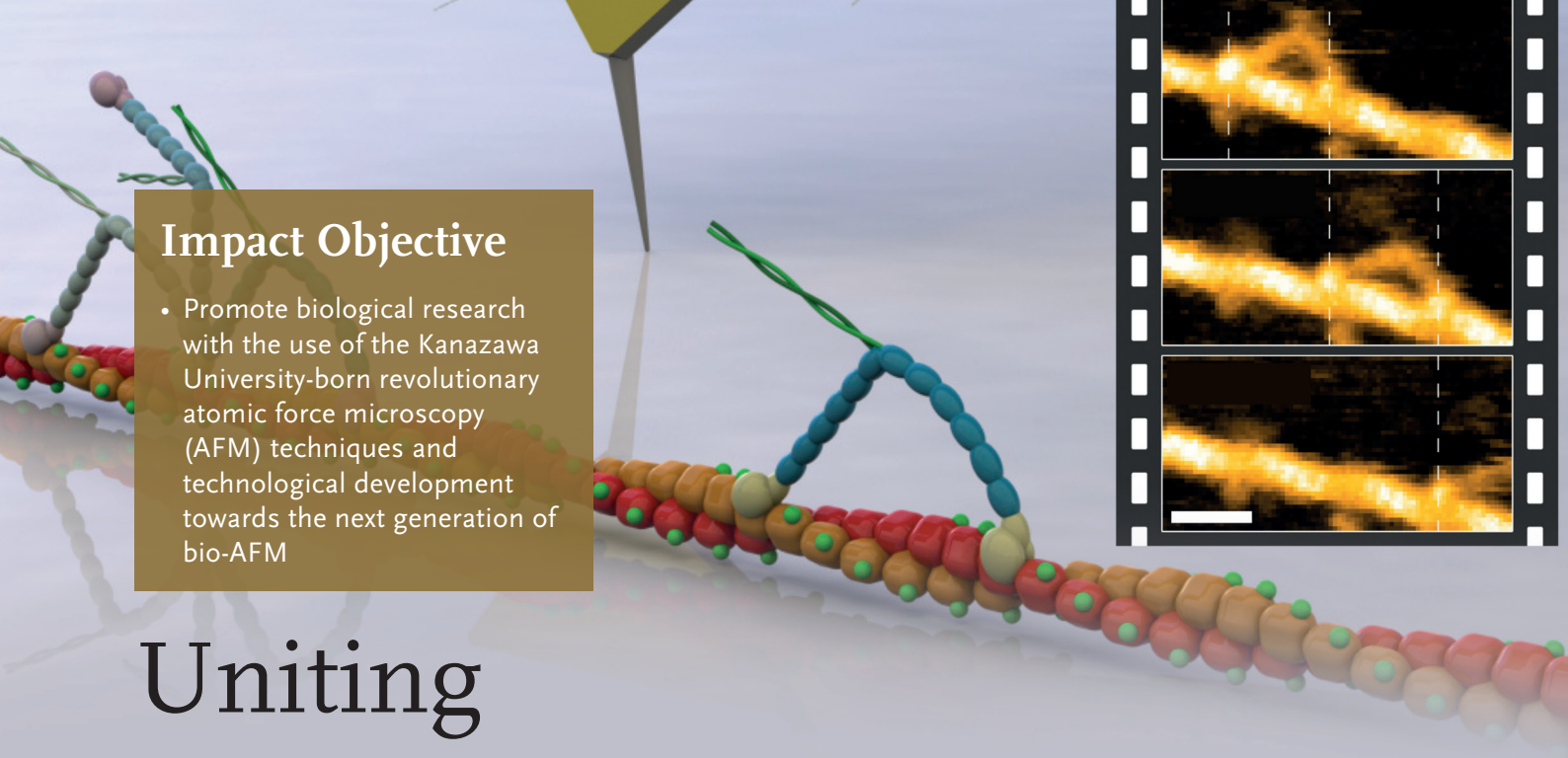


Impact Objective

- Promote biological research with the use of the Kanazawa University-born revolutionary atomic force microscopy (AFM) techniques and technological development towards the next generation of bio-AFM

Uniting biological research

*The four **Heads of Division** in the **Bio-AFM Frontier Research Center** at **Kanazawa University** explain their passion for their work, the principles of the Center, and the future applications of their research*

PROFESSOR TAKAYUKI UCHIHASHI, DIRECTOR OF THE BIO-AFM FRONTIER RESEARCH CENTER AND HEAD OF THE HIGH-SPEED AFM DIVISION

Can you share a little about how your own career has developed?

I helped develop atomic force microscopy (AFM) under the supervision of Professor Seizo Morita. I learned many skills: electronics implementation, software programming and mechanical drawing. During my PhD (Osaka University), I was involved in development of non-contact AFM (NC-AFM) working in an ultrahigh vacuum. I continued research using NC-AFM during my postdoc in the Joint Research Center for Atom Technology in Tsukuba. Here, I gradually shifted my observation target from inorganic to organic materials. When I was subsequently working in Trinity College in Dublin, I met Professor Toshio Ando at a conference in 2002. His presentation about high-speed AFM (HS-AFM) had a striking impact on me as I had never imagined that AFM could achieve such fast imaging. I then decided to change my research focus to biophysics using HS-AFM. After joining Professor Ando's lab at Kanazawa University, I gained experience in the establishment of HS-AFM and played a direct role in some developments. I am very proud of what we have contributed to biological issues using HS-AFM.

What is the impact of the work that is under way in the High-speed AFM Division?

The mission of our Division is the development of novel instruments based on the HS-AFM technique and using these in the Imaging Research Division. We are always trying to refine the present HS-AFM technique to improve imaging speed and invasiveness to render it a more versatile tool. We also develop basic techniques for functional extensions of HS-AFM. For example, we created a wide-area imaging technique for live-cell imaging, temperature control systems, and interactive-mode HS-AFM, which can manipulate molecules with an external force. Additionally, we are working on combining AFM with super-resolution optical imaging and manipulation techniques. These are a powerful way to expand the usefulness of HS-AFM.

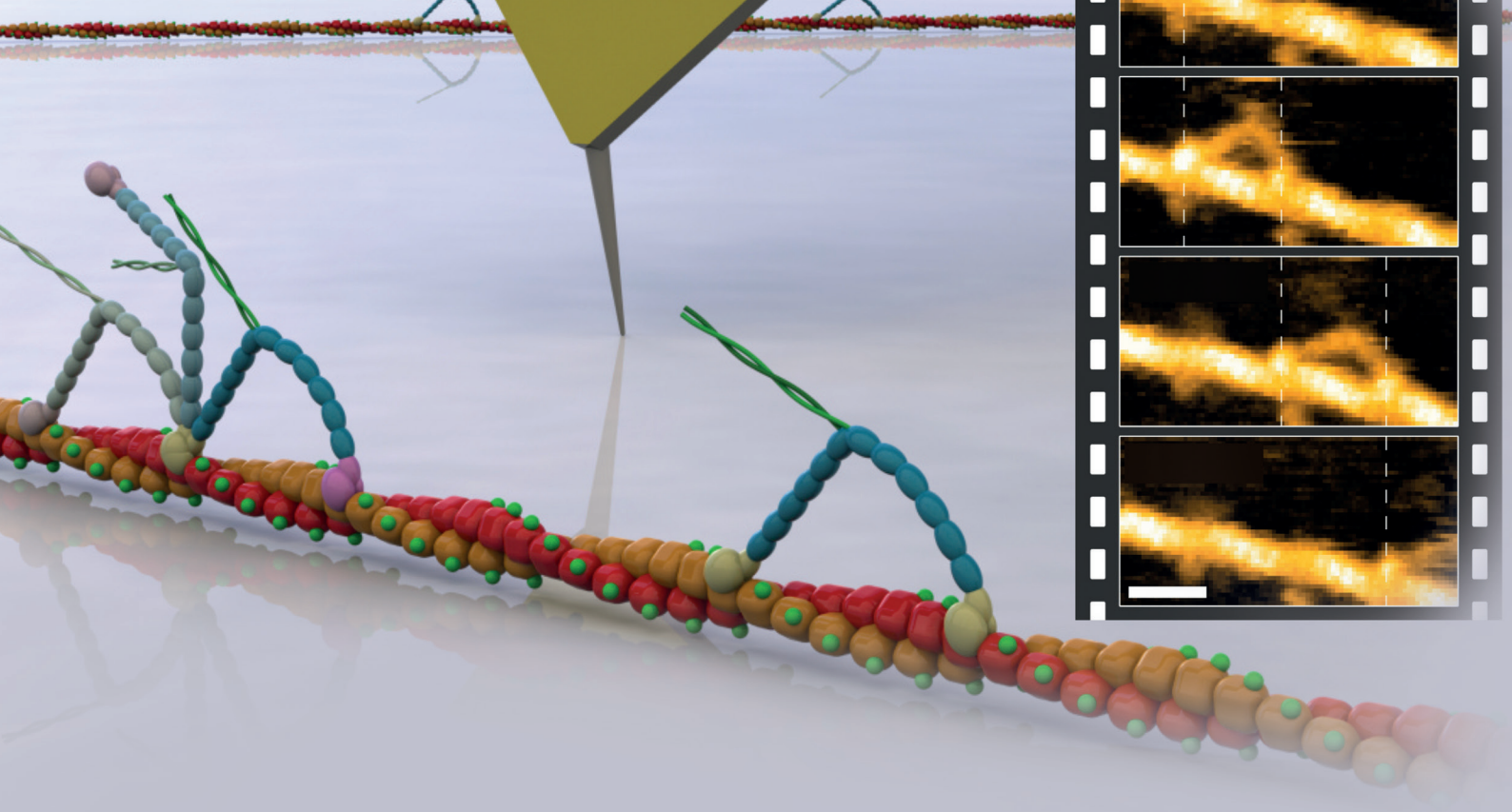
PROFESSOR TOSHIO ANDO, HEAD OF THE IMAGING DIVISION

What are the different areas of expertise of the staff within the Center?

We have staff members with backgrounds in physics, applied physics, physical chemistry, biophysics, cell biology and bacteriology. They are skilled in electronics design, software programming, designing mechanical devices, control and measurement techniques, protein expression and purification, cell cultures, and preparation of organelles.

Have there been any key moments that have changed your career pathway?

I gained my PhD in physics at Waseda University and then worked as a post-doctorate researcher at the University of California, San Francisco. After gaining a tenured position in physics at Kanazawa University I was able to set new research targets. I learned of the existence of AFM from Kazuhiro Oiwa. He talked about an AFM experiment that he was planning to perform on actin-myosin interactions. I then built an AFM instrument combined with a fluorescence microscope and obtained AFM images of myosin. I was quite disappointed by the acquired images because they were no better than electron microscopy. I wanted to see myosin molecules in dynamic action. I started to develop HS-AFM in 1994. This was initially difficult due to lack of funds but this was solved by a large grant from the New Energy Development Organization. My student, Noriyuki Kodera, worked very hard to get HS-AFM images of myosin V and succeeded! We published the paper in PNAS (October 2001). We started to image myosin V interacting with actin filaments, however the filaments broke down during imaging as the tip-sample interaction force was too strong. After struggling with this problem for three years I was able to solve it and we finalised the development of HS-



AFM for practical use in 2008. At last, in 2010, we could watch myosin V walking on an actin filament and we published a paper in *Nature*.

PROFESSOR RICHARD WONG, HEAD OF THE MOLECULE & CELL RESEARCH DIVISION

How has your own research career developed?

I grew up in Hong Kong. To explore the world beyond the 'Pearl of the Orient', I applied and was admitted to University of Tokyo. Initially, my dream was to become a top investment banker! It was during that period I began to appreciate the amazing world inside cells, with all its intrigue and uncharted complication. I was fortunate to start working in a cell biology and anatomy lab led by Nobutaka Hirokawa. The lab was very energetic. People from around the world worked closely together, sharing many experimental tricks and drinking a lot of sake at parties. I enjoyed the lab work and lab life so much that I decided to make it my career.

I moved on to work in Günter Blobel's Lab of Cell Biology at Rockefeller University. Günter is a great mentor. He is energetic and has a lot of motivating ideas, yet is very open-minded and supportive of new pursuits. We discovered that vital role of nuclear pore proteins in chromosome segregation, spindle polarity and centrosome homeostasis during mitosis. I could not have made these findings without the generous support of Günter and Nobutaka, and the valuable help from so many who have worked with me. I am very fortunate.

What kind of research is under way in your Division?

Traffic inside a cell is as intricate as rush hour near any metropolitan area. But drivers know how to follow the roadways and signs to reach their destinations. How do diverse cellular proteins 'read' molecular signposts to find their way? How does the nuclear pore gatekeeper control the trafficking of proteins, RNAs and viruses? Our Division is divided into two groups. Group 1 focuses on the nuclear pore. We are observing nuclear pores in cancer cells using HS-AFM. We wish to expand HS-AFM as a diagnostic tool for use with patients' cells. Group 2 focuses on the bacterial organelle magnetosome, which functions as a cellular compass to navigate along the Earth's magnetic field.

PROFESSOR TAKESHI FUKUMA, HEAD OF THE SUPER-RESOLUTION AFM DIVISION

Is there any work under way in your Division that you would like to share?

We are working on a number of new techniques including HS frequency modulation AFM (FM-AFM), which we recently improved the speed from 1 min/frame to 1 sec/frame. Another is 3D scanning force microscopy (3D-SFM) which enables the visualisation of 3D distributions of hydration structures and flexible molecular chains at a solid-liquid interface. We are now working with three different companies due to this. Furthermore, we have also developed open-loop electric potential microscopy (OL-EPM). This technique is useful in observing local distributions of electrochemical reactions at a solid-liquid interface. We are working with three different companies on different subjects related to the metal corrosion processes. Corrosion is a serious problem in many industrial fields. The annual economic loss caused by corrosion amounts to 3–4 per cent of the GNP of a country. With OL-EPM, we can directly visualise local distribution of active corrosion sites in real time in situ.

How has your research path developed?

I started working on AFM during my postgraduate studies. Initially, I worked on the application of the commercial AFM instruments to the studies on polymeric materials. During my PhD, I studied more advanced commercial instruments: ultrahigh vacuum AFM for studying organic ultrathin films at molecular-scale resolution. As a postdoc, I started to work on improvements to the AFM instruments. During this period, I succeeded in developing liquid-environment FM-AFM that is capable of imaging atomic-scale structures in liquid. This is my biggest achievement so far. Most researchers believed that FM-AFM could not work in liquid. With this success, I went abroad to Trinity College Dublin. There I mainly worked on the development of my own AFM system for liquid-environment applications. I also worked on the applications of the developed system to studies on biological systems.

Focusing Atomic Force Microscopy

*The **Bio-AFM Frontier Research Center** at **Kanazawa University** combines cutting-edge research into developing novel techniques of atomic force microscopy with directing these techniques towards biological applications*

Atomic force microscopy (AFM) is a powerful technique capable of visualising the topography of materials at an atomic resolution. At its most basic, a fine tip is used to probe the surface of the material with detection of tip-surface interaction. This allows the construction of a surface image in very fine resolution not limited by diffraction. Developments made by researchers at Kanazawa University in Japan have refined AFM and allowed its application to a variety of different circumstances and, crucially, to biology.

Initially, probing a wide range of biological samples was difficult as the tip involved in AFM often destroyed the sample. In order to tackle such problems and focus the technology towards end users, the Bio-AFM Frontier Research Center was established in 2010. Led by Professor Takayuki Uchihashi (Director and Head of High-speed AFM), the institute has been working hard on imaging a diverse range of biological scenarios – from cell organelle trafficking to individual protein interactions and membrane interactions. Additionally, the institute works with industrial partners to apply their techniques to current chemical engineering issues such as corrosion of metal surfaces. The Center is split into four divisions – High-speed AFM, Super-resolution AFM, Imaging, and Molecule & Cell Research. Professor Toshio Ando (Former Director, Head of Imaging) explains the basic aim of the Center: 'Our interdisciplinary centre aims for international leadership in the biological AFM technology and the understanding of dynamic biomolecular and cellular processes.'

TECHNICAL TRIUMPHS

The Bio-AFM team have been able to adapt AFM to a variety of different applications and thus achieve both high-resolution and real-time images.

A major advance in this area was made by Ando, Kodera (Member of Imaging) and Uchihashi when they created high-speed AFM (HS-AFM) with a low-invasive imaging capability. The higher speed of this technique allows a more biologically relevant look at the structural changes of a molecule. Alongside this, Professor Takeshi Fukuma (Head of Super-resolution AFM) developed non-contact AFM in which the probing tip does not make direct contact with the surface, rather relying on the effect of hydration forces to measure topography. This prevents the degradation of the sample by the tip, which was particularly common for biological samples. He is developing several branches of AFM to image the solid-liquid interface.

GROUNDBREAKING BIOLOGY

The Bio-AFM Frontier Research Center was primarily founded in order to further the application of these AFM-derived techniques to the life sciences. To this end, Professor Richard Wong is leading the Molecule & Cell Research Division that is investigating two key imaging areas. First is the focus on the nuclear pore and determine how it selects the molecules and proteins that pass through it. Secondly they are investigating the bacterial organelle known as the magnetosome which allows certain bacteria to navigate by the Earth's magnetic field. This organelle contains magnetite crystals surrounded by a lipid bilayer and is thus an ideal structure for investigation through AFM means.

Together, the four Divisions of Bio-AFM are working to develop and hone tools that promise to greatly expand our ability to investigate fine molecular structures. From visualising fundamental atomistic phenomena to uncovering the complexity of protein functions, Bio-AFM will generate new levels of understanding of molecular and atomic interactions.

Project Insights

FUNDING

KAKENHI Basic Research (S) #24227005 (PI, Toshio Ando) – New structural biology to be opened by high-speed atomic force microscopy (2012 – 2017) • JST/CREST (PI, Toshio Ando) – Dynamic structural biology of protein machineries driven by ATP/GTP (2013 – 2019) • KAKENHI Research on Innovative Area #26119003 (PI, Toshio Ando) – Development of advanced high-speed AFM and analysis of proteins' action mechanism (2014 – 2019)

AIMS

Develop technology for atomic/molecular-level analysis • Pioneer biological phenomena and biological samples for bio-AFM studies • Develop instruments for elucidating biological phenomena • Elucidate biological phenomena at the single-molecule level

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BIO

Professor Toshio Ando received a Dc. Sci. degree in Physics from Waseda University, Japan, in 1980. He was a postdoctoral fellow at University of California, San Francisco, before becoming an Assistant Professor at the same institution. He returned to Japan in 1986 to work at Kanazawa University where his research focuses on high-speed AFM and its application to proteins and cells. Ando is the founder of the Bio-AFM Frontier Research Center.