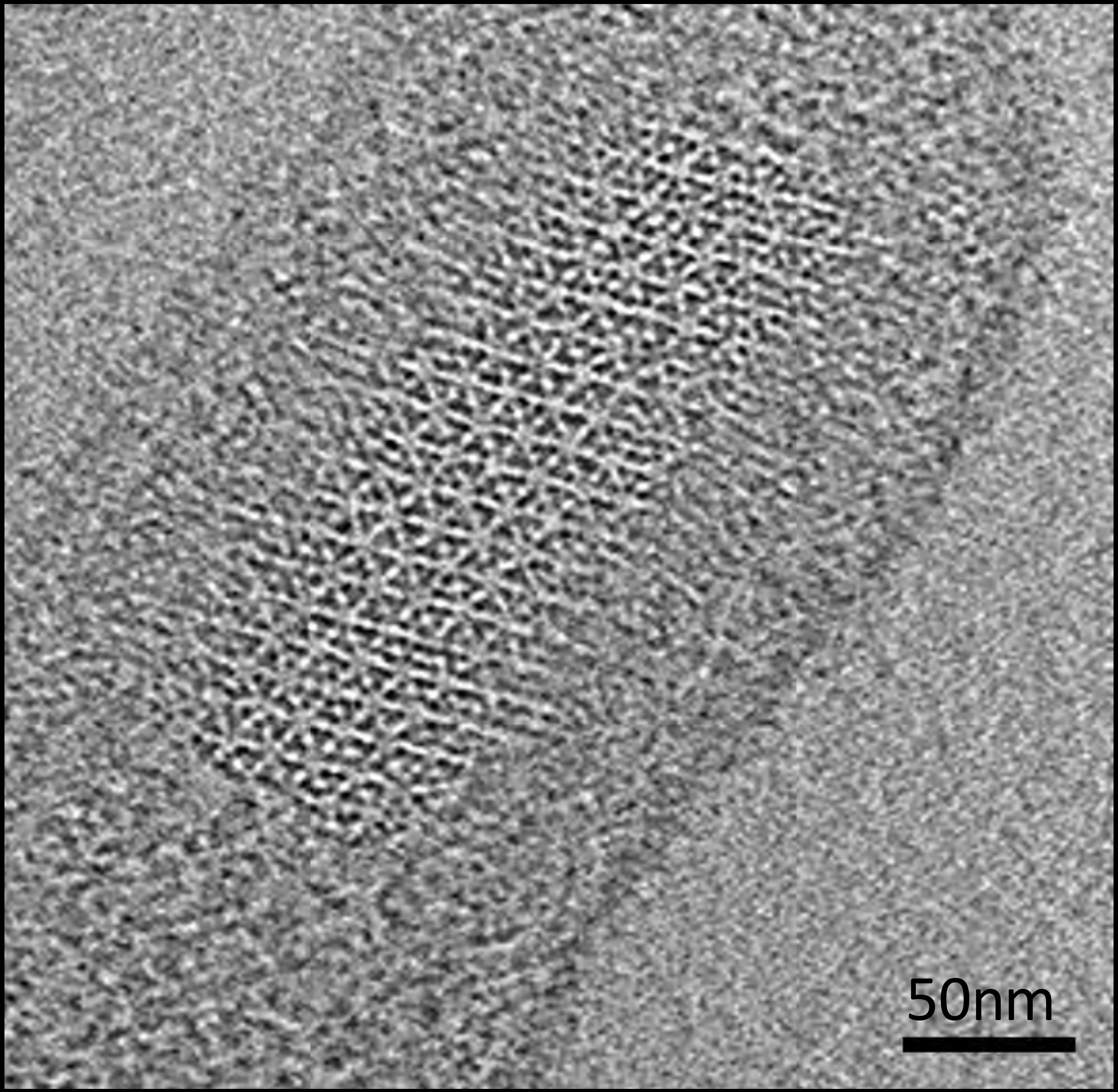
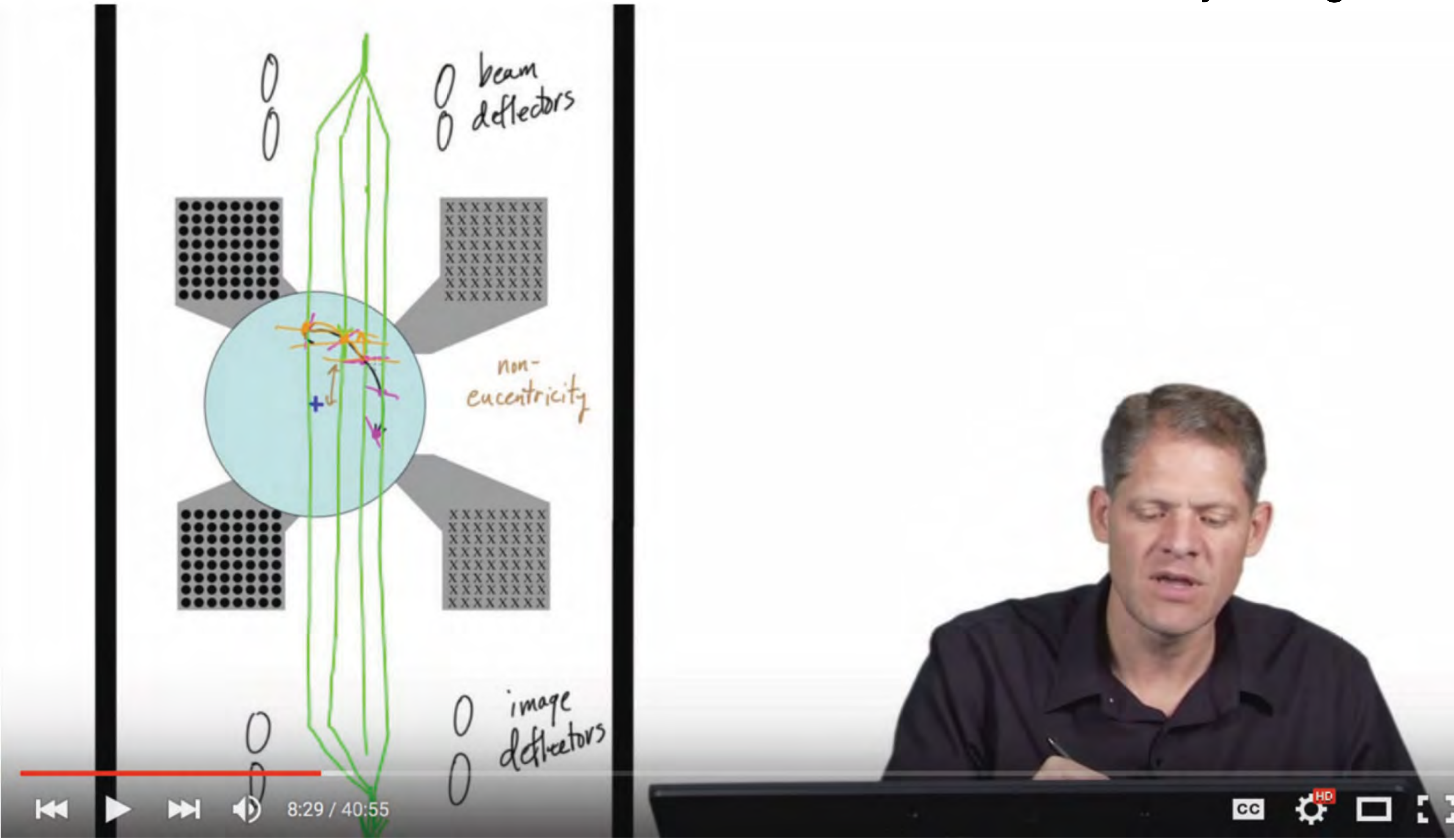


Grant Jensen
Caltech
HHMI

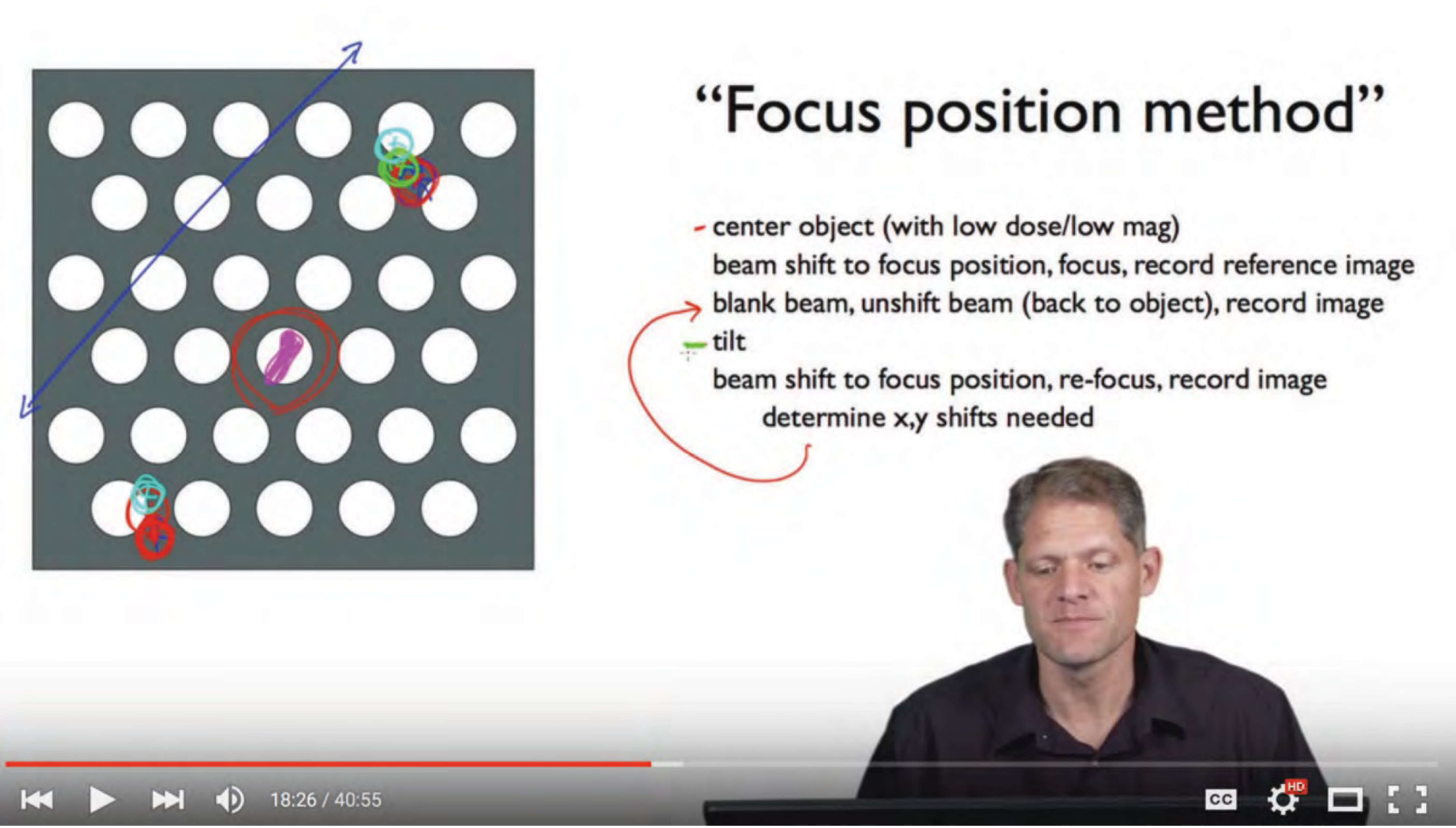
Data collection with today's stages



"Getting Started in Cryo-EM" video series

Three solutions:

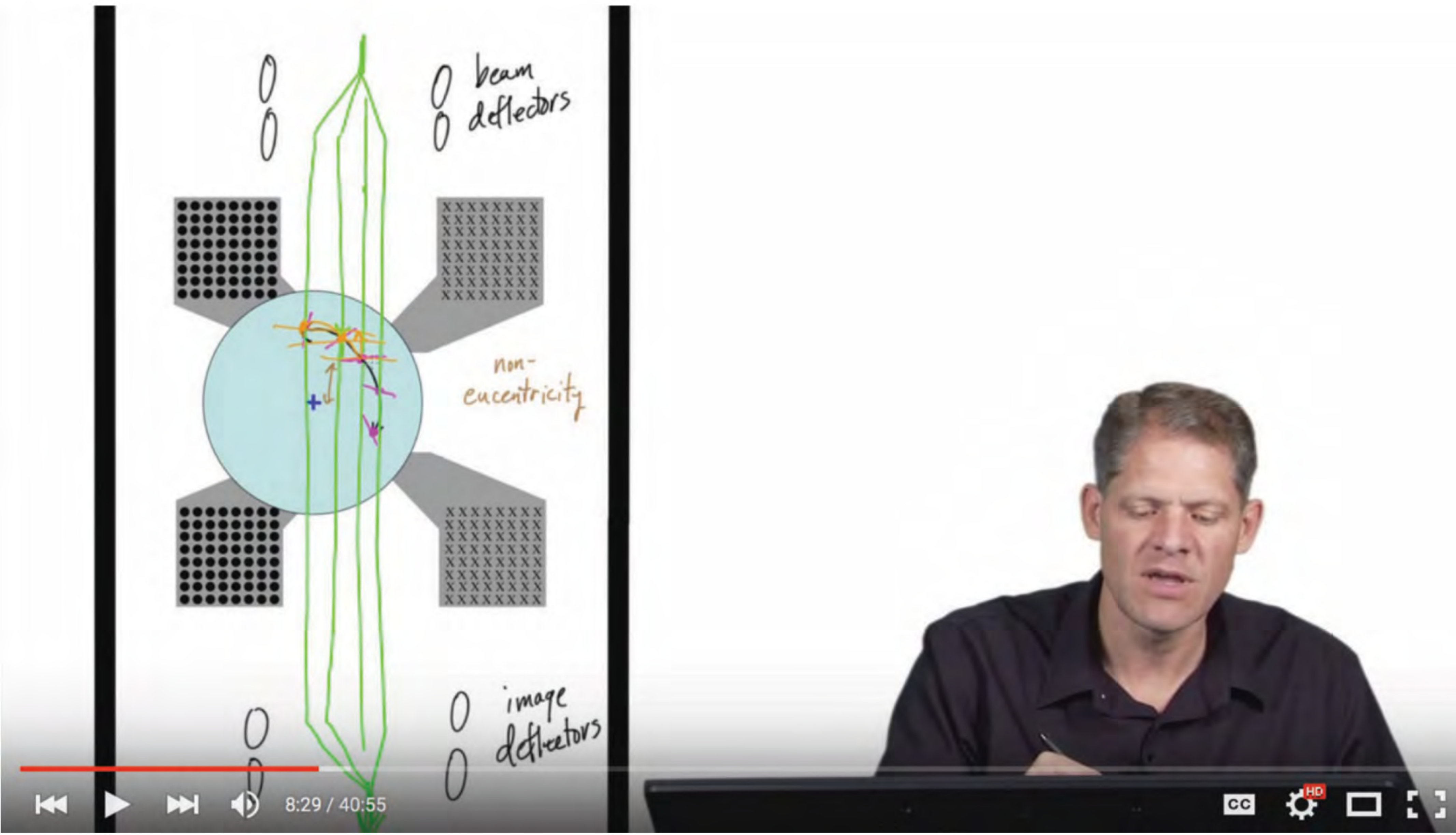
- On-axis focus position



“Focus position method”
- center object (with low dose/low mag)
beam shift to focus position, focus, record reference image
blank beam, unshift beam (back to object), record image
tilt
beam shift to focus position, re-focus, record image
determine x,y shifts needed
18:26 / 40:55
CC
HD

Three solutions:

- On-axis focus position
- Geometric prediction

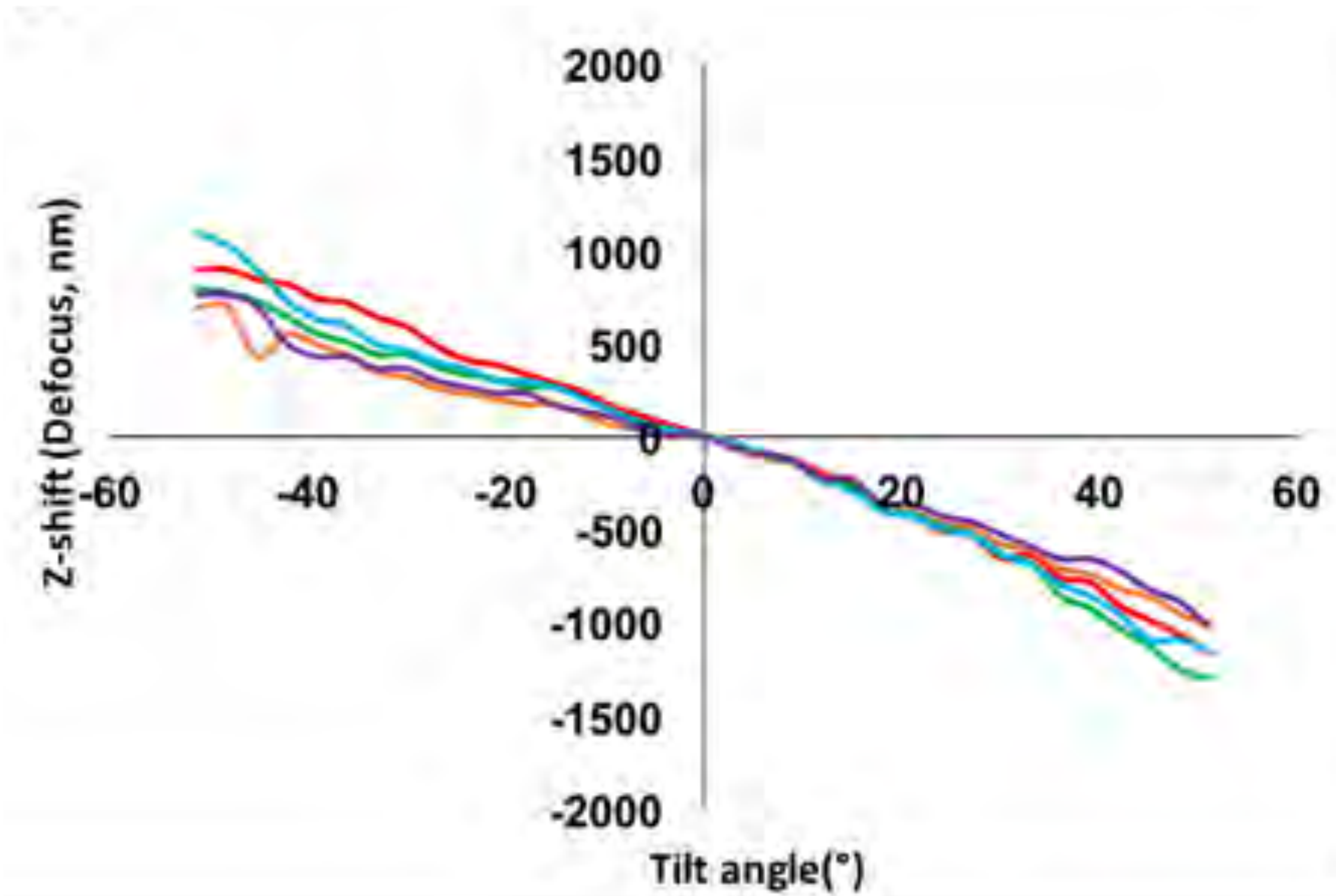


"Getting Started in Cryo-EM" video series

Three solutions:

- On-axis focus position
- Geometric prediction
- Holder calibration

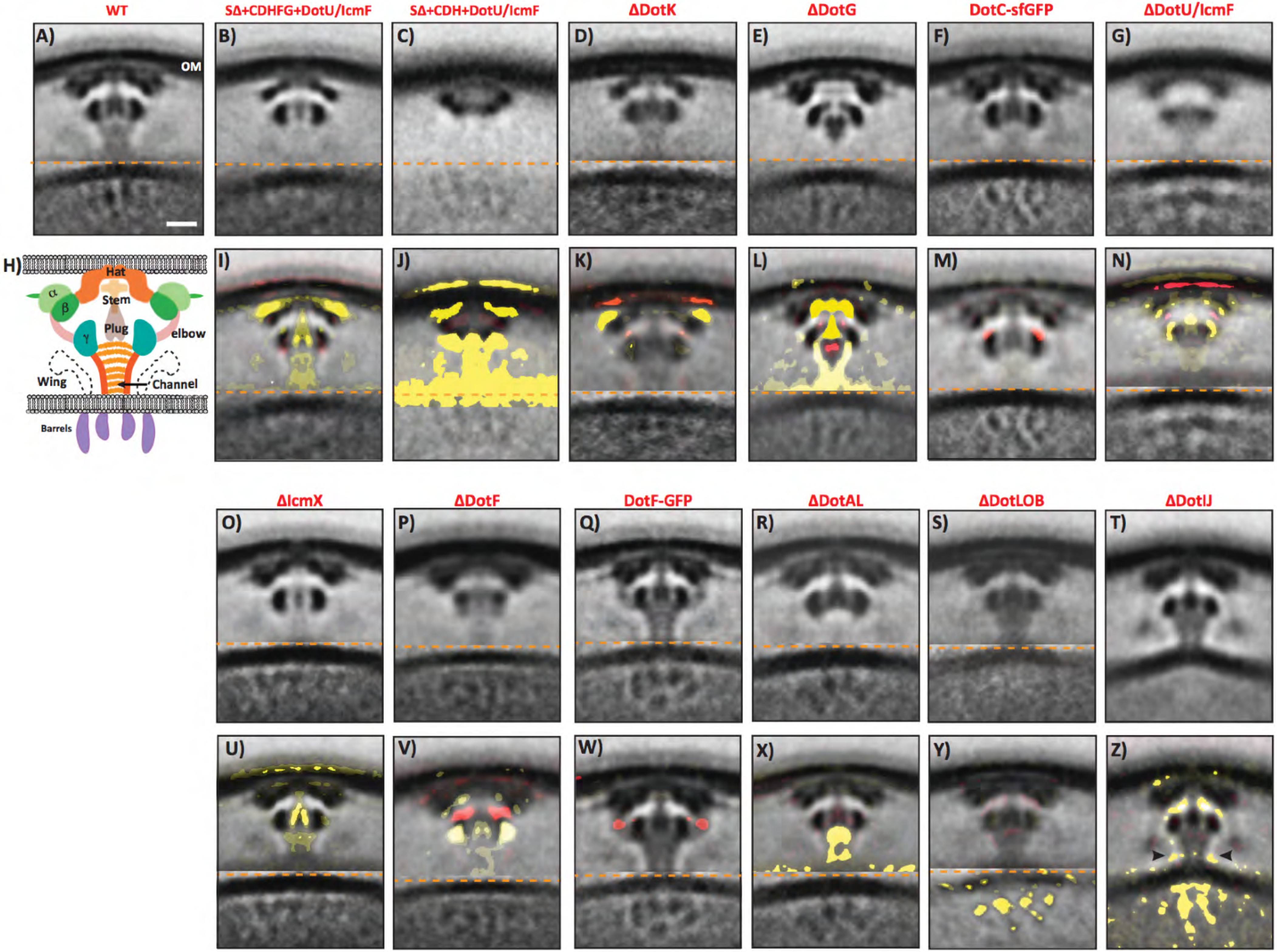
Holder calibration



Key advances enabling fast ECT

- Programmable microscopes
- Digital cameras
- Automatic image alignment, processing pipelines, databases
- Adaptively predictive tracking and focussing or holder calibration

Together it all makes projects requiring thousands of tomograms possible


WT
SΔ+CDHFG+DotU/IcmF
SΔ+CDH+DotU/IcmF
ΔDotK
ΔDotG
DotC-sfGFP
ΔDotU/IcmF
A)
B)
C)
D)
E)
F)
G)
OM
H)
Hat
Stem
Plug
elbow
α
β
γ
Wing
Channel
Barrels
I)
J)
K)
L)
M)
N)
ΔIcmX
ΔDotF
DotF-GFP
ΔDotAL
ΔDotLOB
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O)
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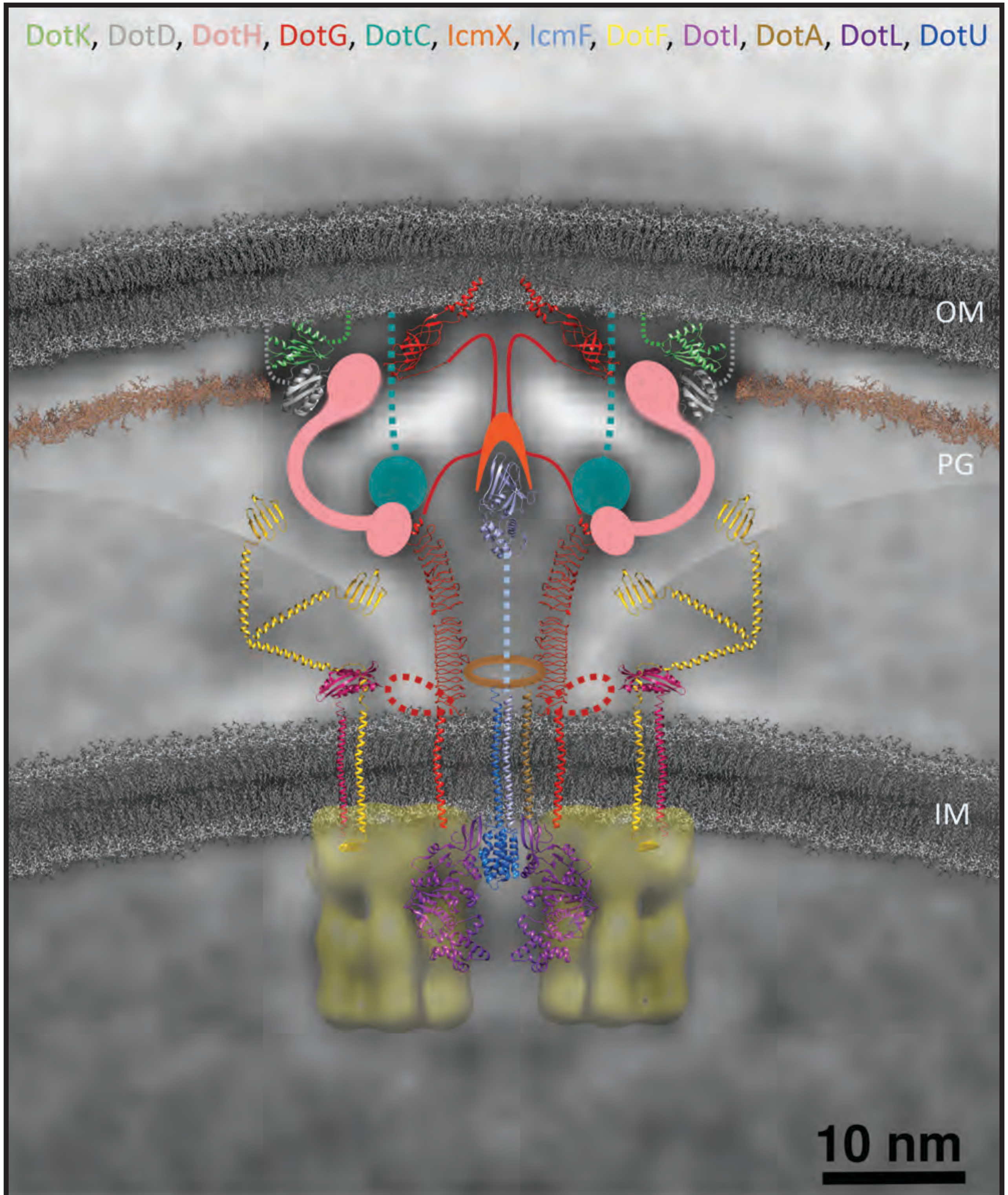
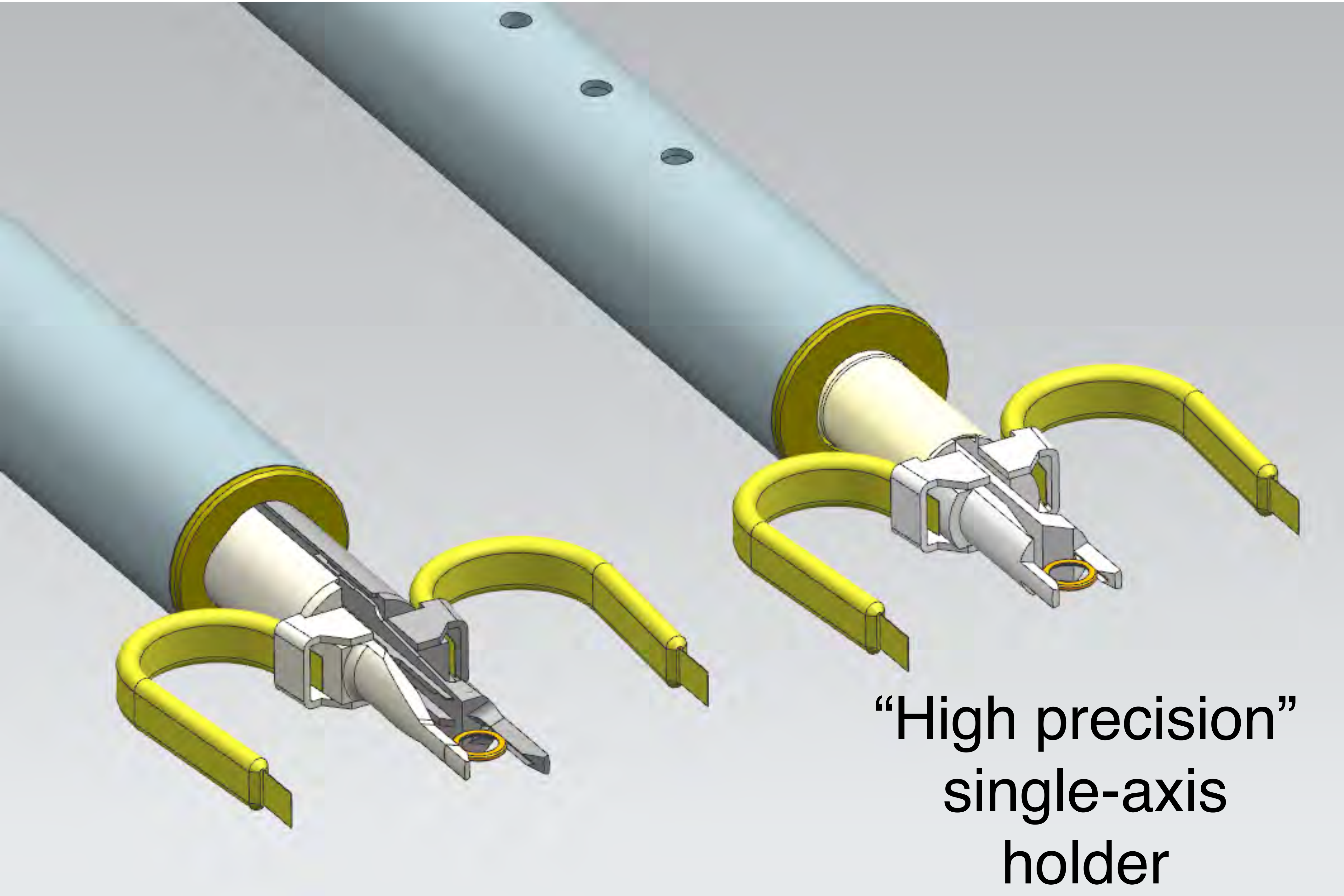
U)
V)
W)
X)
Y)
Z)



Debnath Ghosal

Yiwei Chang
Kwang Cheol Jeong
Joseph Vogel



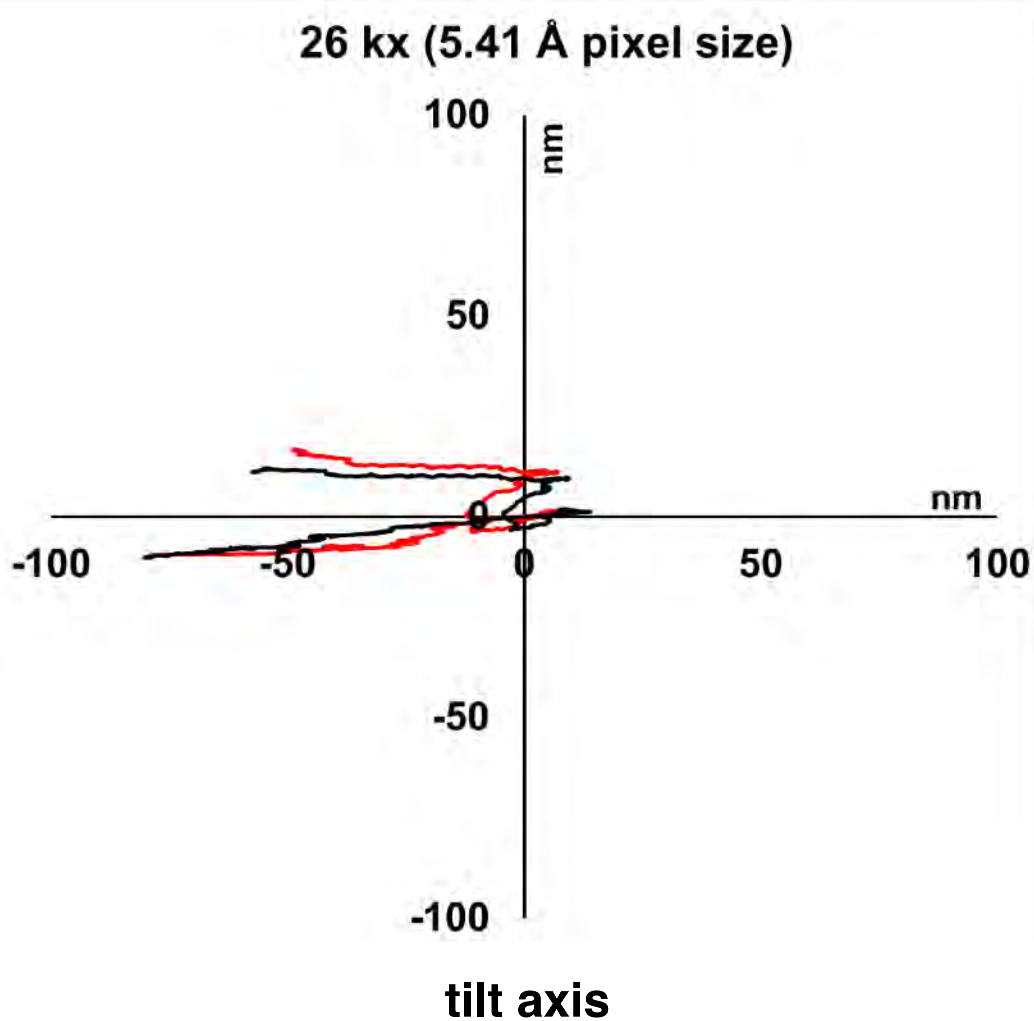
“High precision” single-axis holder

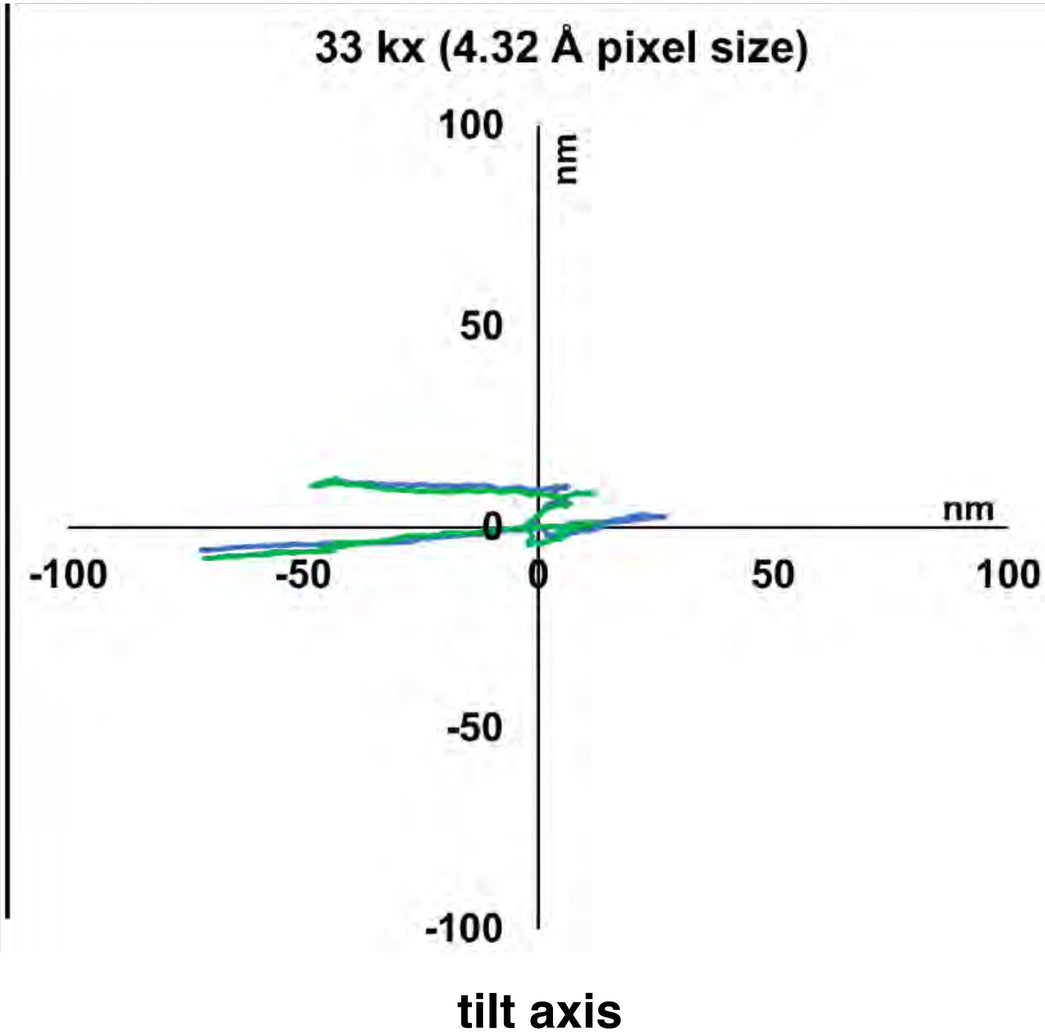
Movie showing continuous tilt series of a bacterial cell taken from -60 to +60 with ~2000 frames

The camera is the limiting component

Nominal magnification	Pixel size (Å)	Exposure time (s)	Total frames
130kx	1.09	12	480
81kx	1.78	20	800
53kx	2.74	50	2000 or less
33kx	4.32	126	5040 or less

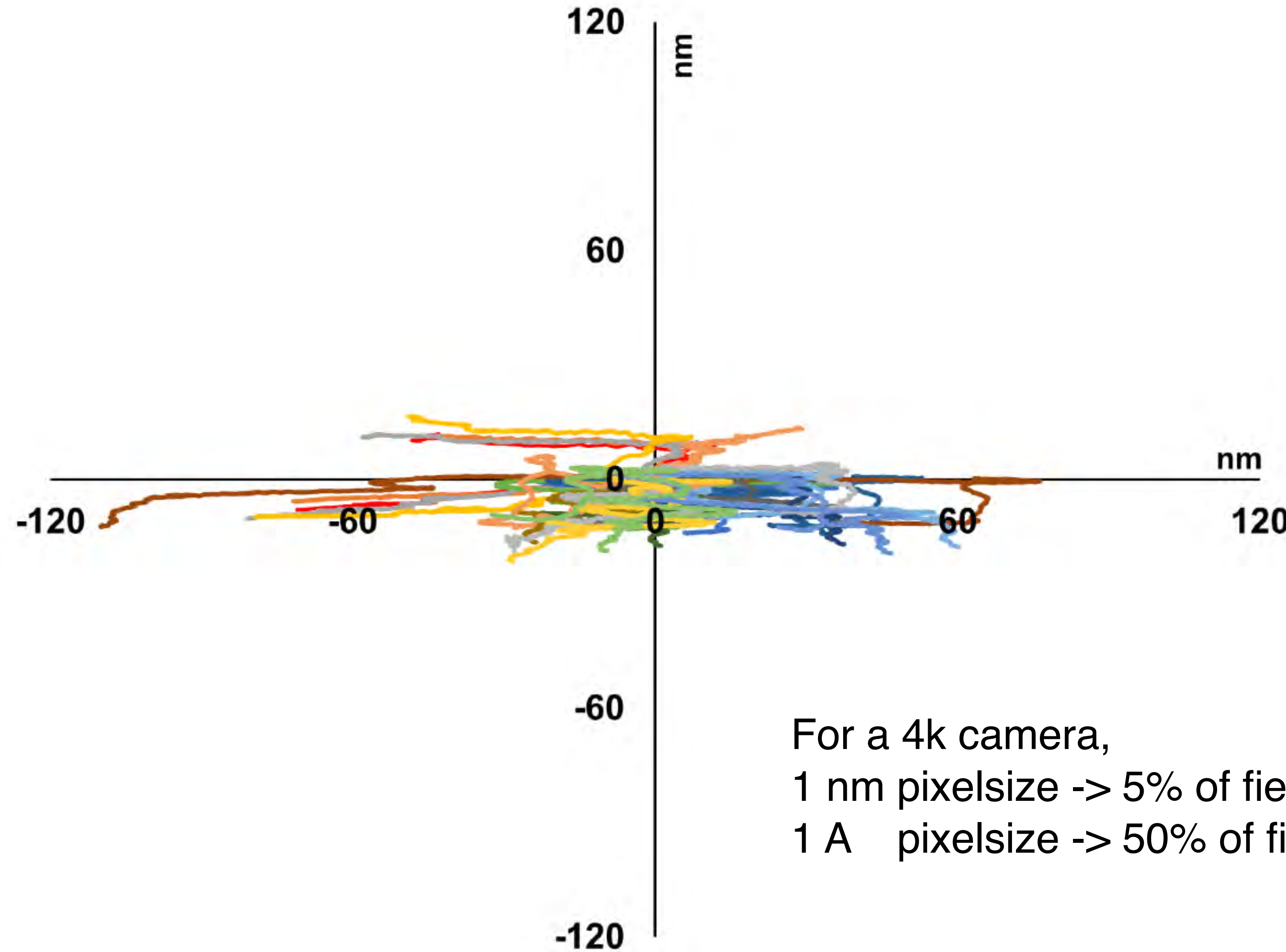
Tilt Series Alignment -60° to +60°





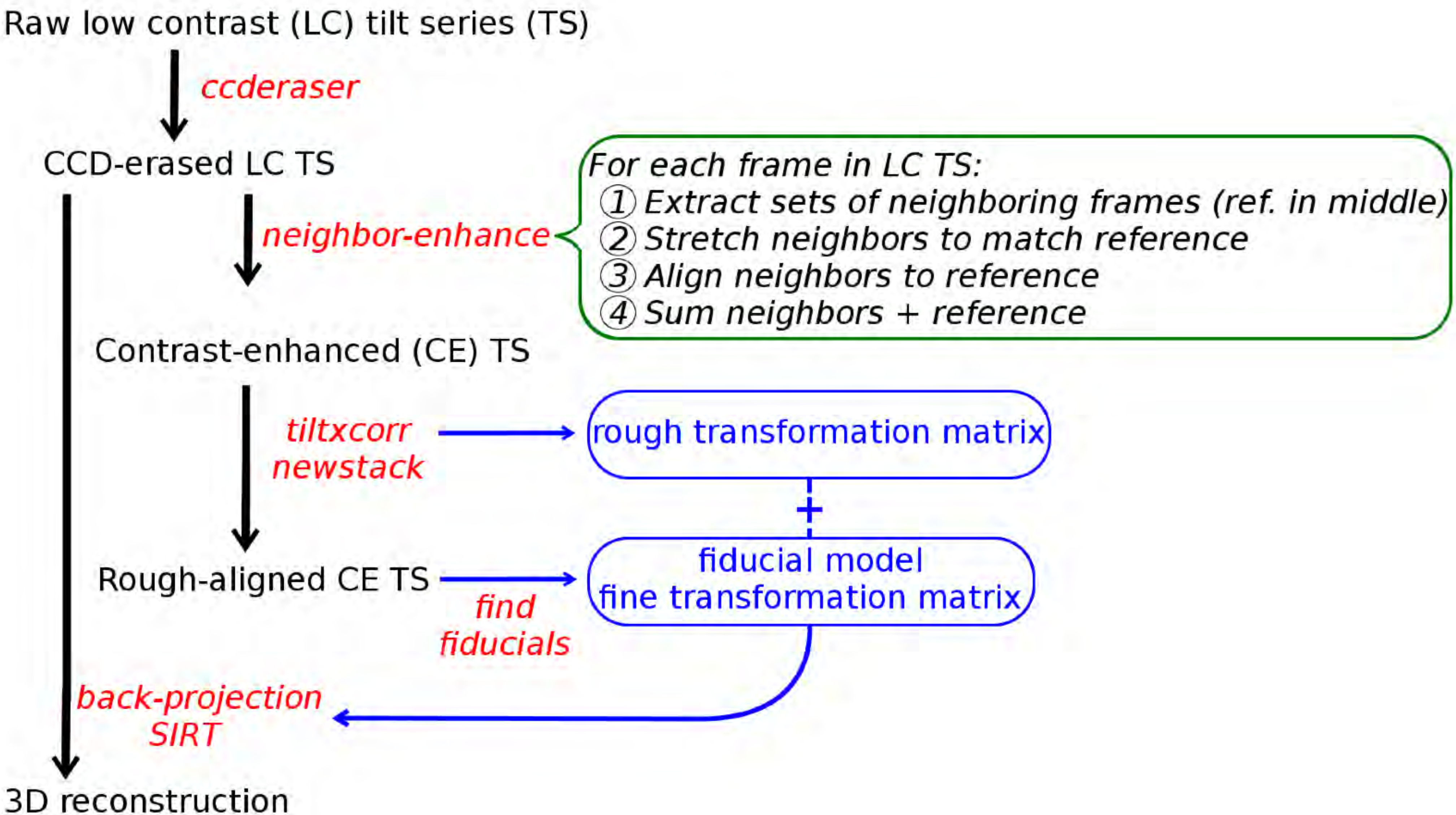
Movie of a bidirectional continuous tilt series

Eucentricity of the high precision stage



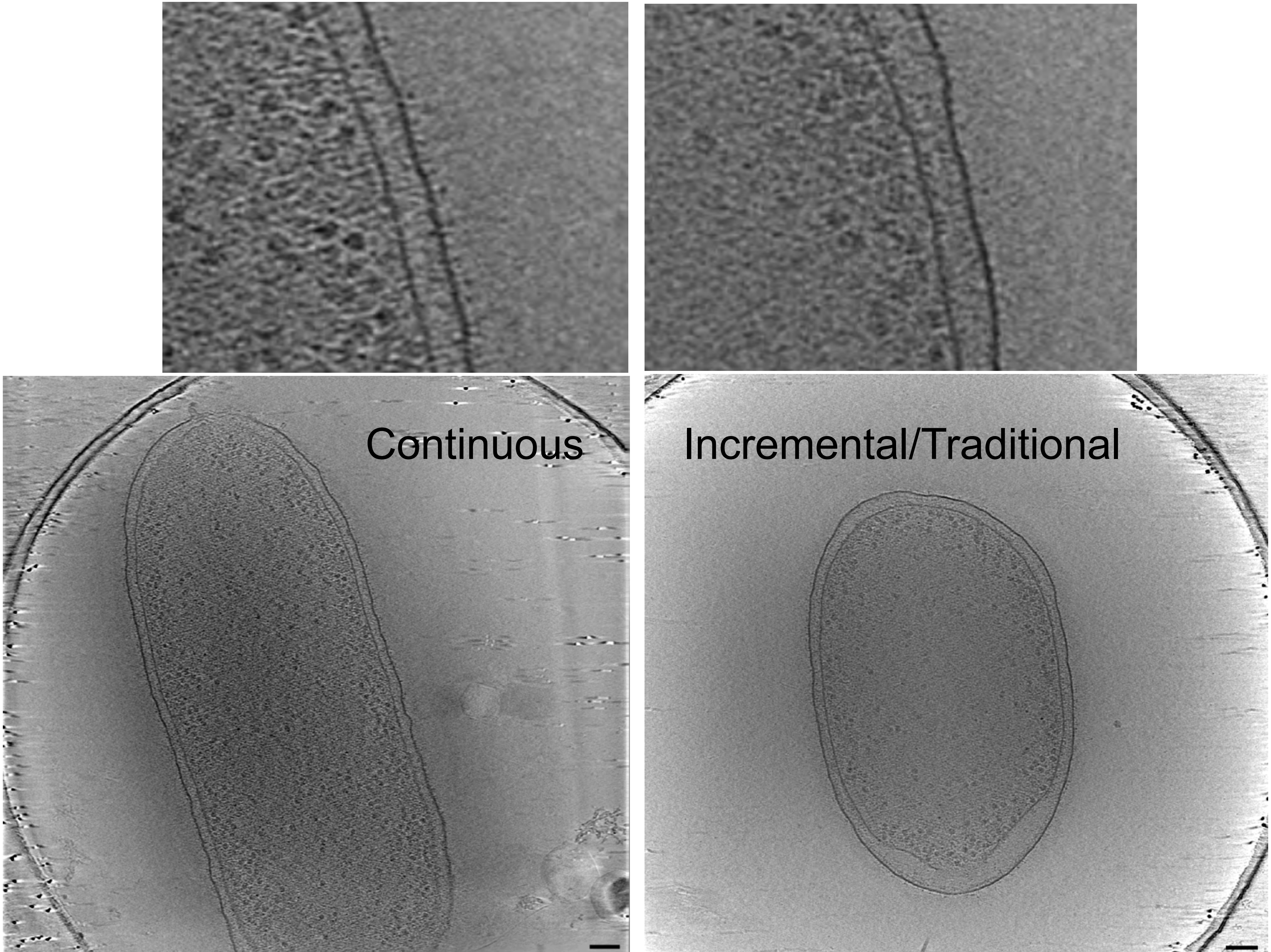
For a 4k camera,
1 nm pixelsize -> 5% of field of view lost
1 A   pixelsize -> 50% of field lost

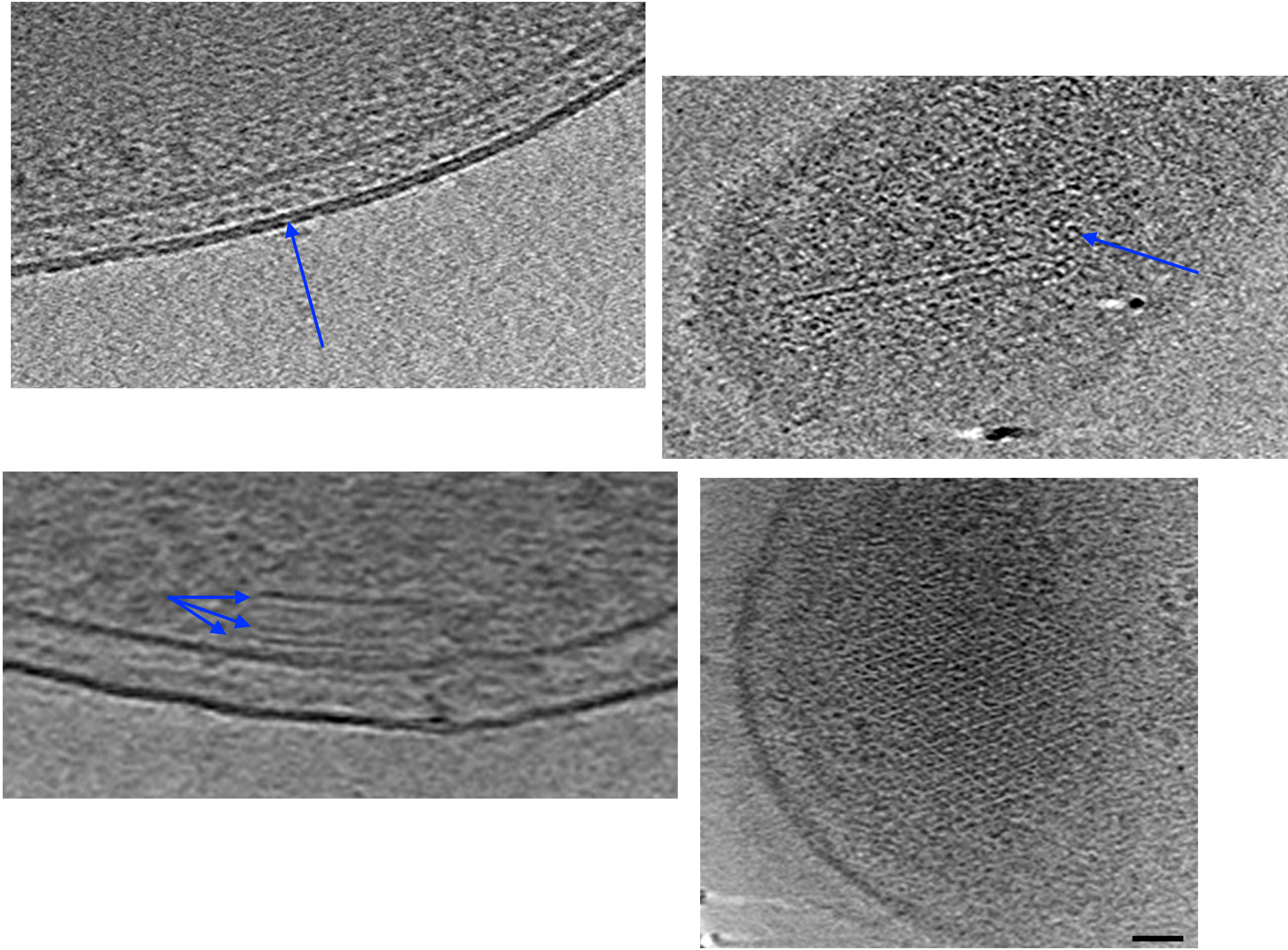
tilt axis

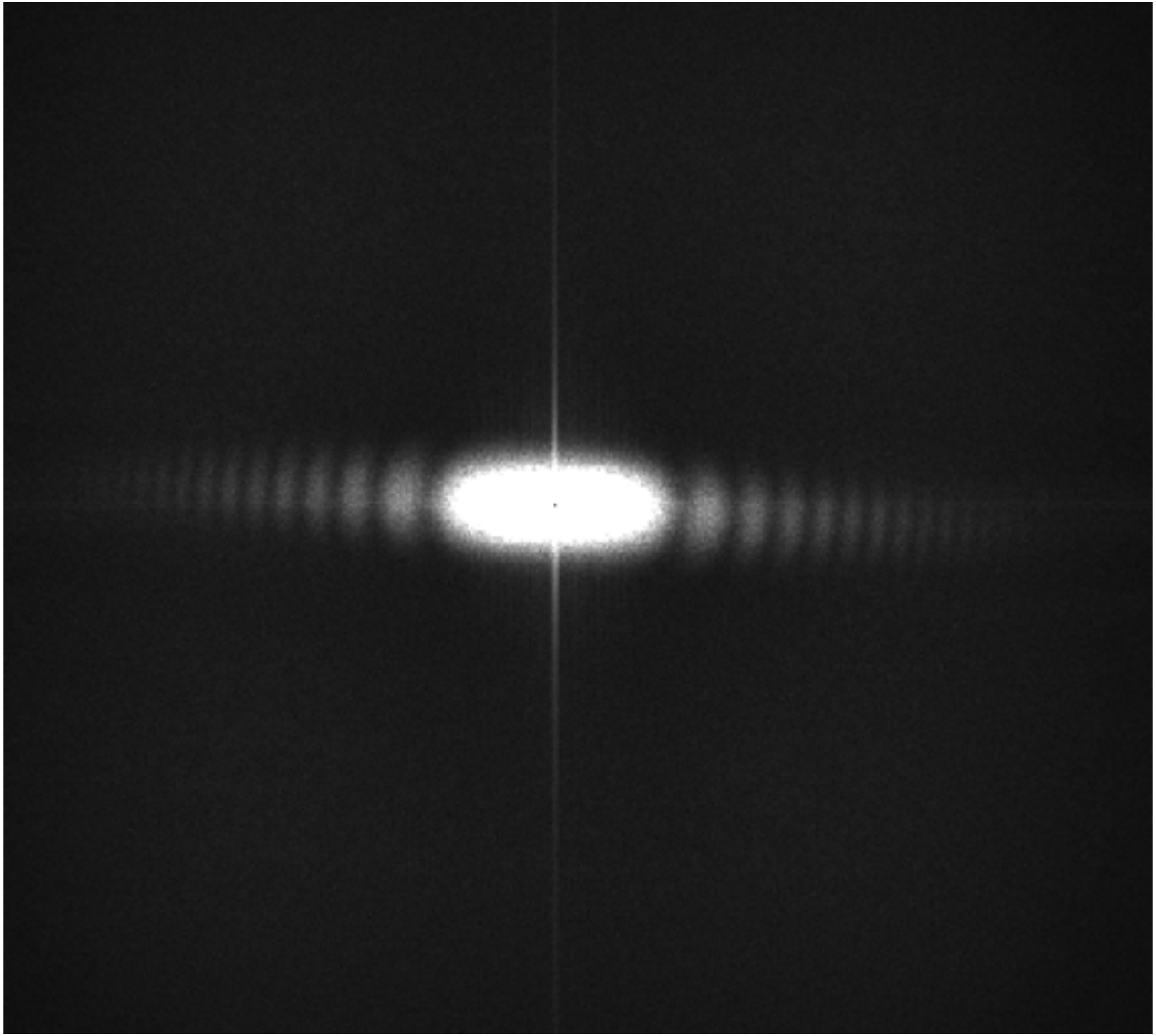

Raw low contrast (LC) tilt series (TS)
ccderaser
CCD-erased LC TS
neighbor-enhance
For each frame in LC TS:
① Extract sets of neighboring frames (ref. in middle)
② Stretch neighbors to match reference
③ Align neighbors to reference
④ Sum neighbors + reference
Contrast-enhanced (CE) TS
tiltxcorr
newstack
rough transformation matrix
+
Rough-aligned CE TS
find
fiducials
fiducial model
fine transformation matrix
back-projection
SIRT
3D reconstruction

Movie of the aligned bidirectional continuous tilt series

Movie of the reconstruction


Continuous
Incremental/Traditional



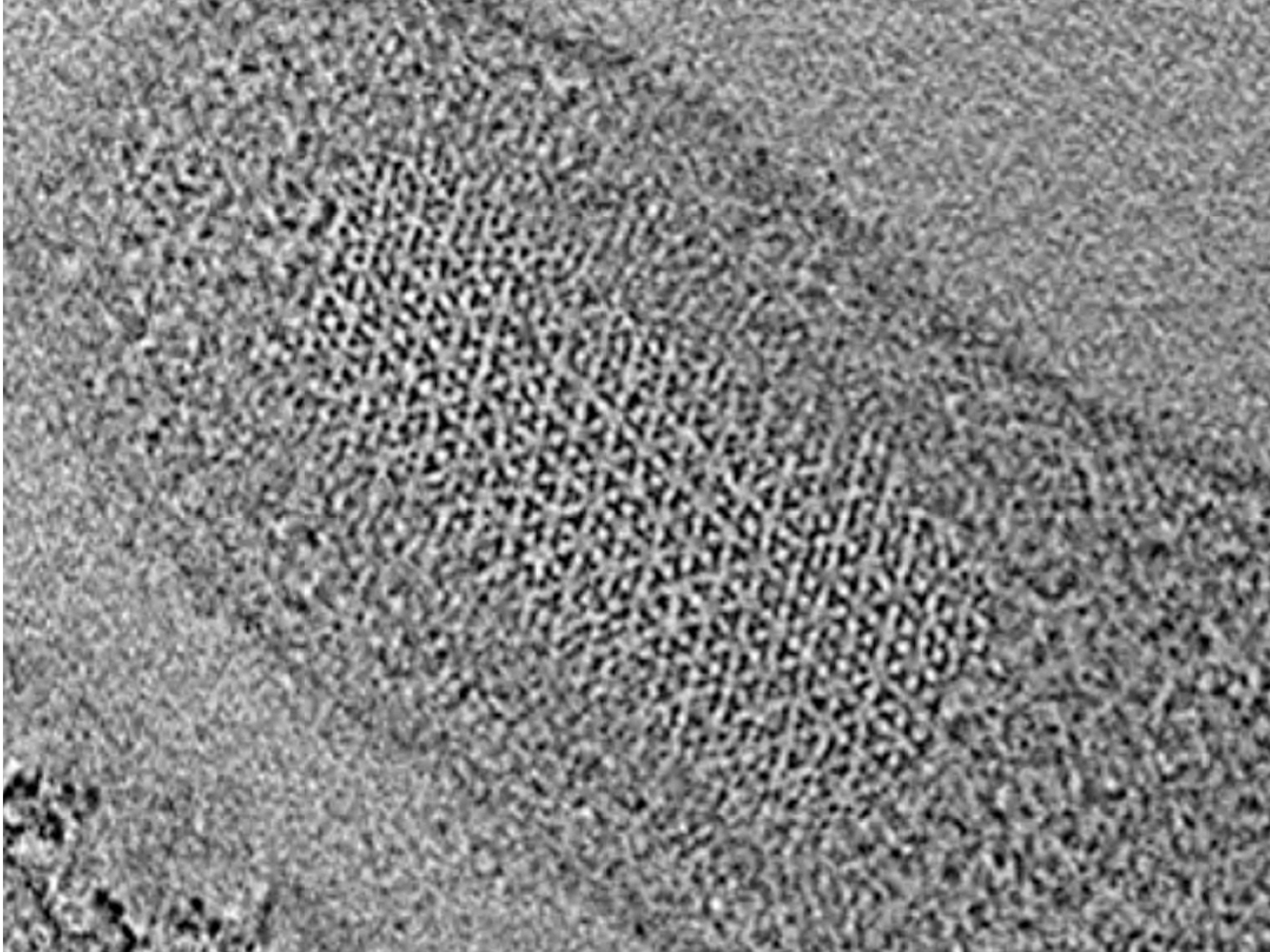


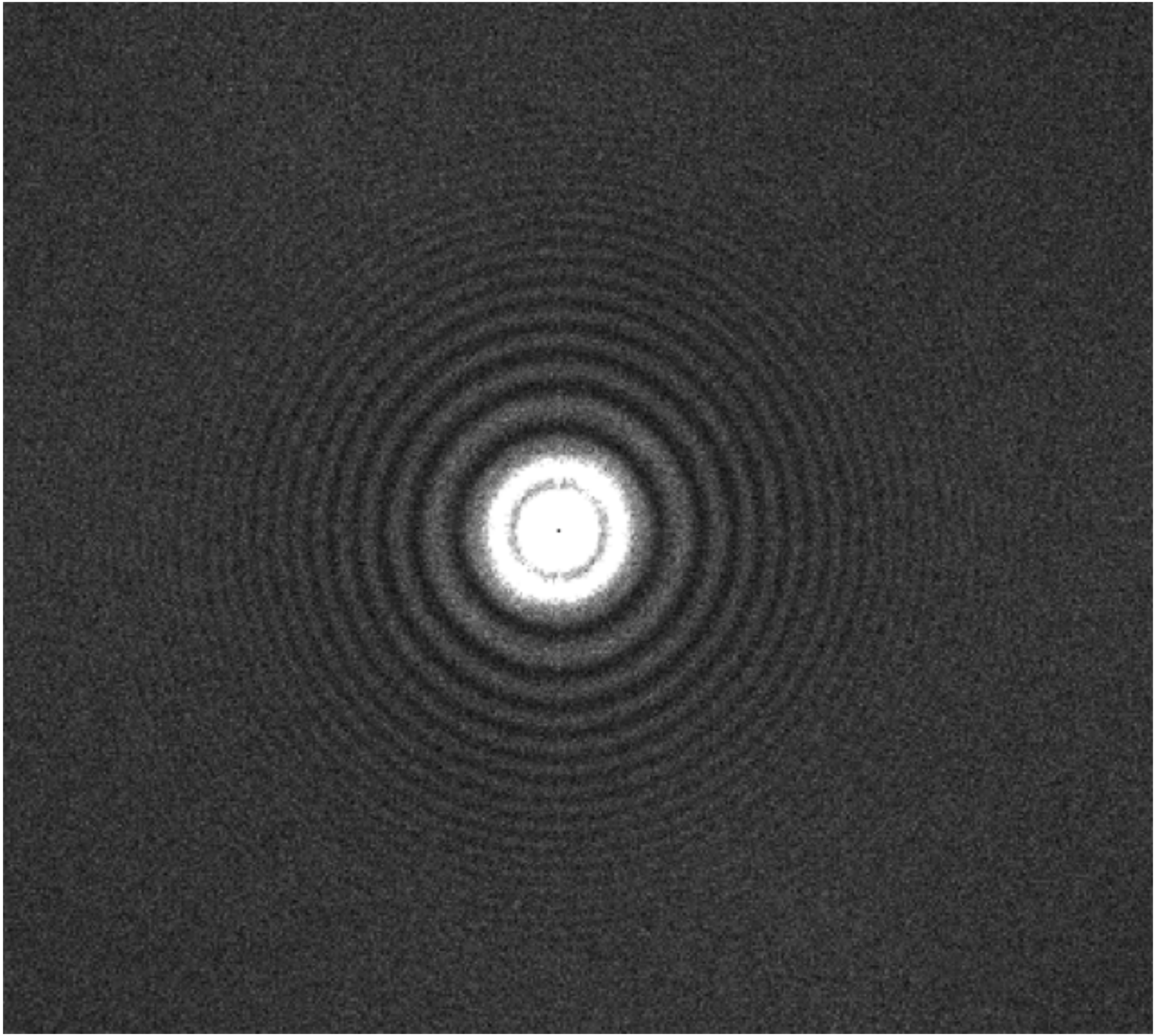
“Fast-incremental”

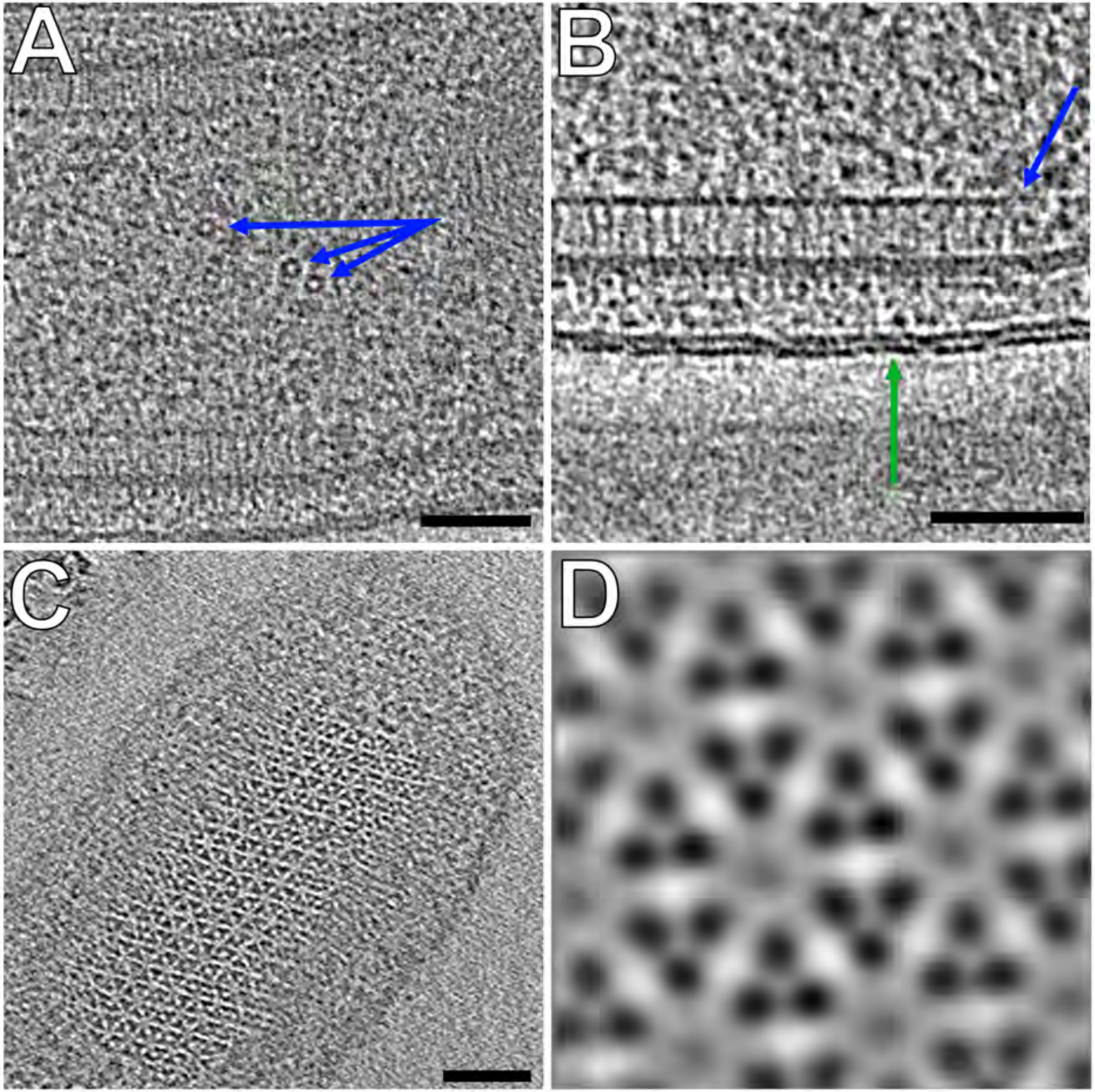
incremental tilting,
but no tracking
or camera read-out

Movie of a unidirectional “fast-incremental” tilt series

Movie of the reconstruction





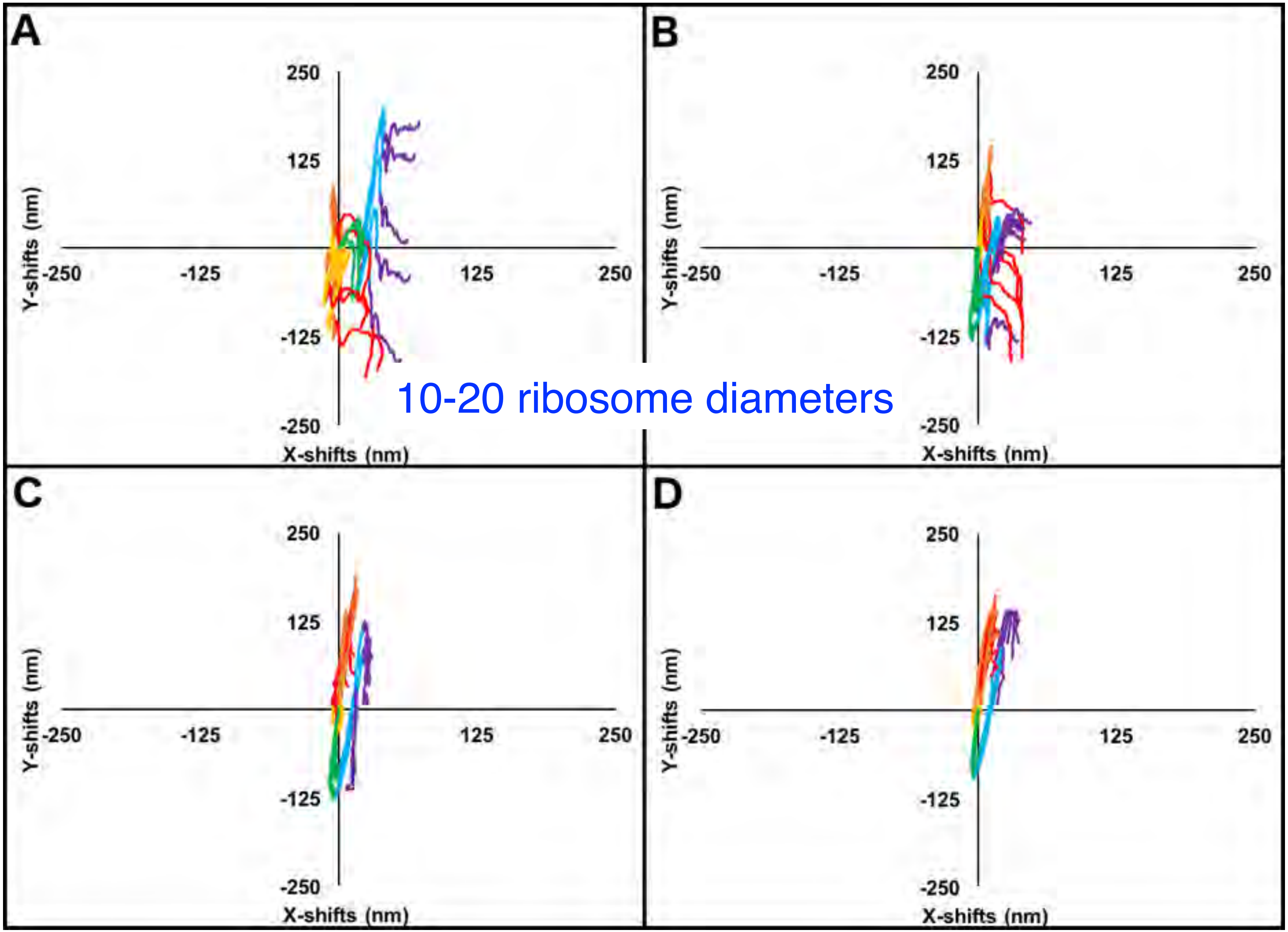

A
B
C
D

Fast incremental tilt series

Tilt scheme	Tilt range	Time spent recording images (s)	Time tilting stage (s)	Camera Latency (s)	Total time (s)	File Format	file size (GB)
Bidirectional	-60 to +60 (start at -18)	41 (1s per tilt)	59	42	142	.tif (LZW compression)	1.90
Dose Symmetric	-60 to +60	41 (1s per tilt)	91	49	181	.tif (LZW compression)	1.95

~2 min

Eucentricity: Dose-symmetric Fast Incremental



Georges Chreifi

Songye Chen

David Mastronarde

Fast tilt-series will obviously advance tomography, but what about single particle analysis?

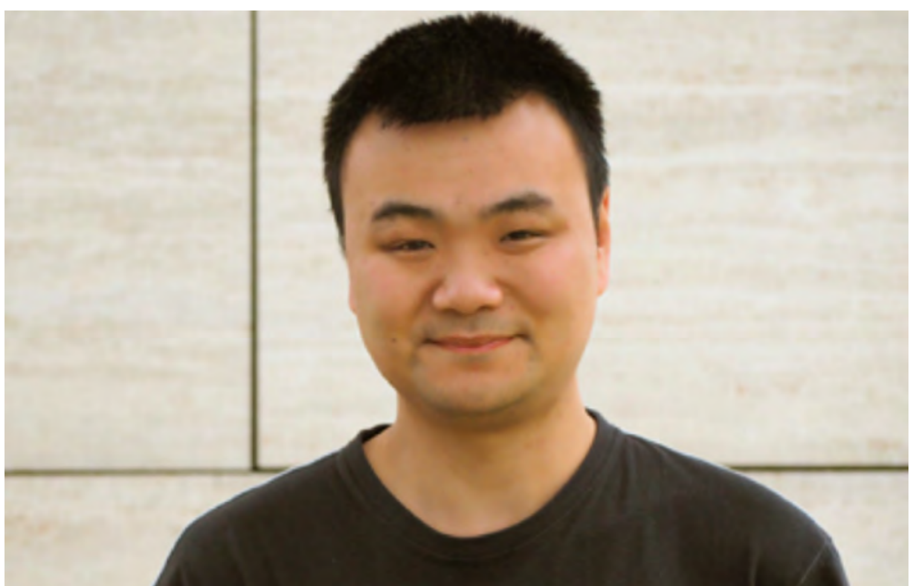
For a given dose, assuming one can align the images, and that tilted images are as good as untilted, you get more information from a tilt series than a single projection (the "dose fractionation theorem")

Fast tilt-series may therefore frequently supplement single particle projections

- disambiguate conformational changes from differences in orientation
- start alignment searches very near the true 3-D orientation
- generate unbiased initial models
- detect and exclude particles on the air/water interface (which are likely damaged)
- improve per-particle CTF-refinement by determining each particle's z-coordinate within the ice
- discover helical parameters quickly at outset of project

For everything too small for
single particle reconstruction,

We propose tomography of nanocrystals



Qing Yao

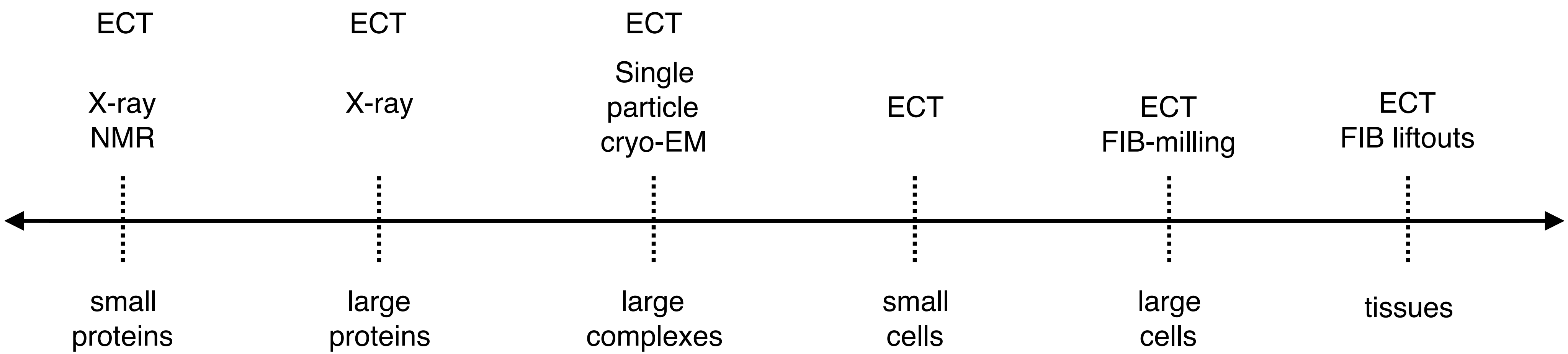
Tomography of nanocrystals

movie of tilt-series of a protein nanocrystal

movie of the reconstruction

Electron tomography of nanocrystals

- Experimentally-determined phases with no need for heavy atom derivatives
- Can use tiny, poorly-ordered crystals
- Less material required (membrane proteins?)
- Can resolve twinning and joints
- Can correct for bends
- Cheaper (a few M$ microscope instead of few hundred-M$ synchrotron)



1. Rapid tilt series
2. Tomography of nanocrystals

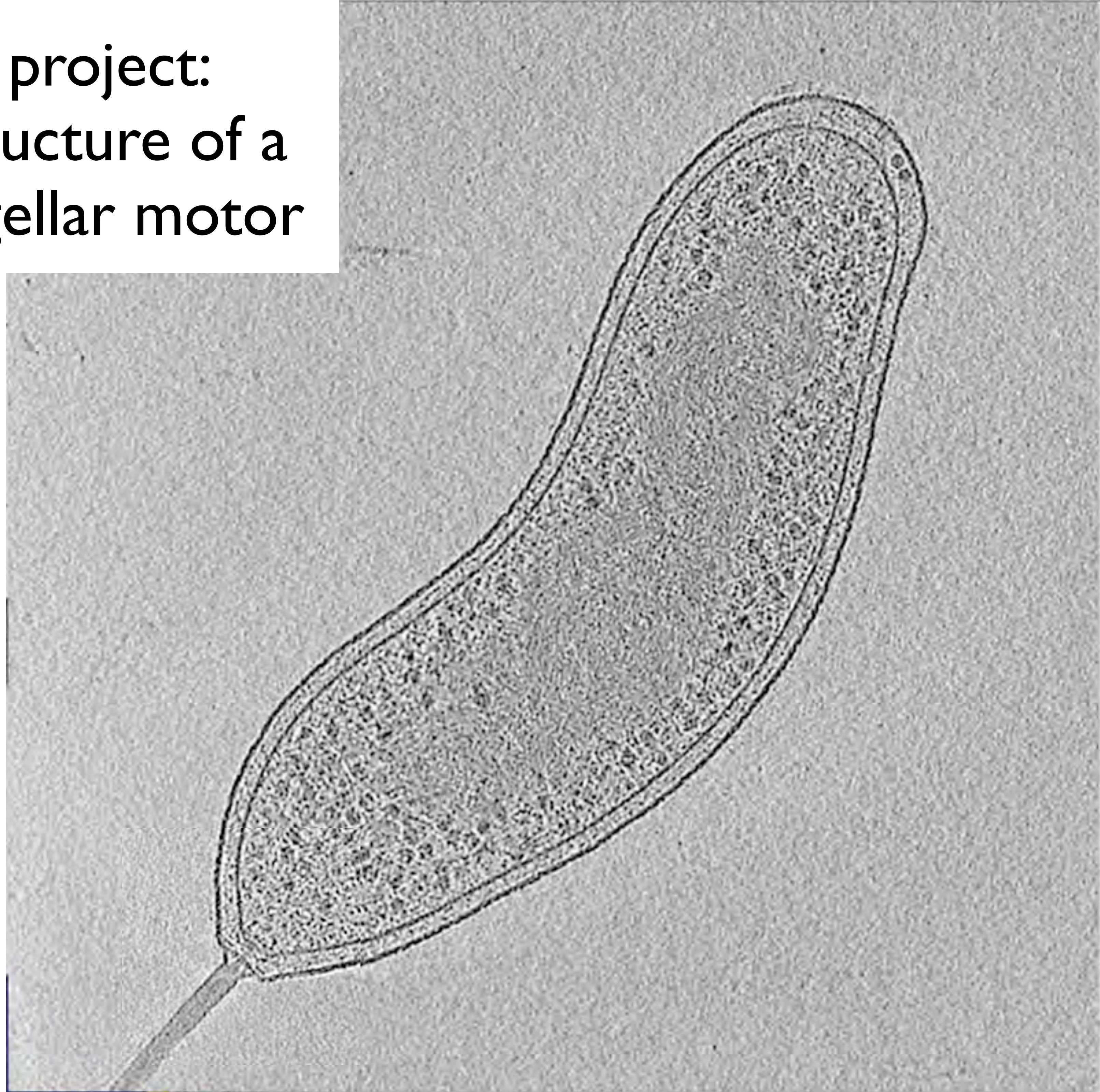
The future of structural biology
is FULL of cryotomography

Claim rests on two principles:

1. Electrons are better than X-rays
2. 3D is better than 2D

Everything else is a technical challenge waiting to be solved

Example project: High res structure of a bacterial flagellar motor



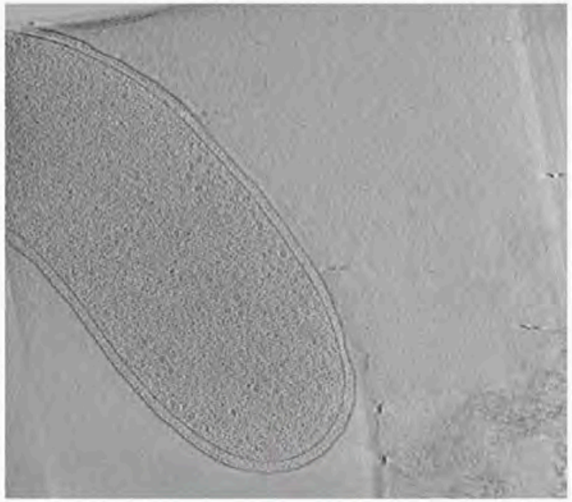
What we need:

- Software to automatically find tomography targets
- Direct detectors/drives that stream continuously with no latency
- Software that picks and tracks *every gold bead* in *every image every time*
- Software to automatically find particles of interest in tomograms
- Ten days on the microscope (assuming 8 motors/cell, 500 tomograms/day, 40k particles needed)

Vibrio cholerae

← Return to database

Share:



0:09 / 0:25

Tilt Series date: September 9th 2015

Data Taken By: Yiwei Chang

Species / Specimen: *Vibrio cholerae*

Strain: O395-N1

Tilt Series Setting: single axis, tilt range: (-60°, 60°), step: 1°, constant angular increment, dosage: 180eV/Å^2, defocus: -8μm, magnification: 27500x.

Microscope: Caltech Polara

Acquisition Software: UCSFTomo

Processing Software Used: Raptor

Notes: Tilt series notes: Classical strain with ctxA deletion
Cell harbors pMT5 plasmid (inducible toxT)

Download files

#	Name	Size	Type	Download
1	20150909_AK_pMT15_10009.mrc	3.45 GB	Tilt series	DOWNLOAD
2	20150909_AK_pMT15_10009_full.rec	534.53 MB	Reconstruction	DOWNLOAD
3	keymov_yc2015-09-09-9.mp4	17.43 MB	Key movie	DOWNLOAD
4	keymov_yc2015-09-09-9.flv	56.36 MB	Key movie	DOWNLOAD
5	keyimg_yc2015-09-09-9.jpg	1.04 MB	Key image	DOWNLOAD

Georges Chreifi
Songye Chen
Qing Yao
Sara Weaver
Yiwei Chang

David Mastronarde