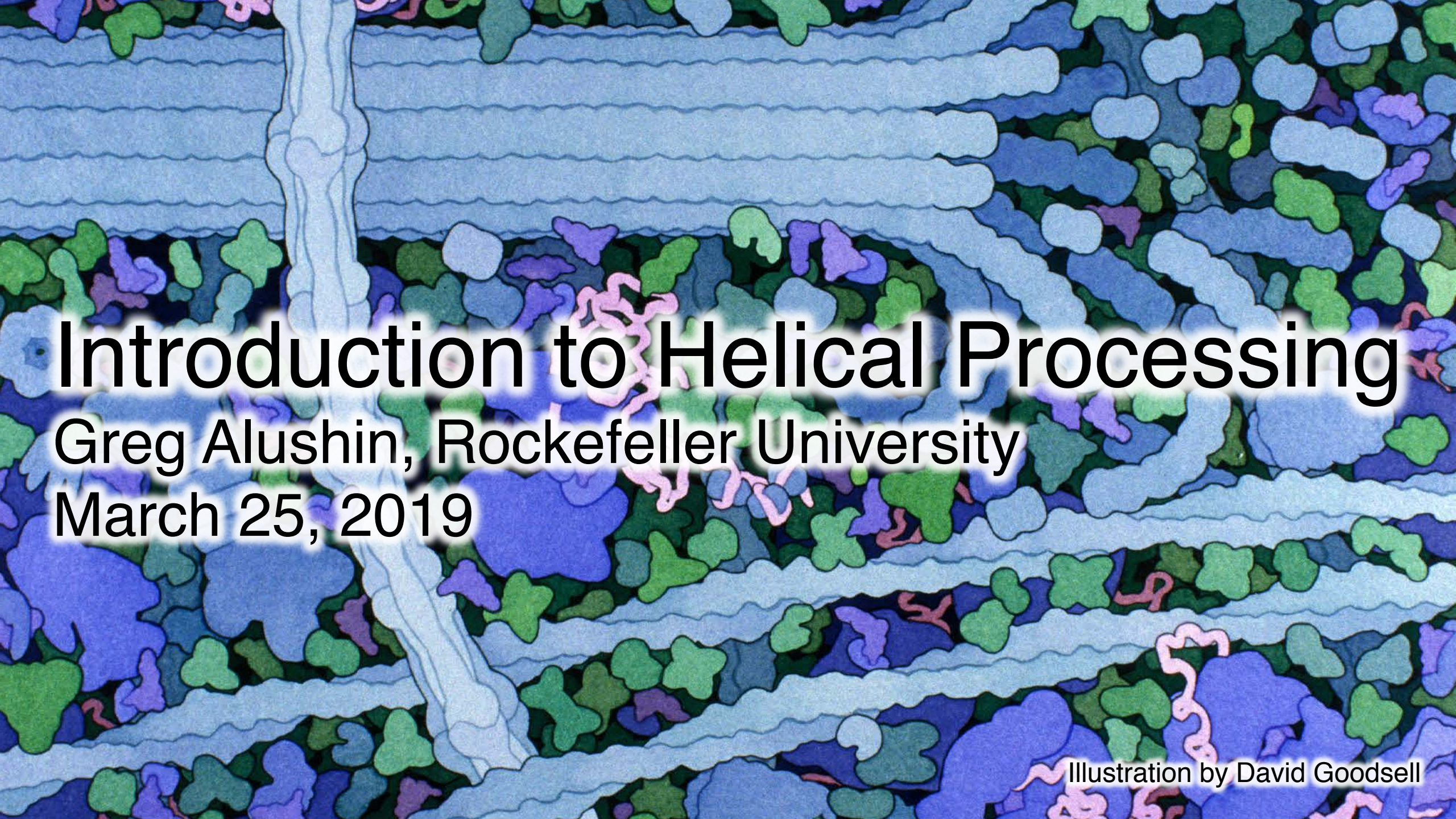
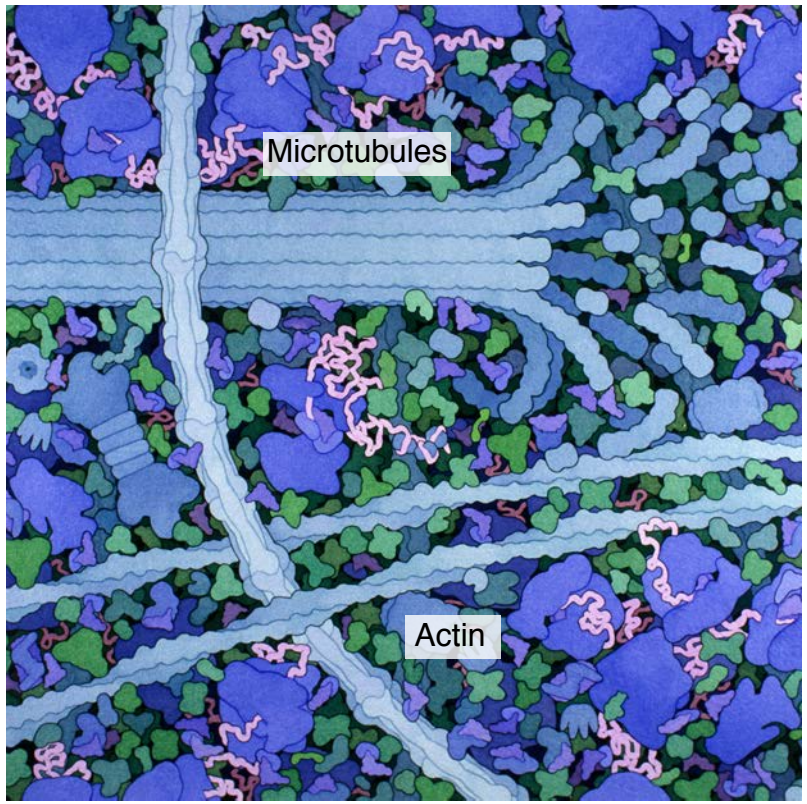


Introduction to Helical Processing

Greg Alushin, Rockefeller University

March 25, 2019

Illustration by David Goodsell



Why cryo-EM of helical filaments?

-They are very common in biology!

-They cannot be crystallized (incompatible with point-group symmetries of a 3D lattice).

-Helical symmetry is extremely powerful for extracting 3-dimensional information from 2-d projection images.

Today:

-A brief overview of helices and helical image processing
(detailed practical on indexing and processing with Hernando Sosa)

-A survey of problematic cases and how people have dealt with them:
Mostly Microtubules

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Mostly Microtubules

Further reading:

For an in depth, mathematical treatment of helical theory:

Chapter 12, “Electron Crystallography of Helical Structures” by David DeRosier, in Electron Crystallography of Biological Macromolecules. Glaeser et al., Oxford University Press, 2007.

A recent review chapter on modern methods:

Fromm, Sachse. Methods Enzymol. 2016. PMID 27572732

What is a helix?

Spiral staircase in the Presbytere, New Orleans, 1934.
Richard Koch

-A vectorial assembly whose subunit arrangement is described by a 2 parameter screw operator (“rise”, “twist”)

-Can be thought of as a “1.5” d crystal

-Translation coupled to a rotation defines the positions of the asymmetric units (“protomers”)

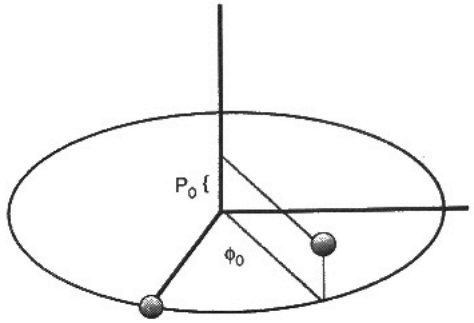
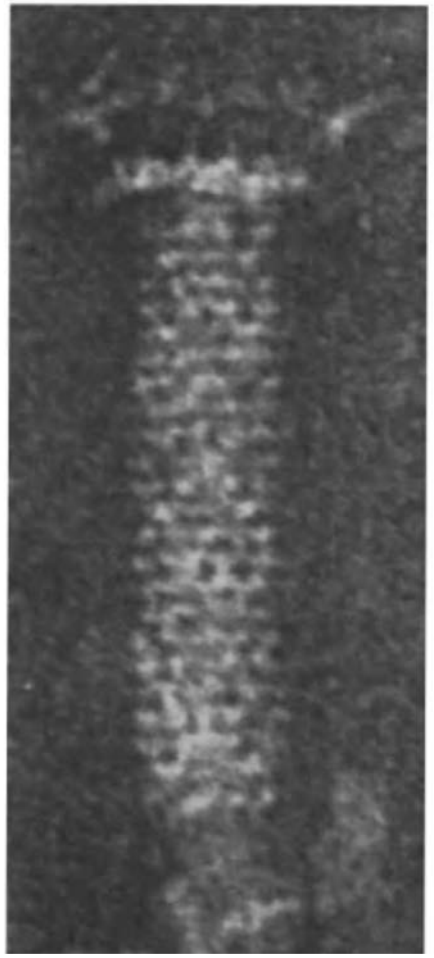
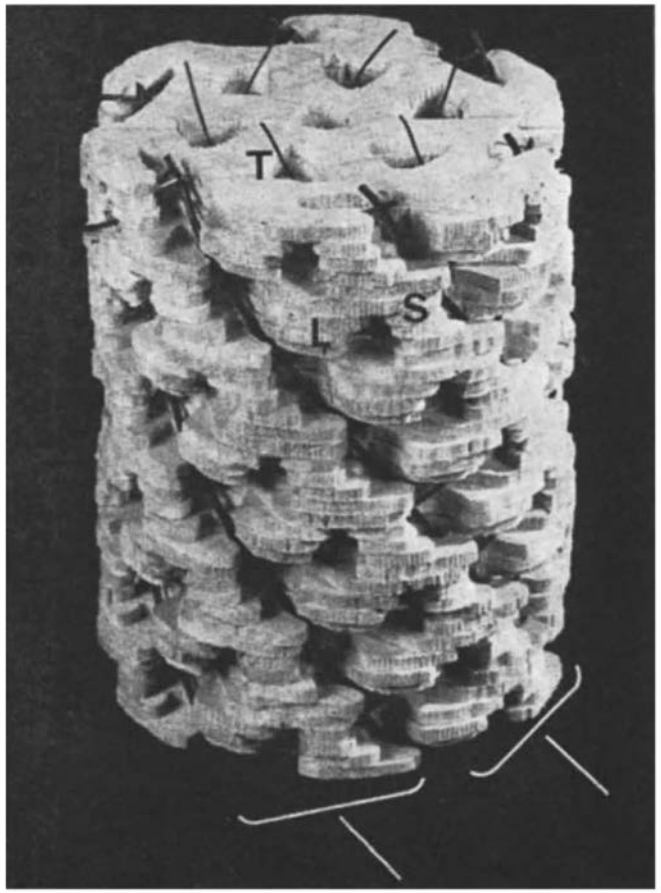


Figure 12.2 “Electron Crystallography of Helical Structures”, DeRosier

The first 3DEM structure ever: T4 bacteriophage tail

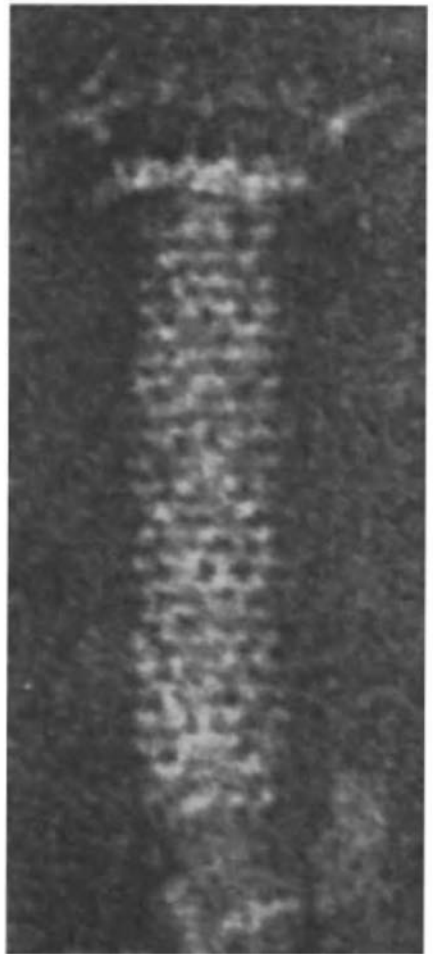




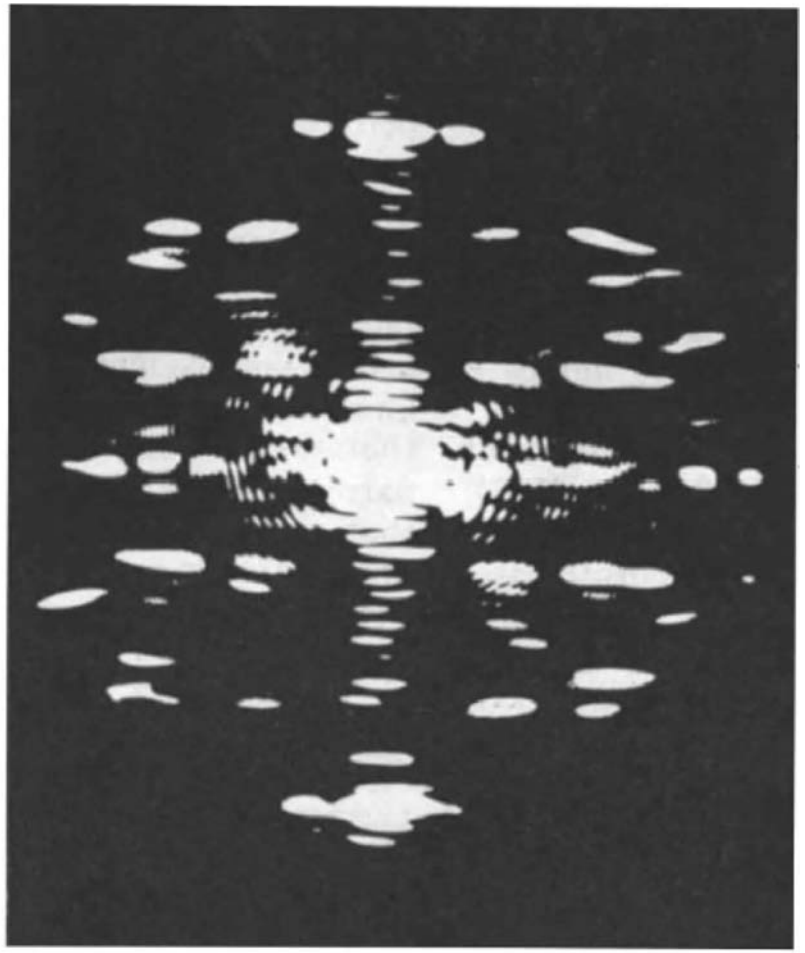
The projection of an object with rotational symmetry is the sum of projections of its asymmetric unit in the various symmetry-related orientations (and positions). In the reconstruction process the three dimensional structure of the asymmetric unit is recovered from the various projections of it which are contained in a single image of the whole object. Looked at in this way, an electron microscope image of the phage tail, which has forty-two subunits in its axial repeat, effectively presents projections of the subunit in twenty-one different orientations, more than enough to reconstruct the structure‡.

DeRosier and Klug. Nature. 1972. PMID 23610788

Historically, this information is extracted from the Fourier Transform ("Fourier-Bessel Reconstruction")



Power Spectrum
of
Fourier Transform



DeRosier and Klug. Nature. 1972. PMID 23610788

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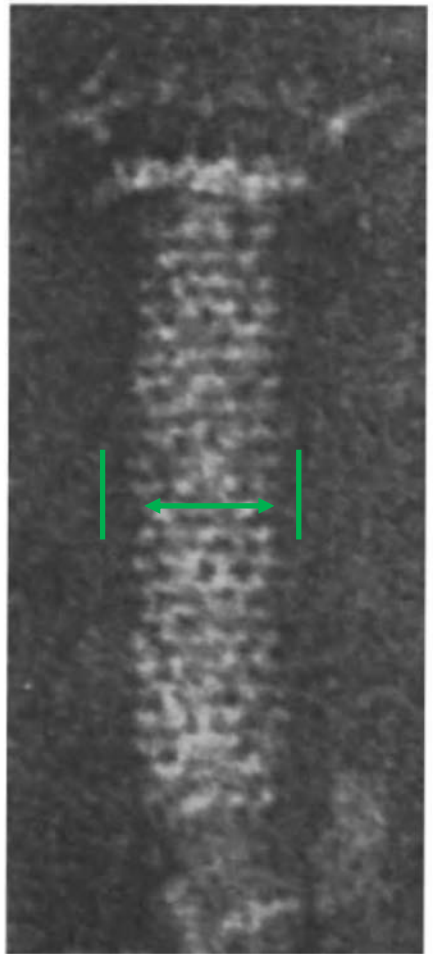
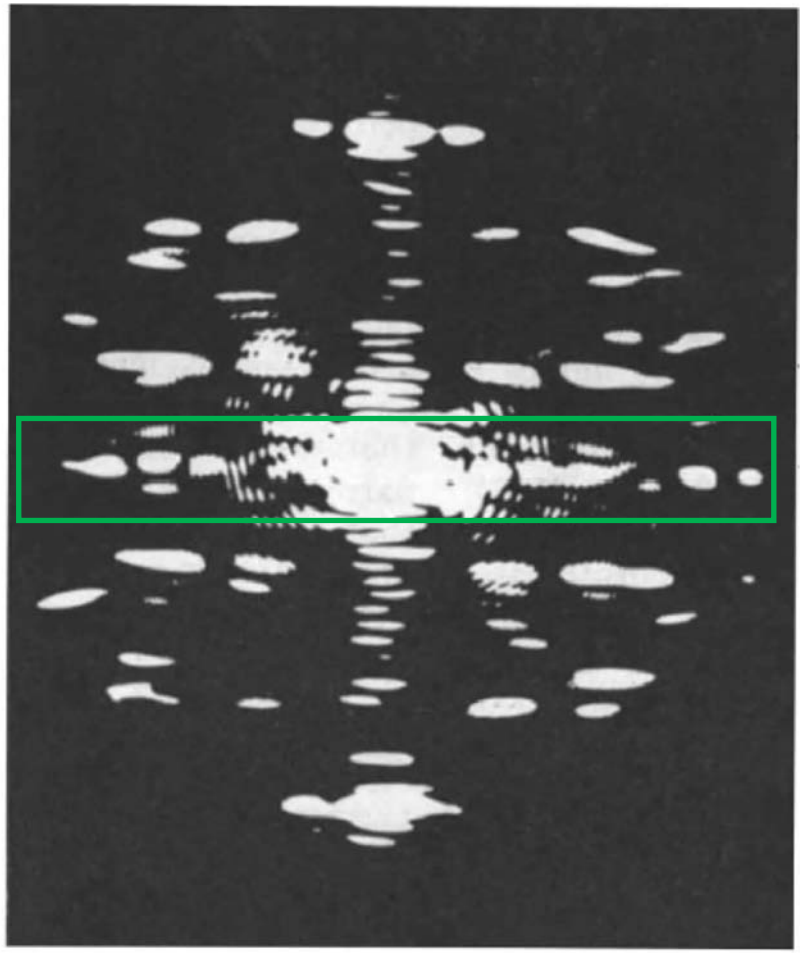


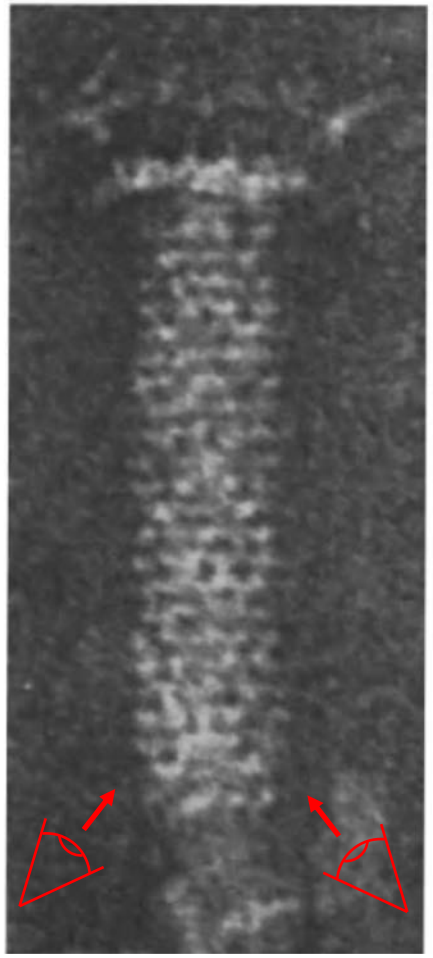
Power Spectrum
of
Fourier Transform



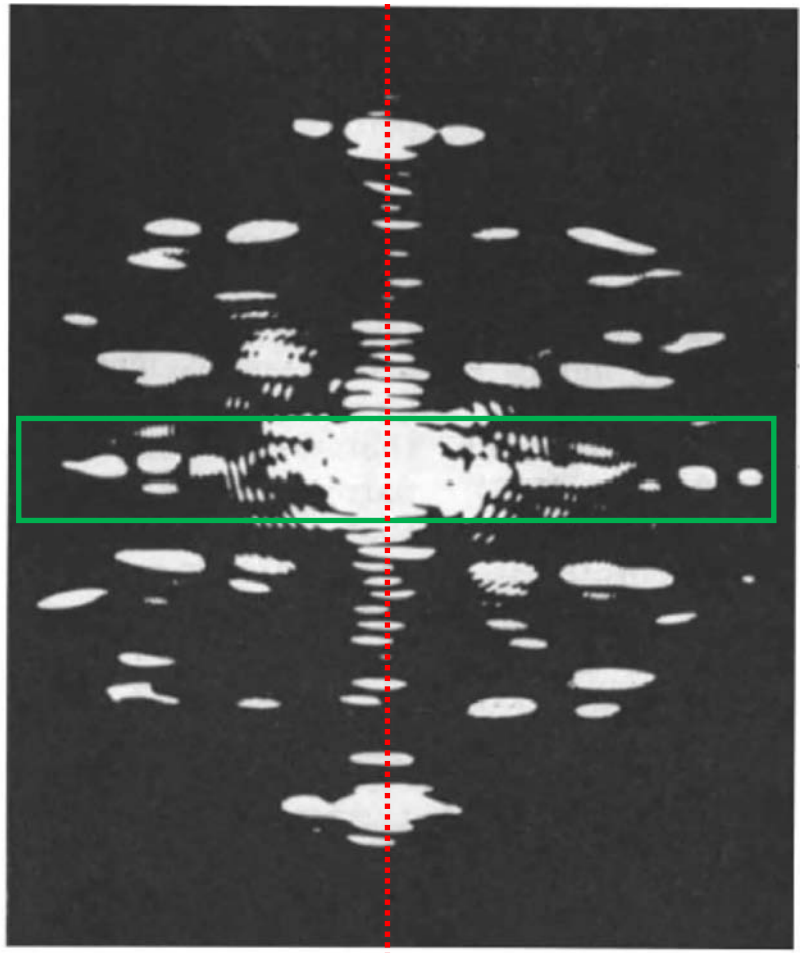
Equator: contains information on radial density distribution of "tube".

DeRosier and Klug. Nature. 1972. PMID 23610788

Historically, this information is extracted from the Fourier Transform ("Fourier-Bessel Reconstruction")



Power Spectrum
of
Fourier Transform

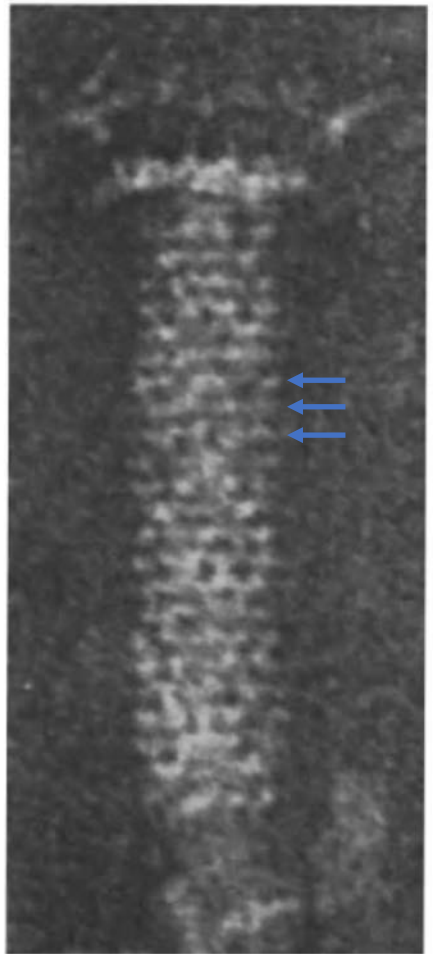


Equator: contains information on radial density distribution of "tube".

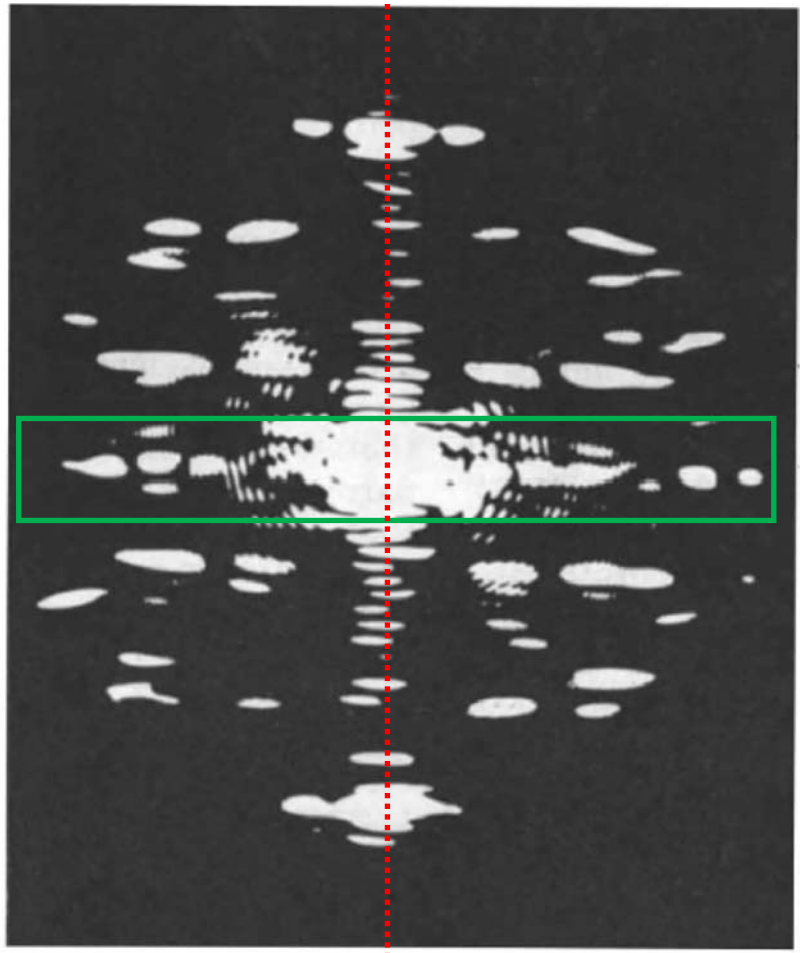
DeRosier and Klug. Nature. 1972. PMID 23610788

Meridian: Divides power spectrum into signal from front and back of helix (n.b. both sides contribute to both halves). Should be symmetric; if not, symptomatic of out-of-plane tilt or damage.

Historically, this information is extracted from the Fourier Transform ("Fourier-Bessel Reconstruction")



Power Spectrum
of
Fourier Transform



Layer lines:
Contain information on axial density distribution / subunit spacing along helix

Equator: contains information on radial density distribution of "tube".

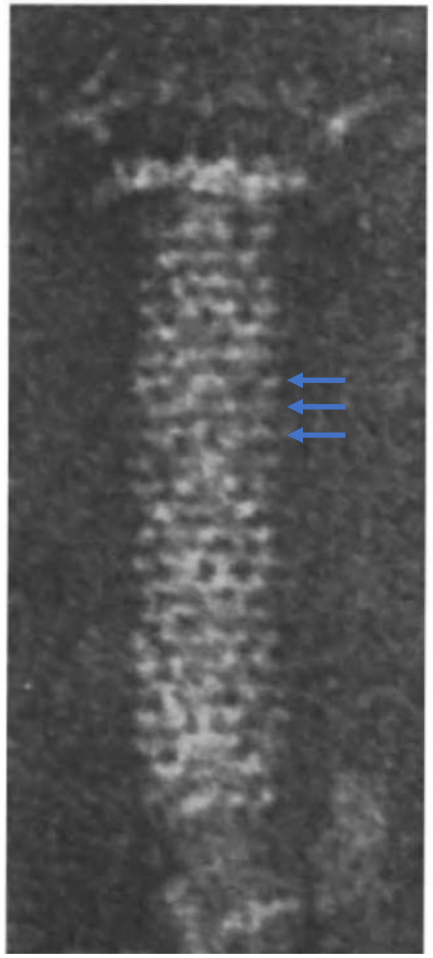
DeRosier and Klug. Nature. 1972. PMID 23610788

Meridian: Divides power spectrum into signal from front and back of helix (n.b. both sides contribute to both halves). Should be symmetric; if not, symptomatic of out-of-plane tilt or damage.

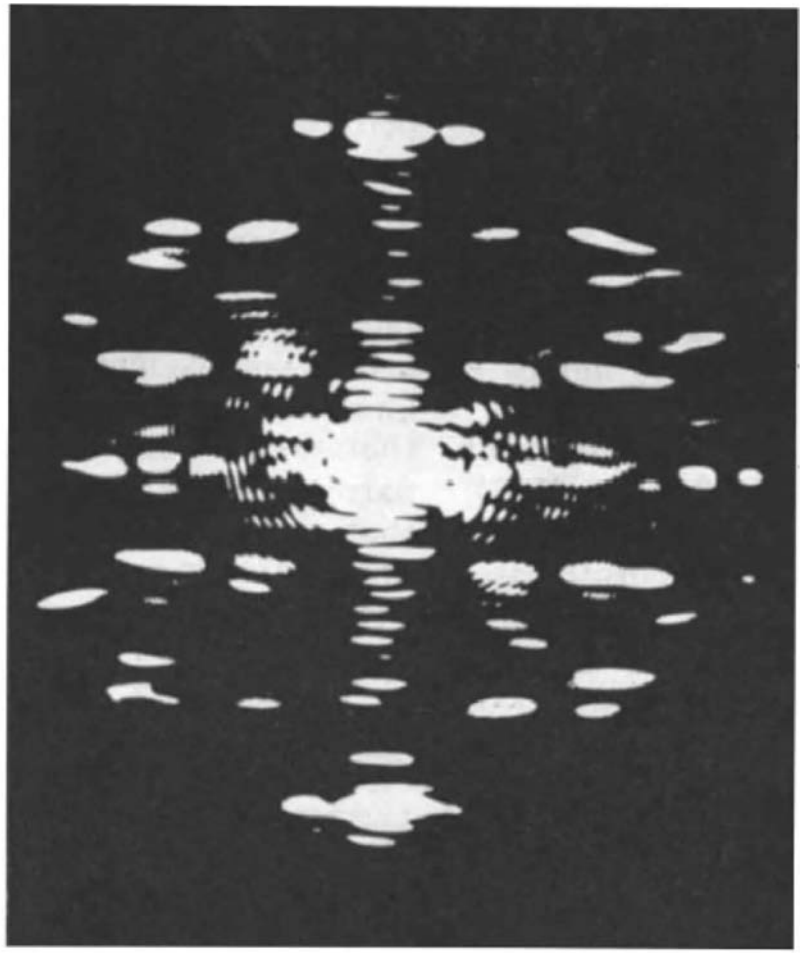
An interlude on layer lines

Why lines (not spots)?

Historically, this information is extracted from the Fourier Transform ("Fourier-Bessel Reconstruction")



Power Spectrum
of
Fourier Transform



-Remember, Fourier transform is 1 / distance

-Indexing the diffraction pattern to estimate helical parameters is still the first (and hardest) step in analyzing a new helix!

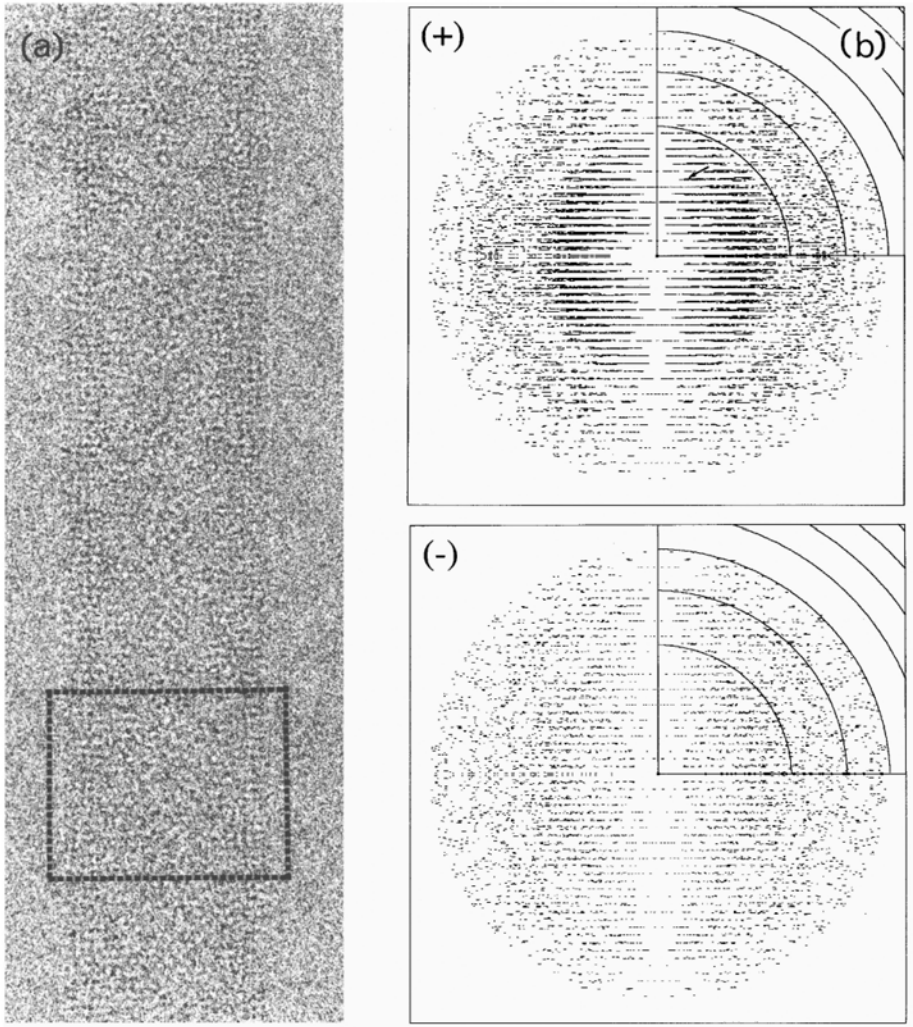
Web tool "helixplorer": http://rico.ibs.fr/helixplorer/

Spring package: http://www.sachse.embl.de/emspring/

DeRosier and Klug. Nature. 1972. PMID 23610788

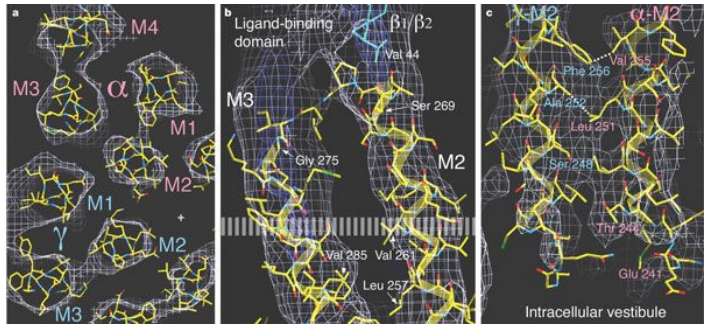
DesFosses…Sachse. JSB. 2014. PMID 24269218

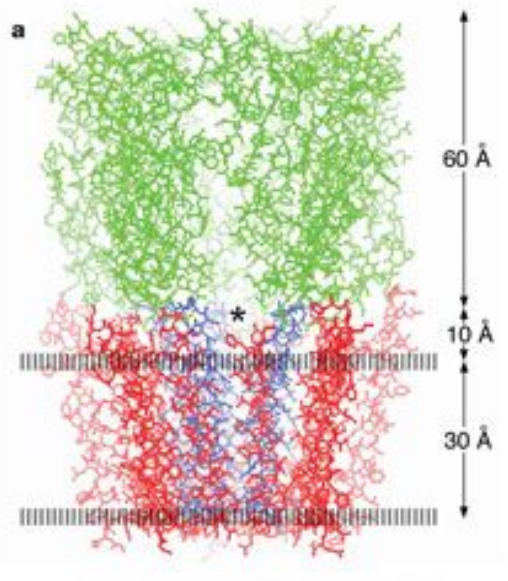
One of the first high-resolution cryo-EM structures: the nicotinic acetylcholine receptor from torpedo ray

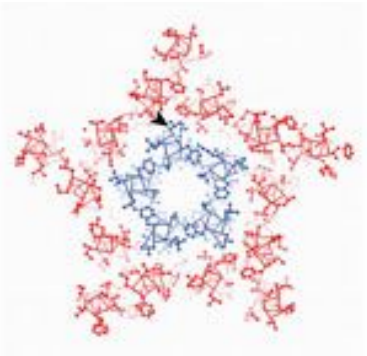


Miyazawa, Fujiyoshi, Stowell, Unwin. JMB. 1999. PMID 10329178

~4 Å structure well before the “resolution revolution”







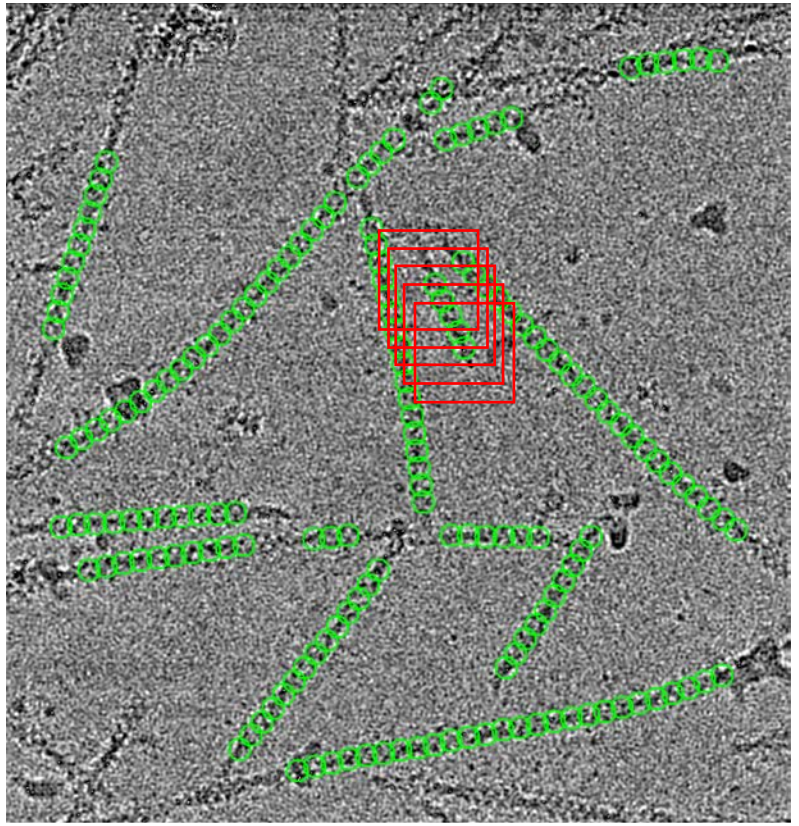
However, this requires essentially perfect images of a perfect sample: usually impossible to achieve in practice.

Miyazawa, Fujiyoshi, Unwin. Nature. 2003. PMID 12827192

See also: Structure of flagellin. Monekura, Yaki-Monekura, Namba. Nature. 2003. PMID 12904785

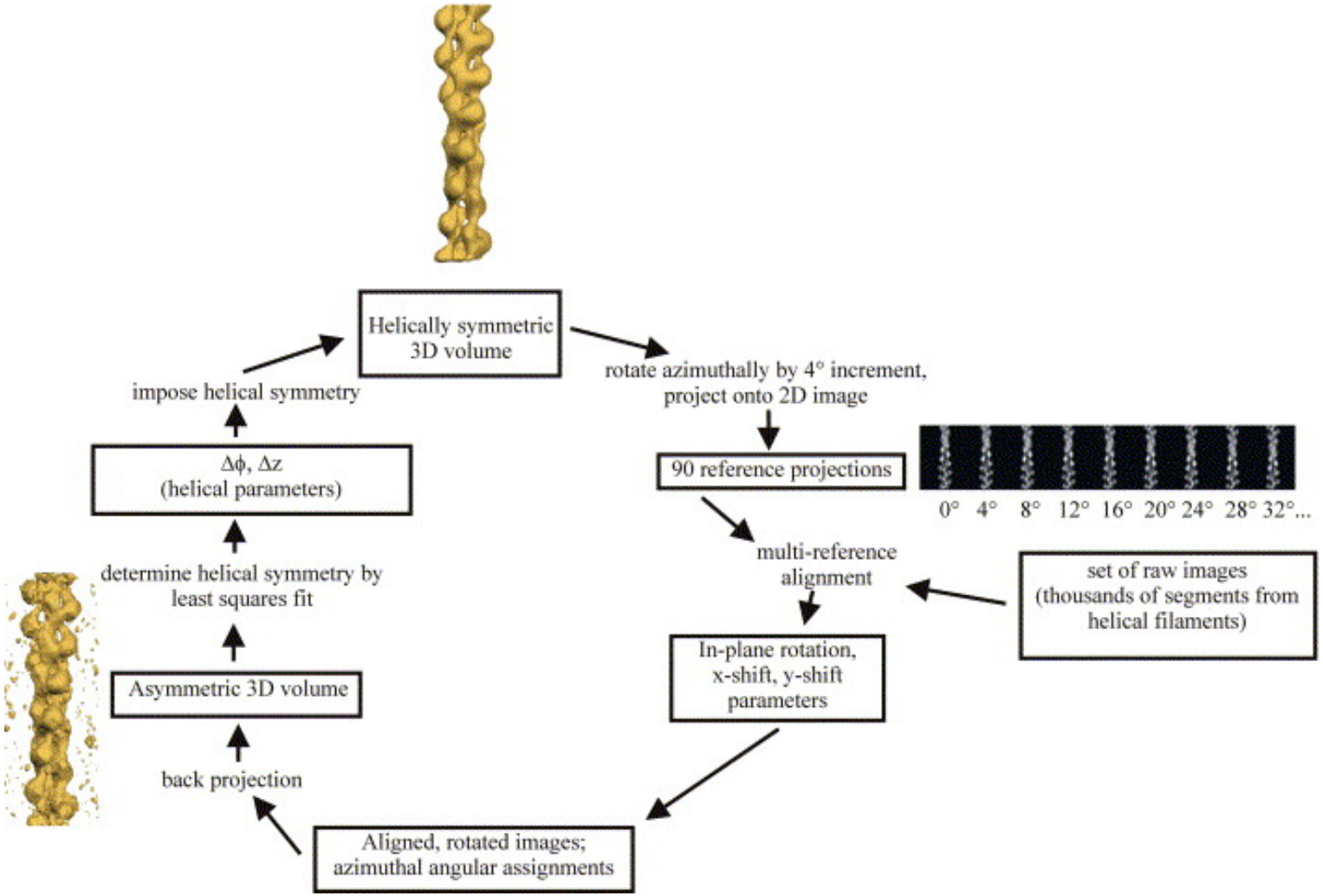
Most modern methods utilize “Iterative Real Space Helical Refinement”, a hybrid with single particle

Pick overlapping segments



Green: centers of all “particles”
Red: examples of boxed regions

Most modern methods utilize “Iterative Real Space Helical Refinement”, a hybrid with single particle



Egelman. JSB. 2007. PMID 16919474

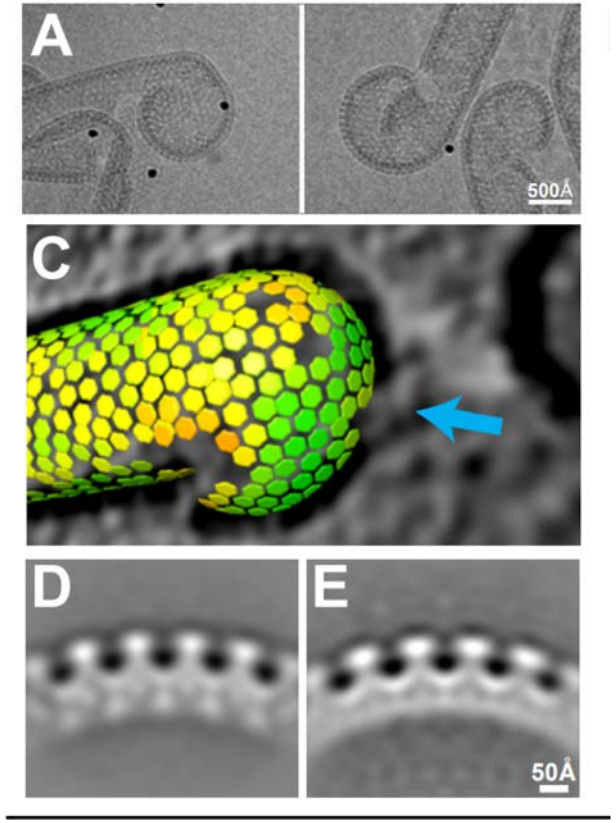
-A reference is required (a featureless cylinder can work in well-behaved cases)

-Helical parameters (rise and twist) are refined through an axial auto-correlation search of an asymmetric reconstruction calculated from the data

-This method is quite sensitive to initial guesses of rise and twist, and can give totally wrong answers! Thus, indexing diffraction pattern is still required.

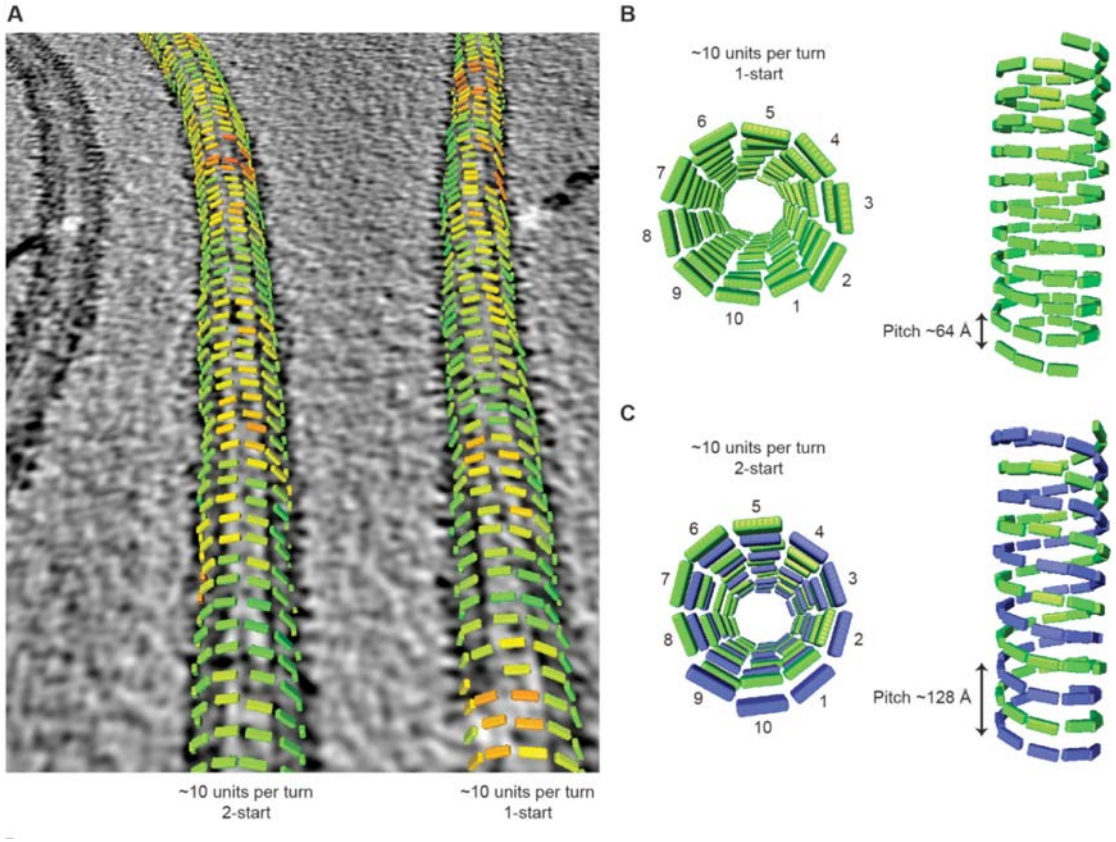
An underappreciated method for unambiguously determining helical parameters: cryo-ET

Retroviral capsid:



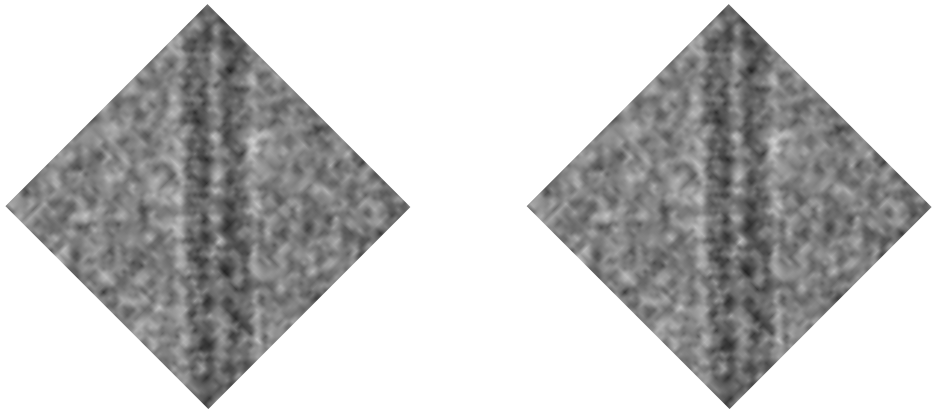
Bharat…Briggs. Nature. 2012. PMID 22722831

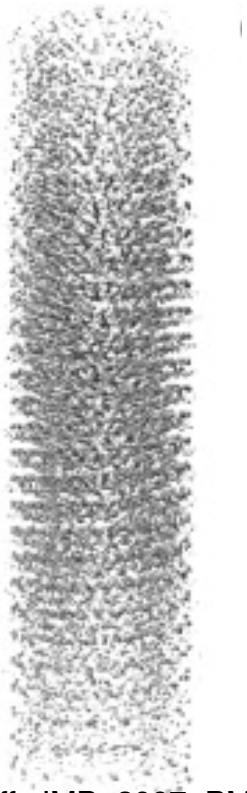
Clatherin adapters:



Skruzny…Briggs, Sachse, Kaksonen. Dev Cell. 2015. PMID 25898165

There are still benefits to working in Fourier space: avoiding interpolation errors in reconstruction



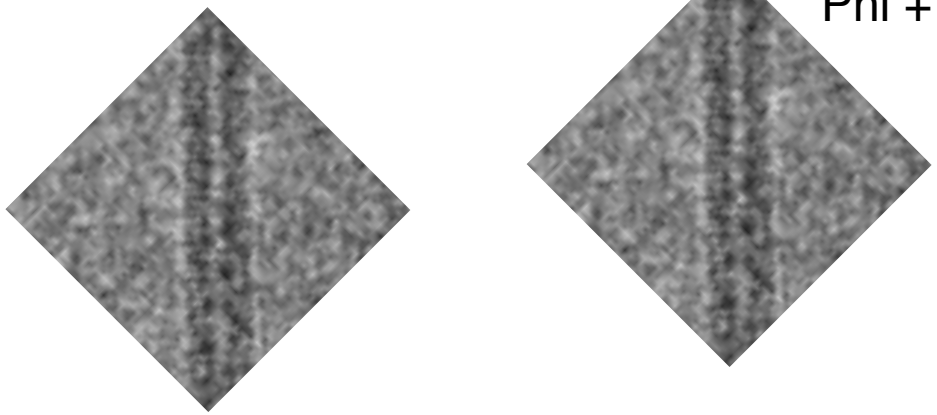


For each segment:

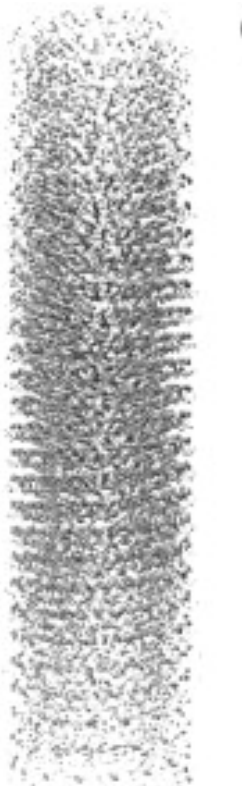
Sachse…Grigorieff. JMB. 2007. PMID 17585939

Insert multiple times into
reconstruction at symmetry
related positions in Fourier space

There are still benefits to working in Fourier space: avoiding interpolation errors in reconstruction



SH_Y + rise
Phi + twist



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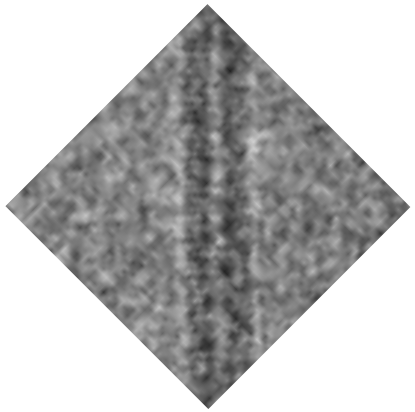
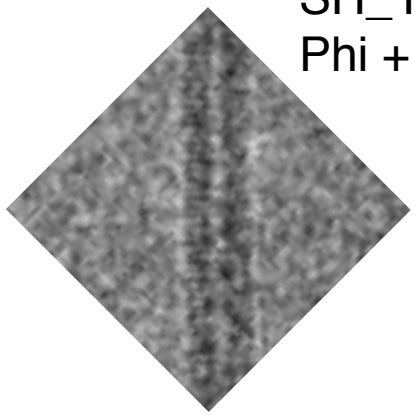
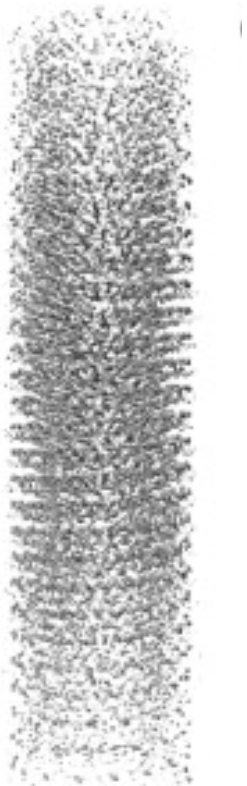




SH_Y + 2*rise
Phi + 2*twist



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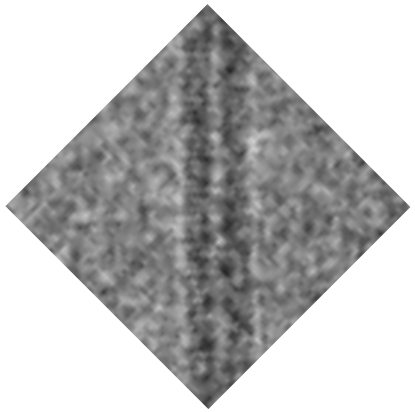
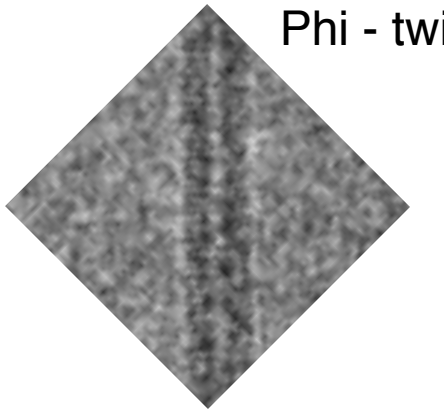
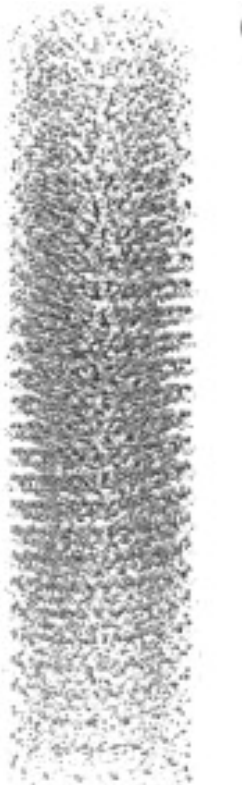
There are still benefits to working in Fourier space: avoiding interpolation errors in reconstruction





SH_Y - rise
Phi - twist



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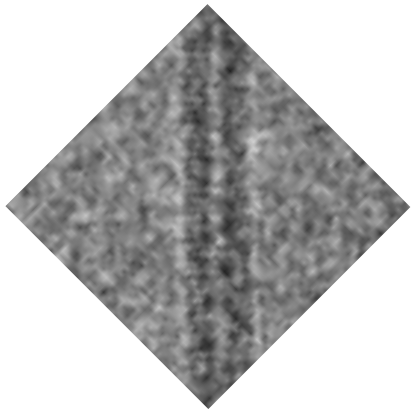
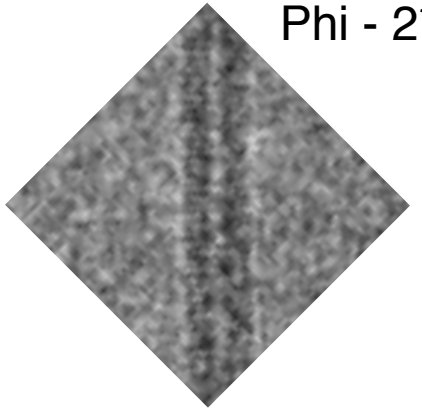
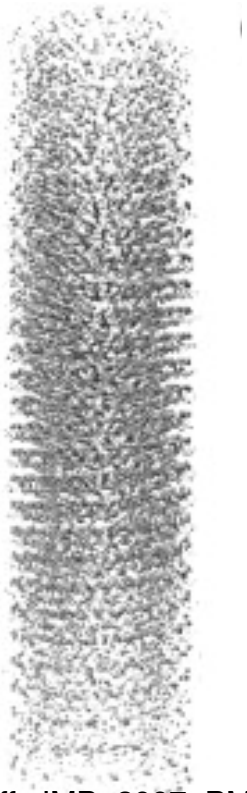




SH_Y - 2*rise
Phi - 2*twist



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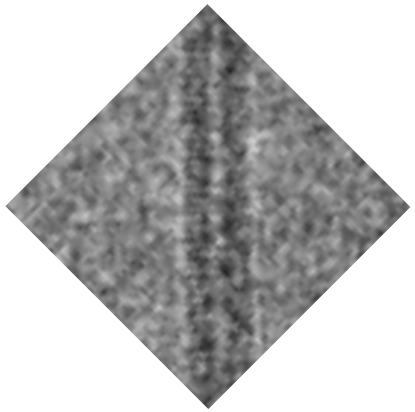
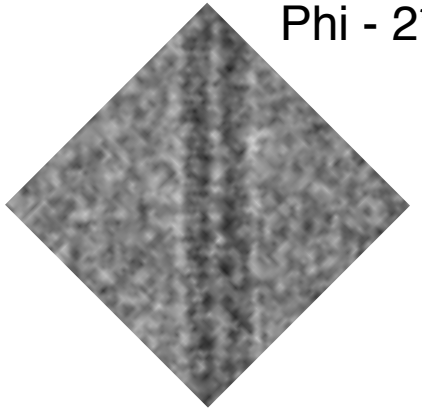
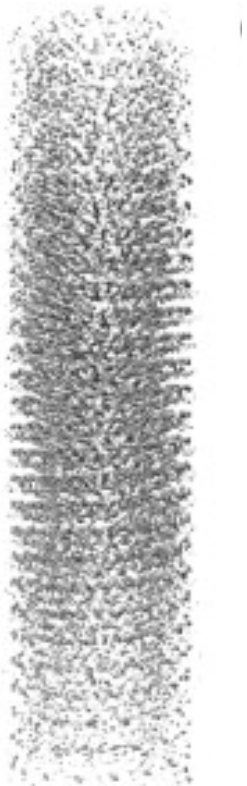
There are still benefits to working in Fourier space: avoiding interpolation errors in reconstruction



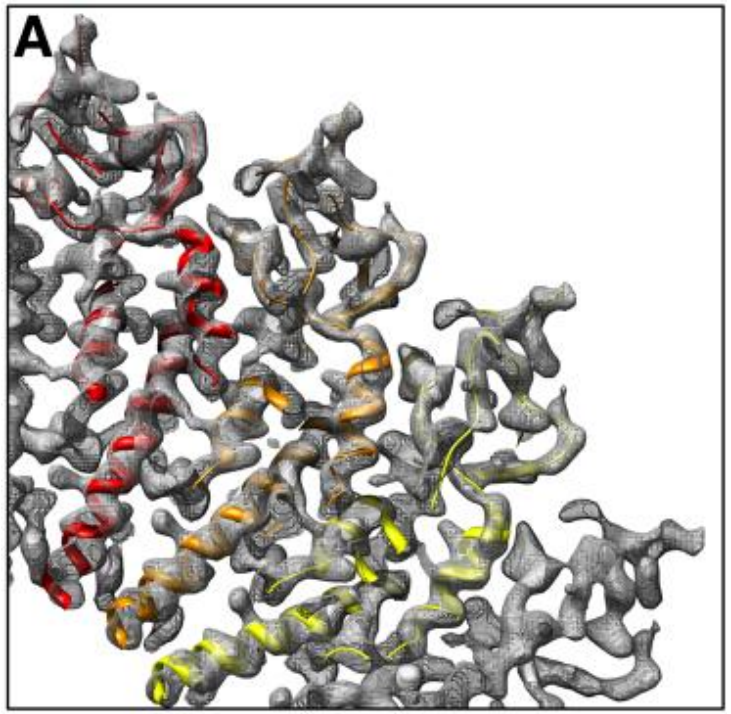


SH_Y - 2*rise
Phi - 2*twist



For each segment:

~4.5 Å resolution

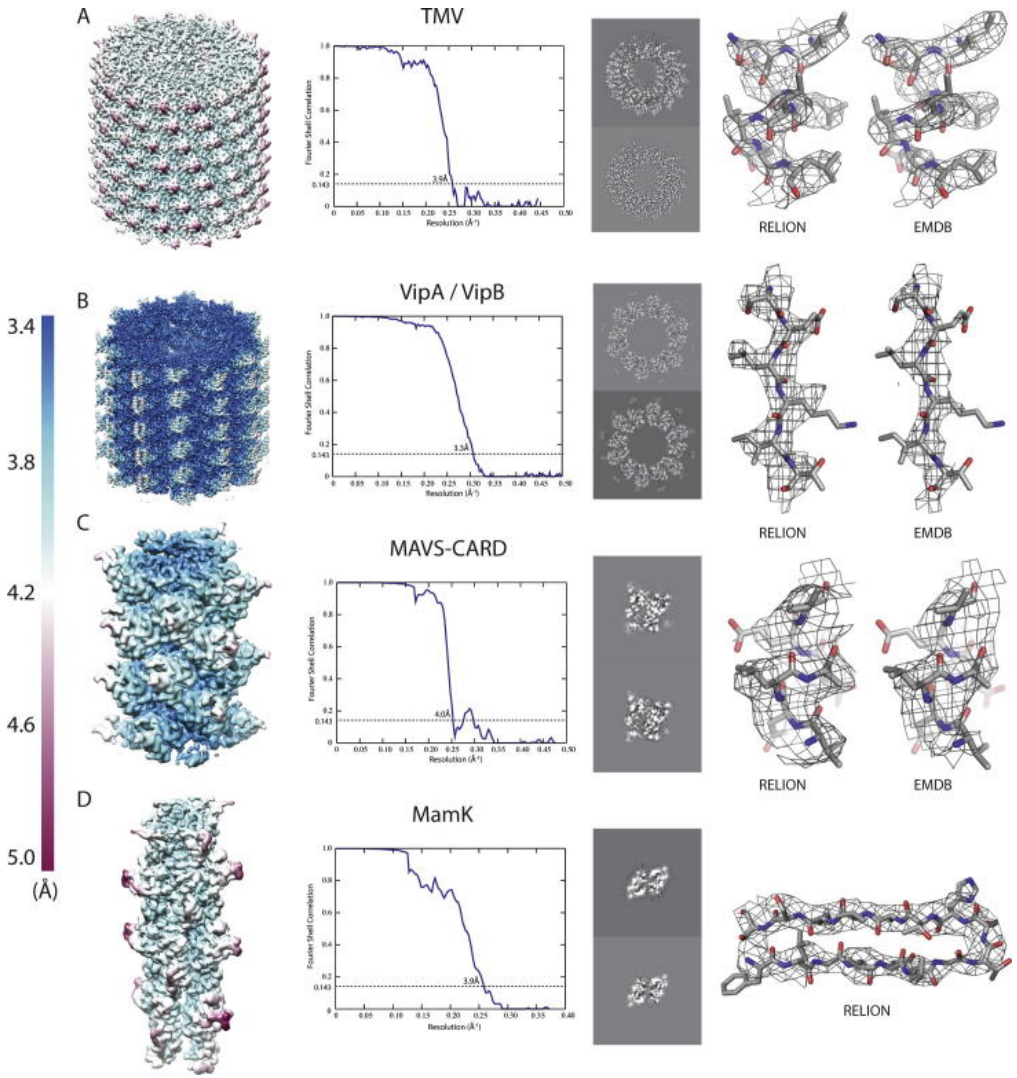


Sachse…Grigorieff. JMB. 2007. PMID 17585939

Insert multiple times into reconstruction at symmetry related positions in Fourier space

Most modern packages (FREALIGN, FREALIX, RELION, SPRING) use this or mathematically equivalent reconstruction procedures.

Practically, I suggest starting with RELION



He, Scheres. JSB. 2017. PMID 28193500

-Has nice graphical user interface

-Has autopicker that (usually) works*

-2D classification, 3D classification, and refinement all have been tuned for helical specimens

-Still includes all the modern features / safeguards of RELION ("gold-standard FSCs", polishing, etc.)

-This is what my lab uses.

*CRYOLO also has explicit helical filament support (machine learning CNN based picker)

Bioarxiv: https://doi.org/10.1101/356584

Part of SPHIRE package: http://sphire.mpg.de/

Questions thus far?

Today:

-A brief overview of helices and helical image processing .
(detailed practical on indexing and processing with Hernando Sosa)

-A survey of problematic cases and how people have dealt with them:
Mostly Microtubules

Problems that may arise:

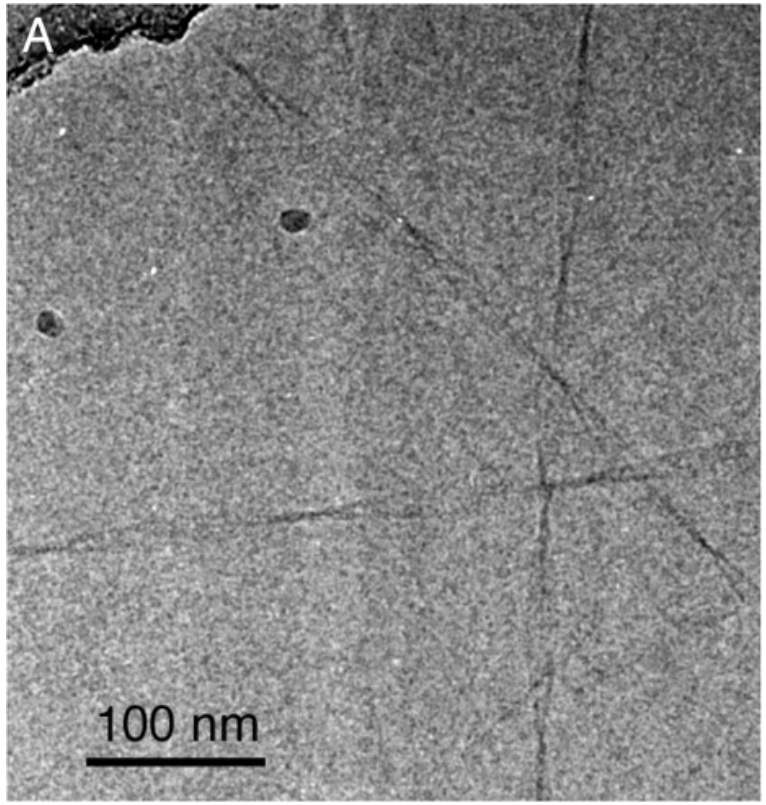
Amyloids:

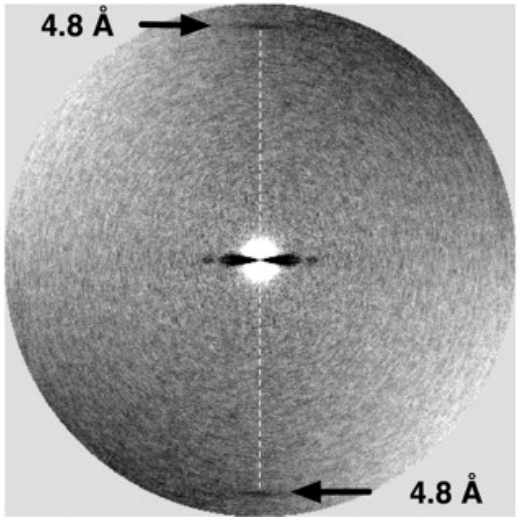
-What if my asymmetric unit is invisible at low resolution?

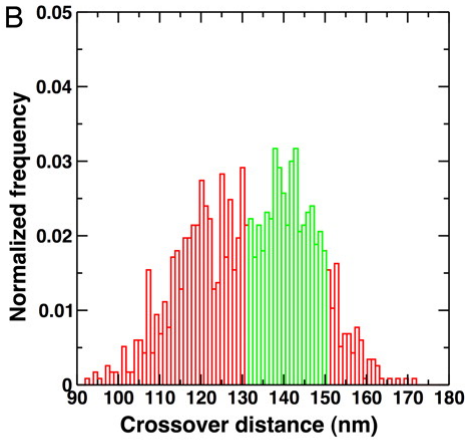
Microtubules:

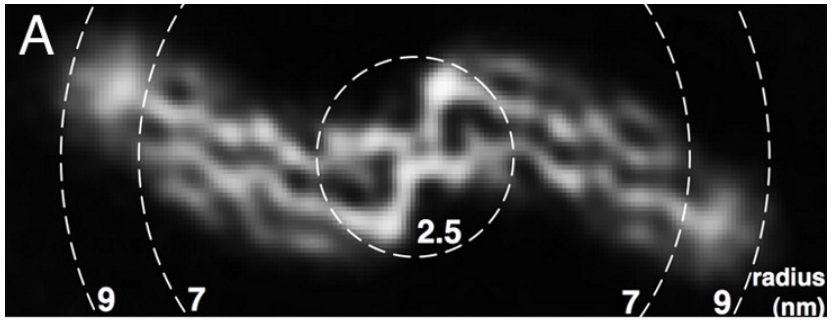
-What if my helix is built from different subunits indistinguishable at low resolution?
-What if my sample contains a mixture of different symmetries?
-What if my helix isn't actually a helix? (pseudo-helical symmetry)
-What if I have two different helices wrapped around one another?
-What if an asymmetric thing binds my helix?

What if my asymmetric unit is invisible at low resolution?





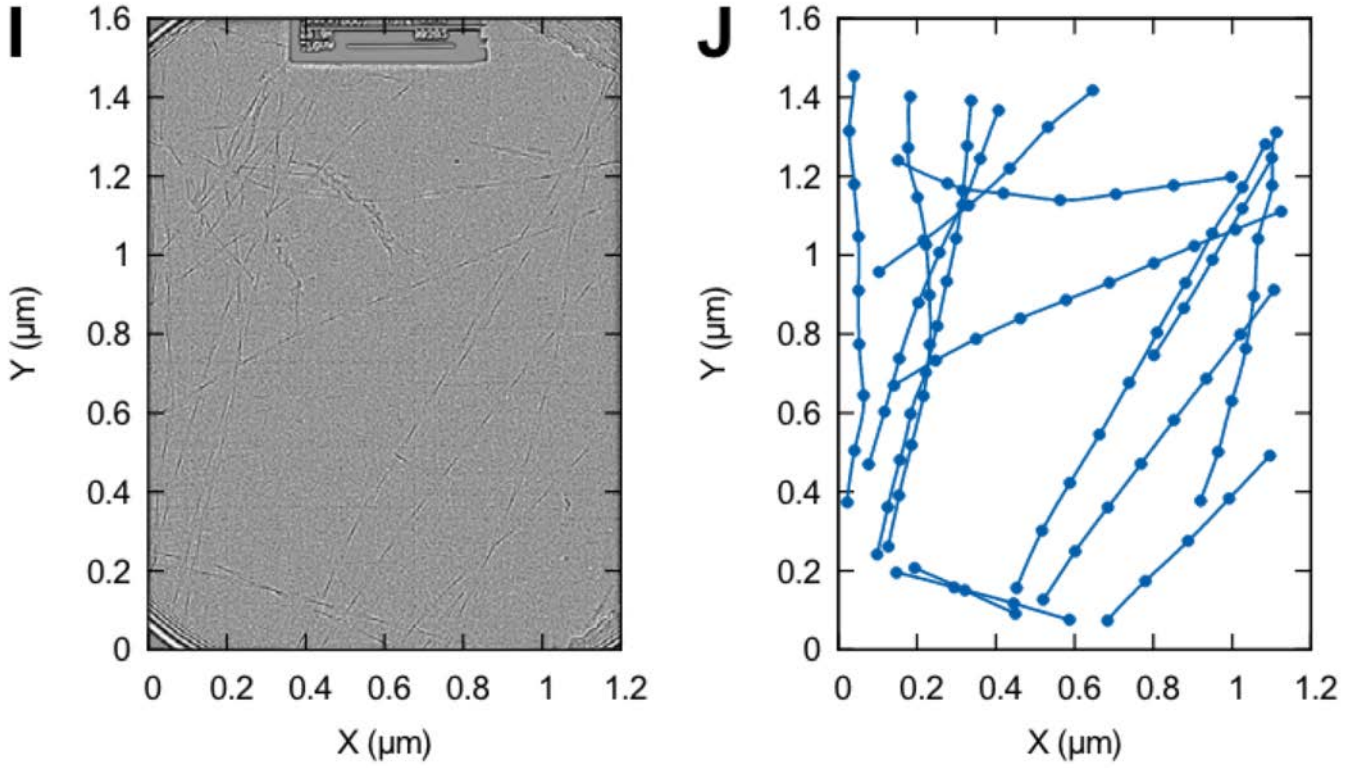


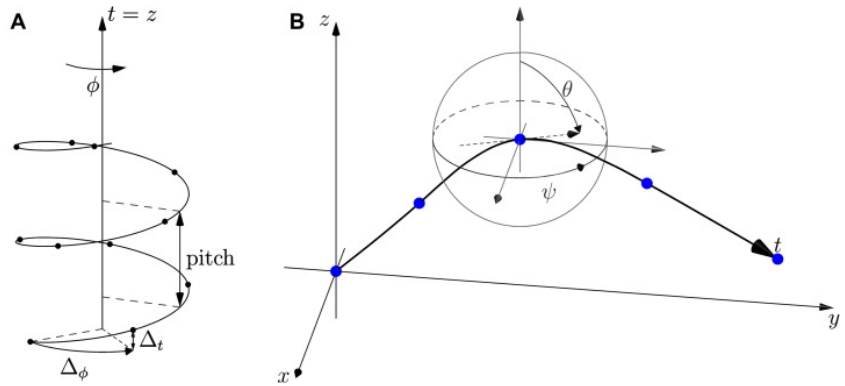


-Can see separation of strands in cross-section, but not the stacking of beta strands.

Sachse, Fandrich, Grigorieff. PNAS. 2008. PMID 18483195

A filament-model based approach: FREALIX



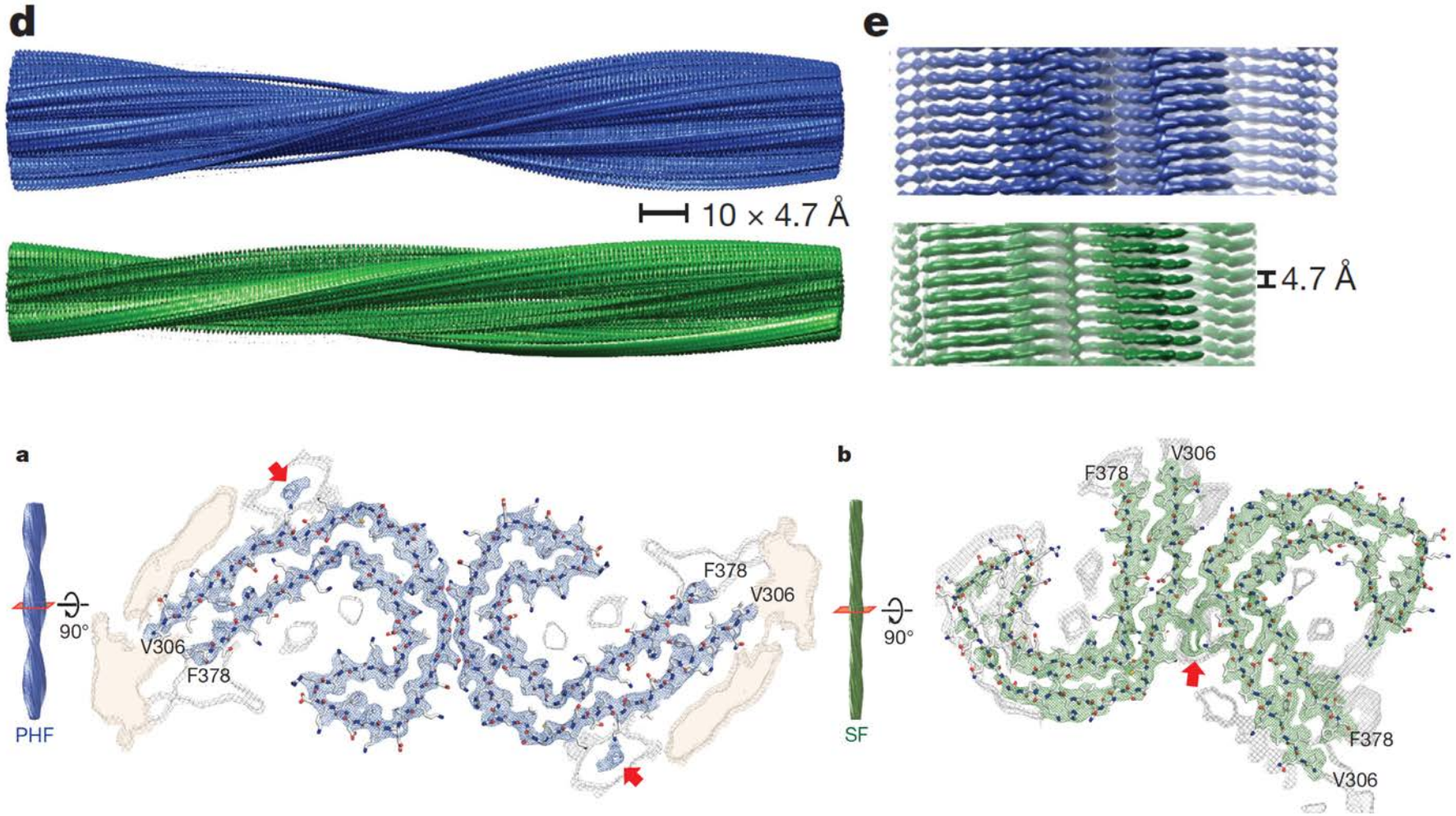


-While a very sophisticated approach was here implemented, not a huge improvement in the reconstructions…

-Nevertheless, the ideas and algorithms are very likely to be useful: I encourage you to read this paper if you want to develop new tools

Rohou and Grigorieff. JSB. 2014. PMID 24657230

Success with RELION: Tau filaments from Alzheimer's patient brain

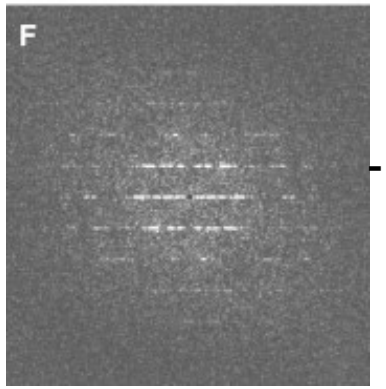


-Direct detector data likely helped (previous examples on film).

-Required modulating regularization parameter (T) in RELION to use high-resolution data for classification.
Dangerous: proceed with caution!

Fitzpatrick…Goedert, Scheres. Nature. 2017. PMID 28678775

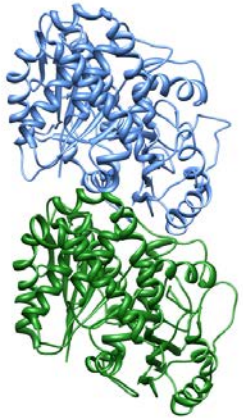
The confounding case of the microtubule



80 Å

1/40 Å^{-1}

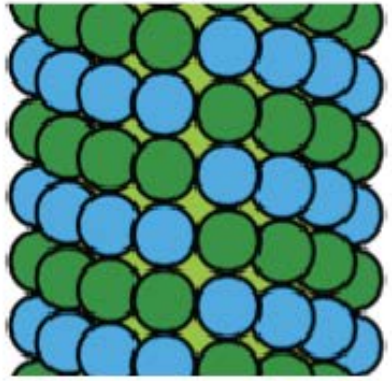
PDB: 1JFF



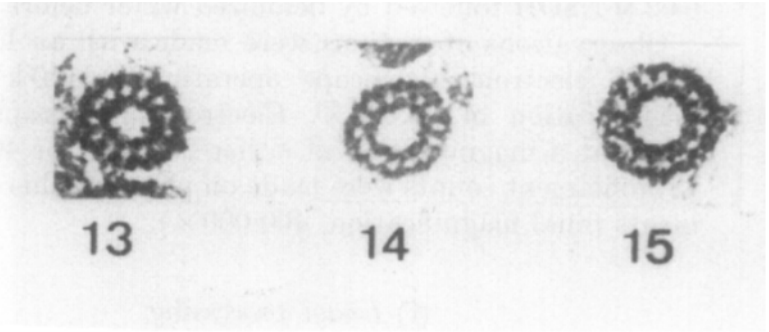
Seam

β

α



Li…Downing. Structure. 2002.
PMID 12377118



Chretien and Wade. JMB. 1990. PMID 2329582

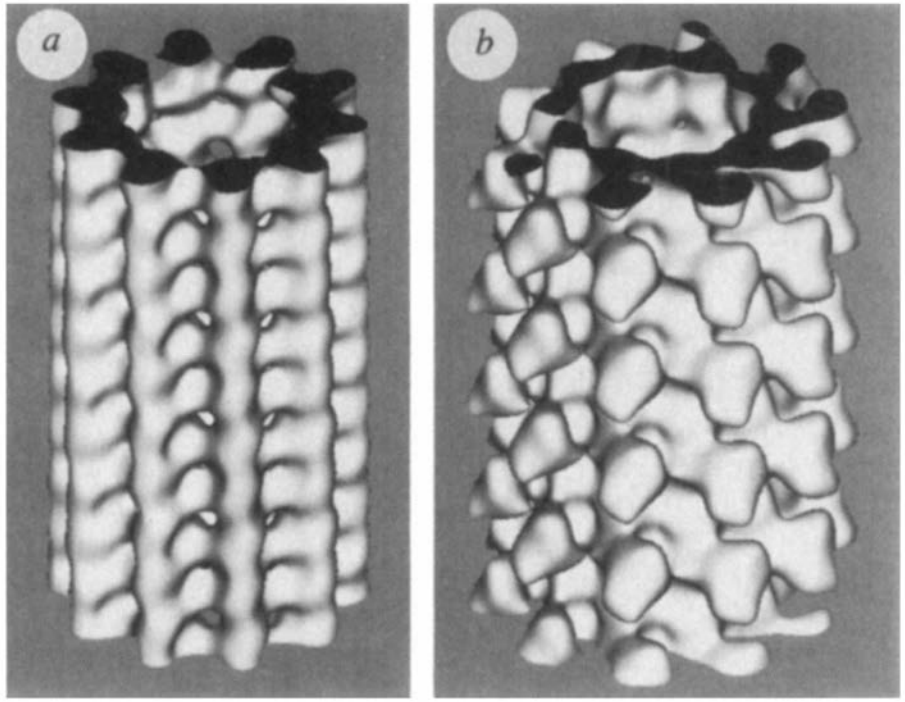
-Composed of 2 subunits (α and β tubulin) that are indistinguishable at low resolutions useful for alignment.

-The native lattice (13 protofilament) features a discontinuity (seam) where heterotypic contacts occur. Not actually a true helix!

-Microtubules assembled in vitro feature different numbers of protofilaments, which co-exist in the same preparation.

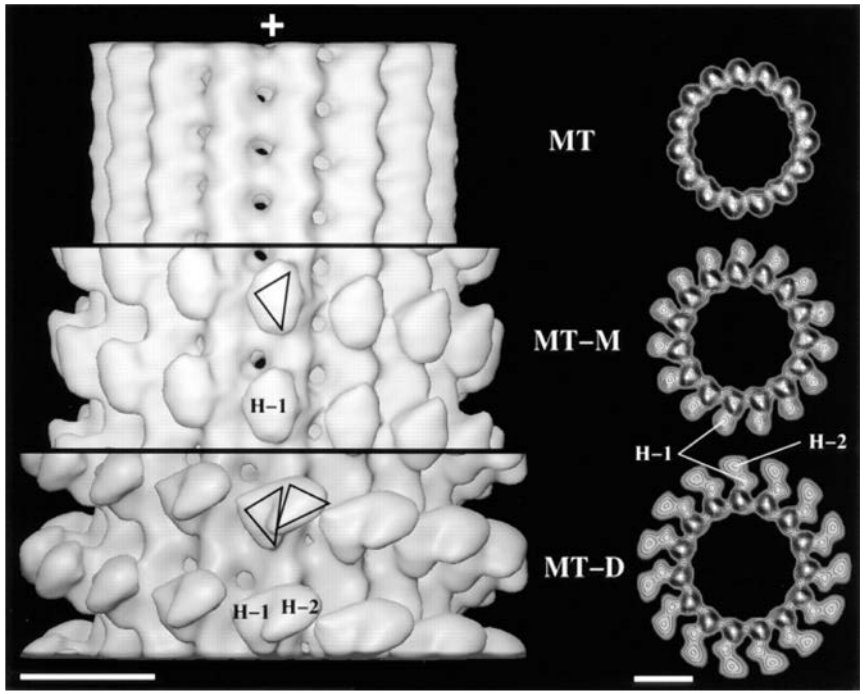
First approach: find microtubules that are true helices

11 pf microtubule



Kikkawa...Hirokawa. Nature. 1995. PMID 7617041

15 pf microtubule (4 start)

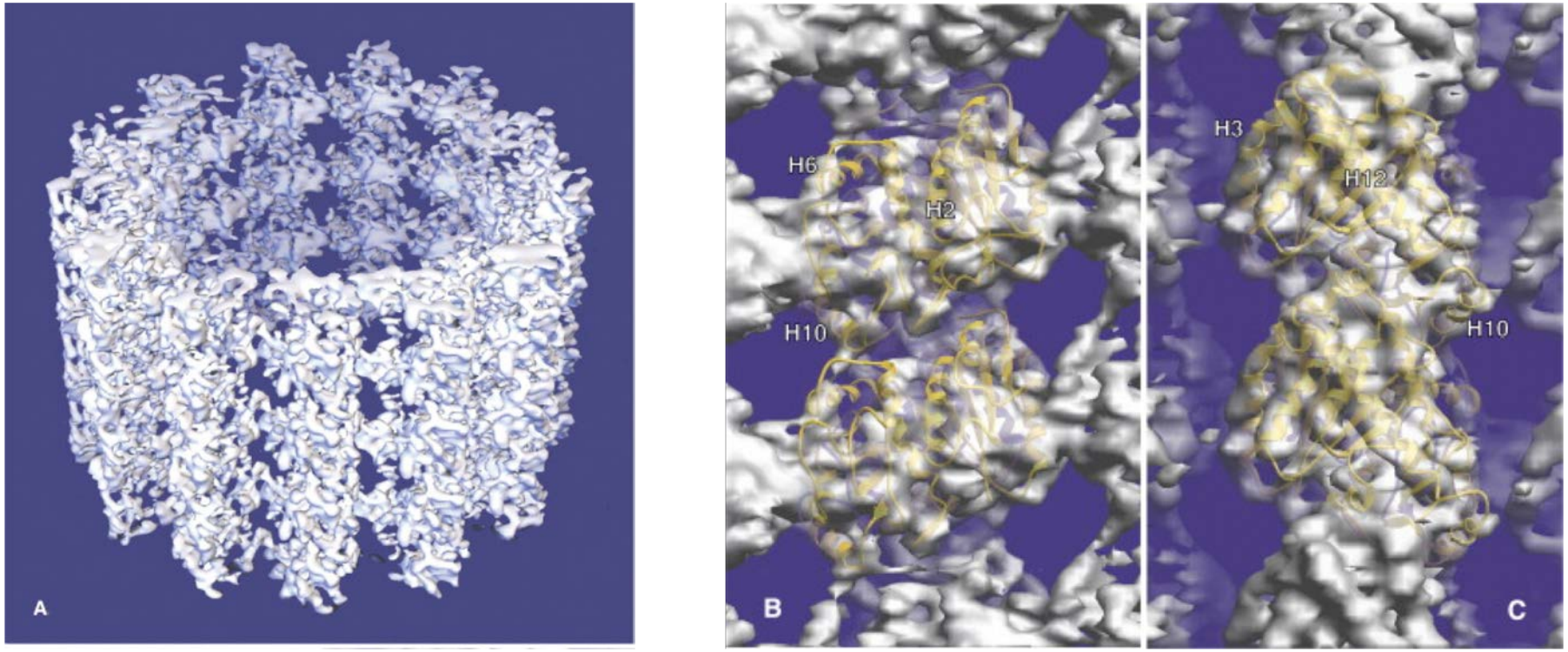


Sosa…Milligan. Cell. 1997. PMID 9244296

-Minority populations of true-helical microtubules could be found through painstaking manual analysis
-Used for early heroic studies of microtubule-kinesin interactions
-Subject to the usual limitations of Fourier-Bessel analysis
-Not the in vivo assembly state

Second approach: ignore the seam

13 pf naked microtubule



Li…Downing. Structure. 2002. PMID 12377118

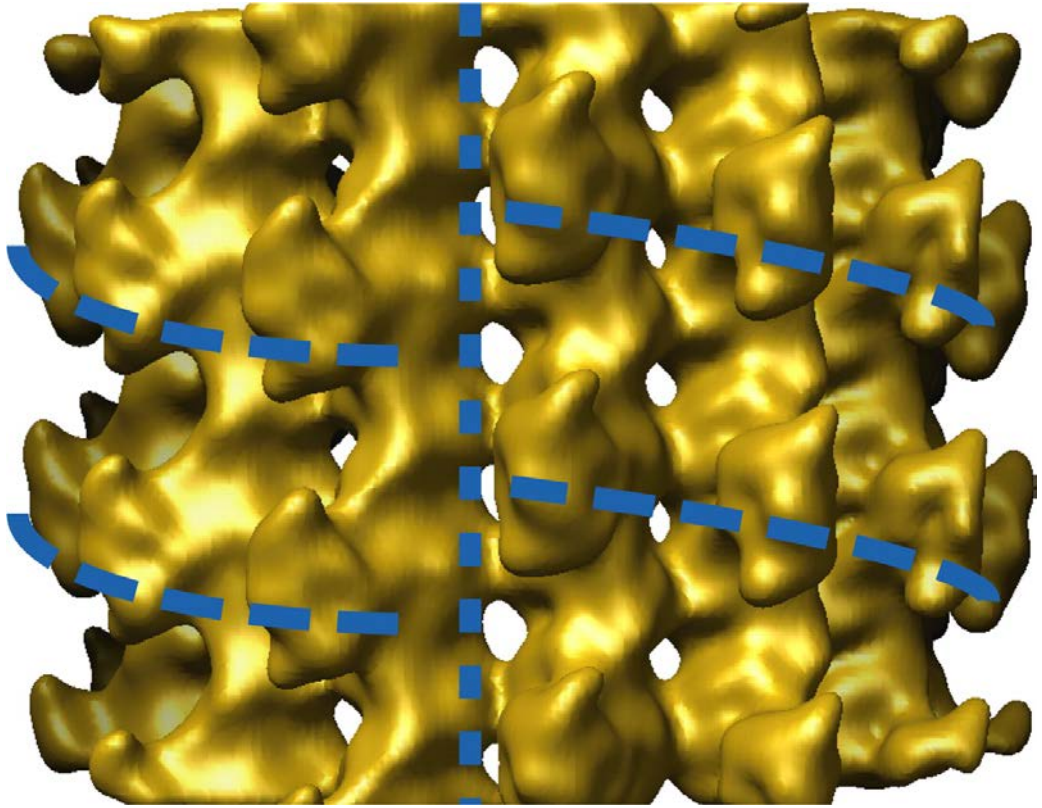
-Majority population 13 pf microtubule reconstructed by averaging together α and β tubulin using real-space, IHRSR-like procedure, reaching sub-nanometer resolution.

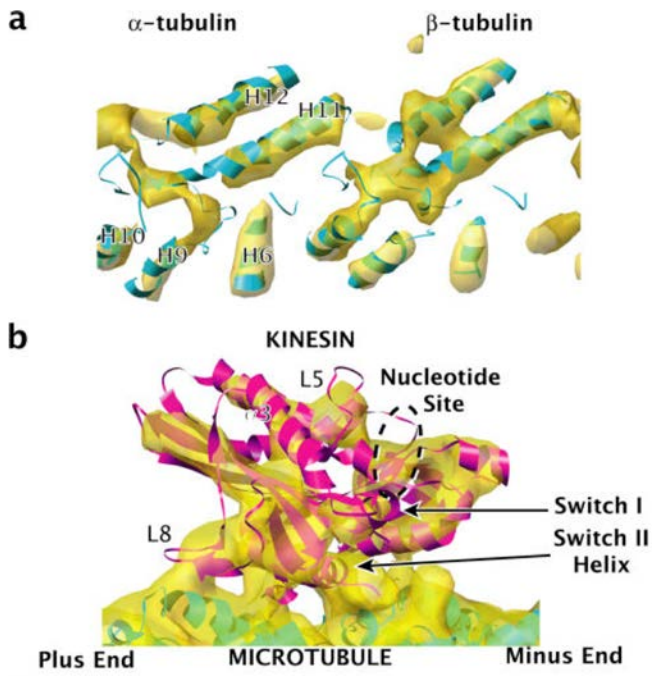
-Note this was considered reasonable after the $\alpha\beta$–dimer was visualized by electron crystallography at atomic resolution and tubulins were found to be very similar.

Electron crystallographic structure of tubulin: Nogales, Wolf, Downing. Nature. 1998. PMID 9428769

Third approach: use a fiducial

13 pf microtubule decorated with kinesin motor domain





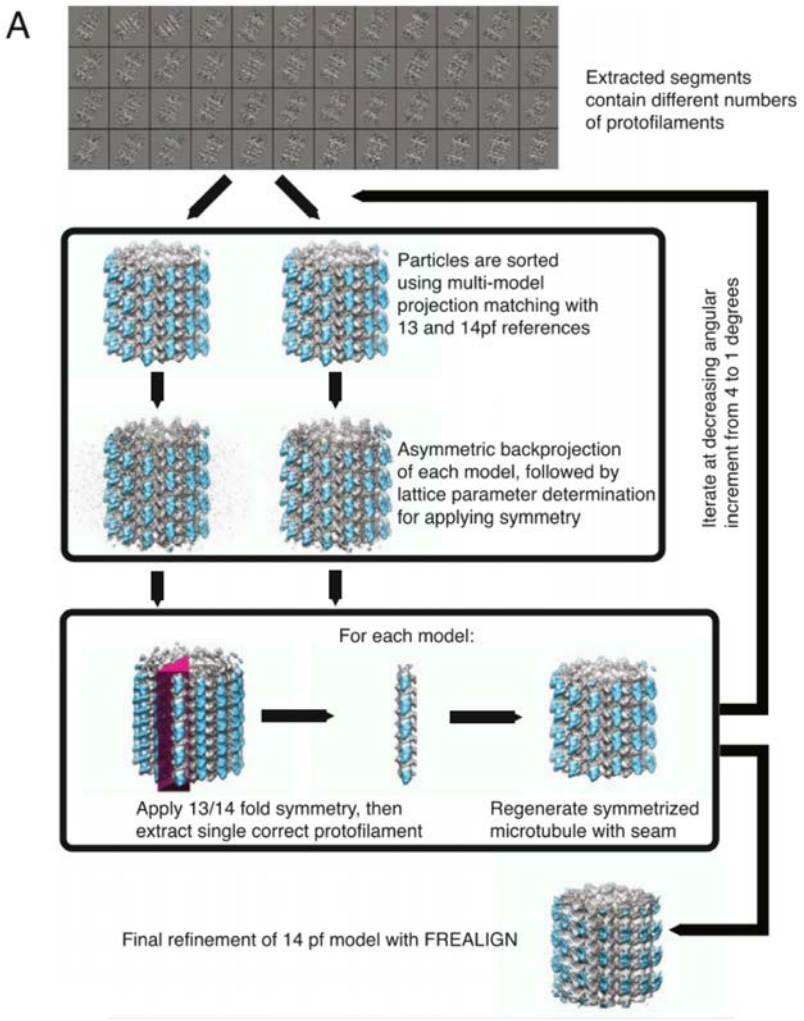
Sindelar and Downing. JCB. 2007. PMID 17470637

-Align kinesin-decorated segments against an asymmetric reference featuring a seam: sufficient signal to find it!

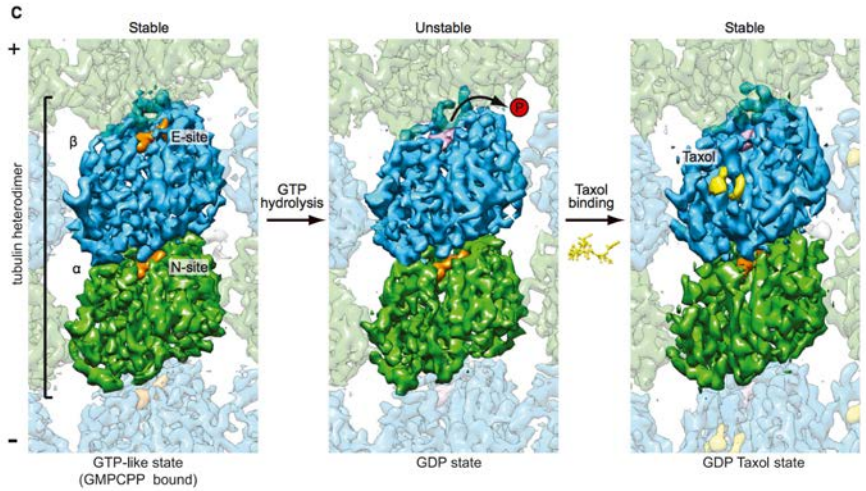
-Rotationally average around filament axis, leaving one “good” protofilament at subnanometer resolution.

Builds upon concepts of: Sosa and Milligan. JMB. 1996. PMID 8709152

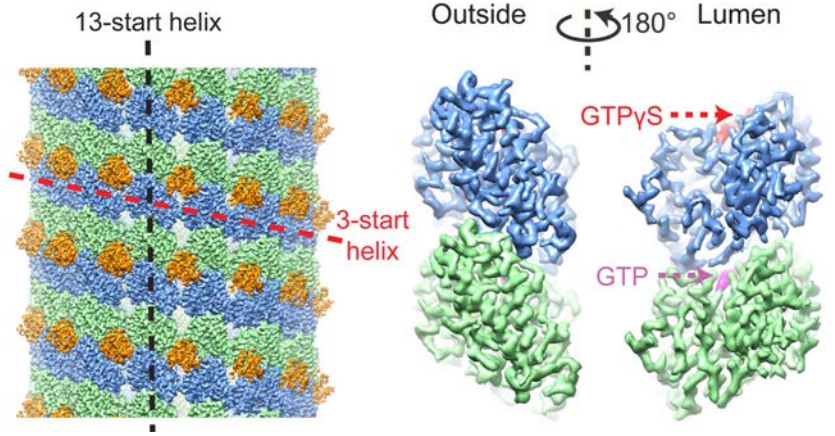
Combining the fiducial concept with 3D classification: first high-resolution structures of microtubules



Kinesin-complexes on film: ~5 Å

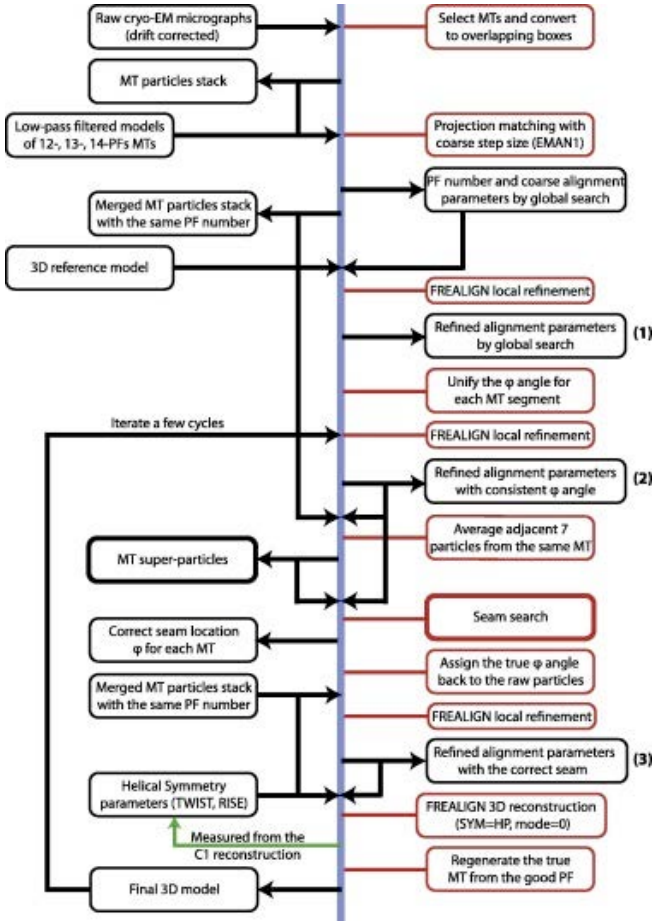


EB-complexes on K2: ~3.5 Å



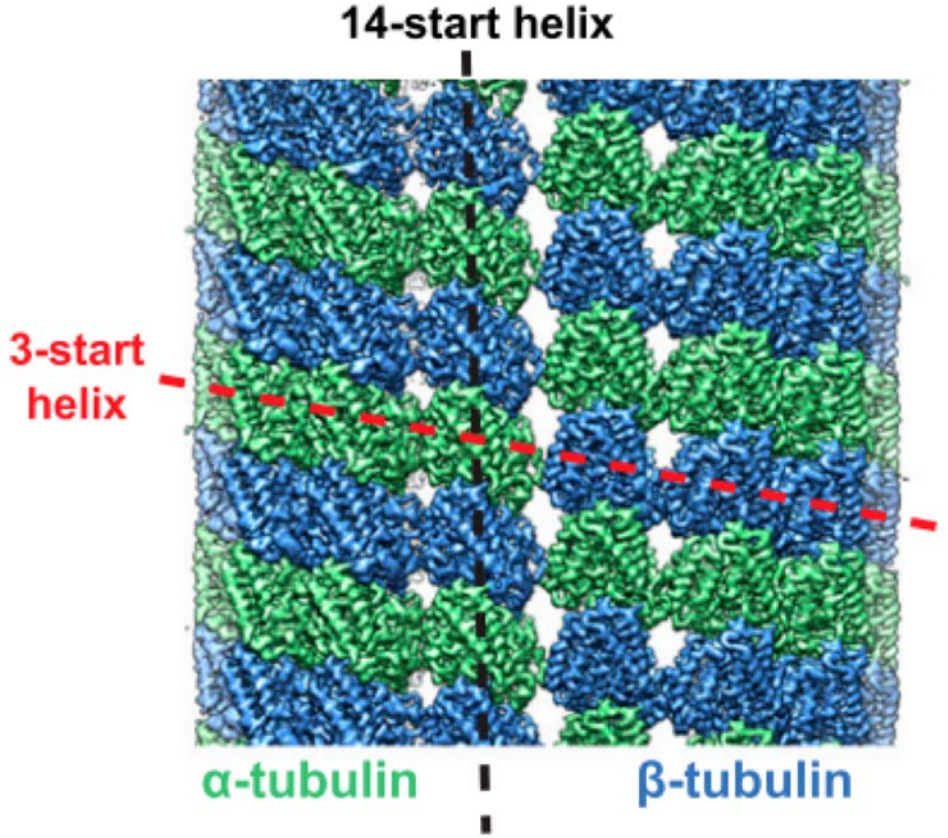
Alushin, Lander, Kellogg...Nogales. Cell. 2014. PMID 24855948
Zhang...Nogales. Cell. 2015. PMID 26234155

Now: no fiducial required.



Zhang, Nogales. JSB. 2015. PMID 26424086

Naked GMPCPP microtubule: 3.6 Å



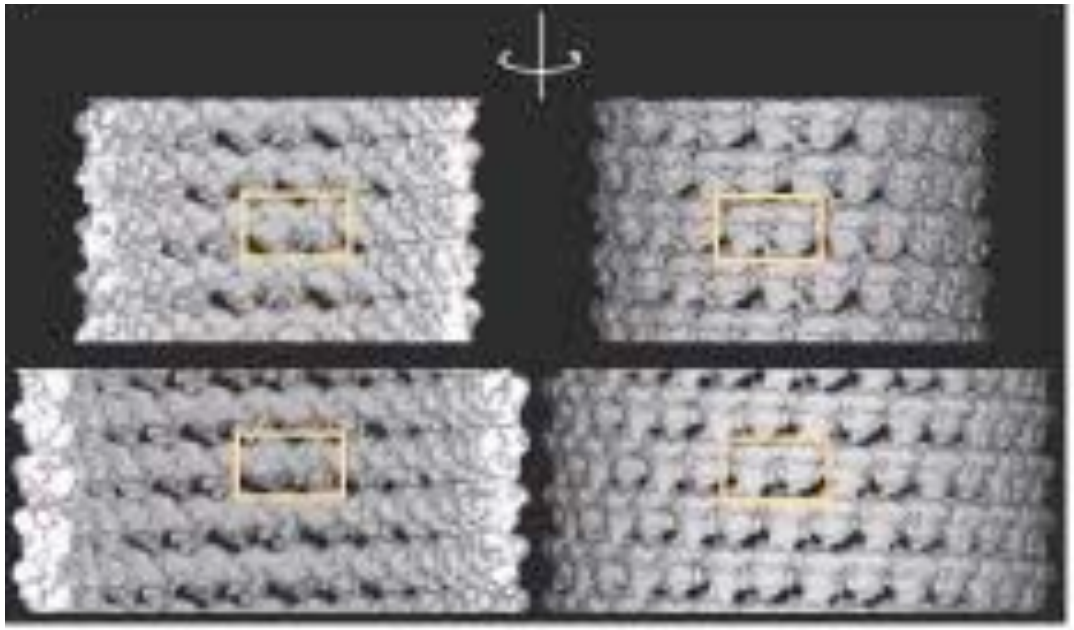
Zhang, LaFrance, Nogales. PNAS. 2018. PMID 29915050

-By testing the two possible registers of each segment shifted by 40 Å along the axial direction, differences between α and β tubulin can be detected.

-Further evidence of the power of direct detectors in allowing weak signal to be exploited.

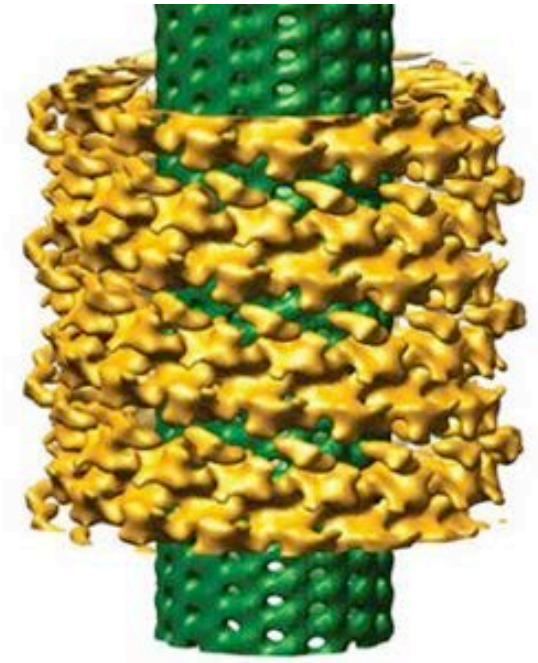
Helices upon helices: the cases of tubulin tubes and the Dam1 complex

GDP / GMPCPP double layered tubes



Wang, Nogales. Nature. 2005. PMID 15959508

DAM1 yeast kinetochore complex



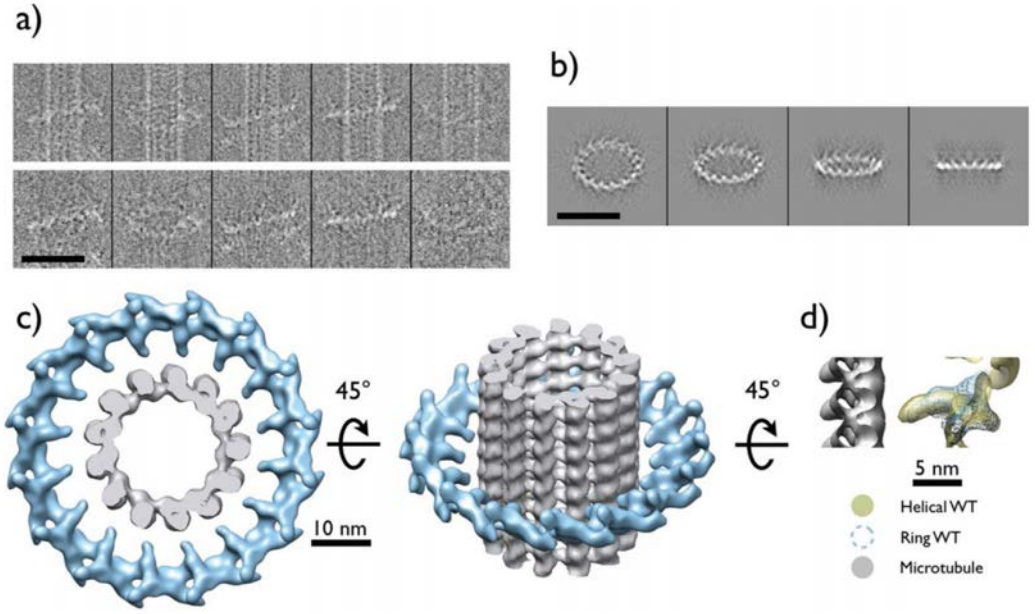
Wang…Nogales. NSMB. 2007. PMID 17643123

-It is sometimes possible to separate the two helical species in Fourier space, even if there are Bessel overlaps in layer lines (similar axial spacing of subunits can cause this, for example). Resolution has been limited.

For theoretical treatment of Bessel overlap: Wang, Nogales. JSB. 2005. PMID 15629658

If symmetries differ or one component is large and asymmetric: use signal subtraction. Case of the DAM1 ring

DAM1 yeast kinetochore complex ring:
native assembly



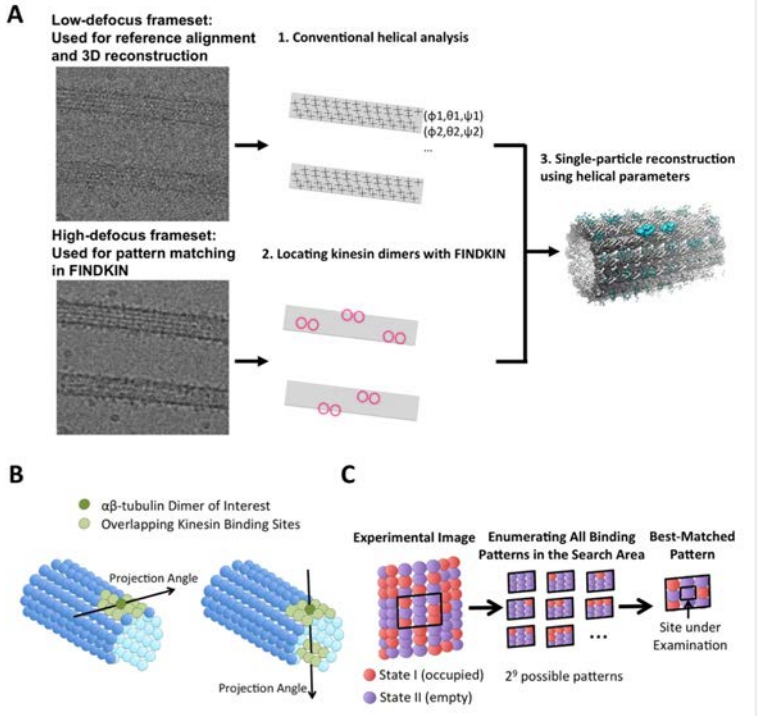
Ramey… Nogales. MBoC. 2011. PMID 21169562

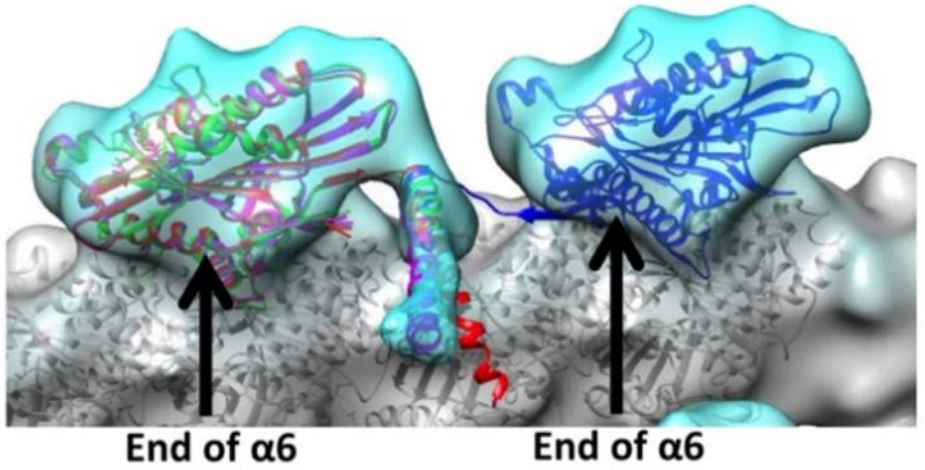
See also helical case: Ramey, Wang, Nogales. JSB. 2009. PMID 19447181

-This approach can work if the structure of the underlying helix bound by another complex is known (n.b., nothing particularly special about a helix, totally analogous in single particle)
-There must be sufficient signal in the other complex to align and reconstruct on its own
-This is probably the only option if there is not a defined binding geometry

If the binding partner is small but the interface is known: a maximalist reference-based approach.

Dimeric kinesin on microtubule



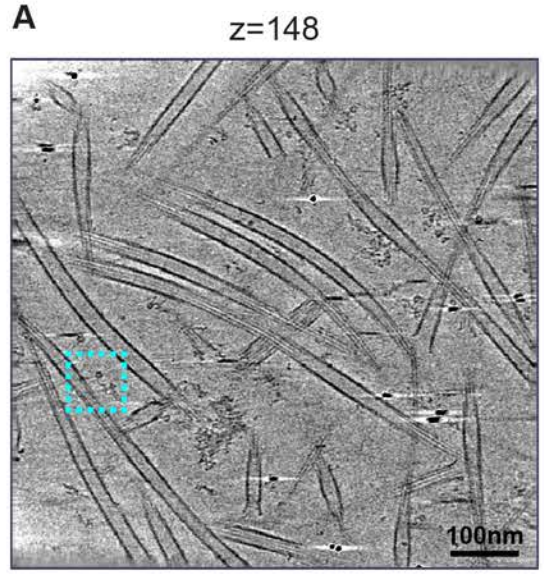


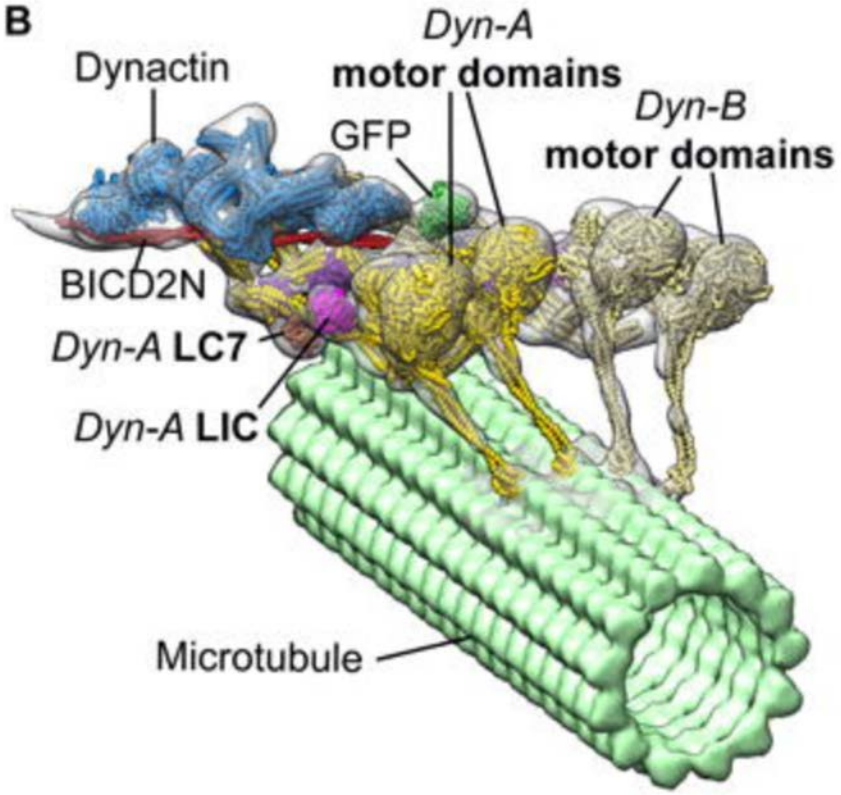
Liu…Sindelar. eLife. 2017. PMID 28504639

-Enumerate all possible combinatorial binding arrangements, then classify with a multi-reference alignment
-First use IHRSR to orient all segments in a common frame of reference, otherwise this would be computationally prohibitive
-Requires a lot of prior information (fairly detailed knowledge of the interface)
-Should be a general strategy applicable to native cytoskeletal motors

Alternatively: use tomography!

Dynein-dynactin complex





Grotjahn…Lander. NSMB. 2018. PMID 29416113

-In principle, does NOT require extensive prior knowledge, but currently does in practice.
-Resolution is currently limited, but this is going to improve, very active area of development.
-A direction I am particularly excited about and one where a lot of my lab's efforts are going.

Questions?