Research Center Project

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

Host institution: Kanazawa University

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

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

Appendix 1　For the prospective center director, fill in the "biographical sketch of prospective center director."

Appendix 2　Provide a reference (recommendation) for the prospective center director (free format not subject to the 3-page limitation).

Prospective administrative director: Seizo Morita, Professor Emeritus of Osaka University（Name, Affiliation）

Appendix 3　For the prospective administrative director, fill in the "biographical sketch of the prospective administrative director."

1) Overall Image of Your Center

* Concisely describe your institute's identity and provide its mission statement as a WPI center.

1. Mission statement

We combine the world's top bio-scanning probe microscopy (SPM) and supramolecular chemistry techniques to develop "nano-endoscopic techniques" that allow us to directly image, analyze, and manipulate the nano-dynamics of proteins and nucleic acids on the cell surface or inside the cell. Furthermore, we use these techniques and multi-scale simulation techniques in a complementary fashion to elucidate the various molecular and cellular dynamics by comparing normal and cancer cells. Based on the developed techniques and knowledge gained through this process, we establish a new academic field, "nano-probe life science", which aims to fundamentally understand and control various life phenomena, including development, disease and aging.

2. Center identity

The most salient feature of NanoLSI is our world's top bio-SPM techniques for nanoscale live imaging of molecular and cellular dynamics, which offer distinctive advantages over other bio-imaging techniques. For example, electron microscopy allows one to image the static ultrastructure of proteins in vacuum, but not to directly image protein dynamics in solution. Live imaging by fluorescence microscopy visualizes the positions of fluorescence-labeled molecules, but does not allow direct imaging of dynamic changes in the protein structure or the positions of unlabeled molecules. To overcome these limitations, Ando developed the high-speed atomic force microscope (AFM) and made it possible to directly image the dynamic structural changes of unlabeled protein molecules in solution (PNAS 2001, 544 citations). In addition, Fukuma developed the liquid-environment frequency modulation AFM (RSI 2005, 197 citations) and achieved true atomic-resolution imaging even in solution. Based on this technique, he also developed three-dimensional AFM (3D-AFM) (PRL 2010, 156 citations) and made it possible to visualize 3D distribution of water and molecular chains at subnanometer resolution. Based on these achievements, Ando, Fukuma and their group members have led the progress in the bio-SPM research field. Therefore, we are confident that we have gathered the world's best members to launch a center of excellence aiming at creating novel bio-SPM techniques for visualizing nano-dynamics of molecules and cells in solution.

To accomplish our mission, NanoLSI has attracted many world's top class scientists with a wealth of experience in oncology, supramolecular chemistry, and mathematical/computing science. The Cancer Research Institute of Kanazawa University, the only joint usage/research center in Japan solely focusing on cancer research, has amassed

superb achievements in the study of cancer stem cells, tumor microenvironment, and molecularly targeted therapy, including the identification of critical molecules involved in leukemia stem cell regulation (Hirao *et al*., Nature 2010, 297 citations). Kanazawa University has also garnered global attention for its advances in supramolecular chemistry, such as the development of novel pillar[n]arenes (Ogoshi *et al*., JACS 2008, 528 citations). Additionally, we have collaborations with outstanding scientists at prestigious universities, including the Adam Foster's group at Aalto University, one of the world's few research groups with a strong track record in AFM simulation in liquids, and the Alexander Mikhailov's group at Fritz Haber Institute that has made great strides in the simulation of complex systems ranging from proteins to cell membranes. The contributions of these scientists will help us in building an in-depth understanding of life phenomena based on the results of nanoscale experiments.

Our greatest, defining strengths are our many accomplished scientists and research achievements across all core disciplines necessary for executing our mission—nanometrology, oncology, supramolecular chemistry, and mathematical/computational science. Another advantage that sets us apart is that the majority of our principal investigators (PIs) are only in their forties, meaning that we can continue to lead the new science of "nano-probe life science".

2) Research Activities

2) -1 Research field

* Write in the target research field(s)
* Describe the importance of the target research field(s), including the domestic and international R&D trends in the field(s) and scientific and/or social significance.
* Describe the value of carrying out research in the field(s) as a WPI center (e.g., Japan's advantages, global impact on science and/or society, future prospects)
* If there are other centers either in Japan or overseas advancing research in fields similar to the center's field(s), please list them. (up to 5 organizations)
* Provide a list of 10 English-written papers that are closely related to the center's project and enclose the PDF files of those papers. Label this bundle of documents "Appendix 8."

1. Name of the target research field: Nano-probe Life Science

We establish a novel interdisciplinary research field "nano-probe life science", where we develop and utilize nano-probe techniques for directly imaging, analyzing and manipulating dynamic behaviors of biomolecules and cells at nanoscale resolution under physiological environments. This new approach will promote the fundamental understanding of the mechanisms underlying diverse life phenomena such as diseases and aging.

2. Importance of the target research field

Domestic/global trends and key challenges in this field

To achieve our mission outlined above, we will seek to resolve the following challenges in the core disciplines of nanometrology, supramolecular chemistry, and oncology.

(1) Challenges in nanometrology: **Nanoscale imaging, analysis, and manipulation of molecular dynamics at the periphery and inside of cells**

Advances in super-resolution fluorescence microscopy have allowed nanoscale imaging dynamics of fluorescence-labeled molecules occuring in the intra-/extracellular spaces (Betzig *et al.*, Science, 346 (2014) 439); however, this technique does not support direct imaging of the target molecules' structural changes or the position of the many

unlabeled molecules. Furthermore, fluorescent tags may interfere with biomolecular function. The microscopic imaging of living cells with advanced atmospheric scanning electron microscopy and polymer membranes have begun to emerge (Takaku *et al.*, PNAS, 110 (2013) 7633), but this approach offers optimal resolution only in the tens of nanometers, and thus, is insufficient to reveal detailed molecular dynamics. Moreover, the electron beams can damage the molecules of interest. The high-speed AFM developed by Ando at NanoLSI has been used to visualize the nano-dynamics of aquaporins on the live bacteria having a relatively hard surface (Yamashita *et al.*, JMB 422 (2012) 300), but it has not yet been successfully used to measure molecular dynamics on the much softer surfaces of eukaryotic cells. Although scanning ion-conductance microscopy (SICM) has been used to image endocytosis and to locate ion channels and receptors on eukaryotic cell surfaces (Korchev *et al.*, Science, 327(2010) 1653), the optimal resolution of this technique is lower than that of AFM and insufficient for imaging the nano-dynamics of receptors and transducers. SICM has been applied in various techniques, including one that uses a nanopipette to inject substances into specific intracellular nano-regions, as well as sample collection and analysis (Mirkin *et al.*, PNAS 104 (2007) 11895). However, SICM has not yet been successfully used to inject molecular machines with high controllability for manipulating protein structures or functions, nor to map the distributions of physical properties, such as pH and oxygen concentration, in liquids using molecular sensors. As these advances and limitations illustrate, progress is being made in the visualization of cell-surface nano-dynamics, but a breakthrough technology is needed for direct imaging of intracellular molecular dynamics. In addition, the development of techniques requiring the fusion of nanometrology with supramolecular chemistry, no matter it be for intracellular or cell-surface applications, is a challenge that has yet to be resolved.

(2) Challenges in supramolecular chemistry: Controlling the position and orientation of molecular sensors/machines to operate them upon nanostructures of interest

Supramolecular chemistry has developed as a field of chemistry for creating new molecular functions through the design of selective intramolecular interactions. Recent research attention has focused on molecular machines, which were the subject of the 2016 Nobel Prize in Chemistry. These nanoscale devices can rotate, reciprocally move, or perform other actions in response to external stimuli. Examples include a molecular elevator that moves up and down in response to pH (Stoddart *et al.*, Science 303 (2004) 1845) and molecular tweezers that open and close in response to light (Aida *et al.*, Nature 440 (2006) 512). However, these highly functional molecular sensors and machines are yet to be translated into practical applications. One barrier is the lack of technology for effectively controlling the position and orientation of these functional molecules so that they can be operated upon nanostructures of interest. Here, SPM techniques could help by offering abilities such as the conveyance of molecules to specific nanoscale regions using a nanopipette, or subnanometer-scale positional control of a molecule affixed to a probe tip. The combination of SPM and supramolecular chemistry promises to pave the way for effectively controlling and operating functional supramolecules upon intracellular and cell-surface nanostructures of interest.

(3) Challenges in oncology: Achieving nanoscale understanding of the cell dynamics of cancer progression

Recent progress in whole-genome sequencing of cancer cells has led to identification of driver oncogenes in many types of cancer. Discovery of drugs that selectively inhibit function of driver gene products has established practice and concept of molecularly targeted therapy of cancer. Despite the progress of recent medical and pharmaceutical

sciences, we have yet sufficiently understood mechanisms of malignant progression of cancer, such as drug resistance and metastasis. This insufficiency is partly because there are no techniques for real-time imaging of nano-dynamic features of non-labeled cells and molecules involved in cancer progression. For example, it is nigh impossible to understand the process leading up to drug resistance without directly imaging the changes that take place inside cells and their environment (e.g., pH, oxygen concentration, osmotic pressure, and amino acid and sugar distributions). The significance of interactions between cancer cells and the microenvironment mediated by growth factors, inflammatory cytokines, and exosomes has been increasingly recognized, but without direct imaging of the molecular and cellular dynamics, it is extremely difficult to elucidate mechanisms of adhesion, migration, and invasion peculiar to cancer cells, through understanding of the changes in inside and surface of cancer cells. Thus, techniques to directly image and analyze molecular cell dynamics at nanoscale resolution are prerequisite to elucidating the unresolved mechanisms of cancer progression.

Scientific/social significance

We seek to tackle the aforementioned challenges in nanometrology, supramolecular chemistry, and oncology through research that expands and combines the knowledge and techniques of each of these disciplines. Instead of just evolving these fields, we will endeavor to pioneer a whole new discipline of “nano-probe life science” that will have enormous implications for the future of science. The knowledge and techniques emerging from our research will pave the way to a fundamental understanding of various biological phenomena that will allow their precise control. Such advances hold immense social significance as they could aid in conquering cancer and other intractable diseases, extending lifespan, and achieving other important health improvements.

3. Reasons why our research is a good match for the WPI initiative

Japan’s competitive edge

Japan’s competitive edge in this research area lies in that it hosts the world’s foremost bio-SPM techniques and the inspired minds that created them. As noted earlier, bio-SPM is the surest path to realizing nano-endoscopic techniques. The most fundamental and critical performance factors of bio-SPM are resolution and speed, and two Japanese scientists from our institute, Fukuma and Ando, have led the world in the ongoing improvement of these factors. Having designed and built their systems from scratch, both scientists have expertise in developing SPM techniques of top-notch resolution and speed. Having a team of scientists of such caliber at one institute is an incredibly strong advantage.

Appeal as global scientific/social challenges

(1) Appeal as a scientific challenge

Origins of material properties and various phenomena can be explained in terms of nanoscale structures formed from atomic and molecular assemblies and their dynamic behaviors. Hence, understanding and controlling nanoscale structures would empower us to manipulate at will all sorts of physical properties and phenomena. This represents the ultimate aim of science—to transcend the boundaries of the established disciplines of physics, chemistry, biology, pharmacy, and medicine—and forms the core concept of nanotechnology. Ever since the

pursuit of nanotechnological research was declared a key pillar of the US strategy by President Clinton in 2000, enormous research as well as financial investments have been made in nanotechnological R&D worldwide. These efforts have blazed new paths toward humankind's understanding and control of natural phenomena at the nanoscale. In the early 2000s, the main focus was on materials and devices, but in the second half of that decade, bioscience became a greater target of exploration. Subsequently, nanoscience encompassed life sciences such as medical and pharmaceutical sciences, evolving into one of the greatest attempts in humankind's unending quest to expand the borders of science and technology.

(2) Appeal as a social challenge

Initially, we will perform detailed comparisons of nanodynamics between normal and cancer cells using our innovative nano-probe techniques. This will lead to fundamental understanding of the mechanisms of basic cellular functions and their cancer-specific abnormalities. Achieving this goal will enable precision control of the various phenomena involved in cancer, and thus make it possible to overcome this intractable disease. Clearly, our research target represents a very important social challenge globally as well as specifically for Japan, where cancer kills roughly one in every three people.

Future prospects for this field

We will endeavor to establish the foundation for nano-probe life science by building techniques for and accruing expertise in the imaging, analysis, and manipulation of life phenomena at the nanoscale. Further in future, those techniques and know-how promise to deepen our understanding and control of not only cancer, but also other life phenomena. Our research field is thus anticipated to pave the way for immense contributions to human health, including lifespan extension and conquering of intractable illnesses such as cancer, heart disease, neurodegenerative disease, and liver disease.

４． Japanese and overseas institutes focusing on similar fields

① **Quantitative Biology Center (QBiC), RIKEN,** investigates and computationally simulates intracellular molecular kinetics at high resolution to deepen our understanding of living systems. Although their research goals are similar to ours, different from us, they mainly use optical microscopy to achieve them.

② **Institute of Transformative Bio-molecules, Nagoya University (ITbM)** develops novel functional molecules to visualize and control living systems. Although they combine chemistry and biology to tackle issues in life sciences as we do, their focus is on animals and plants. In addition, they use optical microscopy for imaging.

③ **Janelia Research Campus, Howard Hughes Medical Institute (HHMI)** focuses on basic medicine, with novel bio-imaging techniques and neuroscience research at the core. In contrast to us, they use optical microscopy as the main imaging technique.

④ **Max Planck Institute for Biophysical Chemistry (MPI-BPC)** combines knowledge in physics, chemistry, and biology to understand the mechanisms by which cells, organelles, and biomolecules express their functions. In contrast to us, they use NMR and optical microscopy as core imaging techniques.

⑤**The Center for Nanophase Materials Sciences (CNMS)** in the USA researches nanotechnology in general and is globally reputed for SPM research. However, their focus is mainly on the materials and energy fields, not

life sciences.

As described above, while there are numerous research institutes aiming to elucidate the principles of the living system, their main imaging tool is optical microscopy. While there are many nanotechnology research centers in the world, none of them is solely focused on the understanding of life phenomena. We will be the first in the world to establish a research institute for understanding living systems using advanced nano-probe techniques and to create the novel academic field “nano-probe life science”.

2)-2 Research objectives

* Describe in a clear and easy-to-understand manner the research objectives that the project seeks to achieve by the end of its grant period (in 10 years). In describing these objectives, the following points should be articulated in an easily understandable manner: The kind of research area(s) you plan to cultivate by, for example, fusing various fields. In that process, what world-level scientific and/or technological issues are you seeking to solve? What will the expected impact of the scientific advances you aim to achieve be on society in the future?
* Describe concretely your research plan to achieve these objectives and any past achievements related to your proposal.

1. Exit goals (10 years from now)

Research goals

Our institute is committed to achieving the following goals by the conclusion of the ten-year period:

- Develop nano-endoscopic techniques enabling direct imaging, analysis, and manipulation of the dynamic behavior of molecules inside and on cells.
- Acquire an accurate understanding of mechanisms of basic cellular functions as well as their cancer-specific abnormalities by using the nano-endoscopic and computer simulation techniques we will develop.
- Pioneer the emerging discipline of “nano-probe life science.”

Global scientific/technological challenges and their social impact

Over the years, various techniques have been developed with the aim of gaining a fundamental understanding of the mechanisms underlying a multitude of life phenomena, including diseases and aging, by directly visualizing molecular and cellular dynamics; however, challenges remain. The resolution of these challenges will allow a fundamental understanding and manipulation/control of the mechanisms of these phenomena. This endeavor has immense significance for society, as it will contribute to human health by conquering intractable diseases and extend life expectancy.

2． Specific plans for research activities and related achievements to date

[Development of technique for measuring nano-dynamics on cell surface and interior]

(Development of nano-imaging techniques)

・**Measurement of the nano-dynamics of cells:** Cell-membrane morphology changes continuously at the nano level in relation to physiological processes, including cell migration and endocytosis. Direct imaging of such changes using conventional techniques has been very difficult. The rapid advancements in bio-SPM indicate that such imaging is on the verge of break-through. Core members of NanoLSI have played a leading role in the development of this technology. Ando increased the operation speed of AFM in solution by several hundred-fold (PNAS 98 (2001) 12468; Prog. Suf. Sci. 83 (2008) 337), enabling direct imaging of protein dynamics at the nano

level (Figs. 1a and 1b) (Nature 468 (2010) 72; Science 333 (2011) 755). Furthermore, they successfully expanded the high-speed AFM scan range, enabling direct imaging of dynamic morphological changes in nerve-cell endings (Fig. 1c) (Sci. Rep. 5 (2015) 8724). However, in high-speed AFM, the probe exerts a force on the sample, and thus, often does not allow for nondestructive imaging of the very soft upper surface of eukaryocytes. Therefore, scanning ion conductance microscopy (SICM) currently attracts attention. In this technique, a nanopipette is scanned without touching the sample, allowing nondestructive imaging of eukaryocytes (Nat. Methods, 6 (2009) 279). Korchev established the basics of SICM and were the first to demonstrate its usefulness in live-cell imaging (Fig. 2). Takahashi of the Fukuma group collaborated with Korchev to combine SICM with electrochemical measurement techniques, and enabled to locally measure oxygen, neurotransmitters, and ATP (ACS Nano, 10 (2016) 3214). Furthermore, Watanabe of the Ando group developed high-speed SICM by combining it with high-speed AFM. He developed a technique allowing direct imaging of nanomorphological changes in cells at ≥1 frame/s. In NanoLSI, the SICM techniques of the Ando, Fukuma, and Korchev groups are combined, and with further development, the measurement of cellular nano-dynamics will be realized.

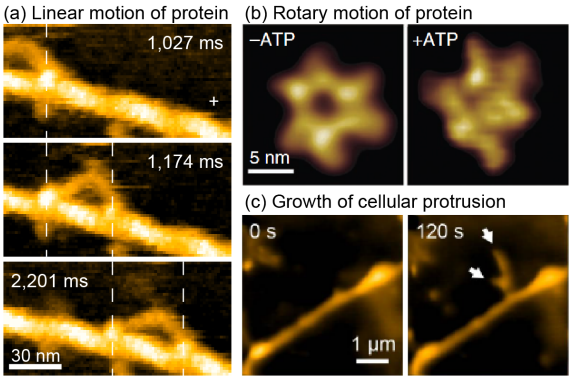

Fig. 1: Nanoscale life phenomena visualized by high-speed AFM. (a) Myosin V walking along the actin filament. (b) Rotary catalysis of rotor-less F1-ATPase. (c) Filopodia growth from living nerve cell.

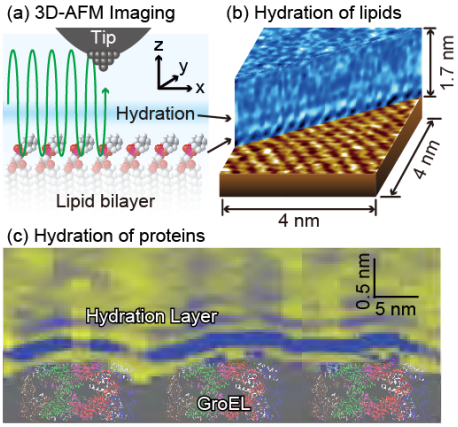

Fig. 3: Subnanoscale 3D distribution of water and surface structures of biomolecules visualized by 3D-AFM. (a) 3D-AFM. (b) Lipid/water interface. (c) GroEL/water inteface.

・**Measurement of nano-dynamics on cell surfaces:** In the cell membrane, various proteins such as receptors and channel proteins are present that allow intra/extracellular signaling and substance transport and are often closely related to cancer development and progression. Conventional techniques cannot satisfactorily visualize protein dynamics on live-cell surfaces at the nano level. Exceptional results have been obtained by Ando, who successfully used high-speed AFM to image aquaporin nano-dynamics on the live bacteria having a relatively hard surface for a cell (JMB 422 (2012) 300). However, high-speed AFM currently is not up to soft eucaryocyte surfaces. While high-speed SICM allows the imaging of eukaryocyte surface structures, its spatial resolution is not sufficient for imaging protein dynamics at the nano level. In addition, to accurately understand transport phenomena, apart from the transporters, one needs to understand the distribution of water

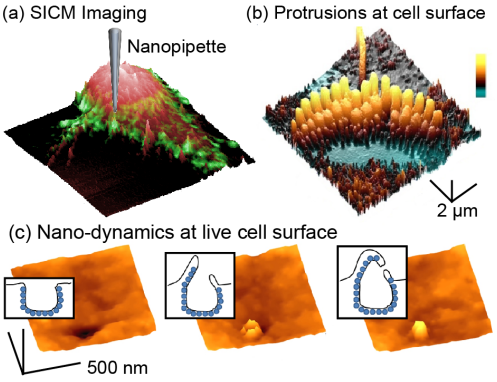

Fig. 2: Nanoscale structures and dynamics on cell surfaces visualized by SICM. (a) SICM imaging. (b) Protrusions at inner hair cell. (c) Formation of a protrusion before clathrin coated pit closure.

molecules, ions, and small organic molecules that are transported. This distribution presumably occurs in a 3D space of several nanometers thick near the interface. With conventional two-dimensional (2D) SPM, such distributions cannot be captured; therefore, this study focuses on 3D-AFM. Fukuma enabled in-liquid operation of frequency modulation AFM (FM-AFM), highest-resolution AFM technique, and enabled true atomic-resolution FM-AFM imaging in liquids for the first time (App. Phys. Lett. 87 (2005) 034101). Based on this technique, he also developed 3D-AFM and enabled direct imaging of 3D distribution of hydration structures and flexible surface structures of biomolecules with subnanoscale resolution (Fig. 3) (Phys. Rev. Lett. 104 (2010) 016101). Foster was the first to succeed in reproducing a 3D-AFM measurement using atomic-level molecular dynamics simulation, making an important step towards the full understanding of the measurement principle and quantitative calculation of hydration structures from the measured 3D-AFM data (Phys. Rev. B 92 (2015) 155412). Similar to SICM, in 3D-AFM, the probe makes hopping movements in the Z direction, enabling minimally invasive measurements. However, the current observation speed of 1 min/frame is not adequate. By combining the high-speed scanning techniques of Ando, 3D-AFM of Fukuma, and the simulation techniques of Foster, we aim to achieve non-invasive, high-speed 3D nano-dynamics measurements at live-cell surfaces.

・**Visualization of intracellular nano-dynamics (nano-endoscopic observation):** The behavior of proteins and nucleic acids on the surfaces and in the interiors of organelles such as nuclei and mitochondria plays important roles in signal transduction through vacuoles and in physiological processes such as transportation. Direct visualization of these phenomena at the nano level is unachievable by conventional techniques. To explore this uncharted realm, we combine the 3D-AFM technique of Fukuma, the high-speed AFM technique of Ando, and the long-probe fabrication technique of Korchev and create a nano-endoscopic imaging technique. In hydration-structure measurement by 3D-AFM, the probe is scanned in a 3D space such that the probe penetrates the hydration structure and the vertical force applied to the probe apex is recorded to visualize 3D water distribution. Similarly, if we can penetrate the membrane at the cell surface with an acceptable perturbation to the cell activities using a very thin and long probe, we should be able to scan the probe in a 3D space including the cell interiors for visualizing the nano-dynamics happening in the scanned region (Fig. 4a). Korchev have established fabrication technique of a glass long probe in an effort to improve the performance of SICM. He has succeeded in inserting it into live cells to inject substances. In the meanwhile, Ando have established a technique for fabricating an electron beam deposited (EBD) carbon tip with a nanoscale asperity on an arbitrary location under scanning electron microscope observation (Sci. Rep. 5 (2015) 8724). We combine these techniques to prepare a glass long probe with a nanoscale asperity. Using this probe with high-speed 3D-AFM, we achieve high-speed 3D intracellular imaging with nanoscale resolution. Preliminary experimental results have proven its potential; we successfully imaged 3D molecular adsorption structures showing complicated, non-

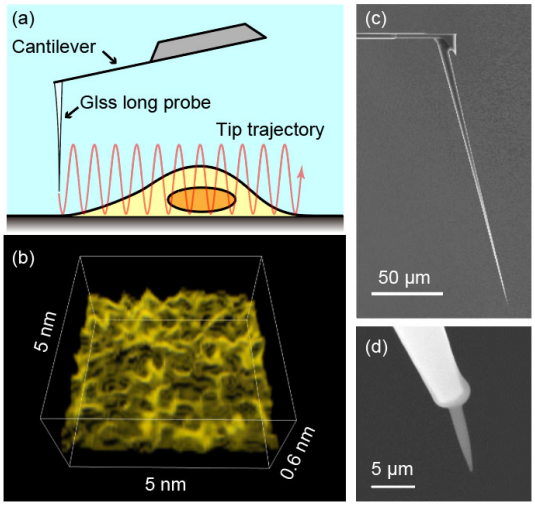

Fig. 4: (a) Nano-endoscopic imaging of inside a cell. (b) 3D-AFM image of molecular adsorption structures obtained at a solid/liquid interface. (c) Glass probe attached to an AFM cantilever. (d) EBD tip fabricated on a glass probe.

uniform distribution with subnanometer resolution (Fig. 4b). We also fabricated a glass long probe with an EBD tip at its end (Figs. 4c and 4d) and successfully applied it to true atomic-resolution imaging in liquids. If it becomes possible to control the probe position in cells, high-speed 2D/3D imaging of nano-dynamics at the surface of organelles should also become possible. One result that supports this possibility is already obtained by Wong, where he directly visualized the dynamics of the pores and proteins around them on extracted nuclei using high-speed AFM (ACS Nano (2017) in press).

(Development of nano-endoscopic analysis and manipulation techniques)

Based on the nano-endoscopic imaging technique, nano-endoscopic analysis and manipulation techniques will be developed.

・**Injection and sampling of substances using a nanopipette:** If substances can be injected into specific near-surface or intracellular nano-regions, nano-dynamics induced by the injection can be measured directly using nano-imaging techniques. To date, there are only limited number of research groups who have successfully injected substances into intra/extracellular nano-regions using nanopipettes and examined the reactions by fluorescence microscopy. The Korchev group is one of the few research groups. We combine this technique with the nano-endoscopic imaging technique to enable direct visualization of nano-dynamics that cannot be imaged by fluorescence microscopy. Moreover, sampling from specific nano-regions, which will allow high-precision analysis of the molecules and ions present, has recently become possible. Takahashi of the Fukuma group have achieved local sampling of cell cytoplasm for mRNA analysis. The combination of these leading nanopipette and nano-imaging techniques will make nano-endoscopic analysis possible.

・**Analysis of nano-distribution of physical properties using a molecular sensor:** Intra/extracellular pH and oxygen concentration are closely related to not only cancer development but also the function of drugs and drug delivery systems. The spatial resolution of conventional techniques including fluorescence sensors is not necessarily sufficient for imaging nanoscale distributions of these physical properties. We aim to solve this problem by combining bio-SPM and supramolecular chemistry techniques. We will develop a molecular sensor that changes its structure in response to pH or oxygen concentration in a liquid environment using our expertise in supramolecular chemistry. By attaching the sensor to an end of a nanopipette for SICM, distributions can be detected based on changes in the ion current passing through the pipette. By combining this nanopipette technique with 3D-AFM, the probe apex can be precisely positioned to a specific nano-region. Researchers at NanoLSI (Ogoshi, Maeda, Akine and MacLachlan) have successfully developed functional molecules that can change their structures in response to various chemical stimuli including pH and oxygen concentration changes. Combining their expertise and the aforementioned bio-SPM techniques, we accomplish the attachment of the molecular sensor to a nanopipette apex. While it has been a great challenge to control the orientation of a nano-sized molecular sensor and directly attach it to the nanopipette apex, there are substantial number of reports on the attachment of nanorods at the apex of an SPM probe by scanning electron microscopy or electrophoresis. Therefore, we employ supramolecular chemistry techniques to design and synthesize a supramolecular nanorod with a molecular sensor integrated at its end. We further attach this nanorod to the apex of a nanopipette. This markedly increases the ability to control the position and orientation of the molecular sensors. The methods described above will enable

measuring the distribution of physical properties inside and outside the cells.

・**Nano-manipulation using molecular machines:** As mentioned above, receptors and channel proteins in the cell membrane are closely related to the mechanisms underlying cancer development and progression. Thus, a detailed understanding and precise control of their functions should contribute to overcoming cancer. So far, the effects of drugs on the protein functions have often been investigated by the fluorescence microscopy imaging of the cellular or molecular responses induced by the addition of drugs into the cell culture solution. However, in such experiments, either of the methods for giving a stimulus or detecting response does not have nanoscale locality. Thus, even when the molecules involved are known, it is difficult to know how these molecules behave in real space and how these behaviors affect the structure and functions of the targeted protein. In NanoLSI, we detect such responses at the nano level using the nano-endoscopic imaging and analysis techniques. To give a stimulus with a nanoscale locality, we use molecular machines that change their structures in response to various external stimuli, such as changes in temperature, electric potential or light irradiation. There are two approaches to let these molecular machines act on specific proteins inside or outside of cells. The first is to synthesize molecular machines with specific target-protein binding sites and inject them into specific intra/extracellular nano-regions using nanopipette. This allows selective and local stimulation of proteins with a precision of several tens of nanometers. For the recognition of the target proteins, we use molecular structures of the recognition part of biomolecules with a help of a library that has been established in previous studies using biochemical methodology. We can arbitrarily control the timing of stimulation by applying an external stimulus. However, to investigate the effects of such artificially synthesized molecules *in vivo*, expert knowledge of pharmacology is essential. Nakajima's group have achieved an enhanced understanding of the factors determining drug response and toxicity as well as the underlying mechanisms by using molecular biological approaches (Annu. Rev. Pharmacol. Toxicol. 53 (2013) 377). Moreover, the dynamics/kinetics of molecules in the body have been quantitatively analyzed, and this has been extended to functional analysis of factors that regulate the dynamics/kinetics of molecules. The interaction of the molecular machines with the target proteins and the cellular response induced by their introduction into a cell are being analyzed from the viewpoints of pharmacology and toxicity, which contributes to the optimization of the molecular design. The second approach is to further improve the locality of the stimulation to the scale of several nanometers. This approach, in which a molecular machine is attached to a nanorod and then to an AFM probe end, is similar to that used for the measurements of distribution of physical properties mentioned above. Consequently, the orientation of the molecular machine in the targeted intra/extracellular site can be controlled, allowing it to act on a specific site with nano level precision. Supramolecular chemistry researchers at NanoLSI have developed molecules that change structures in response to various external stimuli (Fig. 5) (JACS 130 (2008) 5022; Nature Chem. 6 (2014) 429; JACS 139 (2017) 4631). By combining such techniques, various molecular machines that function as molecular tweezers and scissors will be developed. NanoLSI takes full advantage of these supramolecular chemistry techniques to achieve nano-endoscopic manipulation.

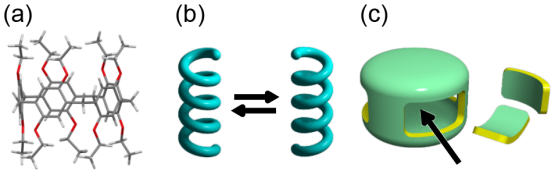

Fig. 5: Functional supramolecular structures. (a) Cyclic compounds for molecular recognition. (b) Dynamic structural control of helical molecules. (c) Molecular containers with caps for storing molecules.

[Nano-level understanding of the mechanisms of basic cellular functions using expertise in cancer research]
(Understanding measurement principles of newly-developed nano-probe techniques and life phenomena by means of mathematical/computational sciences)

・**Verifying and understanding the principles of nano-probe measurement techniques:** In general, the accuracy and reliability of a newly-developed measurement method should be verified before it is used for practical applications. Such verifications typically involve comparison with results obtained by an existing method and/or measurement of model samples with known properties. However, both of these approaches are often difficult for an atomic- or molecular-scale measurement technique. As an alternative solution, simulation techniques are becoming increasingly important owing to rapid advances in mathematical/computational sciences. However, very few research groups have experience in reproducing SPM measurements at the atomic/molecular level *in silico* and verifying the principles behind them. The Foster group is one of these few groups and has many experiences in joint research with SPM researchers from all over the world (Nat. Commun. 7 (2016) 12711; Nat. Commun. 7 (2016) 11559; Nat. Commun. 6 (2015) 8098). So far, he reproduced the 3D hydration-structure measurements reported by Fukuma using the atomic-level molecular dynamics simulation, making an important step towards the full understanding of the measurement principle (Phys. Rev. B 92 (2015) 155412). At NanoLSI, we reproduce nano-endoscopic imaging in 3D spaces, including the cell surface and interior, using computer simulation, and study the principles in detail. In particular, we clarify the effect of the probe on the measurement, the relationship between relaxation time and scanning speed, and the optimal measurement conditions for accurate measurement of life phenomena.

・**Understanding life phenomena:** Regardless of the measurement technique, we obtain information on the structure and properties of targets through probe–target interaction. Therefore, unless we fully understand the nature of the probe and interaction, even a clear atomic-resolution AFM image may not provide a complete understanding of the structure of the target. Simulation techniques help in bridging this gap between the measured data and the real-space model. For example, Fukuma recently developed high-speed FM-AFM and visualized calcite dissolution processes with true atomic resolution yet these images alone were not sufficient to fully understand the atomistic model of the dissolution process. In cooperation with Foster, they performed density functional theory and molecular dynamics calculations for numerous possible models and finally determined a unique atomistic model that is consistent with the experimental results. Similarly, at NanoLSI, we use such atomistic simulation techniques for reproducing the measurements of 3D distribution of water, ions and local surface structures. However, simulating the dynamics of an entire protein, organelle, or cell using this method is not practical because of the high computational cost. This problem will be solved by the coarse-grained modeling approach, where certain number of amino acids or molecules are treated as a cluster. Mikhailov, an expert in the simulation of complex systems (Nat. Phys. 6 (2010) 544; Science 264 (1994) 223), will use various mathematical approaches for modeling the formation and other collective behaviors of complex systems from simple elements and element–element interactions, and reproduce measured nano-dynamics of proteins, organelles and cells to elucidate the underlying mechanisms.

(Nano-level understanding of basic cellular functions using expertise in cancer research and innovative nano-probe techniques)

At NanoLSI, we will image, analyze and manipulate nano-dynamics of unlabeled nucleic acids, metabolites, proteins and organelles in normal and cancer cells using nanoendoscopic technologies. This will allow us to understand the mechanisms of basic cellular functions and their cancer-specific abnormalities.

Stem cells are defined as cells that have the ability to perpetuate undifferentiated status through self-renewal, and develop into mature cells through differentiation. The group of Hirao aims to elucidate the essential nature of stem cells, and has identified that inhibition of cell cycle transition, the removable of reactive oxygen species, and the activation of a nutrient starvation signals are important for regulation of the stemness and self-renewal of hematopoietic and leukemia stem cells (Nat. Med. 12 (2006) 446; Cell Stem Cell, 1 (2007) 101; Nature 463 (2010) 676). Therefore, precise measurement of the redox state and the distribution of nutrients such as glucose and amino acids in cells will enable us to elucidate the essence of the stem cell properties. Use of molecular probes that detect pH and nutrient distribution, and real-time imaging of the dynamics of molecules in normal stem and mature cells by high-speed/high-resolution SPM will pave the way to novel stem-cell control systems. In addition, comparative analyses between normal and cancer stem cells would lead to identification of cancer stem cell specific metabolic abnormality.

Molecular modifications to either DNA or histones affect the structure and function of chromatin in nuclei, leading to change of gene expressions. Inside nuclei, chromatins are considered to be separated by insulator molecules, making up different domains. In this project, we will visualize chromatin domains by nanoendoscopic technology to understand how cell type-specific gene expression patterns are controlled. Furthermore, since global changes in the epigenetic landscape are a hallmark of cancer, we will extend this approach to cancer cells. We will understand mechanisms how malignant properties are acquired in a process of tumorigenesis in aspect of chromatin architecture.

Growth factors play important roles in cell proliferation and development of normal tissues and organs. Matsumoto has played a pioneering role in physiology, drug discovery and medical application of hepatocyte growth factor (HGF) (Cell 67 (1991) 901; J. Gastroentel. Hepatol. 26 (2011) 188; Nat. Commun. 6 (2015) 6737). Recently, his group succeeded in visualization of the dynamic structural change for HGF receptor activation by high-speed AFM. This is the first to capture the dynamics of receptor activation, and the structures are unexpected. His group will verify: (1) structural dynamics and a new mechanism for transmembrane growth factor receptor activation, (2) aberrant molecular dynamics and activation mechanisms of mutant receptors found in cancer patients, and (3) structure-based peptide drug discovery by artificial regulation of the receptor activation.

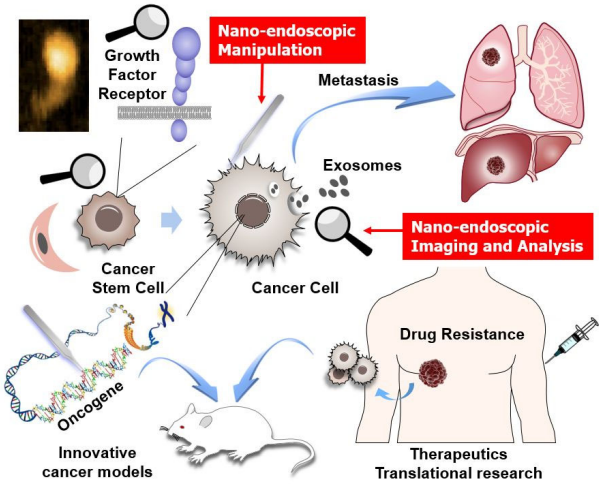

Fig. 6: Comprehensive research on cancer progression using nano-endoscopic techniques.

Exosomes released by cells are small (30–100-nm diameter) vesicles containing lipids, proteins, RNAs, etc. and participate in cell-cell communications, which contribute to variety of cellular functions and phenomena.

Hanayama has performed outstanding works on molecular mechanisms for the association between apoptosis and autoimmune disease, and has identified molecules involved in the formation, release, encapsulation, and uptake of exosomes (Nature 417 (2002) 182; Science 305 (2004) 1147; Cell 140 (2010) 619). In this project, by visualizing and analyzing the function and dynamics of exosomes at the single-particle level using SPM, Hanayama's group will elucidate the principle mechanism of action of exosomes and develop technique to control exosomes. As cancer cell-derived exosomes contain molecules closely involved in angiogenesis, immune-evasion, and metastasis, thereby participate in the formation of microenvironment promotive for cancer progression, these research will contribute to elucidate the detailed mechanisms of cancer progression mediated by exosomes.

For the development and regeneration of the normal epithelial organs, appropriate control of proliferation of normal stem cell is critical. Although the possible molecular mechanism for morphogenesis and regeneration has been elucidated by genome-wide analyses, precise morphological changes at nano-scale has not been investigated. It has been shown that the molecular processes of morphogenesis/regeneration are similar to that of cancer progression, and Oshima has developed various cancer models (Cell 87 (1996) 803; Cell 92 (1998) 645; EMBO J. 27 (2008) 1671). Oshima's group will examine dynamic changes of intracellular ultrastructures at nano scale in the intestinal stem cells during the above process with using cancer cells as references. Based on this project, we expect that dynamic change at nano-level in morphogenesis as well as tumorigenesis will be understood.

Molecular-targeted cancer therapy has changed the concepts and practice of cancer treatment. However, even when molecular-targeted therapies yield a favorable response, relapse due to drug resistance is a major problem. Yano has analyzed the mechanism of the resistance in lung cancer, and showed that growth factor-induced receptor activation leads to resistance against molecular-targeted drugs (Cancer Res. 68 (2008) 9479; Nat. Commun. 6 (2015) 8792; Cancer Discovery 7 (2016) 754). Yano's group will apply AFM to analysis of the dynamics of molecules involved in resistance signals and search for therapeutic targets. Using SPM techniques for patient-derived unlabeled molecules and cells, aberrant signal characteristics, sensitivity, and resistant responses are analyzed. The nano-manipulation technique to overcome resistance will be tested.

Thus, by collaborations with experts in cell biology, cancer biology and nanotechnology, we shall build a path to the future of life science and a new era of cancer biology.

2)-3 Project management

* Describe the center's research organization (including its research, support and administrative components) and your concept for building and staffing the organization.
* Describe your concrete plan for achieving the center's final staffing goal, including steps and timetables.
* If the center will form linkage with other institutions, domestic and/or foreign, by establishing satellite functions, provide the name(s) of the partner institution(s), and describe their roles, personnel composition and structure, and collaborative framework with the center project (e.g., contracts to be concluded, schemes for resource transfer).

* If the center will form linkage with other institutions, domestic and/or foreign, without establishing satellite functions, provide the names of the partner institutions and describe their roles and linkages within the center project.
* List in Appendix 4 the principal investigators who are expected to join the center. If it there are any changes from the list you submitted for the first screening, please state the changes and give your reasons for them.
* Provide a biographical sketch for each principal investigator using Appendix 5.
* List in the Appendix 6 the personnel making up center, including researchers and other center staffs, satellites, partner institutions. Use Excel format when preparing the list.
* Regarding the researchers invited from abroad or from other Japanese institutions, attach a letter from each of them indicating their intent to join the center project (free format). Label this bundle of documents "Appendix 7."

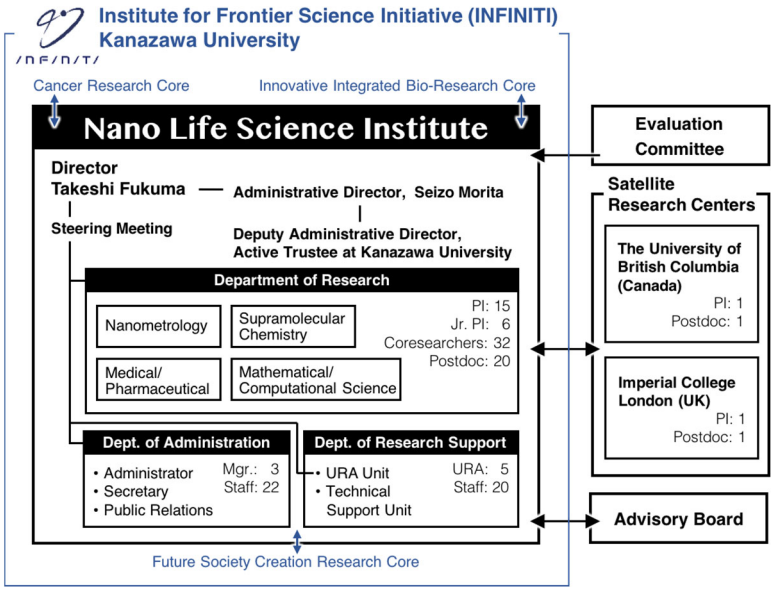

Fig. 7: Organization of NanoLSI

[The concept for NanoLSI]

The center will be comprised of a 77-person Department of Research, a 25-person Department of Administration, and a 25-person Department of Research Support.

At the time the center is established, 16 principal investigators will be appointed. These PIs will engage in research activities alongside the faculty at Kanazawa University, thereby bringing the total number of research department staff at the outset to 50. Furthermore, 20 administrative and Research Support staff will also be transferred to the center at the outset. Within 3 years, the center will reach its final staffing level of 127 persons.

Satellite Research Centers will be promptly established in Europe (the UK) and North America (Canada) in order to accelerate international joint research, train human resources through exchanges of young scientists, and improve international visibility.

i. Center in Europe: Imperial College London, London, UK: Professor Korchev as PI
ii. Center in North America: University of British Columbia, Vancouver, Canada: Professor MacLachlan as PI

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

* Paste on table a) of Appendix 6

	At beginning of project	At end of FY 2017	Final goal (Date: Mar.,2021)
Researchers from within the host institution	12	12	12
Foreign researchers invited from abroad	4	5	7
Researchers invited from other Japanese institutions	0	2	4
Total principal investigators	16	19	23

b) Total members

* Paste on table b) of Appendix 6

			At beginning of project		At end of FY2017		Final goal (Date: Mar.,2021)	
			Number of persons	%	Number of persons	%	Number of persons	%
Researchers			48		65		77	
	Overseas researchers		9	18.8	19	28.1	25	32.5
	Female researchers		5	10.4	12	18.5	16	20.8
	Principal investigators		16		19		23	
		Overseas PIs	5	31.3	6	27.8	8	31.8
		Female PIs	1	6.3	1	5.3	2	8.7
	Other researchers		32		46		54	
		Overseas researchers	4	12.5	13	28.3	17	31.5
		Female researchers	4	12.5	11	23.9	14	25.9
Research support staffs			15		15		25	
Administrative staffs			5		17		25	
Total number of people who form the "core" of the research center			68		97		127	

2)-4 Securing research funding

Past record

* Give the total amount of research funding (e.g., competitive funding) secured by the principal investigators who will join the center project. Itemize by fiscal year (FY2012-2016).

FY	2012	2013	2014	2015	2016
Amount	572	594	668	749	708

(Million Yen)

Prospects after establishment of the center

* Based on the past record, describe the concrete prospects for securing resources that match or exceed the WPI project grant.
* Calculate the total amount of research funding (e.g. competitive funding) based on the percentage of time that the researchers will devote to research activities at the center vis-à-vis the total time they spend on research activities ("Effort" in Appendix 5). Be sure that the prospects (FY2017-2021) are realistically based on the past record.

Within the first two years, the 16 PIs will have collectively secured an average of 650 million yen per year (after effort conversion) for external research funding;furthermore, by adding in the funds secured by other scientists who plan to participate in the center's research, the total figure would amount to 770 million yen. Further funding is anticipated with the addition of six junior PIs with high potential immediately after the center is established, as well as the appointment of staff specializing in fundraising support. In addition, the host institution will provide the equivalent of 540 million yen per year in research project expenses, usage of facilities and equipment, among others. Overall, the center secure 1.31 billion yen in FY2017-2018 and 1.41 billion yen in FY2019 -2020. This exceeds the project grant being requested.

3) Interdisciplinary Research

* Describe why interdisciplinary research is necessary and important in the target field(s) and what new field(s) can be expected to be created by way of this project. Describe your concrete strategy for advancing such interdisciplinary research.

1. Necessity and significance of interdisciplinary research

We aim to gain a fundamental understanding of the mechanisms of basic cellular functions at the atomic or submolecular levels by comparing nanodynamics inside normal and cancer cells. To this end, we will expand and combine our world's top bio-SPM and supramolecular chemistry techniques to take a dramatic technological leap toward the creation of nano-endoscopic techniques for direct imaging, analysis, and manipulation of nano-dynamics of proteins, nucleic acids, and other molecules inside and on the surface of cells, as well as nano-distributions of pH and oxygen concentration. Naturally, this work will require collaborations with oncology specialists in the medical and pharmaceutical sciences. Moreover, partnering with simulation experts will be essential for accurately understanding atomic and molecular dynamics from experimental results. As this illustrates, the path toward our goal will require an all-out effort that draws upon the techniques and knowledge of bio-SPM, supramolecular chemistry, medical and pharmaceutical sciences, and mathematical and computational sciences. This work will lead to the establishment of the new interdisciplinary science field of "nano-probe life science".

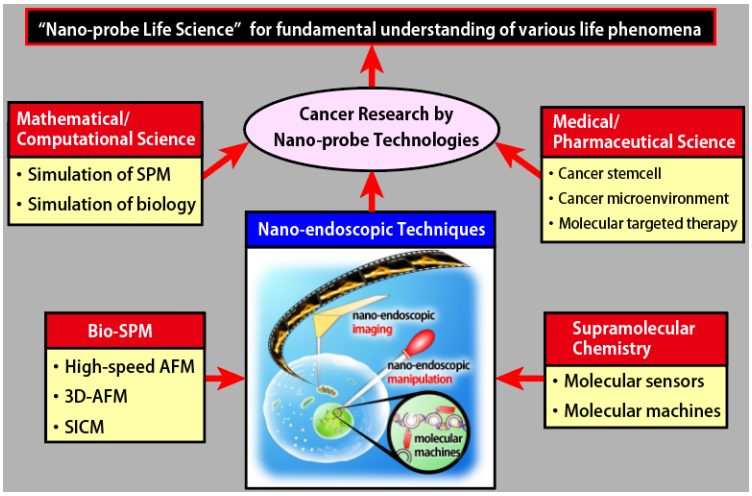

Fig. 8: Strategy for interdisciplinary research at NanoLSI.

2. Strategy for interdisciplinary research

In our research, there are two key challenges that are tackled with by combining expertise in multiple disciplines. The strategies for achieving this goal are outlined below.

[Development of nano-endoscopic techniques through combination of bio-SPM and supramolecular chemistry techniques]

We will realize nano-endoscopic analysis for visualizing the nano-distribution of pH, oxygen concentration, and other properties that significantly influence molecular and cellular dynamics, using SPM probes fitted with highly environmental-responsive molecular sensors. Simultaneously, we seek to achieve nano-endoscopic manipulation of nano-structures using probes fitted with molecular machines (molecular tweezers, scissors, etc.) offering high-level control functionality. Furthermore, we will develop a mode of combined measurement whereby molecular machines are injected into nano-regions with a nanopipette and a nano-probe will be used for live imaging of the molecular and cellular dynamics induced by stimuli from the molecular machines. In this way, we combine the expertise in the two disciplines, nanometrology and supramolecular chemistry.

[Understanding of mechanisms of basic cellular functions and their cancer-specific abnormalities using nano-endoscopic and simulation techniques]

The success of nanometrology depends on not only the performance of the instruments, but also the operating skills of their users and sample preparation conditions. In NanoLSI, SPM researchers will work on the optimization of the operating conditions while medical/pharmaceutical researchers will optimize the sample preparation

procedures. In the meanwhile, simulations necessary for proper interpretation of the measured results are performed by the following two researchers having different expertise in mathematical/computational sciences; Foster, specialized in the atomistic simulation of molecular dynamics and in *ab initio* calculation (Phys. Rev. Lett. 102 (2009) 126807; Phys. Rev. Lett. 93 (2004) 187202), and Mikhailov, focusing on simulation of complicated systems using coarse-grained modeling (Nat. Phys. 6 (2010) 544; Science 264 (1994) 223). The comparative examination of our experimental/simulation results and development of a nanoscale understanding of cancer-relate phenomena will involve pooling the knowledge of SPM, medical/pharmaceutical science, and mathematical/computational science researchers and repeated discussions.

[Formation of international alliance for "nanoprobe life science"]

We will establish an international alliance for the new research field "nanoprobe life science" to achieve the following three goals.

(Complementary use of various bio-imaging techniques)

Although nanoprobe technology is unique and powerful, it is not the only tool for bio-imaging. There are many other technologies and they are developing very rapidly. The complementary use of these different technologies will be a key to achieve fundamental understandings of various life phenomena. To achieve this goal, we will form an international alliance with other life science institutes having world-leading bio-imaging technologies. Tight collaborations through this alliance network will also allow us to keep monitoring the progress of other bio-imaging technologies and to adjust the plan for the development of our nanoprobe technologies. In this way, we will ensure that our technologies always stay at the frontier of bio-imaging.

(Complementary use of various expertise in molecular cell biology)

At NanoLSI, we have gathered various experts in each important cancer research area as well as in basic medical and pharmaceutical science. However, fundamental understanding of basic cellular functions requires broad range of expertise so that we may need to expand the range of our expertise. To this end, we will flexibly expand the international alliance network and, for a particularly important research area, we may recruit a talented researcher to join us as a Junior PI or a PI. The formation of international alliance should help us to meet and find a right person to join our research team.

(Widespread use of nanoprobe technologies for life science research)

Ultimate goal of our institute is to achieve nano-level understandings on various life phenomena using advanced nanoprobe technologies. Considering the wide variety of life phenomena, this goal cannot be achieved only by our institute. Thus, we aim to open our nanoprobe technologies to all life science researchers in the world. However, newly developed methods and instruments are not necessarily usable by non-experts and often need improvements in usability and applicability. Thus, we initially open our technologies to the limited members in the international alliance so that we can obtained detailed reports from them. Based on the feedback, we will improve our technologies to establish them as common tools for nanoprobe life science research.

4) International Research Environment

4)-1 System for advancing international research

* Describe your concrete plan for building an international research center including the makeup of its foreign researchers, establishment of oversea satellites, and provision of researcher exchanges. Please include a timetable for this plan.

* Describe concretely your strategy for staffing foreign researchers (e.g., postdoc positions) through open international solicitations. Describe the procedures you will use to do so.

1. Concrete plan for building an international research center

	2017	2018	2019
NanoLSI	-Employ 64 researchers for start-up (16 PIs incl. 5 from overseas, 2 Junior PIs, 46 other researchers incl. 13 from overseas) -Hosting a symposium in Tokyo -Holding 3 seminars	-Holding 12 seminars -Employ newly 2 Junior PIs incl. 1 from overseas; 4 Junior PIs incl. 1 from overseas in all -Employ newly 5 other researchers incl. 3 from overseas; 51 other researchers incl. 16 from overseas in all	-Holding 12 seminars -Employ newly 2 Junior PIs incl. 1 from overseas; 6 Junior PIs incl. 2 from overseas in all -Employ newly 3 other researchers incl. 1 from overseas; 54 other researchers incl. 17 from overseas in all
Satellite research centers	-Agreement with Imperial College London -Employ 2 Postdoc	-Agreement with University of British Columbia -Exchange of young scientists -Hosting an international symposium in Europe	-Exchange of young scientists -Hosting an international symposium in North America

Sixteen PIs will be hired when the center is established. During open recruitment for these positions, these positions will be advertised widely using a range of methods. This procedure will be repeated in FY2019 and thereafter in order to bring the center to full operation, with the recruitment drive occurring earlier than scheduled when possible.

4) -2 Establishment of international research environment

* Describe your concrete strategy for establishing an international research environment and administration system, and the support system to be provided for researchers from overseas.
* Describe your strategy, procedure and timing for periodically holding international research conferences or symposiums (at least once a year).
* Describe your measures to ensure that top-caliber researchers from around the world can work comfortably in carrying out their research within a competitive international environment.

1. Establishment of an International research center and administrative system, and provision of support for overseas researchers

[Establishment of an international research environment by radicalizing existing systems for university reform] Kanazawa University is working toward creating an international research environment and strengthening its research support system. Its unique Research Professor System and Center of Excellence system (Chozen Project) provide resources for building such research environment, while its Cross-appointment System enables flexible utilization of human resources. These unique systems enable the establishment of a world-class research environment, and through them, the university is able to engage 6 world-class scientists from abroad, including a Nobel laureate in chemistry. The new research center will further intensify these systems, which have already achieved excellent results, and will implement the following measures in order to allow the introduction of fresh blood from outside, and to enhance dynamic collaboration with other institutions.

- Launch other unique COE for enhancing transdisciplinary and trans-institutional research.
- Provide a competitive salary for all researchers and staff in NanoLSI.
- Provide funding for the employment of post-doctoral researchers, technical staff, and research lab secretaries.
- Employ university research administrators (URAs) who, as assistants to the center director, will support scientist recruitment and research funding acquisition.
- Utilize the excellent research networks of research professors invited from overseas to send

young scientists and students to overseas research centers, thereby creating an environment that supports the cultivation of pioneering international scientists and enhances the visibility of the center's research.

- Through international recruitment, hire young scientists who represent the next generation of leaders in their field as junior PIs, and use the tenure-track system to train and retain them as core staff who will lead the center in the future.
- At all times, assign 20 young staff to the department of administration, in order to introduce international administrative systems and staff culture in NanoLSI to the host institution as a whole through its staff rotation.

[Establishment of international research support system] In order to establish a world-class research support system that is capable of quickly implementing the center director's principles and vision, Kanazawa University will employ an administrative director with experience in international research and management of large-scale research projects. the university will also relocate administrative staff from Kanazawa University who are proficient in English and administrative tasks, and will enhance staffing through international recruitment in order to establish an all-English work environment.

In addition to the administrative department, the university will establish a research support department staffed with personnel (URAs and technical staff) who are highly skilled in specialized fields as well as proficient in English. After the WPI program ends, this department will be succeeded by International Research Support Center, which continues to offer high skilled technical and research supports.

[Establishment of a family support system] Spouses will be given priority in the hiring of positions within the university that match their qualifications. In addition, the university will work with the existing school affiliated with the university—an unusual program on the national level which offers a comprehensive preschool through high school education—to develop a special educational program for the children of the center's staff.

2. International research conferences

[Lightening scientist workloads and connecting with business networks through regular conferences organized by specialized support staff] A large-scale international symposium will be hosted once a year, revolving around the main research center and the satellite centers in Europe and North America. A kick-off symposium will be held in Tokyo in March 2018. Monthly international seminars will also be held with the aim of sharing research findings between fields and further promoting interdisciplinary research. Administrative staff specializing in public relations and event planning will handle all aspects of these events (including those hosted internationally) from planning to logistics, with guidance from the center director and PIs.

5) Center Management

5) -1 Operational management

* Describe the role of the center director.
* Describe the role of the administrative director.
* Concretely describe your concept for establishing an administrative organization.
* Concretely describe the center's decision-making system.
* Concretely describe how authority is allocated between the center director and the host institution.
* Concretely describe how the center will adopt a rigorous system for evaluating research and will introduce a system for merit-based compensation (e.g. annual salary scheme). Please describe your procedures and timing for operationalizing these systems.

1. Roles of the center and administrative directors

[Role of Center Director: Creating a new field of academic research] The aim of the center is to create a new interdisciplinary research field called Nanoprobe Life Science, which integrates nanometrology, cancer research, supramolecular chemistry, and computational science in order to elucidate the mechanisms behind a variety of vital phenomena. To achieve this goal, the center director will draw on his own background as a leader in various research fields to develop a vision for this interdisciplinary research. The center director will have authority to determine strategies and all other matters necessary for achieving this vision as well as deciding on the center's direction.

[Role of the administrative director: Achieving the vision] Based on the center director's strong initiative, the administrative director will work to realize the center director's vision, and will develope a research environment that enables the achievement of this vision in the most appropriate way. Kanazawa University will invite Osaka University Professor Seizo Morita to server as administrative director because of his deep understanding of the research areas this center will focus on as well as his abilities to succeed in a competitive international environment and to manage large-scale research projects.

2. Concept for establishing an administrative organization

[Basic concept] Kanazawa University's top priority in establishing an administrative department is to create an effective, prompt, and flexible support system so that scientists can focus on their research activities. To that end, the University will establish a separate research support department staffed with highly specialized personnel. This support system will be continuously reevaluated and improved in order to provide scientists with optimal support. Furthermore, to ensure effective operation, the administrative and research support departments will coordinate extensively with the university's administrative headquarters and departmental administrative offices, thereby drawing on the knowledge and knowhow of the entire university.

[Appointment of a current trustee as deputy administrative director: Building close ties with the university's executive office] A current trustee in the university's executive office will be appointed as deputy administrative director, serving directly under the administrative director. While always prioritizing the center director's vision and the administrative director's approach to managing the center, the deputy will build close ties with the host institution's current executive office, thus enabling various issues to be addressed and ensuring that the center runs smoothly.

3. Decision-making system

[Establishment of a steering meeting] the center director will have authority over all aspects of the center's operation, with the exception of his own dismissal and salary. However, in order to ensure optimal cooperation with the administrative director and PIs and appropriate operation of the center, the center director will establish a steering meeting consisting of the Center Director, Administrative Director, Deputy Administrative Director, URA Unit Leader, technical support unit leader, and other staff appointed by the center director. The steering meeting will have jurisdiction over research, personnel affairs, budgets, and self-assessments. The establishment of just one such meeting ensures that information will be centralized, the center director will be able to implement top-down management, and the number of meetings will be minimized, thereby ensuring that research activities are prioritized

[Establishment of an advisory board] The Center Director will establish an advisory board consisting of the president of Kanazawa University, leading international scientists in related fields, and world-class research center managers. The center director will be able to request advisory support and receive advice from this board at any time regarding interdisciplinary research and operation of the center.

4. Allocation of authority between the center director and the host institution

The center director will have complete authority over decisions related to the center's research strategy, as well as the acquisition and allocation of budgetary resources, personnel, space, and other research resources as well as all other matters. To this end, Kanazawa University will conform to its Commitment, and will proactively reassess existing regulations and develop new systems. Furthermore, to provide foundational support for the center's initiatives, the university will have the authority and responsibility to allocate part of its budget to the center. It will also review and audit the management of the center.

5. Research Evaluation system and flexibly salary system

An annual salary system will be applied to all center personnel, including faculty, administrative department staff, and research support department staff. Their pay scale will be higher than that of other university personnel. At the time of hiring, researchers will present a five-year research plan, and their progress will be evaluated yearly on the basis of this plan. The center director will appoint an evaluation working group to each researcher, consisting of 2 PIs or Junior PIs, 1 researcher working in a related field at Kanazawa University or elsewhere, and other individuals, as deemed necessary. The center management will be evaluated biennially by an external Evaluation Committee that comprises at least 10 world-class scientists. This evaluation committee will assess initiatives to increase interdisciplinary and international research, the level of research achievements, center management, the performance and treatment of staff scientists, and the screening of Junior PIs for tenure positions. The results of these evaluations will be used to improve the center's management.

5) -2 Research environment

* Concretely describe how equipment and facilities, including laboratory space, will be provided in a manner appropriate for a top world-level research center. Include your procedure and timing.
* Concretely describe how the center will provide an environment in which researchers can work comfortably on their research by being exempted from duties other than research and related educational activities, and how they will be provided adequate staff support to handle paperwork and other administrative functions. Include your procedure and timing.
* Concretely describe how the center will arrange for its researchers to participate in the education of graduate students.

1. Provision of research facilities and equipment

The center will be part of the Institute for Frontier Science Initiative, which occupies a research building with 2,100 square meters of total floor space. This research building features open laboratories and common spaces that encourage intellectual cross-pollination, thereby promoting interdisciplinary research. 12 PIs at Kanazawa University currently have approximately 2,850 square meters of research space at their disposal; and in addition, the university has prioritized the center in its facilities and equipment plan, which will provide a new research building with 7,190 square meters of total floor space.

2. Streamlining of procedures and strengthening of support staff

[Streamlining of procedures and allocation of outstanding staff: Freeing scientists from administrative duties] In order To quickly achieve the center director's vision, cumbersome procedures will be eliminated, and

administrative staff will be granted extensive discretionary powers. In addition, staff will be allocated to provide "one-stop support" for all procedures and paperwork related to employment and daily life. To further enable the center director and PIs to focus on research, an adequate number of secretaries with appropriate skills will be transferred to the center. These measures will be implemented by the end of FY2018 in order to release researchers from administrative duties as quickly as possible.

[Establishment of Department of Research Support: Support from specialized staff] In addition to the administrative department, the university will establish a research support department staffed by personnel with highly specialized skills. This department will include URA and technical support units. The URA unit will provide support in attaining international grants, recruiting scientists, promoting relationships with industries and other entities, and facilitating interdisciplinary research. The Technical Support Unit will handle research measurement and analysis requiring advanced technical skills and provide technical guidance to young scientists and graduate students.

3. Participation in graduate student education

A new "WPI Education Program (temporary name)" will be established to allow PIs and Junior PIs to participate in graduate student education.

5) -3 Establishing the center in sync with organizational restructuring

* Concretely describe the host institution's organizational reform that will be synchronized with the establishment of the center.
* Describe measures that will be taken by the host institution to sustain the center's operation after the WPI funding ends. Also describe how the host institution will promote the centers autonomy after WPI funding ends and how it will over the mid-to-long term restructure its existing organization in ways that give the center a permanent place within its organization.

1. Integration of the research center with an existing organization and measures to sustain independent operation after WPI funding ends

As described in section 2 of the Host Institution's Commitment, the goal of the Institute for Frontier Science Initiative is to strategically advance interdisciplinary research. The institute, which has led the university's strategic organizational reforms, is a permanent organization. Placing the new research center within the Institute for Frontier Science Initiative likewise situates it clearly as a permanent organization.

2. Plan for restructuring the existing organization over the mid-to-long term

NanoLSI will develop new systems and management methods in order to build an international research environment and flexible management system. The most successful of these initiatives will be incorporated into mid-to-long-term goals and plans, and will be adopted by the host institution as a whole. This will contribute greatly to the reform of Japan's university conventional system overall.