Progress towards collecting tilt-series in seconds rather than minutes with a new “high precision” stage

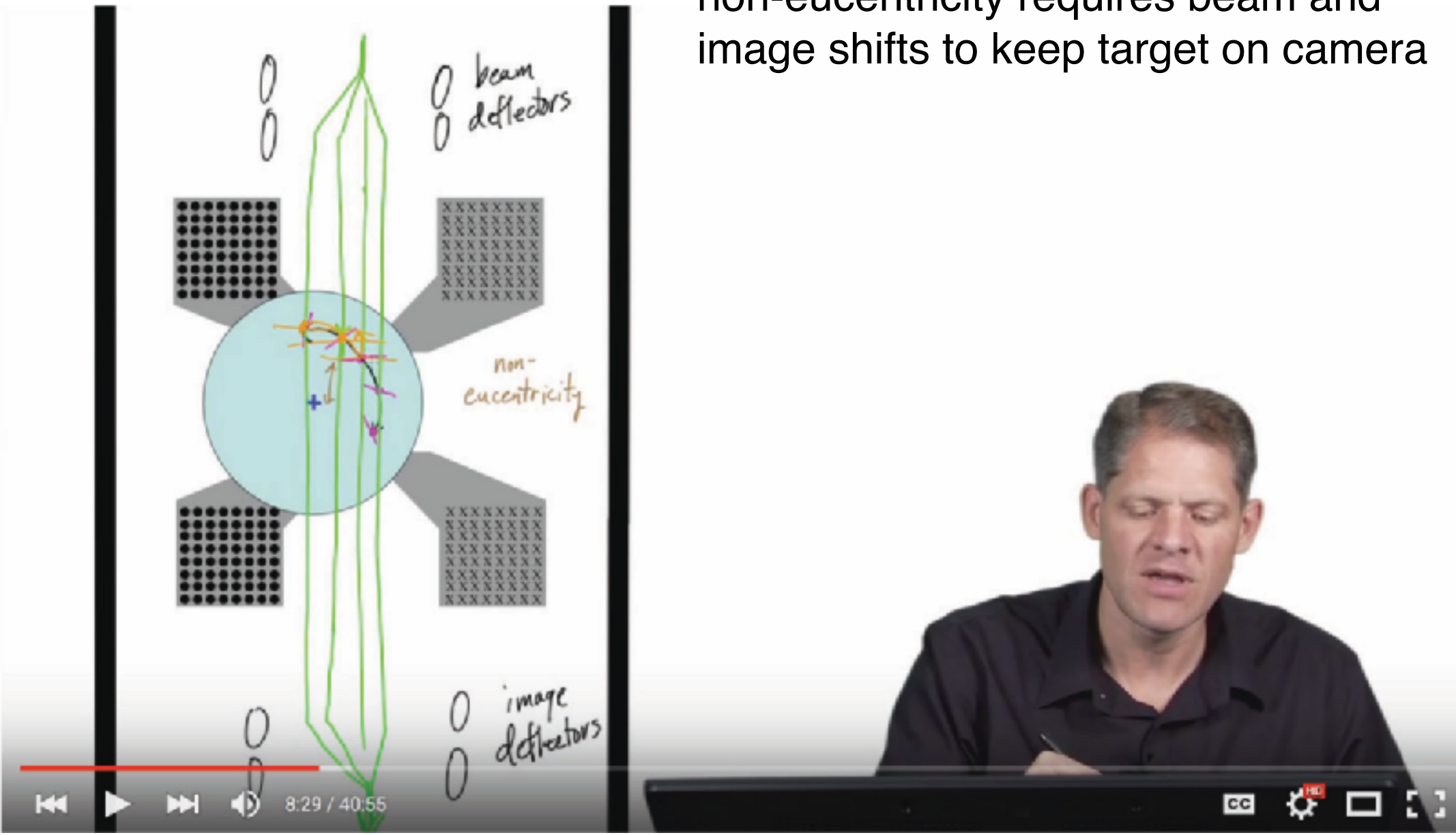
or,

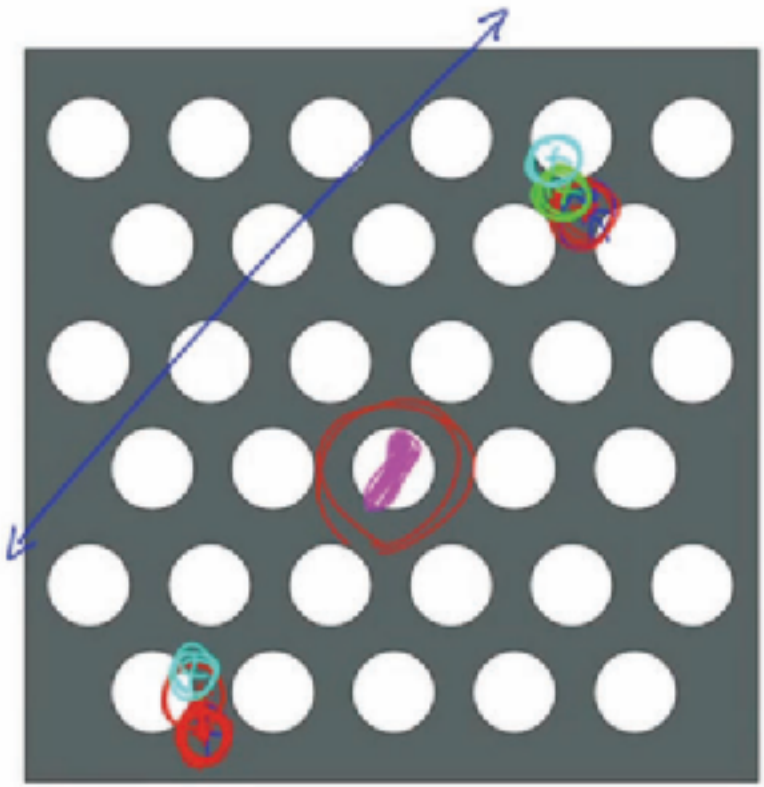
The future of structural biology:

ALL cryotomography, ALL the time

Grant Jensen
Caltech
HHMI

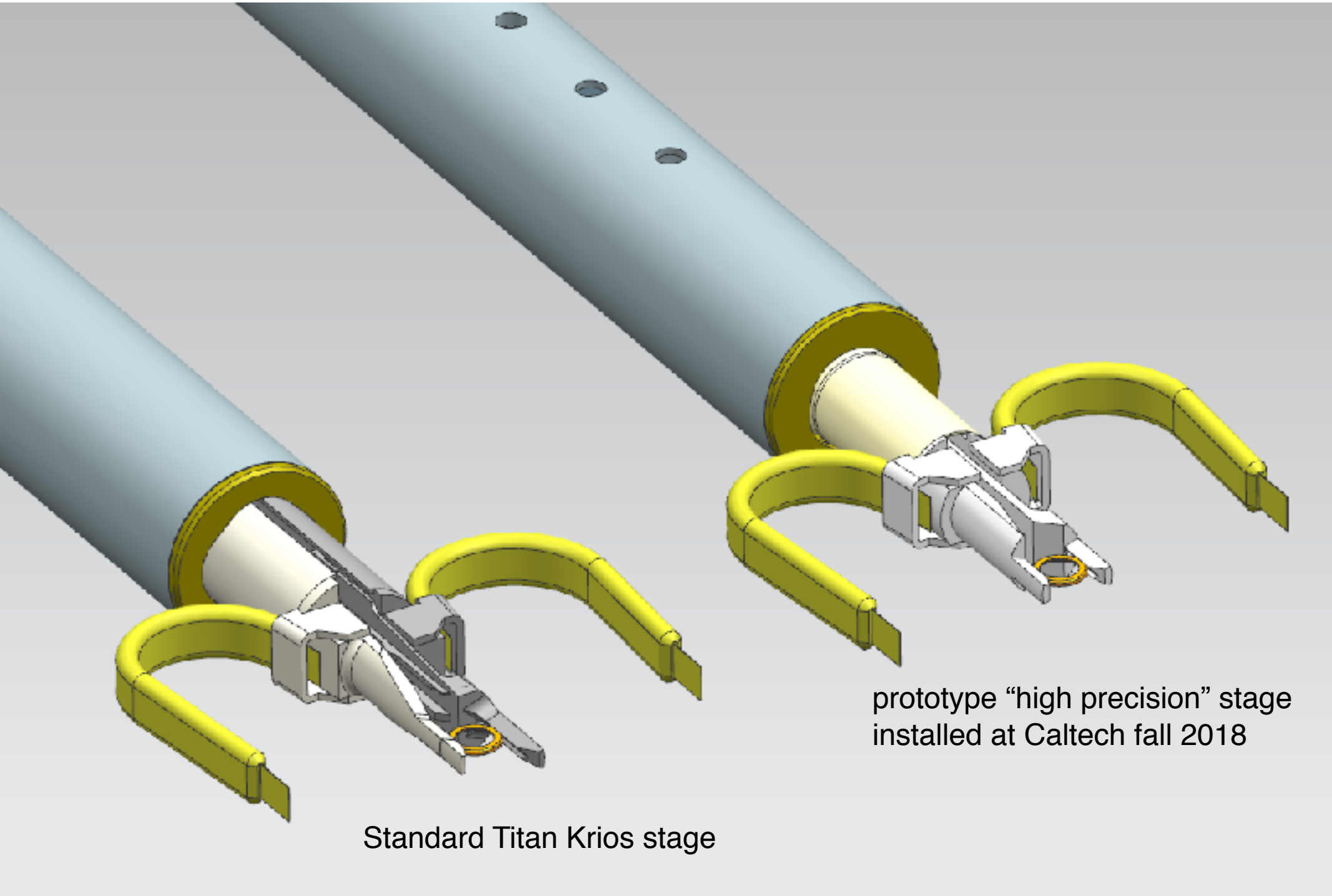
Data collection with today's stages: non-eucentricity requires beam and image shifts to keep target on camera





"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

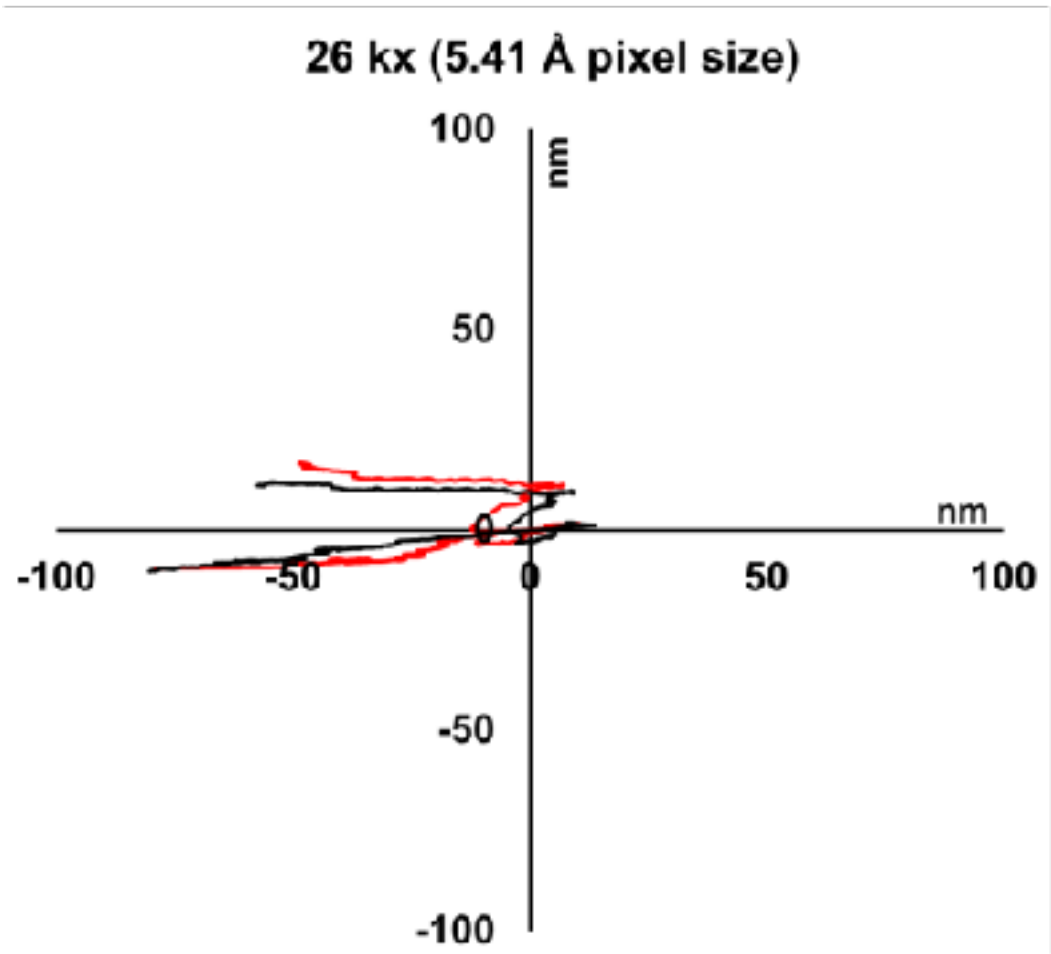

prototype “high precision” stage
installed at Caltech fall 2018
Standard Titan Krios stage

example fast tilt series where camera was set to record images continuously while sample was rolled from -60 to 60

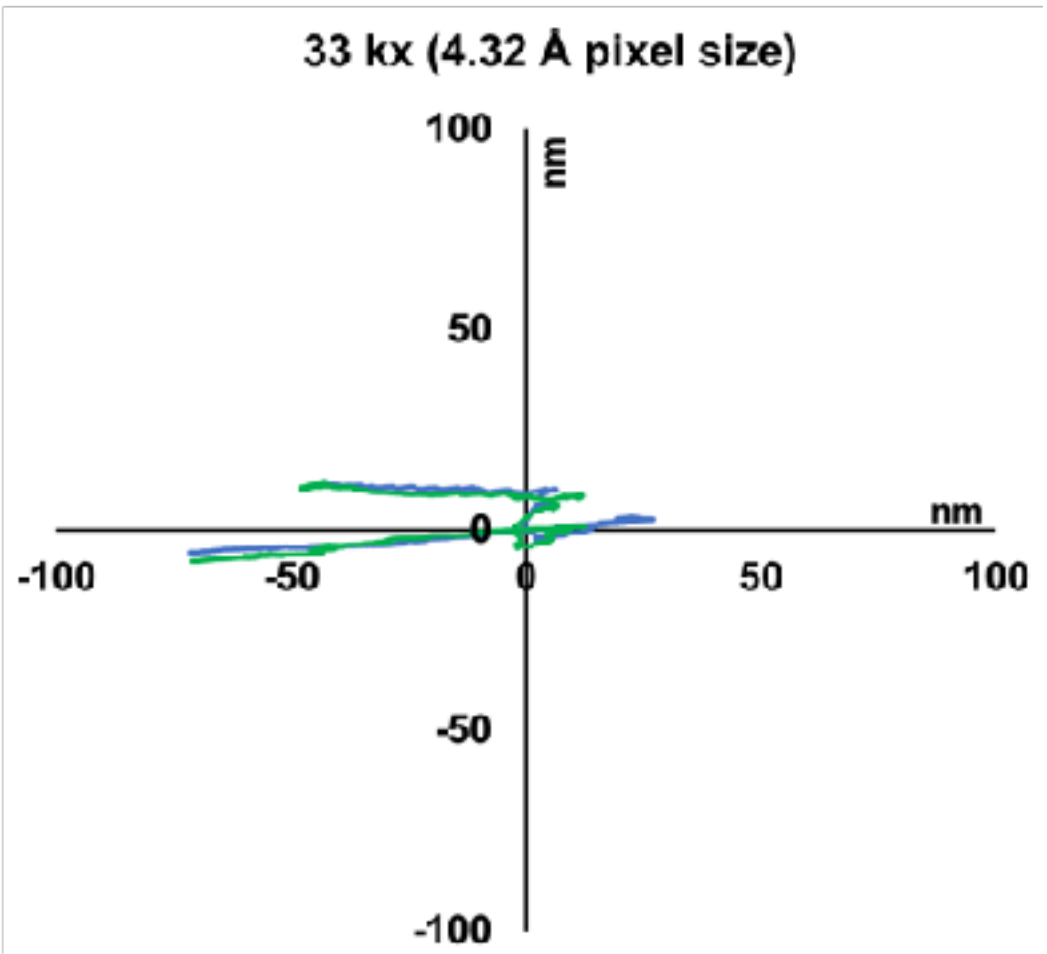
The camera is the limiting component

Nominal magnification	Pixel size (Å)	Exposure time (s)	Total frames	Total time per tilt-series (min)
33kx	4.32	126	5040 or less	9.7
53kx	2.74	50	2000 or less	7.6
81kx	1.78	20	800	6.7
130kx	1.09	12	480	5.0

Tilt Series Alignment -60° to +60°



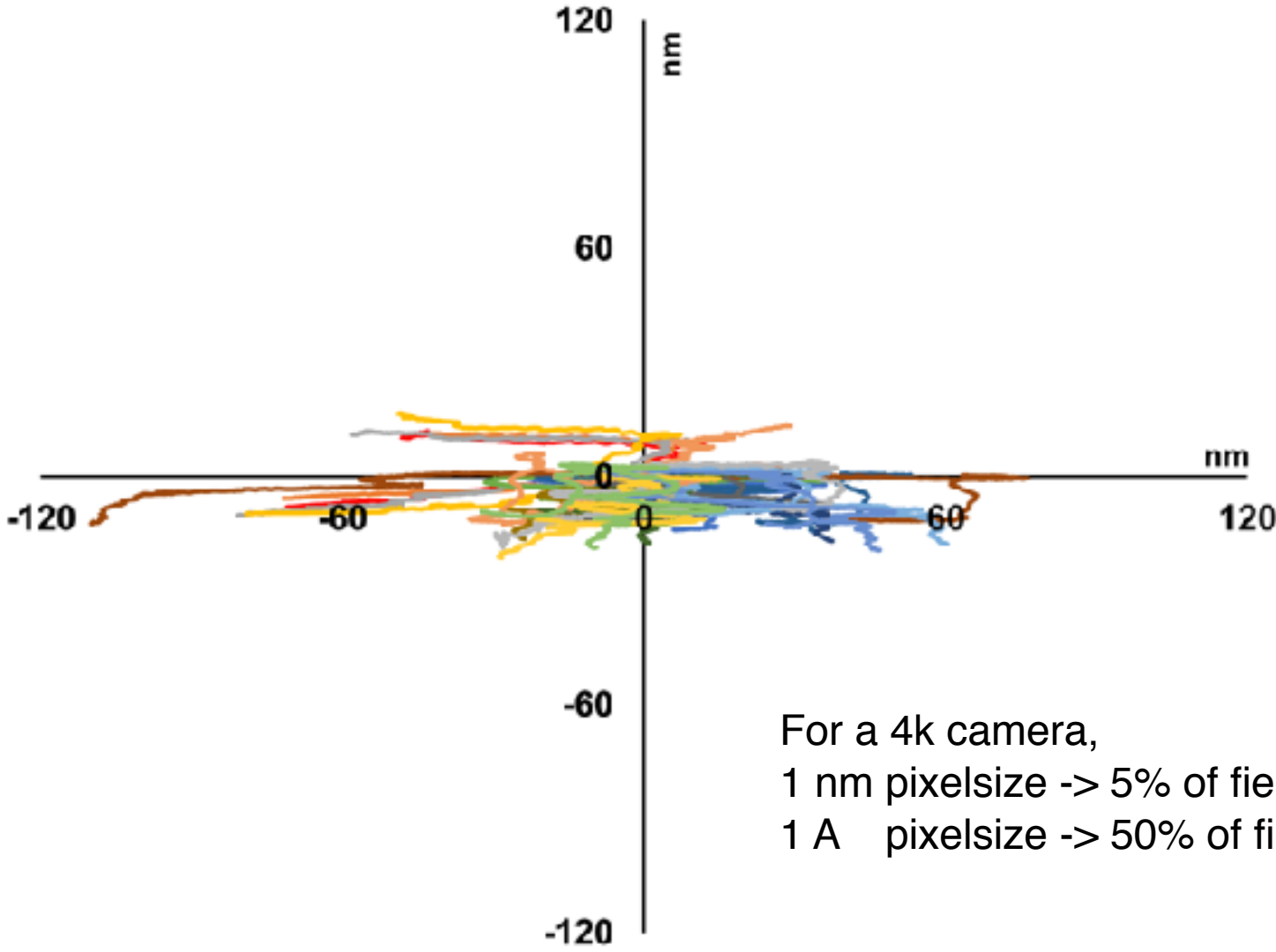
tilt axis



tilt axis

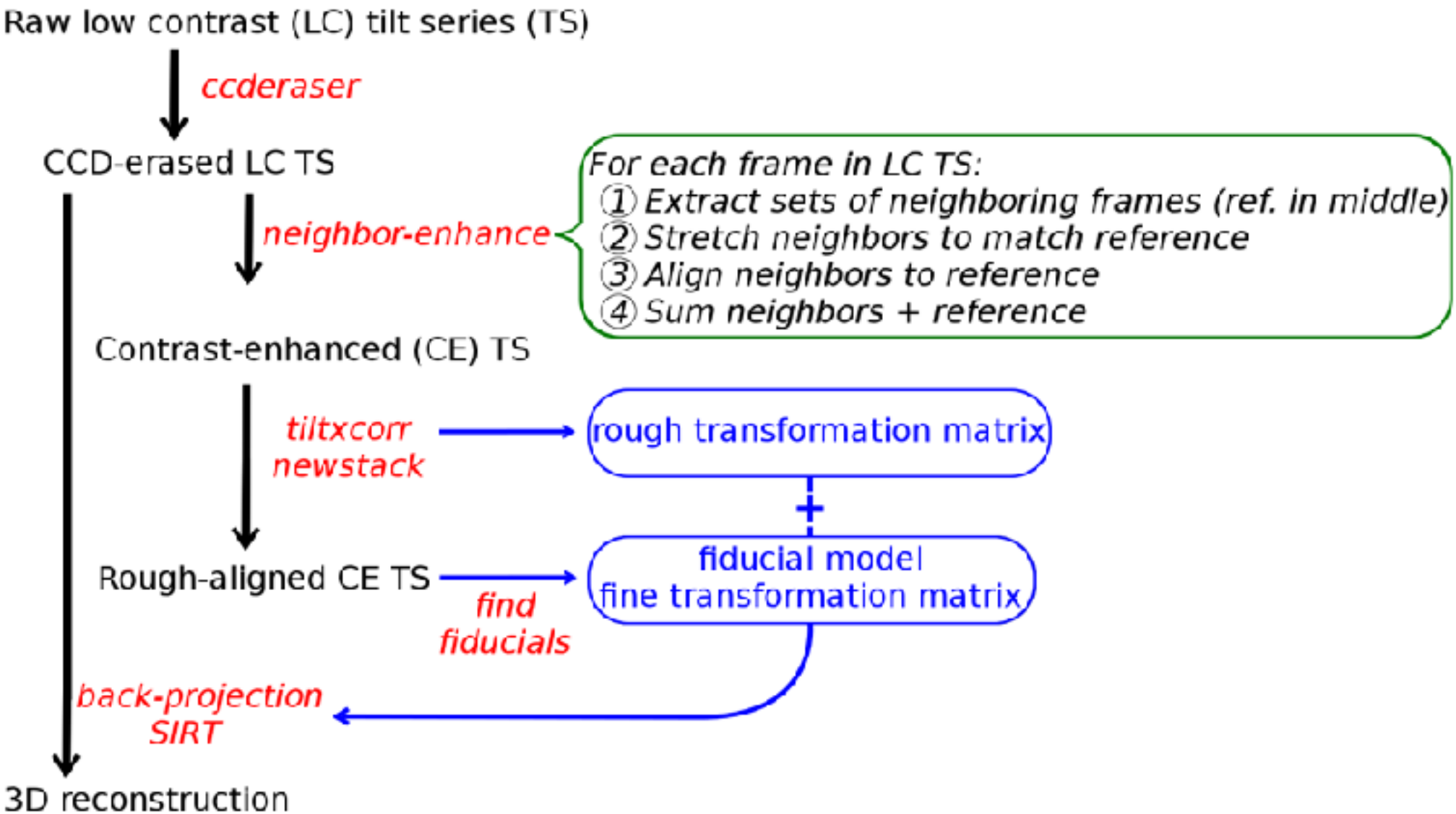
example fast tilt-series

Eucentricity of the high precision stage



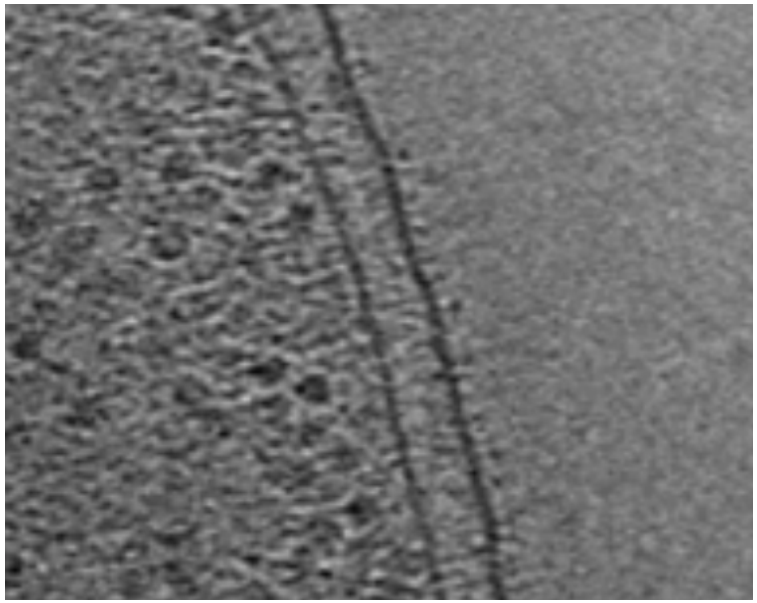
For a 4k camera,
1 nm pixelsize -> 5% of field of view lost
1 A pixelsize -> 50% of field lost

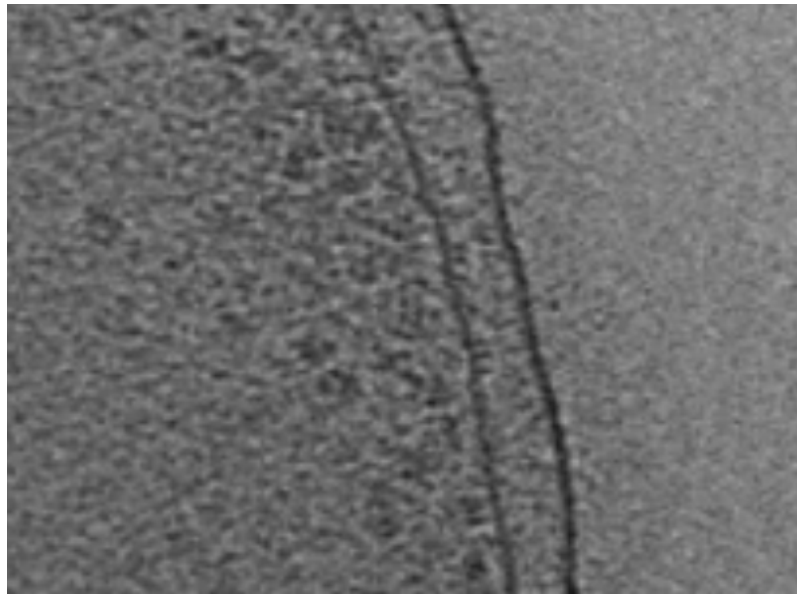
Image processing flowchart

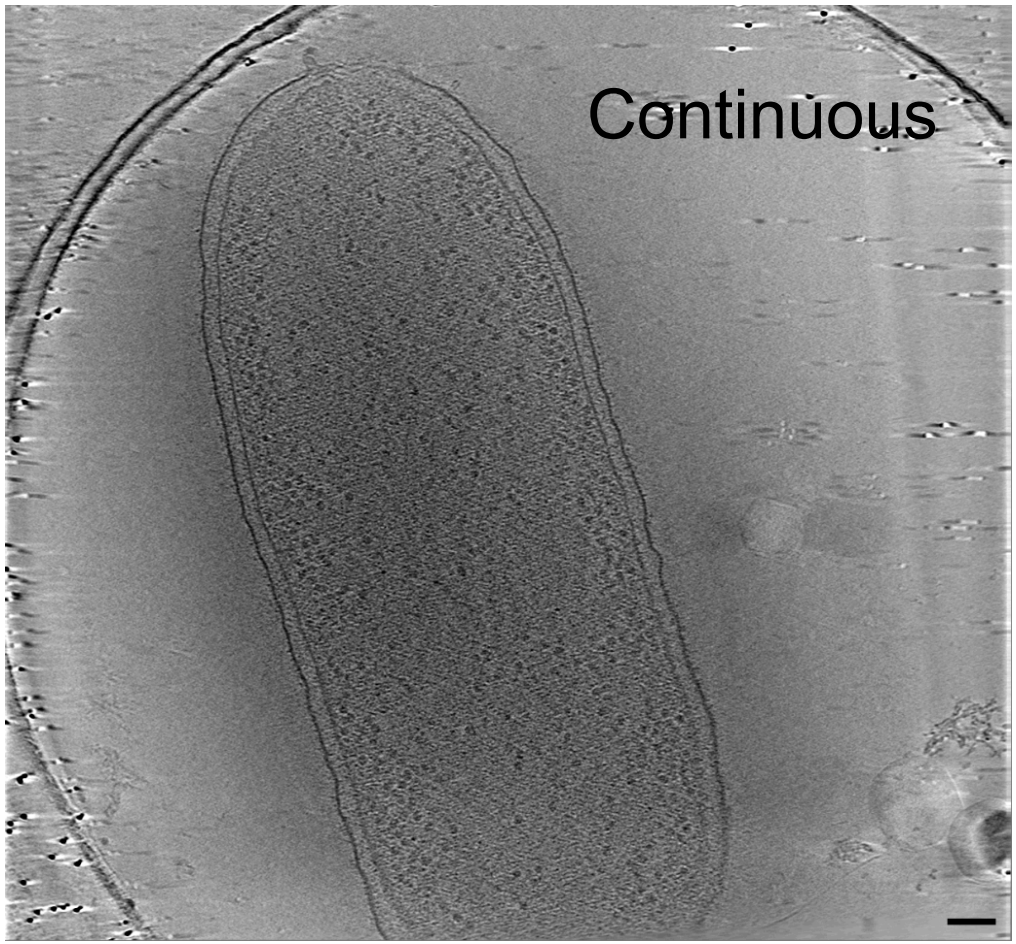


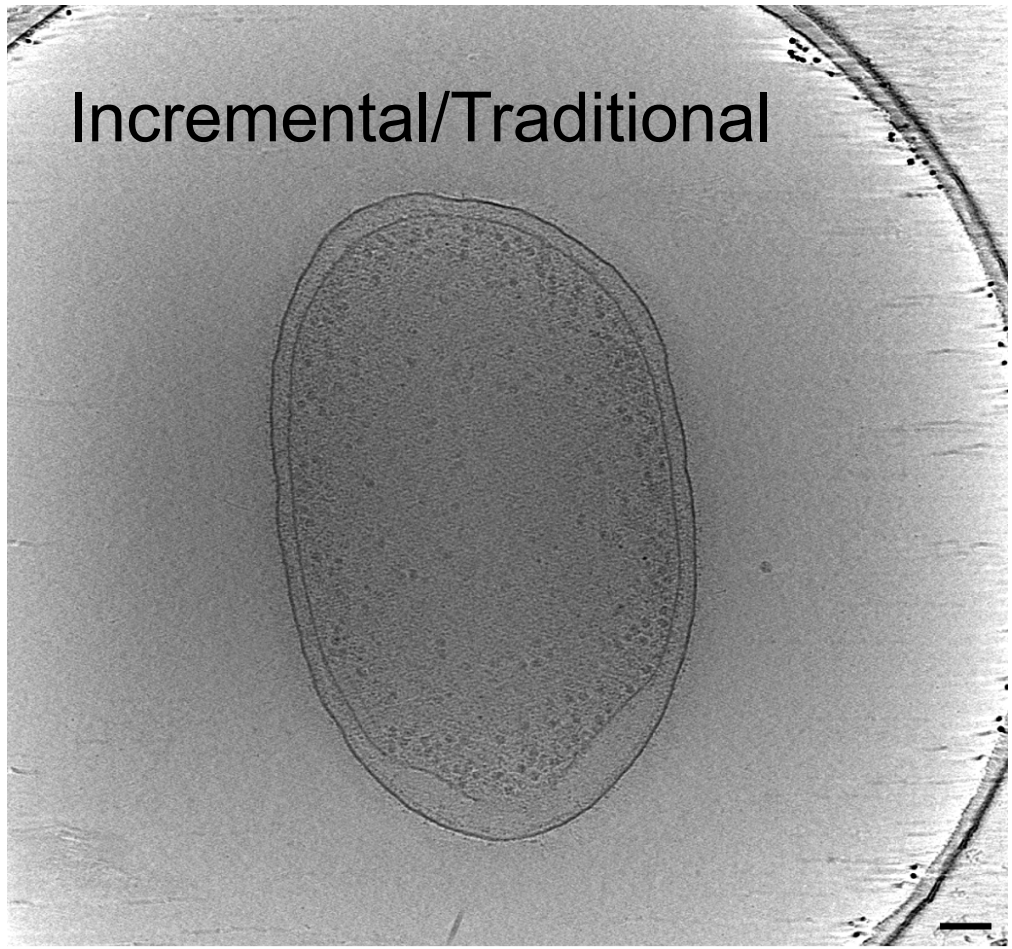
movie

reconstruction

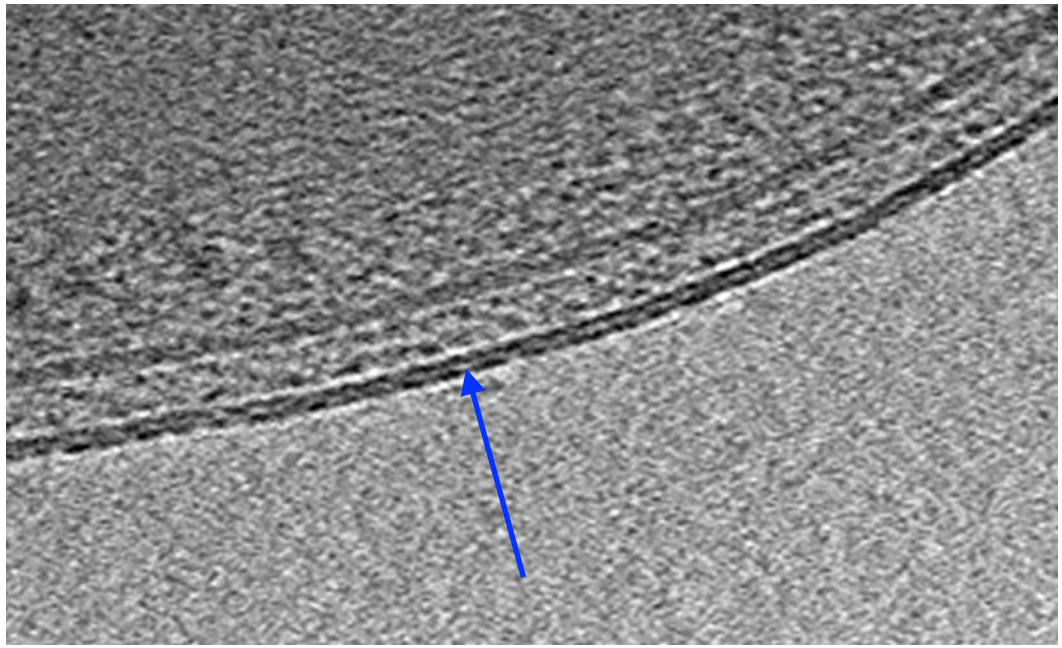


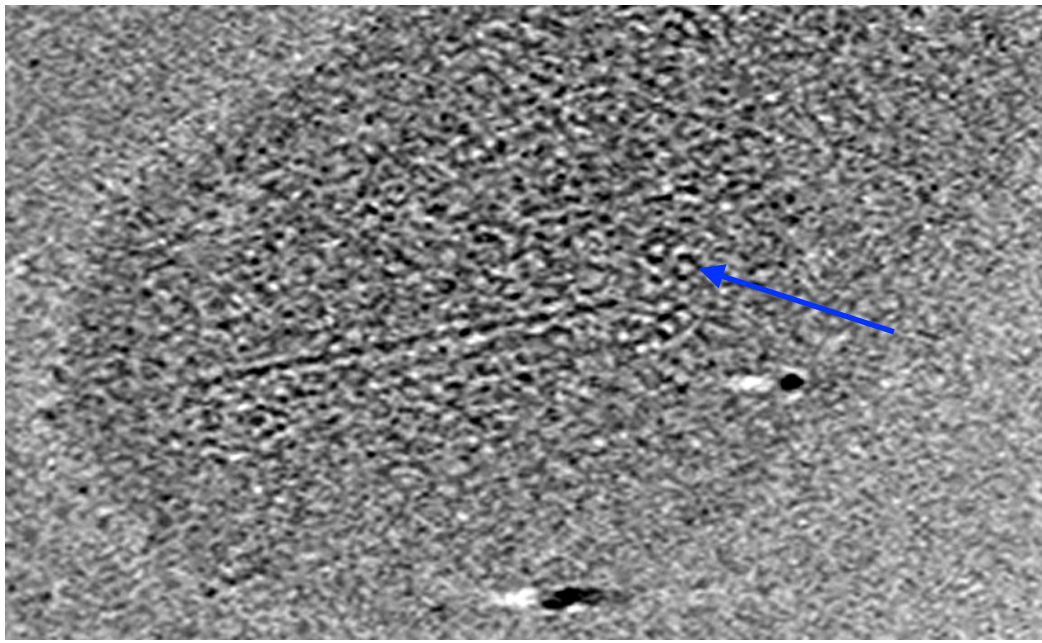


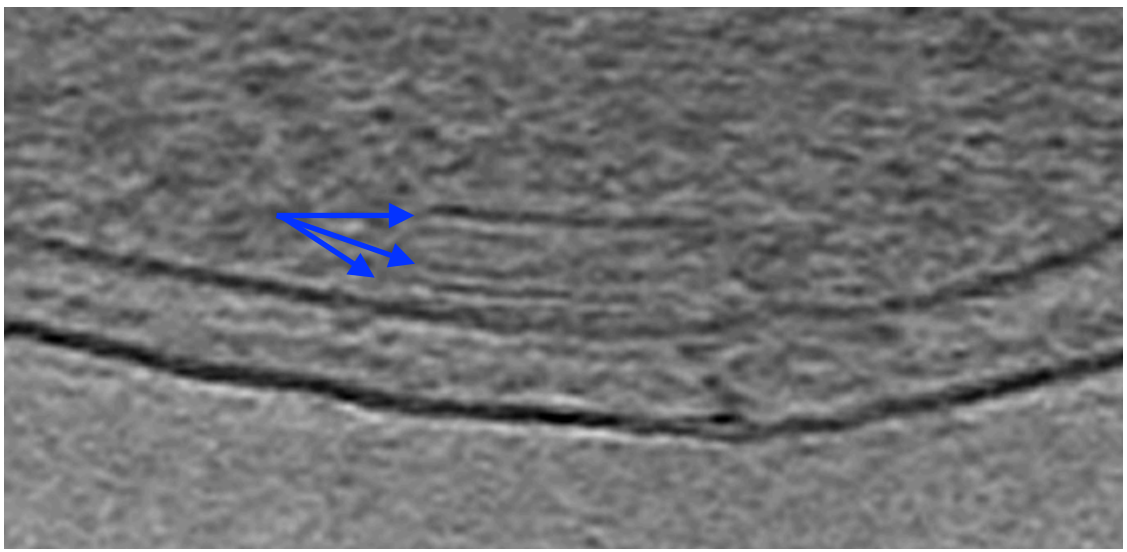

Continuous

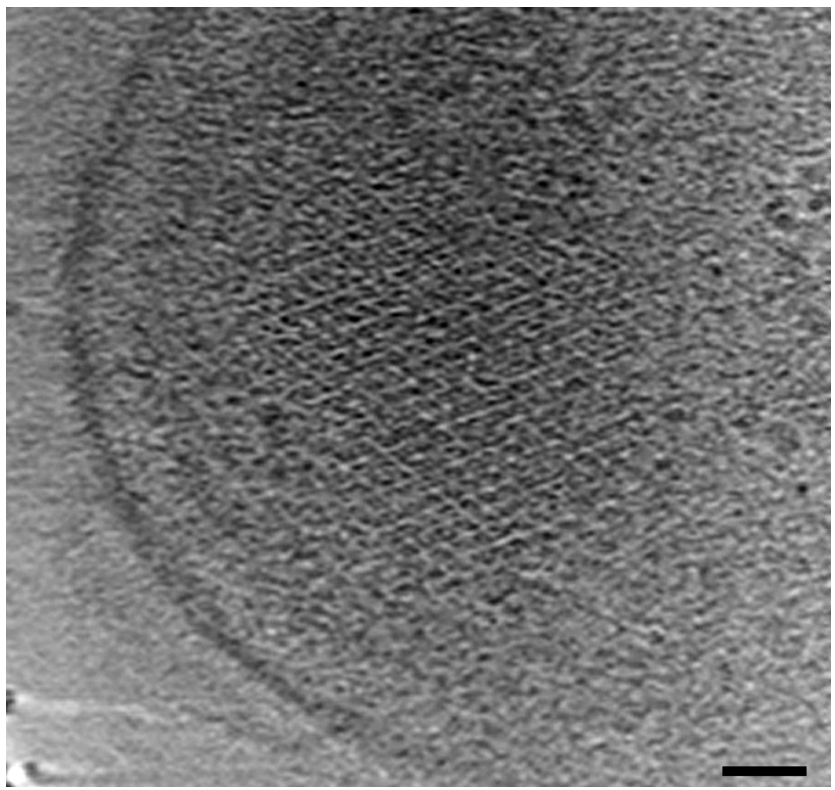

Incremental/Traditional

Example features in tomograms









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

For a given dose, assuming one can align the images, you get more information from a tilt series than a single projection (the "dose fractionation theorem")

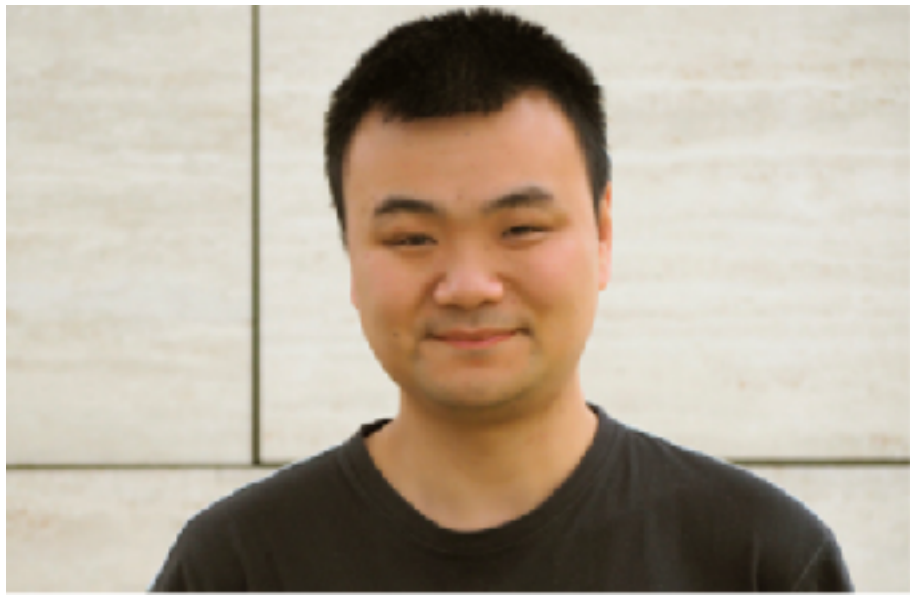
Fast tilt-series will therefore eventually prove more useful than a single projection

- Better classification
- Better initial models
- Better initial alignments

(or perhaps a hybrid approach will be best, where an initial projection image will be obtained with ~10 e/A^2, then a quick tilt series will be obtained with an additional ~90 e/A^2 to classify the particle and estimate orientation)

For everything too small for
single particle reconstruction,

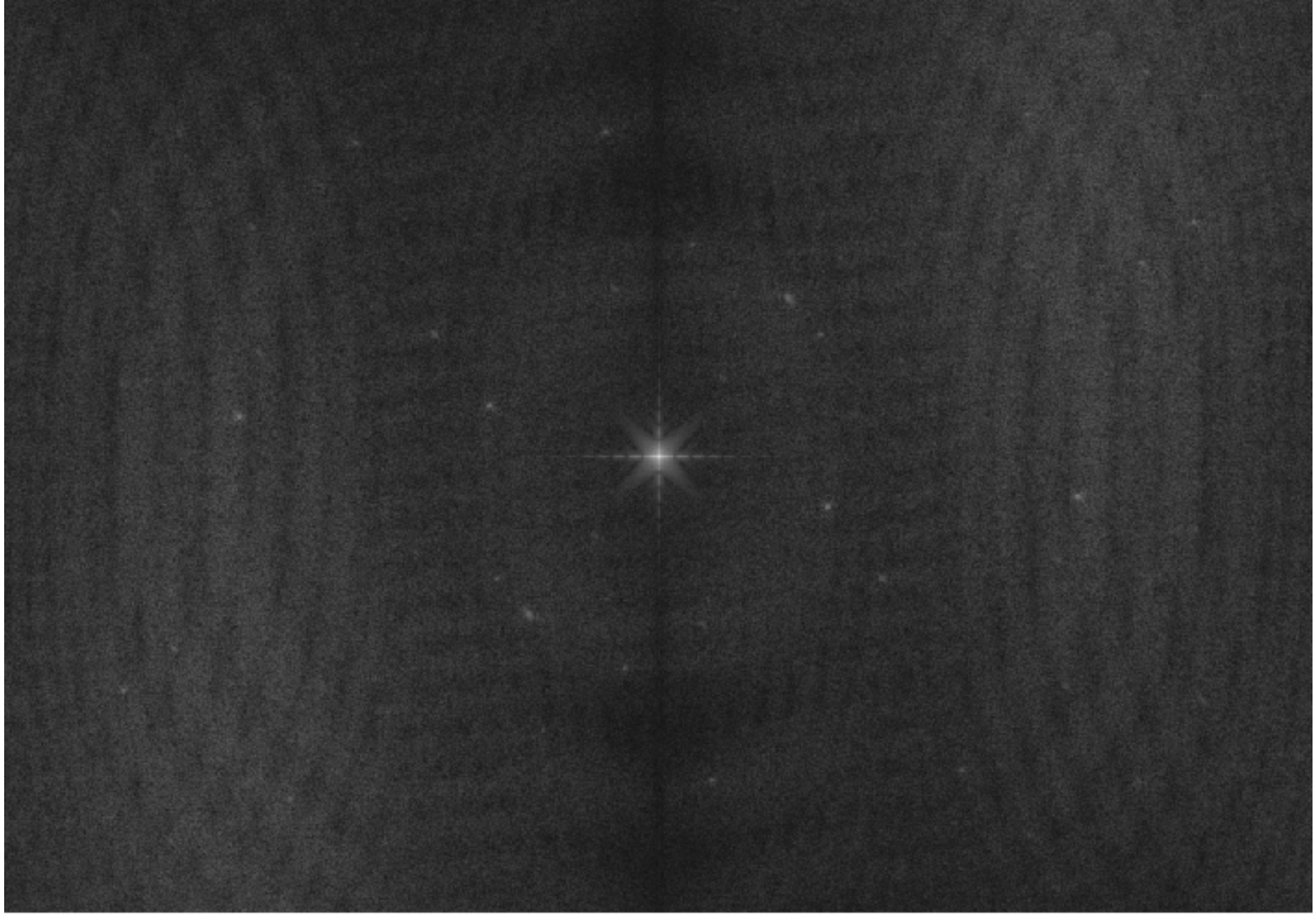
We introduce tomography of nanocrystals



original idea from
Dr. Qing Yao

movie of tilt-series of nanocrystal

movie of tomogram of nanocrystal



Example slice of 3D Fourier transform of nanocrystal tomogram

Why electron tomography of nanocrystals will replace X-ray crystallography

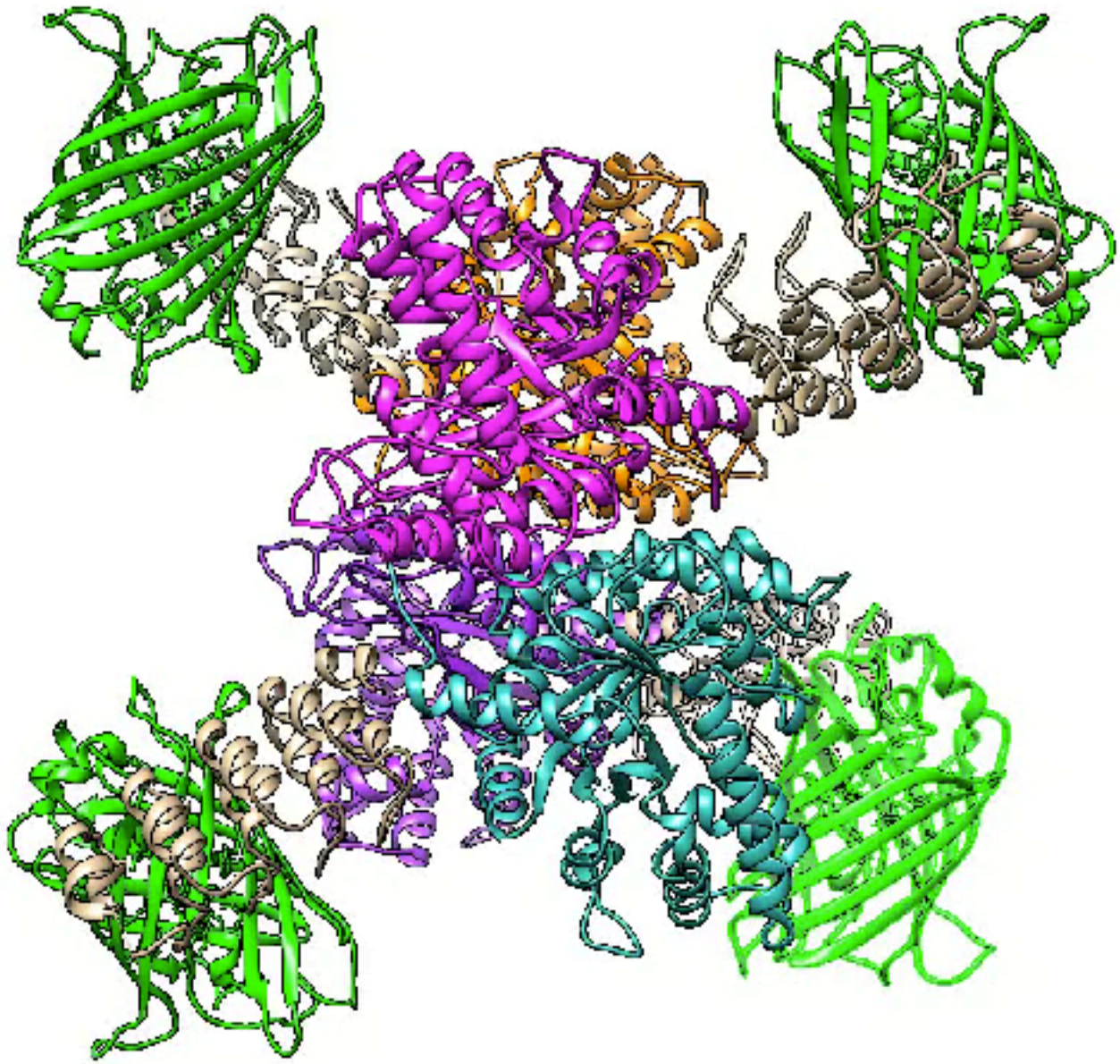
- Fast, accurate phases with no need for heavy atom derivatives
- Uses crystals only a few hundred nanometers thick (which are thought to be much more frequently obtainable than large crystals)
- Can resolve twinning and joints
- Can correct for bends
- Cheaper (a few M$ microscope instead of few hundred-M$ (?) synchrotron)

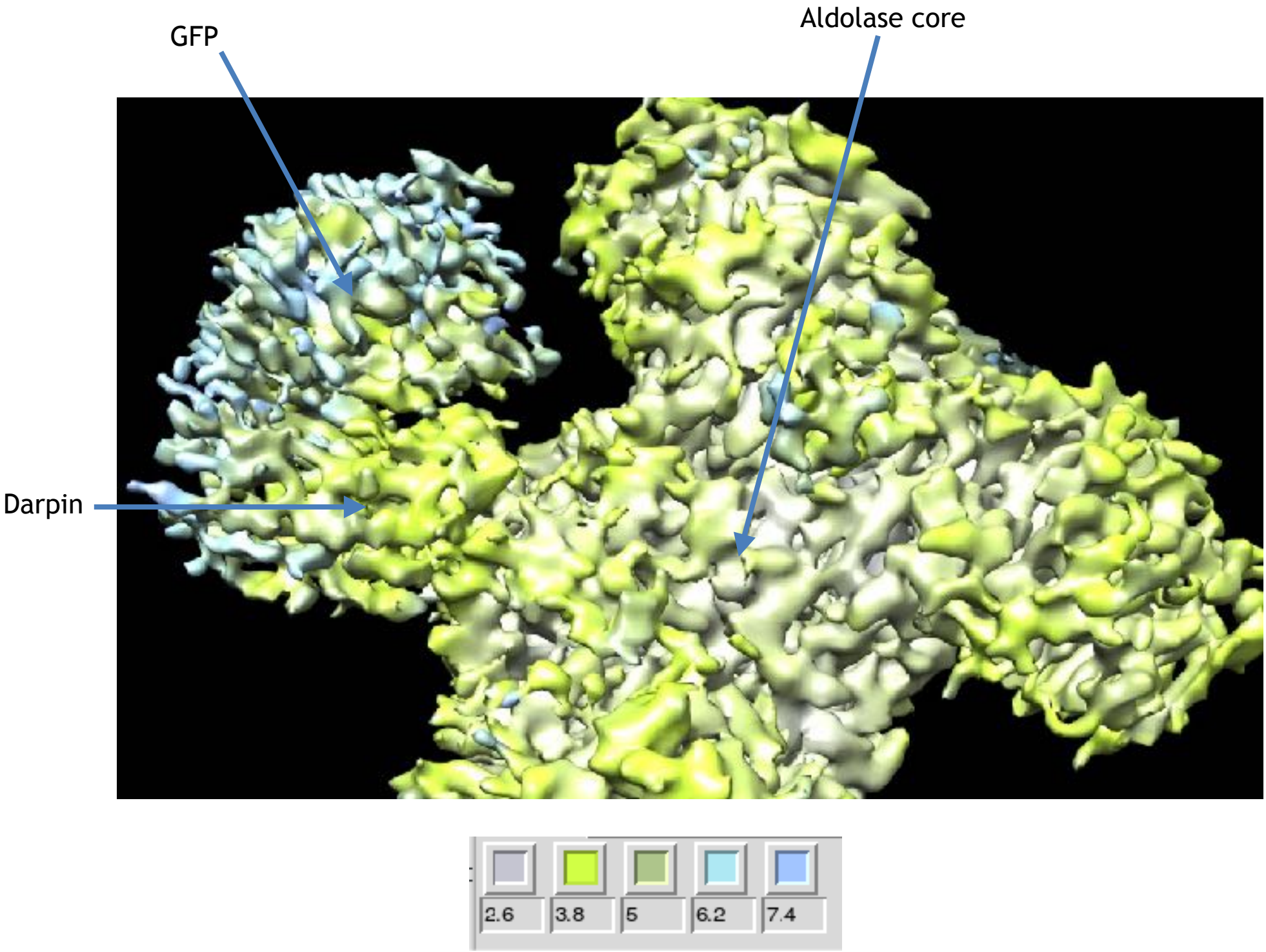
Finally, for everything
(1) too small for single particle analysis and
(2) that won't crystallize

We are developing "platforms"

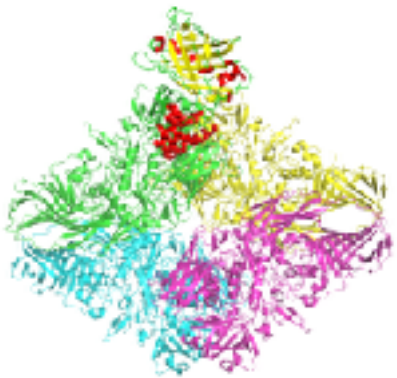
(large complexes with selectable, rigid adaptors to bind any small macromolecule of interest, making a complete complex large enough for single particle approaches)

see also recent Yeates paper PNAS Feb 2018

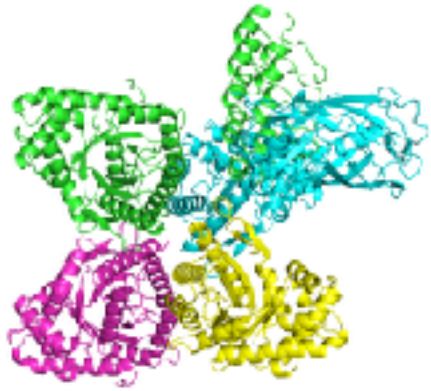



GFP
Aldolase core
Darpin
2.6
3.8
5
6.2
7.4

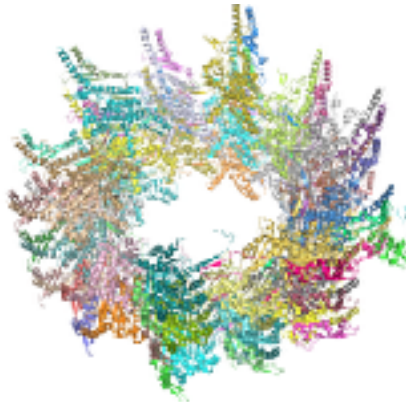
Potential platforms we have tried so far



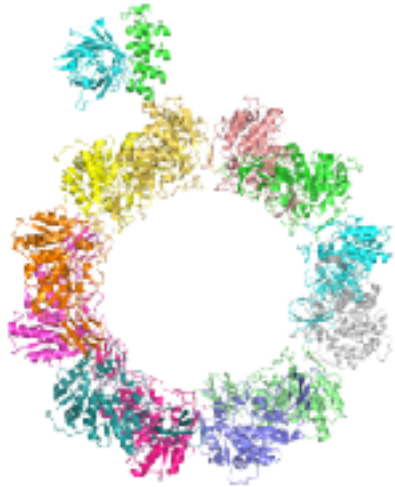
ß-galactosidase



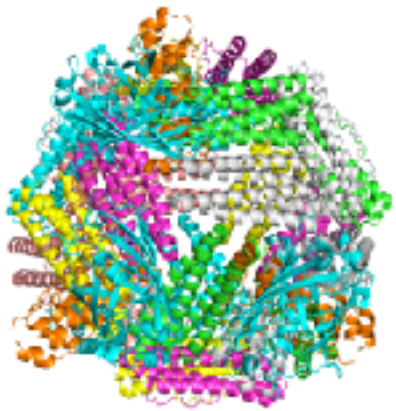
Aldolase



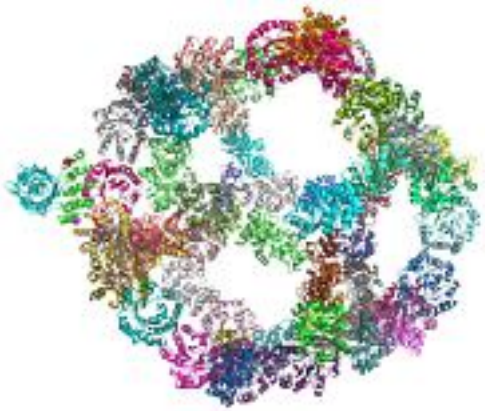
vipA/vipB



TibC

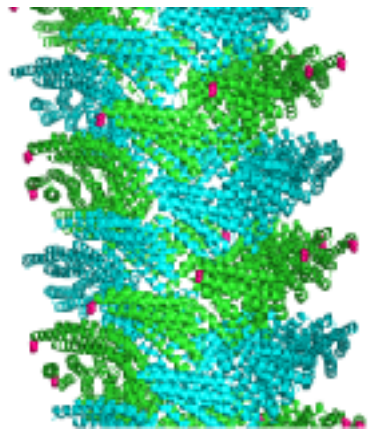


Ferritin



Artificial cage

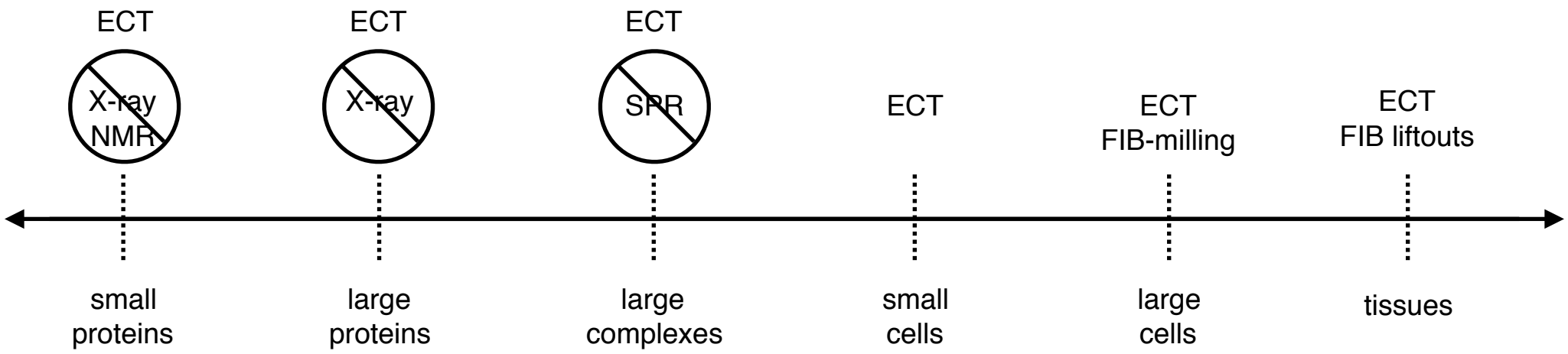
T33 nanocage



DHR tube

The future of structural biology:

ALL cryotomography, ALL the time



Enabling technologies:

1. Rapid tilt series
2. Tomography of nanocrystals

What we need most

- Direct detectors that finish operations faster
- Software to automatically find tomography targets
- Software that picks and tracks *every gold bead* in *every image* *every time* across 1000-frame tilt-series in minutes
- Software to automatically find particles of interest in tomograms
- Software to extract phases from large 3D Fourier transforms of nanocrystal tomograms

movie showing automatic fiducial marker picking - sometimes fails

Georges Chreifi
Songye Chen
Qing Yao
Sara Weaver
Yiwei Chang

special thanks to
David Mastronarde