World Premier International Research Center Initiative (WPI)
FY 2021 WPI Project Progress Report (The center selected in and before FY2020)

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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 2022) situation of your WPI center.
* So as to execute this fiscal year's follow-up review on the "last" center project plan, prepare this report based on it.
* Use yen (¥) when writing monetary amounts in the report. If an exchange rate is used to calculate the yen amount, give the rate.

➢ Prepare this report within 10-20 pages (excluding the appendices, and including Summary of State of WPI Center Project Progress (within 2 pages)).

Summary of State of WPI Center Project Progress (write within **2 pages**)

1. Research Progress

At NanoLSI, we have been working on three major projects: (1) the development of novel nanoprobe technologies especially for live-cell imaging, (2) nano-level understanding of basic cellular functions and cancer, and (3) the establishment of a new research field "nanoprobe life science" (Fig. 1).

(1) Development of novel nanoprobe technologies

Imaging at surfaces and inside of living cells: So far, we have largely demonstrated the proof of concept of these new techniques and are now improving their speed, resolution, and functionality. **Fukuma** optimized the design of needle probes and cell membrane penetration conditions (***Sci. Rep.*** **2021**) and published the first paper on intra-cellular imaging by nanoendoscopy (***Sci. Adv.*** **2021**). **Ando & Watanabe** developed a high-speed SICM technique to visualize dynamic changes in the nanoscale surface structures and elasticity distribution at live cell surfaces and clarified their dependence on the cancer progression induced by gene alterations (***Biomaterials*** **2022**).

Nanoendoscopic analysis and manipulation: We tackled the development of fundamental technologies of nanoendoscopy enabling us to analyze and manipulate the spatiotemporal dynamics of chemicals and physicochemical properties at a subcellular scale. Using SICM, **Korchev & Takahashi** succeeded in the delivery of chemicals at a specific position in a cell and the visualization of heterogeneous stiffness of the cytoskeleton occurring at the nanoscale (***Nanoscale*** **2021**). **Ogoshi** synthesized a new artificial biosensor with a high binding constant (ca. 700 times higher than the previous biosensor) toward a vitamin metabolite. **Akine, Maeda, & MacLachlan** developed new molecular sensors and machines. **Arai** generated the library of near infrared light absorbing dyes for nano-manipulation to reveal and control cellular functions in live cells (***ACS Nano*** **2022**).

Modeling & simulation for nano life science: To provide a theoretical understanding of the AFM data, several mathematical models have been developed for multiscale structures such as proteins, cell membranes, chromosomes, and cell populations. **Foster & Hall** developed a new coarse-grained model of cell membrane dynamics to understand AFM data *(**BPPB*** **2022***)*. **Sumikama** developed a new theory based on polymer physics for 3D-AFM and applied it to chromosome models during inter and mitotic phases. **Flechsig** developed a unique method to reconstruct full 3D atomistic structures from AFM topographic images (***PLoS Comput. Biol.*** **2022**). **Okuda** proposed a new conceptual model that explains how cells collectively migrate as a cluster in 3D space (***Biophys J*** **2022**) .

(2) Nano-level understanding of cellular functions and cancer

Bio-SPM research by the life science PIs using HS-AFM, 3D-AFM, or bio-SICM is becoming increasingly productive. Meanwhile, based on the roadmaps presented last year, we actively explored various applications of the newly developed live-cell imaging techniques.

Basic cell functions: Using HS-AFM, **Matsumoto & Shibata** revealed a dynamic structure for the whole HGF-MET complex and acquired a variety of scaffold proteins that bind to MET (***Nat Commun 2021***, ***iScience*** **2021**). **Wong** visualized autophagy induction by a nuclear pore complex protein TPR (***Autophagy*** **2021**) and also captured the interaction between SARS-CoV-2 spike and ACE2 receptor through a collaboration with **Ando & Hanayama** (***J Extracell Vesicles*** **2021**). Using 3D-

AFM, **Hanayama & Fukuma** obtained detailed information on the structural and mechanical characteristics of extracellular vesicles (***Nanoscale* 2021**). **Toda & Fukuma** started a new project to visualize the intracellular part of a cell-cell junction with In-Cell AFM technology.

Cancer research: **Oshima** demonstrated that non-metastatic cells can metastasize with metastatic cells by a "polyclonal metastasis" mechanism (***Nat Commun* 2021**), and found specific physical characteristics of the metastatic organoid cells by SICM (***Biomaterials* 2022**). **Yano** discovered a novel therapeutic approach of *ALK*-rearranged lung cancer with p53 co-mutations (***Clin Cancer Res* 2021**), and **Hirao** found important autophagic regulations for stem cell homeostasis (***Sci Rep* 2021**). **Nakajima** developed DNA aptamers to suppress cancer cell proliferation (***ACS Appl Mater Inter* 2022**). These results have promoted further collaborations with experts in nanotechnology, leading to a deep understanding of the nature of cancer-specific abnormalities.

(3) Establishment of the novel research field "Nanoprobe Life Science"

Extending Capabilities of Various Bio-SPM Techniques: To maintain our current world-leading position in Nanoprobe Life Science, we have been improving the performance and functionality of our cutting-edge bio-SPM technologies. **Ando & Kodera** developed several high-speed AFM (HS-AFM) techniques to further improve the scanning speed and low-invasiveness: an only trace imaging combined with dynamic feedback control, a differential-based ultrafast amplitude detector (***Appl. Phys. Lett.* 2021**), an ultrafast piezoelectric Z-scanner with a resonance frequency above 1.1 MHz (***Rev. Sci. Instrum.* 2022**), a resonance-controller for the Z-scanner, a new excitation system for ultra-small cantilevers. Through these activities, we are now approaching the final goal of achieving V_{max} around 100 fps. **Shibata & Matsumoto** developed a new technique to identify molecular species in samples composed of heterogeneous molecular species by using a macrocyclic peptide-conjugated tip and phase contrast imaging (***ACS Appl. Mater. Interfaces* 2021**).

Bio-SPM Collaborative Research on Various Life Phenomena: To lead the development of the Nanoprobe Life Science field, we worked on various transdisciplinary collaborations among the four major disciplines: nanometrology, life science, supramolecular chemistry and computational science. The published examples include Bio-SPM studies of channelrhodopsins (***Cell* 2022**), intrinsically disordered proteins (***Nat. Nanotech.* 2021**), Mycoplasma's internal motors (***mBio* 2021**) and endogenous production of H_2O_2 in living cells (***Biosens. Bioelectron.* 2021**); SPM studies of organic molecular systems such as cyclic polymers **(*J. Am. Chem. Soc.* 2021)** and 2D carbon allotrope (***Science* 2021**); and development of an AI-based SPM data analysis method (***ACS Nano* 2022**).

2. Generating Fused Disciplines

Both top-down and bottom-up approaches have been taken to promote fused disciplines. The top-down set out and executed three priority research themes, and the bottom-up supported interdisciplinary research by teams consisting of young researchers.

3. Realizing an International Research Environment

The total number of papers by 16 PIs in 2017-2021 was 591, of which 272 (46%) were internationally co-authored papers. The total number of papers co-authored by one of the four overseas PIs and the resident Japanese researchers in NanoLSI has reached 17 since 2017. Various measures have been executed such as outreach programs for external researchers, mobility and career path for young researchers, diversity of the NanoLSI researchers, and support for young foreign researchers to acquire research funds.

4. Making Organizational Reforms

The successful reforms of NanoLSI have been continued such as research professorships for concentrating on research, a rigorous evaluation-based salary system, integrated management of NanoLSI and the Graduate School "Division of Nano Life Science," the tenure-track junior PI program, and English-based administration.

5. Efforts to Secure the Center's Future Development over the Mid- to Long-term

The total amount of external funds acquired in FY2021 by 81 NanoLSI researchers was 1,288 million yen (1,005 million yen in FY2020). Deployment of 22 tenured or tenure-track researchers at NanoLSI has been completed.

6. Others

The textbook "High-Speed Atomic Force Microscopy in Biology" written by Prof. Toshio Ando was published by Springer Nature.

* Describe clearly and concisely the progress being made by the WPI center project from the viewpoints below.
- In addressing the below-listed 1-6 viewpoints, place emphasis on the following:
  (1) Whether research is being carried out at a top world-level (including whether research advances are being made by fusingdisciplines).
  (2) Whether a proactive effort continues to be made to establish itself as a "truly" world premier international research center.
  (3) Whether a steadfast effort is being made to secure the center's future development over the mid- to long-term.

1. Advancing Research of the Highest Global Level

* Among the research results achieved by the center, concretely describe those that are at the world's highest level. In Appendix 1, list the center's research papers published in 2021.
* Regarding the criteria used when evaluating the world level of center, note any updated results using your previous evaluation criteria and methods or any improvements you have made to those criteria and methods.

Outline

Continued from the last year, we worked on the three projects shown in Fig. 1.

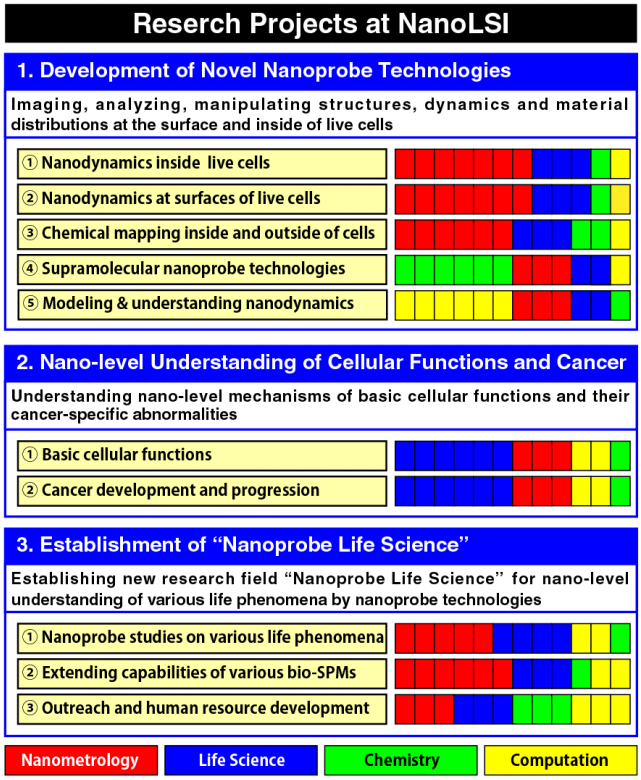

Fig. 1: Research projects at NanoLSI and contributions from the four major disciplines to each project.

(1) We have been developing bio-SPM technologies for visualizing structures, dynamics, and material distribution at the surfaces and inside living cells. So far, we have largely demonstrated their proof of concepts. In particular, we published the first paper on intra-cellular imaging by nanoendoscopy AFM (***Sci. Adv.*** **2021**), highlighting the success of our flagship project. In addition, we are making significant efforts to improve the speed, resolution, and functionalities of these techniques. One of the achievements is the 700-fold sensitivity improvement of our originally designed metabolite sensor.

(2) We have been investigating nanoscale mechanisms of cellular functions and cancer using bio-SPM techniques. Life science research with our world-leading bio-SPM technologies such as HS-AFM, 3D-AFM, and bio-SICM by life science PIs is becoming increasingly productive. In particular, even the cancer researchers and pharmaceutical scientists, who initially had great difficulties finding nanoscale research targets, now started to produce impactful findings. In addition, based on the roadmaps presented in the last site visit, we made considerable efforts to apply the developed techniques to life science research. The first example of such application was published in FY2021: the elucidation of nanoscale changes in the cell surface mechanics and dynamics induced by the cancer progression revealed by HS-AFM (***Biomaterials*** **2022**).

(3) We aim to establish the "Nanoprobe Life Science" field by creating a world-leading center for bio-SPM collaborations. Despite the COVID-19 outbreak, we actively performed bio-SPM collaborations with external researchers and published many impactful papers. Meanwhile, we added a new subject in project (3): "② Extending capabilities of various bio-SPMs," where we aim to improve the performance and functionality of our unique bio-SPM technologies. In particular, we aim to improve the speed of HS-AFM from 10 fps to 100 fps to visualize even faster dynamic events. With these efforts, we will maintain a world-leading position in the bio-SPM research field.

Achievements in FY2021 are summarized as follows.
- Papers: 132 (42% internationally co-authored; 33 with an IF > 10; 54 with an IF > 7),
- Invited talks in int'l meetings: 48,
- Funding: ¥1,207,926,629 overall (30 grants > ¥10,000,000).

These achievements are of the highest global level for an institute with 81 researchers (as of March 2022).

(1) Development of techniques for measuring nanodynamics on the cell surface and interior

(Development of nano-imaging techniques)

·Measurement of nanodynamics on cell surfaces:

We developed a method to simultaneously measure dynamic changes in topography and mechanical properties of living cells based on our high-speed scanning ion conductance microscopy (HS-SICM) technique. We applied this technique to genotype-defined mouse intestinal tumor-derived cells to investigate the relationships between gene mutations and colon cancer phenotypes (Fig. 2a). The tumor cells with mutations of Apc (A), Kras (K), Tgfbr2 (T), Trp53 (P), and Fbxw7 (F) in various combinations were examined in our study. We found that high-metastatic cancer-derived cells carrying AKT mutations (AKT, AKTP, and AKTPF) formed ridge-like structures on their plasma membrane with actively changing in their cell volume, which were not found in low-metastatic and adenoma-derived cells. Furthermore, the elastic modulus was significantly lower in the metastatic AKT-type cancer cells than those obtained in other genotype cells (Fig. 2b). Surprisingly, a principal component analysis using RNAseq-expression profiles showed distributions similar to those in physical properties, suggesting a correlation between genetic alterations and physical properties (Fig. 2c, d). We also confirmed that such malignant cell-specific physical properties were found in human colon cancer-derived cells. These results demonstrated that the HS-SICM analysis is useful as a novel diagnostic strategy for predicting the metastatic ability of cancer cells (***Biomaterials*** **2022**). Now we are trying to extend our nanomechanical measurement with HS-SICM to 3D organoids that are significantly fragile cells compared to 2D cultured cells in physiological conditions.

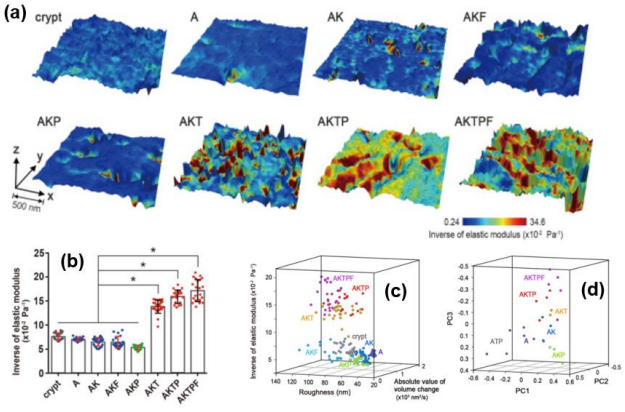

Fig. 2: (a) Topography and elastic modulus mapping in the respective genotype cells. (b) Averaged-inverse elastic modulus (IE) of the representative genotype cells. 3D representation of physical properties, such as IE surface roughness, and the volume change (c), and the principal component analysis (d) for the representative genotype cells.

Previously, we reported the development of a new method to stably observe the living cell surface using AFM and Micro Porous Silicon Nitride Membrane (MPM), a Si3N4 membrane with 100-200 nm thickness and 1-5 μm diameter holes. We successfully observed protrusions of less than 10 nm in diameter on the living colon cancer cell surface. Last fiscal year, we investigated the nanoscale effect of chemical fixation reagents on the colon cancer cell surface using the MPM method. We tested commonly used fixation reagents, 2% glutaraldehyde, 4% paraformaldehyde, and 100% methanol. After treatment with all these fixation reagents, we found that most of all small protrusions disappeared, and there were only large protrusions whose size was 10 - 50 nm on the cell. We also measured the distances between membrane molecules before, after, and during fixation, and we concluded these large protrusions were created by aggregating membrane proteins by fixation reagent (paper submitted, Fig. 3).

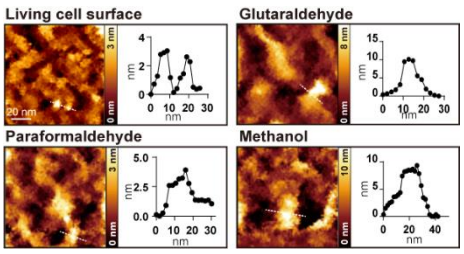

Fig. 3: AFM observation of colon cancer cell surface using Micro Porous Silicon Nitride Membrane. 100 x 100 nm scale. Height profile is along the white line of the left panel.

·Visualization of intracellular nanodynamics (nano-endoscopic observation):

(i) Nanoendoscopy AFM: AFM is the only method that allows label-free imaging of biomolecular nanodynamics. However, such imaging has been impossible inside living cells. To overcome this limitation, we have been developing a nanoprobe technique referred to as "nanoendoscopy", where we insert a needle probe into a living cell and perform 2D/3D-AFM of intra-cellular nanodynamics. So far, we have established the fabrication method of a needle probe and optimized the conditions for inserting it into a living cell (***Sci. Rep.*** **2021**). We succeeded in 3D imaging of whole cell structures and actin fibers, and 2D imaging of nanodynamics at the inner surface of the cell bottom membrane.

In FY2021, we finally summarized these results and published the first paper on nanoendoscopy AFM in ***Sci. Adv.*** **2021**. In addition, Kundan (Fukuma G) and Hirata (Cancer Research Inst.)

succeeded in mapping nanoscale distribution of nuclear surface elasticity in living cells. They newly found that the nucleus becomes stiffer with metastatic progression of the cancer cells, which may explain how metastatic cells can survive under a high pressure during cell invasion through a narrow gap. In another project, Shirokawa (Fukuma G) and Franz (Jr. PI) successfully visualized nanoscale structures of focal adhesion in living cells. We plan to image their formation and disassembling by AFM and TIRF microscopy. While AFM can visualize nanoscale structural changes, TIRF microscopy can visualize trajectories of important proteins (e.g., vinculins and integrins) moving into or out of the focal adhesion. With a help of Fujiwara (iCeMS), an expert in single molecule microscopy, we have installed a TIRF setup and are now testing the simultaneous imaging with these two techniques.

For visualizing nanodynamics, Kodera (PI) started to test a combination of nanoendoscopy and HS-AFM. In addition, Miyata (Jr. PI) has been developing a new AD/DA interface for a fast FPGA and reduced the AD-to-DA latency from 500 ns to 200 ns. For deriving nanomechanical properties from the in-cell AFM data, we started to explore various possibilities, including simulation by Sugita (RIKEN) and Foster (PI), and mathematical modeling by Okuda (Jr. PI). The other on-going projects of biological applications include in-cell observations of cell-cell junctions with Toda (Jr. PI) and dynamics at nuclear surfaces with Wong (PI).

(ii) Deroofed cells: Franz (Jr. PI) is developing experimental tools to expose the intracellular space for nanoprobe exploration with the aim of observing dynamic molecular processes directly within their native intracellular environment with high spatial and temporal resolution. In one approach cells are gently de-roofed by microsonication, which selectively removes some organelles (nucleus, Golgi etc.) but retains others (basal actin cortex, actin stress fibers, focal adhesions), that can then be imaged with nanometer resolution by HS-AFM. An optimized high-aspect ratio long-tip AFM probe design permits fast imaging of comparatively corrugated cellular samples, while a newly developed ultra-large HS-AFM scanner system (40 × 40 μm^2) can image even large cellular organelles in a single scan with nanometer resolution. Using this approach, conformational changes of individual myosin motor proteins driving ATP-stimulated actin stress fiber contraction could be visualized for the first time. Since the exposed intracellular multi-protein complexes and organelles are often structurally fragile, contact-free SICM scanning was also recently adapted to image dynamic contractions of the actomyosin cytoskeleton in collaboration with Korchev G (PI). Both HS-AFM and SICM are also used to quantitatively map mechanical changes during myosin-driven actin cytoskeleton remodeling. Currently, different non-mechanical methods for minimally destructive cell de-roofing are being developed using pore-forming peptides, phospholipases, and light-activatable membrane intercalators.

(Development of nano-endoscopic analysis and manipulation techniques)

·Injection and sampling of substances using a nanopipette:

Nanopipettes can deliver chemicals directly into or on the cell surface by voltage control. To elucidate the intracellular phenomena, e.g. organelle–organelle interaction or chemical responses, Takahashi et al. have developed an auto chemical delivery technology to deliver the chemical at a specific position of the cell. By combining this technology with machine learning, it is possible to perform experiments ranging from long-term (several hours and days) to short-term (ms level), which are difficult for humans. They already established an SICM-fluorescence microscope hybrid system where the conventional fluorescence imaging is coupled with the nanopipette device. However, the fluorescence intensity-based imaging suffers from the quantitative analysis. A collaborative team (Arai and Takahashi) started to construct the robust fluorescence lifetime imaging (FLIM) based SICM system toward a more quantitative analysis of mRNA and metabolites.

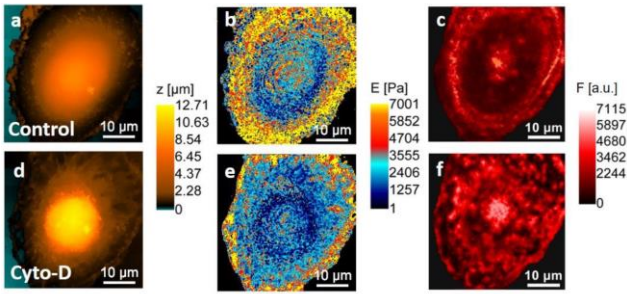

Fig. 4: a, b, c - topography map, mechanical properties and confocal image of actin filaments of living HT-1080 cell before Cyto-D treatment (30 µM), respectively; d, e, f -topography map, mechanical properties and confocal image of actin filaments of living HT-1080 cell after Cyto-D treatment (30 µM).

·Analysis of nano-distribution of physical properties using a molecular sensor:

Cell stiffness is an important factor for cell mobility and function. However, it is still difficult to measure the subcellular site of

stiffness. SICM is an effective tool for characterization of the stiffness with subcellular resolution and low invasiveness. Takahashi et al. tested the possibility of characterizing the stiffness of the cytoskeleton underlying the cell membrane and succeeded in visualizing nanoscale heterogeneous stiffness, which is related to the cytoskeleton distribution on the cell (Fig. 4) (***Nanoscale* 2021**). They plan to investigate the relationship between the injection of some inhibitors with the changing in stiffness.

We are developing molecular sensors that can selectively recognize target compounds using our expertise in supramolecular chemistry. By attaching the sensor to the end of a nanopipette for SICM, distributions can be detected based on changes in the ion current passing through the pipette. Ogoshi et al. have already succeeded in the selective recognition of 1-MNA (1-methylnicotinamide), which is one of the oncometabolites, by using a water soluble pillar[6]arene derivative. Based on molecular design of pillar[n]arenes, they further developed a new biosensor, which can detect a vitamin metabolite with a high binding constant. Maeda et al. modified hydrophilic polymers with the pillar[6]arene-based molecular sensor and demonstrated that the overall sensitivity of the sensor molecule could be improved by densely attaching them on the polymer. They also developed a helical polymer-based chiral sensor that can visually detect chirality and enantiomeric excess of chiral amines based on spring-like conformational change (***Sci. Adv.* 2021 & *Chem. Commun.* 2021**). Akine et al. have developed a new receptor molecule that can bind lactate ion (***Dalton Trans* 2021**), which is known to be an important molecule in cancer. The receptor molecule binds various kinds of carboxylate ions via coordination bond formation. The binding constant for lactate ion was around 10^5 M^{-1} with moderate enantioselectivity R/S = 1.6. A new cage molecule that can bind alkali metal ions was also developed. The host molecule showed a selectivity for larger metal ions, e.g., K^+/Na^+ = 34 (***Inorg. Chem.* 2021**). MacLachlan et al. have been developing supramolecular complexes of platinum that can respond to stimuli and change their color and/or luminescence. Crown ethers were appended to the exterior of luminescent, phenylpyridine-based macrocycles. When these bind to alkali metal ions, they undergo a change in luminescence and color that can be measured (***Inorg. Chem.* 2022**). By changing the chemical structure of the macrocycle, this concept may be adapted for measuring other biologically-relevant ions and analytes within cells.

·Nano-manipulation using molecular machines:

To achieve effective molecular machines for nano-manipulation, the manner of stimulation must be extended. Arai et al. devoted intensive efforts to generate a library of functional photothermal dyes toward a near-infrared (NIR)-operated supramolecular machinery system. They successfully found a promising dye that can be used for stimulation (***ACS Nano* 2022**). Furthermore, it was found that a specific NIR absorbing dye was able to alter artificial lipid dynamics, providing a basis for developing future molecular machines (Fig. 5). This system could be applied for the on-demand release of acetylcholine at a highly localized place in the living cell.

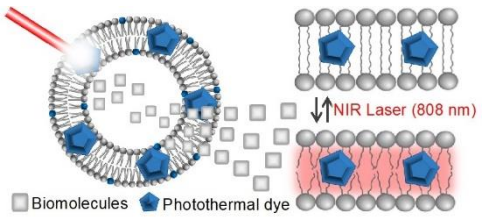

Fig. 5: A NIR laser operated system enabling on-demand release of biomolecules. Working principle to alter the lipid dynamics using a NIR dye.

Akine et al. have developed a new calixarene-based molecule that can be used for dynamic covalent assembly of organic structures. The conformational change by Na^+ binding regulated well the structure of the assembly; the self-assembly by imine formation afforded a dimeric cage in the presence of Na^+ whereas a macrocyclic trimer was formed in the absence of Na^+ (***Chem. Commun.* 2021**). They also developed a new helical molecule that shows an unprecedented time-dependent structural change. When the molecule loses chiral auxiliaries via a six-step ligand exchange reaction, the right-handed structure changed to the left-handed form before racemization (***PNAS* 2022**). This would be a good candidate as a molecular scaffold to enable time-dependent functions. Ogoshi et al. have developed pillar[n]arene-based molecular machines, such as chiral pillar[n]arene nanotubes (***Chem. Sci.* 2021**), photo- (***ACS Nano* 2021**) and vapor-responsive materials (***Chem. Sci.*, 2022**), and CPL controllable chiral assemblies (***Chem. Sci.* 2022**). Chiral transfer systems based on the planar chirality of pillar[5]arene were also produced. Maeda et al. developed versatile methods to synthesize end-functionalized helical polymers (***JACS* 2021 & *Angew. Chem. Int. Ed.*, 2021 & 2022**). These polymers would be easily immobilized on the surface of probes to be used as functional nanoprobes because they are rigid and can present significant changes in helical pitch in response to various external stimuli such as ions and temperature.

(Understanding measurement principles of newly-developed nano-probe techniques and life phenomena by means of mathematical/computational sciences)

･Multiscale modeling at the nano life science interface (Hall, Foster)

The Adam S. Foster Laboratory uses a range of simulation methodologies (including machine learning, molecular dynamics and Brownian dynamics approaches) to investigate various problems at the nano-life science interface. Recent work has focused on using convolutional neural networks to analyze and interpret the molecular nature of adsorbate interrogated by high-resolution AFM measurements and the use of all-atom molecular dynamics to predict hydration patterns on biopolymers such as chitin and cellulose. Within the Foster laboratory, Assist. Prof. Damien Hall employs multiscale modeling to investigate both fundamental and disease-related life processes at the cellular to molecular level of detail. He is currently engaged with the modeling of AFM-based cell membrane topology, elasticity and penetration measurements (***BPPB* 2022**) and is also attempting a mathematical assignment of cancer versus healthy cells based on SPM measurements. The group is also conducting simulations of intracellular diffusion in complex cellular fluids as well as using multiscale cell automata models to investigate the cell life cycle in terms of growth, division and epigenetic transmission stages. (Fig. 6)

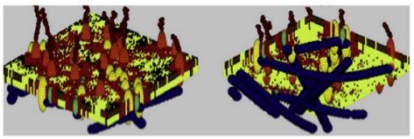

Fig. 6: Modeling and simulation of bio-SPM experiments; Model of the cell membrane based on a meso- to nano-scale approximation of the components (lipids, membrane proteins, surface carbohydrate and underlying cytoskeleton).

･Developing a theory of 3D-AFM and performing molecular dynamics simulations zooming into AFM images (Sumikama)

Modeling and simulation of bio-SPM experiments: We have developed a theory for calculating three-dimensional force microscopy (3D-AFM) by using a polymer simulation and applied it to chromosome models in the inter- and mitotic phase. By changing the scanning speed in the simulation, it was predicted that there exists an optimum velocity range for scanning, which is approximately ten times the most probable speed of molecular motions. It was also applied to the systems mimicking cytoskeleton fibers and carbon nanotubes, with which experiments have been done. The resultant 3D-AFM images are similar to those experimentally obtained, validating the theory.

Understanding life phenomena: To reveal the details of the 3D-AFM images and HS-AFM movies in the atomic level, all-atom molecular dynamics (MD) simulations were performed. The systems simulated were the following: 1) association dynamics of phospholipase A_2 to the cell membrane to clarify the detailed binding mechanism observed by HS-AFM (Fig. 7), 2) Annexin V to reveal the hydration structure measured by 3D-AFM, 3) aggregated structure of pillararene on mica observed by AFM, 4) pillararene and a guest molecule interaction observed by AFM.

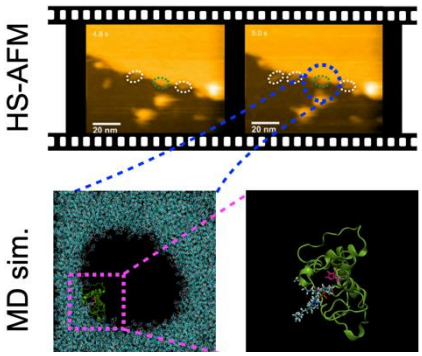

Fig. 7: Binding structure of phospholipase A_2 to the cell membrane.

･Simulation atomic force microscopy and multi-scale modelling of biological processes (Flechsig)

To understand biological processes relevant to life science, Flechsig combines multi-scale modelling of biological processes and simulation AFM in collaborative projects with all fields at NanoLSI. Significant progress has recently been achieved by developing the mathematical and computational methods to reconstruct full 3D atomistic structures from resolution-limited AFM topographic images (***PLoS Comput. Biol.* 2022**). Further achievements include the molecular-level explanation of basement membrane laminin structural

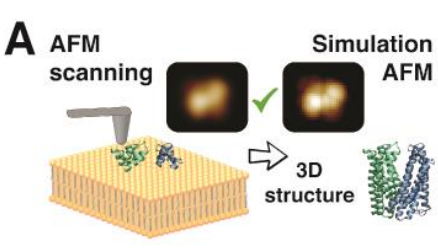

Fig. 8: Schematics of simulation AFM to reconstruct 3D atomistic structures from experimental images.

dynamics seen under HS-AFM, the single-molecule explanation of protein inhibition in cancer research (CYP24A1 protein), and the interpretation of HS-AFM observations for cancer-related MET-receptor dynamics. Significant progress has also been made in understanding functional dynamics of EEA1, important to better explain processes in membrane trafficking, and towards understanding photo-controlled modulation of membrane dynamics for controlled release of bioactive cargos from nanovesicles. (Fig. 8)

·Mechanics of collective cell migration in 3D tissue (Okuda)

Okuda (Jr. PI) proposed a simple mathematical model that explains how cells collectively migrate as a cluster in 3D space (***Biophys J* 2022**). Cells generate contractile and adhesive forces on their surfaces, which are simply expressed in the model by interfacial tension at cell-cell boundaries. Numerical simulations showed that interfacial tension enables cells to migrate as a cluster (Fig. 9). The mechanism is that interfacial tension induces unidirectional flow of each cell surface from front to rear along the cluster surface. The model also explains the variety of cell migratory modes observed in embryogenesis and cancer metastasis. These results show that interfacial tension at cell-cell boundaries plays key roles in the wide range of cell migrations in living systems.

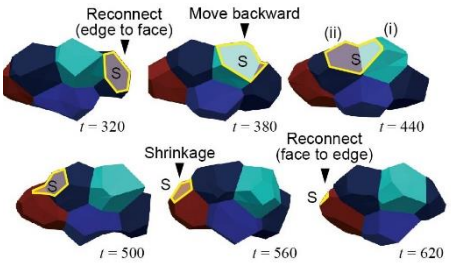

Fig. 9: Collective cell migration in 3D tissue. Cell-cell interface directionally flows from the front to the rear within the cluster.

(2) Nano-level understanding of basic cellular functions and cancer-specific abnormalities

·Cell membrane receptor engineering and dynamics (Matsumoto)

Matsumoto et al. have been investigating functions, structures, and engineering of growth factor receptor, focusing on the MET/HGF receptor. In FY2021, they contributed to Lasso-Graft protein engineering. In this technology, a variety of scaffold proteins grafted with MET-binding cyclic peptide newly acquired the ability to bind to MET (***Nat Commun* 2021**) and a scaffold protein capable of activating MET could be designed (***iScience* 2021**). For structural understanding of growth factor receptor activation, MET and HGF-MET complex were purified from living cells and subjected to HS-AFM analysis. The HS-AFM analysis revealed HGF-MET interactions and dynamic structures for HGF-MET (2:2) complex (Fig. 10). HS-AFM analysis facilitates an understanding of the dynamic mechanism of receptor activation in living cells.

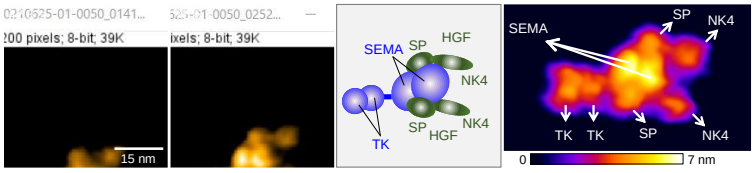

Fig. 10: Dynamic structure for whole HGF-MET complex in HS-AFM.

·Intracellular trafficking (Wong)

The control of intracellular traffic is critical for cell growth and differentiation. Nuclear pore complexes (NPCs) are multi-protein turnstiles that regulate nucleo-cytoplasmic traffic. Recently, Wong et al. visualized the native NPC and further succeeded in the observation of a single filament inside the inner ring of the nuclear pore by HS-AFM. NPC protein TPR regulated autophagy induction in a brain tumor-ependymoma (***Autophagy* 2021**). NPC proteins can undergo liquid–liquid phase separation and form liquid droplets, we recently developed a light-switching dipyrene probe (Pyr-A) to detect biomolecular phase separation (***iScience* 2021**). Besides, NPCs can participate in gene regulation by physically interacting with the genome and cofunction with histones, histone modifiers, or

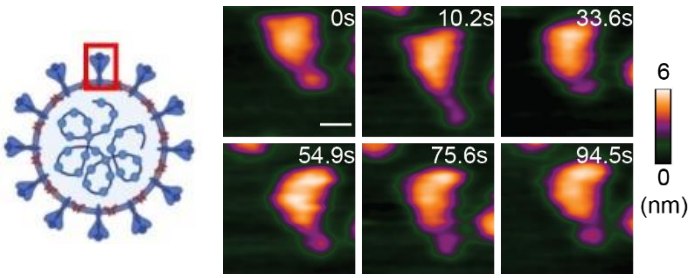

Fig. 11: HS-AFM scanning revealed a flexible S stalk and RBD.

chromatin remodelers. Using HS-AFM, Wong et al. also found that spatiotemporal dynamics of histone Protein H2A involution by DNA inchworming (***J Phys Chem Lett*** **2021**). Moreover, the host NPC trafficking system is often hijacked by viruses (including SARS-CoV-2) to accomplish their replication and to suppress the host immune response. Interestingly, in a pilot interdisciplinary project with the Hanayama and Ando groups, using HS-AFM, we captured SARS-CoV-2 spike and its interaction with ACE2 receptor and small extracellular vesicles (***J Extracell Vesicles*** **2021**) (Fig. 11).

·Cell communications via extracellular vesicles (Hanayama)

Hanayama et al. found novel mechanisms that promote metastasis and myelosuppression by tumor-derived extracellular vesicles (EVs). Specifically, they found that EVs from malignant osteosarcoma inhibited osteoclast maturation by transferring miR-146a-5p, which enhanced distant metastasis (***Front Oncol*** **2021**). They also found that EVs from leukemia cells promote tumor survival and myelosuppression by releasing their DNA (***Cell Death Dis*** **2021**). To unravel the new aspects of EV functions, they applied a 3D-AFM force mapping technique to get more detailed information on the structural and mechanical characteristics of EVs from osteosarcoma cells, and unexpectedly found a non-homogeneous and bumpy structure with distinct local regions bulging out from the surface (***Nanoscale*** **2021**) (Fig. 12). This can be attributable to the presence of membrane nano-domains, where specific proteins might be accumulated, including proteins responsible for elastic fiber formation such as fibulins, fibronectins, and integrins. As a result, metastatic tumor cells became softer than non-malignant cells, which would be convenient for tumor metastasis. In addition, using HS-AFM, they showed that EVs are involved in the formation of transthyretin amyloids in the disease called hereditary (variant) transthyretin amyloidosis (***Front Mol Biosci*** **2022**).

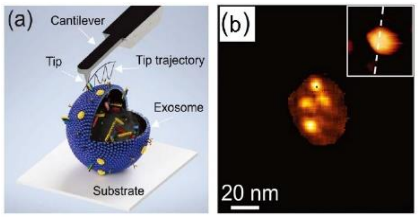

Fig. 12: (a) 3D-AFM force mapping of EV. (b) Presence of distinct local domains bulging out from EV surface.

·Morphogenesis-bottom up & mechanical approach (Toda)

Toda et al. have been working on the mechanisms of how cells organize complex multicellular structures observed in animal tissues. In FY2021, they have developed an in vitro model system where cells secrete and sense artificial signaling proteins in 3D spheroid culture. They analyzed tissue patterning processes by soluble signaling proteins and found that coupling cell signaling with cell adhesion is sufficient to generate distinct multicellular domains as a tissue pattern. In addition, to study how adhesion molecules form a clear tissue boundary, they started a new collaboration with the Fukuma group to visualize the intracellular part of the cell-cell junction with In-Cell AFM technology. They are developing an original cell culture system that allows them to access the cell-cell junction with a thin AFM cantilever.

·Transcriptional regulation & epigenetics (Miyanari)

Miyanari et al. have been studying roles of chromatin dynamics in transcriptional regulation, which is crucial for cell lineage allocation in mammalian development. Nuclear localization of each chromosome within nucleus is one of key determinants to regulate global transcription activity. They developed a new method (WE3D-FISH) to visualize chromosome territories and transcription activities simultaneously in mouse early embryos (***Methods Mol Bio*** **2021**) (Fig. 13). They also identified several novel regulators of chromatin accessibility through a genome-wide CRISPR screening approach (submitted). By considering these results, they started a project to uncover multimodal epigenetic signatures at the single cell level.

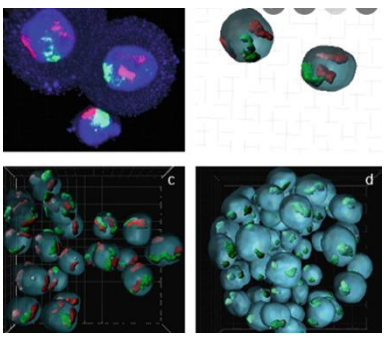

Fig. 13: Images of WE3D-FISH using mouse early embryos

·Stem Cell Fate (Hirao)

Hirao et al. have been investigating molecular mechanisms of stem cell fate decision by metabolic regulation in relation to anabolic and catabolic balance. They found critical roles of autophagy, a

representative catabolic regulation, in protecting HSCs against insults during the early neonatal stage, which is essential for healthy long-term hematopoiesis (***Sci Rep* 2021**). Such a catabolic status also contributes to stem-like status, such as dormancy and stress resistance, in malignant tissue. They have discovered that the endo-lysosomal activity is critical for malignant status in leukemia and glioma cells, and that lysosome is an important target for a novel therapeutic strategy (submitted). Based on these results, they have started collaboration with the Arai and Takahashi groups to discover novel indicators or biomarkers of metabolic status by using novel metabolic sensors combined with nano-pipette technology.

·Oncogenes and Cancer Cell Dynamics (Oshima)

Oshima et al. previously established intestinal tumor-derived organoids that carried various combinations of driver mutations including APC (A), KRAS (K), TGFBR2 (T), FBXW7 (F), and TP53 (P) genes (***Cancer Res* 2018**). Using these organoid systems, they established liver metastasis models by transplantation of the organoids to the spleen. Using this system, they demonstrated that non-metastatic cells can metastasize with metastatic cells by a "polyclonal metastasis" mechanism (Fig. 14) (***Nat Commun* 2021**). Moreover, in collaboration with the Watanabe group at Bio-SPM, they examined the physical properties of the established organoid cell surface using high-speed (HS)-scanning ion conductance microscope (SICM), and found specific physical characteristics in the metastatic organoid cells, such as actively moving micro-ridge structures with significantly softer membrane properties (Fig. 15) (***Biomaterials* 2022**). This is the first evidence of the defined genotype-linked physical properties of malignant cells, which expands our knowledge of the mechanisms of cancer progression.

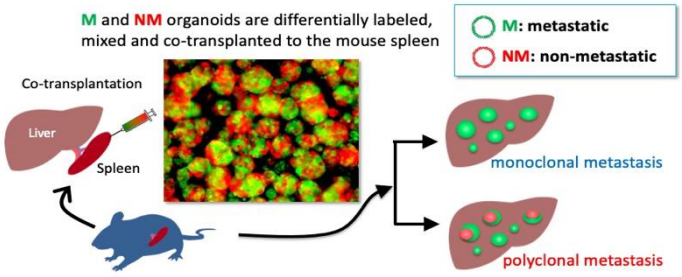

Fig. 14: Polyclonal metastasis of non-metastatic tumor organoids (red) with metastatic organoids (green).

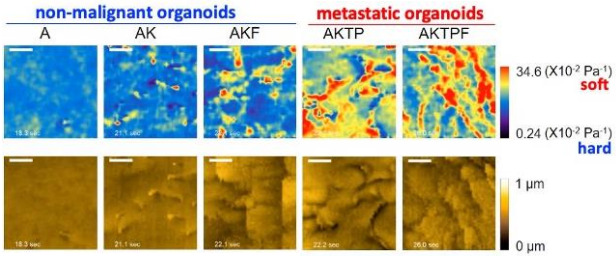

Fig. 15: Physical properties of non-malignant and metastatic organoid cell surface.

·Development of DNA Aptamers as Anti-cancer Molecules (Nakajima)

Nakajima et al. have been working on enzymes metabolizing exogenous and endogenous compounds. In FY2021, they characterized novel substrate specificities of enzymes metabolizing drugs including anti-cancer agents (***Annu Rev Pharmacol Toxicol* 2022**). In addition, they successfully developed DNA aptamer molecules that inhibit CYP24A1, an enzyme degrading anti-proliferative vitamin D_3. By collaboration with the Kodera group, they clarified the molecular dynamics and inhibition mechanism of the DNA aptamer against CYP24A1 by HS-AFM. Interestingly, the DNA aptamer significantly enhanced the vitamin D_3-mediated inhibition of cancer cell proliferation (***ACS Appl Mater Inter* 2022**).

·Development of Cancer Therapeutic Technology (Yano)

Co-existing p53 loss of function mutations caused resistance to ALK inhibitor-induced apoptosis and was associated with worse progression-free survival of *ALK*-rearranged lung cancer patients. Moreover, a proteasome inhibitor markedly induced apoptosis in the ALK inhibitor-treated cells by increasing the expression of a pro-apoptotic

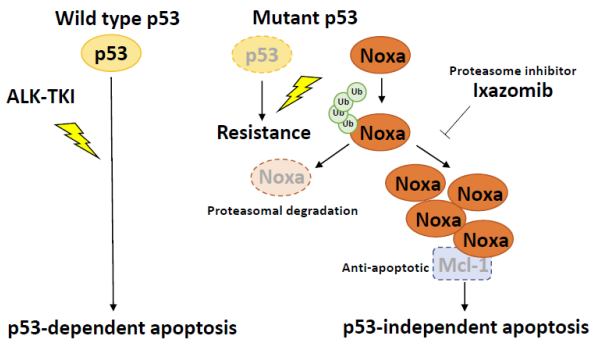

Fig. 16: A proteasome inhibitor increased the expression of a pro-apoptotic protein, Noxa, which bound to an anti-apoptotic protein Mcl1, and induced apoptosis in ALK inhibitor treated *ALK*-rearranged tumor cells.

protein, Noxa, which bound to an anti-apoptotic protein Mcl-1 (Fig. 16), suggesting therapeutic potential in *ALK*-rearranged lung cancer with p53 co-mutations (***Clin Cancer Res* 2021**).

(3) Establishment of a New Research Field: Nanoprobe Life Science (Further improvement of high-speed AFM technologies)

Further improvement of high-speed AFM (HS-AFM) technologies will allow us to study a wider range of biological phenomena. Concerning the speed performance of HS-AFM, the highest possible imaging rate of our HS-AFM (V_{max}) is generally around 10 fps for purified protein molecules, restricting the range of observable molecular processes. V_{max} depends not only on the speed performance of rate-limiting instrumental devices but also on the level of tip disturbance to fragile biological samples. Therefore, we have been exploring methods to minimize tip disturbance. As described in the last progress report, we invented a new X-scanning mode named "the only trace imaging (OTI)" mode that can increase V_{max} by ~2.5-times (see the black and blue lines in Fig. 17a) (***Rev. Sci. Instrum.* 2021**). This year, further improvements on the OTI imaging and the X-scanner were achieved (not disclosed at present). We have also been improving the basic speed performance of HS-AFM by increasing the speed performance of key devices included in HS-AFM. In FY2021, we reported a differential-based ultrafast amplitude detector with zero intrinsic latency based on the basic trigonometric theorem, called DB-A^2 (Fig. 17d, ***Appl. Phys. Lett.* 2021**) and an ultrafast piezoelectric Z-scanner with a resonance frequency above 1.1 MHz, a highest record, using a new piezo support method called "four-vertex support" (Fig. 17e, ***Rev. Sci. Instrum.* 2022**). The new Z-scanner nearly reached the fastest performance that can theoretically be reached with a piezo actuator. However, a faster Z-scanner is further desired. To this end, we invented an electronic circuit called "Resonance-controller" that can control not only the resonant frequency but also the quality factor of a Z-scanner. Using this new controller, we could achieve a Z-scanner with a pseudo resonance frequency of ~2 MHz without changing the mechanical part of the Z-scanner (***Patent application number:* 2022-141581**). Regarding the development of ultra-small cantilevers, we worked to improve the efficiency of cantilever excitation. We designed a new cantilever holder in which a piezo for cantilever excitation was placed so that the vibrations from the excitation piezo are transmitted more directly to the cantilever. This change allowed us to oscillate the ultra-small cantilever even at ~10 MHz with sufficient amplitude for observing most of biological samples. Through these activities, we are now approaching the final goal of achieving V_{max} around 100 fps.

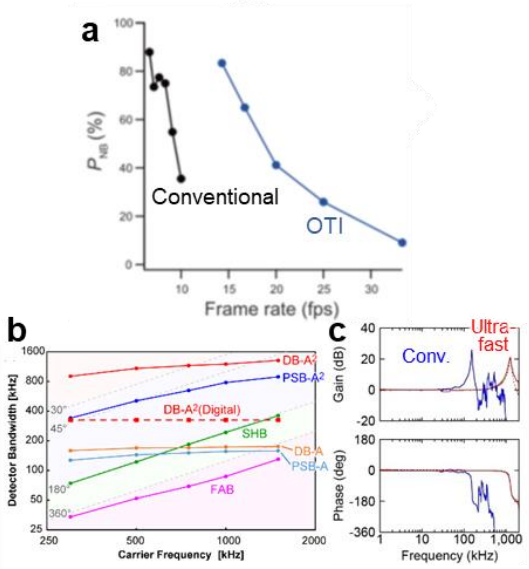

Fig. 17: (a) Probability at which MTs are not broken during successive imaging for 10 s. (b) Comparison of the detector bandwidth of the amplitude detectors. DB-A^2 has the widest detection bandwidth (red). (c) Frequency responses of the ultrafast (red) and conventional (blue) Z-scanners.

We have also been working on the functional extension of HS-AFM. This year, we developed a thermostatic chamber that can cover the mechanical part of HS-AFM to observe temperature-sensitive samples such as living cells. The temperature control ranges from room temperature to ~50°C. The usefulness of this thermostatic chamber was confirmed by the HS-AFM observation of the helicase and nuclease

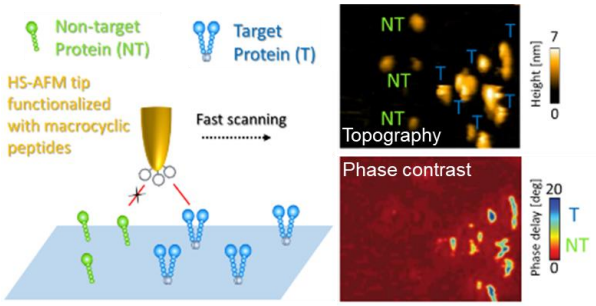

Fig. 18: Molecular recognition by HS-AFM phase contrast imaging by using a macrocyclic peptide-conjugated tip.

activities of CRISPR-Cas3, which is attracting attention as a new genome editing protein, whose optimal enzyme activity is seen around 37°C (***Nat. Commun.*** **2022**). This thermostatic chamber would be an essential tool for observing living cells by HS-AFM. One of the drawbacks of AFM imaging is the inability to identify molecular species in samples composed of heterogeneous molecular species. To overcome this drawback, we reported a method of molecular identification HS-AFM imaging by using a macrocyclic peptide-conjugated tip and phase contrast imaging (***ACS Appl. Mater. Interfaces*** **2021**). To demonstrate the versatility of this method, we showed real-time and real-space recognition HS-AFM imaging for human hepatocyte growth factor receptor (Fig. 18). This new recognition imaging would be applicable to recognition of only the target proteins without any labeling, even on living cells.

(Nanoprobe studies on various life phenomena)

i) Structural and dynamics analysis of intrinsically disordered proteins by high-speed atomic force microscopy (*Nat. Nanotech.*, 2021, IF: 31.538**)**

Many proteins (IDPs) contain intrinsically disordered regions (IDRs) lacking stable secondary and tertiary structure. And yet, they can carry out vital cellular functions. Highly flexible IDRs dynamically explore a wide structural space, an underlying feature of the unique properties and mechanisms of IDPs' function and regulation. Therefore, revealing the dynamic structure of IDPs is essential in understanding the mechanisms. However, this is considerably difficult due to paucity of appropriate techniques. Thus, we explored the potential of HS-AFM to quantitatively delineate the dynamic structure of IDPs. First, we obtained corroborative evidence that the mean end-to-end distance of fully disordered IDRs, $<R_{2D}>$ follows the power law, $<R_{2D}> = \beta_0 \times N_{aa}^{\nu}$ (N_{aa}: the number of amino acids contained in such IDRs; the values of β_0 and ν were determined from HS-AFM images), enabling the determination of N_{aa} from captured images. By exploiting this law and the capability of HS-AFM to observe dynamic structural transitions, we succeeded in quantitative characterizations of IDPs' structure and dynamics, as exemplified in the case of virus phosphoprotein PNT (Fig. 19). As seen there, detailed dynamic structural features were quantitatively delineated, infeasible with other methods.

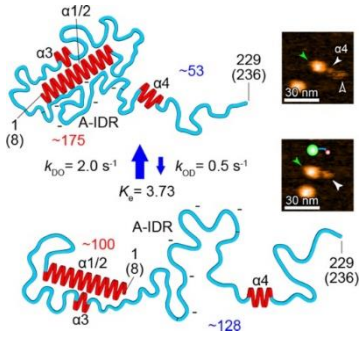

Fig. 19: Dynamic structure of PNT determined by HS-AFM

ii) Internal motor movements of *Mycoplasma mobile* visualized by HS-AFM (*mBio*, 2021, IF: 7.867**)**

Mycoplasma mobile, a parasitic bacterium, glides on the surfaces of animal cells. This process is driven by the force generated through ATP hydrolysis on an internal structure. However, the spatial and temporal behaviors of the internal structures in living cells are unclear. In this study, by scanning the surface of a cell with a nano-meter level AFM probe, we succeeded in visualizing the internal structures "through the cell membrane". The internal structures are observed as chain-like structures, each of which was formed by a series of small particles (Fig. 20). The particles are 2 nm in height and aligned mostly along the cell axis with a pitch of 31.5 nm, consistent with previously reported features based on electron microscopy. In the presence of sodium azide, the average speed of particle movements was reduced, suggesting that movement is linked to ATP hydrolysis. HS-AFM analysis revealed that the particles moved approximately 9 nm to the right of the gliding direction and 2 nm to the cytoplasmic side within 330 ms, then return to their original position, based on ATP hydrolysis. Consequently, this study has provided a more detailed understanding of the gliding mechanism of *Mycoplasma mobile.* This was a collaboration with Prof. Makoto Miyata (Osaka City University) which started through the Bio-SPM Summer School.

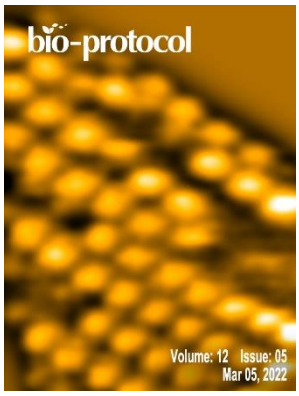

Fig. 20: Internal structures of Mycoplasma mobile visualized by HS-AFM. The image was selected for the journal cover page of "bio-protocol".

iii) Plasma membrane anchored nanosensor for quantifying endogenous production of H2O2 in living cells (*Biosensors and Bioelectronics,* 2021, IF: 10.257**)**

Hydrogen peroxide (H_2O_2), is a secondary messenger produced on the outer cell surface that is pivotal for the modulation of various cellular activities, including proliferation, signal transduction, and apoptosis. At present, experimental techniques for accurate quantification of endogenous H_2O_2 are still a work in progress. In this study, we designed a new nanosensor based on conjugated gold nanostructures that can be anchored to the outer plasma membrane of any type of cell to quantify concentrations of endogenous H_2O_2 using surface enhanced Raman spectroscopy (SERS). The nanosensor is localized at nanometer distance from the cell surface, namely it can probe the very shallow region of the extracellular milieu in which physiologically meaningful variations of H_2O_2 occur. In addition, we combined the new SERS analysis to advanced methods of redox biology for calculation of H_2O_2 gradients across the cell membrane.

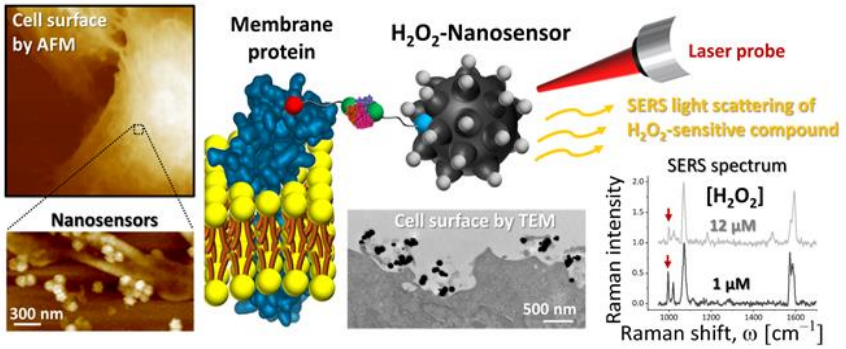

Fig. 21: Schematic illustration and experimental evidences (i.e., AFM and TEM) of the nanosensor anchoring to the cell surface. H_2O_2-sensitive compound is detected by SERS.

iv) Structural basis for channel conduction in the pump-like channelrhodopsin ChRmine (*Cell* 2022, IF: 41.584**)**

ChRmine, a recently discovered pump-like cation-conducting channelrhodopsin, exhibits puzzling properties (large photocurrents, red-shifted spectrum, and extreme light sensitivity) that have created new opportunities in optogenetics. ChRmine and its homologs function as ion channels but, by primary sequence, more closely resemble ion pump rhodopsins; mechanisms for passive channel conduction in this family have remained mysterious. Here, we present the cryo-EM structure and HS-AFM images of ChRmine, revealing architectural features atypical for channelrhodopsins: trimeric assembly, a short transmembrane helix-3, a twisting extracellular loop-1, large vestibules within the monomer, and an opening at the trimer interface (Fig. 22). We applied this structure to design three proteins suitable for fundamental neuroscience investigations. These results illuminate the conduction and gating of pump-like channelrhodopsins and point the way toward further structure-guided creation of channelrhodopsins for applications across biology.

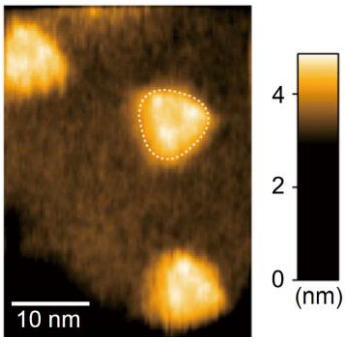

Fig. 22: HS-AFM images showing the formation of ChRmine trimer in the lipid membrane.

v) Direct electrochemical visualization of the orthogonal charge separation in anatase nanotube photoanodes for water splitting (*ACS Catalysis* 2022, IF: 13.084**)**

Photoelectrochemical (PEC) water splitting is an important and rapidly developing technology that produces H_2 as a renewable resource, but local surface investigations remain a major challenge. Using scanning electrochemical cell microscopy (SECCM), the PEC catalytic effects of anatase TiO_2-nanotube arrays grown on Ti felt are explored. The SECCM imaging is performed both perpendicular and parallel to the nanotube growth direction. In contrast to bulk cyclic voltammetry measurements, SECCM measures only the upper region of the nanotubes that remain in contact with the electrolyte, which provides a better understanding of the phenomena connected to the longitudinal charge transport.

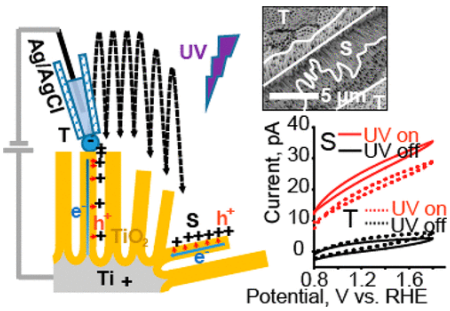

Fig. 23: Schematic of SECCM characterization of the photogenerated hole diffusion.

Despite the presence of regions with higher and lower photocurrent, the PEC reactivities of the nanotube tops and walls are roughly comparable with each other. The data support the model of orthogonal electron–hole separation. This model facilitates the photogenerated hole diffusion over the short distance to the electrolyte interface due to the sufficient transport of photoexcited electrons along the long axial direction of TiO_2 nanotubes and is often applied to one-dimensional systems.

vi) A novel three-state elevator mechanism in the citrate transporter CitS (*Proc. Natl. Acad. Sci. USA* 2022, IF: 11.205**)**

The secondary active transporter CitS shuttles citrate across the cytoplasmic membrane of Gram-negative bacteria by coupling substrate translocation to the transport of 2 Na^+ ions. We used High Speed-Atomic Force Microscopy (HS-AFM) for real-time visualisation of the transport cycle at the level of single transporters. Unexpectedly, instead of a bimodal height distribution for the up and down states, the experiments revealed movements between three distinguishable states, with protrusions of ~0.5 nm, ~1.0 nm and ~1.6 nm above the membrane. Furthermore, the real-time measurements showed that the individual protomers of the CitS dimer move up and down independently. A three-state elevator model of independently operating protomers resembles the mechanism proposed for the aspartate transporter Glt_{Ph}. Since CitS and Glt_{Ph} are structurally unrelated, we are exploring the possibility that the three-state elevators of these two transporters have evolved independently.

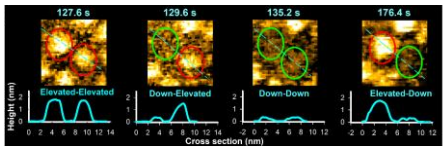

Fig. 24 HS-AFM imaging of CitS transporting domain elevator motion.

vii) Understanding the polymerization of diphenylacetylenes with tantalum(V) chloride and cocatalysts: Production of cyclic poly(diphenylacetylene)s by low-valent tantalum species generated in situ (*J. Am. Chem. Soc.* 2021, IF: 15.419**)**

Cyclic polymers often show different unique properties from common linear polymers due to their topological feature. However, synthesis of cyclic polymers is still challenging. We showed that low-valent tantalum species formed by reduction of $TaCl_5$ by $^nBu_4Sn$ or other co-catalysts induced the polymerization of diphenylacetylenes to produce unprecedented cyclic poly(diphenylacetylene)s. The formation of a cyclic structure of the resulting polymers were clearly visualized by detailed AFM observations (Fig. 25). Based on the experimental results together with established organotantalum chemistry, we proposed an insertion ring-expansion polymerization mechanism through the formation of tantalacyclopentadiene intermediates, rather than the previously considered methathesis mechanism.

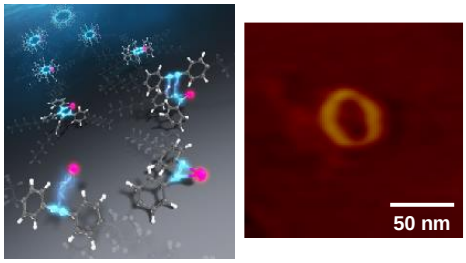

Fig. 25: Formation of cyclic π-conjugated polymers, poly(diphenylacetylene)s.

viii) A Nonbenzenoid Two-Dimensional Carbon Allotrope (*Science* 2021, IF: 41.845**)**

Due to their unique structure and amazing physicochemical properties including high chemical inertness, large specific surface area, high electric conductivity, mechanical flexibility, and biocompatibility, 2D carbon materials hold great potential for bioelectronic implants. However, the quest for the reliable synthesis of planar sp^2-hybridized carbon allotropes other than graphene, such as graphenylene and biphenylene networks, remains challenging given the lack of reliable protocols for generating nonhexagonal rings during the in-plane tiling of carbon atoms. In this work, we demonstrate the bottom-up growth of an ultraflat biphenylene network with periodically arranged four-, six-, and eight-membered rings of sp^2-hybridized carbon atoms through an on-surface interpolymer dehydrofluorination (HF-zipping) reaction. The characterization of this biphenylene network by scanning probe methods reveals that it is metallic rather than dielectric and could act as a conducting

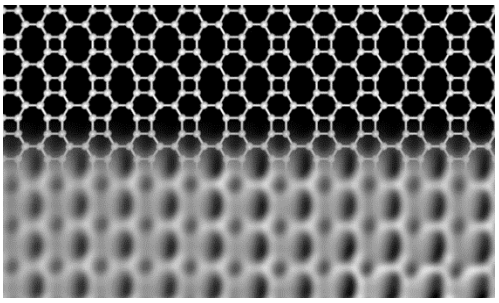

Fig. 26: Simulated atomic structure (top) and measured Scanning Probe image (bottom) for the biphenylene network.

wire in future bioelectronic applications.

ix) Electrostatic Discovery Atomic Force Microscopy (*ACS Nano* 2022, IF: 15.881**)**

While offering high resolution atomic and electronic structure, scanning probe microscopy techniques have encountered greater challenges in providing reliable electrostatic characterization on the same scale. In this work, we offer electrostatic discovery atomic force microscopy, a machine learning-based method which provides immediate maps of the electrostatic potential directly from atomic force microscopy images with functionalized tips. We apply this to characterize the electrostatic properties of a variety of molecular systems and compare directly to reference simulations, demonstrating good agreement. This approach offers reliable atomic scale electrostatic maps on any system with minimal computational overhead. We are now further developing this approach, both in terms of quantitative accuracy and in its application to biomolecular systems.

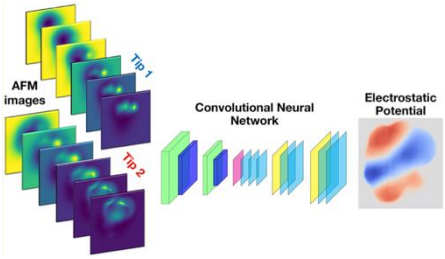

Fig. 27: Schematic of the electrostatic discovery method, where experimental Atomic Force Microscopy images from two tips are converted by a Convolutional Neural Network into predictions of molecular electrostatic potential

2. Generating Fused Disciplines

* Describe the content of measures taken by the center to advance research by fusing disciplines. For example, measures that facilitate doing joint research by researchers in differing fields. If any, describe the interdisciplinary research/fused discipline that have resulted from your efforts to generate fused disciplines. You may refer to the research results described concretely in "1. Advancing Research of the Highest Global Level."

We have made various efforts to promote interdisciplinary research among the four fields, i.e., nanometrology, life science, supramolecular chemistry, and computational science as described below.

Top-down approach

The future planning board sets out three priority research themes, and the PIs that make up the future planning board act as research coordinators to develop interdisciplinary research initiatives in a top-down manner. The three priority research themes are as follows:

- Intracellular imaging and cell monitoring after observation;
- Local stimulation/manipulation and chemical mapping (inside cells and cell surface);
- Cell surface imaging and cell-cell communication.

For each priority research theme, research expenses of 4 million yen were provided from the on-campus COE research expenses provided by the University Headquarters, and personnel expenses of 6 million yen were provided from the WPI subsidy.

Bottom-up approach

In order to support interdisciplinary research by teams consisting of young researchers, a bottom-up interdisciplinary research promotion grant was set up from the WPI subsidy, and a total of 19.95 million yen was provided for 11 research projects through application and selection. Of the 11 research projects supported, one is a research project led by a graduate student in the doctorate program of the Division of Nano Life Science. For each research project, the PI corresponding to the research project acts as a supervisor, and at the beginning of the year following the support, a research report meeting attended by all PIs is held to give advice on future research development.

T-meetings, luncheon webinars, and a poster session

In order to promote interdisciplinary research, T-meetings were held 16 times in FY2021 by combining two research teams out of those (22 teams in total) individually led by 16 PIs and 6 Jr. PIs from different disciplines to allow them to introduce their research. Five of these T-meetings were conducted in combination with a research team led by an overseas PI and a team by a resident PI in NanoLSI. In addition, regarding the research reports of young researchers, 7 online luncheon webinars were held for reports and discussion. In April 2022, an on-site poster session was held with 29 posters exhibited to encourage interdisciplinary research by forming research teams especially consisting of young researchers (except for 6 Jr. PIs), following lifting of the new corona pandemic prevention measures.

3. Realizing an International Research Environment

* Describe what's been accomplished in the efforts to raise the center's recognition as a genuine globally visible research institute, along with innovative efforts proactively being taken in accordance with the development stage of the center, including the following points, for example:
- Efforts being developed based on the analysis of number and state of world-leading, frontline researchers (in Appendix 2); exchanges with overseas entities (in Appendix 4); number and state of visiting researchers (in Appendix 5)
- Proactive efforts to raise the level of the center's international recognition
- Efforts to make the center into one that attracts excellent young researchers from around the world (such as efforts fostering young researchers and contributing to advancing their career paths)

Total number of papers by PIs, Top 1% papers, Top 10% papers, internationally co-authored papers in 2017-2021

During the five years from 2017, when NanoLSI was established, to 2021, the total number of papers by 16 PIs was 591, of which 272 (46%) were internationally co-authored papers. Of the total 591 papers, the Top 1% papers were 26 (4.4%) and the Top 10% papers were 193 (32.7%). The number of Top 1% papers with field-weighted Citation Impact corrections was 7 (1.2%), and the number of Top 10% papers, 90 (15.2%).

Number of co-authored papers with overseas PIs from 2017 to 2021

The total number of papers co-authored by one of the four overseas PIs and the resident Japanese researchers in NanoLSI was 17 in the five years from 2017 to 2021. Of these, 6 papers were published with Prof. Yuri Korchev (nanometrology) of Imperial College London, UK, an overseas satellite, and one paper with Prof. Mark MacLachlan (supramolecular chemistry), the University of British Columbia, Canada, also an overseas satellite. In addition, a total of 9 papers were published with Prof. Adam Foster (computational science) of Aalto University, Finland, and 1 paper with Prof. Alexander Mikhailov (computational science) of the Fritz Haber Institute of the Max Planck Society, Germany.

New overseas PIs

Prof. Carsten Beta, Professor of Biological Physics at the University of Potsdam, Germany (nanometrology, computational science) has been appointed as the successor to Prof. Alexander Mikhailov, who served as an overseas PI for the past five years. With the approval of the University of Potsdam, an overseas PI contract with Prof. Carsten Beta has been signed. On the occasion of the appointment of the new overseas PI, we conducted a long interview with Prof. Carsten Beta about his research and future prospects, and have published the interview on our podcast.
In addition, new female PIs were solicited internationally for selection. Currently, we are negotiating the contract with the first candidate and Co-PI.

Outreach programs for external researchers

The Bio-SPM Summer School and Bio-SPM Collaborative Research Program aim to introduce and disseminate the scanning probe microscope (Bio-SPM) technology of NanoLSI for life science research to external researchers, leading to joint research. From FY2017 to FY2019, a total of 44 overseas researchers from 17 countries participated. In FY2021, due to the influence of the COVID-19 pandemic that has continued since FY2020, the Bio-SPM Summer School was held exclusively for domestic researchers, and 11 researchers participated. Of the 16 projects adopted by the Collaborative Research Program, 12 joint research projects were conducted with domestic researchers. Implementation of a total of four joint research projects, including projects with overseas researchers, has been carried over to FY2022 and beyond. The NanoLSI International Symposium was held online in March 2022 under the theme of "Understanding Nanoscale Biological Processes in the Cells," where 10 domestic researchers, including NanoLSI researchers, and 4 overseas researchers made presentations, and a total of 166 researchers participated online.

Mobility and career path for young researchers

As of the end of FY2021, the number of postdoctoral researchers (including fixed-term assistant professors), a major part of the researcher mobility, was 28 out of 81 researchers in NanoLSI, and 24 out of 28 were overseas researchers. Five postdoctoral researchers left NanoLSI during FY2021 and are pursuing research careers. Of the five, one has a tenured associate professorship, one has a tenure-track assistant professorship, and two have fixed-term assistant professorships. The other is applying for a new research position.

Diversity of the NanoLSI researchers

As of the end of FY2021, of the 81 NanoLSI researchers, 31 (38%) were overseas researchers, and 15 (19%) were females. We are recruiting a new female PI to improve the proportion of female researchers. In addition, for fixed-term assistant professors, the policy has been changed to prioritize the hiring of Japanese female researchers while continuing the conventional policy of prioritizing the hiring of overseas researchers.

Support for young overseas researchers to acquire research funds

With individual support from the full-time URA of NanoLSI, a total of 16 young overseas researchers have acquired KAKENHI from FY2017 to FY2021. In FY2021, 15 new applications were submitted, and 3 were approved. The total number of KAKENHI acquired by overseas researchers belonging to NanoLSI was 26 from FY2017 to FY2021.

4. Making Organizational Reforms

* Describe the system reforms made to the center's research operation and administrative organization, along with their background and results.
* If innovated system reforms generated by the center have had a ripple effect on other departments of the host institutions or on other research institutions, clearly describe in what ways.
* Describe the center's operation and the host institution's commitment to the system reforms.

Continued implementation of successful reforms of NanoLSI

NanoLSI established successful cases of system reform in the first half of the WPI subsidy period. These are research professorships for concentrating on research, a rigorous evaluation-based salary system, monthly top leaders meeting between the university president and the center director, integrated management of NanoLSI and the Graduate School "Division of Nano Life Science," the tenure-track junior PI program, English-based administration, and "under-one-roof" environment equipped with many Bio-SPMs and high-end microscopes. These successful reforms will be maintained and continued for 5 years in the latter half of the subsidy period.

Horizontal development of the successful reforms of NanoLSI

The Host Institution, Kanazawa University, established the Interfaculty Institute for Frontier Science in April 2022. This organization consists of a total of 13 interdisciplinary research institutes or research centers, aiming to form a new interdisciplinary research base. While maintaining its position as an independent research institute, NanoLSI will contribute to the cross-sectional development of successful reforms in collaboration with the Interfaculty Institute for Frontier Science.

5. Efforts to Secure the Center's Future Development over the Mid- to Long-term

* Address the following items, which are essential to mid- to long-term center development:
- Future prospects with regard to the research plan, research organization and PI composition; prospects for fostering and securing of next-generation researchers
- Prospects for securing resources such as permanent positions and revenues; plan and/or implementation for defining the center's role and/or positioning the center within the host institution's institutional structure
- Measures to sustain the center as a world premier international research center after program funding ends
- Host institution's organizational reforms carried out for the center's autonomous administration simultaneously with the creation of the center.

Re-formation of PIs

At the beginning of the second half of the WPI subsidy period from FY2022, 2 out of 16 PIs were replaced. Prof. Toshio Ando was replaced by Prof. Noriyuki Kodera in the field of nanometrology, and Prof. Alexander Mikhailov was replaced by Prof. Carsten Beta in the field of computational science (see Appendix 2a for the CV of the new PIs). Prof. Ando continues his research at NanoLSI as a "Distinguished Professor of Kanazawa University" in the latter half of the WPI subsidy period.

Deployment of Tenured Researchers

As specified in the Host Institution's commitment document revised after the WPI Program Interim Evaluation and the commitment document signed by the new President, Kanazawa University has already completed the assignment of 22 full-time researchers with 16 tenured and 6 tenure-track positions to NanoLSI. Six tenured positions have been reserved for the tenure-track researchers. From now on, Kanazawa University will continue to maintain the placement of excellent full-time researchers (22 positions) who will lead NanoLSI research for a long period of time beyond the WPI subsidy period.

Amount of External Fund Acquisition in FY2021

The total amount of external funds acquired in FY2021 by 81 NanoLSI researchers was 1,288 million yen (1,005 million yen in the previous fiscal year) (see Appendix 3-1 5. Securing external research funding for details).

6. Others

* Describe what was accomplished in the center's outreach activities last year and how the activities have contributed to enhancing the center's "globally visibility." In Appendix 6, describe concretely the contents of these outreach activities. In Appendix 7, describe media reports or coverage, if any, of the activities.
* In addition to the above 1-5 viewpoints, if there is anything else that deserves mention regarding the center project's progress, note it.

Publication of the world's first HS-AFM textbook

The book "High-Speed Atomic Force Microscopy in Biology" written by Specially Appointed Professor Toshio Ando was published by Springer Nature. This book is the world's first HS-AFM "textbook" that comprehensively explains the principles and technologies of the high-speed atomic force microscope, and has received much attention. The number of individual purchases of eBooks has already reached 707 in seven days from March 28 when the relevant webpage of the publisher was opened.

Press release of research outcomes

In FY2021, a total of 19 press releases about research outcomes were issued, of which 17 were also issued in English. In December 2021, when the paper "Visualizing intracellular nanostructures of living cells by nanoendoscopy-AFM" by Penedo (affiliated to NanoLSI at that time) & Fukuma was published online in Science Advances (DOI: 10.1126/sciadv.abj4990), a press conference was held at NanoLSI. A notable research outcome, the paper "Structural and dynamics analysis of intrinsically disordered proteins by high-speed atomic force microscopy" by Kodera & Ando was published in Nature Nanotechnology, vol 16, 181-189, February 2021.

Media coverage and visitors

In January 2022, the NHK program "Science ZERO" aired a special feature on high-speed AFM developed by Prof. Ando and other NanoLSI researchers, "A live image of the nano movement! A new microscope for observing life." In FY2021, NanoLSI was visited by a total of 132 people, of which 53 were from industry and governments including 34 people from Keidanren, and 79 people from education such as students and teachers.

Interaction between WPI researchers and SSH high school students

NanoLSI held the 10th WPI Science Symposium in December 2021. At this symposium, NanoLSI researchers and other researchers of the WPI Centers evaluated and advised on 6 research presentations and 32 poster presentations by students of the Super Science High School (SSH) in Ishikawa Prefecture, and they discussed with great pleasure the researcher's lives and research. A total of 567 people including 364 high school students were participated. The symposium was highly evaluated by the Ishikawa Prefectural Board of Education and the participating high school students. NanoLSI will continue to plan and implement interactions between WPI researchers and SSH high school students, like having its researchers participate in meetings where students of SSH high schools in Ishikawa Prefecture make presentations of their research.

7. Center’s Response to Results of Last Year’s Follow-up

* Transcribe the item from the “Actions required and recommendations” section in the site visit report and the Follow-up report, then note how the center has responded to them.
* If you have already provided this information, indicate where in the report.

Comment 1 *"Insert clear challenges into the center’s roadmap and continually update them so that NanoLSI continues to develop.”*

In the last site visit, we indicated the roadmaps for the six nanoprobe technology development and seven life science research topics. In response to the above comment, we exchanged opinions with Nakano PO to precisely understand the intention and established a policy to further specify the individual issues written in the roadmaps and keep them updated. So far, the center director has established a system to interview individual PIs annually to evaluate and discuss their research results and plans and determine the budget allocations. The center director will use these opportunities to review and update concrete subjects listed in the roadmaps.

Comment 2 *"Make continuing effort to solicit more robust activities by the foreign PIs at NanoLSI so that their presence becomes more clearly visible.”*

So far, all four foreign PIs have secured research space and personnel in the NanoLSI building, and are now engaged in collaborative research with domestic PIs. Some of the results obtained have already been published or are in preparation for publication. To continue these activities even under the COVID-19 outbreak, we will hold regular online inter-group meetings (T-meetings) between an overseas PI and a domestic PI. These were previously held only when overseas PIs visited Japan. In this way, we will promote publication of the achievements obtained and continue to explore new collaborative research projects. In addition, to avoid abrupt termination of the collaborative projects with Mikhailov PI, who retired in March 2022, we have continued to employ his lab member at NanoLSI. Meanwhile, we have already appointed Prof. Carsten Beta of Potsdam University as the next PI in the computational science field.

Comment 3 *"Make further effort to hire female scientists, especially at the PI level.”*

We have taken this comment very seriously and established the following strategies to increase

the number of female researchers at different career stages. As for the PIs, we made huge efforts to directly recruit famous established PIs, but found them mostly affiliated to multiple institutes. Thus, we decided to issue a wide call for applications from talented young researchers at either professor or associate professor level. Now, we are about to appoint one of the applicants. In addition, we aim to increase by at least two female assistant or associate professors or postdoc researchers every year. Furthermore, we changed the basic policy for recruiting a fixed-term assistant professor. Previously, it was planned to employ only foreign researchers, but we decided to extend this to include Japanese female researchers.

Comment 4 ***"Regarding Kanazawa University's support plan, formalizing the allocation of the promised number of personnel (both research and support) should be done as soon as possible. The support plan for administration is qualitative. A more concrete plan including the number of administrative staff is recommended."***

It is essential to secure tenured posts for NanoLSI researchers to maintain and develop NanoLSI as an internationally top-level research institute over a long period of time. In the last site visit, President Yamazaki of Kanazawa University announced the deployment of twenty-two tenured or tenure track researchers to NanoLSI together with their tenured posts. We are pleased to inform you that these promised deployments have been completed in FY2021. NanoLSI and the university headquarters have agreed to keep on these tenured posts for researchers belonging to NanoLSI while they are involved in graduate or undergraduate education in relevant research fields. These personnel measures will build a sound foundation for NanoLSI in advancing research. President Yamazaki has also agreed to maintain the administrative office and staff both in quality and quantity where all NanoLSI operations are carried out in English, such as research support, daily life support, and various communications from the office. As for quantitative plan to deploy administrative staff at NanoLSI, around 17 staff members will be maintained following the current deployment which enabled NanoLSI to provide necessary and sufficient administrative support for researcher.

Comment 5 ***"Articulate concrete plans for advancing BioSPM technology in the future in order to prevent it from becoming obsolete and losing its cutting edges."***

The biggest strength of our institute is the capability to keep producing the world's top-level unique bio-SPM technologies. Thus, individual bio-SPM researchers have been continuously updating their own development plans. To summarize them, we had intensive discussions among bio-SPM researchers and clarified the major targets. For HS-AFM, we aim to enhance the imaging rate from 10 fps to 100 fps. For 3D-AFM, we aim to visualize not only inside cells but also other 3D biological systems such as organelles and chromosomes. These are just a few examples out of many projects. The center director will review and discuss such development plans in the annual meetings with individual PIs to make sure that they are continuously updated. We added a new subject in the project map shown in Fig 1 as "3②Extending capabilities of various bio-SPMs" to explicitly indicate these developments.

Comment 6 ***"The supramolecular chemistry group has developed some biosensors to monitor cellular metabolites, but unfortunately, their selectivity and sensitivity are not high enough at present to be of practical use in combination with Bio-SPM. In the second half of the grant period, this situation needs to be substantially improved."***

We agree with this comment and will continue to improve the sensitivity and selectivity of the developed sensors. So far, we successfully developed biosensors for cancer-related metabolites such as lactate and 1-MNA. Now, we are exploring their various derivatives to make improvements. For example, we recently succeeded in improving the association constant of the 1-MNA sensor by 700 times and achieved the sensitivity (5.7×10^6 M^{-1}) required for practical use. In addition, we aim to further improve the sensitivity and selectivity by integrating many sensors into a polymer film with a controlled mesh size formed at the nanopipette apex. As for the lactate sensor, to rationally improve the lactate selectivity, we plan to introduce a bowl-shaped motif to interact with the methyl group into the previously developed Zn-based sensor molecule via a linker. Suitable bowl-shaped structures and linkers were selected based on thorough computational molecular modeling studies, and synthetic studies are now in progress.

Comment 7 ***"Human resources will be very important. Recruiting young researchers and getting students involved even at undergraduate levels is recommended."***

We agree that the recruitment of young talented researchers is the key for the sustainable development of our institute. So far, we have introduced various programs to promote external young researchers to visit our institute and perform Bio-SPM experiments (e.g., Bio-SPM summer school, collaborative research program, and visiting fellows program). These events are perfect

opportunities not only for their education but also for recruitment. In fact, we have so far successfully recruited three staff members from among the former attendees. We will continue to make these efforts. As for the involvement of students, we have so far established the graduate school division closely linked with our institute to foster next generation nano life scientists. In addition, most of the PIs also give lectures to undergraduate students in various departments, helping us to recruit them to our graduate school division. Furthermore, we plan to start a new lecture course by our Jr. PIs and other young researchers for undergraduate students.

Comment 8 ***"It may in the future be worthwhile to consider introducing robotics and AI in BioSPM technology so that biologists will be able to use it as easily as conventional microscopes without special training. Broader fields of researchers would then be able to produce a large amount of data from various viewpoints, which would greatly contribute to understanding cell functions."***

We expect that some of the AI-based technologies could be effective in Bio-SPM data analysis and will seriously consider such possibilities in our future development. Examples include super-resolution technology using compressed sensing, 3D feature extraction by AI-based segmentation, and noise removal from signals using statistical analysis. For automation using AI or robots, we will adopt different strategies depending on the purpose. As for the possibility of automation for realizing new measurement concepts, we would like to work on this development ourselves. Examples include large-scale high-resolution imaging by automatic tile scan, and an automatic recognition and extraction of organelles by nanopipettes. On the other hand, for automation to improve usability, we would like to contribute indirectly by transferring our developed technology to manufacturers and cooperating with them in the development of automation technology. We believe that this is the most efficient way to make our techniques accessible by non-expert users.

Appendix 1 FY 2021 List of Center's Research Results and Main Awards

1. Refereed Papers

- List only the Center's papers published in 2021. (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 10), 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) Fan Q., Yan L., Tripp M.W., Krejčí O., Dimosthenous S., Kachel S.R., Chen M., Foster A.S., Koert U., Liljeroth P., Gottfried J.M. "Biphenylene network: A nonbenzenoid carbon allotrope", Science 372 (2021) 852-856 (IF=47.728)

2) Kodera N., Noshiro D., Dora S.K., Mori T., Habchi J., Blocquel D., Gruet A., Dosnon M., Salladini E., Bignon C., Fujioka Y., Oda T., Noda N.N., Sato M., Lotti M., Mizuguchi M., Longhi S., Ando T. "Structural and dynamics analysis of intrinsically disordered proteins by high-speed atomic force microscopy", Nat. Nanotechnol. 16 (2021) 181-189 (IF=39.213)

3) Kezilebieke S., Silveira O.J., Huda M.N., Vaňo V., Aapro M., Ganguli S.C., Lahtinen J., Mansell R., van Dijken S., Foster A.S., Liljeroth P. "Electronic and Magnetic Characterization of Epitaxial CrBr3 Monolayers on a Superconducting Substrate", Adv Mater 33 (2021) 2006850 (IF=30.849)

4) Lim K., Nishide G., Yoshida T., Watanabe-Nakayama T., Kobayashi A., Hazawa M., Hanayama R., Ando T., Wong R.W. "Millisecond dynamic of SARS-CoV-2 spike and its interaction with ACE2 receptor and small extracellular vesicles", J. Extracell. Vesicles 10 (2021) e12170 (IF=25.841)
5) Boott C.E., Soto M.A., Hamad W.Y., MacLachlan M.J. "Shape-Memory Photonic Thermoplastics from Cellulose Nanocrystals", Adv. Funct. Mater. 31 (2021) 2103268 (IF=18.808)
6) Yan L., Silveira O.J., Alldritt B., Krejčí O., Foster A.S., Liljeroth P. "Synthesis and Local Probe Gating of a Monolayer Metal-Organic Framework", Adv. Funct. Mater. 31 (2021) 2100519 (IF=18.808)
7) Chen D., Zhang H., Miyazawa K., Kojima R., Zhang P., Yang L., Sun Q., Shao G., Fukuma T., Gao Y., Nguyen N.-T., Raston C.L., Jia G., Zhao D., Prasad P.N., Wang S., Li Q. "Quasi-solid-state self-assembly of 1D-branched ZnSe/ZnS quantum rods into parallel monorail-like continuous films for solar devices", Nano Energy 89 (2021) 106348 (IF=17.881)
8) Yan L., Silveira O.J., Alldritt B., Kezilebieke S., Foster A.S., Liljeroth P. "Two-Dimensional Metal-Organic Framework on Superconducting NbSe2", ACS Nano 15 (2021) 17813-17819 (IF=15.881)
9) Fa S., Mizobata M., Nagano S., Suetsugu K., Kakuta T., Yamagishi T.-A., Ogoshi T. "Reversible "on/Off" Chiral Amplification of Pillar[5]arene Assemblies by Dual External Stimuli", ACS Nano 15 (2021) 16794-16801 (IF=15.881)
10) Matusovsky O.S., Kodera N., Maceachen C., Ando T., Cheng Y.-S., Rassier D.E. "Millisecond Conformational Dynamics of Skeletal Myosin II Power Stroke Studied by High-Speed Atomic Force Microscopy", ACS Nano 15 (2021) 2229-2239 (IF=15.881)
11) Sueyoshi S., Taniguchi T., Tanaka S., Asakawa H., Nishimura T., Maeda K. "Understanding the Polymerization of Diphenylacetylenes with Tantalum(V) Chloride and Cocatalysts: Production of Cyclic Poly(diphenylacetylene)s by Low-Valent Tantalum Species Generated in Situ", J. Am. Chem. Soc. 143 (2021) 16136-16146 (IF=15.419)
12) Ikai T., Ando M., Ito M., Ishidate R., Suzuki N., Maeda K., Yashima E. "Emergence of Highly Enantioselective Catalytic Activity in a Helical Polymer Mediated by Deracemization of Racemic Pendants", J. Am. Chem. Soc. 143 (2021) 12725-12735 (IF=15.419)
13) Catti L., Narita H., Tanaka Y., Sakai H., Hasobe T., Tkachenko N.V., Yoshizawa M. "Supramolecular Singlet Fission of Pentacene Dimers within Polyaromatic Capsules", J. Am. Chem. Soc. 143 (2021) 9361-9367 (IF=15.419)
14) Echizen K., Taniguchi T., Nishimura T., Maeda K. "Synthesis of Stereoregular Telechelic Poly(phenylacetylene)s: Facile Terminal Chain-End Functionalization of Poly(phenylacetylene)s by Terminative Coupling with Acrylates and Acrylamides in Rhodium-Catalyzed Living Polymerization of Phenylacetylenes", J. Am. Chem. Soc. 143 (2021) 3604-3612 (IF=15.419)
15) Sakamoto S., Taniguchi T., Sakata Y., Akine S., Nishimura T., Maeda K. "Rhodium(I) Complexes Bearing an Aryl-Substituted 1,3,5-Hexatriene Chain: Catalysts for Living Polymerization of Phenylacetylene and Potential Helical Chirality of 1,3,5-Hexatrienes", Angew. Chem. Int. Ed. 60 (2021) 22201-22206 (IF=15.336)
16) Narita H., Catti L., Yoshizawa M. "An Aromatic Micelle-Based Saccharide Cluster with Changeable Fluorescent Color and its Protein Interactions", Angew. Chem. Int. Ed. 60 (2021) 12791-12795 (IF=15.336)

17) Ikai T., Kurake T., Okuda S., Maeda K., Yashima E. "Racemic Monomer-Based One-Handed Helical Polymer Recognizes Enantiomers through Auto-Evolution of Its Helical Handedness Excess", Angew. Chem. Int. Ed. 60 (2021) 4625-4632 (IF=15.336)

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19) Xu J., Yang X., Deng Q., Yang C., Wang D., Jiang G., Yao X., He X., Ding J., Qiang J., Tu J., zhang R., Lei Q.-Y., Shao Z.-M., Bian X., Hu R., Zhang L., Liu S. "TEM8 marks neovasculogenic tumor-initiating cells in triple-negative breast cancer", Nat. Commun. 12 (2021) 4413 (IF=14.919)

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21) Mihara E., Watanabe S., Bashiruddin N.K., Nakamura N., Matoba K., Sano Y., Maini R., Yin Y., Sakai K., Arimori T., Matsumoto K., Suga H., Takagi J. "Lasso-grafting of macrocyclic peptide pharmacophores yields multi-functional proteins", Nat. Commun. 12 (2021) 1543 (IF=14.919)

22) Kok S.Y., Oshima H., Takahashi K., Nakayama M., Murakami K., Ueda H.R., Miyazono K., Oshima M. "Malignant subclone drives metastasis of genetically and phenotypically heterogenous cell clusters through fibrotic niche generation", Nat. Commun. 12 (2021) 863 (IF=14.919)

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118) Dao L., Blaue C., Franz C.M. "Integrin α2β1 as a negative regulator of the laminin receptors α6β1 and α6β4", Micron 148 (2021) 103106 (IF=2.251)

119) Zhang Y., Sato R., Fukami T., Nakano M., Nakajima M. "Pirfenidone 5-hydroxylation is mainly catalysed by CYP1A2 and partly catalysed by CYP2C19 and CYP2D6 in the human liver", Xenobiotica 51 (2021) 1352-1359 (IF=1.908)

120) Bednarska J., Novak P., Korchev Y., Rorsman P., Tarasov A.I., Shevchuk A. "Release of insulin granules by simultaneous, high-speed correlative SICM-FCM", J. Microsc. 282 (2021) 21-29 (IF=1.758)

121) Blanco-Dorado S., Belles Medall M.D., Pascual-Marmaneu O., Campos-Toimil M., Otero-Espinar F.J., Rodríguez-Riego R., Rodríguez-Jato T., Zarra-Ferro I., Lamas M.J., Fernández-Ferreiro A. "Therapeutic drug monitoring of voriconazole: Validation of a high performance liquid chromatography method and comparison with an ARK immunoassay", Euro. J. Hosp. Pharm. Sci. Pra. 28 (2021) 154-159 (IF=1.652)

122) Fukuda S., Ando T. "Faster high-speed atomic force microscopy for imaging of biomolecular processes", Rev. Sci. Instrum. 92 (2021) 33705 (IF=1.523)

2. Review articles

123) Chaudhry M.T., Akine S., Maclachlan M.J. "Contemporary macrocycles for discrete polymetallic complexes: Precise control over structure and function", Chem. Soc. Rev. 50 (2021) 10713-10732 (IF=54.564)

124) Klenerman D., Korchev Y., Novak P., Shevchuk A. "Noncontact Nanoscale Imaging of Cells", Annu. Rev. Anal. Chem. 14 (2021) 347-361 (IF=10.745)

125) Xu G., Wang K., Dong X., Yang L., Ebrahimi M., Jiang H., Wang Q., Ding W. "Review on corrosion resistance of mild steels in liquid aluminum", J. Mater. Sci. Technol. 71 (2021) 12-22 (IF=8.067)

126) Zhou Y., Takahashi Y., Fukuma T., Matsue T. "Scanning electrochemical microscopy for biosurface imaging", Curr. Opin. Electrochem. 29 (2021) 100739 (IF=7.271)

127) Sajidah E.S., Lim K., Wong R.W. "How sars-cov-2 and other viruses build an invasion route to hijack the host nucleocytoplasmic trafficking system", Cells 10 (2021) 1424 (IF=6.6)

128) Kato K., Ohtani S., Fa S., Ogoshi T. "Discrete and Continuous One-Dimensional Channels Based on Pillar[n]arenes", Bull. Chem. Soc. Jpn. 94 (2021) 2319-2328 (IF=5.488)

129) Yashima E., Maeda K. "Helical Polymers with Dynamic and Static Macromolecular Helicity Memory: The Power of Helicity Memory for Helical Polymer Synthesis and Applications", Bull. Chem. Soc. Jpn. 94 (2021) 2637-2661 (IF=5.488)

130) Napolitano L.M.R., Torre V., Marchesi A. "CNG channel structure, function, and gating: a tale of conformational flexibility", Pflug. Arch. Eur. J. Physiol. 473 (2021) 1423-1435 (IF=3.657)

131) Soto M.A., Kandel R., MacLachlan M.J. "Chromic Platinum Complexes Containing Multidentate Ligands", Eur. J. Inorg. Chem. 2021 (2021) 894-906 (IF=2.524)

132) Minato T., Umeda K., Kobayashi K., Araki Y., Konishi H., Ogumi Z., Abe T., Onishi H., Yamada H. "Atomic-level nature of solid/liquid interface for energy conversion revealed by frequency modulation atomic force microscopy", Jpn. J. Appl. Phys. 60 (2021) SE0806 (IF=1.48)

3. Proceedings

None

4. Other English articles

None

B. WPI-related papers

1. Original articles

133) Hangai S., Kawamura T., Kimura Y., Chang C.-Y., Hibino S., Yamamoto D., Nakai Y., Tateishi R., Oshima M., Oshima H., Kodama T., Moriya K., Koike K., Yanai H., Taniguchi T. "Orchestration of myeloid-derived suppressor cells in the tumor microenvironment by ubiquitous cellular protein TCTP released by tumor cells", Nat. Immunol. 22 (2021) 947–957 (IF=25.606)

134) Wang M., Han X., Liu C., Takayama R., Yasugi T., Ei S.-I., Nagayama M., Tanaka Y., Sato M. "Intracellular trafficking of Notch orchestrates temporal dynamics of Notch activity in the fly brain",

Nat. Commun. 12 (2021) 2083 (IF=12.121)

135) Miyata, K., Imai, Y., Hori, S., Nishio, M., Loo, T.M., Okada, R., Yang, L., Nakadai, T., Maruyama, R., Fujii, R., Ueda, K., Jiang, L., Zheng, H., Toyokuni, S., Sakata, T., Shirahige, K., Kojima, R., Nakayama, M., Oshima, M., Nagayama, S., Seimiya, H., Hirota, T., Saya, H., Hara, E., Takahashi, A. "Pericentromeric noncoding RNA changes DNA binding of CTCF and inflammatory gene expression in senescence and cancer", Proc. Natl. Acad. Sci. U. S. A. 118 (2021) e2025647118 (IF=11.205)

136) Kozawa, K., Sekai, M., Ohba, K., Ito, S., Sako, H., Maruyama, T., Kakeno, M., Shirai, T., Kuromiya, K., Kamasaki, T., Kohashi, K., Tanaka, S., Ishikawa, S., Sato, N., Asano, S., Suzuki, H., Tanimura, N., Mukai, Y., Gotoh, N., Tanino, M., Natsuga, K., Soga, T., Nakamura, T., Yabuta, Y., Saitou, M., Ito, T., Matsuura, K., Tsunoda, M., Kikumori, T., Iida, T., Mizutani, Y., Miyai, Y., Kaibuchi, K., Enomoto, A., Fujita, Y. "The CD44/COL17A1 pathway promotes the formation of multilayered, transformed epithelia", Curr. Biol. 31 (2021) 3086-30970000000 (IF=10.834)

137) Biswas F.B., Rahman I.M.M., Nakakubo K., Yunoshita K., Endo M., Nagai K., Mashio A.S., Taniguchi T., Nishimura T., Maeda K., Hasegawa H. "Selective recovery of silver and palladium from acidic waste solutions using dithiocarbamate-functionalized cellulose", Chem. Eng. J. 407 (2021) 127225 (IF=10.652)

138) Biswas F.B., Rahman I.M.M., Nakakubo K., Yunoshita K., Endo M., Mashio A.S., Taniguchi T., Nishimura T., Maeda K., Hasegawa H. "Comparative evaluation of dithiocarbamate-modified cellulose and commercial resins for recovery of precious metals from aqueous matrices", J. Hazard. Mater. 418 (2021) 126308 (IF=10.588)

139) Farinelli G., Di Luca A., Kaila V.R.I., MacLachlan M.J., Tiraferri A. "Fe-chitosan complexes for oxidative degradation of emerging contaminants in water: Structure, activity, and reaction mechanism", J. Hazard. Mater. 408 (2021) 124662 (IF=10.588)

140) Izumi K., Iwamoto H., Yaegashi H., Nohara T., Shigehara K., Kadono Y., Nanjo S., Yamada T., Ohtsubo K., Yano S., Mizokami A. "Androgen replacement therapy for cancer-related symptoms in male: result of prospective randomized trial (ARTFORM study)", J. Cachexia Sarcopenia Muscle 12 (2021) 831-842 (IF=9.802)

141) Tanimura, K., Yamada, T., Horinaka, M., Katayama, Y., Fukui, S., Morimoto, K., Nakano, T., Tokuda, S., Morimoto, Y., Iwasaku, M., Kaneko, Y., Uchino, J., Yoneda, K., Yano, S., Sakai, T., Takayama, K. "Inhibition of c-Jun N-terminal kinase signaling increased apoptosis and prevented the emergence of ALK-TKI-tolerant cells in ALK-rearranged non-small cell lung cancer", Cancer Lett. 522 (2021) 119-128 (IF=8.679)

142) Kusuma S.B.W., Hirose D., Yoshizawa A., Szabó L., Ina D., Wada N., Takahashi K. "Direct Synthesis of Full-Biobased Cellulose Esters from Essential Oil Component α,β-Unsaturated Aldehydes", ACS Sustain. Chem. Eng. 9 (2021) 8450-8457 (IF=8.198)

143) Goto S., Sakoda Y., Adachi K., Sekido Y., Yano S., Eto M., Tamada K. "Enhanced anti-tumor efficacy of IL-7/CCL19-producing human CAR-T cells in orthotopic and patient-derived xenograft tumor models", Cancer Immunol. Immunother. 70 (2021) 2503-2515 (IF=6.968)

144) Fukuda K., Otani S., Takeuchi S., Arai S., Nanjo S., Tanimoto A., Nishiyama A., Naoki K., Yano S. "Trametinib overcomes KRAS-G12V–induced osimertinib resistance in a leptomeningeal

carcinomatosis model of EGFR-mutant lung cancer", Cancer Sci. 112 (2021) 3784-3795 (IF=6.716)

145) Yoshimura, A., Yamada, T., Okuma, Y., Fukuda, A., Watanabe, S., Nishioka, N., Takeda, T., Chihara, Y., Takemoto, S., Harada, T., Hiranuma, O., Shirai, Y., Nishiyama, A., Yano, S., Goto, Y., Shiotsu, S., Kunimasa, K., Morimoto, Y., Iwasaku, M., Kaneko, Y., Uchino, J., Kenmotsu, H., Takahashi, T., Takayama, K. "Impact of tumor programmed death ligand-1 expression on osimertinib efficacy in untreated EGFR-mutated advanced non-small cell lung cancer: a prospective observational study", Transl. Lung Cancer Res. 10 (2021) 3582-3593 (IF=6.498)

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147) Yasuda, H., Ichihara, E., Sakakibara-Konishi, J., Zenke, Y., Takeuchi, S., Morise, M., Hotta, K., Sato, M., Matsumoto, S., Tanimoto, A., Matsuzawa, R., Kiura, K., Takashima, Y., Yano, S., Koyama, J., Fukushima, T., Hamamoto, J., Terai, H., Ikemura, S., Takemura, R., Goto, K., Soejima, K. "A phase I/II study of osimertinib in EGFR exon 20 insertion mutation-positive non-small cell lung cancer", Lung Cancer 162 (2021) 140-146 (IF=5.705)

148) Edwards, C., Klekot, O., Halugan, L., Korchev, Y. "Follow Your Nose: A Key Clue to Understanding and Treating COVID-19", Front. Endocrinol. 12 (2021) 14582-14585 (IF=5.555)

149) Kashida, H., Nishikawa, K., Ito, Y., Murayama, K., Hayashi, I., Kakuta, T., Ogoshi, T., Asanuma, H. "A Pyrene-Modified Serinol Nucleic Acid Nanostructure Converts the Chirality of Threoninol Nucleic Acids into Circularly Polarized Luminescence Signals", Chem.-Eur. J. 27 (2021) 14582-14585 (IF=5.236)

150) Takeuchi S., Yanagitani N., Seto T., Hattori Y., Ohashi K., Morise M., Matsumoto S., Yoh K., Goto K., Nishio M., Takahara S., Kawakami T., Imai Y., Yoshimura K., Tanimoto A., Nishiyama A., Murayama T., Yano S. "Phase 1/2 study of alectinib in RET-rearranged previously-treated non-small cell lung cancer (ALL-RET)", Transl. Lung Cancer Res. 10 (2021) 314-325 (IF=5.132)

151) Sivakumar A., Dhas S.S.J., Almansour A.I., Kumar R.S., Arumugam N., Prabhu S., Sivashanmugan K., Ramesh R., Dhas S.A.M.B. "Shock waves induced enhancement of electrochemical properties of CoFe2O4 nanoparticles for energy storage applications", Surf. Interfaces 27 (2021) 101539 (IF=4.837)

152) Uramoto H., Takiguchi T., Koizumi T., Tanimoto A., Hayashi R., Nakazawa Y., Ito K.-I., Nakada M., Hirono Y., Nishino Y., Yano S. "Multi-institutional survey of malignant pleural mesothelioma patients in the Hokushin region", J. Cancer Res. Clin. Oncol. 148 (2021) 1153-1158 (IF=4.553)

153) Yamada, Y., Miwa, Y., Toyoda, Y., Yamaguchi, T., Akine, S., Tanaka, K. "Synthesis of a monocationic μ-nitrido-bridged iron porphycene dimer and its methane oxidation activity", Dalton Trans. 50 (2021) 16775-16781 (IF=4.39)

154) Fleming, D.G., MacFarlane, W.A., Xiao, J., Arseneau, D.J., Kiefl, R.F., Cao, Y., Wang, P.-X., MacLachlan, M.J., Bridges, M.D. "Level Crossing Resonance Studies of the Muoniated Cyclohexadienyl Radical (MuĊ6H6) Interacting with Uncapped Gold Nanoparticles in Porous Silica Hosts", J. Phys. Chem. C 125 (2021) 21410-21423 (IF=4.126)

155) Maynard S.A., Pchelintseva E., Zwi-Dantsis L., Nagelkerke A., Gopal S., Korchev Y.E., Shevchuk A., Stevens M.M. "IL-1β mediated nanoscale surface clustering of integrin α5β1 regulates the adhesion of mesenchymal stem cells", Sci Rep 11 (2021) 6890 (IF=3.998)

156) Yamamura, H., Hagiwara, T., Hayashi, Y., Osawa, K., Kato, H., Katsu, T., Masuda, K., Sumino, A., Yamashita, H., Jinno, R., Abe, M., Miyagawa, A. "Antibacterial Activity of Membrane-Permeabilizing Bactericidal Cyclodextrin Derivatives", ACS Omega 6 (2021) 31831-31842 (IF=3.512)

157) Sato S., Tanimoto A., Yanagimura N., Suzuki C., Takumi Y., Nishiyama A., Yamashita K., Takeuchi S., Ohtsubo K., Makino T., Yoshida Y., Hirono Y., Hayashi R., Koizumi T., Nakazawa Y., Ito K.-I., Motoo Y., Uramoto H., Nakada M., Nishino Y., Yano S. "Multi-institutional survey of cancer disparities in disabled patients in the region of northwestern Japan", Int. J. Clin. Oncol. 26 (2021) 1009-1014 (IF=2.879)

158) Nishiyama A., Staub Y., Suga Y., Fujita M., Tanimoto A., Ohtsubo K., Yano S. "Sarcopenia may influence the prognosis in advanced thyroid cancer patients treated with molecular targeted therapy", In Vivo 35 (2021) 401-410 (IF=2.155)

159) Ohtsubo K., Yamashita K., Yanagimura N., Suzuki C., Tanimoto A., Nishiyama A., Takeuchi S., Iwaki N., Kawano M., Izumozaki A., Inoue D., Gabata T., Ikeda H., Watanabe M., Yano S. "Multiple malignant lymphomas of the bile duct developing after spontaneous regression of an autoimmune pancreatitis-like mass", Intern. Med. 60 (2021) 409-415 (IF=1.271)

2. Review articles

160) Yoshihara, M., Mizutani, S., Kato, Y., Matsumoto, K., Mizutani, E., Mizutani, H., Fujimoto, H., Osuka, S., Kajiyama, H. "Recent insights into human endometrial peptidases in blastocyst implantation via shedding of microvesicles", Int. J. Mol. Sci. 22 (2021) 13479 (IF=5.923)

3. Proceedings

None

4. Other English articles

161) Oshima, H., Ju, X., Echizen, K., Han, T.-S., Oshima, M. "The role of inflammation in gastric tumorigenesis", Research and Clinical Applications of Targeting Gastric Neoplasms (2021) 25-42 (IF=N/A)

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

- List up to 10 main presentations during FY 2021 in order from most recent.
- For each, write the date(s), lecturer/presenter's name, presentation title, and conference name.

Date(s)	Lecturer/Presenter's name	Presentation title	Conference name
2022/3/30	Takeshi Fukuma, Kazuki Miyata, Yuta Kawagoe, Naoyuki Miyashita, Tomoki Nakagawa	Atomic-scale structures and dynamics at the growing calcite step edges investigated by high-speed frequency modulation atomic force microscopy	Faraday Discussion - Understanding Crystallization
2021/12/18	Shigehisa Akine	Dynamic control of coordination chirality of triple-helical metallocryptands	The International Chemical Congress of Pacific Basin Societies 2021 (Pacifichem2021)
2021/11/18	Miki Nakajima, Masataka Nakano	Epitranscriptional regulation of drug-metabolizing enzymes.	36th Japanese Society for the Study of Xenobiotics.
2021/10/25	Takeshi Fukuma	Visualizing Inside of 3D Self-Organizing Systems by 3D-AFM	AVS 67 Virtual Symposium
2021/8/13	Yasufumi Takahashi	Nanoscale electrochemical imaging for Visualizing the Heterogeneous Catalytic Activity	ISE Annual Meeting
2021/7/13	Tomoki Ogoshi	Pillar-Shaped Macrocyclic Compounds "Pillar[n]arenes": from Simple Molecular Receptors to Supramolecular Assemblies	ISMSC-2021 (Online)
2021/7/6	Atsushi Hirao	Cell fate decision by metabolic regulation in hematopoietic stem cell	The 39th Sapporo International Cancer Symposium

		homeostasis and leukemogenesis.	
2021/7/6	Masanobu Oshima	Malignant cancer cells drive polyclonal metastasis through fibrotic niche generation	The 39th Sapporo International Cancer Symposium
2021/6/30	Adam S. Foster	Automated Structure Discovery in Atomic Force Microscopy	ISPM
2021/4/21	Mark MacLachlan	New Photonic Materials from Cellulose Nanocrystals	Materials Research Society (MRS) Spring Meeting

3. Major Awards

- List up to 10 main awards received during FY 2021 in order from the most recent.
- For each, write the date issued, the recipient's name, and the name of award.
- In case of multiple recipients, underline those affiliated with the center.

Date	Recipient's name	Name of award
2022/3/17	Toshio Ando	The 53rd Naito Foundation Merit Award
2022/2/21	Tomoki Ogoshi	SPSJ Science Award 2021
2022/1/28	Yasufumi Takahashi	The 16th Wakashachi Incentive Award (Grand Prize)
2022/1/7	Rikinari Hanayama	JB Reviewer Award
2021/5/19	Toshio Ando	Ishikawa Television Award

Appendix 2　FY 2021 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 FY2021> **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	45	Nano Life Science Institute, Kanazawa University	Doctor of Engineering, Electrical engineering, Nanometrology	90	October, 2017	usually stays at the institute	
Toshio Ando ※1	71	Nano Life Science Institute, Kanazawa University	Doctor of Science, Biophysics and Nano-Bioscience	90	October, 2017	usually stays at the institute	
Yuri Korchev	61	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	58	Nano Life Science Institute, Kanazawa University	Doctor of Medicine, Stem Cell Biology	90	October, 2017	usually stays at the institute	

Masanobu Oshima	60	Nano Life Science Institute, Kanazawa University	D.V.M., Ph.D., Cancer research, Genetics for Cancer modeling	90	October, 2017	usually stays at the institute	
Seiji Yano	56	University Hospital, Kanazawa University	MD, PhD, Medical Oncology, Circumvention of targeted drug resistance	50	October, 2017	usually stays at the institute	
Kunio Matsumoto	63	Cancer Research Institute, Kanazawa University	Doctor of Philosophy, Biological Chemistry, Tumor Biology	50	October, 2017	usually stays at the institute	
Rikinari Hanayama	47	Nano Life Science Institute, Kanazawa University	MD, PhD, Immunology, Cell Biology	90	October, 2017	usually stays at the institute	
Richard W. Wong	47	Nano Life Science Institute, Kanazawa University	Doctor of Medicine, Molecular cell biology	90	October, 2017	usually stays at the institute	
Miki Nakajima	52	Nano Life Science Institute, Kanazawa University	Doctor of Pharmaceutical Sciences, Drug Metabolism and Toxicology, Clinical Pharmacology	90	October, 2017	usually stays at the institute	
Shigehisa Akine	49	Nano Life Science Institute, Kanazawa University	Doctor of Science, Supramolecular chemistry, Coordination chemistry	90	October, 2017	usually stays at the institute	

Katsuhiro Maeda	51	Nano Life Science Institute, Kanazawa University	Doctor of Engineering, Polymer chemistry	90	October, 2017	usually stays at the institute	
Tomoki Ogoshi	45	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.	
Mark MacLachlan	48	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	46	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 ※2	71	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 57 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 working for the center vis-à-vis his/her total working hours.

※1 Upon Professor Ando having been awarded the honorary position of Kanazawa University Distinguished Professor, Professor Kodera will replace him as a PI from April 2022 for generational shift to cover the later half of the WPI grand period and beyond.

※2 Professor Mikhailov requested to decline an overseas PI position due to physical difficulties in traveling to Japan, because of his advanced age and the risks involved from the COVID-19 pandemic, therefore Professor Carsten Beta (University of Potsdam, Germany) will be an overseas PI from April 2022.

Principal investigators unable to participate in project in FY 2021

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

Appendix 2a Biographical Sketch of a New Principal Investigator

(within 3 pages per person)

Name (Age) Carsten Beta * (47)

Affiliation and position (Position title, department, organization, etc.)

Professor, Chair of Biological Physics, University of Potsdam, Institute of Physics and Astronomy, Germany

Academic degree and specialty

Doctor of Natural Sciences, Specialty: Biophysics, Pattern Formation

Effort **20%**

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

Research and education history

(Education)

2001 Diploma in chemistry, Universität Karlsruhe

2004 Dr. rer. nat, Fritz-Haber-Institute of the Max Planck Society and Freie Universität Berlin

(Professional Background)

2005 Post-doctoral research fellow, Cornell University and University of California, San Diego, USA

2005 – 2007 Group Leader, Max Planck Institute for Dynamics und Self-Organization, Department of Fluid Dynamics, Pattern Formation and Biocomplexity, Göttingen

2007 – 2009 Junior professor of Biological Physics (W1, assistant professor), U Potsdam

2009 – 2017 Professor of Biological Physics (W2, associate professor), U Potsdam

since 10/2017 Professor of Biological Physics (W3, full professor), U Potsdam

Achievements and highlights of past research activities

1. Cell motility and chemotaxis: My group has established long-standing expertise in quantitative migration studies of both amoeboid cells (***Eur. J. Cell Biol.*** 2006, citation 138; ***EPL*** 2010, citation 50; ***Phys. Rev. Lett.*** 2012, citation 22) and bacterial swimmers (***Biophys. J.*** 2013, citation 70), where we have identified novel swimming modes (***Sci. Rep.*** 2017, citation 26) and chemotaxis strategies (***Sci. Adv.*** 2020, citation 7). We rely on optical imaging, dedicated microfluidic tools (***Anal. Chem.*** 2007, citation 51), and custom-made cell segmentation and tracking algorithms (***PLOS Comput. Biol.*** 2021) to perform and analyze our live-cell experiments.

2. Nonlinear dynamics and pattern formation: I have worked on pattern formation and feedback control in different physicochemical reaction-diffusion systems (***Phys. Rev. E*** 2003, citation 150; ***Phys. Rev. Lett.*** 2005, citation 39). My group used concepts from this field to study the response time scales of the actin cytoskeleton (***PNAS*** 2013, citation 43; ***Phys. Rev. Lett.*** 2016, citation 7) and the dynamics of cortical actin waves in motile amoeboid cells (***Annu. Rev. Cond. Mat. Phys.*** 2017, citation 33), relying on both experiments (***J. Cell Sci.*** 2014, citation 7; ***PNAS*** 2020, citation 13) and modeling (***PLOS One*** 2018, citation 24).

Achievements

(1) International influence * Describe the kind of attributes listed below.

a) Recipient of international awards: None.

b) Member of a scholarly academy in a major country:
Member of the council (Vorstandsrat) of the German Physical Society (since 10/2021)
Member of the American Physical Society
Member of the Berlin Center for Studies of Complex Chemical Systems

c) Guest speaker or chair of related international conference and/or director or honorary member of a major international academic society in the subject field
International Conference Engineering of Chemical Complexity 2019 (conference chair)
Annual Physics of Cancer Symposium 2017 (co-organizer)
Annual International Dictyostelium Conference 2014 (co-organizer) and others.

(Selected invited talks)

1. Biohybrid active matter — The complex dance of motile cells with passive micro-cargo, 5th Venice Meeting on Fluctuations in Small Complex Systems, Venice, Italy, Oct. 7, 2021
2. Dynamical wave patterns in the cortex of motile amoeboid cells, Conference on Advances in Pattern Formation, Sede Boqer, Israel, Feb. 18, 2019
3. A bacterial swimmer with decisional freedom between two alternative movement strategies, Workshop on Stochastic Dynamics, Buenos Aires, Argentina, Mar. 20, 2017
4. Cortical wave patterns in giant *Dictyostelium* cells, 9th EAI International Conference on Bio-inspired Information and Communication Technologies, New York City, USA, Dec. 3, 2015
5. Spatiotemporal patterns in the actin cortex of motile cells, Gordon Research Conference on Oscillations & Dynamic Instabilities in Chemical Systems, Girona, Spain, July 16, 2014

d) Editor of an international academic journal
Biosensors (member of the editorial board)
International Journal of Molecular Sciences (member of the editorial board)

e) Peer reviewer for an overseas competitive research program (etc.)
Deutsche Forschungsgemeinschaft (DFG, Germany), European Research Council (ERC), Agence Nationale de la Recherche (ANR, France), German-Israeli Foundation for Scientific Research and Development (GIF), Fonds de la Recherche Scientifique (FNRS, Belgium), and others.

(2) Receipt of major large-scale competitive funds (over the past 5 years)

1. DFG Collaborative Research Center 1294 (318763901), Project B02: Inferring the dynamics underlying protrusion-driven cell motility, since Jul. 2021, funding for 1 PhD student, travel and consumables, PI (together with Prof. M. Holschneider, U Potsdam)
2. DFG Collaborative Research Center 1294 (318763901), Project B07: Inferring active particle dynamics by data assimilation, since Jul. 2021, funding for 2 PhD students, travel and consumables, PI (together with Dr. R. Großmann, U Potsdam, and Prof. M. Opper, TU Berlin)
3. EFRE Program Support of Infrastructure for Research, Development, and Innovation (85045803): A maskless aligner for microfabrication, Aug. 2020, EUR 142,800, PI
4. DFG Individual Research Grant (BE 3978/13-1): How confinement impacts flagellar dynamics and motility of bacteria, since Sept. 2020, funding for 1 PhD student, travel and consumables, PI (together with Prof. K. Thormann, U Gießen)

5. DFG Individual Research Grant (BE 3978/3-3): A quantitative study of eukaryotic chemotaxis – motile amoeboid cells as microtransporters, since Jan. 2019, funding for 1 PhD student, travel and consumables, PI
6. BMBF Ideenwettbewerb "Neue Produkte für die Bioökonomie" – IBÖ (031B0653): A paper-based electrochemical rapid test system for drinking water analytics (PeTrA), July 2018 – Oct. 2020, funding for 1 Postdoc, 1 PhD student, travel and consumables, coordinator and PI

(3) Major publications (Titles of major publications, year of publication, journal name, number of citations)

(out of more than 70 peer reviewed publications)

1. Flemming, S., Font, F., Alonso, S., and Beta, C. (2020). How cortical waves drive fission of motile cells. ***PNAS***, 117:6330-6338., citation 13.
2. Alirezaeizanjani, Z., Großmann, R., Pfeifer, V., Hintsche, M., Beta, C. (2020). Chemotaxis strategies of bacteria with multiple run modes. ***Sci. Adv.***, 6:eaaz6153., citation 7
3. Hsu, H.-F., Bodenschatz, E., Westendorf, C., Gholami, A., Pumir, A., Tarantola, M., and Beta, C. (2017). Variability and order in cytoskeletal dynamics of motile amoeboid cells. ***Phys. Rev. Lett.***, 119:148101., citation 4
4. Negrete Jr., J., Pumir, A., Hsu, H.-F., Westendorf, C., Tarantola, M., Beta, C., and Bodenschatz, E. (2016). Noisy oscillations in the actin cytoskeleton of chemotactic amoeba. ***Phys. Rev. Lett.***,117:148102., citation 7
5. Westendorf, C., Negrete, J., Bae, J.A., Sandmann, R., Bodenschatz, E., and Beta, C. (2013). Actin cytoskeleton of chemotactic amoebae operates close to the onset of oscillations. ***PNAS***, 110:3853-3858., citation 43
6. Theves, M., Taktikos, J., Zaburdaev, V., Stark, H., and Beta, C. (2013). A bacterial swimmer with two alternating speeds of propagation. ***Biophys. J.***, 105:1915-1924., citation 70
7. Amselem, G., Theves, M., Bae, A., Beta, C., and Bodenschatz, E. (2012). Control parameter description of eukaryotic chemotaxis. ***Phys. Rev. Lett.***, 109:108103., citation 22
8. Beta, C., Wyatt, D., Rappel, W.-J., and Bodenschatz, E. (2007). Flow-photolysis for spatio-temporal stimulation of single cells. ***Anal. Chem.***, 79:3940-39944., citation 51
9. Varela, H., Beta, C., Bonnefont, A., and Krischer, K. (2005). Transitions to electrochemical turbulence. ***Phys. Rev. Lett.***, 94:174104., citation 39
10. Beta, C., Moula, M.G., Mikhailov, A.S., Rotermund, H.H., and Ertl G. (2004). Excitable CO oxidation on Pt(110) under nonuniform coupling. ***Phys. Rev. Lett.***, 93:188302., citation 38

(4) Others (Other achievements indicative of the PI's qualification as a top-world researcher, if any.)

1. Excellence in Teaching Award, Faculty of Science, U Potsdam (2020)
2. Fellow of the Studienstiftung des Deutschen Volkes (German Academic Merit Foundation)
3. Procter & Gamble Förderpreis (2000)

Appendix 3-1 FY 2021 Records of Center Activities

1. Researchers and center staff, satellites, partner institutions

1-1. Number of researchers in the "core" established within the host institution

- Regarding the number of researchers at the Center, fill in the table in Appendix 3-1a.

Special mention

- Enter matters warranting special mention, such as concrete plans for achieving the Center's goals, established schedules for employing main researchers, particularly principal investigators.
- As background to how the Center is working on the global circulation of world's best brains, give good examples, if any, of how career paths are being established for the Center's researchers; that is, from which top-world research institutions do researchers come to the Center and to which research institutions do the Center's researchers go, and how long are their stays at those institutions.

1-2. Satellites and partner institutions

- List the satellite and partner institutions in the table below.
- Indicate newly added and deleted institutions in the "Notes" column.
- If satellite institutions have been established overseas, describe by satellite the Center's achievements in coauthored papers and researcher exchanges in Appendix 4.

<Satellite institutions>

Institution name	Principal Investigator(s), if any	Notes
Imperial College London	Yuri Korchev	Established the Agreement in January 2019 (The effective date is January 15 2019).
University of British Columbia	Mark MacLachlan	Established the Agreement in October 2018 (The effective date is April 2019).

< Partner institutions>

Institution name	Principal Investigator(s), if any	Notes
RIKEN Center for Biosystems Dynamics Research		Established the collaborative research agreement in May 2018
Nikon Instech Co., Ltd.		Established the collaborative research agreement in May 2019
MicroBiology Research Center for Sustinability, Tsukuba University		Established the collaborative research agreement in June 2019
National Institutes for Quantum Science and Technology (QST), Institute of Quantum Life Science		NEW Established the collaborative research agreement in June 2021

2. Holding international research meetings

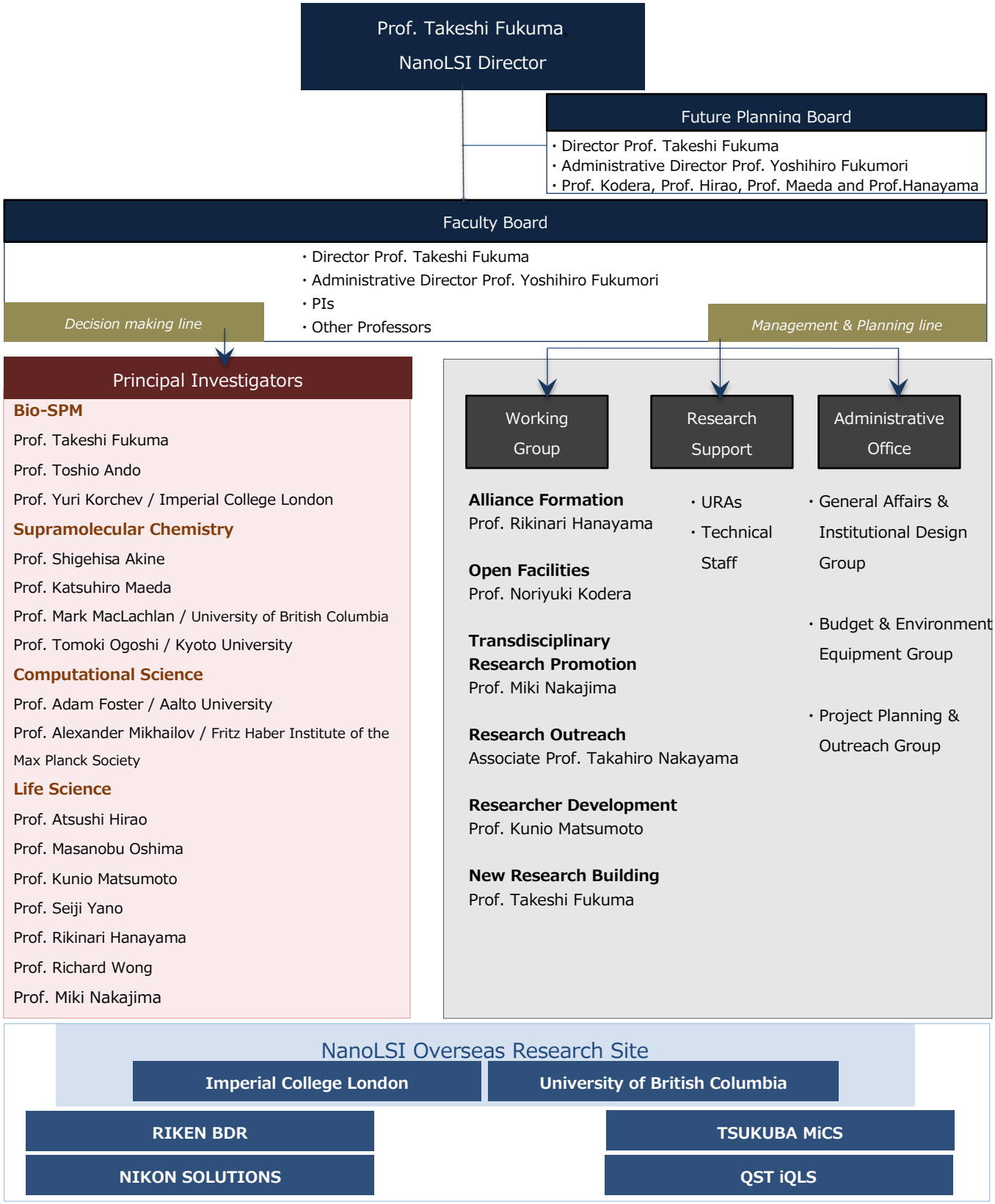
- Indicate the number of international research conferences or symposiums held in FY2021 and give up to three examples of the most representative ones using the table below.

FY 2021: 2 meetings

Major examples (meeting titles and places held)	Number of participants
5th NanoLSI Symposium – Understanding Nanoscale Biological Processes in the Cells, On-site (limited): NanoLSI Main Conference Room / Online (Zoom)	From domestic institutions: 143 From overseas institutions: 23
International Symposium on Tumor Biology in Kanazawa 2021, On-site: NanoLSI Main Conference Room / Online (Zoom)	From domestic institutions: 140 From overseas institutions: 5

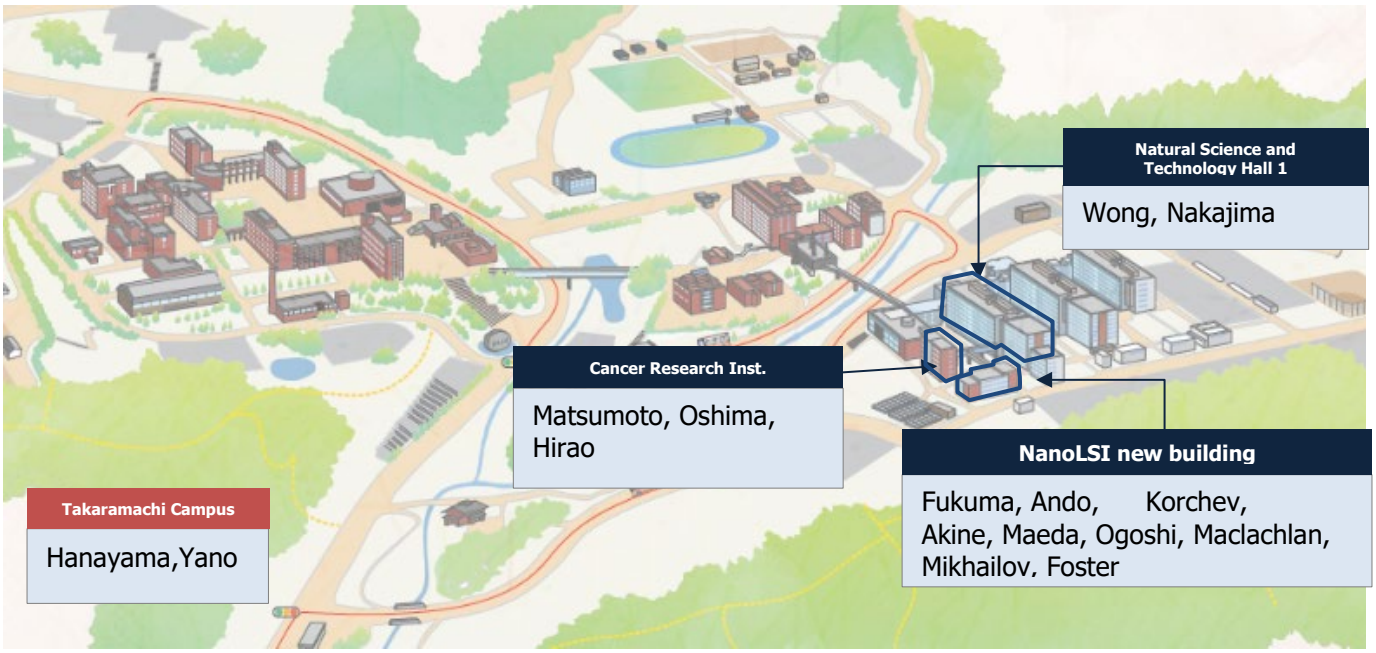
3. Diagram of management system

- Diagram the center's management system and its position within the host institution in an easily understood manner.
- If any new 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).



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.

5. Securing external research funding*

External research funding secured in FY2021

※Including Indirect funding

	number	amount(JPY)
■Grants-in-Aid for Scientific Research	123	438,862,644
■Commissioned research projects	41	431,819,020
■Joint research projects	18	69,379,899
■Donations	166	267,865,066
Total	348	1,207,926,629

- Describe external funding warranting special mention. Include the name and total amount of each grant.

* External research funding includes "KAKENHI," funding for "commissioned research projects," "joint research projects," and for others (donations, etc.) as listed under "Research projects" in Appendix 3-2, Project Expenditures.

Appendix 3-1a FY 2021 Records of Center Activities

Researchers and other center staff

Number of researchers and other center staff

* Fill in the number of researchers and other center staff 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 2021	Final goal (Date: March, 2023)
Researchers from within the host institution	12	11	12
Researchers invited from overseas	4	4	4
Researchers invited from other Japanese institutions	0	1	1
Total principal investigators	16	16	17

b) Total members

	At the beginning of project		At the end of FY 2021		Final goal (Date: March, 2023)	
	Number of persons	%	Number of persons	%	Number of persons	%
Researchers	49		81		88	
Overseas researchers	8	16	31	38	38	43
Female researchers	6	12	15	19	17	19
Principal investigators	16		16		17	
Overseas PIs	5	31	5	31	6	35
Female PIs	1	6	1	6	2	12
Other researchers	27		37		38	
Overseas researchers	1	4	2	5	3	8
Female researchers	5	19	7	19	7	18
Postdocs	6		28		33	
Overseas postdocs	2	33	24	86	29	88
Female postdocs	0	0	7	25	8	24
Research support staffs	8		41		32	
Administrative staffs	13		17		18	
Total number of people who form the "core" of the research center	70		139		138	

Appendix 3-2 Project Expenditures

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	34.8	19.7
	Principal investigators (no. of persons):12	163.3	45.0
	Junior Principal investigators (no. of persons):6	65.8	57.5
	Other researchers (no. of persons):64	294.1	189.5
	Research support staff (no. of persons):9	40.7	39.0
	Administrative staff (no. of persons):16	106.3	56.1
	Remuneration for RA(Research Assistant)	13.0	13.0
	Subtotal	718	419.8
Project activities	Gratuities and honoraria paid to invited principal investigators (no. of persons):3	3.6	3.6
	Research startup cost (no. of persons):10	35.3	35.3
	Cost of satellite organizations (no. of satellite organizations):2	20.8	20.8
	Cost of international symposiums (no. of symposiums):1	4.6	4.6
	Facility expenses	24.6	2.8
	Cost of consumables	28.8	28.8
	Cost of utilities	22.5	22.2
	Other costs	57.9	55.4
	Subtotal	198.1	173.5
Travel	Domestic travel costs	1.8	1.8
	Overseas travel costs	0	0
	Travel and accommodations cost for invited scientists		
	(no. of domestic scientists):0	0	0
	(no. of overseas scientists):1	1.4	1.4
	Travel cost for scientists on transfer		
	(no. of domestic scientists):2	0.2	0.2
	(no. of overseas scientists):0	0	0
	Subtotal	3.4	3.4
Equipment	Depreciation of buildings	0.1	0.1
	Depreciation of equipment	1.5	1.5
	Subtotal	1.6	1.6
Research projects (Detail items must be fixed)	Project supported by other government subsidies, etc. *1	113.2	0
	KAKENHI	261.2	0
	Commissioned research projects, etc.	247.9	0
	Joint research projects	29.5	0
	Ohers (donations, etc.)	39.1	0
	Subtotal	690.9	0
Total		1,612.0	598.3

Costs (Million yens)

WPI grant in FY 2021	700
Costs of establishing and maintaining facilities	2.7
Establishing new facilities	0
Repairing facilities	0
Others	2.7
Costs of equipment procured	74.9
Atomic Force Microscopy System	44.5
AFM-Head	10
Osmium coating system	4.1
Others	16.3

*1. Management Expenses Grants (including Management Enhancements Promotion Expenses (機能強化経費)), subsidies including 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.
*3 The amounts of "Research projects" represent the actual expenditure in FY2021, not accounting depreciation concerning equipment purchased by "Research projects" budget.

*1 運営費交付金（機能強化経費を含む）、国立大学改革強化推進補助金等の補助金、間接経費、その他大学独自の取組による学内リソースの配分等による財源
*2 科研費、受託研究費、共同研究費等によって人件費、旅費、設備備品等費を支出している場合も、その額は「研究プロジェクト費」として計上すること
*3「研究プロジェクト費」は減価償却費を含まない実支出額を計上している

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):3		
	Subtotal	16.3	16.3
Project activities	Subtotal	2.0	2.0
Travel	Subtotal	0	0
Equipment	Subtotal	0	0
Research projects	Subtotal	0	0
Total		18.3	18.3

Appendix 4 FY 2021 Status of Collaboration with Overseas Satellites

1. Coauthored Papers

- List the refereed papers published in FY 2021 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. Italicize the names of authors affiliated with overseas satellite institutions.
- For reference write the Appendix 1 item number in parentheses after the item number in the blocks below. Let it free, if the paper is published in between Jan.-Mar. 2022 and not described in Appendix 1.

Overseas Satellite 1 **Imperial College London** (Total: 3 papers)

1) (Appendix 1 #116) Sato K., Sato F., Kumano M., Kamijo T., Sato T., Zhou Y., *Korchev Y.*, Fukuma T., Fujimura T., Takahashi Y. "Electrochemical Quantitative Evaluation of the Surface Charge of a Poly(1-Vinylimidazole) Multilayer Film and Application to Nanopore pH Sensor", Electroanalysis 33 (2021) 1633-1638 (IF=3.223)

2) Vaneev A.N., Gorelkin P.V., Krasnovskaya O.O., Akasov R.A., Spector D.V., Lopatukhina E.V., Timoshenko R.V., Garanina A.S., Zhang Y., Salikhov S.V., Edwards C.R.W., Klyachko N.L., Takahashi Y., Majouga A.G., *Korchev Y.E.*, Erofeev A.S. "In Vitro/ in Vivo Electrochemical Detection of Pt(II) Species", Anal. Chem. 94 (2022) 4901-4905 (IF=6.986)

3) Makarova M.V., Amano F., Nomura S., Tateishi C., Fukuma T., Takahashi Y., *Korchev Y.E.* "Direct Electrochemical Visualization of the Orthogonal Charge Separation in Anatase Nanotube Photoanodes for Water Splitting", ACS Catal. 12 (2022) 1201-1208 (IF=13.084)

Overseas Satellite 2 **University of British Columbia** (Total: 1 paper)

1) (Appendix 1 #123) Chaudhry M.T., Akine S., *Maclachlan M.J.* "Contemporary macrocycles for discrete polymetallic complexes: Precise control over structure and function", Chem. Soc. Rev. 50 (2021) 10713-10732 (IF=54.564)

2. Status of Researcher Exchanges

- Using the below tables, indicate the number and length of researcher exchanges in FY 2021. 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

<To satellite>

	Under 1 week	From 1 week to 1 month	From 1 month to 3 months	3 months or longer	Total
FY2021					0
					0

<From satellite>

	Under 1 week	From 1 week to 1 month	From 1 month to 3 months	3 months or longer	Total
FY2021					0
					0

Overseas Satellite 2: University of British Columbia

<To satellite>

	Under 1 week	From 1 week to 1 month	From 1 month to 3 months	3 months or longer	Total
FY2021					0
					0

<From satellite>

	Under 1 week	From 1 week to 1 month	From 1 month to 3 months	3 months or longer	Total
FY2021					0
					0

Appendix 5 FY 2021 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: 2

	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	Alexander S. Mikhailov	72	NanoLSI PI / Guest Professor, Fritz Haber Institute of the Max Planck Society	Germany	Doctor of Science/ Theoretical Physics, Chemical Physics, Biophysics	International Solvay Chair in Chemistry (2009)	2021/11/4-12/25 2022/2/16-3/14	Participation as principal investigator, organize the 5th NanoLSI Symposium
2	Sarah Stainer	28	PhD student / Johannes Kepler University	Austria	PhD student / Johannes Kepler University / Studies Molecular Biology	Master of Science・Johannes Kepler University published paper 'Localization and Quantification of clinically relevant Binding Epitopes on Red Blood Cell Membranes using AFM recognition imaging' (2018)	2021/11/21-2022/2/24	Participation as cooperate Researcher, short term stay for research in the ANDO raboratory NanoLSI
3								
4								
5								
6								
7								
8								
9								
10								

Appendix 6 FY2021 State of Outreach Activities

* Fill in the numbers of activities and times held during FY2021 by each activity.
* Describe the outreach activities in the "6. Others" of Progress Report, including those stated below that warrant special mention.

Activities	FY2021 (number of activities, times held)
PR brochure, pamphlet	*NanoLSI Leaflet (1) (JP) *WPI Pamphlet (1) (EN&JP) *AERA separate printing (1) (Total: 3)
Lectures, seminars for general public	*NanoLSI Open Seminar (7) (1 by Ueno, 1 by Flechsig, 1 by Kim, 1 by Fujiwara, 1 by Smalyukh, 1 by Kumagai & Nishida and 1 by Puppulin) (Total: 7)
Teaching, experiments, training for elementary, secondary and high school students	*Lectures for Kanazawa University Global Science Campus (9/19) (Arai) *Lectures for Kanazawa University Jr.Dr. Ikuseijuku (11/21) (Fukumori) *NanoLSI Open House for Kanazawa Izumigaoka high school (facility tour and discussions with researchers) (3/25) (Total: 3)
Open houses	*Rigakuno-Hiroba (8/7)(Kodera, Shibata, Watanabe) *NanoLSI facility tour for Kanazawa University Global Science Campus (9/19) (Fukumori) *Campus Visit of Kanazawa University (11/6) (Miyata) * NanoLSI facility tour for Kanazawa University Jr.Dr. Ikuseijuku (11/21) (Fukumori) *NanoLSI facility tour for the Chairs of KEIDANREN (11/18) *Visits from Industry (10/21, 3/17) (Total: 7)
Participating, exhibiting in events	*An exhibition booth at the Annexed Exhibition of the Molecular Biology Society of Japan (12/1-3) *10th WPI Science Symposium (Lectures mainly for SSH students, research presentation contest for SSH students) (12/18) (Total: 2)
Press releases	Ogoshi, Lim&Wong (x2), Sumikama, Kodera & Ando (x2), Mikhailov, Takahashi & Makarova (x2), Yoshida & Hanayama, Maeda, Akine & MacLachlan, Fukuma, Umeda & Kodera, Ayhan & Maeda, Puppulin & Shibata, Wang & Oshima, Sakata & Akine, Kawahara & Hanayama (Total: 19)
Others（Video, TV program, crowdfunding, podcast, technical book）	*Video of NanoLSI introduction (2) (EN/JP) *TV program (2) "Science Zero", NHK Kanazawa "Kaganoto Evening" *Crowdfunding project to implement the "Cancer Research Early Exposure Program" (7/2-8/29) *Podcast (4) (1 by MacLachlan, 1 by Foster, 1 by Korchev, 1 by Mikhailov *Book on high-speed atomic force microscopy aimed at students and biologists who want to use HS-AFM in their research (by Ando). (Total: 10)

*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 above, 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 needed.

Outreach Activities and Their Results

List up to three of the Center's outreach activities carried out in FY 2021 that have contributed to enhancing the brand or recognition of your Center and/or the brand of the overall WPI program, and describe its concrete contents and effect in narrative style. (Where possible, indicate the results in concrete numbers.)

Examples:
- As a result of using a new OO press-release method, a OO% increase in media coverage was obtained over the previous year.
- By holding seminars for the public that include people from industry, requests for joint research were received from companies.
- We changed our public relations media. As a resulting of using OO to disseminate information, a OO% increase in inquiries from researchers was obtained over the previous year.
- As a result of vigorously carrying out OO outreach activity, ¥OO in external funding was acquired.

Award of a "2022 HFSP Program Grant"

A successful research proposal, "Super-resolution multifunctional scanning ion conductance microscopy: tapping the cell's energy grid," based on a joint research project that was adopted by "NanoLSI Bio-SPM Collaborative Research," was announced on March 15, 2022, as a highly prestigious international

collaborative research grant, a "2022 HFSP Program Grant". "NanoLSI Bio-SPM Collaborative Research" is one of the outreach activities, "NanoLSI Open Facility Programs," targeting researchers.

Publication of the world's first HS-AFM textbook "High-Speed Atomic Force Microscopy in Biology"

The book "High-Speed Atomic Force Microscopy in Biology" written by Specially Appointed Professor Toshio Ando was published by Springer Nature. This book is the world's first HS-AFM "textbook" that comprehensively explains the principles and technologies of the high-speed atomic force microscope, and has received much attention. The number of individual purchases of the eBook has already reached 707 in seven days from March 28 when the relevant webpage of the publisher was opened. Due to its great impact on academia, the publication of this book is an important achievement that will contribute to the establishment of the discipline, Nanoprobe Life Science, over a long period of time.

It started in 2018 with the aim of improving the visibility of NanoLSI and building a global research network in the field of bioimaging, and has supported 57 research projects in total, steadily expanding the research network. The success of the supported project in the "2022 HFSP Program Grant" application demonstrates the high effectiveness of the efforts and further improvement of the presence of NanoLSI in academia.

The 10th World Premier International Research Center Initiative Program (WPI) Science Symposium: Establishing collaborations with high schools designated by the JST Super Science High Schools, the Ishikawa Prefectural Board of Education and the high schools participating in the JST/Kanazawa University Global Science Campus (KU-GSC)

The 10th World Premier International Research Center Initiative Program (WPI) Science Symposium was held at the ISHIKAWA ONGAKUDO on December 18, 2021, with the full cooperation of all WPI Centers and the Center for World Premier International Research Center Initiative (WPI Program Center), JSPS. Prior to the Symposium, NanoLSI worked closely with the designated schools of the Ishikawa Super Science High School and the Ishikawa Prefectural Board of Education since 2019 to carefully plan the Symposium, to build an organizational process, and to realize a symposium in which high school students would be willing to participate. As a result, 364 students and 203 teachers participated from SSH and the high schools participating in the Kanazawa University Global Science Campus (JST commissioned project, KU-GSC).

After the Symposium, NanoLSI accepted a visit from one SSH school and also received a request for a visit from another SSH school next year. In addition, in a collaboration with the Cancer Research Institute, Kanazawa University, and KU-GSC, which began during preparations for this Symposium, the "Cancer Research Early Exposure Program," a research experience program for high school students who hope to become researchers, will be held in August, 2022 (also to be held in 2023 and beyond). Preparations are currently underway.

This symposium was a great success, leading to a contribution unique to our world-class research center for fostering potentially outstanding young scientists in the local community.

Appendix 7 FY 2021 List of Project's Media Coverage

* List and describe media coverage (e.g., articles published, programs aired) in FY2021.

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

	Date	Types of Media (e.g., newspaper, magazine, television)	Description
1	2021.4.16	Website (1), Newspaper (1), Television (1)	Award: Prof. Toshio Ando, the 44th Ishikawa Television Award (Hokuriku Chunichi Shinbun, Ishikawa Television)
2	2021.4.28-6.2	Website (15), Newspaper (2)	Research result: unraveling DNA packaging, Assist. Prof. Keesiang Lim, Prof. Richard Wong, J Phys Chem Lett (Hokkoku Shinbun, Kagaku Shinbun)
3	2021.5.24-5.25	Website (4)	Research result: novel carbon materials by carbonisation of aromatic molecules with three-dimensional structures, Prof. Tomoki Ogoshi, Communications Chemistry (Biglobe, etc.)
4	2021.5.29	Newspaper (1)	Research result: Gliding structure in Mycoplasma mobile revealed, Prof. Noriyuki Kodera, Prof. Toshio Ando, mBio (Hokkoku Shinbun).
5	2021.6.27-7.7	Website (3), Newspaper (4)	Research result: Conductance selectivity of Na+ across the K+ channel, Assist. Prof. Takashi Sumikama, PNAS (Hokkoku Shinbun, Chunichi Shinbun, Hokuriku Chunichi Shinbun x2).
6	2021.7.13-8.31	Website (17)	Research result: Regulators for extracellular vesicle production, Assist. Prof. Takeshi Yoshida, Prof. Rikinari Hanayama, Scientific Reports.
7	2021.7.17-9.3	Website (17)	Research result: Understanding a nanomuscle,Prof. Alexander Mikhailov, PNAS.
8	2021.7.19-7.20	Website (6)	Research result: The mathematics of repulsion for new graphene catalysts, Prof. Yasufumi Takahashi, Assist. Prof. Marina Makarova, Carbon.
9	2021.7.20	Website (4)	Research result: Mycoplasma mobile moves into overdrive: twin motor modified from ATP synthase discovered, Prof. Noriyuki Kodera, Prof. Toshio Ando, mBio .
10	2021.9.1-9.22	Website (11)	Research result: Color coding molecular mirror images, Prof. Katsuhiro Maeda, Assist. Prof. Daisuke Hirose, Tatsuya Nishimura, Science Advances.
11	2021.10.1-10.6	Website (18)	Review: Metallic complexes made from cyclic molecules, Prof. Shigehisa Akine, Prof. Mark MacLachlan, Chem. Soc. Rev., Advance Article.
12	2021.11.22-2022.2.4	Website (18)	Research result: Speeding up atomic force microscopy, Assist. Prof. Kenichi Umeda, Prof. Noriyuki Kodera, Appl. Phys. Lett.
13	2021.12.23-2022.2.18	Website (68), Newspaper (3), Television (1)	Research result: Endoscopy of a living cell on the nanoscale, Assist. Prof. Keisuke Miyazawa, Dr. Hirotoshi Furusho, Assist. Prof. Takehiko Ichikawa, Assist. Prof. Kazuki Miyata, Prof. Takeshi Fukuma, Science Advances (Hokkoku Shinbun, Hokuriku Chunishi Shinbun, Yomiuri Shinbun, NHK Kanazawa).

Appendix 7 FY 2021 List of Project's Media Coverage

* List and describe media coverage (e.g., articles published, programs aired) in FY2021.

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

	Date	Types of Media (e.g., newspaper, magazine, television)	Description
14	2021.12.8-12.23	Website (14), Newspaper (2), Television (4)	Research result: First point of attack: Understanding the entry mechanism of SARS-CoV-2 into human cells, Assist. Prof. Keesiang Lim, Assist. Prof. Takeshi Yoshida, Assoc. Prof. Takahiro Nakayama, Assist. Prof. Akiko Kobayashi, Assoc. Prof. Masaharu Hazawa, Prof. Rikinari Hanayama, Prof. Toshio Ando, Prof. Richard Wong, Extracellular Vesicles
15	2021.12.8-12.14	Website (54)	Event: The 10th WPI Science Symposium, Ishikawa Ongakudo, Dec. 18, 2021
16	2021.12.17	Website (11)	Research result: Helical structures visualized, Assist. Prof. Ayhan Yurtsever, Assist. Prof. Sandip Das, Assoc. Prof Tatsuya Nishimura, Assist. Prof. Daisuke Hirose, Assist. Prof. Kazuki Miyata, Assist. Prof. Ayumi Sumino, Prof. Takeshi Fukuma, Prof. Katsuhiro Maeda (Chemical Communications).
17	2021.12.17	Website (12)	Research result: Small but mighty: Identifying nanosized molecules using atomic force microscopy, Assist. Prof. Leonardo Puppulin, Assoc. Prof. Katsuya Sakai, Prof. Noriyuki Kodera, Assist. Prof. Kenichi Umeda, Assist. Prof. Ayumi Sumino, Dr. Wei Weilin, Prof. Takeshi Fukuma, Prof. Kunio Matsumoto, Prof. Mikihiro Shibata, ACS Appl. Mater. Interfaces.
18	2021.12.23	Website (13)	Research result: Gut feeling: Visualizing intestinal tumors, Assist. Prof. Dong Wang, Assist. Prof. Linhao Sun, Assoc. Prof. Satoru Okuda, Assoc. Prof. Mizuho Nakayama, Assoc. Prof. Hiroko Oshima, Prof. Toshio Ando, Assoc. Prof. Shinji Watanabe, Prof. Masanobu Oshima, Biomaterials.
19	2022.1.16	Television (2)	TV program: Science ZERO, NHK, Jan-16, Kaga-Noto Evening, NHK (feature trailer for Science ZERO, Jan-14), Prof. Mikihiro Shibata (studio), Prof. Toshio Ando, Prof. Richard Wong, Prof. Kunio Matsumoto (VTR)
20	2022.2.2	Newspaper (1)	Award: Prof. Toshio Ando, the 53rd Naito Memorial Award (Hokkoku Shinbun)
21	2022.2.4-2.21	Website (15), Newspaper (1)	Research result: Charge separation imaging on the surfaces of titanium dioxide photoelectrocatalytic nanotubes, Prof. Yasufumi Takahashi, Assist. Prof. Marina Makarova, Prof. Yuri Korchev, et al. (Nikkan Kogyo Shimbun)
22	2022.3.9-3.15	Website (12), Newspaper (1)	Research result: Changing the handedness of molecules, Prof. Shigehisa Akine, Assoc. Prof. Yoko Sakata, et al. (Hokkoku Shinbun)
23	2022.3.28-3.29	Website (12)	Research result: Biomolecular insights into protein-insolubility-related disease