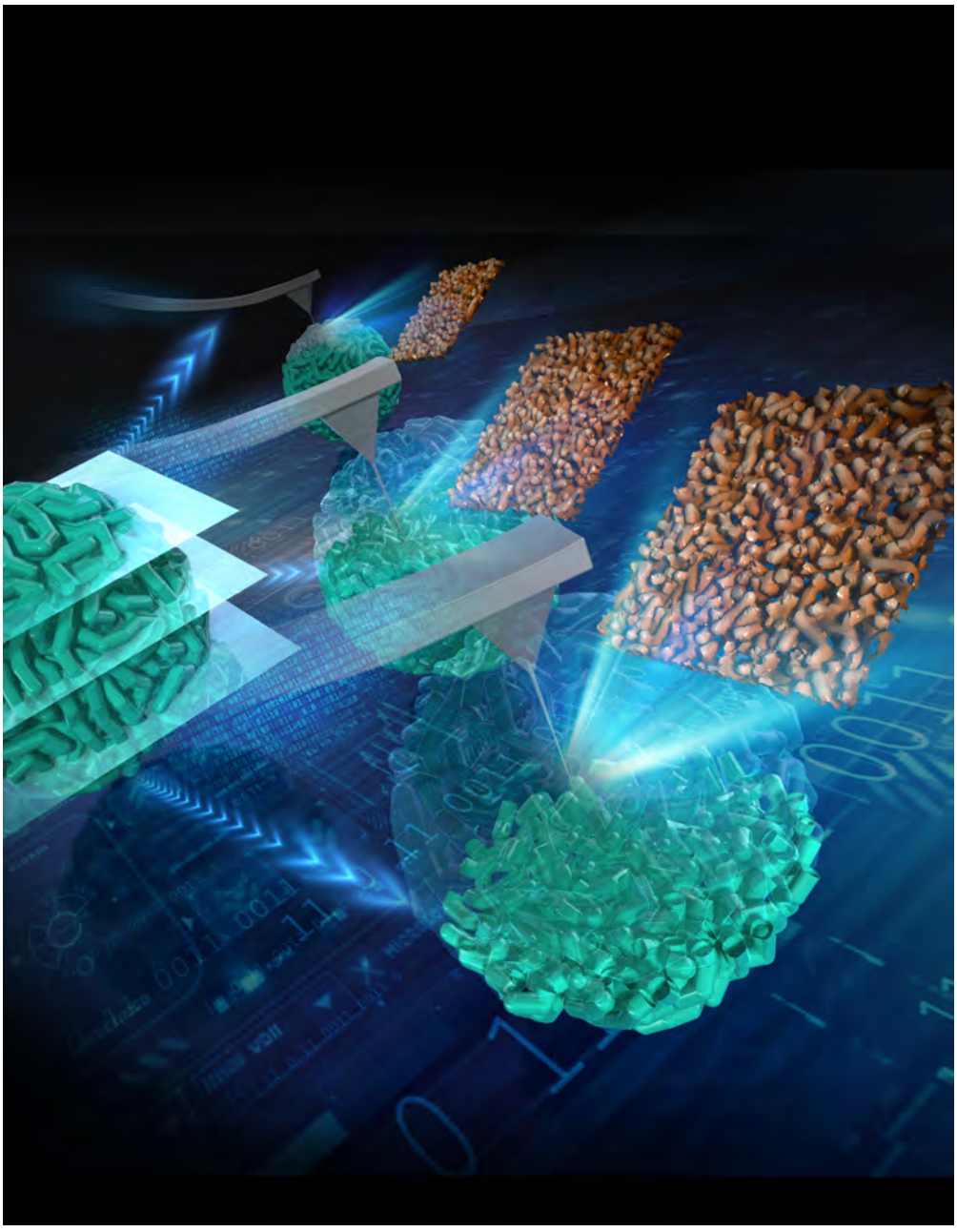
Computational Biophysics of Atomic Force Microscopy



Organizers: Takashi Sumikama, Damien Hall, Holger Flechsig
NanoLSI, Kanazawa University

Support: International Union for Pure and Applied Biophysics (IUPAB)
Nano Life Science Institute (WPI-NanoLSI), Kanazawa University

Venue: Main Conference Room, NanoLSI Building 4th floor, Kanazawa University, Kakuma Campus & Online via Zoom

Administration Contact: nanolsi-office@adm.kanazawa-u.ac.jp

Cover page image credit: Takashi Sumikama, *J Phys Chem Lett (2022)*

List of Content

About NanoLSI

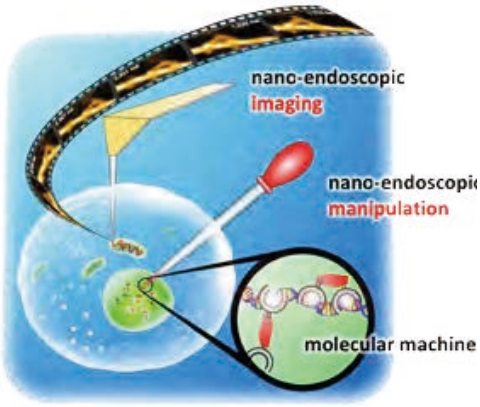
Visualizing small things leads to big discoveries.

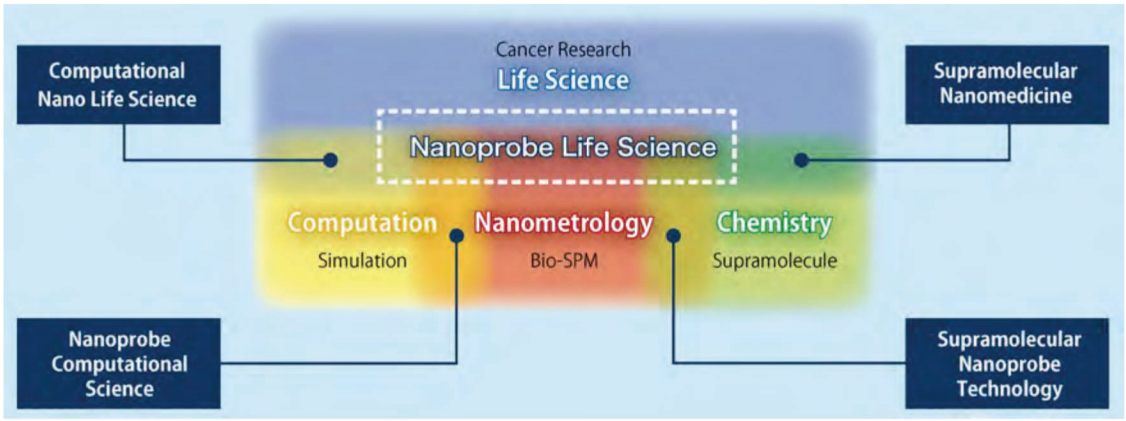
Nano Life Science Institute (NanoLSI), Kanazawa University is a research center established in 2017 as part of the World Premier International Research Center Initiative of the Ministry of Education, Culture, Sports, Science and Technology. The objective of this initiative is to form world-tier research centers.

NanoLSI combines the foremost knowledge of bio-scanning probe microscopy to establish 'nano-endoscopic techniques' to directly image, analyze, and manipulate biomolecules for insights into mechanisms governing life phenomena such as diseases.

WPI NanoLSI

Director: Takeshi Fukuma





About IUPAB

The International Union for Pure and Applied Biophysics (IUPAB) was formed in Stockholm in 1961 as the International Organisation for Pure and Applied Biophysics. It was established as the International Union in 1966, when it became a member of the ICSU (International Council for Science) family. Affiliated to it are the national adhering bodies of 61 countries . Its function as the world's chief biophysics organization is to support research and teaching in biophysics in all corners of the world. Aside from promoting and sponsoring workshops and initiatives its principal regular activity is the triennial International Congresses and General Assemblies.

The 21st World Triennial IUPAB Congress will be held in Kyoto in 2024. The 21st IUPAB Congress of 2024 will be merged with the BSJ annual meeting as a joint congress, which will offer participants great opportunities to exchange cutting-edge research ideas with globally leading scientists. The joint congress will also bring blended flavors of biophysics from each region of the world, in particular, biophysics in Japan and Asian-Oceanian countries.

Website http://iupab.org/about-iupab/

Special Issue in Biophysical Reviews

Biophysical Reviews is the official journal of IUPAB and publishes critical and timely reviews from key figures in the field of biophysics. Interested authors are encouraged to discuss the possibility of contributing a review with the Editor-in-Chief. Biophysical Reviews covers the entire field of biophysics, generally defined as the science of describing and defining biological phenomenon using the concepts and the techniques of physics.

This includes but is not limited by such areas as bioinformatics, biophysical methods and instrumentation, medical biophysics, biosystems, cell biophysics and organisation, macromolecules: dynamics, structures and interactions, and membrane biophysics, channels and transportation.

Collated and edited by the three conference organizers (Takashi Sumikama, Damien Hall and Holger Flechsig) a Special Issue of Biophysical Reviews dedicated to the Computational Biophysics of Atomic Force Microscopy is scheduled to be published in December of 2023. Based around the contributions from the workshop (but also accepting submissions from outside interested parties) those wishing to participate are asked to contact

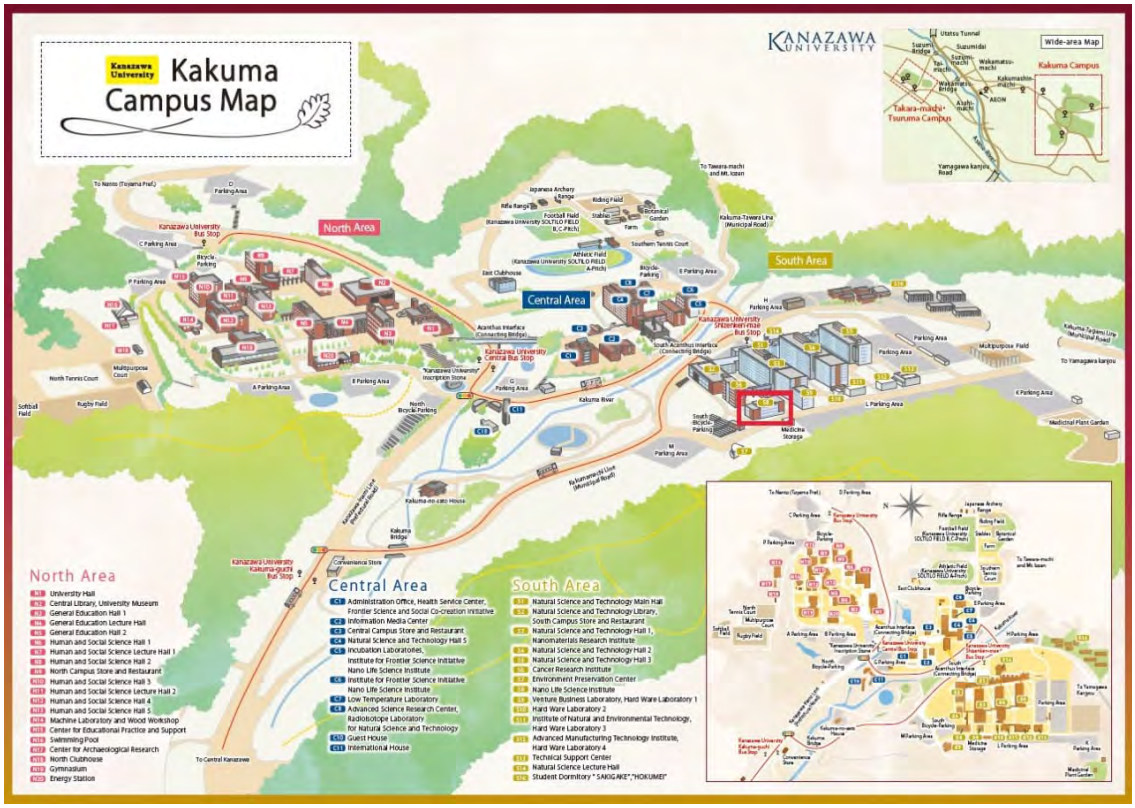
Takashi Sumikama sumi@staff.kanazawa-u.ac.jp
Damien Hall hall.damien@staff.kanazawa-u.ac.jp
Holger Flechsig flechsig@staff.kanazawa-u.ac.jp

Journal Website https://www.springer.com/journal/12551

Access to the NanoLSI building at Kakuma Campus

For information how to access Kanazawa university (Kakuma campus) from Kanazawa station please check the website https://nanolsi.kanazawa-u.ac.jp/en/access/

The Nano Life Science Institute is the building framed in red in the map below.
The conference venue is located on the 4th floor.



Helpful Information

TAXI companies

Ishikawa Kintetsu Taxi	phone 076-221-3265
Fuji Taxi	phone 076-237-1020
Kamome Taxi	phone 076-231-1128

NanoLSI Administration

Office hours:	8:30am-5pm
Contact:	nanolsi-office@adm.kanazawa-u.ac.jp

Wi-Fi Access at NanoLSI

Wi-Fi access is available via the Eduroam service. We will provide individual login credentials with a note of instructions on how to connect at the workshop reception desk. In case of trouble we are happy to help.

Catering and Workshop Banquet

Mixer on the 1st day will be at the *Yabutan* restaurant located on the 2nd floor in the *Natural Science and Technology Library Hall* on Kakuma campus. Attendance fee for the Mixer event is **4,000 Yen**.
For **Lunch on the 2nd day** we will provide lunch boxes.

The **Workshop Banquet on the 2nd day** will be held at the *Shogyotei* restaurant (http://www.asadaya.co.jp/shougyotei/).
A chartered bus will bring all participants to the Banquet venue.

WORKSHOP SCHEDULE

DAY 1 MONDAY – September 19th

Session theme: *Introduction to AFM experiment, Theory and Simulation*
Session chair: Takashi Sumikama

1:00 pm — Poster/Paper display setup (continues for 3 days)

Introduction to Workshop by NanoLSI Director
3:00 pm — **Takeshi Fukuma** (Director of NanoLSI, Kanazawa University)
Introduction to research activities at Nano Life Science Institute (WPI-NanoLSI)

First Keynote Lecture (Toshio Ando)
(Chairperson Introduction)
3:30 pm — **Toshio Ando** (Special Professor, Kanazawa University)
KEYNOTE — *High-speed atomic force microscopy in biology*
4:30 pm — Break

4:45 pm — **Shoji Takada** (Kyoto University)
Coarse-grained biomolecular simulations for geometric modeling of HS-AFM data
5:15 pm — **George Heath** (Leeds University)
Extracting high-resolution information through image analysis

6:30 – Mixer Event at Yabutan Restaurant
IUPAB Travel Fellowship Presentation

DAY 2 TUESDAY – September 20th

Session theme: *Experimental Overview*
Session chair: Damien Hall

9:00 am — **Takayuki Uchihashi** (Nagoya University)
Local mechanical indentation combined with high-speed AFM
9:30 am — **Ayhan Yurtsever** (NanoLSI, Kanazawa University)
3D-AFM investigations of solvation structures on polysaccharide-based nanocrystals and self-assembled peptide nanostructures
10:00 am — **Shingo Fukuda** (NanoLSI, Kanazawa University)
Control systems for high-speed AFM and its advances toward ultra-high-speed AFM
10:30 am — **Noriyuki Kodera** (NanoLSI, Kanazawa University)
Historical description of some of the famous model systems studied in AFM developments and current limitations in high-speed bioAFM

11:15 – 12:15 pm Lunch

Session theme: *Computation and Theory Overview (1)*
Session chair: Holger Flechsig

12:30 pm — **Tamiki Komatsuzaki** (WPI ICReDD & Hokkaido University)
Single Molecule Biophysics: What do Single Molecules Experience its Energy Landscape?
1:00 pm — **Damien Hall** (NanoLSI, Kanazawa University)
High speed atomic force microscopy of dynamic samples

1:30 pm **Takashi Sumikama** (NanoLSI, Kanazawa University)
Computation of three-dimensional atomic force microscopy images

2:00 pm **Holger Flechsig** (NanoLSI, Kanazawa University)
Molecular modeling of protein machinery and simulation atomic force microscopy for interpretation of limited-resolution Bio-AFM imaging

2:30 pm **Florence Tama** (WPI ITbM & Nagoya University)
Integrative modeling to characterize the structure and dynamics of biomolecules

Session theme: *Computation and Theory Overview (2)*
Session chair: Takashi Sumikama

3:15 pm **Adam Foster** (Aalto University)
Machine learning in scanning probe microscopy

3:45 pm **Niko Oinonen** (Aalto University)
Machine learning models and data representations for atomic force microscopy images

4:15 pm **Fabio Priante** (Aalto University)
Neural network potentials for molecular dynamics

4:45 pm **Orlando Silveira** (Aalto University)
Simulations of high-resolution Scanning Probe Microscopy

5:15 pm **Filippo Canova** (Nanolayers Inc.)
Theoretical methods for non-contact atomic force microscopy

5:45 pm Break

Second Keynote Lecture (Toshio Yanagida)
(Introduction by Chairperson Takehiko Ichikawa)

6:00 pm **Toshio Yanagida** (Osaka University)
KEYNOTE *Biomolecular motors convert information into motion: the secret of living organisms' extraordinary energy savings*

7:10 pm Chartered bus to banquet venue
7:30 – 10:00 pm Workshop Banquet at Shogyotei Restaurant

DAY 3 Wednesday – September 21st

Session theme: *Computation and Theory Overview (3)*
Session chair: Damien Hall, Holger Flechsig

10:00 am **Thomas Huber** (Australian National University)
Protein structures are predictable. How?

10:30 am **Satoru Okuda** (NanoLSI, Kanazawa University)
Computational simulations of cell and tissue dynamics

11:00 am **Kenichi Umeda** (NanoLSI, Kanazawa University)
Controlling and data acquisition methods for high-speed AFM & development of next-generation high-speed AFM

11:30 am **Romain Amyot** (NanoLSI, Kanazawa University)
BioAFMviewer: a tutorial for simulated AFM scanning

12:00 – 12:30 **Closing Remarks**
Holger Flechsig – *Scientific Summary*
Takashi Sumikama – *Conference Summary (Activities and Awards)*
Damien Hall – *Special Issue in Biophysical Reviews*

Presentation Abstracts

Introduction to Research Activities at Nano Life Science Institute (WPI-NanoLSI)

Takeshi Fukuma

WPI Nano Life Science Institute, Kanazawa University
fukuma_atsign_staff.kanazawa-u.ac.jp

Nano Life Science Institute was established in 2017 as one of the centers for World Premier International Research Center Initiative (WPI) program. At NanoLSI, we aim to develop novel nanoprobe technologies for visualizing unseen nanoscale biological phenomena and elucidating underlying mechanisms. Major SPM techniques that we have been developing include high-speed atomic force microscopy (HS-AFM) (Ando and Kodera groups), frequency modulation AFM (FM-AFM) and three-dimensional AFM (3D-AFM (Fukuma group), and high-speed and functional scanning conductance microscopy (Korchev and Ando groups). With these techniques, we have been investigating various functions of proteins, DNAs and cells and the related diseases. In the first part of this talk, I would like to provide an overview of our research activities at NanoLSI.

In the second half of the talk, I would like to present our recent work on the development and applications of nanoendoscopy AFM [1]. Atomic force microscopy (AFM) is the only technique that allows label-free imaging of nanoscale biomolecular dynamics, playing a crucial role in solving biological questions that cannot be addressed by other major bioimaging tools (fluorescence or electron microscopy). However, such imaging is possible only for systems either extracted from cells or reconstructed on solid substrates. Thus, nanodynamics inside living cells largely remain inaccessible with the current nanoimaging techniques. Here, we overcome this limitation by nanoendoscopy-AFM, where a needle-like nanoprobe is inserted into a living cell, presenting actin fiber three-dimensional (3D) maps, and 2D nanodynamics of the membrane inner scaffold, resulting in undetectable changes in cell viability. Unlike previous AFM methods, the nanoprobe directly accesses the target intracellular components, exploiting all the AFM capabilities, such as high-resolution imaging, nanomechanical mapping, and molecular recognition. These features should greatly expand the range of intracellular structures observable in living cells.

1. Penedo, M.; Miyazawa, K.; Okano, N.; Furusho, H.; Ichikawa, T.; Alam Mohammad, S.; Miyata, K.; Nakamura, C.; Fukuma, T., Visualizing intracellular nanostructures of living cells by nanoendoscopy-AFM. Science Advances 2021, 7 (52), eabj4990.

KEYNOTE

High-speed atomic force microscopy in biology

Toshio Ando

Distinguished Professor, Kanazawa University
tando_atsign_staff.kanazawa-u.ac.jp

High-speed (HS-) AFM has now established a unique position in experimental biophysics, as the sole tool able to present real motion pictures of the biological nanoworld [1]. Its history can be traced back to the early 1990th when I and Paul Hansma started developing HS-AFM independently [2]. Around that time, pioneers were exploring potentials of newly emerged AFM in biological studies; they attempted not only high-resolution topography imaging but also recognition and localization of specific molecules as well as force measurements to estimate the strength of intra- and intermolecular bonds at the single-molecule level and the elasticity of biological surfaces. In contrast to these pioneering works, the development of practically useful HS-AFM went through with hitches and therefore progressed slowly. Among several issues to solve, the most difficult issue was no damages of fragile molecules during their fast imaging. By great fortune, this issue was solved by a flash of a new idea, leading to the establishment of HS-AFM in 2008 [3].

HS-AFM studies of biological samples have been published more than 300 to this day. Among these, the study of walking myosin V [4] is a milestone in the history of bio-AFM research. This study is a paragon illustrating the power of HS-AFM. The HS-AFM study of the rotary motor F_1-ATPase brought a revelation [5]. Interestingly, the initial aim of this study was simple; visualizing conformational changes of this protein during the ATPase reaction. However, the protruding central shaft hampered clear imaging of the stator ring due to the tip size effect. Then, the stator ring alone was imaged, leading to a surprising discovery. As seen in the program of this workshop, we have now been experiencing interactions between HS-AFM experiments and theoretical analyses (molecular dynamics simulations and reconstruction of dynamic pseudo-AFM images). This scientific endeavor and synergy will unleash technical restrictions and burgeon into more profound understanding of the functional mechanism of proteins.

1. Ando T. (2022) *High-speed atomic force microscopy in biology*. 319 pages, Springer Berlin (Verlag), ISBN 978-3-662-64783-7.
2. Ando T, Kodera N, Takai E, Maruyama D, Saito K, Toda A. (2001) A high-speed atomic force microscope for studying biological macromolecules. *Proc Natl Acad Sci U S A* **98**, 2468–12472.
3. Ando T, Uchihashi T, Fukuma T. (2008) High-speed atomic force microscopy for nano-visualization of dynamic biomolecular processes. *Prog Surf Sci* **83**, 337–437.
4. Kodera N, Yamamoto D, Ishikawa R, Ando T. (2010) Video imaging of walking myosin V by high-speed atomic force microscopy. *Nature* **468**, 72–76.
5. Uchihashi T, Iino R, Ando T, Noji H. (2011) High-speed atomic force microscopy reveals rotary catalysis of rotorless F_1-ATPase. *Science* **333**, 755–758.

Coarse-grained biomolecular simulations for geometric modeling of HS-AFM data

Shoji Takada

Professor, Department of Biophysics, Graduate School of Science, Kyoto University
takada_atsign_biophys.kyoto-u.ac.jp

High-speed AFM is a unique and prominent method to observe structural dynamics of biomolecules at single molecular level at neat physiological condition. However, since AFM measures the surface envelop at which the probe tip interacts with target molecules, the structure underneath the surface need to be interpreted.

To interpret static AFM images, several types of geometric modeling have been utilized as concise means. When we have prior structure information on the target biomolecules, we can fit them into the AFM-measured surface envelop, assuming the shape of the probe tip. We can apply either the rigid-body fitting to a fairly rigid molecule (1), or the flexible fitting to more flexible targets (2,3). For the case that we do not have prior structure information of the target, we can employ the erosion/dilation processing to convert between the AFM-measured surface envelop and the target molecular surface envelop, assuming the shape of the tip (4). Furthermore, we can apply the *de novo* blind tip reconstruction method to infer the shape of the tip solely from the measured AFM data.

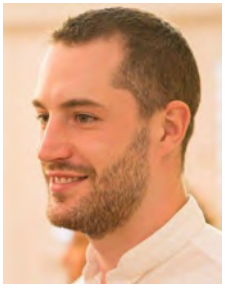
Since HS-AFM movie provides molecular motions, such motions are also expected to be interpreted. Coarse-grained biomolecular simulations enable us to simulate large-scale molecular motions near the time-scale of the HS-AFM measurement, thus providing complementary information. We developed two types of Bayesian inference approaches to assimilate the HS-AFM movie data with coarse-grained molecular simulations; the particle filter method (5) and the hidden Markov modeling. These methods give general frameworks to integrate the HS-AFM movie with computer simulations.

(1) T Niina, Y Matsunaga, S Takada (2021) PLoS Computational Biology 17 (7), e1009215
(2) T Niina, S Fuchigami, S Takada (2020) Journal of Chemical Theory and Computation 16 1349-1358.
(3) H Koide, N Kodera, S Bisht, S Takada, T Terakawa (2021) PLoS computational biology 17 (7), e1009265S
(4) J.S. Villarrubia (1997) J. Res. Nat. Inst. Stand. Tech. 102 425.
(5) Fuchigami, T Niina, S Takada (2020) Journal of Chemical Theory and Computation 16, 6609-6619

Extracting High-Resolution Information Through Image Analysis

George Heath

University Academic Fellow, University of Leeds
g.r.heath_atsign_leeds.ac.uk



Understanding the structure and dynamics of biomolecules at the single molecule level is vital to advancing our knowledge of medicine and diseases, yet there are few techniques which can directly capture these movements at the relevant spatiotemporal scales. High-Speed AFM allows dynamic molecules to be studied at high resolution and in ambient conditions to produce large volumes of data at rates >100,000Hz for many seconds up to hours. Each pixel/data point contains rich data about structure and dynamics of the surface being studied and therefore data processing and analysis needs to be designed for high throughput whilst also taking careful consideration to the noise, drift, varying tip convolution and molecule dynamics that may be present.

Here, I will give short history of digital image analysis and then describe how image data is stored in general, how pixelated image data is created from AFM experiments and different ways it can be interrogated. I will then build on this to show methods to improvement AFM spatial resolution using Localization AFM, an image analysis method which takes concepts from super resolution microscopy. By applying localization algorithms to AFM data, Localization AFM significantly increases resolution beyond the limits set by the tip radius. This technique enables the calculation of high-resolution maps from either images of many molecules or many images of a single molecule acquired over time, facilitating single-molecule structural analysis. Localization AFM is a post-acquisition image reconstruction method that can be applied to any biomolecular AFM dataset.

References

1. Heath G R, Kots E., Robertson J L, Lansky S, Khelashvili G, Weinstein H & Scheuring S. Localization Atomic Force Microscopy. *Nature*, 594, 385–390, (2021).

Local mechanical indentation combined with high-speed AFM

Takayuki Uchihashi

Department of Physics, Nagoya University/ExCELLS

uchihast@d.phys.nagoya-u.ac.jp

Atomic force microscopy (AFM) can not only visualize the surface structure of a sample but also manipulate the local structure of the sample, such as indentation or fracture by applying a controlled force from the probe to a local area of the sample. In the first part, an overview is given on the methods and analysis of nanoindentation and mechanical property measurements by conventional AFM. In the second part, I will present recent developments and applications of a high-speed AFM combined with local indentation and force curve measurement.

HS-AFM is widely used for the observation of single protein dynamics and visualizing surface structures of biological samples. So far, HS-AFM is mainly used for topographical imaging. However, recent developments have allowed us to apply a force from the probe to a local position of the sample during HS-AFM imaging and continuously monitor subsequent changes in the sample. This mode, called the interactive mode, was used to apply a force to a localized position of a biological sample to disrupt its structure, examine the detailed structure of broken fragments [1] and assess the fragility of the biological sample [2]. It has also been applied to artificial supramolecular nanofibers, successfully monitoring fiber digestion by the AFM probe and subsequent self-repair processes and manipulating local structures [3]. Furthermore, recent in-line measurement of force curves during HS-AFM imaging has enabled quantitatively assessment of mechanical properties by acquiring force curves, in addition to applying indentations at local positions on the sample. The in-line-force-curve mode was applied to the local mechanical indentation of microtubules to observe the formation of tubulin dimer defects in microtubules, and the binding energy between tubulins was experimentally evaluated from the hysteresis of the force curve during the indentation process on the deforming microtubules [4].

Taking this in-line force mapping a step further, a force mapping mode was developed that simultaneously records force information and topography. Depending on the size of the force map, a single image frame may be recorded in as low as 3 s. To demonstrate this high-speed force mapping, we apply it to two types of bacteria: B. subtilis and E. coli. For the former, we will present the dynamic breakdown of cell walls upon lysozyme addition. It was found that the cell wall stiffness is reduced up to a factor of 10 within typically 10 min. In the case of E. coli, we observed the growth of a single bacterium and its subsequent division not only with HS-AFM force mapping but also with optical microscopy. It was possible to capture the formation of the septum, which was significantly stiffer than the rest of the cell.

These examples show that HS-AFM force mapping can be used to study dynamic phenomena also from a viewpoint of mechanical properties.

1. T. Haruyama, T. Uchihashi, Y. Yamada, N. Kodera, T. Ando and H.Konno (2018) Negatively charged lipids are essential for functional and structural switch of human 2-Cys peroxiredoxin II", *J. Mol. Biol.* **430**, 602-610.
2. M. Owa, et al (2019) Inner lumen proteins stabilize doublet microtubules in cilia and flagella", *Nat. Commu.* 10, 1143.
3. T. Fukui, T. Uchihashi ,N. Sasaki, H. Watanabe, M. Takeuchi, K. Sugiyasu (2018) Direct observation and manipulation of supramolecular polymerization by using high-speed atomic force microscopy, *Angew. Chem. Int. Ed.* **57**, 15465-15470.
4. C. Gasner and T. Uchihashi (2019) Microtubule self-healing and defect creation investigated by in-line force measurements during high-speed atomic force microscopy, *Nanoscale* **11**, 125-135.

3D-AFM investigations of solvation structures on polysaccharide-based nanocrystals and self-assembled peptide nanostructures

Ayhan Yurtsever

Assistant Professor, WPI-NanoLSI
yurtsever_atsign_staff.kanazawa-u.ac.jp

The atomic force microscope (AFM) is mainly known for its high-resolution imaging capabilities, but it also offers the possibility of measuring the interaction forces and energies between the tip and surfaces, thereby providing more insights into the underlying mechanisms of many biological and physical processes down to the single atom/molecule level. In this seminar, I will talk about the AFM force measurements, quantification of forces, and 3D force mapping methods. The results of three-dimensional AFM (3D-AFM) measurements on the characterization of 3D local hydration structures at the chitin/cellulose nanocrystals-water interfaces will be presented and discussed. Also, I will talk about the 3D-AFM investigations of solvation structures on biomolecules (peptides) and biomolecular assemblies on graphite/HOPG.

1. Yurtsever et al. 2021. Structural and mechanical characteristics of exosomes from osteosarcoma cells explored by 3D-atomic force microscopy. Nanoscale 13, 6661.
2. Yurtsever et al. 2022. Probing the structural details of chitin nanocrystal-water interfaces by three-dimensional atomic force microscopy. Small Methods 2200320.

Control systems for high-speed AFM and its advances toward ultra-high-speed AFM

Shingo Fukuda

Assistant professor, WPI-NanoLSI Kanazawa University, Japan
shingofukuda_atsign_staff.kanazawa-u.ac.jp

High-speed atomic force microscopy (HS-AFM) has been established by tremendous efforts, which introduces a new dimension to structural biology, enabling the direct observation of biological systems ranging from purified proteins to live-cells [1,2]. In the HS-AFM operating in the amplitude modulation (tapping) mode, a cantilever is excited at its near-resonance frequency. The resulting oscillating tip is intermittently contacting the surface, which consequently damps the oscillation amplitude. The surface topography is reconstructed by maintaining the cantilever amplitude constant (the set point value) by the feedback regulation for the Z-axis while scanning XY plane. In the first half of this talk, we describe basic control systems used for HS-AFM; how the cantilever and the sample stage are regulated to accurately acquire the topographic features of sample. We also introduce key developments including the dynamic PID controller [3] and the inverse-based feedforward damping method [4], to realize the gentle and precise scanning. In the second half of this talk, we will discuss our latest progress on further speeding up of HS-AFM to meet the increasing demands for the great expansion of observable dynamic molecular processes. We implemented the only trace imaging mode [5] and the dynamic PID control method with lower threshold. These developments can lower the feedback control error and thereby enhance the imaging rate. Further we implemented fast-axis (X-axis) scanning methods that allow the linear motion without any undesired vibrations even at rates of 100 frames/seconds. By the advent of these technologies, the maximum imaging rate of HS-AFM is now pushed beyond video rate. Such an ultra-high-speed imaging capability can resolve the molecular dynamics at unpreceded resolutions and deepen our understanding on the biological phenomena.

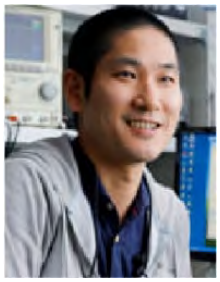
References

[1] T. Ando, *High-speed atomic force microscopy in biology*, Splinger Berlin, Heidelberg (2022)
[2] T. Ando, T. Uchihashi, T. Fukuma, *Prog. Surf. Sci*. 83: 337-437 (2008)
[3] N. Kodera, M. Sakashita, T. Ando, *Rev. Sci. Instrum.*,77, 083704 (2006)
[4] Yang Li, John Bechhoefer, *Rev. Sci. Instrum.*. 78, 013702 (2007)
[5] S. Fukuda, T. Ando, *Rev. Sci. Instrum,*. 92, 033705 (2021)

Historical description of some of the famous model systems studied in AFM
Developments and current limitations in high-speed bioAFM

Noriyuki Kodera

Professor, WPI-NanoLSI, Kanazawa University
nkodera_atsign_staff.kanazawa-u.ac.jp



High-speed atomic force microscopy (HS-AFM) allowed us to directly visualize the dynamic behaviors of biological molecules in action at nanometer spatial and sub-second temporal resolution. The power of HS-AFM has been demonstrated by an increasing number of imaging studies on biological molecules [1,2].

In the first half of the presentation, I will introduce some of the famous model systems that have been captured by HS-AFM so far, and then present the recent results obtained in our research group (factor pool formation around the ribosomal P-stalk [3], determination of the molecular shape of an actin-binding protein CAP1 [4] and interactions between CAP1 and actin filaments, and long-range dsDNA degradations by CRISPR-Cas3 [5]).

In recent years, we have been carrying out research and development to improve the temporal resolution of HS-AFM system, in order to apply HS-AFM to a wider range of biological phenomena. In the second half of the presentation, developments and current limitations in high-speed bioAFM will be explained.

References

1. T. Ando, High-speed atomic force microscopy, *Curr. Opin. Chem. Biol.* **51,** 105-122 (2019).
2. G. R. Heath & S. Scheuring, Advances in high-speed atomic force microscopy (HS-AFM) reveal dynamics of transmembrane channels and transporters, *Curr. Opin. Struct. Biol.* **57,** 93 (2019).
3. H. Imai, T. Uchiumi & N. Kodera, Direct visualization of translational GTPase factor pool formed around the archaeal ribosomal P-stalk by high-speed AFM, *PNAS* **117,** 32386-32394 (2020).
4. N. Kodera, H. Abe, PDN. Nguyen, S. Ono, Native cyclase-associated protein and actin from *Xenopus laevis* oocytes form a unique 4:4 complex with a tripartite structure, *J. Biol. Chem.* **296,** 100649 (2021).
5. K. Yoshimi, K. Takeshita, N. Kodera, S. Shibumura, Y. Yamauchi, M. Omatsu, K. Umeda, Y. Kunihiro, M. Yamamoto & T. Mashimo, Dynamic mechanisms of CRISPR interference by *Escherichia coli* CRISPR-Cas3, *Nat. Commun.* (Accepted).

Single Molecule Biophysics: What do Single Molecules Experience its Energy Landscape?

Tamiki Komatsuzaki

Research Center of Mathematics for Social Creativity,
Research Institute for Electronic Science, Hokkaido University, Japan

Graduate School of Chemical Sciences and Engineering Materials
Chemistry and Engineering Course, Hokkaido University, Japan

Institute for Chemical Reaction Design and Discovery (WPI-ICReDD),
Hokkaido University, Japan

SANKEN, Osaka University, Japan

Institute for Open and Transdisciplinary Research Initiatives, Osaka University, Japan

tamiki_atsign_es.hokudai.ac.jp

How can one learn from single molecule time series concerning the underlying conformation network or energy landscape? What a single molecule actually experiences is not so well recognized, even while this theme can be one of the most striking consequences people can unveil from single molecule biology. In part 1 of my talk I will briefly talk about the mathematical background to derive the underlying energy landscape solely from single molecule time series. The core in constructing the reaction network from single-molecule time-series is the application of rate-distortion theory in information theory, which naturally considers the effects of measurement noise and sampling error, in combination with the quantification of the FRET efficiency-dependent photobleaching behavior. In part 2 of my talk I will talk about hierarchical features of reaction network and energy landscape for the folding/unfolding behavior of adenylate kinase, which are respectively elucidated in terms of single-molecule fluorescence resonance energy transfer (smFRET) measurements. Energy landscapes are constructed as a function of observation time scale, revealing multiple partially folded conformations at small time scales that are situated in a superbasin. Because the photobleaching time scale is dependent on the conformational state of the protein, possible nonequilibrium features are discussed, and a statistical test for violation of the detailed balance condition is developed based on the state sequences.

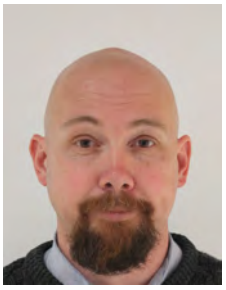
References:

1) James Nicholas Taylor, Menahem Pirchi, Gilad Haran, Tamiki Komatsuzaki, “Deciphering hierarchical features in the energy landscape of adenylate kinase folding/unfolding” *The Journal of Chemical Physics* 148, 123325 (2018)
2) Meysam Tavakoli, J. Nicholas Taylor, Chun-Biu Li, Tamiki Komatsuzaki, Steve Pressé *Advances in Chemical Physics: "Single Molecule Data Analysis: An Introduction"* Edited by Stuart A. Rice, Aaron R. Dinner (Wiley)
3) T. Komatsuzaki, S. Takahashi, M. Kawakami, Haw Yang, Robert Silbey. (Edition)Single Molecule Biophysics: Experiment and Theory. *Advances in Chemical Physics* 146, (2012) John-Wiley& Sons, Inc. ISBN: 978-1-1-180-5780-3.
4) T. Komatsuzaki, R. S. Berry, D.M. Leitner. (Edition)"Advancing Theory for Kinetics and Dynamics of Complex, Many-Dimensional Systems: Clusters and Proteins" *Advances in Chemical Physics* 145, (2011) John-Wiley & Sons, Inc. ISBN: 978-0-470-64371-6 (2011).

High Speed Atomic Force Microscopy of Dynamic Samples

Damien Hall

Assistant Professor, Kanazawa University
hall.damien_atsign_staff.kanazawa-u.ac.jp



In the analysis of static samples High Speed Atomic Force Microscopy (HS-AFM) is capable of providing nanometer resolution (or better) in both the lateral (xy) and vertical (z) planes (**1**). However, biological samples are often not static, with significant movement of the sample potentially occurring over the time scale of the HS-AFM measurement. In such cases two problems can arise that relate to (i.) assignment of the individual components observed within the image, and (ii.) image distortion caused by incongruities between redistribution and measurement timescales (**2**). In the first part of my lecture, I will provide a basic overview of coarse-grained approaches capable of describing the dynamics of biological processes such as diffusion in the cellular membrane, motor protein migration along protein fibers, and protein binding and unbinding to adsorbed DNA. In the second part, I will describe methods for overcoming both the assignment problem and for assessing the degree of distortion imparted by differences in the rate of sample redistribution and AFM measurement. To help in the performance of these methods I introduce and describe two executable software tools HS-AFM UGOKU (User Graphical Optimization tool developed at Kanazawa University) (**2**) and HS-AFM MIREBA (Methodology for Inferring REsolution in Biological Applications) (**3**).

1. Ando T. (2022) *High-speed atomic force microscopy in biology*. 319 pages, Springer Berlin (Verlag), ISBN 978-3-662-64783-7.
2. Hall, D. and Foster A.S. (2022) Practical considerations for feature assignment in high-speed AFM of live cell membranes. Biophysics and Physicobiology. e190016, 1-21
3. Hall, D. and Foster A.S. (2022) Quantification of dynamic cell membrane features by high-speed atomic force microscopy. (Manuscript in preparation).

Computation of three-dimensional atomic force microscopy images

Takashi Sumikama

PRESTO researcher, Japan Science and Technology Agency
Assistant professor, Kanazawa University
sumi_atsign_staff.kanazawa-u.ac.jp

Three-dimensional atomic force microscope (3D-AFM) is a technology to resolve molecular distributions by scanning a probe in the *x*, *y*, and *z*-directions[1], which was originally developed to view solvent molecules at the solid-liquid interfaces. Recently, it was extended to the imaging of intracellular biomolecules with thin and long probes, and it was shown to visualize nanostructures of biopolymers even inside cells[2].

This talk will begin with an introduction to the calculation of 3D-AFM images of solvent molecules at the solid-liquid interface[3,4]. The free energy or potential mean force (PMF) profiles on the solid surface are computed using the umbrella sampling or perturbation method, and the derivative of PMF yields the so-called force-distance curves at a given *x* and *y* position. Merging force-distance curves at all *x*, *y* positions gives a 3D-AFM image. Note that this methodology, computing the free energy, assumes that solvent molecules move much faster than the penetrating probe and distribute almost all possible arrangements that they can take.

On the other hand, the motions of some biopolymers, especially large complexes, are not as fast as the velocity of penetrating probe, and thus they cannot take all possible configurations during probe penetration. That is, biomolecules do not reach equilibrium during probe scanning. Accordingly, the above approach to compute force-distance curves is not applicable to biopolymers.

In this talk, I will also present our recently developed method for computing 3D-AFM images of biopolymers in such non-equilibrium processes[5]. The biopolymers and probes are modeled by the bead-spring polymer model. The force-distance curves are computed by using the Jarzynski equality, which relates external work done while scanning to the free energy difference. Some biopolymer structures are actually resolved in the computed 3D-AFM images. Finally, I will talk about some comparisons with experiments to validate this computational method.

[1] T. Fukuma & R. Garcia, *ACS Nano* 12, 11785 (2018).
[2] M. Penedo et al., *Sci. Adv.* 7, eabj4990 (2021).
[3] M. Watkins & A. L. Shluger, *Phys. Rev. Lett.* 105, 196101 (2010).
[4] B. Reischl, M. Watkins, & A. S. Foster, *J Chem. Theory Comput.* 9, 600 (2013).
[5] T. Sumikama et al., *J Phys. Chem. Lett.* 13, 5365 (2022).

Molecular modeling of protein machinery and simulation atomic force microscopy for interpretation of limited-resolution Bio-AFM imaging

Holger Flechsig

Assistant Professor, Nano Life Science Institute (WPI NanoLSI), Kanazawa University
flechsig_atsign_staff.kanazawa-u.ac.jp

Proteins operating as molecular machines and motors are fundame n biological cells. Understanding their functional principles is therefore or nanotechnology applications such as the design of artificial molecular motors. High-speed atomic force microscopy (HS-AFM) has provided unprecedented insights into motor working cycles by monitoring functional conformational dynamics in real time. Nonetheless, limitations in the spatio-temporal resolution of imaging prevent detailed understanding from experiments. In this situation, multi-scale molecular modelling plays an important role to complement observations.

In the first part of my talk, I will briefly review self-organization in complex systems, the emergence of collective dynamics and dimensionality-reduction, and the relation to proteins and their evolution towards functionality [1]. I will also explain how simple mechanical models help to understand important aspects of design and functional principles of molecular motors, including sophisticated phenomena of allostery [2], and show that molecular movies of entire operation cycles can be recorded with reasonable computational expense [3].

The second part of my talk will focus on the application of mathematical modelling and simulations of biomolecular dynamics to facilitate the interpretation of HS-AFM imaging. Employing models of various complexity, large progress in understanding AFM data has been made. An important role plays simulation atomic force microscopy which we developed and distributed within the freely available BioAFMviewer software [4,5], since it allows to correlate high-resolution 3D molecular conformations with measured AFM topographies of protein surfaces. I will present applications to AFM images ranging from single molecular machines, protein filaments, to large-scale assemblies of protein lattices, and show how the obtained full atomistic information advances the molecular understanding beyond original topographic AFM imaging.

[1] H. Flechsig, A.S. Mikhailov. 2019. Simple mechanics of protein machines. **J Roy Soc Interface** 16: 20190244.
[2] H. Flechsig. 2017. Design of elastic networks with evolutionary optimized long-range communication as mechanical models of allosteric proteins. **Biophys J** 113: 558-571.
[3] H. Flechsig, A.S. Mikhailov. 2010. Tracing entire operation cycles of molecular motor hepatitis C virus helicase in structurally resolved dynamical simulations. **Proc Natl Acad Sci USA** 107: 20875-20880.
[4] R. Amyot, H. Flechsig. 2020. BioAFMviewer: an interactive interface for simulated AFM scanning of biomolecular structures and dynamics. **PLoS Comput Biol.** 16: e 1008444.
[5] R. Amyot, A. Marchesi, C.M. Franz, I. Casuso, H. Flechsig. 2022. Simulation atomic force microscopy for atomic reconstruction of biomolecular structures from resolution-limited experimental images. **PLoS Comput Biol.** 18: e1009970.

Integrative modeling to characterize the structure and dynamics of biomolecules

Florence Tama

Professor, Department of Physics, Graduate School of Science & Institute of Transformative Bio-Molecules, Nagoya University, Japan
Team Leader, RIKEN Center for Computational Science, Japan
florence.tama_atsign_nagoya-u.jp

The exploration of molecular motions by simulation approaches has provided significant insights into biomolecules' structure, function, and dynamics. Because biomolecular structures are complexes and dynamic, interpretation of simulation results can be challenging. Therefore, to characterize essential features regarding the dynamics of these biomolecules, methods such as principal component analysis or normal mode analysis (NMA) have been employed to reduce the problem's dimensionality. These methods often focus on low-frequency (energy) modes, as those are known to describe quite well experimentally observed functional motions. As experimental data does not always provide high-resolution information, hybrid and integrative modeling methods that combine computational molecular mechanics simulations with experimental data have been developed to describe the structure and dynamics of biomolecules. One example is the integration of NMA with experimental data such as 2D and 3D cryo-electron microscopy or small-angle scattering data. Such integrative modeling methods have also been extended to describe conformational states observed by AFM experiments. For example, starting from a known 3D structure, one can generate a coarse-grained representation of the biomolecule, and the shape of this representation can be optimized, via Monte Carlo sampling, for a given AFM image. As a result, a low-resolution 3D model can be built from the 2D AFM image. Therefore, such optimizations can be performed for a set of AFM images to describe in terms of 3D models the conformational variety embedded within the 2D AFM data. Such an approach was used to annotate the different conformational states of the ClpB chaperone observed experimentally.

References

F. Tama, O. Miyashita, and CL. Brooks, III. **Flexible multi-scale fitting of atomic structures into low-resolution electron density maps with elastic network normal mode analysis**. *J. Mol. Biol*. **2004** 337: 985-999

Q Jin, CO Sorzano, JM de la Rosa-Trevín, JR Bilbao-Castro, R. Núñez-Ramírez, O. Llorca , F. Tama, S. Jonić. **Iterative elastic 3D-to-2D alignment method using normal modes for studying structural dynamics of large macromolecular complexes.** *Structure* **2014** 22:496-506

R Vuillemot, O Miyashita, F Tama, I Rouiller and S Jonic. NMMD: Efficient cryo-EM flexible fitting based on simultaneous Normal Mode and Molecular Dynamics atomic displacements. J. Mol. Biol. 2022

B. Dasgupta, O. Miyashita & F. Tama. **Reconstruction of Low-resolution Molecular Structures from Simulated Atomic Force Microscopy Images**. *BBA – General Subjects* **2020**

B. Dasgupta, O. Miyashita, T. Uchihashi, and F. Tama. **Reconstruction of three-dimensional conformations of bacterial ClpB from high-speed atomic- force-microscopy images**. *Frontiers Mol. BioSci.* ***2021***

Machine Learning in Scanning Probe Microscopy

Adam Foster

Professor, Aalto University
adam.foster_atsign_aalto.fi

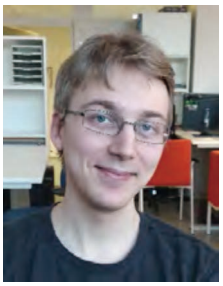
Machine learning methods are increasingly being applied to data challenges in Scanning Probe Microscopy (SPM). In particular, the success of deep learning in image recognition tasks has led to their application to the analysis of SPM images, especially in the context of surface feature characterisation and techniques for autonomously-driven SPM [1]. In this work, we first outline some of the key concepts in applying machine learning methodologies, before exploring the potential for using deep learning to aid in the interpretation of high resolution SPM images [2]. In general, we find high success rates in predicting the atomic and chemical structure of molecules, and the method can also be used for quantitative predictions of electrostatic properties [3]. This approach opens the door to apply high-resolution AFM to a large variety of systems for which routine atomic and chemical structural resolution on the level of individual objects/molecules would be a major breakthrough. We also look at applications of similar approaches to the imaging of biomaterials and AFM imaging in liquids [4]. More generally, we develop some of the ideas behind a future of *active learning* measurements, introducing methods to automate tip preparation [5] and atomic manipulation.

[1] O.M. Gordon and P.J. Moriarty, Mach. Learn.: Sci. Technol. 1, 023001 (2020).
[2] B. Alldritt, P. Hapala, N. Oinonen, F. Urtev, O. Krejci, F. F. Canova, J. Kannala, F. Schulz, P. Liljeroth, and A. S. Foster, Sci. Adv. 6 (2020) eaay6913
[3] Niko Oinonen, Chen Xu, Benjamin Alldritt, Filippo Federici Canova, Fedor Urtev, Ondřej Krejčí, Juho Kannala, Peter Liljeroth and Adam S. Foster, ACS Nano 16 (2022) 89
[4] Yashasvi S. Ranawat, Ygor M. Jaques and Adam S. Foster, Nanoscale Adv. 3 (2021) 3447
[5] Benjamin Alldritt, Fedor Urtev, Niko Oinonen, Markus Aapro, Juho Kannala, Peter Liljeroth and Adam S. Foster, Comp. Phys. Comm. 273 (2022) 108258

Machine learning models and data representations for atomic force microscopy images

Niko Oinonen

Doctoral Candidate, Aalto University
niko.oinonen_atsign_aalto.fi



Machine learning is proving to be an invaluable tool in better interpretation of high-resolution atomic force microscopy (AFM) images [1-4]. In order to make use of machine learning models, the data has to be cast in a suitable form for a given choice of a model. Here I will discuss two different ways of representing molecular systems as a learning target for machine learning from AFM images, image descriptors and graphs, and correspondingly two different types of machine learning models that make use of these representations, convolutional neural networks and graph neural networks. Finally, I present an application of these techniques in a model that constructs a molecule graph from an AFM image [5].

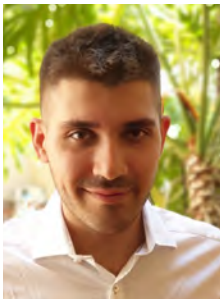
[1] O. Gordon, P. Moriarty, “Machine learning at the (sub)atomic scale: next generation scanning probe microscopy”, Mach. Learn.: Sci. Technol. 1 023001, 2020.
[2] B. Alldritt et al., “Automated structure discovery in atomic force microscopy”, Sci. Adv. Vol. 6, 9 eaay6913, 2020.
[3] N. Oinonen et al., “Electrostatic Discovery Atomic Force Microscopy”, ACS Nano 16, 1, 89–97, 2022.
[4] J. Carracedo-Cosme, R. Pérez, “Molecular Identification with Atomic Force Microscopy and Conditional Generative Adversarial Networks”, arXiv:2205.00447, 2022.
[5] N. Oinonen et al., “Molecule graph reconstruction from Atomic Force Microscopy images with machine learning”, MRS Bulletin, 2022.

Neural Network Potentials for Molecular Dynamics

Fabio Priante

PhD student, Aalto University

fabio.priante_atsign_aalto.fi



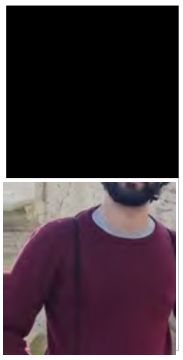
The first Molecular Dynamics (MD) simulations were carried out in the 1950s [1,2], following the development of early electronic computers. Since then, MD has become an essential investigative tool in a wide range of scientific fields, from material science to biology. Designing a MD simulation requires the choice of a potential, or force field (FF), which defines how the atoms in a system will interact with each other. In principle, this can be done very accurately using ab-initio techniques [3], where interatomic forces are calculated after solving the electronic structure of the frozen nuclei. However, this is feasible only for small systems (up to a few hundreds of atoms), and for limited timescales. To simulate more complex scenarios, one has to resort to classical FFs, which trade-off accuracy for computational speed.
In the last decade, thanks to machine learning, many efforts have been made to overcome these limitations, in an attempt to reach close to ab-initio accuracy at a fraction of the computational cost. In particular, the development of neural network potentials (NNP) has paved the way for a new revolution in the field.
In this talk, I will provide an historical perspective on the methods used to construct and parametrize FFs for MD. Particular attention will be given to the recent machine learning approaches, up to equivariant NNP [4], which constitute the current state of the art. I will discuss current and future applications of NNP, and, finally, I will present the development of a NNP for water molecules on a Cu surface, taken from an ongoing research effort.

[1] Fermi E., Pasta J., Ulam S., Los Alamos report LA-1940 (1955).
[2] *Alder, B. J., Wainwright, T. E. Studies in Molecular Dynamics. I. General Method. J Chem Phys.* ***31*** *(2): 459–466 (1959).*
[3] *Car, R., Parrinello, M. Unified Approach for Molecular Dynamics and Density-Functional Theory. Phys Rev Lett.* ***55*** *(22): 2471–2474 (1985).*
[4] Batzner, S., Musaelian, A., Sun, L. *et al.* E(3)-equivariant graph neural networks for data-efficient and accurate interatomic potentials. *Nat Commun* 13, 2453 (2022).

Simulations of high-resolution Scanning Probe Microscopy

Orlando José Silveira Júnior

Post-doc, Aalto University
orlando.silveirajunior_atsign_aalto.fi



Scanning Tunneling Microscopy (STM) and Atomic Force Microscopy (AFM) are scanning probe techniques that form images of surfaces using a physical probe that scans the sample. The characterization of atomic clusters [1], one and two-dimensional organic materials [2], and the understanding of on-surface chemical reactions [3], among others, have been advanced due to the unprecedented spatial resolution that can be achieved with STM and AFM. In parallel to these experimental techniques, the simulation of high-resolution images using top notch electronic structure computational packages have been also demonstrated to be an essential tool for fully understanding, for example, the geometry and atomic configurations of the materials characterized by the Scanning Probe Microscopy. In this talk, we will first outline the recent computational *ab initio* methods used to explore the electronic properties of any nano material. We will then discuss traditional simple models used to simulate STM images that consider a rigid tip apex, useful for cases where the tip is far from the surface. Later we will go through recent modern approaches that consider the probe particle relaxation, as well as the electronics wave functions of the tip and the sample [4,5]. We will show that these models can explain experimentally observed features and greatly aid the characterization of the materials sampled.

[1] M. Emmrich, F. Huber, F. Pielmeier, J. Welker, M. Schneiderbauer, T. Hofmann, D. Meuer, S. Polesya, S. Mankovsky, D. Kodderitzsch, H. Ebert, and F. J. Giessibl, Science 348, 308 (2015).
[2] K. Sun, O. J. Silveira, Y. Ma, Y. Hasegawa, M. Matsumoto, S. Kera, O. Krejčí, A. S. Foster, and S. Kawai, https://arxiv.org/abs/2111.10124 (Accepted in Nature Chemistry).
[3] L. Gross, N. Moll, G. Meyer, R. Ebel, W. M. Abdel-Mageed, and M. Jaspars, Nat. Chem. 2, 821 (2010).
[4] O. Krejčí, P. Hapala, M. Ondráček, and P. Jelínek, Phys. Rev. B 95, 045407 (2017)
[5] P. Hapala, G. Kichin, C. Wagner, F. S. Tautz, R. Temirov, and P. Jelínek, Phys. Rev. B 90, 085421 (2014)

Theoretical methods for non-contact atomic force microscopy

Filippo Federici Canova

CTO, Nanolayers Research Computing Ltd.
felix_atsign_nanolayers.com

Non-contact atomic force microscopes (ncAFM) are capable of *imaging* samples at extremely high resolution, enabling us to *see* the very atomic fabric of surfaces [1]. Unfortunately, these microscopes have quite little in common with optical microscopes we are all familiar with. The probe is usually an atomically sharp tip, rather than our eyes, and the sampling mechanism relies on interatomic forces between the tip and the sample, rather than light diffusing from it; the instrument itself is a very complex piece of machinery, with several electronic feedback controls acting on the probe dynamics. While it is often easy to understand the patterns seen in ncAFM images qualitatively (mostly because the nature of the sample is already known before the experiment begins), the correct interpretation of defects and subtle features in the measured signals requires extensive theoretical work [2]. In general, ncAFM simulations require a model for the tip-sample atomic interactions, and a model of the instrument response to those interactions [3], making this a multiscale modelling effort.
Here we will give a broad overview of the theoretical methods involved in the interpretation of ncAFM images, including classical and ab-initio techniques to calculate tip-sample interactions, and circuit-level instrument simulators, and show how they were applied to solve research problems [4, 5].

[1] T. R. Albrecht, P. Grütter, D. Horne and D. Rugar: "Frequency modulation detection using high-Q cantilevers for enhanced force microscope sensitivity",
Journal of Applied Physics **69** (2), 668 (1991)

[2] F. Schulz, J. Ritala, O. Krejčí, A. P. Seitsonen, A. S. Foster and P. Liljeroth: “Elemental Identification by Combining Atomic Force Microscopy and Kelvin Probe Force Microscopy”,
ACS Nano **12** (6), 5274 (2018)

[3] J. Tracey, F. Federici Canova, O. Keisanen, D. Z. Gao, P. Spijker, B. Reischl, A. S. Foster: “Flexible and modular virtual scanning probe microscope”,
Computer Physics Communications **196** 429 (2015)

[4] S. Kawai, F. Federici Canova, Th. Glatzel, A. S. Foster, and E. Meyer: “Atomic-scale dissipation processes in dynamic force spectroscopy”,
Physical Review B **84** 115415 (2011)

[5] F. Federici Canova, S. Kawai, Ch. de Capitani, K. Kan’no, Th. Glatzel, B. Such, A. S. Foster and E. Meyer, “Energy Loss Triggered by Atomic-Scale Lateral Force”,
Physical Review Letters **110** 203203 (2013)

KEYNOTE

Biomolecular motors convert information into motion: the secret of living organisms' extraordinary energy savings

Toshio Yanagida

Professor, Osaka University

Using optical microscopy and high-speed AFM, we have analyzed how myosin motors that work in muscles, convert the chemical energy of ATP into mechanical energy. We found that the myosin motor generates movement by selecting forward movement from random Brownian motion. That is, it shows that the movement is caused by Brownian motion, and the chemical energy of ATP is not used to cause movement, but to select forward movement from random Brownian motion (acquisition of information). That is, the myosin motor converts information into movement. This discussion has been made a lot in thought experiments of Maxwell's demons, but it is surprising that it was discovered that the demons are actually energized and worked in living things. This is a big discovery from an information point of view. That is, it is suggested that the relationship of 1 bit (information) = 0.7 k_BT (energy) actually holds for biomolecules. Since 1 bit = 10^9 k_BT is required in the electronic circuit of a computer, biomolecules are processing information with energy saving by orders of magnitude. The cells work at approximately 10 picowatts. Even with the latest ICs, this energy can only process 1 bit per second. On the other hand, the biomolecule algorithm using Brownian motion can process 1 gigabit / sec and is reasonable. Recently, it has been reported that many biomolecules work using Brownian motion, and our discovery has the essence of information processing in living organisms.

1. Fujita K, Ohmachi M, Ikezaki K, Yanagida T, Iwaki M. 2019. Direct visualization of human myosin II force generation using DNA origami-based thick filaments. Commun. Biol. 2, 437.
2. Kitamura K, Tokunaga M, Iwane A, Yanagida T. 1999. A single myosin head moves along an actin filament with regular steps of 5.3 nanometers. Nature 397, 129.
3. Funatsu T, Harada Y, Tokunaga M, Saito K, Yanagida T. 1995. Imaging of single fluorescent molecules and individual ATP turnovers by single myosin molecules in aqueous solution. Nature 374, 555.

Protein structures are predictable. How?

Thomas Huber

Professor, Australian National University
Research School of Chemistry
t.huber_atsign_anu.edu.au

Proteins are arguably the most important macromolecules in organisms. They are one of the most abundant class of organic molecules in all life forms and have essential roles in a diverse range of cellular function. The function of a protein is largely dictated by their chemical composition, the primary sequence of amino acids, and the 3-dimensional structure the polypeptide chain adopts in a cellular environment. We therefore typically assume that by knowing the 3D structure of a protein we are in a better position to understand (biological) function.
The premise of this talk is that protein structures are predictable and also computable by application of fundamental principles. In my talk I will discuss the difference between predictability and computability, explain some of the fundamental principles to compute protein structures efficiently and illustrate some more popular approaches. I will further demonstrate how sparse experimental data, in example pseudocontact shifts from NMR measurements, can be used to aid 3D structure computations, and conclude with potential pitfalls one can encounter when over-interpreting experimental data from a protein system as rigid 3D structure.

References

Leman, J.K. *et al.* Macromolecular modeling and design in Rosetta: recent methods and frameworks. *Nat Methods* **17,** 665–680. https://doi.org/10.1038/s41592-020-0848-2

Pan, B.-B. et al. 3D structure determination of a protein in living cells using paramagnetic NMR spectroscopy. *Chem.Comm.* **52** 10237-10240. https://doi.org/10.1039/c6cc05490k

Schmitz, C. et al. Protein structure determination from pseudocontact shifts using ROSETTA. *J. Mol. Biol.* **416** 668-677. https://doi.org/10.1016/j.jmb.2011.12.056

Computational simulations of cell and tissue dynamics

Satoru Okuda

Associate Professor, WPI-NanoLSI, Kanazawa University
satokuda_atsign_staff.kanazawa-u.ac.jp

In multicellular organisms, the multiscale dynamics of cells from the molecular to the organ level are coordinated with each other, causing functional structures and behaviors as a system. To address the underlying mechanisms, mechanics-based computational simulations have been the focus of efforts. Because of the large gap from the molecular to the organ scale, the targets of computational methods vary greatly depending on the structure and spatio-temporal scale. In general, each method has an elementary structure describing the cell structures, which determines the characteristic length scale of each method. For example, molecular dynamics methods, which have been used for proteins, are based on each atom or each population of atoms. Continuum membrane models, which have been used for organelles and cell membranes, are based on a continuum element of cell membranes. Vertex models, which have been used for multicellular tissues, are based on each cell. These methods are powerful tool to predict structures, dynamics, interactions, and properties of each target. However, because of the variety of cellular structures and the very broad spatio-temporal scale of their dynamics, the phenomena that can be handled by existing methods remain very limited. In particular, few methods can applied to mesoscopic-scale phenomena such as intracellular structures such as organelles and tissues composed of cells and extracellular matrix. In this lecture, the mechanics-based computational methods for such mesoscopic cell dynamics will be presented, along with the author's research.

References

1. Martin Karplus, Petsko A Gregory, "Molecular dynamics simulations in biology." Nature 347.6294 (1990): 631-639.
2. Masashi Tachikawa, Atsushi Mochizuki, Golgi apparatus self-organizes into the characteristic shape via postmitotic reassembly dynamics. A Proceedings of the National Academy of Sciences of the United States of America 114(20) 5177-5182, 2017.
3. Satoru Okuda, Yasuhiro Inoue, Mototsugu Eiraku, Taiji Adachi, Yoshiki Sasai, "Vertex dynamics simulations of viscosity-dependent deformation during tissue morphogenesis," Biomechanics and Modeling in Mechanobiology 14(2), 413-425 (2015)

Controlling and Data Acquisition Methods for High-Speed AFM & Development of Next-Generation High-Speed AFM

Kenichi Umeda

Assistant Professor at WPI NanoLSI, Kanazawa University
umeda.k_atsign_staff.kanazawa-u.ac.jp

In the first section, I will explain about the detail specification of controlling, data acquisition and analysis method of high-speed atomic force microscopy (HS-AFM) while comparing the normal AFM instruments. In most of the commercial AFM, digital signal processor (DSP) is used for controlling AD/DA boards while our HS-AFM instrument does not used DSP to reduce the machine cost while increasing the scanning bandwidth. This is important for increasing the number of the instruments and facilitates the collaboration researches. For HS-AFM, since a vast of frames is saved in the storage, an automation of image processing (e.g., constant color scaling with respect the substrate and polynomial flattening filter) is important.
In the second section, I will explain about recent progress in the development of high-speed AFM in terms of high-speed, quantitativeness, and functionality so that it can be applied to more diverse range of bioresearches. For measuring the molecular distance quantitatively, the nonlinearity and memory effect of the piezo scanner causes 20~30% errors. I developed nonlinear feedforward system to compensate the non-ideal characteristics. I also developed zero-latency amplitude detection method to increase the scanning bandwidth tremendously [1]. These components will be useful for further advancement of HS-AFM researches.

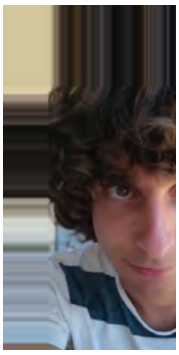
References

[1] Kenichi Umeda, et al., *Applied Physics Letters*, 119, 181602-1–5 (2021).

BioAFMviewer: A tutorial for simulated AFM scanning

Romain Amyot

PostDoc, Nano Life Science Institute (WPI-NanoLSI),
Kanazawa University
romain-amyot_atsign_staff.kanazawa-u.ac.jp



Atomic force microscopy (AFM) allows to visualize the dynamics of single biomolecules during their activity. The analysis and interpretation of experimental results remains challenging, because the resolution of obtained images or *molecular movies* is far from perfect. On the other side, high-resolution static structures of most proteins are known and their conformational dynamics can be computed in molecular simulations. This enormous amount of available data offers a great opportunity to better understand the outcome of resolution-limited scanning experiments. Our software provides the computational package towards this goal. The BioAFMviewer computationally emulates the scanning of any biomolecular structure to produce graphical images that mimic the outcome of AFM experiments. This makes the comparison of all available structural data and computational *molecular movies* to AFM results possible. An automatized fitting process is including as well, bringing the comparison to a higher degree. Through a concise tutorial, we will explore all the functionalities of the BioAFMviewer. From basic topography analysis to complex atomic reconstruction, we will learn how this new computational tool can improve the understanding of experimental images.

References

[1] Romain Aymot and Holger Flechsig. 2020. "BioAFMviewer: An interactive interface for simulated AFM scanning of biomolecular structures and dynamics." PLoS computational biology 16, e1008444.
[2] Romain Amyot et al. 2022. "Simulation atomic force microscopy for atomic reconstruction of biomolecular structures from resolution-limited experimental images." PLoS computational biology 18, e1009970.

IUPAB Travel Fellowship Awards

IUPAB granted eight travel fellowship awards to attend the workshop.

Domestic Fellowship winners – 300 EUR each

Yui Kanaoka
Nagoya University, Japan

Jiaqi Wang
Hokkaido University, Japan

Keiju Suda
Kyushu University, Japan

Saahil Acharya
Okinawa Institute of Science and Technology (OIST), Japan

Dr. Yuya Hanazono
Tokyo Medical and Dental University, Japan

International Fellowship winners – 1000 EUR each

Joohyun Lee
Sungkyunkwan University, South Korea

Dr. Witchukorn Phuthong
Kasetsart University, Thailand

Dr. Nguyen Thanh Hoa
Hanoi University of Science & Technology, Vietnam

Final Remarks

We thank all participants for attending the workshop. We invite you to join our mailing list to receive announcements for upcoming workshops. Please use the following registration form: https://forms.gle/VMa7rfMoJtCFQ5Gq7

We are looking forward seeing you in future events!