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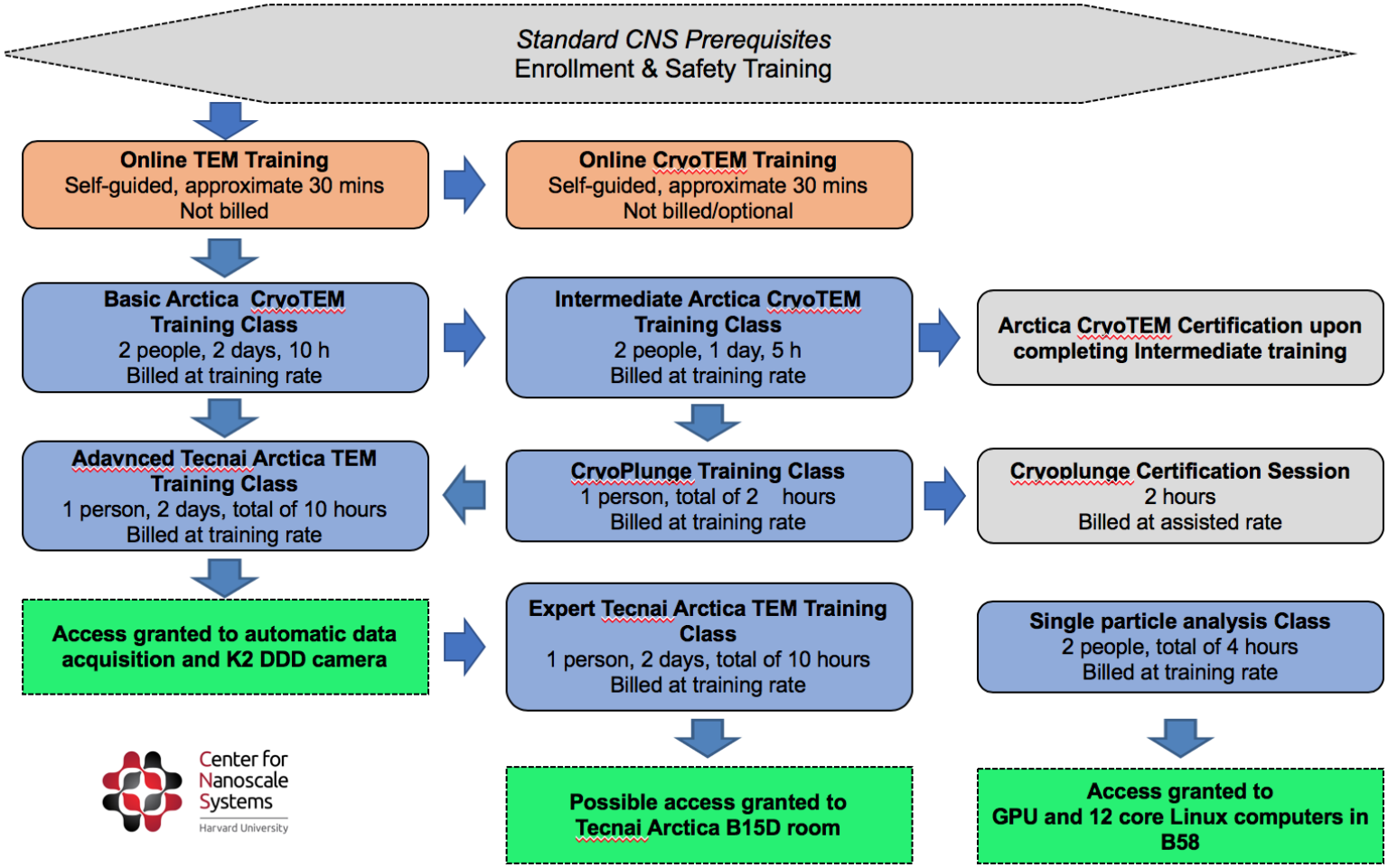
The Tecnai Arctica (TEM-9) Cryo-EM workflow

Contact: Svetla Stoilova-McPhie, PhD
Advanced bioimaging scientist
stoilovamcphie@fas.harvard.edu

The Arctica Cryo-EM training chart



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.

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The Tecnai Arctica workflow

The Tecnai Arctica is a 200 kV, FEG emission microscope that is fully automated for high-resolution 3D characterization of biological samples.

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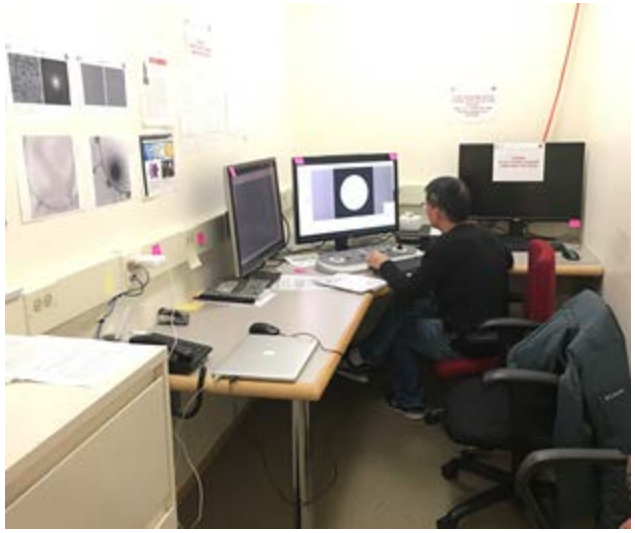

Control room in B58A/LISE for the Tecnai Arctica.

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

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

Access for certified CNS users is granted after GO5 training led by Sandra Nakasone.

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The Arctica Cryo-EM training workflow

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**

The Arctica Cryo-EM training workflow

4. Single particle data collection – B58A (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

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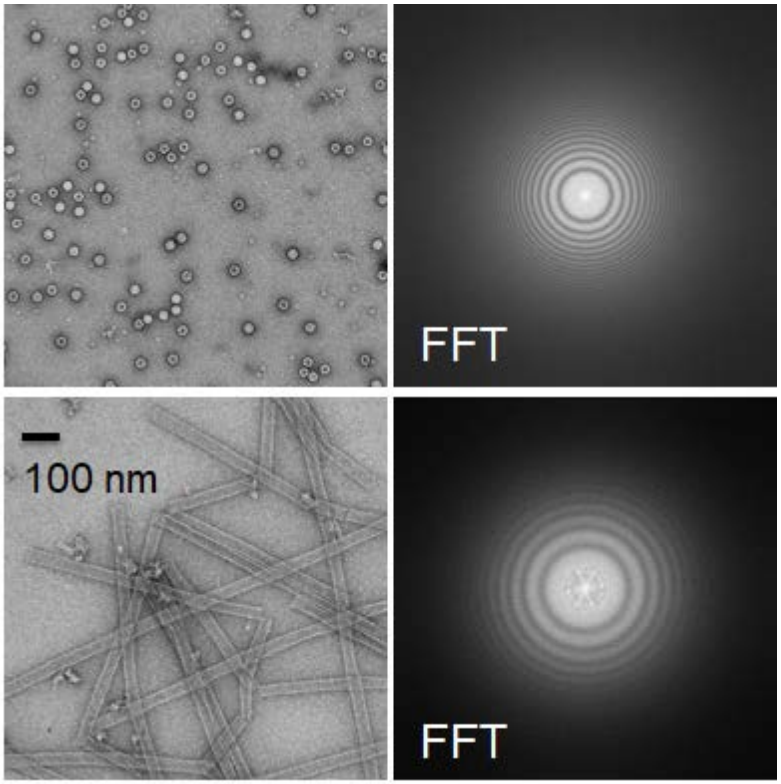
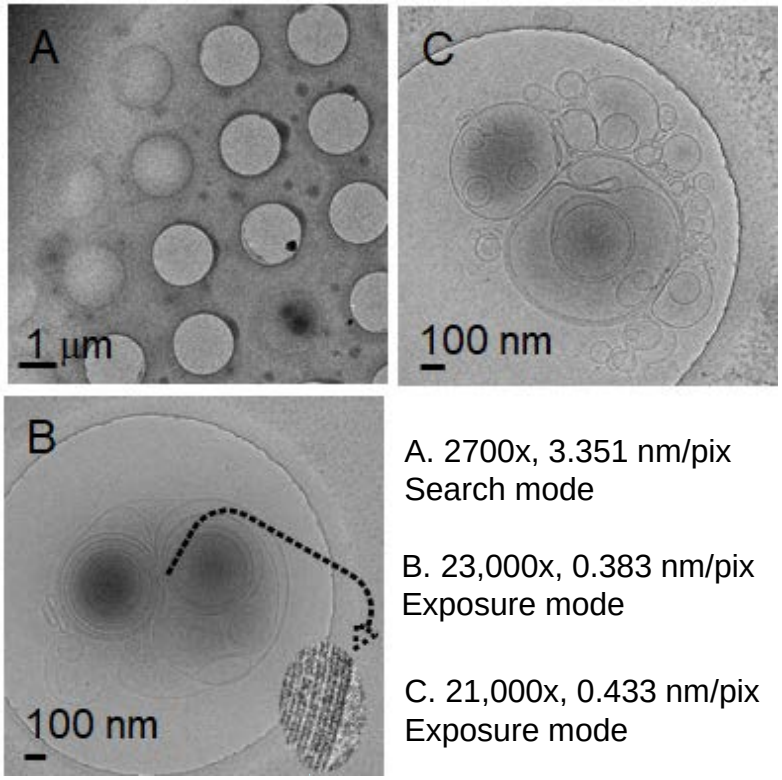
Basic and Intermediate Arctica Cryo-EM courses





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

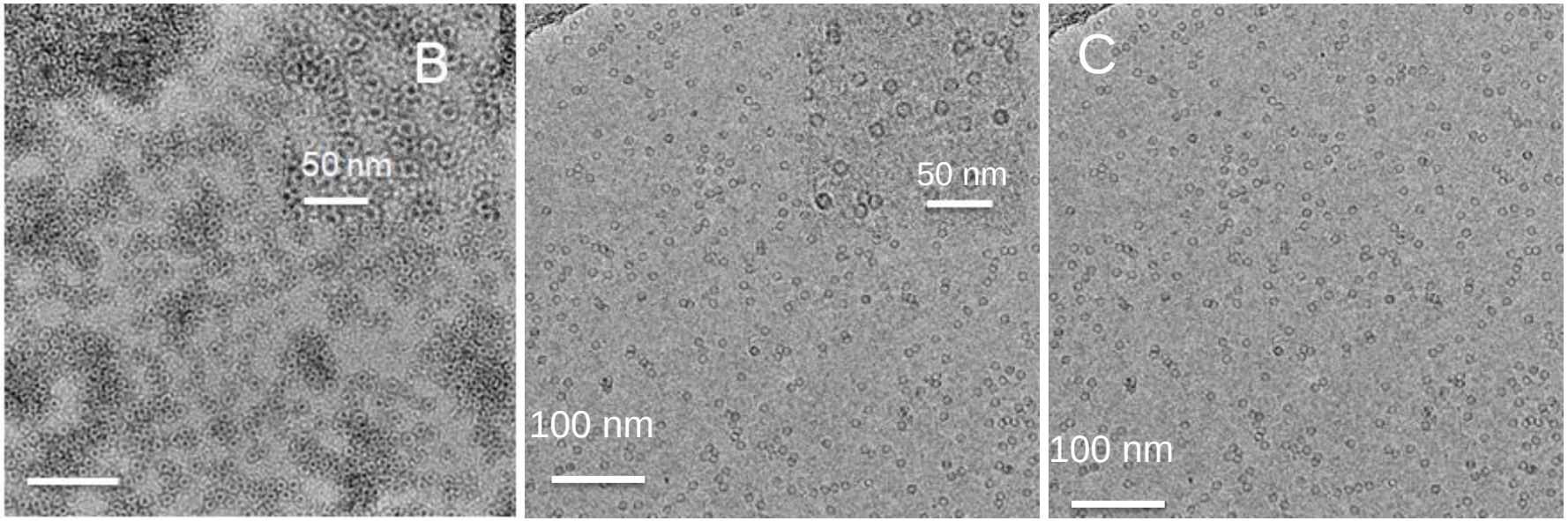
Basic Arctica Cryo-EM training

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

Advanced Arctica Cryo-EM course



Apoferritin 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^{-} $Å^{2}$).

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^{-} $Å^{2}$).