

Kanazawa University WPI-NanoLSI 2022 Academic Year Bio-SPMs Collaborative Research Application Guidelines

The Nano Life Science Institute (NanoLSI), Institute for Frontier Science Initiative, Kanazawa University, is calling for applications for Bio-SPMs collaborative research.

1. Aims

The research teams in Kanazawa University have engaged in the pioneering development of original Bio-SPM technologies, including Super-resolution AFM (FM-AFM/3D-AFM), High-speed AFM, and Scanning Ion Conductance Microscope (SICM), and applied them to the life sciences.

WPI-NanoLSI, Kanazawa University, aims to contribute to the development of the nano life sciences by promoting collaborative research using these Bio-SPMs, beyond the boundaries of individual universities and research institutions. Here, we call for applications for collaborative research projects that are carried out by the applicants using our Bio-SPMs, with the cooperation of the NanoLSI faculty members. This year, we also call for applications for collaborative research projects in which AFM is used for cell measurement.

* Please note that this is a call for collaborative research projects, not a call for contract analysis.

2. Application Eligibility

Applicants must be researchers or engineers from national public or private universities, national research institutes, private companies, overseas research institutes, etc. Please note that students such as graduate students and undergraduate students are not eligible as applicants, but can be included in the research project team. Depending on the applicant's affiliated institution, a collaborative research contract could be required.

3. Research Period

For applicants adopted in 1st or 2nd call, from the date of approval to March 31, 2023.

For applicants adopted in 3rd call, research can be carried out after March 31, 2023

Note: you should inform us of the period during which you intend to perform the research within one month of notification of approval.

4. Type of Collaborative Research

1) Collaborative Research by visits to NanoLSI

Applicants adopted visit WPI-NanoLSI and carry out a research project, with the cooperation of the NanoLSI faculty members.

2) Collaborative Research by sending samples

Applicants adopted does not visit WPI-NanoLSI. Samples are sent to WPI-NanoLSI and researchers in charge at WPI-NanoLSI conduct experiments. Please note that this option is limited to cases where visiting WPI-NanoLSI is difficult due to the COVID-19-related issues. In addition, the applicant is supposed to obtain permission from the researcher in charge before application.

5. How to Apply

Prepare the following application materials (i.e., Form 1 and curriculum vitae). If applicants have discussed research topics, research schedule, schedule for visiting NanoLSI, etc. with the NanoLSI faculty member(s), indicate these things in Form 1.

[Application Materials]

(Form 1)	"2022 Academic Year Bio-SPMs Collaborative Research, Application and Collaborative Researcher Approval Form"
(Arbitrary Format)	"Curriculum vitae for the principal investigator (applicant) only"
*Form 1 can be downloaded from the Bio-SPMs Collaborative Research website: https://nanolsi.kanazawa-u.ac.jp/en/research/applications/bio-spm/	

[Submission Deadlines]

Applications must reach us by

1st in 2022: May 31, 2022 (Monday), 17:00 JST.

2nd in 2022: July 29, 2022 (Friday), 17:00 JST

3rd in 2022: October 31, 2022 (Monday), 17:00 JST

1st in 2023 will be May 31, 2023 (Wednesday), 17:00 JST

For urgent cases, applications will be accepted regardless of the deadline. In such cases, contact the person in charge of "5. Inquiries Regarding Research Contents."

[Submission Destination]

Nano Life Science Institute, Kanazawa University,
Kakuma-Machi, Kanazawa, 920-1192, Japan.
Tel.: +81-76-234-3866, Fax: +81-76-234-4559
E-mail: bio-spmscr_nano@ml.kanazawa-u.ac.jp

6. Inquiries Regarding Research Content

Send an inquiry to the e-mail address below. The inquiry should include the type of Bio-SPMs you wish to use (either Super-resolution AFM [FM-AFM/3D-AFM], High-speed AFM, SICM, or AFM for cell measurement). The reply will come from the person in charge of the specific Bio-SPM technology.

E-mail: bio-spmscr_nano@ml.kanazawa-u.ac.jp

7. Decisions

After review by the Expert Committee of NanoLSI, Kanazawa University, the director of NanoLSI will decide which applications will be approved. The applicants will be informed of the decision.

8. Research Report

An approved researcher has an obligation to report the research results when the research period is over. Using Forms 2 and 3, prepare and submit the research report. The deadline is May 8, 2023. The summary of research results (Form 3) will be publicly posted on the NanoLSI Bio-SPMs Collaborative Research website in 2023 academic year.

(Form 2)	"2022 Academic Year Bio-SPMs Collaborative Research, Research Report"
(Form 3)	"2022 Academic Year Bio-SPMs Collaborative Research, Research Report Summary"
*Forms 2 and 3 can be downloaded from the Bio-SPMs Collaborative Research website: https://nanolsi.kanazawa-u.ac.jp/en/research/applications/bio-spm/	

9. When research papers are published

In order to measure the effectiveness of this Bio-SPMs collaborative research activity, we would like to identify research papers published through collaborative research carried out at NanoLSI. Therefore, approved researchers should inform us when research papers are published. In addition, for three years after the end of research period, we will contact all approved researchers every June via email for an update on any of newly published papers.

10. Others

- (a) Research applications that match our policy described in "1. Aims" will be preferentially approved. It is preferable that applicants have already conducted sample preparations and preliminary experiments, for example, biochemical experiments, test of observation conditions, and microscopic analysis by optical microscope, electron microscope, SPM, etc. However, applications without any preliminary results will also be approved depending on the contents.
- (b) If your samples or methods are quite new to Bio-SPM measurements, we will select your proposal as a "Preliminary Investigation". In Preliminary Investigations, Experts of Bio-SPM will perform some experiments to establish the measurement conditions. When the measurement conditions for your proposal are determined, you can start the full research program.
- (c) When a submitted application is approved, the principal investigator (PI) responsible for the collaborative research and the collaborative partner researcher(s) will have the status of WPI-

NanoLSI collaborator(s) of Kanazawa University. All members who participate in the approved research project can stay at Kakuma Guest House (<http://questhouse.w3.kanazawa-u.ac.jp/index.html>). If no room is available at Kakuma Guest House, stay at hotels in downtown.

- (d) If you cannot cover the travel expenses, including transportation and accommodation for staying at Kanazawa University, mention it in the appropriate part of Form 1. Travel expenses will be supported up to 200,000 JPY for domestic applicants and 350,000 JPY for overseas applicants based on the travel expense regulations of Kanazawa University after review by the Expert Committee of NanoLSI. In the case of collaborative research by sending samples, WPI-NanoLSI will bear the shipping costs for the samples.
- (e) For outstanding proposals related to cancer research, travel expenses will be supported up to 200,000 JPY for domestic applicants and 350,000 JPY for overseas applicants by the Cancer Research Institute, Kanazawa University based on the travel expense regulations of Kanazawa University. When publishing the results, support by Kanazawa University should be acknowledged thus: "This work was partly supported by Extramural Collaborative Research Grant of Cancer Research Institute, Kanazawa University."
- (f) Graduate and undergraduate students can also participate in a collaborative research team. In such cases, approval from their supervisor is absolutely necessary. The approval form is available in Form 1: "2022 Academic Year Bio-SPMs Collaborative Research, Application and Collaborative Researcher Approval Form." Travel expenses can be covered only for graduate students, but not for undergraduate students.
- (g) If the supervisor of the student collaborative researchers changes, a new approval from the new supervisor would be necessary. In such cases, contact the person in charge of the Bio-SPMs Collaborative Research (E-mail: bio-spmscr_nano@ml.kanazawa-u.ac.jp).

[Supplementary Information]

- Regarding the Birth date, Age, and Gender fields in the Application
These fields are necessary for producing statistics for the evaluation report by the Ministry of Education, Culture, Sports, Science and Technology, in which the ages and genders of collaborative researchers must be presented. The information provided will not influence any decisions. In addition, personal information provided will be handled with the utmost caution.
- Regarding Filling out the Application
Add more spaces freely if not enough space is available in the application form. The number of pages can be increased.

[Overview of each Bio-SPM technology]

● Super-resolution AFM (FM-AFM & 3D-AFM)

FM-AFM (Frequency-modulation Atomic Force Microscope) can visualize subnanometer-scale surface structures of biomolecules in solution. Combined with 3D scanning technique, it can also visualize 3D distribution of hydration and flexible surface structures at solid-liquid interfaces. The imaging rate of FM-AFM and 3D-AFM is typically 1 min/frame. The optimal spatial resolution of the instrument is 0.3 nm in the lateral direction and 0.01 nm in the vertical direction. In the case of biomolecular imaging, the practical resolution is mostly determined by the fluctuation of the surface structures rather than the instruments. For more details, see the following articles:

1. H. Asakawa, S. Yoshioka, K. Nishimura, T. Fukuma, "Spatial Distribution of Lipid Headgroups and Water Molecules at Membrane/Water Interfaces Visualized by Three-Dimensional Scanning Force Microscopy", *ACS Nano* 6, 9013-9020 (2012).
2. H. Asakawa, K. Ikegami, M. Setou, N. Watanabe, M. Tsukada, T. Fukuma, "Submolecular-Scale Imaging of α -Helices and C-Terminal Domains of Tubulins by Frequency Modulation Atomic Force Microscopy in Liquid", *Biophys. J.* 101, 1270-1276 (2011).
3. T. Fukuma, "Water distribution at solid/liquid interfaces visualized by frequency modulation atomic force microscopy", *Sci. Technol. Adv. Mater.* 11, 033003 (18 pages) (2010).

● High-speed AFM (HS-AFM)

HS-AFM (High-speed Atomic Force Microscope) can visualize moving objects in solution. Its temporal resolution is typically 100 ms/frame, while the spatial resolution is 2-3 nm in the lateral direction and 0.15 nm in the vertical direction. When it is applied to protein molecules in action, the acquired HS-AFM images can provide a significant insight into how the molecules function. For more details, see the following review articles:

1. T. Ando, T. Uchihashi, S. Scheuring, "Filming biomolecular processes by high-speed atomic force microscopy", *Chem. Rev.* 114, 3120-3188 (2014).
2. T. Ando, T. Uchihashi, N. Kodera, "High-speed AFM and applications to biomolecular systems", *Annu. Rev. Biophys.* 42, 393-414 (2013).
3. T. Uchihashi, N. Kodera, T. Ando, "Guide to video recording of structure dynamics and dynamic processes of proteins by high-speed atomic force microscopy", *Nature Protocols* 7, 1193-1206 (2012).

● Scanning Ion Conductance Microscopy (SICM)

SICM has a unique measurement principle and provides unprecedented opportunity that enables submicroscale functional imaging of single live cells by a combination of nanoscale local stimulation and noncontact topography imaging. The imaging rate of SICM is 30-300 s/frame. Spatial resolution of the instrument is 10 nm in the lateral direction and 5 nm in the vertical direction. For more details, see the following articles:

1. P. Novak, C. Li, A. I. Shevchuk, R. Stepanyan, M. Caldwell, S. Hughes, T. G. Smart, J. Gorelik, V. P. Ostanin, M. J. Lab, G. W. J. Moss, G. I. Frolenkov, D. Klenerman, and Y. E. Korchev, "Nanoscale live-cell imaging using hopping probe ion conductance microscopy", *Nat. Methods* 6, 279-281 (2009).
2. V. O. Nikolaev, A. Moshkov, A. R. Lyon, M. Miragoli, P. Novak, H. Paur, M. J. Lohse, Y. E. Korchev, S. E. Harding, and J. Gorelik, "beta(2)-Adrenergic Receptor Redistribution in Heart Failure Changes cAMP Compartmentation", *Science* 327, 1653-1657 (2010).
3. Y. Zhou, M. Saito, T. Miyamoto, P. Novak, A. Shevchuk, Y. Korchev, T. Fukuma, Y. Takahashi, "Nanoscale Imaging of Primary Cilia with Scanning Ion Conductance Microscopy," *Anal. Chem.* 90, 2891-2895 (2018).

● AFM for Cell Measurement

Based on high-speed AFM or 3D-AFM, NanoLSI is developing AFM technologies for measuring the structure, dynamics or mechanical properties of the surface or inside of cells at a nano scale. High-speed AFM successfully visualized the surface structure of bacteria at a molecular scale and nano-motion of the terminal portion of nerve cells. Based on 3D-AFM, we developed a nanoendoscopy-AFM technique. Using this technique, we succeeded in three-dimensional observation of cell nucleus or actin fibers inside live cells, the measurement of two-dimensional nanodynamics of inner scaffold of plasma membrane, and the measurement of the surface stiffness of cell nucleus. For more details, see the following articles:

1. H. Yamashita, A. Taoka; T. Uchihashi, T. Asano, T. Ando, Y. Fukumori, "Single-molecule imaging on living bacterial cell surface by high-speed AFM", *J. Mol. Biol.* 422 (2), 300-9 (2012).
2. M. Shibata, T. Uchihashi, T. Ando, R. Yasuda, "Long-tip high-speed atomic force microscopy for nanometer-scale imaging in live cells", *Sci. Rep.*, 5, 8724 (2015).
3. M. Penedo, K. Miyazawa, N. Okano, Furusho, H. Ichikawa, T, S. Alam Mohammad, K. Miyata, C. Nakamura, T. Fukuma, "Visualizing intracellular nanostructures of living cells by nanoendoscopy-AFM", *Sci. Adv.* 7 (52), eabj4990 (2021).
4. K. Kobayashi, N. Kodera, T. Kasai, YO. Tahara, T. Toyonaga, M. Mizutani, I. Fujiwara, T. Ando, M. Miyata. "Movements of Mycoplasma mobile gliding machinery detected by high-speed atomic force microscopy", *mBio* 12: e00040-21 (2021).