

## Stanford-SLAC Cryo-EM Center (S<sup>2</sup>C<sup>2</sup>)

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The missions of the Stanford-SLAC Cryo-EM Center (S<sup>2</sup>C<sup>2</sup>) [1] are (i) to provide the scientific community access to state-of-the-art cryo-EM instrumentation for high resolution imaging, and (ii) to train scientists to become independent cryo-EM investigators. Our Center plans to set up four cryo-electron microscopes in the new Arrillaga Science Center on the SLAC campus of Stanford University. Two Titan Krios electron microscopes will be installed in the Fall 2019 while additional electron microscopes will be acquired in 2020. The Center will also be equipped with cryo- specimen preparation devices, data quality assessment tools for on-the-fly evaluation, and image data storage and compute capacity on a short-term basis. Our activities are opened to researchers from diverse backgrounds and institutions.

Currently, we have opened up our highly productive Stanford-SLAC CryoEM facilities [2] for the S<sup>2</sup>C<sup>2</sup> user program on a limited basis, using our existing Titan Krios and Talos-Artica microscopes. Access to the S<sup>2</sup>C<sup>2</sup> is obtained through an open application process based on scientific merit [3]. All user project applications are reviewed by an independent panel of cryo-EM specialists three times a year and are ranked upon (i) evidence that the specimens are ready for near atomic resolution structure determination, and (ii) demonstrated expertise in performing image reconstruction with the acquired image data. Those who are not granted instrument time are encouraged to perform further experiments to demonstrate the feasibility of generating a low-to-moderate resolution image or reconstruction using other facilities. If needed, we offer advice and on-site training in cryo-specimen preparation and screening.

We provide expert staff that trains, assists and advises users, both on-site and remotely, and are available for consultation beforehand and in a follow-up program. We have developed an eLogbook that facilitates the record keeping of all experimental parameters of the microscopes. The eLogbook provides a portal from which users can keep metadata of their EM session that are archived along with the image data. These include software used to record data, pixel size, dose rate, number of frames, phase plate usage, energy filter slit, aperture sizes, spot size, mode of imaging (single particle, tomography, electron diffraction) etc. The eLogbook also provides an interface from which users can change, add and delete collaborators themselves so that they may have access to the raw (and preprocessed) data. In the event that the users participate in remote data collection, they can also modify permissions to enable collaborators to monitor or control the microscopes remotely. This can all be done from a web browser without the need of additional software.

We also provide near real-time feedback of data quality, allowing users to optimize the time that they have been assigned on the microscopes. Acquired images are instantaneously transferred to our data center via a high speed network and automatically organized in experimental directories that only the user and authorized collaborators have access to. As such, the data is immediately available for download while the data collection progresses. In addition, every image that is obtained is also processed for frame alignment (using MotionCor2) [4], contrast transfer function calculation (before and after alignment) as well as automated particle picking (using Relion 3's LoG picker) [5]. This on-the-fly processing provides in near real-time (1-5 minutes after data collection) feedback to users via a private SLACK channel. All calculations are stored in the eLogbook so that histograms and time series plots of image resolution, phase plate changes and drift values can be conveniently tracked. The SLACK app can be downloaded onto a mobile device or viewed through any web browser.

We cross-train scientists who want to incorporate cryo-EM into their own research portfolios [6]. Our training is targeted at a wide variety of skill levels and we include short-term and long-term in-residence training programs. Our training program covers the full spectrum of single particle cryoEM workflows from cryo-specimen preparation to structure validation. Each of our short-term training workshops spans 3-4 days and is widely advertised. The workshop lectures are open to registered participants, in person or via video conference, while the practical components are limited to fewer trainees. These workshops are taught by our in-house staff scientists as well as outside experts. We envision these workshops to become a feeder mechanism for individual in-residence cross-training projects and will also simultaneously serve to provide a baseline level of training expertise that may become a prerequisite for the in-residence training. Broad topics of past and planned workshops include (i) cryo-specimen preparation and electron microscope operation, (ii) image processing and reconstruction, (iii) cryo-EM map-based modeling and structure validation, and (iv) specialized cryo-EM technology developments.

In-residence training requests can be applied via our web site [7]. Our selection criteria include a commitment from the applicant that she/he plans to become cryo-EM competent and is ready to be trained from cryo-specimen preparation to data collection and image reconstruction. Depending on the individual's prior experience, the curriculum of training will vary. Presently, our targeted trainees will be those who plan to use cryo-EM routinely in a laboratory of a Project Leader on a long-term basis. This criterion will benefit the individual PI's laboratory as well as the Project Participants.

## References

- [1] <https://cryoem.slac.stanford.edu/s2c2/>
- [2] <https://sites.slac.stanford.edu/cryo-em/>
- [3] <https://cryoem.slac.stanford.edu/s2c2/project-requests>
- [4] S Q Zheng et al., *Nat Methods* **14**(4): 331-332 (2017).
- [5] J Zivanov et al., *Elife* **7**: e42166 (2018).
- [6] <https://cryoem.slac.stanford.edu/s2c2/training/workshops>
- [7] <https://cryoem.slac.stanford.edu/s2c2/cryoem-training-application>
- [8] The authors acknowledge the support of the National NIH-Funded Cryo-EM Centers: Transformative High Resolution Cryo-Electron Microscopy (grant number U24GM129564).