PSD '20 -- Xray lecture 4

- Laue conditions
- Fourier Transform
- The reciprocal lattice
- data collection

Fourier Transform

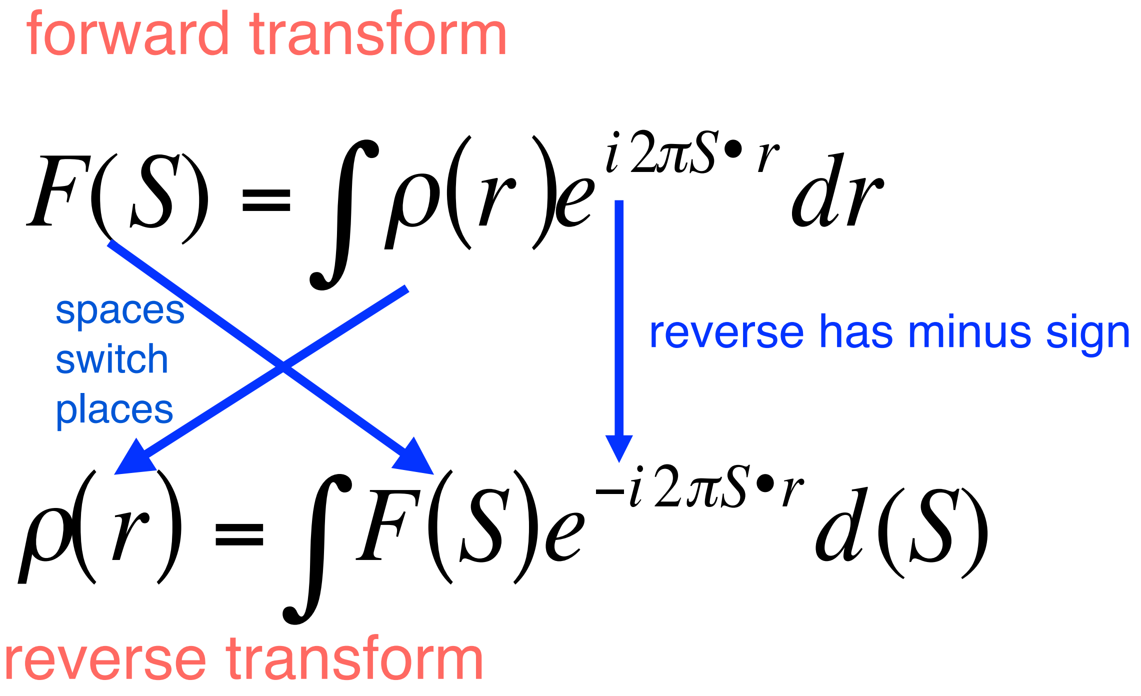
The **Fourier Transform** is a conversion of one space into another space with reciprocal units.

$$F(S) = \int \rho(r) e^{i2\pi S \bullet r} dr$$

The Fourier transform is what Xrays *do* when they scatter from the crystal.

Inverse Fourier Transform

For every Fourier Transform, there exists an inverse Fourier Transform which converts the reciprocal space back to real space.



forward transform

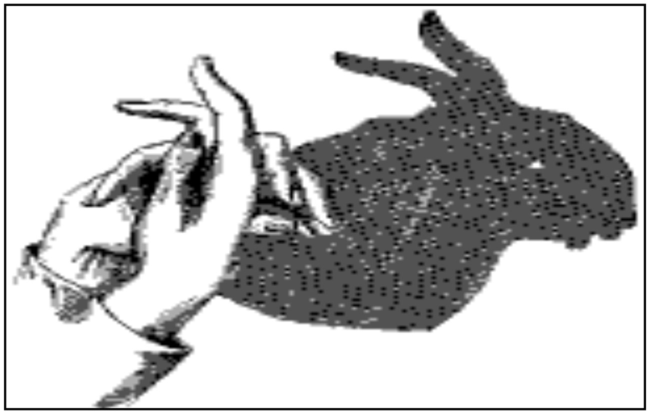
$$F(S) = \int \rho(r) e^{i2\pi S\bullet r} dr$$

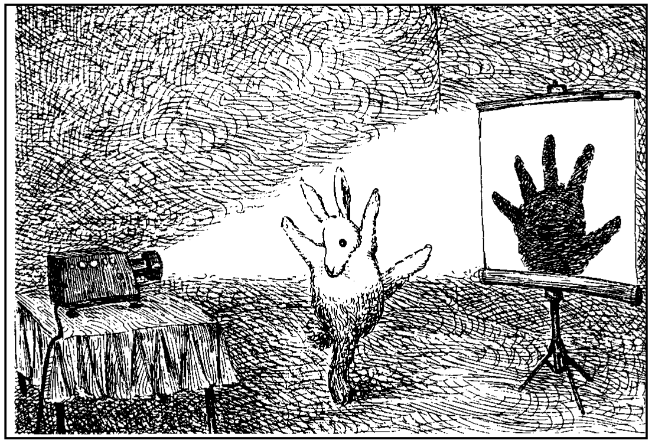
spaces switch places

reverse has minus sign

$$\rho(r) = \int F(S) e^{-i2\pi S\bullet r} d(S)$$

reverse transform





The Inverse Fourier Transform is the electron density that explains the scattering.

Inverse Fourier transform

forward FT $$F(S) = \int \rho(r) e^{i2\pi S \bullet r} dr$$

inverse FT $$\rho(r) = \int F(S) e^{-i2\pi S \bullet r} dS$$

Inverse Fourier transform(proof)

Substitute the *forward transform* into the *inverse transform*. Reduce it. You get *identity*.

$$\rho(r) = \int\left[\int \rho(q) e^{i2\pi S \bullet q} dq\right] e^{-i2\pi S \bullet r} dS$$

For all q ≠ r, the integral is over e^{i} from 0 to 2π, which equals 0.

For q = r, the integral is over e^{0} from 0 to 2π, which equals 1
(actually, 2π, but we correct for that)

Since only q=r matters, we take ρ(r) out of the integral. The equation reduces to an identity …

ρ(r) = ρ(r)

Bragg's law and resolution

Resolution is the spacing between Bragg planes, ***d***

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

Higher resolution means smaller **d**. Good crystal structures have high resolution (less than 2Å). Resolution between 2 and 3Å is "medium" resolution. d > 3 is low resolution.

Crystal planes with higher numbers have smaller d, therefore higher resolution. Therefore reflections with high numbers are high resolution. i.e. F(31 21 0) or F(0 45 0)

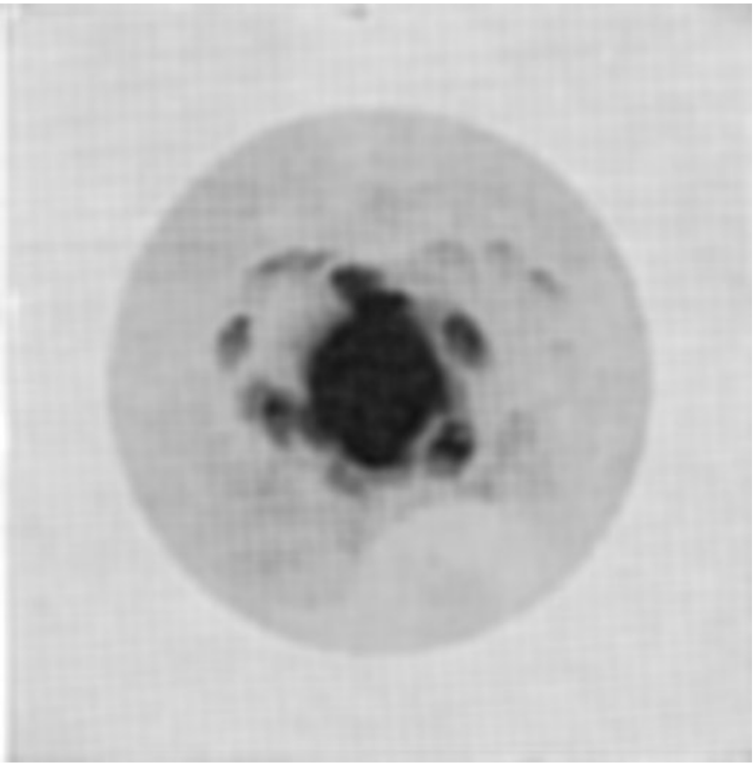
d is defined by λ and Θ. Low Θ means low resolution. High Θ means high resolution.

Given a fixed Θ, lower wavelength λ implies higher resolution. However, λ is fixed for the duration of the experiment.

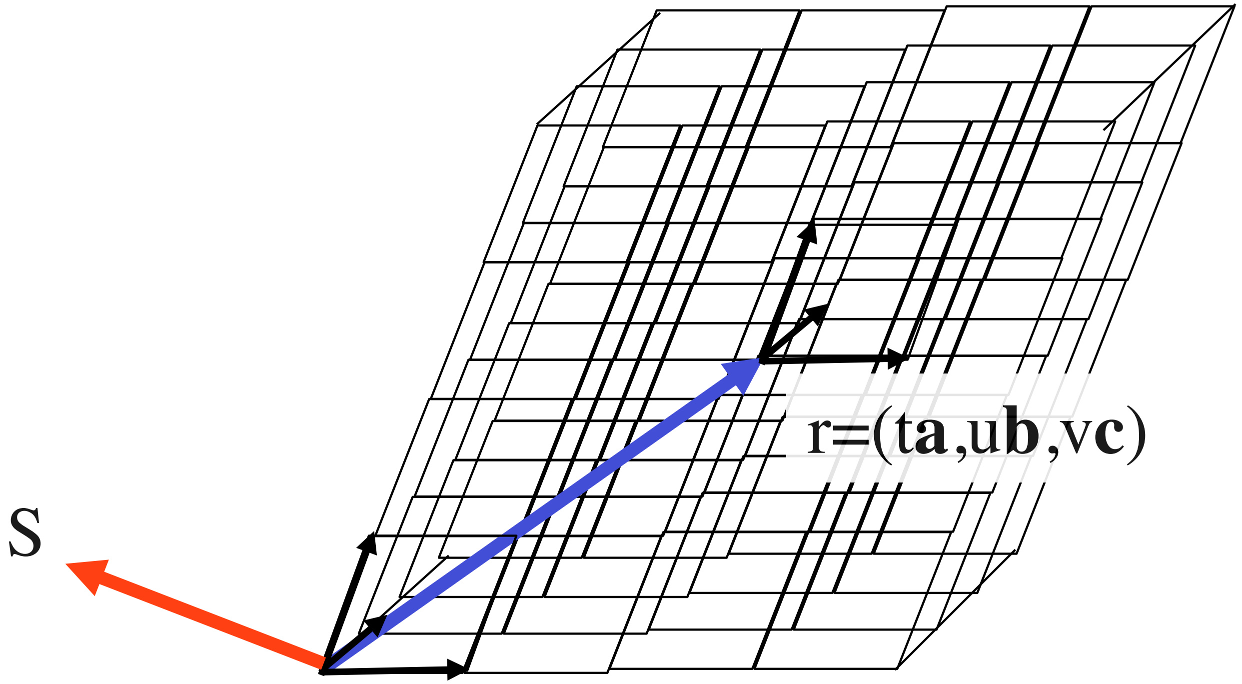
Laue Conditions

or “Bragg planes are Crystal Planes, revisited”

Max von Laue in 1912 reasoned that periodic structures should scatter light only at certain magic angles, and scattering would cancel out at all other angles. His theory was eventually proven true. Spots on the film appear evenly-spaced (not in angle space, but in the space of the *sine* of the angle).



Phase shifts in the crystal lattice must be integer multiples of 2pi, or there is no diffraction



$$\rho(r) = \rho(\boldsymbol{r} + t\mathbf{a} + u\mathbf{b} + v\mathbf{c})$$

phase shift= $2\pi S \bullet r = 2\pi(S \bullet t\mathbf{a} + S \bullet u\mathbf{b} + S \bullet v\mathbf{c})$

where a,b,and c are the unit cell axes and t,u, and v are integers.

What **S** are allowed?

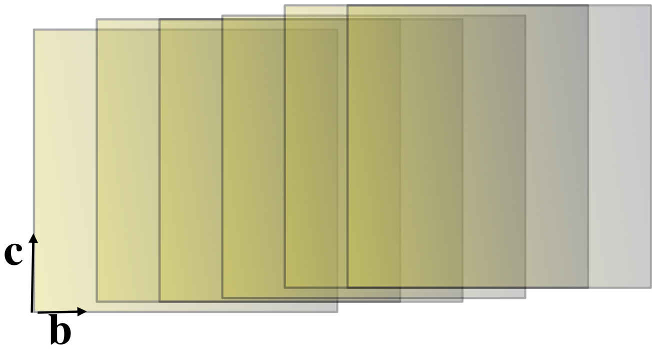
phase of arbitrary origin

$$e^{i2\pi(S\bullet t\mathbf{a}+S\bullet u\mathbf{b}+S\bullet v\mathbf{c})} = e^{i\underline{2\pi S\bullet t}\mathbf{a}}e^{i2\pi S\bullet u\mathbf{b}}e^{i2\pi S\bullet v\mathbf{c}}$$

- Any integer multiple 2π is equal to a full 360° phase shift and interferes constructively.
- All three dot products, S•**a** , S•**b** , S•**c** must be integers, because if they were not integers, then different origins (t,u,v) would have different phases.

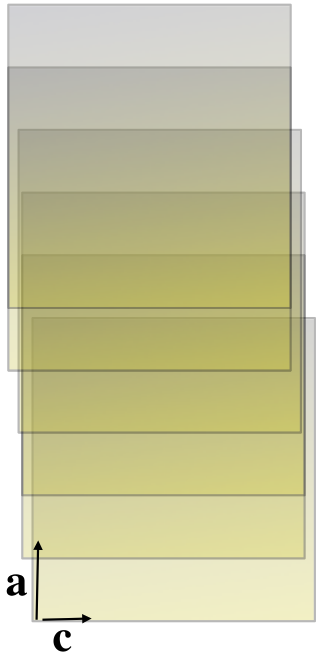
Allowed values of S are evenly spaced in 3 directions

Integer values of S•**a**



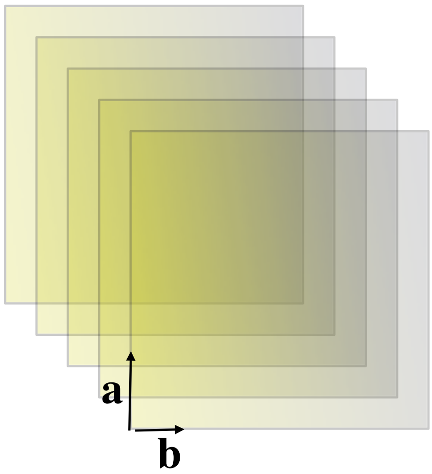
∩

Integer values of S•**b**



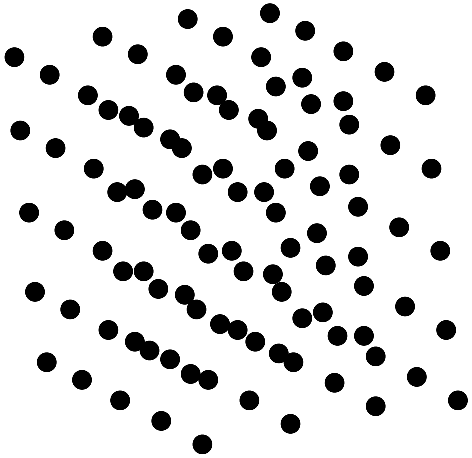
∩

Integer values of S•**c**



The intersection of planes perpendicular to **a**, **b** and **c**, becomes...

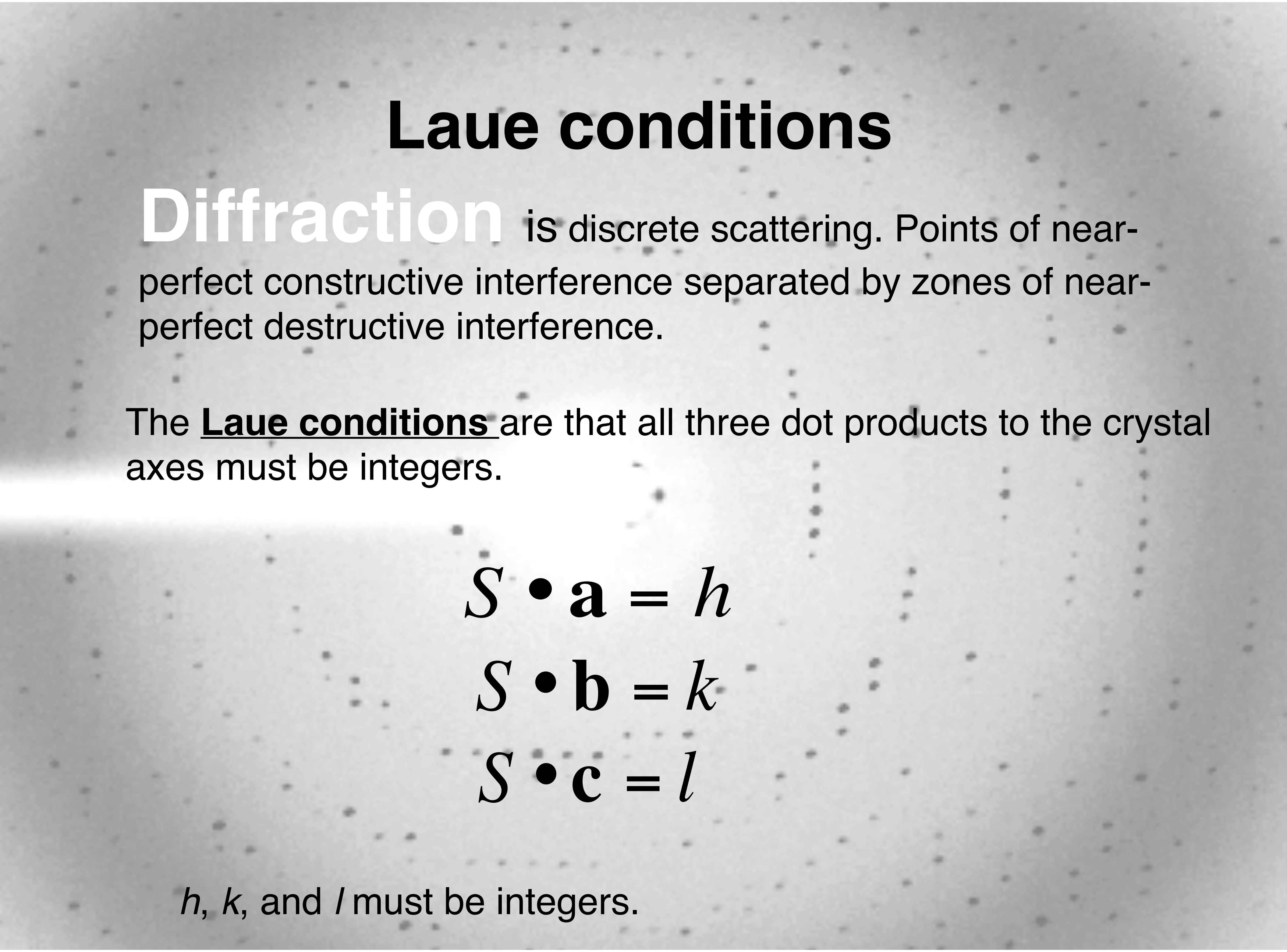
=



… **points** of diffraction, in **S** space.

Points in diffraction space *correspond to* Bragg planes in real space.

Laue conditions



Diffraction is discrete scattering. Points of near-perfect constructive interference separated by zones of near-perfect destructive interference.

The **Laue conditions** are that all three dot products to the crystal axes must be integers.

$$S \bullet \mathbf{a} = h$$

$$S \bullet \mathbf{b} = k$$

$$S \bullet \mathbf{c} = l$$

h, *k*, and *l* must be integers.

Units of Reciprocal space, a*, b* and c*

The reciprocal lattice axes are the shortest non-zero vectors S for which the Laue conditions hold:
In other words...

$$\mathbf{a}^{*} \bullet \mathbf{a} = 1$$

$$\mathbf{b}^{*} \bullet \mathbf{b} = 1$$

$$\mathbf{c}^{*} \bullet \mathbf{c} = 1$$

reciprocal lattice axes

complete Laue conditions

$$\mathbf{a}^{*} \bullet \mathbf{a} = 1 \qquad \mathbf{b}^{*} \bullet \mathbf{a} = 0 \qquad \mathbf{c}^{*} \bullet \mathbf{a} = 0$$

$$\mathbf{a}^{*} \bullet \mathbf{b} = 0 \qquad \mathbf{b}^{*} \bullet \mathbf{b} = 1 \qquad \mathbf{c}^{*} \bullet \mathbf{b} = 0$$

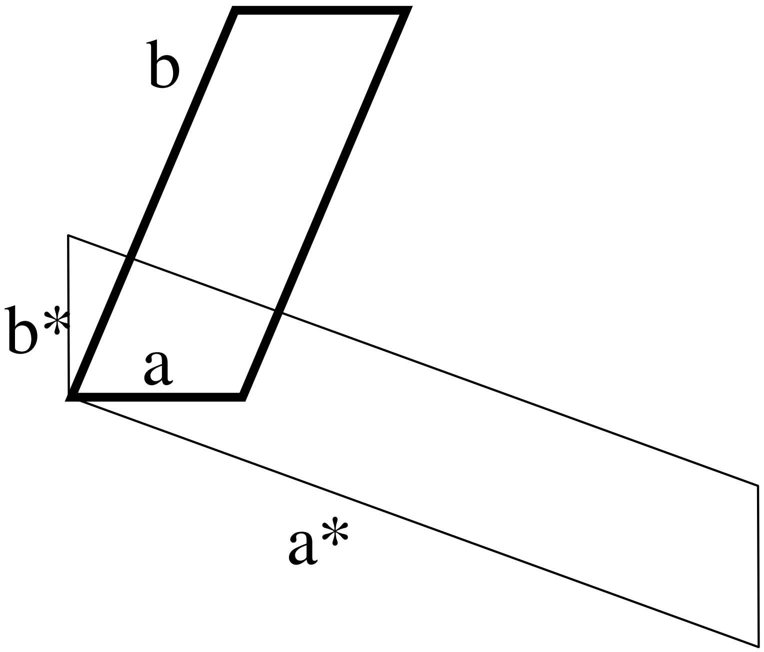
$$\mathbf{a}^{*} \bullet \mathbf{c} = 0 \qquad \mathbf{b}^{*} \bullet \mathbf{c} = 0 \qquad \mathbf{c}^{*} \bullet \mathbf{c} = 1$$

$S = (h\mathbf{a^*}+k\mathbf{b^*}+l\mathbf{c^*})$

Where the reflection are....

$$S = (h\mathbf{a^*}+k\mathbf{b^*}+l\mathbf{c^*})$$

Real cell relations reciprocal cell



Inequalities are opposite:

If a < b, then a* > b*

Cell axes are normals.

$a^* \perp b \quad a^* \perp c$

$b^* \perp a \quad b^* \perp c$

$c^* \perp a \quad c^* \perp b$

Note: real and reciprocal cell are different spaces with different units and different origins, but they are both "attached" to the crystal and rotate with the crystal.

Orthorhombic cell, all 90° angles

$$\mathbf{a}^{*} \bullet \mathbf{a} = 1$$

$$\mathbf{b}^{*} \bullet \mathbf{b} = 1$$

$$\mathbf{c}^{*} \bullet \mathbf{c} = 1$$

If the axes are 90° apart, then **a*** is parallel to **a**, and so on. So the dot-product is just the product of the lengths. Therefore, the length of **a*** is 1/l**a**l, and so on.

la*l = 1/lal

lb*l = 1/lbl

lc*l = 1/lcl

Proof of phase calculation using Miller indexes

The only allowed values of S, according to Laue

$$S = (h\mathbf{a}^*+k\mathbf{b}^*+l\mathbf{c}^*)$$

$v =$ Å coordinates $x\mathbf{a} + y\mathbf{b} + z\mathbf{c}$

$$\begin{aligned}\alpha &= 2\pi S\bullet v = \\ &= 2\pi(h\mathbf{a}^*+k\mathbf{b}^*+l\mathbf{c}^*)\bullet(x\mathbf{a}+y\mathbf{b}+z\mathbf{c}) \\ &= 2\pi(hx+ky+lz) \\ &= 2\pi\mathbf{h}\bullet\mathbf{r}\end{aligned}$$

No abc. No a*b*c*. Axes cancel out!

where, $\mathbf{h}=(h,k,l)$ = Miller indeces of a reflection

$\mathbf{r}=(x,y,z)$ = *fractional coordinates* of an atom

Proof of Miller indeces phase calculation

- From Laue conditions:

$$\begin{array}{lll} \mathbf{a}^* \bullet \mathbf{a} = 1 & \mathbf{b}^* \bullet \mathbf{a} = 0 & \mathbf{c}^* \bullet \mathbf{a} = 0 \\ \mathbf{a}^* \bullet \mathbf{b} = 0 & \mathbf{b}^* \bullet \mathbf{b} = 1 & \mathbf{c}^* \bullet \mathbf{b} = 0 \\ \mathbf{a}^* \bullet \mathbf{c} = 0 & \mathbf{b}^* \bullet \mathbf{c} = 0 & \mathbf{c}^* \bullet \mathbf{c} = 1 \end{array}$$

- Let $\mathbf{S} = h\mathbf{a}^* + k\mathbf{b}^* + l\mathbf{c}^*$

reciprocal space coordinates hkl

- Let $\mathbf{v} = x\mathbf{a} + y\mathbf{b} + z\mathbf{c}$

real space fractional coordinates xyz

- Multiplying out the dot-product,

$$\text{phase} = 2\pi\mathbf{S}\bullet\mathbf{v} = 2\pi(h\mathbf{a}^* + k\mathbf{b}^* + l\mathbf{c}^*)\bullet(x\mathbf{a} + y\mathbf{b} + z\mathbf{c})$$

$$= 2\pi(hx\underbrace{\mathbf{a}^*\bullet\mathbf{a}}_{1} + hy\underbrace{\mathbf{a}^*\bullet\mathbf{b}}_{0} + hz\underbrace{\mathbf{a}^*\bullet\mathbf{c}}_{0} + kx\underbrace{\mathbf{b}^*\bullet\mathbf{a}}_{0} + ky\underbrace{\mathbf{b}^*\bullet\mathbf{b}}_{1} + kz\underbrace{\mathbf{b}^*\bullet\mathbf{c}}_{0} + lx\underbrace{\mathbf{c}^*\bullet\mathbf{a}}_{0} + ly\underbrace{\mathbf{c}^*\bullet\mathbf{b}}_{0} + lz\underbrace{\mathbf{c}^*\bullet\mathbf{c}}_{1})$$

$$= 2\pi(hx + ky + lz)$$

$$= 2\pi\mathbf{h}\bullet\mathbf{r}$$

The forward transform

$$F(h,k,l) = \sum \rho(x,y,z)\, e^{i2\pi(hx + ky + lz)}$$

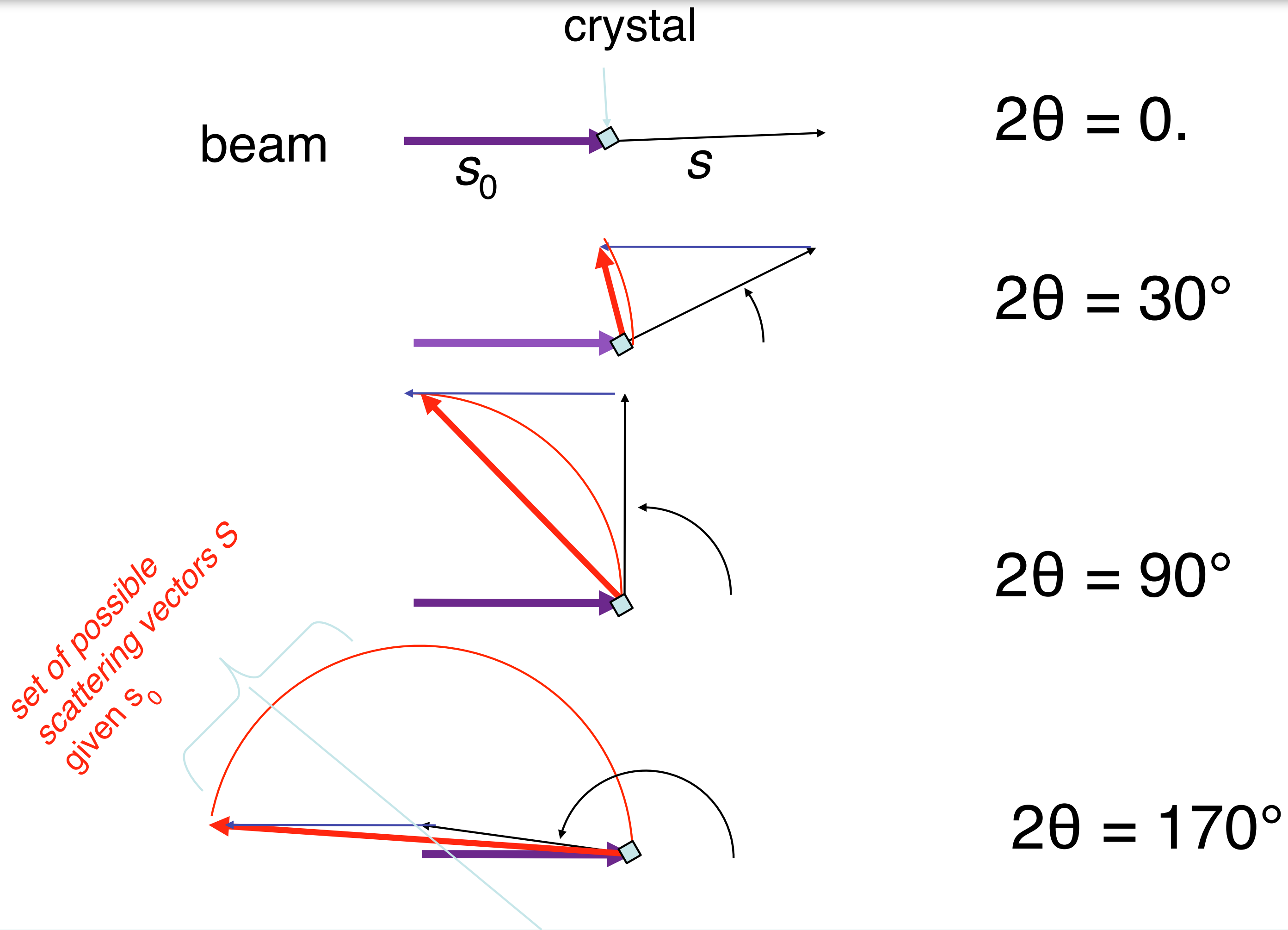
The reverse transform

$$\rho(x,y,z) = \sum F(h,k,l)\, e^{-i2\pi(hx + ky + lz)}$$

Ewald sphere

…is the part of reciprocal space you see on the detector without moving the beam

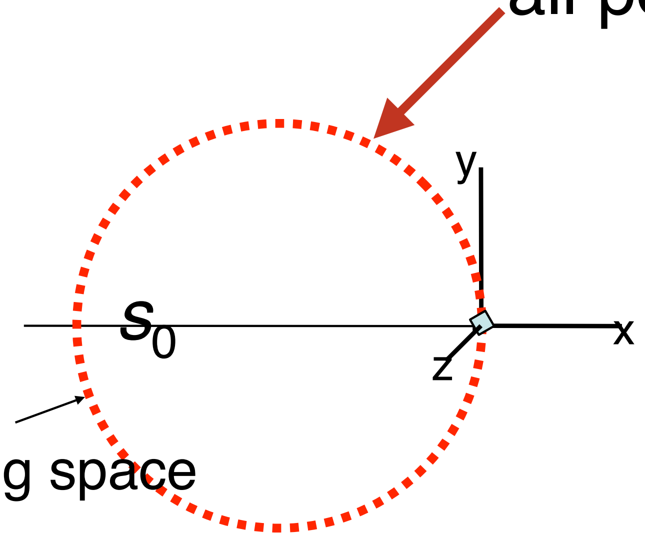
Given θ where is S?



For a given direction of the incoming x-rays, the Ewald sphere is the *set of possible scattering vectors S* given s_0.

The radius of the sphere is 1/λ (reciprocal units, of course)

The **Ewald sphere** is defined as the the locus of **all possible scattering vectors** (**S**) when the beam (**s$_0$**) is fixed.



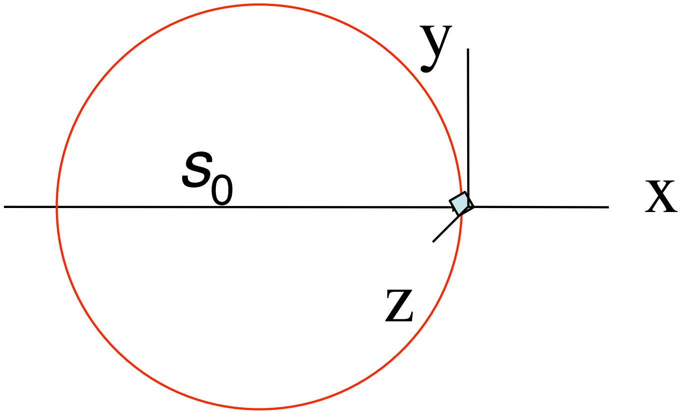
all possible *S*

This sphere is in scattering space (*reciprocal space*).

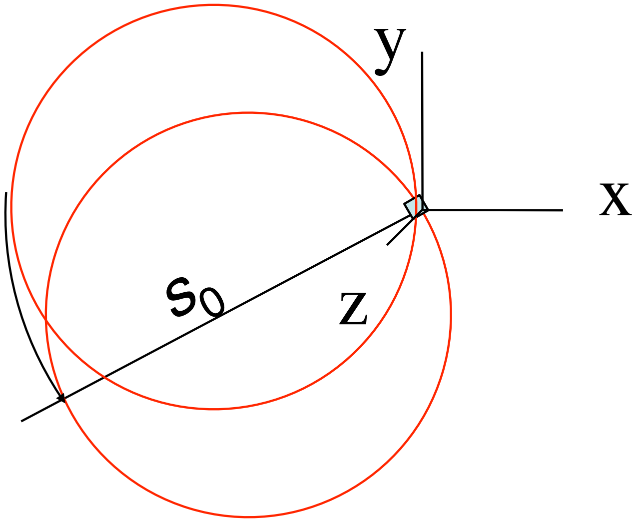
Crystal position defines the coordinate axes.

If a scattered wave has S on the Ewald sphere, it is visible on the film/detector.

Moving the beam (or the crystal) samples all reciprocal space



For a given direction of the incoming x-rays, the set of possible scattering vectors *S* is the surface of a *sphere* of radius 1/λ, passing through the crystallographic origin.

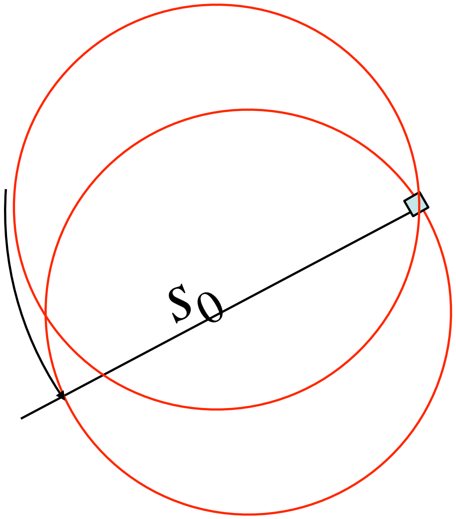


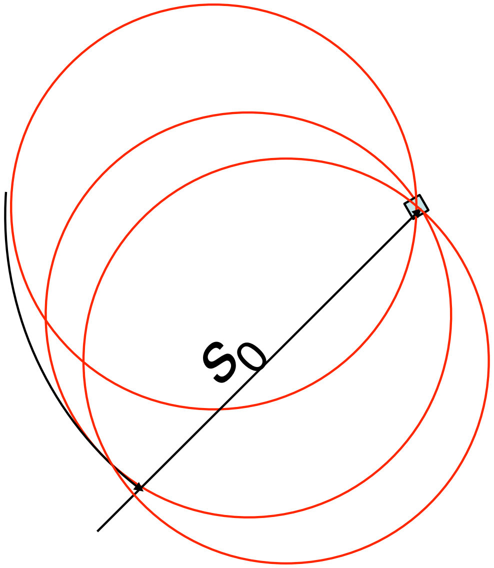
Keeping the crystal fixed, we rotate the X-ray source. The *Ewald Sphere* moves in parallel with the X-ray source. The new set of *S* vectors describe the phase vs. direction of scatter for that position of the source.

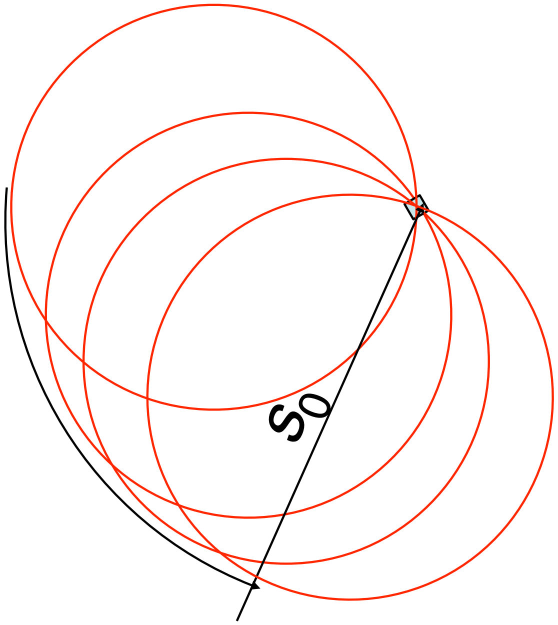
reciprocal space

Moving the beam. Crystal fixed.

but we don't really do it this way.

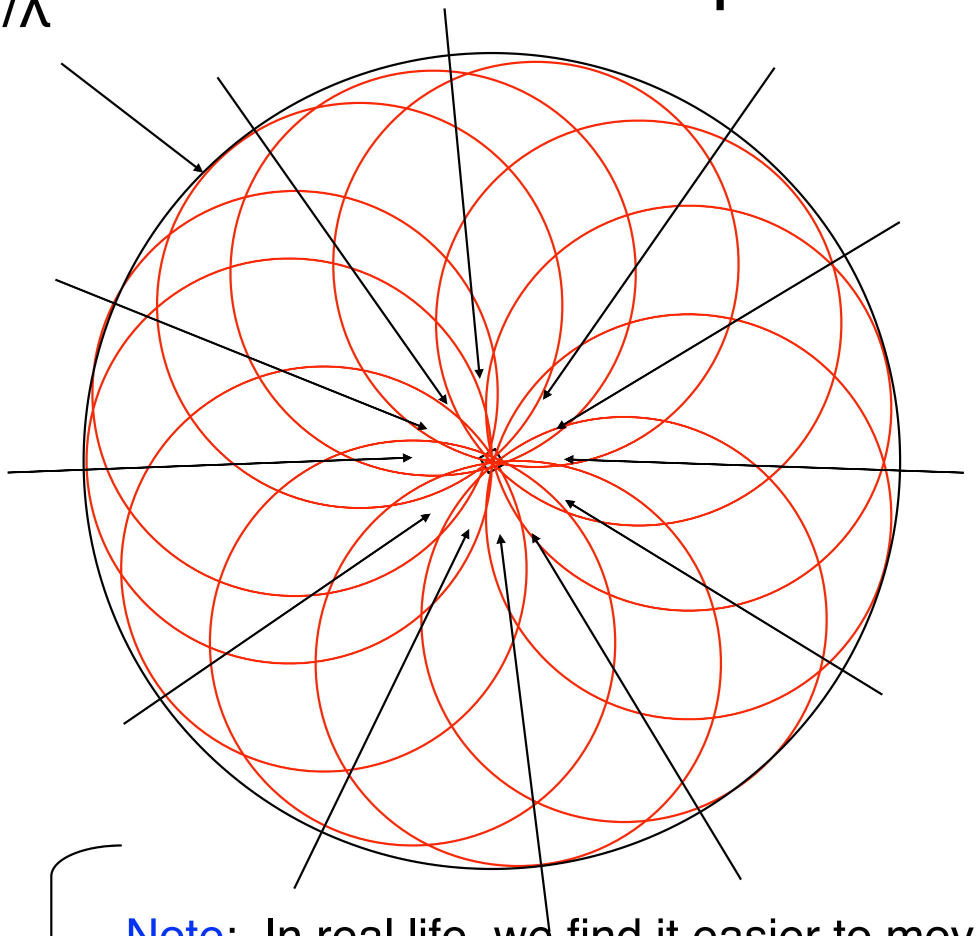






By moving the X-ray source relative to the crystal, we can sample every possible *S*

The visible part of reciprocal space depends on the diffraction limit.



limit of all visible reciprocal space. radius = 2/λ

The set of all vectors *S* (red), given all possible directions of the beam (black arrows), is called reciprocal space.

Note: In real life, we find it easier to move the crystal, not the beam.

The reciprocal lattice moves with the crystal.

Watch the data collection movie. See the intersection of a sphere and a lattice?

http://ucxray.berkeley.edu/~jamesh/movies/diffraction.mpeg

Summary: properties of the reciprocal lattice

- Spacing in reciprocal space is inverse of real space.

- Reciprocal lattice is fixed relative to the crystal and rotates with the crystal.

- The Ewald sphere defines which reciprocal lattice points (reflections) that are visible to the detector for a given orientation of the crystal and beam position.

- Direction of reciprocal axes are orthogonal to real cell axes. i.e. $\mathbf{a}^* \perp \mathbf{b}$ and $\mathbf{a}^* \perp \mathbf{c}$

- Using fractional coordinates and Miller indeces in the Fourier transform means wavelength and cell dimensions drop out.

Data collection

Data collection

- Xray data collection is the process of **rotating the crystal in the beam** while taking **snapshots** of the diffraction pattern.
- Rotating the crystal rotates the reciprocal lattice through the (fixed) Ewald sphere. When a reflection passes through the Ewald sphere it satisfies reflection geometry and a spot appears on the film.
- The location of the spot on the film (or detector) determines the Bragg planes from which it reflected (h,k,l)

- The intensity F^2 of the spot on the film (determined by **densitometry**, background correction and symmetry averaging) is proportional to the degree of electron density variation in the direction perpendicular to the Bragg planes and at resolution *d*.
- Xray data consists of **h, k, l, F and sigma**. Sigma is the standard deviation of F over several measurements.

F = Square-root of the measured intensity of each reflection.

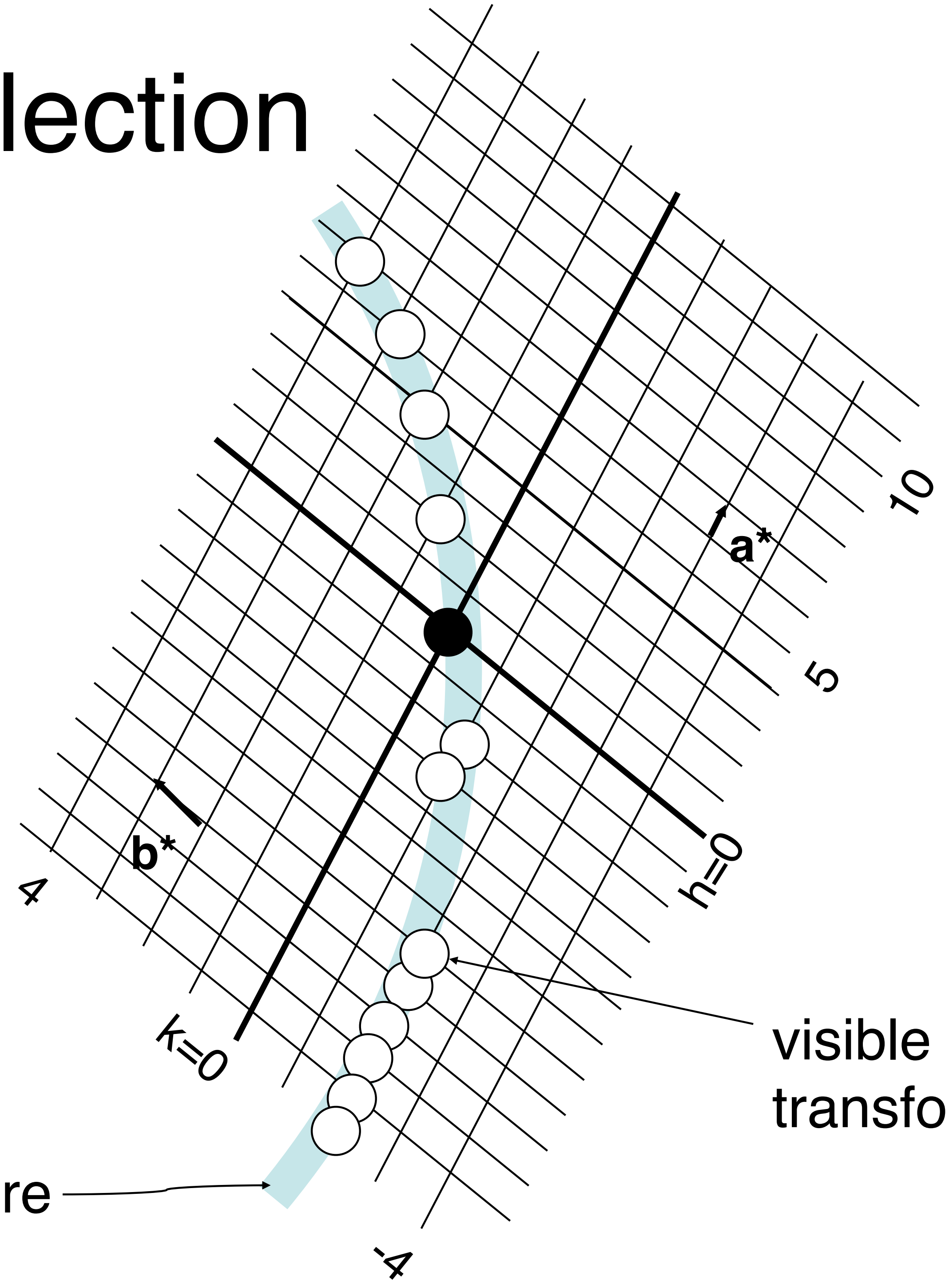
h, k,l = Indeces of Scattering vector (Miller indeces)

sigma = standard deviation over syms

h k l F sigma

data collection

film 1

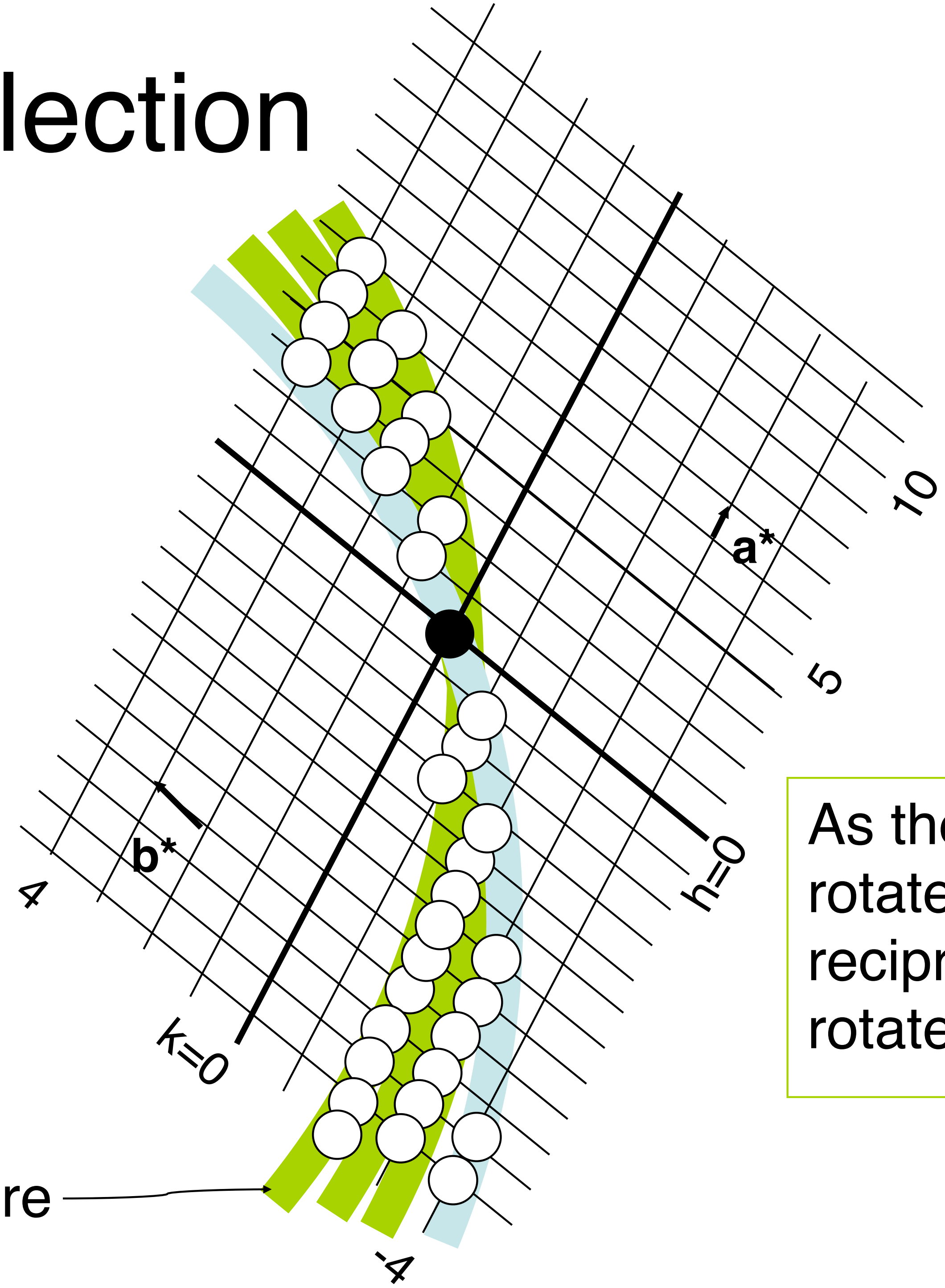


visible part of transform

Ewald sphere

data collection

film 4

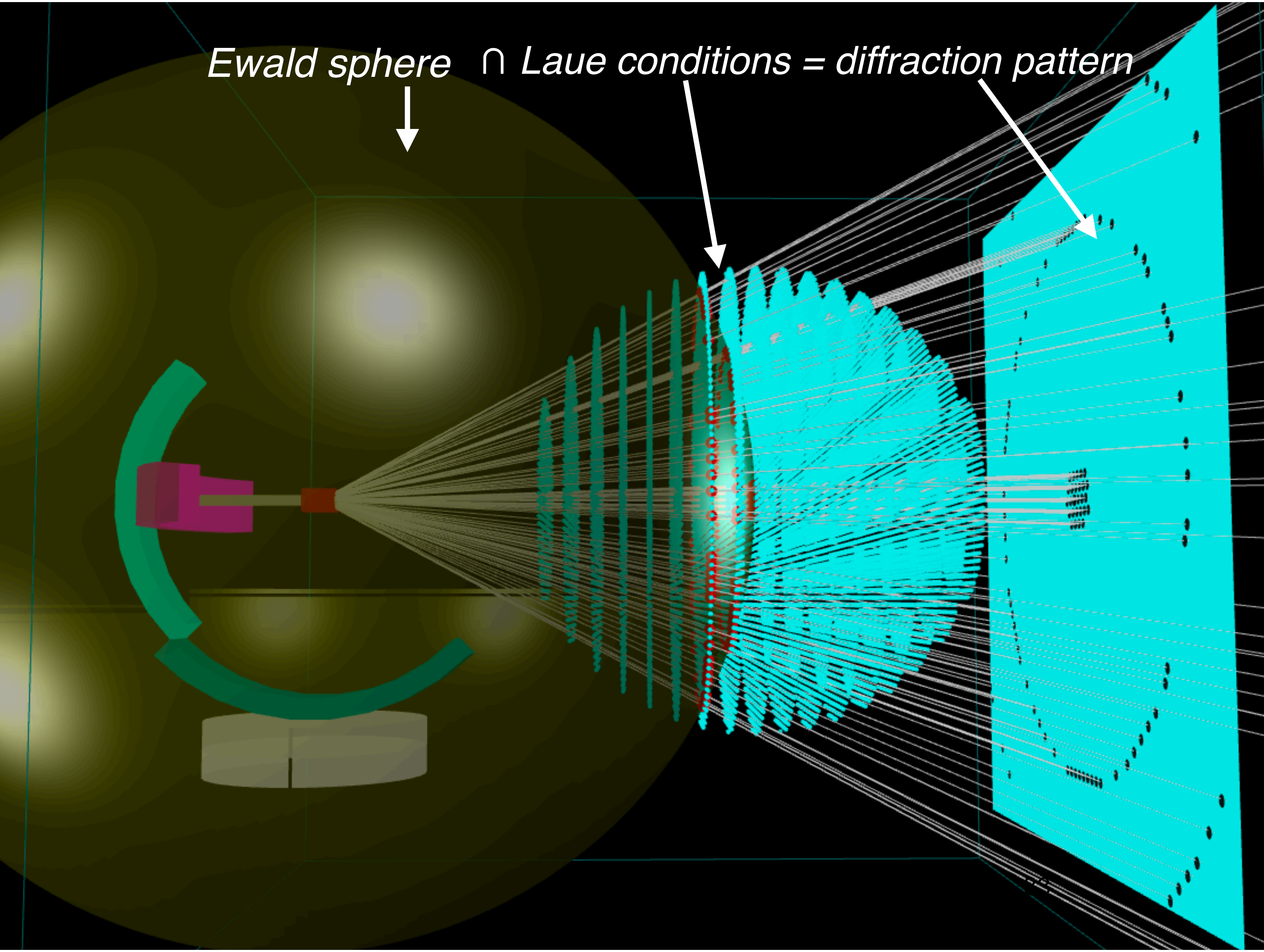


Ewald sphere

As the crystal is rotates, the reciprocal lattice rotates.

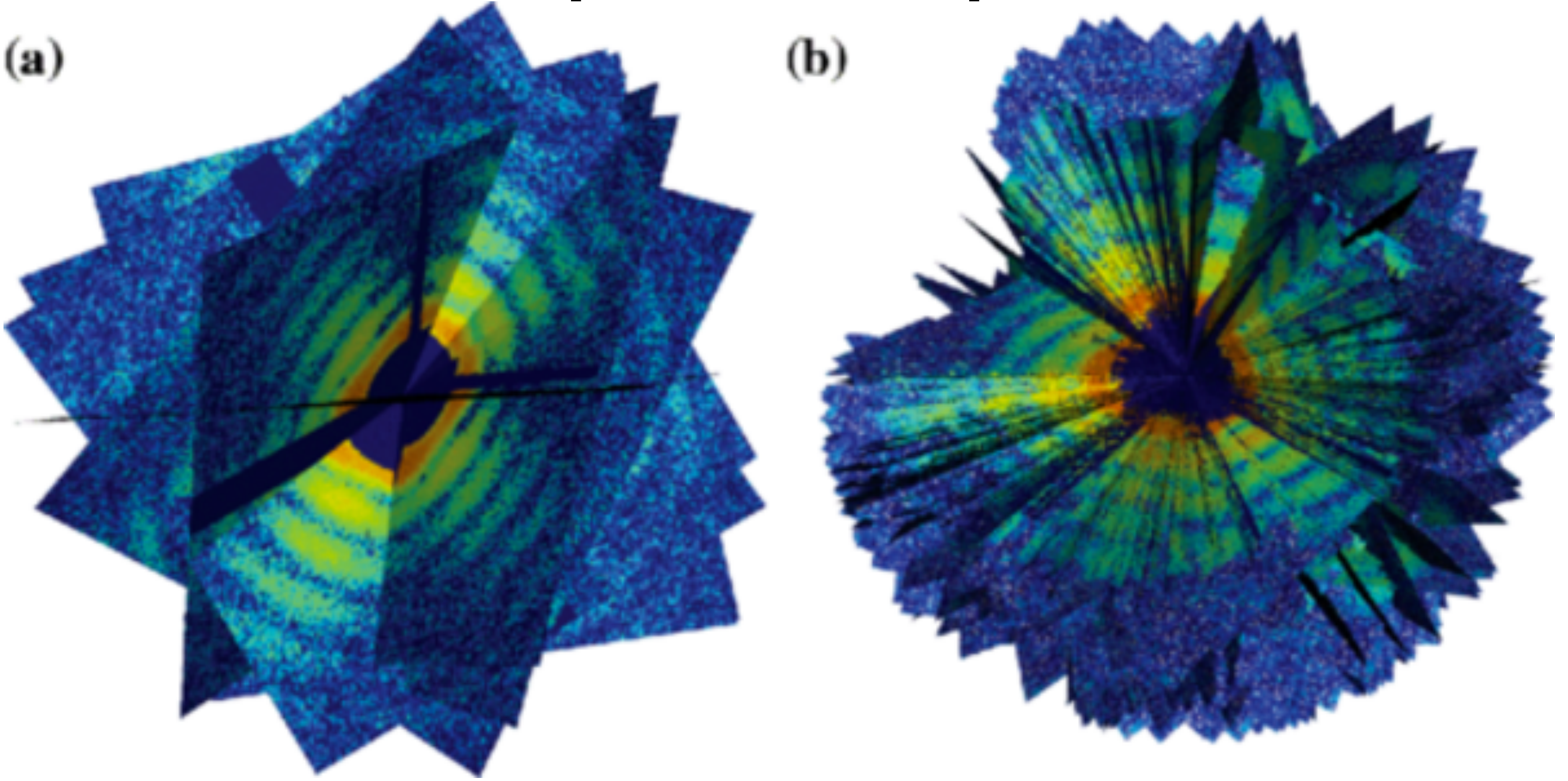
Exercise: Xrayview

- Install Xrayview (http://www.phillipslab.org/downloads)
- Play with settings for until cell lengths.
- Play with orientation, wavelength.
- Note how the *Ewald sphere is a window to the Laue conditions*.
- Reflections that appear on the film must satisfy both Laue conditions and Ewald sphere (reflection geometry).

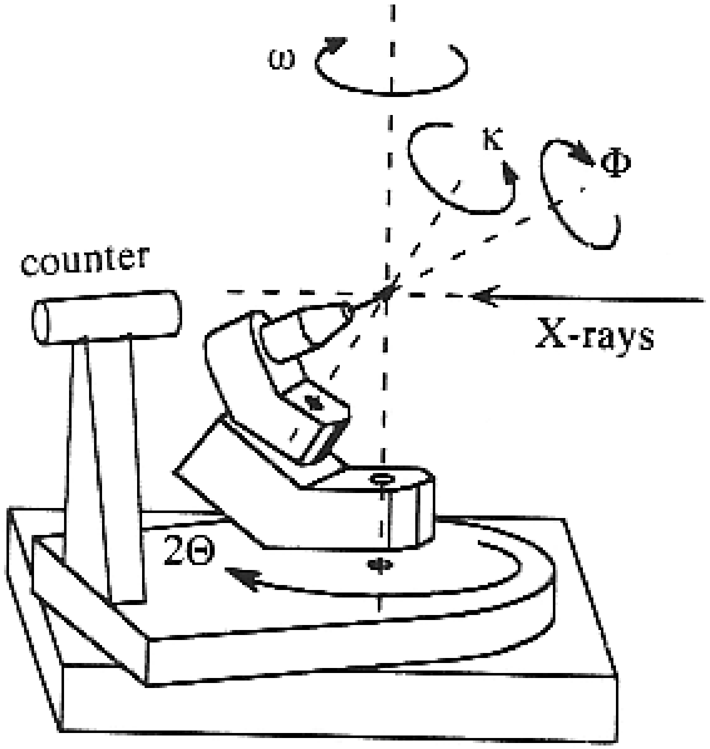


Ewald sphere ∩ Laue conditions = diffraction pattern

Each image is a slice of 3D reciprocal space



Diffractometers yesterday and today



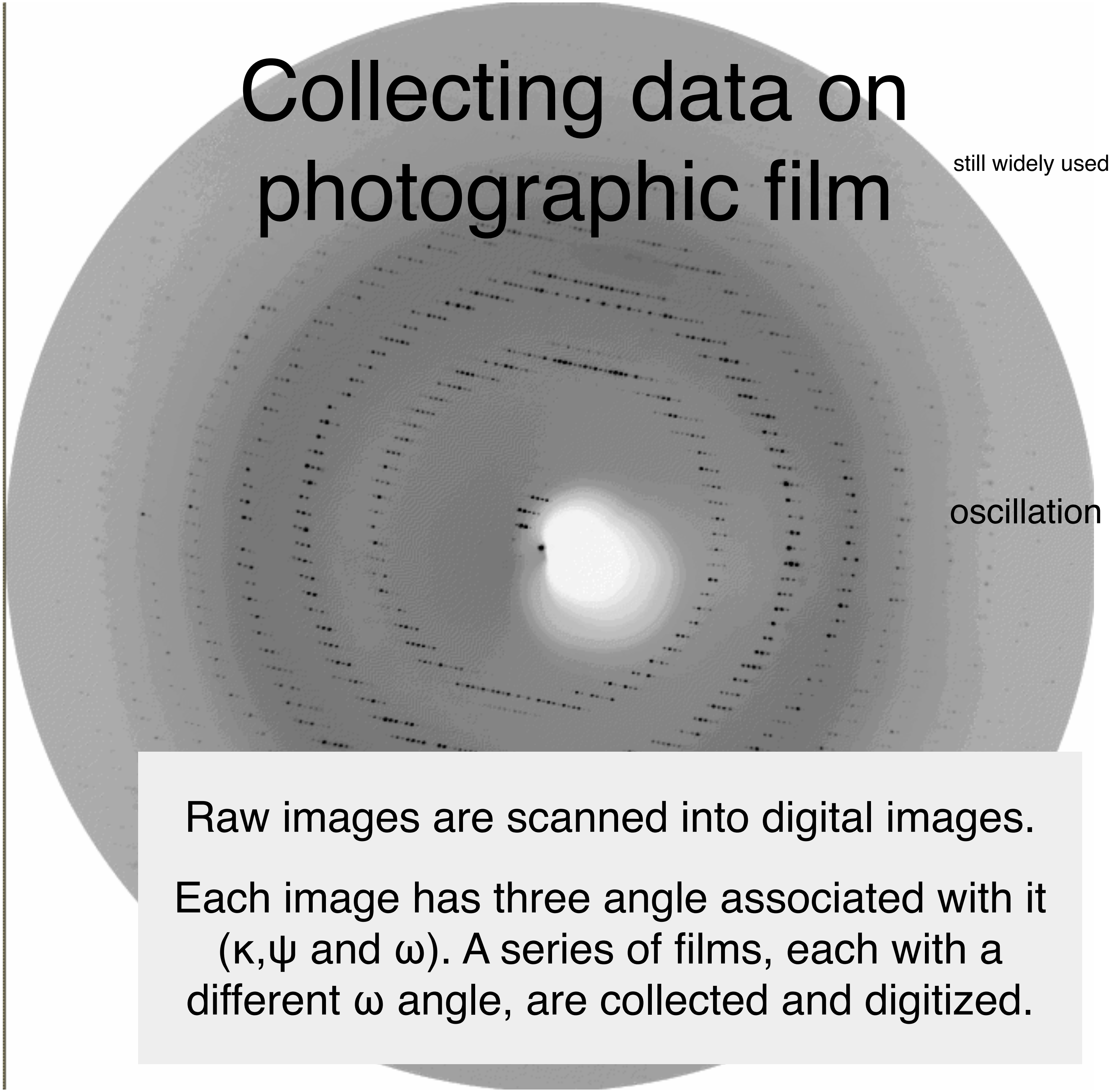
Counter moves in 2θ.

Crystal moves in 3 angles ω,κ, and ψ

Single photon counter (photo multiplier tube)

Collecting data on photographic film

still widely used!



oscillation image

Raw images are scanned into digital images.

Each image has three angle associated with it (κ,ψ and ω). A series of films, each with a different ω angle, are collected and digitized.

Image plates

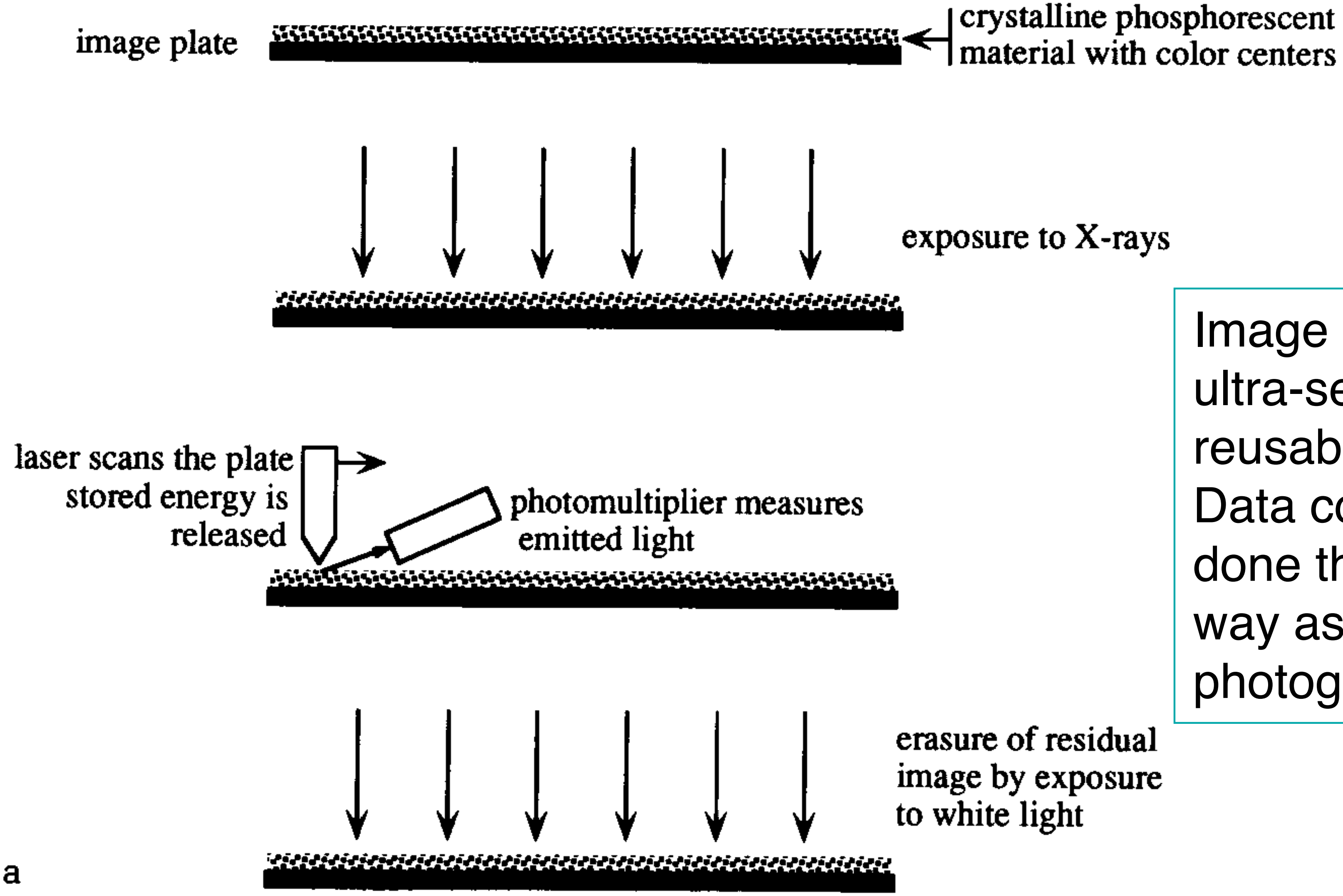
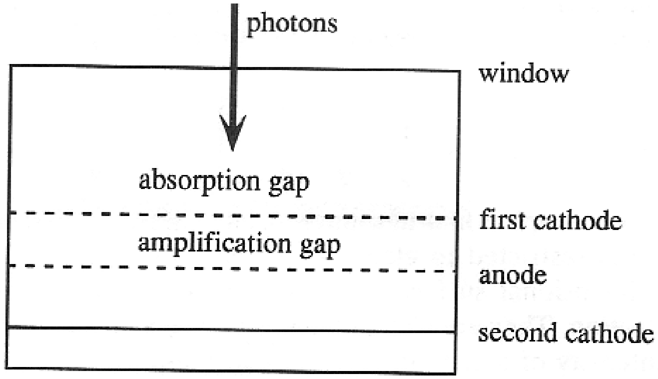
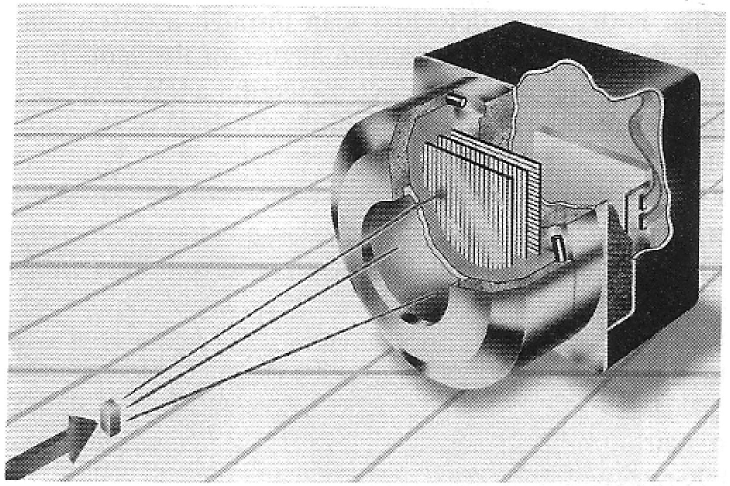


Image plates are ultra-sensitive, reusable films. Data collection is done the same way as for photographic film.

CCD area detectors



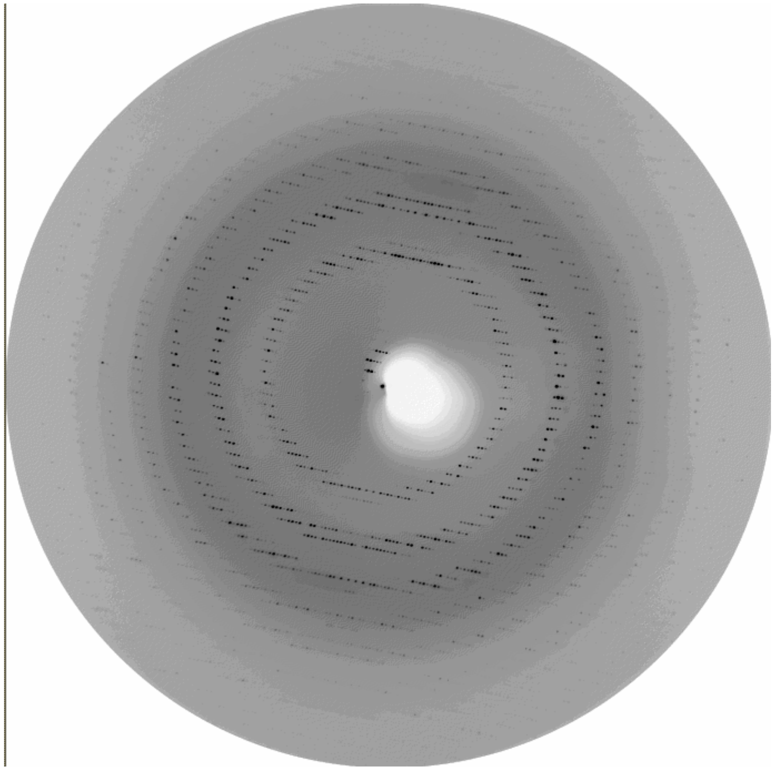
a



b

Position sensitive X-ray detectors give a 3D image of each spot, where film or image plates give 2D images.

Data reduction



raw images

- indexing
- background estimation
- integration of spots
- merging of partials
- scaling
- merging of syms

software

hkl	F	σ
200	99.0	0.2
210	65.1	0.3
201	78.5	0.2
220	6.3	0.1
221	19.9	0.2
222	88.1	0.2

...

reduced data

The Data reduction process: images to hkl F σ

•**indexing** = finding the location of each reciprocol lattice point HKL

•**background estimation** = like subtracting the baseline, in 2D

•**integration of spots** = intensity is proportional to F^2

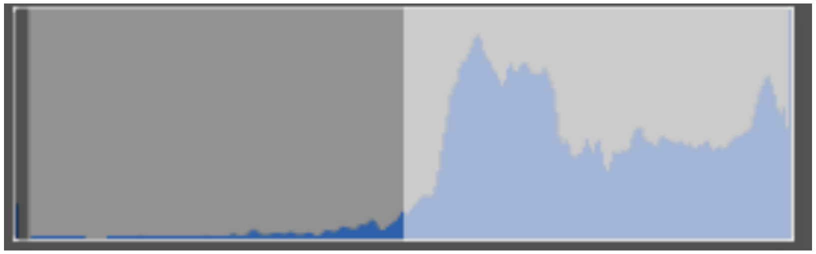
•**merging of partials** = One reflection may be split between two films.

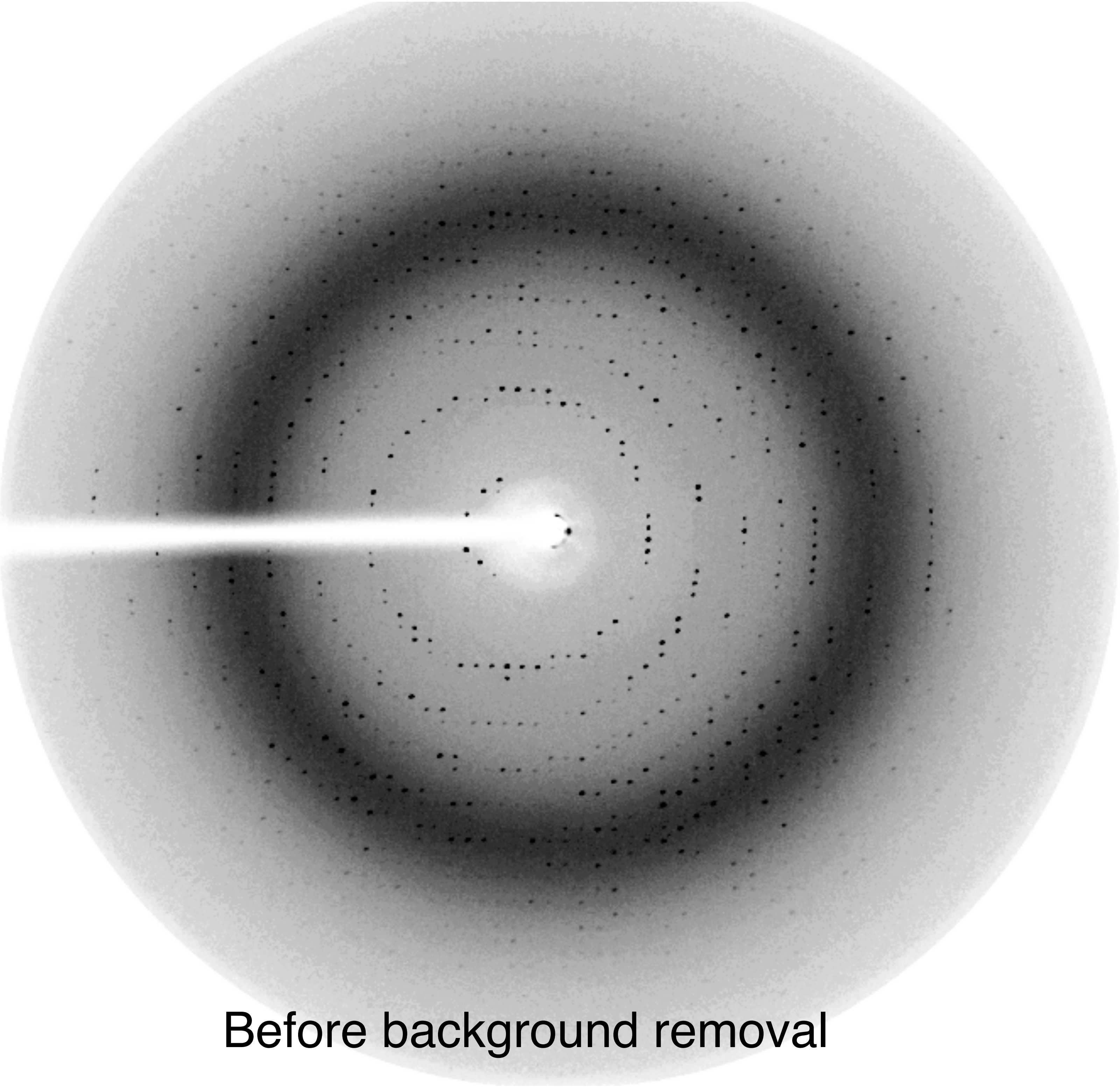
•**Scaling** = If there is significant decay, then data is scaled in blocks of time.

•**Averaging of syms** = Symmetry-related reflections are averaged

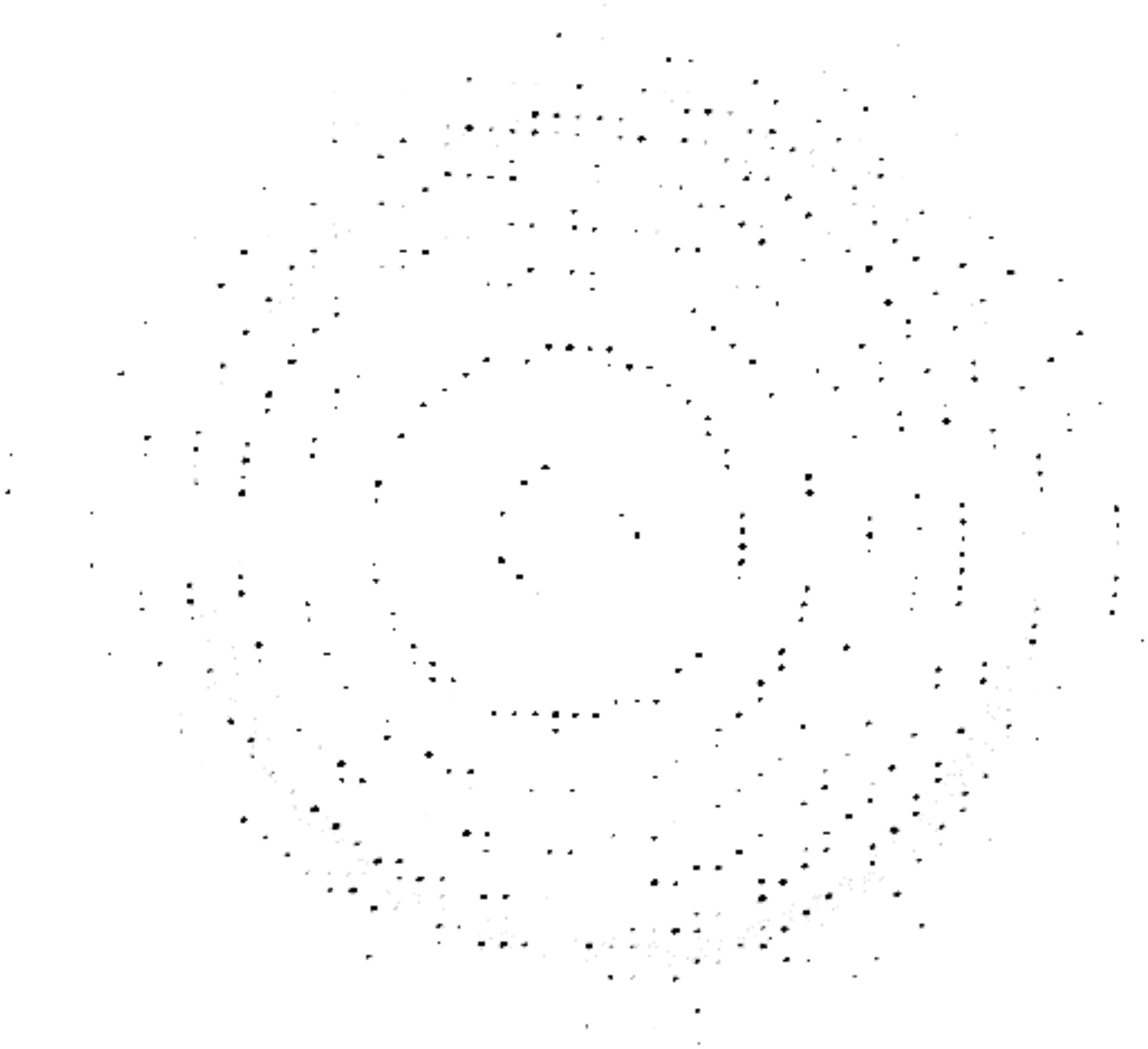
Background removal

- Identify the location of each spot
- Sum the average intensity around the spot.
- Subtract the average intensity.
- Integrate over the spot to get F_{hkl}





Before background removal

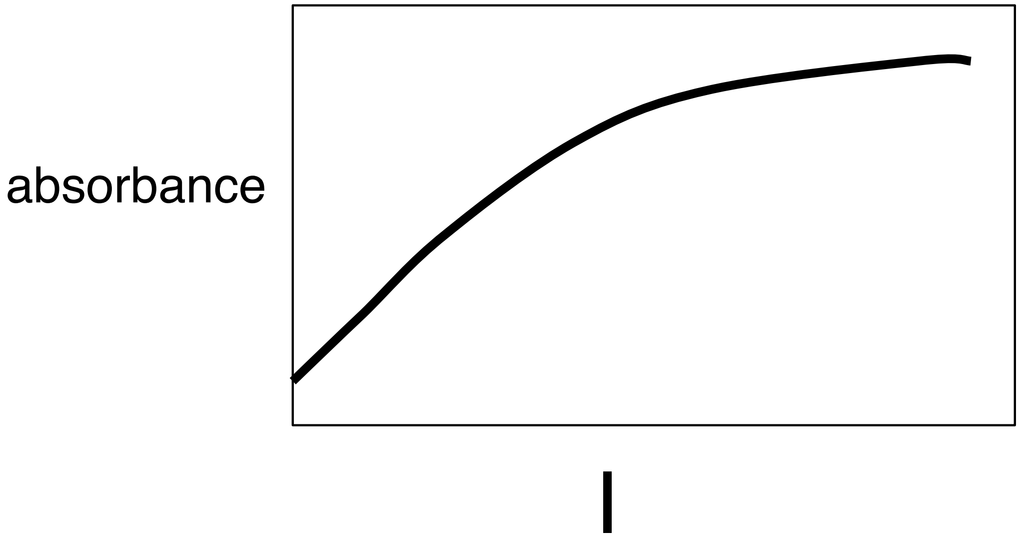


After background removal

Calibrating the film or detector.

For photographic film, or any type of X-ray counter, a calibration curve has been pre-calculated.

The pixels are counted, multiplied by “I” from the calibration curve, to get I(hkl) for each spot.



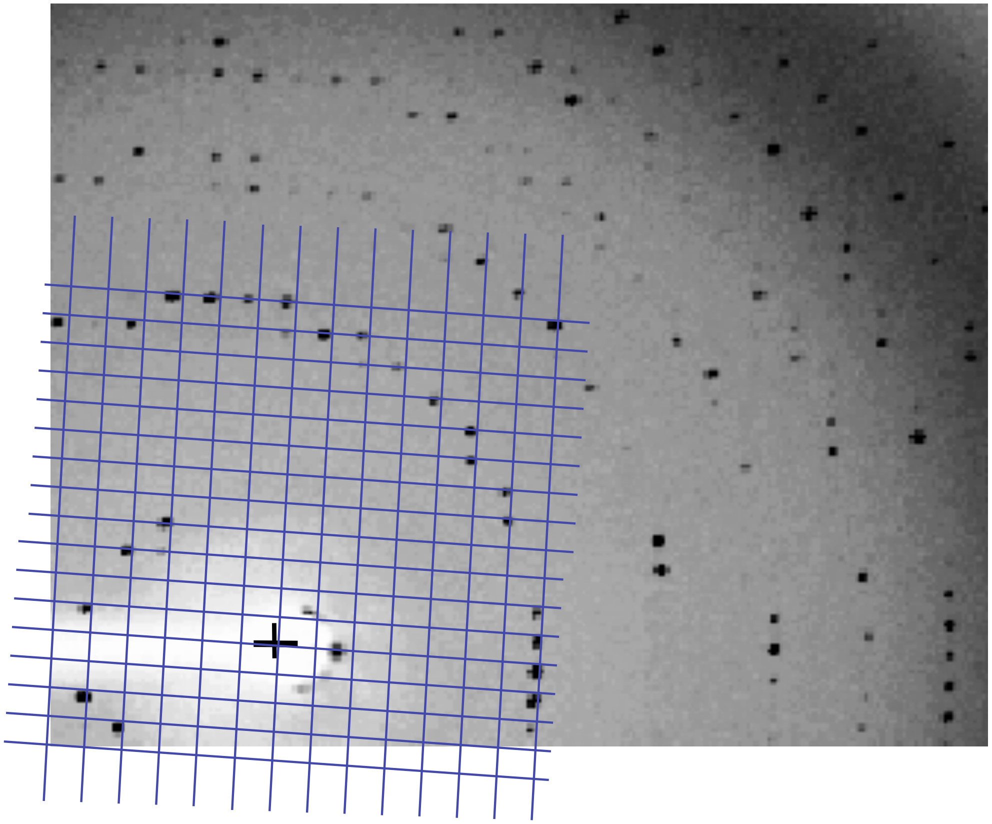
Indexing the data

A reciprocal lattice is initialized using the known cell dimensions.

Spots are predicted to be at the places where the lattice intersects the Ewald sphere.

A systematic search (rotation of the lattice) is done until the predictions match the observations.

Small refinements in the beam position might be required.

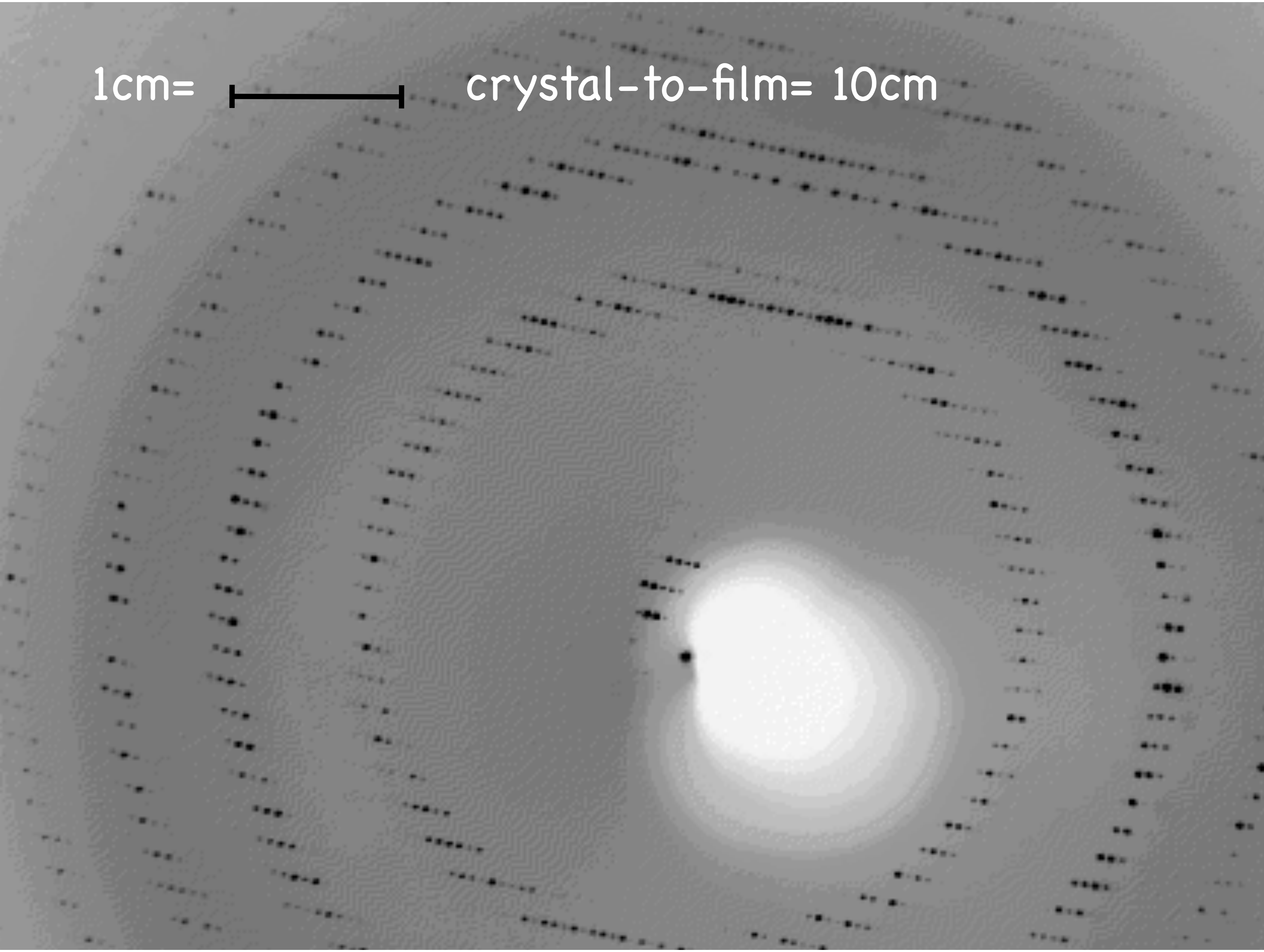


When the solution is found, every spot has an index (hkl).

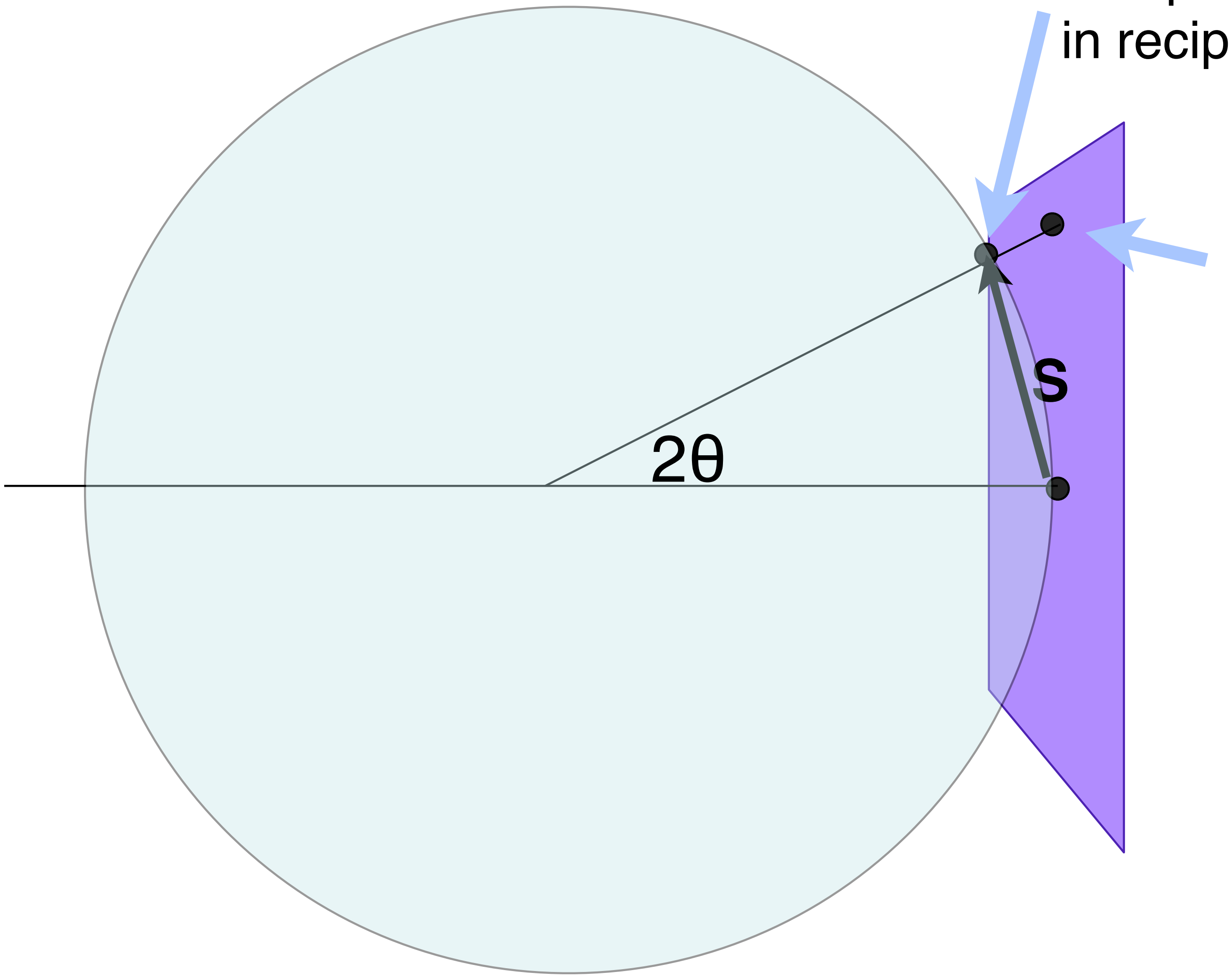
Exercise 3: Determine the unit cell dimensions from the diffraction pattern (due Nov 2)

Draw/write on these pages. Save as PDF. upload to
http://www.bioinfo.rpi.edu/bystrc/courses/bcbp4870/homework.html

- d = spacing between spots in a row, in mm.
- L = Crystal-to-film distance in mm = 100
- Bragg angle $\theta = \tan^{-1}(d/L)/2$
- $|a| = \lambda/(2\sin(\theta))$
- $|a| \approx \lambda L/d$
- Calculate $|a|$, $|b|$ and γ^*


1cm=
crystal-to-film= 10cm

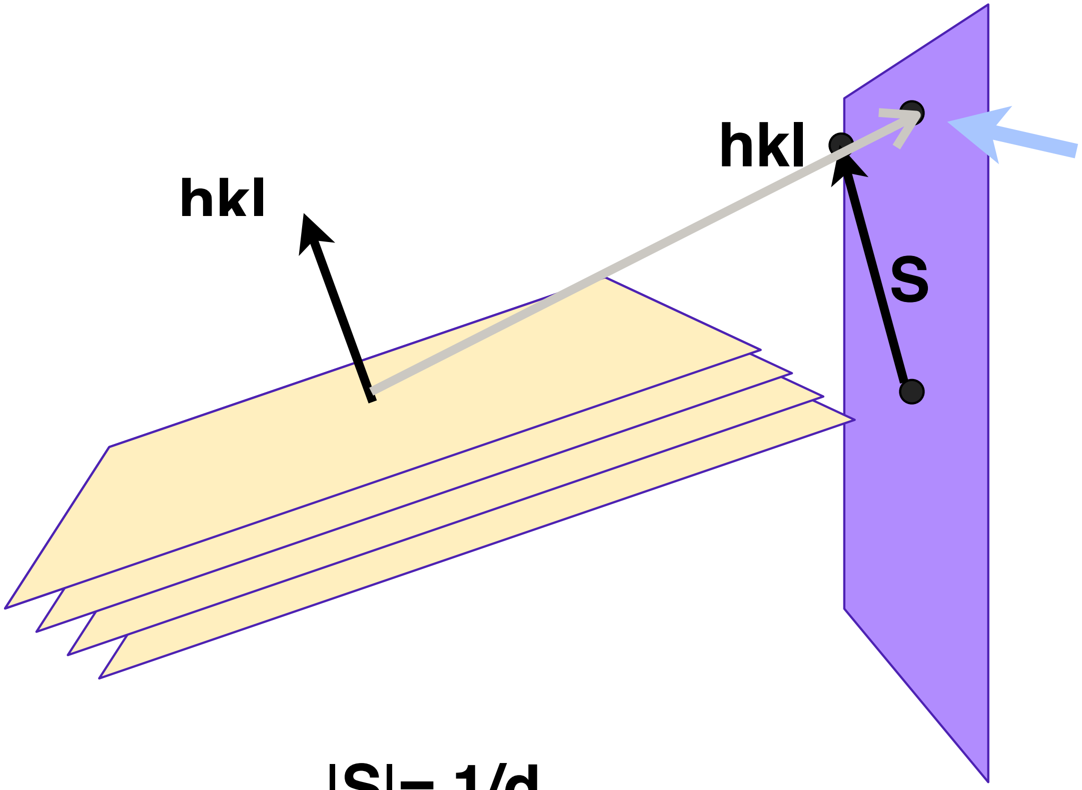
Calculating reciprocal lattice position from film and beam position



true position of spot in reciprocal space

position of spot on film

Relationship between a spot on the film and the crystal planes



position of spot
on film

|S|= 1/d

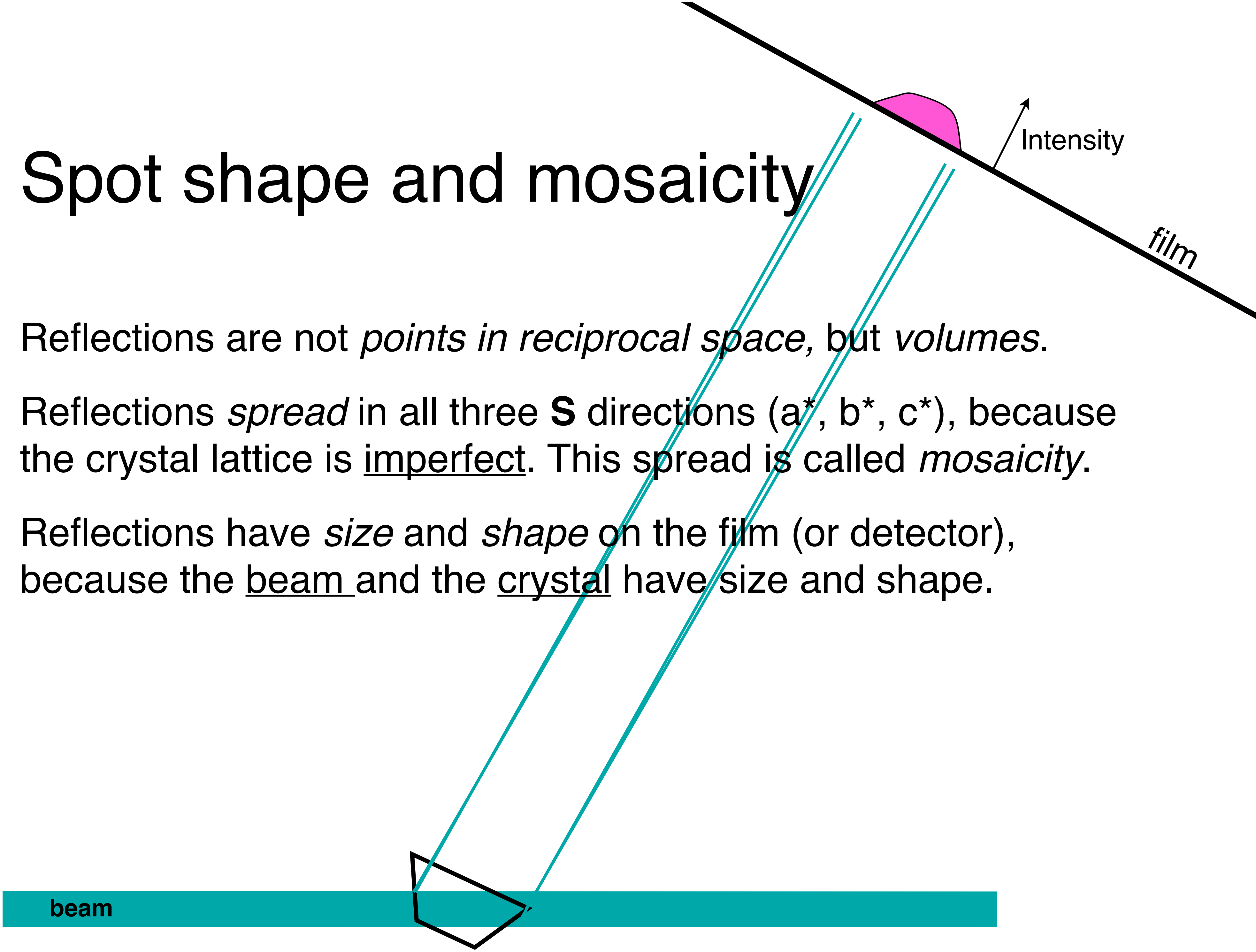
S= ha* + kb* + lc*

Spot shape and mosaicity

Reflections are not *points in reciprocal space,* but *volumes*.

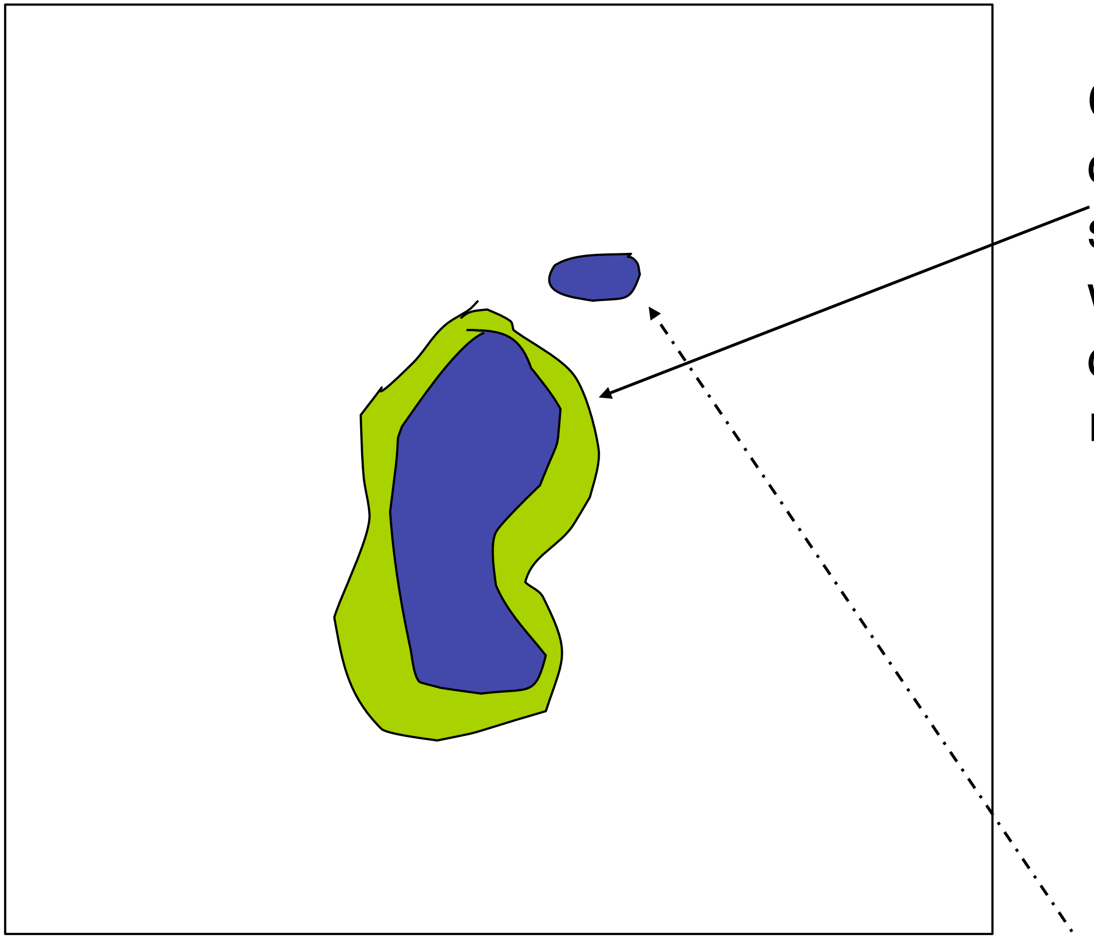
Reflections *spread* in all three **S** directions (a*, b*, c*), because the crystal lattice is imperfect. This spread is called *mosaicity*.

Reflections have *size* and *shape* on the film (or detector), because the beam and the crystal have size and shape.



The spot shape is an image of the intersection of the beam and crystal.

Spot profiles in 3D^{-1}



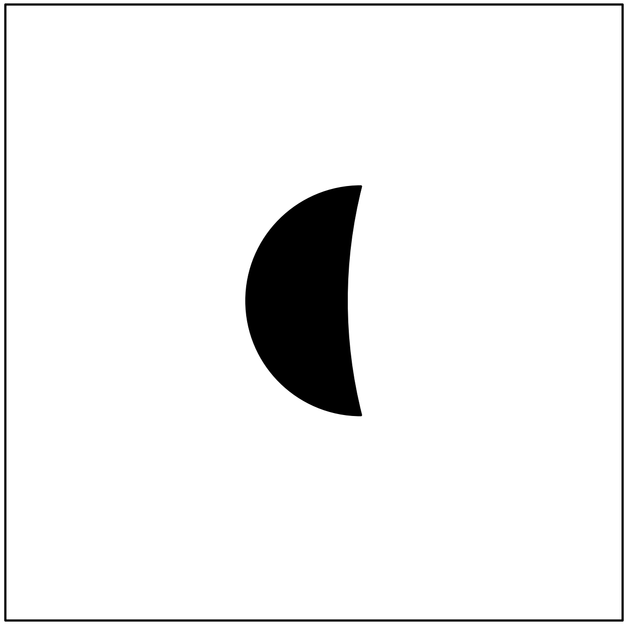
Green is the “profile” of the average spot, summed over all spots within a range in diffractometer coords κ, 2θ,ω

Intensity of each spot (blue) is the integrated only *within the spot profile.*

This prevents counting spurious data like this.

Merging partial reflections

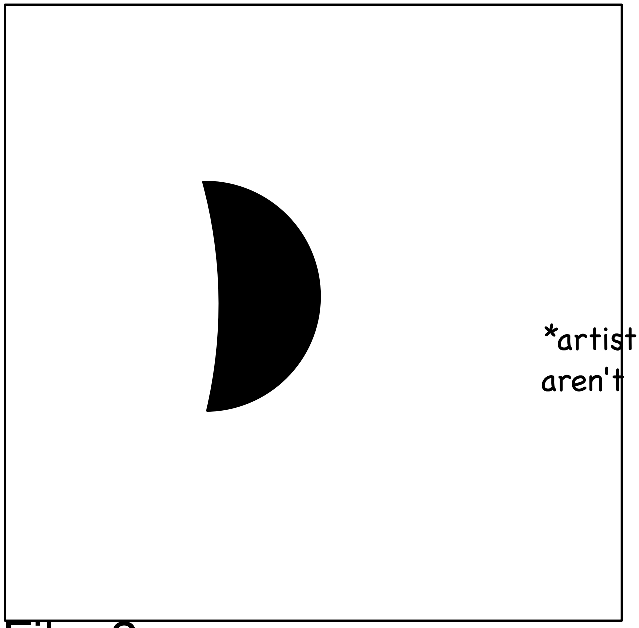
If films were switched while a spot was on the Ewald sphere, both copies (“partials” are summed together to get I(hkl).



Film 1

ω=153.0°

First half of spot hits the Ewald sphere.



Film 2

ω=153.5°

Other half of spot passes through.

*artistic license. Partials aren't really half-moons.

Scaling syms within a dataset

Reflections may have errors in amplitude within a dataset because:

- Xray intensity varied.
- Film/detector sensitivity varied.
- Crystal orientation/ cross section varied with w.
- Crystal decayed over time.
- Exposure time varied.
- Background radiation varied.

Scaling assumes:

(1) Symmetry-related reflections have the same amplitude

(2) Reflections that were collected together are scaled together (i.e. applied the same scale factor).

Quality of the data set =

Should be < 2%

a sym op

$$R_{sym} = \frac{\sum_{hkl} \left| w_{hkl} F(hkl) - w_{R\bullet(hkl)} F\left(\overset{\nwarrow}{R}\bullet(hkl)\right) \right|}{\sum_{hkl} \left| w_{hkl} F(hkl) \right|}$$

Example Structure Factor file

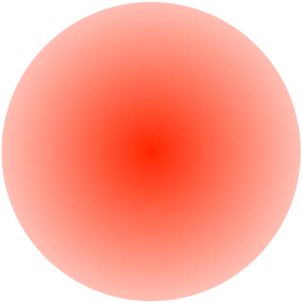
```
data_r1pkqsf
#------------------------------------------------------------
_audit.revision_id        1_0
_audit.creation_date      2003-07-15
_audit.update_record      'Initial release'
#
loop_
_refln.wavelength_id
_refln.crystal_id
_refln.index_h
_refln.index_k
_refln.index_l
_refln.F_meas_au
_refln.F_meas_sigma_au
_refln.status
1 1  -39    0   26       70.300       34.700    0
1 1  -39    0   27      158.300       25.740    0
1 1  -39    1    1      156.000       15.800    0
1 1  -39    1   25       54.100       23.690    0
1 1  -39    1   26      201.400       11.450    0
1 1  -39    2   25      151.900       11.970    0
1 1  -39    3   22      202.800       22.730    0
1 1  -38    0   26       75.900       37.400    0
```

Structure Factors are deposited in the PDB (www.rcsb.org) along with the atomic coordinates.

Data collection process

- Data starts out as a set of images.
- Data reduction is the process by which reflections are extracted from images.
- Considerations for background, spot shape, partial spots, detector sensitivity, and multiple copies.
- Rsym is a measure of dataset quality. Lower is better. Average I/sigma is another measure.

Scattering factor of an atom



An atom is a spherically symmetrical cloud of electron density which is densest in the center.

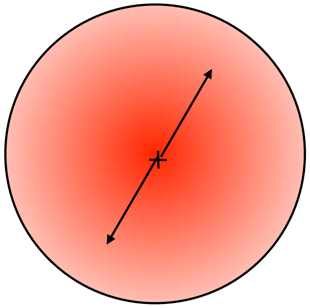
By integrating over the electron cloud, we get the Fourier transform of the atom.

$$f(S) = \int_{atom} \rho(r) e^{i2\pi S \bullet r} dr$$

If we define *r* to be a vector relative to the center of the atom, then *f*(S) can be thought of as a single wave coming from the center of the atom.

Scattering from any centro-symmetric object (like an atom) behaves like a point

Phase shift for all points ***r*** relative to the center but not at the center cancel out because
sin(–2πS•**r**) = –sin(2πS•(–**r**))



Proof

$$e^{i2\pi S\bullet r} = \cos 2\pi S\bullet r + i\sin 2\pi S\bullet r$$

$$+ \qquad e^{i2\pi S\bullet (-r)} = \cos 2\pi S\bullet r - i\sin 2\pi S\bullet r$$

$$e^{i2\pi S\bullet r} + e^{i2\pi S\bullet (-r)} = 2\cos 2\pi S\bullet r$$

……………………………

net sine part is zero!

Fourier transform with atomic scattering factors f(g)

$$F(S) = \sum_{atoms\ g} f(g)\, e^{i2\pi S \bullet r_g}$$

f(g) is a positive real quantity that depends on the number of electrons in the atom and the length of S.

Same equation, using fractional coordinates ***x***=(x,y,z) and Miller indeces ***h*** = (h,k,l):

$$F(h) = \sum_{atoms\ g} f(g)\, e^{i2\pi h \bullet x_g}$$

*confused? See Slide 18

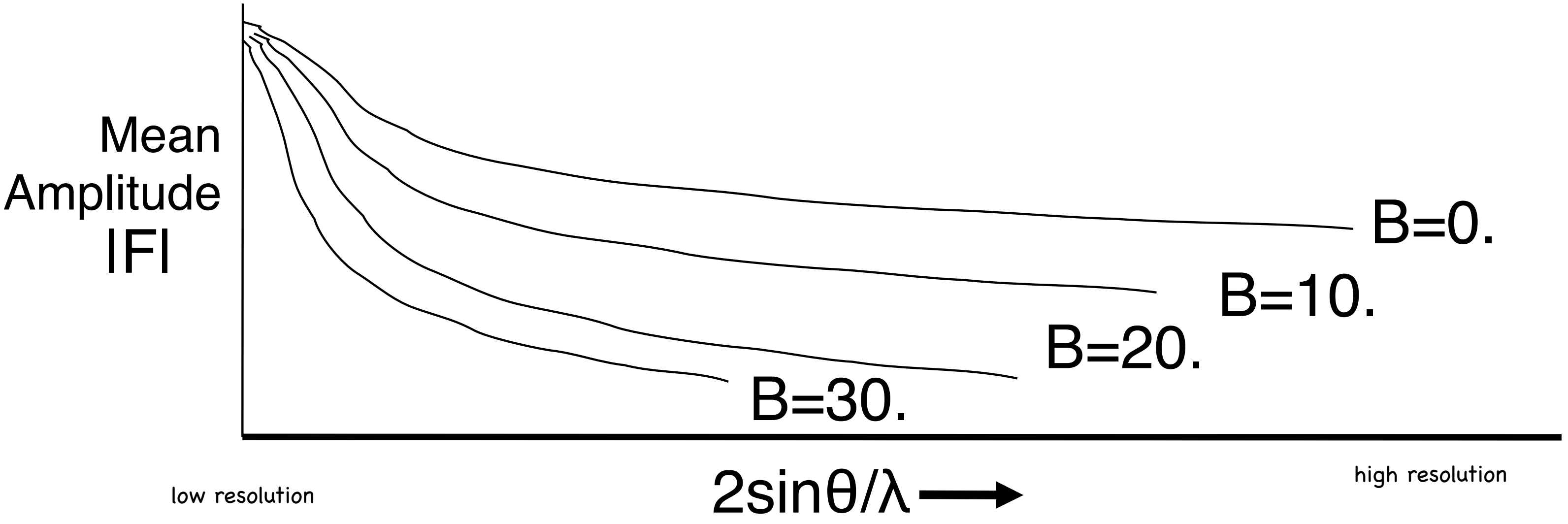
Atomic B-factors

The amplitude of an atom depends on how much it moves.

The sharper the electron density distribution, the broader the scattering factor. The temperature factor, *B*, modifies the scattering factor by spreading out the electron density.

Correction factor for atomic scattering factor based on motion: $e^{-B\frac{\sin^2\theta}{\lambda^2}}$

$2\sin\theta/\lambda = |\mathbf{S}|^2$



Did you know...? **B**-factors are called the "Temperature factors", even though *temperature* has little to do with it!

The Fourier transform with B-factors

$$F(h) = \sum_{atoms\ g} f(g) e^{-B_g \frac{\sin^2\theta}{\lambda^2}} e^{i2\pi h \bullet x_g}$$

Every atom has a B

So…

Structure factors can be calculated from atoms

- Requires x,y,z and B for each atom.
- Called F_{calc} ,or F_{c}, $F_{c}(hkl)$, $F_{c}(\mathbf{h})$

$$F_{c}(h) = \sum_{atoms\ g} f(g) e^{-B_g \frac{\sin^2\theta}{\lambda^2}} e^{i2\pi h \bullet x_g}$$

got enough jargon?......

$F_c(h)$.

Let's call them

"Structure factors"

Review

- What is a reflection?
- In what way is the reciprocal space of a lattice also a lattice?
- What is **a***?
- What are the Laue conditions?
- How are a*, b* and c* related to a, b and c?
- Can you draw Bragg planes in 2D?
- How does the Ewald sphere move when you rotate the beam around the crystal?
- How does the reciprocal lattice rotate when you rotate the crystal around the beam?
- How are crystal planes (Bragg planes) named?
- What does resolution mean with regard to a reflection?

Review

- What kind of data are collected during Xray data collection?
- What is “data reduction”?
- What is “scaling”?
- What does R_{sym} measure?
- As B-factor goes up, the atom contributes more to high resolution data? or less?
- What is an atomic scattering factor?
- How do you determine the cell dimensions given the diffraction pattern?
- What is mosaicity? What causes it?