World Premier International Research Center Initiative (WPI)
FY 2022 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	Masafumi Iwami (as of April 2023)

Common instructions:
* Unless otherwise specified, prepare this report based on the current (31 March 2023) 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: After establishing proof of principle, we made great efforts to extend the capabilities of the newly developed live-cell imaging techniques and to develop practical techniques for life science research. **Fukuma** extended the imaging capability of 3D nanoendoscopy AFM from the imaging of static structures to dynamic processes such as growth dynamics of focal adhesions and actin fibers. Using SICM, **Watanabe** succeeded in visualizing nanoparticle-cell interactions (***Small* 2022**) and established a method to observe nanodynamics of physical properties at the basal surfaces of spherical organoids through a collaboration with **Oshima** (***Small* 2023**).

Nanoendoscopic analysis and manipulation: We devoted continuous effort to develop fundamental technologies relating to nanoendoscopic analysis and manipulation. **Takahashi** established a technique for injecting reagents into cells using a glass nanopipette and developed a technology for automatic cell identification using machine learning and for automatic collection at the single-cell level. **Arai** demonstrated local heating and thermometry at the single cell level (***ACS Nano* 2022 & *Materials Today Bio* 2022**) and established a methodology to manipulate the dynamics of lipid membranes. **Akine, Ogoshi, Maeda, & MacLachlan** developed new cyclic or helical compounds that could be utilized as molecular sensors and machines.

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 (***J Phys Chem Lett* 2022**). **Flechsig** developed a unique method to reconstruct full 3D atomistic structures from AFM topographic images (***PLoS Comput. Biol.* 2022, *ACS Appl Mater Interfaces 2022, PLoS ONE* 2022, *ACS Nano* 2023**). **Okuda** proposed new mathematical models that describe the dynamics of stress fibers, cell membrane, cell adhesion, and collective cell migration (***Biophys J* 2022**, ***Euro Phys J E* 2022**, ***iScience* 2023, *Phys Rev E* 2023**).

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

Life science research using HS-AFM, 3D-AFM, or bio-SICM continued to produce many impactful publications. Meanwhile, we also actively explored applications of the newly developed live-cell imaging techniques in molecular cell biology and cancer research.

Basic cell functions: Using HS-AFM and protein engineering technology with MET-binding peptides, **Matsumoto & Shibata** created a designer receptor agonist capable of dimerizing and activating MET receptors (***Nat Biomed Eng*, 2023**). **Wong, Hanayama & Ando** discovered that the structure of small extracellular vesicles (sEVs) is substantially altered at high temperature, high pH, or hypertonic conditions and that the spherical shape of the sEVs is maintained in acidic or hypotonic environments (***J Extracell Vesicles* 2022**). Using HS-AFM, **Hanayama & Kodera** found how extracellular vesicles contribute to the aggregation and deposition of transthyretin in amyloidosis (***Front Mol Biosci* 2022**). **Toda & Watanabe** started a new project to study how chimeric

cadherins change cell membrane dynamics to induce cell sorting by using scanning ion conductance microscope (SICM). **Miyanari** has succeeded in simultaneously visualizing chromatin accessibility and epigenetic modifications; both are key chromatin signatures in the regulation of transcription (***Methods Mol Bio*** **2023**).

Cancer research: **Oshima & Watanabe** found that metastatic malignant cells have a specific morphology of micro-ridge-like structures with active movement on the membrane surface by using SICM (***Biomaterials*** **2022**). They also found that the basal surface of metastatic intestinal tumor organoids showed similar ridge-like structures and softer cell membranes (***Small*** **2023**). **Nakajima** found that the DNA aptamer significantly enhanced the vitamin D_3-mediated inhibition of cancer cell proliferation (***ACS Appl Mater Inter*** **2022**). **Hirao** established that endo-lysosomal activity as a metabolic biomarker of malignancy and an important therapeutic target for brain tumors (***Cancer Sci.*** **2022**). **Yano** developed a therapeutic approach to ALK-rearranged lung cancer targeting with a transcription factor STAT3 (***NPJ Precis Oncol.*** **2022**). They also found that deficiency of the splicing factor RBM10 limits EGFR inhibitor response in EGFR-mutant lung cancer (***J Clin Invest.*** **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 & Shibata** developed several high-speed AFM (HS-AFM) techniques to further improve the scanning speed, low-invasiveness and assay system: a mass-controller for the cantilever to achieve higher resonant frequency, a new optical system to obtain a more accurate deflection signal of a small cantilever, and a new AFM observation substrate using pillar[5]arene molecules. For high-resolution SICM imaging of live cell surfaces with a high S/N ratio, **Takahashi** developed a method for controlling the inner/outer diameter ratio of glass capillaries, and **Watanabe** developed an ultra-low-noise wide-bandwidth transimpedance amplifier. Meanwhile, **Fukuma** continued to expand the application area of 3D-AFM. In addition to the hydration structures and living cells, he succeeded in visualizing the internal structure of chromosomes using an originally developed carbon nanotube probe.

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 pH around phycosphere (***ISME Journal*** **2022**), cellulose and chitin structures and hydration (***Sci. Adv.*** **2022 &** ***Small Methods*** **2022**), water-driven structuring of adenine assemblies (***JACS*** **2022**), cellular dynamics induced by the interaction with phospholipid nanoparticles (***Small*** **2022**), DNA-edit dynamics by CRISPR-Cas3 and Staphylococcus aureus Cas9 **(*Nat. Commun.* 2022 & *ACS Nano* 2023)**, and the structure and the function of nucleolar protein, PQBP5 (***Nat. Commun.*** **2023**).

2. Generating Fused Disciplines

Both top-down and bottom-up approaches have been continuously 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 2017-2022 was 697, of which 317 (45.5%) were internationally co-authored papers. The total number of papers co-authored by one of the four overseas PIs with resident researchers in NanoLSI has reached 24 since 2017. Various measures have been executed such as outreach programs for external researchers, mobility and career path for young 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

Roadmaps on 6 nanotechnology and 7 life sciences have been updated. The total amount of external funds acquired in FY2022 by 82 NanoLSI researchers was 1,356 million yen (1,288 million yen in FY2021). Policy and practice of fostering next-generation researchers have been featured.

6. Others

Outreach activities have been described, such as press releases of research outcomes, media coverage, visitors to NanoLSI, and approaches to high school students.

* 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 fusing disciplines).
  (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 2022.
* 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

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

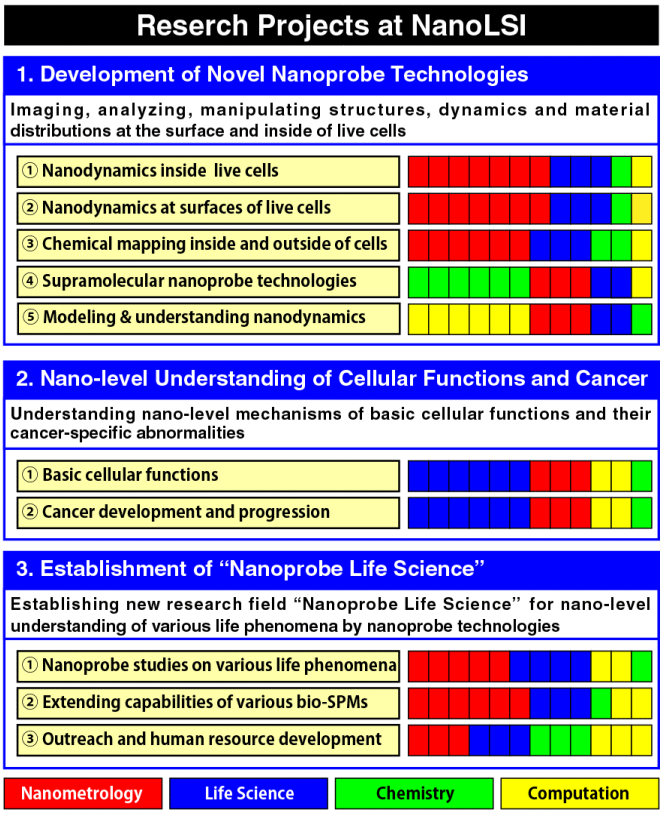


Fig. 1: Research projects at NanoLSI and contributions from the four major disciplines to each project.

(1) We have been developing Bio-SPM technologies for visualizing the structures, dynamics and material distribution inside and at the surfaces of living cells. We had largely completed proof of principle during the previous fiscal year. In FY2022, our focus has shifted to extending the performance and functionalities of the newly developed techniques and the development of practical application techniques for life science research. For example, we have successfully extended the imaging capability of nanoendoscopy AFM from in-cell imaging of static structures to dynamic processes such as the growth of focal adhesions and actin fibers. Furthermore, we have developed a sample preparation technique enabling SICM imaging of cellular surfaces constituting a spherical organoid (***Small* 2022**).

(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 (e.g., HS-AFM, 3D-AFM and bio-SICM) continued to produce impactful publications. Examples include HS-AFM studies on the dynamics of MET receptor agonists (***Nat Biomed Eng* 2023**) and extracellular vesicles (***J Extracell Vesicles* 2022**), and 3D-AFM studies on molecular-scale surface structures and hydration of chitin and cellulose nanofibers (***Small Methods* 2022 & *Sci. Adv.* 2022**). Meanwhile, we have actively explored applications of the newly developed live-cell imaging techniques to different life science disciplines. Examples include SICM studies on the changes in cell surface dynamics and mechanics with cancer progression (***Small 2022* & *Small 2023***), and AFM studies on protein clustering on a cell surface induced by chemical fixation (***Comm Biol* 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 pandemic, we made our best efforts to organize various symposiums, seminars and a summer school in person. In addition, all the overseas PIs managed to visit NanoLSI and engaged in collaborative research. Furthermore, we actively performed bio-SPM collaborations with external researchers and published many impactful papers. Now, the number of participants for the summer school and accepted proposals for the Bio-SPM collaborative research program have recovered to the levels before the COVID-19 pandemic. To continue to lead the bio-SPM research community, we have been working on extending the capabilities of our world-leading bio-SPM techniques. For example, we continued to improve the speed of HS-AFM to achieve 100 fps. In addition, we started to explore possibilities of 3D-AFM imaging of various 3D biological systems such as chromosomes.

Achievements in FY2022 are summarized as follows.
- Papers: 129 (41.1 % internationally co-authored; 46 with an IF > 10; 66 with an IF > 7),
- Invited talks in int'l meetings: 70,

- Funding: ¥1,356,624,224 overall (36grants > ¥10,000,000).
These achievements are of the highest global level for an institute with 82 researchers (as of March 2023).

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

(Development of nano-imaging techniques)

･Measurement of nanodynamics on cell surfaces:

In 2021, Watanabe *et al.* developed a method to measure dynamic changes in nanomechanical properties of living cells based on high-speed scanning ion conductance microscopy (HS-SICM) and investigated the relationships between gene mutations, colon cancer phenotypes and nanomechanical properties in genotype-defined mouse intestinal tumor-derived cells. In FY2022, his group not only applied this technique to investigate nanoparticle-cell interactions (***Small* 2022**) but also extended the capability of HS-SICM nanomechanical measurement from 2D-cultured cells to 3D-tissure-like cellular structures to gain further insight into the nanomechanical properties of cells. The 3D cultured organoids were prepared so that the basal surface (BS) faces toward the outside surface of organoid cells and is accessible to the SICM probe. In addition, 3D living organoids of genotype-defined metastatic intestinal cells were partially embedded into collagen gel to immobilize and stabilize the organoid structures for long-term time-lapse HS-SICM measurement (Fig. 2a). In this setup, they succeeded in measuring the long-term dynamics of subcellular structures, such as ridge-like, stress-fiber, and local elastic modulus distributions on BS (Fig. 2b). Furthermore, they demonstrated the possibility that not only the averaged elastic modulus of cells (Fig. 2c) but also local correlations between topography and elastic modulus mapping provide a physical marker to categorize cancer progression (Fig. 2d) (***Small* 2023**).

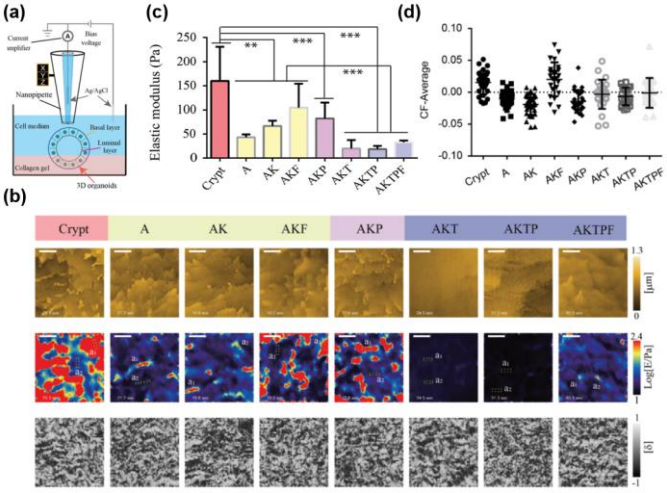


Fig. 2: (a) HS-SICM setup for 3D cell measurement. (b) Snapshots of time-lapse images of height and elastic modulus in genotype-defined (A, AK, AKF, AKP, AKT, AKTP, and AKTPF) 3D organoids of metastasis cancer cells and normal Crypt cells. (c) Elastic modulus of various 3D organoids. (d) Local correlation factor for various 3D organoids.

Ichikawa *et al.*, following on from last year, conducted cell surface observation using AFM. By culturing cells on the microporous silicon nitride membrane (MPM) and observing the cell surface through the 3 or 5 mm diameter holes of MPM, they successfully observed protrusions of less than 10 nm in diameter on the living colon cancer cell surface (collaboration with Prof. Oshima, Kanazawa University Cancer Research Institute). They investigated the nanoscale effect of chemical fixation reagents on the colon cancer cell surface using this technique. They tested commonly used fixation reagents, such as 2% glutaraldehyde, 4% paraformaldehyde, and 100% methanol. After treatment with all these fixation reagents, they found that most of the small protrusions on the living cell surface disappeared, and there were only large protrusions whose size was 10 - 50 nm on the cell. They also measured the distances between membrane molecules before, after, and during fixation, and concluded that these large protrusions were created by aggregating membrane proteins which move freely to some extent in the presence of fixation reagent. These results have been published (***Comm Biol* 2022**). Ichikawa *et al.* also investigated the binding affinity of Cassiicolin 1 (Cas1) and Cassiicolin 2 (Cas2), which are toxins causing Corynespora leaf fall disease, to the phospholipid. They directly observe the Cas1 and Cas2 binding to the phospholipid layer on mica and the lipid layer degradation using high-speed AFM. They found that Cas1 has a low affinity to the neutral phospholipid (1,2-dipalmitoyl-sn-glycero-3-phosphocoline, DPPC) but high affinity and degradation to negative phospholipid (1,2-dipalmitoyl-sn-glycero-3-phosphate, DPPA), glycerolipids (monogalactosyldiacylglycerol) and sterols (stigmasterol, sitosterol, MGDG). Cas2 has a high affinity to betaine lipid (1,2-dipalmitoyl-sn-glycero-3-O-4'-[N,N,N-trimethyl]-betaine, DGTS-d9) in addition to DPPA, MGDG, and sterols. They also confirm these properties with a confocal microscope using GFP-Cas1 and rhodamine-stained phospholipids. They further observed the Cas1-treated leaf with cryo-SEM and found that purified Cas1 caused lesions to the rubber leaf PB 255 clone, but Cas2 did not

cause large lesions. These data have been published (***Phytopathology* 2022**).

·Visualization of intracellular nanodynamics (nano-endoscopic observation):
(i) Nanoendoscopy AFM: In this WPI project, Fukuma *et al.* developed nanoendoscopy AFM, where a long needle probe is inserted into a live cell for the measurement of intracellular nanodynamics and nanomechanics. Last year, they reported in-cell imaging of the focal adhesion (FA) and measurement of nuclear stiffening caused by cancer progression. This year, they made significant progress in these projects.

As for the FA imaging, they succeeded in the in-cell imaging of the growth of the FA and actin fibers by nanoendoscopy AFM and confocal fluorescent microscopy (FM). These images reveal that the FA was initially thick but became thinner as they grew. In addition, the actin fiber was initially in contact with the upper cell membrane and detached during the fiber growth. Furthermore, the paxillin distribution was broadened to fill the gap between the newly formed actin fiber and the cell membrane. These findings were obtained owing to the 3D imaging capability of 3D-AFM with a high vertical resolution. As shown here, they have extended the capability of nanoendoscopy AFM from imaging a static structure to dynamic processes in a live cell.

As for the nuclear stiffening, they continued to work on the in-cell nuclear elasticity measurements and confirmed the nuclear stiffening with cancer progression for different cell lines and different softness of the underlying substrate. In parallel, they performed various biochemical analyses to determine the cause of the stiffening. The flow cytometry and Western blotting revealed that lamin A, B1/2, and C expression levels do not increase. The fluorescent microscopy analyses suggested that the total DNA amount or histone modifications did not change. Finally, the MNase analysis revealed a clear increase in the DNA compaction level with cancer progression. Based on this finding, they are now preparing cells with different DNA compaction levels to clarify their correlation with nuclear elasticity. Nuclear stiffness is strongly related to various diseases known as nuclear envelopathies and cell aging, and the method developed here can greatly help understand these phenomena.
(ii) Deroofed cells: Molecular-resolution analysis of intracellular structures and organelles can benefit from prior removal of the plasma membrane to enable direct nanoprobe/sample contact. Franz (Jr. PI) is developing experimental tools for opening the cell interior for SPM exploration, while maintaining the functionality of the exposed intracellular protein complexes. For instance, microsonication-based cell de-roofing methods were used for nanostructural and biomechanical characterization of contracting actomyosin stress fibers. Complementary experiments using both AFM and SICM imaging and elasticity mapping revealed complex patterns of stiffness changes along contracting actin stress fibers. Furthermore, the contribution of different myosin II isoforms on stress fiber ultrastructure and contraction mechanics were evaluated using a panel of myosin knockout cells. While mechanical de-roofing methods are suitable for exposing relatively stable intracellular compartments like actin stress fibers, more delicate intracellular protein assemblies and organelles require non-mechanical methods for minimally invasive cell de-roofing. Experiments in the group show that short treatment with bee venom phospholipase A2 introduces transient micrometer-sized pores into the plasma membrane of living cells, providing temporary windows into the cell interior for nanoprobe exploration. Using such enzymatic cell membrane digestion in combination with high-aspect-ratio AFM tips, it has now become possible to image intracellular components, including dynamic submembranous cytoskeletal networks or the nuclear lamina, with nanometer-resolution directly within living cells.

(Development of nano-endoscopic analysis and manipulation techniques)

·Injection and sampling of substances using a nanopipette:

Takahashi *et al.* established a technique for injecting reagents into cells using a glass nanopipette to administer reagents such as inositol phospholipid to organelles, and observed changes in the dynamics of the organelles (Fig. 3). In this process, they established a technique to control the dosage of the reagent by applying a potential to the electrode in the glass nanopipette and inducing electroosmotic flow. Furthermore, they developed a technology for automatic cell identification using machine learning and have already established a technology for automatic collection at the single-cell level. In order to apply this

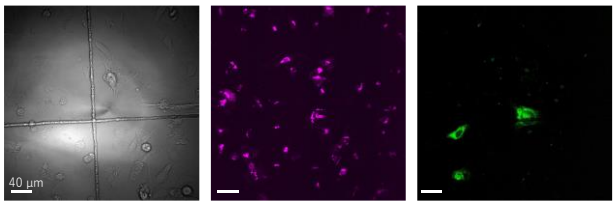


Fig. 3: Intracellular injection of inositol phospholipids by nanopipette. Confocal microscope image of Mouse Embryonic Fibroblast (MEF) cells, (left) bright field image, (center) Golgi apparatus labeled with fluorescent tag, (right) BODYPY-labeled inositol phospholipids.

technology to organelle pick-up, they are currently establishing a technology that enables observation of organelles without staining, such as holographic microscopy, and a collection technology using a glass nanopipette. A collaborative team (Arai and Takahashi) constructed the microscopic system coupling robust fluorescence lifetime imaging (FLIM) with a nanopipette for the combination with metabolomics and transcriptomics. So far, they have succeeded in accessing a single cell, followed by mRNA amplification.

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

They are developing unique molecular sensors that can selectively recognize target compounds using their expertise in supramolecular chemistry. Ogoshi *et al.* have already succeeded in the selective recognition of 1-MNA (1-methylnicotinamide), which is one of the oncometabolites, by using a carboxylate-modified pillar[6]arene derivative. They have successfully improved the binding constant ca. 700-fold by changing the interaction sites.

·Nano-manipulation using molecular machines:

To expand the variety of ways for nano-manipulation, Arai *et al.* generated a library of functional chemical dyes toward a near-infrared (NIR)-driven supramolecular machine system. More specifically, using photothermal and thermosensitive dyes, they demonstrated local heating and thermometry at the single cell level (Fig. 4) (***ACS Nano* 2022 & *Materials Today Bio* 2022**). Furthermore, they established a methodology to manipulate the dynamics of lipid membrane using local heating at the molecular level, leading to a milestone in the development of future molecular machines.

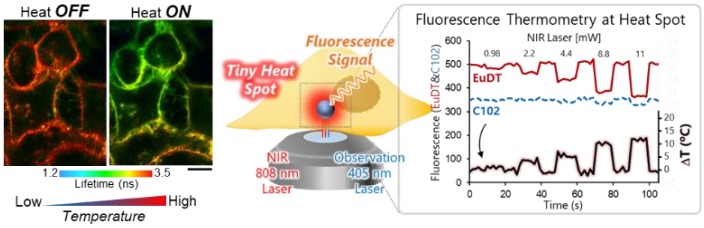


Fig. 4: Local thermometry (FLIM) and heating at subcellular scale.

Akine *et al.* have developed a new helical molecule that shows a unique time-dependent chirality change. The molecule can bind a chiral amine molecule at the constituent cobalt(III) ions to give a right-handed helix, which was inverted to the left-handed form before racemization upon the six-step amine exchange reaction with piperidine (***PNAS* 2022**). This would be a good candidate as a molecular scaffold to enable time-dependent functions. They also developed two types of metal-containing rotaxane molecules that could be utilized as a molecular machine due to their dynamic nature. One is obtained from a metal-containing dumbbell molecule and a crown ether derivative. The dynamic nature of the rotaxane can be easily modulated by additives (***Angew. Chem. Int. Ed.* 2023**). The other was obtained from a metallomacrocycle and a dumbbell molecule (***Dalton Trans.* 2022**). They also developed new amphiphilic metal complexes that can form a monolayer at the air-water interface. The chiroptical properties can be modulated by changing the surface pressure at the interface (***Dalton Trans.* 2022**). Ogoshi *et al.* have achieved pillar[n]arene-based chiral supramolecular assemblies (***Chem. Sci.* 2022 ×2, *Angew. Chem. Int. Ed.* 2022, *Nat. Comm.* 2022**), tubes and cavitands by covalent bonds (***Cell Rep. Phys. Sci.* 2022 & *JACS* 2022**), and ring-opening polymerization by pillar[5]arene crystals (***Angew. Chem. Int. Ed.* 2022 & *Chem. Sci.* 2022**). Maeda *et al.* have developed functional helical polymers (***J. Mater. Chem. C* 2022 & *Angew. Chem. Int. Ed.* 2023**). They also developed facile and versatile methods to synthesize end-functionalized helical polymers (***Angew. Chem. Int. Ed.* 2022**), which could be used to immobilize stimuli-responsive helical polymers on the surface of probes to be used as functional nanoprobes. MacLachlan *et al.* have developed a family of platinum-containing macrocycles functionalized with crown ether-like receptors. When these molecules bind to guests, such as alkali cations, they change color and luminescence, demonstrating their potential as molecular sensors (***Angew. Chem. Int. Ed.* 2023 & *Inorg. Chem.* 2022**). The size of the cavity in the molecules can be tuned using redox chemistry, allowing one to switch the selectivity of the molecule in situ. MacLachlan and Akine *et al.* developed Schiff-base macrocycles with well-defined cavities and investigated the selective guest binding of organic cations in the interior (***Org. Biomol. Chem.* 2022**).

(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)

Foster *et al.* 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. 5).

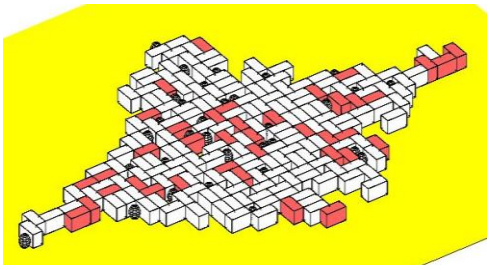


Fig. 5: Modeling cellular growth and transfer of epigenetic elements within growing colonies of the budding yeast *Saccharomyces cerevisiae* (white/red = +/- epigenetic factor).

·Developing a chromosome model and analyses on HS-AFM movies (Sumikama)

They have published a paper describing a theory for the computation of 3D-AFM images of biopolymers such as chromosomes (***JPCL 2022***). To apply this theory to chromosome models and compare their simulated 3D-AFM images with experimental ones, it is necessary to develop more realistic chromosome models that account for heterochromatin and euchromatin. As such, they also developed a classification method called SCN of heterochromatin and euchromatin based on Hi-C experiments (Fig. 6), which is now on ***bioRxiv***. This classification further provides a mechanistic insight into the differences in contact probability between heterochromatin and euchromatin: euchromatin has more contact than heterochromatin (Fig. 6).

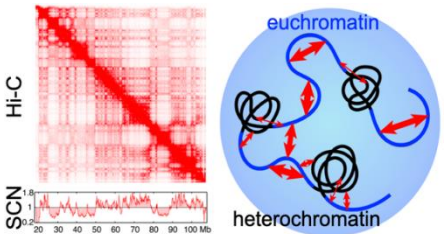


Fig. 6: A classification method (SCN) based on the Hi-C map (left). Schematic representation of heterochromatin and euchromatin (right).

To advance HS-AFM research to the next stage, it is important to theoretically analyze HS-AFM movies to reveal the biological function from the dynamics of molecules. Therefore, they analyzed HS-AFM movies of 1) CaMKII, 2) AMPA receptor, 3) TRPV1 channel, and 4) Na^+ channel based on statistical mechanics. Molecular dynamics simulations also help to validate HS-AFM measurements at the atomic level, and this has been performed on 5) AMPA receptor, 6) nucleosome, 7) Na^+ channel, and 8) lipase systems.

·Reconstruction of 3D atomistic biomolecular dynamics from HS-AFM imaging (Flechsig)

Their previously developed methods of data-driven simulation AFM and automatized fitting (***PLoS Comput Biol* 2022**) have been applied to provide atomistic level interpretation of HS-AFM experiments (Fig. 7) for 1) the aptamer-Cyp24 protein complex (cancer research) (***ACS Appl Mater Interfaces* 2022**), 2) Annexin V lattices (***PLoS ONE* 2022**), 3) TMEM16 membrane transporters, 4) EML4-ALK protein complex (cancer research), 5) Cas9-RNA-DNA endonuclease (***ACS Nano* 2023**), 6) MET-receptors (cancer research), 7) E6AP ligase (***bioRxiv* 2022**). Moreover, multiscale molecular dynamics simulations provide atomistic explanation of basement membrane laminin-integrin dynamics observed under HS-AFM. AI based reconstruction of the 200 nm long atomic structure of EEA1 protein and molecular dynamics simulations complement HS-AFM experiments to understand self-organization and biomechanical properties of the endosomal membrane. Furthermore, an electrostatic model to understand sample-substrate interactions in AFM and predict stability of experimental observations was developed and implemented as software tools.

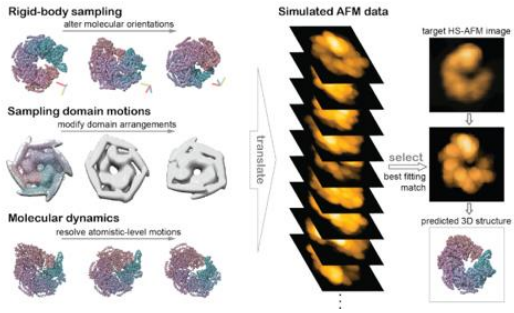


Fig. 7: Concept of inferring 3D atomistic biomolecular dynamics from resolution-limited AFM topographic imaging.

·Modeling multiscale cell dynamics from cytoskeletal to multicellular systems (Okuda)

Okuda (Jr. PI) proposed new mathematical models that describe the dynamics of stress fiber, cell membrane, cell adhesion, and collective cell migration. A developed 3D vertex model explained how cells collectively migrate as a cluster in 3D space as well as the variety of cell migratory modes observed in embryogenesis and cancer metastasis (***Biophys J* 2022**). Moreover, a simple mathematical model revealed that alpha-actinin plays a key role in inducing viscous frictions between actin filaments within a stress fiber to propagate traction forces (***iScience* 2023**). Furthermore, novel computational methods, referred to as a nonconservative fluid membrane model, succeeded in simulating long-term cell dynamics (***EPJE* 2022**) as well as adhesive cell dynamics (***PRE* 2023**). The integrated model revealed novel states of adhesive cell dynamics on a substrate under shear flow (Fig. 8).

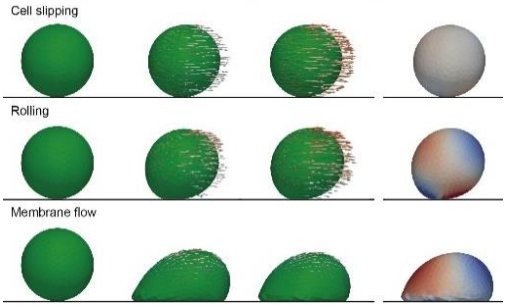


Fig. 8: Novel states of adhesive cell dynamics on substrate under shear flow.

(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 1) dynamic structures for growth factor receptor activation and 2) creation and application of growth factor receptor agonists, focusing on MET/HGF receptor. In FY2022, they created a designer receptor agonist capable of dimerizing and activating MET receptor, using protein engineering technology with MET-binding peptides (Fig. 9) (***Nat Biomed Eng* 2023**). The MET-activating agonists showed outstanding blood stability or the ability to cross the blood-brain-barrier in mice, indicating the creation of a high-performance biological drug for therapeutic purpose. The HS-AFM analysis revealed that the MET-agonist induced MET receptor dimerization/activation due to its bivalent properties (Fig. 9).

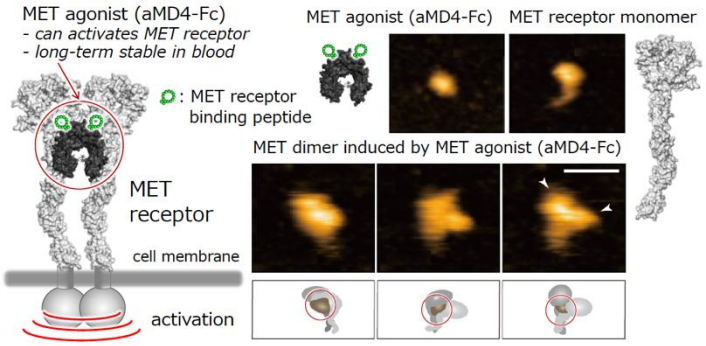


Fig. 9: MET receptor activation by newly created designer receptor agonist (aMD4-Fc).

·Intracellular trafficking (Wong)

The control of intracellular traffic is critical for cell growth and differentiation (***Stem Cells* 2022**). Nuclear pore complexes (NPCs) are multi-protein turnstiles that regulate nucleo-cytoplasmic traffic. Recently, Wong *et al.* developed novel aminocyclopropenone 1n (ACP-1n) and investigated its biological effects on epigenetic "readers" of histone acetylation, the bromodomain-containing protein 4 (BRD4) functions. ACP-1n blocked BRD4 functions by preventing its phase separation ability both in vitro and in vivo, attenuating the expression levels of BRD4-driven MYC. Notably, ACP-1n significantly reduced the nuclear size with concomitant suppression of the level of the NPC protein NUP210. Furthermore, NUP210 is in a BRD4-dependent manner and silencing of NUP210 was sufficient to decrease nucleus size and cellular growth. Their findings highlighted an aminocyclopropenone compound as a novel therapeutic drug blocking BRD4 assembly, thereby preventing BRD4-driven oncogenic functions in cancer cells. (***Cells* 2022**). In an interdisciplinary project with the Hanayama and Ando groups, using HS-AFM, they evaluated nanotopological changes of small

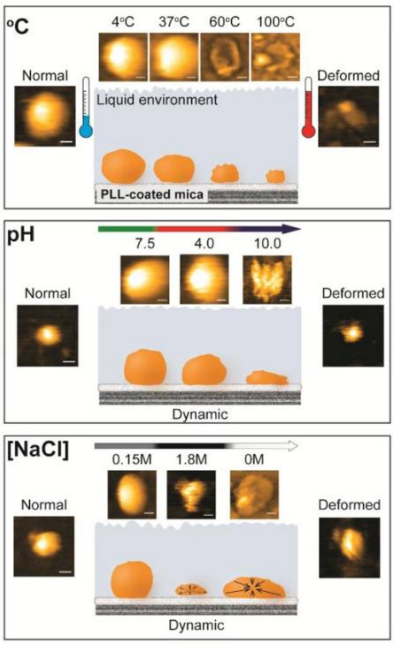


Fig. 10: HS-AFM imaging represents the continuity of the nanotopological dynamics of sEV in response to different physicochemical parameters.

extracellular vesicles (sEVs) with respect to different physicochemical stresses including thermal stress, pH, and osmotic stress. The sEV structure is severely altered at high-temperature, high-pH, or hypertonic conditions. Surprisingly, the spherical shape of the sEVs is maintained in acidic or hypotonic environments (***J Extracell Vesicles* 2022**) (Fig. 10). In the near future, they may also be able to perform HS-AFM imaging to trace the non-canonical nuclear transport of sEV contents.

·Cell communications via extracellular vesicles (Hanayama)

Hereditary transthyretin amyloidosis, which is caused by variants in the transthyretin (TTR) gene, leads to TTR amyloid deposits in multiple organs. Using HS-AFM, Hanayama *et al.* showed that extracellular vesicles (EVs) are involved in the formation of TTR amyloid deposits on the membrane of EVs, as well as the deposition of TTR amyloid in cells, suggesting that TTR in serum-derived EVs is a potential target for future amyloidosis diagnosis and therapy (***Front Mol Biosci* 2022**) (Fig. 11). In addition, they established engineered EVs that prevent SARS-CoV-2 infection by conjugating anti-spike nanobody and IFN-beta to EVs (***Pharm Res* 2022**).

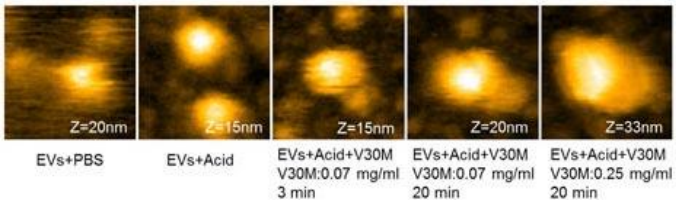


Fig. 11: The height of the EVs increased in a V30M-TTR-dependent manner due to the formation of TTR amyloid deposits on EVs.

·Morphogenesis - bottom up & mechanical approach (Toda)

Toda *et al.* have been working on the mechanisms of how interacting cells organize tissue morphologies. In FY2022, they engineered chimeric Cadherin proteins in which the cadherin intracellular domain is replaced with specific cytoskeletal regulators and showed that cytoskeletal activity at the Cadherin tail can determine whether cells are sorted into the center or to an outer layer of the cell aggregate. Now they have started a collaboration with the Watanabe group using scanning ion conductance microscope (SICM) to study how chimeric cadherins change cell membrane dynamics to induce cell sorting. They are also studying the mechanisms of how the diffusion of the signaling protein Wnt is regulated to generate stable tissue patterns. They developed a protein detection system that can distinguish a soluble form and membrane-tethered form of Wnt and are testing which proteins can tether Wnt on the cell surface to control its diffusion.

·Transcriptional regulation & epigenetics (Miyanari)

Miyanari *et al.* have been studying roles for chromatin dynamics in transcriptional regulation, which is crucial for cell lineage allocation in mammalian development. They have succeeded in simultaneously visualizing chromatin accessibility and epigenetic modifications; both are key chromatin signatures in the regulation of transcription (***Methods Mol Bio* 2023**). They also developed a functional peptide binder to survivin, a regulator of chromosome segregation and universal tumor antigen. They found that the binder can selectively inhibit proliferation of surviving positive cells by inducing apoptosis, and significantly suppressed tumor growth in xenograft mice (***Bioconjug Chem*. 2022**).

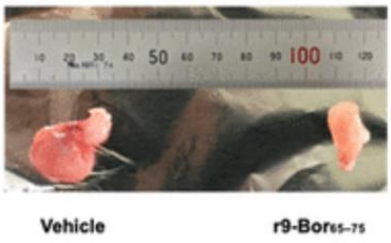


Fig. 12: Anticancer effect of the survivin binder on xenograft nude mouse model.

·Oncogenes and Cancer Cell Dynamics (Oshima)

Oshima *et al.* previously established intestinal tumor-derived organoid lines with defined genetic alterations and malignant phenotypes. Using the organoid lines, they examined apical cell surface structures and physical properties at nanoscale by high-speed (HS)-SICM. Notably, they found that metastatic malignant cells have a specific morphology of micro-ridge-like structures with active movement on the membrane surface (***Biomaterials* 2022**). Moreover, the cell surface of metastatic cells

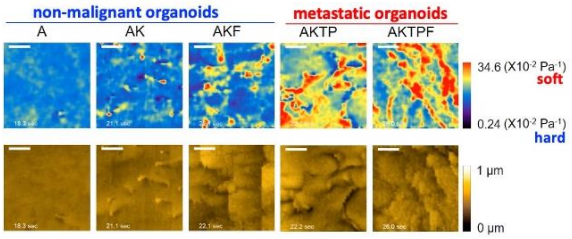


Fig. 13: Physical properties of non-malignant and metastatic organoid cell surface.

showed significantly softer characteristics compared to that of non-metastatic cells (Fig. 13). They next developed a unique organoid culture system to examine the basal surface of cells by HS-SICM. In this system, half of the organoids are embedded in collagen (Fig. 2a). Interestingly, the basal surface of metastatic intestinal tumor organoids showed similar ridge-like structures and softer cell membranes (***Small*** **2023**). These data will contribute to understanding the mechanisms of malignant cancer cells for invasion, migration, and metastasis.

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

Nakajima *et al.* have 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**). They have also obtained preliminary data from an in vivo study using cancer-bearing mice, showing the therapeutic potential of the aptamers for cancer. In addition, they have explored DNA aptamer molecules targeting ADAR, an enzyme catalyzing RNA editing, the abnormal expression of which is relevant for cancer development.

·Development of Diagnostic and Therapeutic Technology (Hirao)

Hirao *et al.* have recently elucidated molecular mechanisms that regulate cell differentiation and stress responses under microenvironmental influence (***Leukemia*** **2022**, ***Commun Biol***. **2022**). They have also uncovered the significance of endo-lysosomal activity as a metabolic biomarker of malignancy, and an important therapeutic target for brain tumors (***Cancer Sci.*** **2022**). To advance innovative imaging technology, they evaluated the performance of a novel sensor for the detection of 1-MNA, a metabolite associated with malignant properties in various types of cancer. The sensor demonstrated notable advantages over the current sensor, P6A (CA), including the quantification of 1-MNA secreted into the cell culture supernate (Fig. 14). These findings are expected to further the development of nano-pipette imaging technology, leading to a deeper understanding of the mechanisms that regulate malignant traits in cancer cells.

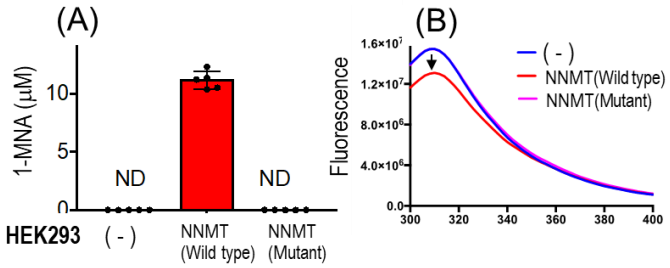


Fig. 14: Detection of 1-MNA secreted from 293 cells expressing NNMT by LC-MS/MS (A) and fluorescence quenching of the sensor (B).

·Development of Therapeutic Approach to Lung Cancer (Yano)

Yano *et al.* developed a therapeutic approach to ALK-rearranged lung cancer targeting with a transcription factor STAT3 (***NPJ Precis Oncol*** **2022**) Ca. They found that the mechanisms underlying adaptive cancer cell survival in residual tumors of ALK-rearranged lung cancer were predominantly dependent on the activity of a transcription factor STAT3 and subsequent transcriptional regulation of apoptosis (Fig. 15). They also found that inactivating genetic alteration of the mRNA splicing factor RNA-binding motif 10 (RBM10) that co-occurs with mutant EGFR decreased efficacy of EGFR inhibitor in lung cancer by inhibiting apoptosis (***J Clin Invest*** **2022**). These findings show new therapeutic approach consisting of ALK- or EGFR-inhibitors with apoptosis inducing drugs.

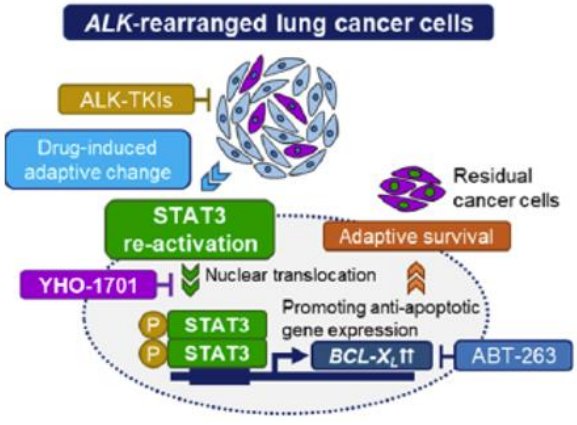


Fig. 15: STAT3 re-activation emerges adaptive survived lung cancer cells treated with ALK inhibitors.

(3) Establishment of a New Research Field: Nanoprobe Life Science (Further improvement of Bio-SPM technologies)

·HS-AFM technology

To expand the range of biological samples and dynamic phenomena that can be studied with HS-

AFM, further improvement of the low-disturbance and high-speed performance of HS-AFM is essential. However, the improvement capacity of instrumental components is already close to their limit. Therefore, HS-AFM group has been attempting to develop new scanning and control methods, while improving hardware components: scanners, amplitude detectors and small cantilevers. Fortunately, Ando *et al*. have made a breakthrough, i.e., retrace imaging during backward X-scanning is more disturbing to the sample because the feedback error signal during retrace imaging does not faithfully reflect the excessive tip–sample interaction. Based on this finding, they combined the Only Trace Imaging (OTI) mode and the dynamic PID control and succeeded in increasing the imaging rate from 10 fps to 50 fps. Furthermore, they worked on improving the optical system of HS-AFM to obtain a more accurate deflection signal of a small cantilever. By developing an evaluation system, the area of a focusing laser spot on the cantilever could be quantitatively measured. So far, Kodera *et al*. successfully reduced the area of the laser spot to ~29% of the conventional one by changing the laser diode and collimation lens. For producing smaller cantilevers with higher f_c and small spring constants, they have started a collaboration with a cantilever manufacturer.

Increasing the choice of AFM observation substrates is important for applying HS-AFM to a wide range of biological systems. In collaboration with the researchers of supramolecular chemistry, Shibata *et al*. prepared a new AFM substrate by immobilizing pillar[5]arene molecules on the mica surface. The pentagonal tubular structure of pillar[5]arene whose top and bottom surface have positive charges allowed DNA to be loosely immobilized on the substrate. Using this new substrate, they succeeded in capturing the nucleosome sliding of H2A.Z, a histone variant associated with various biological processes at 0.3 s (Fig. 16) (***Nano Letters* 2022**).

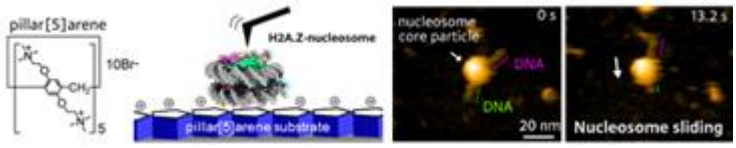


Fig. 16: Further improvement of HS-AFM. HS-AFM images of H2A.Z-nucleosome on the pillar[5]arene-modified mica surface.

·Scanning ion conductance microscopy (SICM)

Takahashi *et al.* have developed a method for controlling the inner/outer diameter ratio of glass capillaries for high-resolution scanning ion conductance microscopy, and established a method for fabricating nanopipettes with a radius of 20 nm or less. This new nanopipette fabrication method provides a simple but highly reproducible method of adjusting the inner/outer diameter ratio of a glass capillary by preheating the nanopipette prior to extension. The new capillary is capable of measuring ion currents with excellent S/N and has successfully visualized the dynamic cell surface topographic change related to endocytosis.

Watanabe *et al.* developed an ultra-low-noise wide-bandwidth transimpedance amplifier (TIA) to improve S/N in SICM measurements. A design of a low input capacitance interface and two-stage opamp configuration provides extremely low current noise performance in frequencies higher than 10 kHz. The background root-mean-square (RMS) noise of the TIA reaches ~3.5 pA at a bandwidth of 100 kHz, corresponding to ~30 percent of RMS current noise of commercially available low-noise TIAs for SICM use. In addition, extended input current range up to ~34 nA at a transimpedance gain of 1 GW assures the capability of developed TIA for most SICM applications. Such excellent performance of developed TIA will push the limits of the temporal resolution of SICM for visualizing dynamic structural changes on a cellular surface that are difficult with current instruments.

·3D-AFM imaging of Various 3D Self-Organizing Systems

Fukuma *et al.* have been developing 3D-AFM techniques to visualize the inside of various 3D self-organizing systems (3D-SOS). They developed 3D-AFM in 2010 and enabled the visualization of 3D hydration structures. Based on this technique, they developed 3D nanoendoscopy AFM and enabled the visualization of intra-cellular structures using a micrometer-scale needle probe in the past WPI project period. They are now exploring possibilities to visualize various 3D-SOSs with a thickness between hydration structures and living cells with various needle probes. For example, they developed a carbon nanotube (CNT) probe and applied it to visualize the inside of chromosomes. Their MD simulation results suggest that the obtained force contrasts largely represent the chromatin density distribution. They plan to use this technique for investigating the formation mechanism of the chromosome structure and its abnormalities caused by diseases or aging.

(Nanoprobe studies on various life phenomena)

i) Quantification of phycosphere pH of marine nano-phytoplankton species at a single cell level (*ISME Journal*, 2022, IF: 11.217**) (Zhang, Korchev)**

Marine phytoplankton are at the base of the food chain and their productivity ultimately determines the maintenance of fisheries. It is well documented that ocean acidification is profoundly altering a range of phytoplankton processes including nutrient acquisition, biogenic calcification and silicification. However, the assessment and prediction of the impacts of ocean acidification on phytoplankton remains challenging, due to a poor knowledge of processes occurring in the phycosphere surrounding cells. In this study, they employ a novel nano-technology pH probe for a high spatial (50 nm) and temporal resolution (2 ms) *in situ* quantification of phycosphere pH of marine nano- and micro-phytoplankton species at a single cell level (Fig. 17). For the first time, they show that the phycosphere pH is consistently higher than bulk seawater, which is amplified under ocean acidification conditions. They also demonstrate that a phycosphere pH increase can significantly alter the chemistry of iron, a limiting trace metal nutrient in about 40% of surface oceans.

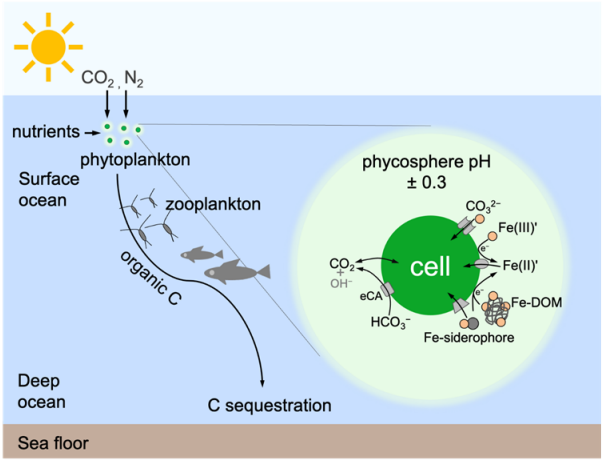


Fig. 17: Impact of an increase of pH in the phycosphere driven by algal uptake of CO_2 and extracellular enzymatic transformation of HCO_3- and a pH decrease driven by CO_2 release from the phytoplankton cell on Fe speciation and its availability to marine phytoplankton.

ii) Molecular insights on the crystalline cellulose/chitin-water interfaces via three-dimensional atomic force microscopy (*Science Advances*, 2022, IF: 14.98; ***Small Methods*, 2022,** IF: 15.367**) (Yurtsever, Miyata, Miyazawa, MacLachlan, Foster, Fukuma)**

Cellulose/chitin, a renewable structural biopolymer, is ubiquitous in nature and is the basic reinforcement component of the natural hierarchical structures of living plants, bacteria, tunicates, and many other organisms. However, a detailed picture of the crystalline cellulose/chitin surface at the molecular level is still unavailable. In this study, using AFM and molecular dynamics simulations, they revealed the molecular details of the cellulose/chitin chain arrangements on the surfaces of individual cellulose/chitin nanocrystals in water. They found substantial differences in the molecular details of water structure at different interfaces, reflecting the heterogeneous nature of the interactions between cellulose/chitin nanocrystals and water at the molecular level (Fig. 18). The inhomogeneous existence of these structured water layers on different crystalline planes may affect the cellulose/chitin nanocrystal surface adsorption and interaction behavior; thus, the degradation of cellulose/chitin by cellulase/chitinase-degrading enzymes could be affected differently.

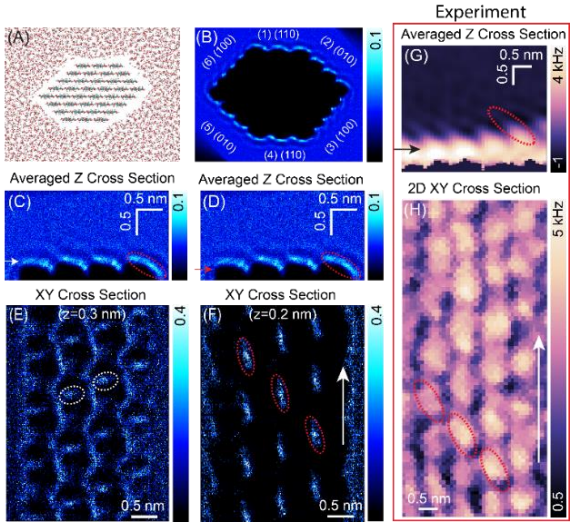


Fig. 18: Water-density distribution around different crystalline planes of a cellulose nanocrystal surface.

iii) Water dimer-driven DNA base superstructure with mismatched hydrogen bonding (*Journal of the American Chemical Society*, 2022, IF: 16,383**) (Foster)**

The existence of water dimers in equilibrium water vapor at room temperature and their anomalous properties revealed by recent studies suggest the benchmark role of water dimers in both experiment and theory. However, there has been a limited observation of individual water dimers due to the challenge of water separation and generation at the single-molecule level. Here, we use SPM to achieve real-space imaging of individual confined water dimers embedded inside a self-assembled layer of a DNA base, adenine, on a silver surface. The hydration of the adenine layers by these water dimers causes a local surface chiral inversion in such a way that the neighboring homochiral adenine molecules become heterochiral after hydration, resulting in a mismatched hydrogen-bond pattern

between neighboring adenine molecules. The observation of single confined water dimers offers an unprecedented approach to studying the fundamental forms of water clusters and their interaction with the local biological environment (Fig. 19).

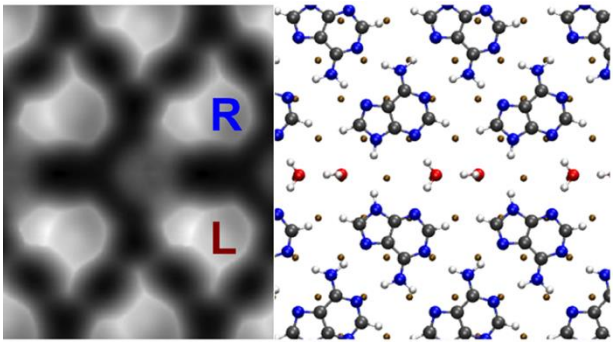


Fig. 19: Simulated AFM image of the hydrated adenine structure.

iv) In situ visualization of dynamic cellular effects of phospholipid nanoparticles via high-speed scanning ion conductance microscopy (*Small,* 2022, IF: 15.153**) (Sun, Watanabe)**

Phospholipid nanoparticles have been actively employed in numerous biomedical applications. A key factor in ensuring effective and safe applications of these nanomaterials is the regulation of their interactions with target cells, which is significantly dependent on an in-depth understanding of the nanoparticle-cell interactions. To date, most studies investigating these nano-bio interactions have been performed under static conditions. It is, however, noteworthy that such nanoparticle-cell interactions are highly dynamic. To gain a deeper insight into the cellular effects of phospholipid nanoparticles, they investigated the dynamic cellular effects of sub-100 nm phospholipid nanoparticles using high-speed scanning ion conductance microscopy. It was revealed that upon introduction into the cellular environment, within a short timescale of hundreds of seconds, phospholipid nanoparticles can selectively modulate the edge motility and surface roughness of healthy fibroblast and cancerous epithelial cells. Furthermore, the dynamic deformation profiles of these cells can be selectively altered in the presence of phospholipid nanoparticles. This work should shed further light on real-time nanoparticle-cell interactions for improved formulation of phospholipid nanoparticles for numerous bioapplications.

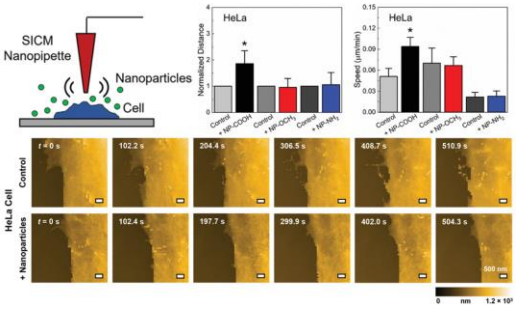


Fig. 20: Nanoparticle-cell interactions measured by HS-SICM

v) Dynamic mechanisms of CRISPR interference by *Escherichia coli* CRISPR-Cas3 (*Nature Communications,* 2022, IF: 17.694**) (Kodera, Umeda)**

Genome editing is a very useful technology not only for basic research to elucidate life phenomena but also for a wide range of other fields such as improving the efficiency of bioproduction in industry, breeding in the agricultural and fishery industries, and developing gene therapy and new drugs in the medical field. A new genome editing tool using CRISPR-Cas3 has unique features that make long-range unidirectional deletions of genome-DNA and have fewer off-target problems compared with the widely used CRISPR-Cas9 system. Despite many studies using cryo-electron microscopy and single-molecule FRET, the precise mechanism of genome editing by CRISPR-Cas3 remained elusive. In this study, in collaboration with researchers of the University of Tokyo and RIKEN, they succeeded in reconstructing the CRISPR-Cas3 system *in vitro* and clarifying the detailed mechanism of genome editing by CRISPR-Cas3. Notably, using high-speed atomic force microscopy (HS-AFM), a series of videos could be obtained of CRISPR-Cas3 in action from target search and binding to DNA degradation including the events of DNA-reeling and unreeling (Fig. 21). The dynamic information obtained from this study provides important and fundamental knowledge for the

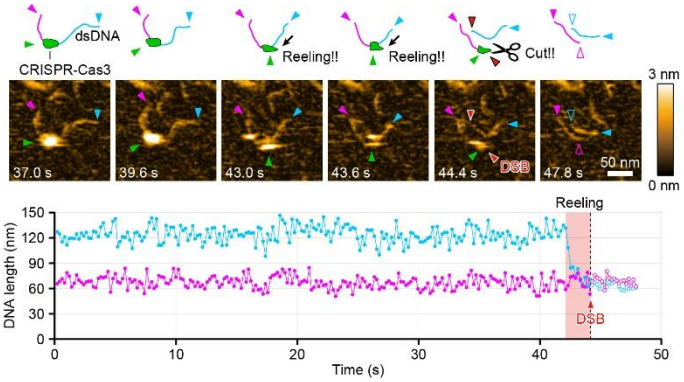


Fig. 21 Direct observation of the long-range unidirectional deletions of DNA by CRISPR-Cas3, using HS-AFM. A Cascade-Cas3 complex bound to the target site (green) reels the upstream long strand side (blue). After reeling DNA, a double-strand break (DSB) is introduced (red). Bottom graph shows a time course of the DNA lengths (short side, magenta; long side, blue).

development of CRISPR-Cas3 into a more efficient and accurate genome editing technology.

vi) Structure and functional role of a nucleolar protein PQBP5 (*Nature Communications,* 2023, IF:17.694**) (Umeda, Kodera, Ando)**

The nucleolus is a membrane-less organelle where rDNA is transcribed to pre-rRNA, which is processed and used for constructing ribosomes. PQBP5 was found to be one of the major nucleolus proteins related to PolyQ diseases but its structure and function have long been elusive. Using HS-AFM, they found that PQBP5 is an IDP with a C-terminal globule and an N-terminal IDR undergoing order-disorder transitions (Fig. 22a). RNA interacts with the IDR at its distal and proximal regions when these regions transiently form small globules (Fig. 22b, c). Super-resolution fluorescence microscopy revealed that PQBP5 forms a basket-like structure surrounded by (and partially colocalized with) other IDPs containing low complexity sequences, fibrillarin and nucleolin (Fig. 22d). Therefore, the PQBP5's basket-like structure seems to function as an anchor for assembly and reassembly of other nucleolar proteins. This was further supported by the imaging of HeLa cells subjected to osmotic stress, where the nucleolus was dissolved by dispersion of fibrillarin, nucleolin, and other proteins whereas PQBP5 remained in the nucleolus.

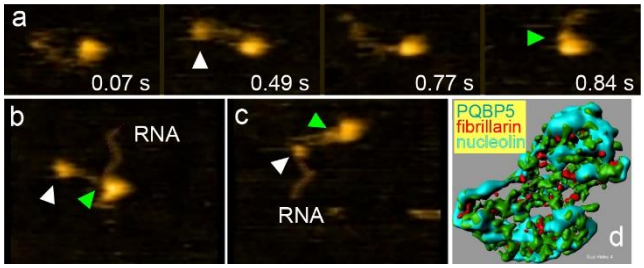


Fig. 22: HS-AFM images of PQBP5 alone (a), PQBP5 bound to RNA (b, c), and localization of three nucleolar proteins (d).

vii) Dynamics of target DNA binding and cleavage by Staphylococcus aureus Cas9 as revealed by high-speed atomic force microscopy (*ACS Nano,* 2023, IF:18.027**) (Puppulin, Sumino, Marchesi, Flechsig, Kodera, Shibata)**

Programmable DNA binding and cleavage by CRISPR-Cas9 has revolutionized life sciences. However, the off-target cleavage observed in DNA sequences with some homology to the target still represents the major limitation for more widespread use of Cas9 in biology and medicine. For this reason, complete understanding of the dynamics of DNA binding, interrogation and cleavage by Cas9 is crucial to improve the efficiency of genome editing. Here, they use HS-AFM to investigate *Staphylococcus aureus* Cas9 (SaCas9) and its dynamics of DNA binding and cleavage. Upon binding to single-guide RNA (sgRNA), SaCas9 forms a close bilobed structure that also transiently and flexibly adopts an open configuration. The SaCas9-mediated DNA cleavage is characterized by release of cleaved DNA and immediate dissociation (Fig. 23). The direct visualization of the process by sequential topographic images suggests that SaCas9-sgRNA binds to the target sequence first, while the following binding of the PAM is accompanied by local DNA bending and formation of the stable complex. Collectively, their HS-AFM data reveal a potential and unexpected behavior of SaCas9 during the search for DNA targets.

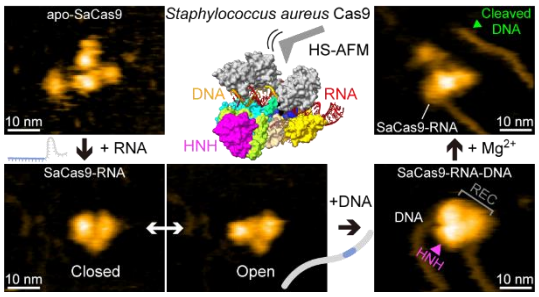


Fig. 23: HS-AFM images of SaCas9 in action on mica surface.

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 taken the following measures to create an interdisciplinary research domain consisting of four fields, i.e. nanometrology, life science, supramolecular chemistry, and mathematical computational science.

Top-down approach

From the second half of FY2020, we have continued to carry out interdisciplinary research that addresses the three priority research themes set out by the NanoLSI Future Planning Board. The three priority research themes are as follows:

- Intracellular imaging and cell monitoring after treatments for observation (coordinators, PIs Fukuma and Wong);
- Local stimulation/manipulation and chemical mapping (inside cells and cell surface) (coordinators, PIs Hirao and Maeda);
- Cell surface imaging and cell-cell communication (coordinators, PIs Oshima and Hanayama).

For each priority research theme, research expenses of 4 million yen were provided from the on-campus COE research funds from 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 19.7 million yen was provided for 13 research projects via application and selection. Of the 13 research projects supported, one is a research project led by a graduate student in the Doctoral Level Section of Integrated Course, 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 fiscal year following the support, a research report meeting attended by all PIs and Professors is held to give advice on future research development.

T-meetings

In order to promote interdisciplinary research, T-meetings were held 38 times in FY2022 by combining two research teams out of those (23 teams in total) individually led by 16 PIs, one associate PI and 6 Jr. PIs from different disciplines to allow them to introduce their research. Twelve of these T-meetings were conducted in combination with a research team led by an overseas PI and a team by a resident PI or a Jr. PI in NanoLSI.

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 2017-2022

During the six years from 2017, when NanoLSI was established, to 2022, the total number of papers authored by PIs was 697, of which 317 (45.5%) were internationally co-authored. Of the total 697 papers, twenty-eight were in the top 1% (3.8%) and 219 in the top 10% (29.5%) in terms of citations. The number of top 1% papers based on field-weighted Citation Impact corrections was 7 (0.9%), and the number of top 10% papers was 99 (13.3%).

In 2022, the total number of papers published by 16 PIs was 106, of which 45 (42.5%) were internationally co-authored. Of the total 106 papers, two were in the top 1% (1.9%), and 26 were in the top 10% (24.5%). The number of top 1% papers based on field-weighted Citation Impact corrections was 0 (0%), and the number of top 10% papers, 9 (8.5%).

Number of co-authored papers with overseas PIs in 2017-2022

The total number of papers co-authored by one of the four overseas PIs with resident researchers in NanoLSI was 24 in the six years from 2017 to 2022. Of these, 9 papers were published with Prof.

Yuri Korchev (nanometrology) of Imperial College London, UK, an overseas satellite, and 4 with Prof. Mark MacLachlan (supramolecular chemistry), the University of British Columbia, Canada, also an overseas satellite. In addition, a total of 12 papers were published with Prof. Adam Foster (computational science) of Aalto University, Finland, and one with Prof. Alexander Mikhailov (former overseas PI, computational science) of the Fritz Haber Institute of the Max Planck Society, Germany, or with his successor, Prof. Carsten Beta (computational science and biophysics), the University of Potsdam, Germany. Of the total of 24 papers, two were published with both Prof. MacLachlan and Prof. Foster.

Seven co-authored papers were published in 2022 alone: 3 with Prof. Korchev, one with Prof. MacLachlan, one with Prof. Foster, and 2 were published with both Prof. MacLachlan and Prof. Foster.

Outreach programs for external researchers in FY2017-FY2022

The Bio-SPM Summer School and Bio-SPM Collaborative Research Program aim to invite external researchers to disseminate the scanning probe microscope (Bio-SPM) technology of NanoLSI, leading to joint research. In FY2022, when the Bio-SPM Summer School was held in August, it was difficult for overseas researchers to come to Japan due to COVID-19, so only one overseas researcher from one country participated. On the other hand, 17 Japanese researchers participated. In the Bio-SPM Collaborative Research Program, a total of 5 joint research projects with overseas researchers from 5 countries were conducted in FY2022. As a cumulative result from FY2017 to FY2022, a total of 30 overseas researchers from 16 countries participated in the Bio-SPM Summer School. In addition, a total of 65 Japanese researchers participated. It should be noted that in FY2020 and FY2021, overseas researchers were not invited due to COVID-19. In the Bio-SPM Collaborative Research Program, a total of 22 joint research projects with overseas researchers from 14 countries were conducted from FY2017 to FY2022.

The NanoLSI Visiting Fellows Program aims to invite PI-level life science researchers and their subordinate researchers from overseas to conduct researcher exchanges and joint research and to establish an organizational cooperative relationship between NanoLSI and the relevant research institutions. In this Visiting Fellows Program, one research group led by Dr. Borja Ibarra Urruela, IMDEA, Spain was invited in FY2022. In addition, the invitation of one research group has been carried forward to FY2023. A total of 3 research groups were invited from FY2017 to FY2022.

Support for young overseas researchers to acquire research funds in FY2017 to FY2022

With individual support from the full-time URA of NanoLSI, a total of 18 young overseas researchers have acquired a total of 25 KAKENHI grants from FY2017 to FY2022. In FY2022 alone, young overseas researchers belonging to NanoLSI submitted 9 new applications for KAKENHI, of which 3 were approved.

Mobility and career path for young researchers

As of the end of FY2022, the number of postdoctoral researchers (including fixed-term assistant professors) was 28 out of 82 researchers in total in NanoLSI, and 21 out of 28 were overseas researchers. Eight postdoctoral researchers (including fixed-term assistant professors) left NanoLSI during FY2022. Of these, one has acquired a tenured associate professorship, two have tenure-track assistant professorships, and one a research fellow position. The remaining two have been applying for researcher positions.

Results of overseas researcher visits in FY2022

Society has been recovering from the impact of COVID-19, and the ban on visa-free travel to Japan was lifted from the second half of FY2022. In conjunction with this, visits of overseas researchers to NanoLSI have also been recovering. In FY2022, 39 researchers from 17 countries visited NanoLSI for a total of 1011 person-days.

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, the rigorous evaluation-based salary system, the tenure-track junior PI program, integrated management of NanoLSI and the Graduate School "Division of Nano Life Science," English-based administration, planning and setting up various research meetings by researchers and administrative staff working together to promote

interdisciplinary research, outreach programs to promote joint research with external researchers, and the implementation of the PDCA cycle based on external evaluation (WPI Program Committee evaluation). These successful reforms will be maintained and continued for 5 years in the latter half of the subsidy period.

Expected ripple effect on the host institution

In FY2023, Kanazawa University will begin construction of a new research facility, the "Facility for Future Co-Creation" (provisional name), which is scheduled to be completed by the end of the fiscal year. The facility will be based on the fundamental strategy of NanoLSI, i.e. to further develop the excellence of specific research areas where it has strengths, to reinforce joint research to promote interdisciplinary research and to gather together researchers under one roof, and also adding industry-academia collaboration and social implementation. Thus, we aim to create new social value by strategically and in an integrated manner reinforcing the process of social implementation of various discoveries resulting from Kanazawa University's distinctive research strengths. At the same time, the basic concept of the facility is to function as a place for interaction where researchers/staff and organizations related to the facility gather and where researchers can fully demonstrate their individuality and capabilities. It is planned to make full use of NanoLSI's experience and success stories will be used in the development and execution of mechanisms and programs to promote exchanges in different fields.

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.

Research plan, research organization and PI composition

Regarding the NanoLSI research and development plan, 6 nanotechnology and 7 life sciences roadmaps have been updated, clarifying the challenges to be addressed. Concerning the research organization, the structure with 16 PIs is maintained, and by positioning Prof. Ando (age 72), who is no longer a PI, as a Distinguished Professor of Kanazawa University, we institutionalized a university-wide mechanism to allow distinguished professors, including Prof. Ando, to continue their research until the age of 75. In addition, the Center Director, the Administrative Director and four PIs who are the core members of NanoLSI operations hold an intensive discussion at the Future Planning Board positioned as the steering committee of NanoLSI, which maintains the balance between top-down and bottom-up operations.

Fostering and securing of next-generation researchers

See Appendix 3-1 FY2022 Records of Center Activities "Special mention".

Career path after graduation

As of April, 2023, the first two students (both Japanese) have graduated from the Doctoral Level Section of Integrated Course, Division of Nano Life Science. They obtained positions as researchers at a private company (Sumitomo Chemical Co., Ltd., Kureha Co., Ltd.). It is expected that three more students will graduate as of October, 2023. All three are foreign, wishing to get researcher positions at an academic institution such as a university. A follow-up survey of their career paths will be conducted.

Positioning NanoLSI within the host institution

NanoLSI is positioned as an independent research institute in the statutes of Kanazawa University. In addition, in the university statutes, it is clearly stated that "special measures can be applied to the operation of the Nano Life Science Institute in order to promote the establishment of an independent research entity," and it is assured that NanoLSI is maintained and continued as a world-class research entity.

The host institution's commitment to NanoLSI after the WPI grant ends

In the report at the FY2022 WPI Program Committee Meeting, President Wada, Kanazawa University, announced that even after the end of the WPI subsidy period, almost the same commitment as now will continue regarding provision of budgets, preferential treatment on personnel affairs, and infrastructure maintenance and development.

Amount of external fund acquisition in FY2022

The total amount of external funds acquired in FY2022 by 82 NanoLSI researchers was 1,356

million Yen (1,208 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.

Press release of research outcomes

In FY2022, 21 press releases concerning research outcomes were issued, of which 17 were also issued in English. Among the research outcomes publicized, an article by PI Matsumoto, “Designing receptor agonists with enhanced pharmacokinetics by grafting macrocyclic peptides into fragment crystallizable regions,” was published in a very high-ranking journal, Nature Biomedical Engineering (IF: 29.234)/vol. 7/164-176 (2023). In addition, an article by Jr. PI Arai, “Modulation of Local Cellar Activities using a Photothermal Dye-Based Subcellular-Sized Heat Spot,” was published in ACS Nano (IF: 18.027)/2022.16.6/9004-9018.

Media coverage

NHK Kanazawa and NHK BS1 broadcast the program “Open the future! Ishikawa's Forefront of Science,” featuring the high-speed atomic force microscope developed by Prof. Ando. Research of NanoLSI was introduced and featured in the April 2023 enlarged issue "WPI Special Vol. 3" of the monthly magazine, Junior Aera with support of JSPS/WPI Program Center. In 2022, a new podcast was launched, introducing the personalities and research content of 15 NanoLSI researchers.

Visitors to NanoLSI

In FY2022, a total of 8 groups from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) visited NanoLSI, including a visit by Hideyuki Tanaka, Senior Vice Minister. In addition, a total of 12 groups from other ministries and universities visited NanoLSI and meaningful discussions were held on the roles and know-how of administrative departments in the formation of research entities.

Approach to high school students

Concerning interactions of the super science high schools designated by MEXT of Ishikawa Prefecture, students from Kanazawa Izumigaoka High School gave research presentations in English and young NanoLSI researchers commented and advised on these research presentations. In addition, NanoLSI and Cancer Research Institute (CRI), Kanazawa University, jointly planned and implemented an outreach program for high school students. This “Cancer Research Early Exposure Program” is a program in which researchers from NanoLSI and CRI serve as instructors and are aimed at excellent high school students who aspire to become cancer researchers and allowed them to experience practical experiments. In 2022, a total of 39 high school students participated in the elective program of 11 events during 4 days. In launching this program, CRI and NanoLSI jointly carried out crowdfunding and received donations of 3.14 million yen from 156 people.

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 ***"Every effort that NanoLSI is currently making appears to be in good shape and is strongly encouraged to continue. It is very good to hear the strong commitment of support from Prof. Wada, the new president of Kanazawa University. Center Director Fukuma has slightly changed gears to a more top-down governance so as to support mission-oriented research subjects, which will be important to achieve the Center’s final goals. At the same time, bottom-up research activities emerging from young researchers will also be extremely useful in producing unexpected cutting-edges in "Nanoprobe Life Science." The leadership of the Director in balancing the top-down and bottom-up trajectories will be very much appreciated. Strategic approaches to involve more early-stage students and female researchers should be continued. In the future, it will also be good to see industry collaborations and perhaps an expansion to non-biological sciences."***

Thank you very much for the favorable and encouraging comments. We agree that balancing the top-down and bottom-up approaches is extremely important. Thus, we will continue to keep

monitoring and adjusting this. For example, we started the top-down type transdisciplinary research promotion grant (TDRP-G) in FY2021 in addition to the bottom-up type. This top-down grant has successfully promoted biological applications of the newly developed live-cell SPM techniques. Meanwhile, we removed the selection rule to prioritize the proposals in line with the WPI missions for the bottom-up grant in FY2022. This change has promoted the exploration of a wide range of transdisciplinary research collaborations. In this way, we promote mission-oriented projects through the top-down approach while pioneering new possibilities through the bottom-up approach.

We will also continue to enhance the involvement of undergraduate students and female researchers. As for students, the PIs have lecture courses in undergraduate schools of various disciplines, including physics, engineering, chemistry, biology, and medical and pharmaceutical sciences. Thus, many undergraduate students in these schools join NanoLSI research groups every year. In addition, we recently set up a new lecture course on nano life science by Jr. PIs for undergraduate students in the biological science and technology school. In this way, we aim to increase the number of students interested in this field and make it easier for them to join our institute. As for female researchers, we have successfully increased the female proportion from 10% to 20% in the past three years using various strategies. For example, we started to encourage hiring not only foreign but also Japanese female researchers. As far as we can maintain the foreign researcher proportion over 30%, we will continue this policy to further increase the proportion of female scientists.

SPM is a fundamental technology applicable to various research areas. Thus, we have been exploring its applications not only to basic life science but also to industrial collaborations and non-biological sciences. Examples of recent industrial collaborations include studies on metal corrosion by an in-liquid potential measurement technique (***JPCC*** **2023**) and polymer brush and surfactants by 3D-AFM (***ACS ANM*** **2021***, **ACS AMI*** **2022**). Meanwhile, in this WPI project, we have developed various live-cell bio-SPM techniques and pioneered their applications in molecular cell biology and medical sciences, which should lead to future collaborations with private companies in the life science area.

So far, we have established an excellent environment for transdisciplinary research among nanometrology, supramolecular chemistry, life science and computational science. While this WPI project aims to combine all four disciplines to perform "nanoprobe life science" research, this environment has also motivated us to explore non-biological transdisciplinary research. For example, SPM, chemistry and computational science groups investigated nanoscale structures and functions of biomaterials (***Small Methods*** **2022***, **Sci. Adv.*** **2022**) and helical polymers (***JACS*** **2021***, **Chem. Commun.*** **2021**). Taking advantage of this environment and the bottom-up TDRP-G system, we will continue to explore a wide range of research subjects.

Appendix 1　FY 2022 List of Center's Research Results and Main Awards

1. Refereed Papers

- List only the Center's papers published in 2022. (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 (the Basic and Generic Research Division at present) 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) (or DOI number), 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.)
   - The papers should be divided into A or B categories on separate sheets, not divided by 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. Shi TH, Nagata Y, Akine S, Ohtani S, Kato K, Ogoshi T. A Twisted Chiral Cavitand with 5-Fold Symmetry and Its Length-Selective Binding Properties. J Am Chem Soc. 2022;144(51):23677–84. (IF=16.383)
2. Shi Y, Soto MA, MacLachlan MJ. Self-Assembled Gels of Cellulose Nanocrystals for Diffusion-Controlled Color Switching. ACS Appl Nano Mater. 2022;5(12):17819–27. (IF=6.14)
3. Shi TH, Fa S, Nagata Y, Wada K, Ohtani S, Kato K, Ogoshi T. Discrete chiral organic nanotubes by stacking pillar[5]arenes using covalent linkages. Cell Reports Phys Sci. 2022;3(12). (IF=7.832)

4. Biswas FB, Das S, Nishimura T, Endo M, Fukuda M, Morita F, Mashio AS, Taniguchi T, Maeda K, Hasegawa H. Functionalized polyvinyl alcohol aerogel for efficient and selective removal of arsenite from aqueous matrices. Chem Eng J. 2022;450. (IF=16.744)
5. Kato K, Maeda K, Mizuno M, Nishina Y, Fa S, Ohtani S, Ogoshi T. Room-Temperature Ring-Opening Polymerization of δ-Valerolactone and ϵ-Caprolactone Caused by Uptake into Porous Pillar[5]arene Crystals. Angew Chemie - Int Ed. 2022;61(50). (IF=16.823)
6. Ogane T, Noshiro D, Ando T, Yamashita A, Sugita Y, Matsunaga Y. Development of hidden Markov modeling method for molecular orientations and structure estimation from high-speed atomic force microscopy time-series images. PLoS Comput Biol. 2022;18(12). (IF=4.799)
7. Yamada R, Trang TN, Flechsig H, Takeda T, Kodera N, Konno H. Importance of annexin V N-terminus for 2D crystal formation and quick purification protocol of recombinant annexin V. PLoS One. 2022;17(12 Decembe). (IF=3.752)
8. Chen IJ, Aapro M, Kipnis A, Ilin A, Liljeroth P, Foster AS. Precise atom manipulation through deep reinforcement learning. Nat Commun. 2022;13(1). (IF=17.694)
9. Fa S, Shi TH, Akama S, Adachi K, Wada K, Tanaka S, Oyama N, Kato K, Ohtani S, Nagata Y, Akine S, Ogoshi T. Real-time chirality transfer monitoring from statistically random to discrete homochiral nanotubes. Nat Commun. 2022;13(1). (IF=17.694)
10. Fujimura Y, Kawakatsu T, Morimoto M, Asakawa H, Nakagawa K, Yoshioka T. Study for removing of silica nanoparticle in pure isopropyl alcohol with a cation exchange membrane. J Mol Liq. 2022;367. (IF=6.633)
11. Yoshimi K, Takeshita K, Kodera N, Shibumura S, Yamauchi Y, Omatsu M, Umeda K, Kunihiro Y, Yamamoto M, Mashimo T. Dynamic mechanisms of CRISPR interference by Escherichia coli CRISPR-Cas3. Nat Commun. 2022;13(1). (IF=17.694)
12. Liu X, Yamazaki T, Kwon HY, Arai S, Chang YT. A palette of site-specific organelle fluorescent thermometers. Mater Today Bio. 2022;16. (IF=10.761)
13. Watanabe S, Nihongaki Y, Itoh K, Uyama T, Toda S, Watanabe S, Inoue T. Defunctionalizing intracellular organelles such as mitochondria and peroxisomes with engineered phospholipase A/acyltransferases. Nat Commun. 2022;13(1). (IF=17.694)
14. Shirasaki T, Yamagoe S, Shimakami T, Murai K, Imamura R, Ishii KA, Takayama H, Matsumoto Y, Tajima-Shirasaki N, Nagata N, Takamura T, Honda M. Leukocyte cell-derived chemotaxin 2 is an antiviral regulator acting through the proto-oncogene MET. Nat Commun. 2022;13(1). (IF=17.694)
15. Ichikawa T, Wang D, Miyazawa K, Miyata K, Oshima M, Fukuma T. Chemical fixation creates nanoscale clusters on the cell surface by aggregating membrane proteins. Commun Biol. 2022;5(1). (IF=6.548)
16. Yanagimura N, Takeuchi S, Fukuda K, Arai S, Tanimoto A, Nishiyama A, Ogo N, Takahashi H, Asai A, Watanabe S, Kikuchi T, Yano S. STAT3 inhibition suppresses adaptive survival of ALK-rearranged lung cancer cells through transcriptional modulation of apoptosis. npj Precis Oncol. 2022;6(1). (IF=10.123)
17. Hiraiwa M, Fukasawa K, Iezaki T, Sabit H, Horie T, Tokumura K, Iwahashi S, Murata M, Kobayashi M, Suzuki A, Nakada M, Hinoi E. SMURF2 phosphorylation at Thr249 modifies glioma stemness and tumorigenicity by regulating TGF-β receptor stability. Commun Biol. 2022;5(1). (IF=6.548)

18. Tanaka H, Kobayashi Y, Furukawa K, Okayasu Y, Akine S, Yasuda N, Maeda H. π-Stacked Ion Pairs: Tightly Associated Charged Porphyrins in Ordered Arrangement Enabling Radical-Pair Formation. J Am Chem Soc. 2022;144(47):21710–8. (IF=16.383)
19. Nozaki I, Ishikawa N, Miyanari Y, Ogawa K, Tagawa A, Yoshida S, Munekane M, Mishiro K, Toriba A, Nakayama M, Nakayama M, Fuchigami T. Borealin-Derived Peptides as Survivin-Targeting Cancer Imaging and Therapeutic Agents. Bioconjug Chem. 2022;33(11):2149–60. (IF=6.609)
20. Rodríguez R, Rivadulla-Cendal E, Fernández-Míguez M, Fernández B, Maeda K, Quiñoá E, Freire F. Full Control of the Chiral Overpass Effect in Helical Polymers: P/M Screw Sense Induction by Remote Chiral Centers After Bypassing the First Chiral Residue. Angew Chemie - Int Ed. 2022;61(46). (IF=16.823)
21. Akine S, Nomura K, Takahashi M, Sakata Y, Mori T, Nakanishi W, Ariga K. Synthesis of amphiphilic chiral salen complexes and their conformational manipulation at the air-water interface. Dalt Trans. 2022;52(2):260–8. (IF=4.569)
22. Cai S, Kurki L, Xu C, Foster AS, Liljeroth P. Water Dimer-Driven DNA Base Superstructure with Mismatched Hydrogen Bonding. J Am Chem Soc. 2022;144(44):20227–31. (IF=16.383)
23. Kikuchi Y, Toyofuku M, Ichinaka Y, Kiyokawa T, Obana N, Nomura N, Taoka A. Physical Properties and Shifting of the Extracellular Membrane Vesicles Attached to Living Bacterial Cell Surfaces. Microbiol Spectr. 2022;10(6). (IF=9.043)
24. Wu P, Nishimura T, Maeda K, Fujii K, Hayano S, Nageh H, Naga N, Nakano T. Raspberry-like Aggregate Formation of p-Stacked Block Copolymer of, and Methyl Methacrylate Synthesized by Atom-transfer Radical Polymerization. Chem Lett. 2022;51(11):1058–61. (IF=1.715)
25. Sajidah ES, Lim K, Yamano T, Nishide G, Qiu Y, Yoshida T, Wang H, Kobayashi A, Hazawa M, Dewi FRP, Ando T, Wong RW. Spatiotemporal tracking of small extracellular vesicle nanotopology in response to physicochemical stresses revealed by HS-AFM. J Extracell Vesicles. 2022;11(11). (IF=17.337)
26. Isono M, Nakano M, Fukami T, Nakajima M. Adenosine N^6-methylation upregulates the expression of human CYP2B6 by altering the chromatin status. Biochem Pharmacol. 2022;205. (IF=6.1)
27. Kodera N, Ando T. Guide to studying intrinsically disordered proteins by high-speed atomic force microscopy. Methods. 2022;207:44–56. (IF=4.647)
28. Nakakubo K, Endo M, Sakai Y, Biswas FB, Wong KH, Mashio AS, Taniguchi T, Nishimura T, Katsuhiro M, Hasegawa H. Cross-linked dithiocarbamate-modified cellulose with enhanced thermal stability and dispersibility as a sorbent for arsenite removal. Chemosphere. 2022;307. (IF=8.943)
29. Aizawa T, Akine S, Saiki T, Nakamura T, Nabeshima T. Rotaxane formation by an allosteric pseudomacrocyclic anion receptor utilising kinetically labile copper(i) coordination properties. Dalt Trans. 2022;51(45):17277–82. (IF=4.569)
30. Wu P, Pietropaolo A, Fortino M, Shimoda S, Maeda K, Nishimura T, Bando M, Naga N, Nakano T. Non-uniform Self-folding of Helical Poly(fluorenevinylene) Derivatives in the Solid State Leading to Amplified Circular Dichroism and Circularly Polarized Light Emission. Angew Chemie - Int Ed. 2022;61(43). (IF=16.823)

31. Yurtsever A, Wang PX, Priante F, Jaques YM, Miyazawa K, MacLachlan MJ, Foster AS, Fukuma T. Molecular insights on the crystalline cellulose-water interfaces via three-dimensional atomic force microscopy. Sci Adv. 2022;8(41). (IF=14.98)
32. Chaudhry MT, Patrick BO, Akine S, MacLachlan MJ. Noncooperative guest binding by metal-free [2 + 2] Schiff-base macrocycles. Org Biomol Chem. 2022;20(42):8259–68. (IF=3.89)
33. Hino N, Matsuda K, Jikko Y, Maryu G, Sakai K, Imamura R, Tsukiji S, Aoki K, Terai K, Hirashima T, Trepat X, Matsuda M. A feedback loop between lamellipodial extension and HGF-ERK signaling specifies leader cells during collective cell migration. Dev Cell. 2022;57(19):2290-2304.e7. (IF=13.417)
34. Ikarashi T, Nakayama K, Nakajima N, Miyata K, Miyazawa K, Fukuma T. Visualizing Molecular-Scale Adsorption Structures of Anti-freezing Surfactants on Sapphire (0001) Surfaces at Different Concentrations by 3D Scanning Force Microscopy. ACS Appl Mater Interfaces. 2022;14(39):44947–57. (IF=10.383)
35. Liu F, Gledhill M, Tan QG, Zhu K, Zhang Q, Salaün P, Tagliabue A, Zhang Y, Weiss D, Achterberg EP, Achterberg EP, Korchev Y. Phycosphere pH of unicellular nano- and micro- phytoplankton cells and consequences for iron speciation. ISME J. 2022;16(10):2329–36. (IF=11.217)
36. Duss M, Soto MA, Patrick BO, MacLachlan MJ. A Supramolecular Strategy for the Synthesis of Cyclic Oligomers and Polymers by Ring Expansion. Chem - A Eur J. 2022;28(54). (IF=5.02)
37. Kimura K, Yamamori S, Hazawa M, Kobayashi-Sun J, Kondo M, Wong RW, Kobayashi I. Inhibition of Canonical Wnt Signaling Promotes Ex Vivo Maintenance and Proliferation of Hematopoietic Stem Cells in Zebrafish. Stem Cells. 2022;40(9):831–42. (IF=5.845)
38. Pham LT, Peng H, Ueno M, Kohno S, Kasada A, Hosomichi K, Sato T, Kurayoshi K, Kobayashi M, Tadokoro Y, Tajima A, Hirao A. RHEB is a potential therapeutic target in T cell acute lymphoblastic leukemia. Biochem Biophys Res Commun. 2022;621:74–9. (IF=3.322)
39. Yuan J, Jiang L, Nishimura T, Sauvé ER, Hean D, Maeda K, Wolf MO. Effect of Oxidation on the Chiroptical Properties of Sulfur-Bridged Binaphthyl Dimers. J Org Chem. 2022;87(18):12315–22. (IF=4.198)
40. Yamamoto A, Ikarashi T, Fukuma T, Suzuki R, Nakahata M, Miyata K, Tanaka M. Ion-specific nanoscale compaction of cysteine-modified poly(acrylic acid) brushes revealed by 3D scanning force microscopy with frequency modulation detection. Nanoscale Adv. 2022;4(23):5027–36. (IF=5.598)
41. Kato K, Kurakake Y, Ohtani S, Fa S, Gon M, Tanaka K, Ogoshi T. Discrete Macrocycles with Fixed Chirality and Two Distinct Sides: Dipole-Dependent Chiroptical Response. Angew Chemie - Int Ed. 2022;61(37). (IF=16.823)
42. Kato K, Hiroi T, Seto N, Ohtani S, Ogoshi T. Selective Gas Adsorption on Molecular Solids of a Hydrogen-bonding [3.3.3]Propellane. Chem Lett. 2022;51(9):975–7. (IF=1.715)
43. Kato K, Seto N, Chida K, Yoshii T, Mizuno M, Nishihara H, Ohtani S, Ogoshi T. Synthesis of Hexa-Aminated Trinaphtho[3.3.3]propellane and Its Porous Polymer Solids with Alkane Adsorption Properties. Bull Chem Soc Jpn. 2022;95(9):1296–302. (IF=5.121)
44. Kenry, Sun L, Yeo T, Middha E, Gao Y, Lim CT, Watanabe S, Liu B. In Situ Visualization of Dynamic Cellular Effects of Phospholipid Nanoparticles via High-Speed Scanning Ion Conductance Microscopy. Small. 2022;18(37). (IF=15.153)

45. Oinonen N, Kurki L, Ilin A, Foster AS. Molecule graph reconstruction from atomic force microscope images with machine learning. MRS Bull. 2022;47(9):895–905. (IF=4.882)
46. Yurtsever A, Wang PX, Priante F, Morais Jaques Y, Miyata K, MacLachlan MJ, Foster AS, Fukuma T. Probing the Structural Details of Chitin Nanocrystal–Water Interfaces by Three-Dimensional Atomic Force Microscopy. Small Methods. 2022;6(9). (IF=15.364)
47. Kajiwara K, Chen PK, Abe Y, Okuda S, Kon S, Adachi J, Tomonaga T, Fujita Y, Okada M. Src activation in lipid rafts confers epithelial cells with invasive potential to escape from apical extrusion during cell competition. Curr Biol. 2022;32(16):3460-3476.e6. (IF=10.9)
48. Bahador A, Yurtsever A, Amrin A, Kariya S, Umeda J, Shen J, Chen B, Fukuma T, Kondoh K. Room temperature and high-temperature properties of extruded Ti-4Fe-3W/2TiC composites in α+β and β phases. Mater Des. 2022;220. (IF=9.417)
49. Jing Y, Kobayashi M, Vu HT, Kasahara A, Chen X, Pham LT, Kurayoshi K, Tadokoro Y, Ueno M, Todo T, Nakada M, Hirao A. Therapeutic advantage of targeting lysosomal membrane integrity supported by lysophagy in malignant glioma. Cancer Sci. 2022;113(8):2716–26. (IF=6.518)
50. Sun K, Silveira OJ, Saito S, Sagisaka K, Yamaguchi S, Foster AS, Kawai S. Manipulation of Spin Polarization in Boron-Substituted Graphene Nanoribbons. ACS Nano. 2022;16(7):11244–50. (IF=18.027)
51. Szymkowiak JK, Walters CM, Hamad WY, MacLachlan MJ. Tuning the Properties of Chiral Nematic Mesoporous (Organo)silica Through Thiol-Ene Click Chemistry. Eur J Inorg Chem. 2022;2022(20). (IF=2.551)
52. Hirose D, Ogino K, Uematsu K, Maeda K. Enantioseparation on helical poly(diphenylacetylene)s bearing optically-active pendants: Effects of differences in higher-order structures of kinetically-trapped and thermodynamically-stable states on chiral recognition ability. J Chromatogr A. 2022;1675. (IF=4.601)
53. Kuromiya K, Aoki K, Ishibashi K, Yotabun M, Sekai M, Tanimura N, Iijima S, Ishikawa S, Kamasaki T, Akieda Y, Hino N, Fujita Y. Calcium sparks enhance the tissue fluidity within epithelial layers and promote apical extrusion of transformed cells. Cell Rep. 2022;40(2). (IF=9.995)
54. D'Acierno F, Bakrani K, Hamad WY, Michal CA, Maclachlan MJ. Tuning the Optical and Thermal Properties of Both Iridescent and Colorless Cellulose Nanocrystal Films. ACS Sustain Chem Eng. 2022;10(27):8715–24. (IF=9.224)
55. Ngo KX, Nguyen PDN, Furusho H, Miyata M, Shimonaka T, Bao Chau NN, Vinh NP, Nghia NA, Mohammed TO, Ichikawa T, Fukuma T, Quoc NB. Unraveling the Host-Selective Toxic Interaction of Cassiicolin with Lipid Membranes and Its Cytotoxicity. Phytopathology. 2022;112(7):1524–36. (IF=4.01)
56. Suzuki C, Nishiyama A, Arai S, Tange S, Tajima A, Tanimoto A, Fukuda K, Takumi Y, Kotani H, Takeuchi S, Omori K, Yano S. Inhibition of EGFR and MEK surmounts entrectinib resistance in a brain metastasis model of NTRK1-rearranged tumor cells. Cancer Sci. 2022;113(7):2323–35. (IF=6.518)
57. Ferdinandus N, Tan JR, Lim JH, Arai S, Sou K, Lee CK. Squaraine probes for the bimodal staining of lipid droplets and endoplasmic reticulum imaging in live cells. Analyst. 2022;147(15):3570–7. (IF=5.227)

58. Ferdinandus, Suzuki M, Vu CQ, Harada Y, Sarker SR, Ishiwata S, Kitaguchi T, Arai S. Modulation of Local Cellular Activities using a Photothermal Dye-Based Subcellular-Sized Heat Spot. ACS Nano. 2022;16(6):9004–18. (IF=18.027)
59. Echizen K, Taniguchi T, Nishimura T, Maeda K. Well-Controlled Living Polymerization of Phenylacetylenes in Water: Synthesis of Water-Soluble Stereoregular Telechelic Poly(phenylacetylene)s. Angew Chemie - Int Ed. 2022;61(26). (IF=16.823)
60. Sumikama T, Fukuma T, Canova FF, Gao DZ, Penedo M, Miyazawa K, Foster AS. Computed Three-Dimensional Atomic Force Microscopy Images of Biopolymers Using the Jarzynski Equality. J Phys Chem Lett. 2022;13(23):5365–71. (IF=6.888)
61. Sonoi R, Hagihara Y. Quantitative understanding of HepaRG cells during drug-induced intrahepatic cholestasis through changes in bile canaliculi dynamics. Pharmacol Res Perspect. 2022;10(3). (IF=2.963)
62. Okuda S, Sato K. Polarized interfacial tension induces collective migration of cells, as a cluster, in a 3D tissue. Biophys J. 2022;121(10):1856–67. (IF=3.699)
63. Momeni A, Hamad WY, MacLachlan MJ. Using rotation to organize cellulose nanocrystals inside a fiber. Nanoscale. 2022;14(20):7613–20. (IF=8.307)
64. Hirosawa K, Fukami T, Nagaoka M, Nakano M, Nakajima M. Methionine Sulfoxide Reductase A in Human and Mouse Tissues is Responsible for Sulindac Activation, Making a Larger Contribution than the Gut Microbiota[S]. Drug Metab Dispos. 2022;50(5):725–33. (IF=3.579)
65. Morikawa T, Fukami T, Gotoh-Saito S, Nakano M, Nakajima M. PPARα regulates the expression of human arylacetamide deacetylase involved in drug hydrolysis and lipid metabolism. Biochem Pharmacol. 2022;199. (IF=6.1)
66. Yamamoto D, Oshima H, Wang D, Takeda H, Kita K, Lei X, Nakayama M, Murakami K, Ohama T, Takemura H, Inaki N, Oshima M. Characterization of RNF43 frameshift mutations that drive Wnt ligand- and R-spondin-dependent colon cancer. J Pathol. 2022;257(1):39–52. (IF=9.883)
67. Biyani M, Yasuda K, Isogai Y, Okamoto Y, Weilin W, Kodera N, Flechsig H, Sakaki T, Nakajima M, Biyani M. Novel DNA Aptamer for CYP24A1 Inhibition with Enhanced Antiproliferative Activity in Cancer Cells. ACS Appl Mater Interfaces. 2022;14(16):18064–78. (IF=10.383)
68. Ito K, Taniguchi T, Nishimura T, Maeda K. Well-Controlled Living Polymerization of N-Propargylamides and Their Derivatives by Rhodium Catalysis. Angew Chemie - Int Ed. 2022;61(17). (IF=16.823)
69. Loos JN, D'Acierno F, Vijay Mody U, MacLachlan MJ. Manipulating the Self-Assembly of Multicomponent Low Molecular Weight Gelators (LMWGs) through Molecular Design. Chempluschem. 2022;87(4). (IF=3.21)
70. Saitou A, Hasegawa Y, Fujitani N, Ariki S, Uehara Y, Hashimoto U, Saito A, Kuronuma K, Matsumoto K, Chiba H, Chiba H, Takahashi M. N-glycosylation regulates MET processing and signaling. Cancer Sci. 2022;113(4):1292–304. (IF=6.518)
71. Hirose D, Nozaki M, Maruta M, Maeda K. Solvent-dependent helix inversion in optically active poly(diphenylacetylene)s and their chiral recognition abilities as chiral stationary phases for high-performance liquid chromatography. Chirality. 2022;34(4):597–608. (IF=2.183)

72. Alldritt B, Urtev F, Oinonen N, Aapro M, Kannala J, Liljeroth P, Foster AS. Automated tip functionalization via machine learning in scanning probe microscopy. Comput Phys Commun. 2022;273. (IF=4.717)
73. Vaneev AN, Gorelkin PV, Krasnovskaya OO, Akasov RA, Spector DV, Lopatukhina EV, Timoshenko RV, Garanina AS, Zhang Y, Salikhov SV, Korchev YE, Erofeev AS. In Vitro/ in Vivo Electrochemical Detection of Pt(II) Species. Anal Chem. 2022;94(12):4901–5. (IF=8.008)
74. Yamaguchi H, Kawahara H, Kodera N, Kumaki A, Tada Y, Tang Z, Sakai K, Ono K, Yamada M, Hanayama R. Extracellular Vesicles Contribute to the Metabolism of Transthyretin Amyloid in Hereditary Transthyretin Amyloidosis. Front Mol Biosci. 2022;9. (IF=6.113)
75. Onishi K, Ohtani S, Kato K, Fa S, Sakata Y, Akine S, Ogasawara M, Asakawa H, Nagano S, Takashima Y, Mizuno M, Ogoshi T. State- and water repellency-controllable molecular glass of pillar[5]arenes with fluoroalkyl groups by guest vapors. Chem Sci. 2022;13(14):4082–7. (IF=9.969)
76. Sakata Y, Chiba S, Akine S. Transient chirality inversion during racemization of a helical cobalt(III) complex. Proc Natl Acad Sci U S A. 2022;119(11). (IF=12.779)
77. Makarova MV, Akaishi Y, Suzuki Y, Rao KS, Yoshimura S, Saito H. High-resolution alternating magnetic force microscopy using an amorphous FeB-based tip driven by an inverse magnetostrictive effect: Imaging of the high-density magnetic recording media. J Magn Magn Mater. 2022;546. (IF=3.097)
78. Umeda K, Kobayashi K, Yamada H. Nanomechanics of self-assembled surfactants revealed by frequency-modulation atomic force microscopy. Nanoscale. 2022;14(12):4626–34. (IF=8.307)
79. Amyot R, Marchesi A, Franz CM, Casuso I, Flechsig H. Simulation atomic force microscopy for atomic reconstruction of biomolecular structures from resolution-limited experimental images. PLoS Comput Biol. 2022;18(3). (IF=4.779)
80. Tanabe M, Hosokawa K, Nguyen MAT, Nakagawa N, Maruyama K, Tsuji N, Urushihara R, Espinoza L, Elbadry MI, Mohiuddin M, Hirao A, Nakao S. The GPI-anchored protein CD109 protects hematopoietic progenitor cells from undergoing erythroid differentiation induced by TGF-β. Leukemia. 2022;36(3):847–55. (IF=12.897)
81. Sharma G, Kato Y, Hachisu A, Ishibashi K, Ninomiya K, Takahashi K, Hirata E, Kuroda K. Synthesis of a cellulose dissolving liquid zwitterion from general and low-cost reagents. Cellulose. 2022;29(5):3017–24. (IF=6.123)
82. Sakamoto S, Taniguchi T, Sakata Y, Akine S, Nishimura T, Maeda K. Synthesis of Pentaarylcyclobutenylrhodium(I) Complexes and Their Reactivity and Initiation Mechanism in Polymerization of Monosubstituted Acetylenes. Organometallics. 2022;41(4):472–9. (IF=3.837)
83. Tarrío JJ, Rodríguez R, Fernández B, Quiñoá E, Freire F. Dissymmetric Chiral Poly(diphenylacetylene)s: Secondary Structure Elucidation and Dynamic Luminescence. Angew Chemie - Int Ed. 2022;61(9). (IF=16.823)
84. Soto MA, Carta V, Cano MT, Andrews RJ, Patrick BO, MacLachlan MJ. Multiresponsive Cyclometalated Crown Ether Bearing a Platinum(II) Metal Center. Inorg Chem. 2022;61(7):2999–3006. (IF=5.436)
85. Kishi KE, Kim YS, Fukuda M, Inoue M, Kusakizako T, Wang PY, Ramakrishnan C, Byrne EFX, Thadhani E, Paggi JM, Deisseroth K, Kato HE. Structural basis for channel conduction in the pump-like channelrhodopsin ChRmine. Cell. 2022;185(4):672-689.e23. (IF=66.85)

86. Nakakubo K, Nishimura T, Biswas FB, Endo M, Wong KH, Mashio AS, Taniguchi T, Nishimura T, Maeda K, Hasegawa H. Speciation analysis of inorganic selenium in wastewater using a highly selective cellulose-based adsorbent via liquid electrode plasma optical emission spectrometry. J Hazard Mater. 2022;424. (IF=14.224)
87. Maity S, Trinco G, Buzón P, Anshari ZR, Kodera N, Ngo KX, Ando T, Slotboom DJ, Roos WH. High-speed atomic force microscopy reveals a three-state elevator mechanism in the citrate transporter CitS. Proc Natl Acad Sci U S A. 2022;119(6). (IF=12.779)
88. Kondo H, Mishiro K, Iwashima Y, Qiu Y, Kobayashi A, Lim K, Domoto T, Minamoto T, Ogawa K, Kunishima M, Hazawa M, Wong RW. Discovery of a Novel Aminocyclopropenone Compound That Inhibits BRD4-Driven Nucleoporin NUP210 Expression and Attenuates Colorectal Cancer Growth. Cells. 2022;11(3). (IF=7.666)
89. Xu YT, Li J, Maclachlan MJ. Stable graphene oxide hydrophobic photonic liquids. Nanoscale Horizons. 2022;7(2):185–91. (IF=11.684)
90. Nakano H, Hamaguchi T, Ikeda T, Watanabe-Nakayama T, Ono K, Yamada M. Inactivation of seeding activity of amyloid β-protein aggregates in vitro. J Neurochem. 2022;160(4):499–516. (IF=5.546)
91. Oinonen N, Xu C, Alldritt B, Canova FF, Urtev F, Cai S, Krejčí O, Kannala J, Liljeroth P, Foster AS. Electrostatic Discovery Atomic Force Microscopy. ACS Nano. 2022;16(1):89–97. (IF=18.027)
92. Makarova MV, Amano F, Nomura S, Tateishi C, Fukuma T, Takahashi Y, Korchev YE. Direct Electrochemical Visualization of the Orthogonal Charge Separation in Anatase Nanotube Photoanodes for Water Splitting. ACS Catal. 2022;12(2):1201–8. (IF=13.7)
93. Miyashita T, Senshu M, Ibi K, Yamanaka H, Nejishima H, Fukami T, Nakajima M. Evaluation of lens opacity due to inhibition of cholesterol biosynthesis using rat lens explant cultures. Toxicology. 2022;465. (IF=4.571)
94. Sivakumar A, Eniya P, Dhas SSJ, Kumar RS, Almansour AI, Sivashanmugan K, Sundar JK, Dhas SAMB. Shock wave induced phase transition from crystalline to the amorphous state of lead nitrate crystals. CrystEngComm. 2022;24(1):52–6. (IF=3.756)
95. Sona S, Hirose D, Kurihara Y, Maeda K. Thermally stable and rewritable circularly polarized luminescent helical poly(diphenylacetylene)s: stabilization of macromolecular helicity memory via reversible ion-pair formation. J Mater Chem C. 2022;11(4):1271–7. (IF=8.067)
96. Morita A, Nakayama M, Wang D, Murakami K, Oshima M. Frequent loss of metastatic ability in subclones of Apc, Kras, Tgfbr2, and Trp53 mutant intestinal tumor organoids. Cancer Sci. 2023;114(4):1437–50. (IF=6.518)
97. Wang D, Nguyen HG, Nakayama M, Oshima H, Sun L, Oshima M, Watanabe S. Mapping Nanomechanical Properties of Basal Surfaces in Metastatic Intestinal 3D Living Organoids with High-Speed Scanning Ion Conductance Microscopy. Small. 2023;19(9). (IF=15.153)
98. Wen H, Li J, Zhang Q, Inose T, Peeters W, Fortuni B, Asakawa H, Masuhara A, Hirai K, Toyouchi S, Fujita Y, Uji-I H. Length-Controllable Gold-Coated Silver Nanowire Probes for High AFM-TERS Scattering Activity. Nano Lett. 2022; 23: 4, 1615–1621. (IF=12.262)

99. Takumi Y, Arai S, Suzuki C, Fukuda K, Nishiyama A, Takeuchi S, Sato H, Matsumoto K, Sugio K, Yano S. MET kinase inhibitor reverses resistance to entrectinib induced by hepatocyte growth factor in tumors with NTRK1 or ROS1 rearrangements. Cancer Med. 2023;12(5):5809–20. (IF=4.711)
100. Yoshimura A, Yamada T, Serizawa M, Uehara H, Tanimura K, Okuma Y, Fukuda A, Watanabe S, Nishioka N, Takeda T, Takahashi T, Takayama K. High levels of AXL expression in untreated EGFR-mutated non-small cell lung cancer negatively impacts the use of osimertinib. Cancer Sci. 2023;114(2):606–18. (IF=6.518)
101. Hirose H, Maekawa M, Ida H, Kuriyama M, Takahashi Y, Futaki S. A noncanonical endocytic pathway is involved in the internalization of 3 μm polystyrene beads into HeLa cells. Biomater Sci. 2022;50. (IF=7.59)
102. Kato K, Ohtani S, Gon M, Tanaka K, Ogoshi T. Cyclic arrays of five pyrenes on one rim of a planar chiral pillar[5]arene. Chem Sci. 2022;75. (IF=9.969)
103. Sakai K, Sugano-Nakamura N, Mihara E, Rojas-Chaverra NM, Watanabe S, Sato H, Imamura R, Voon DCC, Sakai I, Yamasaki C, Takagi J, Matsumoto K. Designing receptor agonists with enhanced pharmacokinetics by grafting macrocyclic peptides into fragment crystallizable regions. Nat Biomed Eng. 2023;7(2):164–76. (IF=29.234)
104. D'Acierno F, Liu L, Nguyen TD, Michal CA, Palma-Dibb RG, Carvalho RM, MacLachlan MJ. Physical and mechanical properties of a dental resin adhesive containing hydrophobic chitin nanocrystals. Dent Mater. 2022;38(12):1855–65. (IF=5.687)
105. Lyu X, Imai S, Yamano T, Hanayama R. Preventing SARS-CoV-2 Infection Using Anti-spike Nanobody-IFN-β Conjugated Exosomes. Pharm Res. 2023;40(4):927-35. (IF=4.58)
106. Ide Y, Manabe Y, Inaba Y, Kinoshita Y, Pirillo J, Hijikata Y, Yoneda T, Shivakumar KI, Tanaka S, Asakawa H, Asakawa H, Inokuma Y. Determination of the critical chain length for macromolecular crystallization using structurally flexible polyketones. Chem Sci. 2022;13(34),9848-54. (IF=9.969)
107. Suzuki K, Nakano M, Nakashima S, Fukami T, Nakajima M. Activation of Inflammation by MCF-7 Cells-Derived Small Extracellular Vesicles (sEV): Comparison of Three Different Isolation Methods of sEV. Pharm Res. 2023;40(4),863-71. (IF=4.58)
108. Tang K, McLeod L, Livis T, West AC, Dawson R, Yu L, Balic JJ, Chonwerawong M, Wray-McCann G, Oshima H, Ferrero RL, Jenkins BJ. Toll-like Receptor 9 Promotes Initiation of Gastric Tumorigenesis by Augmenting Inflammation and Cellular Proliferation. Cell Mol Gastroenterol Hepatol. 2022;14(3):567–86. (IF=8.797)
109. Fa S, Tomita T, Wada K, Yasuhara K, Ohtani S, Kato K, Gon M, Tanaka K, Kakuta T, Yamagishi TA, Yamagishi TA, Ogoshi T. CPL on/off control of an assembled system by water soluble macrocyclic chiral sources with planar chirality. Chem Sci. 2022; 13(29),5846-53. (IF=9.969)
110. Shimizu M, Okamoto C, Umeda K, Watanabe S, Ando T, Kodera N. An ultrafast piezoelectric Z-scanner with a resonance frequency above 1.1 MHz for high-speed atomic force microscopy. Rev Sci Instrum. 2022;93(1). (IF=1.843)
111. Sakaguchi H, Tanimoto A, Sato S, Yanagimura N, Suzuki C, Takumi Y, Nishiyama A, Yamashita K, Takeuchi S, Ohtsubo K, Ohtsubo K, Yano S. Mediastinal Malignant Melanoma Markedly Shrinking in Response to Nivolumab. Intern Med. 2022;61(1):75–9. (IF=1.282)

112. Watanabe-Nakayama T, Ono K. Acquisition and processing of high-speed atomic force microscopy videos for single amyloid aggregate observation. Methods. 2022;197:4–12. (IF=4.647)
113. Makiyama K, Hazawa M, Kobayashi A, Lim K, Voon DC, Wong RW. NSP9 of SARS-CoV-2 attenuates nuclear transport by hampering nucleoporin 62 dynamics and functions in host cells. Biochem Biophys Res Commun. 2022;586:137–42. (IF=3.322)
114. Nagaoka M, Fukami T, Kisui F, Yamada T, Sakai Y, Tashiro K, Ogiso T, Konishi K, Honda S, Hirosawa K, Nakano M, Nakajima M. Arylacetamide deacetylase knockout mice are sensitive to ketoconazole-induced hepatotoxicity and adrenal insufficiency. Biochem Pharmacol. 2022;195. (IF=6.1)
115. Wang D, Sun L, Okuda S, Yamamoto D, Nakayama M, Oshima H, Saito H, Kouyama Y, Mimori K, Ando T, Watanabe S, Oshima M. Nano-scale physical properties characteristic to metastatic intestinal cancer cells identified by high-speed scanning ion conductance microscope. Biomaterials. 2022;280. (IF=15.394)

2. Review articles

116. Ando T. Functional Implications of Dynamic Structures of Intrinsically Disordered Proteins Revealed by High-Speed AFM Imaging. Biomolecules. 2022;12(12). (IF=6.064)
117. Akine S, Miyake H. Stimuli-responsive chirality inversion of metallohelices and related dynamic metal complexes. Coord Chem Rev. 2022;468. (IF=24.833)
118. Sakamoto R, Toyoda R, Jingyan G, Nishina Y, Kamiya K, Nishihara H, Ogoshi T. Coordination chemistry for innovative carbon-related materials. Coord Chem Rev. 2022;466. (IF=24.833)
119. Ohtani S, Kato K, Fa S, Ogoshi T. Host–Guest chemistry based on solid-state pillar[n]arenes. Coord Chem Rev. 2022;462. (IF=24.833)
120. Watanabe-Nakayama T, Ono K. Single-molecule observation of self-propagating amyloid fibrils. Microscopy. 2022;71(3):133–41. (IF=2.072)
121. Nakano M, Nakajima M. Adenosine-to-Inosine RNA Editing and N^6-Methyladenosine Modification Modulating Expression of Drug Metabolizing Enzymes. Drug Metab Dispos. 2022;50(5):624–33. (IF=3.579)
122. Kodera N, Ando T. Visualization of intrinsically disordered proteins by high-speed atomic force microscopy. Curr Opin Struct Biol. 2022;72,260–6. (IF=7.786)
123. Tadokoro Y, Hirao A. The Role of Nutrients in Maintaining Hematopoietic Stem Cells and Healthy Hematopoiesis for Life. Int J Mol Sci. 2022;23(3). (IF=6.208)
124. Kanno Y, Zhou Y, Fukuma T, Takahashi Y. Alkaline Phosphatase-based Electrochemical Analysis for Point-of-Care Testing. Electroanalysis. 2022;34(2):161–7. (IF=3.077)
125. Zhou Y, Sun L, Watanabe S, Ando T. Recent Advances in the Glass Pipet: from Fundament to Applications. Anal Chem. 2022;94(1):324–35. (IF=8.008)
126. Fukami T, Yokoi T, Nakajima M. Non-P450 Drug-Metabolizing Enzymes: Contribution to Drug Disposition, Toxicity, and Development. Vol. 62, Annual Review of Pharmacology and Toxicology. 2022. 405–425 p. (IF=16.459)

127. Nishiyama A, Hattori Y, Takeuchi S, Tanimoto A, Satouchi M, Murayama T, Yano S. Severe Skin Toxicity Caused by Sequential Anti-PD-1 Antibody and Alectinib in Non-small-cell Lung Cancer: A Report of Two Cases and a Literature Review. Intern Med. 2022;61(11):1735–8. (IF=1.282)
128. Kato K, Fa S, Ohtani S, Shi TH, Brouwer AM, Ogoshi T. Noncovalently bound and mechanically interlocked systems using pillar[n]arenes. Chem Soc Rev. 2022; 51(9),3648-87. (IF=60.615)

3. Proceedings.

None

4. Other English articles

129. Toyofuku M, Kikuchi Y, Taoka A. A Single Shot of Vesicles. Microbes Environ. 2022;37(6). (IF=2.596)

B. WPI-related papers

1. Original articles

130. Sugawara S, Okada R, Loo TM, Tanaka H, Miyata K, Chiba M, Kawasaki H, Katoh K, Kaji S, Maezawa Y, Hara E, Takahashi A. RNaseH2A downregulation drives inflammatory gene expression via genomic DNA fragmentation in senescent and cancer cells. Commun Biol. 2022;5(1). (IF=6.548)
131. Washino G, Soto MA, Wolff S, MacLachlan MJ. Preprogrammed assembly of supramolecular polymer networks via the controlled disassembly of a metastable rotaxane. Commun Chem. 2022;5(1). (IF=7.211)
132. Ranawat YS, Jaques YM, Foster AS. Generalised deep-learning workflow for the prediction of hydration layers over surfaces. J Mol Liq. 2022;367. (IF=6.633)
133. Wan J, Monteil CL, Taoka A, Ernie G, Park K, Amor M, Taylor-Cornejo E, Lefevre CT, Komeili A. McaA and McaB control the dynamic positioning of a bacterial magnetic organelle. Nat Commun. 2022;13(1). (IF=17.694)
134. Tanimura K, Yamada T, Okada K, Nakai K, Horinaka M, Katayama Y, Morimoto K, Ogura Y, Takeda T, Shiotsu S, Katayama R, Takayama K. HER3 activation contributes toward the emergence of ALK inhibitor-tolerant cells in ALK-rearranged lung cancer with mesenchymal features. npj Precis Oncol. 2022;6(1). (IF=10.123)
135. Mikami T, Matsumura S, Ichikawa R, Kato R, Uchida J, Nishimura T, Kato T. Bioinspired macromolecular templates for crystallographic orientation control of ZnO thin films through zinc hydroxide carbonate. Polym J. 2022;54(9):1119–26. (IF=3.135)
136. Dawson RE, Deswaerte V, West AC, Tang K, West AJ, Balic JJ, Gearing LJ, Saad MI, Yu L, Wu Y, Tan P, Jenkins BJ. STAT3-mediated upregulation of the AIM2 DNA sensor links innate immunity with cell migration to promote epithelial tumourigenesis. Gut. 2022;71(8):1515–31. (IF=31.795)
137. Nanjo S, Wu W, Karachaliou N, Blakely CM, Suzuki J, Chou YT, Ali SM, Kerr DL, Olivas VR, Shue J, Okimoto RA, Bivona TG. Deficiency of the splicing factor RBM10 limits EGFR inhibitor response in EGFR-mutant lung cancer. J Clin Invest. 2022;132(13). (IF=19.477)

138. Uramoto H, Takiguchi T, Koizumi T, Tanimoto A, Hayashi R, Nakazawa Y, Ito KI, Nakada M, Hirono Y, Nishino Y, Nishino Y, Yano S. Multi-institutional survey of malignant pleural mesothelioma patients in the Hokushin region. J Cancer Res Clin Oncol. 2022;148(5):1153–8. (IF=4.322)
139. Kasai S, Kuwayama N, Motoo Y, Kawashima A, Matsumoto K, Yano S, Matsushima K, Yasumoto K. Dual blockade of MET and VEGFR2 signaling pathways as a potential therapeutic maneuver for peritoneal carcinomatosis in scirrhous gastric cancer. Biochem Biophys Res Commun. 2022;600:80–6. (IF=3.322)
140. Harada Y, Sujino T, Miyamoto K, Nomura E, Yoshimatsu Y, Tanemoto S, Umeda S, Ono K, Mikami Y, Nakamoto N, Kubota Y, Kanai T. Intracellular metabolic adaptation of intraepithelial $CD4^{+}CD8\alpha\alpha^{+}$ T lymphocytes. iScience. 2022;25(4). (IF=6.107)
141. Kawase N, Sugihara A, Kajiwara K, Hiroshima M, Akamatsu K, Nada S, Matsumoto K, Ueda M, Okada M. SRC kinase activator CDCP1 promotes hepatocyte growth factor-induced cell migration/invasion of a subset of breast cancer cells. J Biol Chem. 2022;298(3). (IF=5.485)
142. Katayama Y, Yamada T, Tokuda S, Okura N, Nishioka N, Morimoto K, Tanimura K, Morimoto Y, Iwasaku M, Horinaka M, Yano S, Takayama K. Heterogeneity among tumors with acquired resistance to EGFR tyrosine kinase inhibitors harboring EGFR-T790M mutation in non-small cell lung cancer cells. Cancer Med. 2022;11(4):944–55. (IF=4.711)
143. Uramoto H, Takiguchi T, Koizumi T, Tanimoto A, Hayashi R, Nakazawa Y, Ito KI, Nakada M, Hirono Y, Nishino Y, Nishino Y, Yano S. Multi-institutional survey of thymic carcinoma patients in Hokushin region. J Cancer Res Clin Oncol. 2022;148(2):419–24. (IF=4.322)
144. Sahoo BR, Souders CL, Watanabe-Nakayama T, Deng Z, Linton H, Suladze S, Ivanova MI, Reif B, Ando T, Martyniuk CJ, Martyniuk CJ, Ramamoorthy A. Conformational Tuning of Amylin by Charged Styrene-Maleic-Acid Copolymers. J Mol Biol. 2022;434(2). (IF=6.151)
145. Nakatsumi H, Watanabe S, Gohara K, Kobayashi T, Takeda Y, Kasahara K, Yano S. Methicillin-resistant Staphylococcus aureus Necrotizing Bronchitis after Radiotherapy in Combination with Axitinib. Intern Med. 2022;61(19):2931–4. (IF=1.282)
146. Shibaki R, Kakikawa M. Different effects of magnetic field on drug activity in human uterine sarcoma cell lines MES-SA and MES-SA/Dx5. Electromagn Biol Med. 2022;41(3):343–51. (IF=1.932)
147. Ohta A, Tauchi Y, Hossain F, Sawada Y, Asakawa H, Asakawa T. Effect of Hydrophobic Chain Length on the Antioxidation Properties of Alanyl Tyrosine Dipeptide-type Surfactants. J Oleo Sci. 2022;71(2):215–22. (IF=1.628)
148. Okura E, Nishino Y, Sakashita K, Tanimoto A, Hayashi R, Yoshida Y, Nakada M, Koizumi T, Yano S, Nakazawa Y. Cancer among children, adolescents and young adults in the Hokushin region, Japan, between 2010 and 2015. Jpn J Clin Oncol. 2022;52(1):86–95. (IF=2.925)
149. Matsukawa T, Mizutani S, Matsumoto K, Kato Y, Yoshihara M, Kajiyama H, Shibata K. Placental leucine aminopeptidase as a potential specific urine biomarker for invasive ovarian cancer. J Clin Med. 2022;11(1). (IF=4.964)

2. Review articles

150. Yoshihara M, Mizutani S, Matsumoto K, Kato Y, Masuo Y, Tano S, Mizutani H, Kotani T, Mizutani E, Shibata K, Shibata K, Kajiyama H. Crosstalk between foetal vasoactive peptide hormones and placental

aminopeptidases regulates placental blood flow: Its significance in preeclampsia. Placenta. 2022; 121:32–9. (IF=3.287)

151. Liabeuf D, Oshima M, Stange DE, Sigal M. Stem Cells, Helicobacter pylori, and Mutational Landscape: Utility of Preclinical Models to Understand Carcinogenesis and to Direct Management of Gastric Cancer. Gastroenterology. 2022;162(4):1067–87. (IF=33.883)
152. Taniguchi T. Substituent Effects of Tetracoordinate Boron in Organic Synthesis. Chem - A Eur J. 2022;28(19). (IF=5.02)

3. Proceedings

None

4. Other English articles

None

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

- List up to 10 main presentations during FY 2022 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
2023/2/22	Atsushi Hirao	Cell fate determination mediated by nutrient-derived metabolites in malignant progression.	2023 Normal/Malignant Hematopoiesis and Novel Therapies for Hematologic Malignancies Symposium
2022/11/15	Mark J. MacLachlan	New Photonic Materials Inspired by Nature	2022 Annual Meeting of the Entomological Society of America
2022/11/1	Mikihiro Shibata	Oligomeric states of microbial rhodopsins in lipid bilayer determined by high-speed atomic force microscopy	19th International Conference on Retinal Proteins
2022/10/31	Katsuhiro Maeda	Visualization of Chirality and Enantiomeric Excess of Chiral Amines Using Helical Poly(diphenylacetylene)s	Molecular Chirality Asia 2022
2022/10/18	Ayumi Sumino	Dynamics of scorpion toxin binding to the K+ channel: single-molecule study using high-speed atomic force microscopy (HS-AFM)	2022 East Asian Single-Molecule Biophysics Symposium (EASMB 2022)
2022/8/31	Shigehisa Akine	Ion recognition by metallo-molecular cages and macrocycles with open/close feature	44th International Conference on Coordination Chemistry
2022/7/20	Carsten Beta	How Cortical Wave Patterns Shape Cellular Function	Gordon Research Conference on Oscillations and Dynamic Instabilities in Chemical Systems
2022/7/6	Toshio Ando	ATP energy usage in biomolecular machinery	Gordon Research Conference on single molecule approaches to biology
2022/5/30	Adam S. Foster	Machine Learning in SPM	Nano in Bio 2022
2022/4/11	Masanobu Oshima	Modeling polyclonal metastasis of colon cancer by organoids	115th Annual Meeting of American Association for Cancer Research

3. Major Awards

- List up to 10 main awards received during FY 2022 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
2023/3/15	Toshio Ando	Toray Science and Technology Prize
2022/11/22	Akine Shigehisa	ICPAC KK 2022 Lecture Award (Kuala Lumpur, MALAYSIA)
2022/11/3	Kunio Matsumoto	Kanazawa City Cultural Award
2022/10/18	Yasufumi Takahashi	Masao Horiba Award
2022/9/16	Keisuke Miyazawa	Canon Anelva Prize at IVC-22 for Young Researcher
2022/9/2	Kenichi Umeda	Young Investigator Award, AFM BioMed Conference 2022, Nagoya-Okazaki, Japan
2022/6/25	Satoru Okuda	2021 Seguchi Prize, Bioengineering Division, Japan Society of Mechanical Engineers
2022/6/4	Yoko Sakata	SHGSC Japan Award of Excellence 2022
2022/4/20	Yoko Sakata	The Young Scientists' Award, The Commendation for Science and Technology by the MEXT
2022/4/20	Satoshi Toda	The Young Scientists' Award, The Commendation for Science and Technology by the MEXT

Appendix 2　FY 2022 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 FY2022> **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	46	Nano Life Science Institute, Kanazawa University	Doctor of Engineering, Electrical engineering, Nanometrology	90	October, 2017	usually stays at the institute	
Noriyuki Kodera	44	Nano Life Science Institute, Kanazawa University	Doctor of Science, Biophysics and Nano-Bioscience	90	April, 2022	usually stays at the institute	
Yuri Korchev	62	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, stays 10 days and 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	59	Nano Life Science Institute, Kanazawa University	Doctor of Medicine, Stem Cell Biology	90	October, 2017	usually stays at the institute	
Masanobu Oshima	61	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	57	University Hospital, Kanazawa University	MD, PhD, Medical Oncology, Circumvention of targeted drug resistance	50	October, 2017	usually stays at the institute	
Kunio Matsumoto	64	Cancer Research Institute, Kanazawa University	Doctor of Philosophy, Biological Chemistry, Tumor Biology	50	October, 2017	usually stays at the institute	
Rikinari Hanayama	48	Nano Life Science Institute, Kanazawa University	MD, PhD, Immunology, Cell Biology	90	October, 2017	usually stays at the institute	
Richard W. Wong	48	Nano Life Science Institute, Kanazawa University	Doctor of Medicine, Molecular cell biology	90	October, 2017	usually stays at the institute	
Miki Nakajima	53	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	50	Nano Life Science Institute, Kanazawa University	Doctor of Science, Supramolecular chemistry, Coordination chemistry	90	October, 2017	usually stays at the institute	
Katsuhiro Maeda	52	Nano Life Science Institute, Kanazawa University	Doctor of Engineering, Polymer chemistry	90	October, 2017	usually stays at the institute	
Tomoki Ogoshi	46	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	49	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	-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	47	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	-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
Carsten Beta	48	Biological Physics Group, University of Potsdam	Doctor of Natural Sciences, Biophysics, Pattern formation	20	April, 2022	Under contract, stays at the institute 30 days or more/per fiscal year	-Promotes understanding of the real image from the observation image while working toward the development of new nanoprobe technology -In charge of Selection Committee of Jr.PI -In charge of the 7th NanoLSI International Symposium which will be held in FY2023

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

Principal investigators unable to participate in project in FY 2022

Name	Affiliation (Position title, department, organization)	Starting date of project participation	Reasons	Measures taken
Alexander S. Mikhailov	Department of Physical Chemistry, Fritz Haber Institute of the Max Planck Society	October, 2017	Professor Mikhailov had physical difficulties in traveling to Japan, because of his advanced age.	Professor Carsten Beta (University of Potsdam, Germany) became an overseas PI from April 2022.
Toshio Ando	Nano Life Science Institute, Kanazawa University	October, 2017	Professor Ando was awarded the honorary position of Distinguished Professor, Kanazawa University.	Professor Kodera replaced him as a PI from April 2022 for generational shift to cover the later half of the WPI grand period and beyond.

Appendix 2a Biographical Sketch of a New Principal Investigator

(within 3 pages per person)

Name (Age) Noriyuki Kodera* (44)

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

Professor, WPI Nano Life Science Institute, Kanazawa University

Academic degree and specialty

Doctor of Philosophy in Science, Specialty: Biophysics and Nano-Bioscience

Effort **90%**

* 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)

Mar. 2001 B.Sc. Department of Physics, Faculty of Science, Kanazawa University

Mar. 2003 M.Sc. Division of Mathematical and Physical Sciences, Graduate School of Natural Science and Technology, Kanazawa University

Sep. 2005 Ph.D. Division of Basic Sciences, Graduate School of Natural Science and Technology, Kanazawa University

(Research)

Apr. 2005 – Mar. 2007 Research Fellow (DC2 & PD), The Japan Society for Promotion of Science

Apr. 2007 – Mar. 2010 Postdoctoral Research Fellow, JST-CREST

Apr. 2010 – Sep. 2010 Assistant Professor, Sch. Math. & Phys., Col. Sci. & Eng. Kanazawa University

Oct. 2010 – Jul. 2011 Assistant Professor, Bio-AFM Frontier Research Center, Kanazawa University

Aug. 2011 – Sep. 2017 Associate Professor, Bio-AFM Frontier Research Center, Kanazawa University

Oct. 2017 – Mar. 2018 Associate Professor, WPI-Nano Life Science Institute, Kanazawa University

Apr. 2018 – Present Professor, WPI- Nano Life Science Institute, Kanazawa University

Achievements and highlights of past research activities

1. Development of high-speed atomic force microscope (HS-AFM): I contributed to establish the technical basis for the current HS-AFM (***PNAS*** 2001, citation 846; ***Rev. Sci. Instrum***. 2005, citation 162; ***Rev. Sci. Instrum.*** 2006, citation 149), by which the structural dynamics of proteins can be observed at the sub-second level for the first time (***Annu. Rev. Biophys.*** 2013, citation 190).

2. Biological application studies by HS-AFM: By observing proteins at work with HS-AFM, the functional mechanisms of proteins were deeply understood. Notably, walking myosin V (***Nature*** 2010, citation 644), antibody-antigen interactions (***Nat. Commun.*** 2014, citation 84), cofilin-induced unidirectional cooperative structural changes in actin filaments (***eLife*** 2015, citation 94), DNA cleavage by CRISPR-Cas9 (***Nat. Commun.*** 2017, citation 117) and CRISPR-Cas3 (***Nat. Commun.*** 2022, citation 2), Na^+-induced structural transition of the bacterial flagellar motor protein (***Sci. Adv.*** 2017, citation 39), pooling of the translational GTPases around the ribosomal P-stalk (***PNAS*** 2020, citation 42), and structural dynamics of intrinsically disordered proteins (***Nat. Nanotech.*** 2021, citation 41) were directly visualized. In addition, I established the experimental protocols for HS-AFM (***Nat. Protoc.***

2012, citation 191).

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:

2019 – Present Board member of the Biophysical Society of Japan

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

(Invited talks)

1. Recent progress in high-speed atomic force microscopy technologies, AFM BioMed Conference 2022, Okazaki, Sep 1, 2022
2. Improving the temporal resolution of high-speed AFM, 8th Multifrequency AFM Conference, Online, Oct 29, 2020
3. Direct observation of proteins at work by high-speed atomic force microscopy, Nano In Bio 2016 Le Gosier, France, May 31, 2016
4. Intrinsically disordered proteins studied by high-speed atomic force microscopy, XV. Annual Linz Winter Workshop, Linz, Austria, Feb. 15, 2013
5. Video imaging of walking myosin V by high-speed atomic force microscopy, AFM BioMed Conference, Paris, France, Aug. 24, 2011

d) Editor of an international academic journal

None

e) Peer reviewer for an overseas competitive research program (etc.)

None

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

1. JSPS/MEXT Grants-in-Aid for Scientific Research Grant-in-Aid for Scientific Research (A) #20H00327: Development of the next-generation high-speed AFM and detailed dynamic behavior analysis of biomolecules, Apr. 1, 2020 – Mar. 31, 2024, 44,590,000 JPY, PI
2. JSPS/MEXT Grant-in-Aid for Scientific Research (S) #18H05269: Uncovering the secrets of lipid-transporting ABC proteins, Jun. 11, 2018 – Mar. 31, 2023, 27, 400,000JPY, Co-Investigator
3. JST CREST #JPMJCR1762: Development of Biomolecular 4-dimensional Structure Analysis Methods by Data Assimilation of High-speed Atomic Force Microscopy Single Molecule Measurements, Oct. 2018, Mar. 2023, Takada S.(PI), Kodera N.(GL,51,540,000JPY), Tochio H.(GL), Matsunaga Y.(GL)
4. JSPS/MEXT Grant-in-Aid for Challenging Research (Exploratory) # 17K19345 Direct observation of strain-dependent dynamic behaviors of biomolecules by high-speed AFM, Jun. 30, 2017 – Mar. 31, 2020, 6,500,000 JPY, PI

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

1. Ando T., Kodera N., Takai E., Maruyama D., Saito K., Toda A. A high-speed atomic force microscope for studying biological macromolecules, 2001, ***Proc Natl Acad Sci USA***., 98, 22, citation **794**
2. Kodera, N. Yamashita H., Ando T. Active damping of the scanner for high-speed atomic force microscopy, 2005, ***Rev. Sci. Instrum.,*** 76, 5, 53708, citation **157**
3. Kodera N., Sakashita M., Ando T. Dynamic proportional-integral-differential controller for high-speed atomic force microscopy, 2006, ***Rev. Sci. Instrum.,*** 77, 8, 83704, citation **157**
4. Kodera N., Yamamoto D., Ishikawa R., Ando T. Video imaging of walking myosin v by high-speed atomic force microscopy, 2010, ***Nature***, 468, 7320, citation **586**
5. Uchihashi T., Kodera N., Ando T. Guide to video recording of structure dynamics and dynamic processes of proteins by high-speed atomic force microscopy, 2012, ***Nat. Protoc.,*** 7, 6, citation **177**
6. Ando T., Uchihashi T., Kodera N. High-speed AFM and applications to biomolecular systems, 2013, ***Annu. Rev. Biophys.***, 42, 1, citation **183**
7. Preiner J., Kodera N., Tang Jilin., Ebner A., Brameshuber M., Blaas D., Gelbmann N., Gruber H. J., Ando T., Hinterdorfer, Peter. IgGs are made for walking on bacterial and viral surfaces, 2014, ***Nat Commun.*** 5,4394, citation **81**
8. Ngo K.X., Kodera N., Katayama E., Ando T., Uyeda T.Q. Cofilin-induced unidirectional cooperative conformational changes in actin filaments revealed by high-speed atomic force microscopy, 2015, ***eLife***, 4, e04806, citation **85**
9. Shibata M., Nishimasu H., Kodera N., Hirano S., Ando T., Uchihashi T., Nureki O. Real-space and real-Time dynamics of CRISPR-Cas9 visualized by high-speed atomic force microscopy,2017, ***Nat. Commun.***, 8, 1, 1430, citation **119**
10. Terahara N., Kodera N., Uchihashi T., Ando T., Namba K., Minamino T. Na+-induced structural transition of MotPS for stator assembly of the Bacillus flagellar motor, 2017, ***Sci. Adv.***, 3, 11, eaao4119, citation **30**
11. Imai H., Uchiumi T., Kodera N., Direct visualization of translational GTPase factor pool formed around the archaeal ribosomal P-stalk by high-speed AFM, 2020, ***Proc Natl Acad Sci USA***, 117, 51, citation **14**
12. Kodera N., et al. Structural and dynamics analysis of intrinsically disordered proteins by high-speed atomic force microscopy, 2021, ***Nat. Nanotech.***, 16, 2, citation **31**
13. Umeda K., Okamoto C., Shimizu M., Watanabe S., Ando T., Kodera N. Architecture of zero-latency ultrafast amplitude detector for high-speed atomic force microscopy, 2021, ***Appl. Phys. Lett.*** 119, 18, 181602, citation **3**
14. Shimizu M., Okamoto C., Umeda K., Watanabe S., Ando T., Kodera N. An ultrafast piezoelectric Z-scanner with a resonance frequency above 1.1 MHz for high-speed atomic force microscopy, 2022, ***Rev. Sci. Instrum.,*** 93, 1, 13701, citation **4**

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

1. Feb. 7, 2018, The 14th JSPS Prize, Japan Society for the Promotion of Science

Appendix 2a Biographical Sketch of a New Principal Investigator

(within 3 pages per person)

Name (Age) Carsten Beta * (48)

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:

Chair of the Section "Dynamics and Statistical Physics" of the German Physical Society (since 03/2023)

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

Gordon Research Conference on Oscillations and Instabilities in Chemical Systems 2024 (conference chair)

International Conference Engineering of Chemical Complexity 2019 (conference chair)

Annual Physics of Cancer Symposium 2017 (co-organizer)

(Selected invited talks)

1. How cortical wave patterns shape cellular function, GRC on Oscillations and Instabilities in Chemical Systems, Stonehill College, USA, July 20, 2022
2. 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
3. Dynamical wave patterns in the cortex of motile amoeboid cells, Conference on Advances in Pattern Formation, Sede Boqer, Israel, Feb. 18, 2019
4. A bacterial swimmer with decisional freedom between two alternative movement strategies, Workshop on Stochastic Dynamics, Buenos Aires, Argentina, Mar. 20, 2017
5. 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

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. Yochelis, A., Flemming, S., and Beta, C. (2022). Versatile patterns in the actin cortex of motile cells: Self-organized pulses can coexist with macropinocytic ring-shaped waves. ***Phys. Rev. Lett.***, 129: 088101.
2. Flemming, S., Font, F., Alonso, S., and Beta, C. (2020). How cortical waves drive fission of motile cells. ***PNAS***, 117:6330-6338., citation 19.
3. Alirezaeizanjani, Z., Großmann, R., Pfeifer, V., Hintsche, M., Beta, C. (2020). Chemotaxis strategies of bacteria with multiple run modes. ***Sci. Adv.***, 6:eaaz6153., citation 15
4. 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
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 44
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 83
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 41
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 2022 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.

Fostering and securing of next-generation researchers

The graduate school, "Division of Nano Life Science," which is managed in an integrated manner with NanoLSI, is a notable organization for the long-term continuation of NanoLSI. It guarantees the harmony and synergy between NanoLSI as an independent university research institute and its involvement in education and nurturing future generations of NanoLSI researchers. Further, it vitalizes the research environment through the participation of doctoral course students in NanoLSI activities. The overview and features of the Division of Nano Life Science and the privileges given to the Division of Nano Life Science from the above perspectives are described below.

Brief Overview

The graduate school, "Division of Nano Life Science," fosters excellent graduate students who will establish the novel research field, "Nano Probe Life Science," on the basis of world-level research by NanoLSI. The supervisors for the Division are all world-class researchers of NanoLSI. Key features of the Division are:

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

Current Status

The Division of Nano Life Science started in FY2020 with 12 students of the Master's Level Section of Integrated Course (7 domestic and 5 foreign students from 3 countries) and 10 students of the Doctoral Level Section of Integrated Course (2 domestic and 8 foreign students from 4 countries). As of April 2023, it consists of 24 students of the Master's Level Section of Integrated Course (13 domestic and 11 foreign students from 7 countries) and 32 students of the Doctoral Level Section of Integrated Course (10 domestic and 22 foreign students from 8 countries). To promote interdisciplinary research in the Division of Nano Life Science, all 21 full-time researchers of NanoLSI who have an educational assignment are engaged as educators in the Division of Nano Life Science. In addition, overseas PIs give intensive courses in their own research fields during their stays at NanoLSI. Two mentors selected from different research fields supervise each student as dual mentors. Interdisciplinary research training courses are to be set up along with the NanoLSI research activities such as the Bio-AFM Summer School and the Transdisciplinary Research Promotion Grants. Graduate students in the Division, including the Master's Level Section students, participate in the research of NanoLSI PIs as research assistants. In addition, in order to ensure the operational autonomy of the Division: (1) a NanoLSI professor is assigned to the Head of Division of Nano Life Science, (2) the Administrative Director of NanoLSI supports the Head and acts as the manager of the Division, and (3) the NanoLSI administrative office is in charge of managing the Division in cooperation with the Student Affairs Department of the University headquarters.

Financial support

- Students of the Master's Level Section of Integrated Course of the Division of Nano Life Science are provided with support of 130,000 yen/month (breakdown: 50,000 yen for scholarship + 80,000 yen for

RA salary) and those of the Doctoral Level Section receive 180,000 yen/month (breakdown: 100,000 yen for scholarship + 80,000 yen for RA salary), using the endowments of Kanazawa University.
- Students of the Division of Nano Life Science selected for one of the three graduate school education programs of Kanazawa University (i.e. WISE, FS, and SPRING; all of which are implemented with financial support from government agencies such as MEXT), who are aiming for interdisciplinary research and researcher development across the disciplinary boundaries of each graduate school, are provided with a scholarship of 180,000 yen/month along with a small RA salary and research expenses of 400,000 yen/year. In addition, enrollment and tuition fees are exempted. However, students who have won one of the above-mentioned graduate education program scholarships (see also below) will not be provided with scholarship support from the endowments of Kanazawa University.
WISE: WISE Program for Precision Medicine, Science and Technology
FS: KU Doctoral Fellowship Project for Science and Technology
SPRING: Fellowship Program for Fostering Top Scientists in Fused Disciplines
- Scholarships will be available to all students enrolled in Integrated Course of the Division of Nano Life Science from the endowments of Kanazawa University, from the three graduate education program scholarships, or from external scholarships such as the Government-supported scholarship system for foreign students and JSPS Research Fellowship for Young Scientists. In addition, measures will be taken to provide enrollment fee exemption and full or half tuition fee exemption as far as possible.

Career path after graduation

As of April, 2023, the first two students (both Japanese) have graduated from the Doctoral Level Section of Integrated Course, Division of Nano Life Science. They obtained positions as researchers at a private company (Sumitomo Chemical Co., Ltd., Kureha Co., Ltd.). It is expected that three more students will graduate as of October, 2023. All three are foreign, wishing to get researcher positions at an academic institution such as a university. A follow-up survey of their career paths will be conducted.

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 Sustainability, Tsukuba University		Established the collaborative research agreement in June 2019
National Institutes for Quantum Science and Technology (QST), Institute of Quantum Life Science		Established the collaborative research agreement in June 2021

2. Holding international research meetings

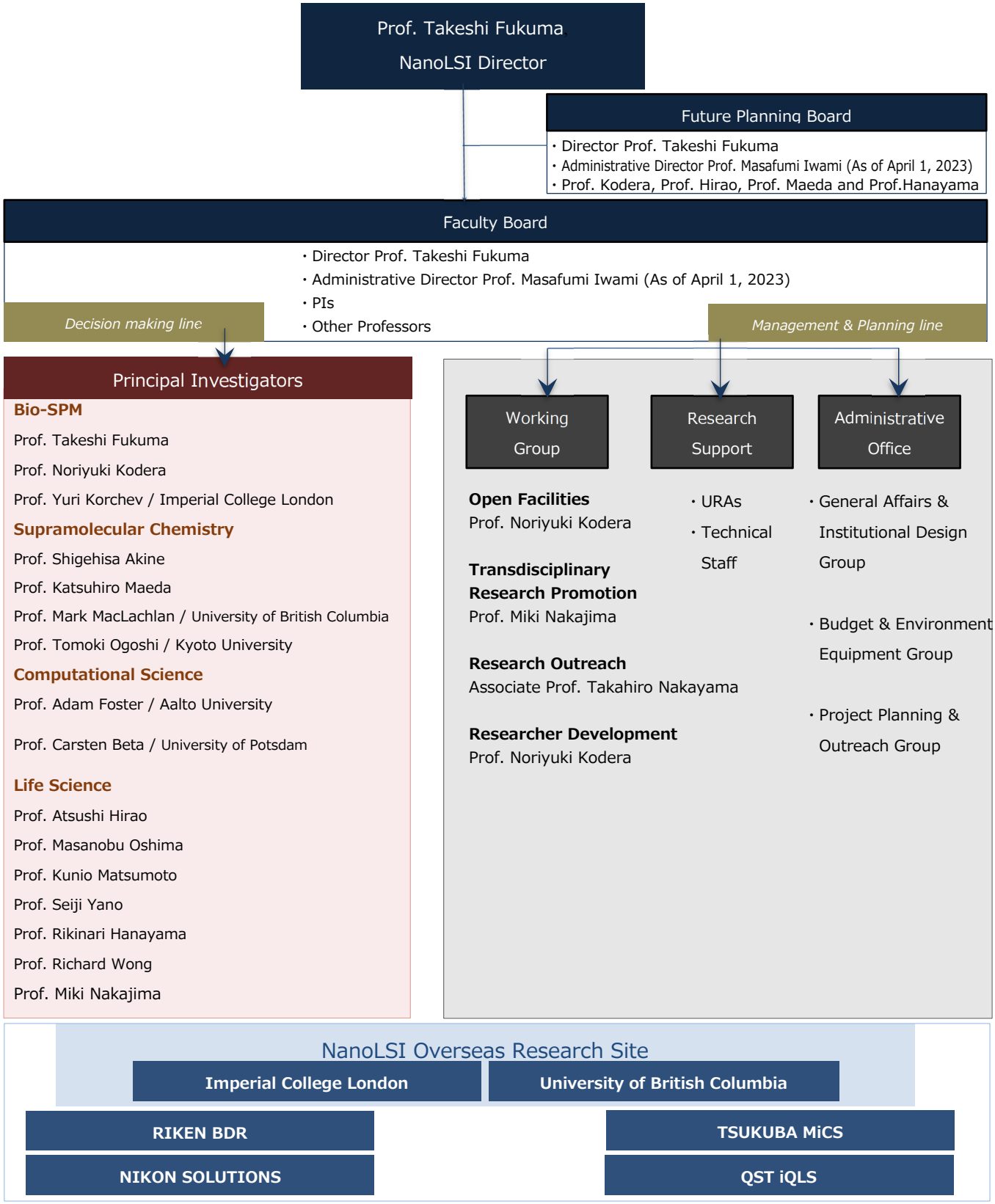
- Indicate the number of international research conferences or symposiums held in FY2022 and give up to three examples of the most representative ones using the table below.

FY 2022: 6 meetings

Major examples (meeting titles and places held)	Number of participants
6th NanoLSI Symposium – Nanoprobe Technology for Understanding Molecular Systems – The ANA Crowne Plaza Hotel Kanazawa, Kanazawa	From domestic institutions: 134 From overseas institutions: 22
Workshop on Computational Biophysics of Atomic Force Microscopy – A Lecture Course Approach Nano Life Science Institute, Kanazawa University, Kanazawa	From domestic institutions: 61 From overseas institutions: 48
Engineering Mechanics of Cell and Tissue Morphogenesis 2022 Nano Life Science Institute, Kanazawa University	From domestic institutions: 54 From overseas institutions: 9

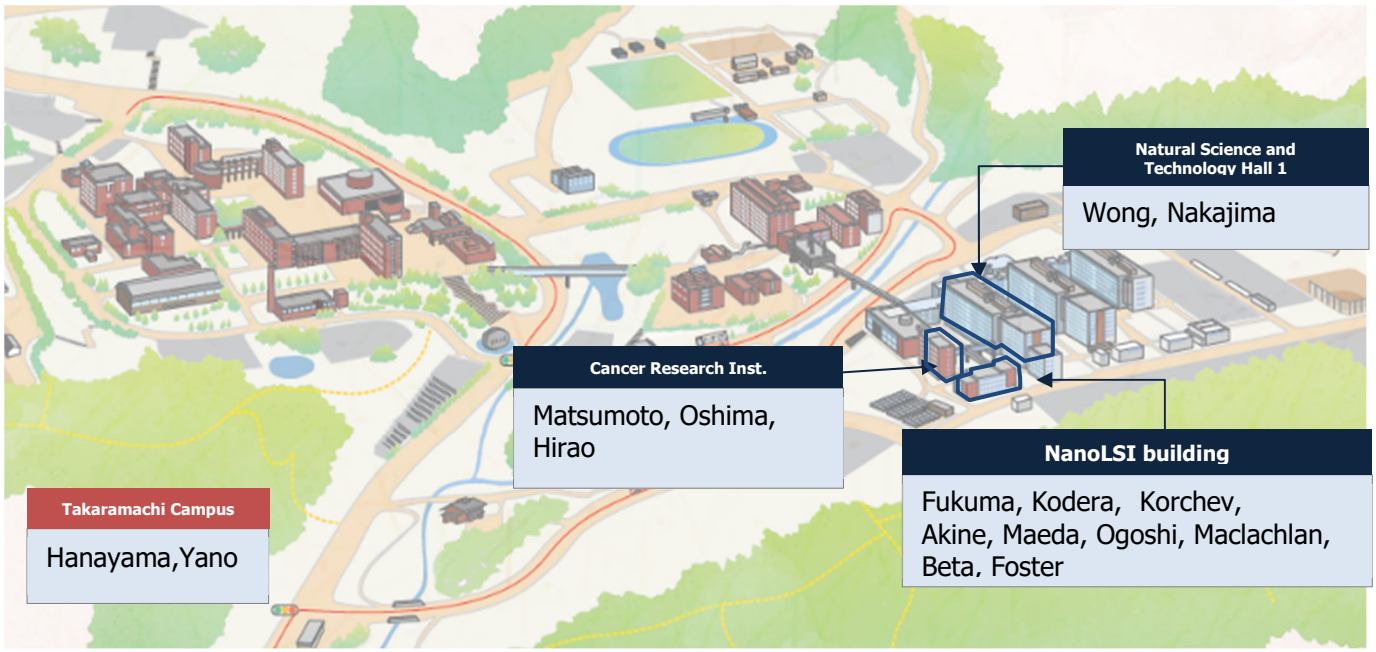
3. Diagram of management system

- 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 building and in other buildings.

5. Securing external research funding*

External research funding secured in FY2022

NanoLSI External Research Funding Secured in FY2022 by type of funding source CONFIDENTIAL

※Including Indirect funding

■Grants-in-Aid for Scientific Research	PI		Jr.PI		Full time researcher		Associated researcher		Total	
	number	amount(JPY)	number	amount(JPY)	number	amount(JPY)	number	amount(JPY)	number	amount(JPY)
Grant-in-Aid for Specially Promoted Research	1	16,380,000							1	16,380,000
Grant-in-Aid for Transformative Research Area(A)	5	33,800,000	4	64,844,000	1	3,380,000	1	5,460,000	11	107,484,000
Grant-in-Aid for Scientific Research (S)	1	6,500,000							1	6,500,000
Grant-in-Aid for Scientific Research (A)	7	72,800,000	2	9,300,000	3	10,848,284	3	1,430,000	15	94,378,284
Grant-in-Aid for Scientific Research (B)	7	20,280,000	6	15,981,000	8	20,280,000	7	19,760,000	28	76,301,000
Grant-in-Aid for Scientific Research (C)	2	325,000	1	260,000	9	14,736,490	12	16,467,420	24	31,788,910
Challenging Research (Pioneering)	1	11,556,358							1	11,556,358
Challenging Research (Exploratory)	6	15,380,952	3	6,258,008	4	9,850,000	5	3,770,000	18	35,258,960
Early-Career Scientists					6	10,212,424	1	3,380,000	7	13,592,424
Grant-in-Aid for Research Activity Start-up					2	2,982,248			2	2,982,248
Grant-in-Aid for Scientific Research on Innovative Areas					1	4,680,000			1	4,680,000
Fund for the Promotion of Joint International Research (Fostering Joint International Research (A))			1	815,763					1	815,763
Fund for the Promotion of Joint International Research (Fostering Joint International Research (B))	1	130,000	1	1,820,000	1	4,182	2	8,405,299	5	10,359,481
Total	31	177,152,310	18	99,278,771	35	76,973,628	31	58,672,719	115	412,077,428
■Commissioned research projects										
AMED Practical Research for Innovative Cancer Control	3	24,580,000					1	16,900,000	4	41,480,000
AMED Advanced Research & Development Programs for Medical Innovation	1	29,900,000							1	29,900,000
AMED Project for Cancer Research and Therapeutic Evolution	2	45,500,000					1	1,950,000	3	47,450,000
AMED Science and Technology Platform Program for Advanced Biological Medicine	1	57,005,000							1	57,005,000
AMED Research on Development of New Drugs	1	1,950,000							1	1,950,000
AMED Research Center Network for Realization of Regenerative Medicine			2	39,442,000					2	39,442,000
AMED Research Program on Hepatitis							2	69,069,000	2	69,069,000
AMED Strategic Research Program for Brain Sciences					1	2,600,000			1	2,600,000
AMED Advanced Research & Development Programs for Medical Innovation (AMED-CREST)	1	5,850,000			1	738,156			2	6,588,156
AMED Cyclic Innovation for Clinical Empowerment	1	2,470,000							1	2,470,000
JST Strategic Basic Research Programs (CREST)	3	104,273,144	1	24,236,863	1	2,600,000			5	131,110,007
JST Strategic Basic Research Programs (PRESTO SAKIGAKE)			1	10,962,221	2	16,713,824	1	13,000,000	4	40,676,045
JST Strategic Basic Research Programs (ACT-X)							1	1,950,000	1	1,950,000
JST Fusion Oriented Research for disruptive Science and Technology			1	7,643,376	1	6,488,442			2	14,131,818
NEDO Research and Development Initiative for Scientific Innovation of New Generation Batteries3					1	15,000,000			1	15,000,000
Others	2	15,135,823	1	2,080,000			11	16,580,046	14	33,795,869
Total	15	286,663,967	6	84,364,460	7	44,140,422	17	119,449,046	45	534,617,895
■Joint research projects										
Joint research projects(New)	13	24,692,616	2	9,000,000	6	10,795,000	2	57,000,000	23	101,487,616
Joint research projects(Carry-forward)	7	26,065,705			1	760,000			8	26,825,705
Total	20	50,758,321	2	9,000,000	7	11,555,000	2	57,000,000	31	128,313,321
■Donations										
Donations(New)	25	4,450,000	12	14,450,000	18	54,705,070	32	9,187,666	87	82,792,736
Donations(Carry-forward)	25	134,401,762	12	15,301,524	18	20,659,773	32	28,459,785	87	198,822,844
Total	50	138,851,762	24	29,751,524	36	75,364,843	64	37,647,451	174	281,615,580

Securing external research funding

	PI		Jr.PI		Full time researcher		Associated researcher		Total	
Total	109	653,426,360	50	222,394,755	84	208,033,893	114	272,769,216	357	1,356,624,224

- 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 2022 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 2022	Final goal (Date: March, 2027)
Researchers from within the host institution	12	11	11
Researchers invited from overseas	4	4	4
Researchers invited from other Japanese institutions	0	1	1
Total principal investigators	16	16	16

b) Total members

	At the beginning of project		At the end of FY 2022		Final goal (Date: March, 2027)	
	Number of persons	%	Number of persons	%	Number of persons	%
Researchers	49		82		85	
Overseas researchers	8	16	28	34	34	40
Female researchers	6	12	16	20	19	22
Principal investigators	16		16		16	
Overseas PIs	5	31	5	31	5	31
Female PIs	1	6	1	6	2	13
Other researchers	27		38		38	
Overseas researchers	1	4	2	5	3	8
Female researchers	5	19	8	21	10	26
Postdocs	6		28		31	
Overseas postdocs	2	33	21	75	26	84
Female postdocs	0	0	7	25	7	23
Research support staffs	8		32		32	
Administrative staffs	13		18		18	
Total number of people who form the "core" of the research center	70		132		135	

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" column may be changed to coincide with the project's actual content.

(Million yens)

Cost items	Details (For Personnel - Equipment please fill in the breakdown of fiscal expenditure, and the income breakdown for Research projects.)	Total costs	Amount covered by WPI funding
Personnel	Center director and administrative director	35.3	19.2
	Principal investigators (no. of persons):11	172.2	38.2
	Associate Principal investigators (no. of persons):1	6.0	6.0
	Junior Principal investigators (no. of persons):6	67.5	58.8
	Other researchers (no. of persons):64	261.4	176.9
	Research support staff (no. of persons):14	55.2	55.2
	Administrative staff (no. of persons):16	109.1	57.6
	Remuneration for RA(Research Assistant)	8.7	8.7
	Labor insurance premium adjustment amount	0.0	0.0
	Subtotal	715.4	420.6
Project activities	Gratuities and honoraria paid to invited principal investigators (no. of persons):3	5.0	5.0
	Research startup cost (no. of persons):10	25.8	25.8
	Cost of satellite organizations (no. of satellite organizations):2	23.9	23.8
	Cost of international symposiums (no. of symposiums):1	10.5	10.5
	Facility expenses	25.7	3.3
	Cost of consumables	15.0	15.0
	Cost of utilities	30.1	28.6
	Other costs	45.2	44.3
	Subtotal	181.2	156.3
Travel	Domestic travel costs	5.6	5.6
	Overseas travel costs	2.4	2.4
	Travel and accommodations cost for invited scientists		
	(no. of domestic scientists):0	0	0
	(no. of overseas scientists):14	10.0	10.0
	Travel cost for scientists on transfer		
	(no. of domestic scientists):1	0.2	0.2
	(no. of overseas scientists):4	1.6	1.6
	Subtotal	19.8	19.8
Equipment	Depreciation of buildings	1.5	1.5
	Depreciation of equipment	168.2	168.2
	Subtotal	169.7	169.7
Research projects (Detail items must be fixed)	Project supported by other government subsidies, etc. *1	110.6	0
	KAKENHI	233.8	0
	Commissioned research projects, etc.	282.6	0
	Joint research projects	48.2	0
	Ohers (donations, etc.)	45.6	0
	Subtotal	720.8	0
Total		1,806.9	766.4

Costs (Million yens)

WPI grant in FY 2022	700
Costs of establishing and maintaining facilities	8.2
Establishing new facilities	1.5
Repairing facilities	3.4
Others	3.3
Costs of equipment procured	168.2
Scanning Electron Microscopy System 1set	60.0
Fluorescence microscope 1set	15.0
Others	93.2

*1. Management Expenses Grants (including Mission Realization Acceleration Expenses (ミッション実現加速化経費), 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.

*1 運営費交付金(ミッション実現加速化経費を含む)、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

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):2		
	Subtotal	18.3	18.3
Project activities	Subtotal	2.3	2.3
Travel	Subtotal	0	0
Equipment	Subtotal	0	0
Research projects	Subtotal	0	0
Total		20.6	20.6

Appendix 4 FY 2022 Status of Collaboration with Overseas Satellites

1. Coauthored Papers

- List the refereed papers published in FY 2022 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. 2023 and not described in Appendix 1.

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

1) Liu F, Gledhill M, Tan QG, Zhu K, Zhang Q, Salaün P, Tagliabue A, Zhang Y, Weiss D, Achterberg EP, Achterberg EP, *Korchev Y.* (Appendix 1 #35) Phycosphere pH of unicellular nano- and micro- phytoplankton cells and consequences for iron speciation. ISME J. 2022;16(10):2329–36.(IF=11.217)

2) Vaneev AN, Gorelkin PV, Krasnovskaya OO, Akasov RA, Spector DV, Lopatukhina EV, Timoshenko RV, Garanina AS, Zhang Y, Salikhov SV, *Korchev YE*, Erofeev AS. (Appendix 1 #73) In Vitro/ in Vivo Electrochemical Detection of Pt(II) Species. Anal Chem. 2022;94(12):4901–5.(IF=8.008)

3)(Appendix 1 #92) Makarova MV, Amano F, Nomura S, Tateishi C, Fukuma T, Takahashi Y, *Korchev YE*. Direct Electrochemical Visualization of the Orthogonal Charge Separation in Anatase Nanotube Photoanodes for Water Splitting. ACS Catal. 2022;12(2):1201–8.(IF=13.7)

Overseas Satellite 2 University of British Columbia (Total: 3 papers)

1) (Appendix 1 #31) Yurtsever A, Wang PX, Priante F, Jaques YM, Miyazawa K, *MacLachlan MJ*, Foster AS, Fukuma T. Molecular insights on the crystalline cellulose-water interfaces via three-dimensional atomic force microscopy. Sci Adv. 2022;8(41).(IF=14.98)

2) Chaudhry MT, Patrick BO, Akine S, *MacLachlan MJ*. (Appendix 1 #32) Noncooperative guest binding by metal-free [2 + 2] Schiff-base macrocycles. Org Biomol Chem. 2022;20(42):8259–68.(IF=3.89)

3) (Appendix 1 #46) Yurtsever A, Wang PX, Priante F, Morais Jaques Y, Miyata K, *MacLachlan MJ*, Foster AS, Fukuma T. Probing the Structural Details of Chitin Nanocrystal–Water Interfaces by Three-Dimensional Atomic Force Microscopy. Small Methods. 2022;6(9).(IF=15.364)

2. Status of Researcher Exchanges

- Using the below tables, indicate the number and length of researcher exchanges in FY 2022. 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
FY2022	0	0	0	0	0
	0	0	1	0	1

<From satellite>

	Under 1 week	From 1 week to 1 month	From 1 month to 3 months	3 months or longer	Total
FY2022	0	1	0	0	1
	0	0	0	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
FY2022	0	0	0	0	0
	0	0	0	0	0

<From satellite>

	Under 1 week	From 1 week to 1 month	From 1 month to 3 months	3 months or longer	Total
FY2022	0	2	0	0	2
	0	0	0	0	0

Appendix 5　FY 2022 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: 39

	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	Eroles Navarro Mar	29	PhD student / National Institute of Health and Medical Research	France	PhD student / Biophysics	•JSPS Postdoctoral Fellowship for North American and European Researchers (Short-Term) (2021)	2022/1/7 -5/8	Short-term stay for JSPS Postdoctoral Fellowship Program for Research in Japan
2	Amyot Romain Felix Emile	29	Researcher, Adhesion and Inflammation Lab (LAI)	France	PhD / Biophysics	BioAFMviewer: An interactive interface for simulated AFM scanning of biomolecular structures and dynamics; PLoS Comput Biol, 16(11), e1008444 (Nov 2020)	2022/10/1 - 2024/9/30	JSPS Postdoctoral Fellowship for Research in Japan (Standard)
3	Moustafa Abdelsamed Moustafa Sakr	47	Assistant professor / Genetic Engineering and Biotechnology institute (GEBRI), University of Sadat	Egypt	Molecular diagnostics and theraputics	The correlation between P53 and COX-2 expression and the pathological alteration in hepatocellular carcinoma; Egyptian Journal of Medical Human Genetics, 23(1) (Feb 2022)	2022/6/1 -12/1	Participation as cooperate researcher
4	Sankar Prasad RATH	50	Professor / Indian Institute of Technology Kanpur	India	PhD / Chemistry	• SERB - Science and Technology Award for Research (SERB-STAR), 2020. • JSPS Invitational Fellowships, Japan, 2020 • Professor R S Varma Memorial Award by Indian Chemical Society, 2020 • C. N. R. Rao National Prize in Chemical Sciences, 2018	2022/6/24	Participation as a speaker in NanoLSI Open Seminar
5	Plaza Garcia Abadillo Ismael	27	Postdoctoral researcher / IMEDIA Nanociencia	Spain	PhD / Optical and Magnetic Tweezers	DNA replication machinery: Insights from in vitro single-molecule approaches; Computational and Structural Biotechnology Journal, 19, 2057-69 (2021)	2022/7/5 -10/5	Short-term stay for joint research
6	Lou Yuting	33	Research Fellow / Mechanobiology Institute, National University of Singapore	Singapore	Doctor of Engineering / Physical, theoretical and computational biology	Interfacial friction and substrate deformation mediate long-range signal propagation in tissues; Biomechanics and Modeling in Mechanobiology 21(3), 1-20 (2022)	2022/7/25 -7/29	Participation in academic seminar
7	U. Valentin NÄ GERL	51	Professor / University of Bordeaux	France	PhD / Neuroscience	Getting sharper: the brain under the spotlight of super-resolution microscopy; Trends in Cell Biology, 33(2), 148-161 (Feb 2023)	2022/8/5	Participation as a speaker in NanoLSI Open Seminar
8	Setareh Sharifi Panah	33	PhD student / University of Potsdam	Germany	PhD student / Biophysics	Biohybrid active matter -- the emergent properties of cell-mediated microtransport; Physical Review Applied, 18(034014) (Sep 2022)	2022/8/22 -9/2	Participation in 10th Bio-SPM Summer School
9	Holuigue Hatice Zohra Michele	26	PhD student / University of Milan, Departement of Physics	Italy	PhD student・Phys2BioMed Early Stage Researcher / Applied Physics	Force Sensing on Cells and Tissues by Atomic Force Microscopy; Sensors 22 (6), 2197 (2022)	2022/9/5 -9/30	Short-term stay for joint research
10	Adam Foster	47	NanoLSI PI / Professor, Aalto University, Department of Applied Physics	Finland	PhD Applied Physics / Surfaces and Interfaces at the Nanoscale	•Väisälä Prize; Surfaces and Interfaces at the Nanoscale (Jan 2009)	①2022/9/11 -9/23 ②2023/2/2 -2/14	①Participation as a speaker in NanoLSI workshop ②Participation as principal investigator
11	Niko Oinonen	28	Doctoral Candidate, Aalto University	Finland	Applied Physics / Surfaces and Interfaces at the Nanoscale	Molecule graph reconstruction from atomic force microscope images with machine learning; MRS Bulletin, 47, 895–905 (Sep 2022)	①2022/9/19 -9/21 ③2023/2/2 -2/14	①Participation as a speaker in NanoLSI workshop ②Participation as a speaker in NanoLSI workshop
12	Orlando Silveira Jr.	32	Postdoctoral Researcher, Aalto University	Finland	Doctoral degree, Natural Sciences, Universidade Federal de Minas Gerais (UFMG)	Manipulation of Spin Polarization in Boron-Substituted Graphene Nanoribbons; ACS Nano, 16(7), 11244-11250 (Jun 2022)	2022/9/19 -9/21	Participation as a speaker in NanoLSI workshop
13	Fabio Priante	28	PhD Graduate Student, Aalto University	Finland	PhD Graduate Student / Aalto University School of Science and Technology	Molecular insights on the crystalline cellulose-water interfaces via three-dimensional atomic force microscopy; SCIENCE ADVANCES, 8(41) (Oct 202)	①2022/9/19 -9/21 ②2023/2/2 -2/14	①Participation as a speaker in NanoLSI workshop ②Participation in NanoLSI workshop
14	Lauri Kurki	25	PhD Graduate Student, Aalto University	Finland	PhD Graduate Student / Aalto University	Water Dimer-Driven DNA Base Superstructure with Mismatched Hydrogen Bonding; J. Am. Chem. Soc. 144(44), 20227–20231 (Nov 2022)	2022/9/19 -9/21	Participation in NanoLSI workshop
15	Witchukorn Phuthong	36	Assistant Proffessor, Kasetsart University, Department of Physics, Faculty of Science	Thailand	PhD / Physics	Bed of Nails Effect in Microneedles: The Finite Element Analysis; The 2nd International Conference for Students in Science and Innovation (Oct 2021)	2022/9/19 -9/21	Participation in NanoLSI workshop
16	Joohyun Lee	32	Graduate student (M.S/Ph.D combined course), Sungkyunkwan University School of Medicine	South Korea	Graduate student (M.S/Ph.D combined course) / Sungkyunkwan University School of Medicine	High resolution cryo-EM structure of the Methanocaldococcus jannaschii small-heat shock protein; The 3rd International Online Conference on Crystals (Jan 2022)	2022/9/19 -9/21	Participation in NanoLSI workshop
17	Nguyen Thanh Hoa	39	Lecturer, Department of Microbiology, Hanoi University of Science and Technology	Vietnam	PhD / Biochemistry - Molecular Biology	Candida albicans Bgl2p, Ecm33p, and Als1p proteins are involved in adhesion to saliva-coated hydroxyapatite; J Oral Microbiol, 13(1), 1879497 (2021)	2022/9/19 -9/21	Participation in NanoLSI workshop
18	Beta Carsten	48	NanoLSI PI / Professor, Institute of Physics and Astronomy, University of Potsdam	Germany	Doctor of Natural Sciences / Biophysics	Controlling turbulence in a surface chemical reaction by time-delay autosynchronization; Physical Review E 67 (4), 046224 (2003)	①2022/9/30 -10/19 ②2023/3/3 - 3/17	①Participation as principal investigator ②Participation as principal investigator
19	Borja Irarra Urruela	50	Assistant research professor, IMDEA Nanociencia	Spain	PhD in Molecular Biology / Biophysics	DNA synthesis determines the binding mode of the human mitochondrial single-stranded DNA-binding protein; Nucleic Acids Res 45 (12), 7237-7248 (2017)	2022/10/3 -10/28	Short-term stay for Visiting Fellows Program
20	Diego Carlero Carnero	30	Researcher, Universidad Autónoma de Madrid	Spain	Biochemistry and Molecular biology	Caracterización del ciclo viral de adenovirus de lagarto y serpiente; Revista Complutense de Ciencias Veterinarias, 9 (2) (July 2015)	2022/10/11 -11/10	Short-term stay for Visiting Fellows Program
21	Koji Itahana	56	Associate Professor, Duke-National University of Singapore (Duke-NUS)	Singapore	PhD / Biophysics	Redox-dependent AMPK inactivation disrupts metabolic adaptation to glucose starvation in xCT-overexpressing cancer cells; J Cell Sci.135:15:jcs259090 (2022)	2022/10/13 -10/14	Participation as a speaker in International Symposium of the Institute Network for Biomedical Sciences
22	Ong Sin Tiong	58	Associate Professor, Duke-National University of Singapore (Duke-NUS)	Singapore	MBChB / Medical Oncology	Therapy Resistance and Disease Progression in CML: Mechanistic Links and Therapeutic Strategies; Curr Hematol Malig Rep 17(6), 181–97 (Dec 2022)	2022/10/13 -10/14	Participation as a speaker in International Symposium of the Institute Network for Biomedical Sciences

23	Enrico Petretto	49	Associate Professor, Duke-National University of Singapore (Duke-NUS)	Singapore	PhD in Biochemistry / Biochemistry	MT-HESS: an efficient Bayesian approach for simultaneous association detection in OMICS datasets, with application to eQTL mapping in multiple tissues; Bioinformatics, pii: btv568 (Oct 2015)	2022/10/13 -10/14	Participation as a speaker in International Symposium of the Institute Network for Biomedical Sciences
24	Ting Wei Liao	30	PhD student, Johns Hopkins University	USA	PhD student / Biophysics	Unravelling the nucleation mechanism of bimetallic nanoparticles with composition-tunable core–shell arrangement; Nanoscale 10 (14), 6684-6694 (2018)	2022/10/19 -11/2	Short-term stay for for learning technical skills
25	Bocanegra Rojo Rebeca	43	Research staff, IMDEA Nanociencia	Spain	PhD / Nanobiomedicine	DNA replication machinery: Insights from in vitro single-molecule approaches; Computational and Structural Biotechnology Journal, 19, 2057-69 (2021)	2022/11/2 -12/2	Short-term stay for Visiting Fellows Program
26	Ricardo Garcia	62	Professor, Instituto de Ciencia de Materiales de Madrid (CSIC), Nanoscience and Nanotechnology	Spain	PhD in Physics / Nanoscience and Nanotechnology	•the Nanotechnology Recognition Award by the American Vacuum Society (2016). •Awarded an ERC Advanced grant (2013) •Technological Innovation Prize (1st), Fundación Madri+d, Madrid Regional Government (2009) •Fellow American Physical Society (Material Physics), American Physical Society. (2007)	2022/11/14 -11/15	Participation as a speaker in 6th NanoLSI symposium
27	Robert Goldman	83	Professor Emeritus, Northwestern University, Feinberg School of Medicine, Department of Cell and Developmental Biology	USA	PhD in Biology / Cell and Developmental Biology	•Doctor of Medical Sciences, honoris causa, Charles University, Prague (2018) •Elected as a foreign member, Finnish Society for Sciences and Letters (2015)	2022/11/14 -11/15	Participation as a speaker in 6th NanoLSI symposium
28	Ohad Medalia	52	Professor, University of Zurich, Departmrnt of Biochemistry	Switzerland	PhD / Biochemistry	•The Alon fellowship (2004) •The MPIB Junior Research Award (2003) •The Rothschild Fellowship (2001) •The Elchanan E. Bondi Memorial Prize for Ph. D. students. (2001) •The European Commission Individual Fellowship (2001) •The Lev Margulis Young Investigators Award of Merit (2000)	2022/11/14 -11/15	Participation as a speaker in 6th NanoLSI symposium
29	Ralf Metzler	54	Professor, University of Potsdam, Institute of Physics & Astronomy, Chair for Theoretical Physics	Germany	PhD / Theoretical Physics	•Feodor Lynen Fellow, Alexander von Humboldt Foundation •Amos de Shalit Fellow, Minerva Foundation •Emmy Noether Fellow, Deutsche Forschungsgemeinschaft •Canada Research Chair in Biological Physics •Finland Distinguished Professor (FiDiPro)	2022/11/14 -11/15	Participation as a speaker in 6th NanoLSI symposium
30	Tsvi Tlusty	53	Distinguished Professor, Ulsan National Institute of Science & Technology (UNIST), Center for Soft and Living Matter, Physics Department	South Korea	PhD in Physics / Physics	•The Morris L. Levinson Prize in Physics (2006)	2022/11/14 -11/15	Participation as a speaker in 6th NanoLSI symposium
31	Oleg Matusovskiy	43	Staff Research Associate, Department of Kinesiology and Physical Education, McGill University	Canada	PhD in Biochemistry /Biochemistry	•Speaker prize award at the Groupe de Recherche Axé sur la Structure des Protéines (GRASP) Symposium (2017) •Travel Fellowships for International Conférences: The Fonds de recherche du Québec Santé (FRSQ), Meakins-Christie Laboratories, McGill University (2012-2016) •Award of Eastern Branch of Russian Academy of Sciences (2011) •INTAS Travel Fellowship, INTAS Conference, Tomsk, Russia (2007) •Award of Science Support Foundation for "Best Ph.D. student of Russian Academy of Science" (2005)	2022/12/5 -12/21	Bio-SPM Collaborative Research Program
32	Mark J. MacLachlan	50	NanoLSI PI / Department of Chemistry, Faculty of Science, The University of British Columbia	Canada	PhD / Materials Chemistry	•Award for Research Excellence in Materials Chemistry (Canadian Society for Chemistry) (2016) •Tier 1 Canada Research Chair in Supramolecular Materials (2015-2022) •Steacie Prize for Natural Sciences (E.W.R. Steacie Memorial Fund) (2014) •Elected Fellow of the Royal Society of Canada (FRSC) (2014) •Rutherford Memorial Medal (Royal Society of Canada) (2013) •Killam Research Prize (UBC) (2011)	①2023/1/16 -1/27 ②2023/3/6 - 3/24	①Participation as principal investigator ②Participation as principal investigator
33	Le Thi Phuong Ngan	31	Researcher, Center for Bioscience and Biotechnology, University of Science, Vietnam National University, Ho Chi Minh City	Vietnam	PhD student / Microbiology, Biotechnology	Influence of N-terminal His-tags on the production of recombinant proteins in the cytoplasm of Bacillus subtilis, Biotechnology Reports 35: e00754. (2022)	2023/2/2 - 2/28	Bio-SPM Collaborative Research Program
34	David Gao		Nanolayers Research Computing Ltd.	UK	PhD / Physics	A mechanism for Frenkel defect creation in amorphous SiO2 facilitated by electron injection (Nov 2016)	2023/2/6 - 2/14	Participation as a speaker in NanoLSI workshop
35	Filippo Federici Canova		Nanolayers Research Computing Ltd.	UK	PhD / Computational Physics	Computed Three-Dimensional Atomic Force Microscopy Images of Biopolymers Using the Jarzynski Equality (June 2022)	2023/2/6 - 2/14	Participation as a speaker in NanoLSI workshop
36	Mingbo Qu	39	Associate Professor, School of Bioengineering, Dalian University of Technology	China	PhD / Biochemical Engineering	The National Natural Science Foundation of China (2022-2025), No. 32170501	2023/3/2 - 3/28	Bio-SPM Collaborative Research Program
37	Alessio Carmignani	33	PhD student, The BioRobotics Institute of Sant'Anna School of Advanced Studies	Italy	PhD student / Molecular Biotechnology	In Vitro and Ex Vivo Investigation of the Effects of Polydopamine Nanoparticle Size on Their Antioxidant and Photothermal Properties: Implications for Biomedical Applications, ACS Appl. Nano Mater, 5, 1702-1713 (Jan 2022)	2023/3/8 - 6/5	Collaborative Research
38	Pedro Gonçalves	36	Assistant professor / the Department of Cell Biology and Anatomy, National Cheng Kung University	Taiwan	PhD / Molecular Pathology and Genetics	Conflict, Competition, and Cooperation Regulate Social Interactions in Filamentous Fungi (2020)	2023/3/15 - 3/16	Participation as a speaker in a seminar
39	Yuri E. Korchev	62	NanoLSI PI / Department of Medicine, Imperial College London,UK	UK	PhD / Biophysics	The role of urea in neuronal degeneration and sensitization: an in vitro model of uremic neuropathy, Molecular Pain, 15 (2019)	2023/3/20 - 3/31	Participation as principal investigator

Appendix 6 FY2022 State of Outreach Activities

* Fill in the numbers of activities and times held during FY2022 by each activity.
* Describe the outreach activities in the "6. Others" of Progress Report, including those stated below that warrant special mention.

Activities	FY2022 (number of activities, times held)
PR brochure, pamphlet	*NanoLSI Leaflet (3) (JP,EN) *WPI Pamphlet (1) (EN&JP) *Nature Index (1)（EN） (Total: 5)
Lectures, seminars for general public	*NanoLSI Open Seminar (16) *Kanazawa University Open Lecture（1 by Hirao） (Total:17)
Teaching, experiments, training for elementary, secondary and high school students	*NanoLSI Research Exchange for Kanazawa Izumigaoka high school (Presentation Workshop) (7/13) *Cancer research Early Exposure Program(8/1-8/5) *Rigakuno-Hiroba（Hands-on Science Seminar for High School Students）(8/8)(Konno, Nakayama) *Lectures for Kanazawa University Global Science Campus (9/23) (Nakayama) *Ogaki Higashi Senior High School of Gifu Prefecture(11/16)(Taoka) (Total: 5)
Open houses	*NanoLSI Open House for Kanazawa University Senior High School (facility tour) (7/21) *NanoLSI Open House for Nanao High School (facility tour and discussions with researchers) (8/19) *NanoLSI facility tour for JSPS Nanoprobe Technology 167(11/12) *NanoLSI facility tour for JST Fusion Oriented REsearch for disruptive Science and Technology program "Jihatsutekina-Yugonoba" (2/21) *Visits by MEXT, other ministries (or national organizations), and university officials （4/25,6/8,6/11,6/13,7/8,9/28,10/11,10/19,10/28,11/17,12/20,12/27,2/13,2/13,2/16,2/24,3/3,3/15,3/15） (Total: 23)
Participating, exhibiting in events	*11th WPI Science Symposium（11/23） *An exhibition booth at the Annexed Exhibition of The 45th Annual Meeting of the Molecular Biology Society of Japan(11/30-12/2) (Total: 2)
Press releases	Nakajima, Okuda, Foster, Foster/Fukuma, Arai, Oshima/Fukuma, Flechsig, Kodera, Matsumoto, Asakawa, MacLachlan/Foster/Fukuma(2), Hanayama/Ando/Wong(2), Miyanari, Oshima, Akine, Shibata(2), Takahashi/Korchev/Fukuma, Fukuma (Total: 21)
Publications of the popular science books	Monthly JuniorAERA, April 2023 enlarged number (WPI featured article Vol. 3)（Kodera）
Others（TV program, podcast, technical book）	*TV program (2) NHK Kanazawa "Open the future! Ishikawa's Forefront of Science", NHK BS1 "Open the future! Ishikawa's Forefront of Science" *Podcast (17) (1 by Beta, 1 by Ando, 1 by Shibata, 1 by Akine, 2 by Flechsig, 1 by Hanayama, 1 by Biyani, 1 by Sato, 1 by Ichikawa, 1 by Arai, 2 by Fukuma, 1 by Yurtsever/Fukuma, 1 by Matsumoto, 1 by Wong, 1 by Kien) (Total: 19)

*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 2022 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.

NanoLSI Outreach Strategy

At the beginning of the launch of the centre, NanoLSI defined a strategy for outreach activities during the WPI grant period. In line with this strategy, we have been progressively dividing our activities into those for the research community and those for the general public, depending on the target audience to be reached.

In the first half of the period, with the enhance the research system as the main focus, activities such as Open Facility Programmes for the research community (Bio-AFM Summer School, Bio-SPM Collaborative Research, Visiting Fellows Programme, et al.) were promoted as the core activities. In the second half of the period, in addition to these, activities for the general public have also been strategically implemented to brand the centre.

While there are methods of conducting activities for the general public that target a wide range of people and are carried out frequently, we have concentrated on activities that focus on raising the quality of the participants' experience in each activity, intending to create a long-lasting word-of-mouth effect.

Specifically, as a world-class research centre located in a regional city, we concentrated on two programmes: a research presentation by Kanazawa Izumigaoka High School, an SSH-designated school, and Cancer research early exposure programme. These activities aim to excite interest in research among young local residents, especially talented high school students who could play a central role in scientific research in the future.

Kanazawa Izumigaoka High School Research Presentation

The research exchange at the Kanazawa Izumigaoka High School research presentations was organised in response to a request for cooperation from the Ishikawa Prefectural Board of Education, which had noted the high effectiveness and influence of the WPI Science Symposium last year, and wanted to see similar initiatives continued as much as possible.

The event was held under arrangements that enabled rich research exchange, with 16 scientists, including overseas researchers, in charge of providing feedback on presentations in English by 38 students on eight themes.

In addition to 47 students and staff from Izumigaoka High School participating on-site, the event was distributed on-demand to 40 students from the high school, their parents, the Ishikawa Prefectural Board of Education and teachers from SSH-designated schools nationwide on a limited subscriber basis.

Due to the positive response to the event, discussions are currently underway on how to continue the event in the following year and beyond.

Cancer Research Early Exposure Programme

The programme began its activities in FY2021 with a crowdfunding initiative in collaboration with Cancer Research Institute, Kanazawa University. The crowdfunding raised over JPY 3.14 million from 156 donors to cover the costs of implementing the programme, and also received support from a company operating events for high school students, a medical equipment trading company and the Japanese branch of a major pharmaceutical company.

The first programme was held in August 2022 under the title 'From Nano to Metre in Cancer Research'. During the first four days, 11 courses of the research experience programme were held, in which students actually conducted experiments in the laboratories of supervising researchers, and on the fifth and final day, three classroom sessions were held on the "Cutting edge and future of life sciences and medical research".

The research experience programme was very intensive, with a maximum of about three students participating in each course and about two researchers providing guidance, emphasising mutual communication. The class sessions were also highly interactive, with the primary objective of discussion with the students rather than one-way lectures. The implementation method was also devised to make the content highly interactive, for example, by walking between the students seated in a circle and conducting a question-and-answer session.

In a questionnaire conducted after the event, the programme received high marks for its exceptional care and attention to detail, while issues such as variations in the difficulty level of the experiments were also identified. These are summarised in the report for crowdfunding donors.

Regarding the burden on researchers, which is a concern when implementing such programmes, the high level of interaction with the students attracted the intellectual interest of the researchers, and there were no complaints about the heavy burden. Rather, positive feedback was received that the high school students' deep interest in basic research was stimulating.

Short-term results include the fact that one of the participants compiled a statement of purpose based on her research experience in this programme and went on to Brown University in the USA with the support

of the Tadashi Yanai Foundation, and that the implementation of this programme led to the establishment of the Satoshi Wada Cancer Fund for cancer-related education and human resource development, among others.

Appendix 7 FY 2022 List of Project's Media Coverage

* List and describe media coverage (e.g., articles published, programs aired) in FY2022.

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

	Date	Types of Media (e.g., newspaper, magazine, television)	Description
1	2022.4.3	Newspaper(1)	Award: Prof. Yasufumi Takahashi, Human Frontier Science Program 2022（Hokkoku shinbun）
2	2022.4-29 - 7.6	website(17),Newspaper(4)	Research result: Promising anticancer molecule identified, Asst. Prof. Madhu Biyani, Prof. Miki Nakajima, ACS Appl. Mater. Interfaces（Kagaku Shinbunm, Hokkoku shinbun, Toyama shinbun, Kitanihon shinbun）
3	2022.5-24 - 6.29	website(13)	Research result: Assigning moving features in high-speed atomic force microscopy, Asst. Prof. Damien Hall, Prof. Adam Foster, Biophysics and Physicobiology
4	2022.6.4	Newspaper(1)	Research result: Polarized interfacial tension induces collective migration of cells, as a cluster, in a 3D tissue, Assoc. Prof. Satoru Okuda, Biophysical Journal（Hokkoku shinbun）
5	2022.6-16 - 7.25	website(16),Newspaper(1)	Research result: Simulating 3D-AFM images for systems not in equilibrium, Asst. Prof. Takashi Sumikama, Prof. Adam Foster, Prof. Takeshi Fukuma, The Journal of Physical Chemistry Letters（Hokkoku shinbun）
6	2022.7-14 - 8.11	website(14),Newspaper(1)	Research result: Heat and manipulate, one cell at a time, Assoc.Prof. Satoshi Arai, Asst. Prof. Cong Quang Vu, ACS Nano（Hokkoku shinbun）
7	2022.6-15 - 8.11	website(14)	Research result: Chemical fixation causes aggregation artefact, Asst. Prof. Takehiko Ichikawa, Prof. Masanobu Oshima, Prof. Takeshi Fukuma, Communications Biology
8	2022.7-6 - 7.6	website(2)	Research result: Revealing atomistic structures behind AFM imaging, Asst. Prof. Holger Flechsig, Post-doc. Romain Amyot, PLOS Computational Biology
9	2022.6.14 - 7.8	website(15),Newspaper(1)	Research result: Elucidating the structure of nanomaterials found in crustaceans, Asst. Prof. Ayhan Yurtsever, Prof. Takeshi Fukuma, Small Methods（Hokkoku shinbun）
10	2022.8.1-8.4	Newspaper(2),Television(2)	Event Report: Cancer research Early Exposure Program （Hokkoku shinbun, Hokuriku chunichi shinbun, NHK Kanazawa, Hokuriku Broadcasting Co.,Ltd. ）
11	2022.8.30 -	website(5),Newspaper(1)	Research result: Dynamic mechanisms of CRISPR interference by Escherichia coli CRISPRCas3, Prof. Kodera, Nature Communications（Hokkoku shinbun）
12	2022.11.8 - 11.9	website(58)	Research result: Biological lasso: Enhanced drug delivery to the brain, Assoc. Prof. Katsuya Sakai, Prof. Kunio Matsumoto, Nature Biomedical Engineering
13	2022.10.17 - 11.4	website(12),Newspaper(1)	Research result: Chemists uncover cracks in the amour of cellulose nanocrystals, Asst. Prof. Ayhan Yurtsever, Prof. Takeshi Fukuma, Science Advances（Hokkoku shinbun）

14	2022.10.17 - 11.5	website(17),Newspaper(2)	Research result: The offshoot of cells visualized in real time, Prof. Rikinari Hanayama, Prof. Toshio Ando, Prof. Richard Wong, Journal of Extracellular Vesicles （Hokkoku shinbun, Nikkan kogyo shinbun）
15	2023.1.18 - 1.24	website(9),Newspaper(1)	Research result: Nanoscopic tool assesses alternative COVID-19 prevention, Asst. Prof. Keesiang Lim, Prof. Rikinari Hanayama, Prof. Toshio Ando, Prof. Richard Wong, Nano Letters （Hokkoku shinbun）
16	2023.1.17 - 1.23	website(13),Newspaper(1)	Research result: Cancer biology: Some cancer cells can lose their capability to migrate, Asst. Prof. Dong Wang, Prof. Masanobu Oshima, Cancer Science （Hokkoku shinbun）
17	2023.2.24 - 2.27	website(13)	Research result: Locking and unlocking molecular structures on demand, Assoc. Prof.Yoko Sakata, Prof. Shigehisa Akine, Angewandte Chemie-International Edition
18	2023.2.28 - 3.6	website(13)	Research result: Biomolecular sliding at the nanoscale, Prof. Mikihiro Shibata, Nano Letters
19	2023.3.6 - 3.14	website(11)	Research result: Detect, bind and cut: biomolecular action at the nanoscale, Prof. Mikihiro Shibata, ACS Nano
20	2023.3.9 - 3.9	website(7),Newspaper(1)	Research result: Scanning probe simultaneously captures structural and ion concentration changes , Prof. Yasufumi Takahashi, Prof. Yuri Korchev, Prof. Takeshi Fukuma, JACS Au （Hokkoku shinbun）
21	2023.3.14 - 3.27	website(5)	Research result: Observation of molecular self-assembly of peptides and understanding the mechanism of seamlessly bridging biology with a technological solid, Asst. Prof. Ayhan Yurtsever, Prof. Takeshi Fukuma, ACS Nano