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

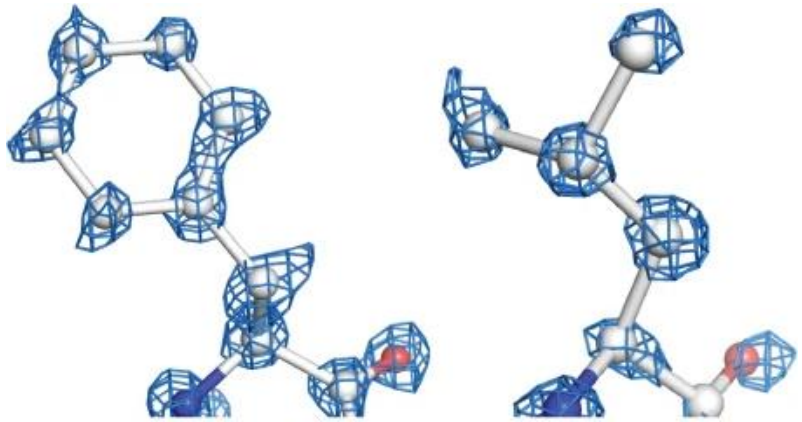
Nikolaus Grigorieff

hhmi | Howard Hughes Medical Institute

UMass Chan MEDICAL SCHOOL

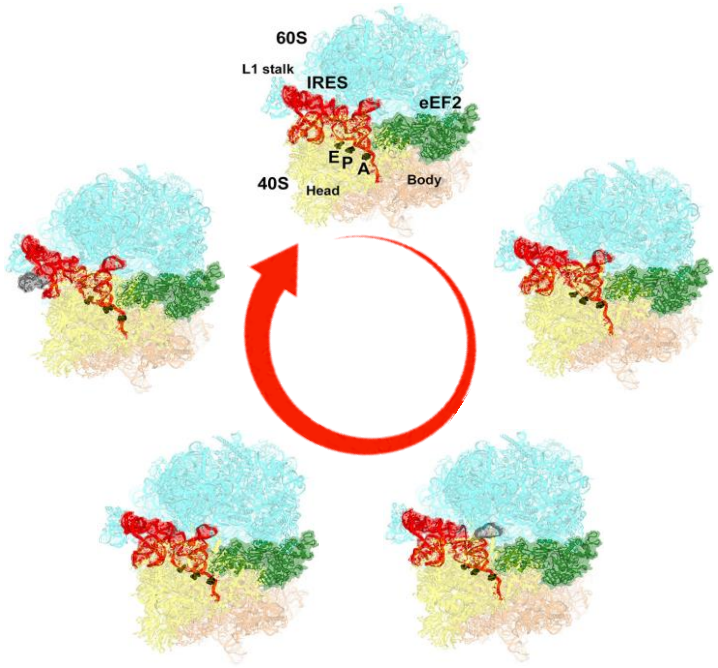
10 Years of Cryo-EM Revolution

Apoferritin at 1.22 Å resolution



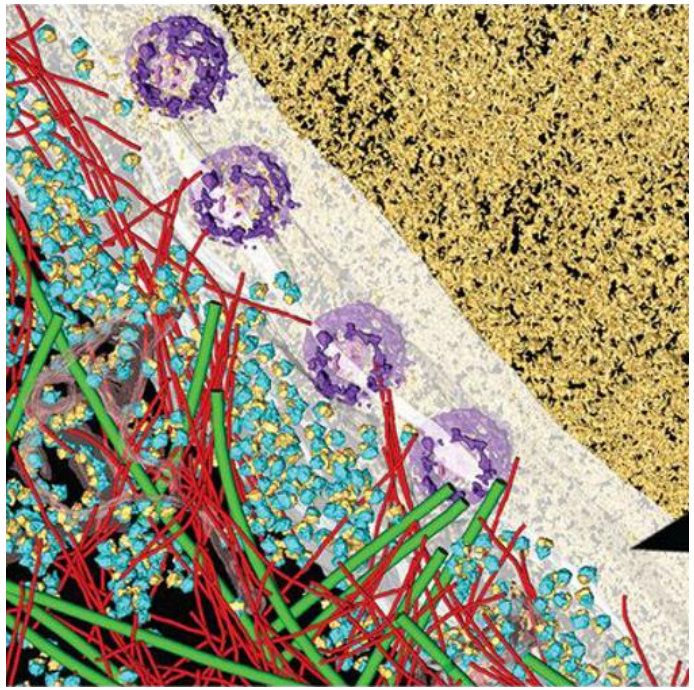
Nakane et al. 2020
(PMID: 33087931)

IRES viral mRNA translocation



Abeyrathne et al. 2016
(PMID: 27159452)

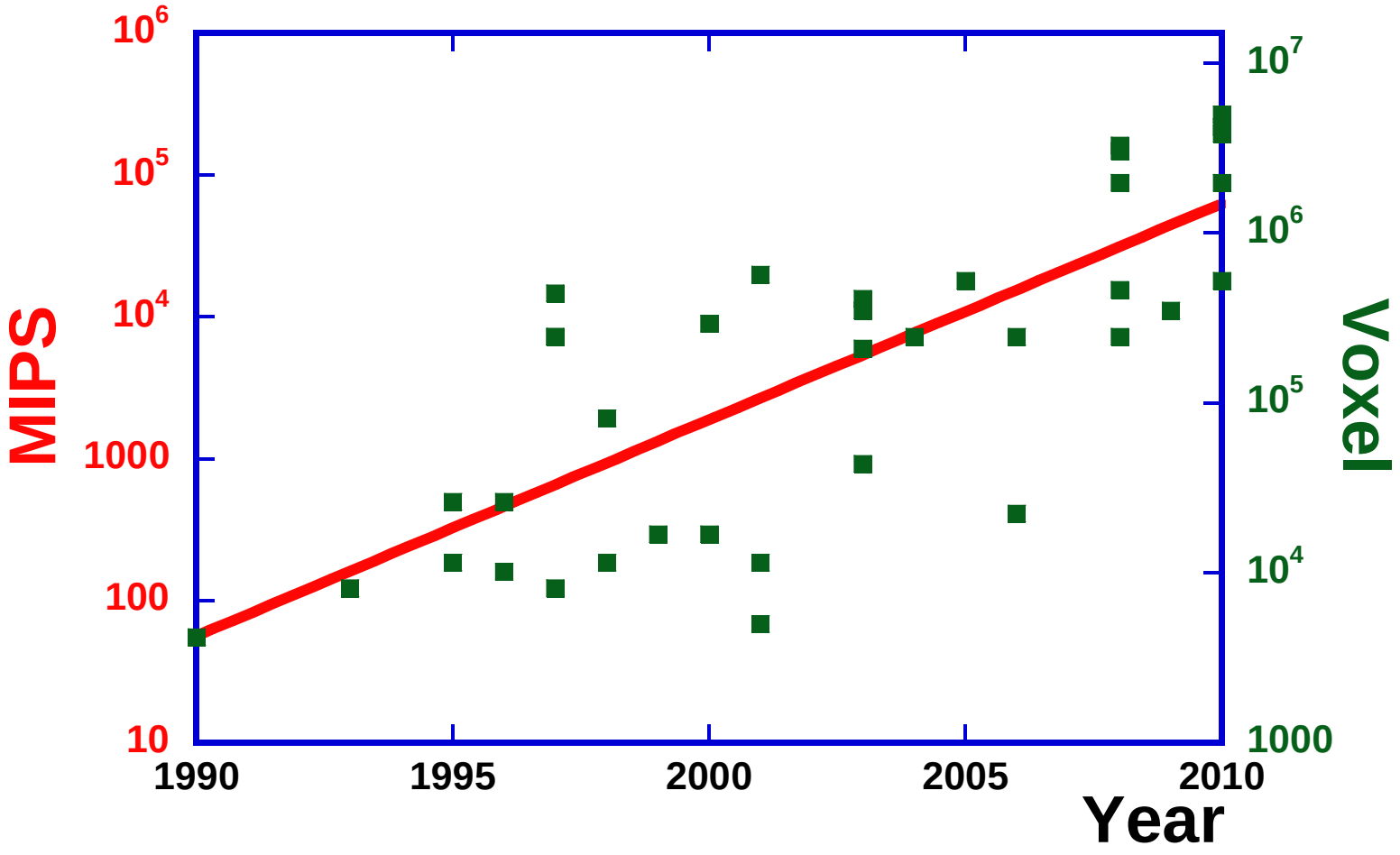
HeLa cell nuclear periphery



Mahamid, Villa et al. 2016
(PMID: 26917770)

More “native”

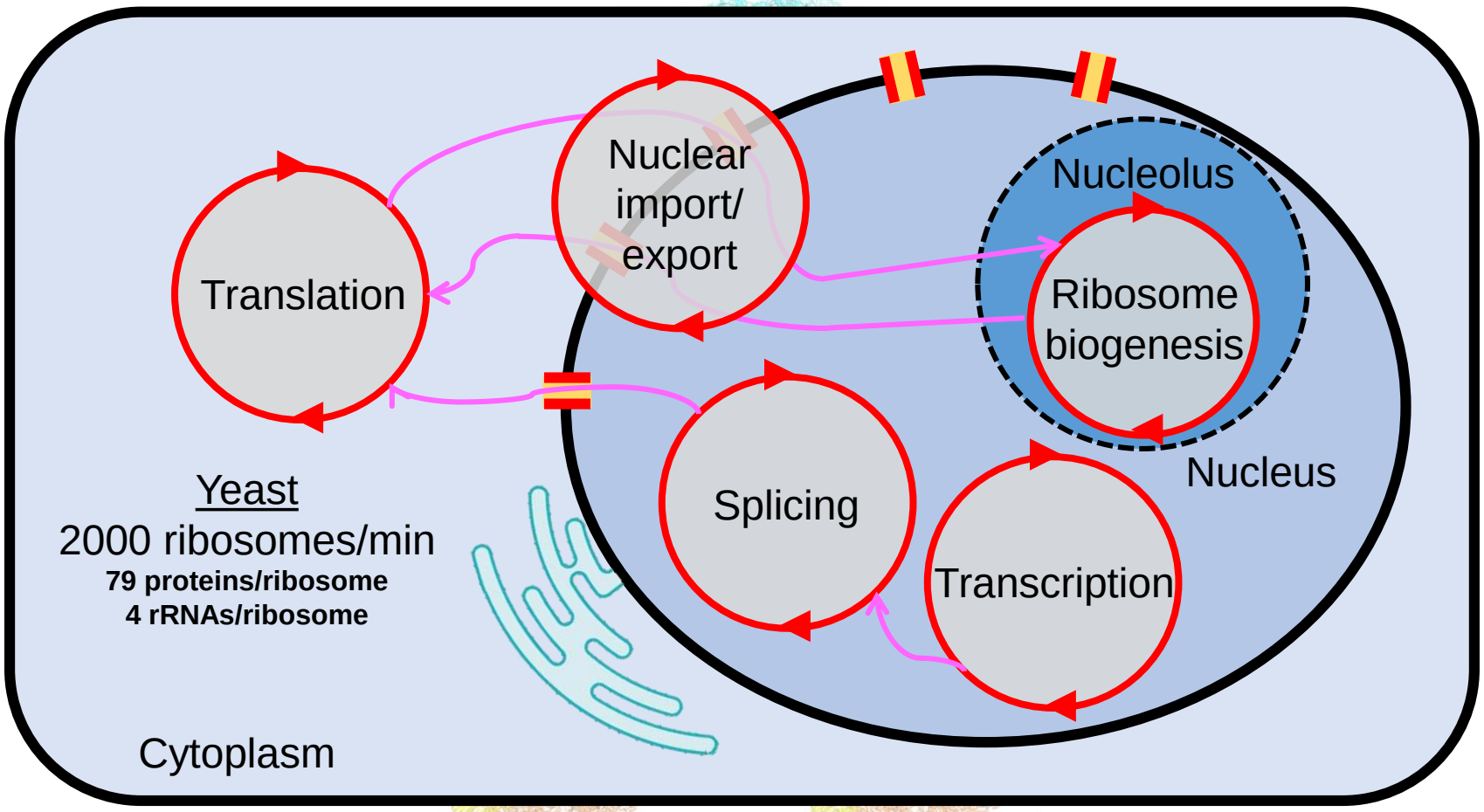
The Revolution Behind the Revolution



Powerful computers / GPUs enable

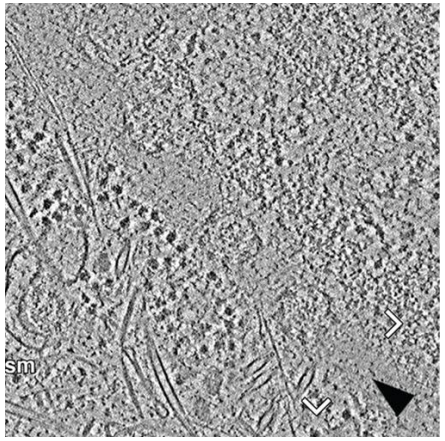
- Direct detectors
  - Fast frame rates
  - Electron counting
- Beam-induced motion correction
  - Movie mode
- New algorithms
  - Maximum-likelihood
  - Machine learning
- High-resolution reconstructions
  - Large datasets
  - Large volumes with many voxels
- Reconstruction of heterogeneous samples
  - Many states from a single sample
  - Modeling of continuous heterogeneity

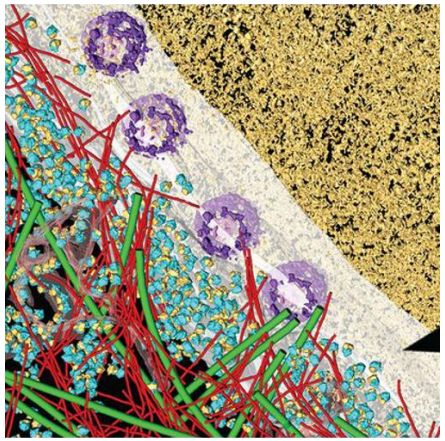
Broader Context

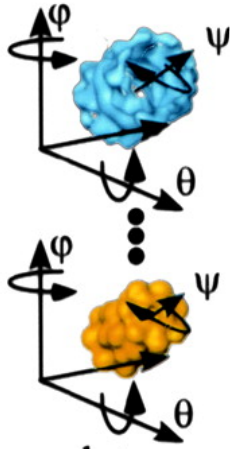


… “an elaborate network of interlocking assembly lines…” Alberts 1998

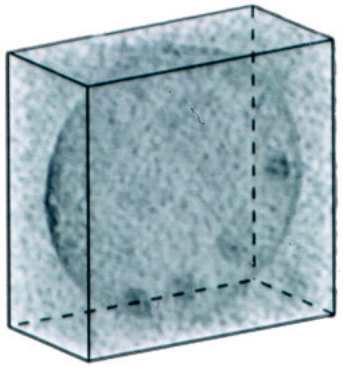
3D Template Matching





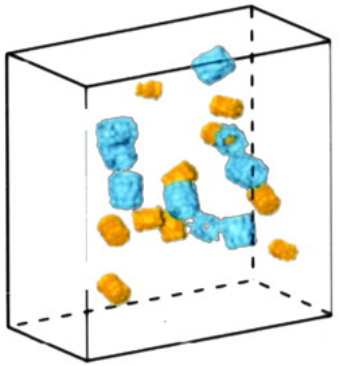


templates



tomogram

Templates match visible features

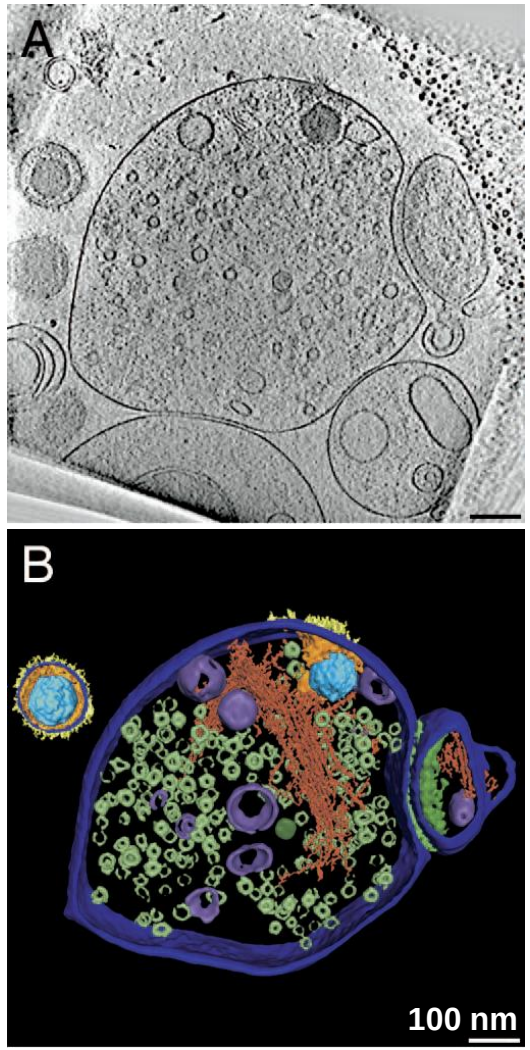


identified molecules

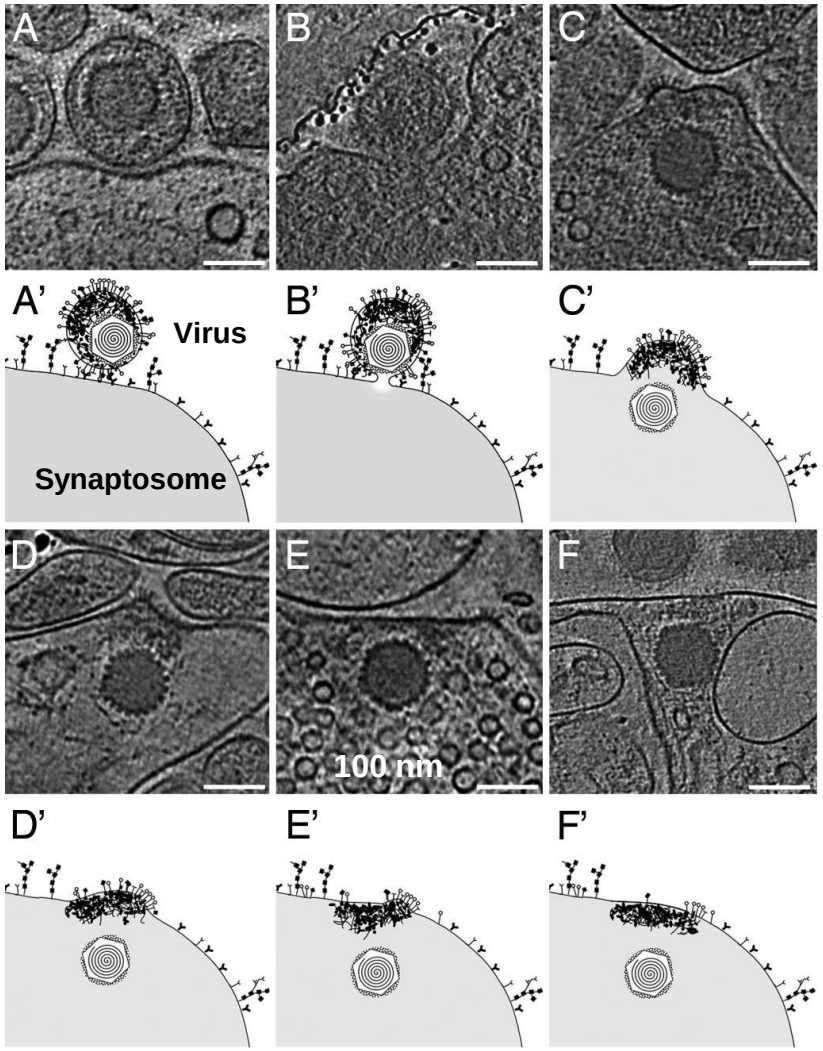
Mahamid et al. 2016 (PMID: 26917770)

Frangakis et al. 2002 (PMID: 12391313)

Dense Density



Herpes virus entering a synaptosome



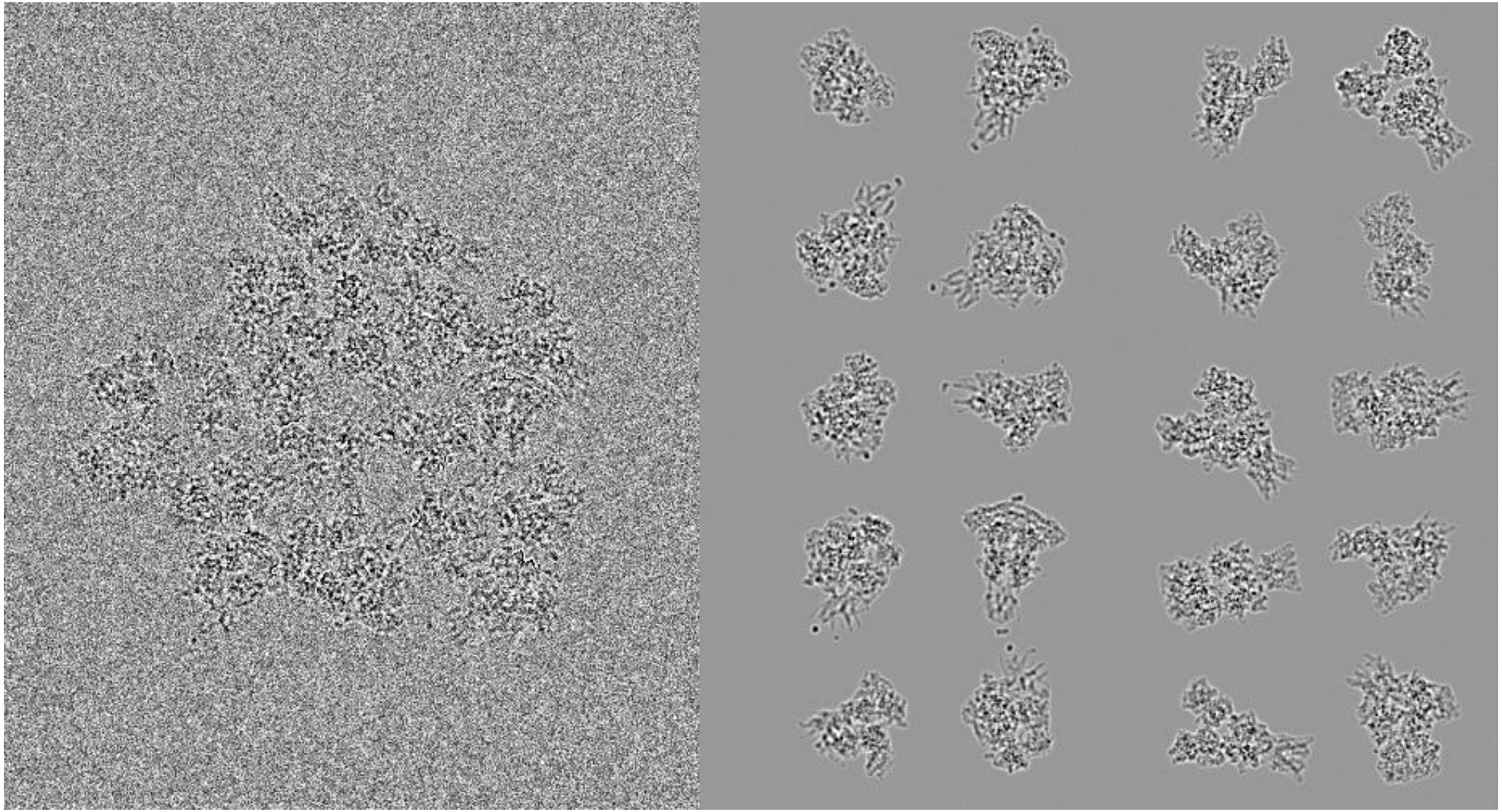
Maurer et al. 2008 (PMID: 18653756)

High Resolution Fingerprints

Close-to-high resolution EM image

NMDA receptor

AMPA receptor

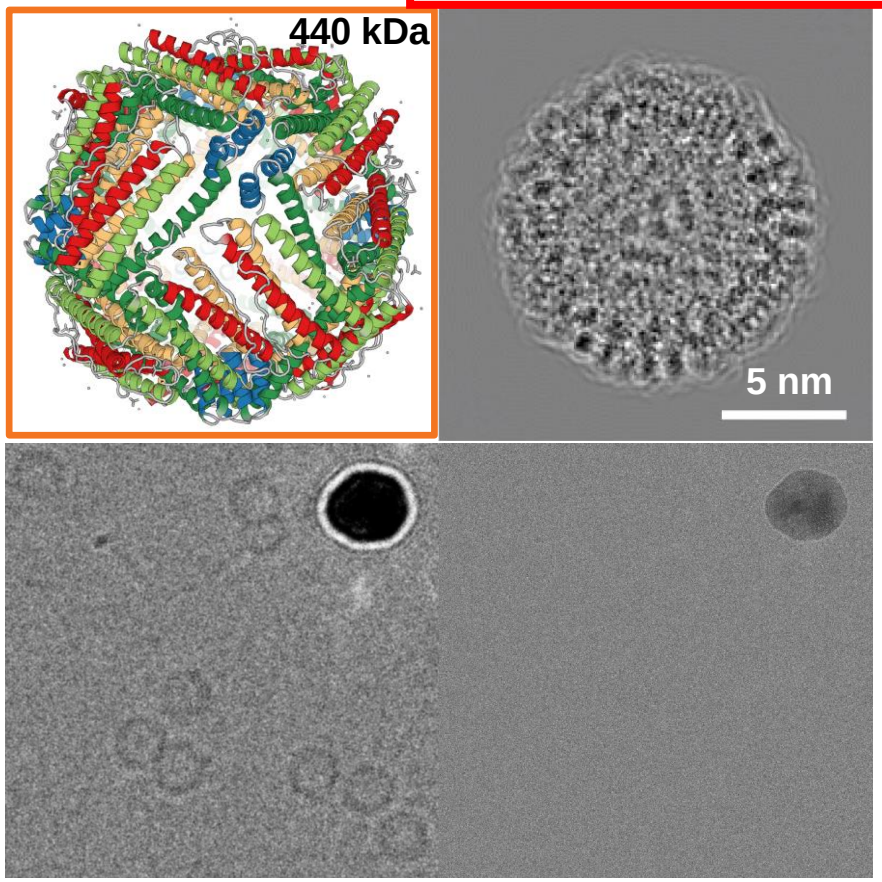


Proof of Principle

Apoferritin

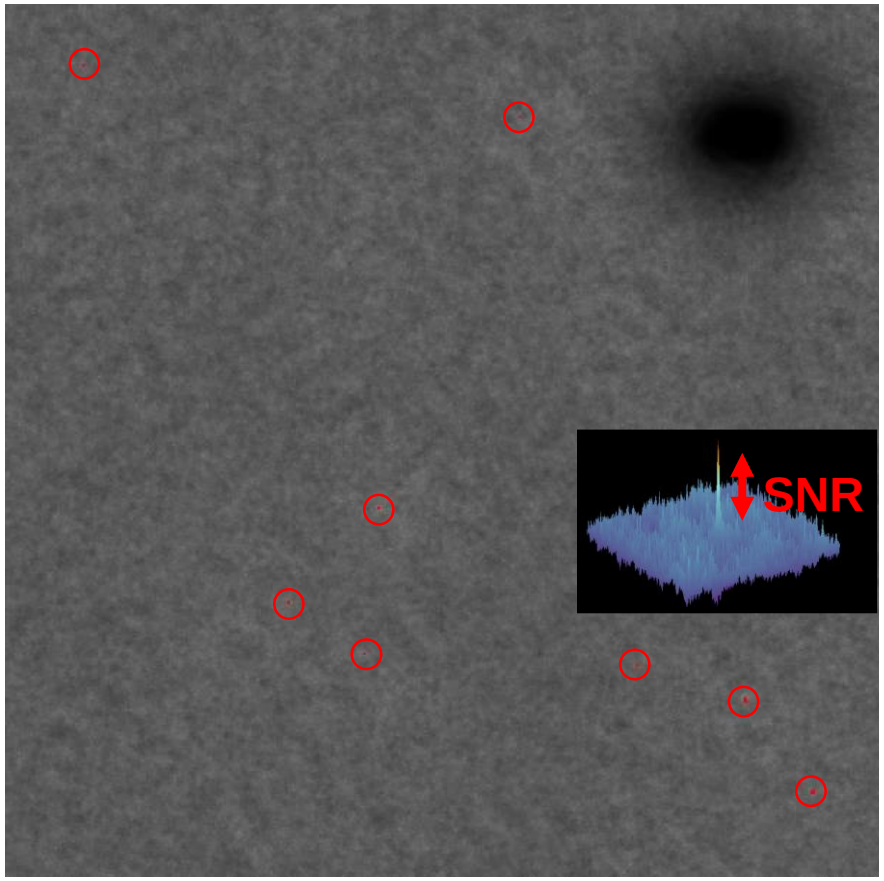
2.4 million projections

Computationally expensive!



Defocused image (control)

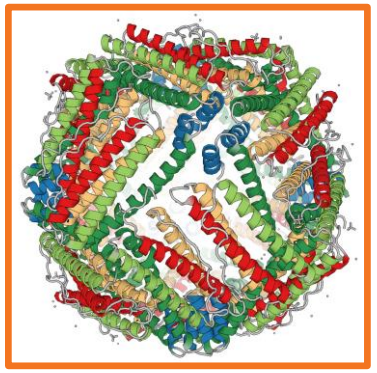
Cryo-EM image



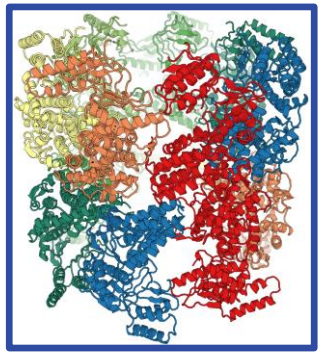
Correlation map
(maximum correlation value at each point)

Rickgauer et al. 2017 (PMID: 28467302)

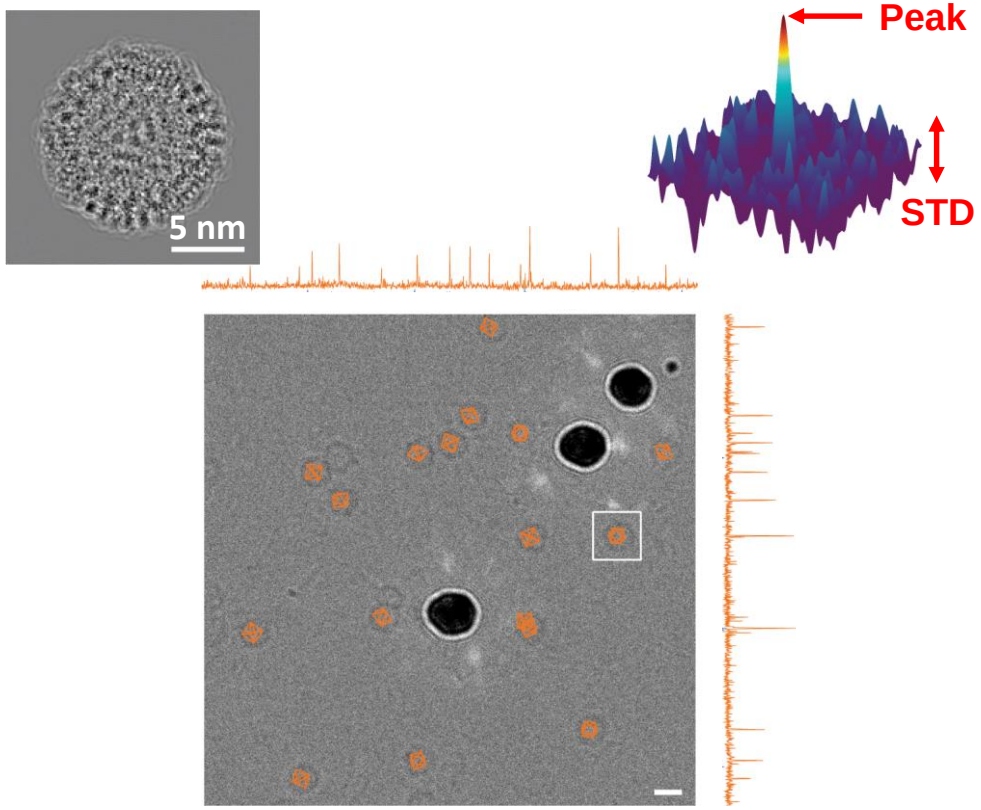
High Specificity



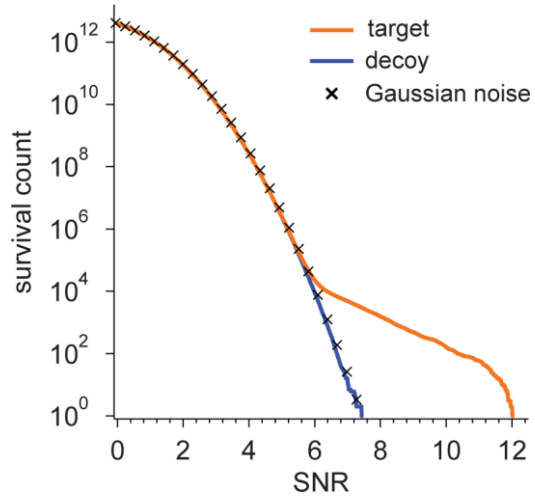
Apoferritin



GroEL (decoy)

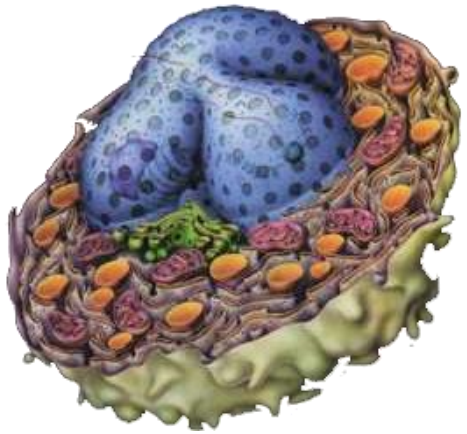


SNR = Peak / STD
≈ 12 (apoferritin)

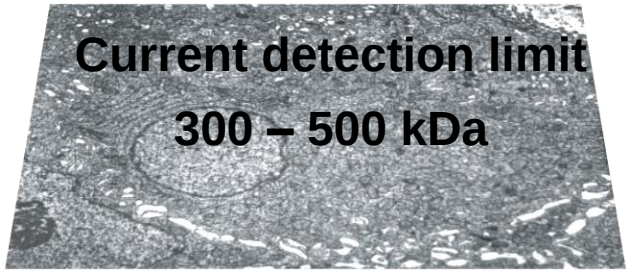


Rickgauer et al. 2017 (PMID: 28467302)

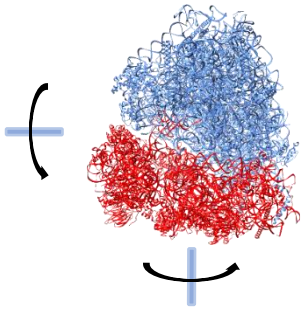
2D Template Matching



One exposure of untilted sample

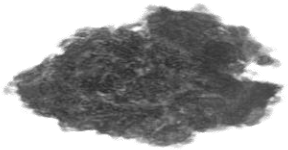


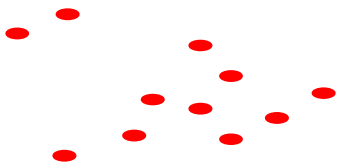
2 – 3 million orientations



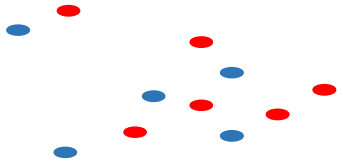
High-resolution template

Projections

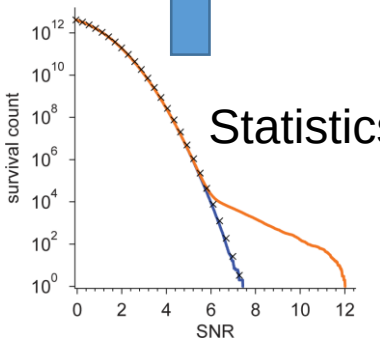




Correlation peaks



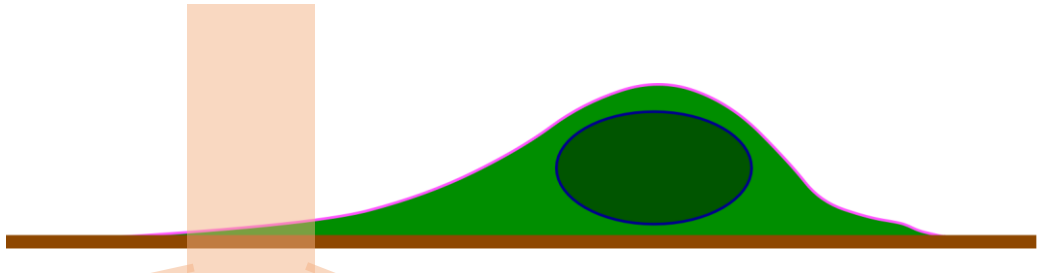
Detected targets

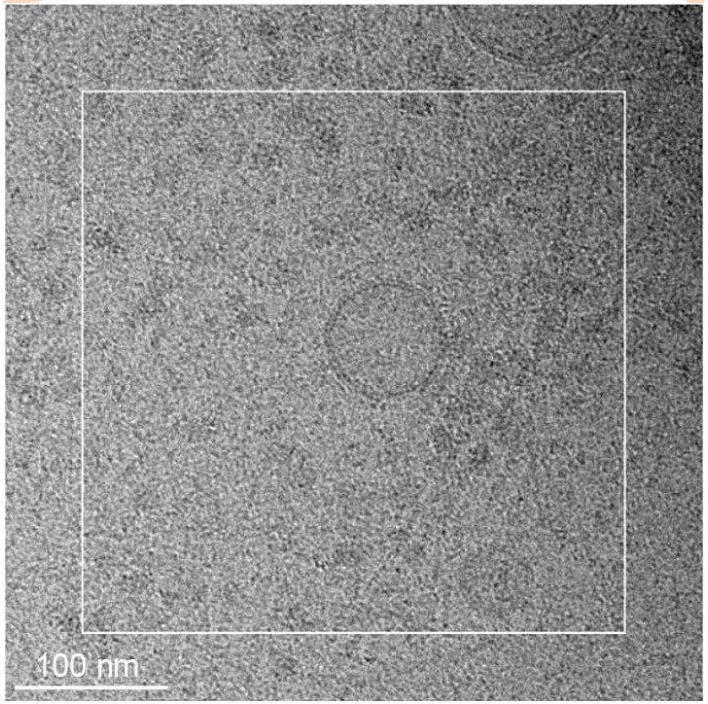


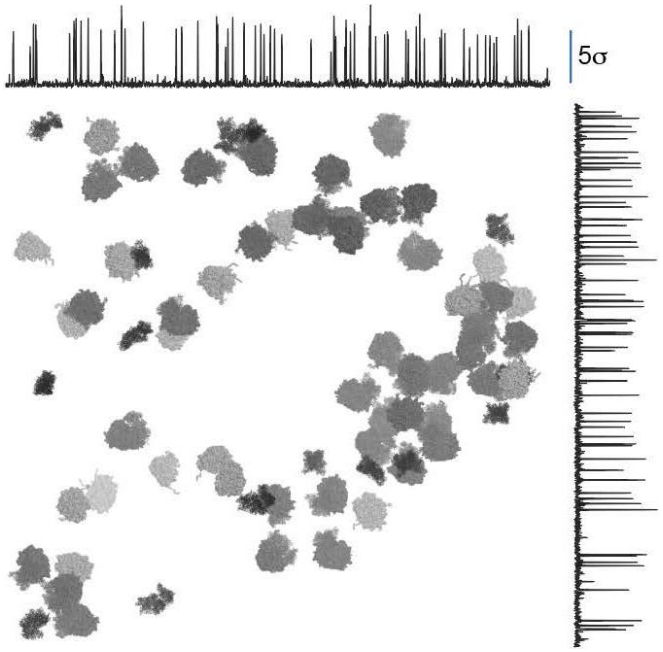
Statistics

Rickgauer et al. 2017 (PMID: 28467302)

Detecting Ribosomes in Cells



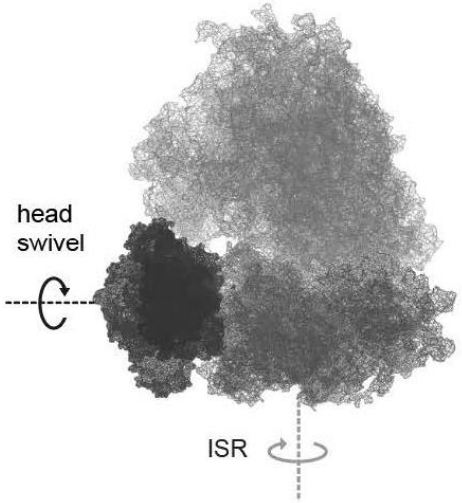




Cells: Mouse embryonic fibroblast

Template: Human 80S ribosome

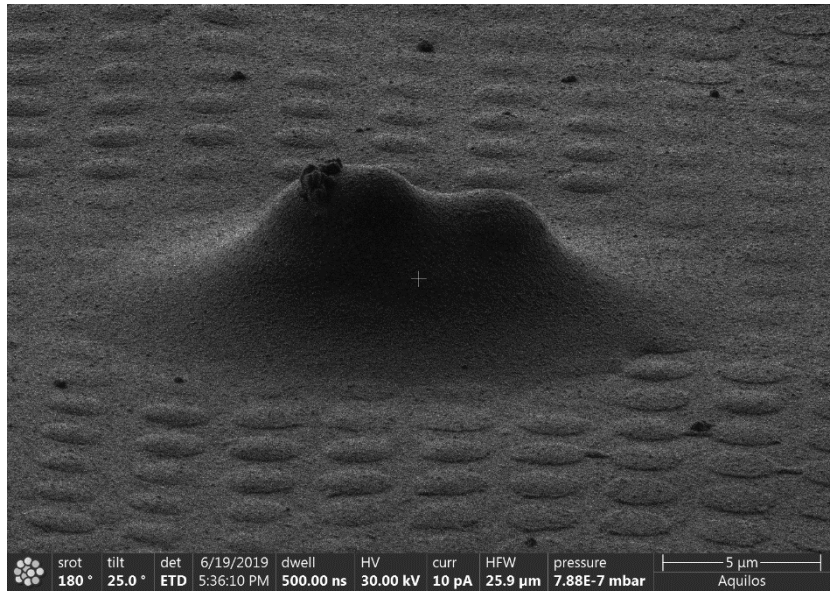
6ek0: 80S ribosome

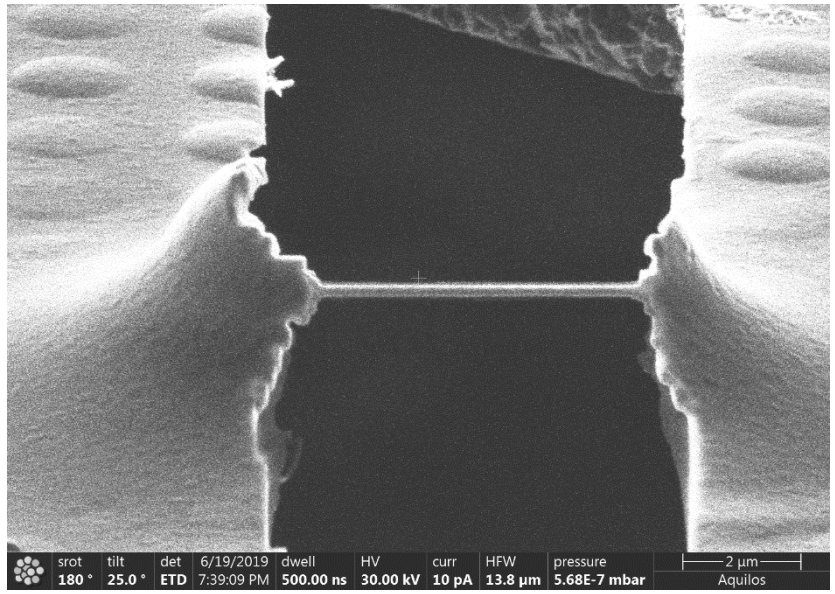


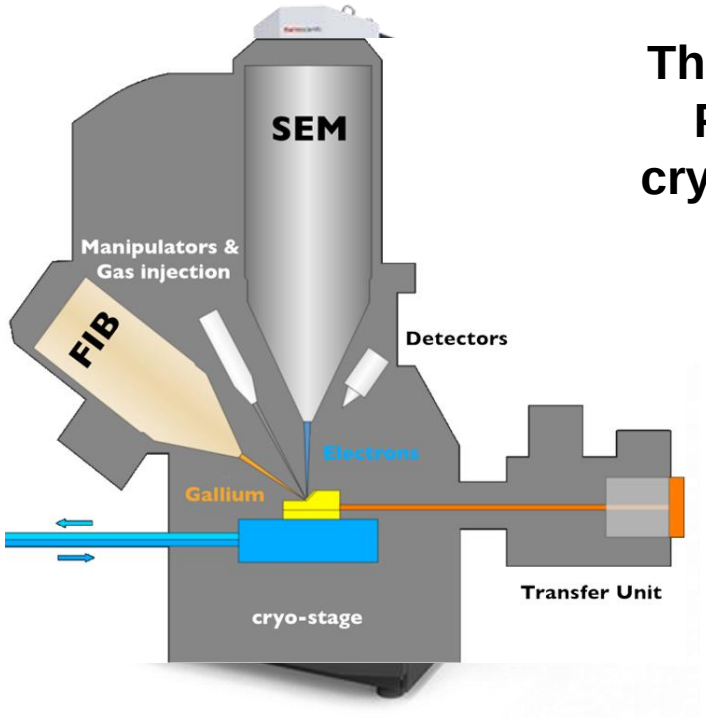
Natchiar et al. 2017
(PMID: 29143818)

Rickgauer et al. 2020 (doi: 10.1101/2020.04.22.053868)

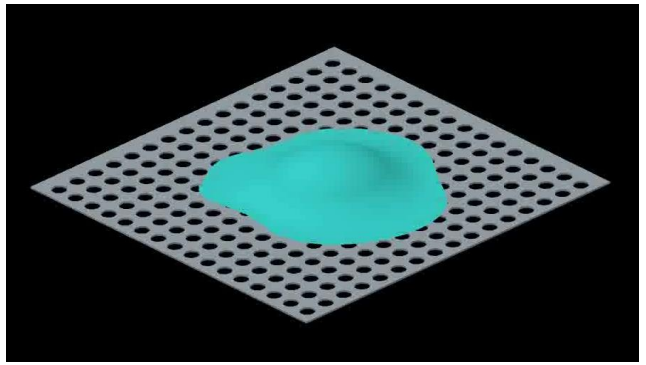
FIB-milling frozen S. cerevisiae







Thermo Fisher Aquilos Focused Ion Beam cryo-milling instrument



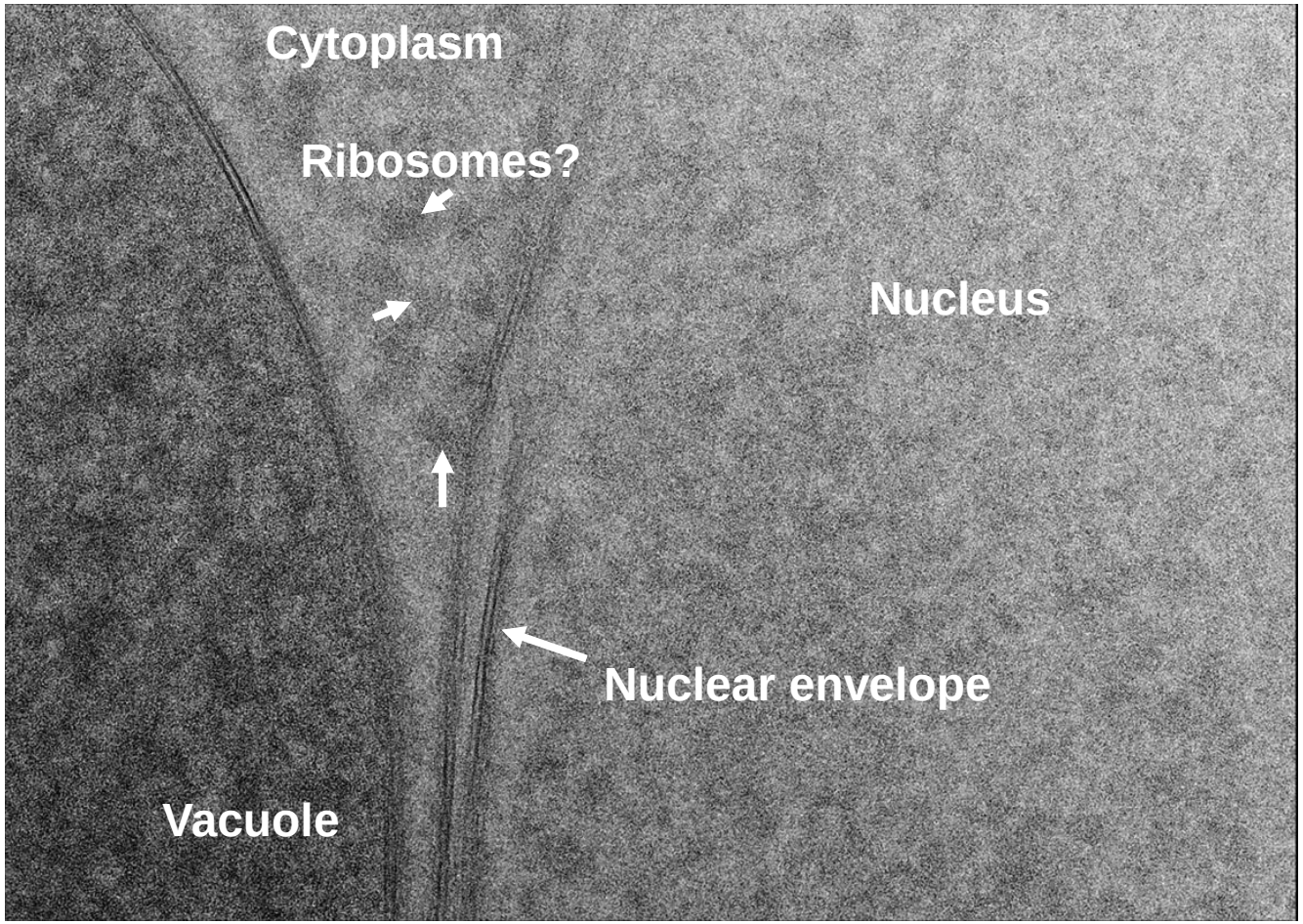
Animation by **Tim Laugks**

MPI of Biochemistry

Martinsried

Lucas et al. 2022 (PMID: 36005291)

Nuclear Periphery

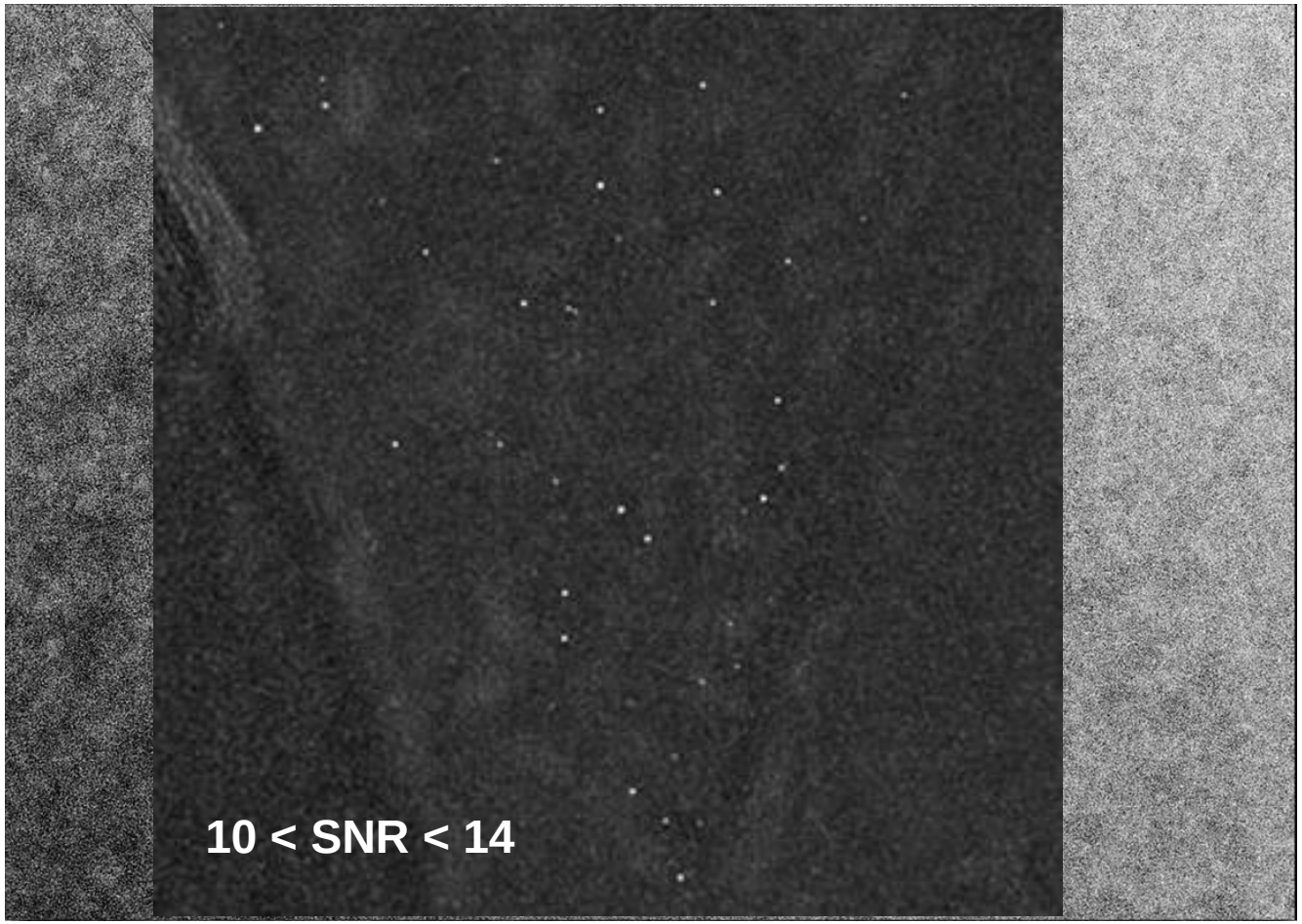


Titan Krios, Gatan K3
Sample thickness: 150 nm

500 Å

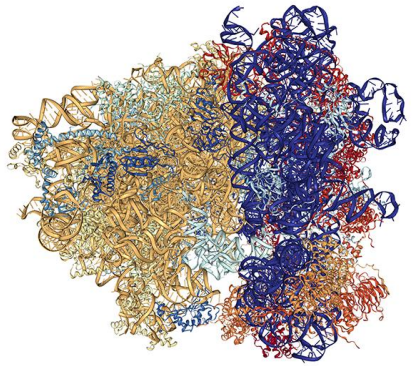
Lucas et al. 2022 (PMID: 36005291)

Template Search



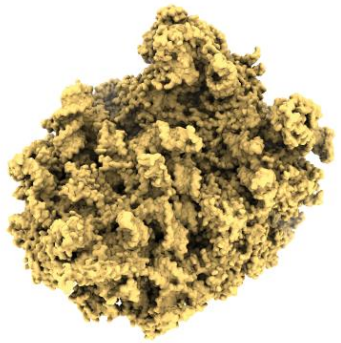
Titan Krios, Gatan K3
Sample thickness: 150 nm

500 Å



6q8y: 60S large ribosomal subunit

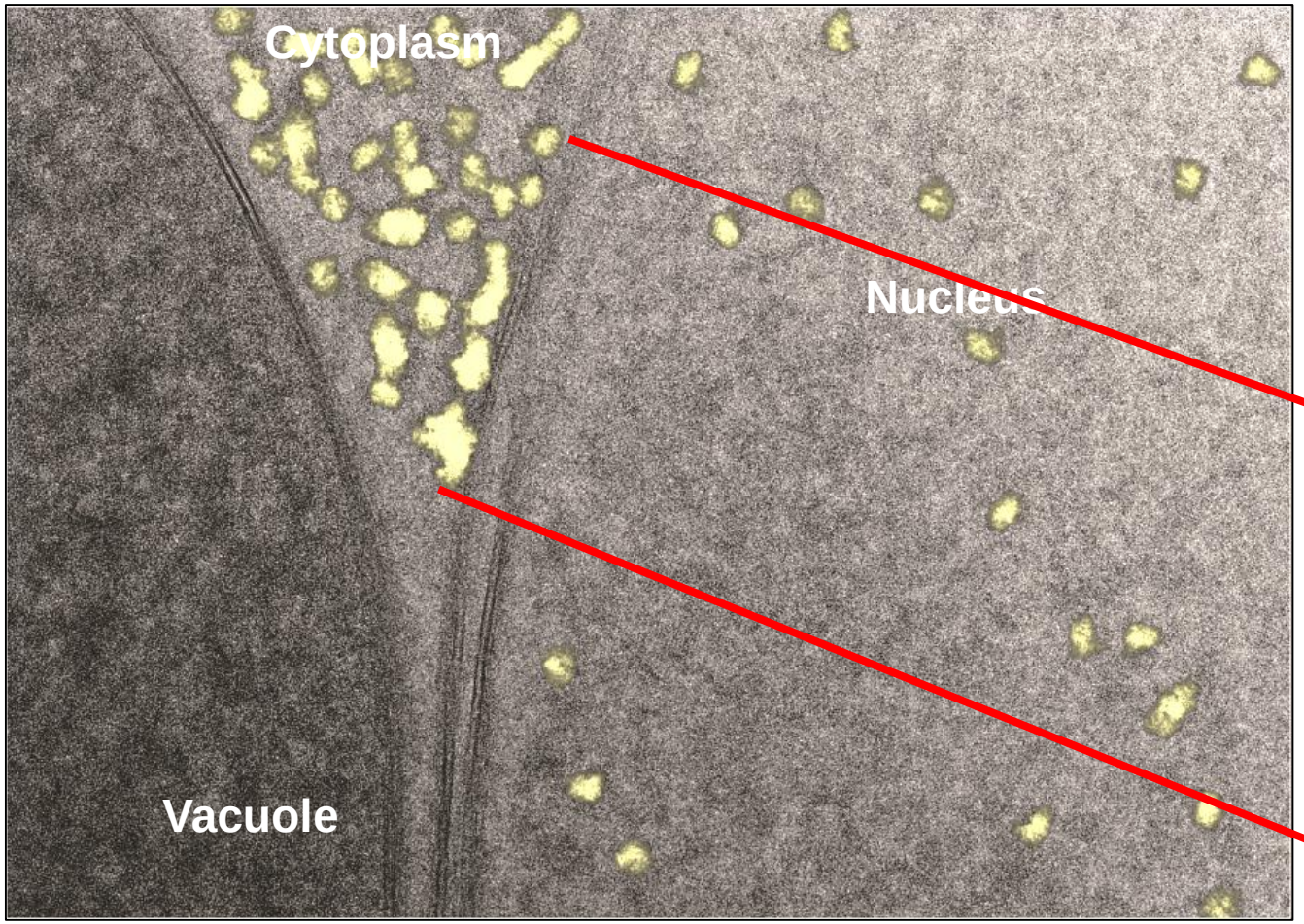
Tesina et al. 2019
(PMID: 30911188)



3D template

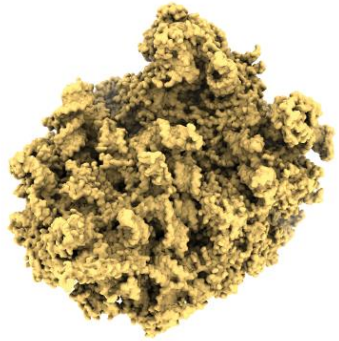
Lucas et al. 2022 (PMID: 36005291)

Detecting 60S Ribosomal Subunits



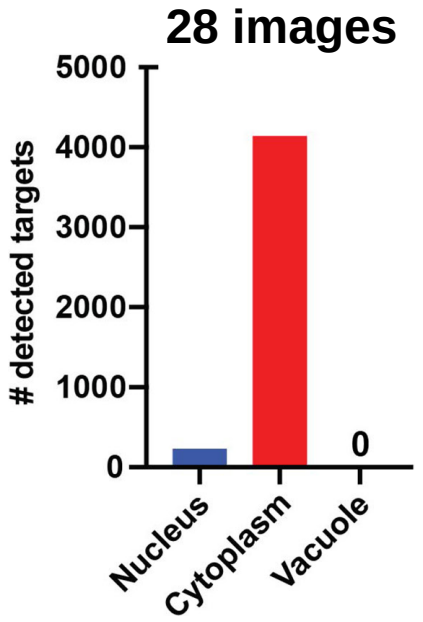
Titan Krios, Gatan K3
Sample thickness: 150 nm

500 Å



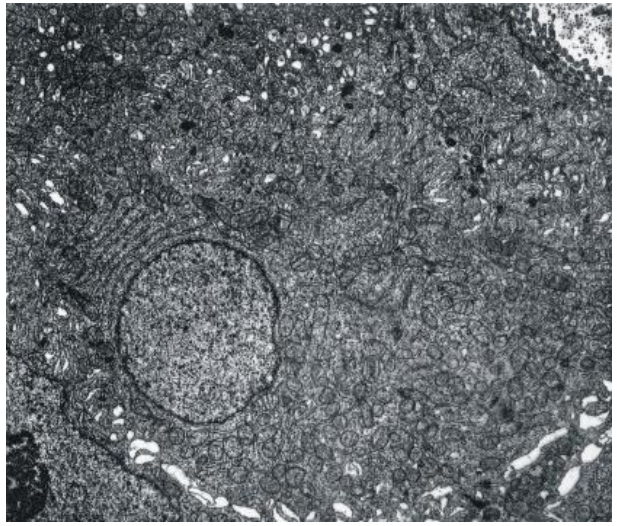
6q8y: 60S large ribosomal subunit

Tesina et al. 2019
(PMID: 30911188)

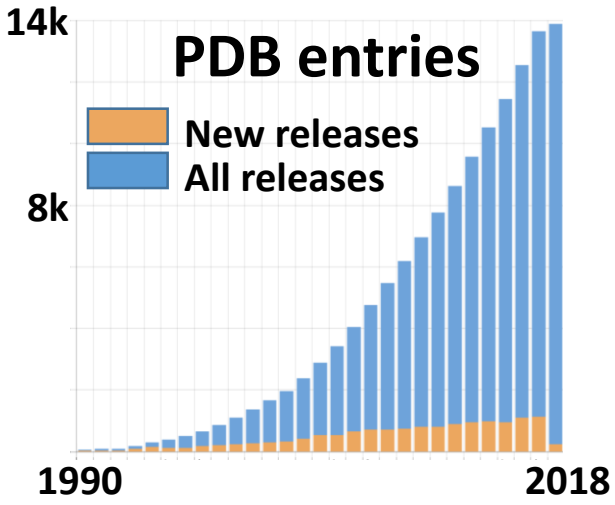


Lucas et al. 2022 (PMID: 36005291)

Prospects



Angstrom Images, LLC



+ AlphaFold 2
(machine learning)

Current molecular weight limit:

- **300 - 500 kDa** orientations not constrained → **60 - 100 kDa** if images perfect
- **100 kDa** with constraints (e.g. membrane)

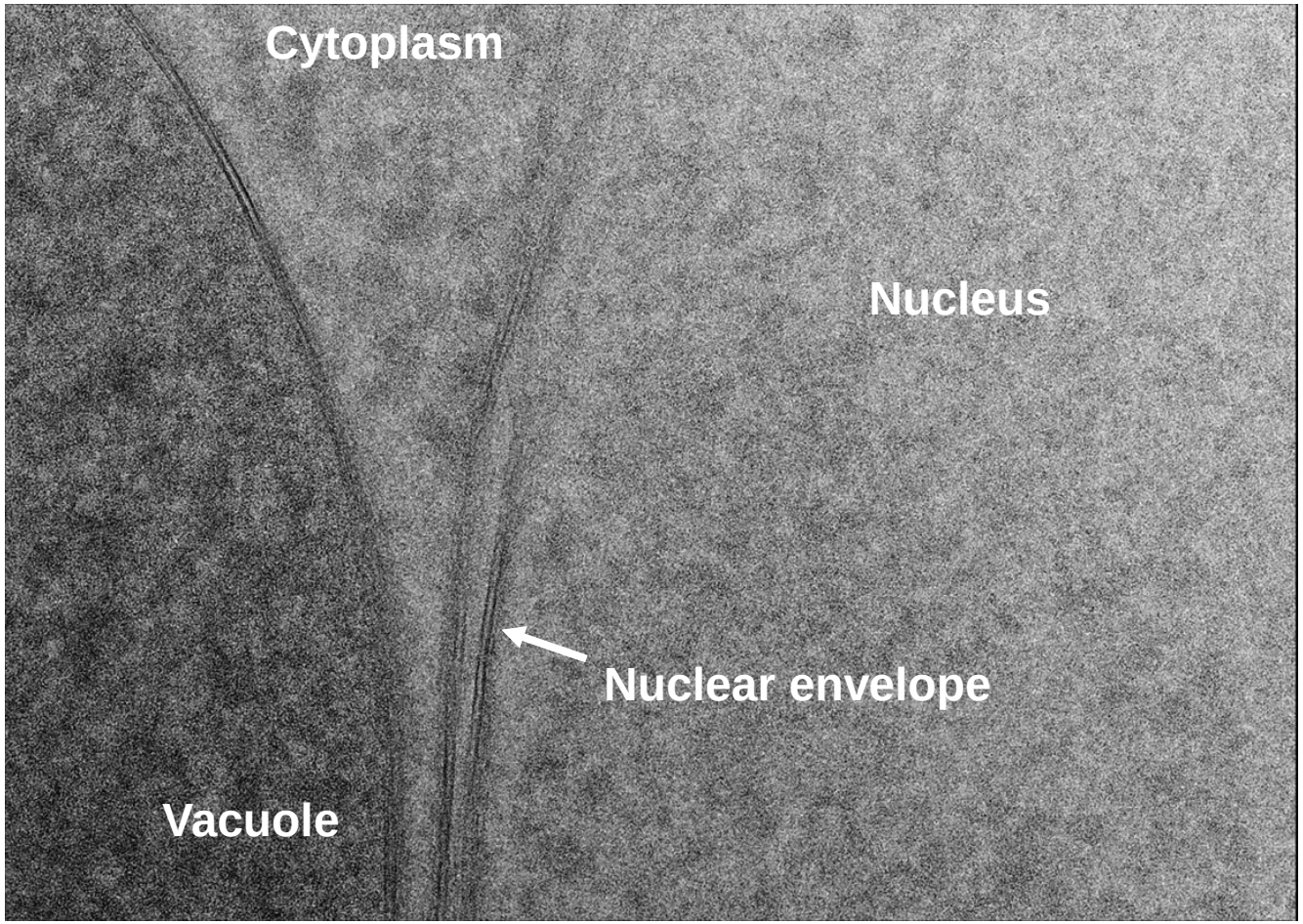
Challenges With 2DTM

- 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

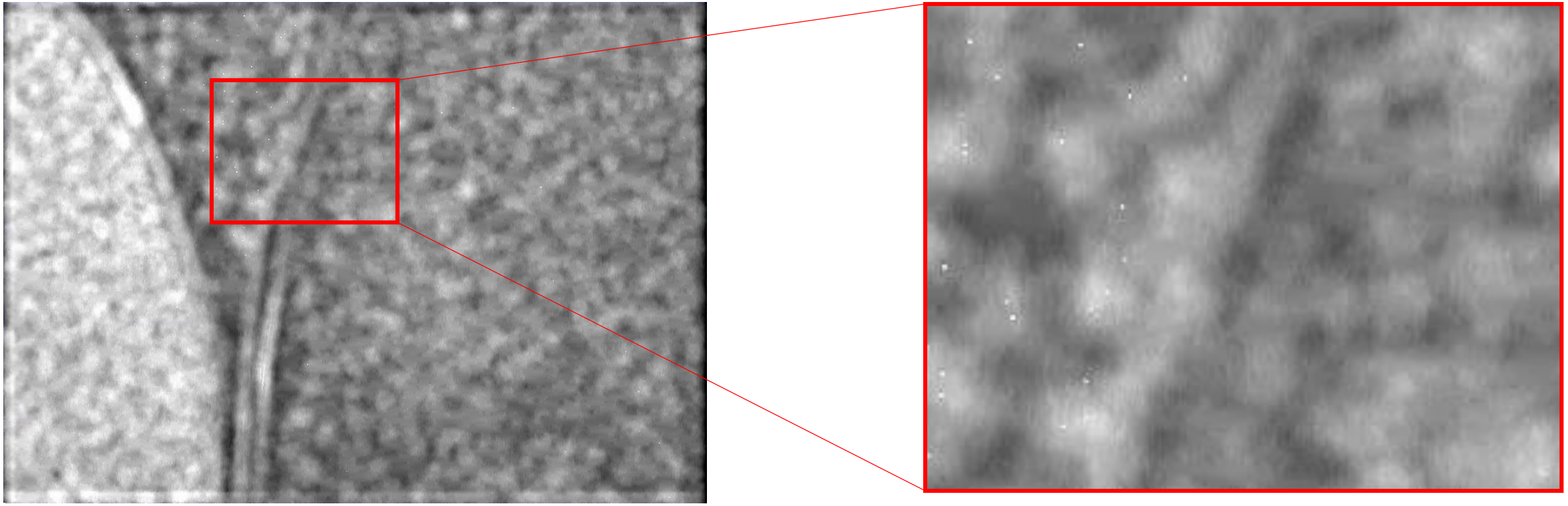


Titan Krios, Gatan K3
Sample thickness: 150 nm

500 Å

Lucas et al. 2022 (PMID: 36005291)

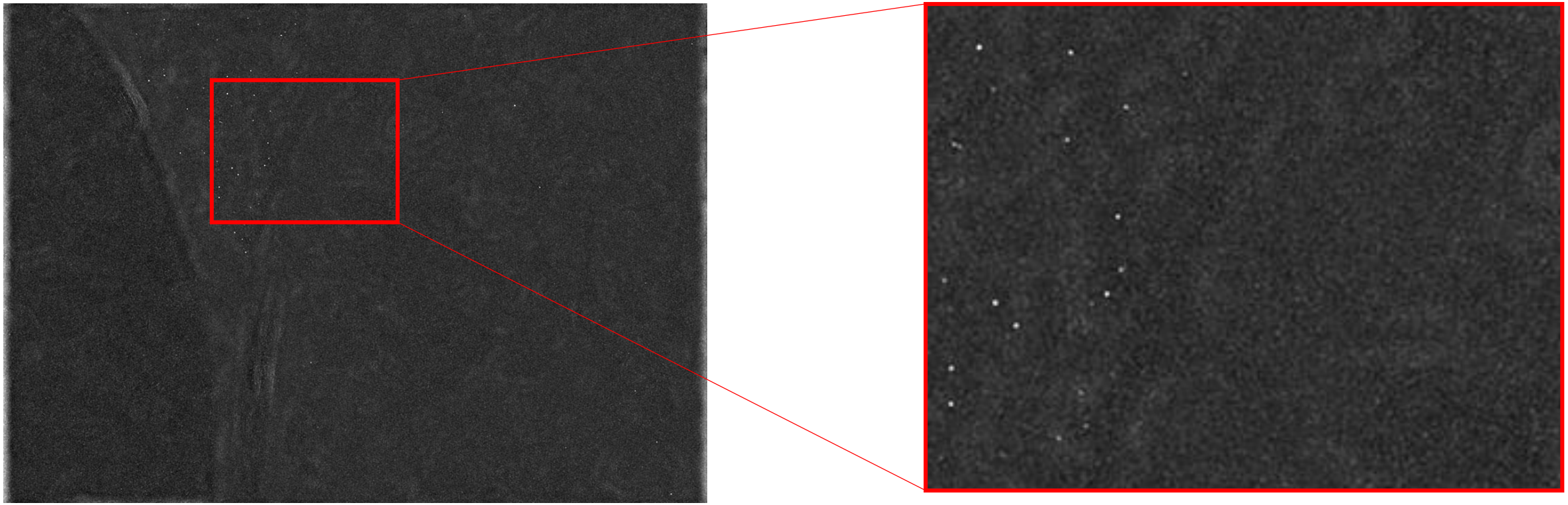
Maximum Intensity Projection (MIP)



MIP

Lucas et al. 2022 (PMID: 36005291)

Scaled MIP



Scaled MIP

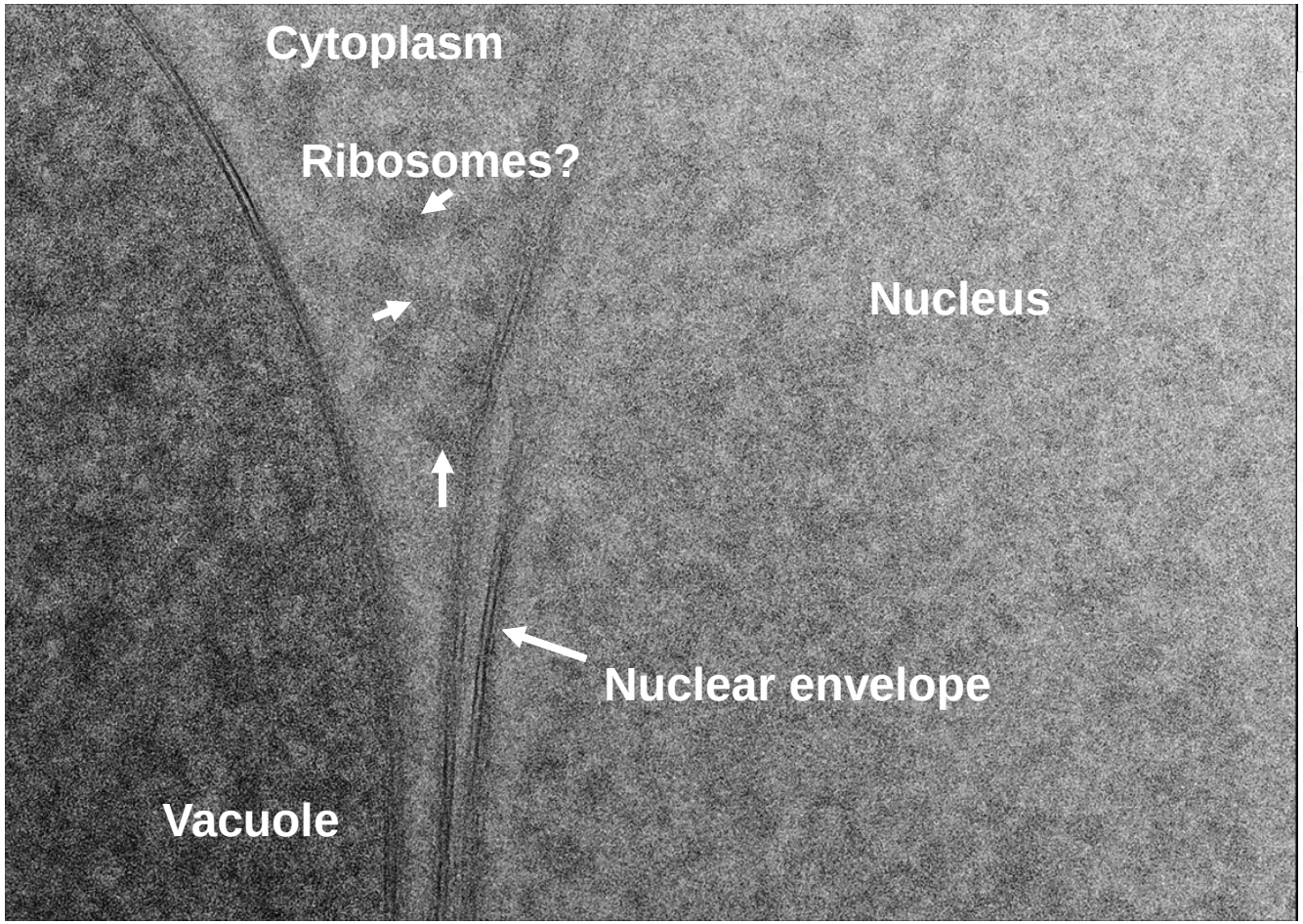
$$scaled\ mip_{xy} \approx \frac{mip(\phi,\theta,\psi,\Delta f)_{xy} - \mu(\phi,\theta,\psi,\Delta f)_{xy}}{\sigma(\phi,\theta,\psi,\Delta f)_{xy}}$$

Lucas et al. 2022 (PMID: 36005291)

Challenges With 2DTM

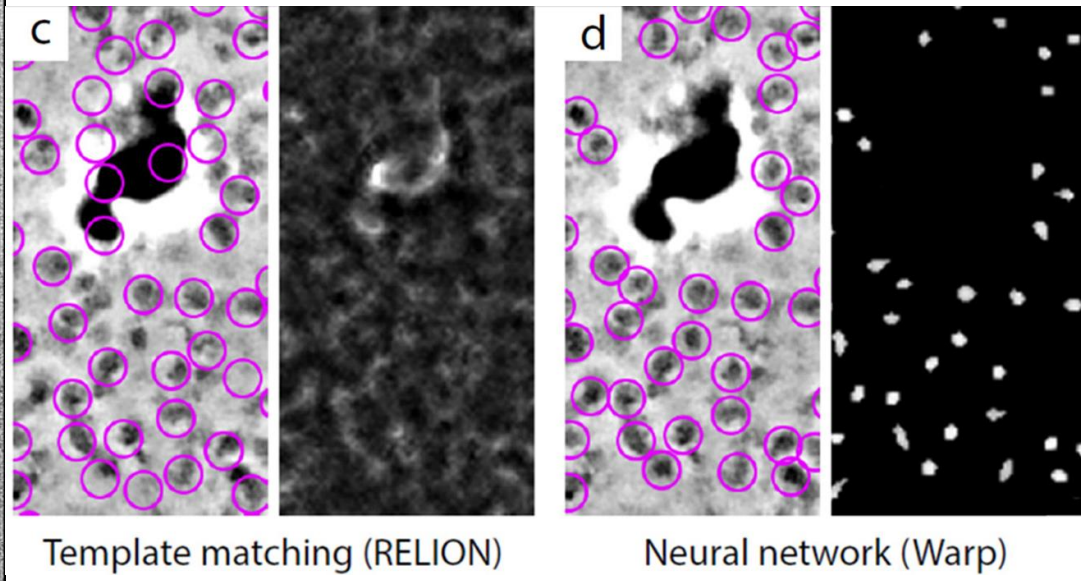
- Sample **inhomogeneity**
- **Low-resolution** contrast
- Targets **too small** / many false negatives
- Templates **mismatch**

Low-Resolution Contrast



Titan Krios, Gatan K3
Sample thickness: 150 nm

500 Å



Glaeser et al. 2021

How to combine these approaches?

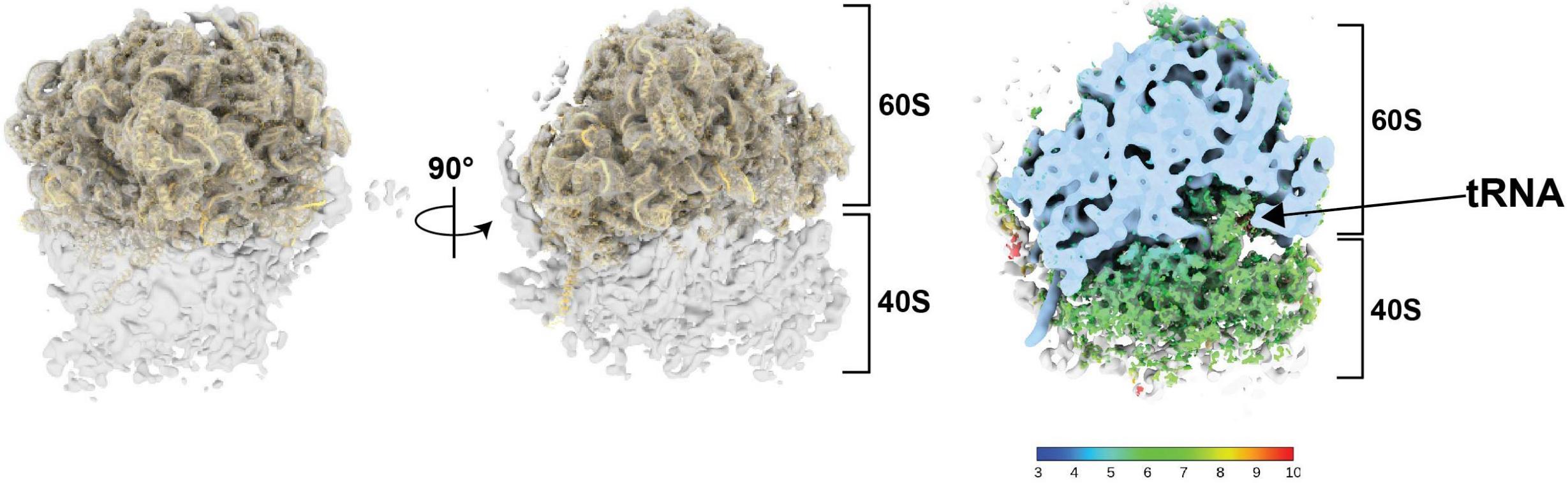
Lucas et al. 2022 (PMID: 36005291)

Challenges With 2DTM

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

Detecting Extra Density

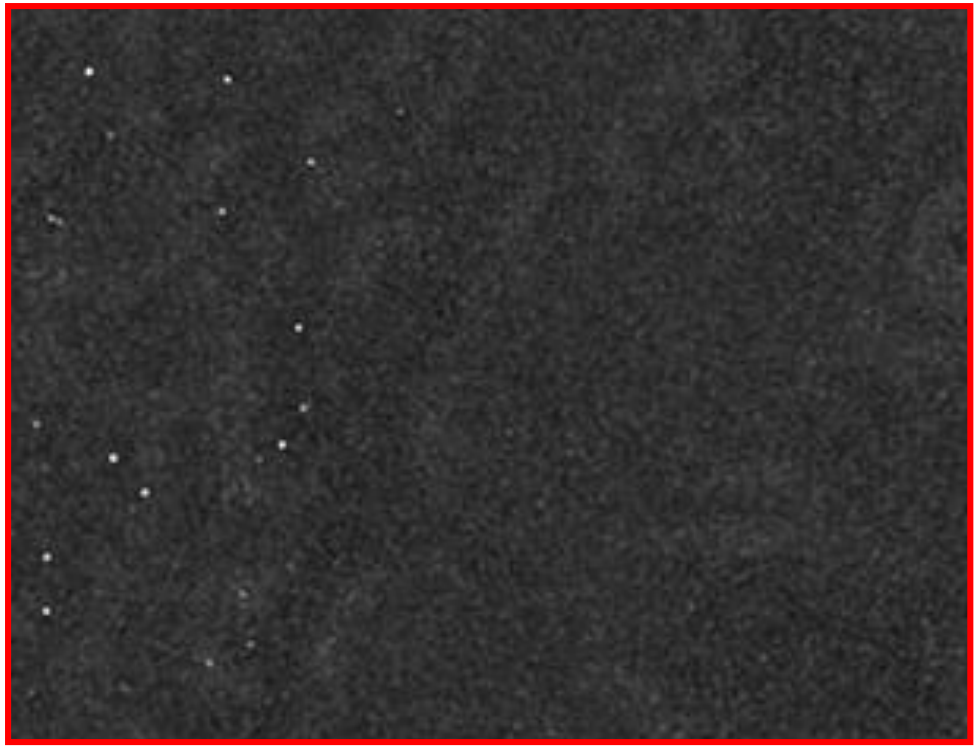
28 micrographs, 3991 detected targets



Lucas et al. 2022 (PMID: 36005291)

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

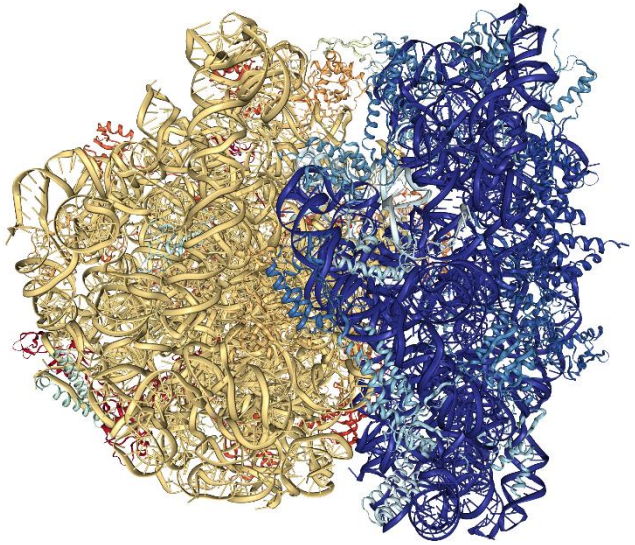


Lucas et al. 2022 (PMID: 36005291)

Combining 2D and 3DTM: Ribosomes in Bacteria

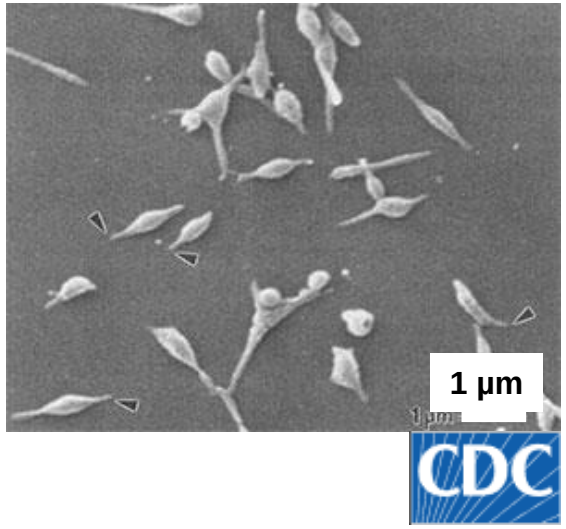
50S large ribosomal subunit
1.3 MDa

30S small ribosomal subunit
0.7 MDa



Bacillus subtilis
70S ribosome

Mycoplasma pneumoniae



Thin enough to be electron transparent

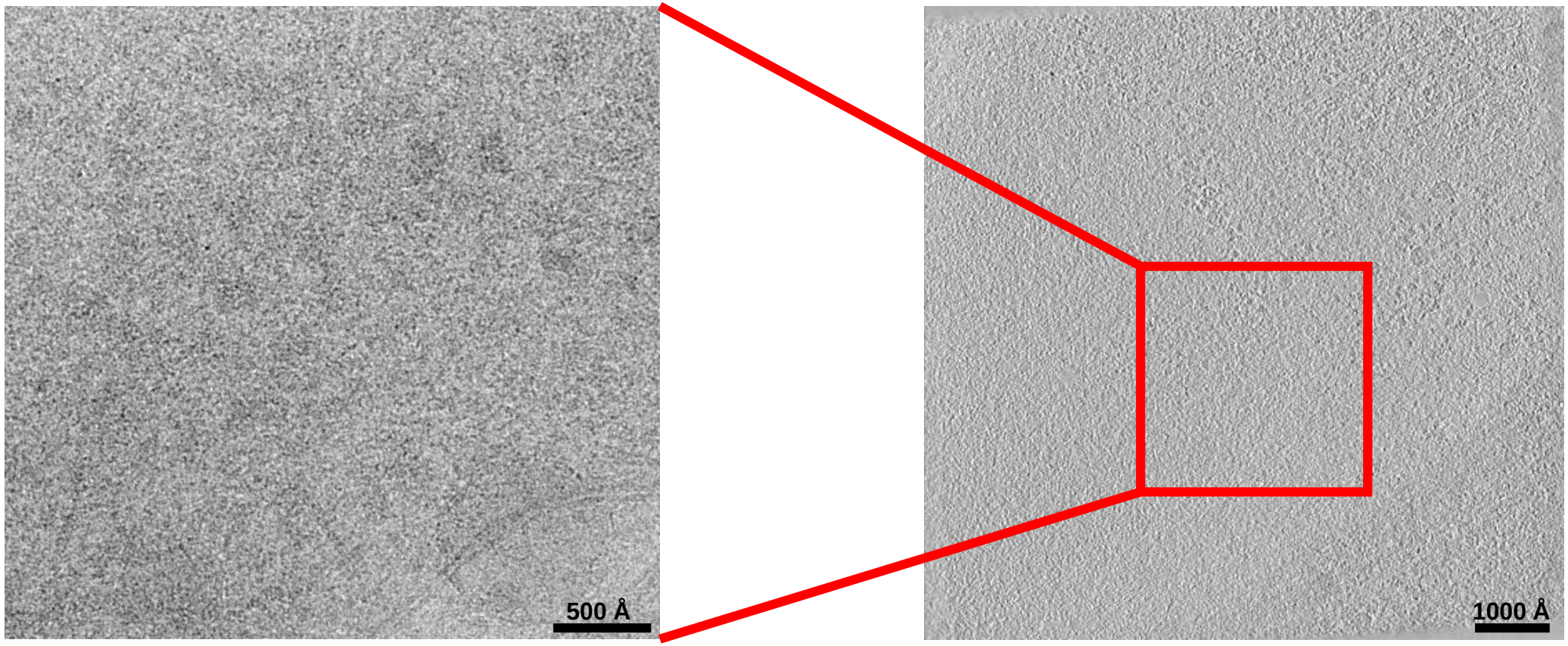
Sohmen et al. 2015 (PMID: 25903689)

Lucas et al. 2021 (PMID: 34114559)

Two Experiments

Single image of untilted sample (30 e/Å^2)

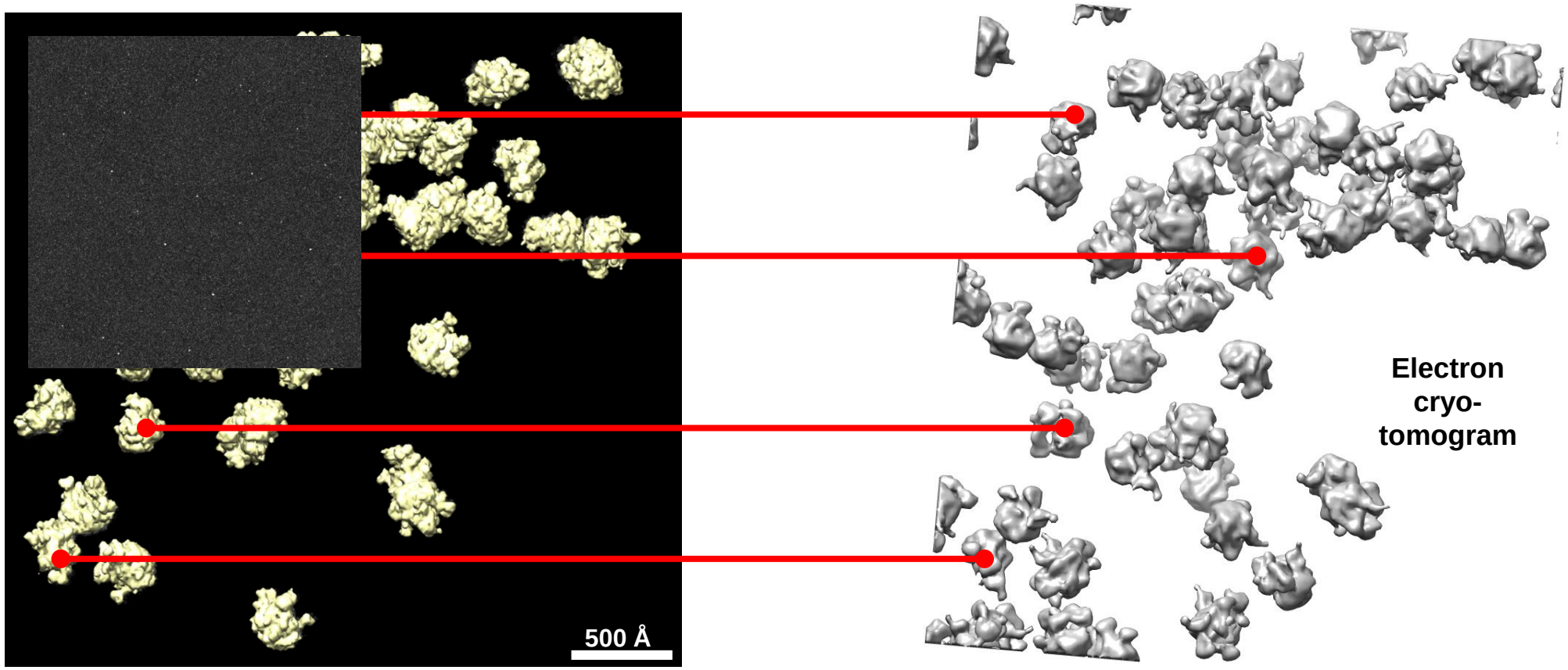
Tomogram (80 e/Å^2)



Sample thickness: 150 nm

Lucas et al. 2021 (PMID: 34114559)

Large Ribosomal Subunits



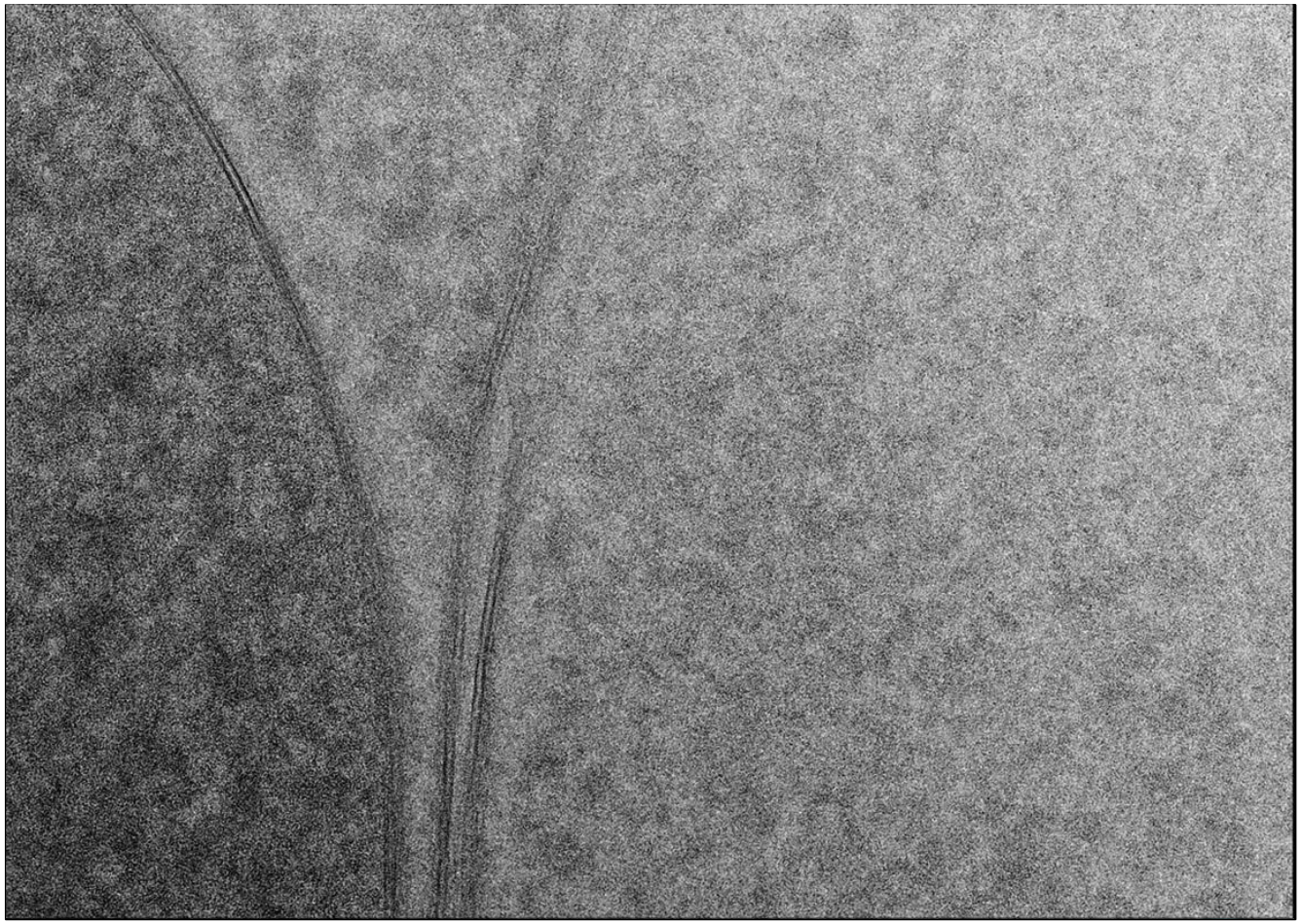
Particle defocus refinement → z coordinates

Lucas et al. 2021 (PMID: 34114559)

Challenges With 2DTM

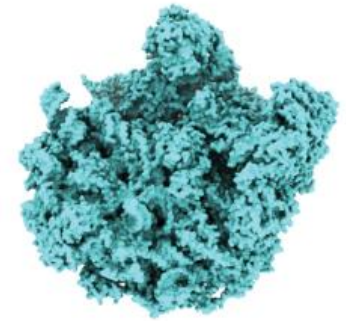
- Sample **inhomogeneity**
- **Low-resolution** contrast
- Targets **too small** / many false negatives
- Templates **mismatch**

Detecting Pre-60S Subunits



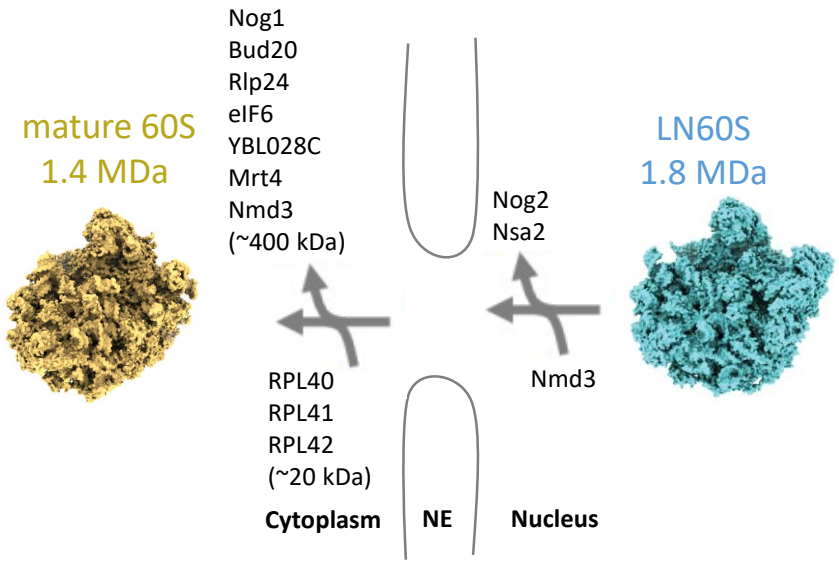
Titan Krios, Gatan K3
Sample thickness: 150 nm

500 Å



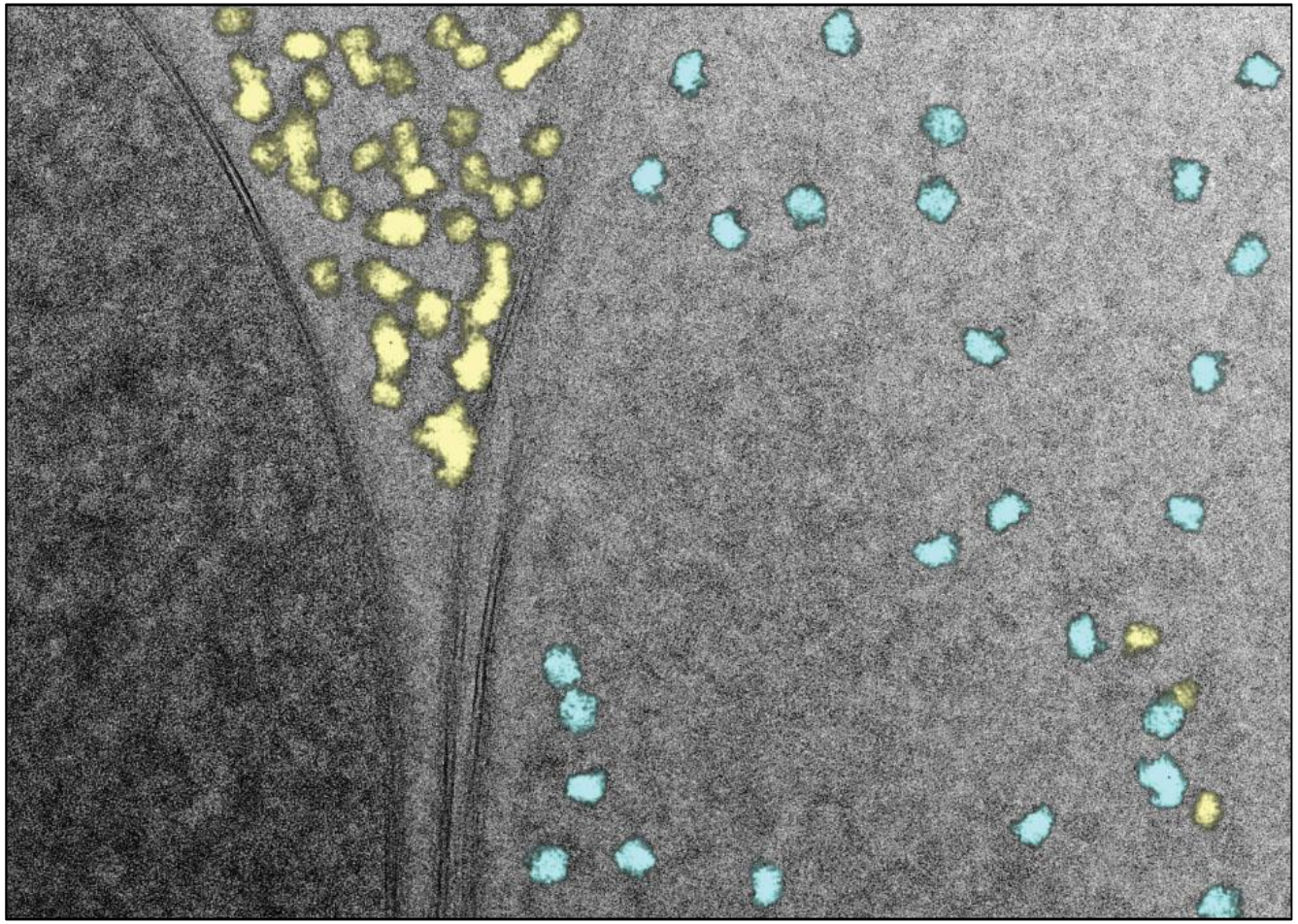
6n8j: late nuclear pre-60S subunit

Zhou et al. 2019
(PMID: 30814529)



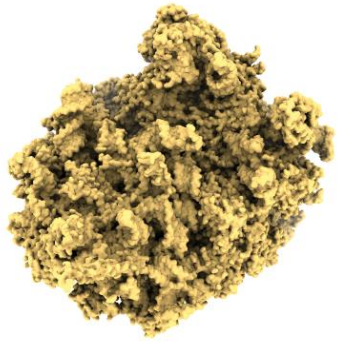
Lucas et al. 2022 (PMID: 36005291)

Distinguishing 60S From Pre-60S



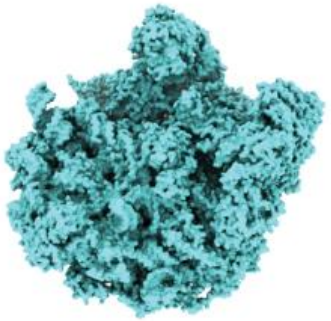
Titan Krios, Gatan K3
Sample thickness: 150 nm

500 Å



6q8y: 60S large ribosomal subunit

Tesina et al. 2019
(PMID: 30911188)



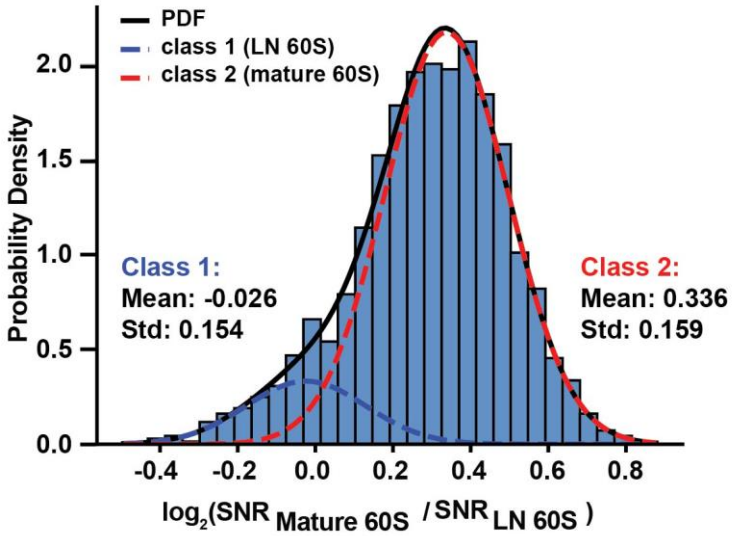
6n8j: late nuclear pre-60S subunit

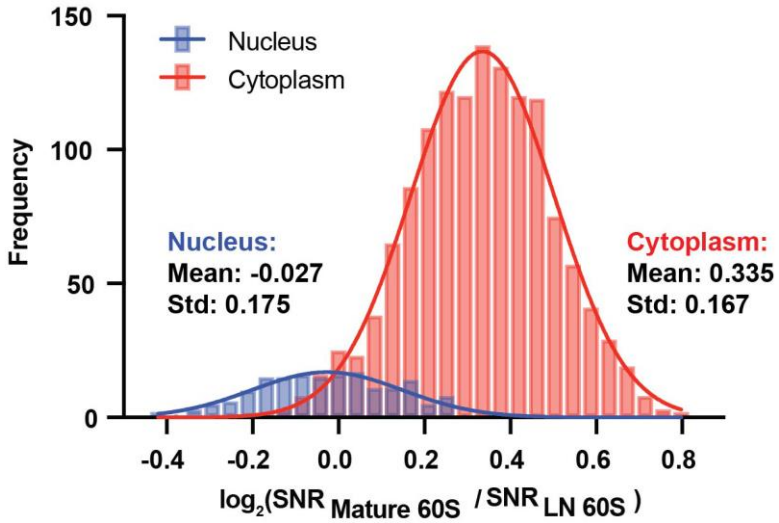
Zhou et al. 2019
(PMID: 30814529)

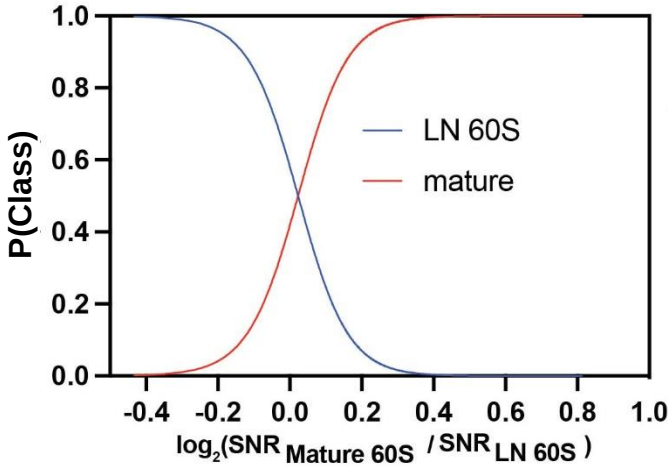
Lucas et al. 2022 (PMID: 36005291)

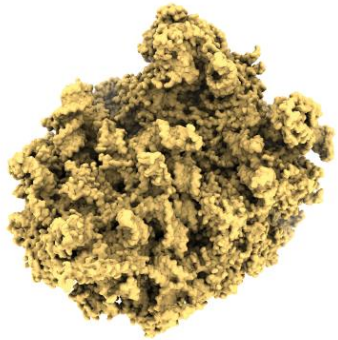
Distinguishing 60S From Pre-60S

28 micrographs, 1531 detected targets



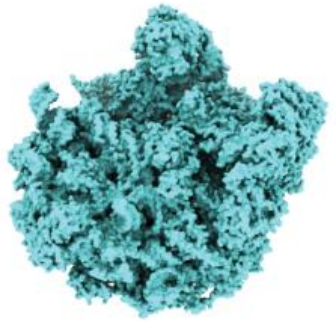






6q8y: 60S large ribosomal subunit

Tesina et al. 2019
(PMID: 30911188)

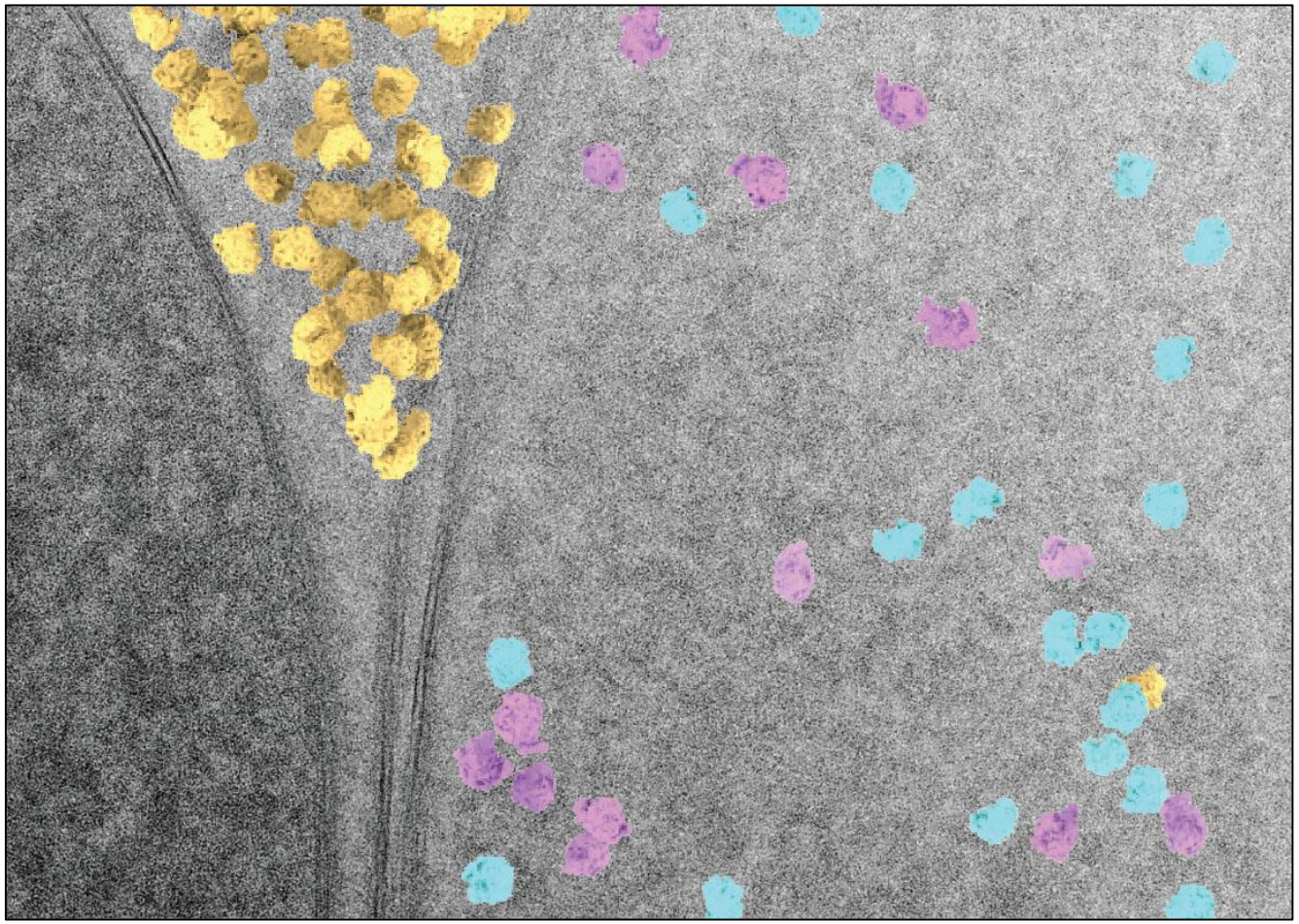


6n8j: late nuclear pre-60S subunit

Zhou et al. 2019
(PMID: 30814529)

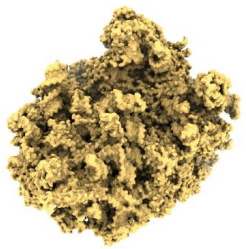
Lucas et al. 2022 (PMID: 36005291)

Distinguishing Pre-60S Intermediates

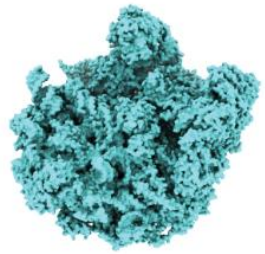


Titan Krios, Gatan K3
Sample thickness: 150 nm

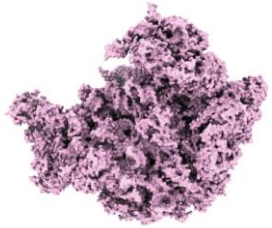
500 Å



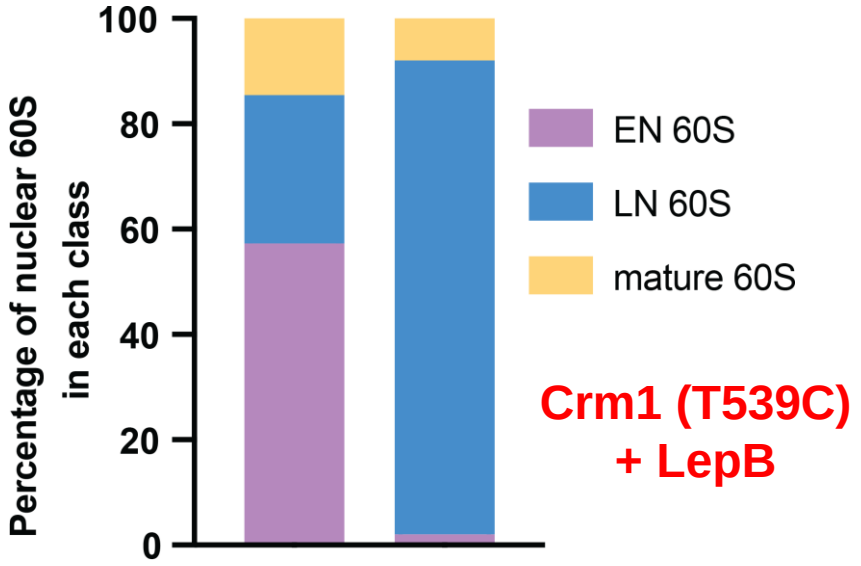
6q8y
Mature 60S



6n8j
Late nuclear
pre-60S

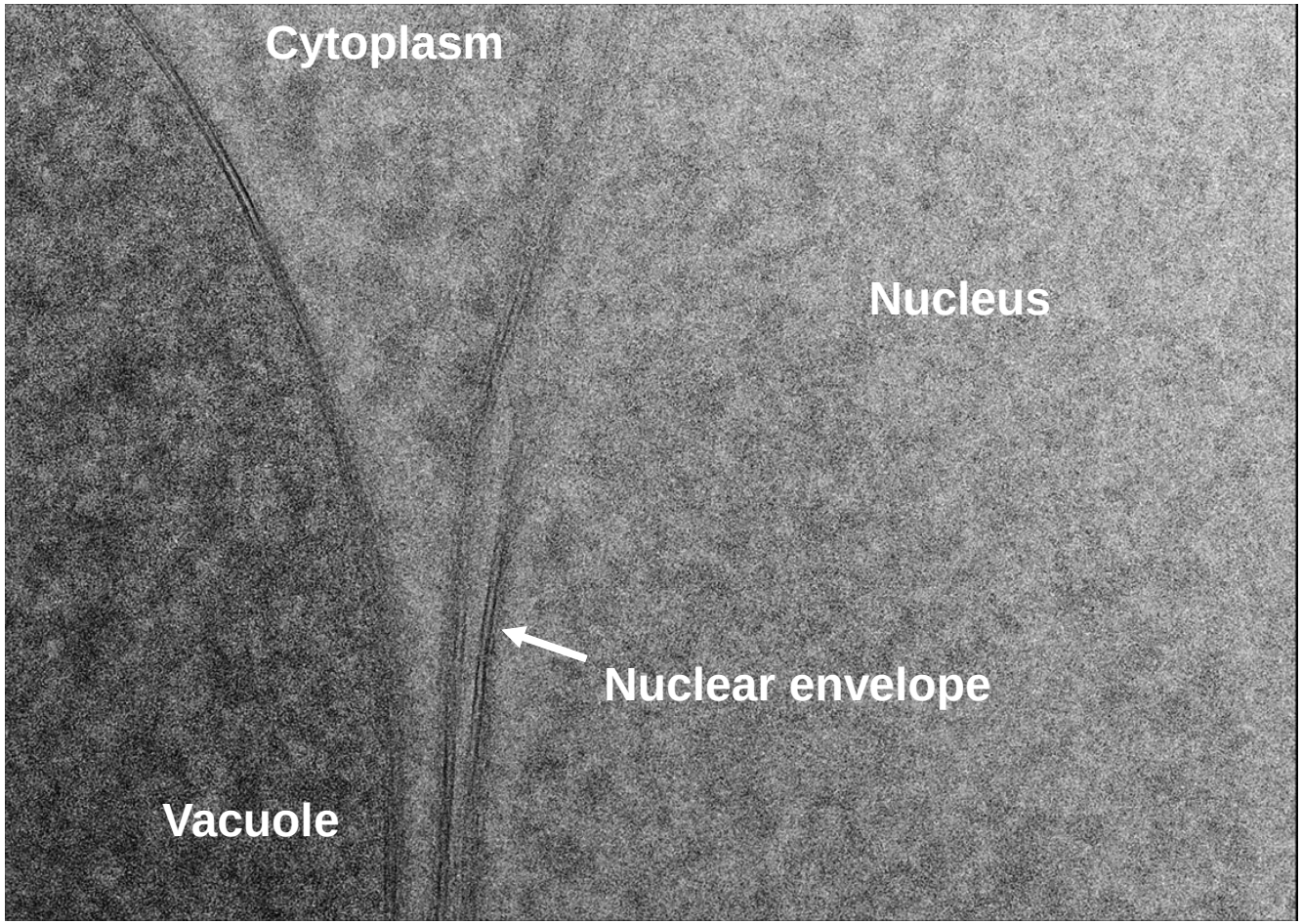


3jct
Early nuclear
pre-60S



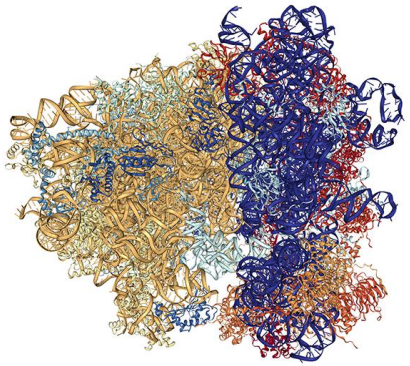
Lucas et al. 2022 (PMID: 36005291)

Refining Atomic Models Against Images?



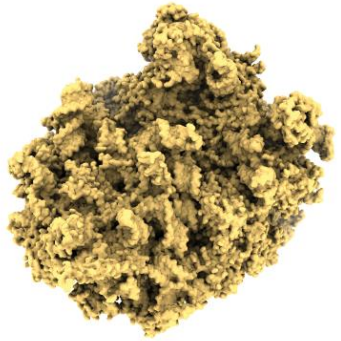
Titan Krios, Gatan K3
Sample thickness: 150 nm

500 Å



6q8y: 60S large ribosomal subunit

Tesina et al. 2019
(PMID: 30911188)



Maximize SNR

Acknowledgements

Financial Support: **HHMI, NIH, CZI**

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