The Tecnai Arctica (TEM-9) Cryo-EM workflow

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Cryo-TEM training involves several levels. The initial level is an overview of the technique and practical considerations. Hands on training follows in a small group environment by experienced staff. Users are allowed then to operate the microscope after passing a basic certification. Experienced users are given 24hr access to complete their projects.
The Tecnai Arctica is a 200 kV, FEG emission microscope that is fully automated for high-resolution 3D characterization of biological samples.

Control room in B58A/LISE for the Tecnai Arctica.

Access for certified CNS users is granted after completing the intermediate training.

Specimen preparation are in GO5/LISE for the Tecnai Arctica.

Access for certified CNS users is granted after GO5 training led by Sandra Nakasone.
1. User register as CNS users and undergo safety training.

2. Optimizing sample with negative staining with TEM-5.

**SSM assisted use with negative staining screening and training**

- Screening of the negatively stained EM grids - sample 1 - 2 EM grids
  - Negative staining training (GO5, 30min – 1 hour).
  - F20 (TEM-5) screening (B15E, 1-2 hours) (**SSM** and **Carolyn Marks**)
- Required training
  - Negative staining training (GO5, 1 hour) **SSM**
  - GO5 training (**Sandra Nakasone**, 2h)

3. Optimizing samples for Cryo-EM.

**SSM assisted use with Cryo-EM sample preparation and screening**

- Preparing Cryo-EM grids (freezing the samples, GO5, 1h), 1 sample 3 – 4 grids.
- Mounting grids in autogrids and loading samples in the Arctica (TEM-5, B15A, 1h)
- Screening of the Cryo-EM grids with the Arctica (TEM-9, B58A, 3 – 4 h)
- Required training
  - Use of the Cryoplunge and freezing of Cryo-EM grids (GO5) **SSM**
  - Basic Cryo-EM Arctica training (TEM-9, B58, two days) **SSM**
  - Intermediate Cryo-EM Arctica training (TEM-9, 1 day) **SSM**

SSM assisted use with Cryo-EM single particle data collection and training.

- Freezing of 8 to 12 grids and store in liquid nitrogen (GO5, 1h)
- Mounting the Cryo-EM grids in the Arcica (TEM-9, B15D, 1h)
- Collecting data with the Arctica (TEM-9, K2 summit camera, movie mode, B58A 5 days – 2 weeks)
- Data processing (B58, 6 – 12 weeks)

- Required training in addition of training in 3.
  - Advanced Cryo-EM Arctica training (automatic data collection, B58A, 1 day)
  - Advanced Cryo-EM Arctica training (K2 usage for data collection)

5. Data/structure analysis and validation – B58 (SSM).

SSM assisted use with Cryo-EM single particle data analysis and training.

- Training protocols for EMAN2 and Relion 3 on stand alone GPU Linux workstation
- Data evaluation with Digital Micrograph or EMAN2 before processing
- Stand alone particle picking algorithms
- Data validation and modelling with UCSF-Chimera
- Required training in addition fo 4. is 2-3 hours assisted use (initially) and 1h consultations, as needed
Basic and Intermediate Arctica Cryo-EM courses

Basic Arctica Cryo-EM training

Negative stained (1% UA) Adeno-associate viruses (AAV) and Microtubules (MT). Learning Low Dose imaging and Fourier transform (FFT)

Two days

Intermediate Arctica Cryo-EM training

Cryo-EM of liposomes. Screening and low-dose imaging. Visualizing the lipid bilayer

One day

A. 2700x, 3.351 nm/pix
Search mode

B. 23,000x, 0.383 nm/pix
Exposure mode

C. 21,000x, 0.433 nm/pix
Exposure mode
Apoferitin from equine spleen

A  Negative staining – Carbon 300 mesh Cu grids

B  Exposure mode – 39,000x magnification, 2.5 Å/pixels. The were acquired for 1 second on an Eagle 4096 x 4096 pixels Eagle CCD camera, at low dose conditions (< 25 e⁻ Å²).

C  Exposure mode – 23,500x magnification, 1.51 Å/pixels. The images were acquired for 10 second on a K2 DDE camera in counting mode, at low dose conditions (< 25 e⁻ Å²).