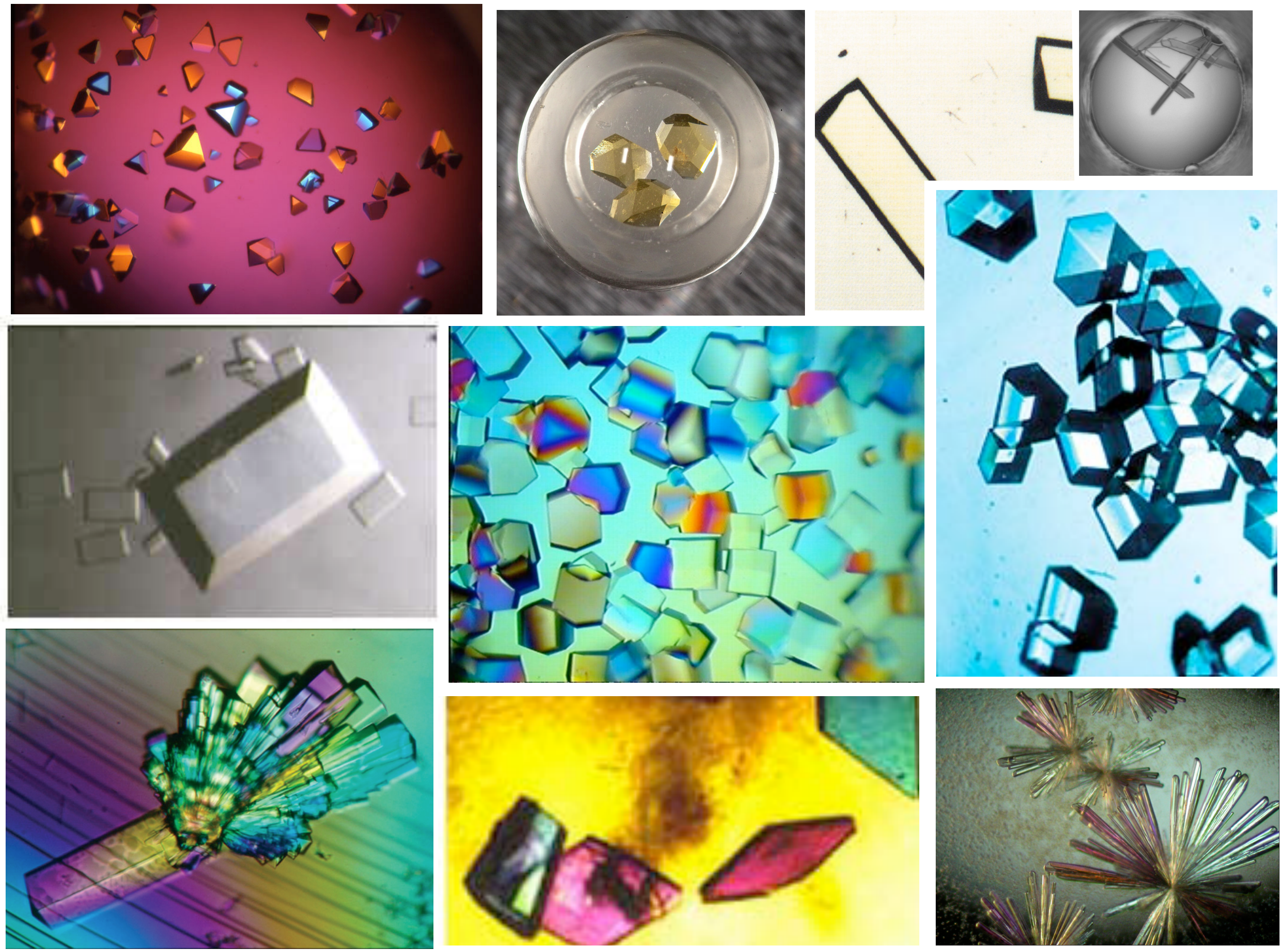
Protein Structure Determination '20

Lecture 3:

Growing crystals.

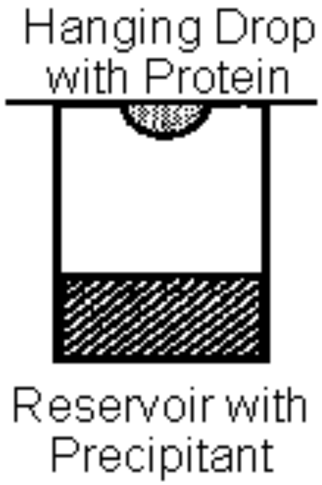
Bragg's Law of Diffraction.

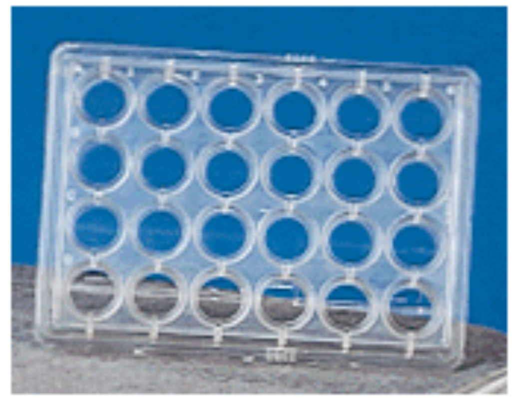
protein crystals



The color you see is "**birefringence**", the wavelength-dependent rotation of polarized light.

vapor diffusion method





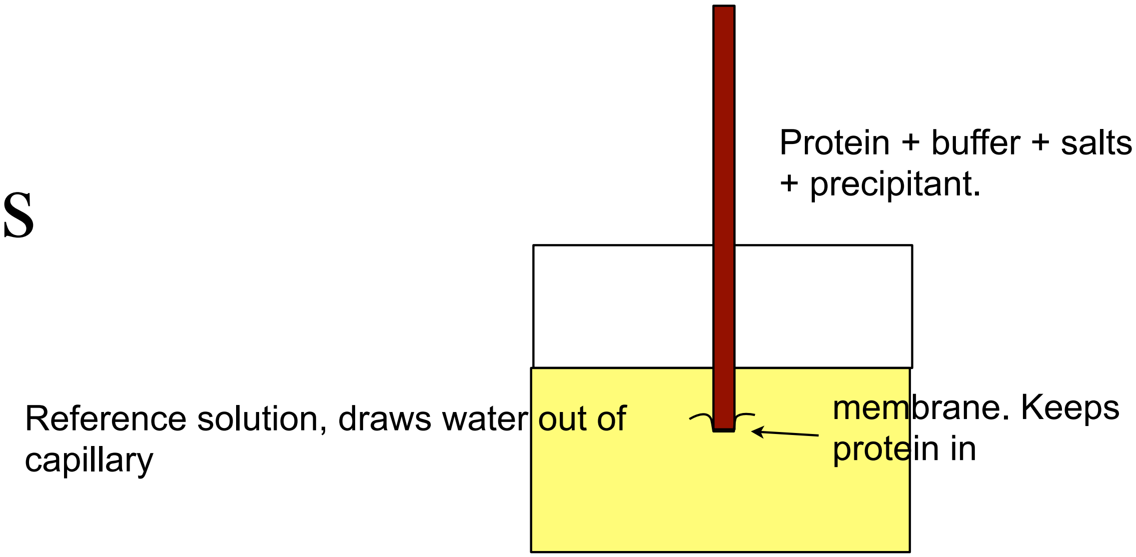
a Linbro plate

- Most popular.
- "Sitting drop" or "Hanging drop".
- Volatiles (i.e. water) evaporate from one surface (drop) and condense on the other (reservoir).
- Drop has *higher* water concentration than reservoir, so drop slowly shrinks.
- Easier to access and mount crystals than the batch-under-oil method.

Other ways to grow crystals

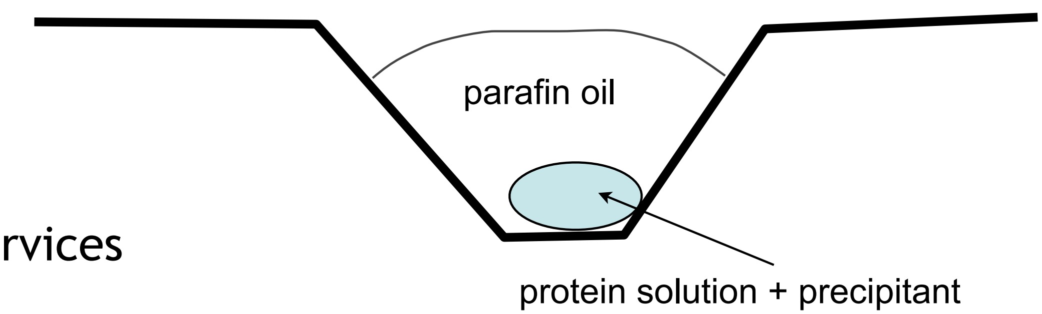
Microdialysis

- Crystals grown in situ
- Not for cryo



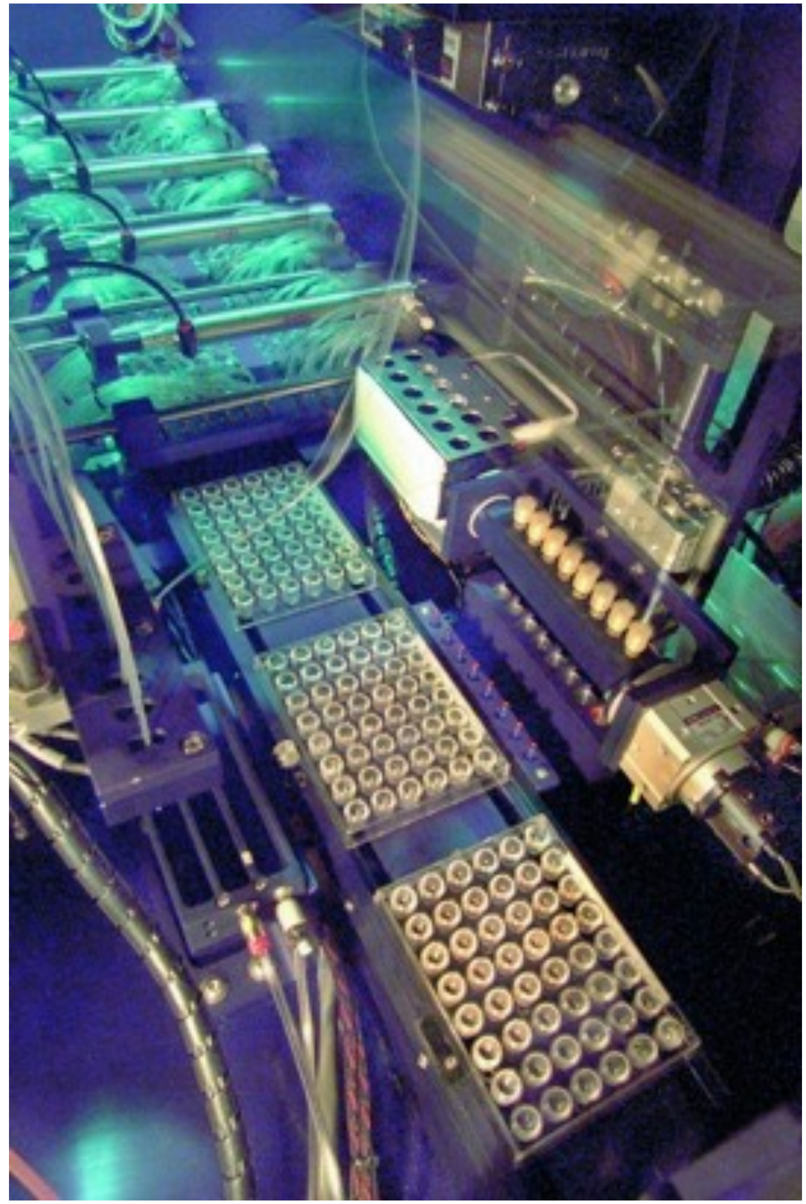
Microbatch under oil

- Amenable to high-throughput
- No “reference solution”
- Easy to access and mount crystals
- Used by high throughput crystallization services



Crystallization robot

High-throughput crystallography labs use pipeting robots to explore thousands of “conditions”. Each condition is a formulation of the **crystal drop** and the **reservoir solution**.



Conditions can have different:

•protein concentration

•pH

•precipitant, precipitant concentration

•detergents

•organic co-solvents

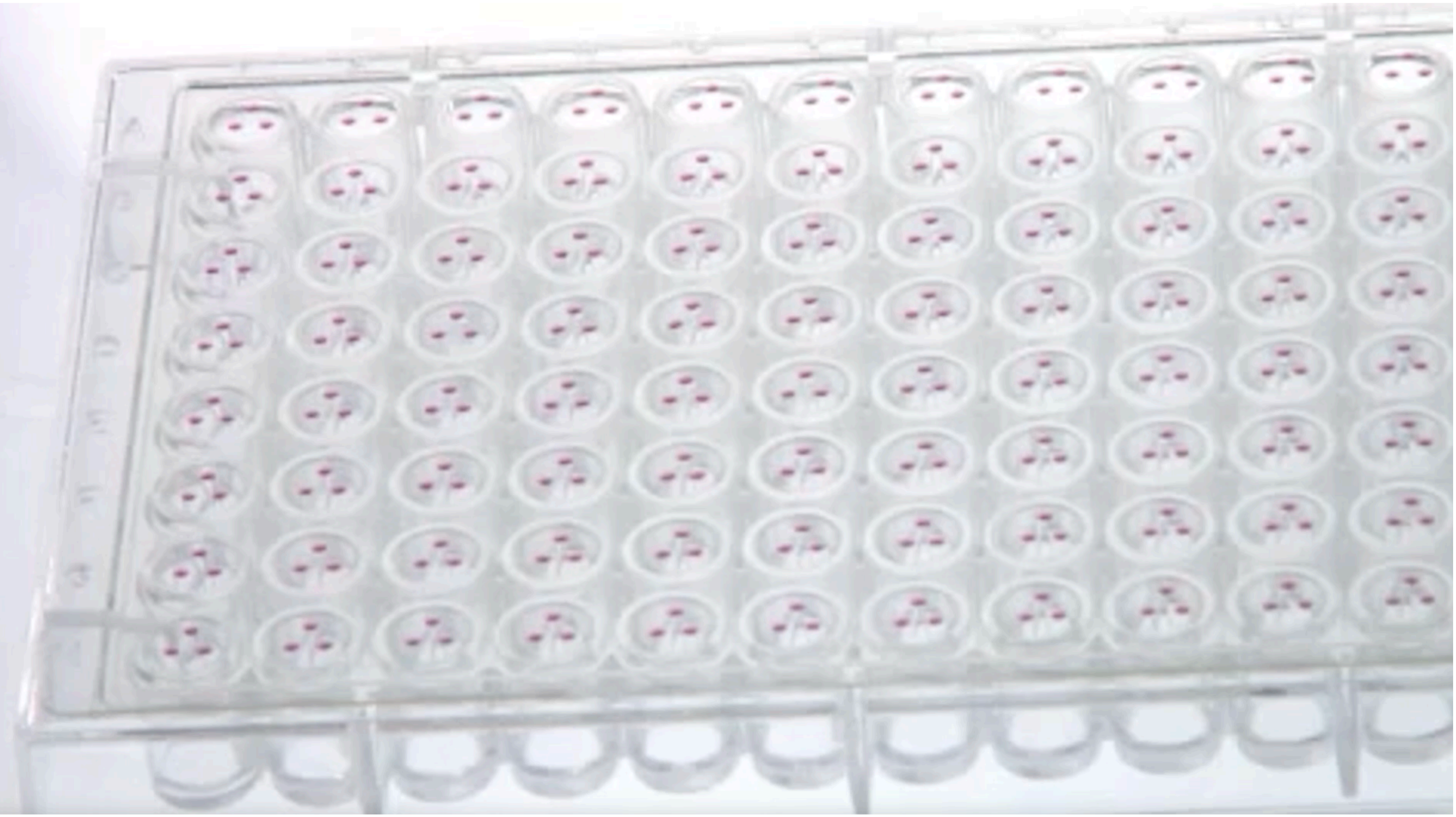
•metal ions

•ligands

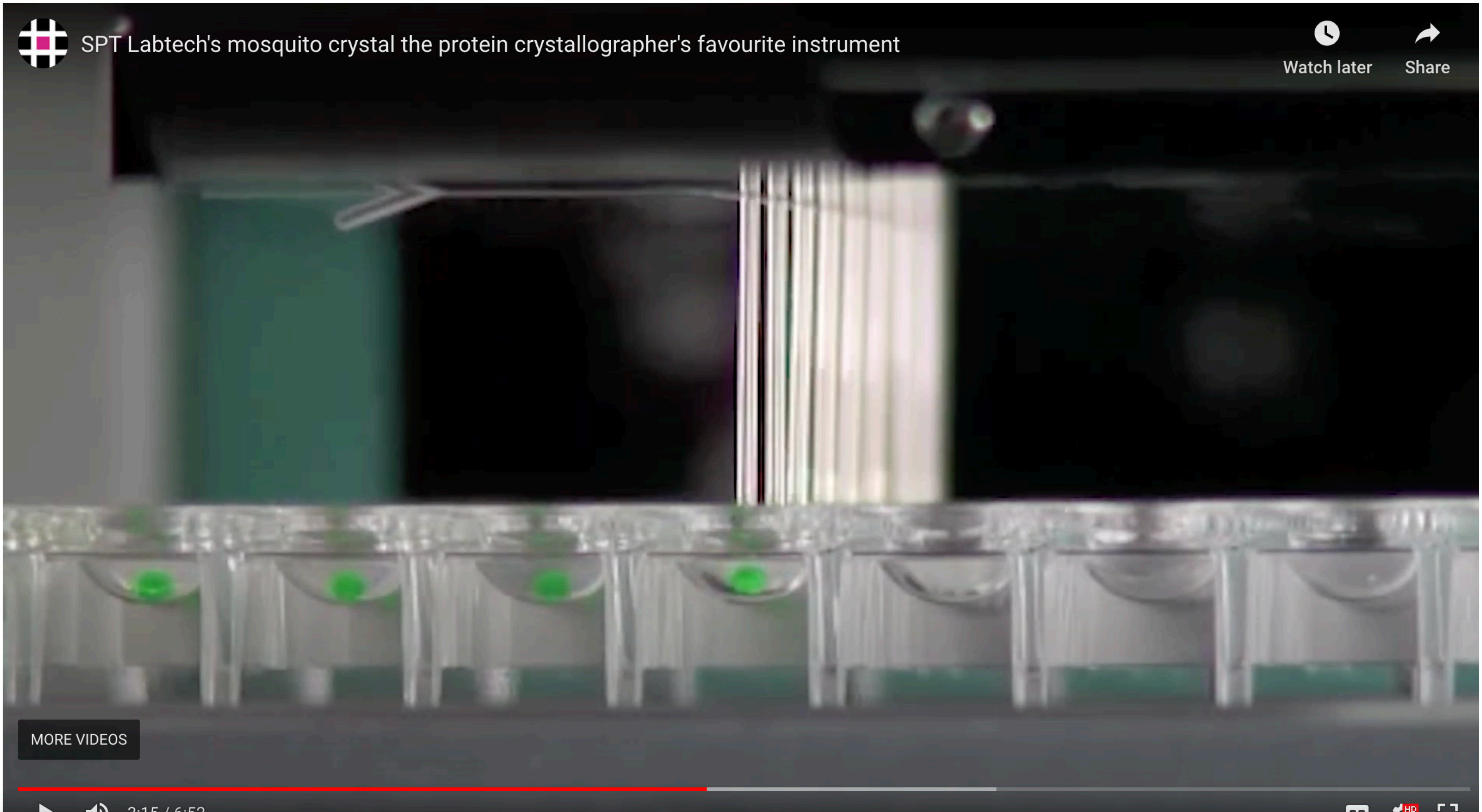
•concentration gradient

The **Hauptman-Woodward Institute** in Buffalo NY will screen 1500 precipitants for under $400.

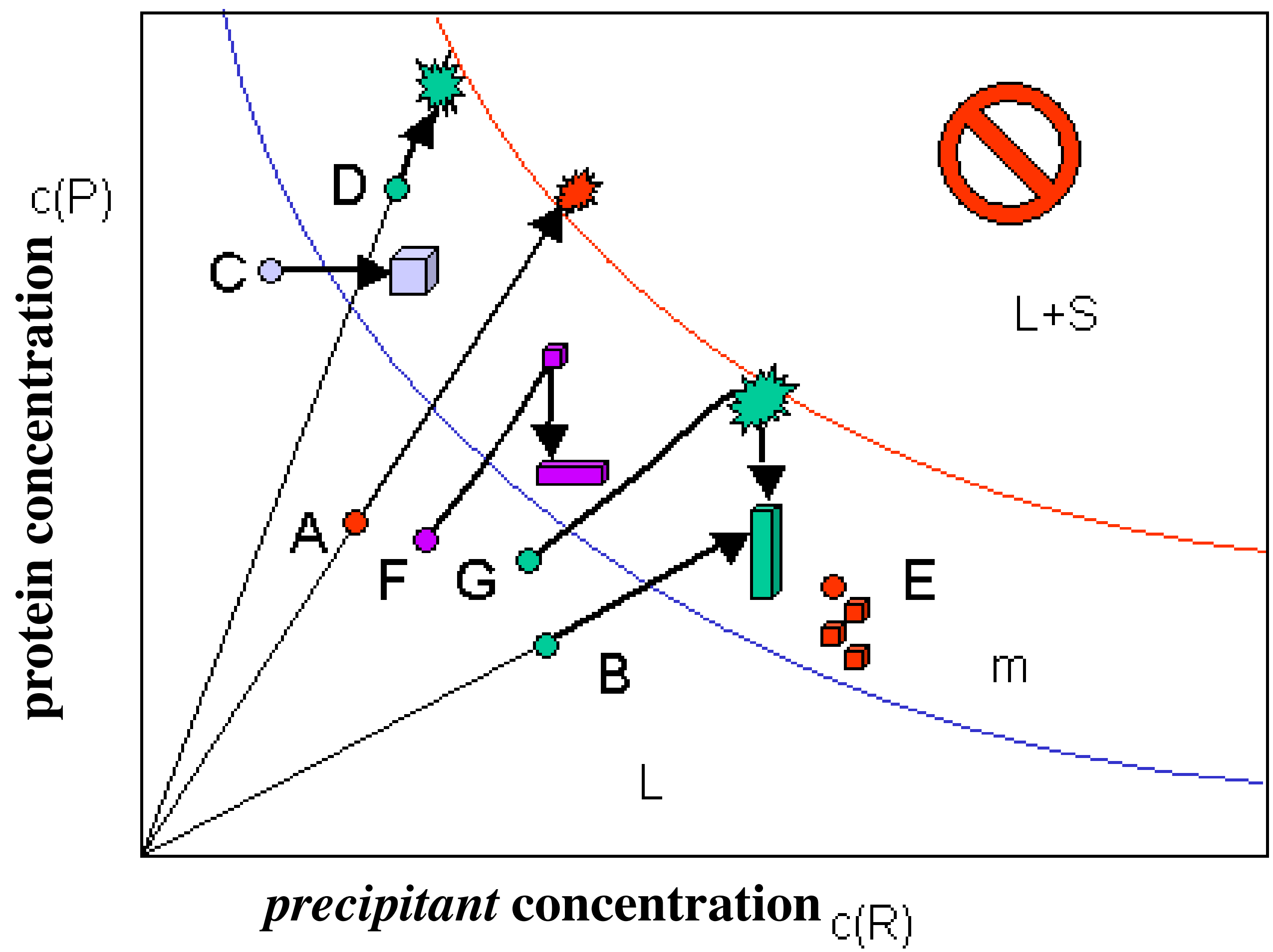
microbatch under oil



robotic liquid handler



Saturation and supersaturation



Arrows indicate different diffusion experients.

A,B,D,F,G. Vapor diffusion.

E. Bulk

C. Microdialysis

L=liquid
S=solid
m=metastable state (supersaturated)

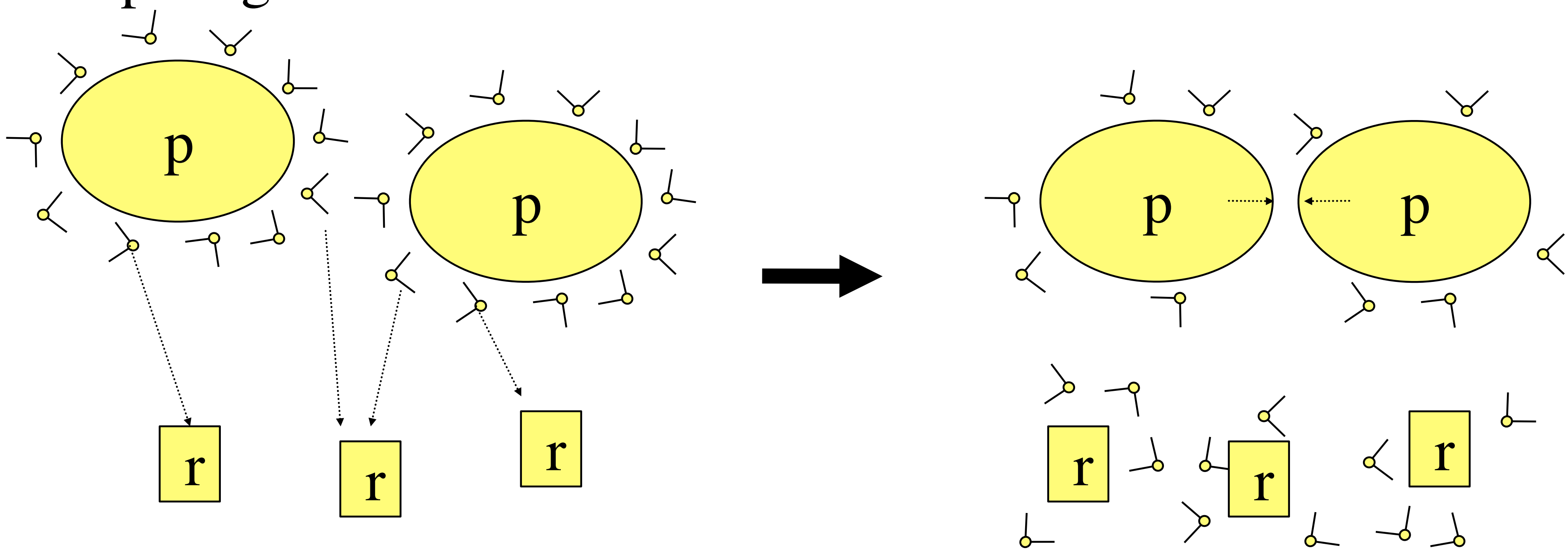
blue line = saturation of protein

red line = supersaturation limit

Crystal growth occurs between these two limits. Above the supersaturation limit, proteins form only disordered precipitate.

precipitants attract water

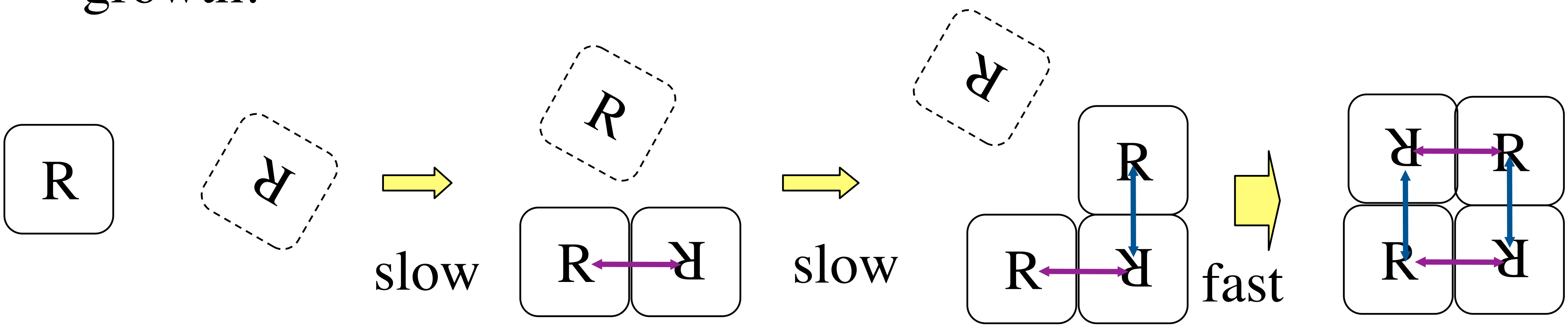
A precipitant (r) causes proteins (p) to stick to each other by competing for solvent.



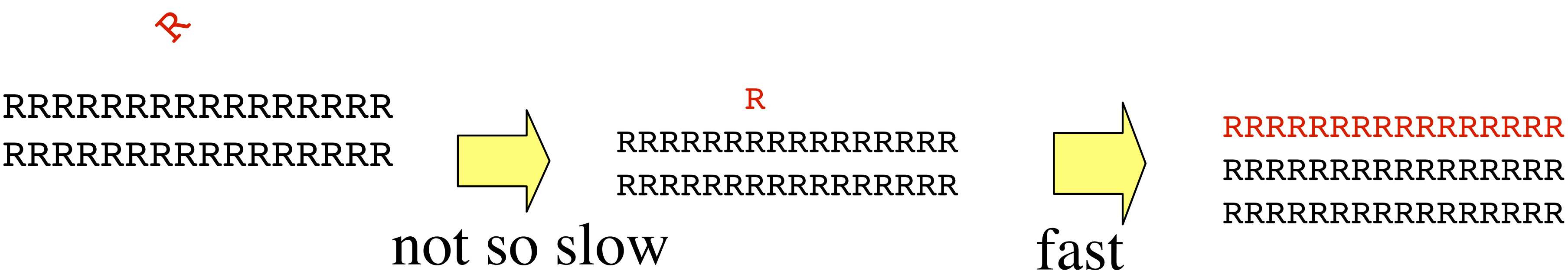
r = EtOH, $(NH_4)_2SO_4$, methylpentanediol, polyethylene glycol, etc

Crystal nucleation

Nucleation takes higher concentration than crystal growth.

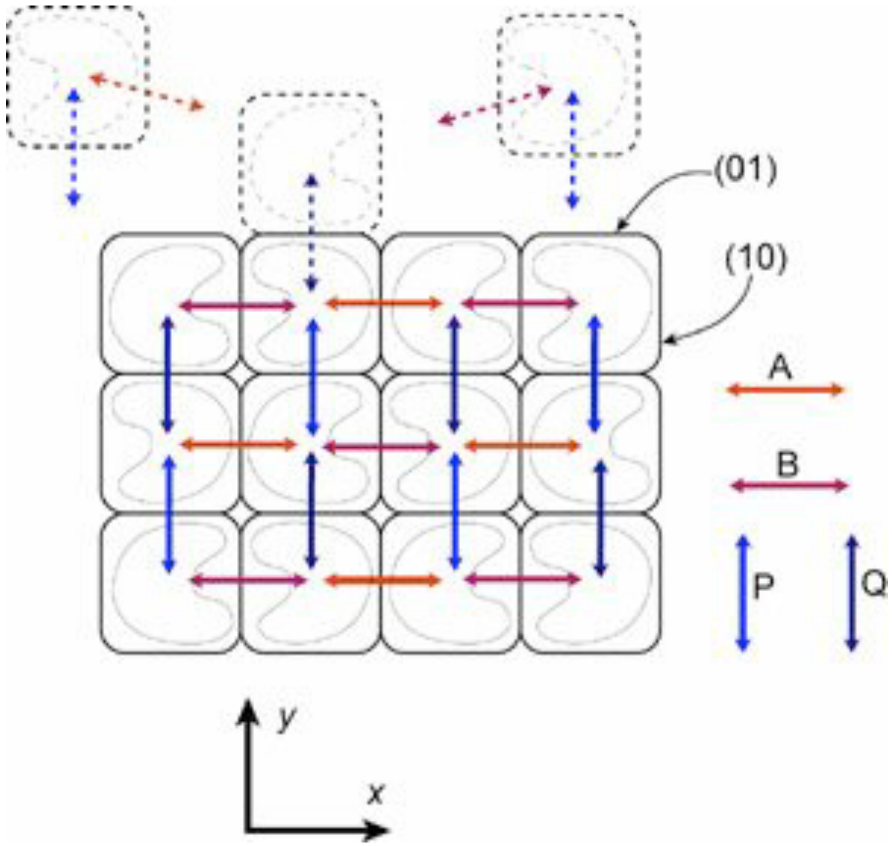


After nucleation, the large size of a face makes the weak bond more likely.



Crystal growth

Determinants of the crystal dimensions

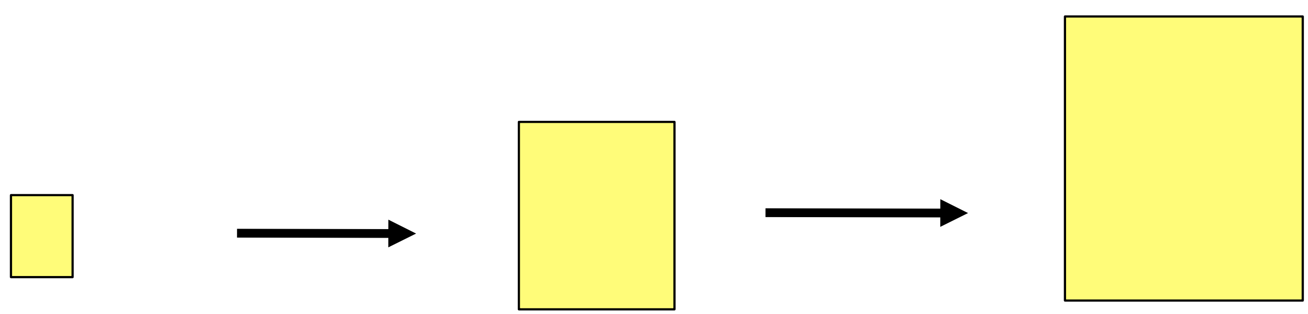


Bonds A,B are stronger than P,Q. Dimensions of crystal at equilibrium are proportional.

More on Periodic Bond Chain theory: http://www.che.utoledo.edu/nadarajah/webpages/PBC.htm

Crystal morphology

Growth in weak-bond directions increases proportional to the size of the face (collision theory).



Weak bonds in Z favor growth in XY, forming “plate” xtal.

Growing cross-section in XY favors growth in Z.

Ratio of cross sections is inverse to ratio of bond strength.

The end of crystal growth

Crystal growth depletes the surrounding solution of protein,

....slowing growth.

...preventing nucleations close to a growing crystal

...concentrating impurities on the surface of the crystal

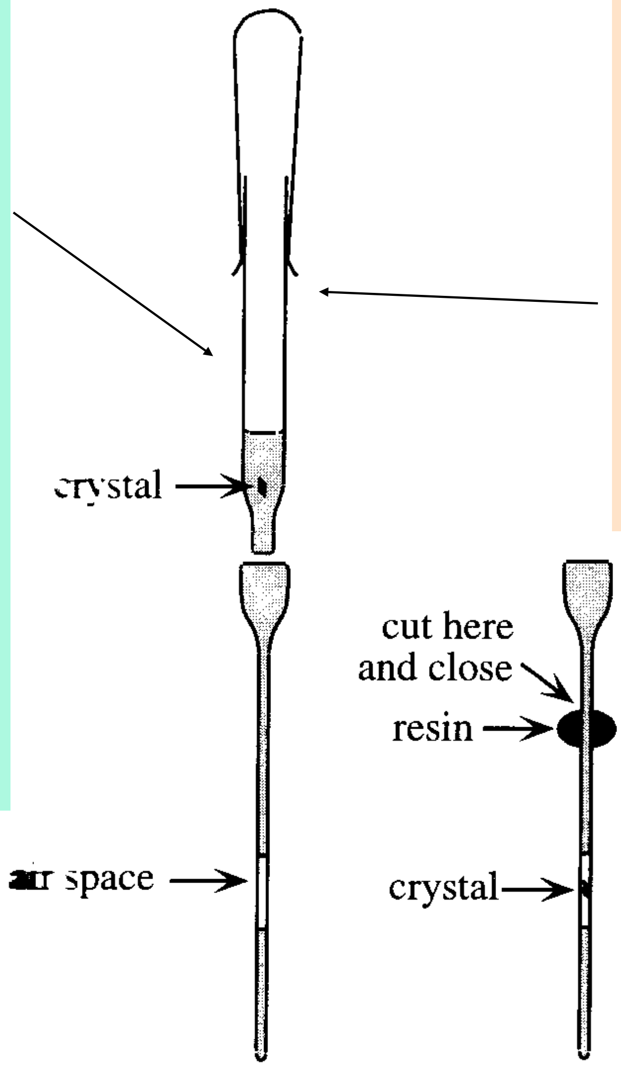
Cobalt impurities in SiO_2 (amethyst) are concentrated in the part of the crystal that formed <u>last</u> (the tip).

mounting crystals the old way

Thin-walled glass capillaries (<1mm in diameter) are filled with “**mother liquor**”(*the fluid in which the crystal was grown*) and a crystal is carefully dropped in. The mother liquor is removed using filter paper cut to fine strips. The crystal sticks to the glass, immobilized.

The xtal remains in vapor diffusion contact with the mother liquor. If not it will dryout and crack.

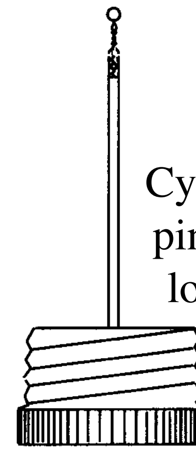
Protein crystals are extremely fragile!!! They may break upon sudden contact with a solid object. Tiny pipets are used to pull crystals from drops.



The new way. Freeze!

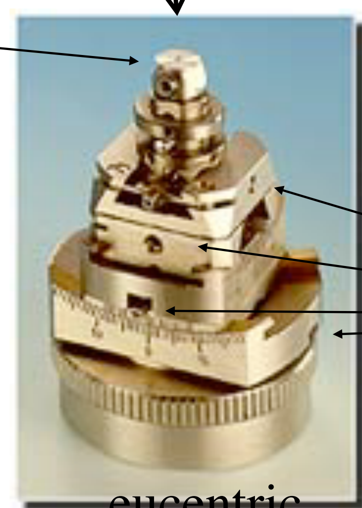
Eliminates X-ray damage to crystal.
Crystals do not "decay" during data collection.

Crystals, mounted on loops, are dipped in liquid N_2 at –70°C.



Cyro-cap. Base, pin, and nylon-loop. 1mm or smaller.

pin mount. Swap for magnetic baseplate for cryo caps.

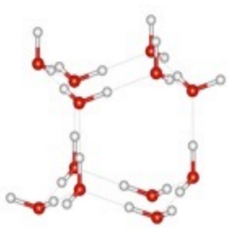


eucentric goniometer head

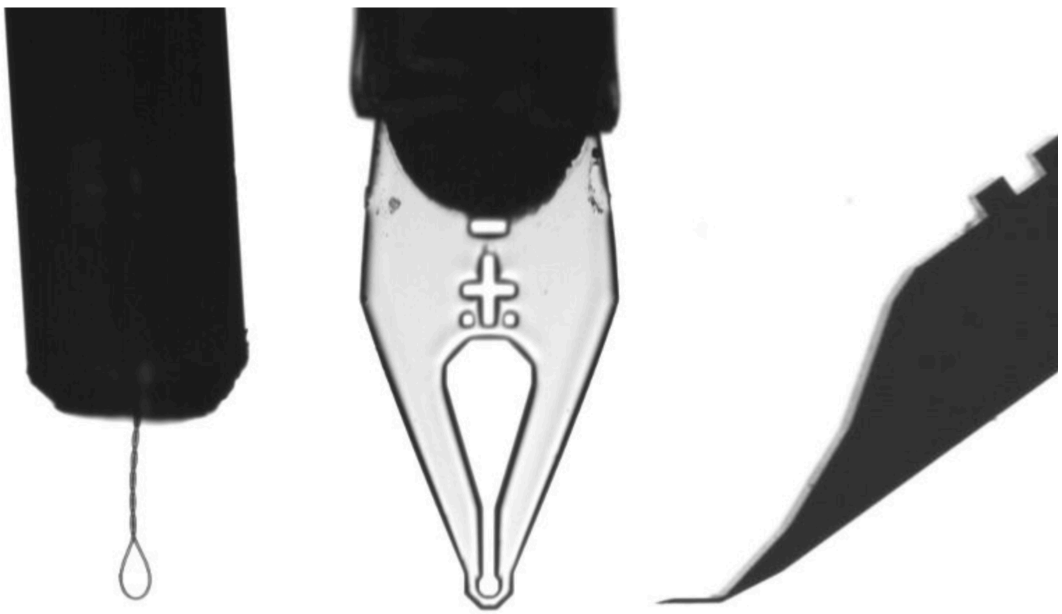
Mounted xtal is attached to a goniometer head for precise adjustment.

Small wrenches fit here, here, here and here to adjust height, position, angle.

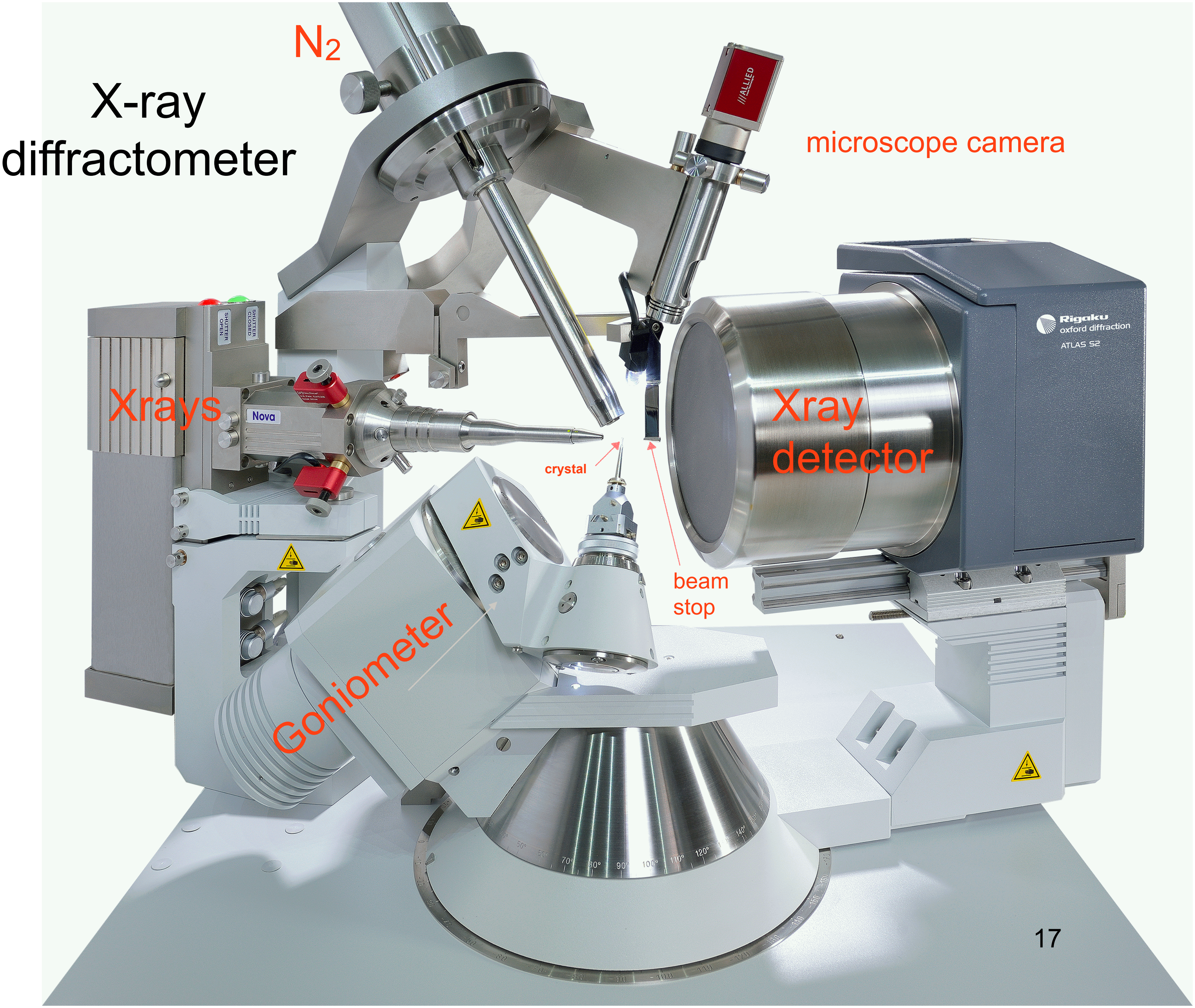
__Crystals must be flash frozen in N_2__ to prevent the formation of hexagonal ice. When freezing in liquid N_2, water glass forms, not hexagonal ice. Water glass does not diffract.



Crystal mounting tools for freezing

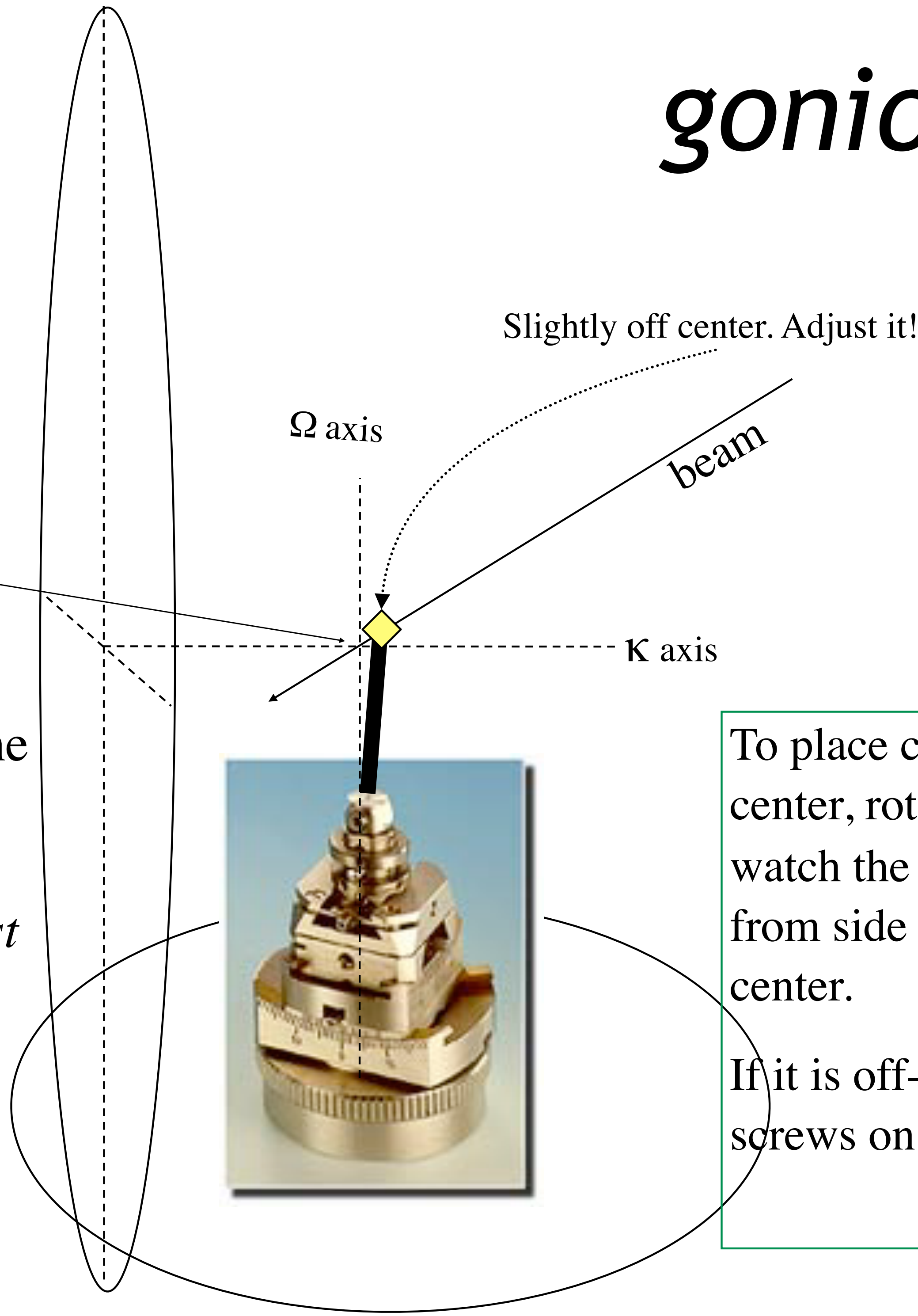


Two-Stage Robotic Crystal Mounting of Protein Crystals for X-Ray Data Collection
In Proc. of the IEEE Int. Conf. on Automation Science and Engineering (CASE'08), Arlington, VA, August 2008, pp.1019-1024


N$_2$
X-ray diffractometer
microscope camera
SHUTTER OPEN
SHUTTER CLOSED
Xrays
Nova
Rigaku oxford diffraction
ATLAS S2
Xray detector
crystal
beam stop
Goniometer

goniometer

"**machine center**" is the intersection of the beam and the two goniostat rotation axes. *The crystal must be at machine center*.

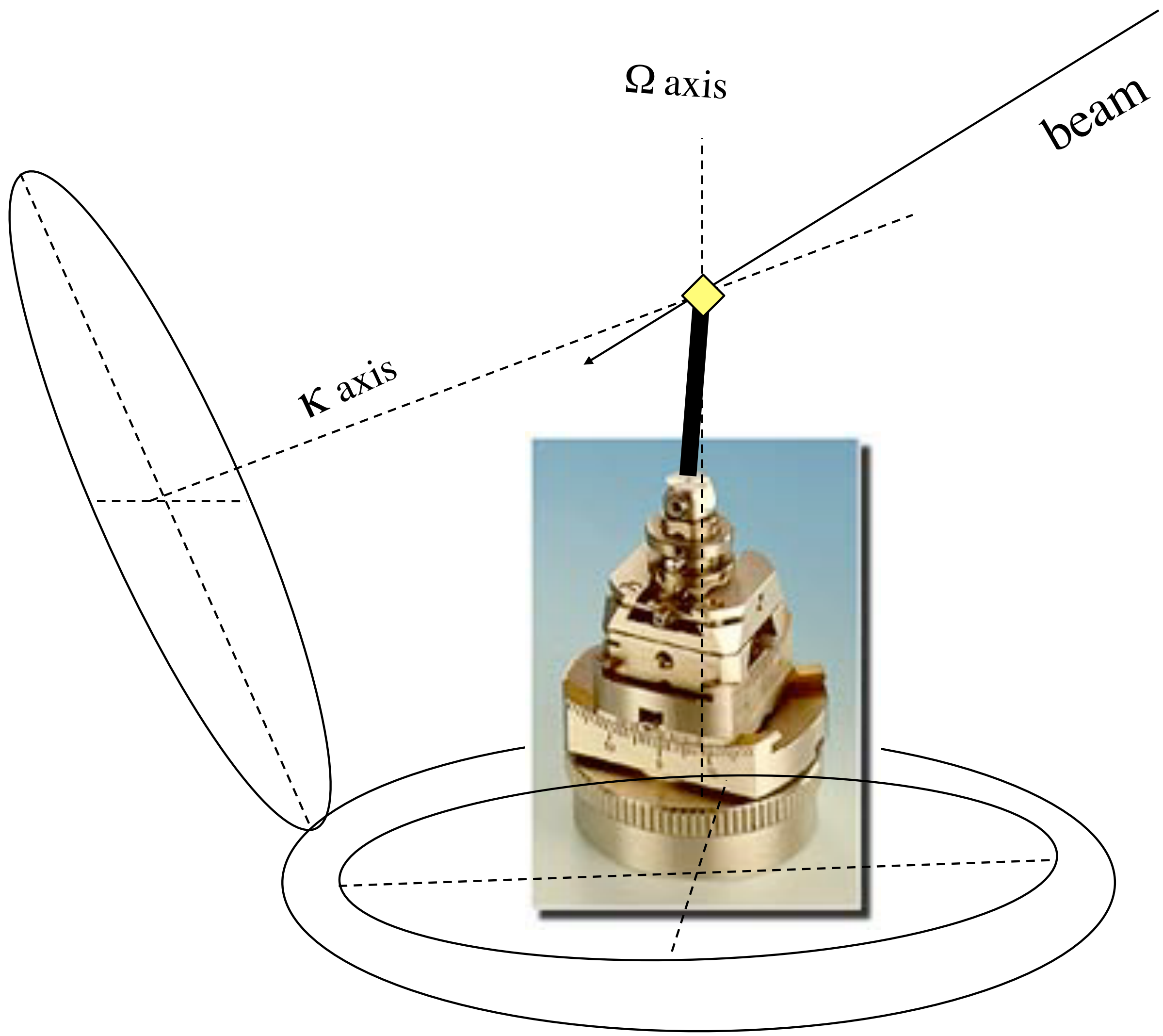


To place crystal at machine center, rotate ω and κ and watch the crystal. If it moves from side to side, it is off center.

If it is off-center, we adjust the screws on the goniometer head.

goniometer

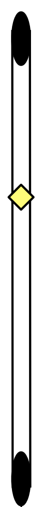
ϰ geometry



Mounted crystal

not freezing

Xtal is mounted in a thin-walled glass capillary tube

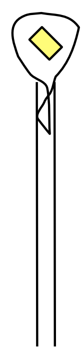


wax

Crystal must be kept at proper humidity and temperature!! Very fragile!

freezing (preferred)

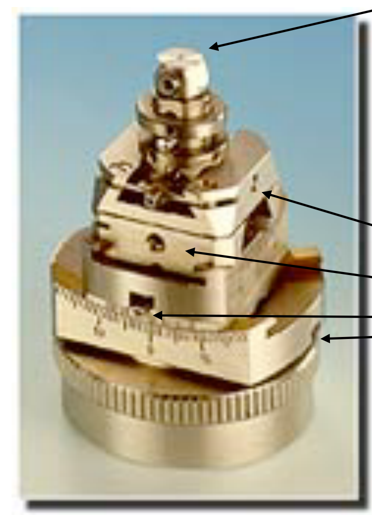
Xtal is mounted on a thin film of water in a wire loop. The loop is fixed to a metal or glass rod.



Must freeze immediately or film will dry out!

Mounted xtal is attached to a goniometer head for precise adjustment.

pin mount. Swap for magnetic baseplate for cryo caps.

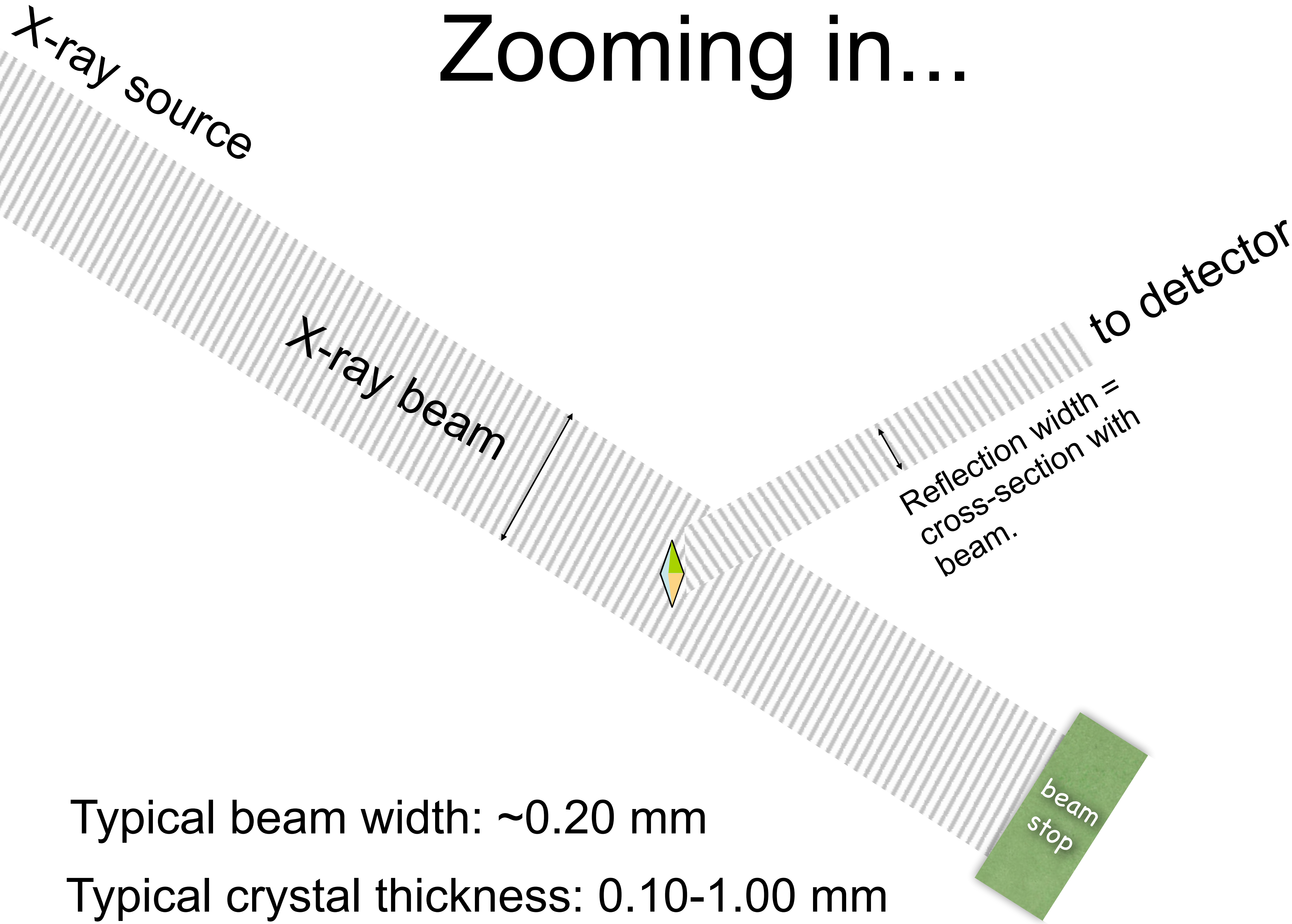


Small wrenches fit here, here, here and here.

eucentric goniometer head

- The crystal must rotate around **machine center**, in the beam.
- The beam must be able “see” the crystal from **all angles**.
- The **orientation** of the crystal axes must be known precisely.
- The beam is wider than the crystal, so the whole crystal is **inside the beam**.
- The interaction of the the crystal with the beam produces "**reflection**" spots.

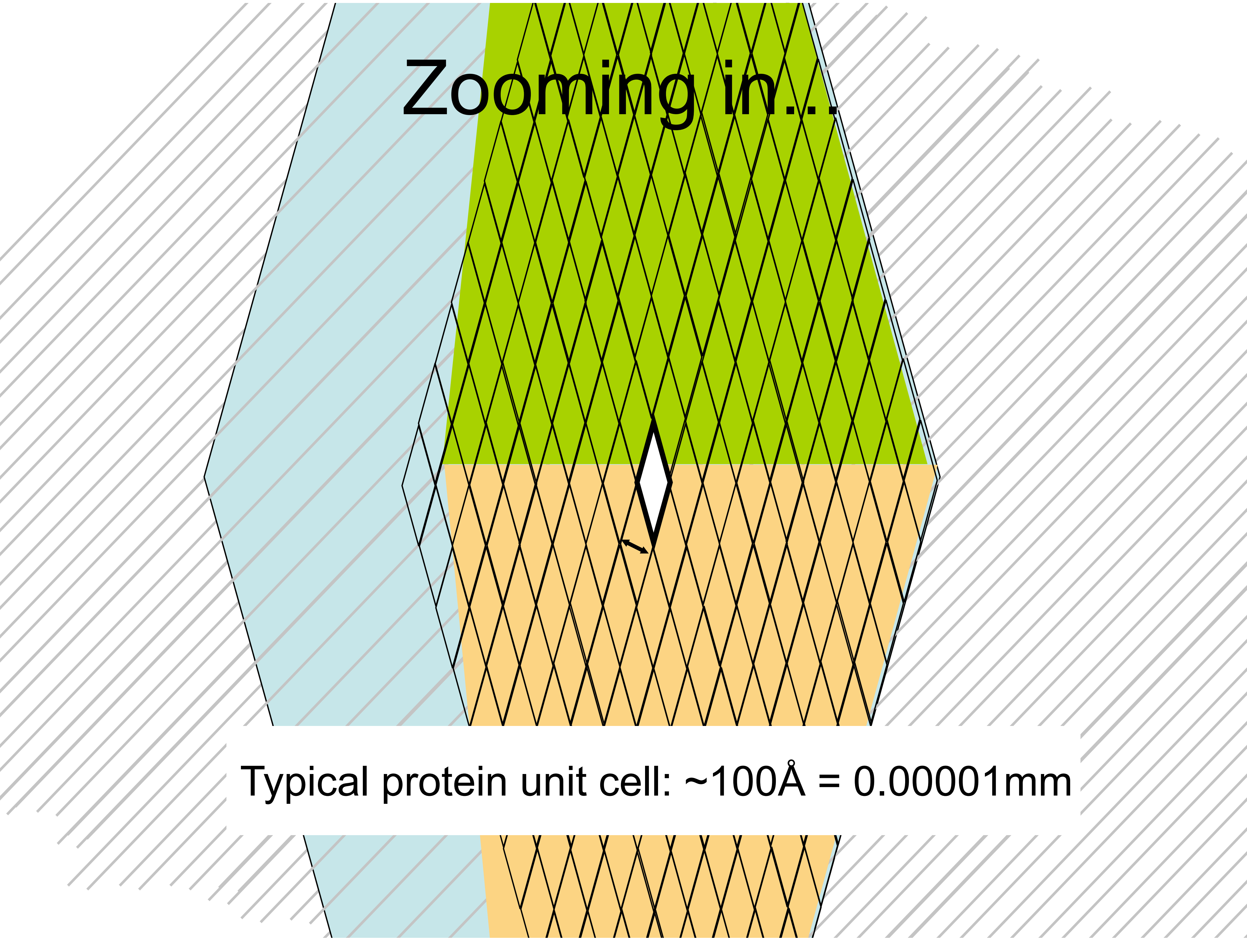
Zooming in...



Typical beam width: ~0.20 mm

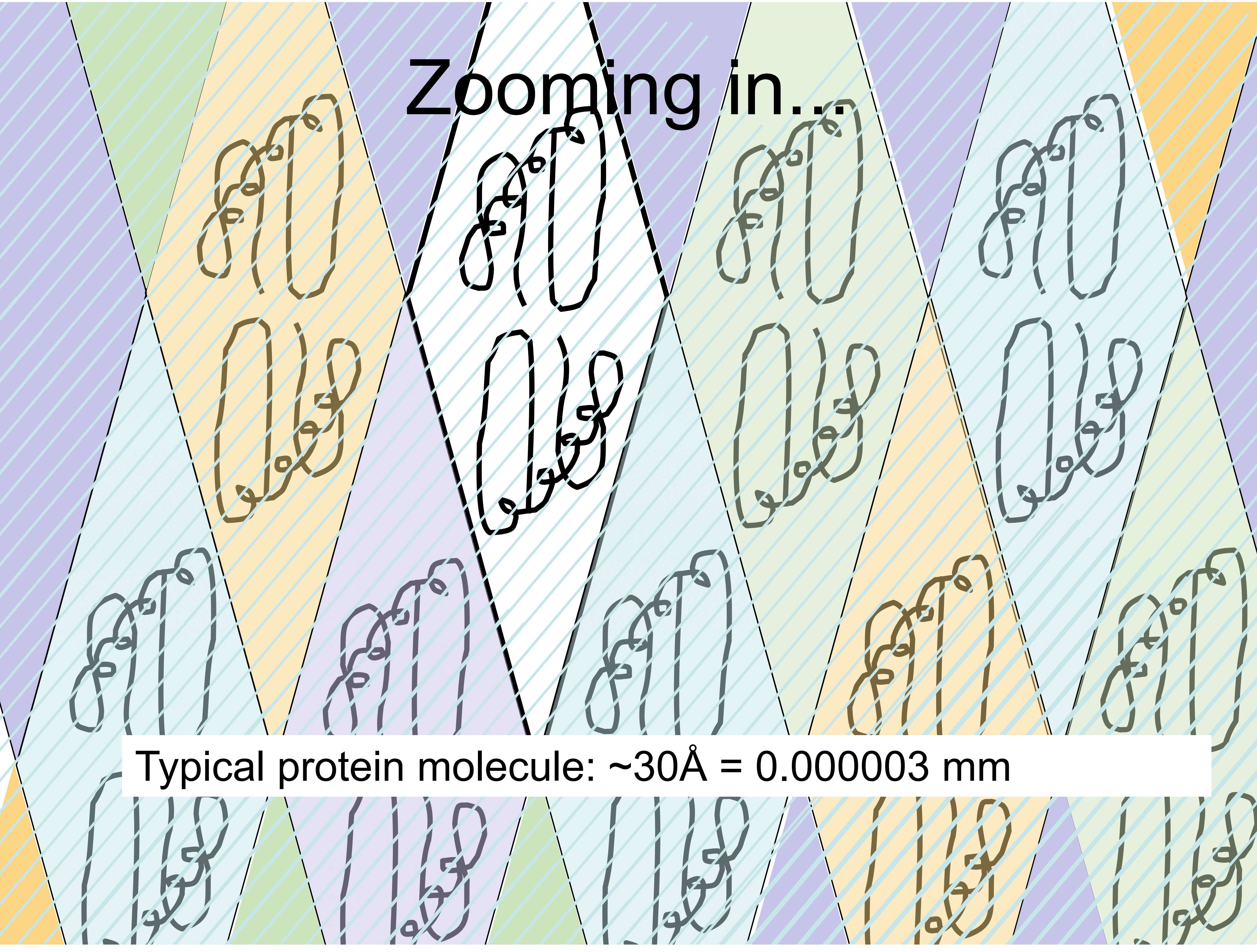
Typical crystal thickness: 0.10-1.00 mm

Zooming in...



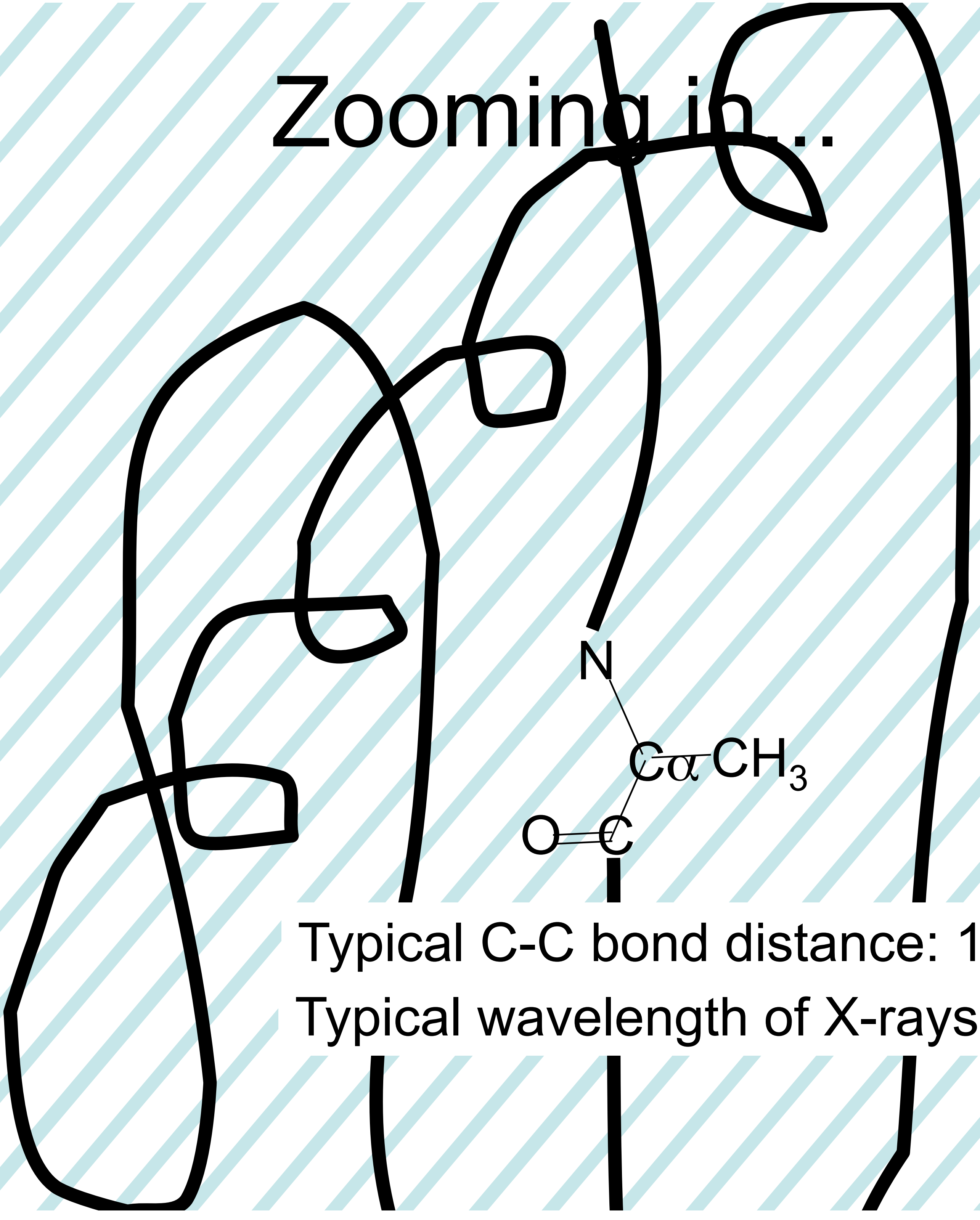
Typical protein unit cell: ~100Å = 0.00001mm

Zooming in...



Typical protein molecule: ~30Å = 0.000003 mm

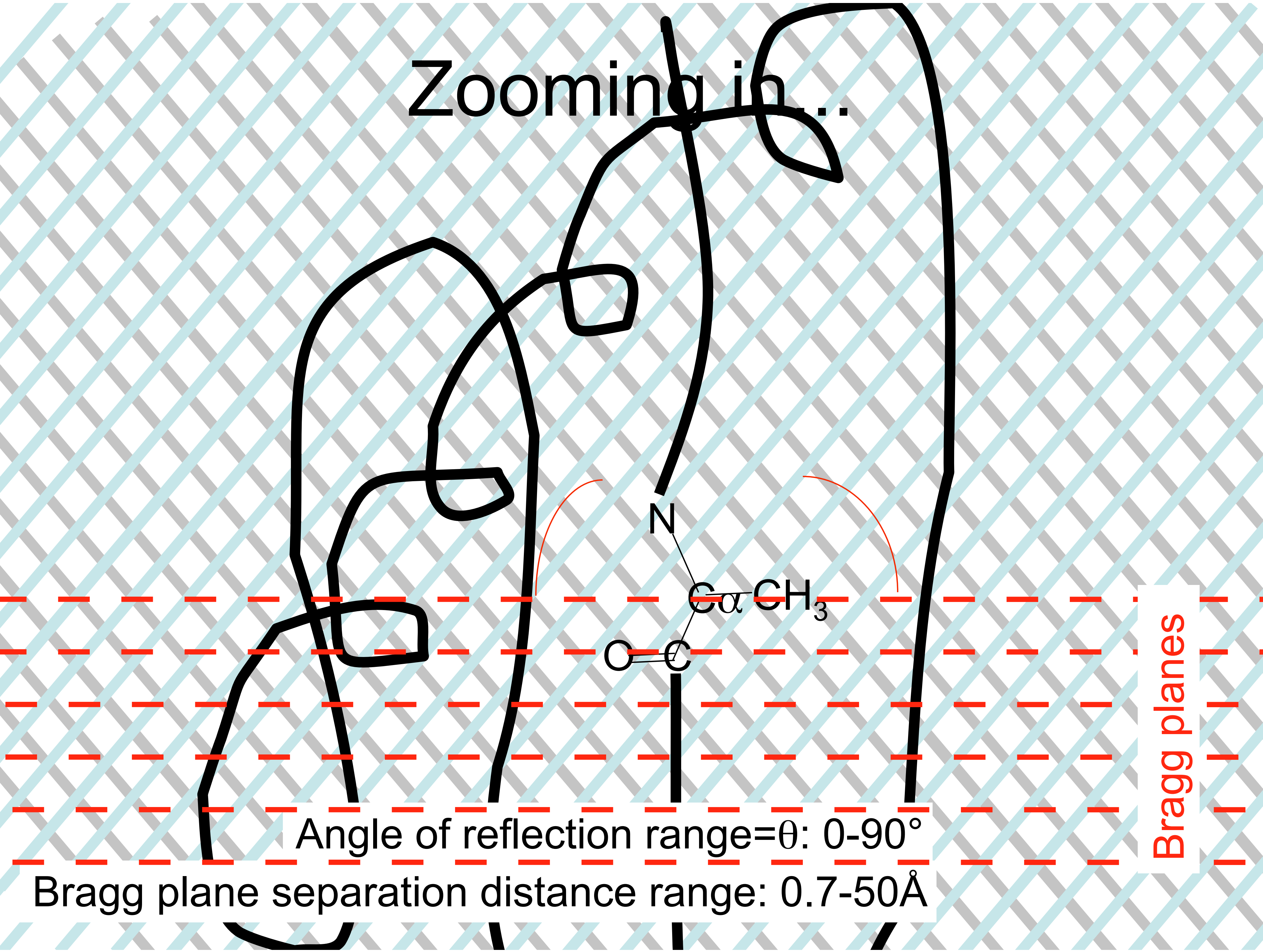
Zooming in...



Typical C-C bond distance: 1.52Å

Typical wavelength of X-rays: 1.5418Å

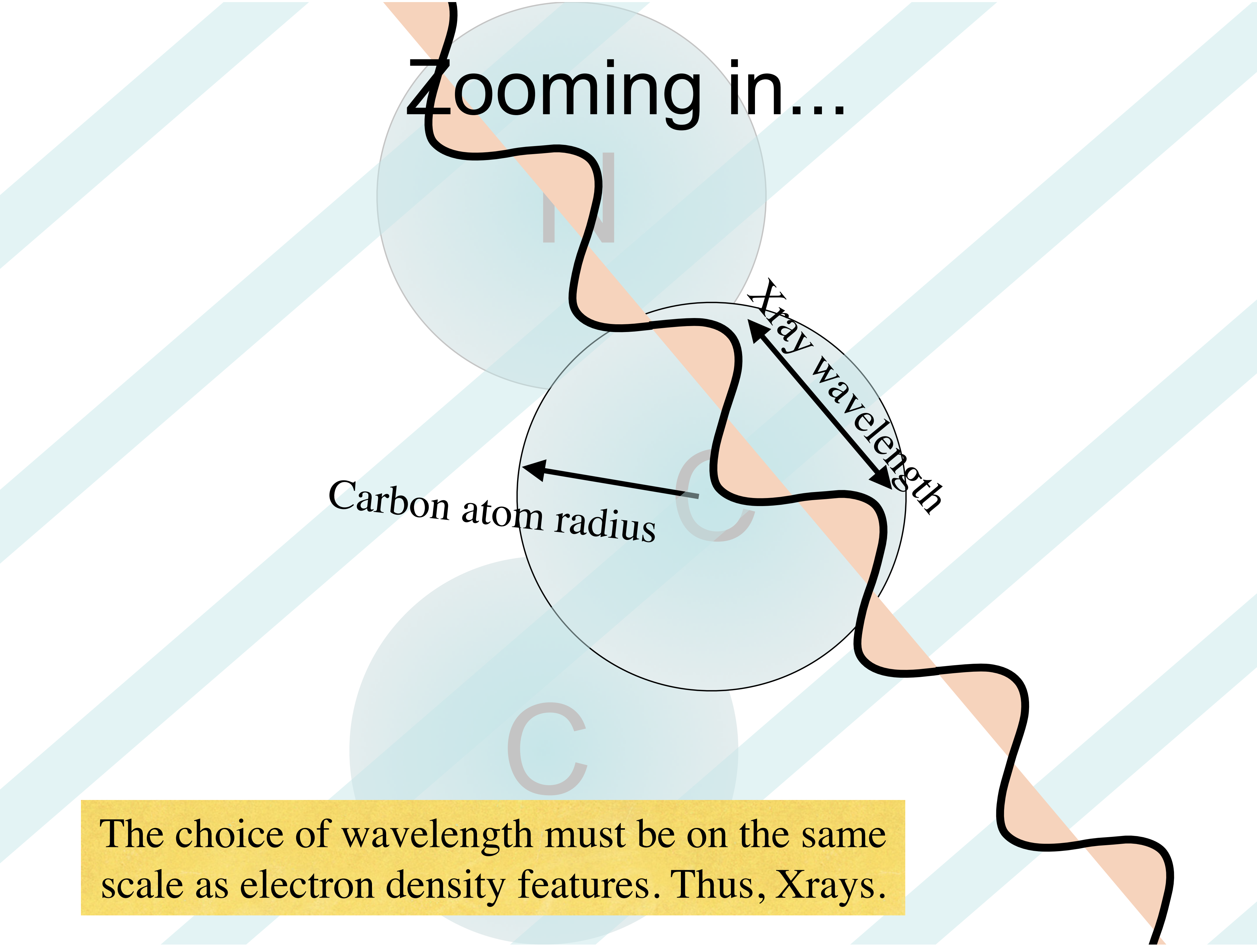
Zooming in…



Angle of reflection range=θ: 0-90°

Bragg plane separation distance range: 0.7-50Å

Zooming in...



The choice of wavelength must be on the same scale as electron density features. Thus, Xrays.

REVIEW

X-rays are plane waves

(even though we sometimes draw them as arrows or lines)

Electrons (not nuclei) scatter X-rays.

Wave summation is interference.

Waves are summed as vectors (amplitude, phase).

Proteins can be crystallized.

Crystal growth starts with nucleation.

Crystal morphology depends on symmetry and crystal contacts.

Crystals are generally frozen.

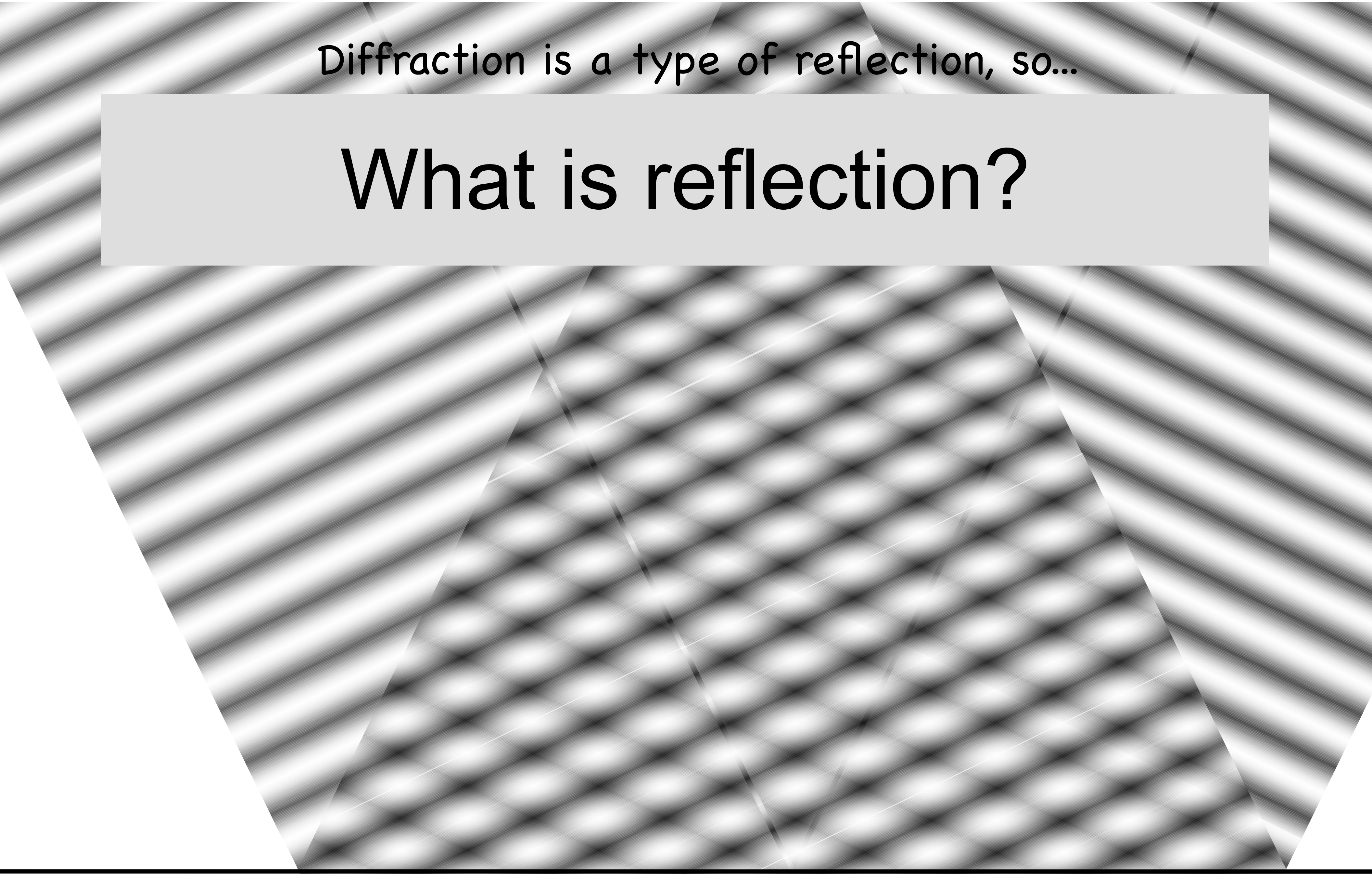
Crystallization is done by robots, these days.

Diffractometry is also done by a robot.

How do you get diffraction when the wavelength of the light is much smaller than the roughness of the surface?

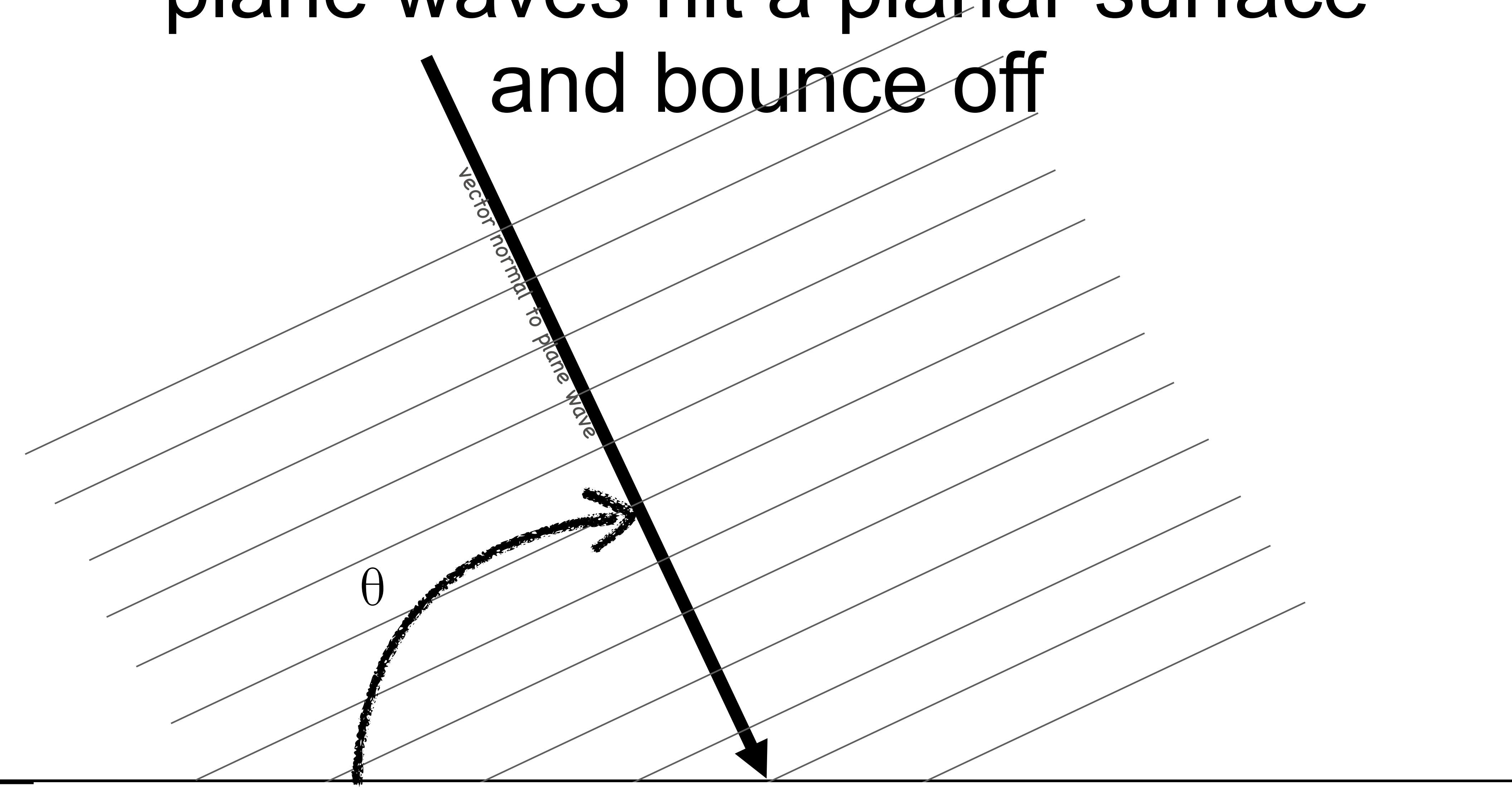
Diffraction is a type of reflection, so…

What is reflection?



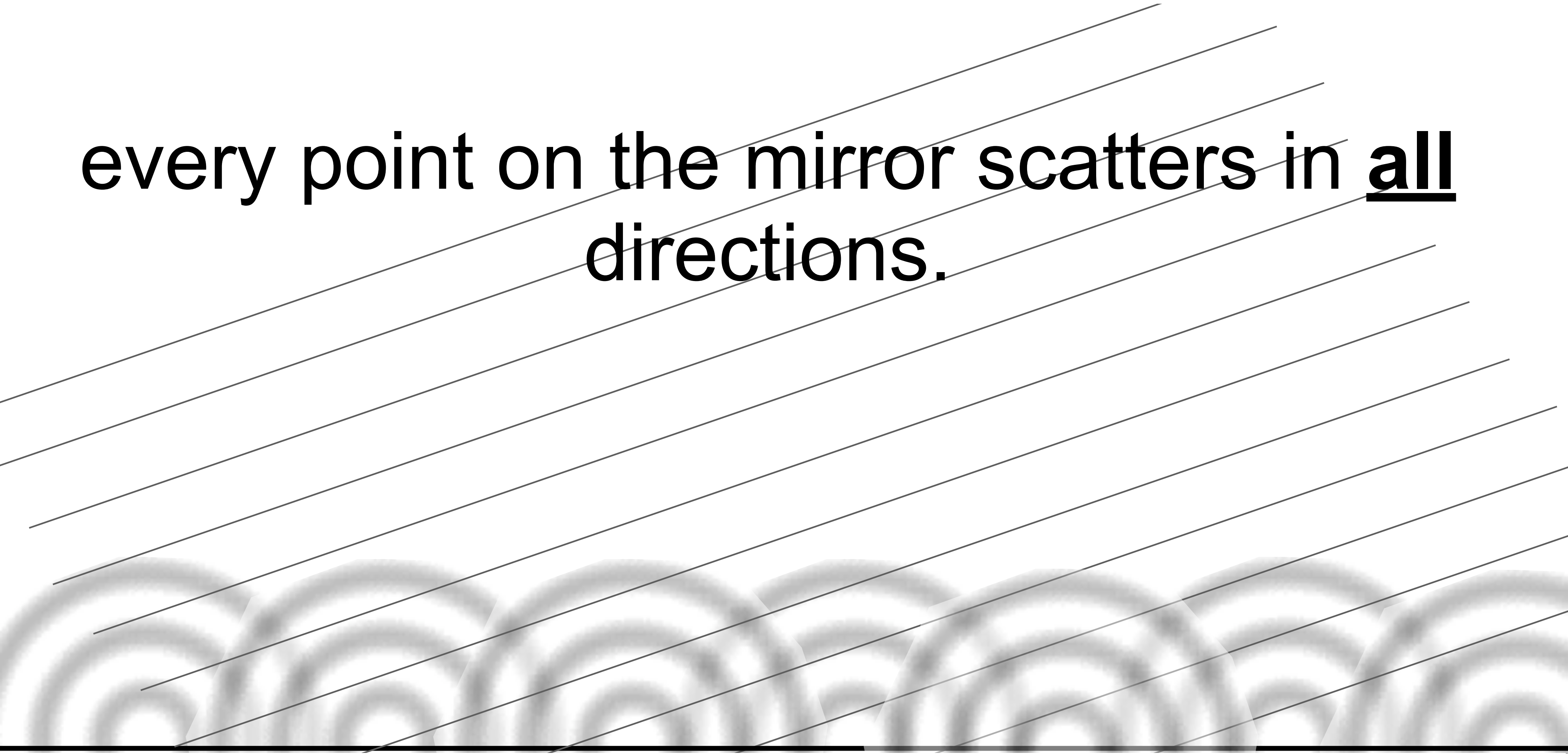
Why does light scatter at only one angle from a smooth surface?

Consider the following: plane waves hit a planar surface and bounce off



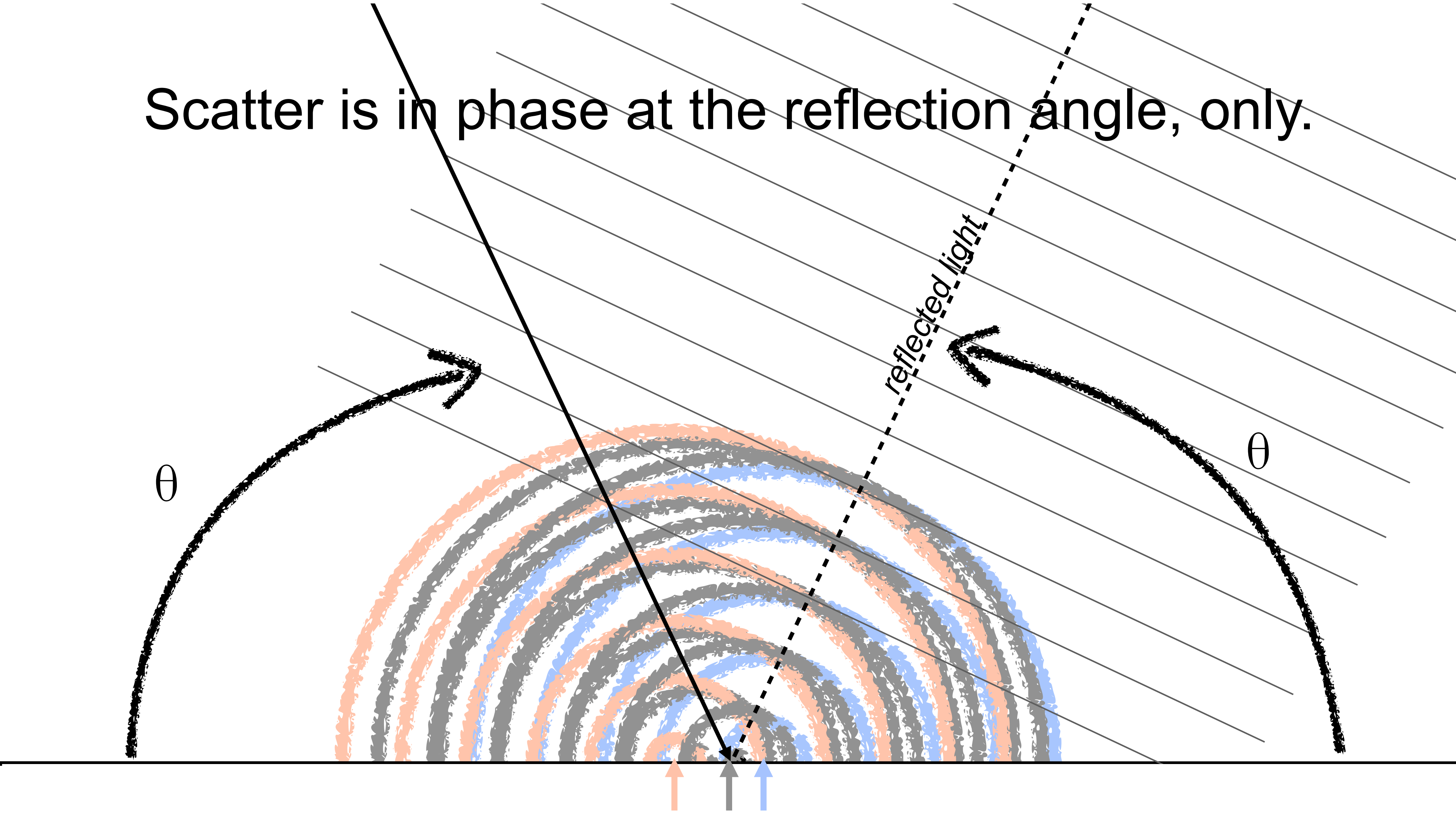
The parallel lines represent the <u>crests</u> of waves.
Normal vector is the direction of travel.

every point on the mirror scatters in **<u>all</u>** directions.



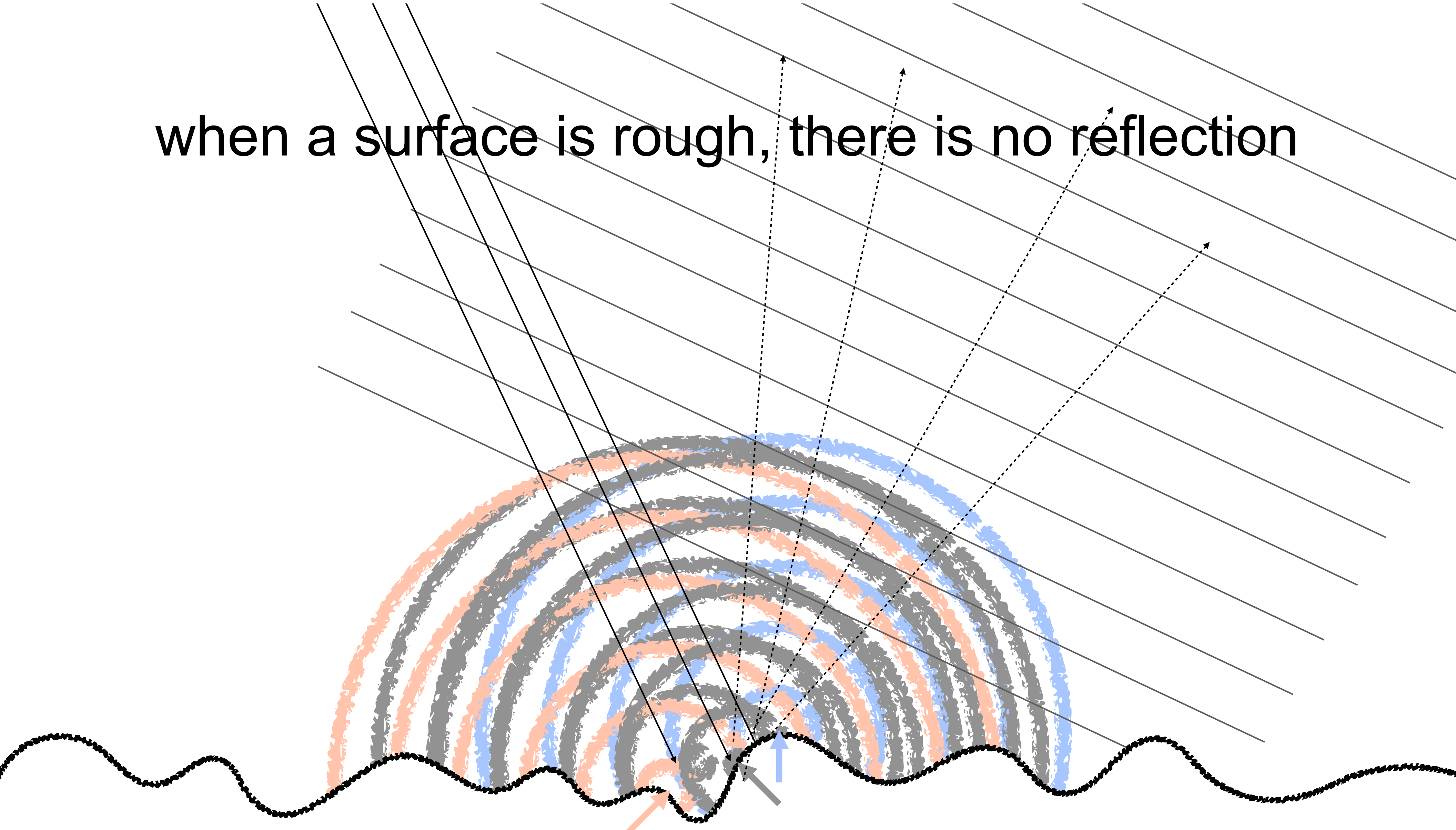
This plane wave (frozen in time) sends out scattered waves from each point on the mirror, in all directions, but they are <u>out of phase with each other.</u>

Scatter is in phase at the reflection angle, only.



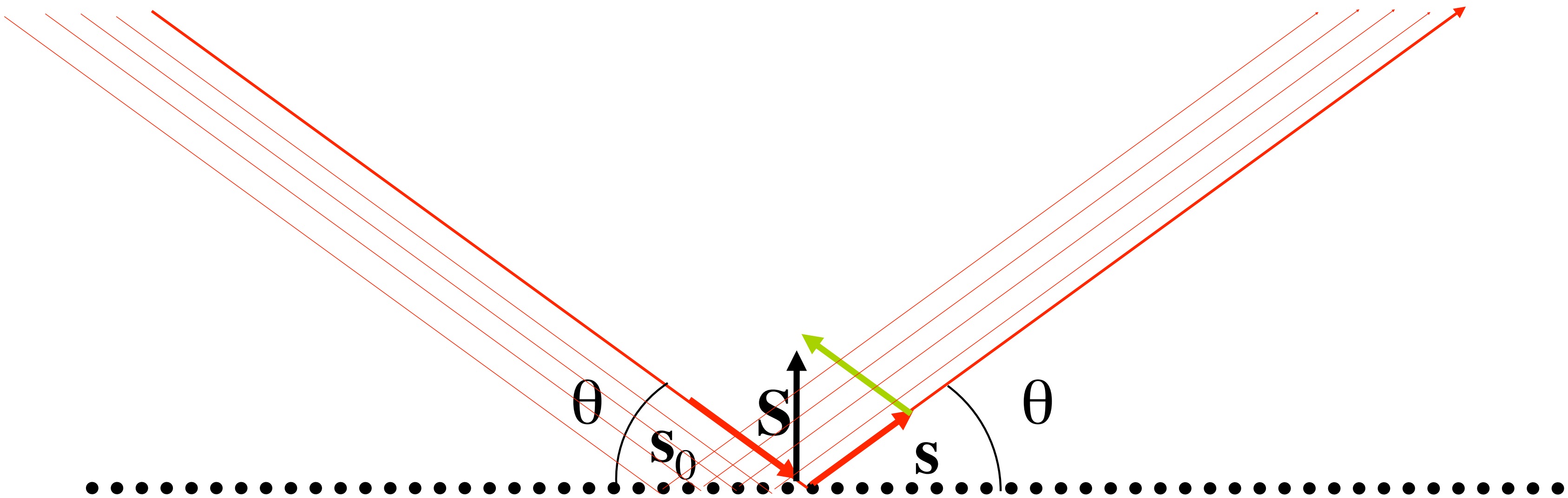
The combined waves from nearby points on a plane interfere *destructively* in all directions **but one**, which is the *reflection angle*.

when a surface is rough, there is no reflection



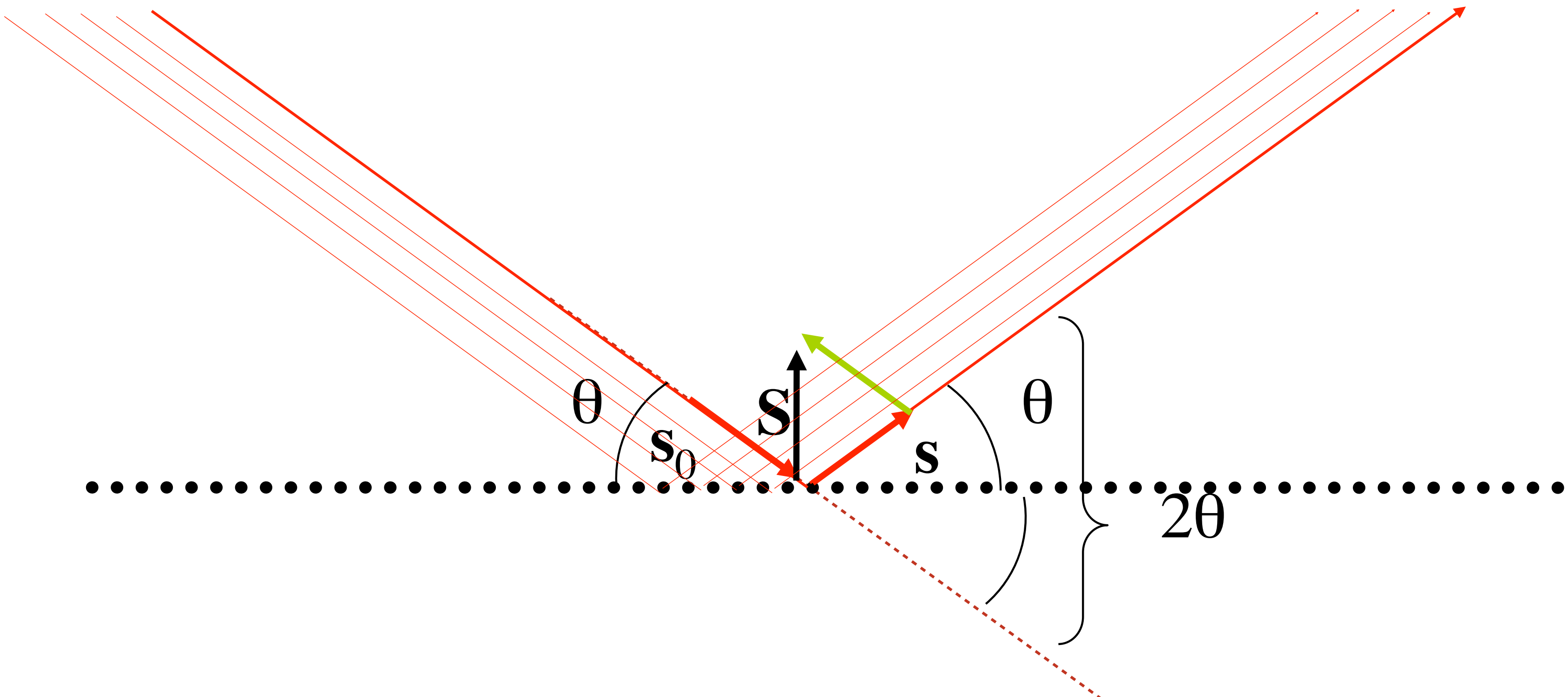
If the points of scatter do not fall on a plane, then there is no consistent angle of constructive interference.

When the angle of incidence equals the angle of scatter, all rays travel the same distance.



...and at any other angle ≠ θ , path lengths are different, therefore interference is destructive.

If the reflection angle = θ

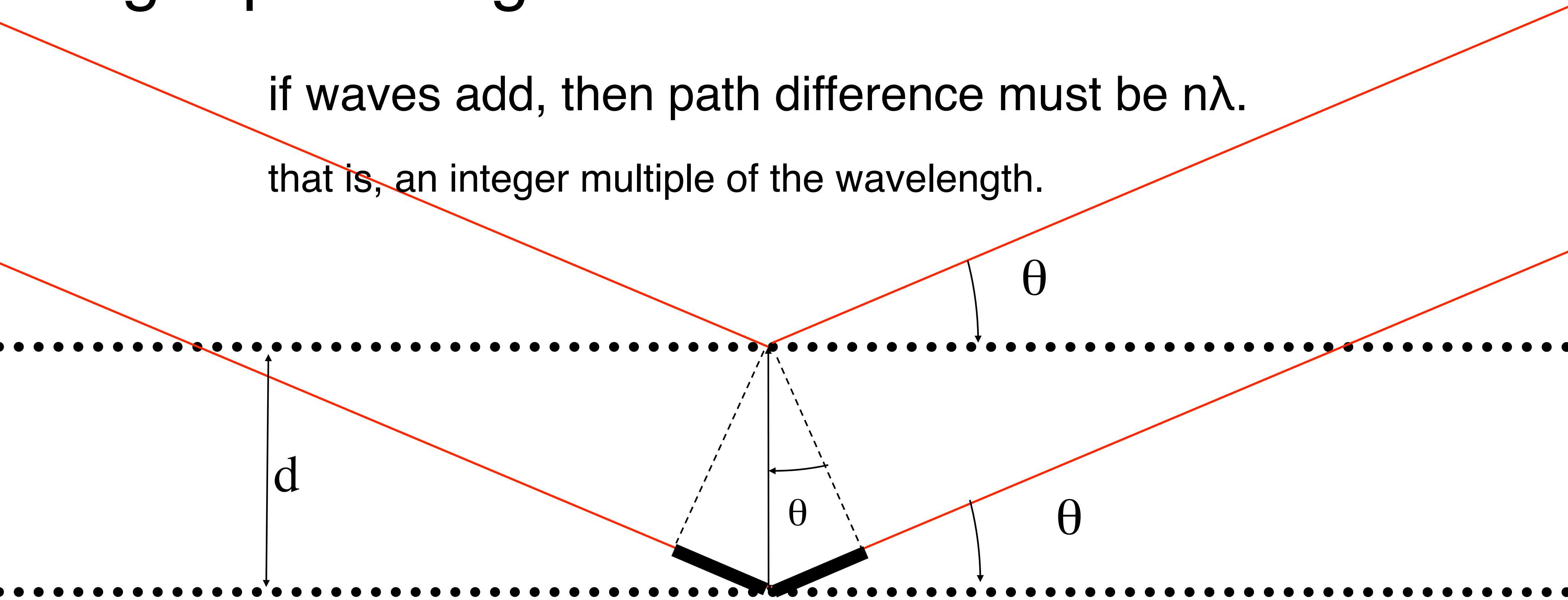


Then the scattering angle is 2θ

Integer path length differences leads to diffraction

if waves add, then path difference must be $n\lambda$.

that is, an integer multiple of the wavelength.

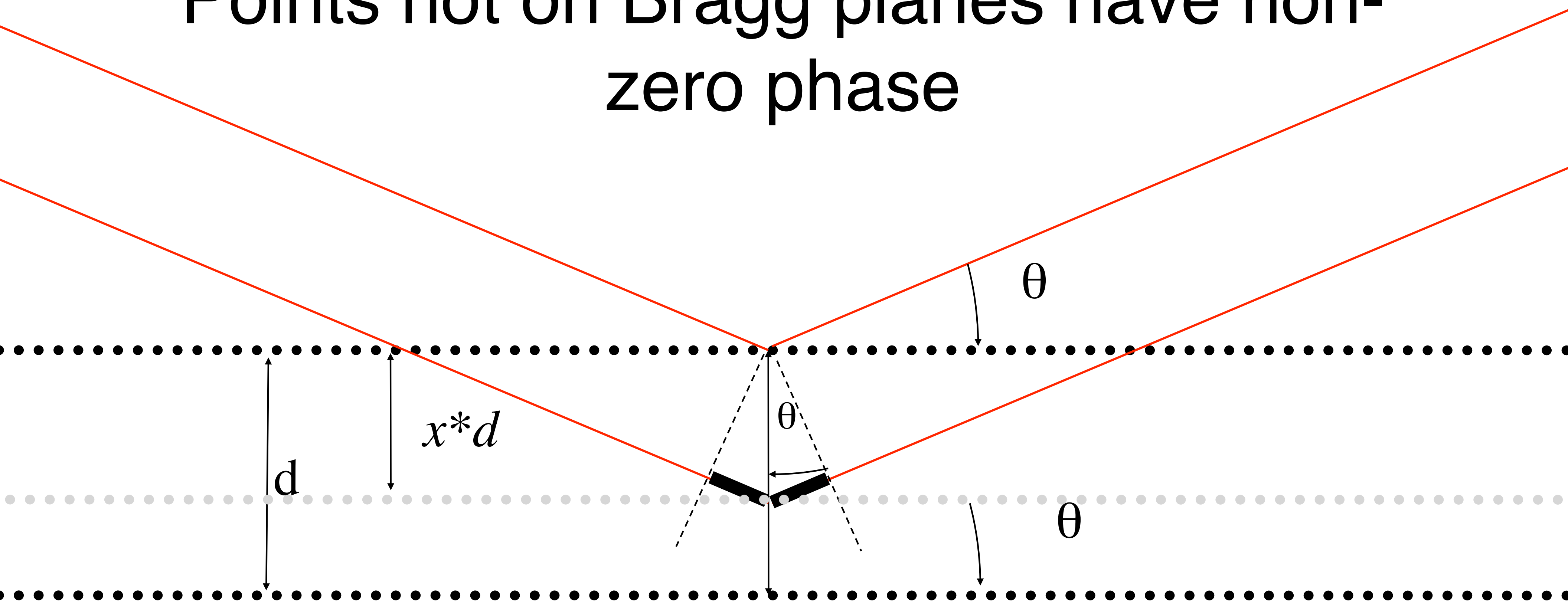


path difference=the two bold lines = $2d \sin\theta$

Let path difference equal integer multiple of the wavelength, and you get

Bragg's law, $n\lambda=2d\sin\theta$

Points not on Bragg planes have non-zero phase



x = fraction of distance d

Path difference = $2(x^*d)\sin\theta$

Phase difference = $2\pi\, 2(x^*d)\sin\theta / \lambda$ radians

but, since $d=\lambda/2\sin\theta$

therefore, phase difference in radians = $2\pi x$

Review

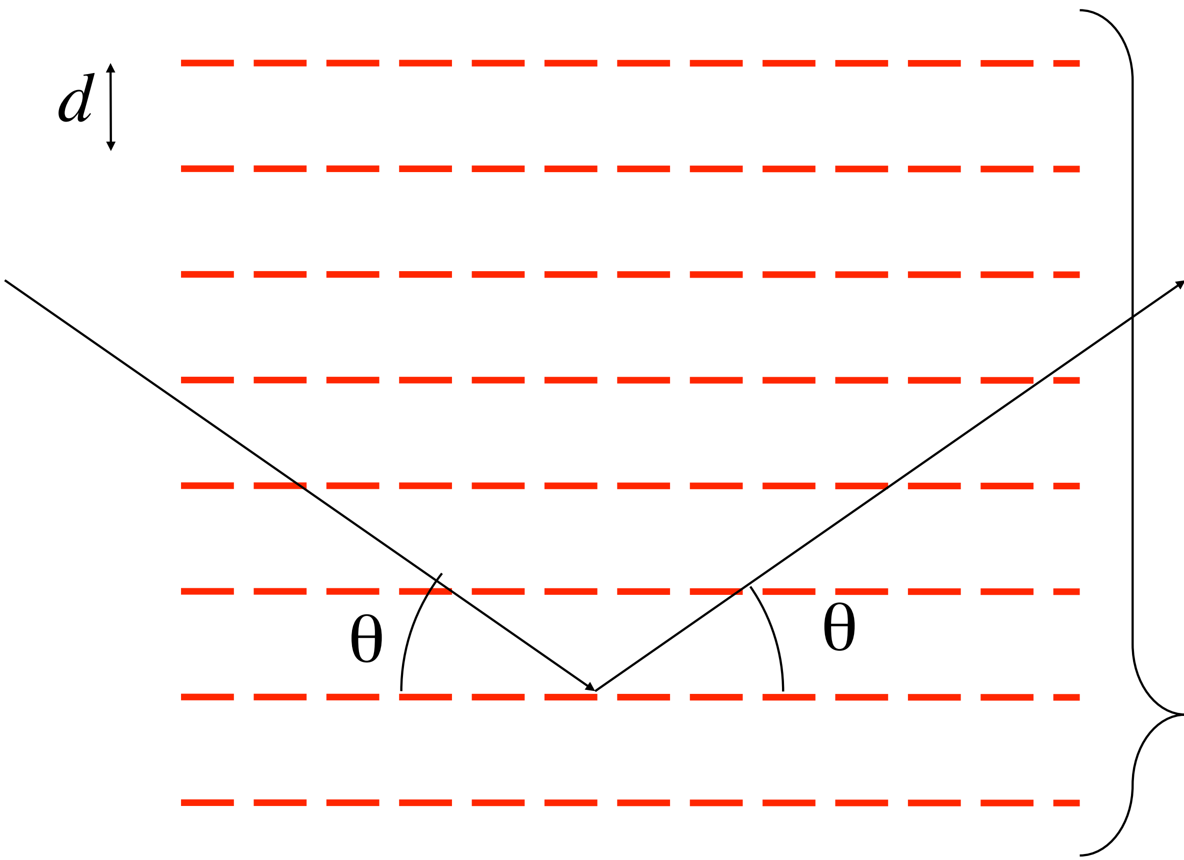
Mirrors reflect light because all points on the surface scatter in phase at only the reflection angle.

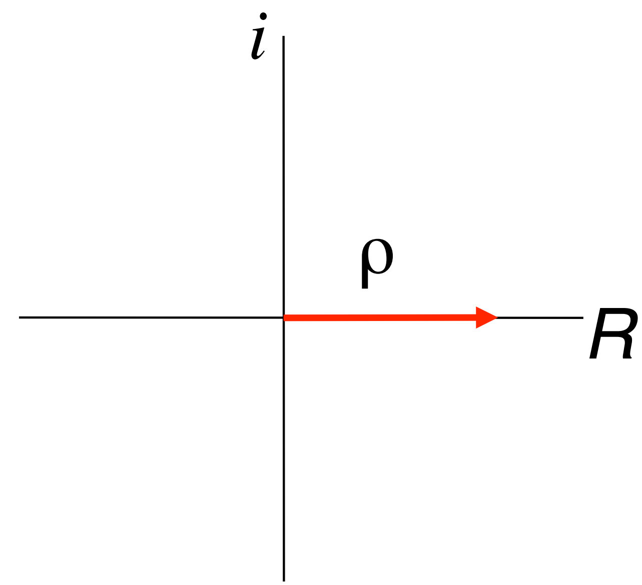
Bragg's law says that repeating planes of atoms separated by distance **d** reflect monochromatic light of wavelength λ at specific reflection angles θ.

Bragg's Law: $n\lambda=2d\sin\theta$

The Fourier transform of Bragg planes

(1) All Bragg planes scatter in phase.
(2) Bragg planes through the origin have phase =0
(3) The amplitude from Bragg planes is the sum of the density

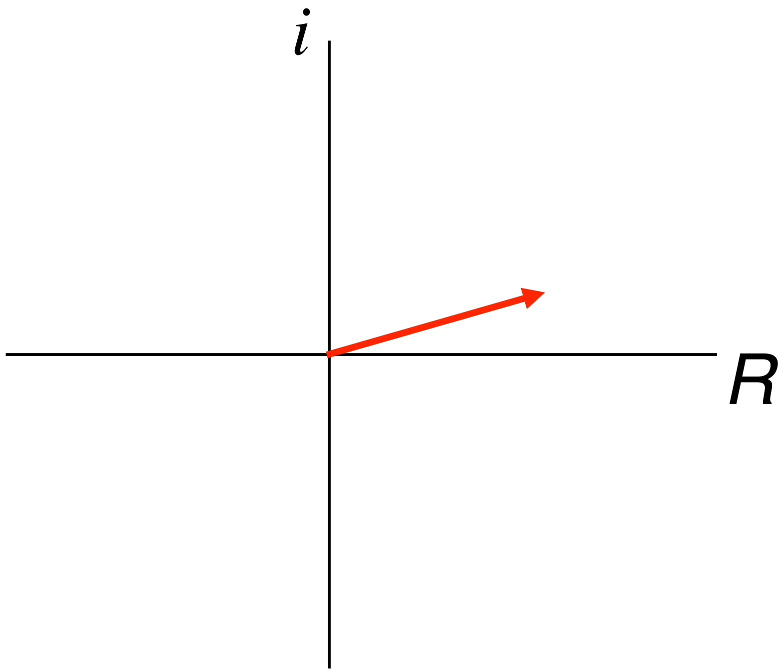


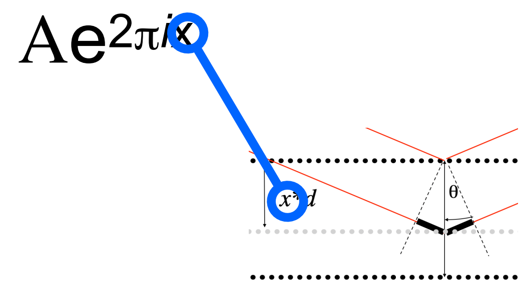


Amplitude ρ is proportional to the total number of e^- on all of these planes.

reminder

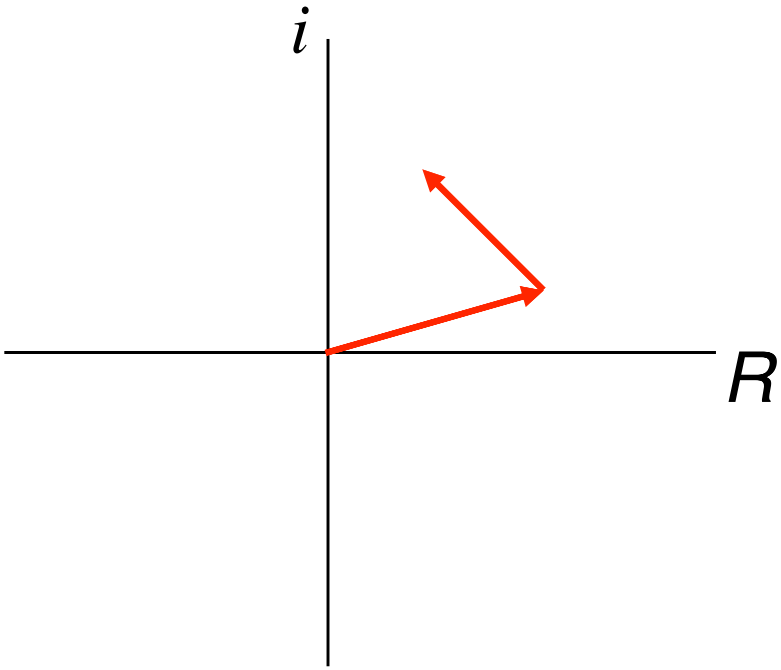
this is a wave





reminder

this is wave summation

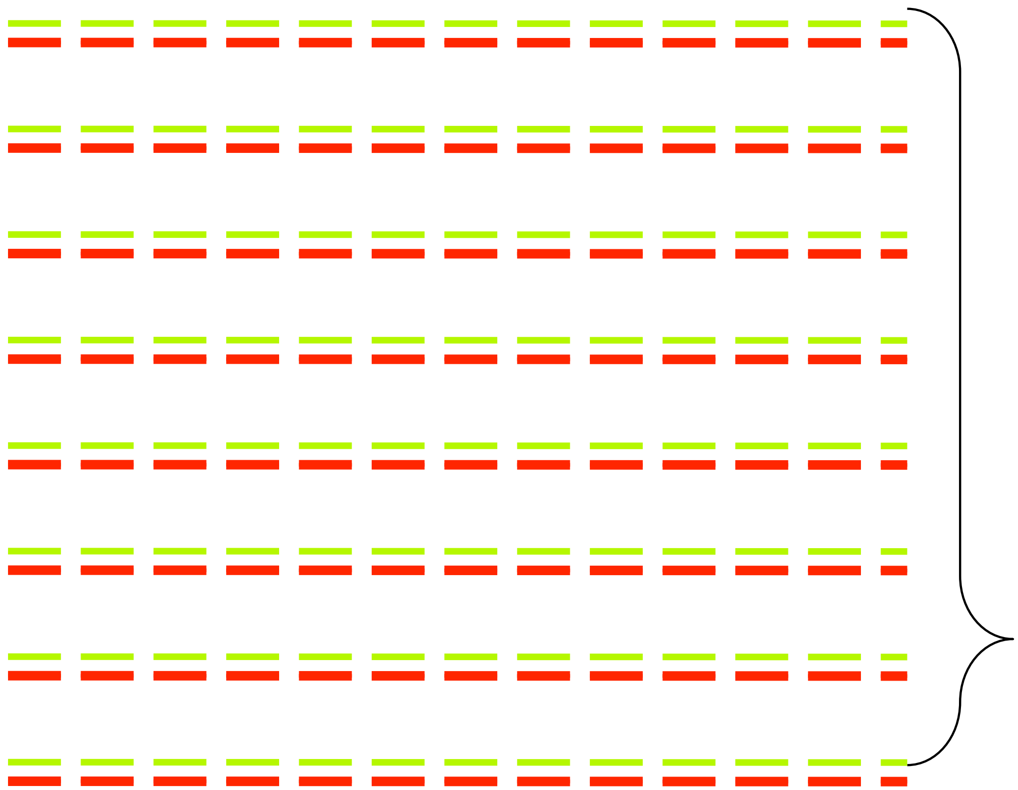


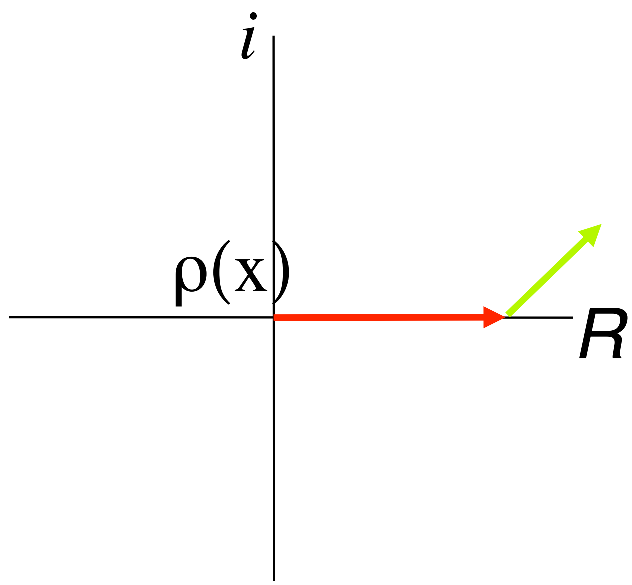
$$A_1 e^{2\pi i x_1} + A_2 e^{2\pi i x_2}$$

$$(A_1\cos(2\pi x_1), A_1\sin(2\pi x_1)) + (A_2\cos(2\pi x_2), A_2\sin(2\pi x_2))$$

$$(\, A_1\cos(2\pi x_1)) + A_2\cos(2\pi x_2)), A_1\sin(2\pi x_1) + A_2\sin(2\pi x_2)\,)$$

Bragg planes *offset by fraction x* have phase $2\pi x$

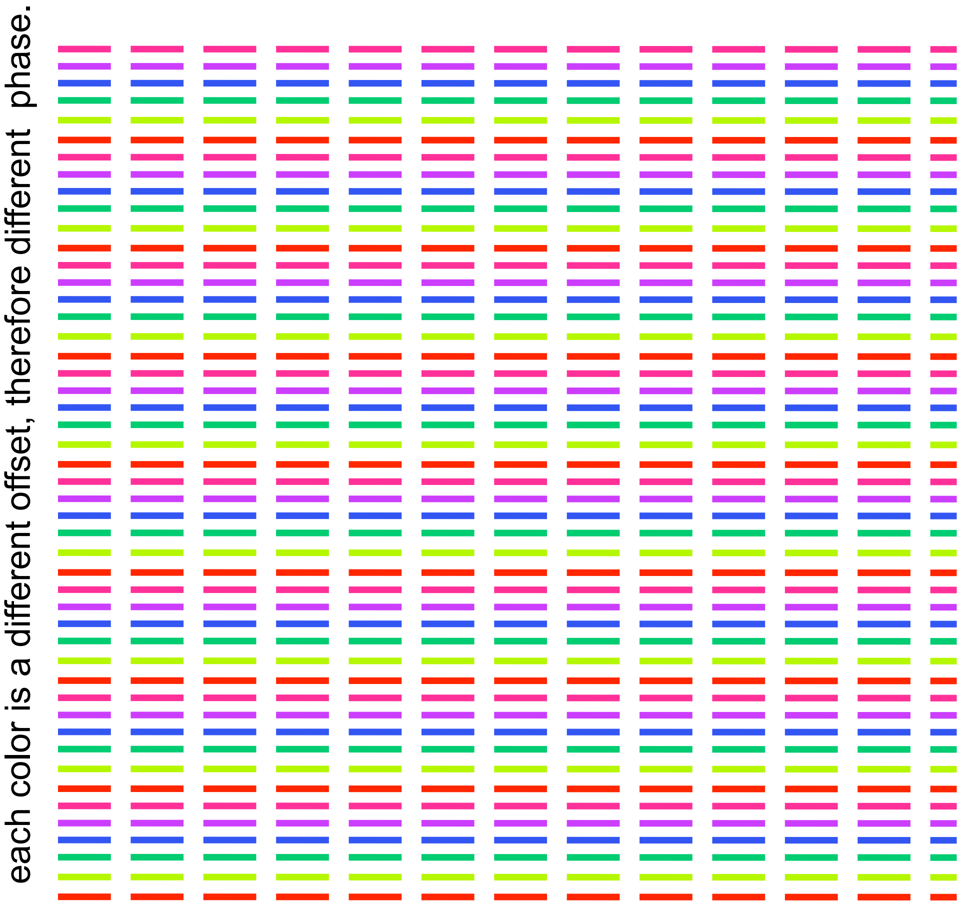


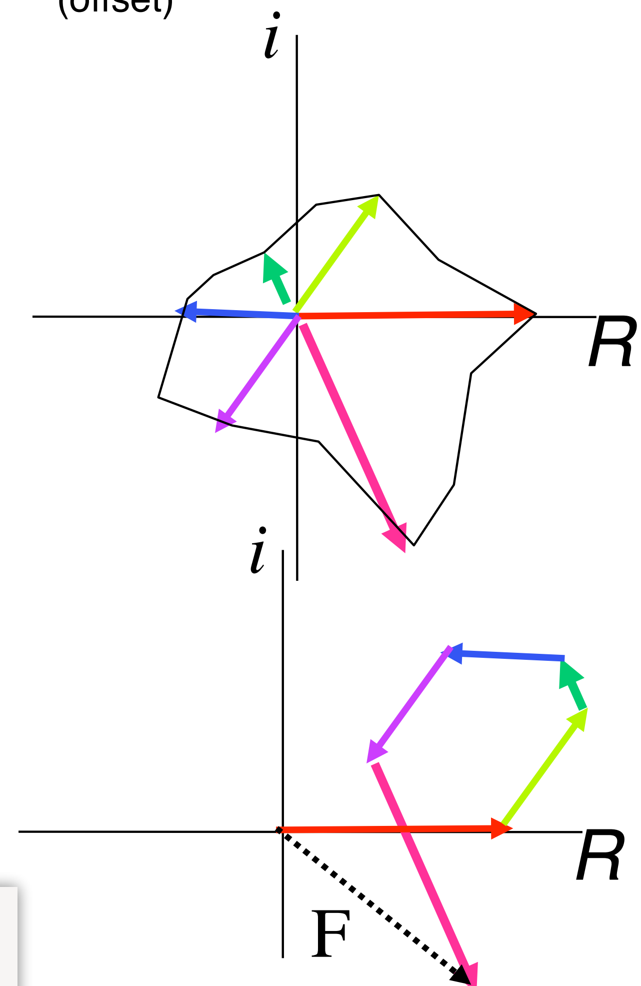


Planes shifted by x = d/6 are phase shifted by 2π/6.

ρ(x) is proportional to the total number of *e-* on these planes.

Integrating offset Bragg planes from x=0 to 1

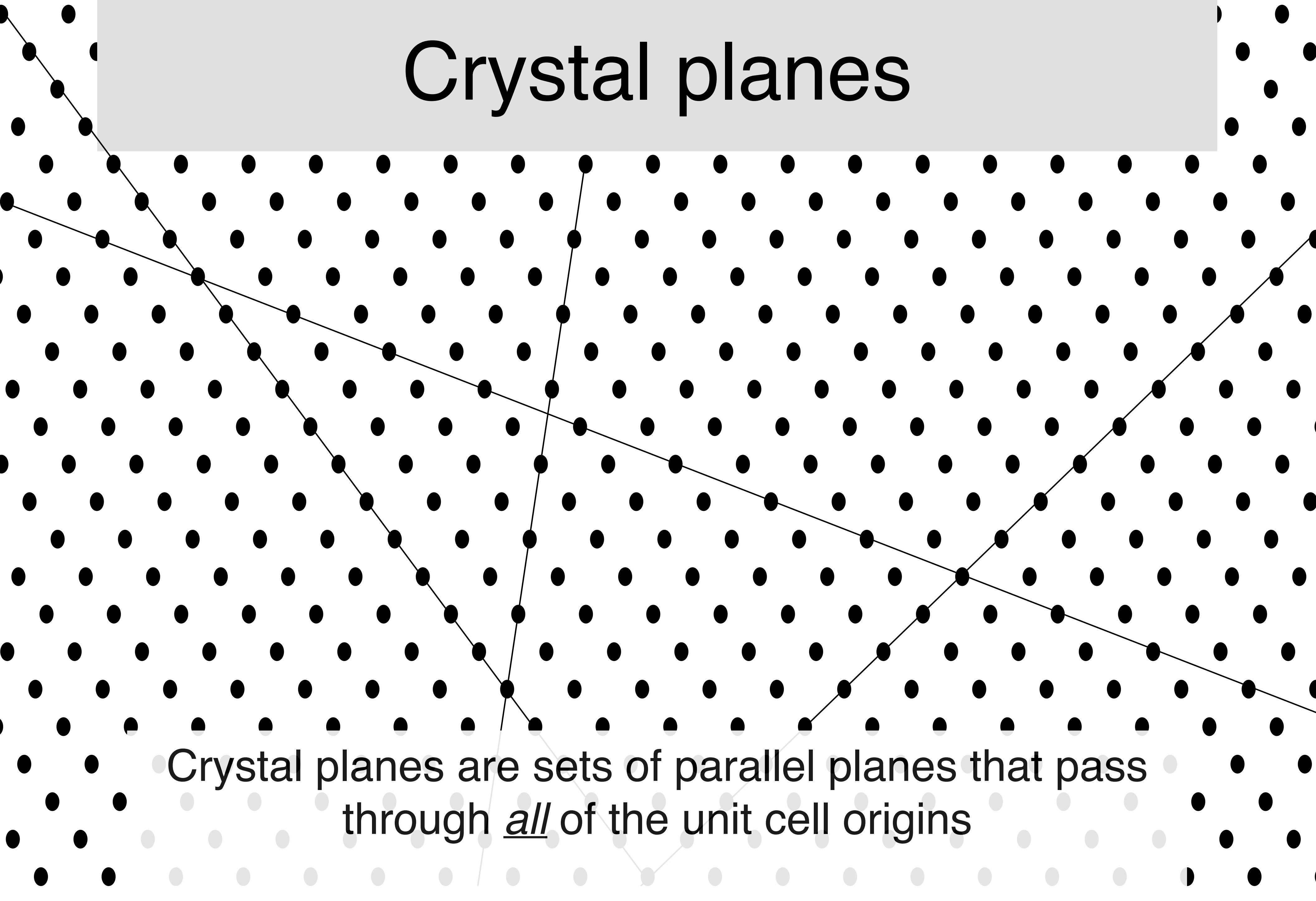




The **total** wave F is the integral over x.

$$F = \int_0^1 \rho(x) e^{2\pi i x} dx$$

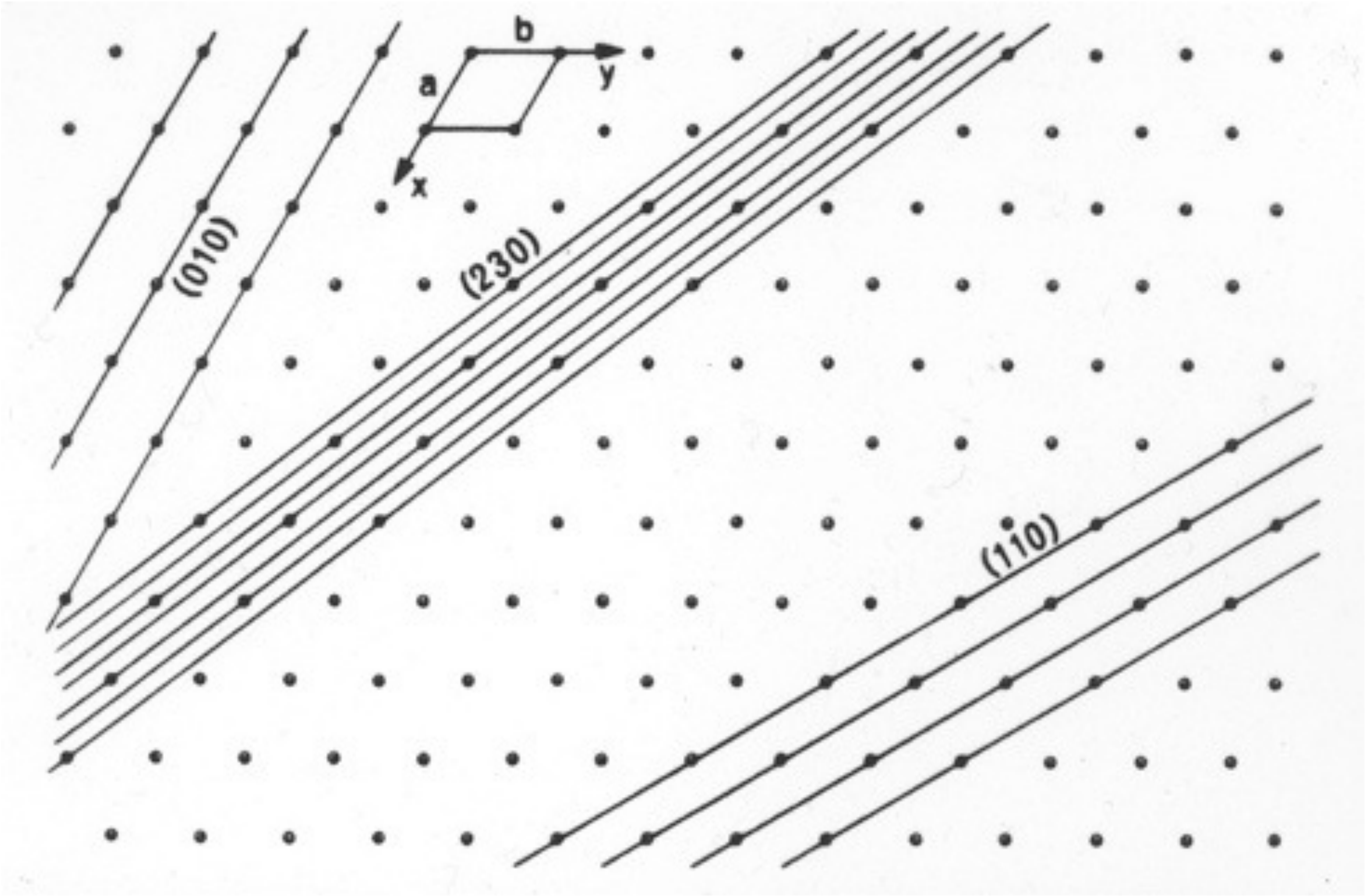
Crystal planes



Crystal planes are sets of parallel planes that pass through *all* of the unit cell origins

Crystal Planes

Crystal planes are numbered according to how they intersect the **crystal axes**.
Looking from the **Origin** to the next plane, where it intersects the **a** axis is at **1/h**, the **b** axis at **1/k** and the **c** axis at **1/l.** The planes are called **(h k l)**.
Each set of Bragg planes defines a single diffracted spot, called a “**reflection**”. Reflections are numbered using (h k l).



NOTE: h k and l are always integers!

How to draw Bragg planes on a lattice

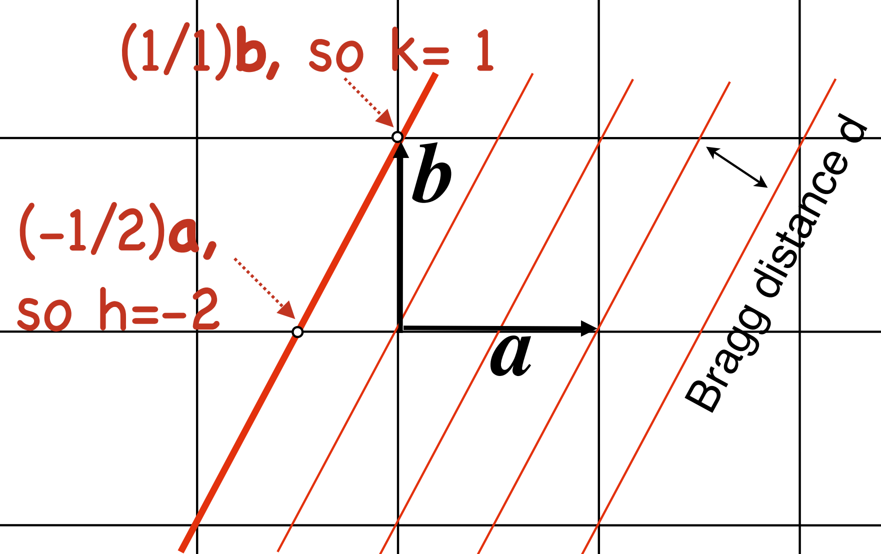
1. Pick (h,k,l) then draw a **plane that intersects** the axes …

$\boldsymbol{a}$ at $1/h$, $\boldsymbol{b}$ at $1/k$, $\boldsymbol{c}$ at $1/l$

2. Draw a parallel plane through the origin.

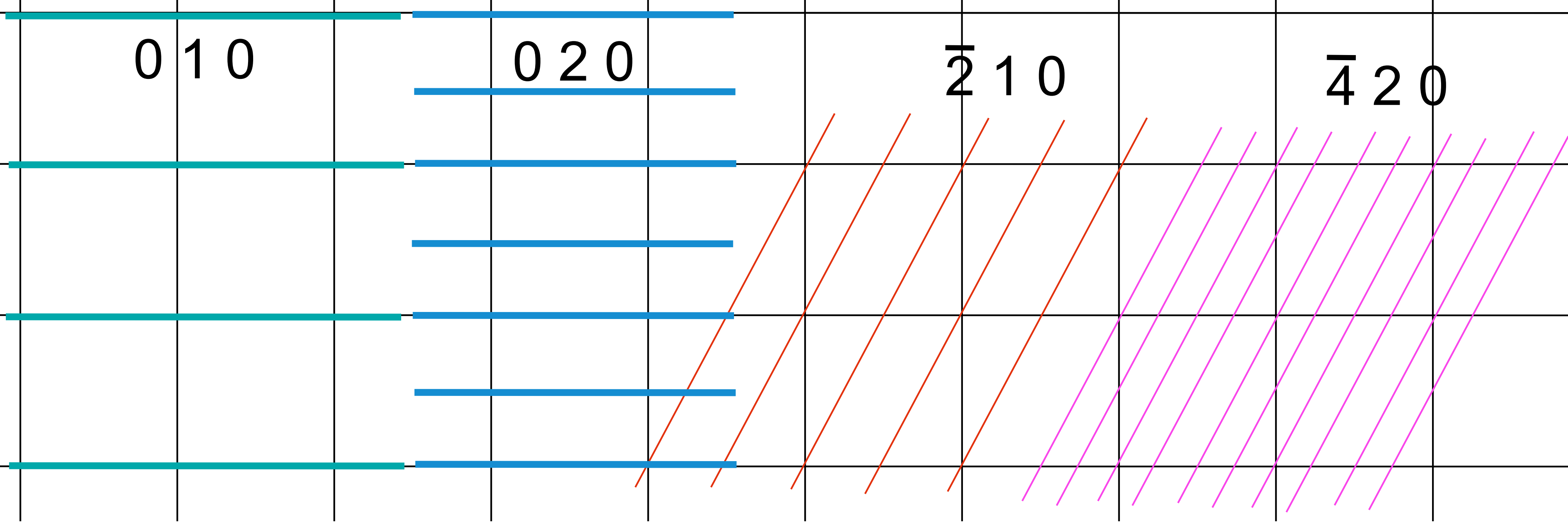
3. Continue drawing equally spaced planes in both directions. *Each origin must have a Bragg plane going though it.*

$\bar{2}\,1\,0$



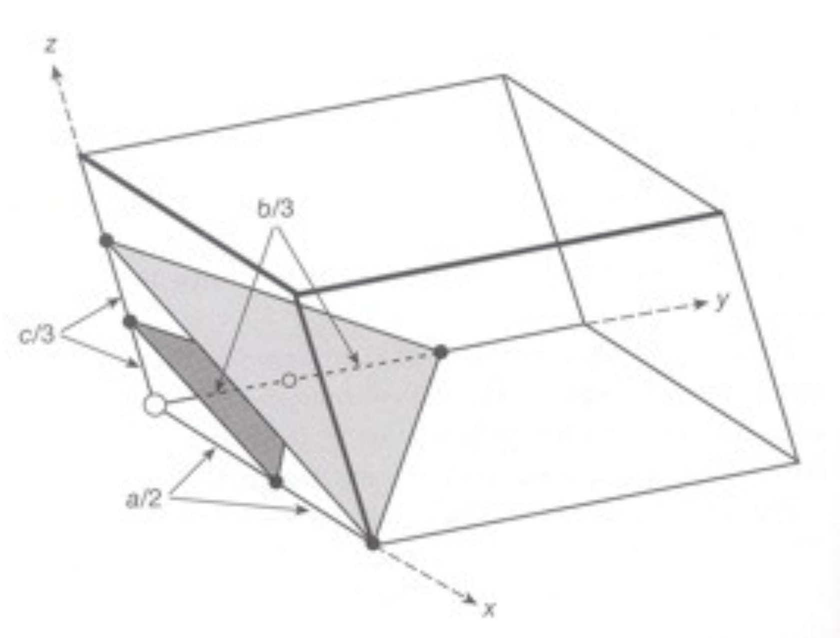
If *hkl* indeces are doubled, the reciprocal space distance is doubled and the Bragg real space distance *d* is halved.

- All unit cell origins have phase zero. But not all phase-zero Bragg planes must go through a unit cell origin. For example, the n=odd Bragg planes for the 0 2 0 reflection does not touch a single unit cell origin.

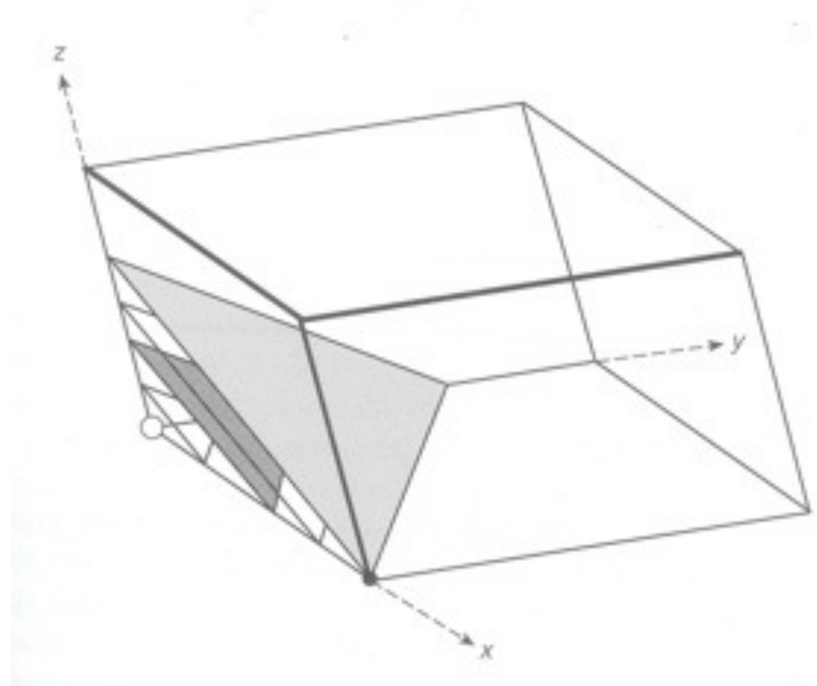


3D Bragg planes/Crystal planes

(2 3 3) Bragg planes



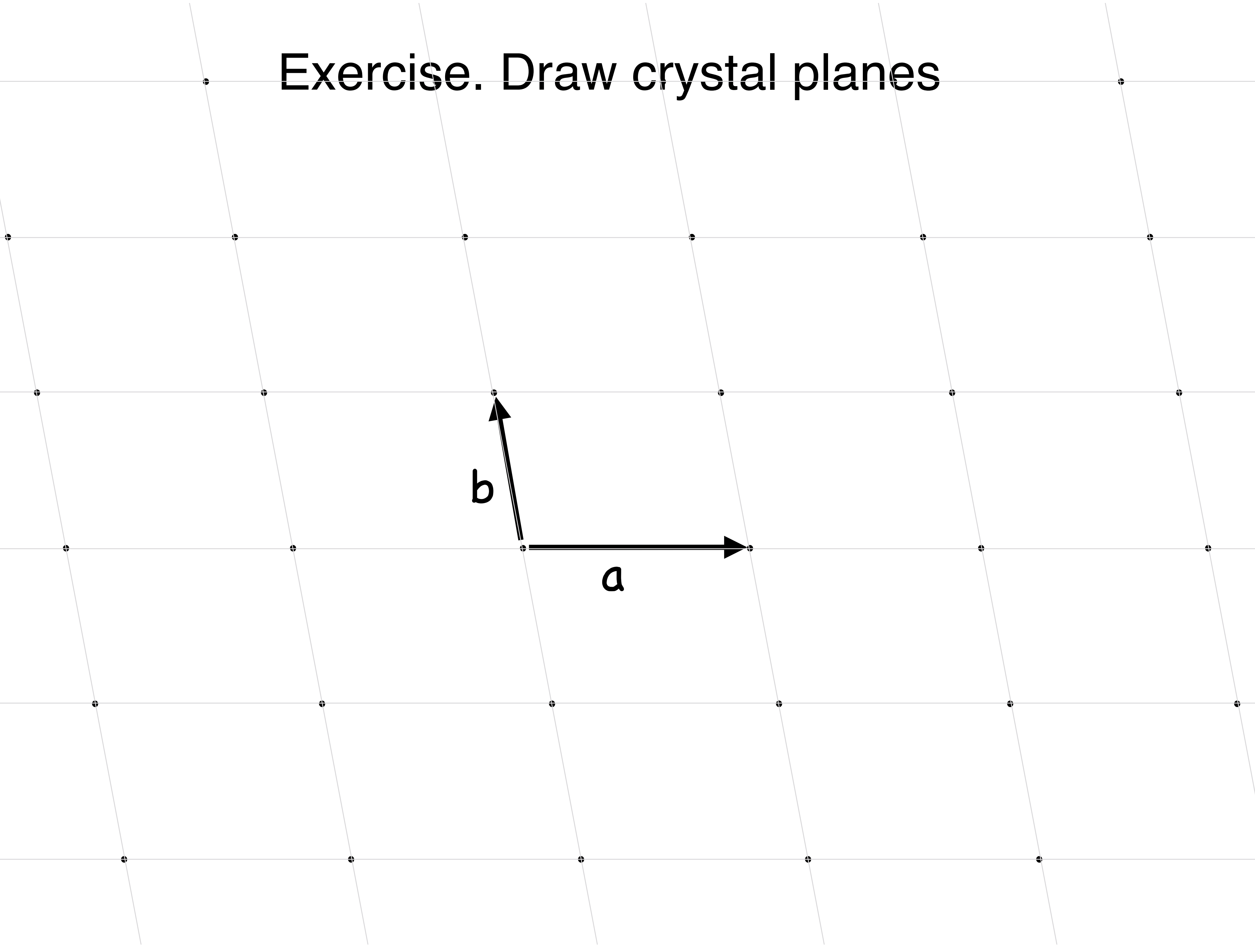
(4 6 6) Bragg planes



Phase-zero planes intersect the cell axes at multiples of fractional coordinates

(1/h,0,0), (0,1/k,0),(0,0,1/l)

Exercise. Draw crystal planes



Proof: The only Bragg planes *of interest are* crystal planes

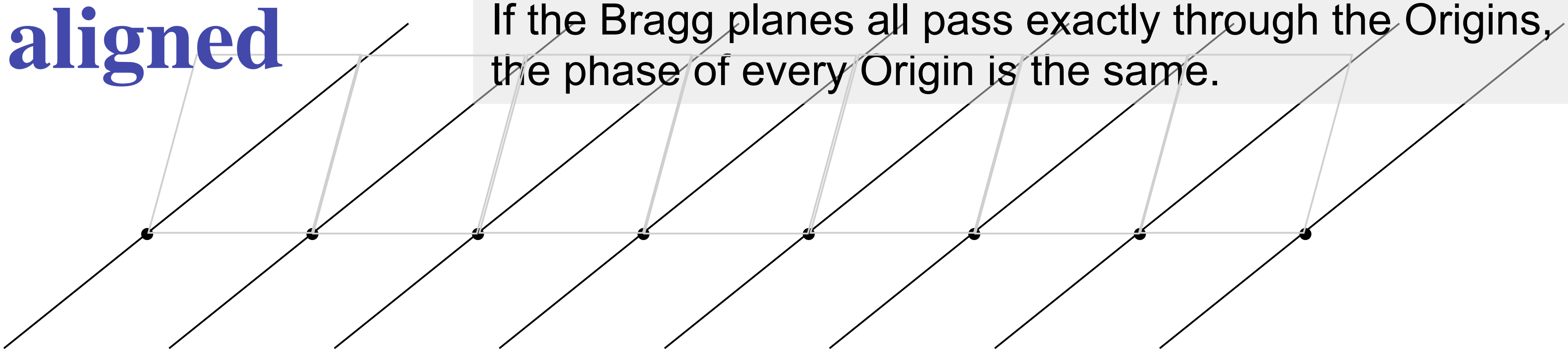
Only crystal planes scatter with non-zero amplitude. Other Bragg planes all have zero amplitide.

Proof: All Bragg planes of phase zero pass through the Origins

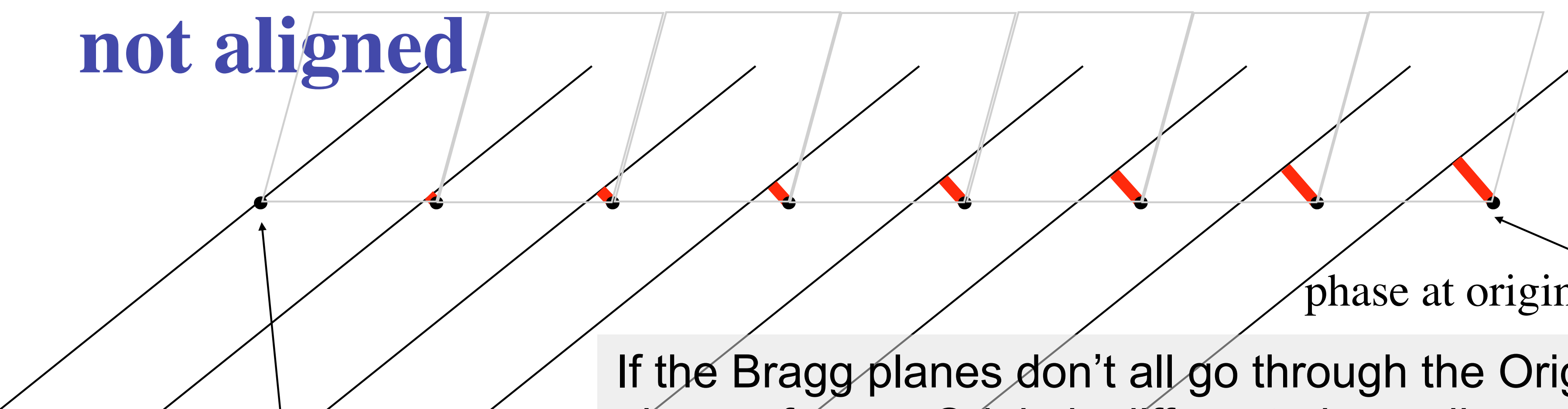
1. Bragg planes are either aligned with the Unit Cell Origins, or they are not.

aligned

If the Bragg planes all pass exactly through the Origins, the phase of every Origin is the same.



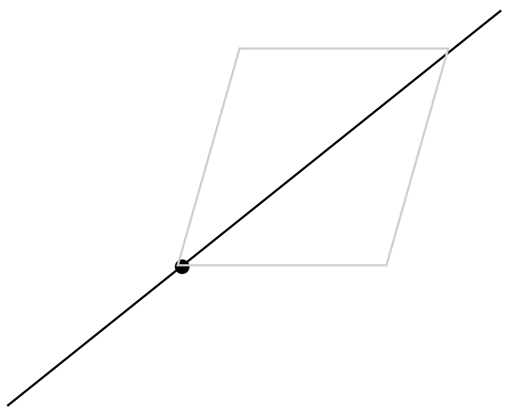
not aligned



If the Bragg planes don’t all go through the Origins, then phase of every Origin is different, depending on the where it is in the crystal.

Proof: All Bragg planes of phase zero pass through the Origins

2. All planes that pass through the Origins have the same number of electrons



The angle and intercept with the Unit Cell determine with atoms are on the plane.

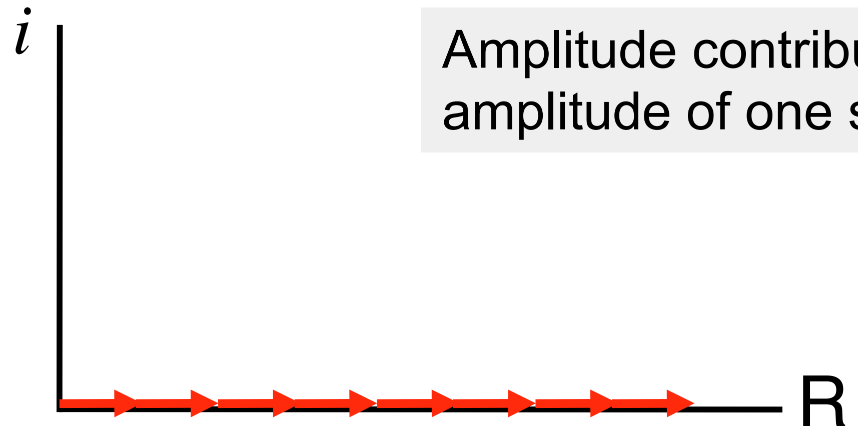
3. All planes that pass through the Origins contribute the same amplitude.

...because amplitude is proportional to number of electrons, and (statement 2).

Proof: All Bragg planes of phase zero pass through the Origins

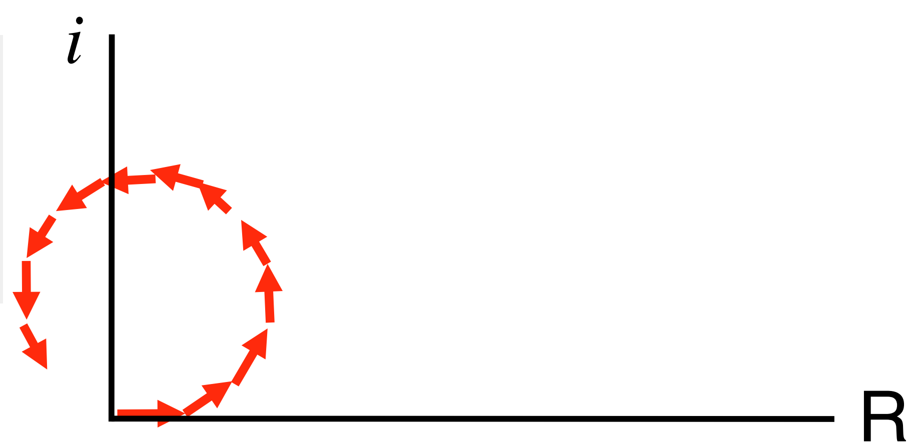
4. Total amplitude is the sum of the amplitudes of the planes if the planes have the same phase.

Amplitude contributed by origin planes is 10K times the amplitude of one such plane, if there are 10K unit cells.



5. Total amplitude is approximately zero if the planes have different phases.

Phase shifts by a constant for each unit cell. Vectors sum in a circle. Summed over 10K unit cells, vector length is small.



Proof: All Bragg planes of phase zero pass through the Origins

6. Any point in the Unit Cell can be the Origin.

7. All equivalent positions by lattice symmetry have the same phase.

Because of statement 6, statements 1-5 apply to any point in the Unit cell.

8. If the Bragg planes do not pass through all Origins, the diffraction amplitude is zero.

Because of statements 7 and

Conclusion: The only Bragg planes that diffract X-rays are the crystal planes.

Crystal planes define a spot in reciprocal space

- Measure d = the distance between planes in Å.
- Calculate θ using Braggs law.

  $n\lambda = 2d \sin(\theta)$, n=1 if d is 1 Bragg distance.

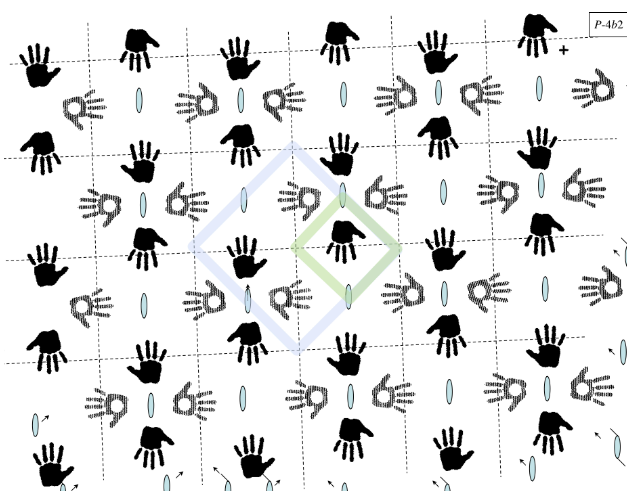
  Therefore $\theta = \sin^{-1}(\lambda/2d)$
- $\mathbf{S} = \mathbf{s} - \mathbf{s}_0$
- $\mathbf{s}$ and $\mathbf{s}_0$ are the same length, so $\mathbf{S}$ is perpendicular to the Bragg planes.
- The length of S, $|S| = 2 \sin(\theta) / \lambda = 1/d$

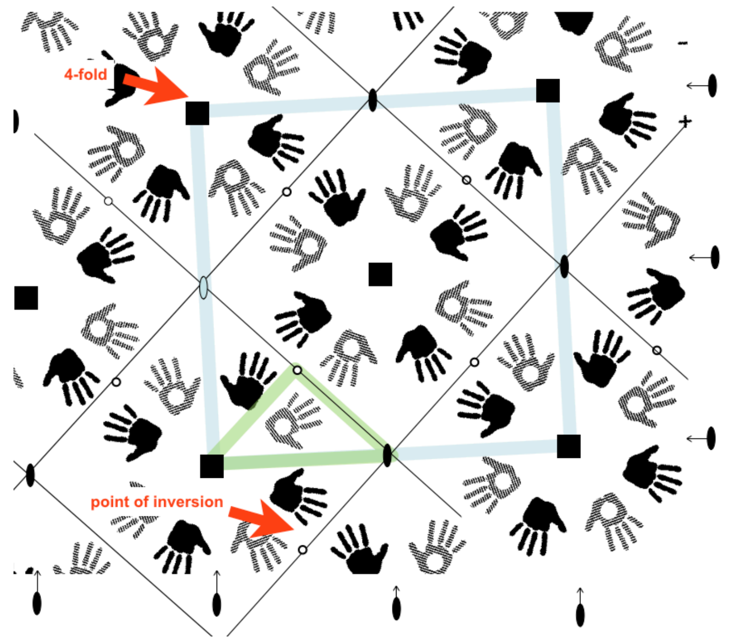
In class: Draw crystal planes (1 1 0), calculate θ, and draw diffraction geometry

In class exercise. Draw crystal planes (-2 -1 0) and draw the direction and length of **S**

Homework 1

- due Thu Oct 29





Review

- Why does a smooth surface reflect light?
- What is Bragg's Law?
- What are Bragg planes?
- What does it mean to talk about Bragg planes that are offset by some distance?
- How does the offset distance translate to a phase?
- What are crystal planes? How are they named?
- Are crystal planes Bragg planes?
- What happens to the total scattered amplitude if the Bragg planes are crystal planes?
- What happens to the total scattered amplitude if the Bragg planes are not crystal planes?