



HIVE Center Winter Training Retreat (virtual)
January 19, 2022
Meeting time: 9 am - 11:30 am PST (12 pm – 2:30 pm EST)

Cryo-ET and Subtomogram Averaging (STA) workshop

Robert Dick, Cornell University

1) General Introduction (~30min)

- a) Basic comparison of single particle (SP) cryo-EM to cryo-ET and STA.
- b) Is cryo-ET and STA the right method for you?
- c) Microscopes, detectors, and energy filters
 - 200 vs 300kV
 - side entry and stability
 - Direct detectors: K2, K3, Falcon 4, etc.

*5-minute break

2) Sample preparation (~30 min total)

- a) Types of samples for cryo-ET vs cryo-EM and STA
 - Sample preparation
 - Purification, screening, SEC, TEM, etc.
- b) Freezing
 - Grid selection
 - Plungers: Manual, Vitrobot, GP2, nano-spray.
 - (GP2 video of backside blotting).
- c) Screening and optimization
 - Initial imaging of samples in ice. Is this grid good enough to collect on?
 - Ice thickness
 - Sample and fiducial (gold) abundance and distribution
 - Keep in mind that a SP project may collect hundreds-thousands of images, we typically collect 25-50 tomograms.
- d) Specialized techniques (~10min)
 - FIB-milling **see paper: 1_Zila_Cell_FIB*

*5-minute break

3) Data collection (~10 min)

- Tilt schemes: **see paper: 2_Turonova_NatCom_DataCollection*
- Defocus

4) Data processing

**see paper: 3_Obr_biorxiv_DataProcessing*

- a) Practical considerations: Infrastructure, cost, etc. (~10 min)
 - Data storage: how much do you need? More than you think!
 - Workstations: GPU, CPU, RAM, considerations (get a big monitor)!
 - Software: sbgrid, dynamo, Etomo and IMOD, MATLAB, RELION, WARP'M

*5-minute break

4) Data processing – continued

b) Overview of workflow (~45 min total)

- Tomogram reconstruction
 - *(Etomo demonstration of a tomogram reconstruction ~15min)
- Particle picking and extraction: Modeling, mapping, templating.
- Alignments: Cleaning, un-binning, repeating
 - *(Demonstration: Dynamo setup and making a mask ~15min)
- Alternative workflows/tools: Particles into warp/m, relion4-beta
- Specialized sorting and analysis: nearest neighbors, tilt-twist, etc.
**see paper: 4_Obr_NatComm_DataProcessing*

5) Discussion and wrap-up (~5 min total)

Resources:

Tutorials

- RELION-4 tutorial: https://relion.readthedocs.io/en/release-4.0/STA_tutorial/index.html
- Dynamo-Tutorial: https://wiki.dynamo.biozentrum.unibas.ch/w/index.php/Walkthrough_for_lattices_on_vesicles
- ETOMO-Tutorial: <https://bio3d.colorado.edu/imod/doc/etomoTutorial.html>
- WARP'M-Tutorial: *Website is currently a security risk!*
- SerialEM (EMBL): <https://blogs.embl.org/cryoem/>
Let's start by sharing our [Krios Quantum K3 manual](#). (download-unzip-click "main.html")
- SerialEM: <https://bio3d.colorado.edu/SerialEM/>

Software/Scripts

- Lattice mapping (place object plugin for Chimera): <https://www2.mrc-lmb.cam.ac.uk/groups/briggs/resources/place-object/>
- SBgrid: <https://sbgrid.org/>
- Nearest neighbors: <https://schurlab.ist.ac.at/downloads/>
- subTOM: <https://subtom.readthedocs.io/en/latest/>
- dynamo2m: <https://github.com/alisterburt/dynamo2m>

Websites

- Where are the microscopes:
https://www.google.com/maps/d/u/0/viewer?mid=1eQ1r8BiDYfaK7D1S9EeFJEgkLggMyoaT&hl=en_US&ll=40.54164018324083%2C-94.4629534929123&z=5
- #teamtomo: <https://teamtomo.org/resources/cryoet-software/m/user-guide.html>

Videos

- Grant Jensen lecture series on Tomography from Caltech on YouTube.
Playlist (Part 5: Tomography videos #33-37)
https://www.youtube.com/watch?v=gDgFbAqdM_c&list=PLhiuGaXIZZenm7lu5qv_A59zEWkRkKbn5&ab_channel=caltech
- John Briggs lecture on Tomography and Subtomogram Averaging from MRC LMB on YouTube.
https://www.youtube.com/watch?v=4Z0sQ_GhBkk&ab_channel=MRCLaboratoryofMolecularBiology

Papers

- Step-by-step guide to efficient subtomogram averaging of virus-like particles with Dynamo (PMID: 34437529)
- A flexible framework for multi-particle refinement in cryo-electron tomography (PMID: 34437530)