

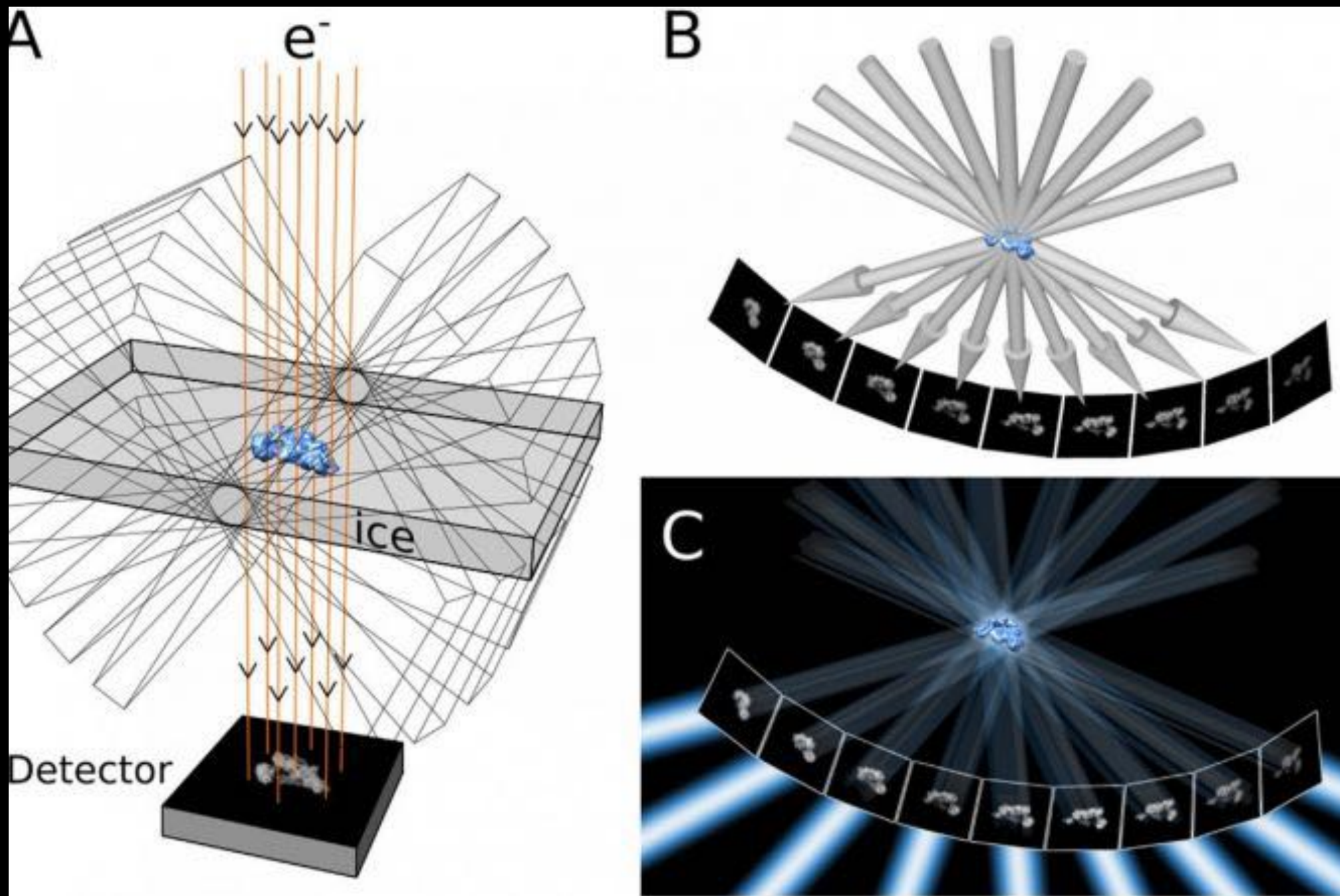


Workshop on challenges for high speed tomography **Liu Lab**

Using high-throughput cryo-ET to visualize bacterial flagellar motor

Shirwei ZHU

Cryo-Electron Tomography

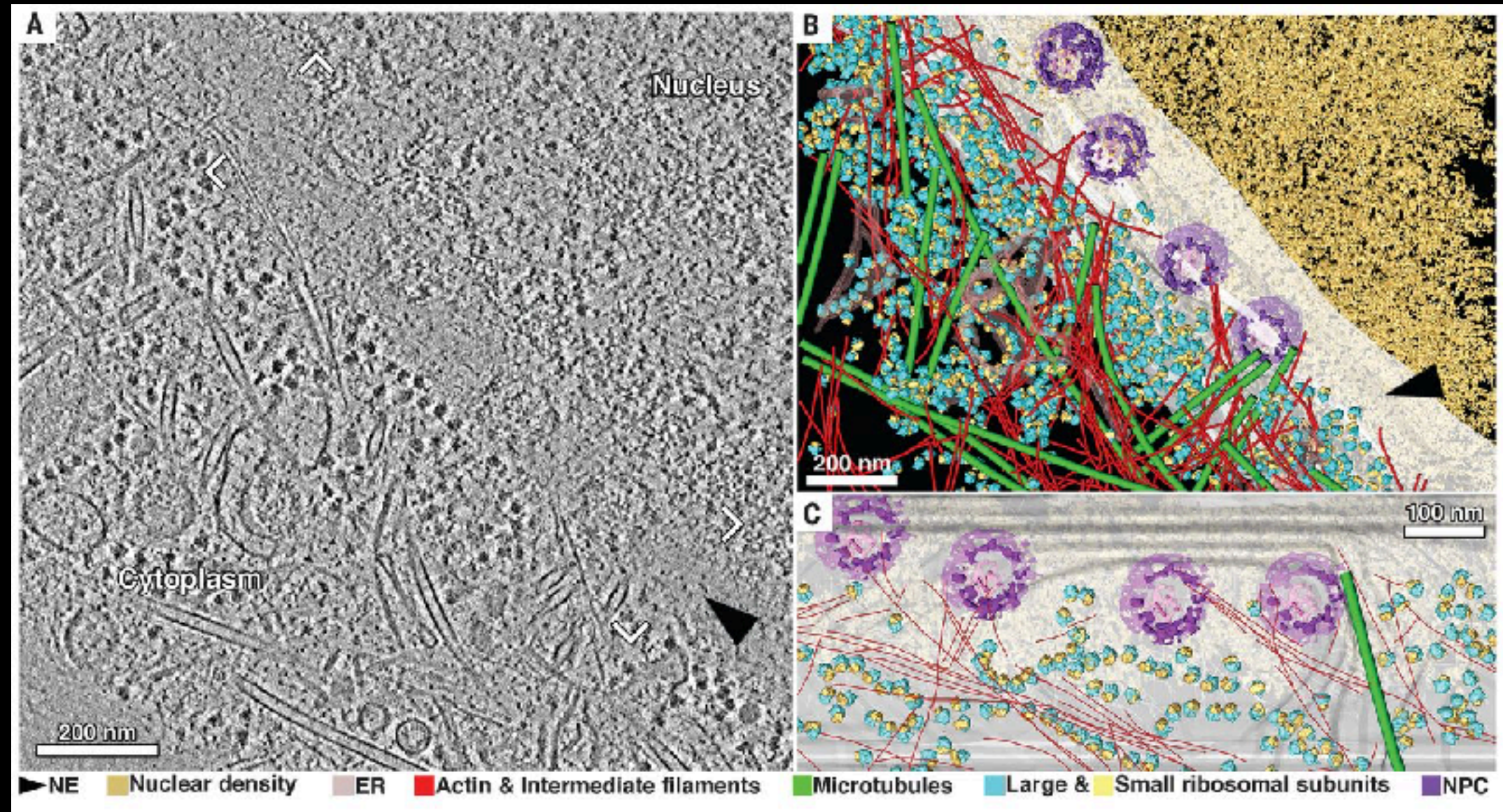


Koning *et al.*, 2018

Cryo-ET can produce a 3D macromolecular and sub-cellular structures in a near-native state.

What is cryo-ET used for?

Visualizing individual organelles within cell.



Mahamid *et al.*, 2016

Generate structural insights at a nanometer resolution
in cellular contexts.

Conditions for the tomogram collection

Titan Krios 300 kV

K2 Camera-4K*4K

SerialEM

Magnification 33,000

-50~+50 with 2°

Total dose 60 e-/Å²

VPP alignment and operation

10 tomograms taken from 6 cells

Time consuming factors: VPP alignment and tilting increase...

Why do we pursue a high speed in tomography?

Time consuming: 12 mins to 40 mins / tomogram;
About 100 tomograms /day in my lab.

How can we improve the performance of cryo-ET?

Softwares:

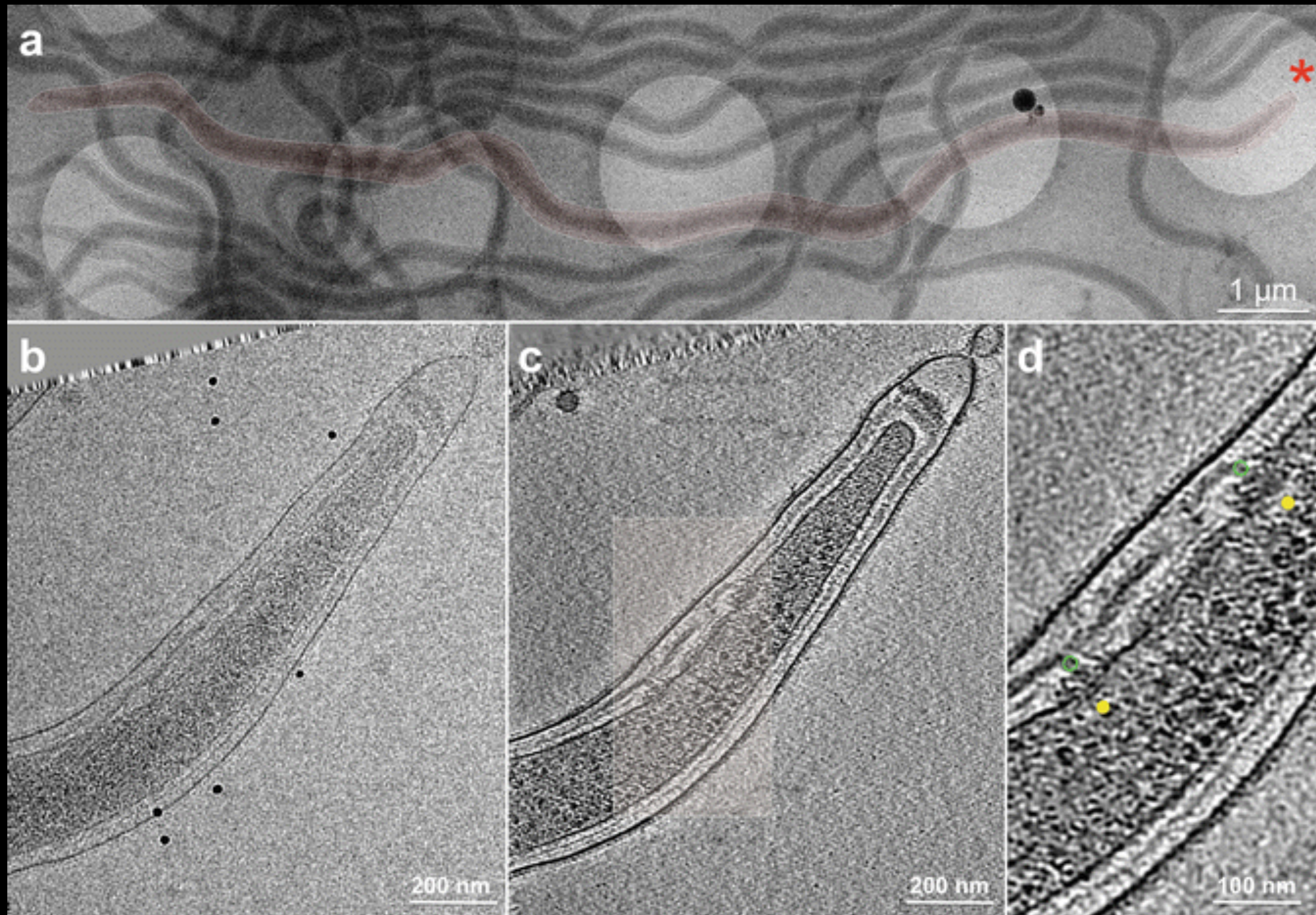
SerialEM is a popular software used for data collection of cryo-ET. It still has rooms to reduce the time.

Research targets:

Ribosome, Virus...these are good targets for sub-tomogram averaging at a high resolution .

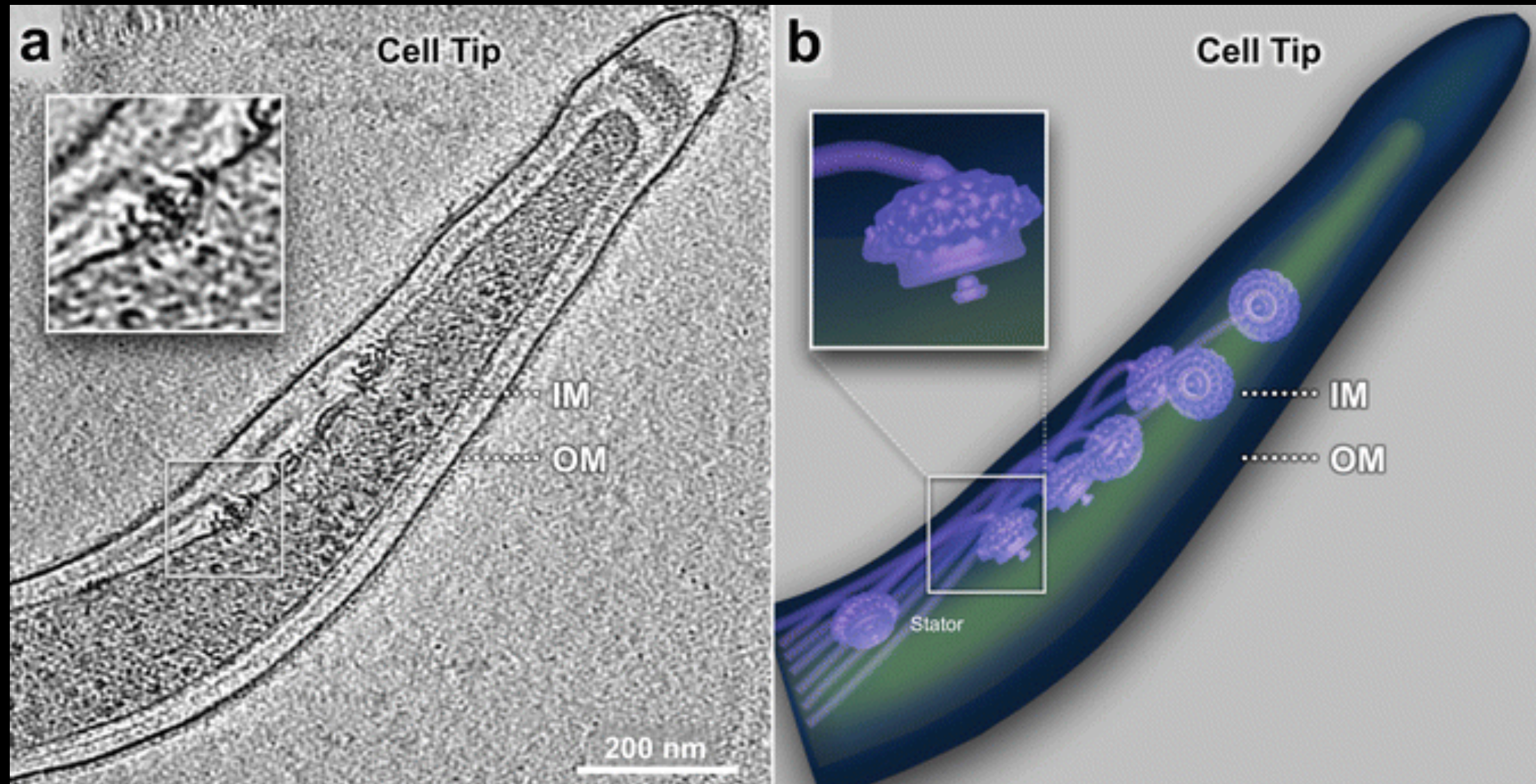
How about the performance of cryo-ET on molecular complexes spanning membrane using Cryo-ET?

How can we improve the performance of cryo-ET on molecular complex?



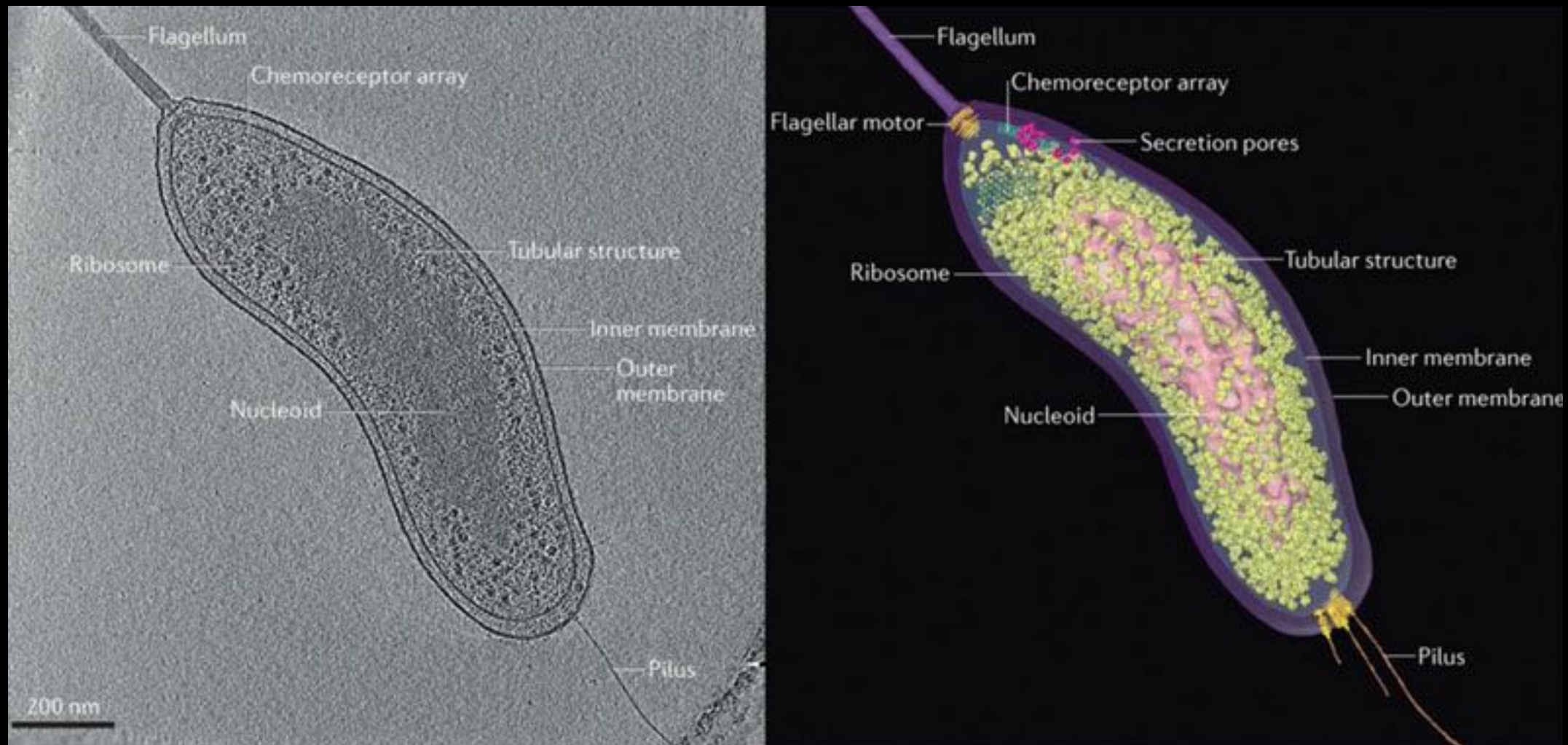
Bacterial flagellar motor is a good target for cryo-ET?

Borrelia has several periplasmic flagella



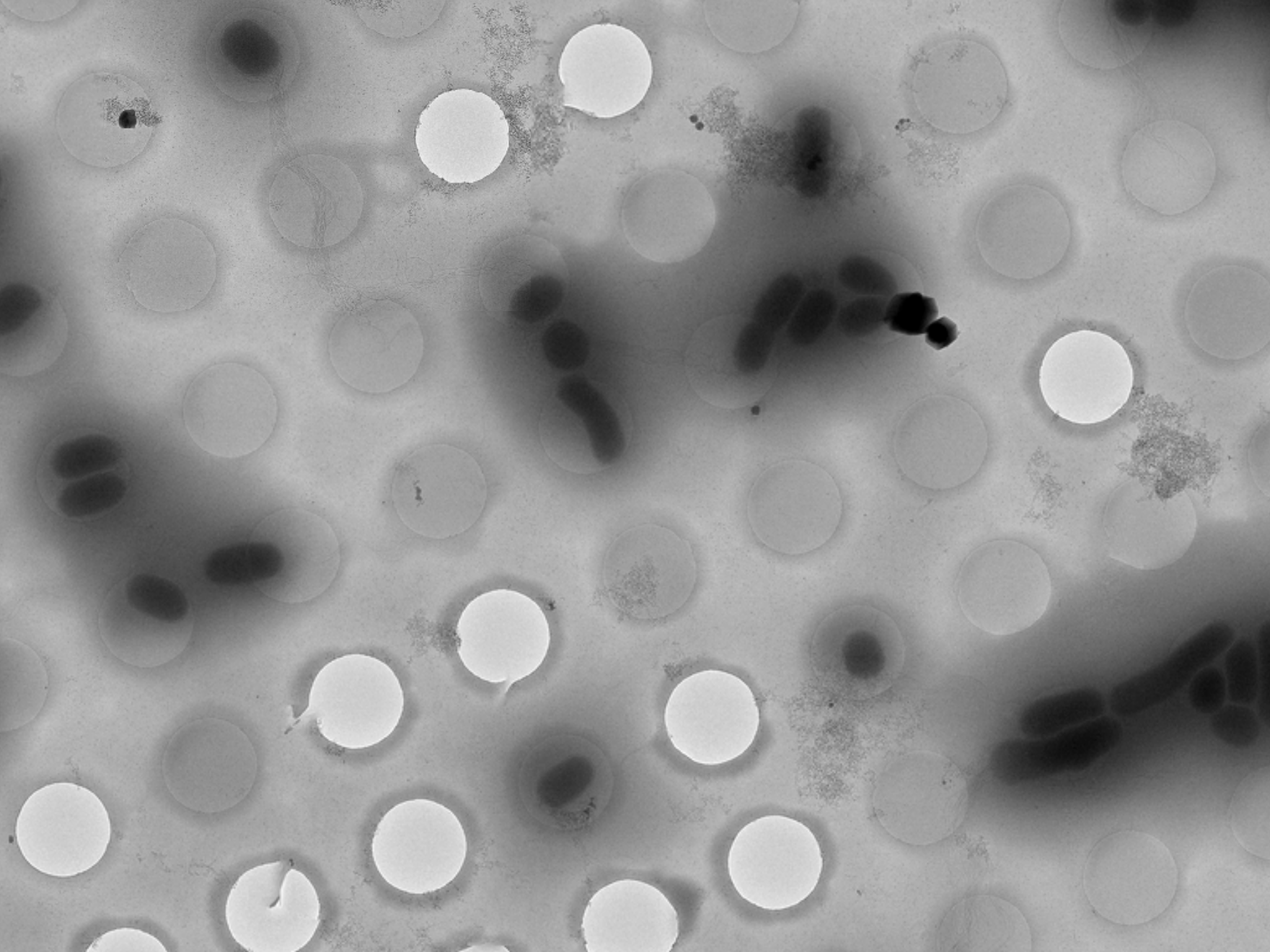
Over 7 motors locate at the cell tips in one tomogram.

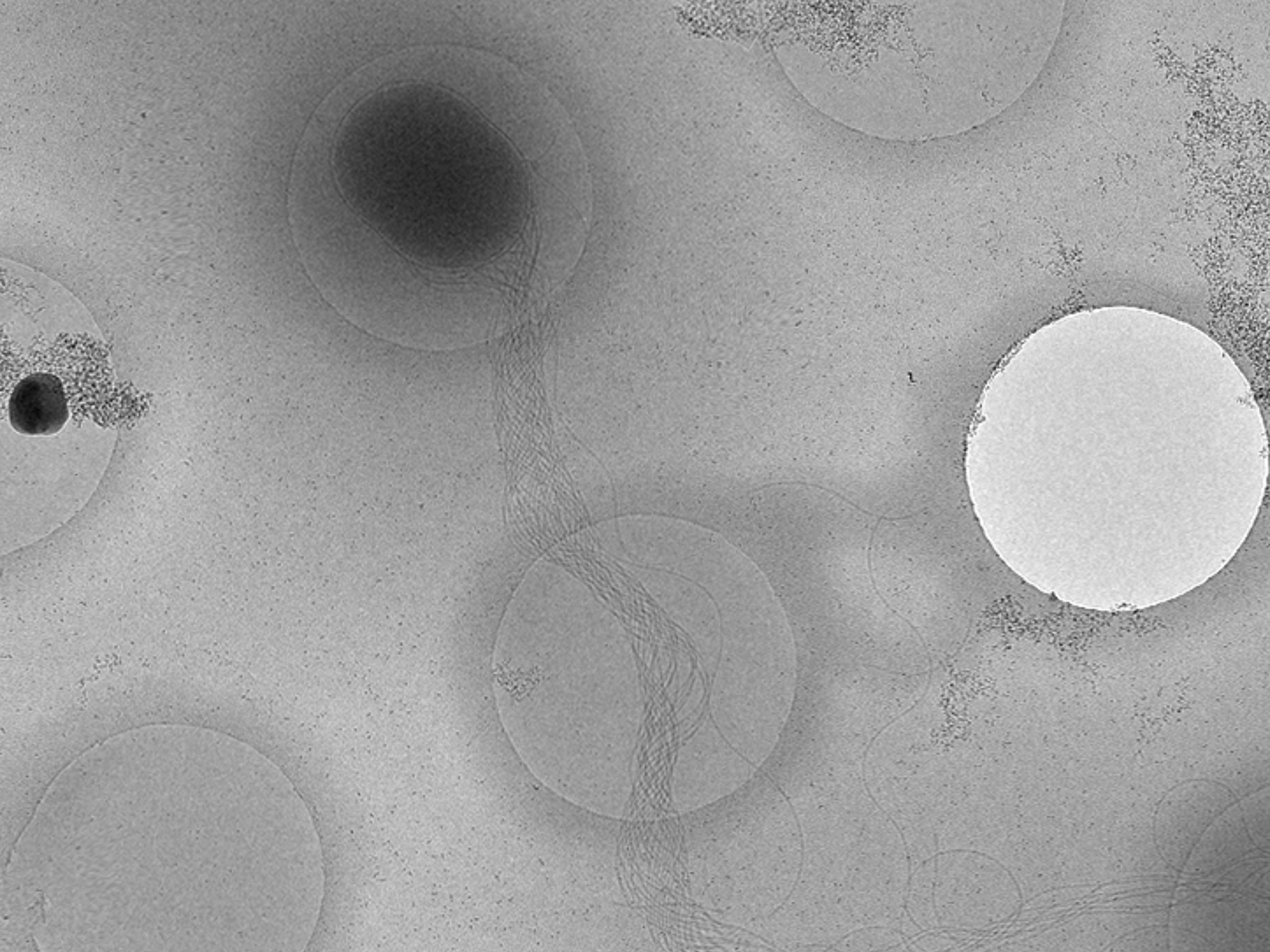
How about external flagella using cryo-ET

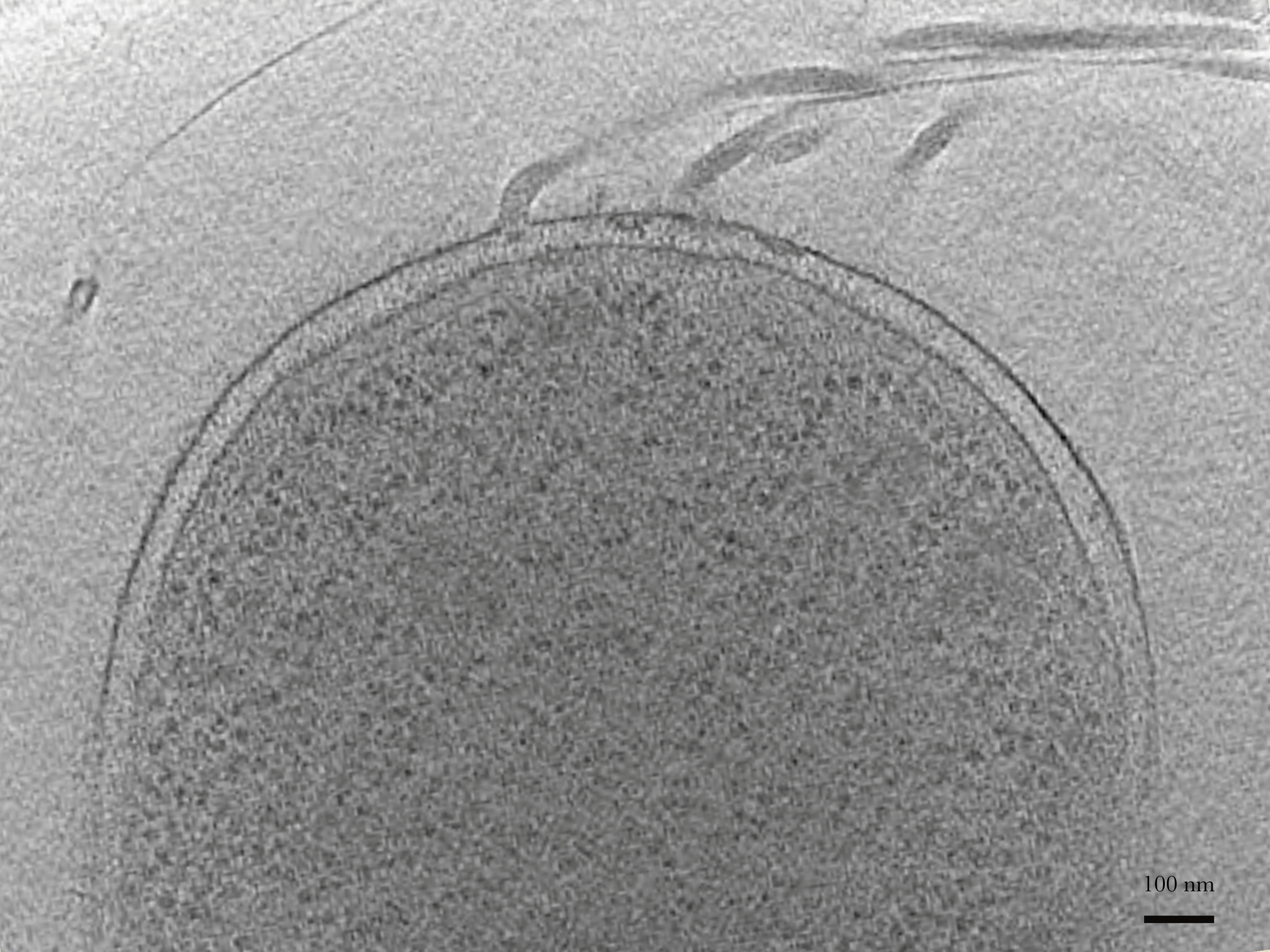


Oikonomou *et al.*, 2016

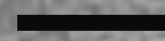
It might not good for sub-tomogram averaging due to limited targets.

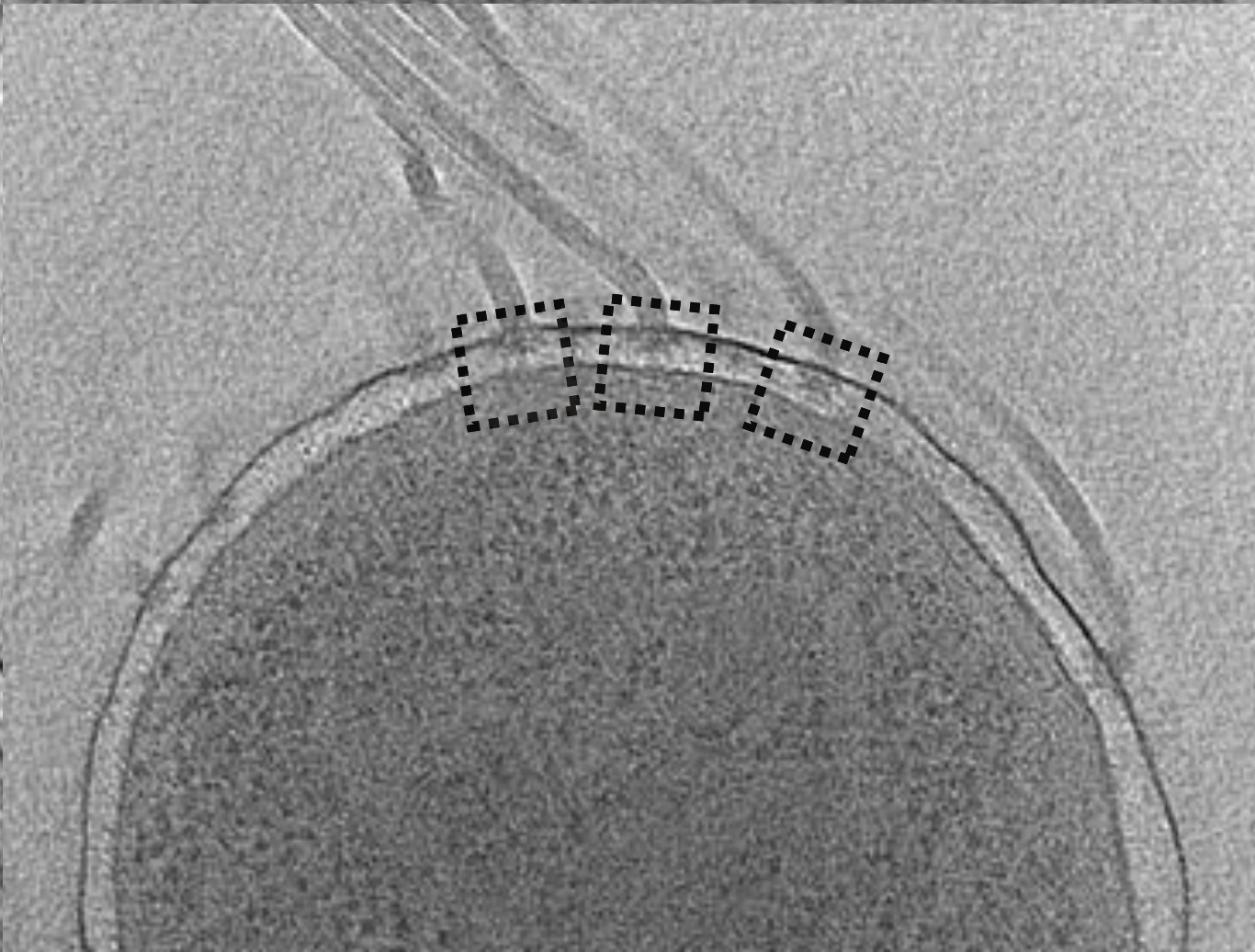
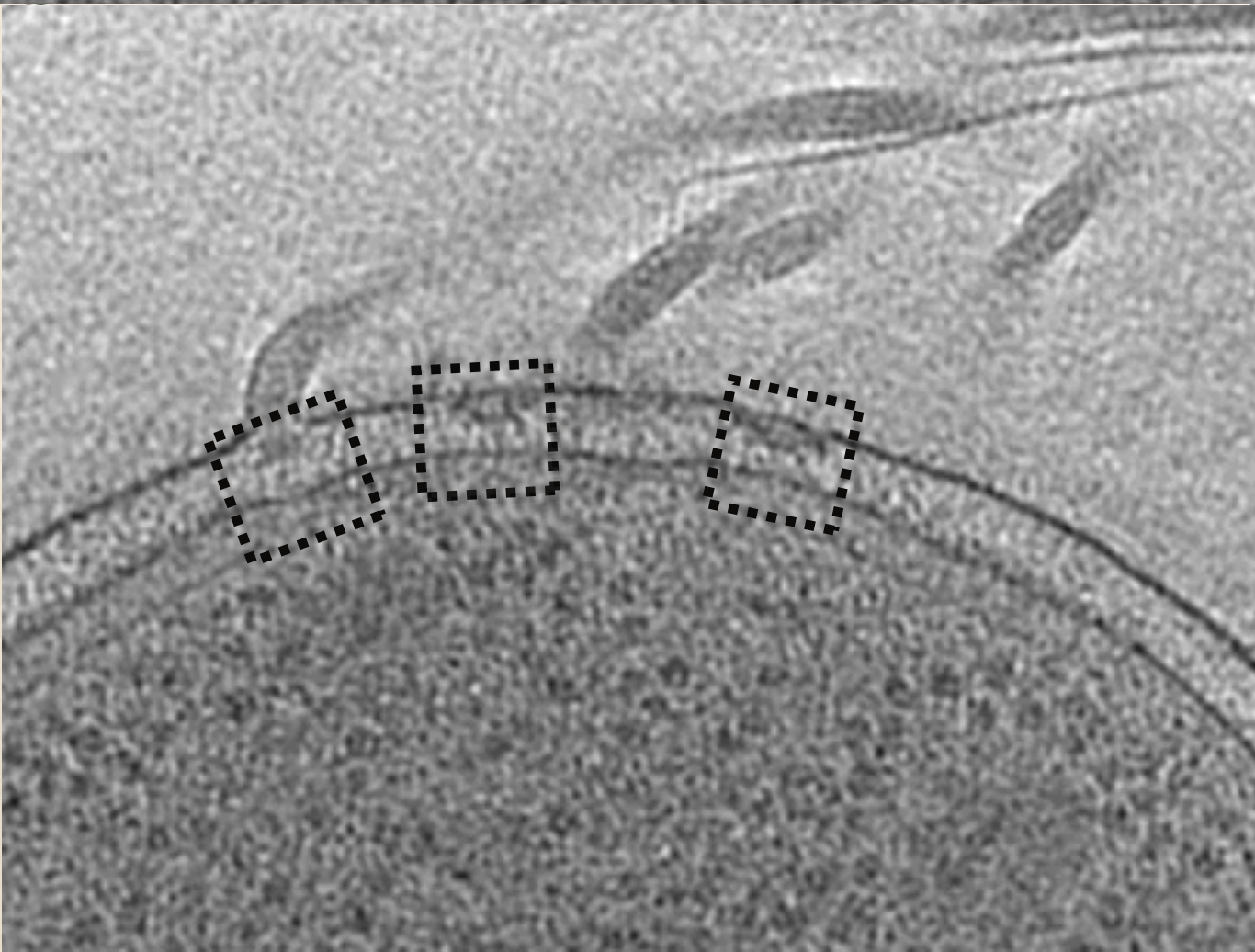




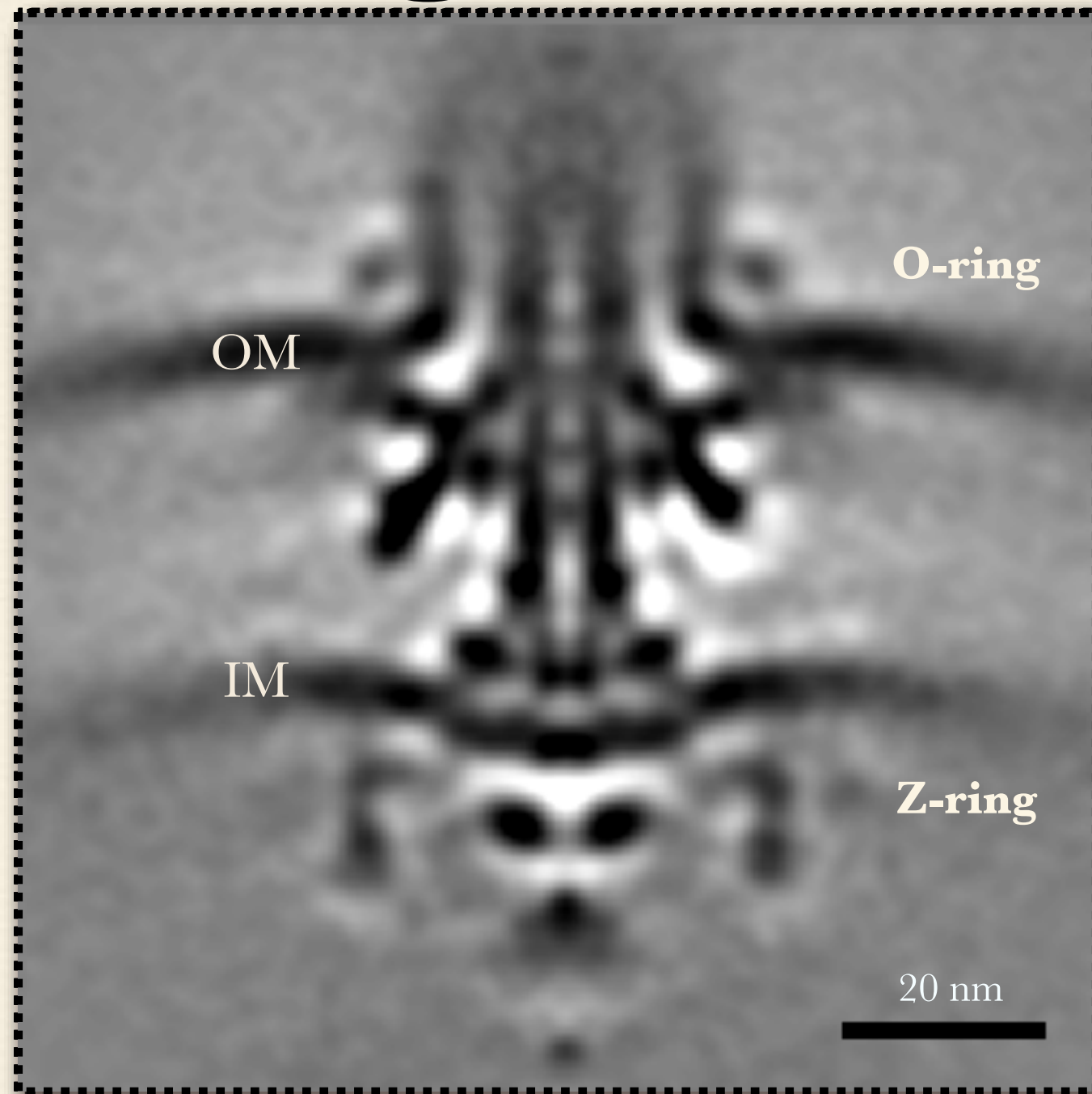


100 nm



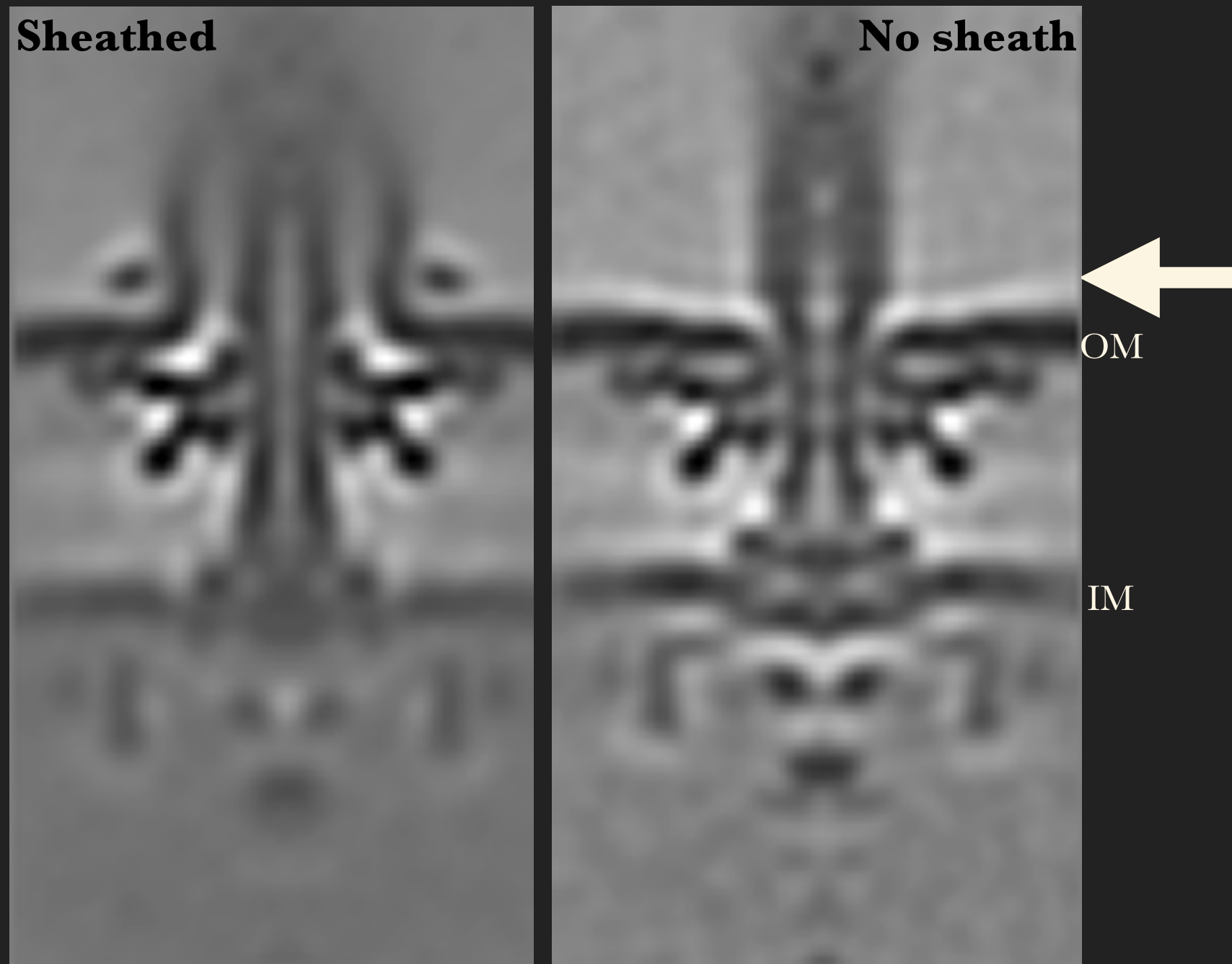


Sub-tomogram Average



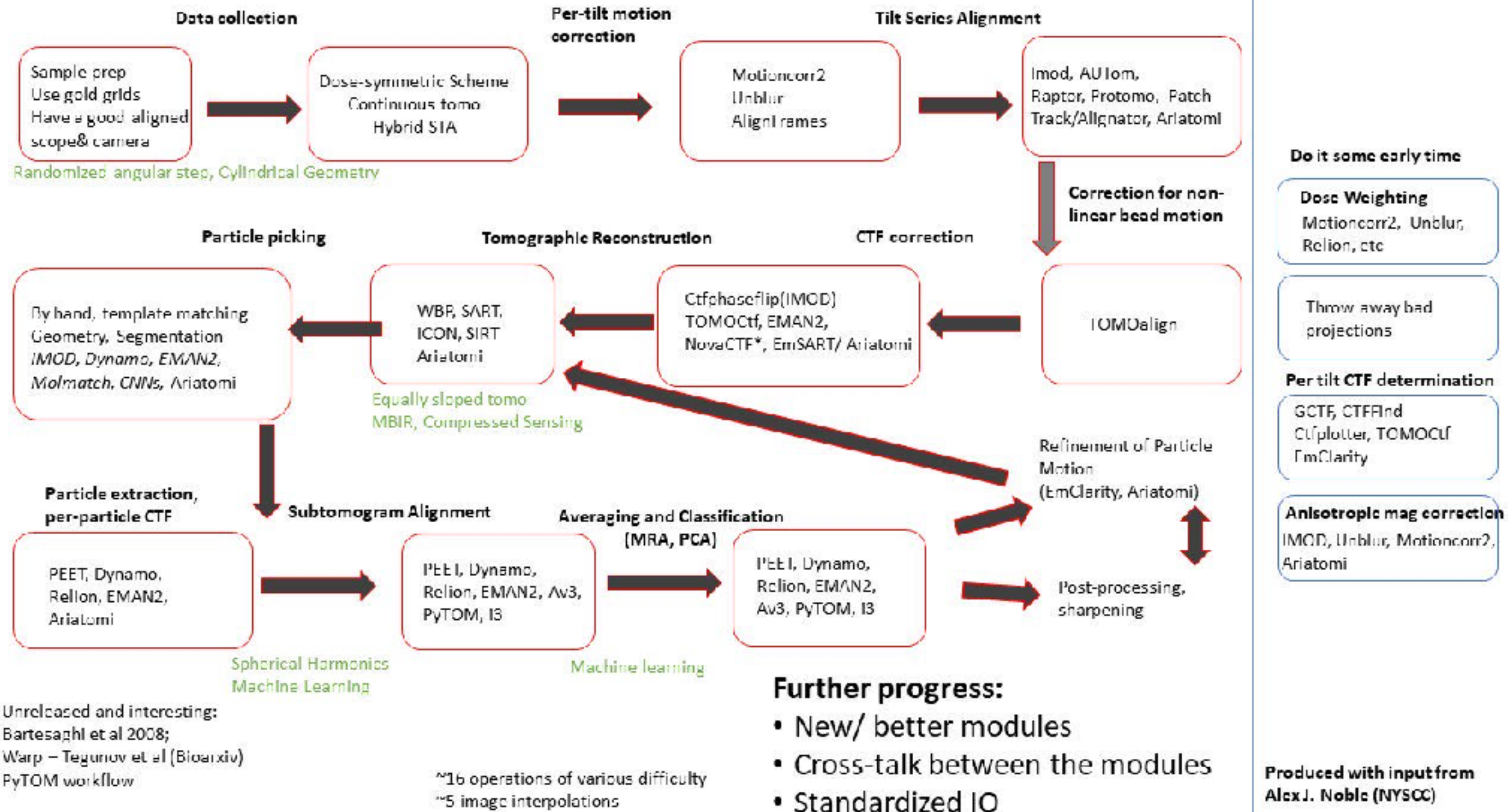
About 13 mins/tomogram

Conformational change between sheathed flagella and non-sheathed flagella



Multiple softwares are required to get a solid subtomogram average.

Multiple software available in image processing



From Misha's Twitter

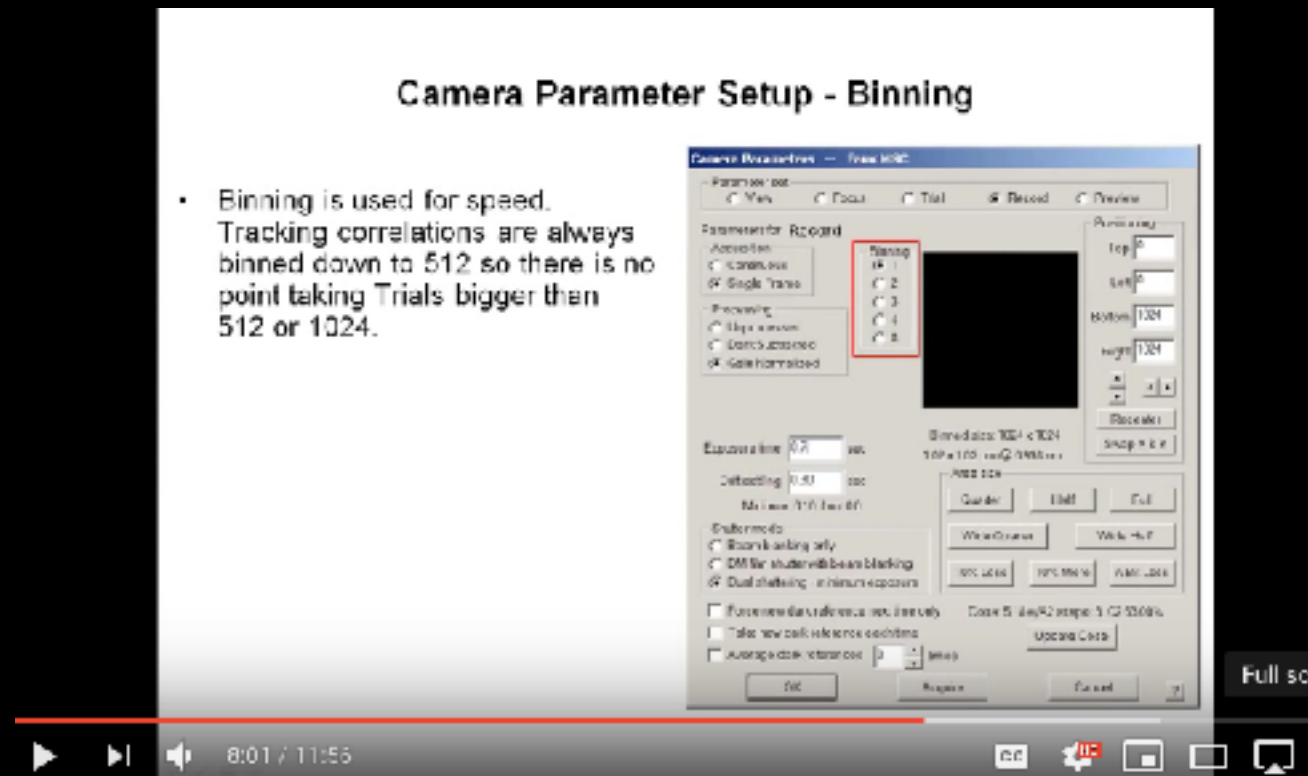
Times are required for mastering these softwares

Real case in one tomogram aiming to high throughput tomography

Process	Software	Time
Data acquisition	SerialEM	12 mins
Processing to the alignment files	Motioncorr2	2 mins
	ETomo	5~8 mins
Tomogram generation	Tomo3d	20~30 secs in bin4
		25 mins in bin1

Practical tips during the data collection on flagellar motor

Tip1: Set binning 4 under Record mode



Saving 80 seconds.

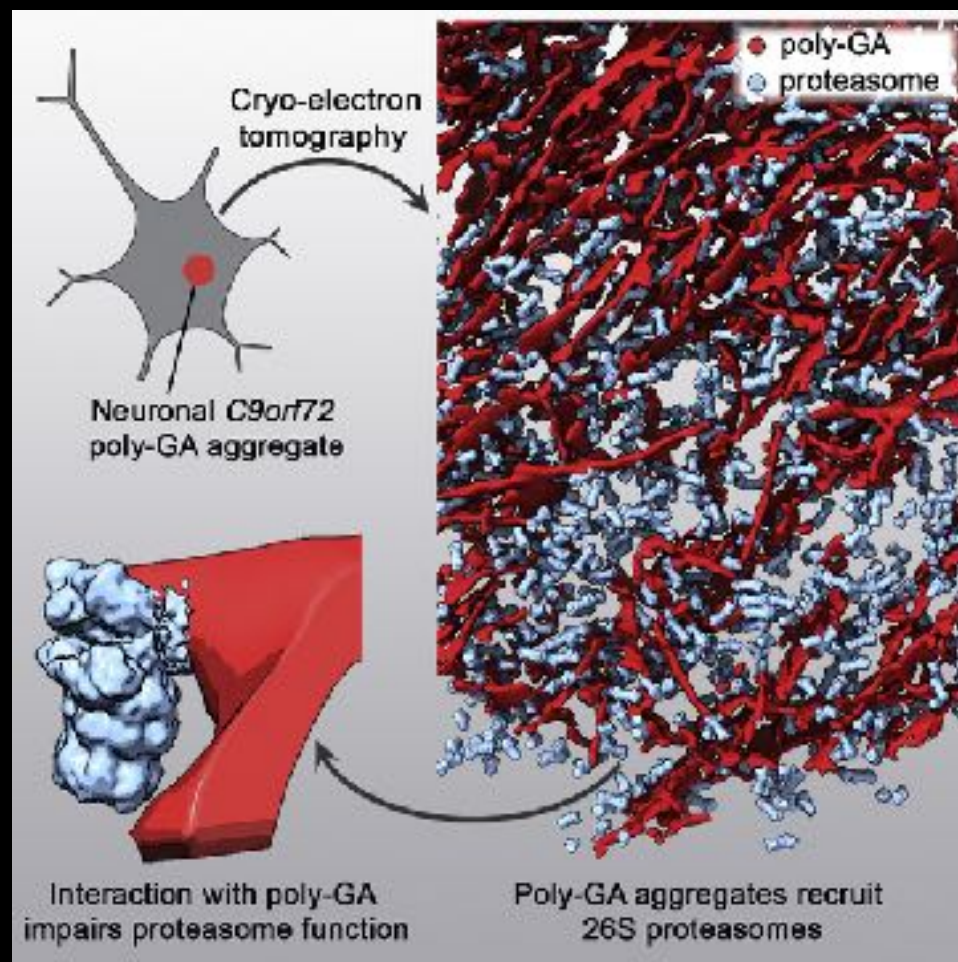
Tip 2: Find the shortest time on Focus and Track
Saving more

Tips.....

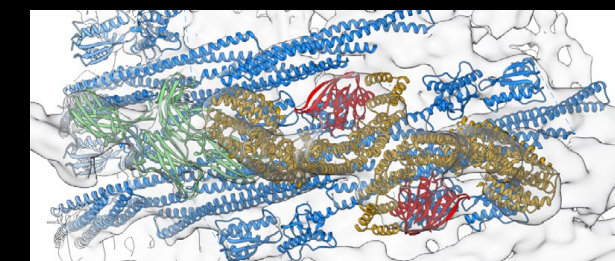
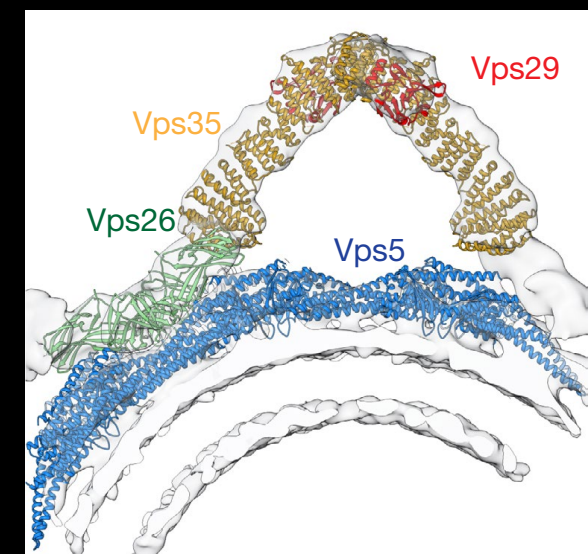
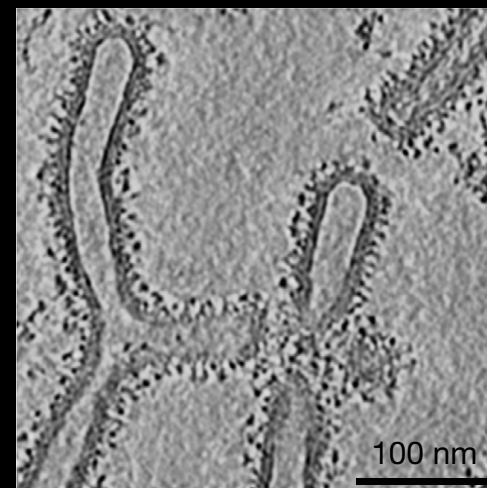
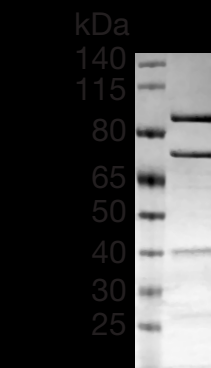
High performance of cryo-ET

Sample, Sample, Sample.

Sample preparation and increase research targets/ tomograms



Guo *et al.*, 2018



Kovtun *et al.*, 2018

“It is very easy to answer many of these
fundamental biological questions; you just look
at the thing!”

–Richard Feynman

Thank you.

Discussions

1, We have been aware that SerialEM is doing autofocus for 2~3 mins. Can we have more room on autofocus steps to push the speed for cryo-ET data collection.

2, Stage movement is continuous without any stop.