

World Premier International Research Center Initiative (WPI)

FY 2017 WPI Project Progress Report

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

Common instructions:

* Unless otherwise specified, prepare this report from the timeline of 31 March 2018.

* So as to base this fiscal year's follow-up review on the "last" center project, please prepare this report from the perspective of the latest project plan.

* Use yen (¥) when writing monetary amounts in the report. If an exchange rate is used to calculate the yen amount, give the rate.

* Please 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

Through intensive discussions between researchers from four major research disciplines (i.e. nanometrology, life science, supramolecular chemistry, and computational science), we clarified specific research subjects and plans to achieve the main goals of this project. Based on these ideas, we performed preliminary studies to explore the possibilities of new interdisciplinary research.

(1) Development of novel nanoprobe technologies

We will develop nanoprobe technologies for imaging, analyzing, and manipulating of nanoscale structures and their dynamic changes at the surfaces and inside of live cells.

- **Scanning probe microscopists** started to develop special nanoprobes required for novel imaging techniques. For imaging inside of live cells, **Fukuma** started to explore various fabrication methods to create a long, thin, and stiff nanoprobe for insertion into an intracellular space without causing significant mechanical stress to the cell. **Ando** developed a nanopipette with a carbon nanotube (CNT) at its end to improve SICM spatial resolution and to obtain clear molecular-resolution images of a live cell surface. **Korchev** developed a nanopipette with an integrated field effect transistor at its end. This nanoprobe will be combined with molecular sensors developed by supramolecular chemists to visualize nanoscale distribution of various ions and molecules near and inside of live cells.

- **Supramolecular chemists** had intensive discussions with life scientists and determined chemicals whose local distribution near and inside of a cell is important for understanding specific biological phenomena. They have already started to explore the possibilities of their design and synthesis. This is a relatively long-term project. As a shorter-term project, they started to explore possibilities to modify and integrate the functional macromolecules that they have been investigating to a nanoprobe. This will give the nanoprobe either a chemical sensitivity or a controllability by an external stimulus. Examples include macrocyclic compounds (**Ogoshi & MacLachlan**), metal complexes (**Akine**), and helical polymers (**Maeda**). Meanwhile, **Takahashi** and **Asakawa**, who are scanning probe microscopists with chemistry backgrounds, performed a detailed methods survey for chemical functionalization of nanoprobes and started discussions with supramolecular chemists on practical ways to apply these methods to their original macromolecules.

- **Computational scientists** performed modeling, simulations, and analysis of complex biological systems and their scanning probe microscopy (SPM) measurements. **Foster** and **Mikhailov** performed simulations of AFM measurements for various structures and dynamic events at solid-liquid interfaces (hydration, crystal dissolution, and motor protein dynamics) and compared to results obtained by the simulations and AFM experiments to understand their origins (*Nano Lett. 2017, Nat. Comm. 2017, ACS Nano 2017*). To further expand their capabilities, they also explored possibilities of modeling more complicated biological phenomena, such as protein dynamics in the cytoplasm (**Mikhailov**) and folding of chromatin fibers (**Sumikama**). In addition, Foster started to develop a large-scale database system for SPM images and spectroscopy data of their sophisticated analysis by a machine-learning approach.

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

- **Basic cell biologists** started to address basic principles of cellular functions, such as cell proliferation, differentiation, and intercellular communication, by observing nanostructures and dynamics inside or outside of cells. **Matsumoto** and **Nakajima** observed real-time images of the association between a growth factor and its receptor or between an RNA editing enzyme and its substrate with AFM researchers, leading to proposals of new activation mechanisms. **Hirao** discovered a novel molecular mechanism that maintained hematopoietic stem cell function and suppressed leukemia onset under high-fat diet (*Cell Stem Cell 2018*). Subsequently, Hirao began development of a label-free whole

bone marrow cell analysis technology to analyze changes in the microenvironment. **Wong** successfully visualized the native nuclear pore complex (NPC) by HS-AFM and uncovered a role for the central channel NPC component in regulating cell differentiation (*EMBO Reports*, 2018). **Hanayama** discovered roles for exosomes in tumor progression and drug resistance. To elucidate the difference between cancer and normal cells, Hanayama started to visualize the dynamics of exosome secretion at the single-particle level with AFM and SICM researchers.

- **Cancer researchers** started interdisciplinary research with scanning probe microscopists and supramolecular chemists to directly observe changes in ultrastructure and intracellular metabolism at the nanoscale during malignant progression. Based on the discovery regarding critical roles of cancer associated gene mutations (*Cancer Res*, 2018), **Oshima** and **Yano** initiated studies to detect morphological changes driven by multiple mutations of cancer driver genes with SPM experts, **Ando** and **Fukuma**. **Hirao** started to develop novel chemical sensors to detect metabolites associated with cancer progression in collaboration with supramolecular chemist, **Ogoshi**. **Wong** promoted the interdisciplinary research for the nuclear pore complex (NPC) of colorectal cancer cells using HS-AFM and clarified the NPC dynamics-regulating mechanism that changed with malignant progression of colon cancer (*ACS Nano*, 2017). **Matsumoto** acquired several macrocyclic peptides that bind to HGF and the MET receptor using a chemical biotechnology technique, which will be useful along with PET diagnostic technology to detect MET receptor activation in cancer tissues.

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

We will establish a new research field "Nanoprobe Life Science", where we aim for nano-level understanding of various life phenomena using nanoprobe technologies. At Kanazawa University, we established the Bio-AFM frontier research center in 2010. Since then, we have made significant efforts to develop innovative AFM technologies and to promote collaborative biological research using the developed techniques. In this WPI project, we will greatly advance the level and extent of these activities. To expand our international collaboration network, we established a system to regularly and officially call for proposals of collaborative research worldwide. We also established an Open Facility with AFM and SICM instruments for common use in these interdisciplinary collaborative research projects. In addition, we will continue to have a summer school every year, where international attendees can use our instruments to investigate their own samples. Through these activities, we will promote high-level research achievements. In this FY, we have directly visualized nanodynamics of various biological systems, such as nuclear pore complexes (*Wong et al., ACS Nano*), CRISPR-Cas9 (*Shibata et al., Nat. Comm.*), and human 2-Cys peroxiredoxin II (*Konno et al., J. Mol. Biol.*) using high-speed AFM and thereby made significant progress in their nano-level understandings. These achievements have provided a great impact in the life science field, leading to the future establishment of this new research field.

2. Fusion of Research Fields

- **NanoLSI international symposium:** To promote the fusion of four different research fields and to enhance the visibility of NanoLSI, we organized a kickoff symposium in Tokyo and a joint symposium, the UBC-KU Joint Symposium, at the University of British Columbia (site of a future satellite center).
- **NanoLSI transdisciplinary research promotion grant:** We designed this to promote the integration of four and possibly more research fields, especially within NanoLSI. Its goal is to develop the new transdisciplinary research field of Nanoprobe Life Science and to develop young researchers in the area.

3. International Research Environment

- **Satellite research center:** We are currently negotiating with two institutions, Imperial College London and University of British Columbia, regarding the contents of the collaborative research agreement. With these agreements, we will employ two postdocs at each institution who will support the PI's research and significantly contribute to our project in visibility and internationalization.
- **NanoLSI fellowship:** We have also introduced a new research fellowship program for foreign researchers featuring joint-use of the most advanced HS-AFM facilities, and we have begun accepting applications from various research fields, including structural biology. Through this program, we will promote our reputation and solidly establish the academic research field of Nano Life Science.

4. Reformation of Organizations and Systems

- **NanoLSI Educational Program:** To utilize the achievements of NanoLSI to develop young researchers of the next generation, NanoLSI has established a new educational program within the Graduate School of Natural Science and Technology. We will also establish a new major program in the Graduate School of Frontier Science Initiative.
- **NanoLSI RP system:** We have introduced a NanoLSI RP system, which will accelerate the establishment of a strong research base to be recognized around the world. Also, we have installed a researcher excellence allowance system, which will achieve a salary system with international standards.

- * Please describe clearly and concisely the progress being made by the WPI center project from the viewpoints below.
- In addressing the below-listed 1-6 criteria, please 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 fields).
 - (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. Conducting research of the highest world level

* Regarding the criteria used when evaluating the world level of center, please note any updated results using your previous evaluation criteria and methods or any improvements you have made to those criteria and methods.

[Research objectives]

At this center, we aim to develop nanoprobe technology and promote nanoscale life science research through the fusion and development of nanometrology, supramolecular chemistry, life science, and computational science with the world’s highest standard as our foundation. Specifically, to further develop the world’s top bio scanning probe microscope (SPM), integration with supramolecular chemistry will allow development of a nanoprobe technology that can observe, analyze, and manipulate the nanodynamics of the surface and interior of live cells. In addition, by complementarily using these technologies alongside mathematical and computational technologies, such as simulation and numerical modeling, we hope to achieve an understanding of the basic functions of cells and cancer-specific abnormalities at a nanoscale level. Using the nanoprobe technology findings created through these activities, we intend to cooperate with life science researchers around the world to pursue nanoscale research of various biological phenomena and develop a new interdisciplinary academic field called “Nanoprobe Life Science.”

[Specific research subjects]

During this fiscal year, we organized study groups, seminars, symposiums, etc., to promote the aforementioned goals and to provide a mutual understanding and discussion between researchers. In addition, we proposed a large number of specific research topics. As shown in Fig. 1, these topics are organized and integrated into three broad topics and nine intermediate topics in order to clarify the research topics to be tackled at the start. These research topics are an integration of the four major research fields mentioned above, and a ratio of the contribution from each field is shown. In the following section, we report the specific plans pertaining to and the progress made in each of these subjects.

[Outline, plan, and progress of each research subject]

(1) Development of Novel Nanoprobe Technologies

In this domain, we develop the world’s top bio SPM, and we integrate it with findings from supramolecular chemistry. In this manner, we develop a new nanoprobe technology to observe, analyze, and manipulate the nanoscale structures, dynamics, and material distributions of the surface and interior of live cells. In addition, by using mathematical and computing sciences, we aim to verify the principles of the new measurement method and promote data analysis to understand life systems through the measured data.

① Visualizing Nanodynamics Inside Live Cells (Main PI : Fukuma) (Objective)

We aim to develop nanoendoscopic imaging technique to visualize nanoscale structures and dynamics inside live cells.

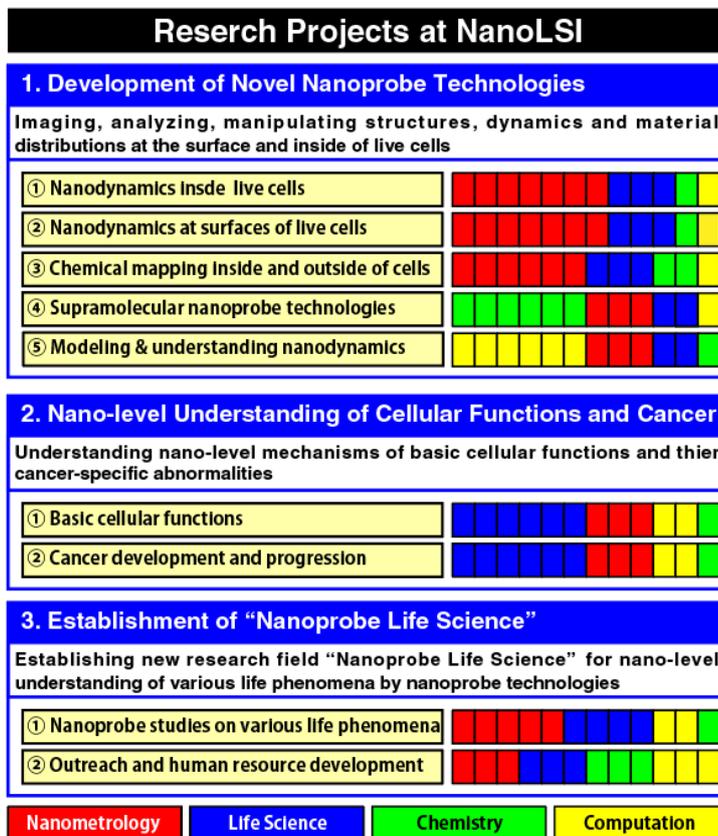


Fig. 1: Research projects at NanoLSI and contributions from the four major disciplines to each project.

(Background and Methods)

We will develop nanoendoscopic imaging technique based on 3D-AFM. In 3D-AFM, a probe is scanned in a 3D interfacial space, and the force applied to the probe apex is recorded to produce a 3D force image representing 3D distributions of water and flexible surface structures. Similarly, by scanning a long nanoprobe in a 3D space, including inside of a live cell, we will visualize nanodynamics of biomolecules and their assemblies in it (Fig. 2).

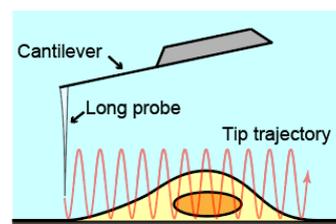


Fig. 2: 3D imaging of a cell

(Subjects and Plans)

Table 1: Research subjects and plans for the development of nanoendoscopic imaging technique

Subjects	FY2017	FY2018	FY2019	FY2020	FY2021	FY2022-2026
Nanoprobe	Long nanoprobe (EBD, glass, CNT etc.)			Functionalized long nanoprobe (molecular machine, molecular sensor)		<ul style="list-style-type: none"> • Applications & improvements (usability, productivity, reproducibility) • Combination with other methods
Microscope	Prototype I (combination of 3D-AFM and OM, large scan, FPGA, Tip scan)		Prototype II (High speed, large capacity, improved force detection & scanning methods)		Prototype III (Usability, analysis software, quantitative capability)	
Measurement Method	3D imaging of chromosomes, nucleus, cells (Regardless of resolution)		3D imaging of chromosomes, nucleus, cells (Spatial resolution < 10 nm)		3D imaging of chromosomes, nucleus, cells (Time resolution: < 10 s/frame)	

(Research Progress)

• Development of the Nanoprobe

To insert a probe into an intracellular space without causing excessive stress to a cell, a long, thin, and stiff nanoprobe should be developed. According to previous studies, the probe should have a maximum diameter of less than 200 nm, a length of several micrometers, and a high stiffness. To satisfy these requirements, we are testing three different types of nanoprobes: electron beam deposited carbon tips, carbon nanotube (CNT) tips, and quartz tips. To date, we have established a reproducible fabrication process of an EBD tip with a maximum 100–200 nm diameter and a 3–5 μm length (Fig. 3a). With the developed probe, we succeeded in imaging and penetrating a live cell surface (Figs. 3b and 3c). We will further develop a longer EBD tip with a reinforced support of its fixed end. In addition, we will explore the possibilities of using a CNT and a quartz nanoprobe.

• Development of the Microscope

We will combine a 3D-AFM head and an inverted optical microscope.

We purchased a commercial optical microscope and began modifying its stage component. For 3D-AFM part, we should newly integrate a large-scale XY closed-loop scanner for a cellular imaging. We purchased a closed-loop XY scanner and began designing the remaining mechanical components. For complicated tip scanning and high-speed and large-scale data recording, we purchased a high-speed FPGA board and began developing firmware to control it. Meanwhile, we also initiated designs of a custom analog-digital IO unit dedicated to nanoendoscopic applications.

• Development of Measurement Methods

To explore the possibilities of 3D-AFM imaging, we will work on four different sample types in parallel. First, we will fabricate model 3D structures consisting of CNTs by FIB-SEM and compare their 3D-AFM and SEM images (Fig. 4a). Second, we will visualize internal chromosomal structures, consisting of ~30 nm diameter folded chromatins, using a CNT probe (Fig. 4b). Third, we will image chromosome configurations in an isolated nucleus (Fig. 4c). Finally, we will insert a probe into the intracellular space and visualize nuclear pore complexes at the surface of a nucleus and chromosome configurations inside the nucleus. By comparing the results obtained by experiments and simulations, we will elucidate nanoscale structures and dynamics of these biomolecular assemblies. To date, we succeeded in imaging surface structures chromosomes (Fig. 4b) in liquid by AFM. We will develop a CNT probe with a diameter of 10–30 nm and a length of 300–500 nm to visualize internal structures of a chromosome.

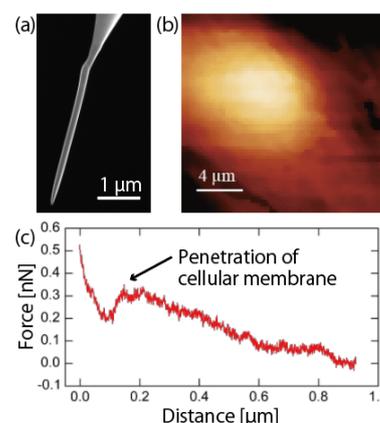


Fig. 3: (a) EBD tip. (b) AFM image of COS7. (c) Penetration of cellular membrane.

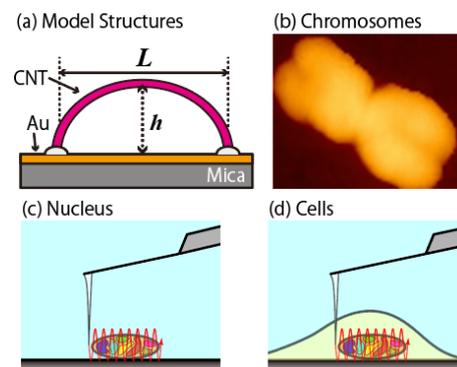


Fig. 4: Nano-level 3D imaging of internal structures of biological systems

(Expected World's Top-level Achievements)

Nanoendoscopic imaging technique will enable various measurements that have been deemed impossible

using the existing technology. For example, direct imaging of the inside of a chromosome, nucleus, or a live cell at a nanoscale (< 10 nm) resolution has been unachievable, even with the current state-of-the-art measurement techniques. Therefore, the measurements will provide valuable insights into the nano-level understanding of various cellular functions, such as gene expression. Furthermore, detailed comparisons between a normal and cancer cell can elucidate the mechanisms of not only the basic cellular functions but also their cancer-specific abnormalities. Therefore, we believe that the outcome of this study will be globally competitive in nanometrology and life science.

② Measuring Nano-dynamics on the Cell Surface (Principal Investigator : Ando) (Objective)

We aim to materialize high-speed scanning ion conductance microscopy (HS-SICM) enabling *in-situ* observation of protein molecules actively functioning on the membrane surface of live cells and intracellular organelles, and in the interior of unroofed/demembrated cells. We will also materialize HS-AFM observations of purified membrane proteins in tiny suspended membranes with physiological ionic environments.

(Background and Methods)

The Ando group developed HS-AFM enabling real-time, high-resolution imaging of individual biological nano-machines during their functional activity. HS-AFM has displayed a great power in visualizing purified protein molecules in action, but it is much less effective for molecules on the eukaryotic membrane. This is due to large deformation of the membrane by contacting with the AFM tip, resulting in blurred, low-resolution images. To overcome this problem, we will develop HS-SICM that possesses high-speed, high-resolution, and non-invasive imaging capabilities. Through these developments and others, we will make possible the observations described in the Objective.

(Subjects and Plans)

Table 2: Research subjects and plans for developing HS-SICM capable of in-situ imaging

Subjects	FY2017	FY2018	FY2019	FY2020	FY2021	FY2022-2026
Nanoprobes	Nanoprobes for high-speed/high-resolution SICM			Optimization of nanoprobes for low impedance and noise		<ul style="list-style-type: none"> • Applications to more complex systems • Imaging capability other than topography • Development into a product
Microscope	Prototype I (higher S/N and bandwidth current detection, faster scanning, etc.)		Prototype II (combining with fluorescence microscope, easier operation, long-time stability, controlling sample cell environment)			
Imaging	Preparation of small suspended membranes. HS-AFM imaging membrane proteins. Test imaging by SICM for improving the microscope		Test imaging: Eukaryotic cells, mitochondria, nuclei, proteins. (sub-molecular resolution, 1 frames/s)	Imaging of various biological samples (sub-molecular resolution, faster imaging, truly non-contact imaging)		

(Research Progress)

• Development of nanoprobes

An SICM probe with a tip pore diameter of 1–2 nm and a wall thickness less than 1 nm (Fig. 5) will be fabricated: A giga-sheal lipid bilayer will be furnished to the tip of a nano-pipette. Following that, a carbon nanotube (CNT) will be inserted into the bilayer, and the bilayer will be polymerized. We succeeded in inserting a CNT into the lipid bilayer formed at the end of a nano-pipette and confirmed an ion current through the CNT. We also surveyed conditions to increase the success rate of CNT insertion. CNTs were not inserted into the bilayers of a photo-polymerizable lipid. We examined other methods for lipid polymerization.

• Development of the microscope

We built a HS-SICM system and performed test measurements for the nano-probes mentioned above. We also studied increases in the signal-to-noise ratio of the ion current detection.

• Development of small suspended membranes

We succeeded in forming two-dimensional (2D) tamavidin crystals directly on the mica surface and in producing many small pores in the 2D crystals. By forming a lipid bilayer on the 2D crystals, we produced tiny suspended membrane regions all over the lipid bilayer and confirmed their rigidity.

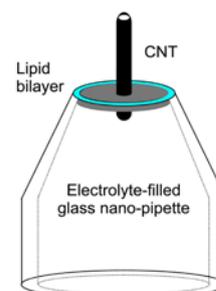


Fig.5: CNT nano-pipette

(Expected achievements of the highest world level)

The Ando group established the world-fastest HS-AFM for biological studies and developed a fast ion-current detection technique. We will include a high-resolution imaging capability to HS-SICM to materialize the observations mentioned in the Objective. The upcoming technology has the potential of surpassing our HS-AFM and having a great impact on life sciences.

③ Material distribution measurement inside and outside of cells (Principal Investigator: Korchev)

(Objective)

We aim to develop a nanopipette-based measurement method that visualizes the biomolecule distribution inside and outside of live cells.

(Methods)

We develop a chemical sensor based on scanning ion conductance microscope (SICM). With SICM, voltage is applied between two electrodes—one placed inside and one outside a probe—and the ionic current is recorded while scanning the sample surface, which allows the nanoscale surface shape of live cells to be visualized. Through regulation of the applied voltage and chemical modification of the nanopipette tip, SICM can be used as a sensor to measure chemical distribution near the cell surface (Fig. 2).

(Subjects and plans)

Table 3: Research subjects and plans for the development of material distribution measurement

Subjects	FY2017	FY2018	FY2019	FY2020	FY2021	FY2022-2026
Chemical sensor	Nanopore sensor (modification, method, principle)			Multi-barrel probe (measure different chemicals simultaneously)		<ul style="list-style-type: none"> • Nanobiopsy technique (part of cell, tissue) • Time-dependent single cell gene expression
Cell function mapping	Nanoparticle uptake, cell interaction, Lipid imaging		Cell metabolites measurement using microelectrodes and ISFETs	Signal molecules measurement using nanopore chemical sensors		
mRNA evaluation	System for picking up small amount of cytosol		Development of high-throughput cytosol collection system	System for PCR in the nanopipette		

To achieve the above objectives, three research subjects are examined (listed in Table 3).

(Research progress)

• Development of a chemical sensor

A selective chemical sensing function is incorporated through chemical modification of the nanopipette tip. Further, ion interactions within the nanopipette's inner wall are measured through changes in the ionic current. For the pH measurement probe, modifications with poly-L-lysine and chitosan have been examined. We detected pH in a range of 5 to 8 with good reproducibility for the chitosan modification. We also developed a FET nanopore sensor (NexFET) that can control the movement speed of specific biomolecules by regulating the thickness of the electric double layer at the nanopipette tip. The NexFET is fabricated in dual-barrel quartz nanopipettes, which uses a pyrolytic carbon deposition in one of the barrels followed by a polypyrrole electrodeposit at the carbon-coated nanopipette tip (Fig. 6, Nat Comm 2017). In the future, we aim to develop a probe that can measure lower pH, and a probe that can measure intracellular glucose and ATP. In addition, by using supramolecular technology, including that which involves pillararene, and chemically modifying the nanopipette tip, we can measure metabolites generated by cancer cells.

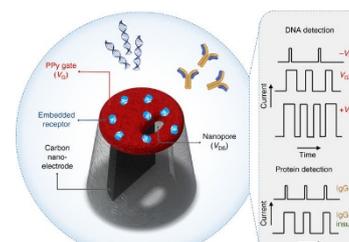


Fig. 6: Principle diagram of NexFET measurement.

• Cell function mapping

By combining SICM with fluorescence microscopy, we identified nanoscale differences in the shape of primary cilia from different cell types (Fig. 7, Anal Chem 2018). We also have initiated studies to visualize the extracellular vesicle (exosome) and phospholipids on the cell surface. At present, we are able to visualize a single exosome, with a diameter of approximately 100 nm and trapped within the cell membrane, at a scan range of $1 \times 1 \mu\text{m}$. In the future, we will visualize the internalization process of the exosome. We are also currently conducting lipid imaging on the cell surface, including phosphatidylserine (PS). Since PS is negatively charged, this charge can be visualized by SICM. We have successfully measured extracellular exposure of PS (outside the cell membrane) during apoptosis, and we are currently attempting time-lapse imaging. We aim to establish a lipid mapping method by the end of 2018.

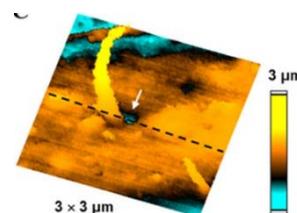


Fig. 7: Primary cilia morphology by SICM

• Evaluation of intracellular mRNA

We have developed a method that can directly perform sequencing without PCR. Briefly, we filled one side of the theta nanopipette with a DNA-containing solution, which is the measurement target, while filling the other side with electrolyte. Subsequently, we applied voltage to move the DNA by electrophoresis and measured the DNA length by the ionic current response. We successfully developed a nanobridge-type nanopore sensor, which uses the nanospace between two barrels (Fig. 8, Nano letters 2017).



Fig. 8: Principle diagram of measurement by a Nanobridge sensor.

(Expected achievement of the world's highest standard)

If the above objectives are achieved, nanoscale measurements of metabolites, hormones, and genes, which were viewed as impossible using previous technologies, will be possible. These studies would be facilitated with our newly developed technologies, allowing the achievement of the world's highest standards in nanometrology and life sciences.

④ Supramolecular nanoprobe development (Principal Investigators: Akine, Maeda, Ogoshi, MacLachlan)

(Objective)

We aim to develop new probes for high-performance nano-endoscopy, which are functionalized with the latest supramolecular technology.

(Methods)

In this study, we develop highly selective probe molecules based on recent advances in supramolecular chemistry and molecular recognition chemistry. Further, we aim to develop nanoprobe with enhanced performance. We develop dual-mode probes functionalized with responsive molecules and probes for molecular observations and manipulations with nanoscale precision.

(Subjects and plans)

Table 4: Research subjects and plans for the development of supramolecular nanoprobe

Subjects	FY2017	FY2018	FY2019	FY2020	FY2021	FY2022-2026
Supramolecular nanoprobe	Design and synthesis of new probe molecules (responsive helical polymer, photoswitching receptor, surface analysis, bioconjugate probes, etc.)			Improvement of new probe molecules (highly sensitive and selective probes, reactive probes, etc.)		<ul style="list-style-type: none"> • Applications & improvements (in vivo sensing) • Switching of dual-mode probes
Nanolevel cancer research	Molecular sensors for biomolecules (glucose, pH, oxygen, lactate, oncometabolites, etc.)			Development of new probes modified with sensors (investigation of sensing ability in cellular environments)		

To achieve the above objectives, we examine two research subjects, as presented in Table 4.

(Research progress)

• Development of fundamental supramolecular nanoprobe technology

To gain a deeper understanding of protein and biomembrane surfaces, analysis of chemical information in addition to morphological information at the nanometer scale is needed. To this end, specialized probe molecules that can detect specific chemical information are necessary. Therefore, the goal here is to identify surface functional groups with high selectivity, sensitivity and nanometer-scale accuracy. It is effective to introduce cyclic molecules, helical polymers, metal complexes, and peptides with highly selective recognition abilities for each target molecule. In previous experiments, we successfully developed polymers with chiral recognition abilities (Maeda et al. J. Am. Chem. Soc. 2018, 140, 3270) and macrocyclic compounds with alkane recognition abilities (Ogoshi et al., Chem. Rev. 2016, 116, 7937), which were studied mainly in homogeneous solutions. Therefore, we will introduce these molecules to a probe tip and study the substrate surfaces (Fig. 9). The ON/OFF switch of molecular recognition ability is also important. At present, different probes need to be used to determine the concentrations of chemical species and surface functional groups following morphological observations of the surface. Thus, we will provide a switching function for the probes that activate their function only when needed. We have successfully developed switching host molecules that change their recognition behavior in response to photoirradiation (Ogoshi et al., J. Am. Chem. Soc. 2018, 140, 1544.), organic anions (Akine et al., Nature Commun. 2017, 8, 16005), and other external stimuli, such as pH and metal ions. In the future, we will develop new molecules that apply this switching technology for the biomolecule recognition

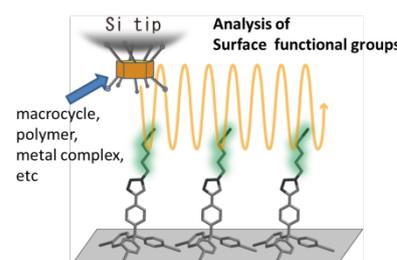


Fig. 9: Development of new selective probe molecules for nanoendoscopy

ability.

• **Development of application technology for nanoscale cancer research**

The region around cancer cells has different chemical properties from that around normal cells. Therefore, it is important to accurately determine the concentration distributions in real-time at the nanometer scale. We will develop highly-sensitive sensors for oxygen, pH, and oncometabolites and will evaluate the local concentrations at the nanometer scale by combining these sensors with the nanoprobe technology. We will also use them to analyze biological samples, such as cells. Presently, we have succeeded in selectively capturing cationic oncometabolites using cyclic molecules, and we are attempting to detect oncometabolites in cancer cells using the nanoprobe technology.

(Expected achievement of the world’s highest standard)

If we achieve the above objectives, we can determine the local concentrations of various substances at high spatial resolutions, which have not been possible using any of the existing technologies. Clarification of these concentration distributions will contribute to a greater understanding of nanoscale substance transport in cancer cells. By comparing cancer cells to normal cells, advancements in the understanding of substance transport in cancer cells and the abnormalities of metabolite production can be promoted. This imaging study is only possible by combining our supramolecular chemistry sciences with nanoprobe technology, which can result in the world's highest standards in nanometrology and life sciences.

⑤ **Elucidating Nanodynamics from Measured Data (Main PIs: Foster, Mikhailov)**
(Objective)

We aim to elucidate nanoscale structures and dynamics of biological systems from images or movies obtained by nanoprobe techniques using mathematical modeling and computational approaches.

(Background and Methods)

In nanoprobe technologies, we obtain images or movies that represent structures and dynamics of a measured objective through its interaction with a probe. Meanwhile, the main purpose of a measurement is to elucidate the structures and dynamics of the measured objective from the measured data, which requires analysis of an inverse problem as illustrated in Fig. 10. This is often challenging especially when we aim to elucidate nanodynamics of a complicated biological system.

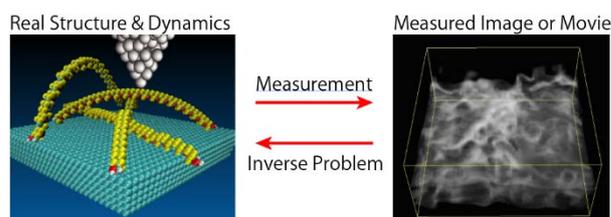


Fig. 10: Inverse problem for understanding real structures and dynamics from measured images or movies.

Here, we overcome this difficulty by combining mathematical modeling and computational approaches.

(Subjects and Plans)

Table 5: Research subjects and plans for analyses of real models from measured data

Subjects	FY2017	FY2018	FY2019	FY2020	FY2021	FY2022-2026
Simulation	Simulations of myosin, ATPases, dynamin, protein machines, lipid bilayers. Simulations of 3D-AFM images of fibrillar structures.			Simulations of allosteric interactions in protein machines, designed structures, probe penetration into a membrane.		Simulations and mathematical modeling of more complex biomolecular systems with higher functionalities.
Math. Modeling	Coarse-grained models for simulations of proteins. Mesoscopic models for protein assemblies. Multi-particle modeling of active colloids and membranes.			Mechanisms of allosteric interactions in protein machines. Design of protein-like machines' structures.		
Machine Learning	Establish AFM image analysis server and metadata protocols for automated categorisation of experimental images.			Develop machine learning toolset for analysis of 3D-AFM images: feature extraction, tip identification, dynamic recognition		

(Research Progress)

• **SPM Simulation and Machine Learning (Foster)**

Generally, local hydration structures at the solid–liquid interface around boundary edges on heterostructures are key to an atomic-level understanding of various physical, chemical and biological processes. Recently, we succeeded in combined SPM experiments and simulations to study the local hydration structures formed on a heterogeneously charged phyllosilicate surface and to resolve the local dissolution mechanism on calcite surfaces (see Fig. 11).

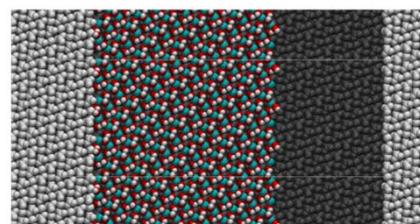


Fig. 11: simulated transition phase during dissolution of calcite.

The methodology and findings could be crucial for the exploration of further functionalities. Alongside this, we have begun our development of the NanoLSI machine learning infrastructure. The first stage is the imaging server, providing automated extraction of experimental metadata, which has

already been setup and is now being tested. This will be used as a platform to develop a novel methodology for solid-liquid simulations in the context of AFM, encapsulating the chemical and atomic structure of the surface, the nature and structure of the solution and the role of the tip in a machine learning (ML) approach.

• Modeling and Simulation of Biological Systems (Mikhailov)

Mathematical modeling and molecular simulations were undertaken in order to interpret the available data and assist in planning further experiments. The research was carried out at three structural levels: (a) single biological nano-machines, (b) aggregates of such machines, and (c) collective multi-particle effects in large populations of them. (a) Combining large-scale molecular dynamics (MD) simulations (in collaboration with Beijing Computational Science Research Center) and coarse-grained elastic-network (EN) modeling, a mechanistic molecular interpretation was found¹ to the results of AFM experiments by T. Ando with coworkers with the rotary motor F₁-ATPase (Fig. 12). Stochastic thermodynamics of an essential chemical nanomachine, the channeling enzyme tryptophan synthase, was completely determined and analyzed². (b) Through a combination of all-atom MD simulations, coarse-grained Go-type modeling and analytical treatment, active dynamics of helical polymer filaments made by dynamin motors was studied (together with the Max Delbrück Center for Molecular Medicine in Berlin) and clustering effects in such filaments, observed in AFM experiments by T. Ando with coworkers, were reproduced (publication in preparation). (c) Hydrodynamic non-equilibrium fluctuation effects of large populations of protein machines in viscoelastic gels (such as the cellular cytoskeleton), in the cytoplasm and in lipid bilayers were explored^{3,4}.

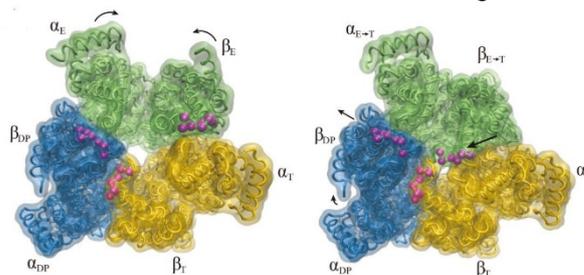


Fig. 12: Coupled domain dynamics from EN simulations of the F1-ATPase ring.

1. L. Dai, H. Flechsig, J. Yu, Biophys. J. 113, 1440 (2017)
2. D. Loutchko, M. Eisbach, A. S. Mikhailov, J. Chem. Phys. 146, 025101 (2017)
3. K. Yasuda, R. Okamoto, S. Komura, A. S. Mikhailov, Europhys. Lett. 117, 38001 (2017)
4. A. S. Mikhailov, Y. Koyano, H. Kitahata, J. Phys. Soc. Jap. (Special Topics), 86, 101013 (2017)

• Modeling of Chromatin Fibers (Sumikama)

As a first step to solve the inverse problem in Fig. 10, a coarse-grained simulation code to visualize the dynamics of chromatin fiber has been developed and fluctuating motion of the smallest chromosome in human genome was simulated (Fig. 13). A code to make more compact form of the chromosome seen in nucleus and methodology to obtain SPM data from the simulation are under development.

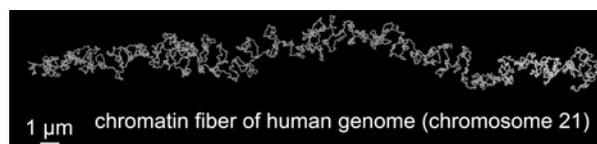


Fig. 13: A snapshot of the chromatin fiber.

(Expected World's Top-level Achievements)

Computational approaches such as simulation and machine learning have been a powerful tool for analyzing SPM data. However, their applications have been mostly limited to relatively simple systems consisting of atoms or small organic molecules. By combining these computational approaches with mathematical modeling techniques, we aim to analyze more complicated biological systems, which will lead to a significant development of an interdisciplinary research field "Nanoprobe Computational Science". As this research is only possible by combining expertise in three different fields, nanometrology, life science and computational science, the outcomes should have a world's top-level competitiveness. In 2017, we published several collaborative works between nanometrology and computational science as listed below.

- E. Holmstrom, S. Ghan, H. Asakawa, Y. Fujita, T. Fukuma, S. Kamimura, T. Ohno, A. S. Foster, J. Phys. Chem. C, **121** (2017) 20790.
- K. Miyata, J. Tracey, K. Miyazawa, V. Haapasilta, P. Spijker, Y. Kawagoe, A. S. Foster, K. Tsukamoto, T. Fukuma, Nano Lett. **17** (2017) 4083.
- K. Miyazawa, M. Watkins, A. L. Shluger, T. Fukuma, Nanotechnology, **90** (2017) 245701.
- S. Kawai, S. Nakatsuka, T. Hatakeyama, R. Pawlak, T. Meier, J. Tracey, E. Meyer, A. S. Foster, Sci. Adv. **4** (2018) eaar7181
- K. Umeda, L. Zivanovic, K. Kobayashi, J. Ritala, P. Spijker, A. S. Foster, H. Yamada, Nat. Commun. **8** 2111 (2017)
- S. Kawai, K. Takahashi, S. Ito, R. Pawlak, T. Meier, P. Spijker, F. F. Canova, J. Tracey, K. Nozaki, A. S. Foster, E. Meyer, ACS Nano **11** 8122 (2017)
- K. Buchmann, N. Hauptmann, A. S. Foster and R. Berndt, Nanotech. **29** (2017) 394004
- A. Richter, V. Haapasilta, C. Venturini, R. Bechstein, A. Gourdon, A. S. Foster and A. Kühnle, Phys. Chem. Chem. Phys. **19** (2017) 15172
- S. Kawai, T. Nishiuchi, T. Kodama, P. Spijker, R. Pawlak, T. Meier, J. Tracey, T. Kubo, E. Meyer, and A. S. Foster, Sci. Adv. **3** (2017) e1603258
- S. Kawasaki, E. Holmström, R. Takahashi, P. Spijker, A. S. Foster, H. Onishi, M. Lippmaa, J. Phys. Chem. C **121** (2017) 2268
- H. Söngen, C. Marutschke, P. Spijker, E. Holmgren, I. Hermes, R. Bechstein, S. Klassen, J. Tracey, A. S. Foster, A. Kühnle, Langmuir **33** (2017) 125

(2) Promotion of Nano Life Science Research

Fundamental understanding of the basic functions of cells and cancer-specific abnormalities at the nano level.

① Understanding basic principle of cellular functions (Principal investigators: Hirao, Hanayama, Matsumoto, Wong, Nakajima)

(Objective)

Understanding the basic principles of cellular functions by observing nanostructures and dynamics inside cells.

(Background and methods)

NanoLSI has decided to engage with high priority on the integration of its flagship research areas “SPM” and “cancer research”. We set out three major objectives to further understand the basic principles of various cellular functions such as cell proliferation, cellular differentiation, and intercellular communication. Our plan is to apply the crowding analytical scanning probe technologies to several cellular nanostructures and dynamics imaging.

(Annual plan)

Table 6: Research subjects and plans for the fundamental cellular functions

Subjects	FY2017	FY2018	FY2019	FY2020	FY2021	FY2022-2026
Cell Proliferation	Validation of the receptor activation mechanism by dynamic molecular imaging and simulation		Receptor mutation in cancer patients from the aspect of molecular dynamics		Nanoscale imaging of cells with EM, AFM & SICM	• Establishment of novel concept & mechanistic insight • Applications to other cellular functions
Cell differentiation	Identification of regulators of cell differentiation		Imaging of exosomal regulators using cancer and normal cells			
Cell-Cell Communication	Visualization of dynamics of exosome secretion & uptake	Functional analysis of regulators for exosome secretion & uptake				

(Research and progress)

• Cell proliferation (Matsumoto and Nakajima)

Cell proliferation can be defined as an increase in the number of cells resulting from the normal, healthy progression by which cells grow and divide. Matsumoto et al. have been investigating the fundamental mechanisms of activation of growth factor, hepatic growth factor (HGF), and its receptor, MET. In this fiscal year, using high-speed AFM, they observed real-time images of the association of HGF and MET proteins, leading to the proposal of a novel model regarding cell signaling. Based on these findings the Matsumoto group has been focusing, presently, on single-molecule imaging in live cells, and on computational science to validate the new model, and on elucidating the allosteric effect through intramolecular electrostatic network changes triggered by HGF-MET bonding.

Nakajima et al. conducted research on the significance of A-to-I RNA editing as a post-transcriptional regulation focusing on anti-cancer drug target molecules. Dihydrofolate reductase (DHFR) is a folic acid metabolizing enzyme associated with the synthesis of DNA necessary for the proliferation of cancer cells, and is the target molecule of the anti-cancer drug, methotrexate. They discovered evidence verifying that A-to-I RNA editing influences drug responsiveness (Pharmacol Ther 181: 13-21, 2018). The Nakajima group collaborates with the Fukuma group to elucidate the molecular mechanisms how A-to-I RNA editing enzymes, ADAR1/2, regulate drug responsiveness by analyzing formation of homo- or hetero-dimer of ADAR protein with SPM technology.

• Cellular differentiation (Hirao and Wong)

Long-term maintenance of tissue homeostasis depends on the precise control of somatic stem cell activity. To maintain tissue homeostasis, a mechanism that appropriately regulates stem cell dynamics and its micro-environment is important. Hirao et al. discovered that Spred1, a member of the Sprouty family of proteins, plays an essential role in maintaining hematopoietic stem cell function and suppressing leukemia onset under high-fat diet (Cell Stem Cell 2018). These discoveries suggest that diet is one factor drastically altering micro-environmental factors surrounding stem cells. To fully understand this stem cell regulation system, the Hirao group collaborated with the RIKEN and JEOL researchers to develop a label-free whole bone marrow cell analysis technology based on intracellular nanostructure and dynamics analysis. By using the scanning electron microscopy that can perform high-speed imaging of wide areas with automatic imaging technology (deep learning), they plan to analyze changes in the bone marrow micro-environment due to nutritional load and the accompanying hematopoietic stem cell abnormalities. The control of intracellular traffic is vital for cell differentiation. Nuclear pore complexes (NPCs) are multi-protein turnstiles that regulate nucleo-cytoplasmic traffic. Recently, Wong et al. successfully visualized the native NPCs by HS-AFM (ACS Nano 2017). They uncovered the role of the central channel NPC component Nup62 in regulating the differentiation state of squamous cell carcinoma (SCC) cells. In

undifferentiated SCC cells, Nup62 transports the transcription factor p63 into the nucleus to facilitate p63 driving genes expression that maintain the proliferative capacity and stemness of SCCs (EMBO Rep.2018). The Wong group collaborated with the Fukuma group, they plan to develop a newfangled FM-AFM to examine 4D-genome-architecture in undifferentiated and differentiated cells respectively. In collaboration with the Ando group, Wong's group will develop a sample preparation technique for observing undifferentiated and differentiated native NPCs from various organoids and tissues via SICM imaging in the near future.

• **Intercellular communication (Hanayama)**

Exosomes are membrane-derived vesicles that have recently been implicated as key mediators of intercellular communication. Hanayama et al. discovered a mechanism in which exosomes derived from human glioma and osteosarcoma function in microglia and osteoclasts and promote angiogenesis and metastasis (paper submission in preparation). They also discovered the role of exosome emissions during anti-cancer drug treatment. Later, they further established a highly sensitive experimental system that could quantify exosome emission (Curr Protoc Cell Biol, 2017). They also identified multiple low-molecular weight compounds that regulate exosome release. Using AFM and SICM, the Hanayama group will visualize and measure the dynamics of constitutive release and exosome enrichment at the single-particle level and elucidate the difference between cancer and normal cells. Currently, they succeeded in label-free detection of exosomes on the cell surface using SICM (Fig. 14); in the future, they will further analyze the dynamics of single particles by SICM live imaging.



Fig.14: Visualization of exosomes on cells by SICM

(Expected achievement of the world's highest standard)

By using an integrated approach with cutting-edge nanoscale imaging technology, we expect to obtain highly valuable information of nanoscopic cellular functions, such as dynamic protein-protein interaction, intracellular localization of metabolites, dynamic regulation of organelle and nanoparticles, and association between non-label stem and niche cells *in vivo*. All this information will provide novel insights to identify core machinery regulating cellular functions at the nano scale. Therefore, we believe that the outcome of these interdisciplinary projects will represent the world's top-level competitiveness in life science.

② **Development of innovative therapeutic technology based on the understanding of cancer progression mechanism (principle investigators: Oshima and Yano)**

(Objective)

We aim to elucidate the nanostructure and dynamic changes associated with cellular transformation and malignant progression of cells. Based on these findings, develop new cancer diagnostic and therapeutic methods.

(Background and methods)

In this study, we will elucidate cell structure abnormalities due to transformation and malignant progression of tumor cells and dynamic changes of pharmacokinetics using high-speed AFM and SICM, as well as promote metabolic measurement with a newly developed nanoprobe. In this manner, we will determine the true nature of "cancerization" and "malignant progression" mechanisms at the nano level. Based on the information obtained from these analyses, we will develop applied studies that contribute to the development of new diagnostic and therapeutic methods.

(Annual plan)

Table 7: Research subjects and plans for the cancer progression and diagnosis/treatment

Subjects	FY2017	FY2018	FY2019	FY2020	FY2021	FY2022-2026
Cancer Progression	Cell surface structure/nuclear pore (AFM, SICM etc.)			Microenvironment/metabolite analysis (cryoSEM, nanoprobe)		<ul style="list-style-type: none"> • Establishment of novel concept & mechanistic insight • Proof of concept for diagnosis and treatment
Cancer Diagnosis	Technology establishment of PET imaging diagnosis for growth factor receptor activation status			Clinical study of PET imaging diagnosis for growth factor receptor activation status		
Cancer Treatment	Cell surface structure/fusion-protein distribution (AFM etc.)			Protein-drug interaction (cryoSEM, nanoprobe)		
Precision Medicine	Function and regulation mechanisms of drug-metabolizing enzymes			Nanoscale imaging of drug-metabolizing enzymes or related proteins (HS-AFM)		

To achieve the above objectives, we will examine the four research subjects shown in Table 7.

(Research progress)

• Elucidation of cancer onset mechanisms (Oshima, Wong, and Nakajima)

Oshima et al. developed genetic models of colorectal cancers, which showed “submucosal infiltration”, “epithelial mesenchymal transition (EMT)”, and “liver metastasis” (Cancer Res, 78: 1334-46, 2018). They also succeeded in developing an EMT-induced 3D culture model of human colorectal cancer cells (Fig. 15). Using these model systems, we started interdisciplinary research using HS-AFM observation with the nanometrology group (Ando and Fukuma) to elucidate the changes in the cell surface structure of cancer cells during progression processes. In this manner, we will determine the genotype-phenotype correlation in cancer cell progression at the nano level.

Wong et al. examined nuclear pore complex (NPC) of colorectal cancer cells using HS-AFM and clarified the NPC dynamics mechanism that changes with the progression of colon cancer (ASC Nano, 11: 5567-5578, 2017) (Fig. 16). We showed chromatin structural changes of colorectal cancers caused by TPR, a nucleoporin (Oncotarget, 2018). In the future, we will determine the importance of nucleoporin in carcinogenesis.

Nakajima et al. found the role of estrogen receptor in the upregulation of cytochrome P450 (CYP1B1), which is an important mechanism of estrogen-dependent carcinogenesis. (Oncotarget 8: 106608-24, 2017). In the future, through interdisciplinary research with the supramolecular chemistry group (Ogoshi et al.), we will develop supramolecules that can easily trap reactive metabolites associated with CYP1B1 that cause adverse effects.

• Development of cancer diagnostic technology (Hirao and Matsumoto)

Hirao et al. performed functional screening of cancer-metabolizing enzymes using the CRISPR/CAS9 library. They found that the therapeutic effect of a tyrosine kinase inhibitor was enhanced through the inhibition of the nicotinamide-metabolizing enzyme. Furthermore, using the nicotinamide metabolite as an index, they performed screening of new therapeutic drugs. Through interdisciplinary research with the supramolecular chemistry group (Ogoshi et al.), we prepared a chemical sensor, and found that Pillararene binds to nicotinamide metabolite. In the future, we will develop a detection system for nicotinamide metabolite by determining the specificity of Pillararene, and develop a diagnostic tool for the visualization of metabolites for the appropriate selection of patients.

Matsumoto et al. acquired cyclic peptides that bind to HGF and the MET receptors using a chemical biotechnology technique with special macrocyclic peptides that bind to the target molecule with high affinity (Nature Commun, 6: 6373, 2015)(Fig. 17). Among these, HGF-inhibiting peptides (HiP-8) specifically bound and inhibited binding to activated two-chain HGF (tcHGF), and accumulated in HGF-producing tumor tissues. In the future, we will continue improving this PET diagnostic technology to detect MET receptor activation in cancer tissues.

Wong et al. showed the NPC changes as a potential new diagnostic marker for colorectal cancer. In the future, we will establish a faster measurement method towards the development of a diagnostic tool for early detection of cancer. We began interdisciplinary research with Yano et al. using integrated analysis to examine abnormalities of phosphorylated kinase activity and transcriptome, furthermore we aim to elucidate the mechanism of drug resistance.

• Development of cancer therapeutic technology (Yano)

Yano et al. clarified the intracellular distribution of oncogenic fused protein using a lung cancer cell line. They developed lung cancer cells that acquired drug resistance and discovered that epithelial mesenchymal transition (EMT) is involved in this resistance. The functional characteristics of these resistant lines will be determined through interdisciplinary research with the nanodynamics group (Fukuma et al.). In the future, we will examine cancer cells with abnormalities in the process of acquisition of drug resistance at the nano level and elucidate the mechanism by which drug resistance is induced.

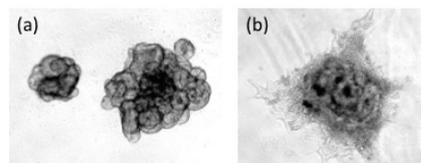


Fig. 15: (a) Human colon cancer organoids, and (b) induction of EMT transformation.

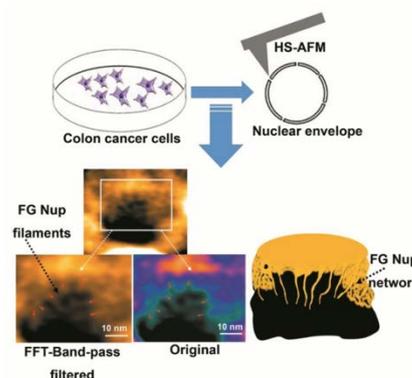


Fig. 16: AFM image of nuclear pore complex (NPC) of human colon cancer cells (ASC Nano, 2017).

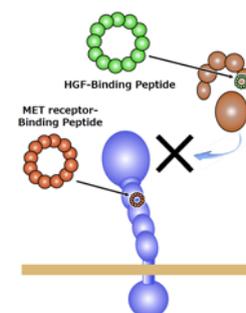


Fig. 17: Macrocyclic peptides binding to HGF or MET (Nat Commun, 2015)

Ando group has been studying streptolysin O (SLO) using high-speed AFM. SLO binds to the cell surface via cholesterol and perforates the membrane, resulting in cell death (Fig. 18). They are now aiming to create drugs commonly applicable for various types of cancers by conjugating SLO mutants to cancer-specific antibodies.

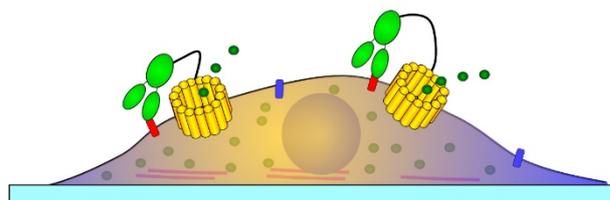


Fig. 18: Membrane perforation by SLO-antibody

• **Personalized medicine (efficacy and toxicity of anti-cancer drug) (Nakajima)**

Drugs are metabolized by enzymes expressed in the liver, where they lose their effects or are activated. The activation of drug-metabolizing enzymes shows large variations and are the main factors for individual differences in drug response. Nakajima et al. found that expression of arylacetamide deacetylase that metabolizes the prostate cancer drug flutamide is regulated by microRNA. In the future, through interdisciplinary research with Ando and Kodera, we will conduct dynamics analysis of drug-metabolizing enzymes at the nano level.

(Expected achievement of the world's highest standard)

Recently, genome and omics analyses are promoted at the single-cell level, and biochemical changes and cell properties relating to tumorigenicity have been demonstrated. However, these changes for malignant progression have not been examined at the nano level. Through the above projects, we would be the first in the world to directly understand the changes in cell structure and intracellular metabolism based on changes in genotype beyond ultrafine morphology. Furthermore, development of diagnostic and therapeutic methods based on these completely new findings should lead to precision medicine at the world's highest standard at the single-cell level well beyond the existing framework.

(3) Establishment of New Research Field: Nanoprobe Life Science

We will establish a novel interdisciplinary research field: Nanoprobe Life Science, where we aim for nano level understanding of various life phenomena using nanoprobe technologies. To this end, it is essential to perform impactful life science research with nanoprobe technology and announce it to researchers all over the world. While the development of the systems, environments and organizations will be discussed later, here we report representative achievements of collaborative works between **nanometrology** and **life science** researchers.

① **High-Speed Atomic Force Microscopy Reveals Loss of Nuclear Pore Resilience as a Dying Code in Colorectal Cancer Cells**

One of the key reasons for cancer death is the highly invasive behavior of cancer cells, which is often due to aggressive metastasis. Metastasis is helped by various growth factors and cytokines secreted from cells of the immune system, which are activated through various signaling pathways. Remarkably, these signaling pathways enter the nucleus through the nuclear pore complex (NPC), which is supposed to act as a doorkeeper for the nucleus. The concomitant assessment of nanoscopic structures and dynamics of NPC has been technically unfeasible via optical microscopes. Therefore, direct visualization of NPC dynamics at nanoscale resolution was considered "mission impossible".

Using the high-speed atomic force microscope developed by Prof. Ando Toshio, for the first time, Wong et al. captured "living" nano-nuclear pores of colon cancer cells (ACS Nano, 2017). We also revealed the first nano dying code – loss of nuclear pore inner channel protein elasticity. The present study is based on the crucial bio-imaging technology developed at NanoLSI. This study has huge implications in medical applications of HS-AFM/FM-AFM, acting as a novel "nano-endoscopy" in order to visualize the molecular dynamics of intra-cellular organelles (such as nucleus and nuclear pores) in cancer cells and other diseases in future.

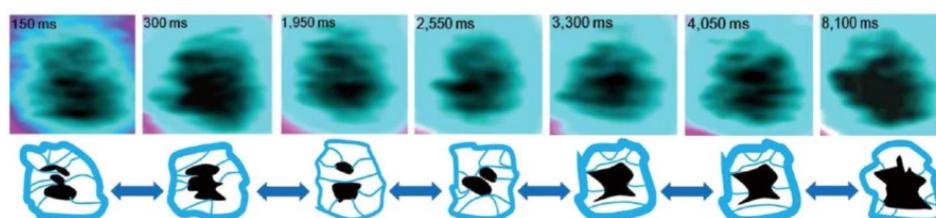


Fig. 19: HSAFM images showing an extension of the FG-Nups in nuclear pore central channel. Different frames show conformational changes of FG threads retracting and diffusing and sometimes forming a network that looks like a cobweb.

M. S. Mohamed, A. Kobayashi, A. Taoka, T. Watanabe-Nakayama, Y. Kikuchi, M. Hazawa, T. Minamoto, Y. Fukumori, N. Kodera, T. Uchihashi, T. Ando, R. W. Wong, *ACS Nano* (IF=13.942) 11, 5567-5578 (2017).

② Real-space and real-time dynamics of CRISPR-Cas9 visualized by high-speed atomic force microscopy

The CRISPR-associated endonuclease Cas9 binds to a guide RNA and cleaves double-stranded DNA with a sequence complementary to the RNA guide. The Cas9–RNA system has been widely used for numerous applications, such as genome editing. Here, we used high-speed atomic force microscopy (HS-AFM) to visualize the real-space and real-time dynamics of CRISPR-Cas9 in action. HS-AFM movies visualized that apo-Cas9 adopts unexpected flexible conformations. On the other hand, Cas9–RNA forms a stable bilobed structure and interrogates a target site on the DNA by three-dimensional diffusion. These movies also provided real-time visualization of the Cas9-mediated DNA cleavage reaction. Notably, the Cas9 HNH nuclease domain fluctuates upon DNA binding, and subsequently adopts an active conformation, where the HNH active site is docked at the cleavage site in the target DNA (Fig. 20). Collectively, our HS-AFM data provide unprecedented details about the functional dynamics of CRISPR-Cas9 and highlight the potential of HS-AFM to elucidate the action mechanisms of RNA-guided effector nucleases from distinct CRISPR-Cas systems.

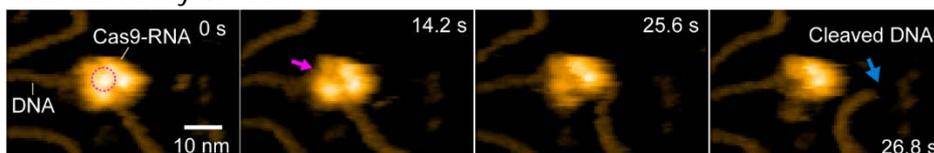


Fig. 20: A sequential HS-AFM images of a Cas9–RNA–DNA complex during DNA cleavage reaction.

M. Shibata, H. Nishimasu, N. Kodera, S. Hirano, T. Ando, T. Uchihashi, O. Nureki, *Nat. Commun.* (IF=12.124) 8, 1430 (2017).

③ Negatively charged lipids are essential for functional and structural switch of human 2-Cys peroxiredoxin II

The function of ubiquitous 2-Cys peroxiredoxins (Prxs) can be converted alternatively from peroxidases to molecular chaperones. This conversion has been reported to occur by the formation of high molecular weight (HMW) complexes upon overoxidation of or ATP/ADP binding to 2-Cys Prxs; however, this mechanism is not well understood. We first demonstrated that upon binding to phosphatidylserine (PS) or phosphatidylglycerol (PG), dimeric human 2-Cys PrxII (hPrxII) is assembled to trefoil-shaped small oligomers (possibly hexamers) with full chaperone and null peroxidase activities. Spherical HMW complexes are formed, only when PS or PG is bound to overoxidized or ATP/ADP-bound hPrxII. The spherical HMW complexes are lipid vesicles covered with trefoil-shaped oligomers arranged in a hexagonal lattice pattern. Thus, these lipids with a net negative charge, which can be supplied by increased membrane trafficking under oxidative stress, are essential for the structural and functional switch of hPrxII.

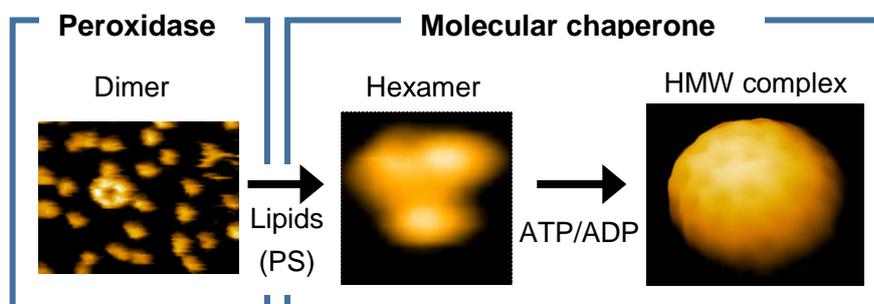


Fig. 21: The oligomeric state conversion of hPrxII by lipids and nucleotide (ADP or ATP)

T. Haruyama, T. Uchihashi, Y. Yamada, N. Kodera, T. Ando, H. Konno, *J. Mol. Biol.* 430, 602-610 (2018).

develop nanoprobe specifically for the new imaging modes. This work will be done in **collaboration with supramolecular chemists**, who will provide functional molecules and methods for their integration into a nanoprobe (③ & ④). Second, bio-SPM researchers will develop microscopes and their control systems for large-scale, high-speed and/or 3D scanning of various nanoprobe (⑤ & ⑥). Finally, the instruments developed will be used for imaging biological systems in **collaboration with life scientists** (⑧). The data obtained will be analyzed in **collaboration with computational scientists** (⑨).

• Fusion of Computational Science and Other Fields

Based on the expertise in mathematical and computational science, we will elucidate mechanisms of both biological phenomena and their nanoprobe measurements. First, computational scientists will work on complicated biological systems modeling. With the developed models, they will reproduce biological phenomena (⑪) and their nanoprobe measurements (⑩). The results obtained will be compared with experimental results (⑨) and/or expectations based on our general understanding of life science (⑫). Furthermore, they will analyze large-scale experimental data consisting of 2D movies and 3D images to elucidate important phenomena that are not obvious to the human eye (⑨). This work will be performed in **collaboration with SPM experimentalists and life scientists**.

• Fusion of Life Science and Other Fields

Based on the expertise in life science, we will elucidate nano-level mechanisms of basic cellular functions and their cancer-specific abnormalities. The life scientists will conduct their studies through complementary use of both nanoprobe techniques and other general analysis techniques (⑬ & ⑭). Some of the experimental results will be compared with those obtained by bio-simulation (⑫). Initially, nanoprobe experiments will be performed primarily using existing techniques (⑮), while the newly developed nanoprobe technologies will be used after a few years (⑧). The data obtained will be analyzed by simulation and machine learning to improve our understanding of biological phenomena (⑯ & ⑰). This work will be performed in **collaboration with SPM experimentalists and computational scientists**.

3. Establishing 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; number and state of visiting researchers; exchanges with overseas entities
- 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)

We have reviewed the previous system reform policies of the host institution and have designed/introduced new systems.

- ① **NanoLSI RP system:** Based on the current RP system at Kanazawa University, we have introduced the NanoLSI RP system and have designated to it all PIs and some associate members in order to establish a solid research base that is recognized around the world. Also, we have introduced a researcher excellence allowance system, which is based strictly on the evaluation of their research by the director of NanoLSI. These systems will meet international standards and promote international mobility among researchers.
- ② **Developing young researchers:** We have introduced a multi-field, multi-mentor system and have designed the NanoLSI transdisciplinary research promotion grant to develop young researchers in the area of Nanoprobe Life Science. Moreover, we have prepared six TT posts for international recruitment of junior PIs. Furthermore, we have begun preparation for the NanoLSI summer school, which will be based on the seven-year AFM summer school experience. Also, we have introduced the NanoLSI educational program, with the establishment of a new major in the Graduate School of Frontier Science Initiative in mind, and are applying for the grant of "Excellent Graduate School Program".
- ③ **Improved research exchange and visibility:** Prior to the kickoff symposium—the 1st NanoLSI International Symposium (NanoLSI 1st), which was held in Tokyo in February—we have held three international symposiums on Bio-SPM, cancer research, and supramolecules. At the UBC-KU joint symposium, which was held at the University of British Columbia in January, where a satellite research center is to be opened, two PIs delivered lectures. Moreover, in FY2017, we organized 11 NanoLSI Open Seminars, which included foreign lecturers from various fields and 215 participants, and we have begun organizing the 2nd NanoLSI international symposium (NanoLSI 2nd) in collaboration with Imperial College London.

4. Reforming the research organization

* 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.

* Please describe the center's operation and the host institution's commitment to the system reforms.

We worked on a NanoLSI system reform that integrated Kanazawa University system reform and reorganization.

① **Management strategy:** As a steering panel for research plans, we have established a Research initiative board consisting of the center director, the administrative director, and four PIs. Additionally, we have installed WG for each priority policy, which enables us to establish an effective decision-making process and to secure prompt implementation.

② **Systematic equipment operation:** Last January, Kanazawa University founded the IT Department which manages all technical staff of the university. This department will support the preparation of a technical environment for the Bio-SPM collaborative research proposal that maximizes the utility of the equipment installed this year. We will issue the first call for proposals in April 2018.

③ **Ripple effect:** NanoLSI was born of efforts toward various system reforms and advanced integration of various research fields at the Institute for Frontier Science Initiative (InFiniti). The path to the establishment of NanoLSI became the model for the emergence of new interdisciplinary research fields. Recently, at Kanazawa University, a new research base—the Nano Material Science Institute (tentative name)—has been envisioned as part of the drastic reorganization currently taking place.

5. Efforts to secure the center's future development over the mid- to long-term

* Please 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 the 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.

The outlook for the research plans is shown in **Section 1**. For strategic management, three activities—interdisciplinary research promotion, development of young researchers, and globalization—were considered essential; with mid- to long-term perspectives in mind, we have designed the system and efforts described in **3**, **4②**, and **7**. The NanoLSI alliance, the NanoLSI fellowship program, the Bio-SPM collaborative research proposal, NanoLSI summer school, and the NanoLSI educational program will surely contribute significantly to these three essential subjects and are directly related to the enrichment of the research environment at NanoLSI. Permanence of NanoLSI as an organization has already been declared through the commitment of the host institution. Presently, from a mid- to long-term perspective, in addition to the development of a global research environment (**3**) and NanoLSI-specific system reform (**4① & ②**), Kanazawa University has reassigned researchers and administrative staff to full-time positions for NanoLSI. Moreover, it secure funding for personnel and research projects and ensure research space to solidify NanoLSI as an independent organization.

6. Others

* In addition to the above 1-5 evaluation items, only if there is anything else that deserves mention regarding the center project's progress, please note it.

Not applicable.

7. Center's response to the follow up results in last year

* Transcribe the item from the "Advice/ recommendations" section in the site visit report and "Actions required and recommendations" in the Follow-up report, then note how the center has responded to them.

* For the center launched in FY 2017, please describe the status of response to the pointed items in "Major points that need to be improved" of "The screening result for WPI centers launched in FY 2017."

* However, if you have already provided this information, please indicate where in the report.

Comment 1. *Bio-imaging is a rapidly developing field, one in which Bio-SPM is not the only tool used for research. Both the progress and competitiveness of the proposed nanoendoscope technique for 3D real-time imaging should be constantly monitored to ensure that it stays at the frontier of bio-imaging over the 10-year period of WPI funding.*

① **Invited presentations by bio-imaging researchers at seminar and symposium:** To keep us updated on the recent progress in various bio-imaging techniques, we regularly invite top bio-imaging researchers to present at our seminar and symposium. Presentations given in this FY are listed below.

- **Cryo-EM** ▶ 2017.11.8-10: Prof. Andre Hoelz (California Institute of Technology, USA)
 - **Fluorescent OM** ▶ 2018.3.9: Prof. Masaru Ishii (Osaka Univ.)
 - **PET Molecular Imaging** ▶ 2018.3.16: Prof. Hidefumi Mukai (Riken.)
- ② **Advisory board members from bio-imaging research field:** To ensure objective opinions from experts on various bio-imaging techniques, we selected top bio-imaging researchers to serve as advisory board members.
- **Bio-SPM:** Peter Hinterdorfer (Univ. of Linz); Ricardo Garcia (CSIC, Spain); Hermann Gaub (LMU, Munchen)
 - **Cryo-EM:** Kei-ichi Namba (Osaka Univ.); Jun-ichi Takagi (Osaka Univ.)
 - **Fluorescent OM:** Yoshie Harada (Osaka Univ.); Michiyuki Matsuda (Kyoto Univ.)
- ③ **NanoLSI alliance:** To enhance communications with other bio-imaging researchers, we make an alliance contract with other life science institutes having a strength in bio-imaging technique. In this FY, we reached an agreement with RIKEN Center for Biosystems Dynamics Research(BDR) toward collaboration (signed in May). We also started talks with the Institute for Integrated Cell-Material Sciences in Kyoto University (iCeMS) for collaboration. We plan to make a similar contract with other institutes in Japan and overseas.

Comment 2. *Although cancer cells are stated to be a major target of the research with Bio-SPM, the Committee members pointed out that using the technique to acquire more information on basic cell biology is needed before applying it to cancer cells. The proposed PIs dealing with cell biology all belong to the Cancer Institute of Kanazawa University. It is highly recommended that basic cell biologists including structure biologists be invited from national or international institutions.*

- ① **Basic cell biologists at NanoLSI:** We would like to clarify that not all of our life science PIs belong to the Cancer Research Institute (CRI). For example, Profs. R. Wong and R. Hanayama are basic cell biologists who do not belong to CRI. In addition to these PIs, we have several experts in basic cell biology, which are listed below.
- **Associate Prof.:** Azuma Taoka
 - **Assistant Profs.:** Masaharu Hazawa, Hironori Kawahara, Tomoyoshi Yamano, Takeshi Yoshida
- ② **Advisory board members from structural biology:** To obtain practical advice from structural biologists of various specialties, we invited leading researchers in structural biology to be members of our advisory board, as listed below.
- **Cryo-EM:** Kei-ichi Namba (Osaka Univ.)
 - **Cryo-EM & X-ray Crystallography:** Jun-ichi Takagi (Osaka Univ.)
- ③ **NanoLSI fellowship program:** We established a research fellowship program whereby we invite various structural biologists—accompanied by their own postdocs, RAs, etc.—to stay for a few months with financial support to perform collaborative life science research using our nanoprobe techniques. For FY2018, we have already begun advertising this position worldwide. This new program will allow us to form tight connections with structural biologists of various specialties, improve the visibility of our institute, and become familiar with the demands for our existing and newly-developed nanoprobe technologies in life science. We are certain that this fellowship program will be more effective than a 10-year contract in promoting our reputation worldwide and establishing the academic field of Nano Life Science.

Comment 3. *This is a very challenging project so it is not entirely clear whether the development of a nanoprobe will necessarily lead to new understanding of life science phenomena or cancer disease modeling. Work on normal cells to define the role of this technology is considered to be very important.*

- ① **Multiple steps towards establishment of novel nanoprobe technologies:** We do not plan to limit the applications of the developed techniques to studies on cancer cells. Instead, we will explore the possibilities of imaging with various scales and complexities. For example, in the case of the nanoendoscopic imaging technique, we will work with four different types of samples: model 3D structures consisting of carbon nanotubes, chromosomes, isolated nuclei, and live cells. More detailed explanations of all nanoprobe technologies are provided in the research progress report at pp. 3-14.
- ② **Comparison between normal and cancer cells:** Even if we can visualize nanodynamics in a normal cell, correlation with specific cellular functions would be difficult. Therefore, we plan to compare the results of measurements taken in both normal and cancer cells. From any differences between them, we will gain important clues for elucidations of the mechanisms of cellular functions and their cancer-specific abnormalities. Based on this idea, we determined two major research foci: a

nano-level understanding of ① basic cellular functions and ② cancer development and progression, as shown in Fig. 1 at page 3. For the detailed plans to achieve these goals, please refer to the research progress report at pp. 10-13.

Comment 4. *It is vital to open the technology up to wider circles of life and biomedical sciences, not necessarily focused on cancer. This may be realized by forming strategic alliances with related research groups and institutions.*

- ① **NanoLSI alliance:** To promote the complementary use of various bio-imaging techniques, we began the formation of the NanoLSI Alliance. This will allow life scientists in other bio-imaging institutes to use our advanced nanoprobe technology for their own research. The progress of this project is described in the response to Comment 1.
- ② **Open facilities:** We established an "Open Facility" policy to promote collaborative work among researchers from various disciplines and countries. To this end, in FY2017, we prepared several bio-SPM instruments for common use. We also hired several technical staff and one URA to support the research activities and management positions.
- ③ **Call for proposals for international collaborative research:** We established a system to regularly call for proposals for collaborative life science research using our nanoprobe technologies. Here, the instruments and staff members of Open Facility will play major roles in the research or administrative activities. So far, we have designed the system, prepared the application forms. We will issue the first call for proposals in April 2018.

Comment 5. *WPI is not only a vehicle for supporting scientific proposals but it is also an instrument for reforming conventional university systems. From this point of view, your proposal places NanoLSI within an already-reformed system at Kanazawa University and does not seem to include any idea of further reforming the university system. How the proposed NanoLSI breaks the university's traditional research culture based on departments, allows the introduction of fresh blood from outside, and enhances dynamic collaboration with other institutions needs to be clarified.*

Activities of NanoLSI, including the NanoLSI RP system (3①), the development of young researchers (3②), NanoLSI-specific system reform (4①), and efforts noted in 5 to plan mid- to long-term development of the research base were all promoted toward the establishment of a global research environment. Moreover, all of these activities are based on an entirely new system design. Especially, the NanoLSI-specific researcher excellence allowance system and the NanoLSI RP system, which are integrated with strict work evaluations from the center director, are new developments in human resource and salary systems. Additionally, the reassignment of researchers and administrative staff is a breakthrough for the university's traditional departmental research culture. Finally, the international research exchange (3③), the Bio-SPM collaborative research proposal program based on open facilities (4②), and efforts discussed in 7 all contribute to the introduction of fresh blood from the outside and to enhancing dynamic collaboration with other institutions. Such bold efforts have a significant impact on the host institution, which is evidenced by a new vision to establish another interdisciplinary research institute, as shown in 4③.

Appendix 1 FY2017 List of Center's Research Results and Main Awards

1. Refereed Papers

- List only the Center's papers published in 2017. (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 the name of his/her WPI center). (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.)

Newly selected centers are to list papers under category C below (in addition to categories A and B above).

C. Previously published important WPI-related papers

List previously published papers that provided the basis for the center's research project plan. (Around 30 papers as a yardstick.)

Note: On 14 December 2011, the Basic Research Promotion Division in MEXT's Research Promotion Bureau circulated an instruction requiring paper authors to include the name or abbreviation of their WPI center among their institutional affiliations. As some WPI-affiliated authors of papers published up to 2011 may not be aware of this requirement, their papers are treated as "WPI-related papers." From 2012, however, the authors' affiliations must be clearly noted and only category A papers will be basically listed.

(2) Method of listing paper

- List only referred papers. Divide them into categories (e.g., original articles, reviews, proceedings).

- For each, write the author name(s); year of publication; journal name, volume, page(s), and article title. Any listing order may be used as long as format is the same. (The names of the center researchers do not need to be underlined.)

- If a paper has many authors (say, more than 20), all of their names do not need to be listed.

- If the papers are written in languages other than English, divide them into paper's categories when listing them.

- Assign a serial number to each paper to be used to identify it throughout the system.

- Order of Listing

A. WPI papers

1. Original articles

2. Review articles

3. Proceedings

4. Other English articles

5. Articles written in other than English

B. WPI-related papers

1. Original articles

2. Review articles

3. Proceedings

4. Other English articles

5. Articles written in other than English

C. Previously published important WPI-related papers

(3) Submission of electronic data

- In addition to the above, provide a .csv file output from the Web of Science (e.g.) or other database giving the paper's raw data including Document ID. (Note: the Document ID is assigned by paper database.)

- These files do not need to be divided into paper categories.

(4) Use in assessments

- The lists of papers will be used in assessing the state of WPI project's progress in FY 2017.

- 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

None

B. WPI-related papers

1. Original article

- 1) Cadinu, P; Nadappuram, BP; Lee, DJ; Sze, JYY; Campolo, G; Zhang, Y; Shevchuk, A; Ladame, S; Albrecht, T; Korchev, Y; Ivanov, AP; Edel, JB "Single Molecule Trapping and Sensing Using Dual Nanopores Separated by a Zeptoliter Nanobridge", Nano Lett. 17 (2017) 6376-6384 (IF=12.712)
- 2) Terpstra, AS; Hamad, WY; MacLachlan, MJ "Photopatterning Freestanding Chiral Nematic Mesoporous Organosilica Films", Adv. Funct. Mater. 27 (2017) 1703346 (IF=12.124)
- 3) 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", *Nat. Commun.* 8 (2017) 1430 (IF=12.124)
- 4) Umeda, K; Zivanovic, L; Kobayashi, K; Ritala, J; Kominami, H; Spijker, P; Foster, AS; Yamada, H "Atomic-resolution three-dimensional hydration structures on a heterogeneously charged surface", *Nat. Commun.* 8 (2017) 2111 (IF=12.124)
 - 5) Jahangiri, A; Nguyen, A; Chandra, A; Sidorov, MK; Yagnik, G; Rick, J; Han, SW; Chen, W; Flanigan, PM; Schneidman-Duhovny, D; Mascharak, S; De Lay, M; Imber, B; Park, CC; Matsumoto, K; Lu, K; Bergers, G; Sali, A; Weiss, WA; Aghi, MK "Cross-activating c-Met/beta 1 integrin complex drives metastasis and invasive resistance in cancer", *Proc. Natl. Acad. Sci. U. S. A.* 114 (2017) E8685-E8694 (IF=9.661)
 - 6) Nakayama, M; Sakai, E; Echizen, K; Yamada, Y; Oshima, H; Han, TS; Ohki, R; Fujii, S; Ochiai, A; Robine, S; Voon, DC; Tanaka, T; Taketo, MM; Oshima, M "Intestinal cancer progression by mutant p53 through the acquisition of invasiveness associated with complex glandular formation", *Oncogene* 36 (2017) 5885-5896 (IF=7.519)
 - 7) Maeda, K; Shimomura, K; Ikai, T; Kanoh, S; Yashima, E "Static Memory of Enantiomeric Helices Induced in a Poly(biphenylacetylene) by a Single Enantiomer Assisted by Temperature- and Solvent-Driven Helix Inversion", *Macromolecules* 50 (2017) 7801-7806 (IF=5.835)
 - 8) Holmstrom, E; Foster, AS "Adsorption of Water onto SrTiO₃ from Periodic Moller-Plesset Second-Order Perturbation Theory", *J. Chem. Theory Comput.* 13 (2017) 6301-6307 (IF=5.245)
 - 9) Cirillo, F; Pellegrino, M; Malivindi, R; Rago, V; Avino, S; Muto, L; Dolce, V; Vivacqua, A; Rigracciolo, DC; De Marco, P; Sebastiani, A; Abonante, S; Nakajima, M; Lappano, R; Maggiolini, M "GPER is involved in the regulation of the estrogen-metabolizing CYP1B1 enzyme in breast cancer", *Oncotarget* 8 (2017) 106608-106624 (IF=5.168)
 - 10) Dong, Y; Furuta, T; Sabit, H; Kitabayashi, T; Jiapaer, S; Kobayashi, M; Ino, Y; Todo, T; Teng, L; Hirao, A; Zhao, SG; Nakada, M "Identification of antipsychotic drug fluspirilene as a potential anti-glioma stem cell drug", *Oncotarget* 8 (2017) 111728-111741 (IF=5.168)
 - 11) Adachi, Y; Watanabe, K; Kita, K; Kitai, H; Kotani, H; Sato, Y; Inase, N; Yano, S; Ebi, H "Resistance mediated by alternative receptor tyrosine kinases in FGFR1-amplified lung cancer", *Carcinogenesis* 38 (2017) 1063-1072 (IF=5.105)
 - 12) Nakanishi, T; Ohno, Y; Aotani, R; Maruyama, S; Shimada, H; Kamo, S; Oshima, H; Oshima, M; Schuetz, JD; Tamai, I "A novel role for OATP2A1/SLCO2A1 in a murine model of colon cancer", *Sci Rep* 7 (2017) 16567 (IF=4.259)
 - 13) Suzuki, Y; Kitahara, S; Suematsu, T; Oshima, M; Sato, Y "Requisite role of vasohibin-2 in spontaneous gastric cancer formation and accumulation of cancer-associated fibroblasts", *Cancer Sci.* 108 (2017) 2342-2351 (IF=3.974)
 - 14) Ogoshi, T; Shimada, Y; Akutsu, T; Yamagishi, T "A rod-like phenolic pi-conjugated polymer with pillar[5] arene units in its main chain", *Polymer* 128 (2017) 325-329 (IF=3.684)
 - 15) Mehr, SHM; Oshima, H; Carta, V; Patrick, BO; White, NG; MacLachlan, MJ "Tuning the tautomeric behavior of tris (salicylaldimines)", *Org. Biomol. Chem.* 15 (2017) 8418-8424 (IF=3.564)
 - 16) Ikai, T; Wada, Y; Awata, S; Yun, C; Maeda, K; Mizuno, M; Swager, TM "Chiral triptycene-pyrene pi-conjugated chromophores with circularly polarized luminescence", *Org. Biomol. Chem.* 15 (2017) 8440-8447 (IF=3.564)
 - 17) Asakawa, H; Inada, N; Hirata, K; Matsui, S; Igarashi, T; Oku, N; Yoshikawa, N; Fukuma, T "Self-assembled monolayers of sulfonate-terminated alkanethiols investigated by frequency modulation atomic force microscopy in liquid", *Nanotechnology* 28 (2017) 455603 (IF=3.44)
 - 18) Kita, K; Arai, S; Nishiyama, A; Taniguchi, H; Fukuda, K; Wang, R; Yamada, T; Takeuchi, S; Tange, S; Tajima, A; Nakada, M; Yasumoto, K; Motoo, Y; Murakami, T; Yano, S "In vivo imaging xenograft models for the evaluation of anti-brain tumor efficacy of targeted drugs", *Cancer Med.* 6 (2017) 2972-2983 (IF=3.362)
 - 19) Yamaura, Y; Tatsumi, N; Takagi, S; Tokumitsu, S; Fukami, T; Tajiri, K; Minemura, M; Yokoi, T; Nakajima, M "Serum microRNA profiles in patients with chronic hepatitis B, chronic hepatitis C, primary biliary cirrhosis, autoimmune hepatitis, nonalcoholic steatohepatitis, or drug-induced liver injury", *Clin. Biochem.* 50 (2017) 1034-1039 (IF=2.434)
 - 20) Mikhailov, AS; Koyano, Y; Kitahata, H "Hydrodynamic Effects in Oscillatory Active Nematics", *J. Phys. Soc. Jpn.* 86 (2017) 101013 (IF=1.45)
 - 21) 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", *Sci. Adv.* 3 (2017) eaao4119 (IF=N/A)

2. Review article

- 22) Imamura, R; Matsumoto, K "Hepatocyte growth factor in physiology and infectious diseases", *Cytokine* 98 (2017) 97-106 (IF=3.488)
- C. Previously published important WPI-related papers
- 23) Dufrene, YF; Ando, T; Garcia, R; Alsteens, D; Martinez-Martin, D; Engel, A; Gerber, C; Muller, DJ "Imaging modes of atomic force microscopy for application in molecular and cell biology", *Nat. Nanotechnol.* 12 (2017) 295-307 (IF=38.986)
- 24) Murray, J; Kim, K; Ogoshi, T; Yao, W; Gibb, BC "The aqueous supramolecular chemistry of cucurbit[n]urils, pillar[n]arenes and deep-cavity cavitands", *Chem. Soc. Rev.* 46 (2017) 2479-2496 (IF=38.618)
- 25) Kon, S; Ishibashi, K; Katoh, H; Kitamoto, S; Shirai, T; Tanaka, S; Kajita, M; Ishikawa, S; Yamauchi, H; Yako, Y; Kamasaki, T; Matsumoto, T; Watanabe, H; Egami, R; Sasaki, A; Nishikawa, A; Kameda, I; Maruyama, T; Narumi, R; Morita, T; Sasaki, Y; Enoki, R; Honma, S; Imamura, H; Oshima, M; Soga, T; Miyazaki, J; Duchen, MR; Nam, JM; Onodera, Y; Yoshioka, S; Kikuta, J; Ishii, M; Imajo, M; Nishida, E; Fujioka, Y; Ohba, Y; Sato, T; Fujita, Y "Cell competition with normal epithelial cells promotes apical extrusion of transformed cells through metabolic changes", *Nat. Cell Biol.* 19 (2017) 500+ (IF=20.06)
- 26) Loo, TM; Kamachi, F; Watanabe, Y; Yoshimoto, S; Kanda, H; Arai, Y; Nakajima-Takagi, Y; Iwama, A; Koga, T; Sugimoto, Y; Ozawa, T; Nakamura, M; Kumagai, M; Watashi, K; Taketo, MM; Aoki, T; Narumiya, S; Oshima, M; Arita, M; Hara, E; Ohtani, N "Gut Microbiota Promotes Obesity-Associated Liver Cancer through PGE(2)-Mediated Suppression of Antitumor Immunity", *Cancer Discov.* 7 (2017) 522-538 (IF=20.011)
- 27) Hiratani, T; Hamad, WY; MacLachlan, MJ "Transparent Depolarizing Organic and Inorganic Films for Optics and Sensors", *Adv. Mater.* 29 (2017) 1606083 (IF=19.791)
- 28) Kawai, S; Takahashi, K; Ito, S; Pawlak, R; Meier, T; Spijker, P; Canova, FF; Tracey, J; Nozaki, K; Foster, AS; Meyer, E "Competing Annulene and Radialene Structures in a Single Anti-Aromatic Molecule Studied by High-Resolution Atomic Force Microscopy", *ACS Nano* 11 (2017) 8122-8130 (IF=13.942)
- 29) Mohamed, MS; Kobayashi, A; Taoka, A; Watanabe-Nakayama, T; Kikuchi, Y; Hazawa, M; Minamoto, T; Fukumori, Y; Kodera, N; Uchihashi, T; Ando, T; Wong, RW "High-Speed Atomic Force Microscopy Reveals Loss of Nuclear Pore Resilience as a Dying Code in Colorectal Cancer Cells", *ACS Nano* 11 (2017) 5567-5578 (IF=13.942)
- 30) Akine, S; Miyashita, M; Nabeshima, T "A Metallo-molecular Cage That Can Close the Apertures with Coordination Bonds", *J. Am. Chem. Soc.* 139 (2017) 4631-4634 (IF=13.858)
- 31) Ogoshi, T; Shimada, Y; Sakata, Y; Akine, S; Yamagishi, TA "Alkane-Shape-Selective Vapochromic Behavior Based on Crystal-State Host-Guest Complexation of Pillar[5]arene Containing One Benzoquinone Unit", *J. Am. Chem. Soc.* 139 (2017) 5664-5667 (IF=13.858)
- 32) Miyata, K; Tracey, J; Miyazawa, K; Haapasilta, V; Spijker, P; Kawagoe, Y; Foster, AS; Tsukamoto, K; Fukuma, T "Dissolution Processes at Step Edges of Calcite in Water Investigated by High-Speed Frequency Modulation Atomic Force Microscopy and Simulation", *Nano Lett.* 17 (2017) 4083-4089 (IF=12.712)
- 33) Sakata, Y; Murata, C; Akine, S "Anion-capped metallohost allows extremely slow guest uptake and on-demand acceleration of guest exchange", *Nat. Commun.* 8 (2017) 16005 (IF=12.124)
- 34) Ren, R; Zhang, YJ; Nadappuram, BP; Akpınar, B; Klenerman, D; Ivanov, AP; Edel, JB; Korchev, Y "Nanopore extended field-effect transistor for selective single-molecule biosensing", *Nat. Commun.* 8 (2017) 586 (IF=12.124)
- 35) Murayama, T; Masuda, T; Afonin, S; Kawano, K; Takatani-Nakase, T; Ida, H; Takahashi, Y; Fukuma, T; Ulrich, AS; Futaki, S "Loosening of Lipid Packing Promotes Oligoarginine Entry into Cells", *Angew. Chem.-Int. Edit.* 56 (2017) 7644-7647 (IF=11.994)
- 36) Vivekananda, U; Novak, P; Bello, OD; Korchev, YE; Krishnakumar, SS; Volynski, KE; Kullmann, DM "Kv1.1 channelopathy abolishes presynaptic spike width modulation by subthreshold somatic depolarization", *Proc. Natl. Acad. Sci. U. S. A.* 114 (2017) 2395-2400 (IF=9.661)
- 37) Tanimoto, A; Takeuchi, SJ; Arai, S; Fukuda, K; Yamada, T; Roca, X; Ong, ST; Yano, S "Histone Deacetylase 3 Inhibition Overcomes BIM Deletion Polymorphism-Mediated Osimertinib Resistance in EGFR-Mutant Lung Cancer", *Clin. Cancer Res.* 23 (2017) 3139-3149 (IF=9.619)
- 38) Lizundia, E; Nguyen, TD; Vilas, JL; Hamad, WY; MacLachlan, MJ "Chiroptical, morphological and conducting properties of chiral nematic mesoporous cellulose/polypyrrole composite films", *J. Mater. Chem. A* 5 (2017) 19184-19194 (IF=8.867)
- 39) Naka, K; Hirao, A "Regulation of Hematopoiesis and Hematological Disease by TGF-beta Family Signaling Molecules", *Cold Spring Harbor Perspect. Biol.* 9 (2017) a027987 (IF=8.769)

- 40) Hazawa, M; Lin, DC; Handral, H; Xu, L; Chen, Y; Jiang, YY; Mayakonda, A; Ding, LW; Meng, X; Sharma, A; Samuel, S; Movahednia, MM; Wong, RW; Yang, H; Tong, C; Koeffler, HP "ZNF750 is a lineage-specific tumour suppressor in squamous cell carcinoma", *Oncogene* 36 (2017) 2243-2254 (IF=7.519)
- 41) West, AC; Tang, K; Tye, H; Yu, L; Deng, N; Najdovska, M; Lin, SJ; Balic, JJ; Okochi-Takada, E; McGuirk, P; Keogh, B; McCormack, W; Bhathal, PS; Reilly, M; Oshima, M; Ushijima, T; Tan, P; Jenkins, BJ "Identification of a TLR2-regulated gene signature associated with tumor cell growth in gastric cancer", *Oncogene* 36 (2017) 5134-5144 (IF=7.519)
- 42) Nguyen, TD; Hamad, WY; MacLachlan, MJ "Near-IR-Sensitive Upconverting Nanostructured Photonic Cellulose Films", *Adv. Opt. Mater.* 5 (2017) 1600514 (IF=6.875)
- 43) Mihara, N; Yamada, Y; Akine, S; Sugimoto, K; Tanaka, K "Electronic perturbation of supramolecular conjugates of porphyrins and phthalocyanines", *Chem. Commun.* 53 (2017) 2230-2232 (IF=6.319)
- 44) Kakuta, T; Yamagishi, T; Ogoshi, T "Supramolecular chemistry of pillar[n] arenes functionalised by a copper(I)-catalysed alkyne-azide cycloaddition "click" reaction", *Chem. Commun.* 53 (2017) 5250-5266 (IF=6.319)
- 45) Sakata, Y; Kobayashi, S; Akine, S "Two-step modulation of ion recognition using a bis(saloph)-macrocylic host having a 24-crown-8-like cavity", *Chem. Commun.* 53 (2017) 6363-6366 (IF=6.319)
- 46) Tsuneishi, C; Koizumi, Y; Sueto, R; Nishiyama, H; Yasuhara, K; Yamagishi, T; Ogoshi, T; Tomita, I; Inagi, S "The controlled synthesis of pillar[6] arene-based hexagonal cylindrical structures on an electrode surface via electrochemical oxidation", *Chem. Commun.* 53 (2017) 7454-7456 (IF=6.319)
- 47) Ogoshi, T; Sueto, R; Hamada, Y; Doitomi, K; Hirao, H; Sakata, Y; Akine, S; Kakuta, T; Yamagishi, T "Alkane-length sorting using activated pillar[5]arene crystals", *Chem. Commun.* 53 (2017) 8577-8580 (IF=6.319)
- 48) Sekino, M; Furutachi, H; Tojo, R; Hishi, A; Kajikawa, H; Suzuki, T; Suzuki, K; Fujinami, S; Akine, S; Sakata, Y; Ohta, T; Hayami, S; Suzuki, M "New mechanistic insights into intramolecular aromatic ligand hydroxylation and benzyl alcohol oxidation initiated by the well-defined (mu-peroxo)diiron(III) complex", *Chem. Commun.* 53 (2017) 8838-8841 (IF=6.319)
- 49) Meseck, GR; Terpstra, AS; MacLachlan, MJ "Liquid crystal templating of nanomaterials with nature's toolbox", *Curr. Opin. Colloid Interface Sci.* 29 (2017) 9-20 (IF=6.136)
- 50) Nanjo, S; Arai, S; Wang, W; Takeuchi, S; Yamada, T; Hata, A; Katakami, N; Okada, Y; Yano, S "MET Copy Number Gain Is Associated with Gefitinib Resistance in Leptomeningeal Carcinomatosis of EGFR-mutant Lung Cancer", *Mol. Cancer Ther.* 16 (2017) 506-515 (IF=5.764)
- 51) Ikai, T; Suzuki, D; Shinohara, K; Maeda, K; Kanoh, S "A cellulose-based chiral fluorescent sensor for aromatic nitro compounds with central, axial and planar chirality", *Polym. Chem.* 8 (2017) 2257-2265 (IF=5.375)
- 52) Ikai, T; Awata, S; Kudo, T; Ishidate, R; Maeda, K; Kanoh, S "Chiral stationary phases consisting of pi-conjugated polymers bearing glucose-linked biphenyl units: reversible switching of resolution abilities based on a coil-to-helix transition", *Polym. Chem.* 8 (2017) 4190-4198 (IF=5.375)

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

- List up to 10 main presentations during FY2017 in order from most recent.
- For each, write the lecturer/presenter's name, presentation title, conference name and date(s)

- 1) **Oshima M**, "Comprehensive phenotype characterization of intestinal tumors with various driver mutations", International Society of Precision Cancer Medicine (ISPCM) Annual Meeting (Busan, Korea), Mar. 17, 2018
- 2) **Adam Foster**, "Molecularly functionalized surfaces and interfaces", DPG Spring Meeting 2018 (Berlin, Germany), Mar. 14, 2018
- 3) **Shigehisa Akine**, "Metal-containing host molecules that can close their apertures", Strasbourg-Kanazawa Universities Joint Symposium on Organic Synthesis (Strasbourg, France), Feb. 8, 2018
- 4) **Toshio Ando**, "Dynamic GroEL-GroES Interaction and Its Dependence on Substrate Protein", XX Linz Winter Workshop (Linz, Austria), Feb. 2-5, 2018
- 5) **Takeshi Fukuma**, "Recent Progress in FM-AFM and 3D-AFM for Visualizing Structures and Dynamics at Solid-Liquid Interfaces", XX. Annual Linz Winter Workshop (Linz, Austria), Feb. 2-5, 2018
- 6) **Atsushi Hirao**, "Regulation of stem cell properties by metabolic signals in hematopoietic neoplasm", JSPS - National University of Singapore (NUS) 2nd Symposium (Kumamoto, Japan), Jan. 18-20, 2018
- 7) **Rikinari HANAYAMA**, "Propagation of disease-related molecules via exosomes", 21st Annual Meeting of the Japanese Society of Cardiovascular Endocrinology and Metabolism (Osaka, Japan), Dec. 10, 2017 (Plenary lecture)
- 8) **Takeshi Fukuma**, "Recent Progress in Liquid-Environment FM-AFM and Its Related Techniques", MRS Fall Meeting 2017 (Boston, USA), Nov. 29, 2017
- 9) **Oshima M**, "Comprehensive phenotypic characterization of intestinal cancer with multiple driver mutations", 48th International Symposium of the Princess Takamatsu Cancer Research Fund (Tokyo, Japan), Nov. 7, 2017
- 10) **Tomoki Ogoshi**, "Synthesis of Pillar-Shaped Macrocyclic Compounds "Pillar[n]arene s" Providing Supramolecular Assemblies", MBLA Lectureship, Ludwig-Maximilians-Universität Hosted by Prof. Paul Knochel (Munich, Germany), Oct. 9, 2017

3. Major Awards

- List up to 10 main awards received during FY2017 in order from the most recent.
- For each, write the recipient's name, name of award, and year issued.
- In case of multiple recipients, underline those affiliated with the center.

- 1) Noriyuki Kodera, The 14th JSPS PRIZE, Dec. 26, 2017
- 2) Takeshi Fukuma, The 71st Hokkoku Culture Prize, Nov. 3, 2017
- 3) Yasufumi Takahashi, The 1st Bioindustry Research Award, Jul. 13, 2017

Appendix 2 FY 2017 List of Principal Investigators

NOTE:

- Underline names of principal investigators who belong to an overseas research institution. Place an asterisk (*) by names of investigators considered to be ranked among world's top researchers.
- In case of researchers not listed in the latest report, attach "Biographical Sketch of a New Principal Investigator".

		<Results at the end of FY2017>				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*	41	Nano Life Science Institute, Institute for Frontier Science Initiative	Doctor of Engineering, Electrical engineering, Nanometrology	90	October, 2017	usually stays at the center	
<u>Adam Stuart Foster*</u>	42	Department of Applied Physics, Aalto University	PhD in Theoretical Solid State Physics	30	October, 2017	Participates at head office (11- day stay until March 31, 2018, and for 30 days/year from April 1 onward (scheduled))	Promotes understanding of the real image from the observation image while working toward the development of new nanoprobe technology
<u>Korchev Yuri*</u>	57	Department of Medicine, Imperial College London	Ph.D. in Biophysics and Cytology, Biophysics	30	October, 2017	Participates at head office (17- day stay until March 31, 2018, and for 30 days/year from April 1 onward (scheduled))	Engaged in measuring the distribution of substances inside and outside the cell while working toward the development of new nanoprobe technology
<u>Mark MacLachlan*</u>	44	Department of Chemistry, University of British Columbia	PhD in Chemistry	10	October, 2017	Participates at head office (15- day stay until March 31, 2018, and for 30 days/year from April 1 onward (scheduled))	Engaged in development of supramolecular nanoprobe while working toward the development of new nanoprobe technology
<u>Alexander S. Mikhailov*</u>	67	Department of Physical Chemistry, Fritz Haber Institute of the Max Planck Society	Doctor of Science, Theoretical Physics, Chemical Physics, Biophysics	40	October, 2017	Participates at head office (59- day stay until March 31, 2018, and for 90 days/year from April 1 onward (scheduled))	Promotes understanding of the real image from the observation image while working toward the development of new nanoprobe technology. In addition, twice supervised a public seminar with researchers invited from overseas, contributing to the promotion of integrated research

Richard W. Wong*	43	Nano Life Science Institute, Institute for Frontier Science Initiative	Doctor of Medicine, Molecular cell biology	90	October, 2017	usually stays at the center	
Toshio ANDO*	67	Nano Life Science Institute, Institute for Frontier Science Initiative	Doctor of Science, Biophysics and Nano-Bioscience	90	October, 2017	usually stays at the center	
Rikinari Hanayama*	43	Nano Life Science Institute, Institute for Frontier Science Initiative	MD, PhD, Immunology, Cell Biology	80	October, 2017	usually stays at the center	
Shigehisa Akine*	45	Nano Life Science Institute, Institute for Frontier Science Initiative	Doctor of Science, Supramolecular chemistry, Coordination	80	October, 2017	usually stays at the center	
Tomoki Ogoshi*	41	Nano Life Science Institute, Institute for Frontier Science Initiative	Doctor of Engineering, Supramolecular Chemistry, Structural Organic Chemistry	80	October, 2017	usually stays at the center	
Katsuhiro Maeda*	47	Nano Life Science Institute, Institute for Frontier Science Initiative	Doctor of Engineering, Polymer chemistry	80	October, 2017	usually stays at the center	
Masanobu Oshima*	56	Nano Life Science Institute, Institute for Frontier Science Initiative	D.V.M., Ph.D., Cancer research, Genetics for Cancer modeling	80	October, 2017	usually stays at the center	
Miki Nakajima*	48	Nano Life Science Institute, Institute for Frontier Science Initiative	Doctor of Pharmaceutical Sciences, Drug Metabolism and Toxicology, Clinical Pharmacology	70	October, 2017	usually stays at the center	
Atsushi Hirao*	54	Cancer Research Institute	Doctor of Medicine, Stem Cell Biology	60	October, 2017	usually stays at the center	

Seiji Yano*	52	Cancer Research Institute	MD, PhD, Medical Oncology, Circumvention of targeted drug resistance	40	October, 2017	usually stays at the center	
Kunio Matsumoto*	59	Nano Life Science Institute, Institute for Frontier Science Initiative	Doctor of Philosophy, Biological Chemistry, Tumor Biology	90	October, 2017	usually stays at the center	

Researchers unable to participate in project in FY 2017

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

N/A

Appendix 3-1 FY 2017 Records of Center Activities

1. Researchers and center staffs, satellites, partner institutions

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

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

Special mention

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

1-2. Satellites and partner institutions

- List the satellite and partner institutions in the table below.
- Indicate newly added and deleted institutions in the "Notes" column.
- If satellite institutions have been established, 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
University of British Columbia	Mark MacLachlan	Currently under negotiation with the
Imperial College London	Yuri Korchev	institute in regards to the contents of the collaborative research agreement

< Partner institutions >

Institution name	Principal Investigator(s), if any	Notes
RIKEN Center for Biosystems Dynamics Research		already agreed to establish the collaborative research agreement (will be signed in May)

2. Securing external research funding*

External research funding secured in FY2017

Total: 518,010,990 yen

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

* External research funding includes "Grant-in-Aid for Scientific Research," funding for "commissioned research projects," and for "joint research projects" as listed under "Research projects" in Appendix 3-2, Project Expenditures.

Grant-in-Aid for Scientific Research(S): 48,620,000 yen

AMED Practical Research for Innovative Cancer Control: 29,998,000 yen

AMED Practical Research for Innovative Cancer Control: 116,953,000 yen

AMED Project for Cancer Research and Therapeutic Evolution: 40,950,000 yen

3. International research conferences or symposiums held to bring world's leading researchers together

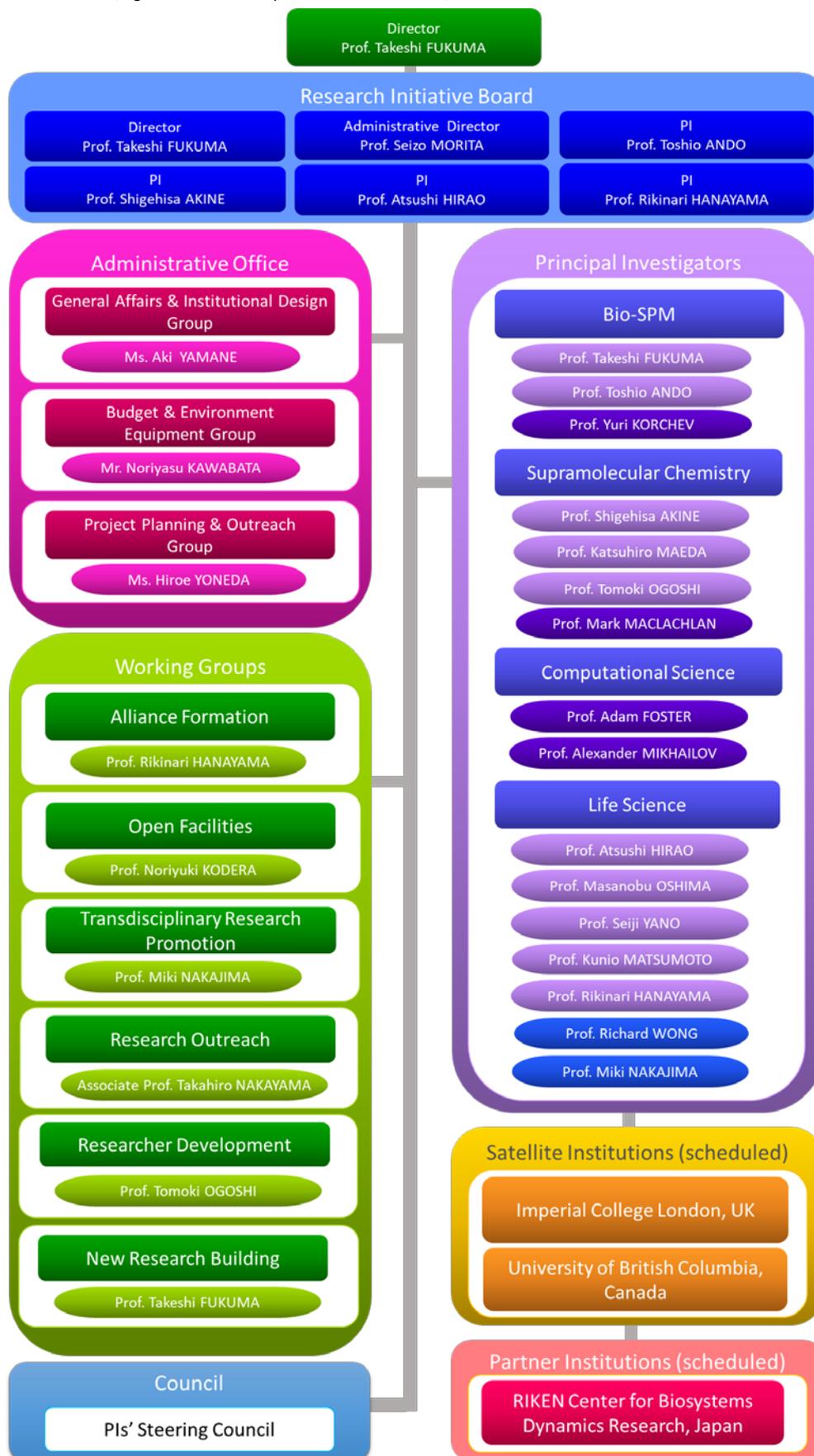
- Indicate the number of international research conferences or symposiums held in FY2017 and give up to three examples of the most representative ones using the table below.

FY 2017: 3 meetings	
Major examples (meeting titles and places held)	Number of participants
3rd International Symposium on Center of Excellence for Innovative Material Sciences Based on Supramolecules The Kanazawa Theatre (Kanazawa) 2017.10.16-17	From domestic institutions: 219 From overseas institutions: 8
International Symposium on Atomic Force Microscopy at Solid-Liquid Interfaces Kanazawa Tokyu Hotel (Kanazawa) 2017.11.8-9	From domestic institutions: 65 From overseas institutions: 11
The 1st NanoLSI International Symposium – Towards Establishment of New Research Field: Nanoprobe Life Science- National Museum of Emerging Science and Innovation (Tokyo) 2018.2.21-22	From domestic institutions: 130 From overseas institutions: 8

4. Center's management system

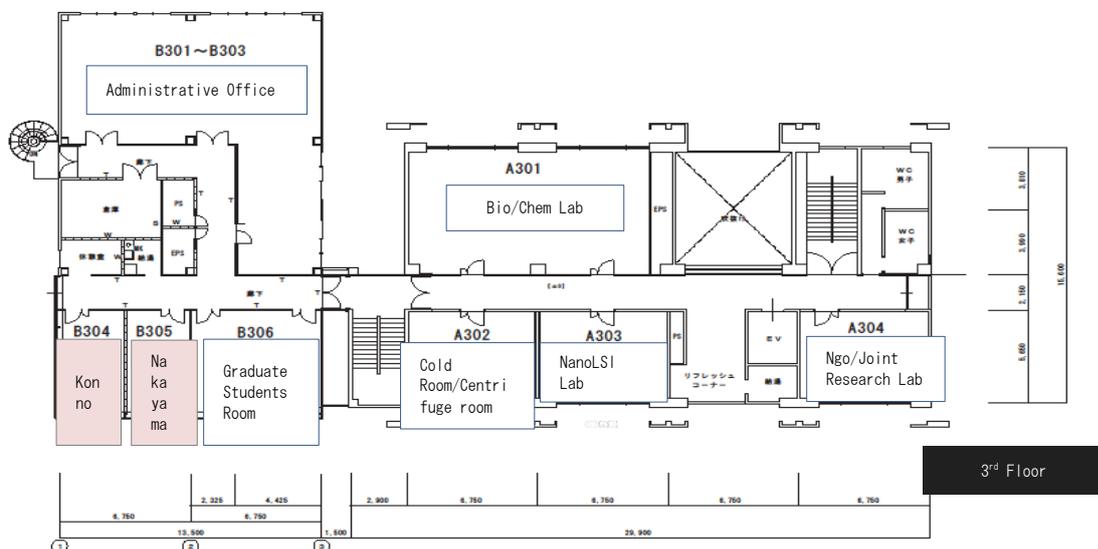
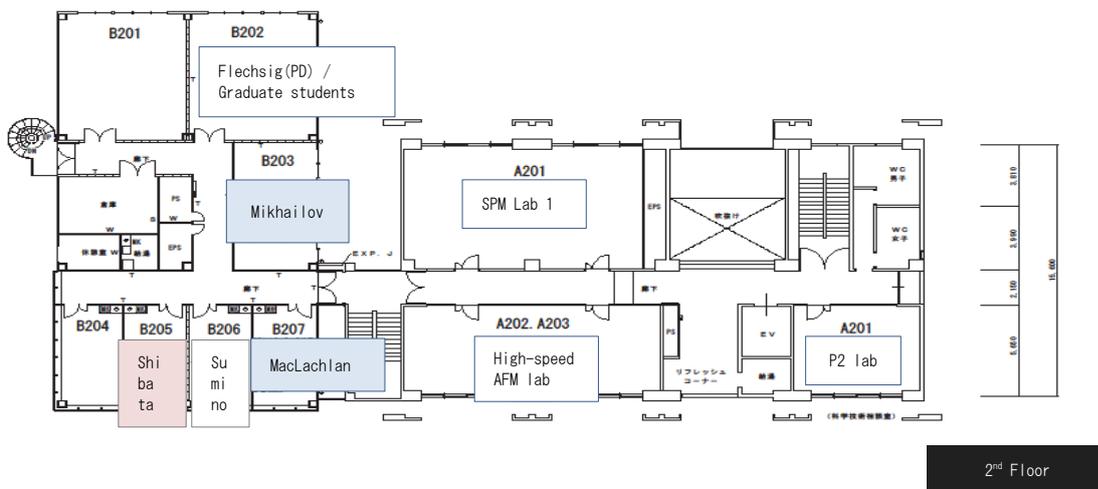
- Please diagram management system in an easily understood manner.

- If any changes have been made in the management system from that in the latest "center project," please describe them. Please 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).



5. Campus Map

- Please draw a simple map of the campus showing where the main office and principle investigator(s) are located.



Appendix 3-1a FY 2017 Records of Center Activities

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

1-1. Number of researchers and other center staffs

* Please fill in the number of researchers and other center staffs in the table below.

* Please describe the final goals for achieving these numbers and dates when they will be achieved.

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

(persons)

	At beginning of project	At end of FY 2017	Final goal (March, 2021)
Researches from within the host institution	12	12	12
Foreign researchers invited from abroad	4	4	5
Researchers invited from other Japanese institutions	0	0	5
Total principal investigators	16	16	22

b) Total members

	At beginning of project		At end of FY2017		Final goal (March, 2021)	
	Number of persons	%	Number of persons	%	Number of persons	%
Researchers	49	/	50	/	76	/
Overseas researchers	7	14.3	8	16.0	23	30.3
Female researchers	6	12.2	6	12.0	16	21.1
Principal investigators	16	/	16	/	22	/
Overseas PIs	5	31.3	5	31.3	6	27.3
Female PIs	1	6.3	1	6.3	2	9.1
Other researchers	33	/	34	/	54	/
Overseas researchers	2	6.1	3	8.8	17	31.5
Female researchers	5	15.2	5	14.7	14	25.9
Research support staffs	8	/	7	/	20	/
Administrative staffs	13	/	18	/	25	/
Total number of people who form the "core" of the research center	70	/	75	/	121	/

Appendix 3-2 Project Expenditures

1) Overall project funding

* In the "Total Cost" 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 and details may be changed to coincide with the project's actual content.

Cost Items	Details (For Personnel - Equipment please fill in the breakdown of fiscal expenditure, and the income breakdown for Research projects.)	(Million yens)		Costs (Million yens)
		Total Costs	Amount covered by WPI funding	
				WPI grant in FY 2017 700
Personnel	Center director and Administrative director	14.1	8.2	
	Principal investigators (no. of persons):11	67.6	22.0	Costs of establishing and maintaining facilities 15.6
	Other researchers (no. of persons):29	53.7	11.6	Establishing new facilities 0
	Administrative staffs (no. of persons):26	49.2	49.2	Repairing facilities 15.4
	Subtotal	184.6	91.0	Others 0.2
Project activities	Gratuities and honoraria paid to invited principal investigators (no. of persons):4	5.3	5.3	Cost of equipment procured 476.4
	Research startup cost (no. of persons):4	29.7	29.7	Cryo-FIB-SEM 129.9
	Cost of international symposiums (no. of symposiums):1	5.7	5.7	(Number of units:1)
	Rental fees for facilities	10.3	0.4	Confocal laser microscopy 75.2
	Cost of consumables	13.9	13.9	(Number of units:2)
	Other costs	53.7	53.7	Others 271.3
	Subtotal	118.6	108.7	
Travel	Domestic travel costs	1.1	1.1	
	Overseas travel costs	0.4	0.4	
	Travel and accommodations cost for invited scientists (no. of domestic scientists):8 (no. of overseas scientists):14	6.7	6.7	
	Travel cost for scientists on secondment (no. of domestic scientists):1 (no. of overseas scientists):0	0.1	0.1	
	Subtotal	8.3	8.3	
Equipment	Depreciation of buildings	0.3	0.3	
	Depreciation of equipment	5.9	5.9	
	Subtotal	6.2	6.2	
Research projects (Detail items must be fixed)	Subsidy from the National Government, etc	112.1	0.0	
	Grants-in-Aid for Scientific Research, etc.	154.2	0.0	
	Commissioned research projects, etc.	251.1	0.0	
	Joint research projects	22.4	0.0	
	Others (donations, etc.)	23.5	0.0	
	Subtotal	563.3	0.0	
	Total	881.0	214.2	

*1. Funding sources that include government subsidies (including Enhancements promotion expenses (機能強化促進経費), National university reform reinforcement promotion subsidy (国立大学改革強化推進補助金) etc.), indirect funding, and allocations from the university's own resources.

*2 When personnel, travel, equipment (etc.) expenses are covered by Grants-in-Aid or under commissioned research projects or joint research projects, the amounts should be entered in the "Research projects" block.

2) Costs of Satellites and Partner institutions

(Million yens)

Cost Items	Details	Total Costs	Amount covered by WPI funding
Personnel	Principal investigators (no. of persons):2	/	/
	Other researchers (no. of persons):0		
	Research support staffs (no. of persons):0		
	Administrative staffs (no. of persons):0		
	Subtotal	0	0
Project activities	Subtotal	21.2	21.2
Travel	Subtotal	2.0	2.0
Equipment	Subtotal	0.0	0.0
Research projects	Subtotal	16.9	0.0
	Total	40.1	23.2

Appendix 4 FY 2017 Status of Collaboration with Overseas Satellites

1. Coauthored Papers

- List the refereed papers published in FY 2017 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. 2018 and not described in Appendix 1.

Overseas Satellite 1 Imperial College London (Total: 1 paper)

- 1) (-) Yuanshu Zhou, Masaki Saito, Takafumi Miyamoto, Pavel Novak, Andrew I Shevchuk, *Yuri E Korchev*, Takeshi Fukuma and Yasufumi Takahashi "Nanoscale Imaging of Primary Cilia with Scanning Ion Conductance Microscopy" *Analytical Chemistry* 2018 90 (4), 2891-2895

- 2)
- 3)
- 4)

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

- 1)
- 2)
- 3)
- 4)

2. Status of Researcher Exchanges

- Using the below tables, indicate the number and length of researcher exchanges in FY 2017. 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
FY2017					

<From satellite>

	Under 1 week	From 1 week to 1 month	From 1 month to 3 months	3 months or longer	Total
FY2017		2			2

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
FY2017	2				2

<From satellite>

	Under 1 week	From 1 week to 1 month	From 1 month to 3 months	3 months or longer	Total
FY2017	1	1			2

Appendix 5 FY 2017 Visit Records of World Top World-level Researchers from Abroad

* If top world-level researchers have visited/ stayed at the Center, please provide information on them in the below table.

* To determine whether the researcher is a "top world-level researcher," please see the standard stipulated in the Application Guideline.

Total: 22

	Name	Age	Affiliation (Position title, department, organization)	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)
1	Yuri Korchev	57	NanoLSI PI / Professor, Imperial College London	PhD in Biophysics and Cytology, Biophysics	Editorial Board Member, Pflügers Archiv - European Journal of Physiology	2017/11/03- 2017/11/13, 2018/02/15- 2018/02/20	participation as principal investigator; invited lecture at International Symposium on Atomic Force Microscopy at Solid-Liquid Interfaces; invited lecture at the 1st NanoLSI International Symposium
2	Michael Higgins	43	Associate Professor, University of Wollongong	PhD/cellular- material interface, nanobionics, electroceuticals and the application of scanning probe microscopy techniques to study biological- material systems	ARC Australian Research Fellowship (2010) Australian Academy of Science International Linkage Award (2010)	2017/11/06- 2017/11/10	invited lecture at International Symposium on Atomic Force Microscopy at Solid-Liquid Interfaces
3	Andre Hoelz	46	Professor, California Institute of Technology	Ph.D. /Structural Biology and Biochemistry	Camille Dreyfus Teacher-Scholar Award (2015); Kimmel Scholar Award, Sidney Kimmel Foundation for Cancer Research (2012); 54th Mallinckrodt Scholar Award, Edward Mallinckrodt, Jr. Foundation (2011); Albert Wyrick V Scholar Award, The V Foundation for Cancer Research (2010)	2017/11/07- 2017/11/11	invited lecture at International Symposium on Atomic Force Microscopy at Solid-Liquid Interfaces
4	Sergei Kalinin	42	Director, Institute for Functional Imaging of Materials, Oak Ridge National Laboratory	Ph.D. /Big data, Scanning Probe Microscopy, Piezoresponse Force Microscopy	IEEE Senior member (2015); Medal for Scanning Probe Microscopy, Royal Microscopical Society (2015); Microscopy Today Innovation Award for "Electrochemical Strain Microscopy" (2011); Roland B. Snow Award, American Ceramics Society (2010); Microscopy Today Innovation Award for "Adaptive Band Excitation Method in Scanning Probe Microscopy" (2010); IEEE-UFFC Ferroelectrics Young Investigator Award (2010); Burton medal for Young Investigator, Microscopy Society of America (2010)	2017/11/07- 2017/11/10	invited lecture at International Symposium on Atomic Force Microscopy at Solid-Liquid Interfaces

	Name	Age	Affiliation (Position title, department, organization)	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)
5	Kislon Voitchovsky	39	Senior Lecturer, Durham University	PhD.	FEMS Awards-EUROMAT (2013); The 2015 Liquids and Complex Fluids Group Prize	2017/11/07- 2017/11/11	invited lecture at International Symposium on Atomic Force Microscopy at Solid-Liquid Interfaces
6	Damien Hall	44	Associate Professor, Institute for Protein Research, Osaka University / Senior Research Fellow, Australian National University	Ph.D. Biochemistry	JST Wakate Assistant Professor, University of Tsukuba (2008-2013)	2018/3/2	lecture at NanoLSI Open Seminar
7	Richard W. Wong	43	NanoLSI PI	Doctor of Medicine, Molecular cell biology	Ambassador of American Society for Cell Biology (2017- 2019); The Royal Society of Medicine, United Kingdom Fellow (2017-)	usually stays at the center	participation as principal investigator
8	Nicholas Barker	48	Institute of Medical Biology, A*STAR, Senior Principal Investigator and Research Director	PhD Cell biology, Tumor biology	Visiting professor at the Center for Regenerative Medicine (CRM) at Edinburgh University; Associate principal investigator with EuroSyStem	2017/10/06- 2017/10/15 2017/10/25	short-term stay for joint research; invited lecture at Kanazawa University Cancer Research Institute International Symposium
9	Mark John Maclachlan	44	NanoLSI PI / Professor, University of British Columbia	PhD in Chemistry	Fellow of the Royal Society of Chemistry (UK) (2016); 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); Rutherford Memorial Medal (Royal Society of Canada) (2013); Killam Award for Excellence in Graduate Student Mentorship (UBC) (2013); Strem Award for Pure or Applied Inorganic Chemistry (Canadian Society for Chemistry) (2013); Killam Research Prize (UBC) (2011)	2017/07/24- 2017/8/10 2017/10/16- 2017/10/21 2018/02/20- 2018/02/28	participation as principal investigator; invited lecture at International Symposium on Center of Excellence for Innovative Material Sciences Based on Supramolecules; invited lecture at the 1st NanoLSI International Symposium
10	Jean-Pierre Sauvage	73	Emeritus professor, University of Strasbourg	PhD Supramolecular chemistry	Nobel Prize in Chemistry(2016); "Grand Prix de la Fondation de la Maison de la Chimie" (2014); "Luigi Tartufari" International Prize, Accademia Nazionale dei Lincei (Italy) (2012); Blaise Pascal Medal 2012 in Chemistry of the European Academy of Sciences	2017/11/5- 2017/11/10	short-term stay for joint research

	Name	Age	Affiliation (Position title, department, organization)	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)
11	Amar Flood	43	Professor, Indiana University	PhD. /Anion Recognition, Molecular Machines and Assemblies, Self-assembly of 2D and 3D Organic Crystals	Luther Dana Waterman Professor (2015); James F. Jackson Professor of Chemistry (2014); Award for Excellence in Physical Organic Chemistry (Wiley) (2011); Cram Lehn Pedersen Award in Supramolecular Chemistry (RSC) (2011)	2018/02/17-2018/02/24	short-term stay for joint research; lecture at NanoLSI Open Seminar; invited lecture at the 1st NanoLSI International Symposium
12	Stephen Goldup	41	Professor, University of Southampton	PhD Supramolecular chemistry	Royal Society of Chemistry Hickinbottom Award 2014 for "pioneering work on the synthesis of rotaxanes"	2017/10/15-2017/10/18	invited lecture at International Symposium on Center of Excellence for Innovative Material Sciences Based on Supramolecules
13	Jay S. Siegel	58	Professor, Tianjin University	Ph.D.	Fellow of Royal Society of Chemistry (2007)	2017/10/15-2017/10/18	invited lecture at International Symposium on Center of Excellence for Innovative Material Sciences Based on Supramolecules
14	Kimoon Kim	63	Professor, Pohang University of Sci. and Tech.	Ph.D. Supramolecular chemistry, Materials Chemistry	Knowledge Creation Award (2014); The 2012 Izatt-Christensen Award, ISMSC (2012); Earl L. Muetterties Memorial Lectureship Award, UC Berkeley (2011)	2017/10/15-2017/10/18	invited lecture at International Symposium on Center of Excellence for Innovative Material Sciences Based on Supramolecules
15	Yoko Yamakoshi	50	Professor, ETH, Zürich	Ph.D.	The Society of Japanese Women Scientists Award (2015); Research Excellence Award from the American Heart Association (2011)	2017/10/15-2017/10/18	invited lecture at International Symposium on Center of Excellence for Innovative Material Sciences Based on Supramolecules
16	Jonathan Nitschke	44	Professor, University of Cambridge	PhD	International Award for Creative Work of the Japan Society of Coordination Chemistry (2016); Bob Hay Lectureship of the Royal Society of Chemistry Macrocycles and Supramolecular Chemistry group (2012); Cram Lehn Pederson Prize in Supramolecular Chemistry (2012)	2017/10/15-2017/10/18	invited lecture at International Symposium on Center of Excellence for Innovative Material Sciences Based on Supramolecules
17	Albert M. Brouwer	57	Professor, University of Amsterdam	Ph.D.	Morino Lectureship (2012)	2017/10/15-2017/10/18	invited lecture at International Symposium on Center of Excellence for Innovative Material Sciences Based on Supramolecules

	Name	Age	Affiliation (Position title, department, organization)	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)
18	Adam Stuart Foster	42	NanoLSI PI / Professor, Aalto University	PhD in Theoretical Solid State Physics	Väisälä prize, The Finnish Academy of Science and Letters (2009)	2017/05/28- 2017/06/07 2017/11/05- 2017/11/15	participation as principal investigator; invited lecture at International Symposium on Atomic Force Microscopy at Solid-Liquid Interfaces
19	Alexander S. Mikhailov	67	NanoLSI PI / Professor, Fritz Haber Institute of the Max Planck Society	Doctor of Science /Theoretical Physics, Chemical Physics, Biophysics	International Solvay Chair in Chemistry (2009)	2017/12/03- 2017/12/17 2018/01/29- 2018/03/28	participation as principal investigator; invited lecture at the 1st NanoLSI International Symposium
20	Jin Yu	41	Beijing Computational Science Research Center	PhD., Physics /Biophysics, modeling and computation Non-equilibrium statistical mechanics	1000-Talent Award for Young Scholar of China(2012)	2018/03/10- 2018/03/14	discussion for joint research; lecture at NanoLSI Open Seminar
21	Jeffrey Noel	64	Max Delbrück Center (MDC) for Molecular Medicine and the Fritz Haber Institute	Ph.D. in Physics	Computing Awards XSEDE Grant MCB140274 for 4.6MSUs (Co-PI) (2014)	2018/3/13	lecture at NanoLSI Open Seminar
22	Mikko Karttunen	50	Professor, Western University	PhD./properties of biological & soft matter using theory and the methods of computational chemistry and physics	Canada Research Chair (Tier 1) (2017)	2018/02/18- 2018/02/22	discussion for joint research; invited lecture at the 1st NanoLSI International Symposium

Appendix 6 FY2017 State of Outreach Activities

* Using the table below, show the achievements of the Center's outreach activities in FY2017(number of activities, times held).

* Describe those activities that have yielded novel results or that warrant special mention in the "Special Achievements" space below.

* In appendix 7, list and describe media coverage (e.g., articles published, programs aired) in FY2017 resulting from press releases and reporting.

Activities	FY2017 (number of activities, times held)
PR brochure, pamphlet	NanoLSI Pamphlet (1), WPI Pamphlet (1), WPI Achievements Pamphlet (1), Nature Index 2018 (1), asia RESEARCH NEWS (1)
Lectures, seminars for general public	Japan Sea Innovation Conference (1)
Teaching, experiments, training for elementary, secondary and high school students	N/A
Science café	N/A
Open houses	International Symposium on Center of Excellence for Innovative Material Sciences Based on Supramolecules (1), International Symposium on Atomic Force Microscopy at Solid-Liquid Interfaces (1), Kanazawa University Cancer Research Institute International Symposium (1), 1st NanoLSI International Symposium (1), Institute for Frontier Science Initiative Symposium (1)
Participating, exhibiting in events	6th WPI Science Symposium (1), AAAS (1)
Press releases	(4): Adoption for WPI, Shibata/Ando/Kodera, Wong/Hazawa, 1st International Symposium

<Special Achievements>

TV Program on Kanazawa University Key Researches "KOKOKARA" (2):Yano, Maeda

Appendix 7 FY 2017 List of Project's Media Coverage

* List and describe media coverage (e.g., articles published, programs aired) in FY2017 resulting from press releases and reporting.

	Date	Types of Media (e.g., newspaper, television)	Description
1	2017.9.26-10.2	Newspaper (9)	Adoption for WPI (<i>Nikkei Shimbun, Nikkei Sangyo Shimbun, Engineering and Construction News, Hokuriku Chunichi Shimbun, Hokkoku Shimbun</i>)
2	2017.10.23	Newspaper (1)	Introduction to NanoLSI by Director Takeshi Fukuma (<i>Hokkoku Shimbun</i>)
3	2017.10.26	Newspaper (2)	Professor Atsushi Hirao talks about his ambitions for nanotechnology research, and news of the International Symposium commemorating the 50th Anniversary of the Cancer Research Institute where Professor Seiji Yano reported his research results (<i>Hokuriku Chunichi Shimbun, Hokkoku Shimbun</i>)
4	2017.10.27-11.4	Newspaper (3)	Director Takeshi Fukuma receiving the Hokkoku Cultural Award (<i>Hokkoku Shimbun</i>)
5	2017.11.10-11.11	Newspaper (1) , Website (7)	News of the results of high-speed AFM research by Associate Professor Mikihiro Shibata and Associate Professor Noriyuki Kodera (<i>Hokkoku Shimbun, Nature Communications, LIVE SCIENCE, News-Medical.Net, Asian Scientist, elementy, bp - Affairs, Nihon Keizai Shimbun</i>)
6	2017.11.12	Radio (1)	Regarding Kanazawa University's WPI adoption (FM Ishikawa)
7	2017.12.7-12.9	Newspaper (1)	Professor Richard Wong and Assistant Professor Hazawa Masaharu elucidate mechanisms inhibiting the proliferation of skin stem cells (<i>Hokkoku Shimbun</i>)
8	2017.12.27	Newspaper (1)	Regarding the 14th Japan Society for the Promotion of Science Award to Associate Professor Noriyuki Kodera (<i>Hokkoku Shimbun</i>)
9	2018.1.13	Newspaper (1)	Research results on genetic variation related to carcinogenesis of colorectal cancer by Professor Masanobu Oshima (<i>Asahi Shimbun</i>)
10	2018.2.13	Newspaper (1) , Website (1)	Regarding the lecture by Professor Takeshi Fukuma and Professor Kunio Matsumoto at the Japan Sea Innovation Conference (<i>Hokkoku Shimbun</i>)
11	2018.2.22-3.9	Newspaper (1) , Website (3)	Regarding the NanoLSI International Symposium (<i>Hokkoku Shimbun, Asahi Shimbun Digital, Kahoku Shimbun Online, ZDNet Japan</i>)
12	2018.3.9	Newspaper (2)	Decision by Hokuriku Bank to award a grant for young researchers to Associate Professor Yasufumi Takahashi, Associate Professor Tatsuki Fukami, and Assistant Professor Masaharu Hazawa (<i>Hokuriku Chunichi Shimbun, Hokkoku Shimbun</i>)
13	2018.3.16	Newspaper (1)	Regarding NanoLSI (<i>The Science News</i>)