

For the long-term survival of NanoLSI as a remarkable organization, harmonization and synergistic effects between NanoLSI's position as an independent research institute within the University and its involvement in graduate school education will revitalize the research environment by fostering future generations of NanoLSI researchers and the involvement of doctorate students in NanoLSI activities via the Graduate School of Frontier Science Initiative, Division of Nano Life Science, an educational unit paired with NanoLSI. Here we provide an overview of this graduate school, its features, and the preferential treatment provided for this graduate school from these.

Brief Overview

The graduate school, "Division of Nano Life Science," fosters excellent graduate students who will establish the new research field, "Nano Probe Life Science", on the basis of world level research conducted by NanoLSI. The supervisors for this graduate school are all world-class researchers who belong to NanoLSI. Features of the Division are as follows:

- Graduate students can carry out their own research independently in the excellent research environment with the latest laboratory equipment in NanoLSI;
- Graduate students are expected to be self-disciplined, and to take part in various research activities in NanoLSI, including attending international symposia, conferences, seminars;
- Graduate students are encouraged to engage in interdisciplinary research such as nanometrology, life sciences, supramolecular chemistry and computational sciences under supervisors in the various fields;
- Graduate students are offered sufficient financial support.

Current Situation

The Division of Nano Life Science started in FY2020 with 12 master course students (7 domestic and 5 foreign students from 3 countries) and 10 doctorate students (2 domestic and 8 foreign students from 4 countries), followed by 9 master course students (6 domestic and 3 foreign students from 3 countries) and 7 doctorate students (4 domestic and 3 foreign students from 2 countries) entering in April FY2021.

To promote interdisciplinary research in the Division of Nano Life Science, all 27 full-time researchers of NanoLSI who have an educational assignment are engaged as educators in the Division of Nano Life Science. In addition, overseas PIs give intensive courses in their own research fields during their stays at NanoLSI. Two mentors selected from different research fields supervise each student as dual mentors. Interdisciplinary research training courses are to be established along with the NanoLSI research activities such as the Bio-SPM Summer School and the Transdisciplinary research promotion grants. Graduate students in the Division, including master course students, participate in the research of NanoLSI PIs as research assistants.

In addition, in order to ensure the operational autonomy of the Division, (1) a NanoLSI PI is assigned to the Head of Division of Nano Life Science, (2) the NanoLSI administrative director supports the Head and acts as manager of the Division, and (3) the NanoLSI administrative office is in charge of managing this Division together with the Student Affairs Department of the University headquarters.

Financial support

A master's student of the Graduate School of Frontier Science Initiative, Division of Nano Life Science is provided with 130,000 yen per month (breakdown: scholarship ¥50,000 + RA salary ¥80,000). A doctorate student receives ¥180,000 per month (breakdown: scholarship ¥100,000 + RA salary ¥80,000).

Students of the Graduate School of Frontier Science Initiative, Division of Nano Life Science who are selected for the WISE Program for Nano-Precision Medicine, Science, and Technology, (a graduate school education program of KU that aims to develop fused research beyond the boundaries of each graduate school discipline and to foster human resources in research), receive a scholarship of the same amount as above; admission fee and tuition fee exemptions are offered.

Students of the Graduate School of Frontier Science Initiative, Division of Nano Life Science selected for the Fellowship Program for Fostering Top Scientists in Fused Disciplines (a fellowship program aiming to create an environment where excellent doctorate students can concentrate on their research to create a "new fused science field, Made-in-Japan,") receive a scholarship of the same amount as above and a research budget of ¥400,000 per year.

Overseas students, including those of the Graduate School of Frontier Science Initiative, Division of Nano Life Science, normally receive full or half tuition fee exemption.

Measures are taken to ensure that a scholarship is given to every student belonging to the Graduate School of Frontier Science Initiative, Division of Nano Life Science by support directly from KU and/or in combination with a scholarship for fostering future researchers from external organizations such as the JSPS Research Fellowship for Young Scientists.

10. Center's Response to Results of FY 2020 Follow-up (including Site Visit Results)

* Describe the Center's response to results of FY 2020 follow-up. Note: If you have already provided this information, please indicate where in the report.

Responses to Site Visit Report

Comment 1 ***"Create a strategic plan and roadmaps for the latter half of the funding period and beyond."***

To create the strategic plan and roadmaps, we had intensive discussions among all the members including young scientists and established PIs from different disciplines. Although the overall strategy and plan are summarized in Sec. 7-1, here we provide some additional explanations.

Roadmap for Technological Development: The cell analysis technologies that we are developing are listed in Fig. 18 in Sec. 7-1. For each technology, we have created a roadmap. An example for the intra-cellular imaging technique "nanoendoscopy" is shown in Fig. 19. The subjects described in red are mostly common to all the technologies as summarized in Fig. 17 in Sec. 7-1. As a first step, we visualize the individual intra-cellular components, then proceed to visualize their dynamics or mechanical properties, and finally investigate the specific life science problem. In the case of the nanoendoscopy, the investigation on the intra-cellular transport mechanism NPCs is one of the focused research topics described in Sec. 7-1.

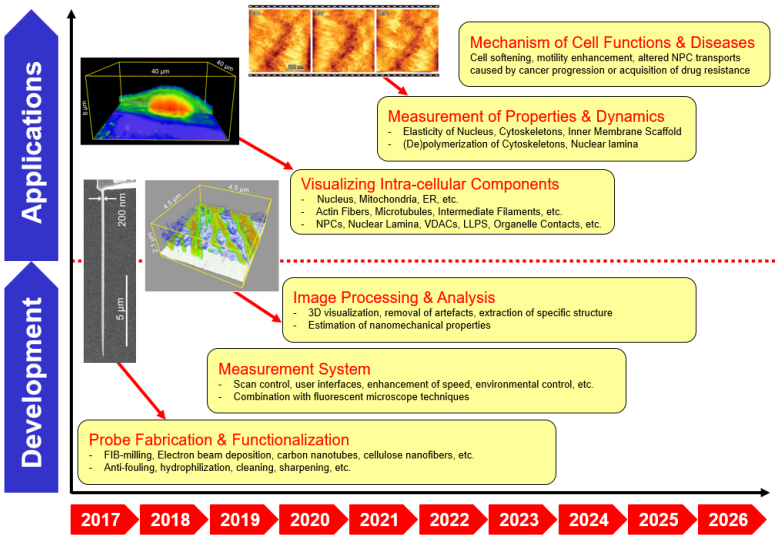

Fig. 19: Roadmap for the development of nanoendoscopy for visualizing intra-cellular nanodynamics inside living cells.

Target Life Science Questions: In Sec. 7-1, we stated that we will focus on several life science questions that are important in different life science disciplines. As an alternative, we also seriously considered the possibility of focusing on a single flagship project. However, we came to the conclusion that this strategy does not fit our institute. As described in Sec. 1, the biggest strength of our institute is the capability of producing various unique Bio-SPM technologies and thereby pioneering new application areas in nanoprobe life science. To take full advantage of this, we decided to follow our original plan to work on several important topics to have a strong impact on different life science disciplines.

The majority of the selected subjects are more or less related to cancer research such as cancer-specific aberrant cell behaviors, metastatic cancer progression, drug resistance, and cell-level cancer diagnosis (Fig. 20). This is also true of the subjects mainly focusing on basic cell functions, where we often compare the behaviors of normal and cancer cells. In this respect, we can say that cancer research is the major focus of our nano life science research projects.

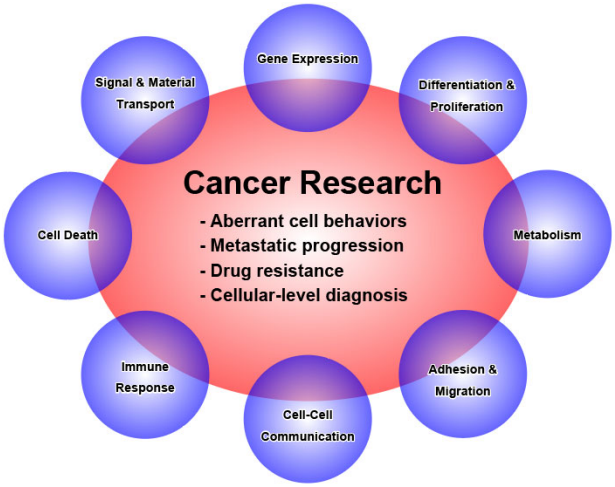

Fig. 20: Correlation between our basic cell biology studies and cancer research. They are closely related and thus will be pushed forward together.

Comment 2 ***"Carefully monitor the influence on cells upon the penetration and high-speed scanning by nano-probes, not just viability."***

Previously, we performed fluorometric cell viability tests by monitoring signals from calcein-AM and propidium iodide (PI) after the 2D and 3D nanoendoscopy measurements. The results successfully demonstrated that these measurements do not lethally damage the cells. However, as pointed out here, this does not mean that there is no influence on cellular activities. To address this issue, we should take two different strategies.

General Tests: As a part of the development of this new method, we should use a few generally accepted

assays to test cellular activities, such as fluorometric tests with calcein-AM and PI. In addition, we have already performed long-term monitoring of the structural changes of the cells after the measurements and confirmed that they can divide into two cells within a similar timescale as unmeasured cells. Furthermore, we are now trying to monitor the changes of stress response factors such as Ca^{2+} during and after the tip penetration and scanning.

Specific Tests: In practice, it is impossible to check all the details of all cellular activities for every measurement. This is also true for any measurement technologies including widely used fluorescent microscopy techniques. Therefore, the best practice is to check the influence on the specific structures or functions that we investigate in each application. For example, we started to use this technique to measure the mechanical properties of the nucleus and to investigate its dependence on cancer progression. For such experiments, we may monitor changes in the important proteins that are related to the cellular mechanics such as actin and tubulin. Therefore, after the abovementioned general tests, we will start to use this method for specific applications, and we will seriously consider appropriate testing or monitoring methods for each application.

Comment 3 ***"Secure positions for active young scientists including junior PIs, and also consider employing more talented researchers in needed areas. They will become the center's next generation."***

Regarding the securing of positions for active young researchers, 6 tenure positions have already been secured at the President's discretion for the 6 Jr. PIs to whom the tenure track is applied. Jr. PIs are key players to promote fused research. For other young researchers, a considerable number of positions will be secured by using NanoLSI's abundant external funding after the WPI grant period ends.

In FY2021, 3 more young researchers will be hired through international open recruitment. Research areas concerned are interdisciplinary ones such as the design and synthesis of chemical sensors, dissection of nuclear events through deep sequencing, cancer stem cell research by applying Bio-SPM. Through new recruitments including those mentioned above, more talented young researchers will be successfully employed.

Comment 4 ***"Keep pursuing international exchanges even amidst the current COVID-19 situation, and further enhance the research activities of foreign PIs."***

The NanoLSI Symposium, held every year since FY2017, was held online in FY2020 due to COVID-19. The themes of the symposium were bio-imaging and sensing and manipulation for medical science; 21 researchers participated from overseas.

Two open seminars were held online by the leading authors of papers at the time they were published.

A video "Virtual Laboratory Tour" was created to virtually inspect the new NanoLSI research building, which was completed in September 2020. The copyright and portrait rights will be settled and the video will be published on the NanoLSI website shortly.

With the mediation of the Canadian Embassy in Japan, a virtual visit of Nano Canada members, a consortium of Canadian nanotechnology companies, was welcomed using the "Virtual Laboratory Tour" video in an online open house format.

In response to the request for the dissemination of research information in foreign countries, where activity restrictions and lockdowns continue, 4 overseas PIs produced podcasts in which each PI presented his research.

Comment 5 ***"Continue endeavoring to improve the center's gender balance."***

Regarding gender balance, new researchers will be selected among female scientists, taking into account the progress of their research in each fiscal year in line with NanoLSI's strategic plan and roadmaps in the second half of the WPI grant period. By this measure, the current number of female scientists, currently 11 out of a total of 83 NanoLSI researchers, will be steadily increased to 13 in FY2021, 15 in FY2022, and 17 in FY2023; the ratio of female researchers at the end of FY2023 is expected to be over 20%. Negotiations have already been completed with two female researchers who will participate in NanoLSI from FY2021.

Furthermore, in order to gain the participation of a new female PI, a working group consisting of the Administrative Director and three PIs has been established. This working group will try to recruit a new female PI on campus or from off-campus through joint appointment contracts.

Responses to WPI Follow up Report

Comment 1 ***"While the achievements and efforts made by NanoLSI are appreciated, the center will soon need to summarize its achievements of the first five years and propose strategies for moving forward towards its final goals. Creating a strategic plan and***

roadmaps for the latter half of the funding period and beyond will be most critical. At the same time, the center is encouraged sharpen even further its fundamental technologies of BioSPM, which are already world-class."

Responses to this comment has been described in the above responses to Comment 1 of the site visit report.

Comment 2 ***"As mentioned above, there is room for improvement regarding the fusion of disciplines, internationalization, and diversity. In particular, many members of the Program Committee pointed out the inappropriate gender balance at NanoLSI. The center should seriously consider ways to improve this situation, and is requested to present a concrete plan for doing so."***

Responses to this comment has been described in the above responses to Comment 3, 4 and 5 of the site visit report.

Appendix 1-1 List of Papers Underscoring Each Research Achievement

* List papers underscoring each research achievement [1] ~ [10] listed in the item 2-1 "Research results to date" of 2. "Advancing Research of the Highest Global Level" (up to 20 papers) and provide a description of the significance of each (within 10 lines).
* For each, write the author name(s); year of publication; journal name, volume, page(s), and article title. Any listing order may be used as long as format is the same. If a paper has many authors, underline those affiliated with the Center.
* If a paper has many authors (say, more than 10), all of their names do not need to be listed.
* Place an asterisk (*) in front of those results that could only have been achieved by a WPI center.

***[1] Development of 2D- and 3D-AFM imaging inside living cells**
***1. Cell penetration efficiency analysis of different atomic force microscopy nanoneedles into living cells**
(*Sci. Rep., 11 (2021) 7756*, **IF: 3.998).**
Marcos Penedo, Tetsuya Shirokawa, Mohammad Shahidul Alam, **Keisuke Miyazawa**, **Takehiko Ichikawa**, **Naoko Okano**, **Hirotoshi Furusho**, Chikashi Nakamura and **Takeshi Fukuma**

Over the last decade, nanoneedle-based systems have demonstrated to be extremely useful in cell biology. They can be used as nanotools for drug delivery, biosensing or biomolecular recognition inside cells; or they can be employed to select and sort in parallel a large number of living cells. When using these nanoprobes, the most important requirement is to minimize the cell damage, reducing the forces and indentation lengths needed to penetrate the cell membrane. This is normally achieved by reducing the diameter of the nanoneedles. However, several studies have shown that nanoneedles with a flat tip display lower penetration forces and indentation lengths. In this work, we have tested different nanoneedle shapes and diameters to reduce the force and the indentation length needed to penetrate the cell membrane, demonstrating that ultra-thin and sharp nanoprobes can further reduce them, consequently minimizing the cell damage.

***[2] Development and application of measuring technologies on the surface of living cells**
***2. Development of high-speed ion conductance microscopy**
(*Rev. Sci. Instrum., 90 (2019) 123704***, IF: 1.480)**
Shinji Watanabe, Satoko Kitazawa, **Linhao Sun**, **Noriyuki Kodera**, and **Toshio Ando**

Scanning ion conductance microscopy (SICM) reveals the surface topography of living cells without damaging them. However, SICM imaging is slow. One image can take a few minutes or more to capture. This is not only inefficient, it's also too slow to image the dynamic behavior of a biological sample. In this study, we developed high-speed SICM, increasing the imaging rate by a factor of 100 without compromising spatial resolution. High-speed SICM increases the chances to capture phenomena occurring in living cell surfaces that no one has ever seen before.

***3. Geometrical Characterization of Glass Nanopipettes with Sub-10 nm Pore Diameter by Transmission Electron Microscopy**
(*Anal. Chem., 92 (2020) 15388 – 15393***, IF: 6.785)**
Kazuki Shigyou, **Linhao Sun**, Riku Yajima, Shohei Takigaura, Masashi Tajima, **Hirotoshi Furusho**, Yousuke Kikuchi, **Keisuke Miyazawa**, **Takeshi Fukuma**, **Azuma Taoka**, **Toshio Ando**, and **Shinji Watanabe**

Glass nanopipettes are widely used for various applications in nanosciences. In most of the applications, it is important to characterize their geometrical parameters, such as the aperture size and the inner cone angle at the tip region. For nanopipettes with sub-10 nm aperture and thin wall thickness, transmission electron microscopy (TEM) must be most instrumental in their precise geometrical measurement. However, this measurement has remained a challenge because heat generated by electron beam irradiation would largely deform sub-10 nm nanopipettes. In this paper, we provide methods for preparing TEM specimens that do not cause deformation of such tiny nanopipettes. This technique will be significant for a quantitative measurement of stiffness and surface charge density of samples using scanning ion conductance microscopy.

***[3] Development of nanoprobe-biosensors for chemical mapping inside and outside of living cells**
***4. High-resolution label-free 3D mapping of extracellular pH of single living cells**
(*Nat. Commun., 10(2019) 5610***, IF: 12.121)**

Yanjun Zhang, **Yasufumi Takahashi**, Sung Pil Hong, Fengjie Liu, Joanna Bednarska, Philip S. Goff, Pavel Novak, Andrew Shevchuk, Sahana Gopal, Iros Barozzi, Luca Magnani, Hideki Sakai, **Yoshimoto Suguru**, Takuto Fujii, Alexander Erofeev, Peter Gorelkin, Alexander Majouga, Dominik J. Weiss, Christopher Edwards, Aleksandar P. Ivanov, David Klenerman, Elena V. Sviderskaya, Joshua B. Edel & **Yuri Korchev**

We Developed nanoprobe-biosensors for chemical mapping inside and outside of living cells.Cellular chemical heterogeneity is a hallmark of cancer. We have recently developed a range of novel nanoprobe-biosnsors for oxygen gradients, ROS species, ATP, and pH biosensing. Importantly these nanoprobes can be integrated with our advanced SICM, which enable us to map intracellular and extracellular chemicals of living cancer cells with higher spatial and temporal resolution.

[4] Further improvement of high-speed AFM technologies
***5. Faster high-speed atomic force microscopy for imaging of biomolecular processes**
(*Rev. Sci. Instrum., 92 (2021)*, 92(3) *#33705*, IF: 1.480)
Shingo Fukuda and **Toshio Ando**

Great expansion of dynamic molecular processes observable with high-speed AFM demands its faster and less disturbing capacity. However, even a 50% improvement in the speed performance imposes tremendous challenges, as the optimization of major rate-limiting components for their fast response is nearly matured. This study took an alternative approach based on a finding that retrace imaging during backward X-scanning produces larger feedback error than trace imaging. The elimination of retrace imaging and the shortened backward scanning period could lower the feedback control error and thereby enhance the imaging rate by ~2.5 times. This "only trace imaging" mode can be easily implemented in any HS-AFM system by minor modifications of the software and hardware.

6. Molecular and Functional Analysis of Pore-Forming Toxin Monalysin From Entomopathogenic Bacterium *Pseudomonas entomophila*
(*Front. Immunol., 11 (2020) 520*, IF: 5.085)
Saori Nonaka, Emil Salim, Koki Kamiya, Aki Hori, Firzan Nainu, Rangga Meidianto Asri, Ayu Masyita, Takumi Nishiuchi, Shoji Takeuchi, **Noriyuki Kodera** and Takayuki Kuraishi

(Development of non-flat AFM substrate)

We developed the AFM substrates made of PDMS (polydimethylsiloxane), a thermosetting biocompatible elastomer, with submicrometer-sized concave and/or convex shapes using by the nano-sphere imprinting method (Issued patent: 677310). Since such substrates may deform biological membranes, one can investigate the phenomena depending on the curvature of biological membranes. To test this substrate, we observed the insertion process of Monalysin, a pore-forming toxin, into a lipid membrane. Monalysin was preferentially inserted into the convex-shaped lipid membrane formed on the PDMS substrate, while no insertion was seen into the flat lipid membrane formed on the mica surface, implying that Monalysin could recognize the target membrane's curvature. As this new AFM substrate can be used to mimic local environments on the organelle surfaces and to induce the deformation on biological molecules, the application area of HS-AFM in biology will be greatly expanded.

[5] Development and application of AFM data analysis technologies using mathematical and computational sciences
***7. Synthesis and Local Probe Gating of a Monolayer Metal-Organic Framework**
(*Adv. Funct. Mater., (2021) 2100519*, IF: 16.836)
Linghao Yan, Orlando J. Silveira, Benjamin Alldritt, Ondřej Krejčí, **Adam S. Foster**, **Peter Liljeroth**

A key step in the development of reliable simulation tools for SPM analysis, is demonstrating their capabilities in a wide variety of systems, from bulk insulators in vacuum to organic molecules in solution. In this work, we use our high-resolution SPM method to interpret experimental images of large-area uniform 2D metal-organic frameworks (MOFs). A 2D monolayer (Cu-dicy- anoanthracene) MOF with long-range order is successfully fabricated on an epitaxial graphene surface, and its structural and electronic properties are studied by low-temperature SPM, complemented by density-functional theory calculations. Access to multiple molecular charge states in the 2D MOF is demonstrated using tip-induced local electric fields. It is expected that a similar strategy could be applied to fabricate and characterize 2D MOFs with exotic, engineered

electronic states.

***8. BioAFMviewer: An interactive interface for simulated AFM scanning of biomolecular structures and dynamics**
(*PLoS Comput. Biol.,16 (2020) e1008444,* IF: 4.700)
Romain Amyot, **Holger Flechsig**

In this work, we report the BioAFMviewer software platform for simulated AFM scanning of biomolecular structures and their conformational dynamics. The development and implementation of computational AFM scanning allows to employ the enormous amount of available high-resolution structural protein data and movies from molecular modelling to significantly improve the interpretation of resolution-limited experimental images. We applied fitting of atomic protein structures to high-speed AFM images and demonstrated how this facilitates the understanding of experimental results. Generally, applications to predict 3D protein structure and conformational changes from high-speed AFM images using computational twin experiments become possible. With its versatile interactive interface and rich functionality, the BioAFMviewer provides a powerful computational tool for the broad Bio-AFM community. Furthermore, its modular architecture allows us to easily implement novel methods and tools to foster our mission of automatized data-driven analysis of AFM images.

***9. High-speed AFM reveals accelerated binding of agitoxin-2 to a K+ channel by induced fit**
(*Science Advances, 5 (2019) eaax0495,* IF: 13.1117)
Ayumi Sumino, **Takashi Sumikama**, Takayuki Uchihashi, Shigetoshi Oiki

Nowadays, various techniques to observe dynamics of single biomolecules have been developed, and movies filmed such molecular dynamics have been increasing year by year. As such, one next significant step is a development of theoretical methods to analyze those movies, which have been lacking so far. In this paper, we successfully established an analysis method by extending the event-oriented analysis, a method authors once developed, to a method including three-point time correlations of the states of biomolecules. This novel method was applied to HS-AFM movies recording the binding/unbinding dynamics of a scorpion toxin to a K^+ channel. The analyses not only clearly showed that these dynamics occur by the so-called induced-fit mechanism, but also enabled us to classify the state of the channel in each frame of the movies to either low- or high-affinity state of the channel to the toxin. Thus, this paper indicated a crucial importance of collaboration among nanometrology, computational and life sciences to understand how biomolecules work.

[6] Promotion of supramolecular chemistry research and its application to life science and SPM nanometrology
10. Pressure-Responsive Hierarchical Chiral Photonic Aerogels
(*Adv. Mater., 31 (2019) 1808186*, IF: 27.398)
Yuanyuan Cao, Lev Lewis, Wadood Y. Hamad, **Mark J. MacLachlan**

Cellulose nanocrystals (CNCs) form a chiral nematic (CN*) liquid crystalline phase in water. Yuanyuan Cao et al. extracted this phase and heated it to form a hydrogel in which the CNCs have a CN* order. When she rapidly froze the hydrogel in liquid nitrogen, ice crystals formed that disrupted the CN* order in some regions. The white, rubbery material becomes colored when it is squeezed because the layers with CN* order adopt a 2D structure. This process was made reversible by embedding an elastomer in the aerogel. As well, the new material was divided into fine pieces to demonstrate a new type of solvent-responsive ink. This discovery of a new type of responsive photonic material could help us construct materials for rapid pressure sensing.

***11. Pillar[6]arene acts as a biosensor for quantitative detection of a vitamin metabolite in crude biological samples**
(*Commun. Chem., 3 (2020) 183,* IF: 4.253)
Masaya Ueno, Takuya Tomita, Hiroshi Arakawa, **Takahiro Kakuta**, Tada-aki Yamagishi, Jumpei Terakawa, Takiko Daikoku, Shin-ichi Horike, **Sha Si**, Kenta Kurayoshi, Chiaki Ito, Atsuko Kasahara, **Yuko Tadokoro**, **Masahiko Kobayashi**, Tsutomu Fukuwatari, Ikumi Tamai, **Atsushi Hirao** & **Tomoki Ogoshi**

Hirao and Ogoshi groups developed new biosensor for quantitative detection of 1-methylnicotinamide (1-

MNA), which produced by the cancer-associated nicotinamide N-methyltransferase (NNMT). Pillar[6]arene, a pillar-shaped macrocyclic compounds with a hexagonal cross-section, forms host–guest complex with 1-MNA. When 1-MNA is bound to pillar[6]arene, the fluorescent response of the latter significantly decreases — an effect that can be exploited as an indicator for the presence or absence of 1-MNA. The P6A fluorescence detection mechanism works even in crude biological samples. Specifically, they were able to detect 1-MNA in urine. By improving the sensitivity and specificity of the biosensors, the detection system should contribute to the development of low-cost, easy, and rapid methods for the detection of human metabolites for diagnosis.

[7] Nano-dynamics research on proteins, DNA and organelle by HS-AFM

***12. Structural and dynamics analysis of intrinsically disordered proteins by high-speed atomic force microscopy**

(*Nat. Nanotech., 16 (2021) 181-189*, IF: 31.538)

Noriyuki Kodera, **Daisuke Noshiro**, Sujit K. Dora, Tetsuya Mori, Johnny Habchi, David Blocquel, Antoine Gruet, Marion Dosnon, Edoardo Salladini, Christophe Bignon, Yuko Fujioka, Takashi Oda, Nobuo N. Noda, Mamoru Sato, Marina Lotti, Mineyuki Mizuguchi, Sonia Longhi & **Toshio Ando**

Intrinsically disordered proteins (IDPs) are ubiquitous proteins that are disordered entirely or partly and play important roles in diverse biological phenomena. Notably, IDPs has also been shown to be key players in cellular liquid-liquid phase separation. Their structure dynamically samples a multitude of conformational states, thus making their structural analysis very difficult with conventional methods such as X-ray crystallography, electron microscopy and NMR spectroscopy. However, in the present study, we demonstrated that HS-AFM is a suitable tool to characterize the structure and dynamics of IDPs. Successive HS-AFM images of an IDP molecule can not only identify constantly folded and constantly disordered regions in the molecule, but can also document disorder-to-order transitions. Moreover, the number of amino acids contained in these disordered regions can be roughly estimated, enabling a semiquantitative, realistic description of the dynamic structure of IDPs.

***13. Phase separation organizes the site of autophagosome formation**

(*Nature, 578 (2021) 301−305*, IF: 42.779)

Yuko Fujioka, Jahangir Md. Alam, **Daisuke Noshiro**, Kazunari Mouri, **Toshio Ando**, Yasushi Okada, Alexander I. May, Roland L. Knorr, Kuninori Suzuki, Yoshinori Ohsumi & Nobuo N. Noda

Autophagosomes, double-membrane vesicles formed de novo upon starvation, deliver cytoplasmic components to lysosomes or vacuoles for their degradation. The formation of the autophagosome precursor (called PAS) comprising several cytosolic Atg proteins is triggered by dephosphorylation (de-Pi) of Atg13 upon starvation. Yet, early PAS formation processes and its physicochemical and functional properties have been elusive, mainly because these Atg proteins are intrinsically disordered, except for dimeric Atg17. HS-AFM imaging revealed (i) folding of Atg13's disordered region into a globule upon phosphorylation, (ii) network-like structure formation by crosslinking of Atg17 molecules by de-Pi Atg13, and (iii) development of a droplet-like structure upon long incubation of de-Pi Atg13 and Atg17-Atg29-Atg31. The liquid droplet formation was also observed in vivo. Thus, the PAS is formed by liquid-liquid phase separation through a weak multivalent binding among the Atg proteins.

14. Dynamic structural states of ClpB involved in its disaggregation function

(*Nat. Commun., 9 (2018) #2147,* IF: 12.121)

Takayuki Uchihashi, Yo-hei Watanabe, Yosuke Nakazaki, Takashi Yamasaki, Hiroki Watanabe, Takahiro Maruno, Kentaro Ishii, Susumu Uchiyama, Chihong Song, Kazuyoshi Murata, Ryota Iino & **Toshio Ando**

The AAA+ ATPase molecular chaperone ClpB disentangles and reactivates aggregated proteins. How this function is achieved has long been elusive. HS-AFM images of ClpB hexamers in the presence of ATP showed large scale conformational changes, from round and spiral rings to twisted-half-spiral rings, and even to open rings, with a frequency similar to the ATPase rate per hexamer. The twisted-half-spiral form (dimer of trimers) was identified in this study for the first time. These structural changes were drastically decreased in a middle-domain mutant that lost disaggregation activity but retained ATPase activity. Thus, ClpB performs protein disaggregation through these structural changes, in particular through large height increase in the spiral and twisted-half spiral rings, which possibly acts to extend and disentangle aggregated proteins trapped in their

central cavity.

***[8] Functional imaging of living cells**
***15. High-Speed SICM for the Visualization of Nanoscale Dynamic Structural Changes in Hippocampal Neurons**
(*Anal. Chem., 92 (2020) 2159–2167*, IF: 6.785)
Yasufumi Takahashi, **Yuanshu Zhou**, Takafumi Miyamoto, Hiroki Higashi, Noritaka Nakamichi, Yuka Takeda, Yukio Kato, **Yuri Korchev**, and **Takeshi Fukuma**

We directly measured HIV-like particle assembly using SICM and found that they can reach full size in 20 s and release in 0.5 to 3 min. Compared to previous estimates, this is more than 10 times faster. In our opinion this is a highly important discovery that GFP tagging affects virus particle assembly and release.

[9] New discoveries on cell biology using Bio-SPM technologies
***16. Spatiotemporally tracking of nano-biofilaments inside the nuclear pore complex core.**
(*Biomaterials, 256 (2020) 120198*, IF: 10.317)
Mahmoud Shaaban Mohamed, Masaharu Hazawa, Akiko Kobayashi, Laurent Guillaud, **Takahiro Watanabe-Nakayama**, **Mizuho Nakayama**, Hanbo Wang, **Noriyuki Kodera**, **Masanobu Oshima**, **Toshio Ando**, **Richard W.Wong**

In this paper, we reported a spatiotemporally tracking of nuclear pore complex (NPC), the nano-gateway between nucleus and cytoplasm. NPC contained intrinsically disordered (non-structured) regions (IDRs) with phenylalanine-glycine (FG) motifs (FG-NUPs) as central selective barrier. Notably, FG-NUPs were overexpressed in various cancers. We showed that the central FG-NUPs assembled via liquid-liquid phase separation (LLPS) of NPCs in colorectal cells and organoids at timescales of ~150 ms using HS-AFM. Using the FG-NUP inhibitor, trans-1,2-cyclohexanediol, we found that central plug size was significantly reduced and incompletely reversible back to filamentous structures in aggressive colon cancer cells and organoids.

***17. High-speed AFM reveals molecular dynamic of human influenza A hemagglutinin and its interaction with exosomes.**
(*Nano Letters, 20 (2020) 6320-6328*, IF: 11.238)
Keesiang Lim, **Noriyuki Kodera**, Hanbo Wang, Mahmoud Shaaban Mohamed, **Masaharu Hazawa**, Akiko Kobayashi, **Takeshi Yoshida**, **Rikinari Hanayama**, **Seiji Yano**, **Toshio Ando**, and **Richard W. Wong**

In this paper, we used HS-AFM to decipher the molecular dynamics of Influenza A hemagglutinin (HA) and its interaction with exosomes. We revealed that the native conformation of HA in the neutral buffer is ellipsoidal, and HA undergoes a conformational change in an acidic buffer. Real-time visualization of the fusogenic transition by HS-AFM suggested that the mechanism is possibly fit to the "uncaging" model, and HA intermediate appeared as Y-shaped. A firm interaction between the HA and exosome in an acidic buffer indicated the insertion of a fusion peptide into the exosomal layer and subsequently destabilizes the layer, resulting in the deformation or rupture of exosomes, releasing exosomal contents. In contrast, the HA–exosome interaction was weak in a neutral buffer because the interaction is mediated by weak bonds between the HA receptor-binding site and receptors on the exosome.

18. Engineering synthetic morphogen systems that can program multicellular patterning
(*Science, 370 (2020) 327-331*, IF: 41.846)
Satoshi Toda, Wesley L. McKeithan, Teemu J. Hakkinen, Pilar Lopez, Ophir D. Klein, Wendell A. Lim

Toda et al have developed a synthetic diffusible cell-cell signaling system called synthetic morphogen that can program de novo tissue patterns. Morphogen is a general term of specialized secreted proteins that diffuse and form a concentration gradient around secreting cells, which will work as positional information for receiver cells to spatially control their cell fate. We explored what features of morphogens are sufficient for positional encoding and successfully converted arbitrary proteins (e.g. GFP) into synthetic morphogens by trapping and sensing of the proteins with synthetic receptor technologies. Synthetic morphogens expressed from a localized source formed a signal gradient with tunability on dynamics and range and can be used to program de novo multidomain tissue patterns. The synthetic biology approach with engineered

cell-cell signaling highlights basic mechanisms of tissue patterning and helps to understand how molecular behaviors lead to macroscale tissue organization.

[10] Progress in cancer research and new achievements by applying SPM technology

19. Spred1 Safeguards Hematopoietic Homeostasis against Diet-Induced Systemic Stress
（*Cell Stem Cell, 22 (2018) 713 – 725*, **IF: 20.860**）

Yuko Tadokoro, Takayuki Hoshii, Satoshi Yamazaki, Koji Eto, Hideo Ema, **Masahiko Kobayashi**, **Masaya Ueno**, Kumiko Ohta, Yuriko Arai, Eiji Hara, Kenichi Harada, **Masanobu Oshima**, **Hiroko Oshima**, Fumio Arai, Akihiko Yoshimura, Hiromitsu Nakauchi, **Atsushi Hirao**

Stem cell self-renewal is critical for tissue homeostasis, and its dysregulation leads to organ failure or tumorigenesis. Hirao's group have revealed that Spred1, a negative regulator of RASMAPK signaling, safeguards hematopoietic stem cell (HSC) homeostasis under high-fat diet (HFD) conditions by modulating HSC self-renewal. At steady-state, Spred1 negatively regulates HSC self-renewal and competitiveness in a manner supported by Rho kinase activity. Spred1 deficiency mitigates HSC failure induced by infection mimetics and prolongs HSC lifespan, but does not trigger leukemogenesis because of compensatory Spred2 upregulation. In contrast, HFD induces ERK hyperactivation and aberrant self-renewal in Spred1-deficient HSCs, resulting in functional failure associated with severe anemia and myeloproliferative neoplasm-like disease. Interestingly, HFD-induced hematopoietic abnormalities are partly due to altered gut microbiota. These findings served important evidence for further analysis to identify functional mediator metabolites that link between gut and bone marrow.

***20. Macrocyclic peptide-based inhibition and imaging of hepatocyte growth factor**
(*Nat. Chem. Biol. 15 (2019) 598 – 606*, IF: 12.587)

Katsuya Sakai, Toby Passioura, **Hiroki Sato**, Kenichiro Ito, Hiroki Furuhashi, Masataka Umitsu, Junichi Takagi, Yukinari Kato, Hidefumi Mukai, Shota Warashina, Maki Zouda, Yasuyoshi Watanabe, **Seiji Yano**, **Mikihiro Shibata**, Hiroaki Suga & **Kunio Matsumoto**

Activation of the MET receptor by HGF (hepatocyte growth factor) closely participates in cancer metastasis and drug resistance. HGF is secreted as an inactive precursor HGF which is activated by proteolytic processing in cancer microenvironments. Thus, selective detection/inhibition of active HGF is definitively important in cancer diagnosis and therapeutics. We generated HiP-8 (HGF-inhibitory Peptide-8), a macrocyclic peptide consisting of 12 amino acids, which selectively recognizes and inhibits active HGF. High-speed AFM analysis demonstrated that HiP-8 inhibits the dynamic change in molecular shape of HGF into static, indicating a unique mechanism that a small molecule inhibits molecular dynamics of target protein. Positron emission tomography using HiP-8 enabled noninvasive visualization and simultaneous inhibition of active HGF in tumors. Selective detection and inhibition of active HGF by HiP-8 may be useful for diagnosis and treatment of cancers.

Appendix 1-2 List of Papers of Representative of Interdisciplinary Research Activities

* List **up to 10 papers** underscoring each interdisciplinary research activity and give brief accounts (within 10 lines).
* For each, write the author name(s); year of publication; journal name, volume, page(s), and article title. Any listing order may be used as long as format is the same. If a paper has many authors, underline those affiliated with the Center.
* If a paper has many authors (say, more than 10), all of their names do not need to be listed.

1. **Nanometrology x Life Science**
"Macrocyclic peptide-based inhibition and imaging of hepatocyte growth factor"
(*Nat. Chem. Biol.*, *15 (2019) 598-606*, IF: 12.587)
Katsuya Sakai, Toby Passioura, Hiroki Sato, Kenichiro Ito, Hiroki Furuhashi, Masataka Umitsu, Junichi Takagi, Yukinari Kato, Hidefumi Mukai, Shota Warashina, Maki Zouda, Yasuyoshi Watanabe, Seiji Yano, Mikihiro Shibata, Hiroaki Suga and Kunio Matsumoto

Through a cross-disciplinary approach, the authors found that HiP-8 (HGF-inhibitory peptide-8), a macrocyclic peptide consisting of 12 amino acids, specifically binds to HGF. Biochemical analysis indicated that HiP-8 binds to HGF through multivalent binding interfaces. High-speed AFM analysis indicated that HiP-8 restricted the dynamic domain movement of HGF into static closed conformations. This study established the novel concept that a small macrocyclic peptide can inhibit the molecular dynamics of a target protein.

2. **Supramolecular Chemistry x Life Science**
"Pillar[6]arene acts as a biosensor for quantitative detection of a vitamin metabolite in crude biological samples"
(*Commun. Chem., 3 (2020) 183*, IF: 4.253)
Masaya Ueno, Takuya Tomita, Hiroshi Arakawa, Takahiro Kakuta, Tada-aki Yamagishi, Jumpei Terakawa, Takiko Daikoku, Shin-ichi Horike, Sha Si, Kenta Kurayoshi, Chiaki Ito, Atsuko Kasahara, Yuko Tadokoro, Masahiko Kobayashi, Tsutomu Fukuwatari, Ikumi Tamai, Atsushi Hirao and Tomoki Ogoshi

This study was supported by the NanoLSI transdisciplinary research promotion grant. Metabolic syndrome is associated with obesity, hypertension, and dyslipidemia, and increased cardiovascular risk. Therefore, quick and accurate measurements of specific metabolites are critical for diagnosis. Through a cross-disciplinary approach, the authors found that water-soluble pillar[5]arene (P5A) forms host–guest complexes with both 1-MNA and nicotinamide, and water-soluble pillar[6]arene (P6A) selectively binds to 1-MNA at the micromolar level. P6A can be used as a "turn-off sensor" by photoinduced electron transfer (detection limit is 4.38×10^{-6} M). Their findings demonstrate that P6A can be used as a biosensor to quantify 1-MNA in crude biological samples.

3. **Nanometrology x Life Science**
"High-Speed AFM Reveals Molecular Dynamics of Human Influenza A Hemagglutinin and Its Interaction with Exosomes"
(*Nano Lett., 20 (2020) 6320-6328*, IF: 11.238)
Keesiang Lim, Noriyuki Kodera, Hanbo Wang, Mahmoud Shaaban Mohamed, Masaharu Hazawa, Akiko Kobayashi, Takeshi Yoshida, Rikinari Hanayama, Seiji Yano, Toshio Ando, and Richard W. Wong

Influenza A hemagglutinin (HA) is one of the crucial virulence factors that mediate host tropism and viral infectivity. Presently, the mechanism of the fusogenic transition of HA remains elusive. Through a cross-disciplinary approach, the authors used high-speed atomic force microscopy (HS-AFM) to decipher the molecular dynamics of HA and its interaction with exosomes and revealed that the native conformation of HA in the neutral buffer is ellipsoidal, and HA undergoes a conformational change in an acidic buffer. Real-time visualization of the fusogenic transition by HS-AFM. These results suggest that the mechanism is possibly fit to the "uncaging" model, and HA intermediate appears as Y-shaped.

4. **Nanometrology x Life Science**
"High-Speed SICM for the Visualization of Nanoscale Dynamic Structural Changes in Hippocampal Neurons"

(*Anal. Chem. 92 (2020) 2159-2167*, IF: 6.785)
Yasufumi Takahashi, Yuanshu Zhou, Takafumi Miyamoto, Hiroki Higashi, Noritaka Nakamichi, Yuka Takeda, Yukio Kato, **Yuri Korchev,** and **Takeshi Fukuma**

This study was collaborated with Imperial College of London. Dynamic reassembly of the cytoskeleton and structural changes represented by dendritic spines, cargo transport, and synapse formation are closely related to memory. However, the visualization of the nanoscale topography is challenging because of the diffraction limit of optical microscopy. Through a cross-disciplinary approach, the authors have developed automation region of interest (AR)-mode SICM and a fast *Z*-axis scanner for improving the temporal resolution of topography imaging. The newly developed algorithm requires half of the scanning time and allows for the visualization of dynamic cargo transport, changes in the volume of dendritic spines and synaptic boutons, and the growth cones of hippocampal neurons without labeling.

5. **Nanometrology x Computational Science**
"Computed Atomic Force Microscopy Images of Chromosomes by Calculating Forces with Oscillating Probes"
(*J. Phys. Chem. C 124 (2020) 2213–2218*, IF: 4.189)
Takashi Sumikama, Adam. S. Foster, and **Takeshi Fukuma**

AFM is a promising tool to visualize biomolecules at the sub-nanometer scale. Experimentally obtained AFM images have been compared with the simulated ones; however, such conventional images of biomolecules were usually computed by calculating equidistance surface from given atomic positions, not by calculating force. Here, the authors use a polymer model of a chromosome, as a representative biomolecule, and the AFM probe, and computed isoforce surfaces upon the fiber. The oscillation of probes utilized in the dynamic mode of AFM measurements was also implemented in the simulation. The computed isoforce images were clearer than the conventional equidistance ones, and a very similar images to isoforce ones were obtained when the diameter of the probe was reduced to approximately 30% in the equidistance images. Thus, the probe was found to approach very close to samples beyond the estimation of the equidistance surface, contributing clear AFM images.

6. **Nanometrology x Computational Science**
"High-speed AFM reveals accelerated binding of agitoxin-2 to a K+ channel by induced fit"
(*Sci. Adv. 5 (2019) eaax0495*, IF: 13.117)
Ayumi Sumino, **Takashi Sumikama**, Takayuki Uchihashi, and Shigetoshi Oiki

Agitoxin-2 (AgTx2) from scorpion venom is a potent blocker of K^+ channels. The docking model has been elucidated, but it remains unclear whether binding dynamics are described by a two-state model (AgTx2-bound and AgTx2-unbound) or a more complicated mechanism. Here, the authors analyzed the binding dynamics of AgTx2 to the KcsA channel using HS-AFM. From images of repeated binding and dissociation of AgTx2 to the channel, single-molecule kinetic analyses using a supercomputer revealed that the affinity of the channel for AgTx2 increased during persistent binding and decreased during persistent dissociation. The authors propose a four-state model, including high- and low-affinity states of the channel, with rate constants. An induced-fit pathway was dominant and accelerated binding 400-fold. This is the first analytical imaging of scorpion toxin binding in real time, which is applicable to various biological dynamics .

7. **Nanometrology x Life Science**
"Spatiotemporally tracking of nano-biofilaments inside the nuclear pore complex core"
(*Biomaterials 256 (2020) 120198*, IF: 10.317)
Mahmoud Shaaban Mohamed Masaharu Hazawa Akiko Kobayashi Laurent Guillaud **Takahiro Watanabe-Nakayama** **Mizuho Nakayama** Hanbo Wang **Noriyuki Kodera** **Masanobu Oshima** **Toshio Ando** and **Richard W. Wong**

In this paper, the authors reported a spatiotemporally tracking of nuclear pore complex (NPC), the nano-gateway between nucleus and cytoplasm. NPC contained intrinsically disordered (non-structured) regions (IDRs) with phenylalanine-glycine (FG) motifs (FG-NUPs) as central selective barrier. Notably, FG-NUPs were overexpressed in various cancers. The authors showed that the central FG-NUPs assembled via liquid-liquid phase separation (LLPS) of NPCs in colorectal cells and

organoids at timescales of ~150 ms using HS-AFM. Using the FG-NUP inhibitor, trans-1,2-cyclohexanediol, the authors found that central plug size was significantly reduced and incompletely reversible back to filamentous structures in aggressive colon cancer cells and organoids.

8. **Nanometrology x Supramolecular Chemistry**
"Ultra-thin, transparent, porous substrates as 3D culture scaffolds for engineering ASC spheroids for high-magnification imaging"
(*J. Mater. Chem. B 8 (2020) 6999-7008*, IF: 5.344)
Yoshitaka Suematsu, Ya An Tsai, Shinji Takeoka, **Clemens M. Franz**, **Satoshi Arai** and Toshinori Fujie

In this study, the authors achieved the preparation of spheroids from adipose-tissue derived stem cells (ASCs) on free-standing porous polymeric ultrathin films ("porous nanosheets"). ASCs migrated on the porous nanosheet, leading to the spontaneous organization of spheroids. The porous nanosheet also provided more than twice the optical transparency in confocal and holographic microscopy observation compared to conventional nanoimprinted substrates for 3D cell culture. The internal structure of the organized spheroids could be clearly observed with 40× magnification. In addition, the engineered spheroids showed bioactivities. Thus, porous nanosheets offer a unique cell culture substrate, not only for engineering 3D cellular organization from stem cells, but also for the toolset toward high resolution imaging using light microscopy. 2 Jr. PIs contributed to this interdisciplinary research through live cell imaging using the holographic and confocal microscopy.

9. **Nanometrology x Supramolecular Chemistry**
"One-step synthesis of one-dimensional supramolecular assemblies composed of helical macromolecular building blocks"
(*J. Am. Chem. Soc., 141 (2019) 13995-14002*, IF: 14.612)
Yuya Wada, Ken-ichi Shinohara, **Hitoshi Asakawa**, Sayaka Matsui, Tetsuya Taima, and Tomoyuki Ikai

This study was supported by the NanoLSI transdisciplinary research promotion grant. The authors demonstrate the one-step synthesis of one-dimensional macromolecular assemblies by simply mixing a glycine-based isocyanide with a nickel catalyst, in which helical constituent polymers are linked end-to-end through multiple hydrogen bonds. Surprisingly, copolymerization with an analogous chiral isocyanide (1 mol %) afforded an almost perfect one-handed helical supramolecular fiber owing to intramolecular/intermolecular dual chiral amplifications. The simplicity and broad applicability of this approach, which can also afford exquisite chiral amplification, enable the creation of a wide variety of functional supramolecular assemblies and provide access to new supramolecular materials.

10. **Nanometrology x Life Science**
Structural and mechanical characteristics of exosomes from osteosarcoma cells explored by 3D-atomic force microscopy
(*Nanoscale,* 13 *(2021)* 6661-6677, IF: 6.895)
Ayhan Yurtsever, **Takeshi Yoshida**, **Arash Badami Behjat**, Yoshihiro Araki, **Rikinari Hanayama** and **Takeshi Fukuma**

The authors employed three-dimensional atomic force microscopy (3D-AFM) to reveal the structural and nanomechanical properties of exosomes at high spatial resolution in physiologically relevant conditions. 3D-AFM force mapping analysis revealed that the exosomes investigated had a considerably high elastic modulus as compared to that obtained for synthetic liposomes. Moreover, malignancy-dependent changes in the exosome mechanical properties were revealed by comparing metastatic and nonmetastatic tumor cell-derived exosomes. The authors found a clear difference in their Young's modulus values, suggesting differences in their protein profiles and other exosomal contents. Therefore, the authors conclude that exosomes derived from metastatic tumor cells carry an exclusive protein content that differs from their nonmetastatic counterparts, and thus they exhibit different mechanical characteristics.

Appendix 1-3
Major Awards, Invited Lectures, Plenary Addresses (etc.) (within 2 pages)

*Prepare the information below during the period from the start of the center through March 2021.

1. Major Awards

*List main internationally-acclaimed awards received/unofficially announced in order from the most recent.
*For each, write the recipient's name, the name of award, and the date issued.
In case of multiple recipients, underline those affiliated with the center.

Date	Recipient's name	Name of award
2020/12/17	Tomoki Ogoshi	17th (FY2020) Japan Society for the Promotion of Science Prize
2020/4/16	Satoru Okuda, Katsuyuki Unoki, Mototsugu Eiraku, Ken-ichi Tsubota	JSME Medal for Outstanding Paper
2020/4/7	Seiji Yano	The Commendation for Science and Technology by the Minister of Education, Culture, Sports, Science and Technology, Awards for Science and Technology
2020/1/29	Yasufumi Takahashi	Nakatani Award（Incentive Prize）
2019/11/8	Takeshi Fukuma	Nanoprobe Technology Committee (JSPS No. 167 Committee) Nanoprobe Technology Award
2019/9/22	Shigehisa Akine	JSCC Award for Creative Work
2019/9/12	Ayumi Sumino	Incentive Award of the 66th Toxin Symposium
2019/5/24	Tomoki Ogoshi	Kao Science Award 2019
2019/4/9	Hitoshi Asakawa	The Commendation for Science and Technology by the Minister of Education, Culture, Sports, Science and Technology, The Young Scientists' Award
2019/2/7	Takeshi Fukuma	15th (FY2018) Japan Society for the Promotion of Science Prize
2018/9/15	Yutaro Yamada, Hiroki Konno, Katsuya Shimabukuro	Biophysics and Physicobiology Editors' Choice Award 2018
2018/5/13	Kazuki Miyata	SSSJ Rising-Researcher Lecture Award
2018/5/13	Kenichi Umeda	SSSJ Young-Researcher Lecture Award
2018/4/18	Mikihiro Shibata	The Commendation for Science and Technology by the Ministry of Education, Culture, Sports, Science and Technology, The Young Scientists' Award
2017/12/26	Noriyuki Kodera	The 13th(FY2017) Japan Society for the Promotion of Science Prize

2. Invited Lectures, Plenary Addresses (etc.) at International Conferences and International Research Meetings

*List up to 20 main presentations in order from most recent.
*For each, write the lecturer/presenter's name, presentation title, conference name and date(s)

Date(s)	Lecturer/Presenter's name	Presentation title	Conference name
2020/12/2	Alexander S. Mikhailov	Simple Mechanics of Protein Machines	20th RIES-HOKUDAI intl. Symposium

2020/11/9	Takeshi Fukuma	Visualizing Inside of 3D Self-Organizing Systems by 3D-AFM	The Virtual Symposium on Scanning Probe Microscopy: Current Status and Future Trends
2020/11/1	Katsuhiro Maeda	Synthesis and Structure of Helical Poly(diphenylacetylene)s	Molecular Chirality Asia 2020
2020/10/29	Noriyuki Kodera	Improving the temporal resolution of high-speed AFM	The 8th Multifrequency AFM Conference
2020/10/27	Toshio Ando	Keynote speech "High-speed AFM for life sciences", **Plenary talk**	The 8th Multifrequency AFM Conference
2020/10/5	Richard Wong	Plenary lecture-The structure and function of nuclear pore complex.	The 4th ICMSTEA (International Conference on Mathematics, Science, Technology, Education and their Application) 2020
2020/7/20	Rikinari Hanayama	High purity isolation and sensitive quantification of extracellular vesicles using affinity to TIM4	International Society for Extracellular Vesicles 2020
2019/12/14	Masanobu Oshima	Multistep tumorigenesis and polyclonal metastasis of colon cancer	24th Annual Meeting of the Korean Society of Cancer Prevention
2019/12/12	Seiji Yano	Drug-tolerant persister cells and AXL	The 24th JFCR-ISCC
2019/7/13	Tomoki Ogoshi	Assembly of Pillar[n]arenes for Molecular Scale Porous Materials	15th International Conference on Calixarenes (Calix 2019)
2019/3/4	Takeshi Fukuma	Three-dimensional AFM imaging of hydration and flexible surface structures at solid-liquid interfaces	APS March Meeting 2019
2019/2/12	Atsushi Hirao	Metabolic Regulation of Stemness in Malignant Hematopoiesis	Eleventh AACR-JCA Joint Conference on Breakthroughs in Cancer Research: Biology to Precision Medicine
2018/10/5	Miki Nakajima	Significance of post-transcriptional regulation of drug-metabolizing enzymes: perspective insight into future pharmacotherapy, **Plenary Lecture**	22nd Microsomal and Drug Oxidation/33rd JSSX
2018/9/10	Toshio Ando	High-speed AFM: Visualizing protein molecules during their functional activity, **Keynote Speech**	19th International Microscopy Congress,
2018/7/31	Mark J. MacLachlan	Coordination Chemistry in Macrocycles, **Plenary Lecture**	ICCC Conference
2018/7/29	Takeshi Fukuma	Visualizing Calcite Growth and Dissolution Processes by High-Speed FM-AFM	Gordon Research Conference on Biomineralization
2018/5/29	Shigehisa Akine	Novel metallo-molecular containers with open/close feature	101st Canadian Chemistry Conference and Exhibition
2018/5/9	Takeshi Fukuma	Recent Progress in Liquid-Environment Frequency Modulation AFM and Its Related Techniques, **Keynote Speech**	The 20th International Scanning Probe Microscopy Conference
2018/4/20	Masanobu Oshima	Comprehensive phenotype characterization of colon cancer with various combination of driver mutations	The 9th International Conference of Asian Pacific Organization for Cancer Prevention
2018/3/14	Adam S. Foster	Molecularly functionalized surfaces and interfaces	DPG Spring Meeting 2018

Appendix 1-4 2020 List of Center's Research Results

Refereed Papers

- List only the Center's papers published in 2020. (Note: The list should be for the calendar year, not the fiscal year.)

(1) Divide the papers into two categories, A and B.
   A. WPI papers
      List papers whose author(s) can be identified as affiliated with the WPI program (e.g., that state "WPI" and the name of the WPI center (WPI-center name)). (Not including papers in which the names of persons affiliated with the WPI program are contained only in acknowledgements.)
   B. WPI-related papers
      List papers related to the WPI program but whose authors are not noted in the institutional affiliations as WPI affiliated. (Including papers whose acknowledgements contain the names of researchers affiliated with the WPI program.)

      Note: On 14 December 2011, the Basic Research Promotion Division in MEXT's Research Promotion Bureau circulated an instruction requiring paper authors to include the name or abbreviation of their WPI center among their institutional affiliations. From 2012, the authors' affiliations must be clearly noted.

(2) Method of listing paper
   - List only refereed papers. Divide them into categories (e.g., original articles, reviews, proceedings).
   - For each, write the author name(s); year of publication; journal name, volume, page(s), and article title. Any listing order may be used as long as format is consistent. (The names of the center researchers do not need to be underlined.)
   - If a paper has many authors (say, more than 20), all of their names do not need to be listed.
   - Assign a serial number to each paper to be used to identify it throughout the report.
   - If the papers are written in languages other than English, underline their serial numbers.
   - Order of Listing
      A. WPI papers
         1. Original articles
         2. Review articles
         3. Proceedings
         4. Other English articles

      B. WPI-related papers
         1. Original articles
         2. Review articles
         3. Proceedings
         4. Other English articles

(3) Submission of electronic data
   - In addition to the above, provide a .csv file output from the Web of Science (e.g.) or other database giving the paper's raw data including Document ID. (Note: the Document ID is assigned by paper database.)
   - These files do not need to be divided into paper categories.

(4) Use in assessments
   - The lists of papers will be used in assessing the state of WPI project's progress.
   - They will be used as reference in analyzing the trends and whole states of research in the said WPI center, not to evaluate individual researcher performance.
   - The special characteristics of each research domain will be considered when conducting assessments.

(5) Additional documents
   - After all documents, including these paper listings, showing the state of research progress have been submitted, additional documents may be requested.

A. WPI papers

1. Original articles

1) Kezilebieke S., Huda M.N., Vaňo V., Aapro M., Ganguli S.C., Silveira O.J., Głodzik S., Foster A.S., Ojanen T., Liljeroth P. "Topological superconductivity in a van der Waals heterostructure", Nature 588 (2020) 424-428 (IF=42.779)

2) Tan S.H., Swathi Y., Tan S., Goh J., Seishima R., Murakami K., Oshima M., Tsuji T., Phuah P., Tan L.T., Wong E., Fatehullah A., Sheng T., Ho S.W.T., Grabsch H.I., Srivastava S., Teh M., Denil S.L.I.J., Mustafah S., Tan P., Shabbir A., So J., Yeoh K.G., Barker N. "AQP5 enriches for stem cells and cancer origins in the distal stomach", Nature 578 (2020) 437-443 (IF=42.779)

3) Fujioka Y., Alam J.M., Noshiro D., Mouri K., Ando T., Okada Y., May A.I., Knorr R.L., Suzuki K., Ohsumi Y., Noda N.N. "Phase separation organizes the site of autophagosome formation", Nature 578 (2020) 301-305 (IF=42.779)

4) Toda S., McKeithan W.L., Hakkinen T.J., Lopez P., Klein O.D., Lim W.A. "Engineering synthetic morphogen systems that can program multicellular patterning", Science 370 (2020) 327-331

(IF=41.846)

5) Cao Y., Wang P.-X., D'Acierno F., Hamad W.Y., Michal C.A., MacLachlan M.J. "Tunable Diffraction Gratings from Biosourced Lyotropic Liquid Crystals", Adv Mater 32 (2020) 1907376 (IF=27.398)
6) Fukuda S., Yan S., Komi Y., Sun M., Gabizon R., Bustamante C. "The Biogenesis of SRP RNA Is Modulated by an RNA Folding Intermediate Attained during Transcription", Mol. Cell 77 (2020) 241-250.e8 (IF=15.584)
7) Nakamura K., Li Q.-Q., Krejčí O., Foster A.S., Sun K., Kawai S., Ito S. "On-Surface Synthesis of a π-Extended Diaza[8]circulene", J. Am. Chem. Soc. 142 (2020) 11363-11369 (IF=14.612)
8) Maeda K., Nozaki M., Hashimoto K., Shimomura K., Hirose D., Nishimura T., Watanabe G., Yashima E. "Helix-Sense-Selective Synthesis of Right- and Left-Handed Helical Luminescent Poly(diphenylacetylene)s with Memory of the Macromolecular Helicity and Their Helical Structures", J. Am. Chem. Soc. 142 (2020) 7668-7682 (IF=14.612)
9) Merget S., Catti L., Piccini G., Tiefenbacher K. "Requirements for Terpene Cyclizations inside the Supramolecular Resorcinarene Capsule: Bound Water and Its Protonation Determine the Catalytic Activity", J. Am. Chem. Soc. 142 (2020) 4400-4410 (IF=14.612)
10) Watanabe-Nakayama T., Nawa M., Konno H., Kodera N., Ando T., Teplow D.B., Ono K. "Self- And Cross-Seeding on α-Synuclein Fibril Growth Kinetics and Structure Observed by High-Speed Atomic Force Microscopy", ACS Nano 14 (2020) 9979-9989 (IF=14.588)
11) Arai S., Takeuchi S., Fukuda K., Taniguchi H., Nishiyama A., Tanimoto A., Satouchi M., Yamashita K., Ohtsubo K., Nanjo S., Kumagai T., Katayama R., Nishio M., Zheng M.-M., Wu Y.-L., Nishihara H., Yamamoto T., Nakada M., Yano S. "Osimertinib Overcomes Alectinib Resistance Caused by Amphiregulin in a Leptomeningeal Carcinomatosis Model of ALK-Rearranged Lung Cancer", J. Thorac. Oncol. 15 (2020) 752-765 (IF=13.357)
12) Kawai S., Krejčí O., Nishiuchi T., Sahara K., Kodama T., Pawlak R., Meyer E., Kubo T., Foster A.S. "Three-dimensional graphene nanoribbons as a framework for molecular assembly and local probe chemistry", Sci. Adv. 6 (2020) eaay8913 (IF=13.117)
13) Alldritt B., Hapala P., Oinonen N., Urtev F., Krejci O., Canova F.F., Kannala J., Schulz F., Liljeroth P., Foster A.S. "Automated structure discovery in atomic force microscopy", Sci. Adv. 6 (2020) : eaay6913 (IF=13.117)
14) Strilets D., Fa S., Hardiagon A., Baaden M., Ogoshi T., Barboiu M. "Biomimetic Approach for Highly Selective Artificial Water Channels Based on Tubular Pillar[5]arene Dimers", Angew. Chem. Int. Ed. 59 (2020) 23213-23219 (IF=12.959)
15) Fa S., Egami K., Adachi K., Kato K., Ogoshi T. "Sequential Chiral Induction and Regulator-Assisted Chiral Memory of Pillar[5]arenes", Angew. Chem. Int. Ed. 59 (2020) 20353-20356 (IF=12.959)
16) Miyairi M., Taniguchi T., Nishimura T., Maeda K. "Revisiting the Polymerization of Diphenylacetylenes with Tungsten(VI) Chloride and Tetraphenyltin: An Alternative Mechanism by a Metathesis Catalytic System", Angew. Chem. Int. Ed. 59 (2020) 14772-14780 (IF=12.959)
17) Mishra S., Mondal A.K., Smolinsky E.Z.B., Naaman R., Maeda K., Nishimura T., Taniguchi T., Yoshida T., Takayama K., Yashima E. "Spin Filtering Along Chiral Polymers", Angew. Chem. Int. Ed. 59 (2020) 14671-14676 (IF=12.959)

18) Dobashi H., Catti L., Tanaka Y., Akita M., Yoshizawa M. "N-Doping of Polyaromatic Capsules: Small Cavity Modification Leads to Large Change in Host–Guest Interactions", Angew. Chem. Int. Ed. 59 (2020) 11881-11885 (IF=12.959)

19) Soto M.A., Carta V., Andrews R.J., Chaudhry M.T., MacLachlan M.J. "Structural Elucidation of Selective Solvatochromism in a Responsive-at-Metal Cyclometalated Platinum(II) Complex", Angew. Chem. Int. Ed. 59 (2020) 10348-10352 (IF=12.959)

20) Fa S., Sakata Y., Akine S., Ogoshi T. "Non-Covalent Interactions Enable the Length-Controlled Generation of Discrete Tubes Capable of Guest Exchange", Angew. Chem. Int. Ed. 59 (2020) 9309-9313 (IF=12.959)

21) Taniguchi T., Yoshida T., Echizen K., Takayama K., Nishimura T., Maeda K. "Facile and Versatile Synthesis of End-Functionalized Poly(phenylacetylene)s: A Multicomponent Catalytic System for Well-Controlled Living Polymerization of Phenylacetylenes", Angew. Chem. Int. Ed. 59 (2020) 8670-8680 (IF=12.959)

22) Zhang D., Soto M.A., Lewis L., Hamad W.Y., MacLachlan M.J. "Host–Guest Chemistry Within Cellulose Nanocrystal Gel Receptors", Angew. Chem. Int. Ed. 59 (2020) 4705-4710 (IF=12.959)

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2. Review articles

None

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169) Turutin A., Temirov A., Kubasov I., Kislyuk A., Malinkovich M., Parkhomenko Y., Erofeev A., Korchev Y. "Nanosized Field-effect Transistor Based on Germanium for Next Generation Biosensors in Scanning Ion-conductance Microscopy", Microsc. Microanal. 26 (2020) 1626-1628 (IF=3.414)

4. Other English articles

None

Appendix 2　FY 2020 List of Principal Investigators

NOTE:

*Underline names of principal investigators who belong to an overseas research institution.

*In the case of researcher(s) not listed in the latest report, attach a "Biographical Sketch of a New Principal Investigator"(Appendix 2a).

*Enter the host institution name and the center name in the footer.

<Results at the end of FY2020> **Principal Investigators Total: 16**

Name	Age	Affiliation (Position title, department, organization)	Academic degree, Specialty	Effort (%)*	Starting date of project participation	Status of project participation (Describe in concrete terms)	Contributions by PIs from overseas research institutions
Center Director Takeshi Fukuma	44	Nano Life Science Institute	Doctor of Engineering, Electrical engineering, Nanometrology	90	October, 2017	usually stays at the institute	
Toshio Ando	70	Nano Life Science Institute	Doctor of Science, Biophysics and Nano-Bioscience	90	October, 2017	usually stays at the institute	
Yuri Korchev	60	Department of Medicine, Imperial College London	Ph.D. in Biophysics and Cytology, Biophysics	30	October, 2017	Under contract, stays at the institute 30 days or more/per fiscal year, but due to COVID-19, participates online	-Engaged in measuring the distribution of substances inside and outside the cell while working toward the development of new nanoprobe technology -In charge of the 2nd NanoLSI International Symposium in London held on November 19, 2018
Atsushi Hirao	57	Cancer Research Institute	Doctor of Medicine, Stem Cell Biology	50	October, 2017	usually stays at the institute	

Masanobu Oshima	59	Nano Life Science Institute	D.V.M., Ph.D., Cancer research, Genetics for Cancer modeling	90	October, 2017	usually stays at the institute	
Seiji Yano	55	Cancer Research Institute	MD, PhD, Medical Oncology, Circumvention of targeted drug resistance	50	October, 2017	usually stays at the institute	
Kunio Matsumoto	62	Nano Life Science Institute	Doctor of Philosophy, Biological Chemistry, Tumor Biology	90	October, 2017	usually stays at the institute	
Rikinari Hanayama	46	Nano Life Science Institute	MD, PhD, Immunology, Cell Biology	90	October, 2017	usually stays at the institute	
Richard W. Wong	46	Nano Life Science Institute	Doctor of Medicine, Molecular cell biology	90	October, 2017	usually stays at the institute	
Miki Nakajima	51	Nano Life Science Institute	Doctor of Pharmaceutical Sciences, Drug Metabolism and Toxicology, Clinical Pharmacology	90	October, 2017	usually stays at the institute	

Shigehisa Akine	48	Nano Life Science Institute	Doctor of Science, Supramolecular chemistry, Coordination chemistry	90	October, 2017	usually stays at the institute	
Katsuhiro Maeda	50	Nano Life Science Institute	Doctor of Engineering, Polymer chemistry	90	October, 2017	usually stays at the institute	
Tomoki Ogoshi	44	Graduate School of Engineering, Kyoto University / Nano Life Science Institute, Kanazawa Univeristy	Doctor of Engineering, Supramolecular Chemistry, Structural Organic Chemistry	20	October, 2017	Works at the institute 20% of the total working days / per year based on the cross-appointment agreement between Kyoto univ. and Kanazawa univ.	-Engaged in development of supramolecular nanoprobes while working toward the development of new nanoprobe technology -Engaged in establishment of Nanoprobe Life Science based on supramolecular chemistry
Mark MacLachlan	47	Department of Chemistry, University of British Columbia	PhD in Chemistry	30	October, 2017	Under contract, stays at the institute 30 days or more/per fiscal year, but due to COVID-19, participates online	-Engaged in development of supramolecular nanoprobes while working toward the development of new nanoprobe technology - In charge of the 3rd NanoLSI International Symposium held on August 8, 2019 at UBC
Adam Stuart Foster	45	Department of Applied Physics, Aalto University	PhD in Theoretical Solid State Physics	30	October, 2017	Under contract, stays at the institute 30 days or more/per fiscal year, but due to COVID-19, participates online	-Promotes understanding of the real image from the observation image while working toward the development of new nanoprobe technology -In charge of NanoLSI Educational Program at the Graduate School - In charge of Selection Committee of Jr.PI

Alexander S. Mikhailov	70	Department of Physical Chemistry, Fritz Haber Institute of the Max Planck Society	Doctor of Science, Theoretical Physics, Chemical Physics, Biophysics	40	October, 2017	Under contract, stays at the institute 90 days or more/per fiscal year, but due to COVID-19, stays 78 days	-Promotes understanding of the real image from the observation image while working toward the development of new nanoprobe technology -In charge of NanoLSI Educational Program at the Graduate School -In charge of Selection Committee of Jr.PI - In charge of the 5th NanoLSI International Symposium which will be held in FY2021

*Percentage of time that the principal investigator devotes to his/her work for the center vis-à-vis his/her total working hours.

Principal investigators unable to participate in project in FY 2020

Name	Affiliation (Position title, department, organization)	Starting date of project participation	Reasons	Measures taken

Appendix 3-1 FY 2020 Records of Center Activities

1. Researchers and other center staffs, satellites, partner institutions

1-1. Number of researchers and other center staffs

* Fill in the number of researchers and other center staffs in the table blow.
* Describe the final goals for achieving these numbers and dates when they will be achieved described in the last "center project."

a) Principal Investigators

(full professors, associate professors or other researchers of comparable standing)

(number of persons)

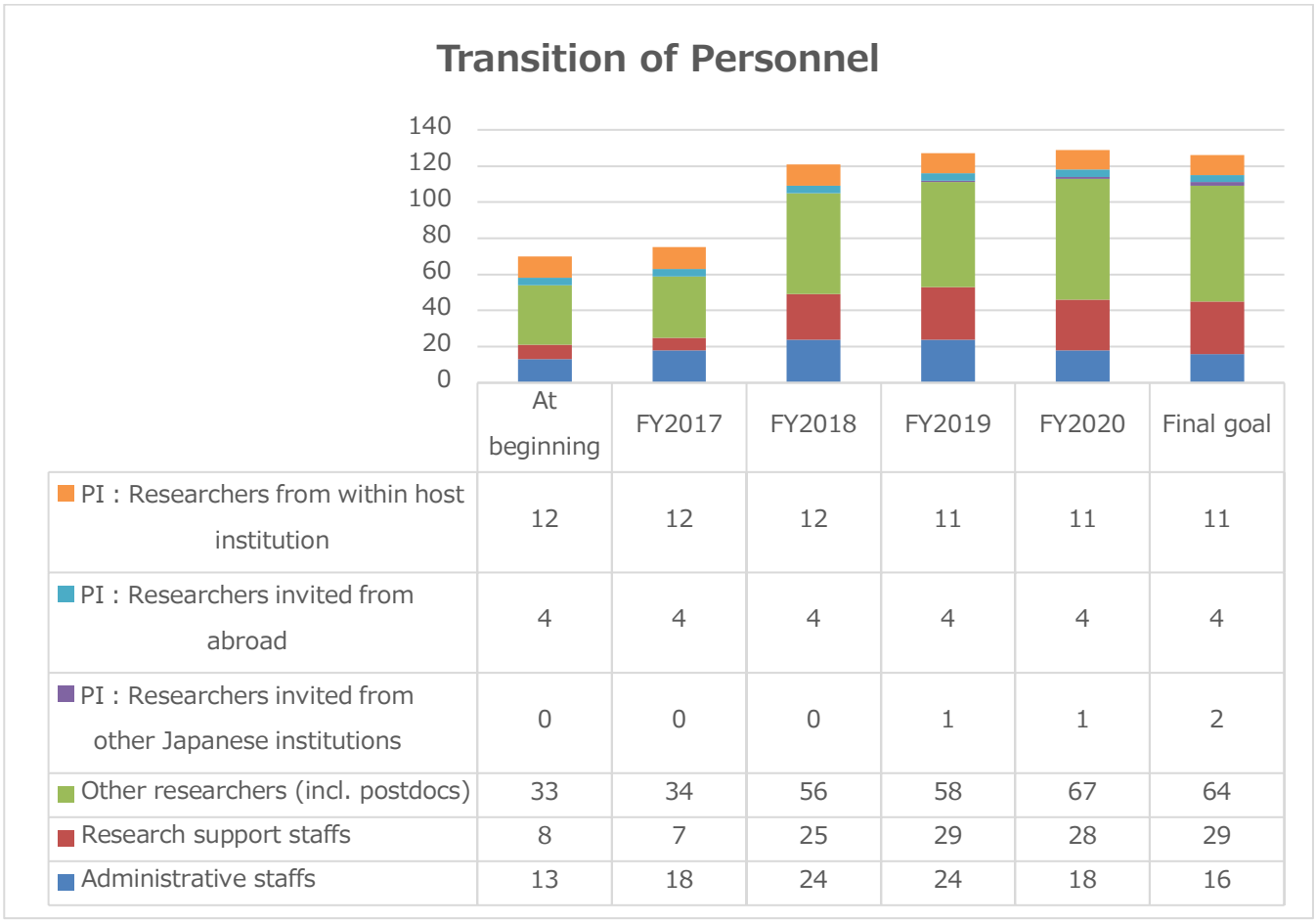
	At the beginning of project	At the end of FY 2020	Final goal (Date:March, 2024)
Researchers from within the host institution	12	11	11
Researchers invited from abroad	4	4	4
Researchers invited from other Japanese institutions	0	1	2
Total principal investigators	16	16	17

b) Total members

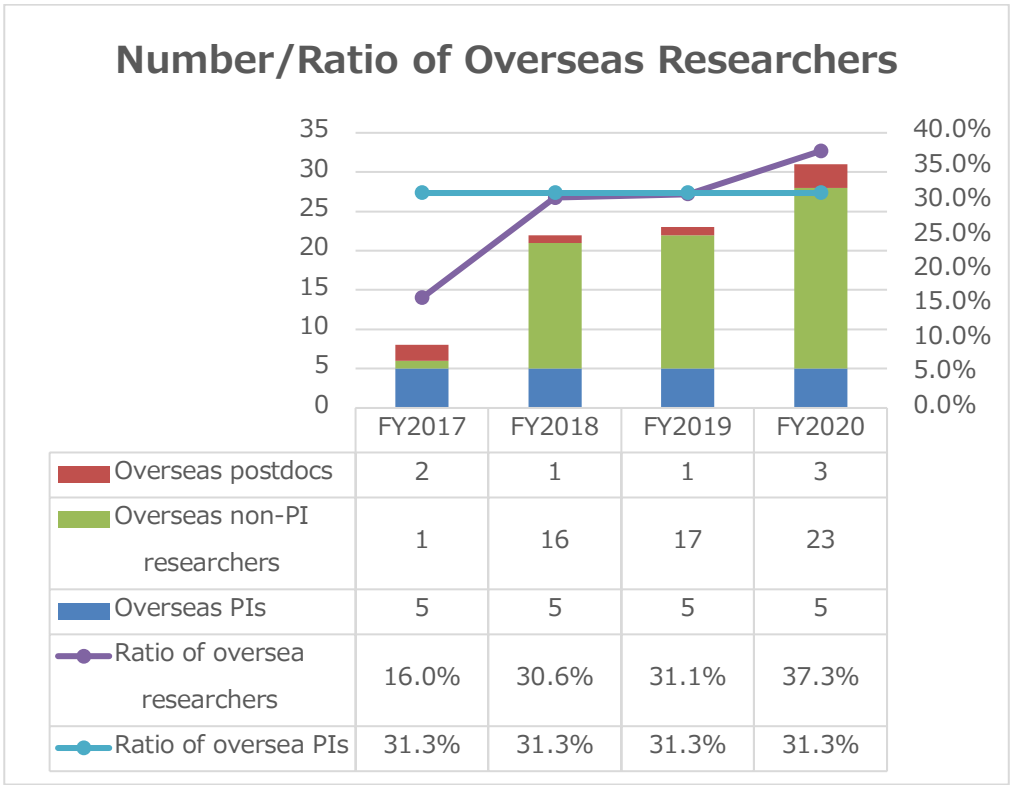
	At the beginning of project		At the end of FY 2020		Final goal (Date:March, 2024)	
	Number of persons	%	Number of persons	%	Number of persons	%
Researchers	49		83		81	
Overseas researchers	8	16	31	37	31	38
Female researchers	6	12	11	13	17	21
Principal investigators	16		16		17	
Overseas PIs	5	31	5	31	5	29
Female PIs	1	6	1	6	2	12
Other researchers	27		64		63	
Overseas researchers	1	4	23	36	25	40
Female researchers	5	19	10	16	15	24
Postdocs	6		3		1	
Overseas postdocs	2	33	3	100	1	100
Female postdocs	0	0	0	0	0	0
Research support staffs	8		28		29	
Administrative staffs	13		18		16	
Total number of people who form the "core" of the research center	70		129		126	

Appendix 3-2　Annual Transition in the Number of Center Personnel

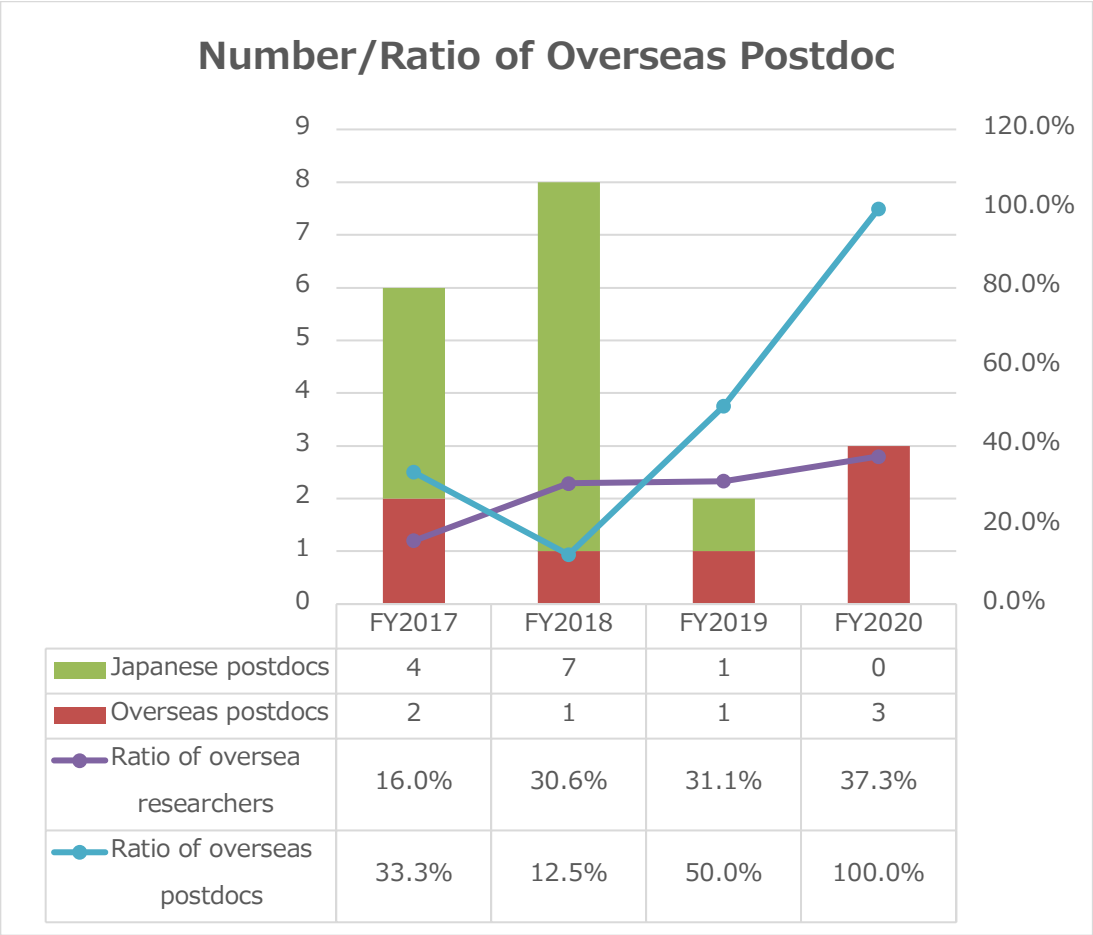
*Make a graph of the annual transition in the number of center personnel since the start of project.



	At beginning	FY2017	FY2018	FY2019	FY2020	Final goal
PI : Researchers from within host institution	12	12	12	11	11	11
PI : Researchers invited from abroad	4	4	4	4	4	4
PI : Researchers invited from other Japanese institutions	0	0	0	1	1	2
Other researchers (incl. postdocs)	33	34	56	58	67	64
Research support staffs	8	7	25	29	28	29
Administrative staffs	13	18	24	24	18	16

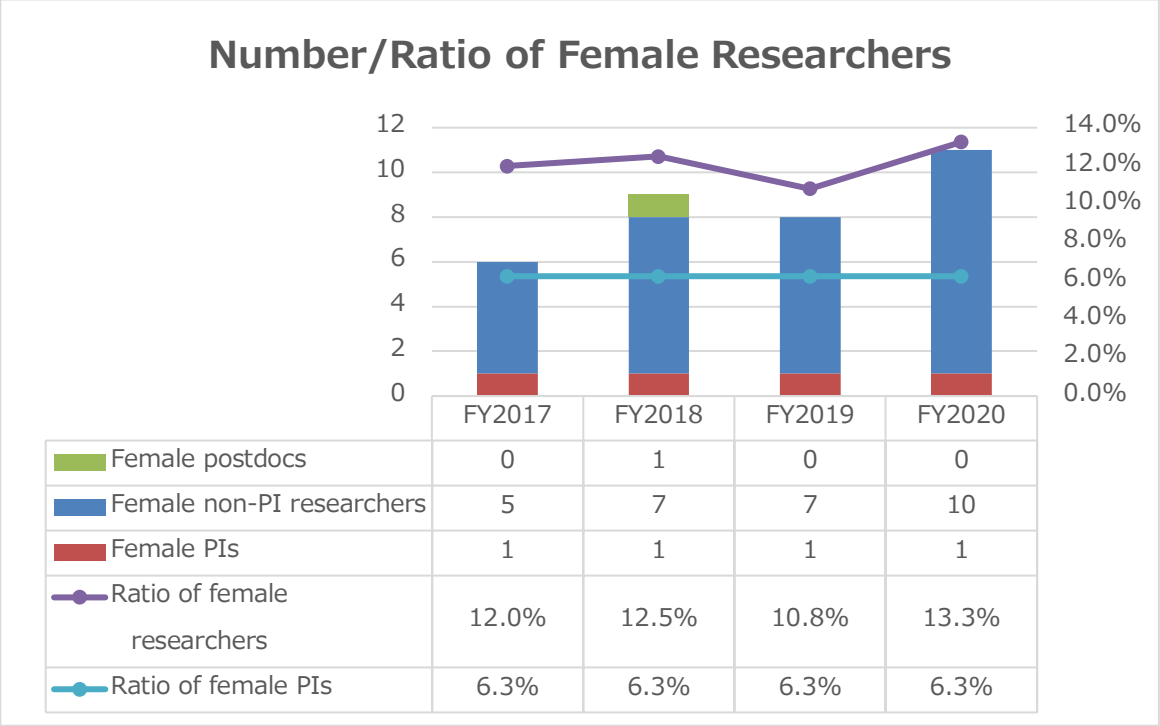


	FY2017	FY2018	FY2019	FY2020
Overseas postdocs	2	1	1	3
Overseas non-PI researchers	1	16	17	23
Overseas PIs	5	5	5	5
Ratio of oversea researchers	16.0%	30.6%	31.1%	37.3%
Ratio of oversea PIs	31.3%	31.3%	31.3%	31.3%



	FY2017	FY2018	FY2019	FY2020
Japanese postdocs	4	7	1	0
Overseas postdocs	2	1	1	3
Ratio of oversea researchers	16.0%	30.6%	31.1%	37.3%
Ratio of overseas postdocs	33.3%	12.5%	50.0%	100.0%

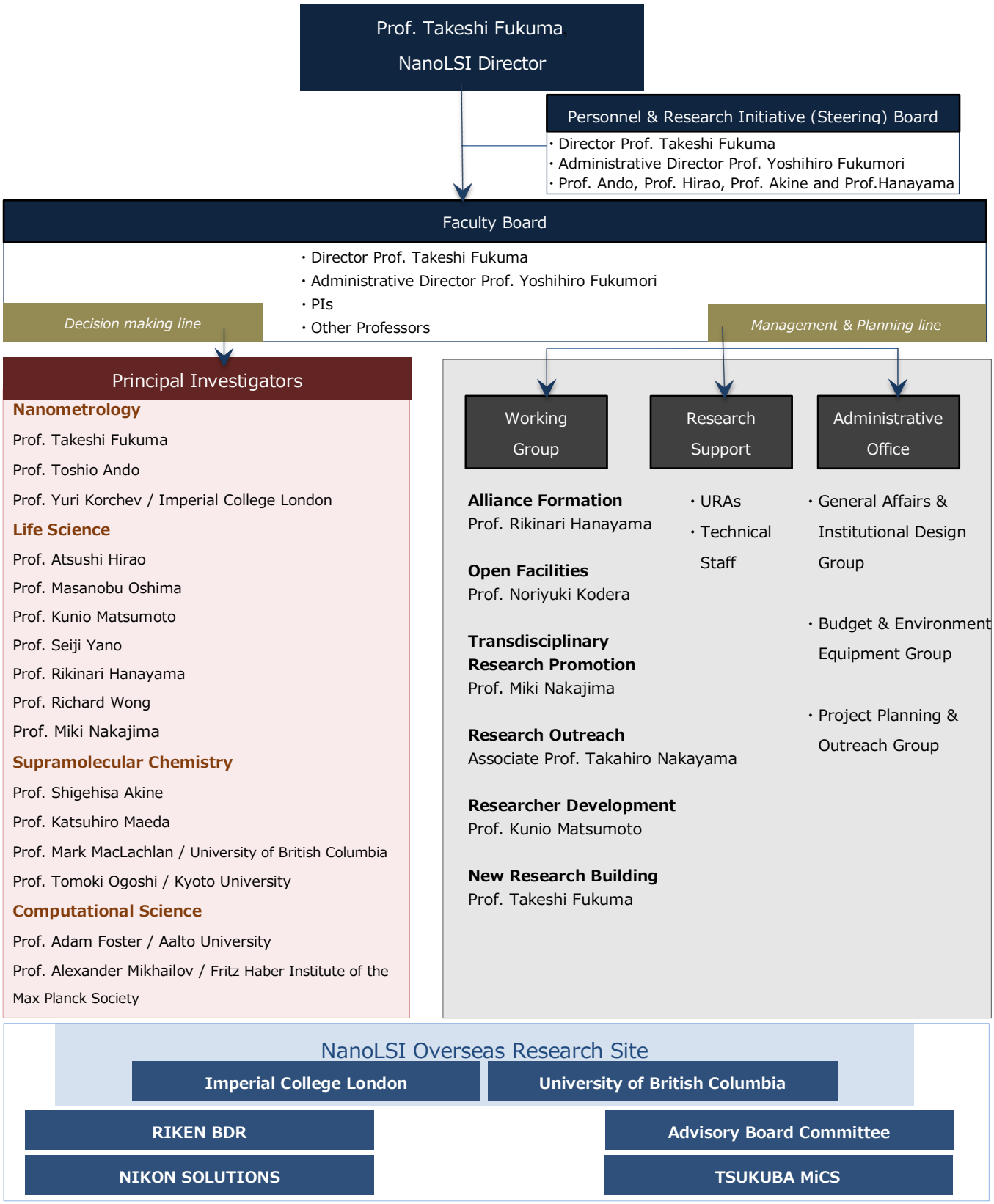
The figure above shows the number of employees in postdoctoral researcher positions. NanoLSI employs overseas postdocs, who have a sufficient level of research experience, as specially-appointed assistant professors, whose three-year term can be renewed. The total number of overseas postdocs in the postdoctoral researcher position and the specially-appointed assistant professor position was 3 in FY2017, 17 in FY2018, 17 in FY2019, and 24 in FY2020. These numbers represent the actual numbers of overseas postdocs employed in NanoLSI.



	FY2017	FY2018	FY2019	FY2020
Female postdocs	0	1	0	0
Female non-PI researchers	5	7	7	10
Female PIs	1	1	1	1
Ratio of female researchers	12.0%	12.5%	10.8%	13.3%
Ratio of female PIs	6.3%	6.3%	6.3%	6.3%

Appendix 3-3　Diagram of Management System

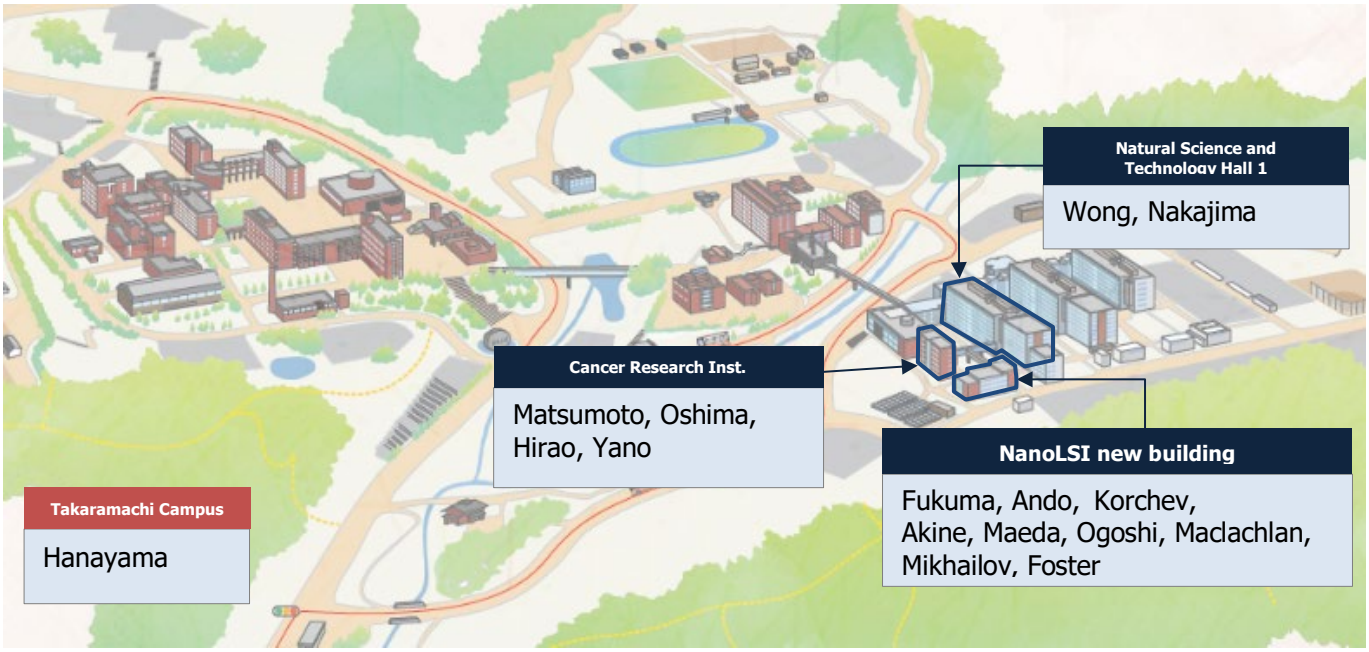
- Diagram the center's management system and its position within the host institution in an easily understood manner.
- If any changes have been made in the management system from that in the latest "center project" last year, describe them. Especially describe any important changes made in such as the center director, administrative director, head of host institution, and officer(s) in charge at the host institution (e.g., executive vice president for research).



At the beginning of FY2020, NanoLSI was officially positioned in the statutes of KU as an independent institute of KU. According to the KU regulation concerned, NanoLSI established Faculty Board consisting of PIs and professors. The faculty board meeting is held every month to build a consensus among the members.

Appendix 3-4　Campus Map

- Draw a simple map of the campus showing where the main office and principal investigator(s) are located.



Wong, Nakajima and Hanayama have their offices and laboratories in both NanoLSI new building and in other buildings.

Appendix 3-5 Project Expenditures in FY2020

1) Overall project funding

* In the "Total costs" column, enter the total amount of funding required to implement the project, without dividing it into funding sources.
* In the "Amount covered by WPI funding" column, enter the amount covered by WPI within the total amount.
* In the "Personnel," "Project activities," "Travel," and "Equipment" blocks, the items of the "Details" culumn may be changed to coincide with the project's actual content.

(Million yens)

Cost items	Details (For Personnel - Equipment please fill in the breakdown of fiscal expenditure, and the income breakdown for Research projects.)	Total costs	Amount covered by WPI funding
Personnel	Center director and administrative director	35.9	21.0
	Principal investigators (no. of persons):12	161.5	50.4
	Junior Principal investigators (no. of persons):6	63.2	55.4
	Other researchers (no. of persons):61	279.4	184.2
	Research support staff (no. of persons):9	40.6	39.1
	Administrative staff (no. of persons):19	106.2	53.0
	Remuneration for RA(Research Assistant)	16.5	16.5
	Subtotal	703.3	419.6
Project activities	Gratuities and honoraria paid to invited principal investigators (no. of persons):3	4.9	4.9
	Research startup cost (no. of persons):12	44.9	44.9
	Cost of satellite organizations (no. of satellite organizations):2	18.6	18.6
	Cost of international symposiums (no. of symposiums):1	5.2	5.2
	Facility expenses	26.3	6.4
	Cost of consumables	64.2	42.8
	Cost of utilities	15.6	6.6
	Other costs	245.3	36.0
	Subtotal	425.0	165.4
Travel	Domestic travel costs	0.7	0.7
	Overseas travel costs	0.0	0.0
	Travel and accommodations cost for invited scientists		
	(no. of domestic scientists):0	0.0	0.0
	(no. of overseas scientists):1	0.6	0.6
	Travel cost for scientists on transfer		
	(no. of domestic scientists):4	0.5	0.5
	(no. of overseas scientists):4	2.1	2.1
	Subtotal	3.9	3.9
Equipment	Depreciation of buildings	0.0	0.0
	Depreciation of equipment	6.3	6.3
	Subtotal	6.3	6.3
Research projects (Detail items must be fixed)	Project supported by other government subsidies, etc. *1	112.2	0.0
	KAKENHI	191.2	0.0
	Commissioned research projects, etc.	175.7	0.0
	Joint research projects	43.0	0.0
	Ohers (donations, etc.)	33.0	0.0
	Subtotal	555.1	0.0
Total		1,693.6	595.2

Costs (Million yens)

WPI grant in FY 2020	700
Costs of establishing and maintaining facilities	0
Establishing new facilities	0
Repairing facilities	0
Others	0
Costs of equipment procured	111.1
Fume hood	56.2
Fluorescence microscope	14.1
Atomic layer deposition equipment	6.8
Temperature regulator	7.9
Ion milling apparatus	14.3
Others	11.8

*1. Funding sources that include government subsidies (including Enhancements promotion expenses (機能強化促進経費), National university reform reinforcement promotion subsidy (国立大学改革強化推進補助金) etc.), indirect funding, and allocations from the university's own resources.
*2 When personnel, travel, equipment (etc.) expenses are covered by KAKENHI or under commissioned research projects or joint research projects, the amounts should be entered in the "Research projects" block.

2) Costs of satellites

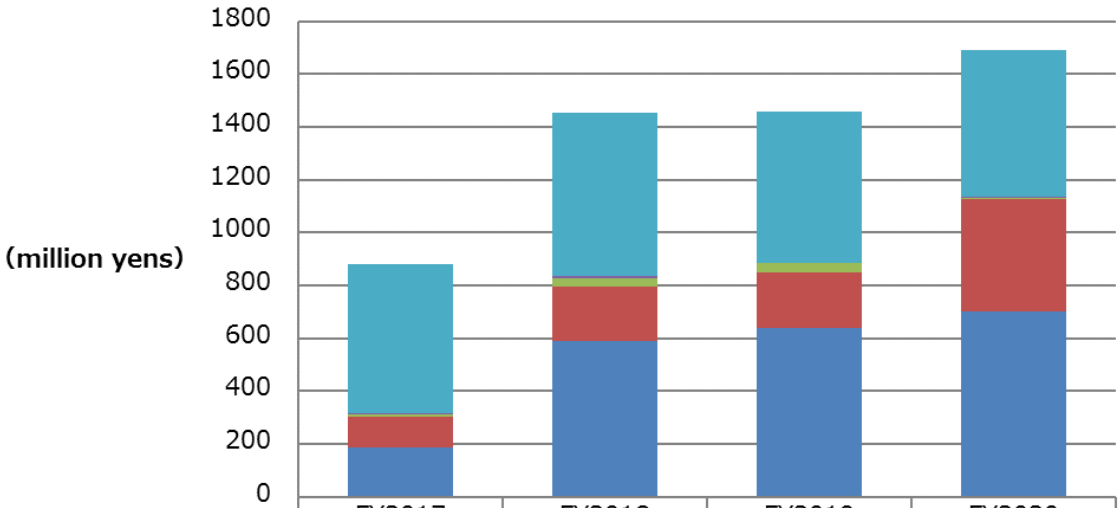
(Million yens)

Cost items	Details	Total costs	Amount covered by WPI funding
Personnel	Principal investigators (no. of persons):1		
	Other researchers (no. of persons):4		
	Subtotal	14.3	14.3
Project activities	Subtotal	1.9	1.9
Travel	Subtotal		
Equipment	Subtotal		
Research projects	Subtotal		
Total		16.2	16.2

Appendix 3-6　Annual Transition in the Amounts of Project Funding

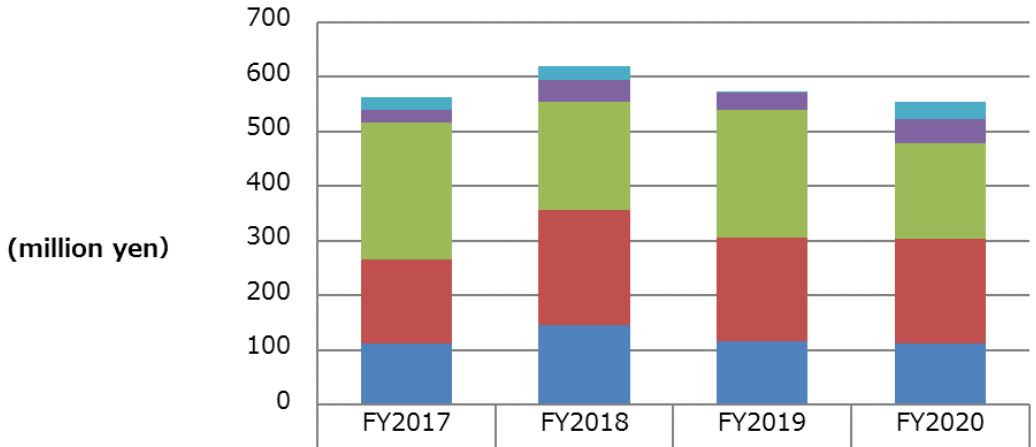
*Make a graph of the transition in the number of overall project funding.

Transition of Project Expenditures



	FY2017	FY2018	FY2019	FY2020
Research Projects	563	619	573	555
Equipment	6	6	3	6
Travel	8	33	33	4
Project Activities	119	207	211	425
Personnel	185	589	640	703

Transition of Research Project Expenditures



	FY2017	FY2018	FY2019	FY2020
Ohers (donations, etc.)	24	26	2	33
Joint research projects	22	38	32	43
Commissioned research projects, etc.	251	198	234	176
KAKENHI	154	212	189	191
Project supported by other government subsidies, etc. *1	112	145	116	112

*1 Definition is as shown in Appendix 3-5 (Project Expenditures)

Appendix 4-1 FY 2020 Status of Collaboration with Overseas Satellites

- If satellite and partner institutions have been established, fill in required items of the form below.

1. Satellites and partner institutions

- List the satellite and partner institutions in the table below (including the domestic satellite institutes).
- Indicate newly added and deleted institutions in the "Notes" column.

<Satellite institutions>

Institution name	Principal Investigator(s), if any	Notes
Imperial College London	Yuri E. Korchev	
University of British Columbia	Mark J. MacLachlan	

< Partner institutions>

Institution name	Principal Investigator(s), if any	Notes

- If overseas satellite institutions have been established, fill in required items on the form below. If overseas satellite institutions have not been established, it is not necessary to complete the form.

2. Coauthored Papers

- List the refereed papers published in FY 2020 that were coauthored between the center's researcher(s) in domestic institution(s) (include satellite institutions) and overseas satellite institution(s). List them by overseas satellite institution in the below blocks.
- Transcribe data in same format as in Appendix 1-4. Italicize the names of authors affiliated with overseas satellite institutions.
- For reference write the Appendix 1-4 item number in parentheses after the item number in the blocks below. Let it free, if the paper is published in between Jan.-Mar. 2021 and not described in Appendix 1-4.

Overseas Satellite 1 **Imperial College London** (Total: 2 papers)

1) (Appendix 1-4 #49) Vaneev A.N., Gorelkin P.V., Garanina A.S., Lopatukhina H.V., Vodopyanov S.S., Alova A.V., Ryabaya O.O., Akasov R.A., Zhang Y., Novak P., Salikhov S.V., Abakumov M.A., Takahashi Y., Edwards C.R.W., Klyachko N.L., Majouga A.G., *Korchev Y.E.*, Erofeev A.S. "In Vitro and in Vivo Electrochemical Measurement of Reactive Oxygen Species after Treatment with Anticancer Drugs", Anal. Chem. 92 (2020) 8010-8014 (IF=6.785)

2) Kolmogorov V., Erofeev A., Woodcock E., Efremov Y., Iakovlev A., Savin N., Alova A., Lavrushkina S., Kireev I., Prelovskaya A., Sviderskaya E., Scaini D., Klyachko N., Timashev P., Takahashi Y., Salikhov S., Parkhomenko Y., Majouga A., Edwards C., Novak P., *Korchev Y.*, and Gorelkin P. "Mapping mechanical properties of living cells at nanoscale using intrinsic nanopipette–sample force interactions", Nanoscale 13 (2021) 6558-6568 (IF=6.895)

Overseas Satellite 2 **University of British Columbia** (Total: 0 papers)

3. Status of Researcher Exchanges

- Using the below tables, indicate the number and length of researcher exchanges in FY 2020. Enter by institution and length of exchange.
- Write the number of principal investigator visits in the top of each space and the number of other researchers in the bottom.

Overseas Satellite 1: Imperial College London

In FY2020, there were no researcher exchanges due to COVID-19.

<To satellite>

	Under 1 week	From 1 week to 1 month	From 1 month to 3 months	3 months or longer	Total
FY2020					0
					0

<From satellite>

	Under 1 week	From 1 week to 1 month	From 1 month to 3 months	3 months or longer	Total
FY2020					0
					0

Overseas Satellite 2: University of British Columbia

In FY2020, there were no researcher exchanges due to COVID-19.

<To satellite>

	Under 1 week	From 1 week to 1 month	From 1 month to 3 months	3 months or longer	Total
FY2020					0
					0

<From satellite>

	Under 1 week	From 1 week to 1 month	From 1 month to 3 months	3 months or longer	Total
FY2020					0
					0

Appendix 4-2 FY 2020 Visit Records of Researchers from Abroad

* If researchers have visited/ stayed at the Center, provide information on them in the below table.
* Enter the host institution name and the center name in the footer.

Total: 6

	Name	Age	Affiliation		Academic degree, specialty	Record of research activities (Awards record, etc.)	Time, duration	Summary of activities during stay at center (e.g., participation as principal investigator; short-term stay for joint research; participation in symposium)
			Position title, department, organization	Country				
1	Arin Marchesi	37	Postdoctoral Fellow, UCSF, Adhesion & Inflammation Lab, Aix-Marseille Université	France	PhD in Neurobiology	Publications: Structural titration of receptor ion channel GLIC gating by HS-AFM	2020/4/1 -present	Assistant professor at NanoLSI
2	Prem Kumar Viji Babu	29	PHD Researcher, Institute of Biophysics, University of Bremen	Germany	PhD, in Natural Sciences		2020/11/13-present	Postdoctoral Researcher at NanoLSI
3	Alexander S. Mikhailov	70	NanoLSI PI / Professor, Fritz Haber Institute of the Max Planck Society	Germany	Doctor of Science/ Theoretical Physics, Chemical Physics, Biophysics	May 2009:Solvay Foundation, Belgium International Solvay Chair in Chemistry 2009 November 1993:Humboldt Foundation, Germany Humboldt Research Award	2020/12/7-2021/3/25	Participation as principal investigator at NanoLSI
4	Feng Li	31	Postdoctoral researcher, Ecole Polytechnique Fédérale de Lausanne (EPFL)	Switzerland	Ph.D, in Chemistry	April 2015-April 2016:JSPS Research Fellowship for Young Scientists (DC2) October 2011-March 2015:Japanese Government (MEXT) Scholarship November 2015: Poster award "Chemistry Letters Young Awards" at 45th Congress of Heterocyclic Chemistry April 2013: Poster award at 4th UK/Japan Conference in Catalytic Asymmetric Synthesis	2020/12/1 -present	Assistant professor at NanoLSI
5	Zhang Yanjun	51	Senior Research Associate, Division of Medicine, Imperial College London	United Kingdom	PhD, in Medicine	January 2019-December 2022: The development of functionalized nanopore extended field effect transistor biosensor and its applications to label-free real-time quantitate detection of single nanoscale brainderived mitochondrial microvesicle, National Natural Science Foundation of China (No. 31870990). April 2014-March 2017: New technical methods for qualitative and quantitative detection of platelet activation and PMPs in stroke prevention, diagnosis and treatment. Tianjin Science and Technology Support Program of China (No.14ZCZDSY00020). April 2013-March 2016: Application HPICM to investigate the relationship between PMP formation and coagulopathy after TBI. Tianjin Natural Science Foundation of China (No.13JCYBJC21900). January 2010-December 2012: Nongenomic effects of aldosterone on regulating renal epithelial sodium channel activity by ATP autocrine / paracrine. National Natural Science Foundation of China (No. 30971184).	2020/12/18 -present	Assosiate professor at NanoLSI
6	Sivashanmugan Kundan	33	Research Fellow, School of Electrical Engineering and Computer Science Oregon State University	United States of America	PhD, in Philosophy	June 2020:Project Awarded by National Institute of Food and Agriculture, USA May 2017:Best Paper Award, IEEE International Conference on Applied System Innovation, Japan November 2014: Selected as Faraday Discussion Candidate, Exploiting Physical Properties of Nanomaterials for Tackling Infectious Diseases-2014, Hanoi, Vietnam September 2013-August 2014:College of Engineering Best International Student Award, National Cheng Kung University, Taiwan September 2014:Best Poster Award, 6th PCGMR/NCKU Symposium on Nano-Technology/Material for Future Devices- 2014, Taiwan	2020/12/18 -present	Assistant professor at NanoLSI

Appendix4-3 Postdoctoral Positions through Open International Solicitations

* In the column of number of applications and number of selection, put the total number (upper), the number and percentage of overseas researchers in the < > brackets (lower).

Fiscal year	number of applications	number of selection
FY 2017	126*	17*
	<88 ,69.8 %>	< 14, 82.4 %>
FY 2018	53*	3*
	<39 , 73.6 %>	< 2, 66.7 %>
FY 2019	34*	11*
	<23 , 67.6%>	<8 , 72.7%>
FY 2020	43*	3*
	<42 , 97.7%>	< 3, 100%>

＊Announced positions were all specially appointed assistant professors in accordance with the host institution, KU's policy of hiring postdoctoral researchers internationally as specially appointed assistant professors.

Kanazawa University

Nano Life Science Institute

Appendix 4-4 Status of Employment of Postdoctoral Researchers

Enter the information below during the period from the start of the center through the end of FY 2020.

- For each person, fill in the spaces to the right. More spaces may be added.
- Leave "Position as of April 2021" blank if unknown.
- Enter the host institution name and the center name in the footer.

Japanese Postdocs

Employment period	Position before employed at WPI center		Next position after WPI center		Position as of April 2021*	
	Position title, organization	Country where the organization is located	Position title, organization	Country where the organization is located	Position title, organization	Country where the organization is located
April 1, 2018 to October 12, 2018.	Postdoctral Researcher, Kanazawa University	Japan	Postdoctoral Associate,Weill Cornell Medicine	USA	Research Associate,Weill Cornell Medicine	USA
October 1, 2018 to March 31, 2019.	PhD Program Student, Kanazawa University	Japan	Assistant Professor, Kanazawa University	Japan	Assistant Professor, Kanazawa University	Japan
January 1, 2019 to october 31, 2019.	Assistant Professor, Kanazawa University	Japan	Assistant Professor, Kanazawa University	Japan	Assistant Professor, Kanazawa University	Japan

Overseas Postdocs

Employment period	Position before employed at WPI center		Next position after WPI center		Position as of April 2021*		Nationality
	Position title, organization	Country where the organization is located	Position title, organization	Country where the organization is located	Position title, organization	Country where the organization is located	
*August 1, 2018 to May 31, 2019.	Postdoctral research assistant, Iowa State University	USA	Assistant Professor,Kyoto University	Japan	Assistant Professor,Kyoto University	Japan	China
*June 1, 2018 to May 31, 2019.	Postdoctral researcher, University of Santiago	Spain	Postdoctral researcher, Rennes Institute of Chemical Sciences, University of Rennes 1	France	Postdoctral researcher, Rennes Institute of Chemical Sciences, University of Rennes 1	France	Spain
*August 20, 2018 to July 31, 2019.	Postdoctral researcher, VU University Amsterdam	Netherlands	Lecturer, China Pharmaceutical University	China	Lecturer, China Pharmaceutical University	China	China
*June 1, 2018 to June 30, 2020.	Postdoctral researcher, Max-Planck-Institute for Iron Research	Germany	Technical Engineer, Shenzhen HUASUAN Tech. Co., Ltd.	China	Technical Engineer, Shenzhen HUASUAN Tech. Co., Ltd.	China	China
*August 1, 2018 to July 31, 2020.	Postdoctral researcher, Swiss Federal Laboratories for Materials Science and Technology	Switzerland	Scientist ,Swiss Federal Institute of Technology in Lausanne	Switzerland	Scientist ,Swiss Federal Institute of Technology in Lausanne	Switzerland	Spain

* Those researchers who were employed as specially appointed assistant professors through international announcements in accordance with the host institution, KU's policy of hiring postdoctoral researchers internationally as specially appointed assistant professors.

Appendix4-5 List of the Cooperative Research Agreements with Overseas Institutions

*Prepare the information below during the period from the beginning of the Center through March 2021.

1. Name of an Agreement: Collaborative Research Agreement
   Dates of an Agreement: January 15, 2019
   Counterpart of an Agreement: Imperial College of Science, Technology and Medicine
   Summary of an Agreement:

Essential points of the Agreement are extracted as follows:

With reference to funding to support the project entitled "Nanopipette biosensors for single-cell analysis, imaging and selective biosensing", (the "Project"), awarded by the Japanese Government under the "World Premier International Research Center Initiative" (WPI), to Kanazawa University and its Nano Life Science Institute, the Parties hereby confirm their intention to regulate their rights and obligations in accordance with the terms and conditions contained in this agreement.

1. DEFINITIONS:
Kanazawa's Supervisor: Professor Takeshi Fukuma, Director, Nano Life Science Institute
Principal Investigator: Professor Yuri Korchev

2. THE PROJECT:
Imperial will carry out the tasks allotted to it in Schedule 2 (research plan of the Project), and will provide the human resources, materials, facilities that are designated as its responsibility in Schedule 2, and this will be limited to the extent allowed by the Financial Contribution paid by Kanazawa. The Project will be managed under the direction and supervision of the Principal Investigator.

3. FINANCIAL CONTRIBUTION:
Kanazawa will pay the Financial Contribution (set out in Schedule 1) to Imperial in accordance with the Detailed Regulation of Accounting attached to this Agreement in Schedule 1 b).

4. USE ABD WXPLOITATION OF INTELLECTUAL PROPERTY:
Any Results which are generated by the Parties jointly and for which it is impossible to segregate each Party's intellectual contribution to the creation of such Results shall be referred to in this Agreement as "Joint Results". Any Joint Owner of any of the Joint Results may commercially exploit the Joint Results upon consultation and agreement with the other Joint Owner. In such circumstances, the Party which is commercially exploiting the Joint Results will pay the other Joint Owner a fair and reasonable royalty rate/revenue on the value of any products or processes commercially exploited by it which incorporate any Joint Results.

2. Name of an Agreement: Funded Project Agreement
   Dates of an Agreement: April 1, 2019
   Counterpart of an Agreement: The University of British Columbia
   Summary of an Agreement:

Essential points of the Agreement are extracted as follows:

A. The Japanese Government has authorized the KU and its Nano Life Science Institute as "World Premier International Research Center Initiative" (WPI), providing grants for the KU in order to accelerate international joint projects, train young scientists, and improve its international visibility towards establishment of a new research field: Nanoprobe Life Science.

B. The KU wishes to set up the funds at UBC for the purpose of supporting Prof. Mark MacLachlan and his laboratory, so as to deem the laboratory as a satellite laboratory of the Nano Life Science Institute.

C. The UBC is willing to accept and administer the funds and to make available for the objectives above its premises and facilities.

2 DIFINITIONS:
In this Agreement, “Proposal” shall mean the project activities attached hereto and marked as Schedule “C” (research plan).

3 UBC OBLIGATIONS:
The UBC will carry out the project activities through Professor Mark MacLachlan and Research Associate in accordance with acceptable research standards, the research policies of the UBC, in accordance with the Proposal and applicable laws of Canada.

4 KU OBLIZATIONS:
The KU shall pay UBC the total sum of <@55,258 for Research Associate’s remuneration plus 11,750 for basic expenditures plus 16,752 for overhead cost> Canadian dollars (CDN$ 83,760) per each fiscal year.

5 PROPERTY RIGHTS:
IP developed jointly by the KU personnel and the UBC personnel shall be jointly owned by the KU and the UBC. The parties hereto shall, in good faith and reasonable manner, negotiate the jurisdiction and the right of handling.

Appendix4-6 Holding International Research Meetings

* Indicate up to two of most representative international research conferences or symposiums each financial year and give the number of participants using the table below.

FY2017-FY2018: 8 meetings

Date	Meeting title and Place held	Number of participants
2017.11.8-9	International Symposium on Atomic Force Microscopy at Solid-Liquid Interfaces Kanazawa Tokyu Hotel, Kanazawa	From domestic institutions: 65 From overseas institutions: 11
2018.11.19	The 2nd NanoLSI International Symposium – Towards Establishment of New Research Field: Nanoprobe Life Science–The Cumberland Hotel, London	From domestic institutions: 16 From overseas institutions: 37

FY2019: 9 meetings

Date	Meeting title and Place held	Number of participants
2019.8.8	The 3rd NanoLSI International Symposium – Supramolecular Chemistry and Nanoprobes in Life Sciences – The University of British Columbia, Vancouver	From domestic institutions: 17 From overseas institutions: 55
2020.1.15-17	MLM2020 "The 1st International Conference on Big Data and Machine Learning in Microscopy" *Organized by Adam Foster(Oversea PI) Kanazawa University Satellite Plaza, Kanazawa	From domestic institutions: 15 From overseas institutions: 25

FY2020: 3meetings

Date	Meeting title and Place held	Number of participants
2020.11.26-27	4th NanoLSI Symposium - Bio-imaging, sensing and manipulation for medical science – Online	From domestic institutions: 253 From overseas institutions: 21
2021.2.23	Kanazawa-UBC Vancouver Online Joint Seminar in Supramolecular Chemistry *Organized by Mark J. MacLachlan (Oversea PI) and Shigehisa Akine（PI） Online	From domestic institutions: 23 From overseas institutions: 17

Appendix 5 List of Achievements of Center's Outreach Activities between FY 2017 – 2020

* Using the table below, show the achievements of the Center's outreach activities from FY2017 through FY2020 (number of activities, times held).
*If there are any rows on activities the center didn't implement, delete that (those) row(s). If you have any activities other than the items stated below, fill in the space between parentheses after "Others" on the bottom with the name of those activities and state the numbers of activities and times held in the space on the right. A row of "Others" can be added, if

Activities	FY2017 (number of activities, times held)	FY2018	FY2019	FY2020
PR brochure, pamphlet	5	6	5	4
Lectures, seminars for the general public	1	4	9	2
Teaching, experiments, training for elementary, secondary and high school students	N/A	2	1	1
Open house	5	N/A	10	7
Participating, exhibiting in events	2	6	4	2
Press releases	4	19	16	23
Others (TV program, Video, Article)	2	1	15	7

Appendix 5 List of Media Coverage of Projects Carried out between FY 2017 – 2020

* Select main items of press releases, media coverage, and reports for FY 2017-2020 (especially by overseas media)

1) Japan

No.	Date	Type of the media (e.g., newspaper, magazine, television)	Description
1	2017.9.26-10.2	Newspaper（9）	Adoption for WPI (Nikkei Shimbun, Nikkei Sangyo Shimbun, Engineering and Construction News, Hokuriku Chunichi Shimbun, Hokkoku Shimbun)
2	2018.9.15-11.2	Newspaper (7)	Public lecture series by Profs. Takeshi Fukuma, Tomoki Ogoshi and Masanobu Oshima (Yomiuri Shimbun)
3	2019.1.17-3.22	Newspaper (5), Television (2)	Research results on origin of resistance to lung-cancer drug by Prof. Seiji Yano and Assoc. Prof. Shinji Takeuchi (Mainichi Shimbun, Yomiuri Shimbun, Nikkei Sangyo Shimbun, Hokkoku Chunichi Shimbun, Hokkoku Shimbun, NHK, Ishikawa Television)
4	2019.5.18-6.6	Newspaper (7), Magazine (1)	Research result on HGF-inhibitory macrocyclic peptide by Assist. Prof. Katsuya Sakai, Assoc. Prof. Mikihiro Shibata and Prof. Kunio Matsumoto (Mainichi Shimbun, Hokuriku Chunichi Shimbun, Nikkei Sangyo Shimbun, Nikkan Kogyo Shimbun, Kagaku Shimbun, Shizuoka Shimbun, Hokkoku Shimbun, Nikkei Biotech Magazine)
5	2020.12.15-12.21	Newspaper (5)	The completion ceremony for the new building of the Nano Life Science Institute (Mainichi shinbun, Hokurikuchunichi shinbun,Hokkoku shinbun, The Daily Engineering & Construction News, THE DAILY INDUSTRIAL NEWS)

2) Overseas

No.	Date	Type of the media (e.g., newspaper, magazine, television)	Description
1	2018.4.27-5.8	Website (56)	Research results on protein stabilizing blood-cell production under dietary stress by Prof. Atsushi Hirao and Assist. Prof. Yuko Tadokoro (The Medical News)
2	2018.6.17	Website (50)	Research results on the role of lipids in facilitating a functional switch between two forms of Peroxiredoxin by Profs. Noriyuki Kodera, Toshio Ando and Assoc. Prof. Hiroki Konno (BioTech Gate)
3	2018.12.28, 2019.1.10	Website (19)	Research results on self-sorting through molecular geometries by Prof. Tomoki Ogoshi, Assist. Prof. Takahiro Kakuta, Assoc. Prof. Hitoshi Asakawa and Prof. Takeshi Fukuma (Phys.org, BioTech Gate, Azonano)
4	2019.1.21-1.30	Website (17)	Research results on a closed cage-like molecule that can be opened by Prof. Shigehisa Akine (Phys.org, Azonano, Nanotechnology Now)

5	2019.1.28, 1.29	Website (18)	Research results on artificial HGF by Prof. Kunio Matsumoto and Assist. Prof. Katsuya Sakai (USA Life Science Database, Phys.org, BioTech Gate, Technology Networks)
6	2019.5.18-6.6	Website (19)	Research result on HGF-inhibitory macrocyclic peptide by Assist. Prof. Katsuya Sakai, Assoc. Prof. Mikihiro Shibata and Prof. Kunio Matsumoto (Phys.org)
7	2019.12.3-12.25	Website (17)	Research result on high resolution electrochemical mapping of hydrogen evolution reaction on transition metal dichalcogenide nanosheets by Assoc. Prof. Yasufumi Takahashi (Azonano, Phys.org)
8	2019.12.3-12.29	Website (30)	Research result on high-resolution label-free 3D mapping of extracellular pH of single living cells by Assoc. Prof. Yasufumi Takahashi and Prof. Yuri Korchev (OPTRONICS ONLINE, Phys.org, The Medical News)
9	2019.12.17	Website (18)	Research result on possible strategy for cancer treatment found in nuclear transport proteins by Assist. Profs. Masaharu Hazawa, Kee Siang Lim, Prof. Richard W. Wong (The Medical News)
10	2019.12.27-2020.1.10	Website (25)	Research result on complete filling of batches of nanopipettes by Dr. Linhao Sun, Dr. Kazuki Shigyou, Prof. Toshio Ando, Assist. Prof. Shinji Watanabe (Phys.org, Chem Europe)
11	2020.6.30-7.6	Website (19)	Research result on Spatiotemporally tracking of nano-biofilaments inside the nuclear pore complex core by Prof. Richard Wong (Pharmabiz.com)
12	2020.7.24-8.3	Website (19)	Research result on Self- and Cross-Seeding on α-Synuclein Fibril Growth Kinetics and Structure Observed by High-Speed Atomic Force Microscopy by Assoc. Prof.Takahiro Nakayama (Phys.org)
13	2020.7.31-8.8	Website (16)	Research result on Wideband Magnetic Excitation System for Atomic Force Microscopy Cantilevers with Megahertz-Order Resonance Frequency by Prof.Takeshi Fukuma (Nanotechnology Now)
14	2020.9.16-10.6	Website (18)	Research result on Transient IGF-1R inhibition combined with osimertinib eradicates AXL-low expressing EGFR mutated lung cancer by Prof. Seiji Yano (Pharmabiz.com)
15	2020.10.15-11.5	Website (17)	Research result on Engineering Synthetic Morphogen Systems that Can Program Multicellular Patterning by Asst. Prof. Satoshi Toda (Jotup)
16	2021.1.4-1.11	Website (20)	Research result on Direct visualization of translational GTPase factor pool formed around the archaeal ribosomal P-stalk by high-speed AFM by Prof. Noriyuki Kodera and Dr. Hirotatsu Imai (Lab Manager)
17	2020.12.4-12.29	Website (20)	Research result on Structural and dynamics analysis of intrinsically disordered proteins by high-speed atomic force microscopy by Prof. Toshio Ando (Phys.org)
18	2021.2.8-2.16	Website (23)	Research result on Malignant subclone drives metastasis of genetically and phenotypically heterogenous cell clusters through fibrotic niche generation by Prof. Masanobu Oshima (GEN)

Appendix6-1 Host Institution's Commitment (Fund, Personnel)

1. Contributions from host institution

(1) Fund, Personnel

* Regarding "Fund" entry, describe with reference to the items in the Progress Report (Jisseki-hokoku-sho) based on Article 12 of the Grant Guidelines (Kofu-yoko).

* Don't include competitive funding obtained by researchers (used as research project funding)

(FY 2017-2020)

<Fund> **(million yen)**

Fiscal Year	2017	2018	2019	2020
Personnel	121	572.7	539.3	490.9
Faculty members	93.5	399.4	393.3	411.4
Full-time	93.5	393.2	393.3	411.4
Concurrent	0	6.2	0	0
Postdocs	0	0	0	0
RA etc.	0	0	0	0
Research support staffs	0	18.8	21.3	7.7
Administrative staffs	27.5	154.5	124.7	71.8
Full-time	27.1	140.5	99.9	71.8
Concurrent	0.4	14	24.8	0
Project activities	0	19	22	239
Travel	0	0	0	0
Equipment	0	0	0	* 697
Research projects	0	67	67	67
Total	121	658.7	628.3	1493.9

<Personnel> **(person)**

Fiscal Year	2017	2018	2019	2020
Personnel	59	60	61	55
Faculty members	39	40	41	42
Full-time	38	39	40	41
Concurrent	1	1	1	1
Postdocs	0	0	0	0
RA etc.	0	0	0	0
Research support staffs	0	3	3	1
Administrative staffs	20	17	17	12
Full-time	**19	16	15	12
Concurrent	***1	1	2	0

* "Equipment" fund of FY2020 consists of the KU's own expense for a new NanoLSI research building and its equipment deployment.

**Of Full-time, 14 are employed with a WPI subsidy (50%) and KU funds (50%).

**Of Full-time, 5 are employed with a WPI subsidy (80%) and KU funds (20%).

***Of Concurrent, one is employed with a WPI subsidy (90%) and KU funds (10%).

Appendix6-1 Host Institution's Commitment

1. Contributions from host institution

(2) Provision of land and/or building(s), lab space, etc.

From October 2017, when NanoLSI was inaugurated, to October 2020, when the new NanoLSI Research Building was completed, the host institution, Kanazawa University (hereafter referred to as KU), provided the temporary 3-story NanoLSI research building with a floor area of 3000 m^2. At the same time, the space charge fee for this research building was exempted. For research space outside of the temporary research building above, researchers who had been working at KU before the inauguration of NanoLSI could continue to use their laboratories and offices until the completion of the new NanoLSI Research Building in October 2020.

The new NanoLSI Research Building has a total floor area of 6840 m^2 and consists of a basement and 4 floors above ground. Researchers in the fields of nanometrology, life science, supramolecular chemistry, and computational science form a research core under one roof. The facility is equipped with 65 scanning probe microscopes, 6 electron microscopes, an animal room and a treatment room, and a total of 3 laboratories that allow P2 level experiments to be performed.

2. System under which the center's director is able to make substantive personnel and budget allocation decisions

The host institution, KU, revised the statutes in April 2020 to make NanoLSI an independent institute, and clarified that the Center Director has personnel rights and budget execution rights related to NanoLSI. Prior to that, KU regarded NanoLSI as an independent institute and effectively approved the Center Director's personnel and budget execution rights.

3. Support for the center director in coordinating with other departments at host institution when recruiting researchers, while giving reasonable regard to the educational and research activities of those departments

NanoLSI has been regarded as an independent institute since the inauguration in FY2017, and researchers have been hired independently through international recruitment. Thus, there is no record of hiring researchers jointly with other institutes/colleges within KU. On the other hand, young NanoLSI researchers have a track record of engaging in graduate school education in other institutes/colleges as a part of their career paths.

4. Revamping host institution's internal systems to allow introducing of new management methods

(e.g., English-language environment, merit-based pay, cross appointment, top-down decision making unfettered by conventional modes of operation)

-Evaluation-dependent annual salary system

Following the success of the evaluation-dependent annual salary system implemented by NanoLSI in FY2018, the host institution, KU, has revised its personnel salary system, combining performance evaluation with the annual salary system, to be applied to all faculty members (researchers) hired in FY2019 and later.

-Organizational reform

The establishment and success of NanoLSI, which was inaugurated in FY2017, helped KU establish the Nanomaterials Research Institute in FY2018 and the Advanced Manufacturing Technology Institute in FY2019. This, along with the reorganization of the existing Cancer

Research Institute, led to the development of a group of institutes featuring fused (transdisciplinary) research, and contributed to the reorganization of KU, which aims at cross-disciplinary education and fused research.

-Research assistant system for master course students

Based on the proposal of the Center Director to apply the research assistant system not only to doctorate students but also to master's students in order to expand financial support for students and foster young researchers, KU revised the relevant regulations and implemented this as a system reform for NanoLSI as well as for the entire university.

-Living support for overseas researchers and their families

The administrative staff experience at NanoLSI summarized the kinds of support provided for overseas researchers and their families in English in a PowerPoint report, made a to-do list, and provided this to KU. KU intends, as a policy, to disseminate the experience and know-how of NanoLSI's support for overseas researchers to the entire KU through rotation of administrative staff.

5. Utilities and other infrastructure support provided by host institution

(*In addition to those listed in the item 1. "Contributions from host institution")

KU exempted the space charge fee of about 20 million yen for the temporary research building (floor area, 3000 m^2) described above. KU provides NanoLSI with 10 million yen per year as a subsidy for utilities and maintenance costs in the new research building (total floor area, 6840 m^2).

6. Support for other types of assistance

-Provision of Budgets

From FY2017 to FY2021, KU provided 60 million yen per year for research, use of which is left to the discretion of the Center Director. KU is also committed to paying 60 million research budgets per year during the second half of the WPI grant period (FY2022-F2026).

-Research Professorship and Allocation of Personnel

The Research Professorship, KU's own research-focused promotion system, was preferentially applied to NanoLSI full-time researchers. At the same time, administrative staff with high English and administrative abilities were prioritized for NanoLSI.

-NanoLSI's New Research Building

With the strong leadership of the President, KU negotiated with MEXT to secure a budget of 1.5 billion yen for the construction of the new NanoLSI Research Building. Together with the contribution of KU, 650 million yen, a total budget of 2.15 billion yen was secured, and the construction of the new NanoLSI Research Building (research space, 6840 m^2) was completed in FY2020.

-Junior PI Program

In order to secure excellent young researchers who are expected to form the next generation, NanoLSI has earmarked 6 tenure posts at the President's discretion for hiring young researchers as tenure-track Jr. PIs.

-Graduate School "Division of Nano Life Science"

In order to foster future generations of NanoLSI researchers, the graduate school enrollment capacity for the entire KU was consolidated and the Graduate School of Frontier Science Initiative, Division of Nano Life Science was launched. In addition, the KU Foundation provided scholarships of ¥50,000 per month for a master's program student and ¥100,000 per month for a doctorate program student of the Graduate School of Frontier Science Initiative, Division of Nano Life Science.

-Independence of NanoLSI

The statutes of KU were revised to make NanoLSI an independent institute and the personnel and budget execution rights of the Center Director were established.

-Top Leaders' Meeting

To support the leadership of the Center Director, a monthly meeting of the President and the Center Director was held to help make quick decisions and implement plans for NanoLSI operations.

Appendix6-2 The Host Institution's Mid-term Plan

* Excerpt the places in the host institution's "Mid-term objectives" and/or "Mid-term plan" that clearly show the positioning of the WPI center within its organization.

Excerpts from the "Kanazawa University Third-term (FY2016-FY2021) Medium-term objectives/Medium-term plans"

"Medium-term objectives"

[5] KU promotes advanced and original research and enhances basic research.

[6] KU aims to strengthen the research implementation mechanism to establish a research institution with the highest international credentials.

"Medium-term plans"

[5-1] Based on the comprehensive research strategy originally formulated by KU during the 2nd Medium-term, KU promotes strong and characteristic research such as research on cancer metastasis/drug resistance mechanisms and advanced medical development to overcome nutrition-related diseases, nanotechnology using innovative atomic force microscopy technology, cultural resource science, and innovative material development by supramolecular chemistry in a systematic and intensive manner through the on-campus COE system (Kanazawa University Chozen and Sakigake projects).

[6-5] With the aim of obtaining a fundamental understanding of the mechanisms of various life phenomena at the nano level, the "Nano Life Science Institute", adopted by the World Premier International Research Center Initiative (WPI) will be established, and a system will be created and operated to fully demonstrate the independent management of the Institute.