Japan-Canada Joint Symposium 2020

“Development and applications of the methods combining interference/phase measurement techniques and analytic techniques”

日本－カナダ　二国間交流シンポジウム 2020

－干渉・位相計測技術と分析技術の融合を目指して－

May 24 (Sunday) – May 27 (Wednesday), 2020

at

International House, Osaka (planned)

Japanese Society of Microscopy (JSM)

Microscopy Society of Canada (MSC)

【はじめに】

日本顕微鏡学会（Japanese Society of Microscopy: JSM）とカナダ顕微鏡学会（Microscopy Society of Canada: MSC）は、両学会に所属する生物・材料系研究者間の相互交流を深め、それにより進歩を続ける電子顕微鏡、並びに科学計測機器のさらなる発展のため、二国間交流シンポジウムを企画しました。初めての試みにあたり、本シンポジウムでは、原子レベルの高分解能法や環境顕微鏡法、そして元素分析技術などの材料系のテーマだけでなく、2017 年のノーベル賞受賞の記憶に新しい生体分子観察法（クライオ電子顕微鏡法）や単粒子解析技術など、生物系テーマを含む広い観点からテーマ・課題を選定し、電子線と物質との相互作用、特に材料科学、デバイス工学、医学、生物学などに共通の課題となっている観察対象へのダメージ、あるいは意図せぬチャージアップなどの影響をも検討することにしました。その結果、日本の得意とする干渉・位相計測技術と、カナダの得意とする分析技術の融合を目指したテーマを選定し、サブテーマ『干渉・位相計測技術と分析技術の融合を目指して』としました。

日本顕微鏡学会第７６回学術講演会と時期を合わせた開催として、より多く、より幅広い分野の研究者の参加と相互交流を目指し、同学術講演会実行委員会とも密接に相談しながら計画を進めました。その結果、1.5 日間のシンポジウム期間中に、21 名の登壇予定者を得ることができ、特にカナダとデンマークから、9 名の参加者となりました。この中には、PosDoc や Dr コースの若手研究者にも加わっていただいており、若手研究者間の交流も期待できるものとしました。

しかしながら、大変残念なことに、本年始めからの新型コロナウイルス感染症の世界的な蔓延により、今回の二国間交流シンポジウムは、講演発表を中止せざるを得ませんでした。そこで、登壇予定者皆さんの予稿原稿や講演プログラムは完成されており準備は整っていましたので、それらを公開することによって、紙上での二国間交流シンポジウムとすることとしました。この冊子は、紙上開催、日本-カナダ交流シンポジウムの予稿集です。

本シンポジウムに参加を予定してくださった研究者の皆さま、本シンポジウム企画に当たりご支援いただきました第 76 回学術講演会実行委員長の高井義造先生と実行委員の皆様方、並びに、本企画にご協力いただいましたすべての皆様方に、誌面を借りて御礼を申し上げます。そして、日本とカナダの両顕微鏡学会の相互交流が今後も継続されることを願っております。

オーガナイザ代表：**森 茂生**

【Preface】

The Japanese Society of Microscopy (JSM) and the Microscopy Society of Canada (MSC) have decided to organize a joint symposium on advanced electron microscopy and its application in order to deepen mutual understanding and scientific interaction between researchers belonging to both societies in biological science and materials science and in order to contribute further development and progress of electron microscopes and scientific measurement instruments. In this first symposium, we selected topics from a broad perspective, not only from materials science fields including atomic level high-resolution microscopy, environmental microscopy, and elemental analysis, but also biological science fields including new biomolecule observation method, i.e., cryo electron microscopy (2017 Nobel Prize), and single particle analyses. During the topic selection, we also considered the interactions between electron beams and substances, the damage and beam induced charging to the observed samples, and in particular common issues affecting materials science, device engineering, medical science, and biological science. As a result, we came to the conclusion that the symposium should focus on "Development and applications of the methods combining interference/phase measurement techniques and analytic techniques," particularly discussing electron interferometry and phase measurement methods that are intensively studied in Japan and analytical and biological microscopies that are intensively studied in Canada.

We decided to hold this symposium jointly with the 76th Annual Meeting of JSM to maximize participation and deepen interaction and discussion between researchers from wide scientific fields. Therefore, we prepared the joint symposium in close collaboration with the JSM Executive Committee of the 2020 Annual Meeting. As a result, for one-and-a-half day symposium, we got twenty-one speakers, including eight from Canada and one from Denmark. Post-doctoral research associated and PhD course students also planned to join this meeting for make an outstanding networking opportunity.

We are regret to report that we cannot hold the joint symposium as planned because of the global spread of new coronavirus infections (COVID-19) starting at the beginning of this year. Instead, we decided to hold the joint symposium virtually on paper. Since the draft manuscripts of all speakers and symposium programs were ready, we decided to make the conference proceeding including them and distribute it to all the planned participants. This booklet is the proceedings of the Japan – Canada Joint Symposium.

We would like to thank all the speakers who scheduled to participate this symposium, Prof. Y. Takai, Chairman of the 76th Annual Meeting Executive Committee, and all the executive committee members, as well as all the people who cooperated with this symposium project. We hope that mutual relation between JSM and MSC will continue and expand.

Representative of the organizers,
Shigeo Mori (Osaka Prefecture University)

【ご挨拶】

日本顕微鏡学会長　幾原雄一

本顕微鏡学会では、大阪で開催される予定であった第７６回学術講演会と併設して、日本顕微鏡学会（Japanese Society of Microscopy: JSM）とカナダ顕微鏡学会（Microscopy Society of Canada: MSC）の二国間交流のシンポジウムの開催を計画していました。このシンポジウムは、現在日本顕微鏡学会が積極的に進めております“国際化へ向けた取り組み”とも合致している上、～干渉・位相計測技術と分析技術の融合を目指して～というタイムリーなテーマが設定されていました。本シンポジウムは、日本学術振興会からの支援も受け、日加二国間のさらなる交流や共同研究の促進が期待されましたが、大変残念なことに、新型コロナウイルス感染症の蔓延により、本シンポジウムにおける講演発表を中止するに至りました。しかし、この記念すべきシンポジウムの企画は是非残したいという関係者の強い要望もあり、この予稿集にみられますように紙上開催は行われました。本シンポジウムをご支援頂きました学術講演会の高井義造実行委員長、本シンポシムの開催にご尽力頂きました森茂生先生、岡田康志先生、原田研先生ら組織委員の皆様、また、カナダ顕微鏡学会の Marek Malac 会長、Dr. Misa Hayashida をはじめとするカナダ側の組織委員の皆様方に、心から感謝申し上げます。これを機に、日本とカナダの両顕微鏡学会の交流がさらに発展することを祈念しております

【Greetings】

Yuichi Ikuhara, President of JSM

The Japanese Society of Microscopy (JSM) planned JSM and the Microscopy Society of Canada (MSC) joint symposium on advanced electron microscopy and its application during the period of the 76th JSM Annual Meeting in Osaka. This symposium was aimed to hold as a part of “Internationalization” that JSM is initiatively promoting, and focused the very timely topics, “Development and applications of the methods combining interference/phase measurement techniques and analytic techniques”. This symposium is supported by JSPS (Japan Society for the Promotion of Science), and has been expected to enhance the international exchange and mutual collaboration between two countries. But, unfortunately, we must cancel this symposium due to the global spread of new coronavirus infections (COVID-19). However, since most of people who concern this symposium hope the content of this symposium should be reserved as our memorial event, we decided to hold this symposium on paper, as can be seen in the present proceedings. I sincerely thank Prof. Yoshizo Takai, the chairman of JSM meeting, Prof. Shigeo Mori, Prof. Yasushi Okada, Dr. Ken Harada and the symposium organizers, and also Prof. Marek Malac, the president of MSC, Dr. Misa Hayashida and

the organizers from Canadian side, as the president of JSM. I really hope that the relationship between JSM and MSC will further continue and enhance our collaboration as a starting point for next step.

Marek Malac, President of MSC

It is an honor to have an opportunity to support and participate in the first Japan-Canada joint symposium. An outstanding scientific program was put together with a great potential to initiate lasting relations among members of the two societies, JSM and MSC. It is with great regret that the symposium cannot take place as planned at Osaka, Japan due to the world wide covid-19 pandemic. I hope that the collaborative spirit and interest in ongoing connections will lead to a joint workshop or symposium in the near future. I would like to thank the organizers of the 76th JSM meeting organizers and to Prof. Takai in particular for their extensive support and understanding. My thanks also belong to Prof. Ikuhara for his acceptance of the joint symposium and the support he kindly provided. Last, but not least, my thanks belong to the symposium organizers, Dr. Harada, Prof. Mori, Prof. Okada and Dr. Hayashida, for their hard work and dedication that ultimately made the symposium possible.

【Contents】

Time Schedule Japan – Canada Joint Symposium

May 24 (Sunday) Welcome Reception	May 25 (Monday) Materials Science Session	May 26 (Tuesday) Biological Science Session	27th (Wednesday) Lab. Tour for Osaka Univ.
	9:00 – 12:00 Morning Session at International House Osaka	8:30 – 11:30 Morning Session at International House Osaka	
			12:00 Meeting at H. Awina Osaka
	12:00 – 13:15 Lunch at Hotel Awina Osaka	11:30 – 13:30 Lunch at Hotel Awina Osaka	12:00 -13:00 Move to Osaka University by Bus
			13:00 – 14:00 Lunch at Osaka University
	13:15 – 16:30 Afternoon Session at International House Osaka		14:10 – 16:30 HVEM Center and Namba Lab.
			16:30 – 18:00 Return to H. Awina Osaka by Bus
17:00 – 19:00 Tutorial and Welcome Reception at Hotel Awina Osaka	19:00 – 21:00 Dinner	18:00 – 20:00 Banquet at Hotel Awina Osaka	19:00 – 21:00 Okonomiyaki Dinner
Accommodation at Hotel Awina Osaka	Accommodation at Hotel Awina Osaka	Accommodation at Hotel Awina Osaka	Accommodation at Hotel Awina Osaka

Symposium location: International House, Osaka (http://www.ih-osaka.or.jp/english/)
Accommodation: Hotel AWINA OSAKA (http://www.awina-osaka.com/)

【2. Program】

2.1 Day 1 (May 25 (Monday), 2020)

Opening Address 09:00 – 09:05

Yuichi Ikuhara (President of JSM)

Materials Science Session 1

Chairperson

Shigeo Mori (Osaka Prefecture University)

Marco Beleggia (Technical University of Denmark)

MS1-01 09:05 – 09:35

Understanding Radiation Damage in Beam-Sensitive TEM Samples

Ray Egerton (University of Alberta)

MS1-02 09:35 – 09:55

Capability of a low-kV TEM in the study of beam sensitive materials

Toshie Yaguchi, Yasuhira Nagakubo, Keiji Tamura, Yasuyuki Nodera, Keisuke Igarashi, Akiko Wakui (Hitachi High-Tech corporation)

MS1-03 09:55 – 10:20

Beam Damage and Sample Preparation in Plasma Focused Ion Beam Microscopy

Peng Dong[1], Ali Allahverdi[2], Sam Norris[1], Hui Yuan[1], Nabil D. Bassim[1] ([1]McMaster University, [2]Iran University)

Short Break 10:20 – 10:30

MS1-04 10:30 – 11:00

Atomic Resolution Dynamic Observations of Grain Boundary and Surface

Yuichi Ikuhara[1,2,3] ([1]University of Tokyo, [2]NSRL, Japan Fine Ceramics Center, [3]WPI-AIMK, Tohoku University)

MS1-05 11:00 – 11:30

Charging of Thin Film Phase Plates in a TEM

Marek Malac[1,2], M. Hayashida[1], R.F. Egerton[2], S. Hettler[3], M. Beleggia[4] ([1]NRC-NANO, [2]Dept. Physics, University of Alberta, [3]INA, University of Zaragoza, [4]DTU)

MS1-06 11:30 – 12:00

Gas electron holography: realisation and implementation

Jes Ærøe Hyllested, G. Prabhu Sai Balasubramanian, E. M. Fiordaliso, **Marco Beleggia**[1] (Technical University of Denmark)

Lunch 12:00 – 13:15

Materials Science Session 2

Chairperson

Ken Harada (CEMS, RIKEN)

Marek Malac (NRC-NANO; University of Alberta)

MS2-07 13:15 – 13:45

Towards Atomic Resolution State Analysis by STEM-EELS

Hiroki Kurata, Mitsutaka Haruta (Institute for Chemical Research, Kyoto University)

MS2-08 13:45 – 14:10

EDS and EELS of Lithium Materials from 0.5 to 30 keV

Raynald Gauvin, Nicolas Brodusch, Frédéric Voisard (McGill University)

MS2-09 14:10 – 14:30

Fusion of Analytical TEM/STEM and in-situ analysis

Hiromi Inada (Hitachi High-Tech Corp.)

MS2-10 14:30 – 14:50

High-precision Phase Analysis of Automatically Collected Electron Holograms

Yoshio Takahashi[1], Tetsuya Akashi[1], Atsuko Sato[2], Yoshiaki Tanigaki[1], Hiroyuki Shinada[1], Yasukazu Murakami[2] ([1]Hitachi, Ltd., [2]Kyushu University)

Short Break 14:50 – 15:00

MS2-11 15:00 – 15:25

Combination of Fluctuation Electron Microscopy and Ptychography for Characterization of Amorphous – Crystalline Mixtures

Arthur Blackburn (University of Victoria)

MS2-12 15:25 – 15:45

Thermal stability and microstructures of the $LiNi_{1/3}Mn_{1/3}Co_{1/3}O_2$ positive electrode for sulfide-based all-solid-state lithium batteries

Hirofumi Tsukasaki, Misae Otoyama, Shigeo Mori, Akitoshi Hayashi, Masahiro Tatsumisago (Osaka Prefecture University)

MS2-13 15:45 – 16:05

Crystallization differences of Al_2O_3 on GaN planes

Emi Kano[1], Kazutaka Mitsuishi[1], Yoshihiro Irokawa[1], Toshihide Nabatame[1], Koji Kimoto[1], Tetsu Kachi[2], Yasuo Koide[1] ([1]National Institute for Materials Science, [2]Nagoya University)

MS2-14 16:05 – 16:30

Higher-order structure of human chromosomes observed by electron tomography and electron diffraction

Misa Hayashida[1], Rinyaporn Phengchat[2], Marek Malac[1], Ken Harada[3], Tetsuya Akashi[4], Nobuko Ohmido[2], Kiichi Fukui[5] ([1]National Research Council Canada, [2]Kobe University, [3]RIKEN, [4]Hitachi, Ltd., [5]Osaka University)

2.2 Day 2 (May 26 (Tuesday), 2020)

Biological Science Session

Chairperson

Yasushi Okada (BRD, RIKEN; University of Tokyo)

Elitza Tocheva (University of British Columbia)

BS-01 08:30 – 09:00

Engineering genetically encoded biosensors of neural activity and metabolism

Robert Campbell[1,2] ([1]University of Alberta, [2]University of Tokyo)

BS-02 09:00 – 09:20

Correlative Light and Electron Microscopy (CLEM) for trace of climbing fiber

Mitsuo Suga, Hideo Nishioka (JEOL Ltd.)

BS-03 09:20 – 09:40

Single-molecule nanoscopy by using cryogenic fluorescence microscopy

Satoru Fujiyoshi (Tokyo Institute of Technology)

BS-04 09:40 – 10:10

Correlation of cryo super-resolution and cryo-electron tomography in bacteria

Danielle Sexton, **Elitza Tocheva** (University of British Columbia)

BS-05 10:10 – 10:30

Near-atomic resolution structures of the doublet microtubules by cryo-EM

Muneyoshi Ichikawa[1,2], Ahmad Khalifa[1], Shintaroh Kubo[3], Daniel Dai[1], Kaustuv Basu[4], Amin Maghrebi[1], Javier Vargas[1], Khanh-Huy Bui[1] ([1]Department of Anatomy and Cell Biology, McGill University, [2]Department of Systems Biology, Graduate School of Biological Sciences, Nara Institute of Science and Technology, [3]Department of Biophysics, Graduate School of Science, Kyoto University, [4]Facility for Electron Microscopy Research, McGill University)

BS-06 10:30 – 11:00

Development and Applications of a New Cryo TEM, JEOL CRYO ARM

Keiichi Namba (Graduate School of Frontier Biosciences, Osaka University)

BS-07 11:00 – 11:30

Cryo-ED and EM for higher-resolution and higher-precision structure analysis

Koji Yonekura[1], Saori Maki Yonekura[1], Tasuku Hamaguchi[1], Hisashi Naitow[1], Kiyofumi Takaba (RIKEN SPring-8 Center)

【3. Abstracts】

3.1　Day 1　(May 25 (Monday), 2020)

Materials Science Session 1

(Chair: Shigeo Mori and Marco Beleggia)

Materials Science Session 2

(Chair: Ken Harada and Marek Malac)

MS1-01

Understanding Radiation Damage in Beam-Sensitive TEM Samples

Ray Egerton[1*]
[1]Physcs Department, University of Alberta, Canada T1W 2E2

To be useful, a physical model for electron-induced radiation damage in beam-sensitive specimens should account for the following observations [1].

Radiolysis is the dominant damage mechanism in organic specimens and, unlike the case of knock-on displacement, there is no primary-energy threshold. In fact, signal/damage ratio is relatively insensitive to electron accelerating voltage between 1kV and 500 kV.

Atomic-scale damage, measured by electron diffraction, is reduced typically by a factor of 10 when the specimen is cooled to around 100 K. Secondary damage, including mass loss, is generally reduced by a larger factor.

The beam sensitivity of organic materials varies by orders of magnitude and is reduced by replacing light atoms with heavier ones. In polymers and organic molecular crystals, radiation sensitivity correlates with melting temperature, with an activation energy of about 8.7 meV.

Encapsulation (in nanotubes, with graphene or by surface coating) reduces mass loss and apparently also structural damage.

Although Coulomb repulsion prevents outrunning the primary stage of radiolysis with electrons, field-emission sources are bright enough to partially outrun mass loss, with negligible temperature rise thanks to the efficient radial conduction of heat away from a STEM probe.

[1] R.F. Egerton, Micron 119, 72-87 (2019).

Acknowledgment: the author is supported by the Natural Sciences and Engineering Research Council of Canada.

MS1-02

Capability of a low-kV TEM in the study of beam sensitive materials

Toshie Yaguchi[1], Yasuhira Nagakubo[1], Keiji Tamura[1], Yasuyuki Nodera[1],
Keisuke Igarashi[1]and Akiko Wakui[1]

[1]Hitachi High-Tech Corporation, 882 Ichige, Hitachinaka-shi, Ibaraki-ken, 312-8504 JAPAN

Low-kV TEMs have been widely used in the biomedical and the soft material fields due to their advantageous features such as high contrast and low-damage imaging. The features are also required in the characterization of composite materials used in the batteries. In order to improve the capability of our 20-120 kV TEM in the study of electron beam sensitive materials, we added some new features. To obtain electron diffraction pattern from the area of 18 nm in diameter at the same low dose condition for TEM observation, a field limiting aperture with the diameter of 1 μm is employed in the standard configuration. In addition, the hollow cone beam dark field TEM image observation system in combination with an atomosphere blocked specimen heating holder is now routinely applicable to the *in-situ* studies such as crystallization process of glass solid electrolytes for all-solid-state lithium batteries [1,2].

Fig.1 shows TEM images of Nafion coated Pt/CB electrocatalyst of fuel cell. A TEM image of nafion with high contrast and low damage is obtained (a), while some damages, indicated by arrows, appear on nafion with electron dose over 7.2×10^6 e/nm^2 (b).

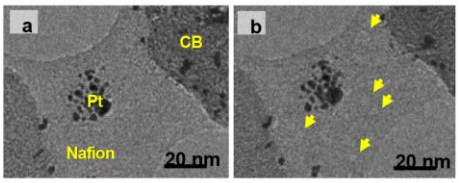

Fig.1 Nafion coated Pt/CB electrocatalyst of fuel cell observed at 120 kV.
(a) Dose:1.7×10^4 e/nm^2 , (b) Dose: 7.2×10^6 e/nm^2

[1] Yaguchi et al., Proceeding of the 75th Annual Meeting of the Japanese Society of Microscopy. p.147. (2019).
[2] Igarashi et al., Proceeding of the 60th Battery Symposium, Japan. p.215. (2019).

MS1-03

Beam Damage and Sample Preparation in Plasma Focused Ion Beam Microscopy

Peng Dong[1*], Ali Allahverdi[2], Sam Norris[1], Hui Yuan[1] and Nabil D. Bassim[1]
[1]Department of Materials Science and Engineering and Canadian Centre for Electron Microscopy, McMaster University, 1280 Main St W, Hamilton, Canada, [2]School of Chemical Engineering, Iran University of Science and Technology, Narmak, Tehran, Iran

Recently, plasma sources that use Xe^+ ions produce both a higher beam current as well as a higher sputtering rate for incident ion than traditional Ga^+ liquid metal ion source [1, 2]. The fundamentals of ion-sample interactions have not been clearly explored and optimization of experimental parameters in order to produce high-quality tomograms is still being performed.

We study the ion-solid interactions for both Xe^+ and Ga^+ sources. The measured amorphization mostly matches the predicted amorphization depth from Monte Carlo simulations. By comparing the interaction depths to the attenuation of the Raman response in of the TO Phonon mode in silicon (520 cm^{-1}) , we note that there are interesting electronic and crystal effects when changing from Ga to Xe, demonstrating a salutary effect from using plasma sources for milling functional materials.

In order to mitigate curtaining effects, we applied a sacrificial silicon mask on top of surface, as indicated by Fig. 1, to develop a planar milling front. We conducted 3D tomography on an OPC mortar specimen and demonstrate the feasibility of acquiring large volume, high resolution tomograms of cementitious materials. (Fig. 1).

[1] Bassim, N. et al., MRS Bulletin **39**. **04**, 317-325 (2014).
[2] Burnett, T.L. et al., Ultramicroscopy, **161,** 119-29 (2016).
Acknowledgements: the authors would like to thank the staff in CCEM.

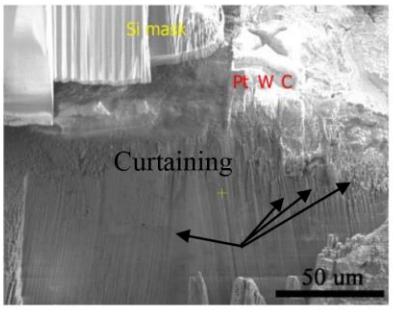

Fig. 1 The schematic of surface quality comparison using Si mask and different protection layers

MS1-04

Atomic Resolution Dynamic Observations of Grain Boundary and Surface

Yuichi Ikuhara[1,3]
[1] IEI, University of Tokyo, 2-11-16 Yayoi, Bunkyo-ku, Tokyo 113-8656, Japan
[2]NSRL, Japan Fine Ceramics Center, Nagoya 456-8587, Japan
[3]WPI-AIMR, Tohoku University, Sendai, 980-8577, Japan

GB fracture in Al_2O_3 is strongly dependent on the GB characters and the dopant segregated at GBs. In order to clarify the atomistic GB fracture mechanism in Al_2O_3 ceramics, Al_2O_3 bicrystals including GBs with specific geometrical configuration were systematically fabricated, and some of them were doped by rare-earth elements. Then, the atomic structures and chemistry in thus fabricated GBs were characterized by atom-resolved STEM, and the dynamic behavior of GB fracture was observed by TEM nanoindentation experiment. The relationship between GB characters, segregated dopants and GB fracture behavior of Al_2O_3 will be discussed in detail. On the other hand, the properties of lithium ion battery (LIB) cathodes strongly depend on the dynamic diffusion of lithium ions during charge/discharge process. Then, direct visualization of lithium site is required to understand the mechanism of the diffusion of lithium ions. In this study, Cs corrected STEM were applied to directly observe the {010} surface, which corresponded to perpendicular to the 1-D diffusion orientation, of the olivine Li_xFePO_4. Commercially available $LiFePO_4$ singe crystals were used for all experiments. The crystals were cut perpendicular to their <010> axis and polished, and the structures of {010} surfaces before and after chemical delithiation were characterized by STEM. It was found that orientation of boundary layers at the $FePO_4/LiFePO_4$ interface gradually changed from lower index planes to higher index planes. The dynamic diffusion mechanism of the lithiation/delithiation from and to the surface will be discussed based on the observation results.

[1] S. Kondo, T. Mitsuma, N. Shibata, Y. Ikuhara, Sci. Adv., 2[11], e1501926(2016).
[2] S. Kondo, A.Ishihara, E.Tochigi, N. Shibata, Y. Ikuhara, Nature Commun., 10:2112 (2019)

MS1-05

Charging of Thin Film Phase Plates in a TEM

M. Malac[1,2], M. Hayashida[1], R.F. Egerton[2], S. Hettler[3], M. Beleggia[4]

[1]NRC-NANO and [2]Dept. Physics, U of Alberta, Edmonton, Canada,[3]INA, University of Zaragoza, Spain, [4]DTU, Copenhagen Denmark.

Irradiation of thin films by high energy electrons can result in local positive or negative charging of the films. When placed in the back focal plane of an imaging lens, such locally charged uniform thin film can act as a phase plate, referred to as hole-free phase plate [1]. The mechanisms responsible for the film charging include trapping of holes left behind by secondary electron emission and a local change in surface potential due to electron stimulated desorption of water from the film surface. We will discuss the various mechanisms, their relative importance and the parameters affecting the magnitude and shape of the phase shift the charged patch imparts on an electron passhing through the thin film. In particular, the film temperature and contaminants present on the film can have large effect on the observed phase shift and on the stability and applicatibility of a hole-free phase plate utilizing such film. When an amorphous carbon film is irradiated, the conductivity of the film itself increases with increased irradiation suggesting possible avenues to reduce charging of sample support for biological samples.

We acknowledge the ongoing support of Dr. S. Motoki, Dr. Y. Konyuba, Dr. Y. Okura, Dr. I.Ishikawa, and Dr. H. Iijima of JEOL Ltd. and Dr. Y. Taniguchi of Hitachi High Technologies Corp.

[1] M. Malac et. al., Ultramicroscopy 118 (2012), 77.

MS1-06

Gas electron holography: realisation and implementation

Jes Ærøe Hyllested[1*], G. Prabhu Sai Balasubramanian[1], E. M. Fiordaliso[1] and Marco Beleggia[1]
[1]DTU Nanolab, Technical University of Denmark, 2800 Kgs. Lyngby, Denmark

Carrying out electron holography (EH) experiments in a gaseous environment is a good idea. Gas-electron interactions promote local chemical/physical transformations of the sample, whose effects can be directly observed with the phase-sensitive eye of EH.

To establish gas-EH in an environmental TEM (ETEM), we have designed and fabricated a new type of biprism and placed it within the pressured portion of the ETEM column. We have assessed its performance with various gasses, H_2, N_2, O_2, by measuring the change in fringe spacing, visibility, and phase sensitivity as a function of pressure. Our findings reveal that gas-EH is a viable setup that opens the door to a whole new class of experiments.

We have then used gas-EH to follow the progressive oxidation of FeO nanoparticles from magnetite to maghemite to hematite, observing the expected change in their magnetic state.

In another experiment, we have deposited a carbon layer from a gas precursor on the shell of GaAs nanowire, used the beam to promote diffusion of C atoms, intended as dopants, into the intrinsic core, and observed a change in the resulting built-in potential.

The combination of EH with liquid-cell holders to measure the charge state of nanoparticles in various liquid environments, and the mean-inner potential of liquids as a function of their molecular weight and ionicity, will be also discussed based on a recent publication [1].

[1] M.N. Yesibolati et al., Phys. Rev. Lett. **in press.** (2020).

MS2-07

Towards Atomic Resolution State Analysis by STEM-EELS

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Electron energy-loss near-edge structures (ELNES) of the inner-shell electron excitation spectra reflect the partial density of states in the unoccupied band, so it can be expected to map the two-dimensional spatial distribution related to a specific electronic state using the STEM-EELS method. However, the intensity of inner-shell electron excitation spectrum is so weak that it is difficult to obtain fine structures with a high S/N ratio while maintaining high spatial resolution. In our previous studies, we have performed state analysis of transition metal oxides with layered structures using site-resolved EELS, in which Jahn-Teller distortion was detected [1] and different covalent bonding properties depending on the atomic sites were revealed [2].

Recently, we have enabled to map electronic state with atomic resolution by applying non-rigid registration technique [3] to many spectrum-image (SI) data using a template matching. Using this technique the hole mapping was performed on a high-Tc superconductor of $L_{2-x}Sr_xCuO_{4\pm\delta}$ [4]. In such a mapping, it has been essential to subtract dark current of CCD correctly [5] because the signal counts of one spectrum in a SI data were extremely low due to the short dwell time for SI acquisition to prevent radiation damage of the specimen. We will show the recent results on atomic resolution state analysis.

[1] M. Haruta et al., Phys. Rev., B**80**, 165123 (2009).
[2] M. Haruta et al., J. Appl. Phys., **114**, 083712 (2013).
[3] L. Jones et al., Adv. Struct. Chem. Imag., **1,** 8 (2015).
[4] M. Haruta et al., Phys. Rev. B**97**, 05139 (2018).
[5] M. Haruta et al., Ultramicroscopy, **207**, 11827 (2019).
Acknowledgments This work was supported by JSPS KAKENHI Grant Numbers 17H02739 and 19H02597.

MS2-08

EDS and EELS of Lithium Materials from 0.5 to 30 keV

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This paper will present state of the art results acquired with the SU-9000 dedicated 30 keV (and less) STEM that has EELS capabilities. It has a resolution of 0,22 nm in bright field STEM without aberration correctors. It is equipped with an Extreme SDD EDS detector that allows Lithium detection. With EELS and EDS, results for Li detection will be presented and the challenges, in regards of quantification and beam damage, will be covered. Examples of EELS analysis at 30 keV for nanomaterials will be presented, including surface plasmon. The SU-9000EA allows to perform electron diffraction and CBED patterns acquired at the nanoscale will be presented.

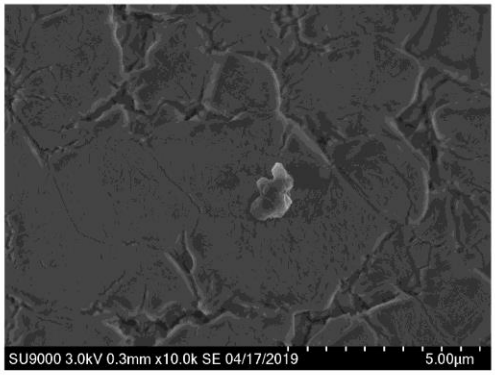

Figure 1. LiCl specimen after the acquisition of a EDS spectrum. 200 S live time.

As an example, figure 1 shows a LiCl specimen after an EDS spectrum was acquired. In order to detect the Li K line after 200 second acquisition time, the specimen had to be damaged by the beam. Understand the beam damage is critical for successful application of EDS for Li based materials.

MS2-09

Fusion of Analytical TEM/STEM and *in-situ* analysis

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[1]Hitachi High-Tech Corp., 1-17-1 Toranomon, Minato-ku, Tokyo Japan,

Hitachi probe aberration corrected CFEG analytical 200kV TEM HF5000 [1] has an open window gas injection inlet that can be chosen MEMS heating holder (Hitachi High-Tech Canada) (Fig.1(a)). Most of the specification remains the same as standard and *in-situ* configuration, so that sharp atomic resolution Pt nano particle on CeO_2 support can be observed (b). Catalyst of Pt/CeO_2 and dispersed carbon *in-situ* observation was studied, Fig.1(c). While heating and inducing O_2 gas, Pt cluseters actively move into carbon and are aggregate in CeO_2 ((d) arrow). Stable specimen heating combined with Cs-correcor, single atom resolved ADF-STEM(e) and SEM(f) images can also be observed that gives an evidence of Pt cluseters dispersion inside and on carbon under the reaction condition. Lastly this technique allows to conduct EDX and EELS under environmental condition.

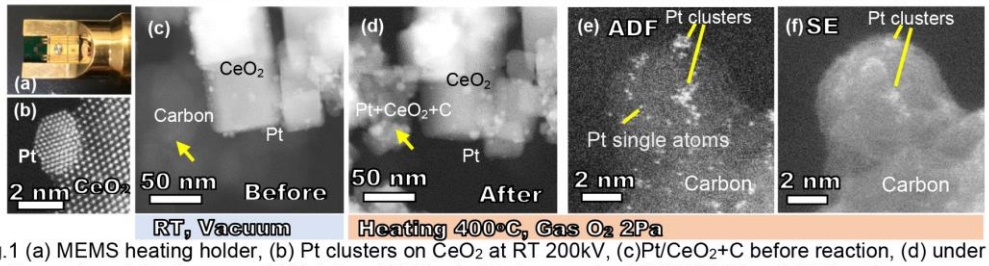

Fig.1 (a) MEMS heating holder, (b) Pt clusters on CeO_2 at RT 200kV, (c)Pt/CeO_2+C before reaction, (d) under heating and gas injection same FoV, (e) and (f) Single Pt atom resolved ADF-STEM and SEM under same condition.

[1] H Inada et al., Microsc. Microanal. **23** 918 (2017).

Acknowledgments The authors acknowledge to Prof. Xi Liu of Shanghai Jiao Tong University for providing the catalyst samples and giving his fruitful comments.

MS2-10

High-precision Phase Analysis of Automatically Collected Electron Holograms

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Atomic resolution electron holography is a powerful tool for investigating electrostatic potential of nanoparticles (including catalyst systems) by detecting phase shift of object waves. For improving the statistical precision of phase analysis, averaging numerous phase data is necessary. We developed a system that collect huge number of holograms from different places of an electron-transparent thin foil with many nanoparticles dispersed on it [1].

We analyzed mean inner potential (MIP) of gold nano-particles on carbon thin foil, which was collected in an 1.2 MV holography TEM. For example, the precision of MIP for one particle was 3.9 V, while it was only 0.45 V by averaging 40 different nanoparticles. As a result, it was clearly shown that MIP decreased gradually with decreasing particle size down to 4 nm, whereas it increased again in the range below 4 nm.

On the other hand, by averaging 10 phase images from the same particle (Fig. 1(a)), it was suggested that the surface was subjected to a significant compressive stress and anomalous decrease in phase (Fig. 1(b)) was observed at the grain boundary, which were probable causes of the size dependency of MIP.

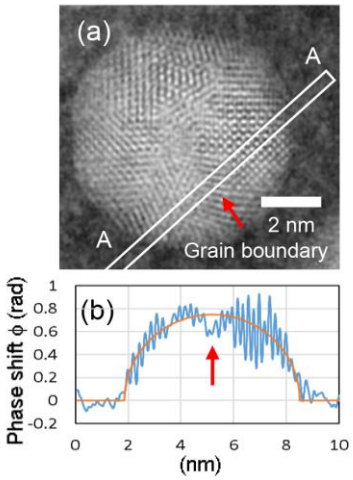

Fig.1. (a) Phase image of Au particle (b) Line profile along A-A.

[1] Y. Takahashi et al., Microscopy (2020), DOI: 10.1093/jmicro/dfaa004.

Acknowledgments This work was supported by JST CREST Grant Number JPMJCR 1664, Japan.

MS2-11

Combination of Fluctuation Electron Microscopy and Ptychography for Characterization of Amorphous – Crystalline Mixtures

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Ptychographic algorithms reconstruct a model of the phase shift imparted on to an electron beam as it passes through a sample from series of diffraction-type patterns obtained by illuminating selected overlapping regions of the sample. This allows high-resolution reconstructions to be made on suitable sample materials. Further image resolution enhancement is possible by extracting and modelling the amorphous backgrounds [1]. In some material mixtures, the medium and long-range order of this nominally amorphous background is of interest. This order influences the structural and electronic material properties and can be characterized by fluctuation electron microscopy (FEM), where the variance of diffraction patterns from small area illumination is studied [2].

Illustration is made of combining the two techniques based on examples of 'amorphous' metal – nanoparticle systems (e.g. Fig.1). Processing the diffraction data in these complementary manners reveals high resolution information from the particles and medium range order information on the amorphous material, which is not readily obvious in reconstructed images. This technique is being directed towards electronic device and polymer material applications.

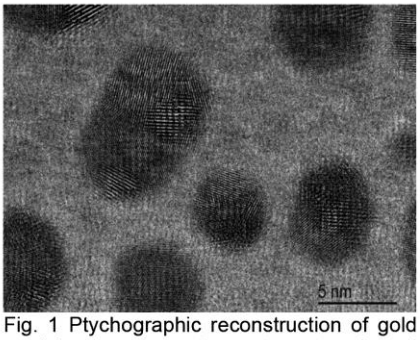

Fig. 1 Ptychographic reconstruction of gold particles on amorphous tungsten based support material imaged at 60 keV, with FEM applied to the support.

[1] Maiden, A. M., et al., Scientific Reports **5**, 14690, (2015)
[2] Voyles, P. M. et al., Ultramicroscopy **93**, 147 (2002).

MS2-12

Thermal Stability and Microstructures of the $LiNi_{1/3}Mn_{1/3}Co_{1/3}O_2$ Positive Electrode for Sulfide-based All-solid-state Lithium Batteries

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The positive electrodes comprising of Li_3PS_4 (LPS) glasses and $LiNi_{1/3}Mn_{1/3}Co_{1/3}O_2$ (NMC) is a promising candidate for all-solid-state lithium batteries owing to excellent charge–discharge cycle characteristics [1]. However, sulfide-based electrolytes have a major drawback of low chemical stability to moisture in air, which affects process cost and thermal stability of all-solid-state batteries [2]. Thus, Li_4SnS_4 (LSS), exhibiting high moisture resistance, could be alternative to LPS [3]. In this study, we investigated thermal stability in LPS–NMC and LSS–NMC composites mainly by DSC measurements and TEM observations.

Figure 1 shows the hollow-cone dark filed (HCDF) image and electron diffraction (ED) pattern for the LPS–NMC composites after heating at 500 °C. In the ED pattern, Debye-Scherrer rings of Li_3PO_4, $CoNi_2S_4$ and MnS are detected. The HCDF image shows these crystalline phases are uniformly distributed as a nanocrystellite, as indicated by the arrows. These suggest LPS and NMC thermally react and decompose by heating. In this presentation, thermal stability of LSS-NMC is also discussed based on the results of LPS-NMC.

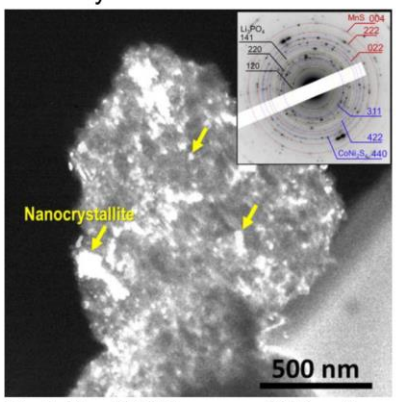

Fig 1. The HCDF image and ED pattern of LPS–NMC composites after heating at 500 °C.

[1] K. Okada et al., Solid State Ionics **255**, 120 (2014).
[2] H. Tsukasaki et al., J. Power Sources **434**, 226714 (2019).
[3] K. Kanazawa et al., Inorg. Chem. **57**, 9925 (2018).

MS2-13

Crystallization differences of Al_2O_3 on GaN planes

Emi Kano[1*], Kazutaka Mitsuishi[1], Yoshihiro Irokawa[1], Toshihide Nabatame[1], Koji Kimoto[1], Tetsu Kachi[2] and Yasuo Koide[1]

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GaN is a promising material for high-power and high frequency devices. Al_2O_3 has a large bandgap and is attractive for gate-structure application with GaN. However, the structure and properties of Al_2O_3/GaN have not been well understood yet. We found that Al_2O_3 layer on GaN (0001) plane crystallized in a transmission electron microscope (TEM). Crystallization of Al_2O_3 can change the device properties [1], which led us to investigate the Al_2O_3 deposited on different GaN planes to control the crystallization.

The Al_2O_3 layer was deposited on three different planes of GaN substrate. As shown in Fig. 1, amorphous Al_2O_3 on GaN (0001) plane started to grow to crystalline γ-phase from Al_2O_3/GaN interface in irradiating the electron beam, while the crystallization of Al_2O_3 on the other GaN planes was suppressed. Al_2O_3 on one GaN plane crystallized only when the focused electron beam was irradiated, while Al_2O_3 on the latter GaN did not crystallize even with focused electron beam irradiation.

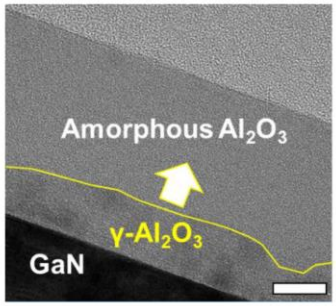

Fig. 1 Cross-sectional TEM image of Al_2O_3 on GaN (0001) plane. Al_2O_3 crystallized from interface between GaN and formed γ-Al_2O_3 structure. Scale bar: 10 nm.

[1] Y. Hori et al., Jpn. J. Appl. Phys. **49**, 080201 (2010).

Acknowledgments This research was supported by Ministry of Education, Culture, Sportsm Science and Technology (MEXT), Japan, through its "Program for research and development of next-generation semiconductor to realize energy-saving society" and "Nanotechnology Platform Project." The authors acknowledge Dr. K. Ito, D. Kikuta and T. Narita in Toyota Central R&D Labs., Inc. for their technical support.

MS2-14

Higher-order structure of human chromosomes observed by electron tomography and electron diffraction

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[1] NRC-NANO, National Research Council, Edmonton, Alberta, Canada, [2] Graduate School of Human development and Environment, Kobe University, Kobe, Hyogo, Japan, [3] RIKEN, Hatoyama, Saitama, Japan, [4] HITACHI, Ltd., Hatoyama Saitama, Japan, [5] Graduate School of Pharmaceutical Science, Osaka University, Suita, Osaka, Japan

The DNA molecule is packaged tightly in chromosomes. The higher order structure is still an enigma although many researchers have studied for over a century. Here we observed an isolated chromosome stained with osmium using electron tomography with 1 MV acceleration voltage. 150-200 nm periodicity perpendicular to a chromosome axis was observed in the tomogram. Both 120-200 nm structures and 30 nm periodicities perpendicular to a chromosome axis were also observed by electron diffraction from unstained chromosomes on a 300 kV TEM. Based on the results, we suggested a new model of chromosome structure in this study.

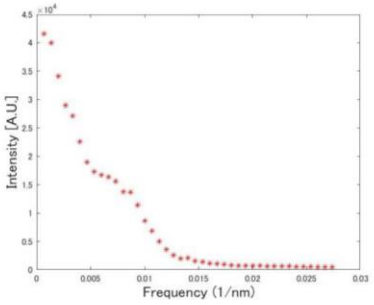

Fig. 1 Line profile from a diffraction pattern in the direction of chromosome axis.

This work was supported by Japan Science and Technology Agency (JST) SICORP (17935614, 2017 to 2020).

Biological Science Session

(Chair: Yasushi Okada and Elitza Tocheva)

BS-01

Engineering Genetically Encoded Biosensors of Neural Activity and Metabolism

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The Campbell research group is focussed on the use of protein engineering for the development of fluorescent protein-based biosensors for imaging of cell signalling and metabolism. Protein engineering, using a combination of rational protein design and directed protein evolution, is the most effective and versatile approach for generating new genetically encoded fluorescent biosensors. By exploiting structure-guided design, combined with iterative cycles of high-throughput fluorescence image-based screening of bacterial colonies, and lower throughput testing of promising variants in mammalian cells, we are developing a growing selection of fluorescent protein-based biosensors with improved properties. In this seminar I will present some of our most recent efforts to engineer an improved generation of biosensors. Specifically, I will provide an update on the expanding palette of calcium ion biosensors, and describe how we are using similar engineering efforts to make biosensors for neurotransmitters, ions, and key metabolites.

BS-02

Correlative Light and Electron Microscopy (CLEM) for trace of climbing fiber

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CLEM (Correlative light & electron microscopy) images the same position of a sample by light and electron microscopy. Optical microscopy (OM) identifies the specific positions of interest with fluorescent markers, and electron microscopy (EM) provides the fine structures of the positions. In this presentation, we show practical method of CLEM, which visualized fine structure of a climbing fiber in a mouse cerebellar cortex.

The sample was fixed and thick slices of it was imaged using confocal microscopy (OM). Then the slices were further fixed with OsO_4, dehydrated, and embedded in epoxy resin. The imaged position by OM was trimmed, and serial sections of 45 nm thick was cut using an ultramicrotome. The sections were further imaged using array tomography [1] by a scanning EM (SEM) and analyzed including deep learning [2].

Three dimensionally (3D) reconstructed climbing fiber in a mouse cerebellar cortex is displayed with dendrites of Purkinje cells (white) in Fig. 1. This is one connecting fiber while the different colors show branches for clarity. Details of CLEM workflow will be explained in the presentation.

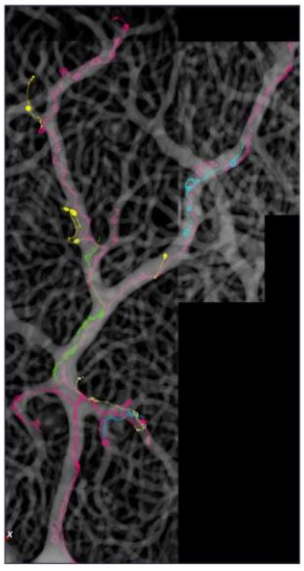

Fig. 1 Three dimensionally reconstructed climbing fiber and dendrites of Purkinje cells.

[1] Micheva and Smith. Neuron 55, 25-36 (2007)
[2] Konishi et al., Microscopy 68, 338-341 (2019).

BS-03

Single-molecule nanoscopy by using cryogenic fluorescence microscopy

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Department of Physics, Tokyo Institute of Technology

Among microscopies, far-field fluorescence microscopy is a unique method to noninvasively image individual molecules in whole cells. If the three-dimensional spatial precision is improved to the angstrom level, various molecular arrangements in the cell can be visualized on an individual basis. We have developed a cryogenic reflecting microscope with a 0.99-numerical aperture and a 0.05-nm imaging stability at a temperature of 1.8 K. With this cryogenic microscope, an individual ATTO647N molecule at 1.8 K was localized with standard errors of 0.53 nm (x), 0.31 nm (y), and 0.90 nm (z).[1] Towards nanoscopy of multiple biomolecules in cell, we demonstrate the cryogenic far-field microscopy of 1.8 K using near-infrared and red fluorophores bonded to double-stranded DNA molecules (10.2 nm length).[2] Although each fluorophore was localized with a 1 nm lateral precision by acquiring an image at one axial position within the focal depth of ±0.7 μm, the distance between the two fluorophores on the lateral plane (D_{XY}) was distributed from 0 to 50 nm. This systematic error was mainly due to detecting with the large focal depth the dipole emission from orientationally fixed fluorophores. Each fluorophore was localized with precisions of ±1 nm (lateral) and simultaneously ±11 nm (axial) by acquiring images every 100 nm in the axial direction from −900 to 900 nm. By correcting the dipole orientation effects, the distribution of D_{XY} was centered around the DNA length with the standard deviation of 5 nm.

[1] T. Furubayashi et al., J. Am. Chem. Soc. **139**, 8990-8994 (2017).
[1] T. Furubayashi et al., J. Phys. Chem. Lett. **10**, 5841-5846 (2019).

BS-04

Correlation of Cryo Super-Resolution and Cryo-Electron Tomography in Bacteria

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[1]Department of Microbiology and Immunology, 2350 Health Sciences Mall, Vancouver, BC, V6T 1Z3, Canada

For decades bacteria were thought of as "bags" of enzymes, in contrast to eukaryotes where intracellular compartmentalization and establishment of large-scale order has been known for a long time. Today we know that bacterial cytoskeletal proteins polymerize into surprisingly diverse superstructures [2]. By bridging the scales between individual diffusing proteins, macromolecular assemblies, and even between two neighboring cells, these superstructures evolved to act in essential cellular processes. Most of the open questions in Bacterial Cell Biology would benefit from resolving the *in vivo* dynamics and superstructure of different functional stages, at macromolecular resolution and in 3-dimensions. While light microscopy (LM) of fluorescently tagged mutants is highly valuable for the investigation of protein localization and dynamics, the resolution power of electron microscopy (EM) is required to elucidate the structure-function relationships of macromolecular complexes *in vivo* and *in vitro*. Therefore, we combine the strengths of LM and EM under cryogenic conditions to characterize the mechanism of secretion in bacteria. To overcome the diffraction limit of standard LM (~250nm), we implement super-resolution LM approaches and combine them with cryo-ET [1].

[1] Chang, Y. W., Chen, S., Tocheva, E. I., et al. *Nat Methods, 11*(7), 737-739. (2014)
[2] Pilhofer, M., & Jensen, G. J. *Curr Opin Cell Biol, 25*(1), 125-133. (2013)

BS-05

Near-atomic resolution structures of the doublet microtubules by cryo-EM

Muneyoshi Ichikawa[1,2], Ahmad Khalifa[1], Shintaroh Kubo[3], Daniel Dai[1], Kaustuv Basu[4], Amin Maghrebi[1], Javier Vargas[1] and Khanh Huy Bui[1*]
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[2]Department of Systems Biology, Graduate School of Biological Sciences, Nara Institute of Science and Technology, Ikoma, Nara 630-0192, Japan,
[3]Department of Biophysics, Graduate School of Science, Kyoto University, 606-8502 Kyoto, Japan,
[4]Facility for Electron Microscopy Research, McGill University, Montréal, QC H3A 0C7, Canada

Cilia and flagella are important organelles of eukaryotes which generate fluid flow around the cells. Cilia and flagella share same axonemal 9+2 structure where nine doublet microtubules are bundled. The doublet microtubules are much more stable compared with singlet microtubules within the cytoplasm. The doublet microtubules were also known to contain numerous protein structures inside its tubulin lattice, termed as microtubule inner proteins (MIPs). Previously, we have reported a subnanometer-resolution structure of the doublet microtubule purified from ciliate *Tetrahymena* [1]. However, the functions and identities of MIPs were yet to be elucidated. Here, we obtained near-atomic resolution structures of the doublet microtubules purified from *Tetrahymena* cilia and *Chlamydomonas* flagella by cryo-electron microscopy. Our near-atomic resolution structures of doublets provided insights into functions and identities of the MIPs.

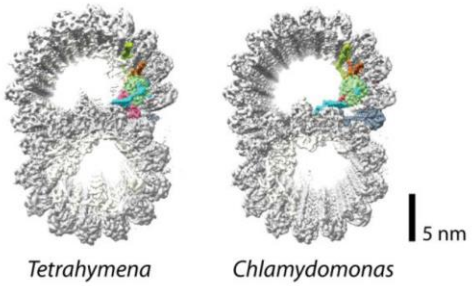

Fig 1. cryo-EM structures of the doublet microtubules purified from cilia and flagella.

[1] Muneyoshi Ichikawa et al., Nature Communications, **8**, 15035 (2017)

BS-06

Development and Applications of a New CryoTEM, JEOL CRYO ARM

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JEOL recently produced an electron cryomicroscope (cryoTEM) named CRYO ARM upon our request in 2010 to develop a high-resolution, high-throughput, user-friendly cryoTEM for structural biology. We used a prototype CRYO ARM 200 completed in early 2017 with a thermal FEG operated at 200 kV and a Gatan K2 camera and obtained a 2.41 Å resolution 3D map of β-galactosidase from 98,158 particle images collected over 3 days. We were also be able to visualize the structures of supercoiled flagellar hook of *Salmonella* and human cardiac muscle thin filament in the Ca^{2+} ON and OFF states, which revealed the molecular mechanism of heart beat. Then, by using CRYO ARM 300, the first commercial version installed at RIKEN SPring-8 Center, we achieved 1.53 Å resolution in mouse apoferritin structure from 120,295 particle images extracted only from 840 images collected over one day. The map shows features of truly atomic resolution (Fig. 1). It is worthy of note that merely the first 56 images of the full data set produced a 1.76 Å resolution map. These results suggest that the cold FEG is quite effective in enhancing high resolution image signal, and a stable cold FEG, such as the one used in CRYO ARM, will be a powerful tool for atomic resolution structural analysis biological macromolecules by single particle image analysis.

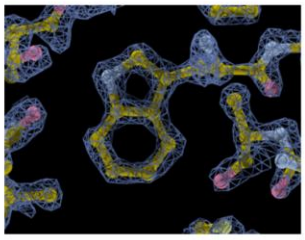

Fig 1. 3D map of a tryptophan in mouse apoferritin at 1.53 Å resolution, showing clear holes in its five- and six-membered rings.

BS-07

Cryo-ED and EM for higher-resolution and higher-precision structure analysis

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We have developed and investigated a new cryo-EM system based on JEOL CRYO ARM 300 for electron 3D crystallography and higher-resolution single particle analysis [1][2].

Diffraction data quality is remarkably improved with electron energy-filtered diffraction (eEFD) and 300 kV electrons. We examined thin 3D crystals of protein complexes, polypeptides and organic molecules whose structures have not been determined.

For single particle cryo-EM, the system provides parallel illumination of an extremely-coherent electron beam from a cold-field emission gun, and boosts image contrast with an in-column energy filter. These features are highly suitable for improving the resolution in single particle reconstructions and decreasing the number of molecular images needed and the absolute value of the estimated B-factor for map sharpening. To facilitate its use, we also developed GUI programs for efficient operation and accurate structure analysis.

In this symposium, we report on the performance of the system for both the techniques.

[1] K. Yonekura, T. Ishikawa, and S. Maki-Yonekura,, *J. Structl. Biol.* **206**, 243-253 (2019).
[2] T. Hamaguchi, S. Maki-Yonekura, H. Naitow, Y. Matsuura, T. Ishikawa, and K. Yonekura, *J. Struct. Biol.* **207**, 40-48 (2019)..

【4. Organizers】

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Yasushi Okada (RIKEN, University of Tokyo)

岡田康志　（理化学研究所、東京大学）

Ken Harada (RIKEN)

原田　研　（理化学研究所）

Marek Malac (National Research Council Canada, University of Alberta)

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【6. Planned Venue Information】

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