Summary of Research Center Project

*Briefly describe the general plan of your project (Compile in English within 2 pages.)

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）

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

1) Overall Image of Your Center

We combine the world's top bio-scanning probe microscopy (SPM) and supramolecular chemistry techniques to develop "nano-endoscopic techniques" that can directly image, analyze, and manipulate the nano-dynamics of proteins and nucleic acids on the surface of or inside cells. With the developed nanoprobe technologies and multi-scale simulation techniques, we will achieve nano-level understanding of basic cellular functions and their cancer-specific abnormalities. Based on the techniques developed and knowledge gained, we establish a new academic field, "nano-probe life science," where we aim to achieve fundamental understandings on various life phenomena using advanced nanoprobe technologies.

2) Research Activities

1. Development of imaging techniques for intra- and extracellular nano-dynamics

Ando improved the speed of atomic force microscopy (AFM) by several hundred-fold to develop high-speed AFM, enabling imaging protein dynamics at nanoscale resolution in solution. Fukuma developed liquid-environment frequency modulation AFM, enabling true atomic-resolution imaging in liquids. Based on this technique, he also developed three-dimensional AFM (3D-AFM) and achieved subnanometer-scale imaging of the 3D distribution of fluctuating water and surface structures at solid-liquid interfaces. Korchev established the basics for scanning ion conductance microscopy (SICM), an SPM technique that utilizes a nano-pipette, and was the first to demonstrate its effectiveness in live cell imaging. These researchers designed and fabricated their unique SPM devices from scratch, and have used them for various nano-biological measurements that were not possible using the preexisting techniques. In NanoLSI, we will foster this ability to create innovative nano-probe techniques. First, we will develop high-speed SICM by combining high-speed AFM and SICM to image the nano-dynamics of cellular structures. We will also develop high-speed 3D-AFM by combining 3D-AFM and high-speed AFM to image 3D distribution of signaling factors that interact with or pass through the receptors or channel proteins on the cell surface. We will combine this technique with Korchev's nano-fabrication technique to develop a nano-endoscopic imaging technique, in which a long glass probe with a high aspect ratio is scanned in a 3D space (inside of a cell). The force or ion current on the probe apex is recorded to visualize various nano-dynamic events occuring inside the cell, including rearrangement of molecular assemblies and material transportation.

2. Development of nano-endoscopic analysis and manipulation techniques

By combining the supramolecular chemistry techniques developed by Ogoshi, Maeda, Akine, and MacLachlan with the aforementioned bio-SPM techniques, we will develop nano-endoscopic analysis and manipulation techniques. We will design and synthesize molecular sensors that undergo a structural transformation in response to changes in pH, or concentration of oxygen species or metabolites in the liquid environment. These molecular sensors will be integrated at the apex of a nano-pipette. The nano-pipette is scanned in the intra- and extracellular spaces with high-speed 3D-AFM to

measure the nanoscale distribution of pH and oxygen concentration at the surface of or inside the cell. Furthermore, we will design and synthesize molecular machines that undergo structural changes in response to external stimuli such as temperature, electric potential, or light and let them act on intracellular or extracellular proteins in two ways. In the first method, molecular machines with a recognition part for a target protein are injected by a nano-pipette. In the other method, a molecular machine is attached to the apex of an AFM probe through a linker molecule and the probe position is controlled such that the molecular machine can act on a target protein. We will measure the dynamic responses of intracellular and extracellular molecules and cells induced by these nano-endoscopic manipulations using the nano-endoscopic imaging and analysis techniques.

3. Fundamental understanding of basic cellular functions and their cancer-specific abnormalities

We utilize nanoendoscopic technologies for fundamental understanding of basic cellular functions. To do so, we image, analyze and manipulate nano-dynamics of unlabeled nucleic acids, metabolites, proteins and organelles in both normal and cancer cells. This will also allow us to understand the mechanisms of cancer-specific abnormalities. Hirao aims to elucidate the essential nature of stem cells by precise measurement of the redox state and the distribution of nutrients such as glucose and amino acids in normal and cancer cells. To understand cell type-specific gene expression patterns, chromatin domains will be visualized. Since global changes in the epigenetic landscape are a hallmark of cancer, we will extend this approach to cancer cells. Matsumoto elucidates the structural changes in growth factor receptor activation involved in cell proliferation and investigates aberrant activation mechanisms of mutant receptors found in cancer patients. Hanayama elucidates the principle mechanism of action of exosomes and develop technique to control exosomes. Oshima investigates dynamic changes of intracellular ultrastructures at nanoscale in the intestinal stem cells and cancer cells. Yano applies AFM to analysis of the dynamics of molecules involved in resistance signals and search for therapeutic targets. Thus, we shall build a path to the future of life science by collaborations with experts in cell biology, cancer biology and nanotechnology.

3) Interdisciplinary Research

We combine the world's top bio-SPM and supramolecular chemistry techniques to develop innovative nano-probe techniques, including nano-endoscopy. With these techniques and multiscale simulations, we fundamentally understand the mechanisms of basic cellular functions and their cancer-specific abnormalities. In this way, we combine our expertise in nanometrology, supramolecular chemistry, mathematical/computational sciences, and medical/pharmaceutical sciences to establish a novel academic research field, "nano-probe life science."

4) International Research Environment

Kanazawa University will locate world-class researchers as PIs and recruit young researchers by radically and flexibly utilizing the personnel and administrative systems. The university will establish a support system so that overseas researchers can exert their full abilities and have a comfortable stay in Kanazawa.

5) Center Management

This center will be established in the Institute for Frontier Science Initiative; it will be qualified for personal and capital source investment. Center Director will have the authority to decide the budget and administrative affairs of the Center.