Detecting Distinct 60S Ribosome Maturation Intermediates in Cells by 2D Template Matching

Nikolaus Grigorieff
10 Years of Cryo-EM Revolution

Apo ferritin at 1.22 Å resolution
Nakane et al. 2020
(PMID: 33087931)

IRES viral mRNA translocation
Abeyrathne et al. 2016
(PMID: 27159452)

HeLa cell nuclear periphery
Mahamid, Villa et al. 2016
(PMID: 26917770)

More “native”
The Revolution Behind the Revolution

Powerful computers / GPUs enable

- Direct detectors
  - Fast frame rates
  - Electron counting
- Beam-induced motion correction
  - Movie mode
- New algorithms
  - Maximum-likelihood
  - Machine learning
- High-resolution reconstructions
  - Large datasets
  - Large volumes with many voxels
- Reconstruction of heterogeneous samples
  - Many states from a single sample
  - Modeling of continuous heterogeneity
…“an elaborate network of interlocking assembly lines…” Alberts 1998
3D Template Matching

Templates match visible features

identified molecules

Mahamid et al. 2016 (PMID: 26917770)
Frangakis et al. 2002 (PMID: 12391313)
Dense Density

Herpes virus entering a synaptosome

Maurer et al. 2008 (PMID: 18653756)
High Resolution Fingerprints

Close-to-focus cryo-EM image

NMDA receptor

AMPA receptor
Proof of Principle

Apoloferitin

2.4 million projections

Computationally expensive!

Defocused image (control)
Cryo-EM image

Correlation map
(maximum correlation value at each point)

Rickgauer et al. 2017 (PMID: 28467302)
High Specificity

SNR = Peak / STD
≈ 12 (apoferritin)

Rickgauer et al. 2017 (PMID: 28467302)
2D Template Matching

One exposure of untilted sample

2 – 3 million orientations

High-resolution template

Projections

Correlation peaks

Detected targets

Statistics

Current detection limit
300 – 500 kDa

Rickgauer et al. 2017 (PMID: 28467302)
Detecting Ribosomes in Cells

Cells: Mouse embryonic fibroblast
Template: Human 80S ribosome

6ek0: 80S ribosome
Natchiar et al. 2017 (PMID: 29143818)

FIB-milling frozen S. cerevisiae

Thermo Fisher Aquilos
Focused Ion Beam
cryo-milling instrument

Animation by Tim Laugks
MPI of Biochemistry
Martinsried

Lucas et al. 2022 (PMID: 36005291)
Nuclear Periphery

Titan Krios, Gatan K3
Sample thickness: 150 nm

Lucas et al. 2022 (PMID: 36005291)
Template Search

Titan Krios, Gatan K3
Sample thickness: 150 nm

10 < SNR < 14

6q8y: 60S large ribosomal subunit
Tesina et al. 2019
(PMID: 30911188)

3D template

Lucas et al. 2022 (PMID: 36005291)
Detecting 60S Ribosomal Subunits

Titan Krios, Gatan K3
Sample thickness: 150 nm

6q8y: 60S large ribosomal subunit
Tesina et al. 2019
(PMID: 30911188)

Lucas et al. 2022 (PMID: 36005291)

28 images
Prospects

Current molecular weight limit:

- **300 - 500 kDa** orientations not constrained
- **100 kDa** with constraints (e.g. membrane)

- **60 - 100 kDa** if images perfect

+ **AlphaFold 2** (machine learning)

PDB entries

- New releases
- All releases
Challenges With 2DTM

- Sample *inhomogeneity*
- **Low-resolution** contrast
- Targets *too small* / many false negatives
- Templates *mismatch*
Challenges With 2DTM

- Sample **inhomogeneity**
- **Low-resolution** contrast
- Targets **too small** / many false negatives
- Templates **mismatch**
Uneven Contrast

Titan Krios, Gatan K3
Sample thickness: 150 nm

Lucas et al. 2022 (PMID: 36005291)
Maximum Intensity Projection (MIP)

Lucas et al. 2022 (PMID: 36005291)
Scaled MIP

\[ \text{scaled mip}_{xy} \approx \frac{\text{mip}(\phi, \theta, \psi, \Delta f)_{xy} - \mu(\phi, \theta, \psi, \Delta f)_{xy}}{\sigma(\phi, \theta, \psi, \Delta f)_{xy}} \]

Lucas et al. 2022 (PMID: 36005291)
Challenges With 2DTM

- Sample inhomogeneity
- Low-resolution contrast
- Targets too small / many false negatives
- Templates mismatch
Low-Resolution Contrast

Titan Krios, Gatan K3
Sample thickness: 150 nm

How to combine these approaches?

Lucas et al. 2022 (PMID: 36005291)

Glaeser et al. 2021
Challenges With 2DTM

- Sample inhomogeneity
- Low-resolution contrast
- Targets too small / many false negatives
- Templates mismatch
Detecting Extra Density

Lucas et al. 2022 (PMID: 36005291)

28 micrographs, 3991 detected targets
Increasing Sensitivity

- Better background models (machine learning)
- Peak profile fitting (auto-correlation function)
- Better templates (accounting for radiation damage, hydration layer, inelastic scattering etc)
- Combining 2D and 3D template matching
Combining 2D and 3DTM: Ribosomes in Bacteria

50S large ribosomal subunit
1.3 MDa

30S small ribosomal subunit
0.7 MDa

Mycoplasma pneumoniae
Thin enough to be electron transparent

Bacillus subtilis
70S ribosome

Sohmen et al. 2015 (PMID: 25903689)

Lucas et al. 2021 (PMID: 34114559)
Two Experiments

Sample thickness: 150 nm

Single image of untilted sample (30 e/Å²)

Tomogram (80 e/Å²)

Lucas et al. 2021 (PMID: 34114559)
Large Ribosomal Subunits

Electron cryo-tomogram

Particle defocus refinement → z coordinates

Lucas et al. 2021 (PMID: 34114559)
Challenges With 2DTM

- Sample **inhomogeneity**
- **Low-resolution** contrast
- Targets **too small** / many false negatives
- Templates **mismatch**
Detecting Pre-60S Subunits

Titan Krios, Gatan K3
Sample thickness: 150 nm

6n8j: late nuclear pre-60S subunit

Zhou et al. 2019 (PMID: 30814529)

Lucas et al. 2022 (PMID: 36005291)
Distinguishing 60S From Pre-60S

Titan Krios, Gatan K3
Sample thickness: 150 nm

6q8y: 60S large ribosomal subunit
Tesina et al. 2019
(PMID: 30911188)

6n8j: late nuclear pre-60S subunit
Zhou et al. 2019
(PMID: 30814529)

Lucas et al. 2022 (PMID: 36005291)
Distinguishing 60S From Pre-60S

28 micrographs, 1531 detected targets

6q8y: 60S large ribosomal subunit
Tesina et al. 2019 (PMID: 30911188)

6n8j: late nuclear pre-60S subunit
Zhou et al. 2019 (PMID: 30814529)

Lucas et al. 2022 (PMID: 36005291)
Distinguishing Pre-60S Intermediates

Titan Krios, Gatan K3
Sample thickness: 150 nm

6q8y
Mature 60S

6n8j
Late nuclear pre-60S

3jct
Early nuclear pre-60S

Percentage of nuclear 60S in each class

Lucas et al. 2022 (PMID: 36005291)

Crm1 (T539C) + LepB
Refining Atomic Models Against Images?

Titan Krios, Gatan K3
Sample thickness: 150 nm

Nuclear envelope

Cytoplasm

Nucleus

Vacuole

6q8y: 60S large ribosomal subunit
Tesina et al. 2019 (PMID: 30911188)

Maximize SNR

500 Å
Acknowledgements

Grigorieff lab
Mike Rigney
Kexin Zhang
Stephen Diggs
Johannes Elferich
Selene Flemming
Ximena Zottig
Lingli Kong
Bronwyn Lucas

Former members
Tim Grant
Alexis Rohou
Ben Himes

UMass Cryo-EM
Chen Xu
KangKang Song
Christna Ouch

Financial Support: HHMI, NIH, CZI