

## **CryoEM Training – Basic**

(Each session is set to 2.5 hours. )

### **Level 1 – Basic TEM – 4 sessions.**

#### Session 1-1

- Prepare continuous carbon film grid
- Introduction to TEM, comparing X-ray method
- Basic TEM operation on CM120 or Tecnai Spirit
- Hardware and Software for Modern TEM system
- Vacuum system & interface

#### Session 1-2

- Specimen rod insertion and retraction
- Tuning microscope (eucentricity, gun alignment, filament saturation, beam-tilt pivot points, rotation center)
- C2 aperture, Stigmation; Obj aperture, stigmation
- Demo and introduce CTF

#### Session 1-3

- Negative stain sample preparation
- Refreshing scope tuning procedure
- Introduce digital cameras – hardware components, background preparation, pixelsize at levels of detector and specimen.
- Introduce DQE, and direct electron detectors
- Negative stain specimen imaging using CCD cameras

#### Session 1-4

- Supervising tuning scope, negative stain preparation and CCD camera preparation
- Assist user practice and answer question they might have.
- Demo cryo cycle to condition the column of scope when done with the session

### **Level 2 – Cryo TEM – 4 sessions.**

#### Session 2-1

- Prepare side-entry cryo holders – warm-up and zeolite cycle
- FEI Vitrobot IV operation
- Explain holey grid pre-treatment – bake, wash & electron irradiation
- Freeze cryoEM grids

#### Session 2-2

- Low Dose TEM operation, explain Low Dose concept, setup.
- Introduce and explain shutter control on TEM system
- CCD imaging with Low Dose setup on Tecnai system

#### Session 2-3

- Cryo grids loading to cryo holder on transfer station
- Cryo holder insertion and retraction
- Supervising user practice on loading grid and insertion of cryo holder to scope
- Demo and explain vacuum recovering and quiet bubbling in the dewa
- Demo whole processing from sample loading to imaging in Low Dose condition. Explain ice condition.

#### Session 2-4

- Supervised practice session for user.
- Debugging ice condition
- Supervise Cryo-cycle
- Supervise Warm-up and bake cryo holder