

# A Tutorial on Helical Structures

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## A tutorial on helical structures

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An example of  
a helical  
structure



There are also helical biological structures:

Rod-shaped viruses

Actin filaments

Microtubules

Intermediate filaments

Myosin filaments

Bacterial pili

### How can we study helical structures?

X-ray fiber diffraction?

1. It is difficult to get good preparations and the analysis is also difficult.

2. It can potentially provide atomic resolution (~3Å) but tobacco mosaic virus is the only structure solved to this resolution using the general method of isomorphous replacement.

### Electron microscopy is the method of choice.

It is routine to achieve a resolution of 20 Å.

1. At this resolution one can visualize subunits and even domains.

2. This resolution is sufficient to permit quite accurate docking of atomic models for the subunits into maps.

Several structures have been solved 8-10 Å allowing one to see alpha helices.

One structure, the acetylcholine receptor has been solved to 4.6 Å, where one might be able to trace the backbone.

We can predict that electron microscopy will continue to be the method of choice for helical particles.

We predict that the methodology will continue to be improved so that near atomic resolution will be possible for at least some structures.

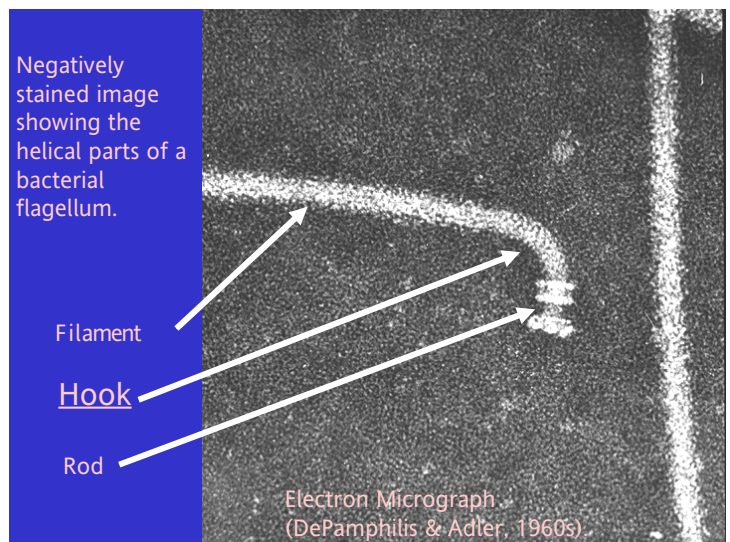
But we must be cautious about making predictions:

"It's hard to make predictions, especially about the future."

Yogi Berra



**Today's example is a helical component of the bacterial flagellum.**



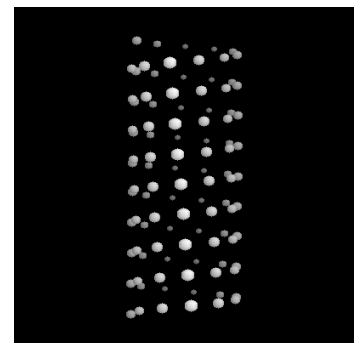
How do we generate a three dimensional map of a structure with helical symmetry?

Whether a structure has helical symmetry of not, we combine images corresponding to different views of the structure.

But helical structures are special. Often only one view is needed! How can that be?

A helical particle consists of a set of identical subunits arranged on a helical lattice.

The subunits are oriented at equally spaced angles.



Thus a single image of a helical structure can have all views of the subunit needed to generate a three dimensional map.

But to produce a 3D map, we have to know the position and angular orientation of every subunit; i.e., we need to go into the helical symmetry rules!



And you need to know where your going when it comes to determining helical symmetry.

You've got to be very careful if you don't know where you're going because you might not get there.  
- Yogi Berra



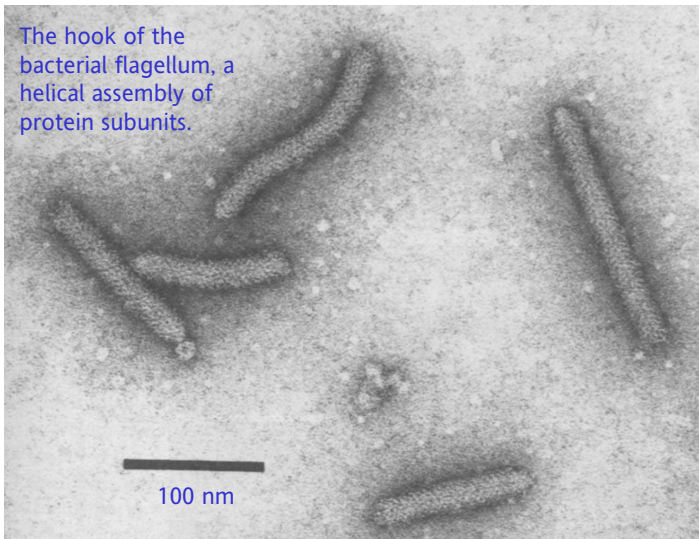
Most of the computer programs needed to convert images into a 3D map are automated or semi-automated, but ...

the key step needed to initiate the use of these computer programs is a determination of the helical symmetry.

We will begin with this.

It looks simple to determine the symmetry of our model just from looking at its image, but how about a real structure?

The hook of the bacterial flagellum, a helical assembly of protein subunits.

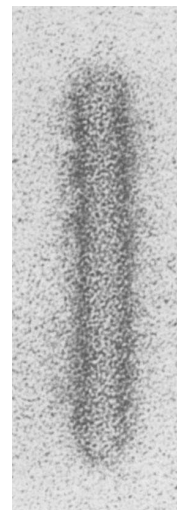
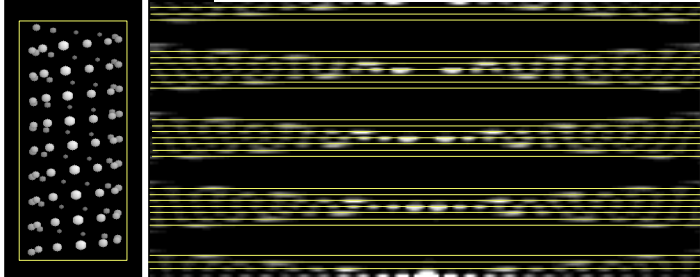


What do we do to determine the helical symmetry if we can't determine it by inspection of the image?

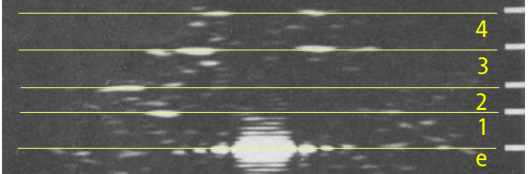
We begin by calculating the Fourier transform of a digitized image.

This is the Fourier transform of our model helix. Only the top half is shown because the bottom half is identical (due to Friedel's law).

The transform of a helical structure consists of strong reflections that lie on a series of regularly spaced lines called **layer lines**.



This is the (whole) Fourier transform of the hook.



There are 4 layer lines plus the equator. There are in fact many more layer lines but they are hidden by noise

To determine the helical symmetry:

1. We determine the positions of all the layer lines.

The axial positions of the layer lines can be selected approximately by eye, but these positions obey strict rules.

Layer lines obey the same positional rules as reflections from a 2D crystal as we shall see.

The axial positions of the layer lines can be selected approximately by eye, but these positions obey strict rules.

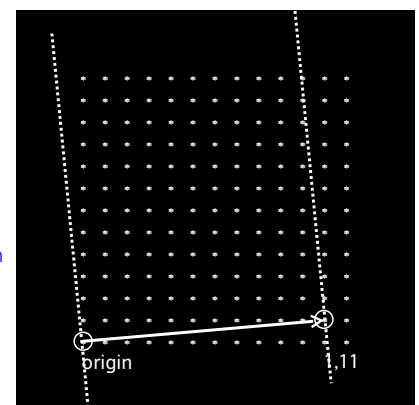
Layer lines obey the same positional rules as reflections from a 2D crystal.

This intimate relationship derives from the intimate relationship between 2D and helical lattices.

In particular, every helical lattice corresponds to some portion of a 2D lattice.

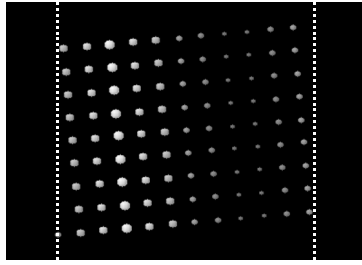
A strip cut from the 2D lattice shown here can be rolled up to give the helical lattice of our model.

- A. We first select a point  $(x,y)$ . For the lattice of the model  $(x,y)=(11,1)$ .
- B. We draw a line from the origin to the point. This line is called the circumferential vector.
- C. We draw two lines that go through the two points and are perpendicular to the circumferential vector.
- D. We cut along the two dotted lines.

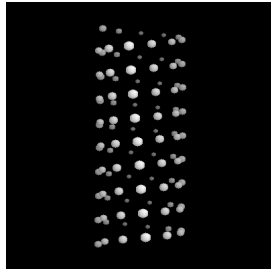


Join the two ends together to generate the helical lattice.

The points in the lattice are rendered in the same way they are rendered in the model helix to provide depth cues.



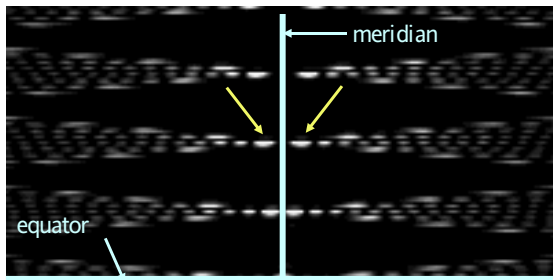
Thus the helical and 2D lattices are closely related as are their transforms.



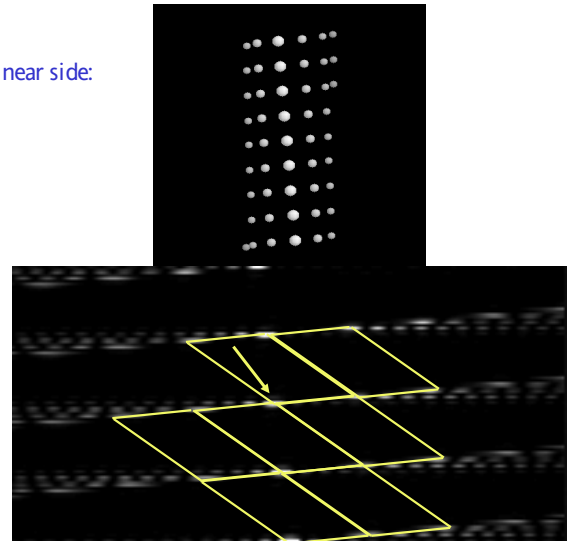
The Fourier transform of a helix looks like the Fourier transform of a 2D lattice but everything appears doubled!

Each layer line has **pairs of spots** placed symmetrically about the meridian.

The **innermost** spots on each layer line form two approximate lattices one arising from the the **near side** of the helix (the side closer to the viewer) and the other from the **far side**.

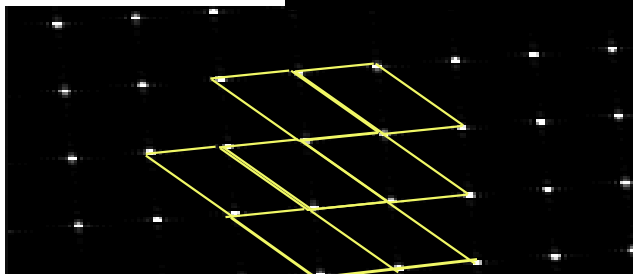
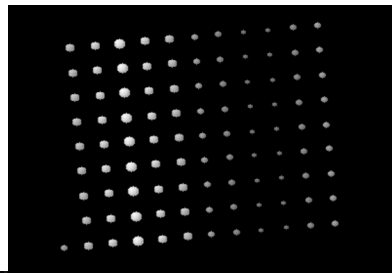


The near side:

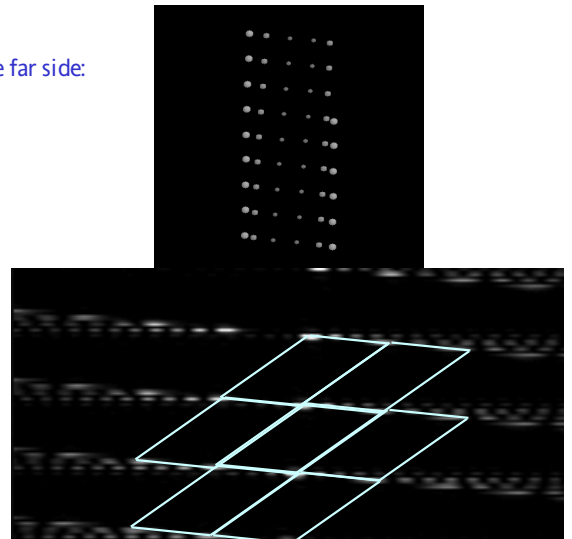


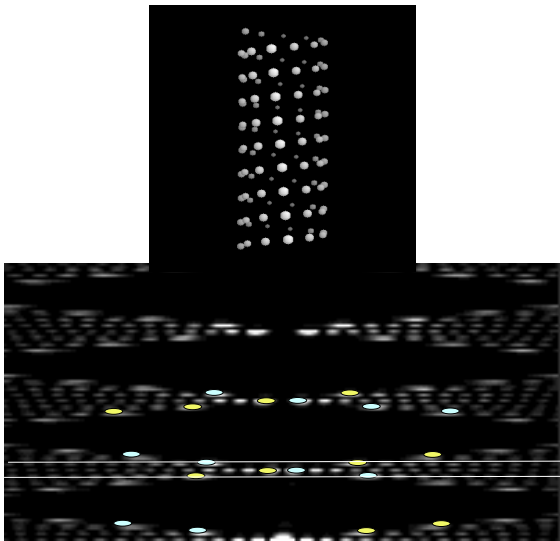
2D lattice

Fourier transform of this lattice.



The far side:





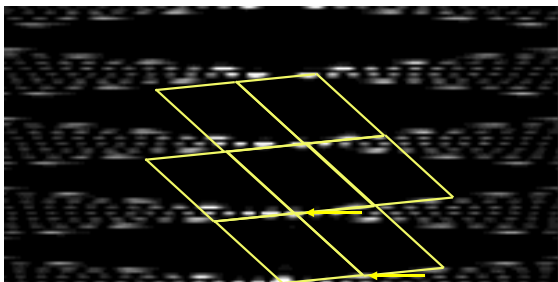
Isn't it hard to figure out which reflections belong to which lattice?

Not necessarily.

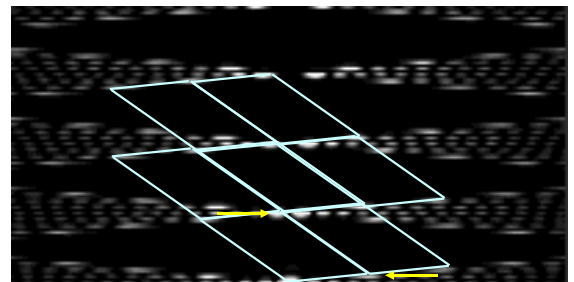
Often there are only two choices.

1. Choose *two layer lines* near the origin.
2. Pick the innermost spot on one side of one of them.
3. Pick one of two innermost spots on the other.
4. Draw the lattice defined by these two spots.

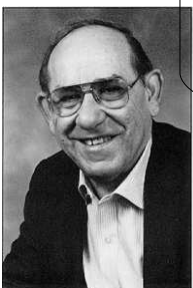
In this case, we chose incorrectly, and the wrong choice results in a lattice that fails to go through all the (first) maxima of the layer lines.



Instead of choosing the right side spot, we try the left-side spot

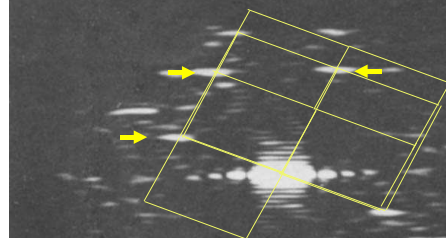


Okay, you say, but let's see if this theoretical strategy works in practice on the hook.



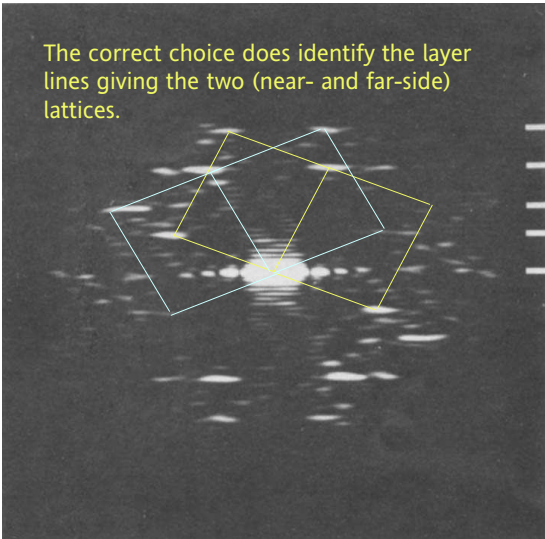
In theory, there is no difference between theory and practice. In practice, there is.

The wrong choice results in a lattice that misses strong layer lines.



The other choice does!

The correct choice does identify the layer lines giving the two (near- and far-side) lattices.



We made too many wrong mistakes.



Luckily, there are only two choices. So usually you can only make one wrong mistake.

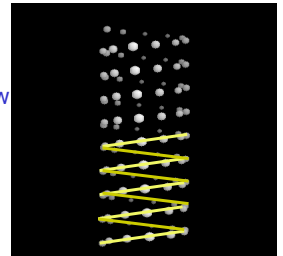
To determine the helical symmetry.

1. We determine the positions of all the layer lines.
2. We next determine the order of each layer line.

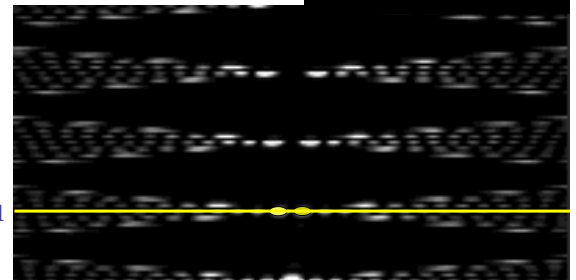
Each **layer line** arises from a **family of helical lattice lines**.

For example, the **family** highlighted in yellow gives rise to the highlighted layer line.

The **order** of the layer line is equal to the number of continuous lines in the family; in this case there is one.

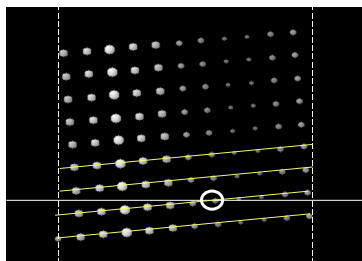


$n=1$

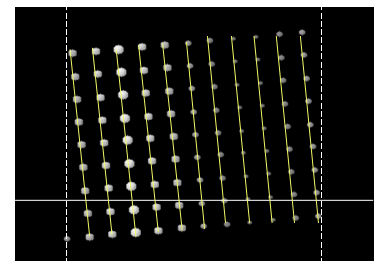


This number is also equal to the number of times the family crosses an equatorial plane or line.

This can be seen most easily in the unrolled helical net.

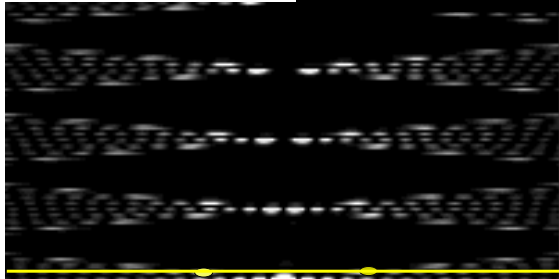
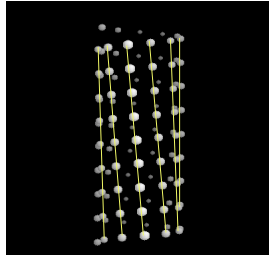


Here is another helical family, which crosses 11 times. Thus its order is  $n=11$ .



Here are the lattice lines drawn onto our model. They are very steep lines. They give rise to the layer line closest to the equator.

The order of this layer line is eleven.



n=11

How does one determine the **order, n**, for each layer line if one knows nothing to start with?

One can get an approximate order of each layer line and one can determine whether the order is odd or even.

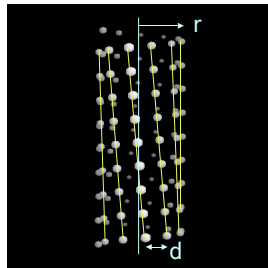
These can be enough to determine the order exactly.

The number of lattice lines (which equals the **order, n**) is equal to the **circumference** of the helix divided by the **distance between lattice lines**:

$$n = 2\pi r / d$$

We can determine the value of  $r$  by measuring the width of the particle.

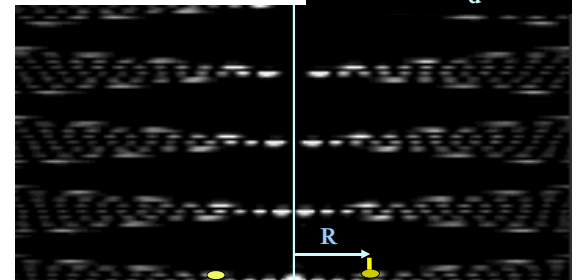
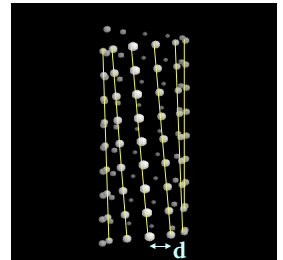
But how can we measure  $d$ ?



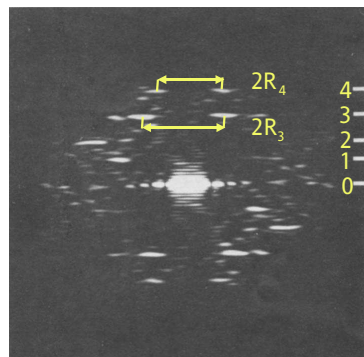
If we measure  $R$ , the distance from the meridian to the first major reflection, we have essentially determined  $d$ ; that is  $d \sim 1/R$ .

A more exact formula is:

$$2\pi r R \sim n + 2 \text{ for } n > 4$$



Because of errors, it is best to estimate the orders of layer lines whose maxima are closest to the meridian; these have the smallest  $n$ .



For layer lines 3 and 4, we measure  $R$ . From the image of the hook, we measure  $r$ .

Here is what was actually determined for the hook:

Layer line #3,  $n = 2\pi r R - 2 = 6.4$  and thus  $n = 6$  or  $7$ .

Layer line #4,  $n = 2\pi r R - 2 = 4.6$  and thus  $n = 4$  or  $5$ .

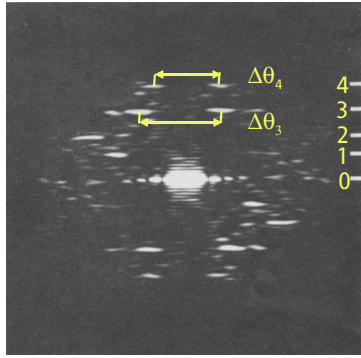
How can one pick between the possibilities?



We can determine whether  $n$  is odd or even and thus we can distinguish between 4 vs 5 or 6 vs 7.

To do so we use the phases determined in the Fourier transforms of the image.

We calculate the phase differences between pairs of reflections symmetric about the meridian.



Thus we measure  $\Delta\theta_3 = \theta_{3,L} - \theta_{3,R}$  for layer line #3 and the same for layer line #4.

If  $\Delta\theta \sim 180^\circ$ ,  $n$  is odd;  
if  $\Delta\theta \sim 0^\circ$ ,  $n$  is even!

Here is what was found:

For layer line #3,  $n = 6$  or 7,

but  $\Delta\theta_3 \sim 11^\circ$  and hence  $n$  is even and  $n=6$ .

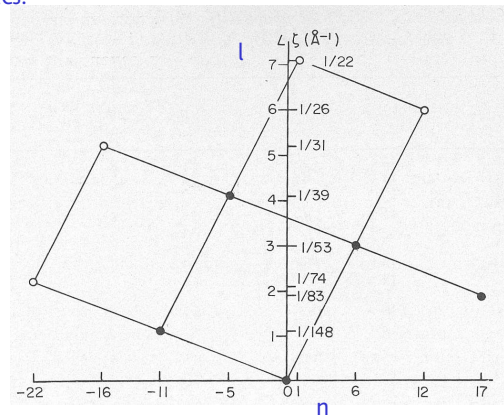
For layer line #4,  $n = 4$  or 5,

but  $\Delta\theta_4 \sim 163^\circ$  and hence  $n$  is odd and  $n=5$ .

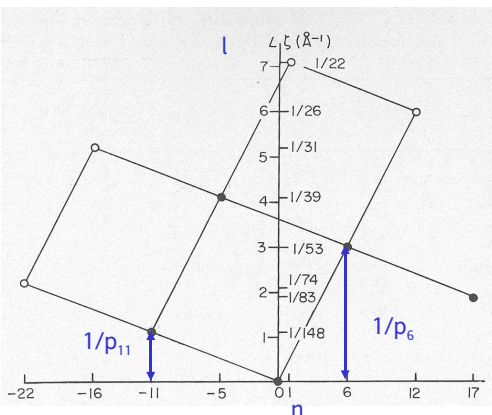
With our measurements of layer line heights and orders, we can draw lattice in which we plot  $n$ , layer line order, vs.  $l$ , layer line height for the layer lines.

These points form a perfect lattice and hence predict the positions and orders of all possible the layer lines arising from this helical symmetry.

Here the “known” 4 layer lines are indicated as filled black circles and a few of the predicted ones are shown as open circles.



The height of each layer line gives us a measure of the pitch,  $p$ , of the corresponding lattice lines and from that we can construct the helical lattice.



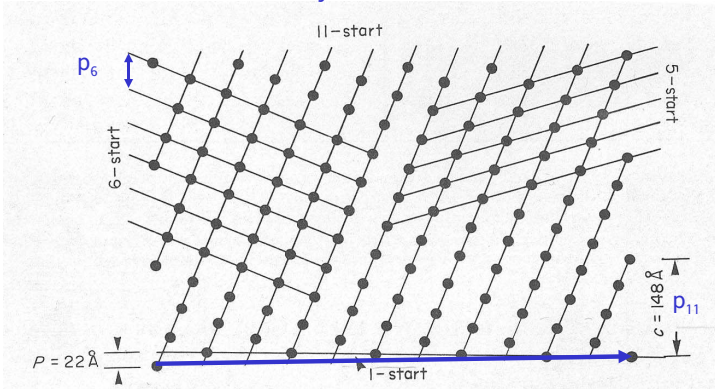
Notice that some of our points in the  $n, l$  plot have negative values of  $n$  (e.g.,  $n=-11$ ).

The convention is that  $n > 0$  refers to right-handed helical families and  $n < 0$  to left-handed families.

For our plot, we have chosen two pairs of helical families  $n = -11$ ,  $l = 1/148 \text{ \AA}$  and  $n = +6$ ,  $l = 1/53 \text{ \AA}$ , but we don't yet know the absolute hand of the structure.

So it could also be that  $n = +11$ ,  $l = 1/148 \text{ \AA}$  and  $n = -6$ ,  $l = 1/53 \text{ \AA}$ .

The helical lattice can be constructed by drawing a circumferential vector. The vector is divided into six parts and six lattice lines having pitch  $p_6$  are drawn in. The same is done for the  $n=11$  family.



The correct lattice is either the one we have drawn or the one with the opposite hand (i.e., its mirror image corresponding to the case in which the signs of the  $n$ 's are reversed).

How do we determine handedness?

1. Get an atomic resolution map and note whether the alpha helices have the correct hand.

2. Look at only the top surface instead of the superposition of top and bottom (e.g., get images of shadowed particles which reveal only one side).

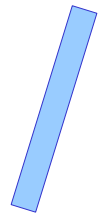
3. Tilt the structure in the microscope.

We will use tilt.

Let us arrange that the top of the helical structure is tilted away from the viewer in the image.

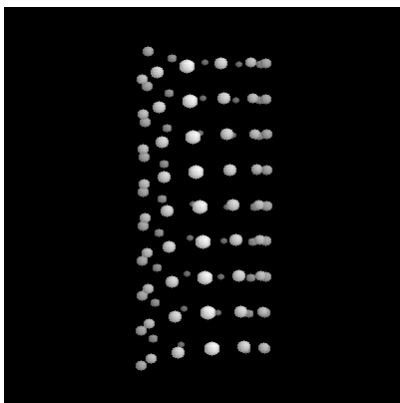


The microscopist



The helical structure

The effect is to scallop the the right-hand sides of right-handed helices and the left-hand side left-handed helices.

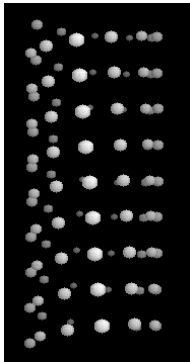


We can use this effect to determine the hands of some of the helical families.

Remember we only have to do so for one layer line since the hands of the others are determined from our  $n, l$  plot.

We see that there are clear rows on the right-side image but not on the left.

This is expected for a right-handed family of helical lattice lines.



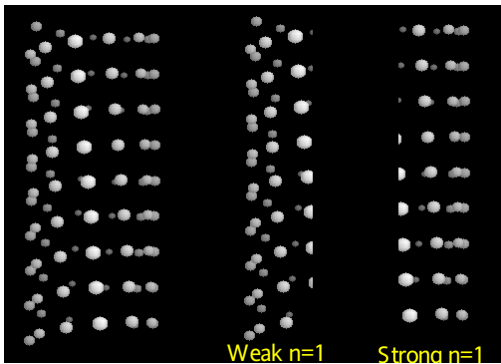
Does this work for real structures?

On some structures one can see scalloping but for most we cannot as is the case for the hook.

To detect the scalloping on each layer line, we resort to Fourier transforms.

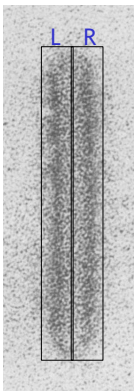


What we expect is the the right hand side of the image will generate a stronger layer line corresponding to the  $n=1$  family than will the left hand side of the image.



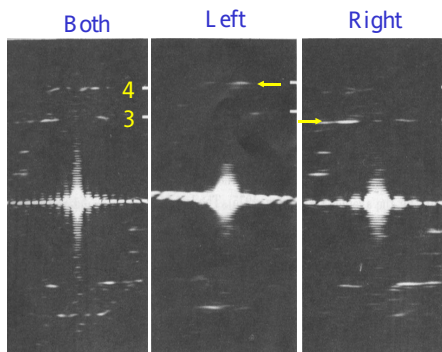
Will this work on our real example, the hook?

Let us look at the Fourier transforms of the left and right hand sides of the image.

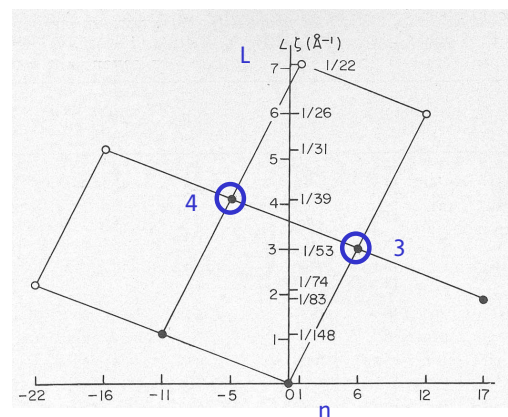


Note that 3 is stronger in R while 4 is stronger in L.

Thus the 3 is right handed and 4 is left handed.



This means that our original assignment was correct.

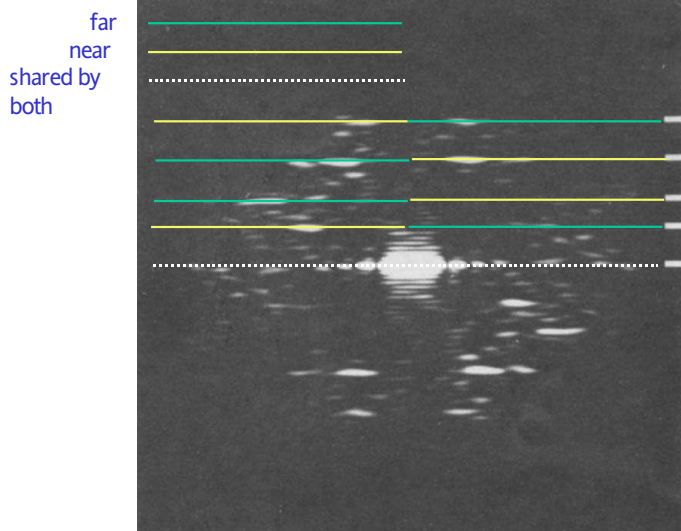


The hardest part of generating a map is done. We have:

1. located the layer lines.
2. determined their axial positions,  $l$ .
3. determined their orders,  $n$ .
4. determined their absolute handedness

From each image we can now generate two independent 3D maps.

One corresponding to the near side of the structure and one to the far side.



Assume we have collected the two (near and far) data sets.

These data sets are known as the big G's:  $G_{n,l}(R)$ .

To generate a 3D map we carry out an inverse Fourier transform (IFT).

The first step is the calculation of little g's:  $g_{n,l}(r)$ .

There is one obtained from every big G.

A little math about Fourier transforms of helices:

The Fourier transform of anything is:

$$F(X,Y,Z) = \iiint \rho(x,y,z) \exp\{2\pi i(xX+yY+zZ)\} dx dy dz$$

If we rewrite this sum using cylindrical coordinates of radius,  $r/R$ , azimuth,  $\theta/\Theta$ , and height,  $z/Z$ , we have the Fourier transform of anything in cylindrical coordinates:

$$F(R,\Theta,Z) = \Sigma G_n(R,Z) \exp\{in(\Theta+\pi/2)\}$$

Where

$$G_n(R,Z) = \iiint \rho(r,\theta,z) J_n(2\pi Rr) r dr \exp(-in\theta) d\theta \exp(2\pi izZ) dz$$

$$F(R,\Theta,Z) = \Sigma G_n(R,Z) \exp\{in(\Theta+\pi/2)\}$$

This equation describes the Fourier transform of a subunit.

All possible values of  $n$  ( $\dots -5, -4, -3, -2, -1, 0, 1, 2, 3, 4, 5, \dots$ ) occur for every possible value of  $Z$ .

When we place the subunit in a helical lattice, the values of  $n$  and  $Z$  are restricted according to our  $n,l$  (or  $n,Z$ ) lattice.

This is analogous to the way that placing a subunit in a crystal restricts the Fourier transform to discrete values; e.g.  $F(h,k,l)$ .

When the anything is a helix, the symmetry causes the Fourier to exist only on layer lines.

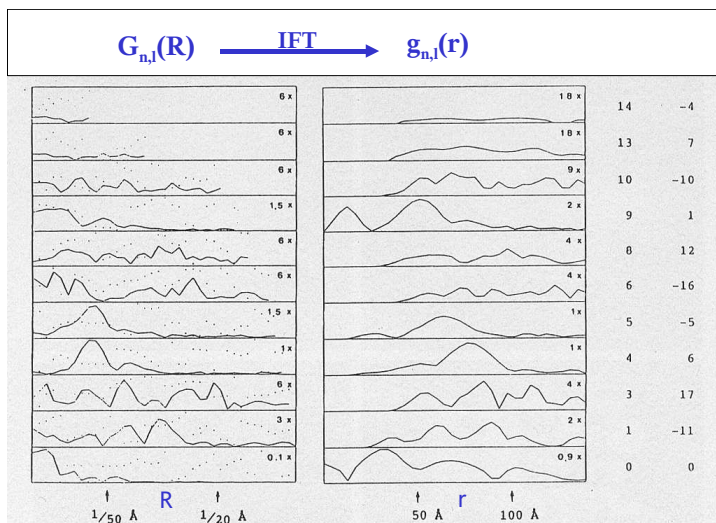
It thus restricts the transform and hence the allowed values of  $G_{n,l}$  in exactly the same way that a 2D crystal restrict the Fourier transform to certain values of X and Y:  $F(h,k)$ . Here h and k correspond to n and l.

For helices then,

$$F(R, \Theta, Z) = G_{n,l}(R) \exp\{in(\Theta + \pi/2)\}$$

and  $F=G$  when  $\Theta = -\pi/2$ .

When we collect data off of the Fourier transforms, we are collecting  $G_{n,l}(R)$ .



The  $g$ 's are finally converted into a 3D map.

There are several sets of programs that collect data and generate 3D maps:

[The Brandeis Helical Package](#)

[The MRC-LMB Helical Package](#)

[Phoelix](#)

These programs will do all the busy work but they will not figure out the helical symmetry;

that is the part you must determine.

Egelman's Program – may change the way we analyze all helical structures!

It cuts the helical structure into many short segments.

Segments are aligned to projections of the reference 3D map.

A new 3D map is computed but it is different from the reference map.

The process is iterated using the new map as reference.

Where do you get a starting 3D reference to align to?

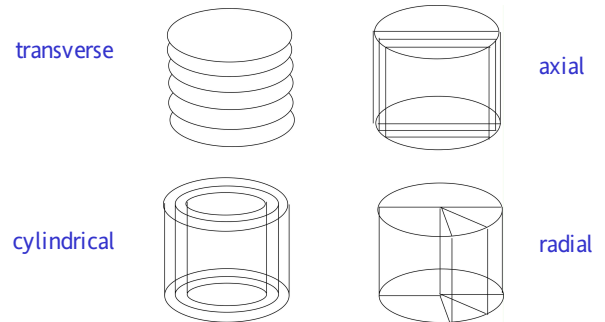
The starting 3D reference needn't be accurate.

A helical object with the wrong symmetry or even a smooth cylinder can work.

Thus, in some cases structures were solved where one knew nothing about the helical symmetry!

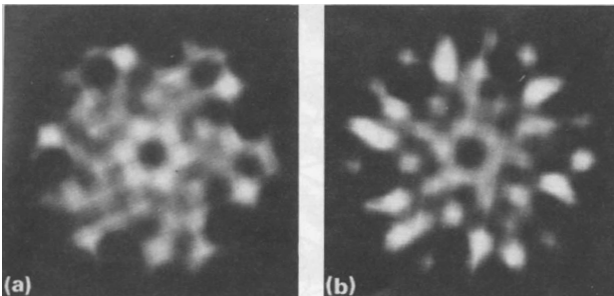
Once we have the 3D map, we look at it in a variety of ways.

We can take slices through the map.



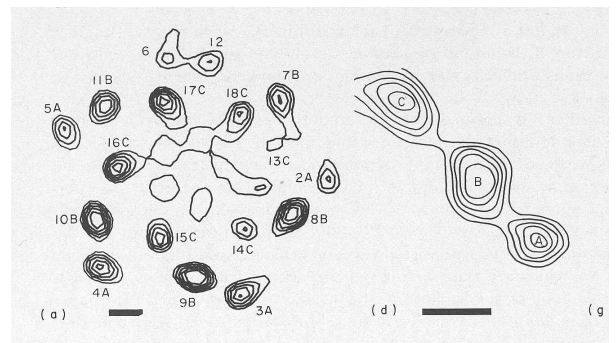
Far side

Near side



Gray scale representations of transverse sections.

Contour plots.



transverse section

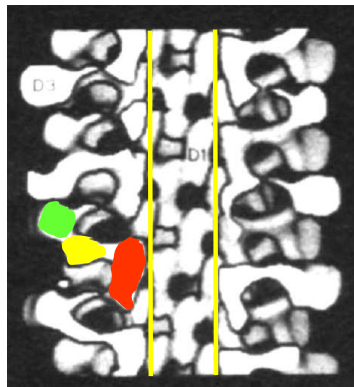
Axial section through part of one subunit

Here is a surface view of the hook map, which has been cut in half.

In it we can see domains!

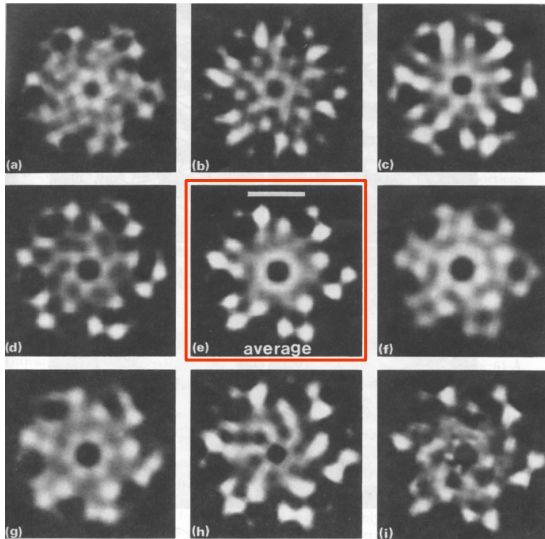
Each subunit has three domains shown here in color.

There is a central 30Å wide channel thought to be used for protein export.



Just as the maps of near and far side differ, so do the maps obtained from different images.

But we can average them to increase the signal-to-noise.



### How can we get higher resolution maps?

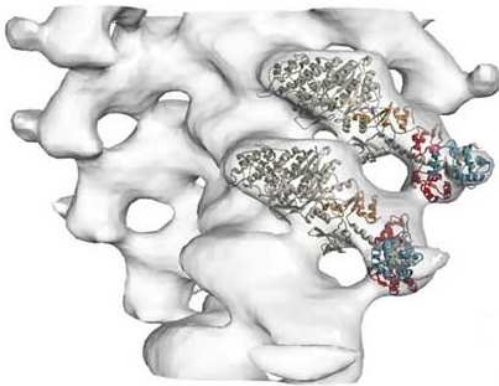
To collect higher resolution data, we must:

More rigorously correct images for all defects (eg., curvature).

Collect all layer lines predicted by the  $n,l$  plot out to the desired resolution.

Align and average data from many images

Or dock atomic models for the subunit into our lower resolution map.

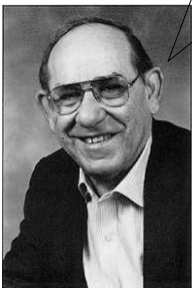


Model of myosin docked into a map of the actin-myosin complex. (Volkman et al.)

Things are looking more promising than ever for high resolution studies of helical structures by EM because fortunately ...

PS

The future ain't what it used to be.



I didn't say everything I said.



JHE IN