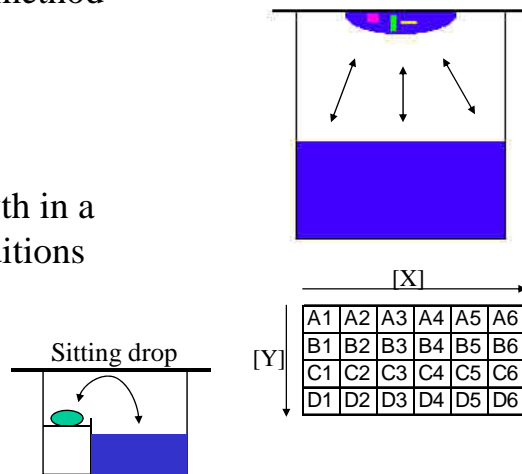
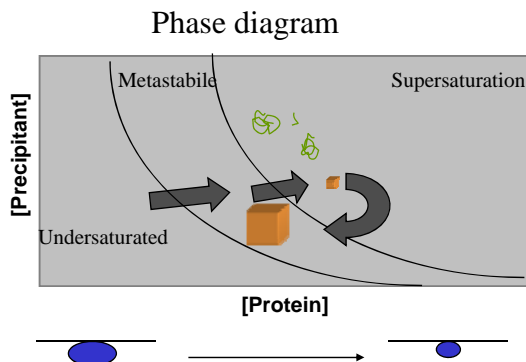


Growing Protein Crystals: by Vapor Diffusion

- Hanging drop method
 - Fast
 - Simple
 - Inexpensive
- ↳ Test for growth in a variety of conditions



Crystal Nucleation & Growth

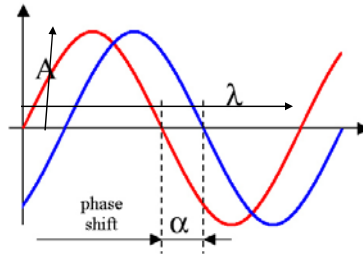


Basic concept:

1. Concentrate solution enough so nucleation occurs in only a few cases
2. Initial growth pulls some protein out of solution
3. Reducing [protein] back into metastable range
4. Grow only a few large crystals

Diffraction

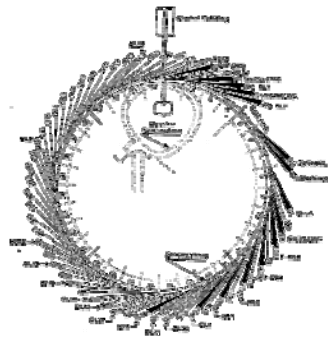
- Light Microscope 400-700 nm
 - uses lense to focus diffracted light
 - into an image
- X-ray crystallography uses X-rays of 0.154 nm (1.54 Å)
 - no x-ray lense available
- Need to know wavelength, for Cu sources its 1.54 Å
- Need to measure Amplitude of Diffracted Reflections
 - Intensity is proportional to A^2
- Need to estimate the relative phase



Synchrotron X-ray Sources

- ❑ Many Synchrotrons world wide
<http://www.esrf.eu/UsersAndScience/Links/Synchrotrons/>

- ❑ A look at the one in Grenoble France



- ❑ Here's a typical beamline at a synchrotron
<http://www.esrf.eu/AboutUs/GuidedTour/Beamline>

X-ray Equipment in Delaware



RU-H3R
X-ray generator

Area Detector



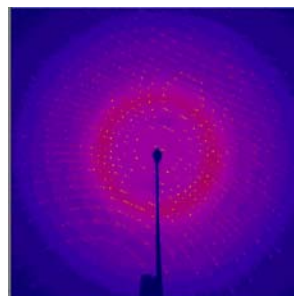
Close-up of Cryo Crystal



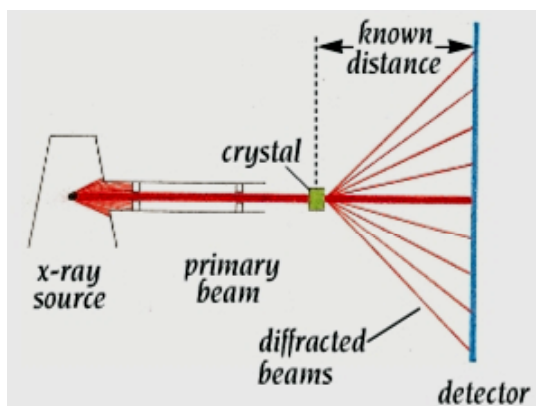
Overview of X-ray Experiment



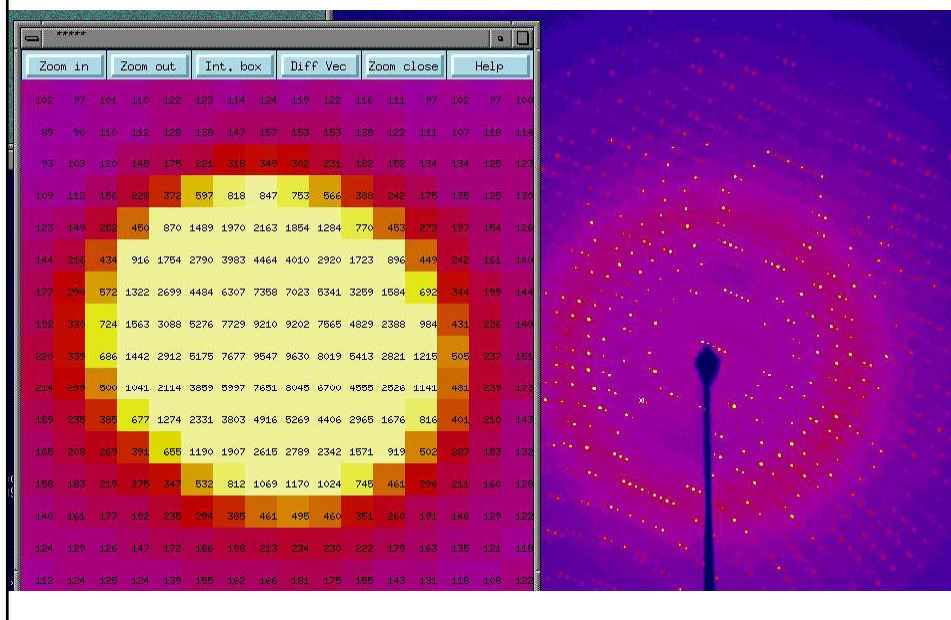
Protein crystal in a loop



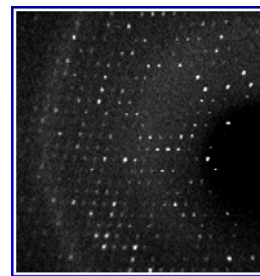
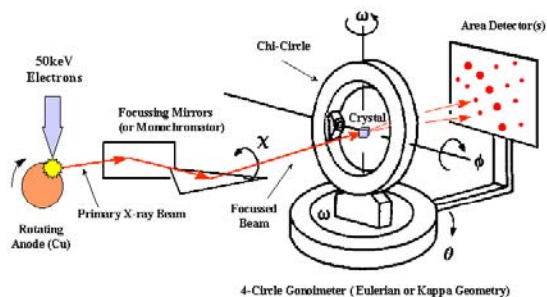
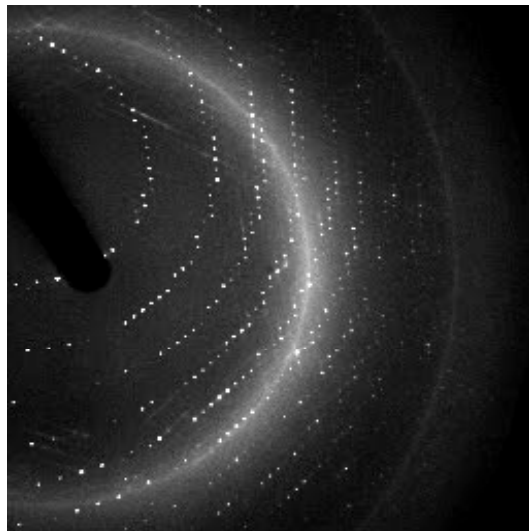
diffraction pattern



Zoom into a single reflection



Movie of a Data Collection



Each reflection has
an hkl index
and a measured intensity

8 reflections selected from a 30,000
reflection data set shown to the right

h	k	l	I
2	0	3	1483.6
3	-1	-3	19999.9
3	-1	-2	6729.6
3	-1	-1	30067.1
3	-1	1	8227.0
3	-1	2	29901.5
3	-1	3	24487.5
3	-1	4	502.1

Each Reflection Index Defines a Set of Parallel Planes that Slice Through the Crystal

Miller indices hkl

Example:
draw parallel planes
that define the single
reflection hkl 2 3 4

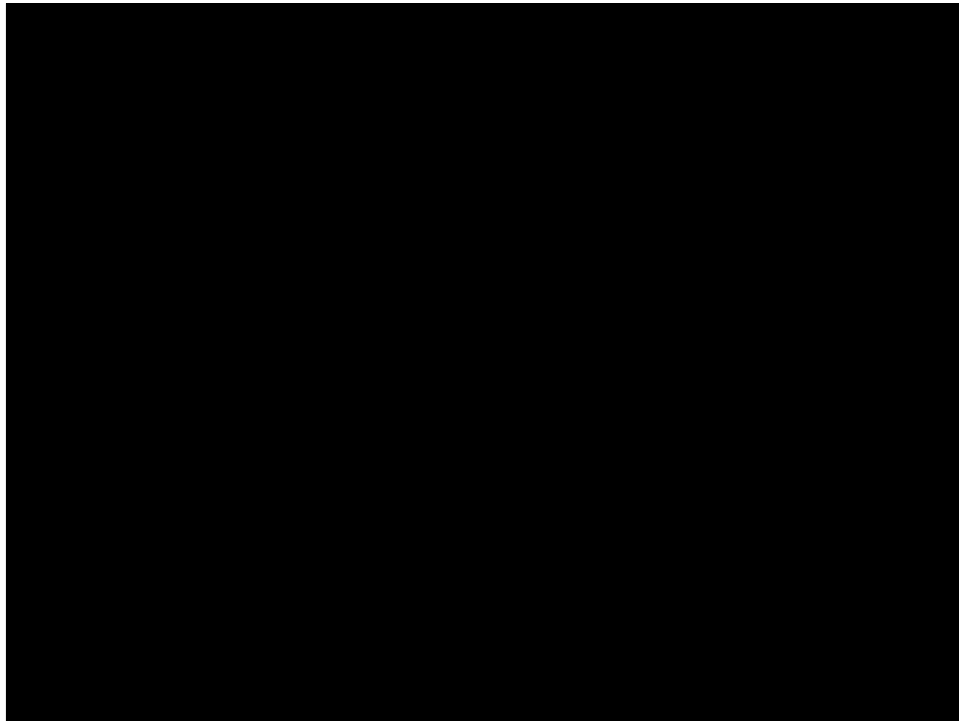
Reciprocal Space	Real Space
h	a
k	b
l	c

Reciprocal space has all reflections out to diffraction limit

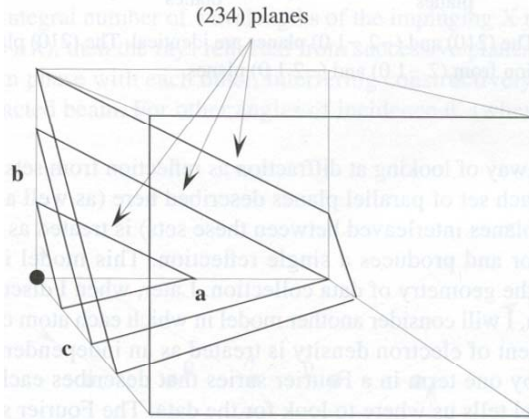
h 0 to 30

k 0 to 35 just a hypothetical example

l 0 to 43



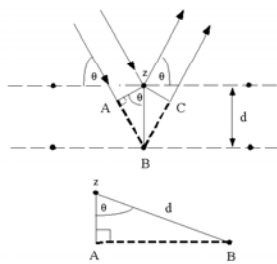
Each reflection defines a set of parallel planes that slice through the crystal



Reciprocal space has all reflections out to diffraction limit
 h 0 to 30
 k 0 to 35 just a hypothetical example
 l 0 to 43

The Diffraction Condition

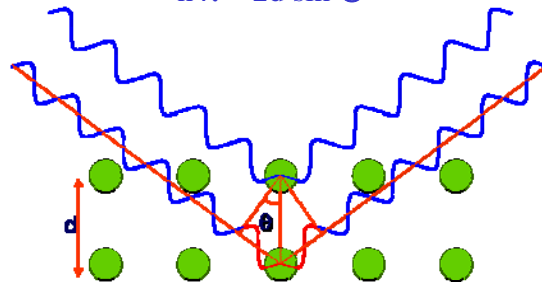
☐ Reflections are the result of constructive interference

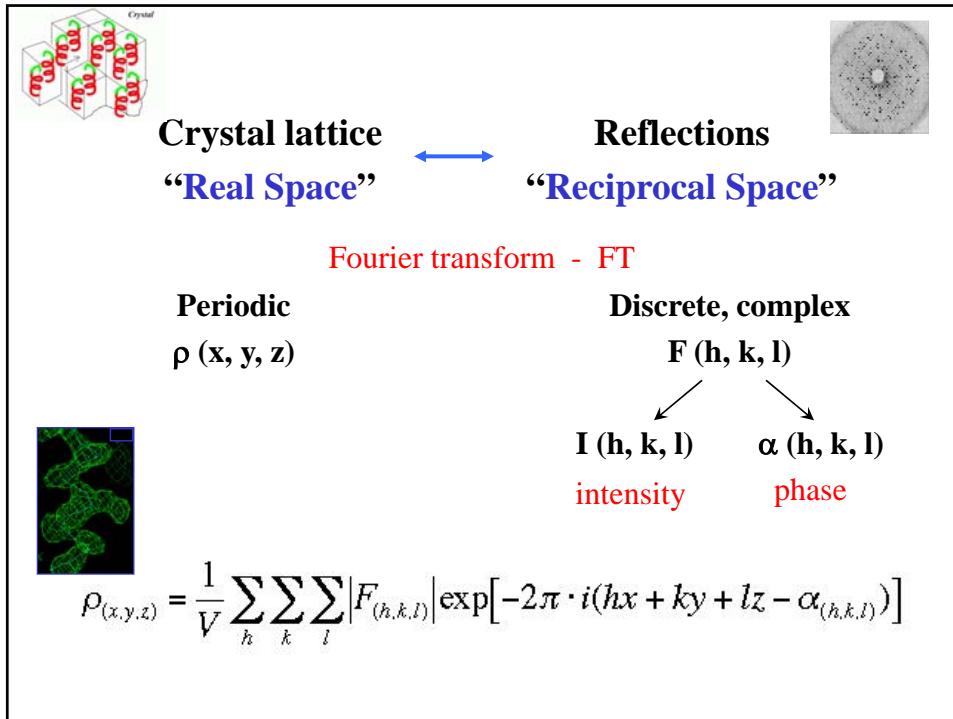


$$\sin \Theta = AB / d$$

$$d \sin \Theta = AB$$

$$n \lambda = 2d \sin \Theta$$



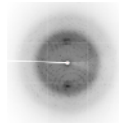


Web resources

- Xrayview – program to view Bragg’s Law in reciprocal space
<http://phillips-lab.biochem.wisc.edu/xrayviewuse.html>
- Crystallography 101 – by Bernhard Rupp
<http://www.ruppweb.org/Xray/101index.html>
- Crystallography Made Crystal Clear – Gale Rhodes
<http://www.usm.maine.edu/~rhodes/CMCC/>

2nd half of class – meet in 312 Drake Hall

- Introduce cryo protectant to your lysozyme crystals
- Pick up and flash freeze crystals under liquid nitrogen
- Collect X-ray diffraction data



- Look at protein models and electron density maps on a graphics terminal

