Introduction to Ruby-Helix & Asymmetric Helical Reconstruction

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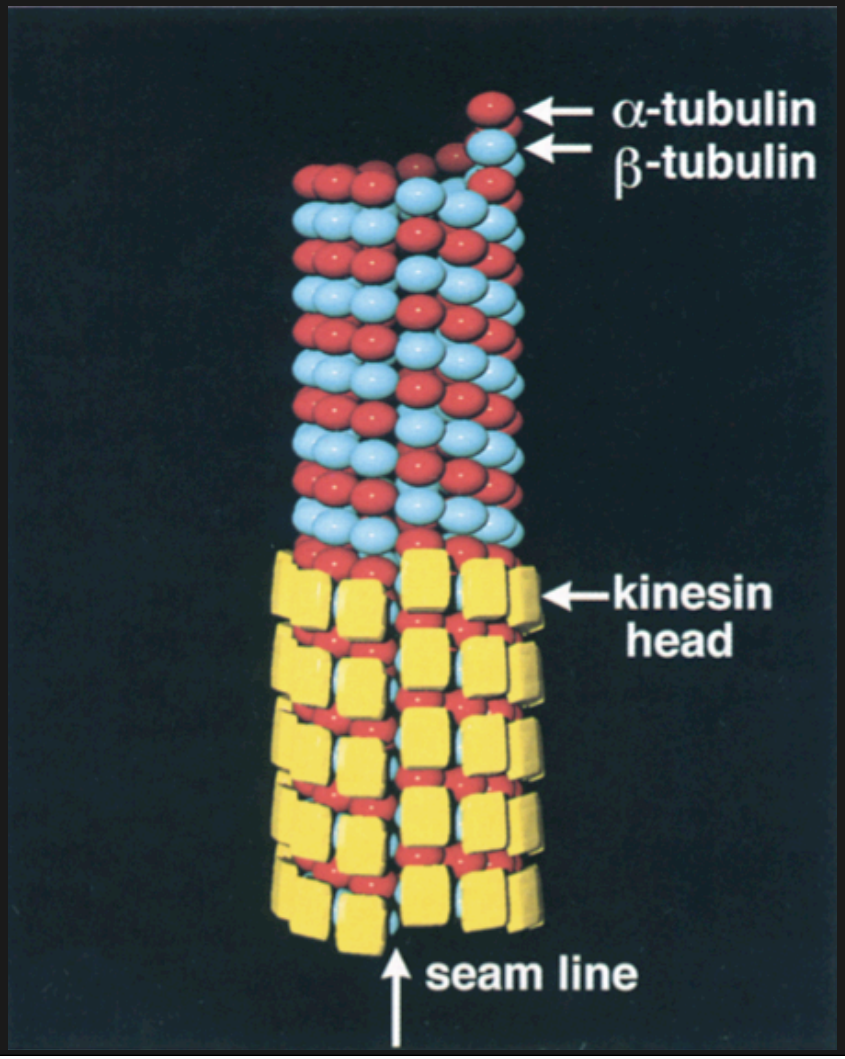
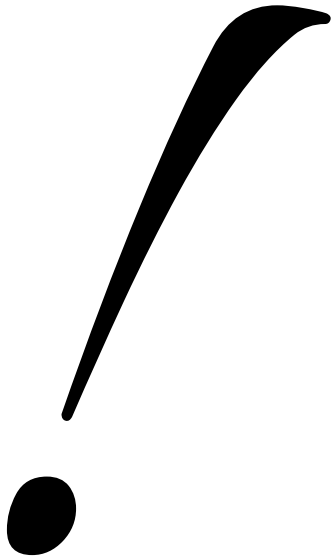
Microtubule is *not* a helical object



1994, JCB, Kikkawa et al


Univ. of Texas, Southwestern
2001 ~ now

Ruby



2003, fall

Overview

- Ruby-Helix
- Conventional helical reconstruction
- Asymmetric helical reconstruction
- (Ruby-Helix demo)

Ruby?

- Interpreted scripting language
- Object-oriented

  Everything is object

- Free
- For detail, see http://www.ruby-lang.org

Ruby is getting popular

- In Amazon.com, ranking (April 6th, 2006)
  - Ruby on Rail: 321

    (11th in computer)
  - Programming Ruby: 667

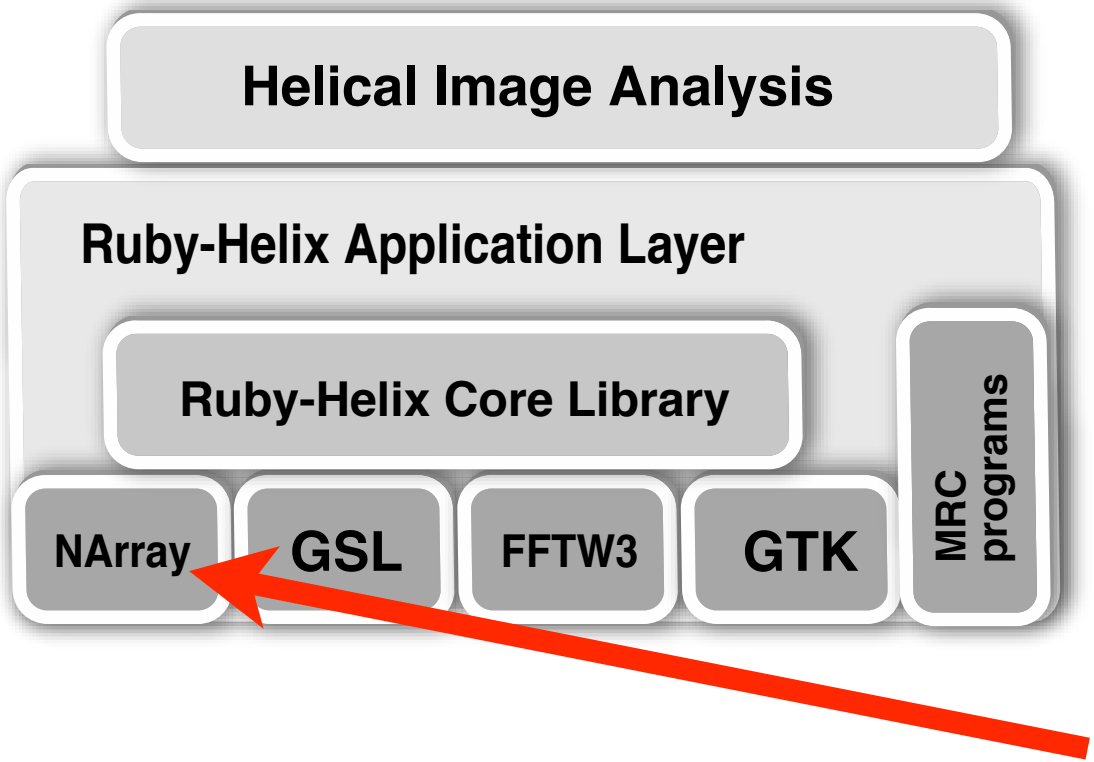
  Learning Perl: 1721

  Learning Python: 5721

Ruby Features

- ★ Simple, easy to learn
- Gigantic set of built-in libraries
- Portable
- Interpreted
- Object oriented
- Very popular for web application scripting (Ruby on Rail)
- ★ Ruby for high-level work, with compute-intensive work in C/C++/Fortran libraries

Ruby-Helix architecture



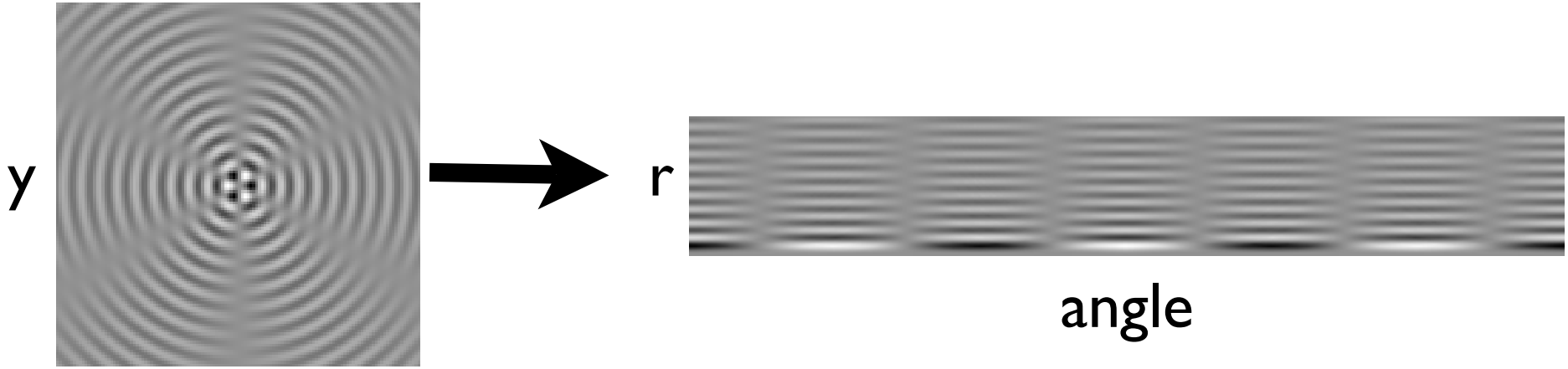
Application Scripts : Ruby

Higer Level Libraries : Ruby

Lower Level Libraries : C

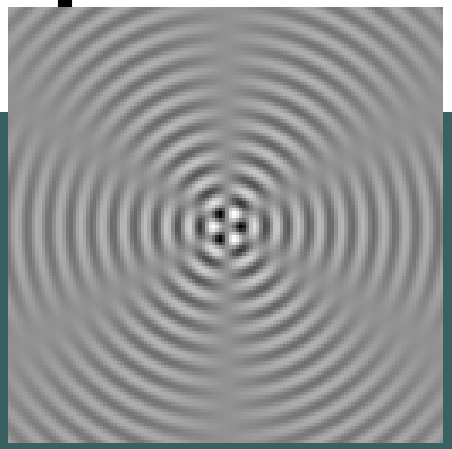
NArray works like Matlab

Sample script: conversion to polar coordinate



$$x = r * \cos(angle)$$
$$y = r * \sin(angle)$$

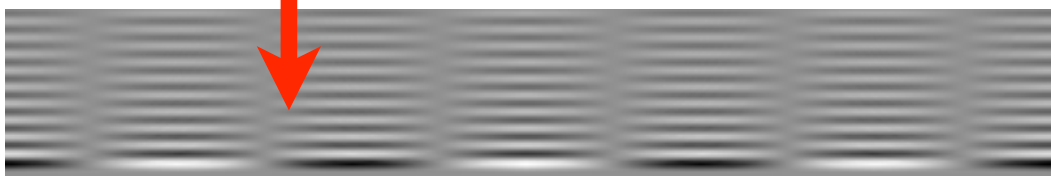
Ruby-Helix script



```
require "mrc.rb"
include NMath
Size = 128

image = NArray.open_mrc("input.mrc")

ang = NArray.sfloat(Size * Math::PI, 1).indgen! / (Size * 0.5 )
r   = NArray.sfloat(1,Size/2).indgen!

x =  r * cos(ang) + Size / 2
y =  r * sin(ang) + Size / 2

image.interpolate2d(x,y).write_mrc("output.mrc")
```

x = r * cos(angle)

y = r * sin(angle)



Conventional

Helical reconstruction

See also a lecture by Moody:

http://www.biomachina.org/courses/structures/07.html

Many biological molecules form helices

- Nucleotide ⇒ DNA
- Actin ⇒ Actin filament
- Tubulin ⇒ Microtubule
- Flagellin ⇒ Flagella
- Membrane Proteins

First 3D reconstruction from EM

JANUARY 13, 1968

Reconstruction of Three Dimensional Structures from Electron Micrographs

by

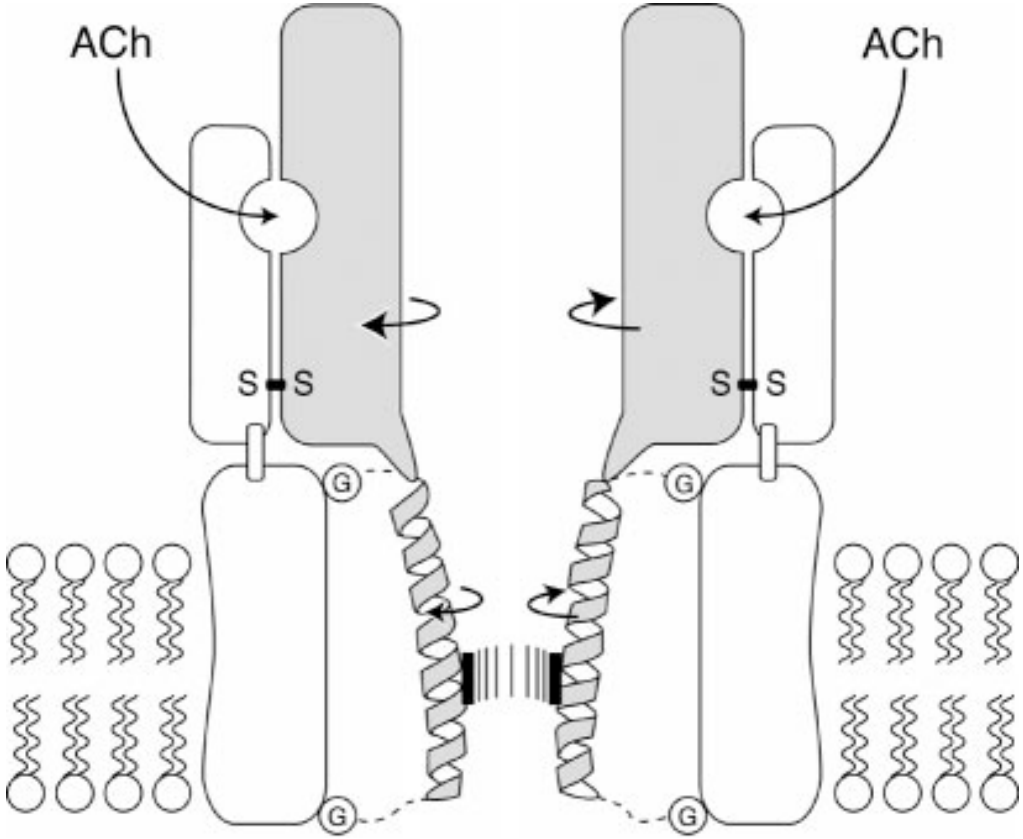
D. J. DE ROSIER
A. KLUG

MRC Laboratory of Molecular Biology, Hills Road, Cambridge

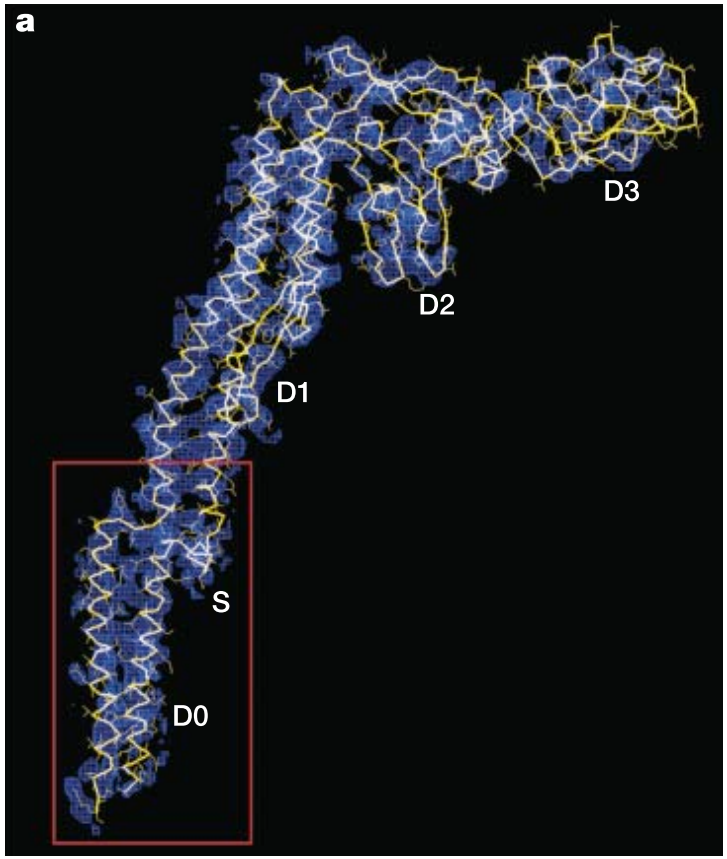
General principles are formulated for the objective recons of a three dimensional object from a set of electron mi images. These principles are applied to the calculation of dimensional density map of the tail of bacteriophage *T4*.

Nature (1968)

Two atomic structures by helical reconstruction in 2003



Acetylcholine receptor

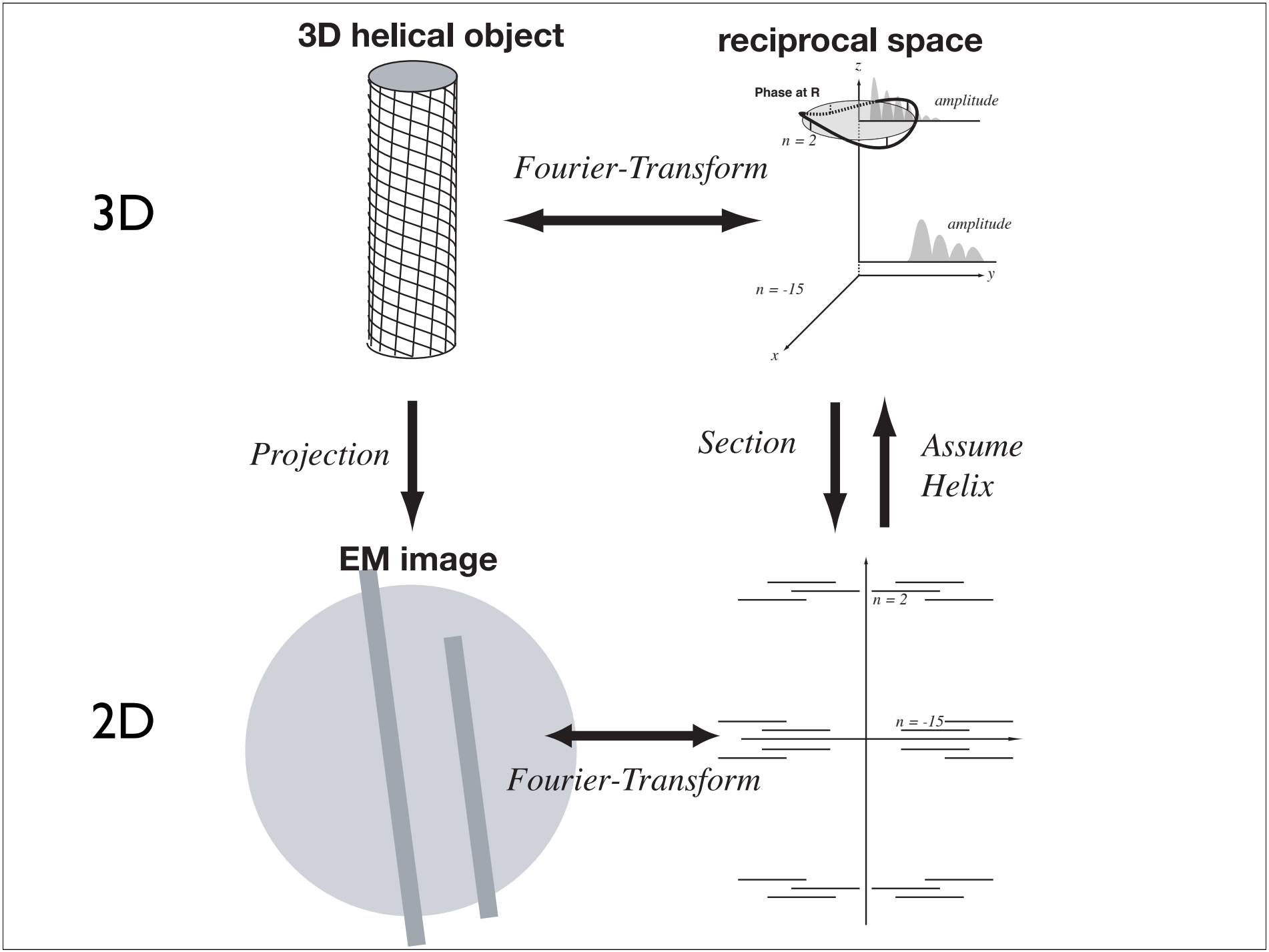


Bacterial flagellar filament

New Techniques

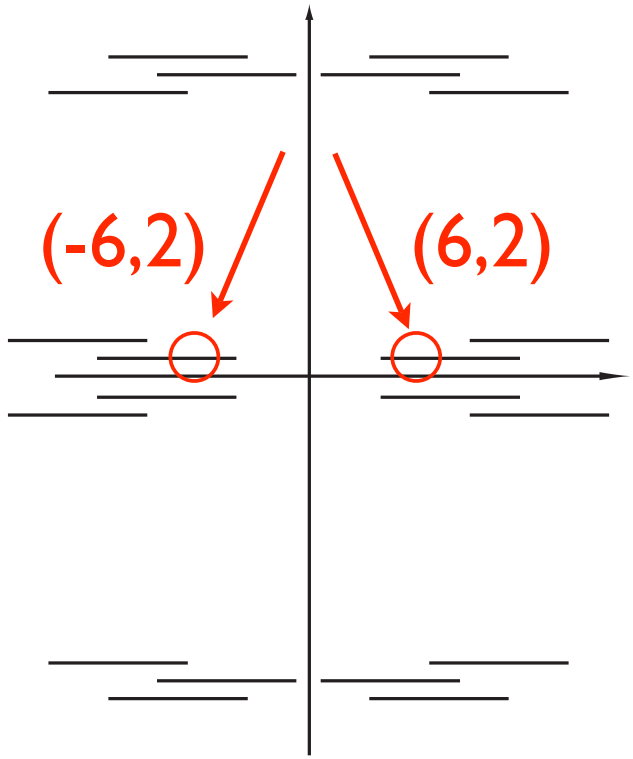
- IHRSR (2000, Egelman)
- Solvent flattening (2000, Yonekura & Toyoshima)
- Asymmetric helical reconstruction (2004, Kikkawa)
- Separation of overlapped layer lines (2005, Wang & Nogales)

Basic idea of helical reconstruction

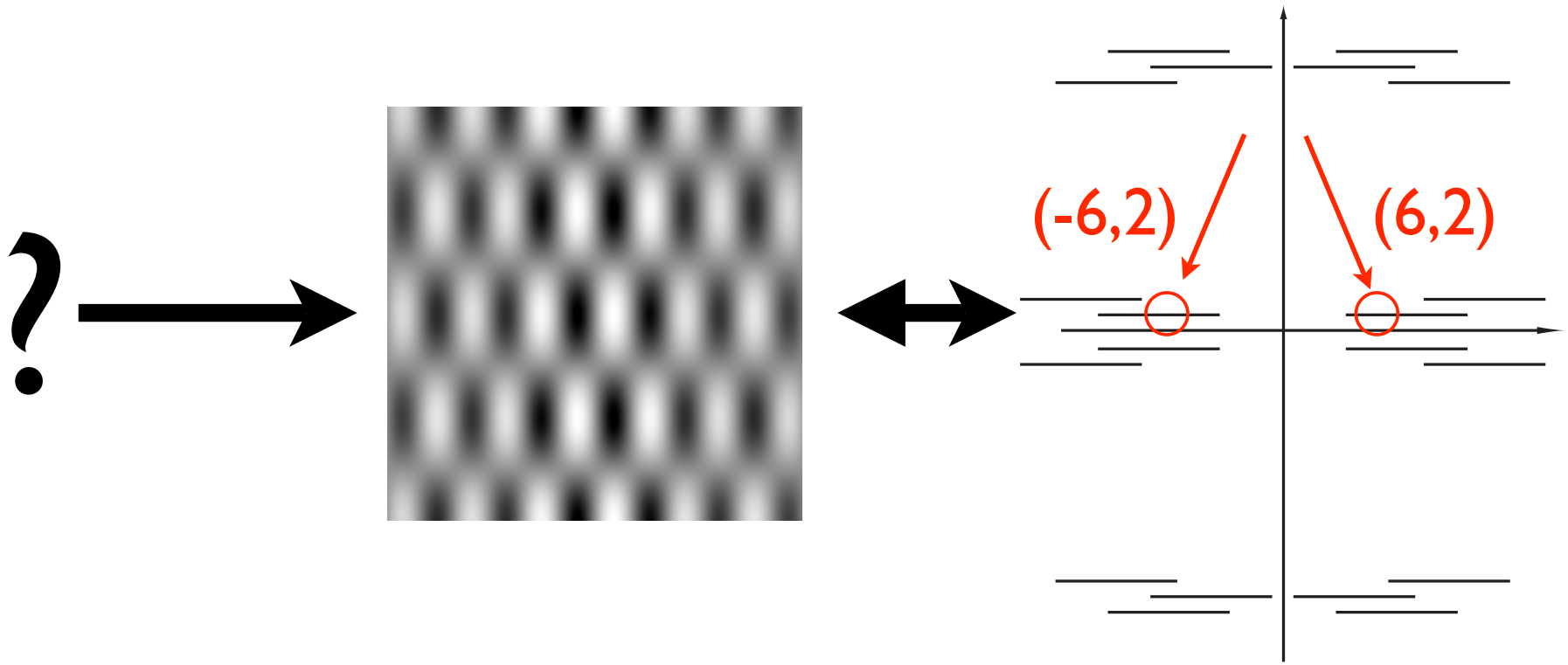

3D helical object
reciprocal space
3D
Phase at R
amplitude
n = 2
Fourier-Transform
amplitude
n = -15
y
x
z
Projection
Section
Assume
Helix
EM image
2D
n = 2
n = -15
Fourier-Transform

What 3D structure generates these spots in Fourier transform?

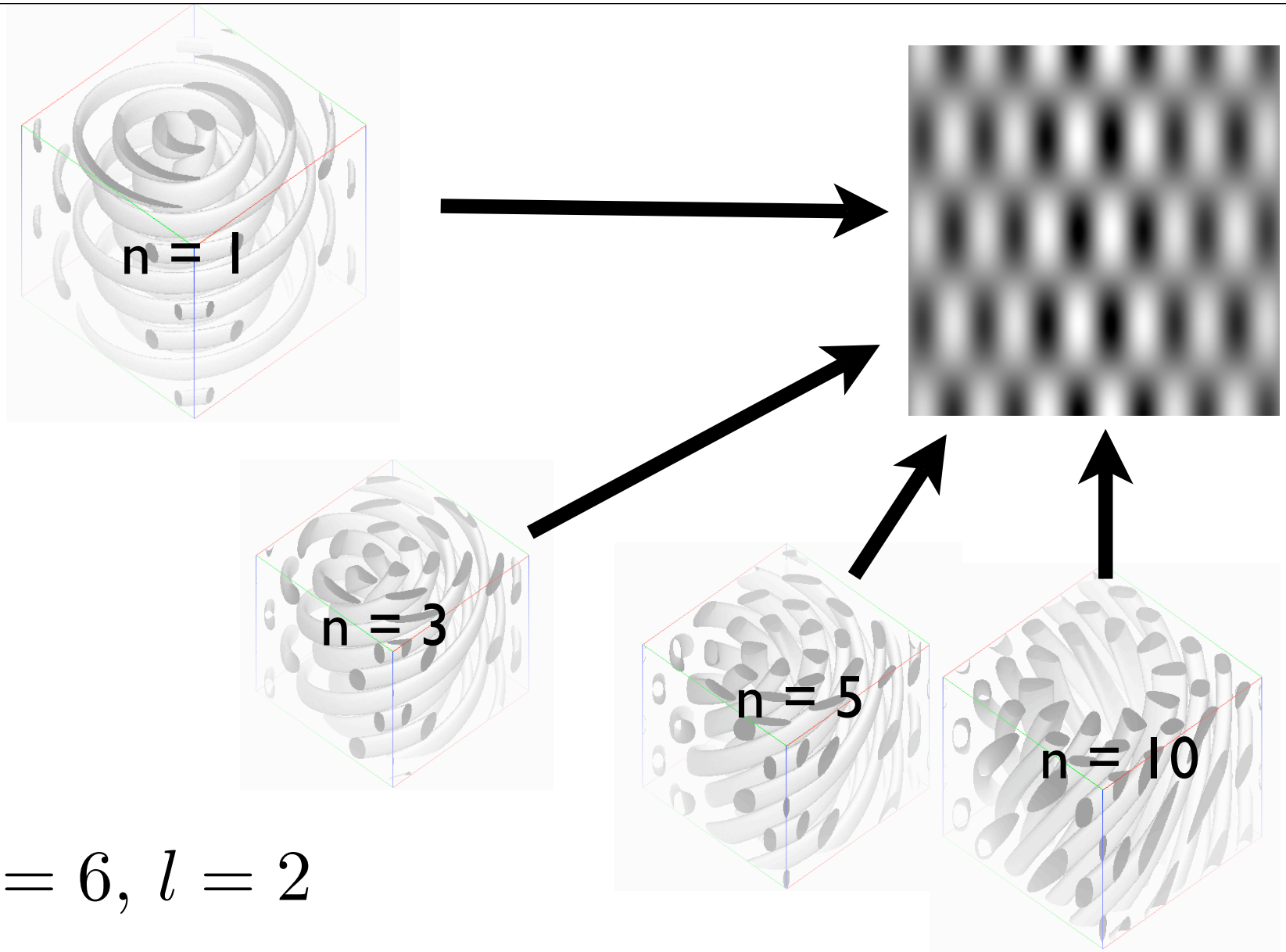
?



Or what 3D structure generates the 2D pattern?



There are many solutions..



$R = 6,\ l = 2$

$$\rho(r, \phi, z) = J_n(2\pi r R)\cos(n\phi - 2\pi l z/c)$$

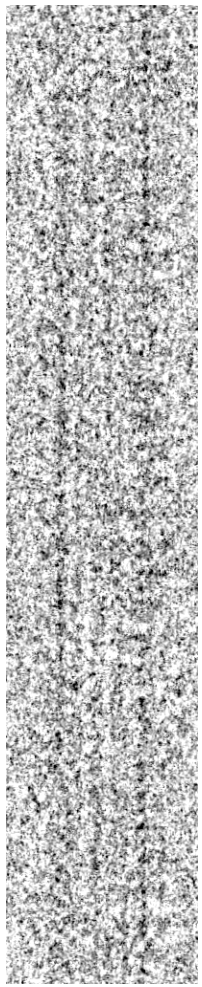
Assigning Bessel orders

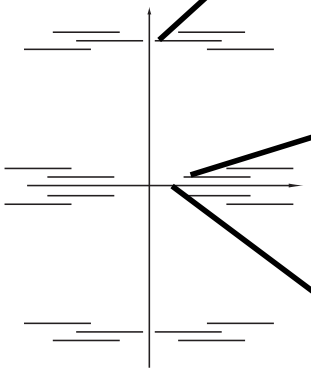
=

guessing the structure

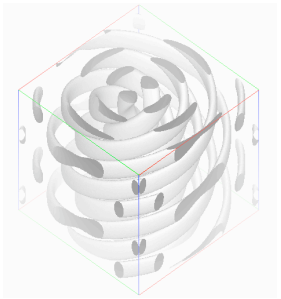
&

interpolating 3D space

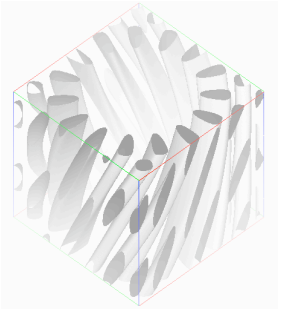




"indexing"



+



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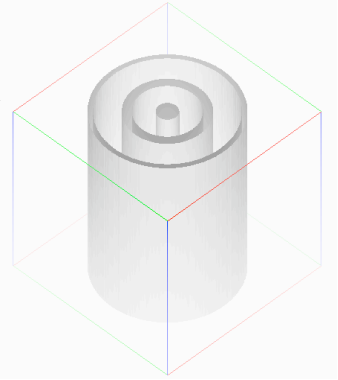
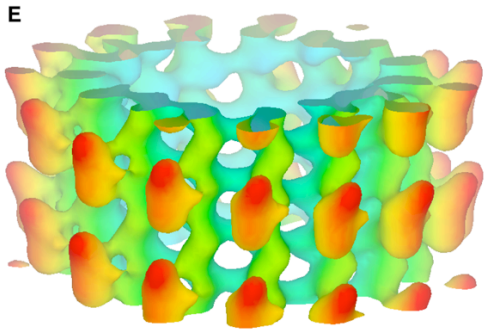


+

.....

sum



Advantages and Disadvantages of Fourier Space

- Advantages

  S/N is higher at lower resolution

  Data size is smaller, faster computation

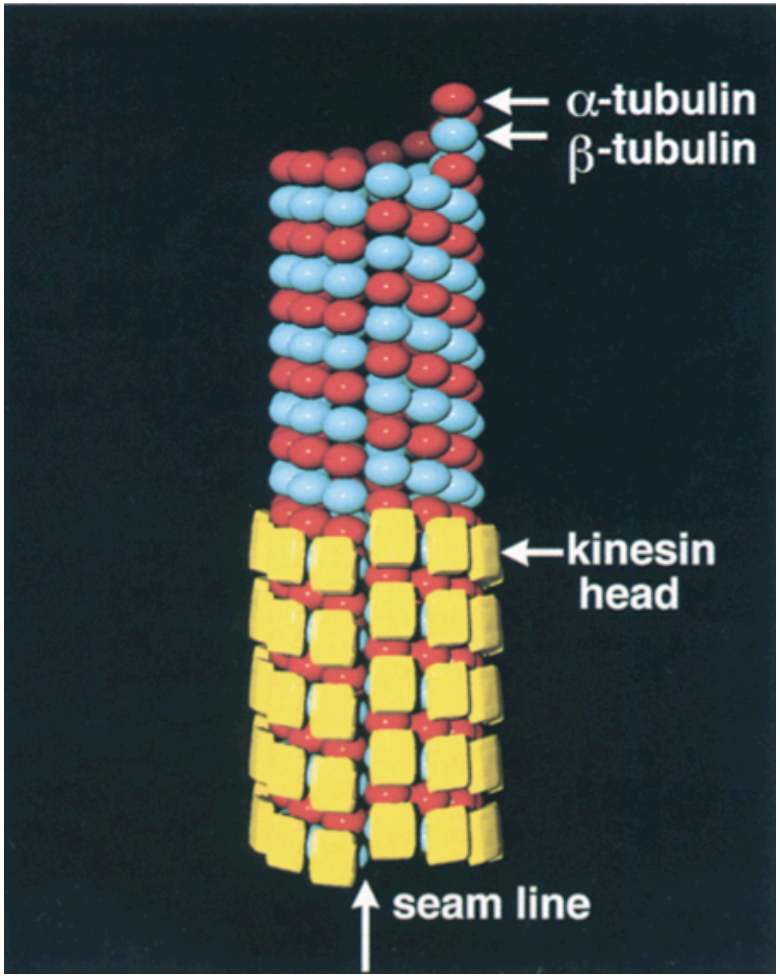
- Disadvantages

  Needs ordered structures

  Needs distortion corrections

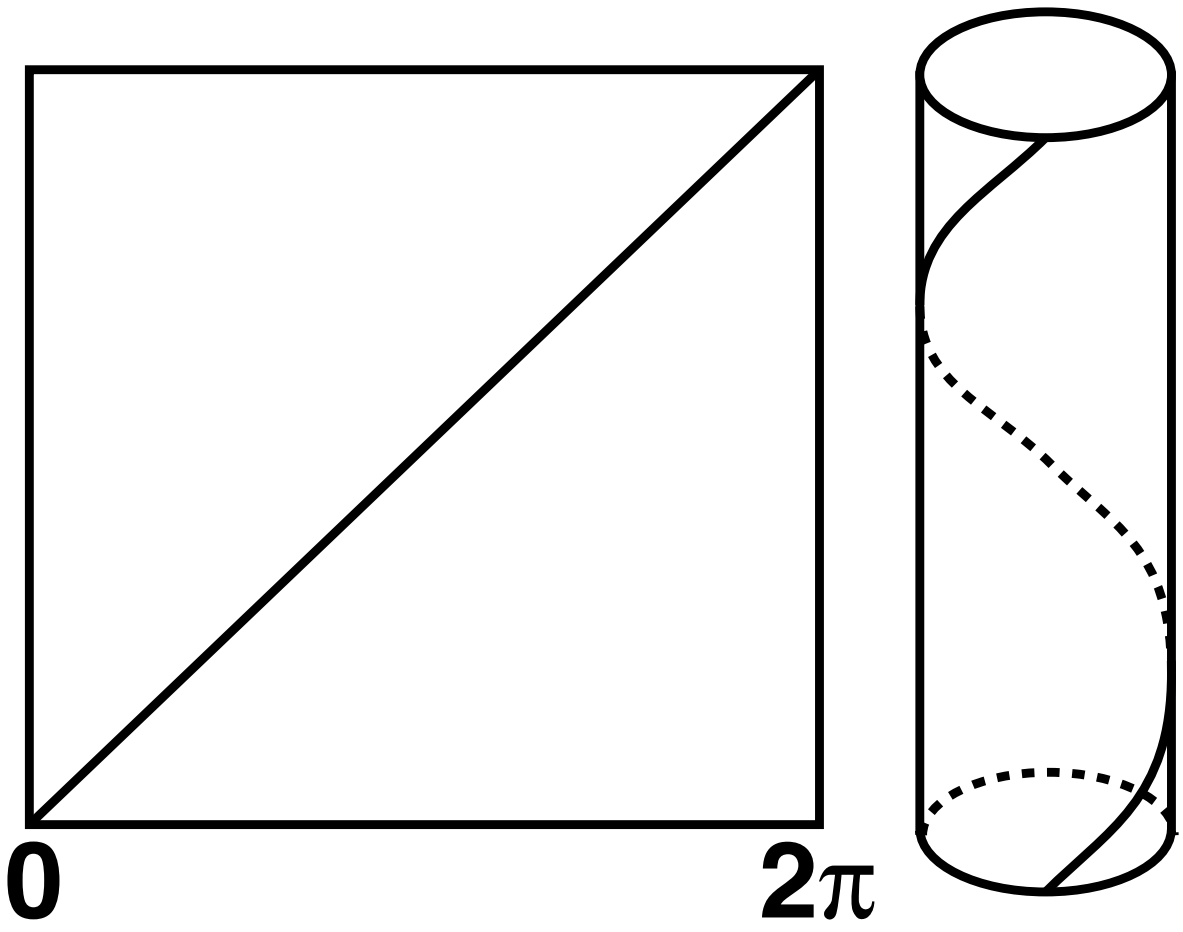
  Indexing is difficult

Microtubule is *not* a helical object

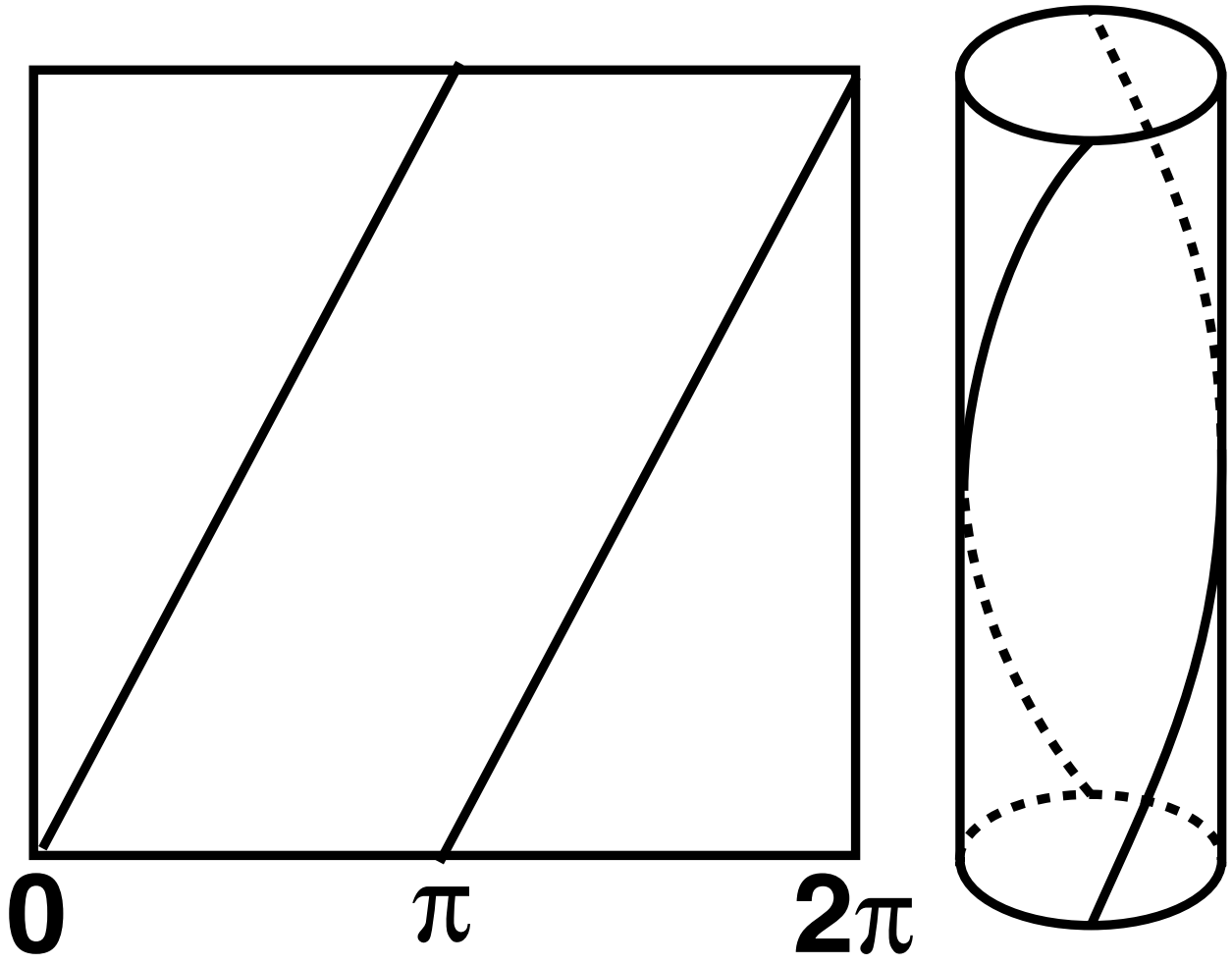


(n,l) can be non-integer!

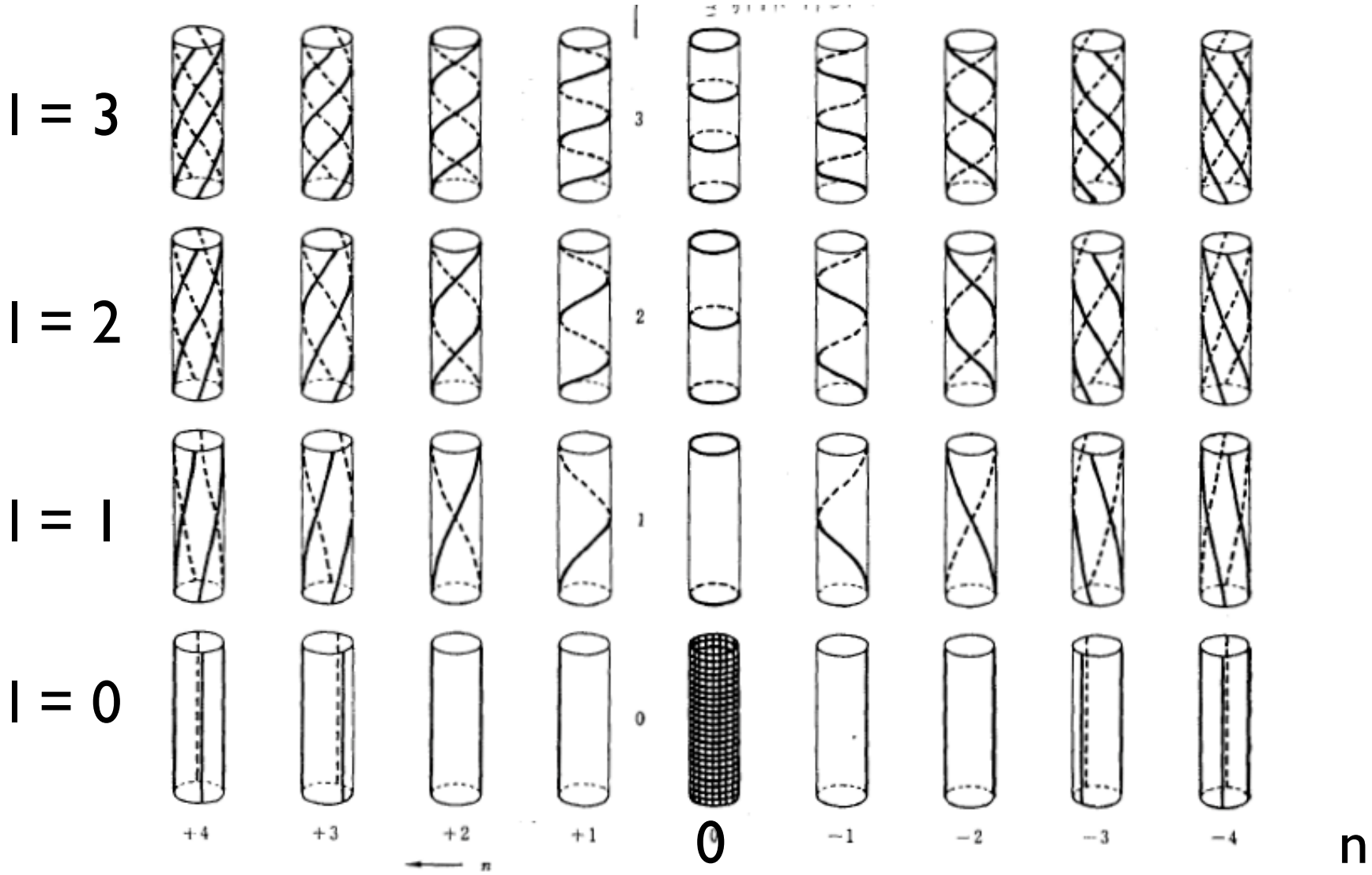
(n=1, l=1)-helix



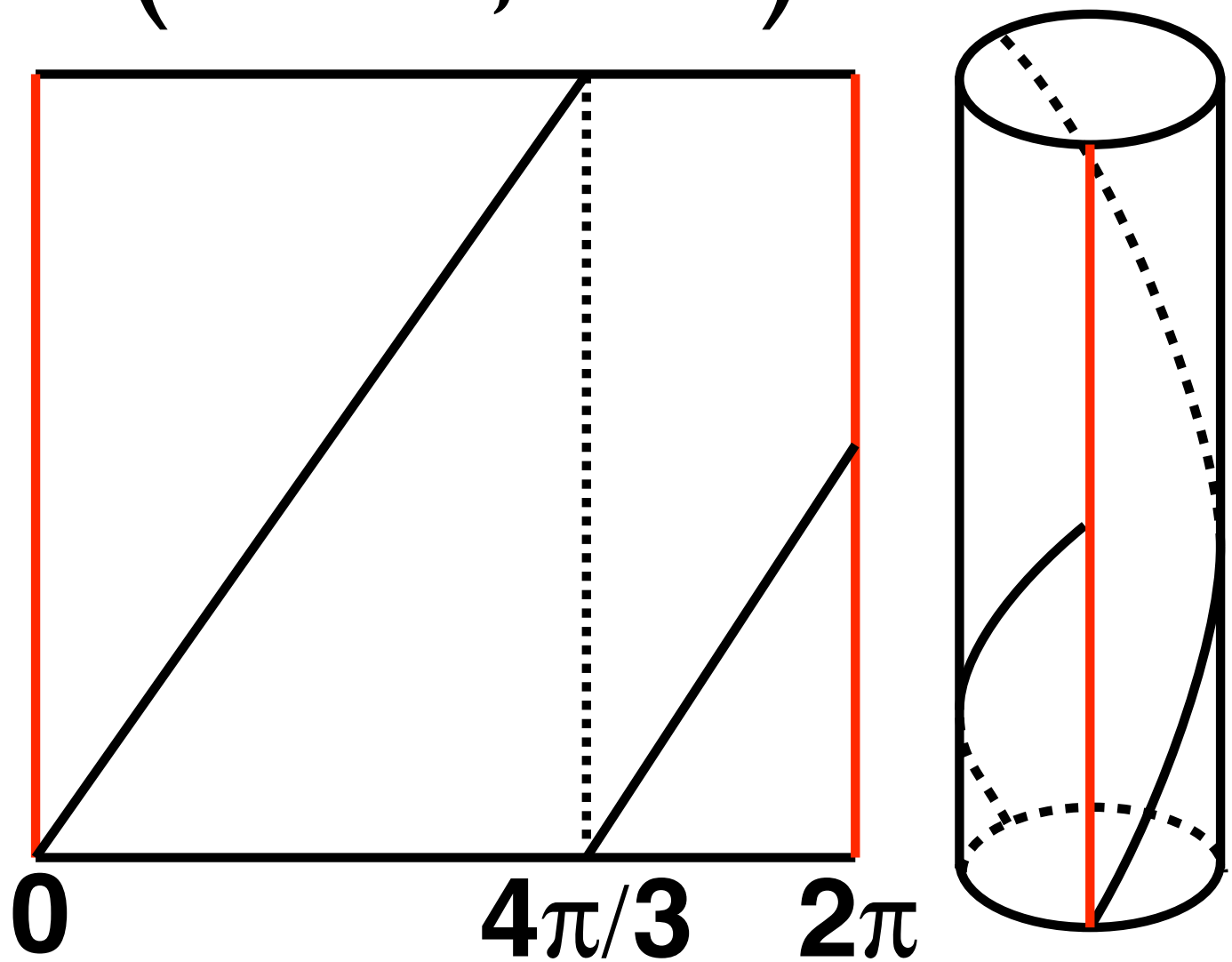
(n=2, l=1)-helix

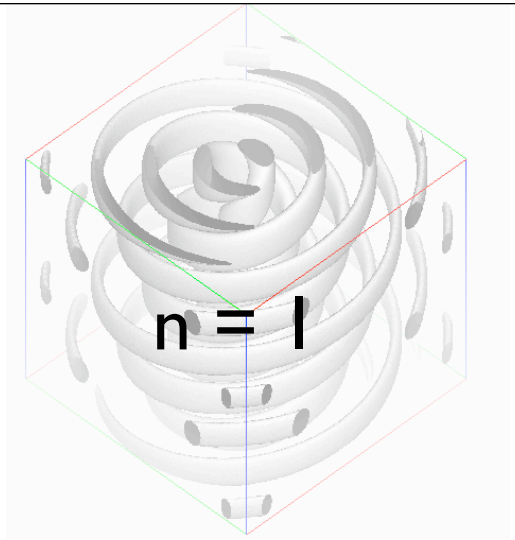


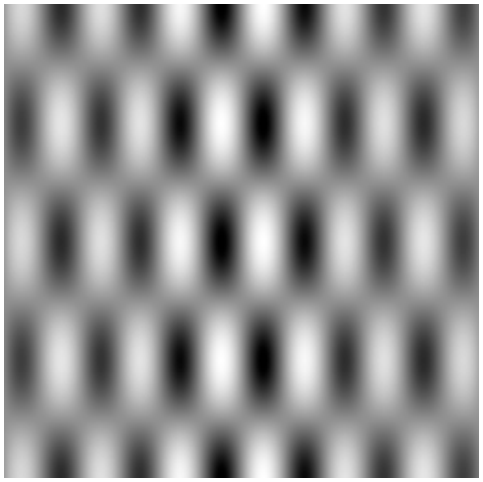
(n,l) helices

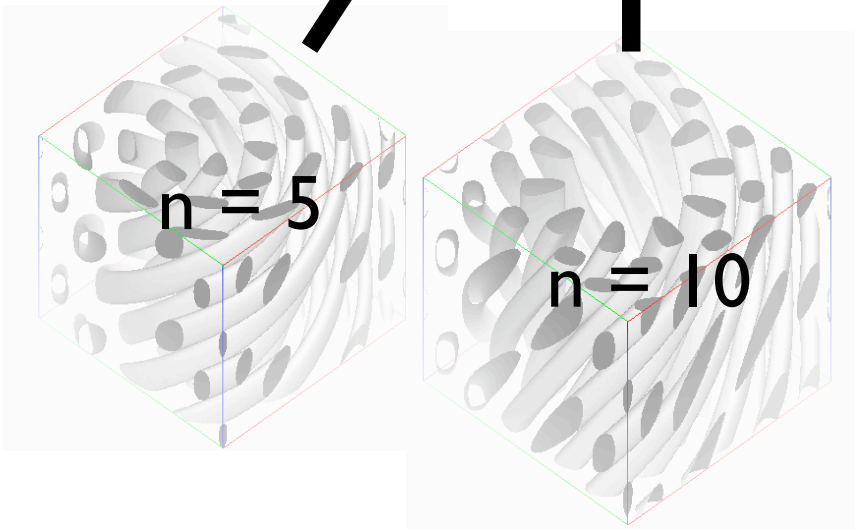


(n=1.5, l=1)-helix







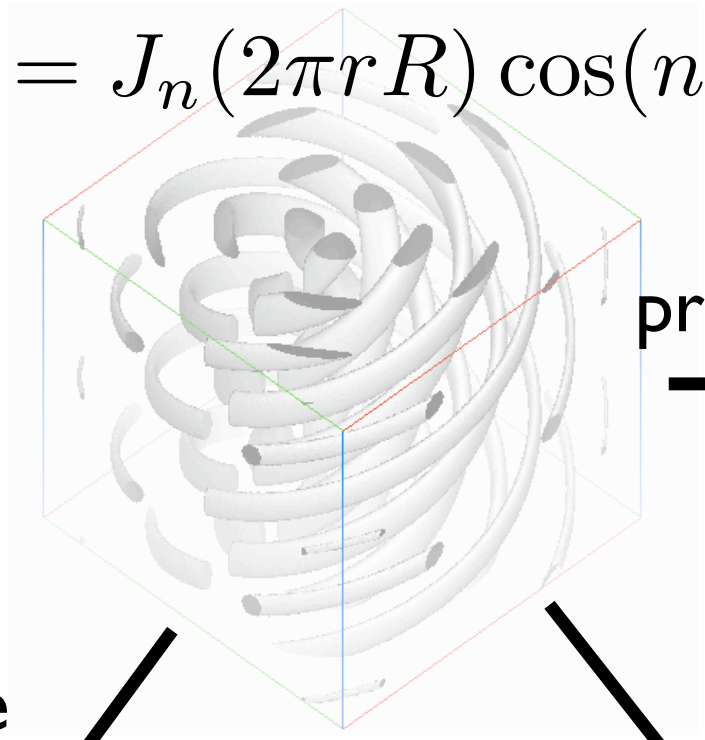


$n = 2.5?$

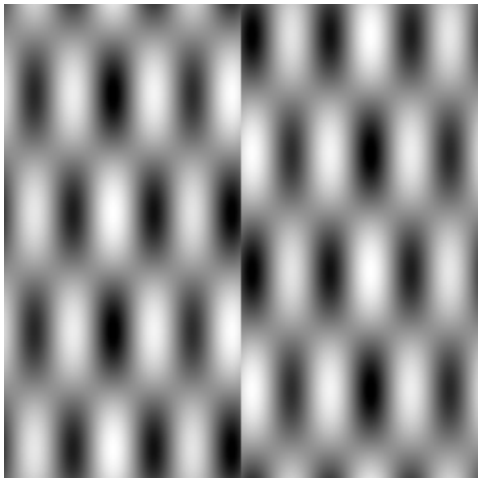
$$R = 6,\ l = 2$$

$$\rho(r, \phi, z) = J_n(2\pi r R)\cos(n\phi - 2\pi l z/c)$$

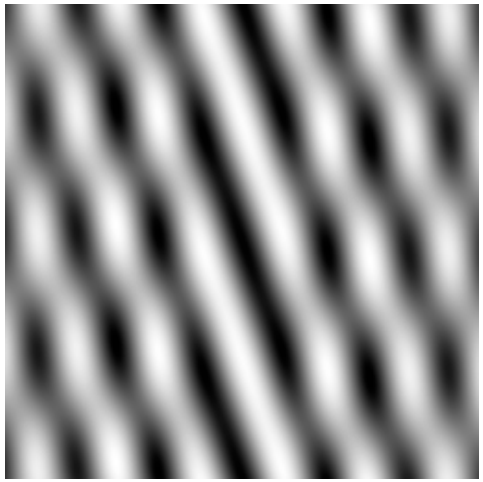
$$\rho(r, \phi, z) = J_n(2\pi r R)\cos(n\phi - 2\pi l z/c)$$



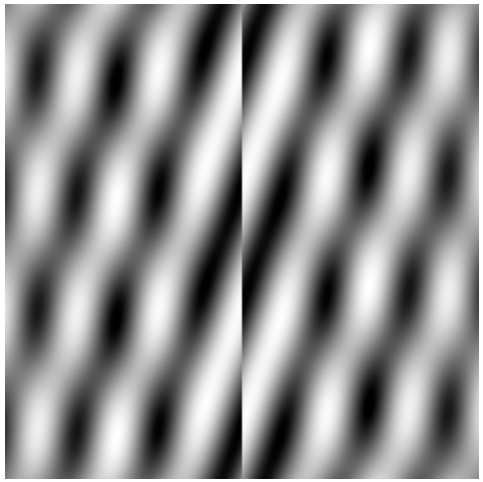
projection



seam free side



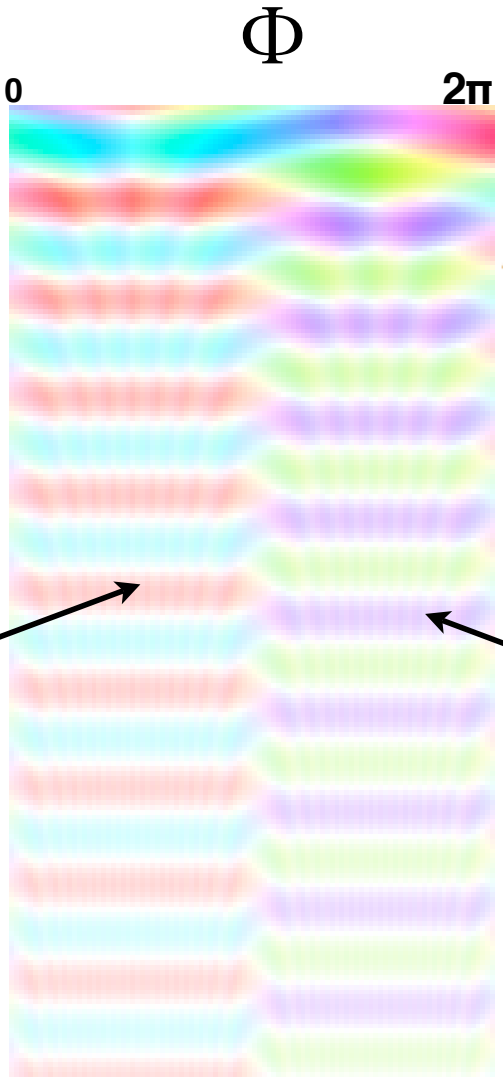
seam side



"M"(asa Kikkawa) Function

$$M_\nu(x,\Phi) = \frac{1}{2\pi}\int_{-\Phi-\frac{\pi}{2}}^{\frac{3\pi}{2}-\Phi} e^{-ix\sin\varphi' + i\nu\varphi'}\, d\varphi'.$$

M-function



$$M_\nu(x,\Phi) = \frac{1}{2\pi}\int_{-\Phi-\frac{\pi}{2}}^{\frac{3\pi}{2}-\Phi} e^{-ix\sin\varphi'+i\nu\varphi'}\,d\varphi'.$$

color saturation = amplitude

color hue = phase

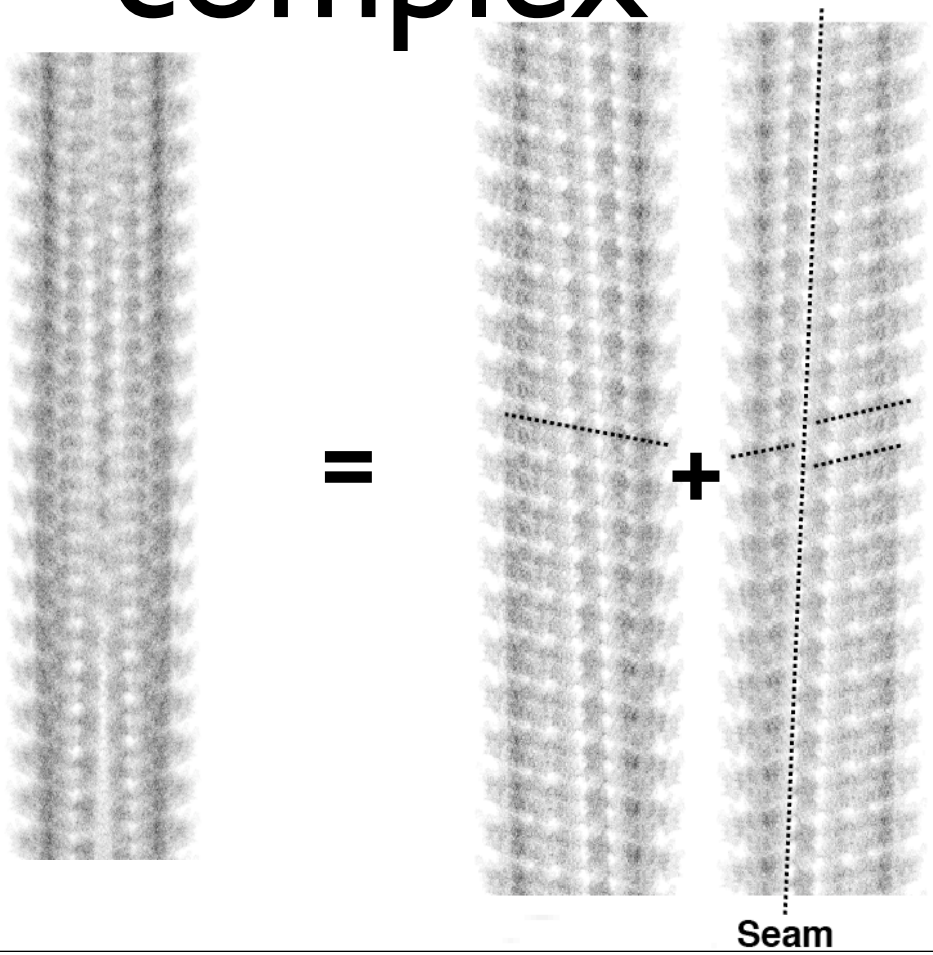
Seam free ~ Jn(x)

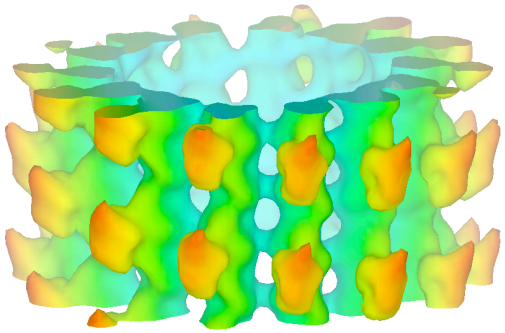
Seam side ~ Yn(x)

M-function ≈
Bessel function

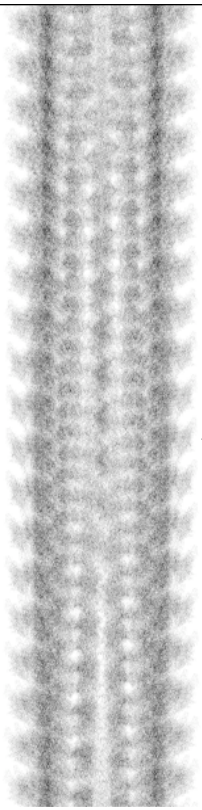
Application to a model image

A model image of the kinesin-microtubule complex



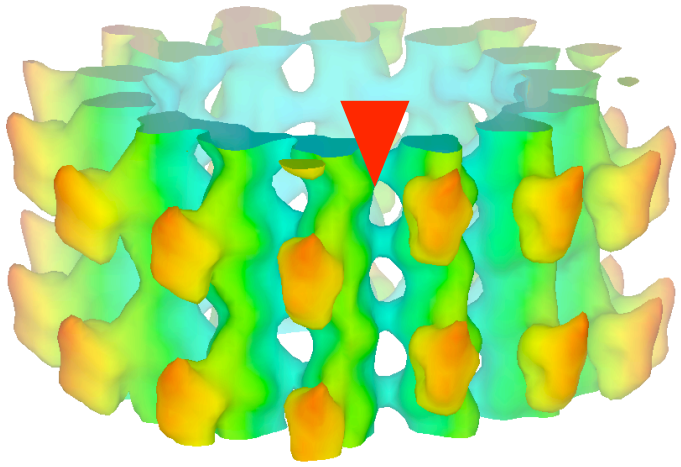


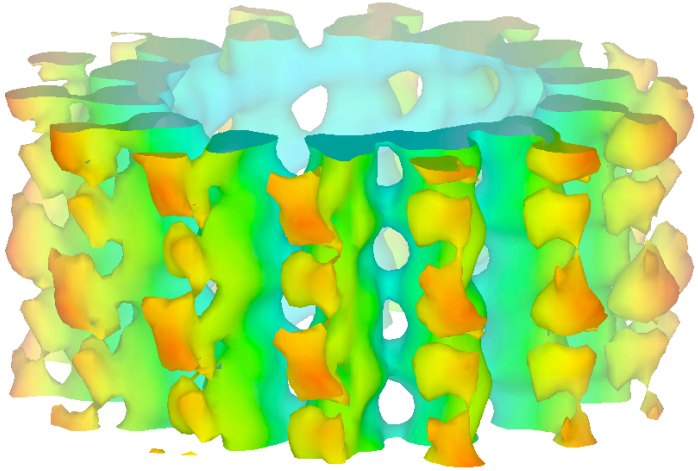
conventional
helical reconstruction



near

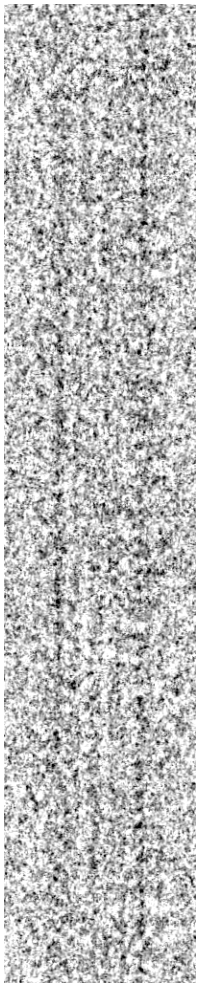
far (seam side)



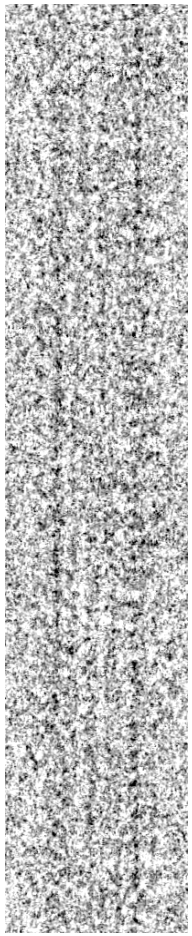


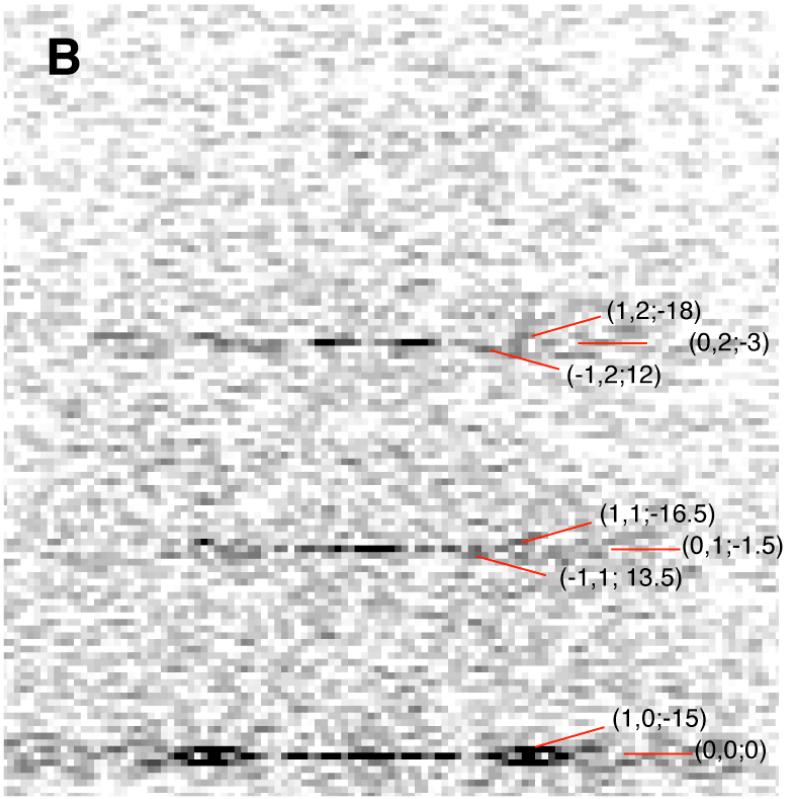
Application to experimental images

Application to the kinesin-microtubule complex

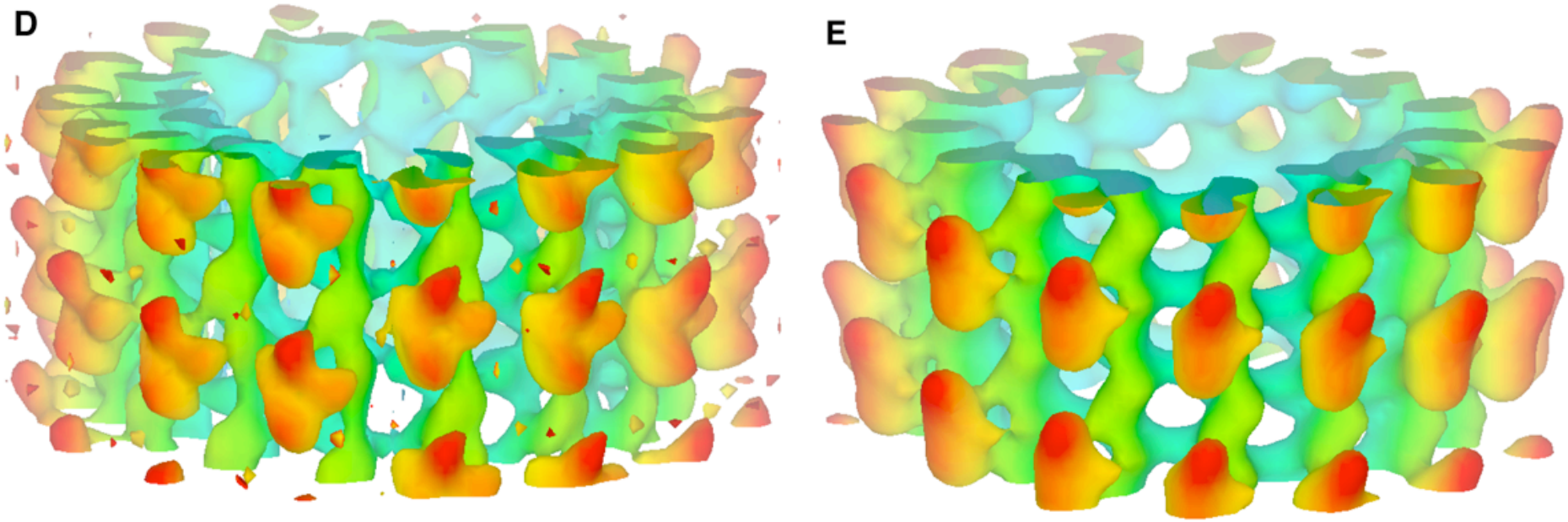


Experimental Image of Kinesin-Microtubule complex





3D reconstruction



asymmetric
from single image
(~150 subunit)

conventional

Applications of the Asymmetric Helical Reconstruction

Applications

- High resolution studies of motor-microtubule complex
- Other less ordered helical structures, such as bacterial flagella and TMV
- Implementation of AHR is being done as a part of Ruby-Helix.

Acknowledgments

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M. Kikkawa

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