



KEK×CRYO-EM ACTIVITY REPORT

VOL.8

April 2025 - March 2026

Cryo-Electron Microscope

KEK Titan Krios

Instrument Name: Titan Krios G4

Acceleration Voltage: 300 kV

Electron Source: XFEG

Stage: AutoGrid-compatible side-entry stage

Phase Plate: Volta Phase Plate

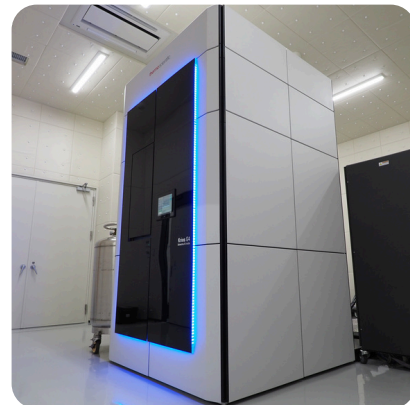
Energy Filter: Selectris-X

Detector 1: Falcon 4i

(*130k = 0.96 Å/px, 165k = 0.75 Å/px, 215k = 0.58 Å/px, 270k = 0.46 Å/px)

Detector 2: Ceta-D

Software: EPU, EPU-D



KEK Talos Arctica

Instrument Name: Talos Arctica G2

Acceleration Voltage: 200 kV

Electron Source: XFEG

Stage: AutoGrid-compatible side-entry stage

Phase Plate: Volta Phase Plate

Former Detector: Falcon 3EC

(*92k = 1.13 Å/px, 120k = 0.88 Å/px, 150k = 0.69 Å/px)

Detector 1: Falcon 4

(*92k = 1.08 Å/px, 120k = 0.84 Å/px, 150k = 0.66 Å/px)

Detector 2: Ceta 16M

Software: EPU, EPU-D



KEK Tundra

Instrument Name: Tundra Cryo-TEM

Acceleration Voltage: 100 kV

Electron Source: XFEG

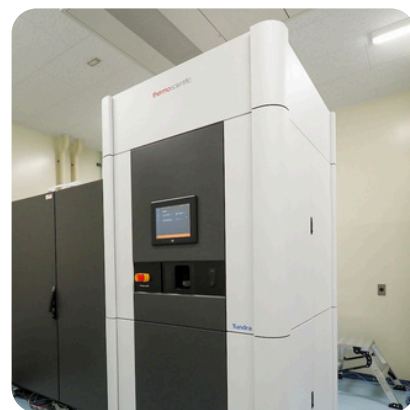
Stage: Computerized specimen cryo-stage

Detector: FalconC

(*120k = 1.9 Å/px, 140k = 1.4 Å/px, 230k = 1.2 Å/px)

Software: EPU

Equipments: Tundra Loading Workstation and Transfer Device



Grid Freezing Device

Model: Vitrobot Mark IV

Glow Discharge Unit

Model: PIB-10

Current: soft mode = ~7mA, hard mode = ~11mA

KEK Cryo-EM Facility Operations

The KEK Cryo-Electron Microscopy Facility operates a Thermo Fisher Scientific 300 kV cryo-EM Titan Krios G4, a 200 kV cryo-EM Talos Arctica G2, and a 100 kV cryo-EM Tundra.

The facility was enhanced in 2025 with the addition of the Thermo Fisher Scientific Tundra system.

1. Provide over 200 days of machine time annually to academic and corporate users.
2. Support grid freezing and data acquisition (including single-particle analysis when needed).
3. Support the adoption of cryo-EM techniques.

The following schedules apply to the Talos Arctica G2 and Titan Krios G4.



Weekly Schedule

Monday: Maintenance

Tuesday: 1-day slot (screening and data collection)

Wednesday: 1-day slot (screening and data collection)

Thursday: 1-day slot (screening and data collection)

Friday–Sunday:

3-day slot (screening and data collection)



Daily Schedule

9:30 - Meeting

Morning - Grid freezing (up to 6 grids)

Afternoon - Screening measurement (approx. 1 hour per grid)

Around 18:00 - Start of overnight or weekend measurements

The next day, KEK Staff will copy the data and send it to the user.



What to Bring

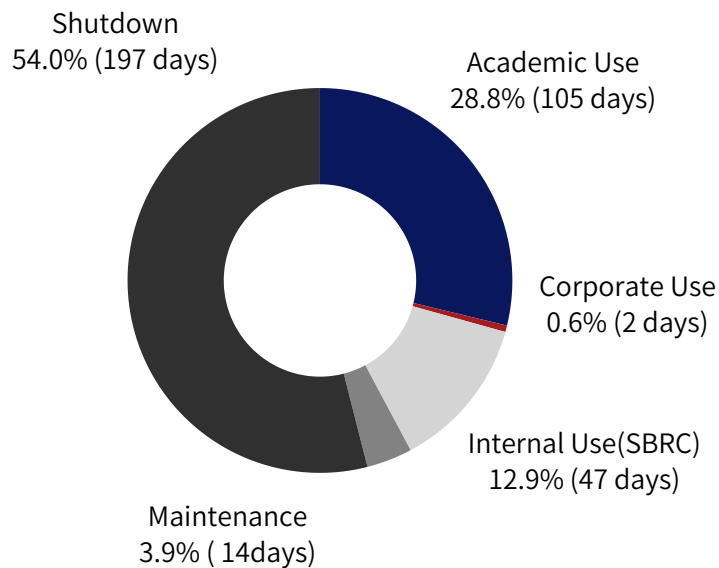
Samples

Dilution buffer

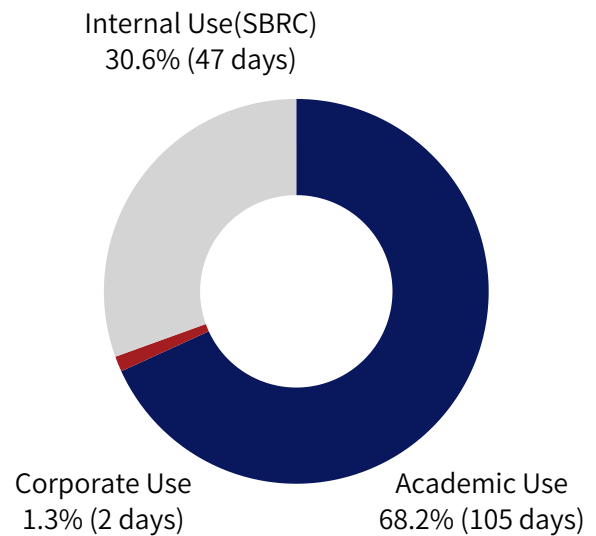
4–8 TB HDD

KEK Cryo-EM Usage Statistics

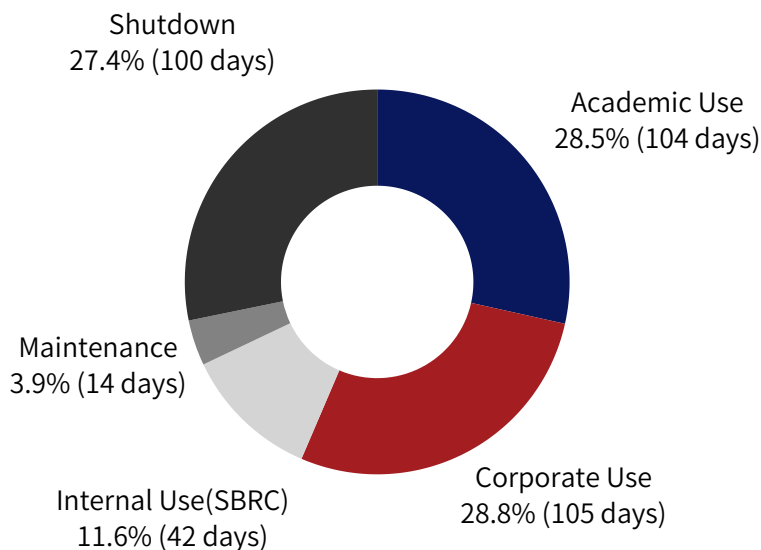
**Talos Equipment Usage Status
2025 (365 days)**



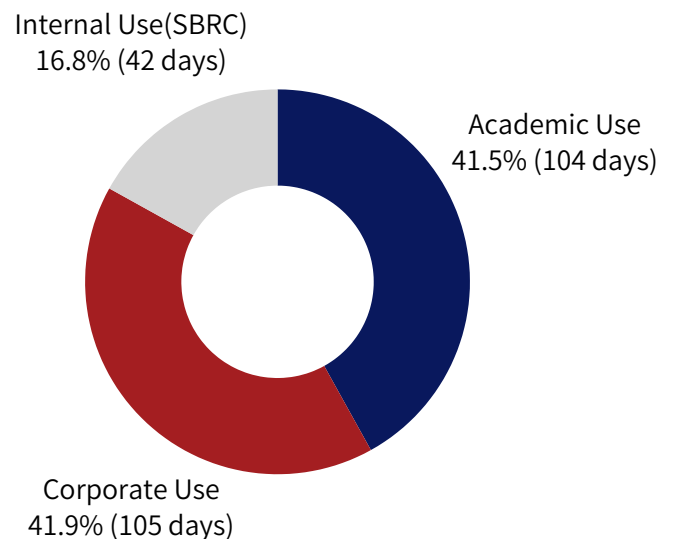
**Talos Equipment Usage Status
(only operating days)
2025 (154 days)**



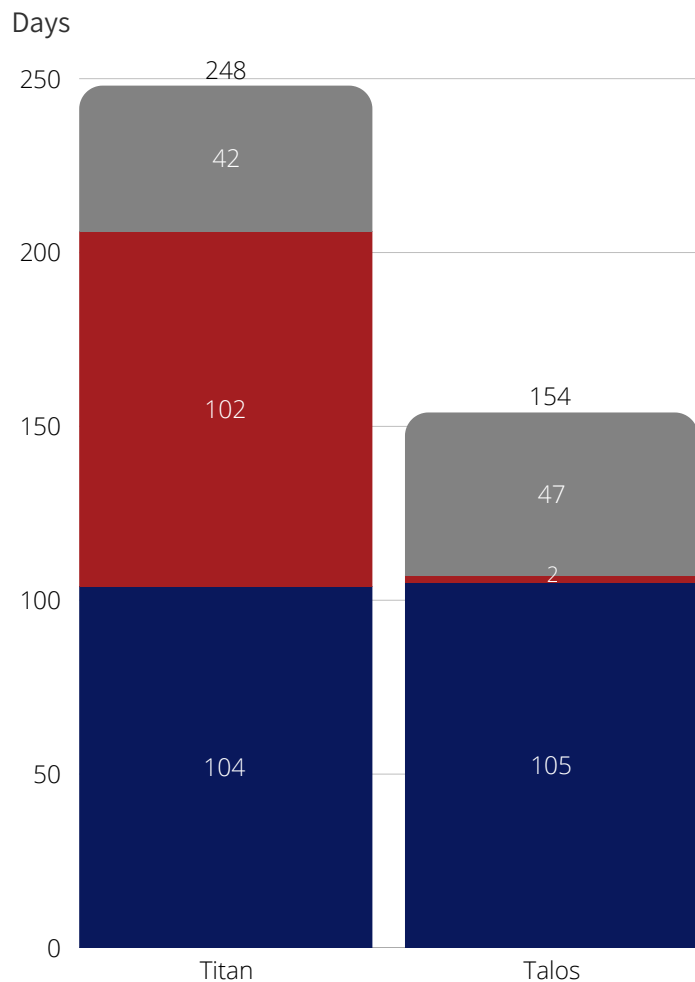
**Titan Equipment Usage Status
2025 (365 days)**



**Titan Equipment Usage Status
(only operating days)
2025 (248 days)**



Titan and Talos Machine Time Allocation (FY2025)



● Academia ● Company ● Internal Use(SBRC)

Note: Figures include data from Vol. 1-7 for annual calculation.
Note: Titan Krios is not included in the FY2022 data as it was under test operation.

Structural Biology Research Center
Institute of Materials Structure Science
High Energy Accelerator Research Organization (KEK)

Installation of a 100 kV Tundra Cryo-TEM and Launch of a Beginner Training Course in Cryo-EM

The Structural Biology Research Center (SBRC) has introduced a 100 kV cryo-transmission electron microscope, the Tundra cryo-TEM (Thermo Fisher Scientific; Figure 1), with support from AMED BINDS (Basis for Supporting Innovative Drug Discovery and Life Science Research program). This addition aims to further expand structural analysis support using cryo-electron microscopy (cryo-EM) and enhance educational opportunities for future users.

SBRC already operates a 300 kV Krios G4 and a 200 kV Talos Arctica G2, primarily to provide microscope time to external users and support image data acquisition and structural analysis—particularly single-particle analysis (SPA)—for many researchers. However, for researchers and students wishing to start using cryo-EM, providing an environment suitable for systematic education and training across the entire workflow—from sample preparation to SPA—is essential. Therefore, the Tundra cryo-TEM was introduced to serve as a new foundation for fostering future cryo-EM users, specifically designated for beginner training, grid evaluation, and basic data acquisition.

Preparation and Installation Process

Preparations began in earnest around February 2025, starting with the technical specifications based on the planned instrument's configuration and requirements. Introducing a large-scale electron microscope requires comprehensive preparation, including contract procedures, technical specifications, installation requirements, construction requests, and schedule coordination. Using the installation process and specifications of the Krios G4 introduced in FY2022 as useful references, we organized the required instrument configuration and installation conditions through discussions with KEK personnel and completed the technical specifications in April 2025 to proceed with the procurement process.

In parallel, we considered the installation site. Stable cryo-EM operation requires minimizing temperature fluctuations, environmental magnetic fields, vibration, and noise. We also accounted for equipment transport routes, the relocation of existing equipment, and user flow. Ultimately, we decided to renovate the former yeast culture room in the cryo-EM experimental building into a new microscope room, EM Room 3.

During FY2025, preparations in EM Room 3 progressed. Floor load capacity, the power supply system, air-conditioning, and the water-cooled chiller installation were carefully addressed. Although the microscope body weighs approximately one ton, floor verification confirmed that the equipment could be installed without additional reinforcement. The power supply system underwent modification work to meet the specific requirements of the microscope and peripheral equipment, without needing a major increase in existing electrical capacity.

Selecting the chiller's site required careful coordination between indoor noise/heat concerns and outdoor piping distance/cooling performance trade-offs. Ultimately, we optimized the installation site and piping route, resolving cooling performance concerns by placing the chiller outdoors. Additionally, because EM Room 3 is located within a regulated genetically modified organism experiment area, we considered creating a new entrance in the corridor-side wall for smoother visitor access. However, this plan was ultimately abandoned because of constraints related to the chiller piping route and the limited space available for the operation desk. These considerations highlight that the installation of a cryo-TEM required not only the placement of the microscope itself, but also careful coordination of the building structure, peripheral facilities, transport routes, and user access.



Following the bidding process from August to September 2025, the procurement of the Tundra cryo-TEM was officially confirmed. Existing equipment relocation and installation work proceeded, with basic room preparation completed by late October 2025. At the end of November, the full equipment set was delivered by two 2-ton trucks, and SBRC members gathered to observe the delivery. The microscope body and electron gun were assembled over the next several days, and beam alignment and performance evaluation were completed by the end of the year. The system was accepted and prepared for full-scale operation on January 31, 2026.

Workflow Characteristics and Course Design

From late December to early January, initial training was provided for the operations team and SBRC members new to cryo-EM, focusing on the basic operations and workflow of the Tundra cryo-TEM. Through hands-on experience, we found that the Tundra cryo-TEM requires an operational workflow that differs significantly from our existing Krios G4 and Talos Arctica G2 systems.

While the Krios G4 and Talos Arctica G2 feature autoloaders that automatically transfer multiple grids, the Tundra cryo-TEM employs a transfer device similar to a conventional side-entry holder, requiring grids to be manually inserted and removed one at a time. The system also includes a dedicated station for loading liquid-nitrogen-cooled grids into the transfer device. Consequently, many steps—from handling grids to loading them into the microscope and removing them after data collection—require particular care. Users must become familiar with these distinctive procedures, making this hands-on, manual workflow highly valuable for beginners to learn the fundamental mechanics of grid handling.

Based on these characteristics, we began designing a beginner training course in February 2026. Rather than just teaching microscope operation, the course aims to help participants understand the complete cryo-EM experimental workflow and how each operation connects to data acquisition and analysis. The course is structured as an intensive two-week program limited to one or two participants per cycle to maximize hands-on learning. This format allows sufficient time for participants to experience both data acquisition and downstream analysis, rather than learning each step only superficially. We also established a framework to provide accommodation support, making it easier for participants from distant institutions to attend.

To support the course, we developed a new training manual (Figure 2A). Using standard samples, we repeatedly tested the full workflow—grid preparation, loading grids into the transfer device, inserting the transfer device into the microscope, setting imaging conditions, and acquiring image data—to identify critical operational points and common difficulties for beginners. We then conducted demonstration training for SBRC members using the draft manual to evaluate the flow of explanations, clarity of the operating procedures, safety considerations, and time allocation. Based on these insights, we refined the structure and wording of the manual. We also prepared an English version to accommodate international trainees.

Launch, Training Content, and Future Outlook

SBRC began accepting applications from external users in May 2026. The training course now operates as a “Cryo-EM internship,” offering an intensive, immersive two-week program with accommodation support tailored specifically for students and early-career researchers. As of July 2026, one participant has already completed the course, and five additional applicants have been confirmed.

This comprehensive training course covers the entire practical workflow, rather than just a limited segment of the experiment (Figure 2B).

It includes:

- Grid preparation and handling
- Microscope operation
- Image data acquisition
- SPA using the acquired dataset
- Model building (to be introduced)

Unlike the annual Cryo-EM beginner course regularly organized by SBRC, the SPA component of this internship allows participants to work directly with datasets they actually acquired using the Tundra cryo-TEM. They perform automated processing using CS-schemes, an SPA workflow developed at SBRC, followed by manual analysis. This dual approach allows trainees to gain a comprehensive overview of the analysis workflow, understand the purpose of each processing step, and learn how to evaluate results. Through this process, trainees acquire the basic judgment skills needed to handle their own datasets in the future.

The first participant provided positive feedback on the training (Figure 2C). The introduction of the Tundra cryo-TEM represents more than just a new instrument at SBRC; it establishes a vital foundation for providing practical learning opportunities to researchers and students entering the field of cryo-EM. Moving forward, we will continue to recruit participants, refine the manual and course content based on feedback, and further develop this framework to foster future cryo-EM users.

Acknowledgements

We would like to thank Dr. Raymond Burton of the National Institute for Physiological Sciences for his support in the pixel size calibration of the Tundra cryo-TEM.

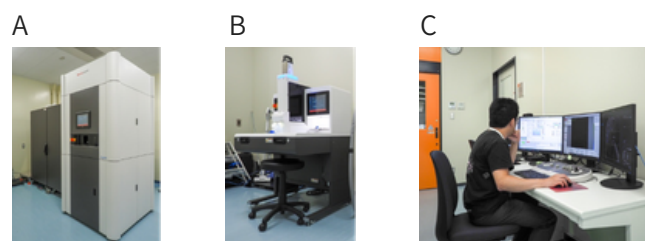


Figure 1.
Tundra cryo-TEM and related equipment

[A] The Tundra cryo-TEM measures 1.1 m (W) × 1.2 m (L) × 2.3 m (H).

[B] A grid is loaded into the transfer device at the loading station and the transfer device is manually inserted into the microscope column through the front loading port of the TEM.

[C] All procedures are monitored and controlled from the control workstation. The photo shows the author operating the Tundra cryo-TEM.

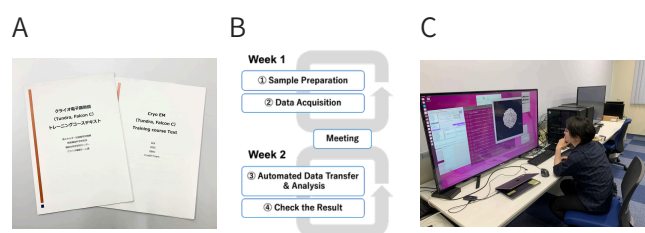


Figure 2.
Training manuals and workflow of the Cryo-EM internship

[A] Training manuals. Japanese and English manuals were prepared.

[B] Two-week workflow of the Cryo-EM internship.

[C] The first participant of the internship program learning SPA using a newly installed analysis workstation.

Yuki Fujii

Researcher



Structural Biology Research Center,
Institute of Materials Structure Science,
High Energy Accelerator Research Organization (KEK)

Cryo-EM Usage Fees

Titan Krios

Academic

Equipment Use: ¥3,000/hour* (Usage unit: 9 or 24 hours/day)

Measurement and Analysis Assistance/Guidance: Free of charge

(装置利用：3,000円/時間＊ (利用単位は9時間/日または24時間/日))

測定解析補助：無料)

Corporate

Equipment Use: ¥15,000/hour (Usage unit: 24 hours/day)

Measurement and Analysis Assistance/Guidance: ¥30,000/hour (Usage unit: 8 hours/day)

(装置利用：15,000円/時間 (利用単位は24時間/日))

測定解析補助：30,000円/時間 (利用単位は8時間/日))

Talos Arctica

Academic

Equipment Use: ¥2,000/hour* (Usage unit: 9 or 24 hours/day)

Measurement and Analysis Assistance/Guidance: Free

(装置利用：2,000円/時間＊ (利用単位は9時間/日または24時間/日))

測定解析補助：無料)

Corporate

Equipment Use: ¥10,000/hour (Usage unit: 24 hours/day)

Measurement and Analysis Assistance/Guidance: ¥30,000/hour (Usage unit: 8 hours/day)

(装置利用：10,000円/時間 (利用単位は24時間/日))

測定解析補助：30,000円/時間 (利用単位は8時間/日))

Optional

Initial Analysis (optional) : ¥125,000 x number of days

初期解析 : 125,000円 x 日数

Detailed Analysis (optional) : ¥250,000 x number of days

詳細解析 : 250,000円 x 日数

*The amount is equivalent to the actual cost of consumables (grids, C-clip, C-clip rings, filter paper, liquid nitrogen, HDD, etc.). We sincerely appreciate the cooperation of our users.

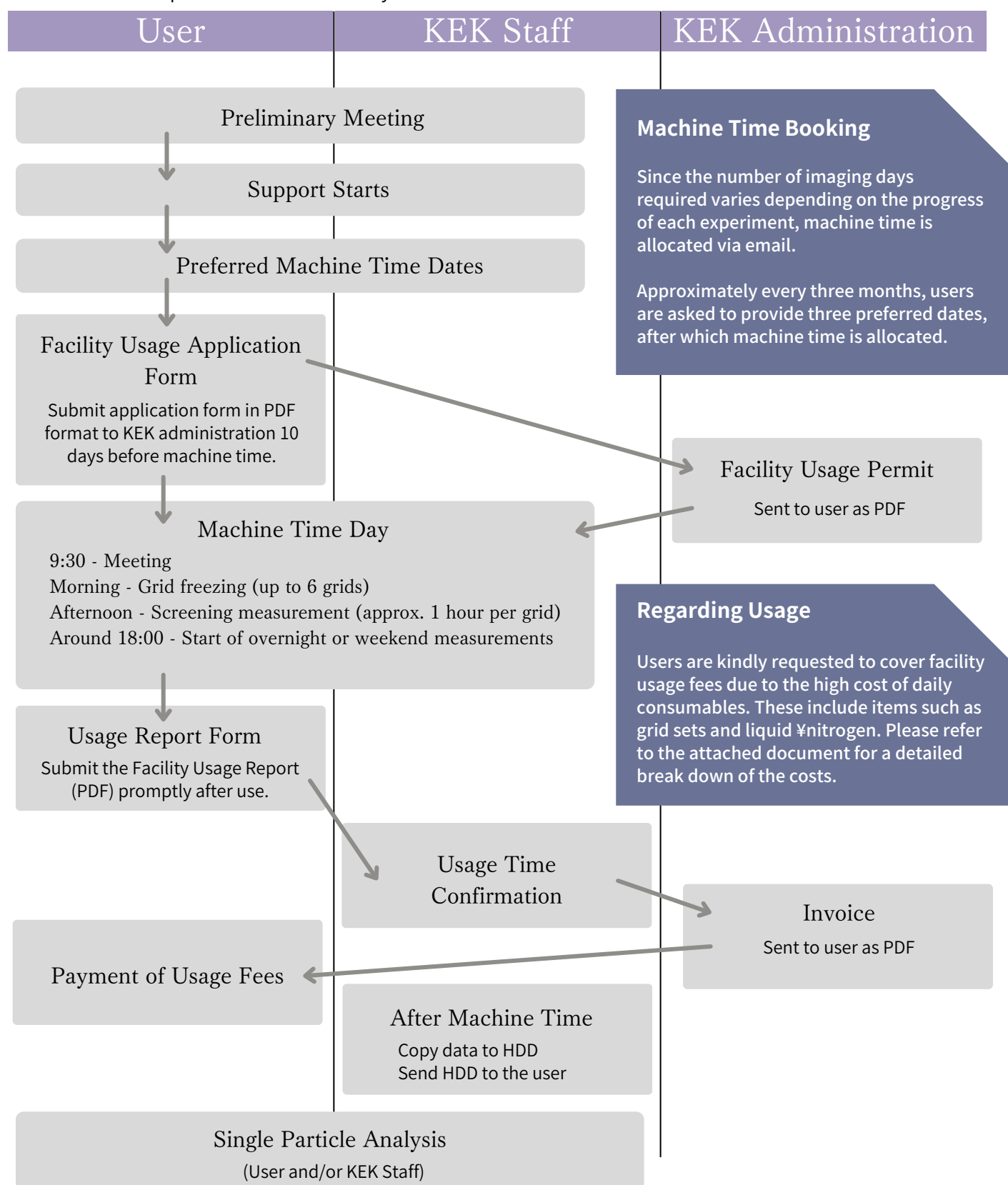
*消耗品実費相当の金額となっています (グリッド・C-clip・C-clipリング・ろ紙・液体窒素・HDDなど)。ユーザーの皆様のご協力に心より感謝申し上げます。

KEK Cryo-EM User Workflow

Because the number of imaging days required varies depending on the progress of each experiment, machine time is allocated via email.

Approximately every three months, users are asked to provide three preferred dates, after which machine time is allocated.

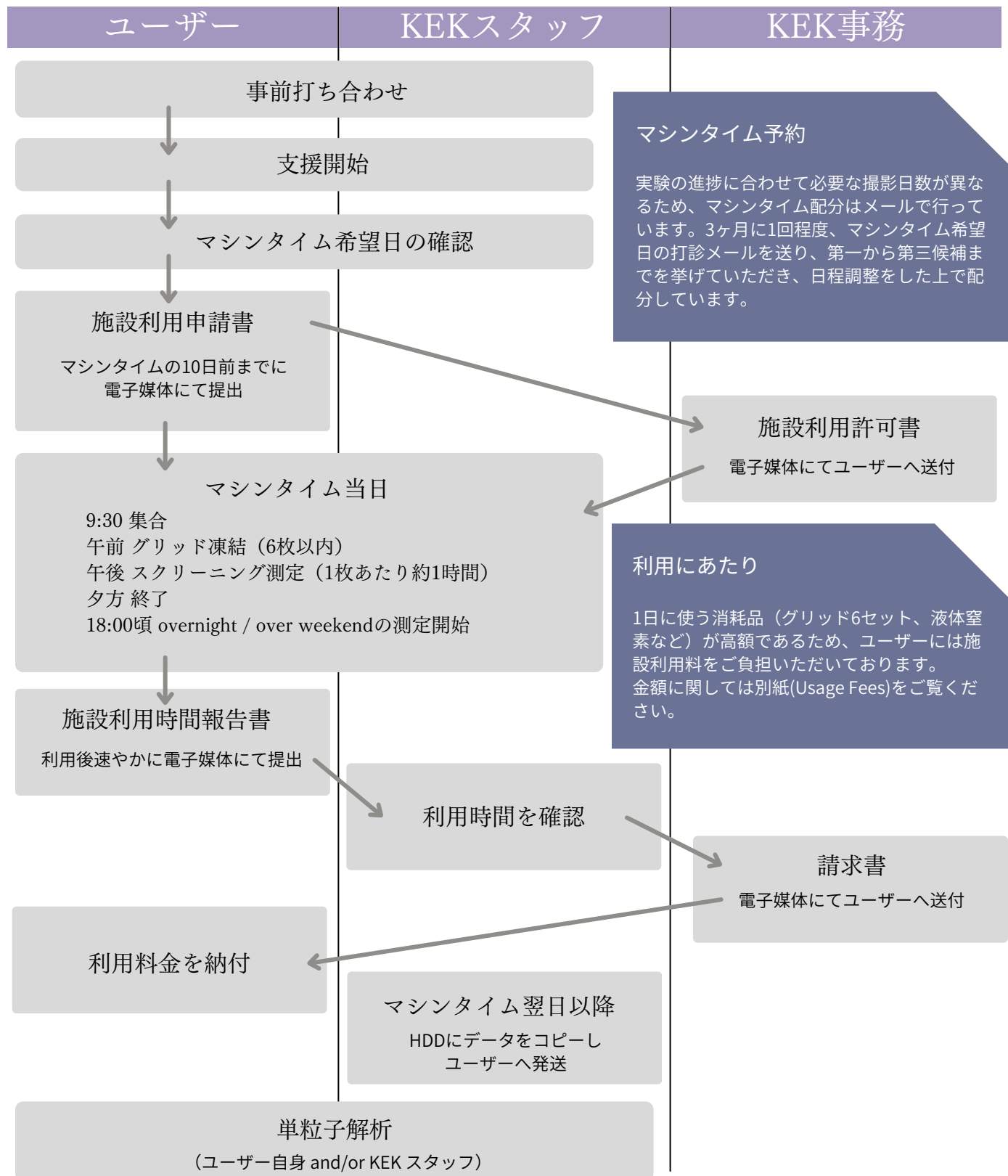
In general, each group is allocated one slot every one to two months. To ensure transparency, all schedules are published on the KEK Cryo-EM website.



KEKクライオ電顕利用の流れ

アカデミアユーザーの初回利用までの流れは「事前打ち合わせ→支援開始→マシンタイム配分→マシンタイム当日」が一般的です。実験の進捗に合わせて必要となる撮影日数が異なるため、マシンタイム配分はメールで行っています。

3ヶ月に1回程度、KEKからユーザー宛にマシンタイム希望日の打診メールを送り、第一から第三候補までを挙げていただき、日程調整をした上でマシンタイムを配分しています。配分は各グループあたり1-2ヶ月に1枠で、透明性を高める目的で全ての予定をKEKクライオ電顕のweb siteで公開しています。





The KEK Cryo-EM Team

Toshiya Senda
Masato Kawasaki
Mikio Tanabe
Toshio Moriya
Tsukasa Nakamura
Yuki Fujii
Takuya Tsubomatsu
Kyoungmoo LEE
Akira Shinoda
Rieko Sukegawa
Chiho Masuda

編集後記

KEK×CRYO-EM ACTIVITY REPORT Vol.8をお手に取っていただき、ありがとうございます。

2025年度は、KEKのクライオ電子顕微鏡にとって新たな一歩となる一年でした。100 kVクライオ電子顕微鏡「Tundra」が新たに加わり、施設の体制もさらに充実しました。また、多くのユーザーの皆様にご利用いただき、2025年度は構造生物学研究センターに700名を超える来訪者をお迎えすることができました。

このような充実した一年を振り返りながら、今年私が出会った、大好きなハワイでの新しい発見について、少しでもご紹介したいと思います。

それは、採れたてのマカダミアナッツです。マカダミアナッツは、外側のとても硬い殻を割ると、さらにもう一枚殻があり、その中に実が入っています。その場で採れたてを味わった方からは、「今まで食べていたマカダミアナッツとはまったく違い、まるでフルーツのようだった」と聞き、とても驚きました。何度もハワイを訪れているにもかかわらず、まだまだ知らない魅力があることを知り、とてもうれしく思いました。

改めまして、本誌の制作にご協力くださった皆様、そして日頃よりKEKをご利用いただいている皆様に、心より感謝申し上げます。

今後ともKEKクライオ電子顕微鏡をよろしくお願いいたします。

A HUI HOU!

(ハワイ語で「また会いましょう」の意味です。)

増田

KEK×CRYO-EM ACTIVITY REPORT

VOL.8 April 2025 - March 2026



KEK：高エネルギー加速器研究機構

IMSS：物質構造科学研究所

Structural Biology Research Center：構造生物学研究センター