Fifth Japan-Canada Microscopy Societies Joint Symposium 2024

第5回 日本-カナダ顕微鏡学会交流シンポジウム 2024

Extending limits of electron beam analysis in nanoscience

ナノサイエンスのための電子顕微鏡による解析限界への挑戦

June 4 - 5, 2024

International Conference Hall, Makuhari Messe
Chiba, Chiba, Japan

June 3 - 4, 2024

Richcraft Hall, Carleton University
Ottawa, Ontario, Canada

Japanese Society of Microscopy (JSM)

Microscopy Society of Canada (MSC)

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Fifth Japan-Canada Microscopy Societies Joint Symposium 2024

	Time (Tokyo)	Speakers	Time (Ottawa)
Session 1	**June 4, Tuesday**	**Makuhari Messe, Tokyo**	**June 3, Monday**
	9:00 ～ 9:30	Connecting	20:00 ～ 20:30
	9:30 ～ 9:35	Opening address: Prof. S. Okabe, President of JSM	20:30 ～ 20:35
	9:35 ～ 10:00	**C-1**: R. Phengchat (NRC-NANO)	20:35 ～ 21:00
	10:00 ～ 10:25	**J-1**: S. Narai (Sumitomo Pharma Co, Ltd.)	21:00 ～ 21:25
	10:25 ～ 10:50	**J-2**: T. Higuchi (Akita Prefectural University)	21:25 ～ 21:50
	10:50 ～ 11:15	**C-2**: M. T. Schreiber (NRC-NANO)	21:50 ～ 22:15
	11:15 ～ 11:40	**J-3**: H. Saito (Kyushu University)	22:15 ～ 22:40
	11:40 ～ 12:00		
	12:00 ～ 13:00	JSM Annual General Meeting	
Session 2	**June 5, Wednesday**	**Carleton University, Ottawa**	**June 4, Tuesday**
	3:30 ～ 4:00	Connecting	14:30 ～ 15:00
	4:00 ～ 4:25	**J-4**: A. Noga (Chuo University)	15:00 ～ 15:25
	4:25 ～ 4:50	**J-5**: S. Kanomi (Tohoku University)	15:25 ～ 15:50
	4:50 ～ 5:15	**J-6**: E. Kano (Nagoya University)	15:50 ～ 16:15
	5:15 ～ 5:40	**C-3**: K. L. Kavanagh (Simon Fraser University)	16:15 ～ 16:40
	5:40 ～ 6:05	**J-7**: H. Tamaki (Nagoya University)	16:40 ～ 17:05
	6:05 ～ 6:30	**C-4**: K. Beyerlein (INRS)	17:05 ～ 17:30
	6:30 ～ 6:55	**C-5**: G. Sciaini, (University of Waterloo)	17:30 ～ 17:55
	6:55 ～ 7:00	Closing address: Prof. N. Braidy, President of MSC	17:55 ～ 18:00
			18:00 ～ 19:30
		MSC Mixer	19:30 ～ 22:00

URL of web-site connection for hybrid meeting

Session 1 June 4, 2024 9:30 – 11:40 am Tokyo time

International Conference Hall, Makuhari Messe, Chiba, Chiba, Japan
https://us02web.zoom.us/j/88007403645?pwd=Mnh2YzZNK2NSd3ZOT3hpQ2l5SDR0UT09
Zoom Meeting ID: **880 0740 3645** Passcode: **398969**

Session 2 June 4, 2024 15:00 – 18:00 pm Ottawa time

Richcraft Hall, Carleton University, Ottawa, Ontario, Canada
Zoom Meeting ID: **829 2537 3231** Passcode: **866652**

【Preface】

Dear Participants,

We are pleased to welcome all of you to participate in the 5th Japan-Canada Microscopy Societies Joint Symposium 2024. This joint symposium began in 2020, has overcome the difficulties of the COVID-19 pandemic and is celebrating its fifth anniversary this year. Furthermore, we are very pleased to have the opportunity to hold this 5th symposium in conjunction with the 80th annual meeting of the Japanese Society of Microscopy (JSM) and the 50th anniversary meeting of Microscopy Society of Canada (MSC).

The theme of this symposium is "Expanding the limits of electron beam analysis in nanoscience," and it brings together with a well-balanced group of young and talented researchers from a wide range of fields, including materials science and biological science. We hope that this will be an opportunity to know the latest research and to deepen discussion.

As the dates of the annual meeting of both societies coincide this year, we decided to hold this joint symposium simultaneously at both venues with the respective society conferences. This new attempt transcends the 13-hour time difference between the countries to make this a truly real joint symposium.

We would like to express our sincere gratitude to Professor Shigeo Okabe of President of JSM, Professor Masahide Yoshikawa of the chairperson of the 80th Meeting of JSM for kindly support to hold this symposium in parallel and providing the venue for Session 1 in Tokyo, Professor Nadi Braidy of President of MSC, Professors Chloe van Oostende and Jeff Fraser of the chairs of the 50th Meeting of MSC for kind support to hold this symposium in parallel and providing the venue for Session 2 in Ottawa. We would also thank to all colleagues who cooperated in planning this symposium, and to all of you who are participating in this symposium.

We hope that the mutual exchange between these two microscopy societies will continue for a long time and contribute to the development of research in microscopy for the next generation.

Sincerely,

Organizers
Ken Harada (RIKEN)
Marek Malac (NRC-NANO and University of Alberta)
Shigeo Mori (Osaka Metropolitan University)
Misa Hayashida (NRC-NANO)

【Greetings】

Dear Esteemed Colleagues,

On behalf of the Japanese Society of Microscopy, it is my great pleasure to welcome all the microscopists, senior and young researchers, and students to the Fifth Annual Joint Workshop of the Microscopy Society of Canada- Société du Microscopie du Canada (MSC-SMC) and the Japan Microscopy Society (JSM), which will be held on June 4th and 5th, 2024 in both Makuhari, Chiba, Japan and Ottawa, Ontario, Canada both in person and online.

This workshop, "Extending limits of electron beam analysis in nanoscience," is designed to update knowledge and information about the latest technologies in electron beam analysis and its applications to nanoscience. Twelve young and talented scientists from Canada and Japan will give lectures on their recent research progress and discuss current situations and future perspectives in the field. This will be an excellent opportunity for the participants to develop networks of young researchers for future collaborations.

The relationship between the two societies, MSC-SMC and JSM, has been strengthened since the start of the joint events in 2020. This year, MSC-SMC celebrates its 50th anniversary, and JSM will hold the memorable 80th Annual Meeting. Furthermore, the schedule of the annual meeting of the two societies happens to be overlapped. The two-day events include both on-site interaction in two meeting places and online access to the event, which will facilitate remote interaction of microscopists in two meetings. This event is expected to set a new stage for the interaction of two microscopic societies.

I look forward to having a great workshop with great scientists from two countries and sharing new and exciting research in electron microscopy.

Yours sincerely,

Shigeo Okabe, (University of Tokyo)
President of The Japanese Society of Microscopy

【Greetings】

Dear Esteemed Colleagues,

I am delighted to extend a heartfelt welcome to all participants of the 5th Annual Joint Symposium between the Journal of Microscopy (JSM) and the Microscopy Society of Canada (MSC). It brings me great pleasure to convene this gathering of our two societies for the fifth time in the framework of the annual meeting of the MSC. This meeting is special as it marks the 50th anniversary of the creation of the MSC.

The previous editions of this event have highlighted our common passion for microscopy and advanced materials R&D together with the complementarity of our microscopy societies. It is now clear that the partnership between our microscopy societies exemplifies the spirit of international collaboration, offering a platform for the exchange of ideas, techniques, and experiences. Let the synergy between our societies offer opportunities for collaboration and mutual growth for scientific discovery and cultural exchange.

Through initiatives such as this symposium, we have the chance to explore new avenues of cooperation, whether through joint research projects, student exchange programs, or collaborative publications. By coming together, we can harness the diverse perspectives and innovative approaches of both Japanese and Canadian microscopy communities to address pressing scientific challenges, new research opportunities and drive technological innovation.

I wish to express my sincerest gratitude to the organizing committee, especially Marek Malak and Misa Hayashida from the MSC and Ken Harada and Shigeo Mori from the JSM, the sponsors of the symposium: JSPS, Soquelec Ltd. / JEOL Canada, Hitachi High-Tech Canada, and all contributors whose dedication has made this symposium possible. Your tireless efforts have laid the groundwork for what promises to be an enriching and intellectually stimulating experience for all involved.

In the spirit of collaboration and camaraderie, I encourage each of us to actively engage in discussions, forge new connections, and foster lasting collaborations. Together, let us seize this opportunity to inspire and be inspired, as we continue our journey of friendship, collaboration, and mutuality, strengthening the bonds between our societies and fostering a future of shared scientific endeavors.

Warm regards,

Nadi Graidy, (Université de Sherbrooke)
President of Microscopy Society Canada

【Scientific Program】

Session 1 **(Tokyo time: Tuesday, June 4, 2024)**

Web connecting 09:00 – 09:30

Opening address 09:30 – 09:35

Prof. Shigeo Okabe (President of JSM)

C-1 09:35 – 10:00

Rinyaporn Phengchat (Nanotechnology Research Centre, National Research of Council)

Visualization of surface structures and protein localization in plant chromosomes

J-1 10:00 – 10:25

Shun Narai (Sumitomo Pharma Co, Ltd.)

Understanding Crystal Surface Molecular Arrangement: Integration of Optical/Scanning Electron Microscopy and 3D ED/MicroED

J-2 10:25 – 10:50

Takumi Higuchi (Akita Prefectural University)

Small-angle beam deflection measurement towards quantum-enhanced electron microscopy

C-2 10:50 – 11:15

Makoto Tokoro Schreiber (NRC-NANO)

The NanoMi open-source electron microscopy platform: progress and hardware implementations

J-3 11:15 – 11:40

Hikaru Saito (Kyushu University)

Momentum-Resolved Cathodoluminescence of a Plasmonic Crystal Containing a Phosphor Thin Film

Session 2 **(Ottawa time: Tuesday, June 4, 2024)**

Web connecting 14:30 – 15:00

J-4 15:00 – 15:25
Akira Noga (Chuo University)
Cryo-electron tomography reveals novel Bld10p/Cep135 filamentous structures contributing to the nine-fold symmetry of centriole

J-5 15:25 – 15:50
Shusuke Kanomi (Tohoku University)
Hierarchical Structures of Semicrystalline Polymers Revealed by Nanodiffraction Imaging Based on Four-dimensional Scanning Transmission Electron Microscopy

J-6 15:50 – 16:15
Emi Kano (Nagoya University)
Atomic resolution analysis of extended defects and their evolution during annealing in Mg ion-implanted GaN

C-3 16:15 – 16:40
Karen L. Kavanagh (Simon Fraser University)
Semiconductor Nanowire Junction Potential Maps

J-7 16:40 – 17:05
Hirokazu Tamaki (Nagoya University)
Wavefield reconstruction using full-field illumination ptychography with a structured electron beam

C-4 17:05 – 17:30
Kenneth Beyerlein (Institut national de la recherche scientifique (INRS))
Streak Imaging in a Dynamic Transmission Electron Microscope

C-5 17:30 – 17:55
German Sciaini, (University of Waterloo)
The Ultrafast Electron Imaging Lab: Where Electrons, Light, Nanofluidics, and People Meet

Closing address 17:55 – 18:00
Prof. Nadi Braidy (President of MSC)

Session 1 C-1

Visualization of surface structures and protein localization in plant chromosomes

Rinyaporn Phengchat[1*], Channarong Sartsanga[2], Toshiyuki Wako[3], Kiichi Fukui[4], Nobuko Ohmido[2],

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[2] *Graduate School of Human Development and Environment, Kobe University, Japan*

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In plant, rod-like structures of chromosomes are formed, similar to animals, through the condensation of interphase chromatin during cell division. In spite of large size and small number per cell, studies of plant chromosome structure are limited due to the complicated procedure for chromosome extraction. The presence of cell wall and polysaccharides in the plant cells can attach to chromosomes and obscure the observation. In addition, a standard procedure for plant chromosome isolation includes ethanol-acetic acid fixation that partially destroy chromosomal proteins, leading to a controversy of the chromosome structures.

In this study, we developed a procedure to extract individual chromosome in solution from barley (Hordeum vulgare) root tips without prior fixation with the ethanol-acetic acid. And we prepared the chromosomes with the application of ionic liquid (IL), instead of drying, for high-resolution microscopic observation. Two types of microscopes were employed to elucidate structures of isolated barley chromosomes. First, helium ion microscope (HIM) was used to visualize chromatin fibers at the chromosome surface. Second, high voltage transmission electron microscope (HVTEM) was used for localizing immunogold labelled Topoisomerase II (Topo II), a major non-histone protein found in both plant and animal chromosomes. HIM observation (Fig. 1) revealed fibrous structures covering the entire length of the barley chromosome. The fibers were fold and randomly connected. Interestingly, unlike at chromosome arms, the chromatin fibers at centromeric region appeared as straight lines stretching toward both arms of the chromosome. Fig.2 shows the localization of Topo II throughout the barley chromosome length. Dissimilar to animal chromosomes where Topo II exhibits an axial localization, Topo II in barley chromosomes localized dispersedly in the arm regions and only accumulated at the two chromosome constrictions; the centromeres and the nucleolus organizing regions; and at the telomeres. Our finding indicates the difference of the chromosome organization in

plants compared to the reported animal chromosomes that may explain the superior genome compaction capability of plant chromosomes.

Acknowledgment: We would like to thank Dr.Kimihiro Norizawa (Nanotechnology Open Facilities Center), Prof. Dr. Kaoru Mitsuoka and Dr.Naoko Kajimura (Research Center for Ultra-High Voltage Electron Microscopy) at Osaka University for their guidance regarding the microscope observation.

Reference

[1] Sartsanga, C., Phengchat, R., et al. *Chromosome Res* **29**, (2021), 81–94.

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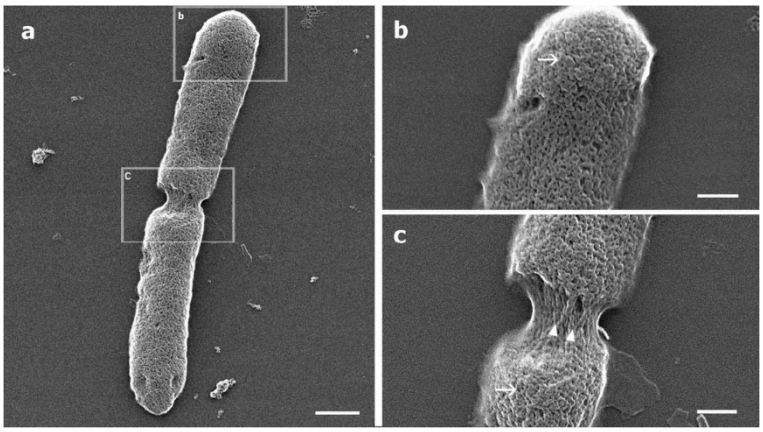

Fig. 1 Barley chromosome observed by HIM. Network of chromatin fibers was observed through the whole length of the chromosome excepted at the centromere where straight chromatin fibers were observed.

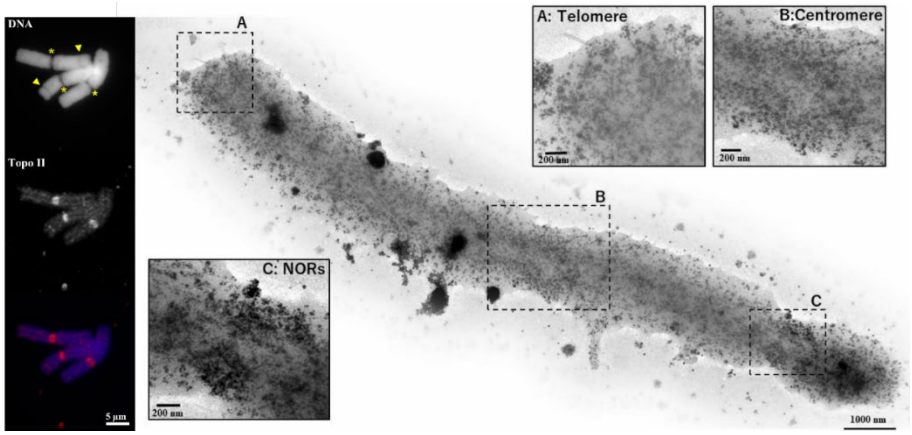

Fig. 2 Distribution of Topo II in metaphase barley chromosomes. Images from both optical microscope and HVTEM showed differential distribution and density of Topo II at telomeres, centromeres, and the NORs compared to arm regions.

Session 1　J-1

Understanding Crystal Surface Molecular Arrangement: Integration of Optical/Scanning Electron Microscopy and 3D ED/MicroED

Shun Narai（奈良井峻）[1*], Koji Yonekura (米倉功治)[2]

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Controlling the physical properties of solid dosage forms, such as solubility and stability, is crucial for maintaining the efficacy, safety, and quality of pharmaceuticals. It is well known that not only the differences in the interior molecular arrangement of active pharmaceutical ingredients but also the surface molecular arrangement can influence their physical properties[1]. Therefore, understanding the molecular arrangement both inside and on the surface of crystals is highly important for controlling the functionality of pharmaceuticals.

In this study, we propose a new method to elucidate the molecular arrangement of both crystal inside and surfaces, combining observation of crystal morphology using optical microscopy and/or scanning electron microscopy, and analysis of crystal structures and crystal lattice orientation using 3D ED (3 Dimensional Electron Diffraction)/MicroED (Microcrystal Electron Diffraction) with transmission electron microscopy. By connecting the crystal lattice orientation, crystal structure, and crystal shape information, the molecular arrangement on the crystal surface can be experimentally determined.

We have validated this method with mefenamic acid crystals. A flat hexagonal crystal shape was observed with optical microscopy (Fig. 1), and the crystal structure was determined by 3D ED/MicroED (Fig. 2). By combining the information on crystal lattice orientation analysed from the UB matrix, a crystal surface model was created (Fig. 3). With this capability, it may eventually be possible to obtain the surface charge distribution on crystal surface through precise analysis of the Coulomb potential, thereby accelerating research on the properties of crystal surfaces in the future.

References:

[1] Molecular Basis of Crystal Morpology-Dependent Adhesion Behavior of mefenamic Acid During Tableting. Vrushali Waknis, Pharmaceutical Research, Vol. **31**, page.160-172 (2014)

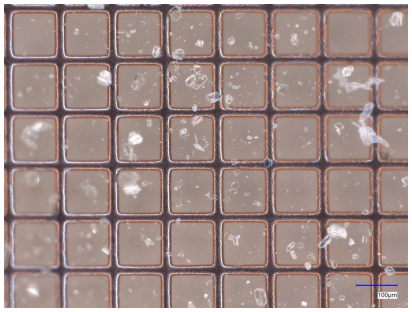

Fig. 1. Mefenamic acid crystals on a sample support grid for MicroED analysis. Most of crystal are flat planner hexagonal shapes.

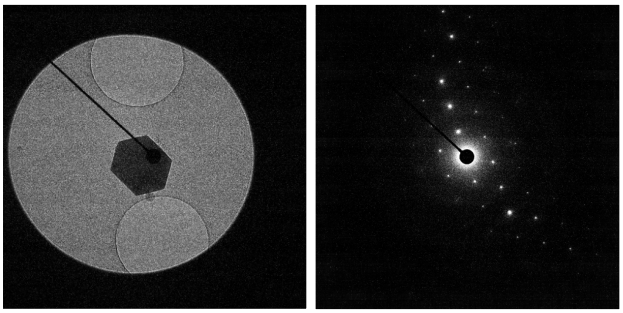

Fig. 2. A transmission electron image and electron diffraction pattern of a mefenamic acid crystal.

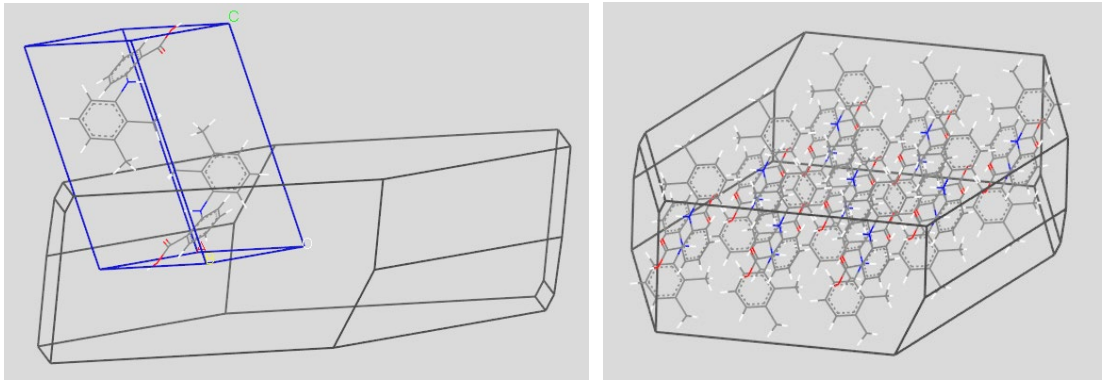

Fig. 3. A combined crystal model incorporating both crystal structure and hexagonal-shaped crystal morphology. The model has revealed that the c-axis of the crystal lattice is nearly vertical to the flat planner of the crystal.

Session 1 J-2

Small-angle beam deflection measurement towards quantum-enhanced electron microscopy

Takumi Higuchi（樋口 琢己）[1], Takeru Nagashima（永嶋 彪流）[1],

Shigeo Miura（三浦 茂男）[1] and Hiroshi Okamoto (岡本 洋) [1*]

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A major problem in electron microscopy of biological and/or soft materials is radiation damage. The dilemma is that a large dose of electrons destroy the specimen, whereas a small dose of electrons result in poor signal-to-noise ratio because of the shot noise. In a limited sense, this problem has been solved by classical averaging methods such as single particle analysis [1]. However, we have no way to improve the resolution of single objects found in, e.g., a thin slice of a frozen cell. Despite the seemingly intractable nature of the problem of radiation damage, quantum metrology offers a valid avenue of attack. A variety of quantum electron microscopy (QEM) schemes have already been proposed [2, 3], which could go beyond the shot noise limit to approach the Heisenberg limit. On the other hand, experimental effort towards QEM is still in its infancy [4, 5].

We work on a QEM scheme based on superconducting qubits [6]. A superconducting qubit is capable of generating a quantum superposition of distinct electromagnetic fields. Hence an electron passing by the qubit gets entangled with the qubit. It is known that such entanglement may be exploited to produce a better resolution. However, the electromagnetic field generated by a qubit tends to be small and deflects the electron beam only slightly. Let the electron wave length and the width of the qubit be λ and w, respectively. The smallest deflection angle we can use is the natural diffraction spread λ/w. Hence, from the experimental standpoint, we should be capable of detecting such a small beam deflection generated by a device placed in a millikelvin environment.

In our proof-of-concept experiment, we design an electron-optical setup for measuring a small-angle deflection. We perform our experiment at room temperature, but the instrument is designed to work in principle at a temperature generated by a dilution refrigerator. In particular, we detect the electrons by the Faraday cup, because, unlike the semiconducting detectors, it could be used at millikelvin temperature.

Figure 1 shows an image of the small-angle measurement apparatus under construction. From the left are the electron gun, an extraction electrode, an $x-y$ deflector, and a fluorescent screen. The ultrahigh vacuum chamber (not shown) enables a use of a field emitter stably. A set of small electrodes will be placed just at the downstream side of the extraction electrodes to simulate a qubit. We plan to replace the fluorescent screen with a pinhole aperture followed by the Faraday cup, to measure the 2D distribution of the electron beam intensity at cryogenic temperatures. We plan to benefit from the open-source NanoMi project [7] when we implement a controller for the $x-y$ deflector.

Acknowledgment:

We thank Dr. Marek Malac, P. Eng. Darren Homeniuk (Nanotechnology Research Centre Canada) and Dr. Ismet I. Kaya (Sabanci University) for useful discussions. This research was supported by JSPS KAKENHI (Grant Number 19K05285).

References:

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[2] P. Kruit et al., Ultramicroscopy **164**, 31 (2016).

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[4] A. E. Turner et al., Phys. Rev. Lett. **127**, 110401 (2021).

[5] S. A. Koppell et al., Ultramicroscopy **207**, 112834 (2019).

[6] H. Okamoto et al., Micron **161**, 103330 (2022).

[7] M. Malac et al., Micron **161**, 103362 (2022).

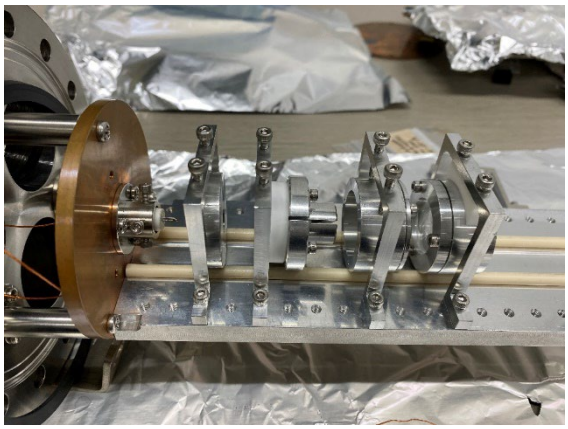

Fig. 1 Electron optical system being assembled. Lenses and other components can be placed freely on our electron optics testbed. In the photo, from left to right, an electron gun, an extractor, a deflector, a grounded mesh, and a fluorescent screen are installed.

Session 1 C-2

The NanoMi open-source electron microscopy platform: progress and hardware implementations

Makoto Tokoro Schreiber[1,2], Marek Malac[1,2], Mark Salomons[1] Darren Homeniuk[1], Sam Ruttiman[1,2], Xuanhao Wang[3], Ka Wing Kwan[1], Olivier Adkin-Kaya[4], Mohammad Kamal[4], Jesus Alejandro Marin Calzada[2], Patrick Price[1], Martin Cloutier[1], Misa Hayashida[1], Ray Egerton[2], Ken Harada[5], Yoshio Takahashi[6], Heiko Müller[7]

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We have been developing a public license electron microscopy platform: NanoMi (ナノ美) [1,2]. The NanoMi platform currently includes electrostatic lenses, deflectors, stigmators, detectors, the associated control electronics, and Python-based software for control of the respective components. All components are designed to be easily manufacturable as well as to be independent of the vacuum envelope of the microscope; so that they may be incorporated into existing vacuum systems including custom-made chambers. With the presently available components, it is possible to build TEM, STEM, SEM, and electron diffraction systems. The components are designed for modest imaging performance with an expected minimum achievable probe size or image resolution of 10 nm. The maximum electron energy is 50 keV. Further information and links to electronic resources, such as the source code of the control software, can be found at nanomi.org [1].

At present, two versions of the NanoMi microscope have been built. The first version (Figure 1a) was used to test and demonstrate the core electron-microscopy capabilities. It uses off-the-shelf ConFlat™ hardware for the vacuum envelope. The second version (Figure 1b) has been designed for integration into an ultrafast laser optics bench to photoemit electron pulses and interact them with optical pulses. This version uses a home-built vacuum chamber that lies horizontally on-top of a standard optics bench and has many ports for laser-optical access. Vacuum pressures of 2×10^{-10} torr have been achieved.

Here we present an overview of the NanoMi platform and the progress we have achieved in constructing functional NanoMi microscopes.

Acknowledgment: Support for the project was provided by NRC-NANO and by NSERC RGPIN-2021-02539

References:

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[2] M. Malac et. al, NanoMi: An open source electron microscope hardware and software platform, *Micron* **163** (2022) 103362.

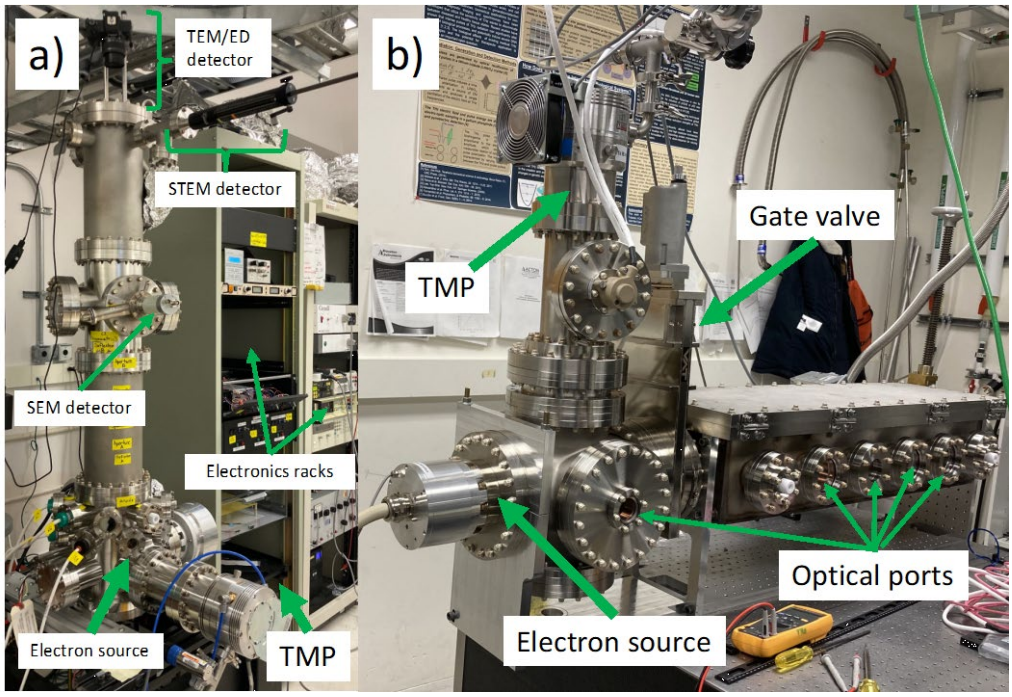

Figure 1. a) NanoMi version one designed for electron-optical flexibility. b) NanoMi version two designed for laser-optical integration.

Session 1 J-3

Momentum-Resolved Cathodoluminescence of a Plasmonic Crystal Containing a Phosphor Thin Film

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Plasmonic resonators have realized remarkable Purcell effect for extremely small emitters as well as quantum emitters due to the small mode volume. Among various structures proposed before, nanoresonator arrays with metal-insulator-metal (MIM) layers [1] seem to have an advantage in a broad range applicability for planer luminescent films including inorganic materials, where the electromagnetic field is strongly concentrated into the gap between the metal layers [1]. The band structures of the MIM resonator arrays are featured by hybridizations of the localized surface plasmon (LSP) modes [2] and the lattice modes [3]. However, it has been unclear how modal symmetry affects such a hybridization especially when it occurs at a specific reciprocal lattice point with a high degree of symmetry such as Γ point. In this study, the hybridized modes at the Γ point were fully characterized by momentum-resolved scanning transmission electron microscopy -cathodoluminescence (CL) as shown in Fig. 1. As a result, existence of a selection rule regarding symmetry is experimentally verified [4].

Then, the above resonator structure was applied to a Zn_2SiO_4 phosphor film (Fig. 2a). CL is powerful to investigate luminescence enhancement at the nanoscale. However, discrimination of coherent and incoherent emissions is often problematic in CL due to the lack of excitation wavelength selectivity. Zn_2SiO_4 has a wide bandgap of ~5 eV [5] and includes impurities or defects causing radiative electron transition (path 1 in Fig. 2b). The electron transition inside the Zn_2SiO_4 can partially be coupled with the plasmon modes. This resonator-mediated emission is path 2. However, lack of the wavelength selectivity in the electron beam excitation brings about the third path in CL, i.e., direct excitation of the plasmon modes (path 3). CL saturation imaging was applied to extract incoherent emission maps without the path 3 contributions, resulting in successful visualization of plasmon-enhanced luminescence at the nanoscale, and demonstration of near-field coupling between the luminescence centers and the plasmon modes.

Acknowledgment: This work was supported by "Advanced Research Infrastructure for Materials and Nanotechnology in Japan (ARIM)" of the Ministry of Education, Culture, Sports, Science and Technology (MEXT), and Japan Society for the Promotion of Science KAKENHI Grant Number 23K17350, 22H05034, 22H01928, 21K18195, 17K14118, and The Murata Science Foundation.

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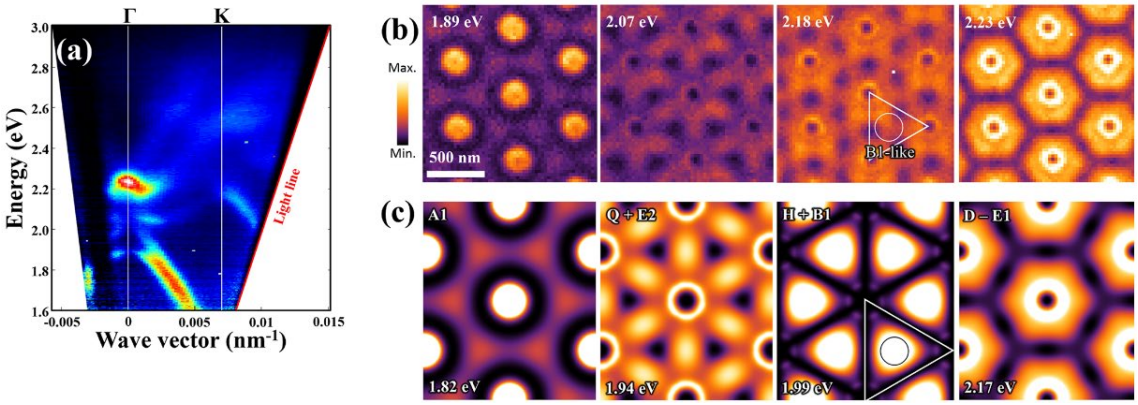

Fig. 1 (a) Momentum-resolved CL spectrum obtained along the Γ−K direction. (b) Momentum-selected and energy-filtered CL maps at the Γ point. (c) Electric field strength $|E_z(x,y)|^2$ simulated by finite-difference time-domain method [4].

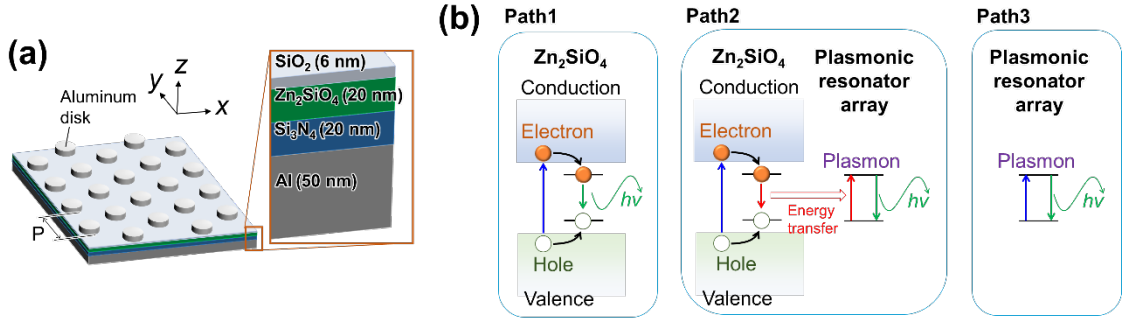

Fig. 2 (a) Schematic drawing of the fabricated phosphor-resonator structure. (b) Schematic drawings of the three possible CL paths at the emission wavelength of 525 nm in the phosphor-resonator structure.

Session 2　J-4

Cryo-electron tomography reveals novel Bld10p/Cep135 filamentous structures contributing to the nine-fold symmetry of centriole

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Centrioles have a unique structure composed of nine rotationally arranged triplet microtubules. This nine-fold symmetrical structure, highly conserved among eukaryotes, functions as both the center of the microtubule network in the cytoplasm and the base of cilia. Functional analysis revealed that a conserved centriolar protein, SAS-6, constitutes the cartwheel, which is a structure considered essential to the establishment of the nine-fold symmetry. However, in *bld12*, a null mutant of SAS-6, 70% of the centrioles still have nine triplet microtubules. Moreover, an engineered SAS-6 that self-assembles into a six-fold symmetrical structure in vitro, tends to produce mostly nine-fold symmetrical cartwheels and centrioles when transformed into *Chlamydomonas* cells [1]. These results suggest that a SAS-6-independent mechanism(s) also functions to determine the nine-fold symmetry.

As previous studies showed that Bld10p (a homolog of mammalian Cep135) is crucial for the centriolar nine-fold symmetry, we carried out functional and structural analyses of this protein [2]. Immuno-electron microscopy revealed linearly arranged Bld10p signals between the bulges (pinheads) connecting the cartwheel spokes and the triplet microtubules. At the same time, thin section electron microscopy revealed novel filamentous structures that crosslink between adjacent pinheads. These structures were not amenable to cryo-electron subtomogram averaging because of their large structural variability; however, we were able to directly observe them by exploiting a Volta Phase Plate (Fig. 1). The positions of these structures coincided with those of the immuno-gold labels to Bld10p, strongly suggesting that Bld10p constitutes them. In strains expressing truncated Bld10p in *bld10bld12* (a double mutant that lacks both Bld10p and SAS-6), the

number of triplets was reduced and the crosslink structures were shorter. These results indicate that part of the Bld10p molecule functions in a cartwheel-independent mechanism to determine the centriolar 9-fold symmetry.

In addition to the Bld10p filamentous structures, our cryo-electron tomography studies have identified other structures in centrioles and cilia. We will also touch on the results of these preliminary observations.

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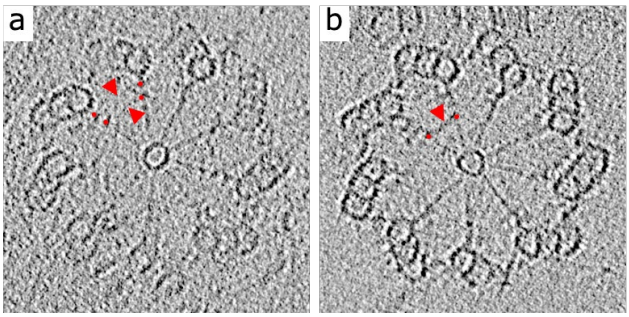

Fig. 1. Novel filamentous structures observed by cryo-electron tomography. There are three kinds of filamentous structures (red arrowheads). Two are located between adjacent pinheads (a) and one is between cartwheel spokes (b). Red points show where filamentous structures connect with pinheads or cartwheel spokes.

Session 2 J-5

Hierarchical Structures of Semicrystalline Polymers Revealed by Nanodiffraction Imaging Based on Four-dimensional Scanning Transmission Electron Microscopy

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Introduction Semicrystalline polymers, with excellent thermal and mechanical properties, are extensively used in various applications. In melt-crystallization, polymer chains fold to form plate-like crystals (lamellar crystals: LCs) with a thickness of about 10 nm, leaving amorphous layers in between. These LCs grow with twist and branch, forming various higher-order structures, such as spherulites. This process results in hierarchical structures inside semicrystalline polymers spanning the sub-nanometer to micrometer scale (Fig. 1). The heterogeneous distribution of LCs, which is closely linked to the properties of polymeric materials, has been a subject of great interest among researchers. However, the structural nature of LCs, particularly the molecular chain orientations inside them, has remained unknown until now.

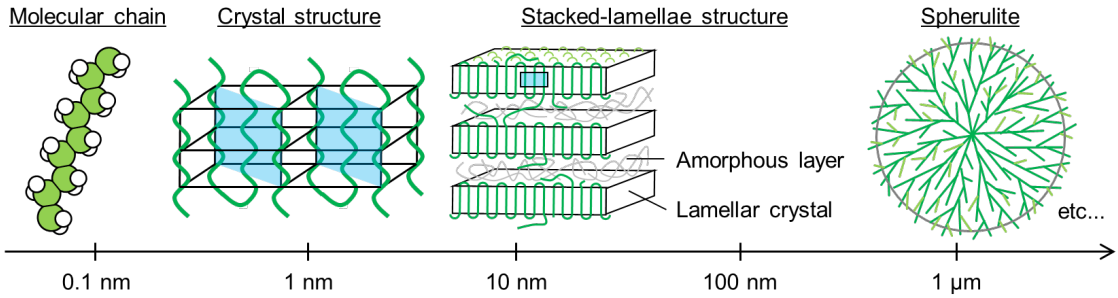

Fig. 1. Hierarchical structure of semicrystalline polymers.

Four-dimensional scanning transmission electron microscopy (4D-STEM) is a technique that scans an electron beam across a sample and acquires electron diffraction (ED) patterns from each scanning position[1]. The position-resolved ED patterns provide high resolution, i.e., nanometer-scale maps of various structural information. While 4D-STEM has been increasingly used for inorganic materials since around 2010, its application to organic materials has been limited due to its sensitivity to electron

irradiation damage. In 2021, we undertook the challenge of exploring 4D-STEM conditions suitable for high-density polyethylene (HDPE) and, for the first time, mapped the molecular chain orientations with 30 nm resolution[2]. The method of mapping crystal structures by 4D-STEM using a quasi-parallel electron beam is called nanodiffraction imaging (NDI). In this study, we used high-resolution NDI to visualize LCs of HDPE and directly determined the molecular chain orientations inside LCs[3].

Results and Discussion An ultrathin (~100 nm) section was prepared from an HDPE film melt-crystallized at 120 °C. A 1.2-nm-diameter electron beam was scanned across the section with a step of 6 nm using a JEM-F200 (JEOL Ltd.; 200 kV). The ED patterns at each point were recorded on a K3 IS (Gatan, Inc.). The intensity of the ED spots was extracted from each pattern, and Fig. 2a was reconstructed, clearly showing the crystalline and amorphous regions as bright and dark, respectively. The ED pattern with 200 spots (Fig. 2b) corresponds to the orange pixel inside the LC numbered as 2 in Fig 2a, indicating that molecular chains were not perpendicular to the LC surface, i.e., chain tilt angle (φ) > 0. Moreover, the φ value map (Fig. 2c) revealed different φ values for the two LCs. These findings are significant as they provide direct evidence of the chain tilt inside LCs, previously ambiguous for semicrystalline polymers. In addition, we will show recent NDI results on different hierarchical structures.

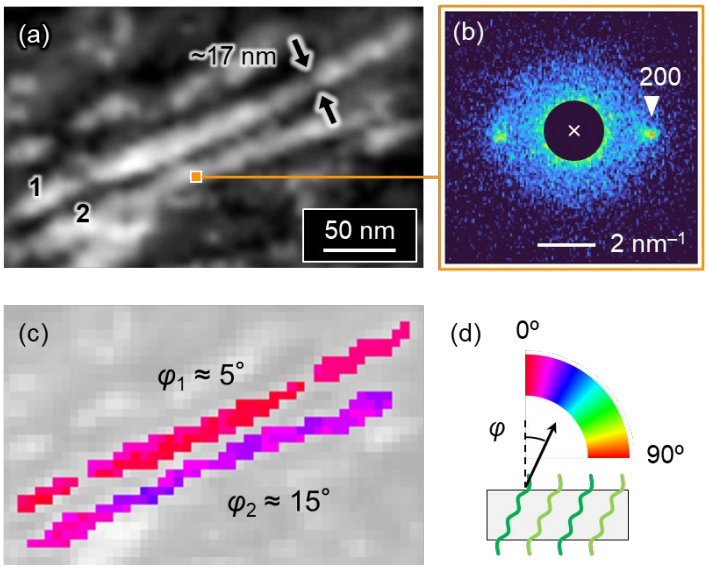

Fig. 2. (a) Reconstructed dark-field scanning transmission electron microscopy image of high-density polyethylene, showing two numbered lamellar crystals (LCs), including an ~17-nm-wide one. (b) Electron diffraction pattern sampled from the orange pixel in the second LC in panel (a). Molecular chains are perpendicular to the line connecting the two 200 spots. (c) Color map of chain tilt angle φ in the same field-of-view as panel (a). (d) A color wheel of φ with the schematic of a LC.

Acknowledgment: This study was supported by the JST CREST program and a JSPS KAKENHI grant. We also thank Mitsubishi Chemical Corporation and JEOL for their support.

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Session 2 J-6

Atomic resolution analysis of extended defects and their evolution during annealing in Mg ion-implanted GaN

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Vertical GaN power devices have been developed as high-efficiency switching devices for high-power applications. Selective area p-type doping is required to unleash the full potential of such devices. For this purpose, Mg ion implantation processes have been developed. A p-type conductivity has been demonstrated in Mg-ion-implanted GaN using ultra-high-pressure annealing (UHPA), where samples are annealed in high pressure N_2 atmosphere of 1 GPa, for acceptor formation. [1]

We utilized transmission electron microscopy (TEM) and atom probe tomography (APT) to conduct atomic-scale investigations of Mg-ion-implanted GaN, aiming to elucidate the crystallographic structures of extended defects and Mg agglomerations formed during post-implantation annealing. Figure 1 shows a HAADF-STEM image of one type of extended defects, specifically interstitial-type dislocation loop observed in the early stage of annealing. APT analyses revealed that Mg atoms tend to agglomerate, forming clusters around such dislocation loops. The concentration of Mg is higher at dislocations with a larger Burgers vector b, indicating that Mg agglomeration is caused by the pressure at these dislocations. [2] The highly Mg-rich regions exhibit a Mg concentration of 1 atomic %, exceeding the solubility limit of Mg in GaN. This indicates that Mg agglomeration may hinder the activation of Mg dopants. Moreover, we demonstrate that the Mg dopants become fully activated after eliminating such extended defects with highly Mg-rich regions, achieved through annealing at longer durations or higher temperatures.

We also investigated the time evolution of extended defects in Mg-ion-implanted

GaN during annealing in N_2 atmospheres at pressures of 2.0 and 0.3 GPa. The annealing durations were 15, 30, 60 and 90 min. Hydrostatic pressure is an important parameter for diffusion of point defect. The diffusion constants of the native defects can be estimated from the coarsening of the extended defects, i.e., an increase in defect size and a decrease in defect density. [3] Our analyses clarify that the diffu1sion constants of native defect and Mg impurity are smaller at the samples annealed at higher N_2 pressure. [4] In samples annealed at 2.0 GPa, defect sizes remained small and the densities remained high. This indicates that coarsening of defects is retarded under the high N_2 pressure annealing conditions. Furthermore, secondary ion mass spectrometry (SIMS) was used to measure the distributions of Mg and H. The H concentration reportedly matches the Mg_{Ga} concentration in GaN after the UHPA process. [5] Figure 2 shows that H concentration in the ion-implanted region was higher in the sample annealed at a higher pressure. We thus infer that the retarded diffusion of V_{Ga} would increase their concentrations in the ion-implanted region, subsequently increasing the Mg_{Ga} concentration, as Mg_{Ga} is produced through the reaction between Mg_i and V_{Ga}.

Acknowledgment: Part of this work was supported by the MEXT "Program for research and development of next-generation semiconductor to realize energy-saving society" (JPJ005357) and the "Program for Creation of Innovative Core Technology for Power Electronics" (JPJ009777).

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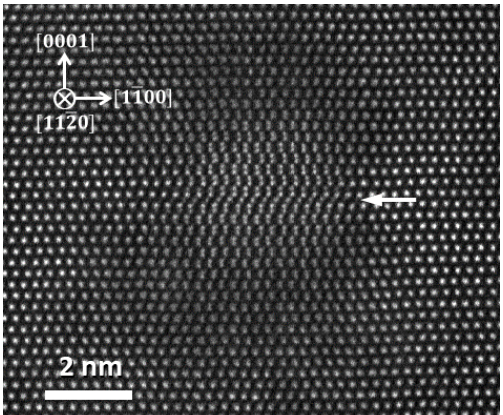

Fig. 1 HAADF-STEM image of interstitial-type dislocation loop. White arrow indicates an extra c-plane of GaN.

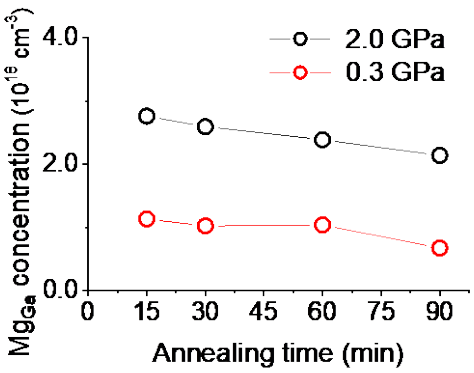

Fig. 2. H concentration, which represent the Mg_{Ga} concentrations, in the ion-implanted region measured by SIMS.

Session 2 C-3

Semiconductor Nanowire Junction Potential Maps

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All electronic devices, such as transistors and sensors, require the fabrication of semiconducting materials with precise control of the electrical carriers present, their type and concentration (electrons and holes). This also assumes that crystalline defects (impurities, stacking faults, precipitates, grain boundaries) have been avoided and that all surfaces are either a passive participant, meaning that they do not dominate the control of electrical current within the device, or are carefully designed to do just that, for example, a field-effect oxide interface. When a device geometry is nanoscale, the detectable presence of an internal electrical junction happens only when surface states and crystal defects are limited. This talk will describe examples of potential maps obtained from a variety of semiconducting nanowire diodes [1-4]. We will see that their junctions are easily mapped in some cases, but not in others. The approach is via the well-established technique known as off-axis electron holography in a transmission electron microscope. The potential within a junction is detected via the change in phase of coherent electrons that pass through a sample. The image below shows an example of a phase map from an axial p-n junction based on an InP heterojunction [1]. The phase change is proportional to the sample thickness and scalar potential consisting of a combination of the bulk mean inner potential and the junction static built-in potential.

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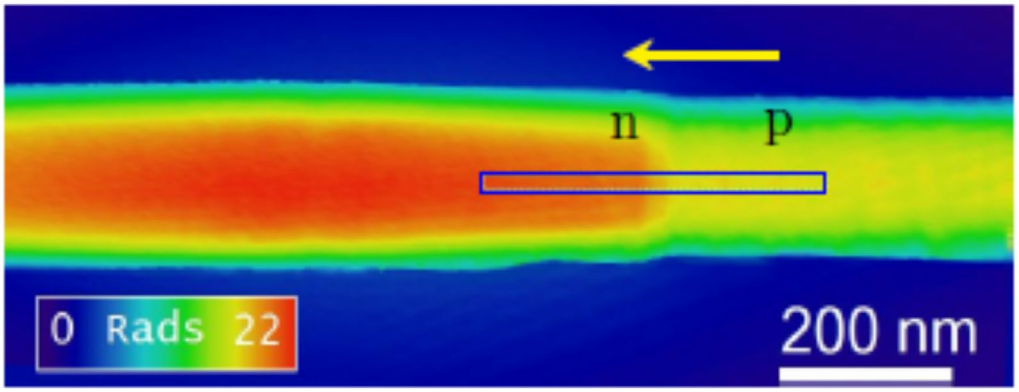

Fig. 1 Phase map from an axial p-n junction within an **InP/GaInP** heterojunction [3].

Session 2 J-7

Wavefield reconstruction using full-field illumination ptychography with a structured electron beam

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Phase information of the electron wave contains quantitative information about the magnetic or electric properties of materials. Ptychography, a wavefield reconstruction technique using far-field or near-field patterns of the electron wave [1][2], has been attracting great interest in the field of electron phase measurement.

In this study, we propose a novel configuration for near-field ptychography with full-field illumination using a structured electron beam, aiming at the accurate and efficient observation of large structures. The proposed method is configured on conventional TEM setups. An electron beam, structured to have non-uniform amplitude and phase distribution, is projected onto the specimen. Then, a series of in-line holograms observed in the near-field region below the specimen are obtained with different illumination positions by scanning the illumination beam. Both the illumination beam wavefield and the complex transmission function of the specimen are reconstructed from the obtained holograms through iterative calculation.

The performance of the proposed method was first evaluated using simulated holograms. Then, its experimental feasibility was evaluated using MgO particles. Figures 1a and 1b show the given amplitude and phase components of the specimen transmission function used in the simulation study. Fig. 1c shows one of the in-line holograms obtained below the specimen. The wavefield of the illumination beam and transmission function of the specimen were reconstructed from the obtained holograms. The reconstructed amplitude (Fig. 1d) and phase (Fig. 1e) of the specimen transmission function show good agreement with the given structure (Figs. 1a and 1b). Experimental evaluation using MgO particles also showed a reasonable phase reconstruction consistent with the particle shape. These results demonstrate the validity of the method.

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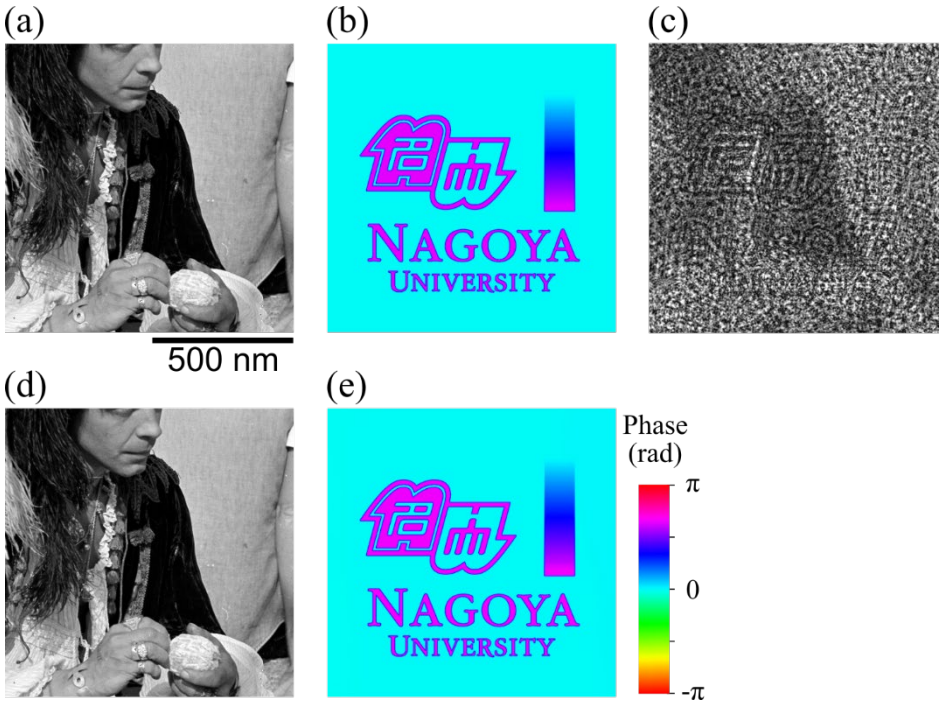

Fig. 1 Reconstruction results from simulated holograms. (a) Amplitude and (b) phase of the given specimen transmission function (ground truth). (c) One of the simulated holograms obtained below the specimen. (d) Amplitude and (e) phase of the reconstructed specimen transmission function.

Session 2 C-4

Streak Imaging in a Dynamic Transmission Electron Microscope

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A suite of high-speed electron microscopy instruments and methods have been developed in the past few decades to watch nanomaterial excitation and dynamics in real time [1]. The dynamic transmission electron microscope (DTEM) is one such instrument that has the unique ability to capture snapshot images of transient and irreversible phase transformations. However, the amount of information that can be obtained about such an event is limited by existing data collection strategies as well as the fact that obtaining a clear image requires the generation of pulses containing > 10^7 electrons [1]. The movie-mode DTEM was developed to allow for multiple images of a single event to be captured by deflecting a rapid sequence of snapshot images onto different regions of the camera. This approach can produce a film consisting of 9 frames and a minimum interframe time interval of 50 nanoseconds [2]. It has been used to track the propagation of phase transformation fronts moving at a speed 1-10 nm/ns, but is not able to capture the early stages of a transformation or fronts moving close to the speed of sound (> 1,000 nm/ns).

We will then present our recent progress in developing different modes of streak imaging to improve the time resolution of a MM-DTEM. Notably, we will demonstrate the application of tomographic compressed sensing image reconstruction [3] to recover a sequence of two-dimensional images of a 1.85-µm-diameter field of view (FOV) with nanoscale spatial resolution, 370-ps inter-frame interval, and 140-frame sequence depth in a 50-ns time window as shown in Fig. 1. We have used the developed streak-mode DTEM (SM-DTEM) to characterize the spatio-temporal evolution of generated high-charge electron pulses [4] and will discuss the observation of a peak in the photoemission at the beginning of the electron pulse (Fig. 2). The new SM-DTEM operation modes have the possibility to rapidly collect sets of images with picosecond time resolution, relieving the constraint on sample stability, and allowing for new in situ experiments that follow the ultrafast material response as it evolves.

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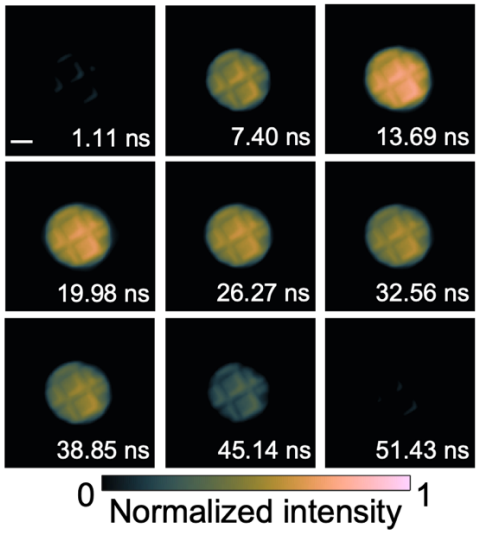

Fig. 1. Selected frames of a 2D SM-DTEM reconstructed scene of 50-ns electron pulse passing through gold cross grating sample.

Scale bar = 500 nm.

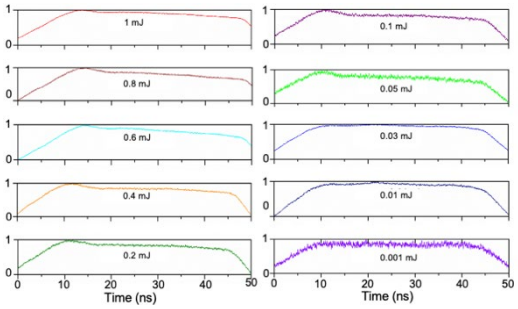

Fig. 2. Streak traces of 50-ns photoemitted electron pulses measured for different UV cathode laser pulse energy.

Session 2 C-5

The Ultrafast Electron Imaging Lab: Where Electrons, Light, Nanofluidics, and People Meet

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One of the paramount experiments in science is the direct observation of atomic motions during chemical reactions and phase transformations. To date, the advent of ultrafast 'structure-sensitive' cameras using ultrashort pulses of electrons or hard X-rays has realized this ambition for crystalline solids. Nevertheless, capturing molecular movies in the liquid phase presents significant challenges; overcoming these would unlock the domain of chemical kinetics for atomic exploration.

In this talk, I will detail the technical breakthroughs that have afforded such precision and give an overview of our team's endeavors at the Ultrafast Electron Imaging Lab (UeIL) to adapt time-resolved ultrafast electron diffraction (UED) and electron microscopy (EM) techniques for liquid samples. With respect to UED, we are actively working to secure resources to build a facility that will open to the research community. For EM, we have devised liquid-phase EM (LPEM) kits[1-4] that outfit all leading transmission electron microscope models.

Figure 1 presents our newly developed, compact, and upgradeable 100-keV UED instrument[5]. Figure 2 showcases the LPEM results obtained for the study of adeno-associated viruses at the Canadian Centre for Electron Microscopy (CCEM) in Hamilton. In partnership with the team of Mark Salomons and Marek Malac at the NRC Nanotechnology Research Centre (NANO) in Edmonton, we are experimenting with hole-free-phase-plate (HFPP) transmission EM to enhance image contrast and resolution in studies of biological specimens. Our goal is to make our LPEM technologies accessible through the two principal EM centres in Canada.

Acknowledgment: The work presented here could not have been possible without the generous financial support provided by the Chan-Zuckerberg Initiative (CZI), the Natural Sciences and Engineering Research Council (NSERC) of Canada, and the Canada Foundation for Innovation (CFI). The University of Waterloo's QNFCF facility was used for the fabrication of UeIL's nanofluidic chips.

The establishment of this infrastructure owes much to the significant contributions from CFREF-TQT, CFI, ISED, the Ontario Ministry of Research & Innovation, and Mike & Ophelia Lazaridis.

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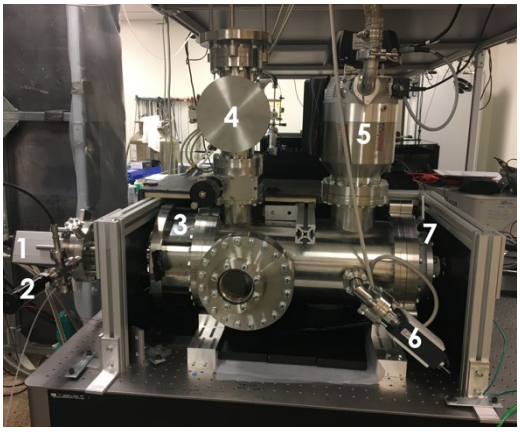

Fig. 1: Photograph of a homemade Ultrafast Electron Diffraction (UED) setup. The primary components include: (1) camera, (2) linear manipulator for the Faraday cup, (3) magnetic lens, (4) sample exchange compartment, (5) turbomolecular pump, (6) vacuum gauge, and (7) high-voltage feedthrough. All principal components were designed at UeIL. This UED setup is slated for commercialization by e-Ray Scientific, a collaborative venture between the teams from Sciaini (University of Waterloo), Siwick (McGill University), and Miller (University of Toronto).

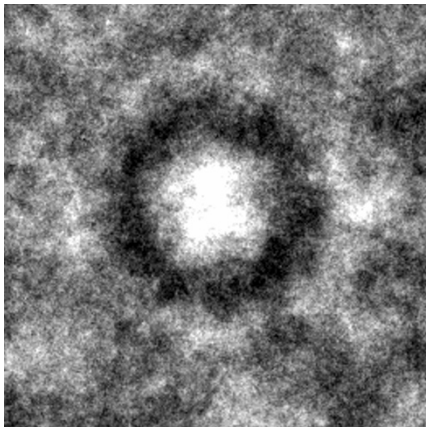

Fig. 2. A zoomed-in TEM image of a single adeno-associated virus (AAV) particle (~ 25 nm in diameter) observed with some degree of defocus using our liquid-phase electron microscopy suite. Acceleration voltage = 200 kV, magnification ≅ 58 kx, electron dose rate ≅ 10 e^{-} $Å^{-2}$ s^{-1}, exposure time = 5 s, liquid layer thickness ≅ 500 nm, window material thickness (total of silicon nitride) = 25 nm.

【Societies】

Japanese Society of Microscopy (JSM)

https://microscopy.or.jp/

Microscopy Society of Canada (MSC)

https://msc-smc.org/

【Venues】

Session 1

International Conference Hall, Makuhari Messe.

2-1, Nakase, Mihama-ku, Chiba-city, 261-8550 Japan

https://www.m-messe.co.jp/organizers/guide/conferencehall/guide_conference

Session 2

Richcraft Hall, Carleton University

9376, University Drive, Ottawa, ON K1S 5B6, Canada

https://carleton.ca/campus/buildings/richcraft-hall/

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