Bee Ling, Shujun, Zhi Yang, Xiaoyu, Li Deng

Collaborators:

Uttam Surana, Hong Hwa Lim, Kazu Maeshima, Sachiko Tamura, Yinyi Huang, Mdm. Loy, Ann Tran, Ping Lee Chong, Jian Shi, Désirée Bock & Martin Pilhofer

Funding: NUS, LHK fund, CBIS, YIA, MOE
A small structural-cell bio groups’ perspective

• A pragmatic case for H-T tomo
• A scientific case for H-T tomo
Timeline (PhD student-driven lab)

• First 2 years for cryomicrotomy skills to start plateauing (inclusive of cryo-EM and image-processing training).

• Next 3+ years to do vast majority of experiments for thesis / paper. How is this time spent?
Time commitment

- Writing
- Sample/Non-EM
- Data collection
- Reconstruction
- TM / STA
Ideal time commitment

- Writing (majority)
- Sample/Non-EM
- TM / STA

Pie chart showing the distribution of time commitment.
Targeting is not trivial

Ladinsky, unpublished
• A pragmatic case for H-T tomo

• A scientific case for H-T tomo
Shujun CAI
Désirée Böck (ETHZ)
Martin Pilhofer (ETHZ)
Nucleosomes: > 1,000 per tomogram
Kinetochore (subcomplex): < 10 per tomogram

Cai Tong NG
Lots of interesting biology is ‘heterogeneous’
Lots of interesting biology is ‘heterogeneous’
Align + Classify

Remap

New mechanisms & hypotheses
Wishlist

• Auto cryo-CLEM & target selection
• High-precision/speed stage
• Fiducialless alignment*
• Instant binning/low-pass filters

* “one-click” and indistinguishable from “manual” reconstructions
New Results

**ETDB-Caltech: a blockchain-based distributed public database for electron tomography**

Davi R Ortega, Catherine M. Oikonomou, H. Jane Ding, Prudence Rees-Lee, Alexandria, Grant J Jensen

doi: https://doi.org/10.1101/453662

This article is a preprint and has not been peer-reviewed [what does this mean?].

Abstract

Three-dimensional electron microscopy techniques like electron tomography provide valuable insights into cellular structures, and present significant challenges for data storage and dissemination. Here we explored a novel method to publicly release more than 11,000 such datasets, more than 30 TB in total, collected by our group. Our
EMPIAR-10227

A collection of yeast cell cryo-ET data

Publication: A collection of yeast cell cryo-ET data
Gan L, Cai S, Ng C, Chen C

Related EMDB entry: EMD-8157
Deposited: 2018-10-03
Released: 2018-11-16
Last modified: 2018-11-21
Dataset size: 1.8 TB
Dataset DOI: 10.6019/EMPIAR-10227

- Image set
  - A collection of yeast cell cryo-ET data
    - Category: tilt series (.mrc files) and tomograms (.rec files)
    - Image format: MRC
    - No. of images or tilt series: 1203
Current practice

Typical cryosection ribbons:
Gold beads spread uniformly on carbon
100 - 150 nm thick

One session 48 hours
3 hours to load grids & locate target positions
45 hours data collection
±60°, 2° incr (60 min / tilt series)
40 - 45 tilt series / session

Reconstruction
20 - 40 minutes, w/ Etomo batch + manual adjustment
Centre for BioImaging Sciences (CBIS)

Vitrobot
Leica UC7/FC7 (> 90% of our samples)

T12 (screening & training)
  CCD camera

Krios
  Falcon 2 & Greg McMullan’s hack
  Volta phase plate
  Leginon & Tomo 4
Journal 1 | Journal 2

Number of Panels/Data

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<th>Year</th>
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<th>Journal 2</th>
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