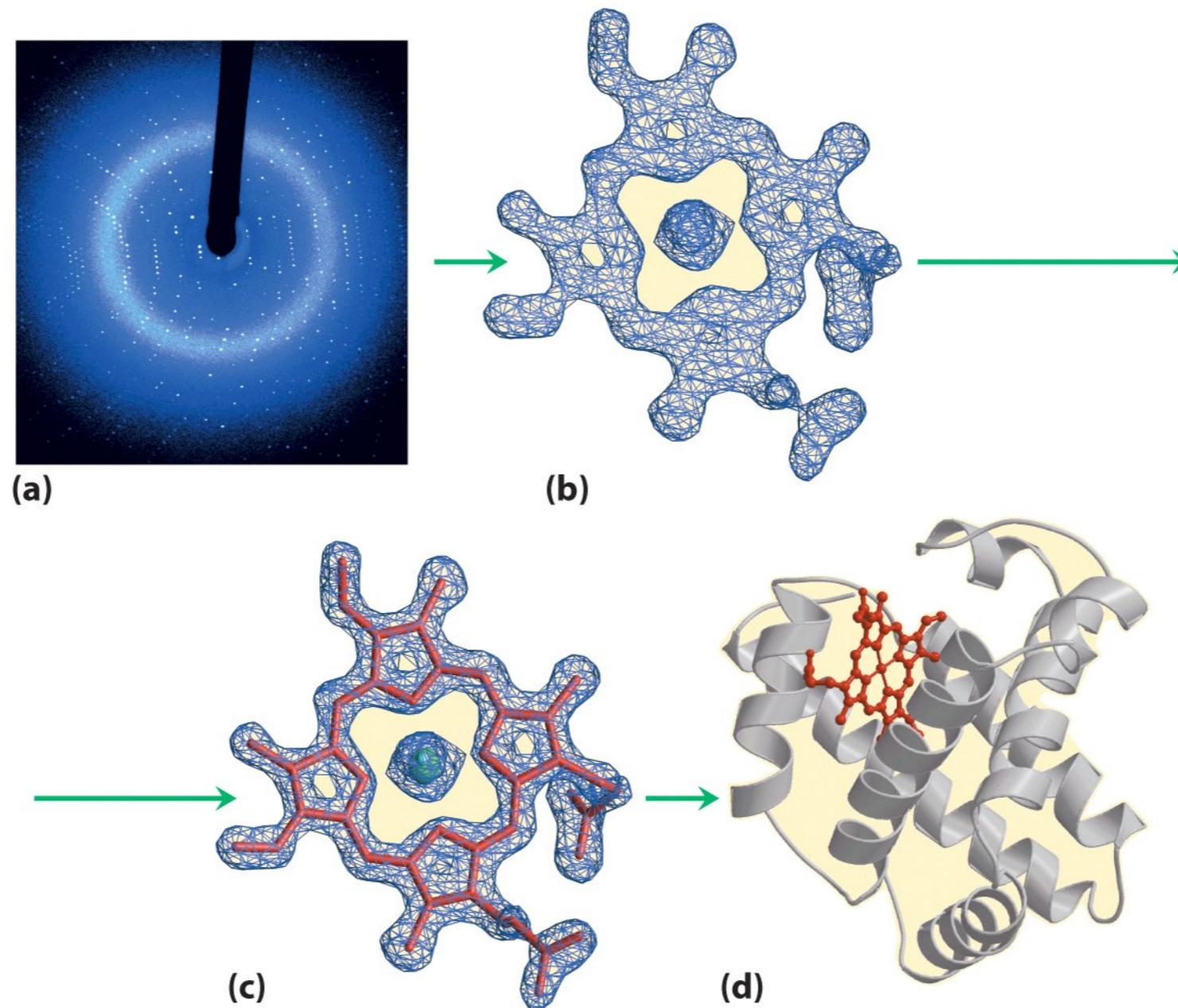


structure determination of proteins with x-rays

a short, but hopefully sufficiently clear overview of x-ray crystallography from the computational chemist's perspective



structure determination of proteins with x-rays

repeat quickly last seminar's material

crystals

x-rays

scattering of atoms, molecules, and crystals

graphics rather than equations

determining structure from scattering (diffraction)

data collection

Fourier transform?

refinement

examples to illustrate the concepts

time-resolved x-ray scattering on photosynthetic reaction center

time-resolved x-ray diffraction on photosynthetic reaction center

structure determination of proteins with x-rays

two main aspects to x-ray crystallography

crystals

x-rays

crystals

asymmetric unit



30 Å

crystals

asymmetric unit



30 Å

symmetry mates

screw axis



crystals

asymmetric unit

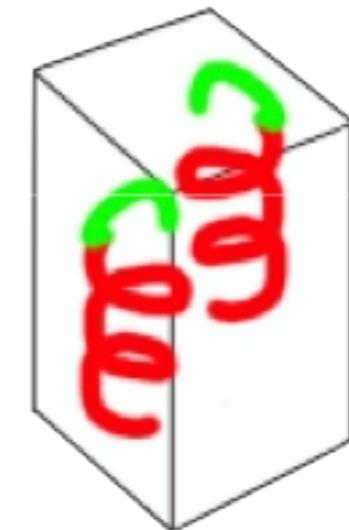


30 Å

symmetry mates
screw axis



unitcell



100 Å

asymmetric unit



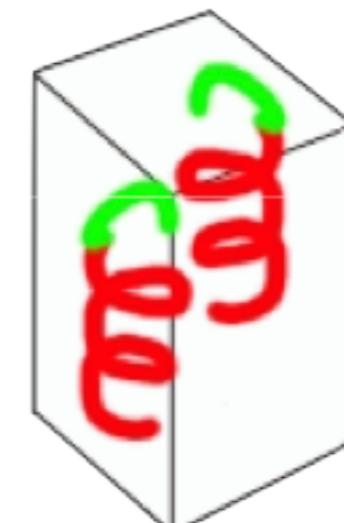
30 Å

symmetry mates

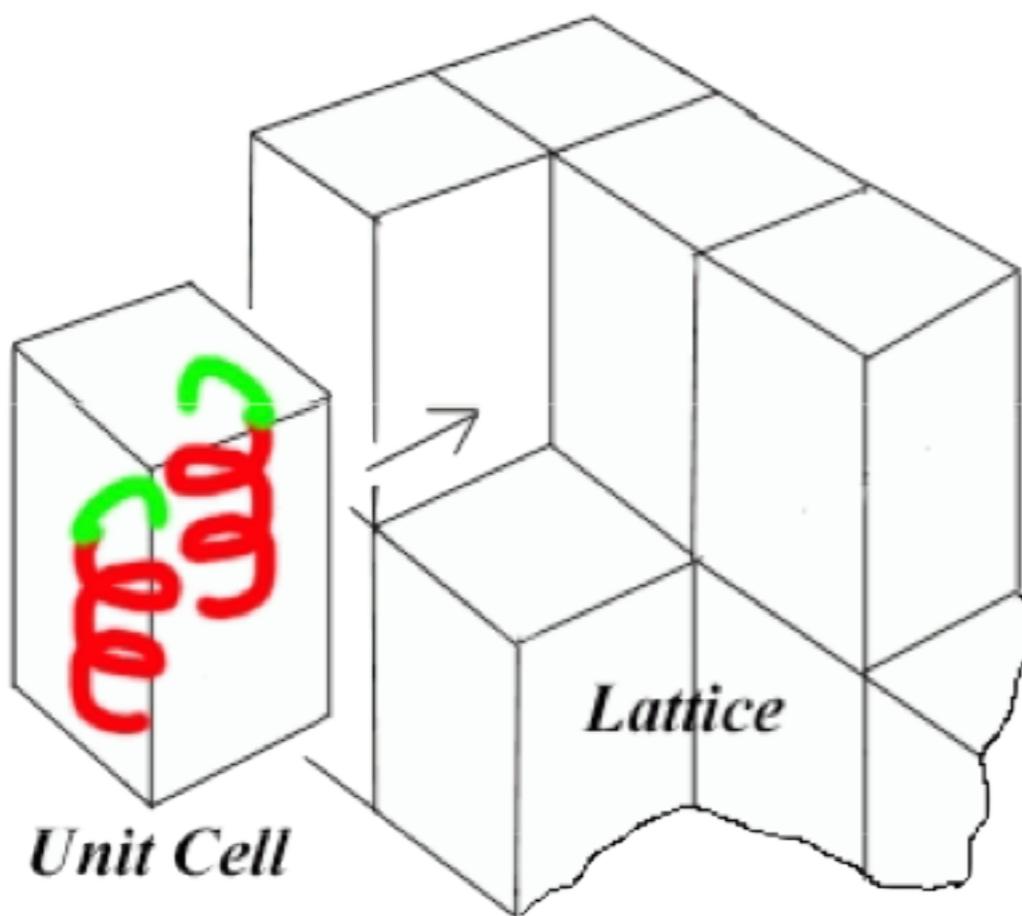
screw axis



unitcell



100 Å



asymmetric unit



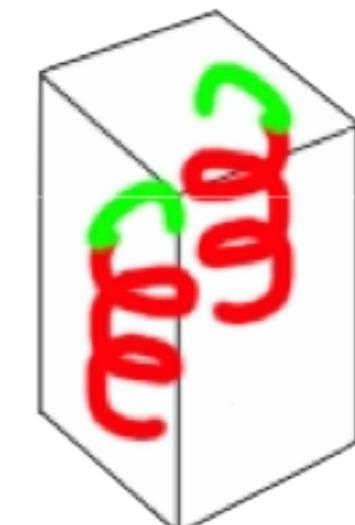
30 Å

symmetry mates

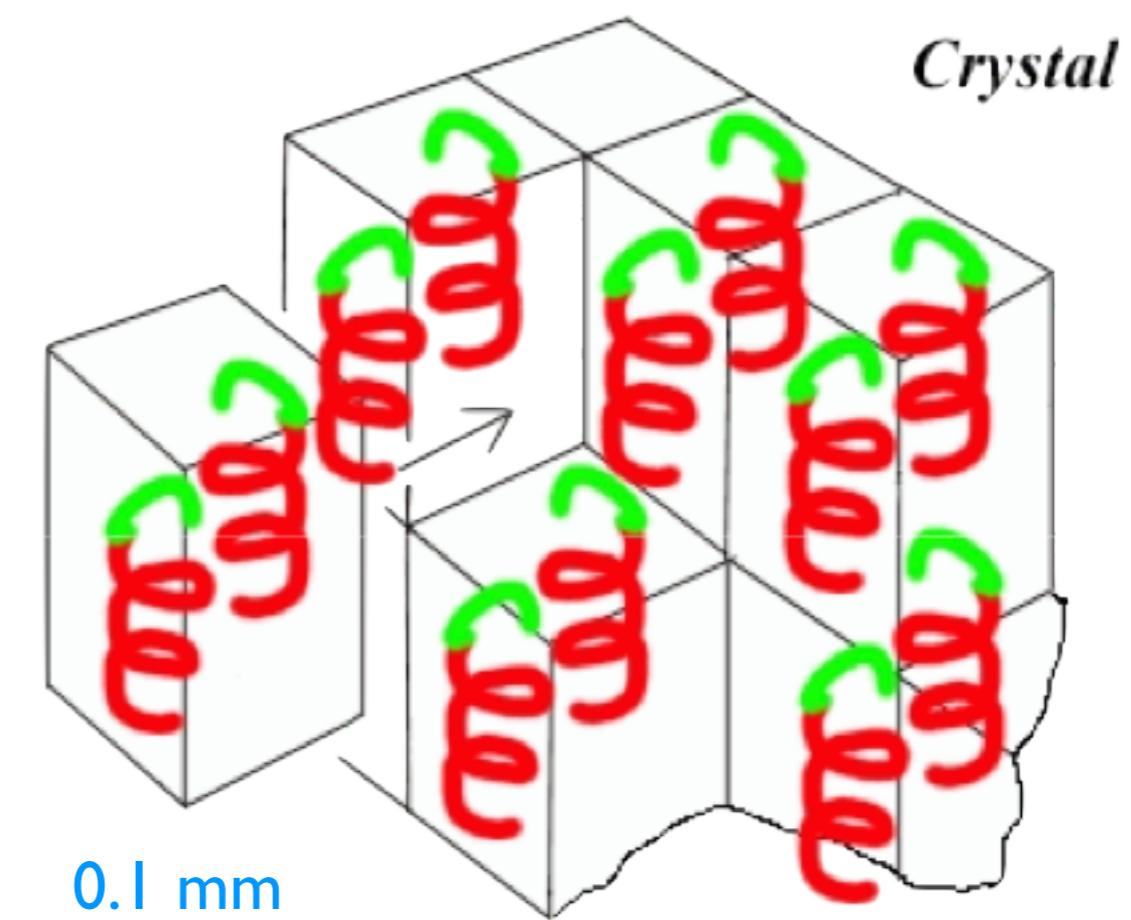
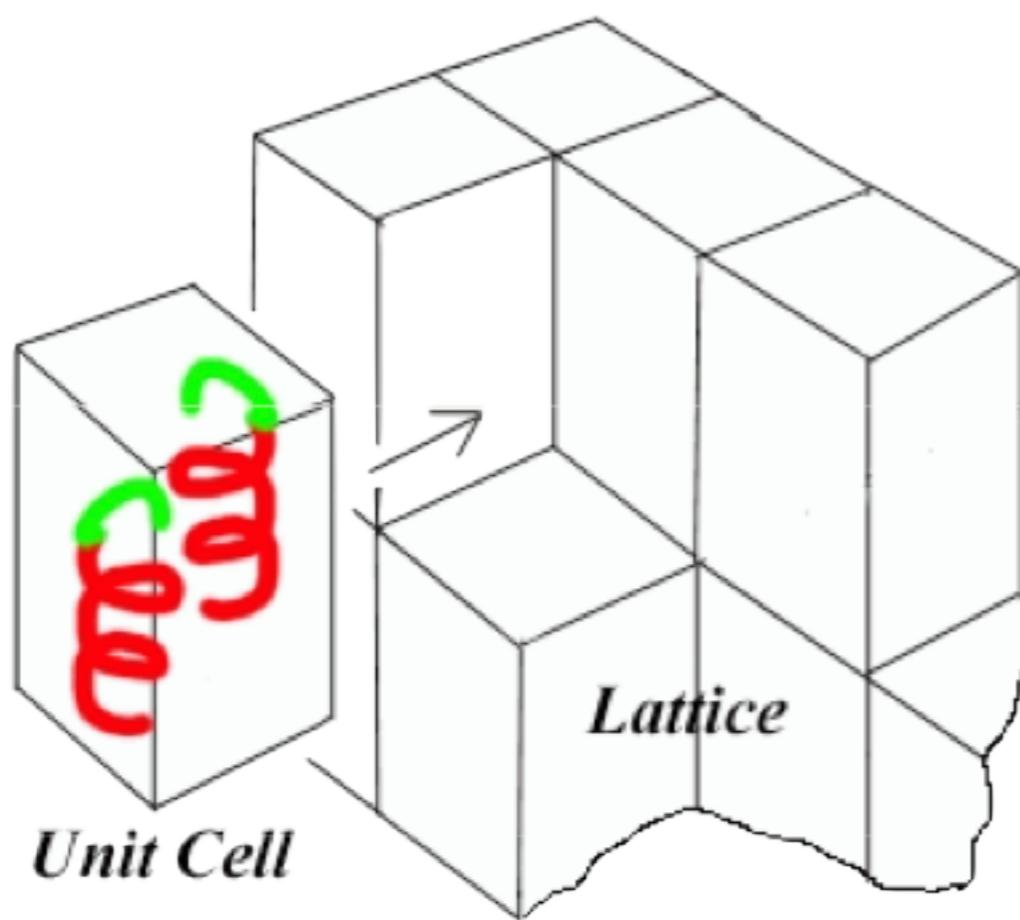
screw axis



unitcell



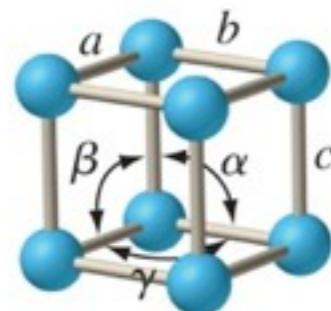
100 Å



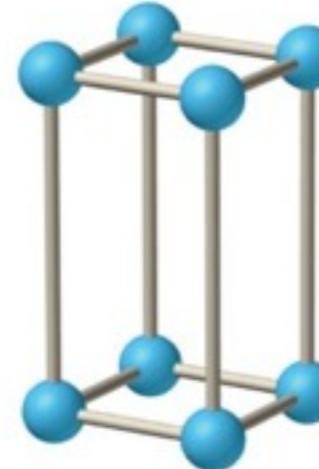
crystals

seven types of crystal lattices

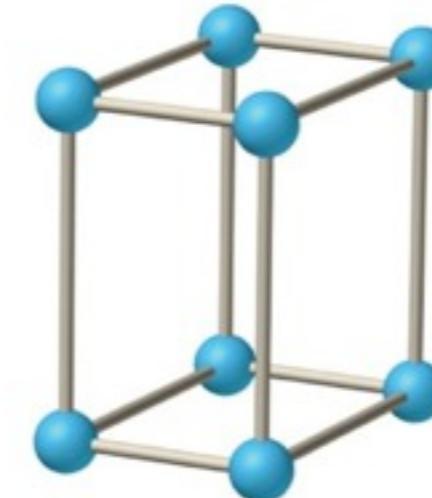
Bravais lattices



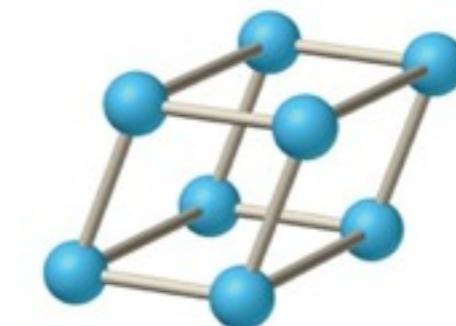
Simple cubic
 $a = b = c$
 $\alpha = \beta = \gamma = 90^\circ$



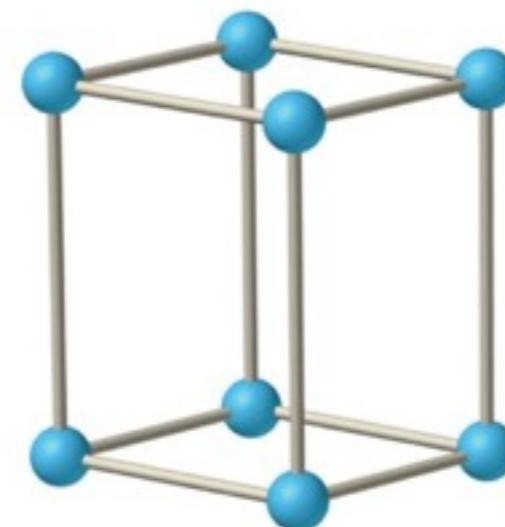
Tetragonal
 $a = b \neq c$
 $\alpha = \beta = \gamma = 90^\circ$



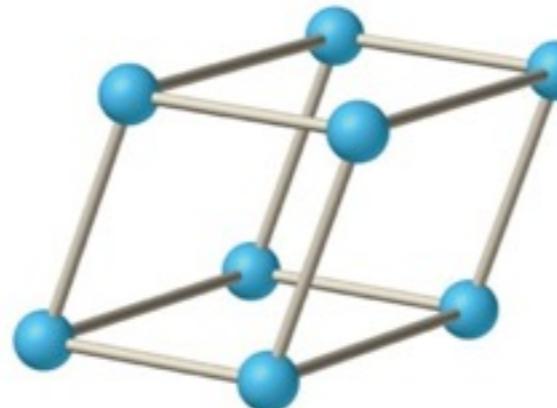
Orthorhombic
 $a \neq b \neq c$
 $\alpha = \beta = \gamma = 90^\circ$



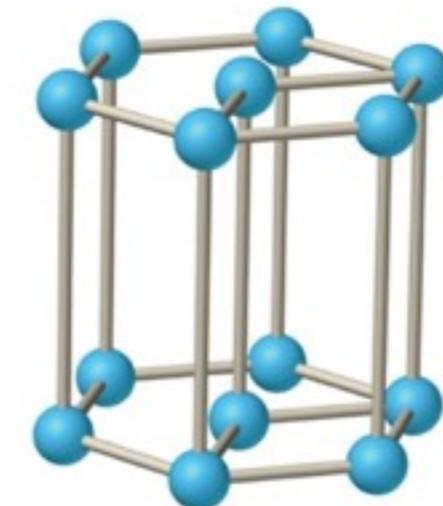
Rhombohedral
 $a = b = c$
 $\alpha = \beta = \gamma \neq 90^\circ$



Monoclinic
 $a \neq b \neq c$
 $\gamma \neq \alpha = \beta = 90^\circ$



Triclinic
 $a \neq b \neq c$
 $\alpha \neq \beta \neq \gamma \neq 90^\circ$

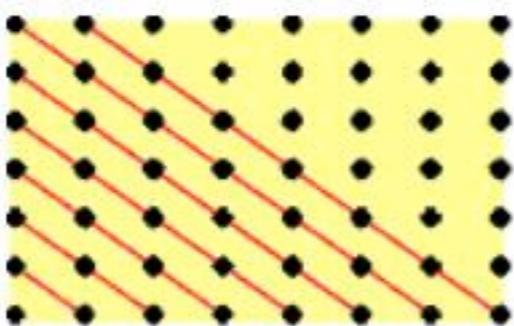


Hexagonal
 $a = b \neq c$
 $\alpha = \beta = 90^\circ, \gamma = 120^\circ$

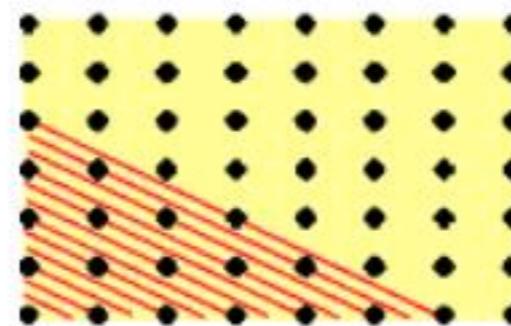
crystals

lattice planes

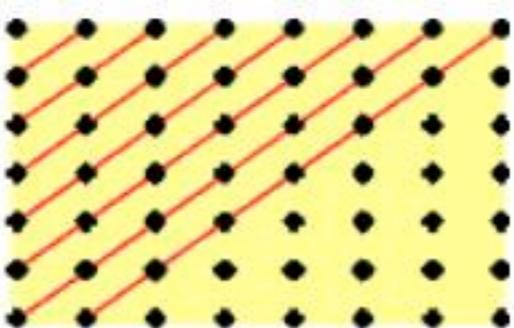
Miller indices (hkl)



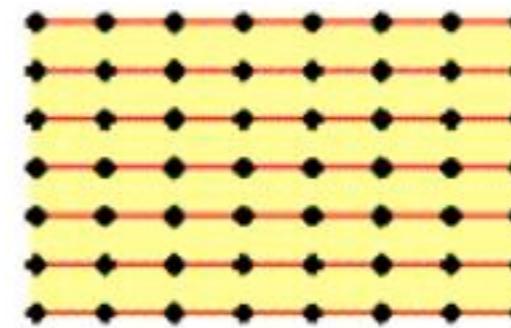
$$(hkl) = (110)$$



$$(hkl) = (230)$$



$$(hkl) = (\bar{1}10)$$



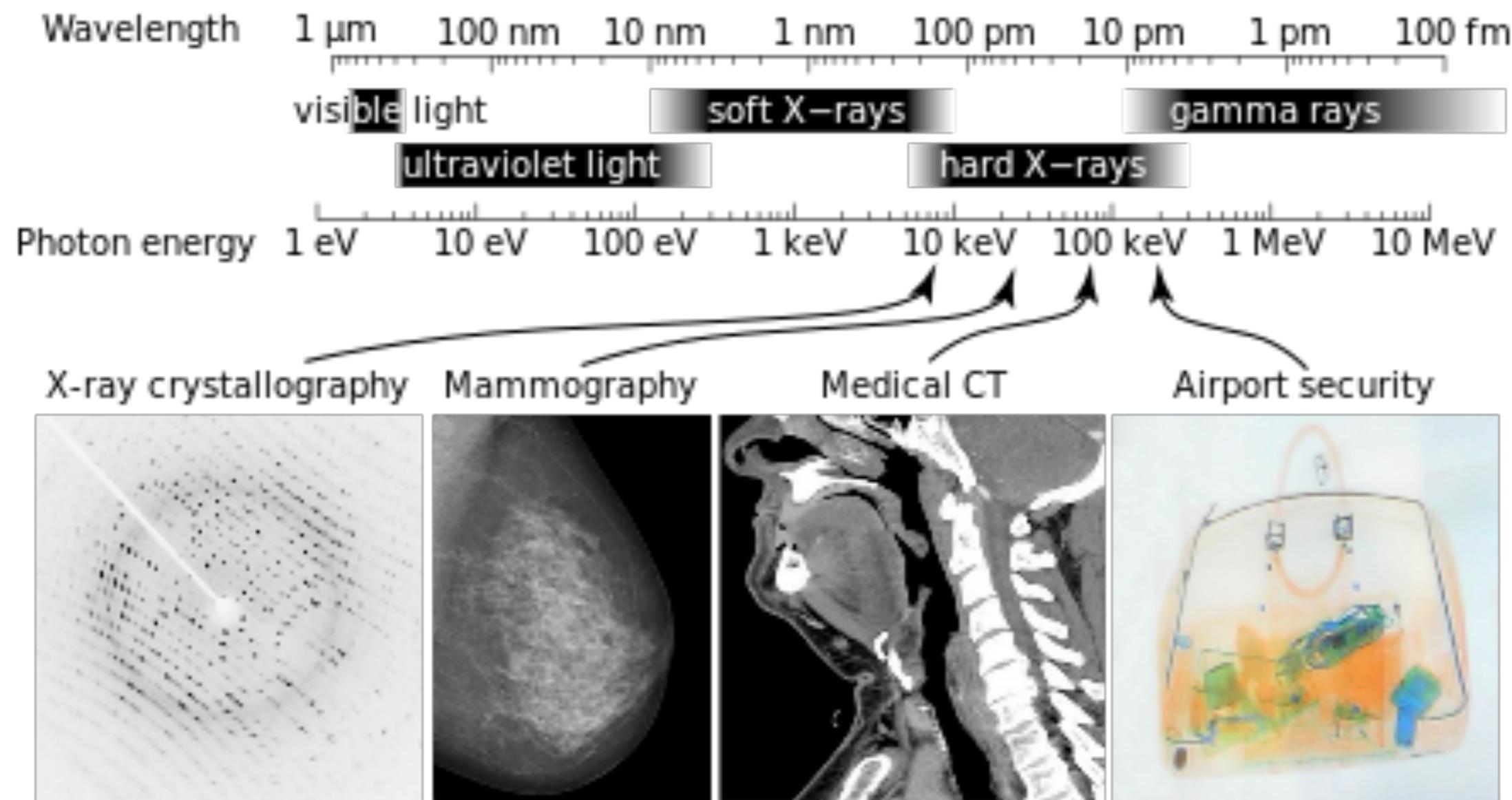
$$(hkl) = (010)$$

separation of planes

$$\frac{1}{d_{hkl}^2} = \frac{h^2}{a^2} + \frac{k^2}{b^2} + \frac{l^2}{c^2}$$

x-rays

electro-magnetic spectrum



X-rays

some characteristics

wavelength

$\sim 0.1 \text{ nm} = 1 \text{ \AA}$

C-C bond $\sim 1.5 \text{ \AA}$

X-rays

some characteristics

wavelength

$\sim 0.1 \text{ nm} = 1 \text{ \AA}$

C-C bond $\sim 1.5 \text{ \AA}$

photon energy

10 keV

1s electron of C: 284 eV

X-rays

some characteristics

wavelength

$\sim 0.1 \text{ nm} = 1 \text{ \AA}$

C-C bond $\sim 1.5 \text{ \AA}$

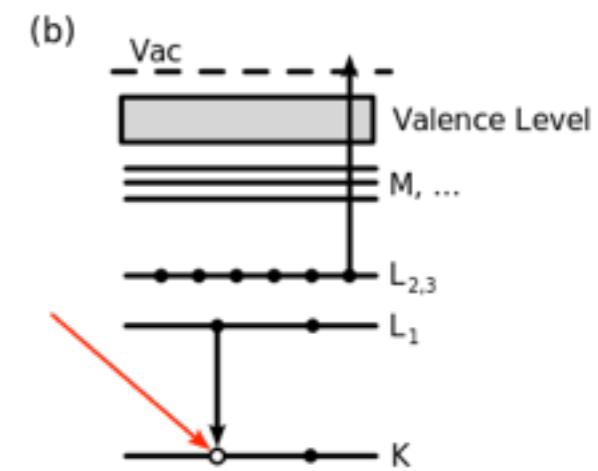
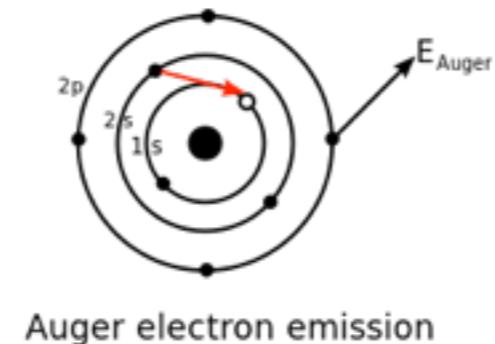
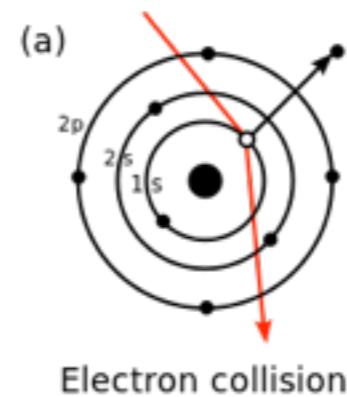
photon energy

10 keV

1s electron of C: 284 eV

inelastic scattering

(core) ionization, Auger decay, radiation damage



X-rays

some characteristics

wavelength

~ 0.1 nm = 1 Å

C-C bond ~ 1.5 Å

photon energy

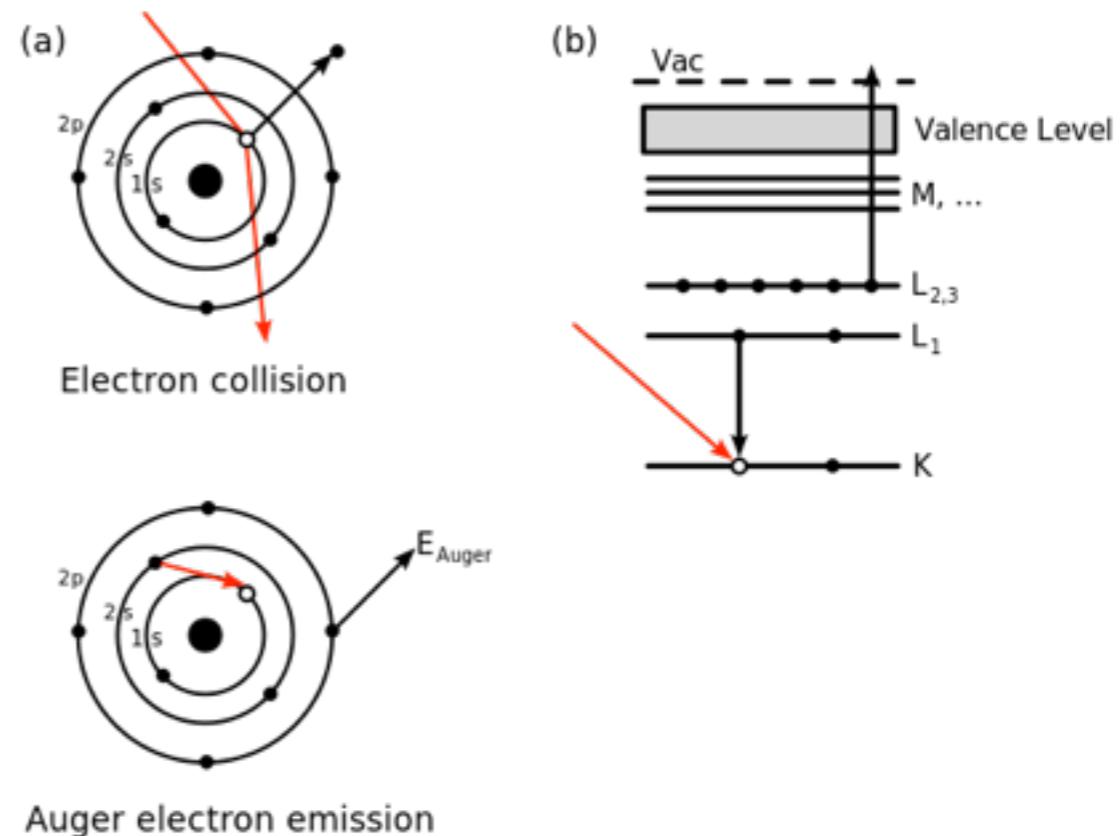
10 keV

1s electron of C: 284 eV

inelastic scattering

(core) ionization, Auger decay, radiation damage

avoid damage: low temperature (77 K)



X-rays

some characteristics

wavelength

~ 0.1 nm = 1 Å

C-C bond ~ 1.5 Å

photon energy

10 keV

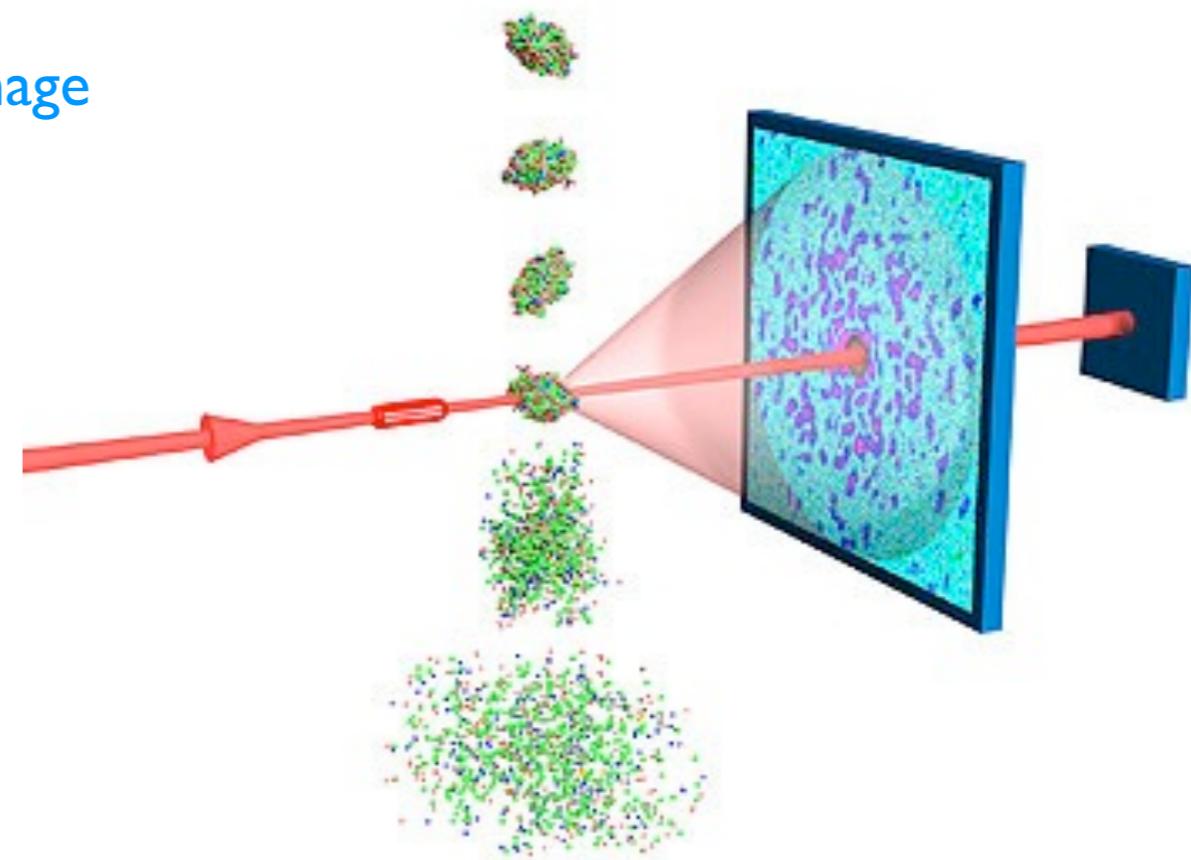
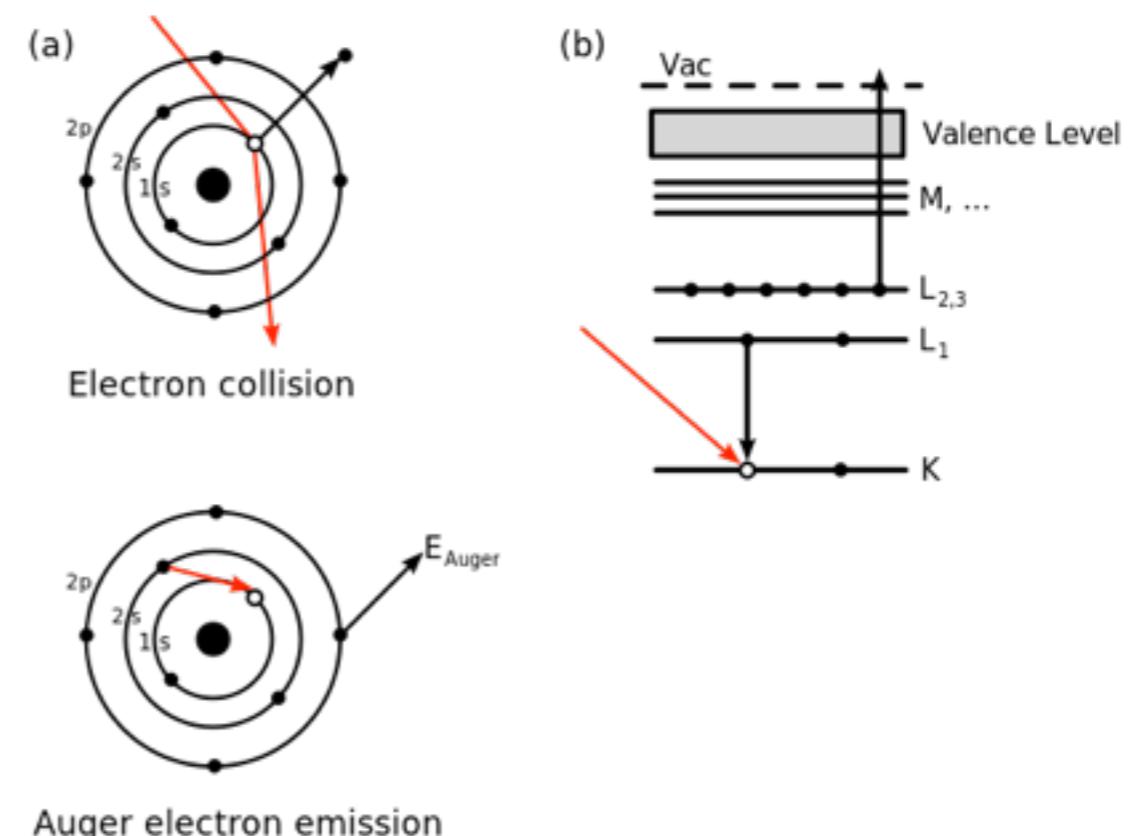
1s electron of C: 284 eV

inelastic scattering

(core) ionization, Auger decay, radiation damage

avoid damage: low temperature (77 K)

outrun damage: diffract & destroy



X-rays

some characteristics

wavelength

~ 0.1 nm = 1 Å

C-C bond ~ 1.5 Å

photon energy

10 keV

1s electron of C: 284 eV

inelastic scattering

(core) ionization, Auger decay, radiation damage

avoid damage: low temperature (77 K)

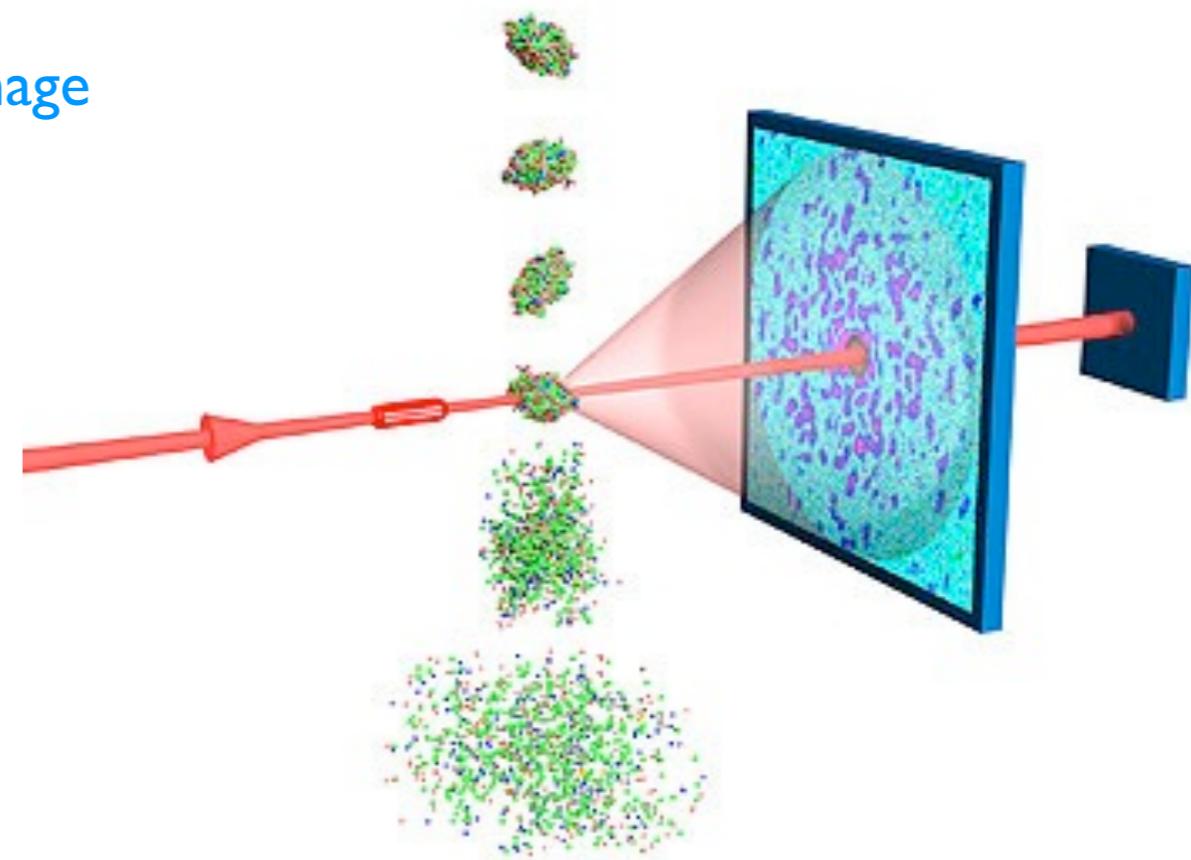
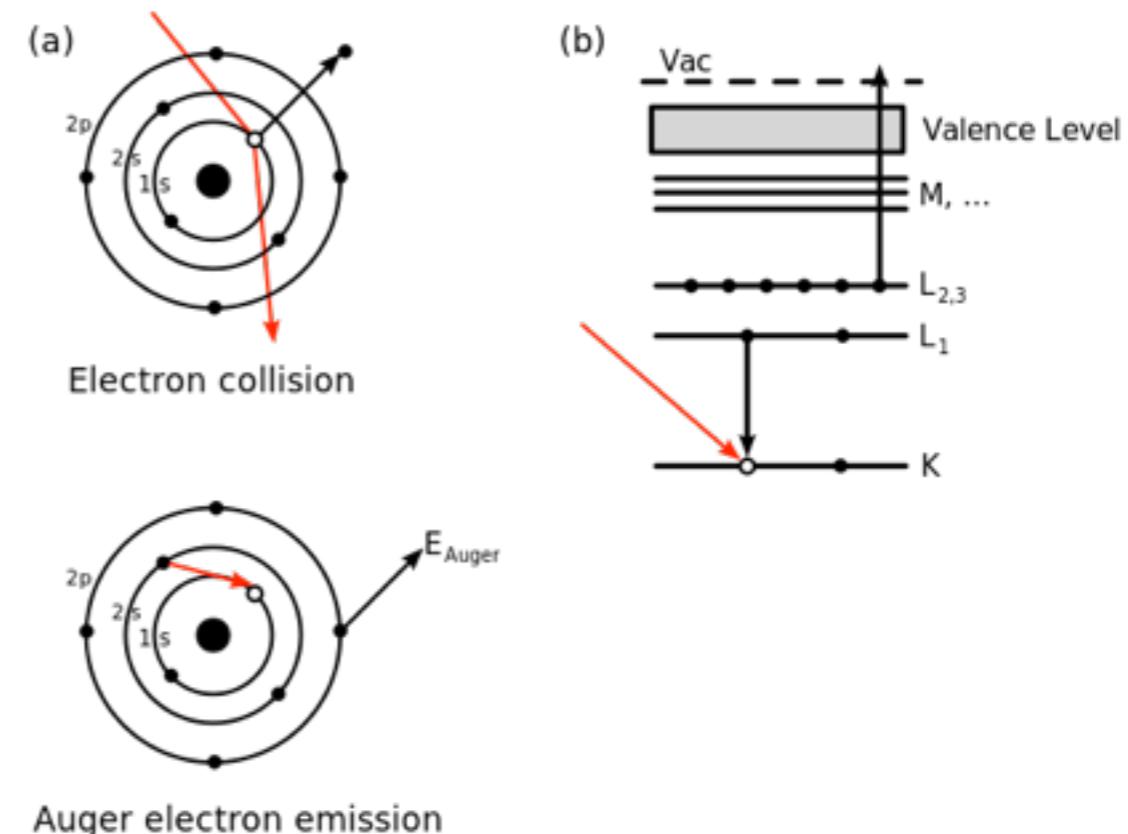
outrun damage: diffract & destroy

elastic scattering

low intensity

small samples

no secondary scattering events



elastic scattering

electron thinks x-rays are oscillating electric fields

much faster (10^{18} Hz) than electron motion in molecules

x-rays think electron stands still

electrons follow the wave and start oscillating at same frequency

elastic scattering

electron thinks x-rays are oscillating electric fields

much faster (10^{18} Hz) than electron motion in molecules

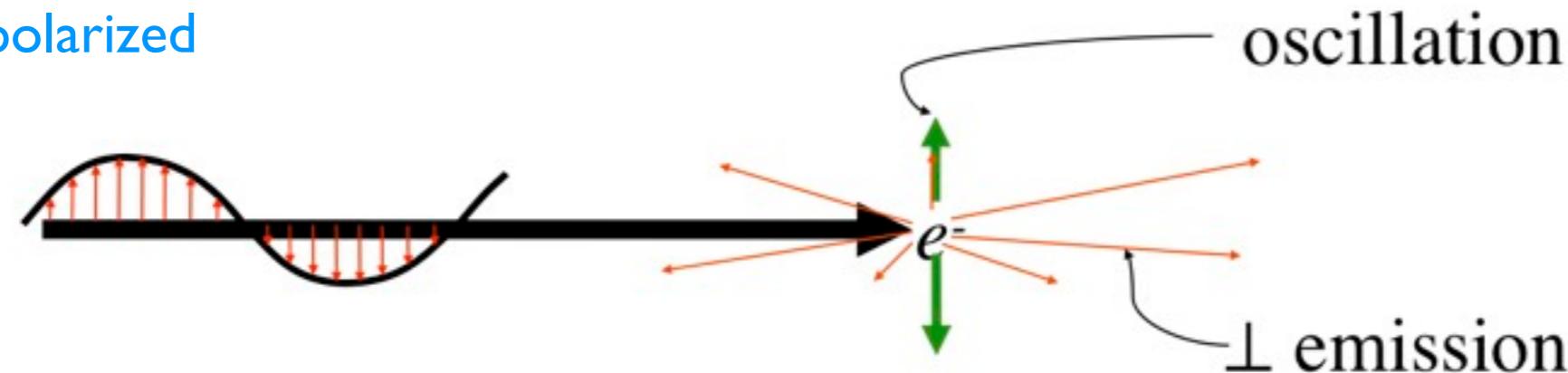
x-rays think electron stands still

electrons follow the wave and start oscillating at same frequency

x-rays induce oscillation of electrons

light emission (Maxwell)

polarized



elastic scattering

electron thinks x-rays are oscillating electric fields

much faster (10^{18} Hz) than electron motion in molecules

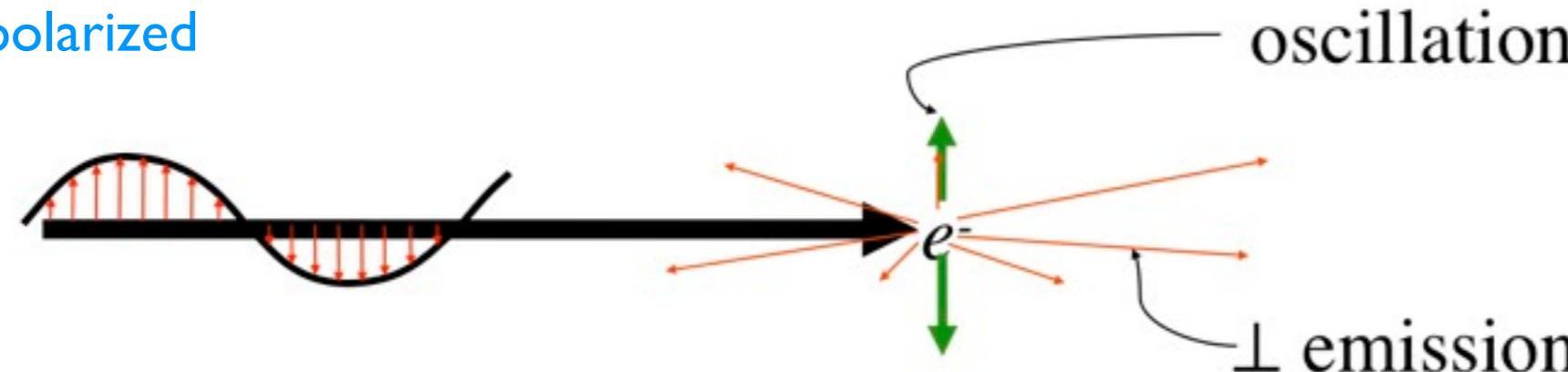
x-rays think electron stands still

electrons follow the wave and start oscillating at same frequency

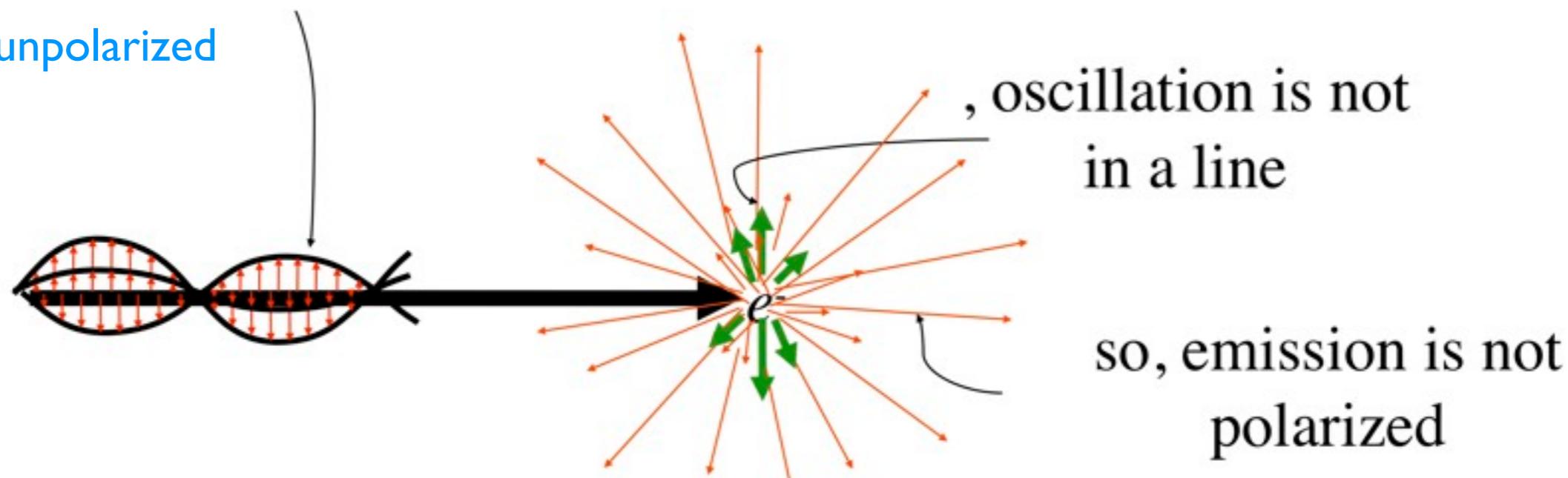
x-rays induce oscillation of electrons

light emission (Maxwell)

polarized



unpolarized

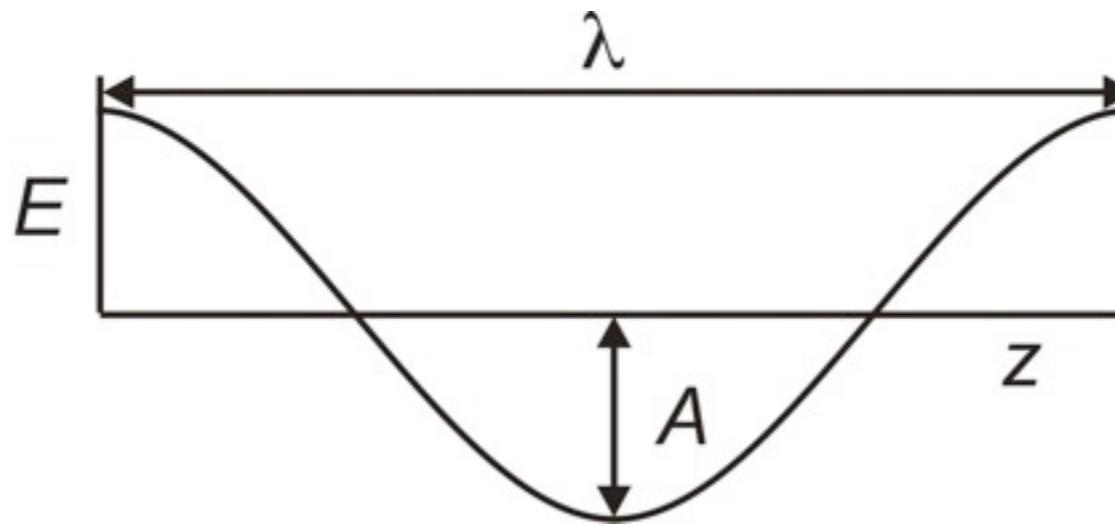


addition of waves

electromagnetic wave

addition of waves

~~electromagnetic~~ wave



distance travelled by wave

$$\Delta z = tc = t\lambda\nu$$

$$E(t = 0; z) = A \cos(2\pi \frac{z}{\lambda})$$

$$E(t; z = 0) = A \cos(2\pi \nu t)$$

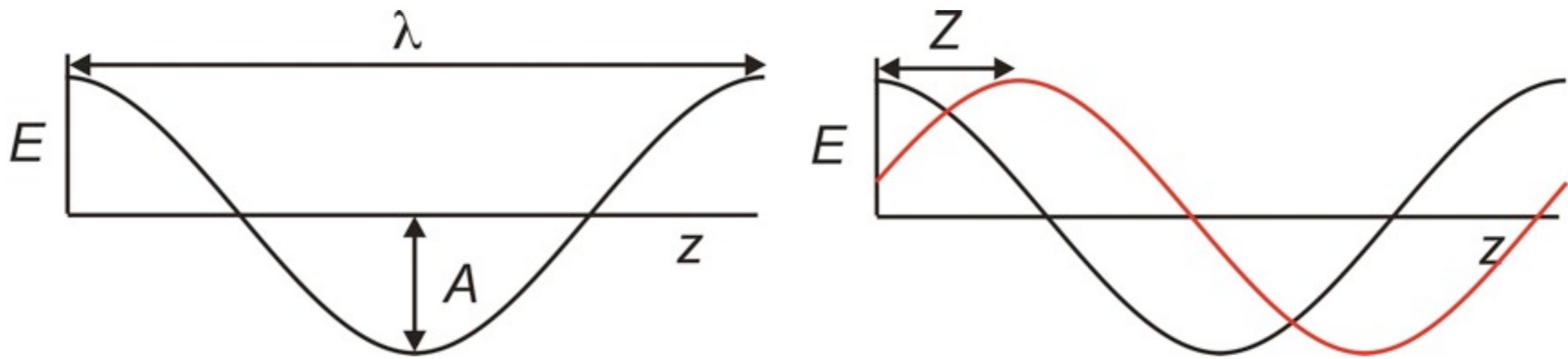
$$E(t; z) = A \cos(2\pi \frac{1}{\lambda} (z - t\lambda\nu))$$

$$= A \cos(2\pi (\frac{z}{\lambda} - \nu t))$$

$$= A \cos(2\pi \nu (t - \frac{z}{c}))$$

addition of waves

new wave



$$E_{\text{old}}(t; z = 0) = A \cos(2\pi\nu t)$$

phase

$$E_{\text{new}}(t; z = 0) = A \cos(2\pi\nu t + \alpha) \quad \alpha = 2\pi Z / \lambda$$

addition of waves

new wave

phase

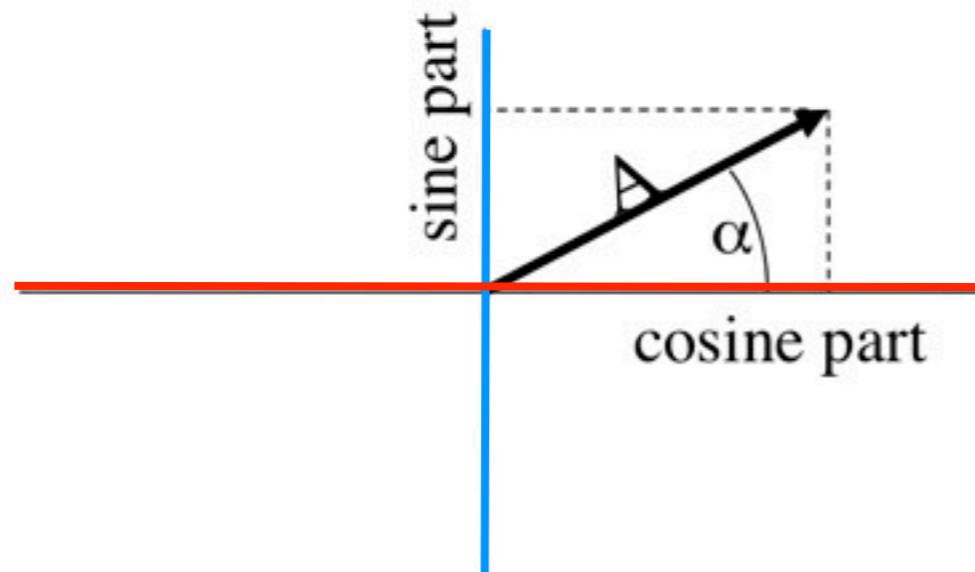
$$\begin{aligned} A \cos(\omega t + \alpha) &= A \cos(\alpha) \cos(\omega t) - A \sin(\alpha) \sin(\omega t) \\ &= \underline{A \cos(\alpha) \cos(\omega t)} + \underline{A \sin(\alpha) \cos(\omega t + \pi/2)} \end{aligned}$$

wave 1, real **wave 2, imaginary**

Argand diagrams

real & imaginary axis

$(A \cos \alpha, A \sin \alpha)$



vector addition

addition of waves

new wave

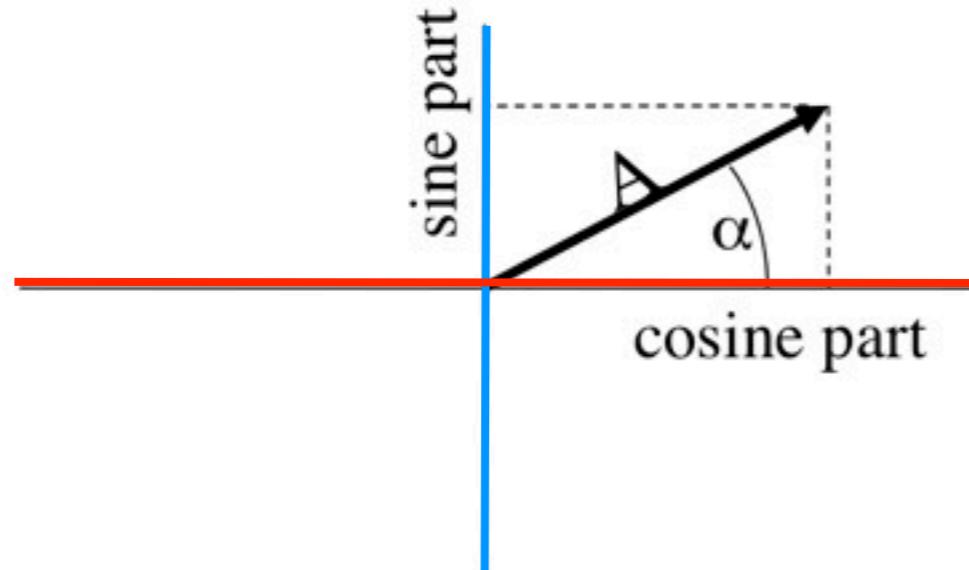
phase

$$\begin{aligned} A \cos(\omega t + \alpha) &= A \cos(\alpha) \cos(\omega t) - A \sin(\alpha) \cos(\omega t + \pi/2) \\ &= (A \cos(\alpha) + i A \sin(\alpha)) \cos(t) \end{aligned}$$

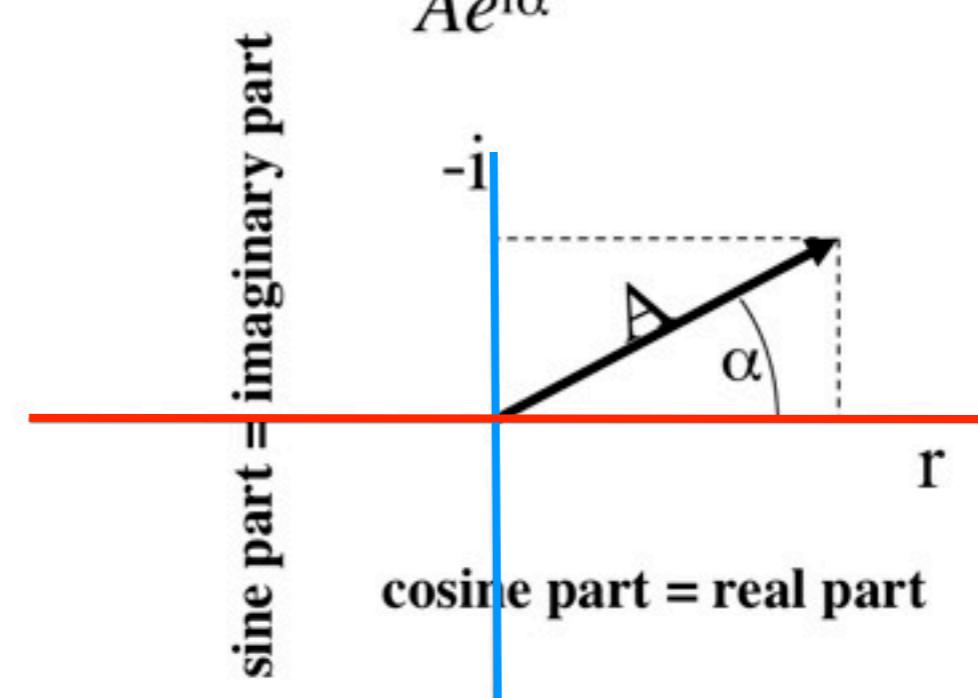
Argand diagrams

real & imaginary axis

$(A \cos \alpha, A \sin \alpha)$

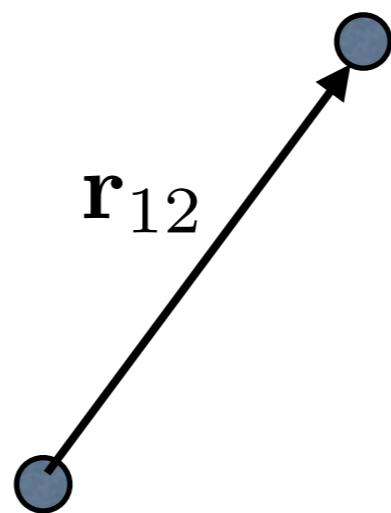


$A e^{i\alpha}$



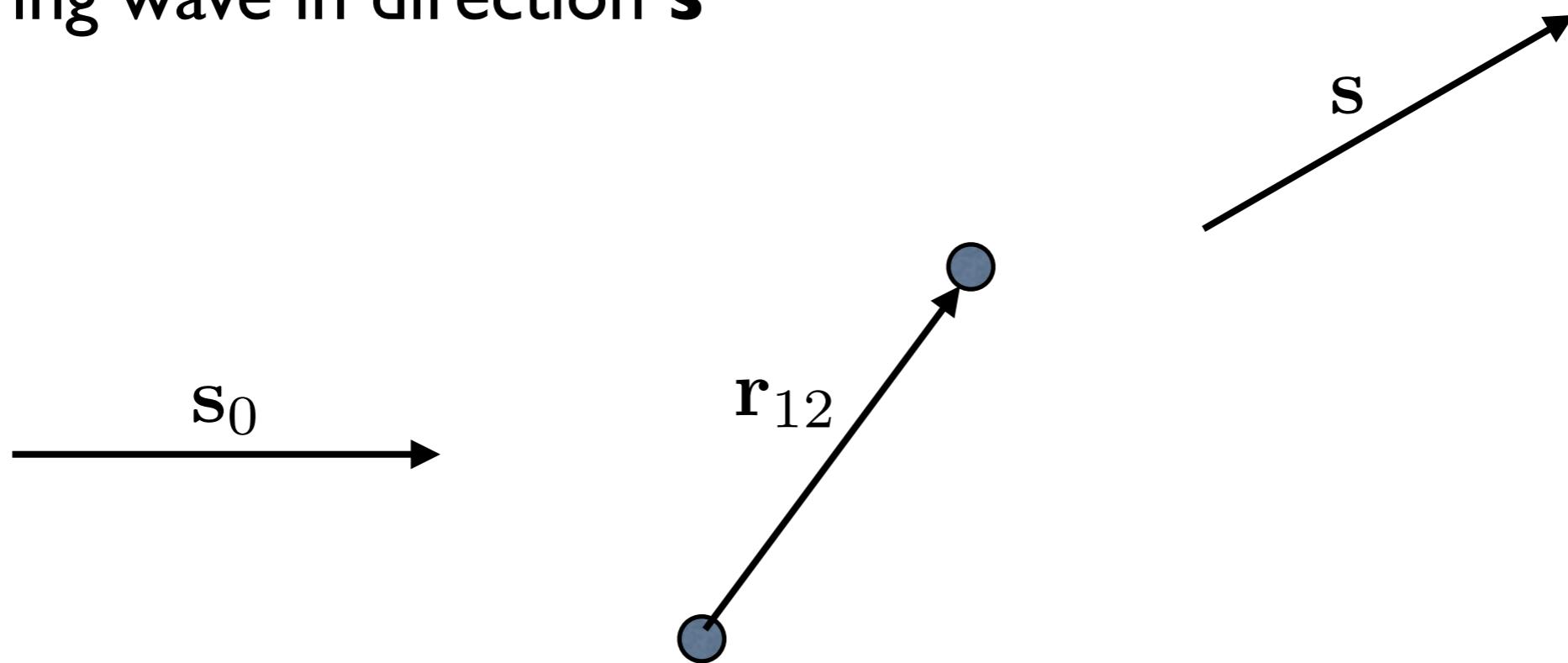
vector addition

scattering with two electrons



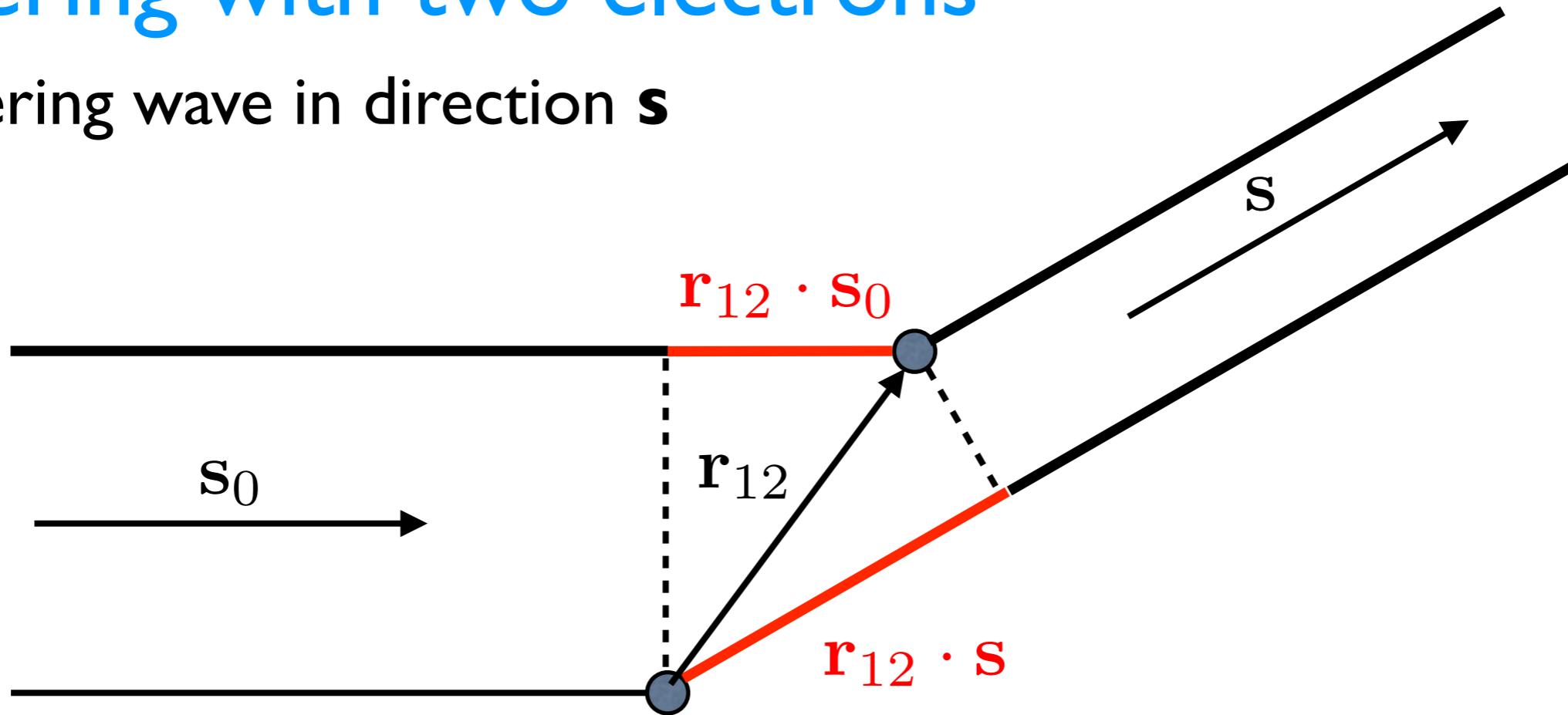
scattering with two electrons

scattering wave in direction \mathbf{s}



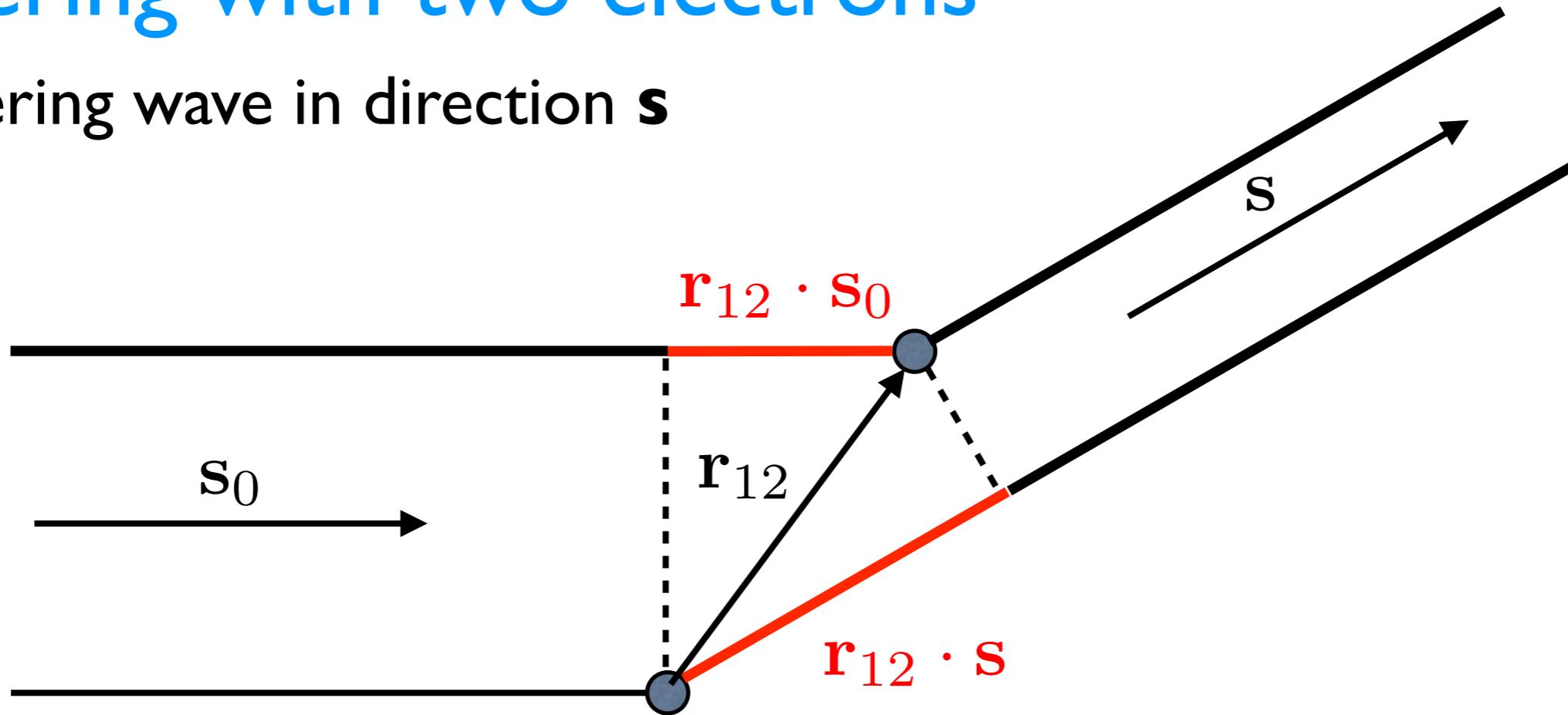
scattering with two electrons

scattering wave in direction \mathbf{s}



scattering with two electrons

scattering wave in direction \mathbf{s}

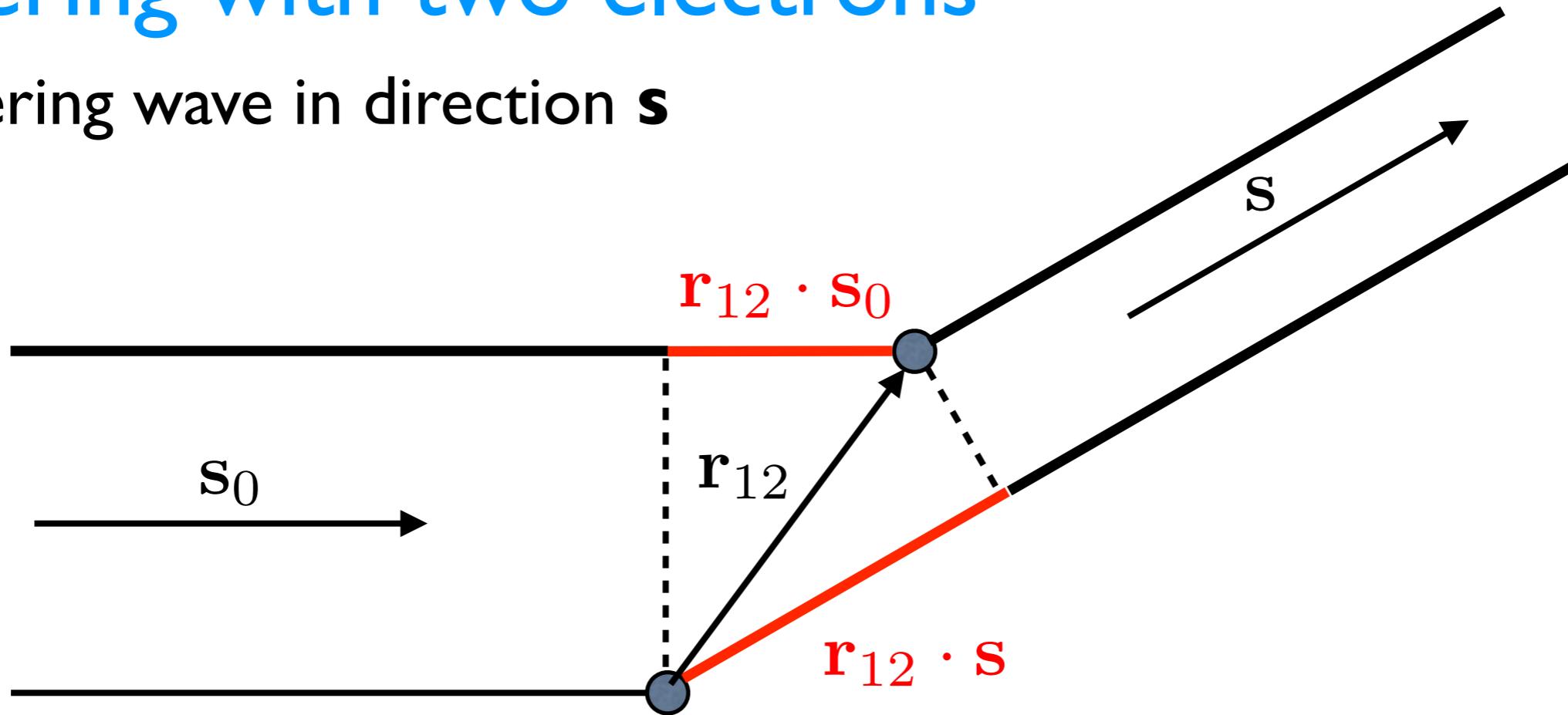


path length difference

$$\Delta = \mathbf{r}_{12} \cdot \mathbf{s} - \mathbf{r}_{12} \cdot \mathbf{s}_0 = \mathbf{r}_{12} \cdot (\mathbf{s} - \mathbf{s}_0)$$

scattering with two electrons

scattering wave in direction \mathbf{s}



path length difference

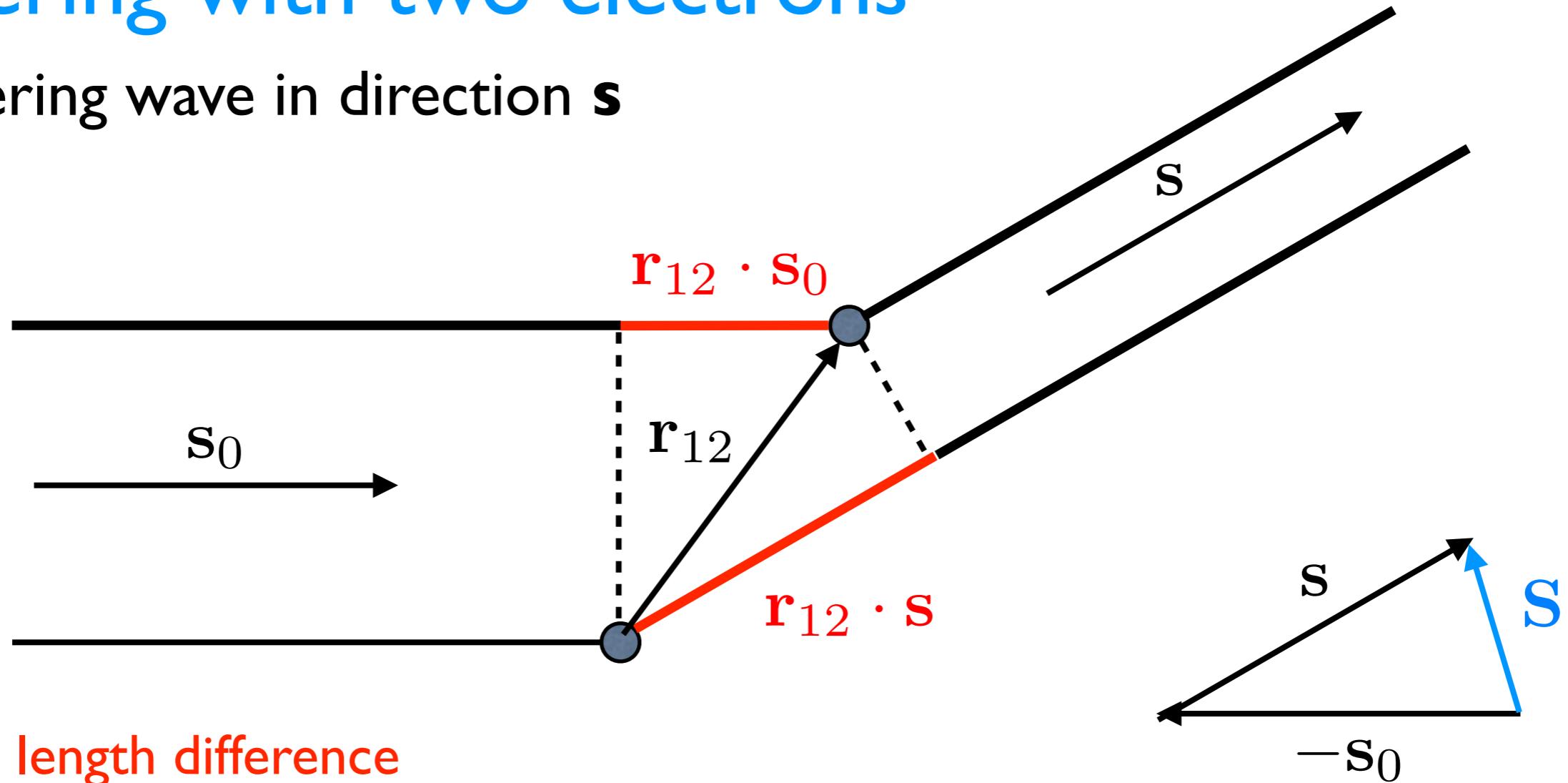
$$\Delta = \mathbf{r}_{12} \cdot \mathbf{s} - \mathbf{r}_{12} \cdot \mathbf{s}_0 = \mathbf{r}_{12} \cdot (\mathbf{s} - \mathbf{s}_0)$$

phase difference

$$\alpha = \frac{2\pi}{\lambda}(\mathbf{r}_{12} \cdot \mathbf{s} - \mathbf{r}_{12} \cdot \mathbf{s}_0) = 2\pi \mathbf{r}_{12} \cdot \mathbf{S}$$

scattering with two electrons

scattering wave in direction \mathbf{s}



path length difference

$$\Delta = \mathbf{r}_{12} \cdot \mathbf{s} - \mathbf{r}_{12} \cdot \mathbf{s}_0 = \mathbf{r}_{12} \cdot (\mathbf{s} - \mathbf{s}_0)$$

phase difference

$$\alpha = \frac{2\pi}{\lambda}(\mathbf{r}_{12} \cdot \mathbf{s} - \mathbf{r}_{12} \cdot \mathbf{s}_0) = 2\pi \mathbf{r}_{12} \cdot \mathbf{S}$$

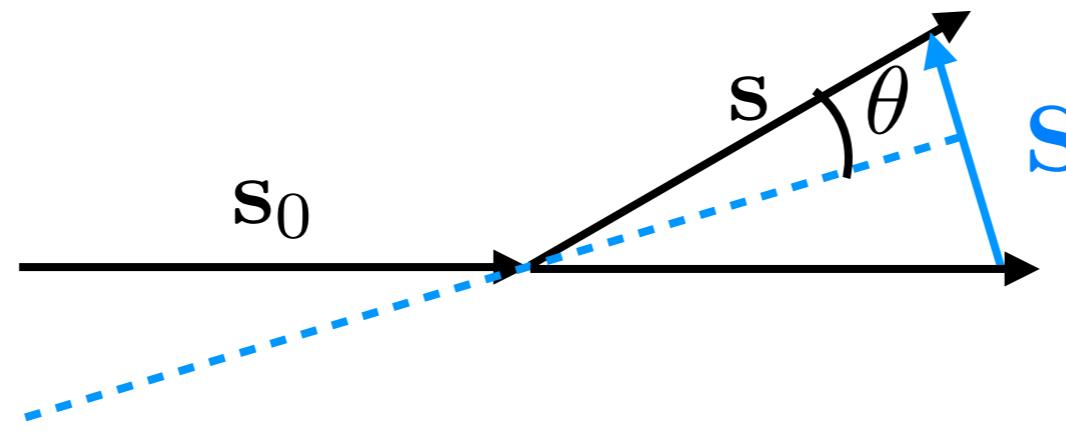
scattering vector
 $\mathbf{S} \equiv (\mathbf{s} - \mathbf{s}_0)/\lambda$

reflection plane

scattering with two electrons

“reflecting” plane

imaginary, but useful later

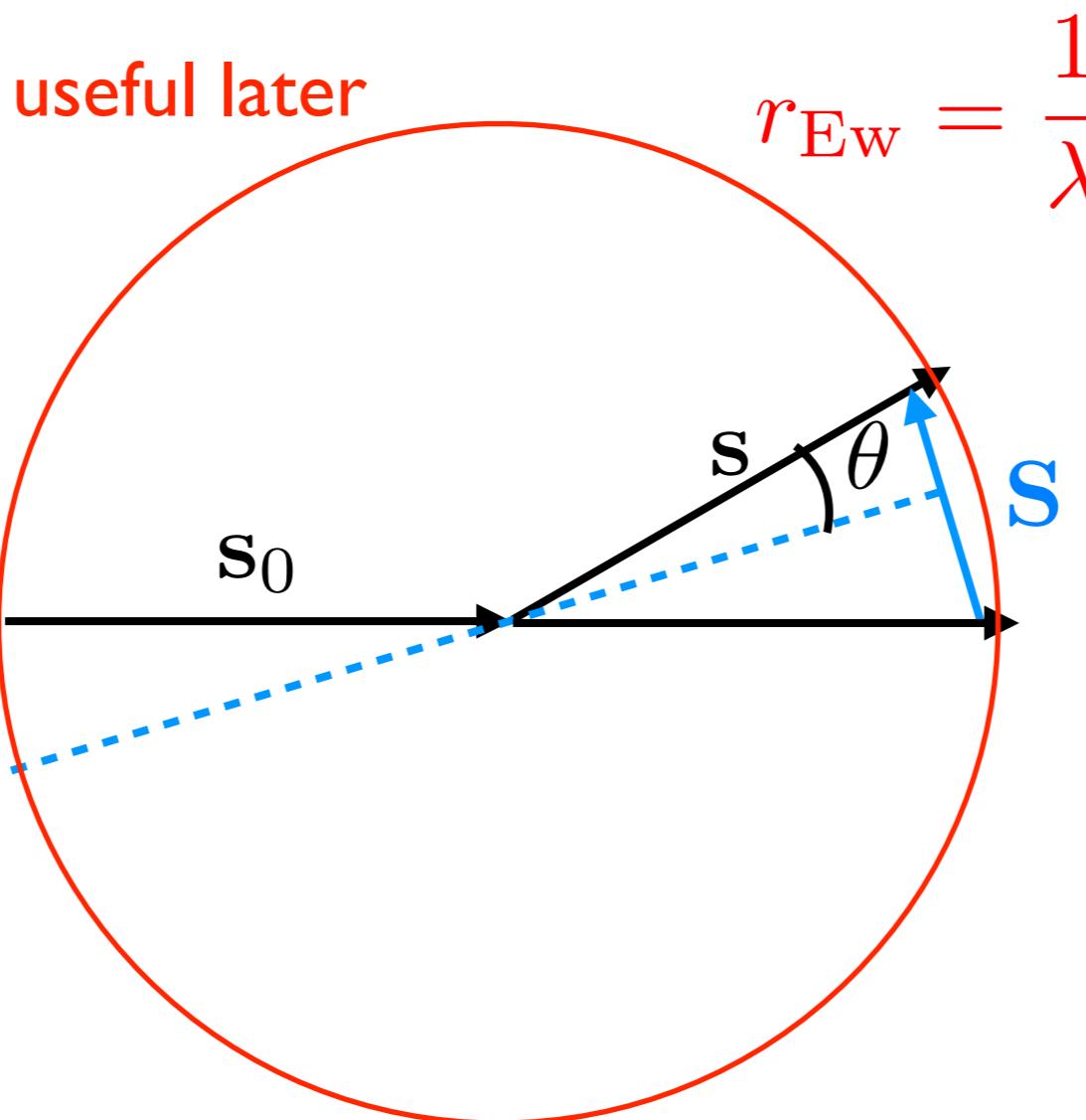


$$|S| = \frac{2 \sin(\theta)}{\lambda}$$

scattering with two electrons

“reflecting” plane

imaginary, but useful later
Ewald sphere



$$|\mathbf{S}| = \frac{2 \sin(\theta)}{\lambda}$$

scattering with two electrons

adding scattered waves

phase difference between x-ray scattered by electron 1 and 2

$$\alpha = 2\pi \mathbf{r}_{12} \cdot \mathbf{S}$$

scattering with two electrons

adding scattered waves

phase difference between x-ray scattered by electron 1 and 2

$$\alpha = 2\pi \mathbf{r}_{12} \cdot \mathbf{S}$$

scattered wave in direction S

scattering amplitudes: electron 1: A_1 , electron 2: A_2

$$\mathbf{F}(\mathbf{s}_0, \mathbf{s}) = A_1 + A_2 \exp[i\alpha]$$

scattering with two electrons

adding scattered waves

phase difference between x-ray scattered by electron 1 and 2

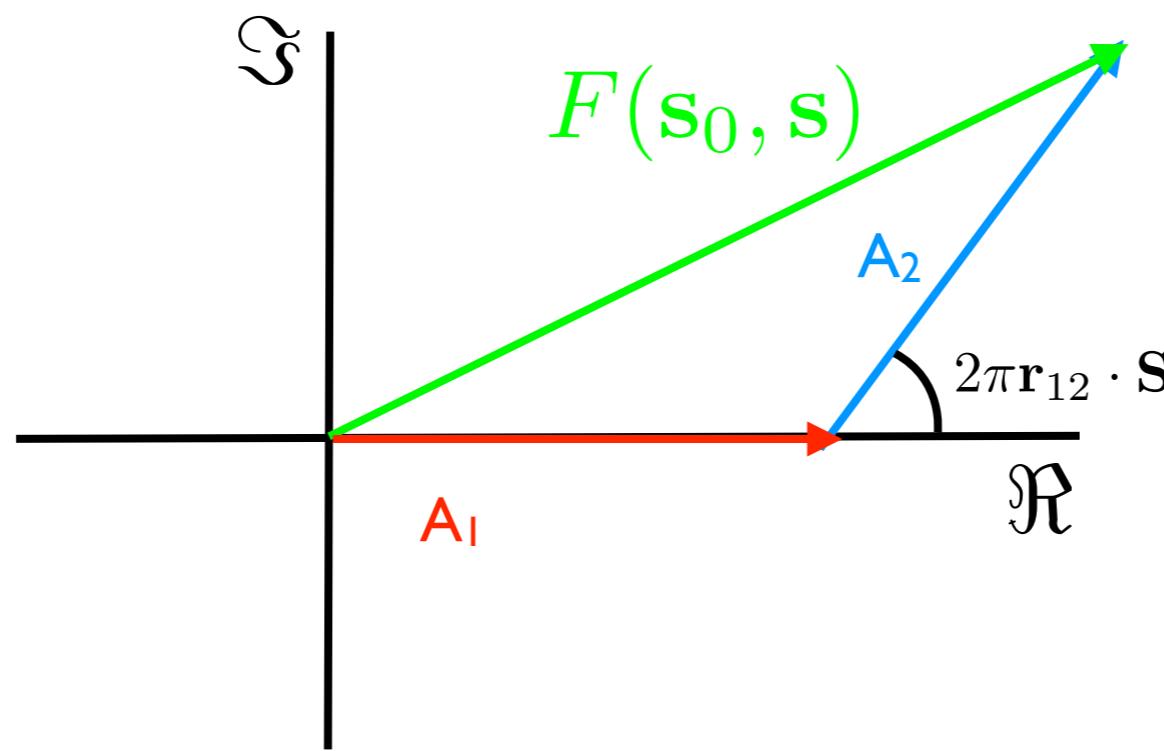
$$\alpha = 2\pi \mathbf{r}_{12} \cdot \mathbf{S}$$

scattered wave in direction S

scattering amplitudes: electron 1: A_1 , electron 2: A_2

$$F(\mathbf{s}_0, \mathbf{s}) = A_1 + A_2 \exp[i\alpha]$$

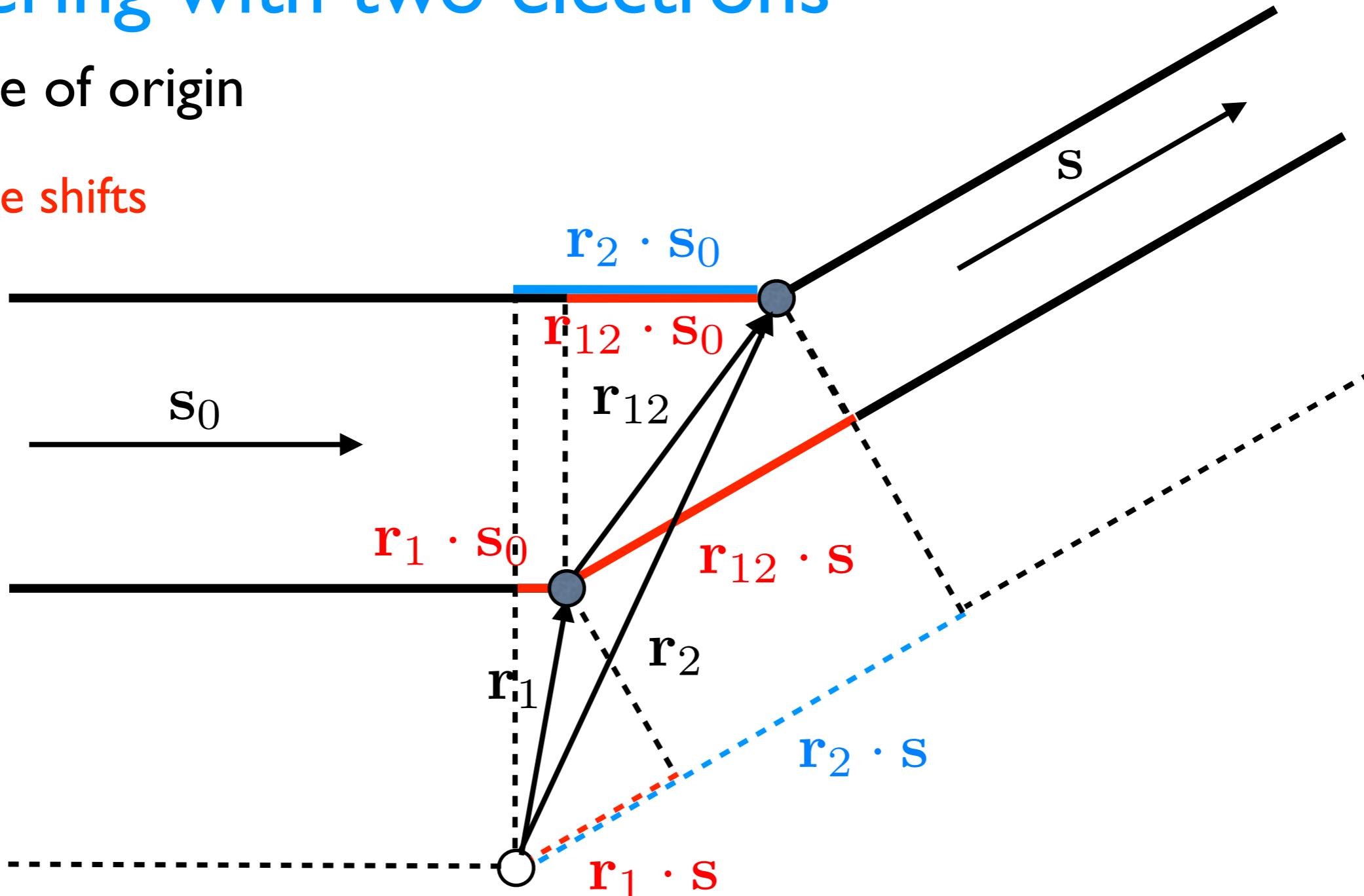
Argand diagram



scattering with two electrons

change of origin

phase shifts



$$\alpha_1 = \frac{2\pi}{\lambda} (r_1 \cdot s - r_1 \cdot s_0) = 2\pi r_1 \cdot S$$

$$\alpha_2 = \frac{2\pi}{\lambda} (r_2 \cdot s - r_2 \cdot s_0) = 2\pi r_2 \cdot S$$

$$= 2\pi(r_1 + r_{12}) \cdot S = \alpha_1 + 2\pi r_{12} \cdot S$$

scattering with electrons

change of origin

phase shifts

shift origin by \mathbf{R} shifts all phases by $2\pi\mathbf{R} \cdot \mathbf{S}$

$$\alpha_2 = 2\pi\mathbf{r}_{12} \cdot \mathbf{S} + 2\pi\mathbf{R} \cdot \mathbf{S}$$

$$\alpha_1 = 0 + 2\pi\mathbf{R} \cdot \mathbf{S}$$

scattering with electrons

change of origin

phase shifts

shift origin by \mathbf{R} shifts all phases by $2\pi\mathbf{R} \cdot \mathbf{S}$

$$\alpha_2 = 2\pi\mathbf{r}_{12} \cdot \mathbf{S} + 2\pi\mathbf{R} \cdot \mathbf{S}$$

$$\alpha_1 = 0 + 2\pi\mathbf{R} \cdot \mathbf{S}$$

scattering with many electrons

scattering of unitcell with N electrons

scattering factor

$$\mathbf{F}(\mathbf{s}_0, \mathbf{s}) = A_1 \exp[i\alpha_1] + A_2 \exp[i\alpha_2] + \dots + A_N \exp[i\alpha_N]$$

$$\mathbf{F}(\mathbf{s}_0, \mathbf{s}) = \sum_k^N A_k \exp[i\alpha_k]$$

scattering with many electrons

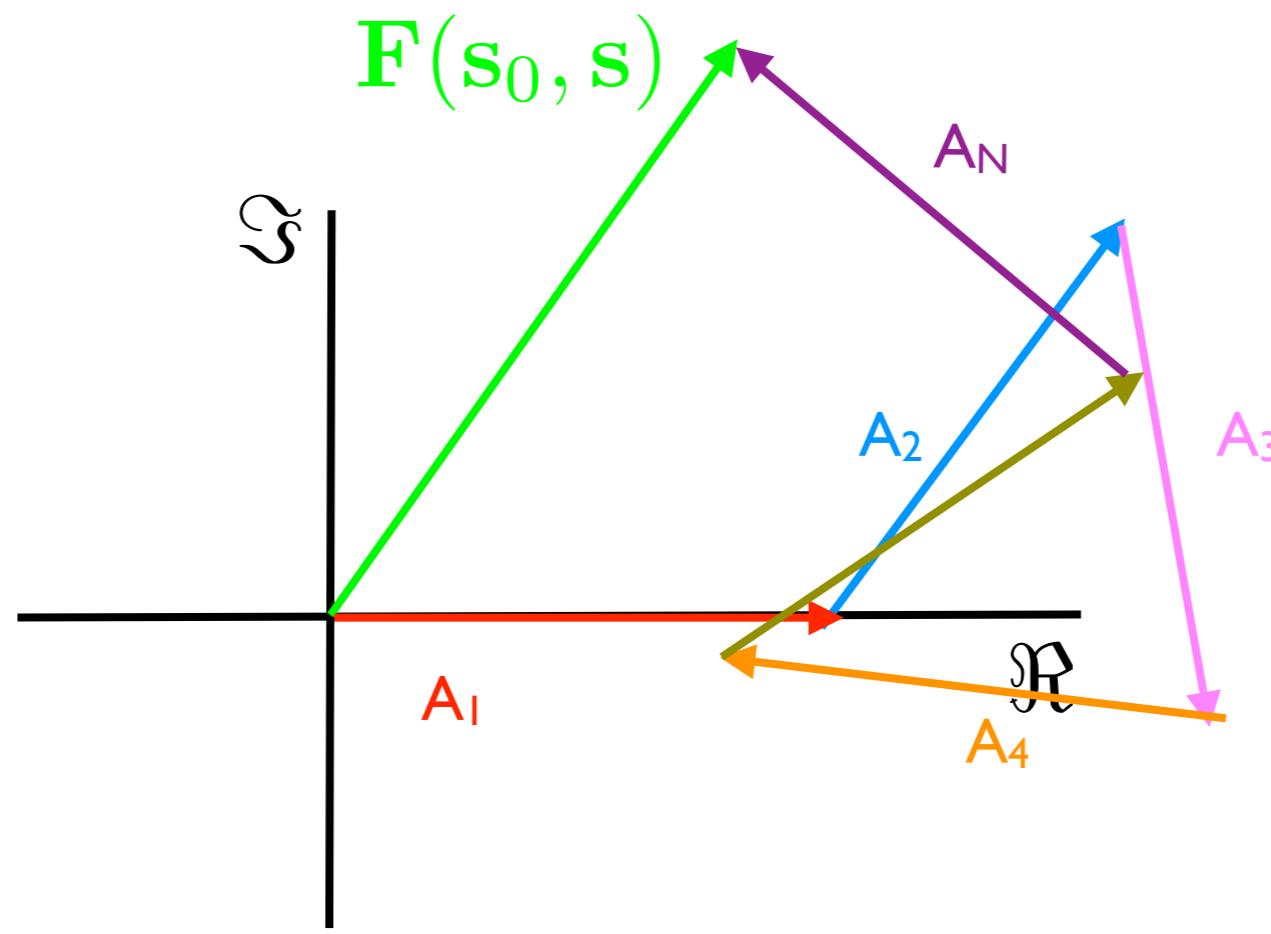
scattering of system with N electrons

scattering factor

$$F(s_0, s) = A_1 \exp[i\alpha_1] + A_2 \exp[i\alpha_2] + \dots + A_N \exp[i\alpha_N]$$

$$F(s_0, s) = \sum_k^N A_k \exp[i\alpha_k]$$

Argand diagram

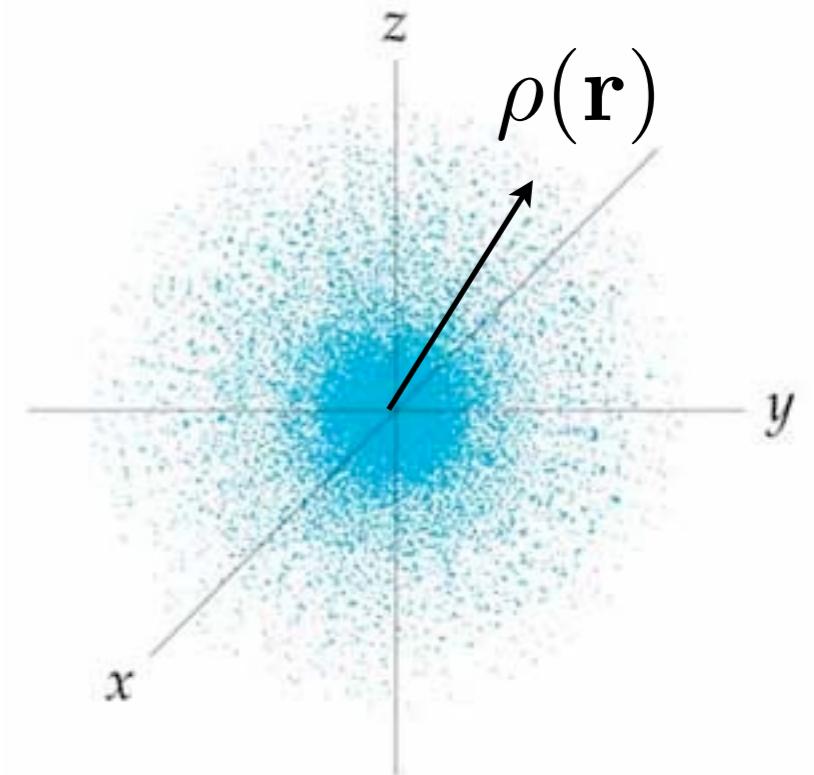


scattering with atoms

scattering of single atom

electrons are ‘free’

$$n_e(\mathbf{r}) = \rho(\mathbf{r})d\mathbf{r}$$



scattering with atoms

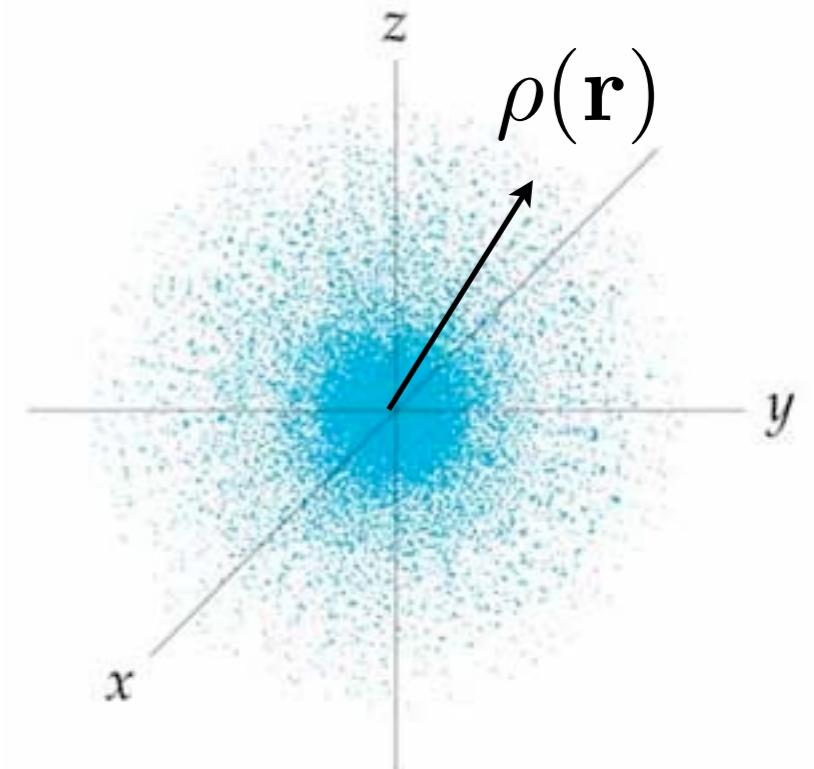
scattering of single atom

electron are ‘free’

$$n_e(\mathbf{r}) = \rho(\mathbf{r})d\mathbf{r}$$

structure factor

$$f = \int_{-\infty}^{\infty} \rho(\mathbf{r}) \exp[2\pi i \mathbf{r} \cdot \mathbf{S}] d\mathbf{r}$$



scattering with atoms

scattering of single atom

electron are ‘free’

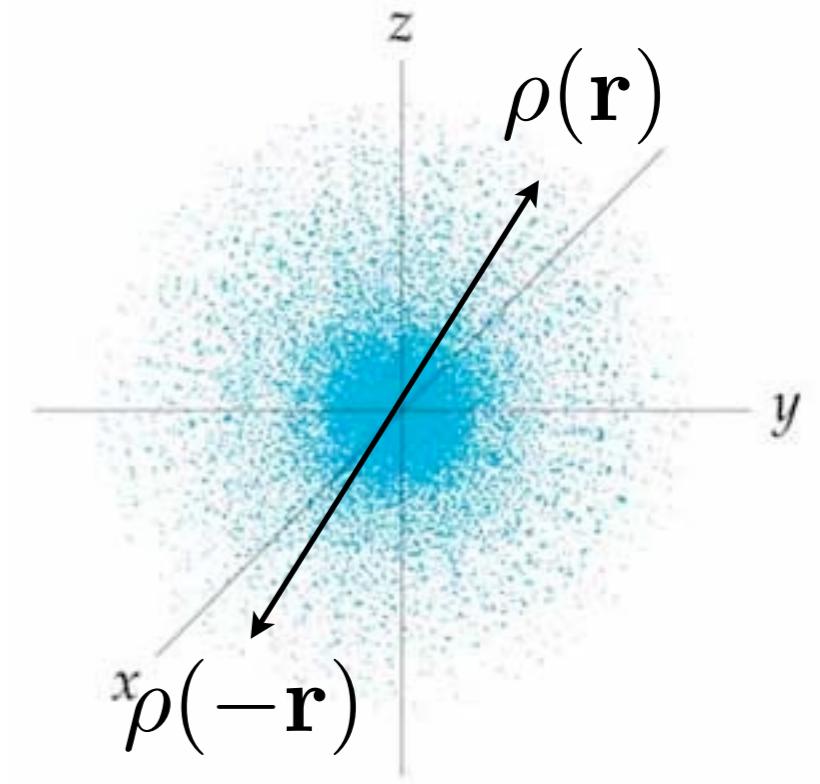
$$n_e(\mathbf{r}) = \rho(\mathbf{r})d\mathbf{r}$$

structure factor

$$f = \int_{-\infty}^{\infty} \rho(\mathbf{r}) \exp[2\pi i \mathbf{r} \cdot \mathbf{S}] d\mathbf{r}$$

spherically symmetric electron cloud

$$\begin{aligned} f &= \int_0^{\infty} \rho(\mathbf{r}) \{ \exp[2\pi i \mathbf{r} \cdot \mathbf{S}] + \exp[-2\pi i \mathbf{r} \cdot \mathbf{S}] \} d\mathbf{r} \\ &= 2 \int_0^{\infty} \rho(\mathbf{r}) \cos(2\pi \mathbf{r} \cdot \mathbf{S}) d\mathbf{r} \end{aligned}$$



scattering with atoms

scattering of single atom

electron are free electrons

$$n_e(\mathbf{r}) = \rho(\mathbf{r})d\mathbf{r}$$

structure factor

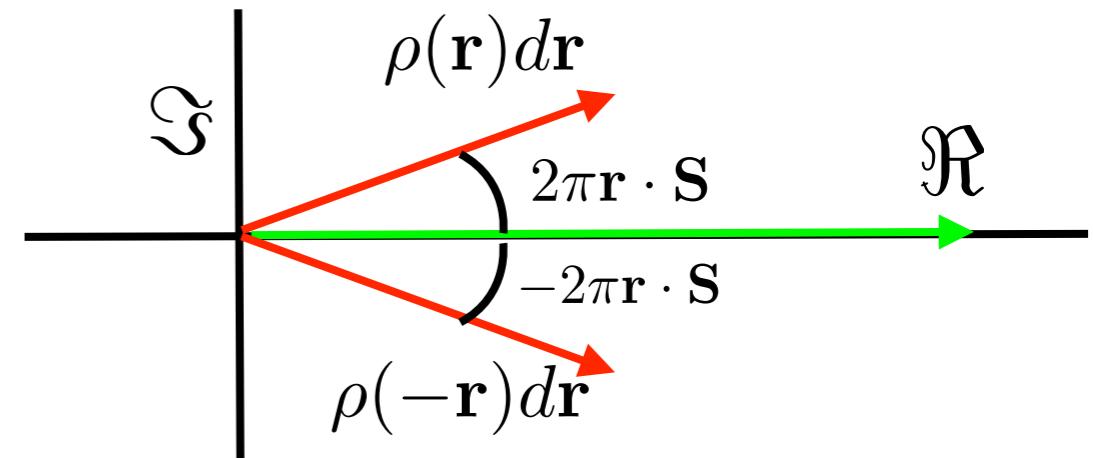
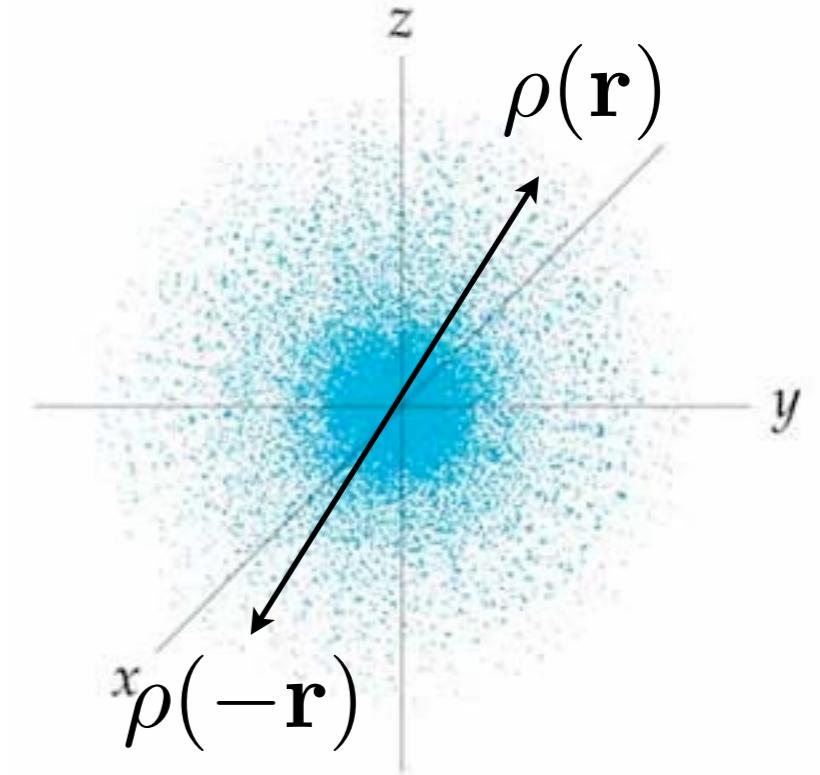
$$f = \int_{-\infty}^{\infty} \rho(\mathbf{r}) \exp[2\pi i \mathbf{r} \cdot \mathbf{S}] d\mathbf{r}$$

spherically symmetric electron cloud

$$f = \int_0^{\infty} \rho(\mathbf{r}) \{ \exp[2\pi i \mathbf{r} \cdot \mathbf{S}] + \exp[-2\pi i \mathbf{r} \cdot \mathbf{S}] \} d\mathbf{r}$$

$$= 2 \int_0^{\infty} \rho(\mathbf{r}) \cos(2\pi \mathbf{r} \cdot \mathbf{S}) d\mathbf{r}$$

atomic structure factor always real!



scattering with atoms

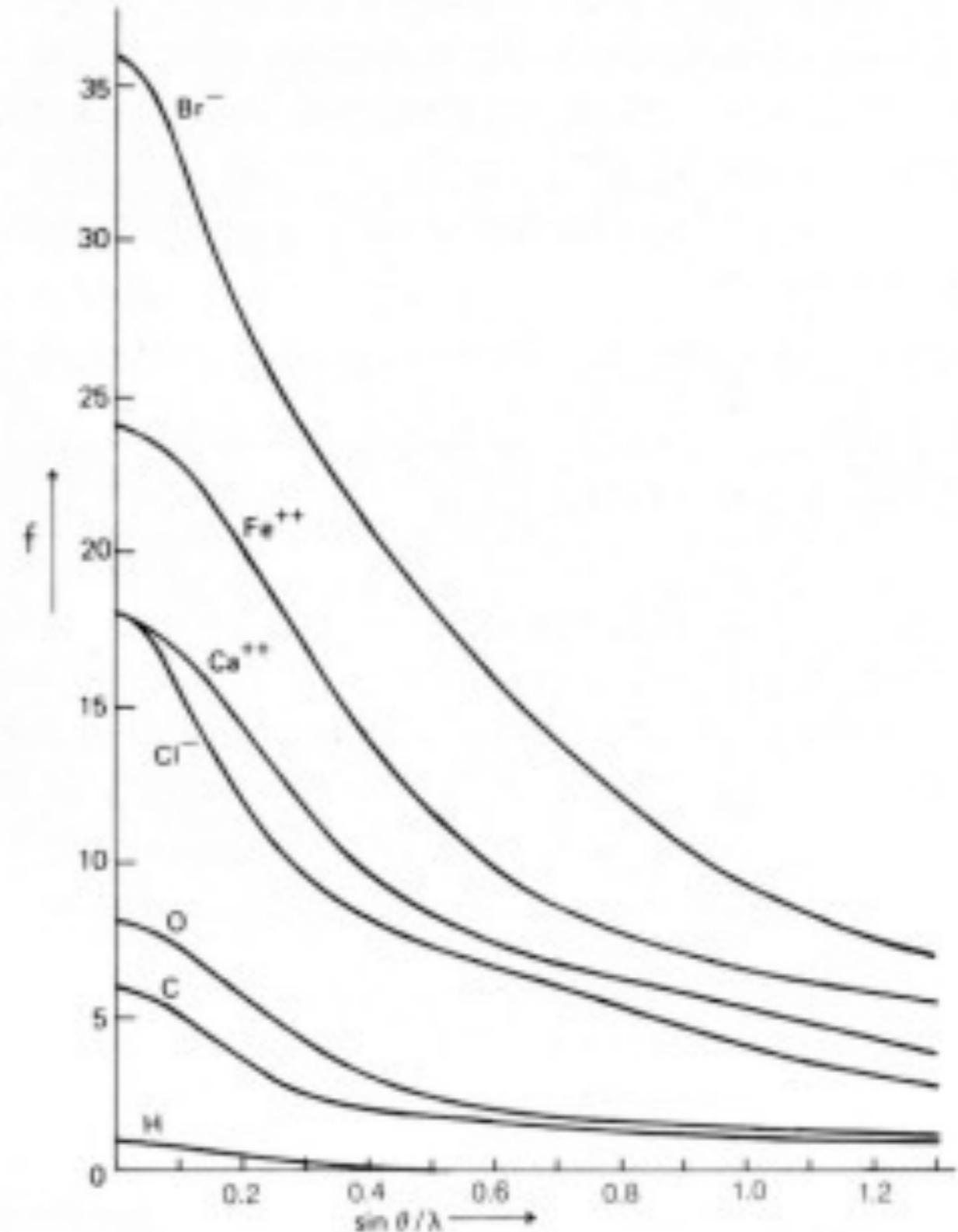
scattering of single atom

structure factor

$$f = \int_{-\infty}^{\infty} \rho(\mathbf{r}) \exp[2\pi i \mathbf{r} \cdot \mathbf{S}] d\mathbf{r}$$

angle dependence

$$|\mathbf{S}| = \frac{2 \sin(\theta)}{\lambda}$$



scattering with single molecules

scattering of atoms in the molecule

$$\mathbf{F}(\mathbf{S}) = \sum_k^N f_k \exp[2\pi i \mathbf{r}_k \cdot \mathbf{S}]$$

scattering with single molecules

scattering of atoms in the molecule

$$\mathbf{F}(\mathbf{S}) = \sum_k^N f_k \exp[2\pi i \mathbf{r}_k \cdot \mathbf{S}]$$

detectors

intensities, but no phases

$$I(\mathbf{S}) = \mathbf{F}^*(\mathbf{S}) \mathbf{F}(\mathbf{S})$$

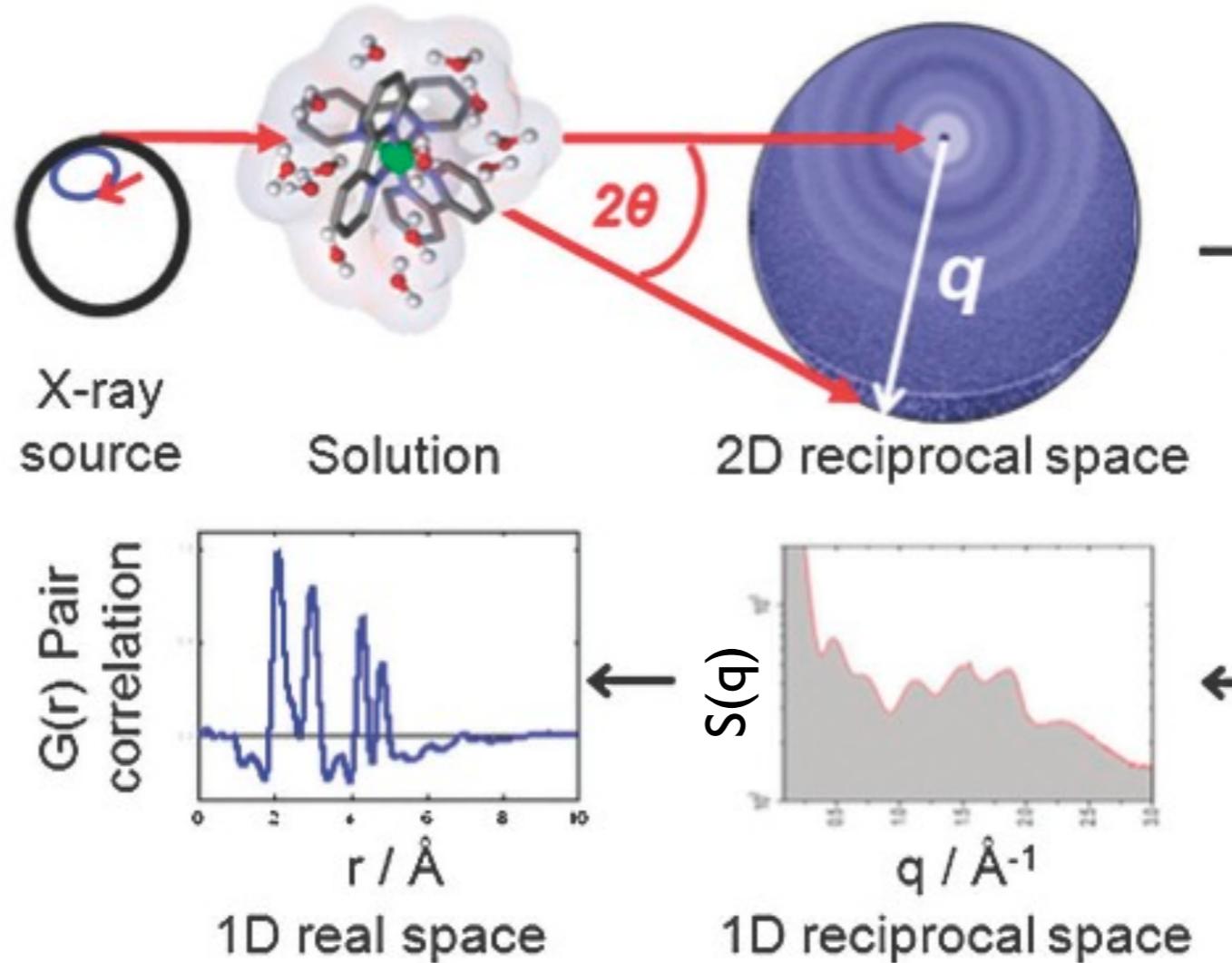
$$= \sum_j^N \sum_k^N f_j f_k \exp[-2\pi i \mathbf{r}_j \cdot \mathbf{S}] \exp[2\pi i \mathbf{r}_k \cdot \mathbf{S}]$$

$$= \sum_j^N \sum_k^N f_j f_k \exp[2\pi i (\mathbf{r}_k - \mathbf{r}_j) \cdot \mathbf{S}]$$

$$= \sum_j^N \sum_k^N f_j f_k \exp[2\pi i (\mathbf{r}_{jk}) \cdot \mathbf{S}]$$

Wide angle x-ray scattering (WAXS)

concept



scattering image

$$I(\mathbf{q}) = \sum_i^N \sum_j^N f_i f_j \exp(i \mathbf{q} \cdot \mathbf{r}_{ij}) \quad q = \frac{4\pi \sin \theta}{\lambda}$$

isotropic samples (average over all orientations): 1D signal

$$I(q) = \langle I(\mathbf{q}) \rangle_{\Omega} = \sum_i^N \sum_j^N f_i f_j \frac{\sin(\mathbf{q} \cdot \mathbf{r}_{ij})}{\mathbf{q} \cdot \mathbf{r}_{ij}}$$

example, anyone?

scattering with crystals

scattering with one atom in the unitcell

unitcell alone

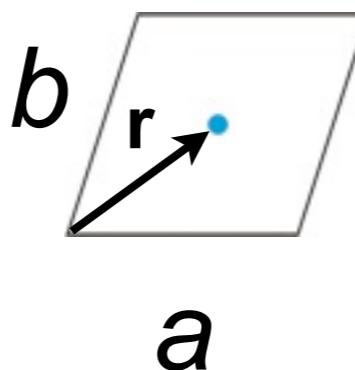
scattering vector

$$F(\mathbf{S}) = f \exp[2\pi i \mathbf{r} \cdot \mathbf{S}] \quad \mathbf{S} \equiv (\mathbf{s} - \mathbf{s}_0)/\lambda$$

position of the single atom with respect to origin

$$\mathbf{r} = \mathbf{a}x + \mathbf{b}y + \mathbf{c}z$$

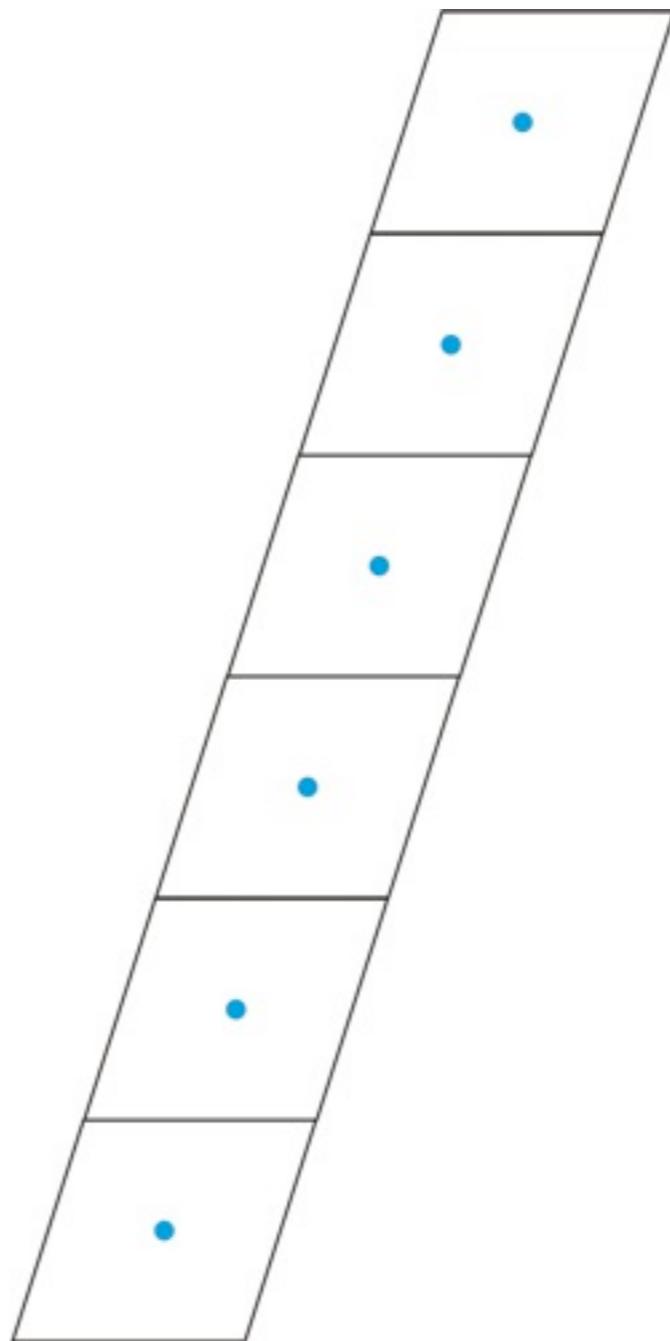
$$\begin{aligned}\mathbf{r} \cdot \mathbf{S} &= (\mathbf{a}x + \mathbf{b}y + \mathbf{c}z) \cdot \mathbf{S} \\ &= \mathbf{a} \cdot \mathbf{S}x + \mathbf{b} \cdot \mathbf{S}y + \mathbf{c} \cdot \mathbf{S}z\end{aligned}$$



scattering with crystals

scattering with one atom in the unitcell

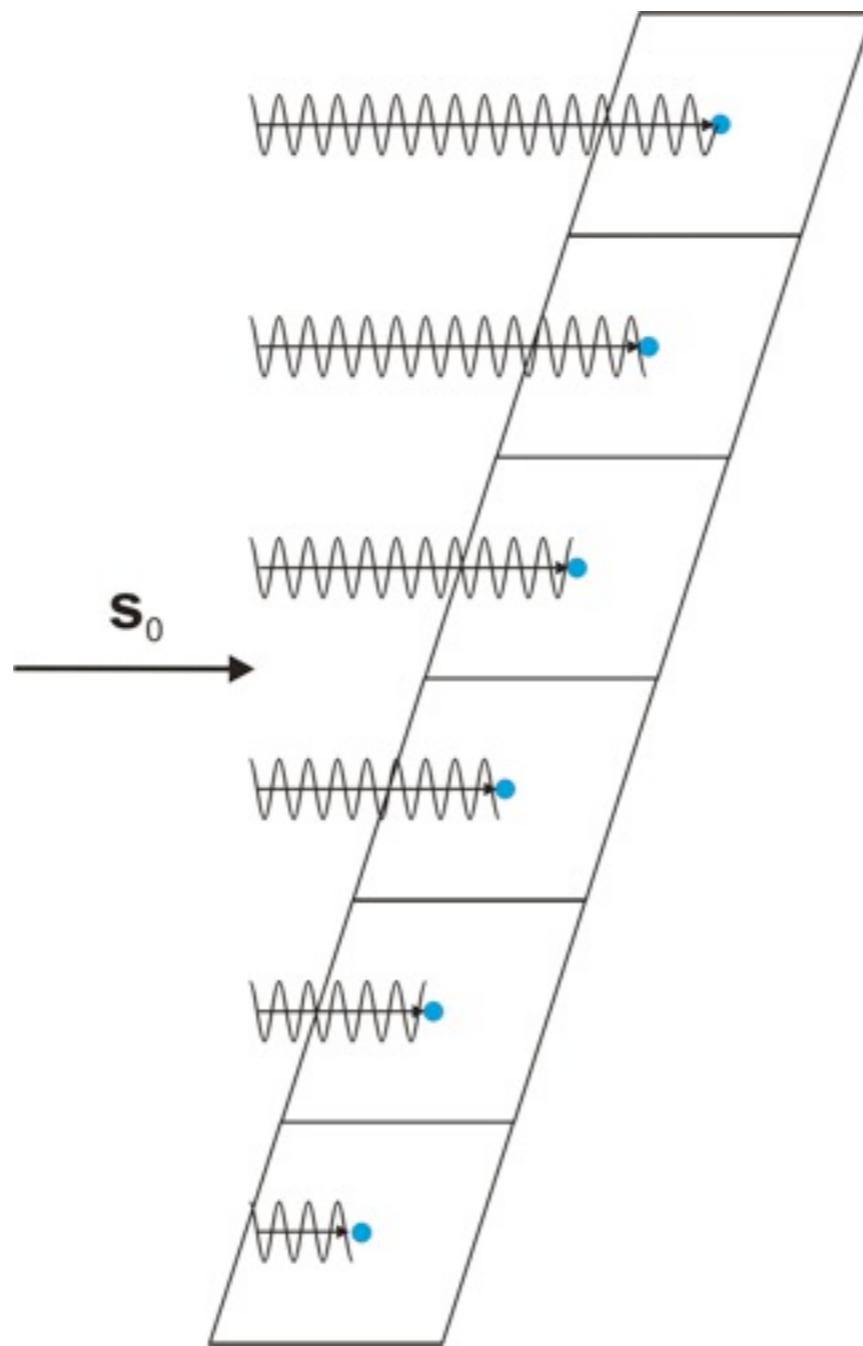
crystal (i.e lattice of unitcells)



scattering with crystals

scattering with one atom in the unitcell

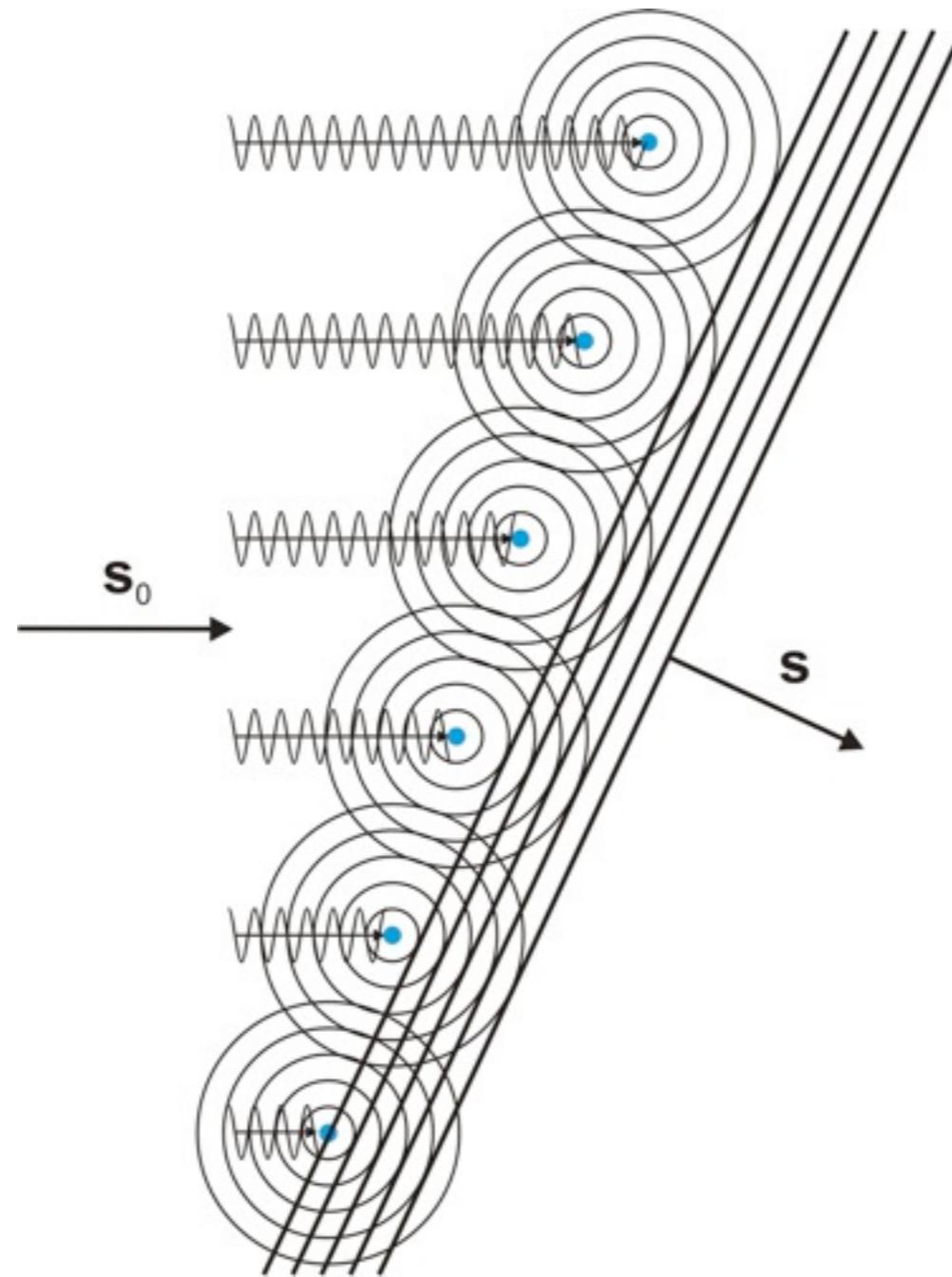
crystal (i.e lattice of unitcells)



scattering with crystals

scattering with one atom in the unitcell

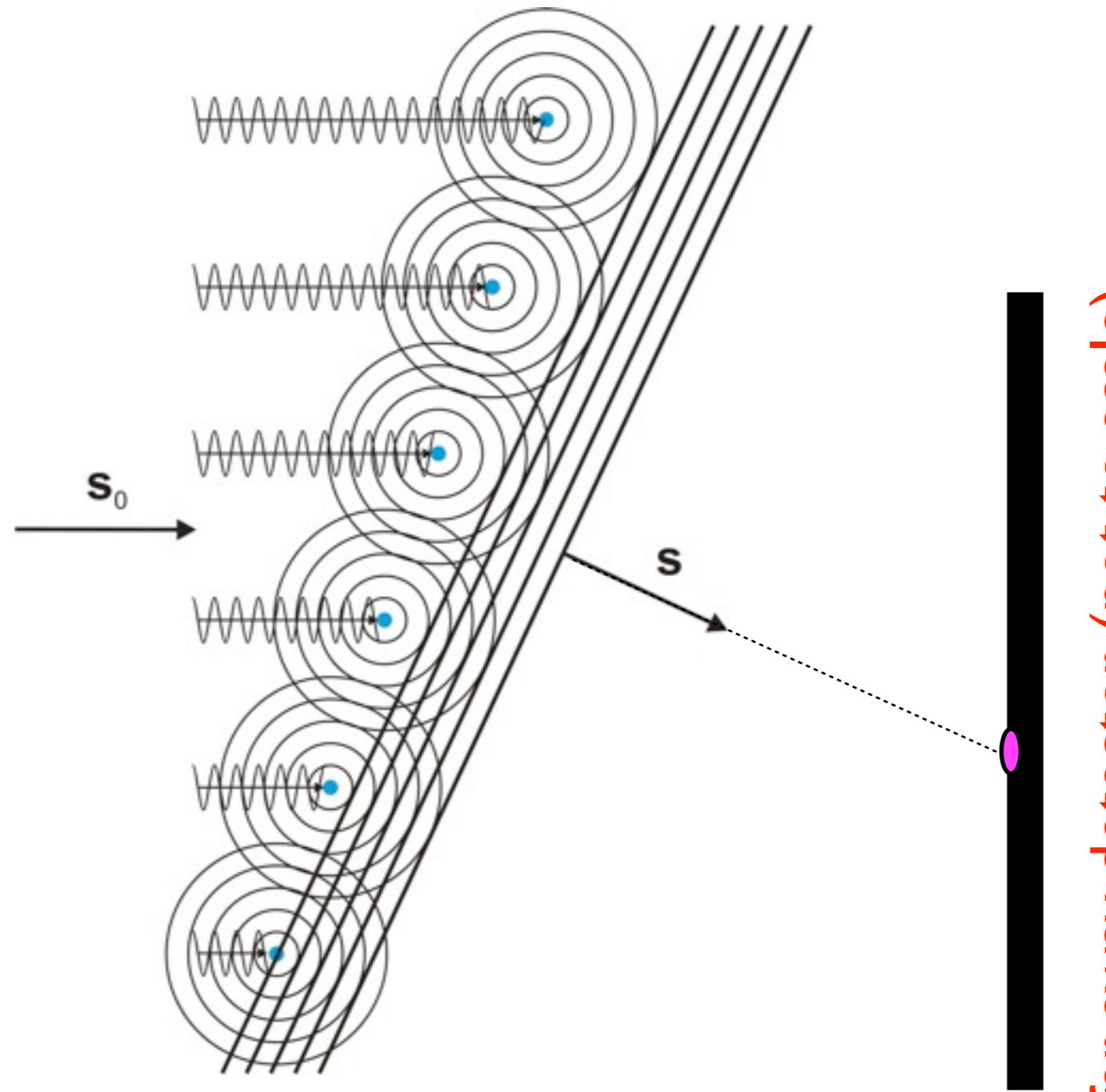
crystal (i.e lattice of unitcells)



scattering with crystals

scattering with one atom in the unitcell

crystal (i.e lattice of unitcells)

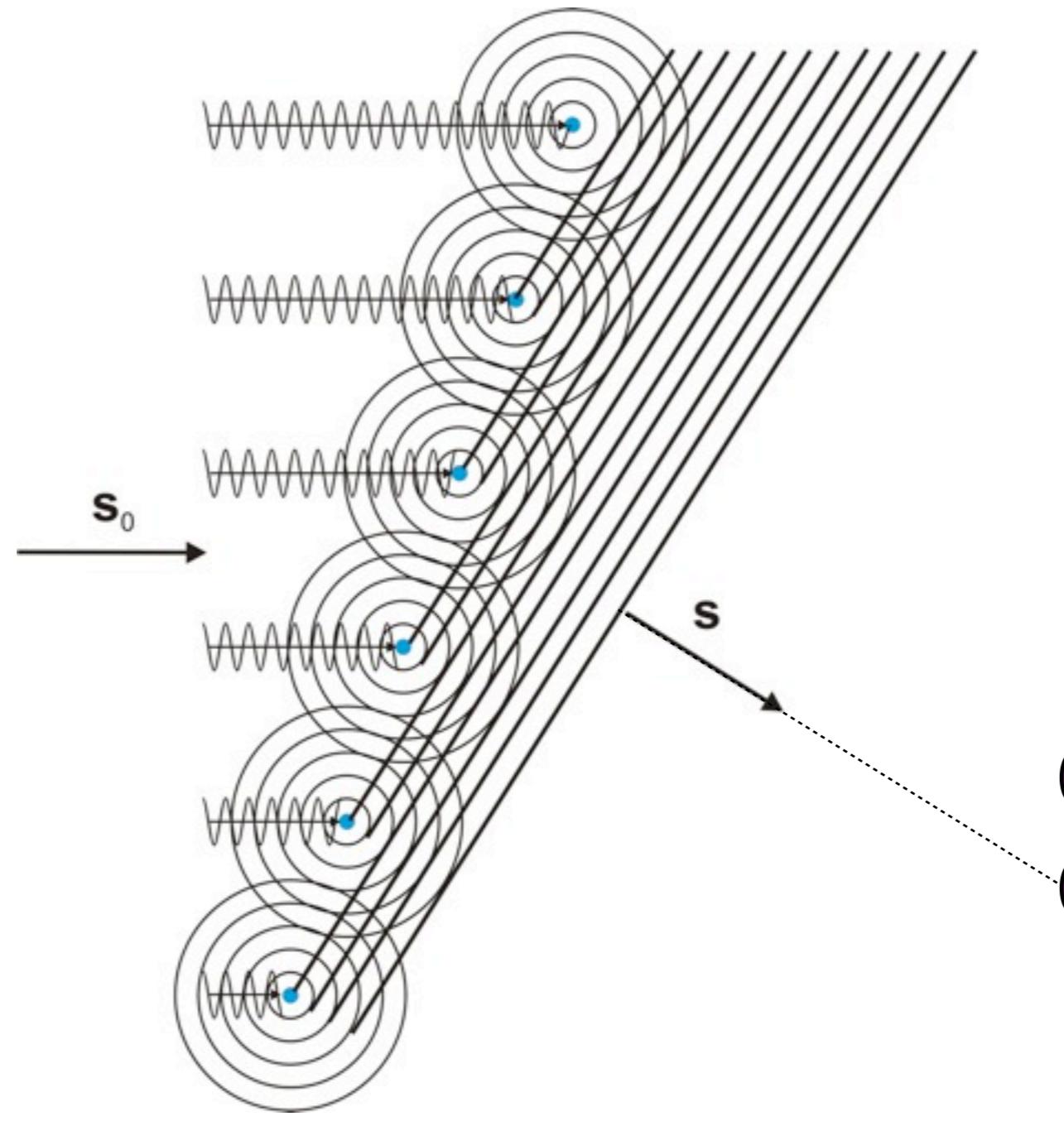


far away detector (not to scale)

scattering with crystals

scattering with one atom in the unitcell

crystal (i.e lattice of unitcells)



far away detector (not to scale)

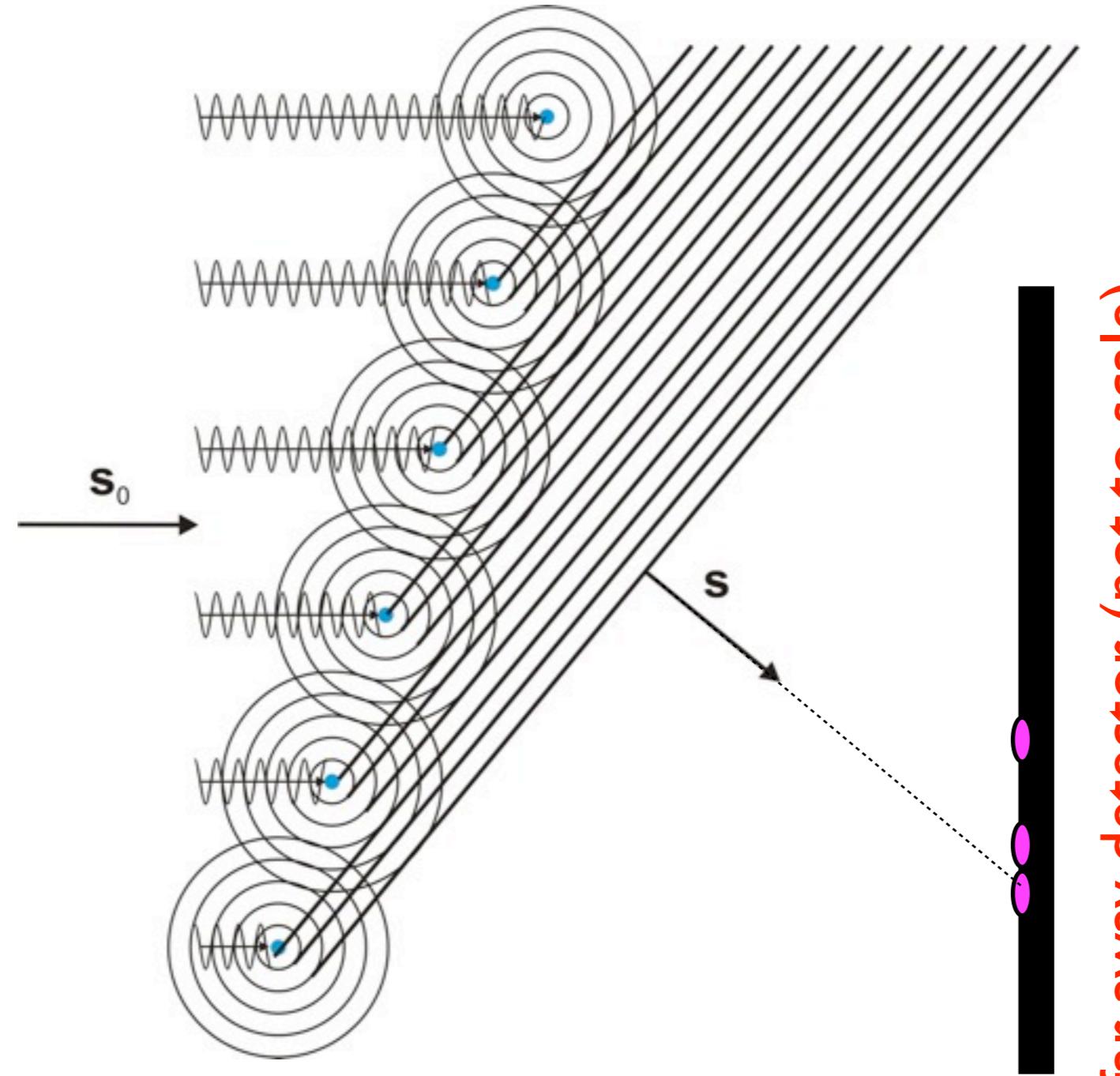
scattering with crystals

scattering with one atom in the unitcell

crystal (i.e lattice of unitcells)

discrete scattering spots: diffraction

reflections



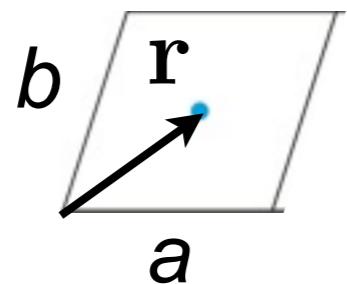
far away detector (not to scale)

scattering with crystals

scattering with one atom in the unitcell

unitcell with lattice point at origin

$$\mathbf{F}(\mathbf{S}) = f \exp[2\pi i \mathbf{r} \cdot \mathbf{S}]$$



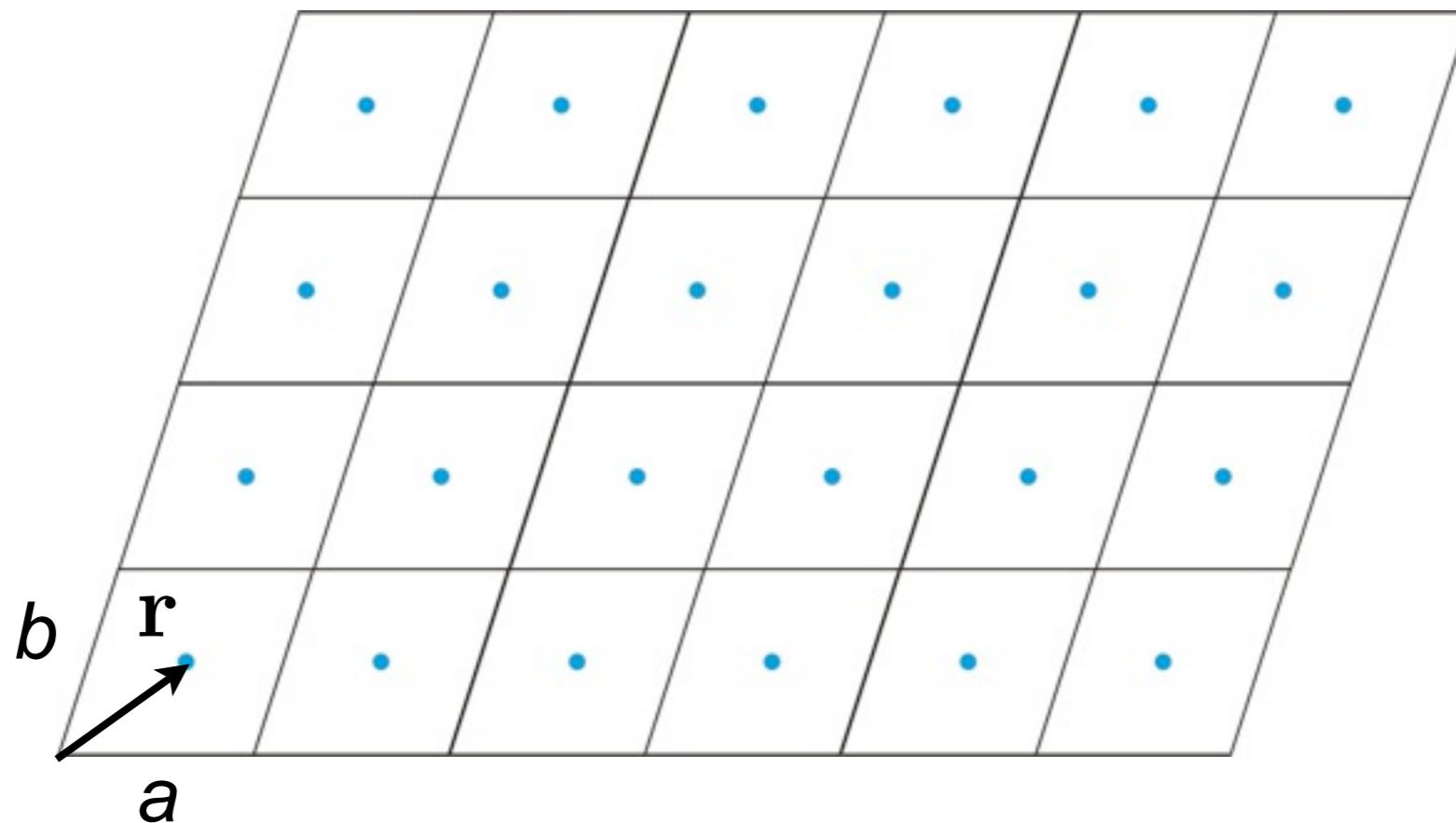
scattering with crystals

scattering with one atom in the unitcell

unitcell with lattice point at origin

$$\mathbf{F}(\mathbf{S}) = f \exp[2\pi i \mathbf{r} \cdot \mathbf{S}]$$

crystal (i.e lattice of unitcells)



scattering with crystals

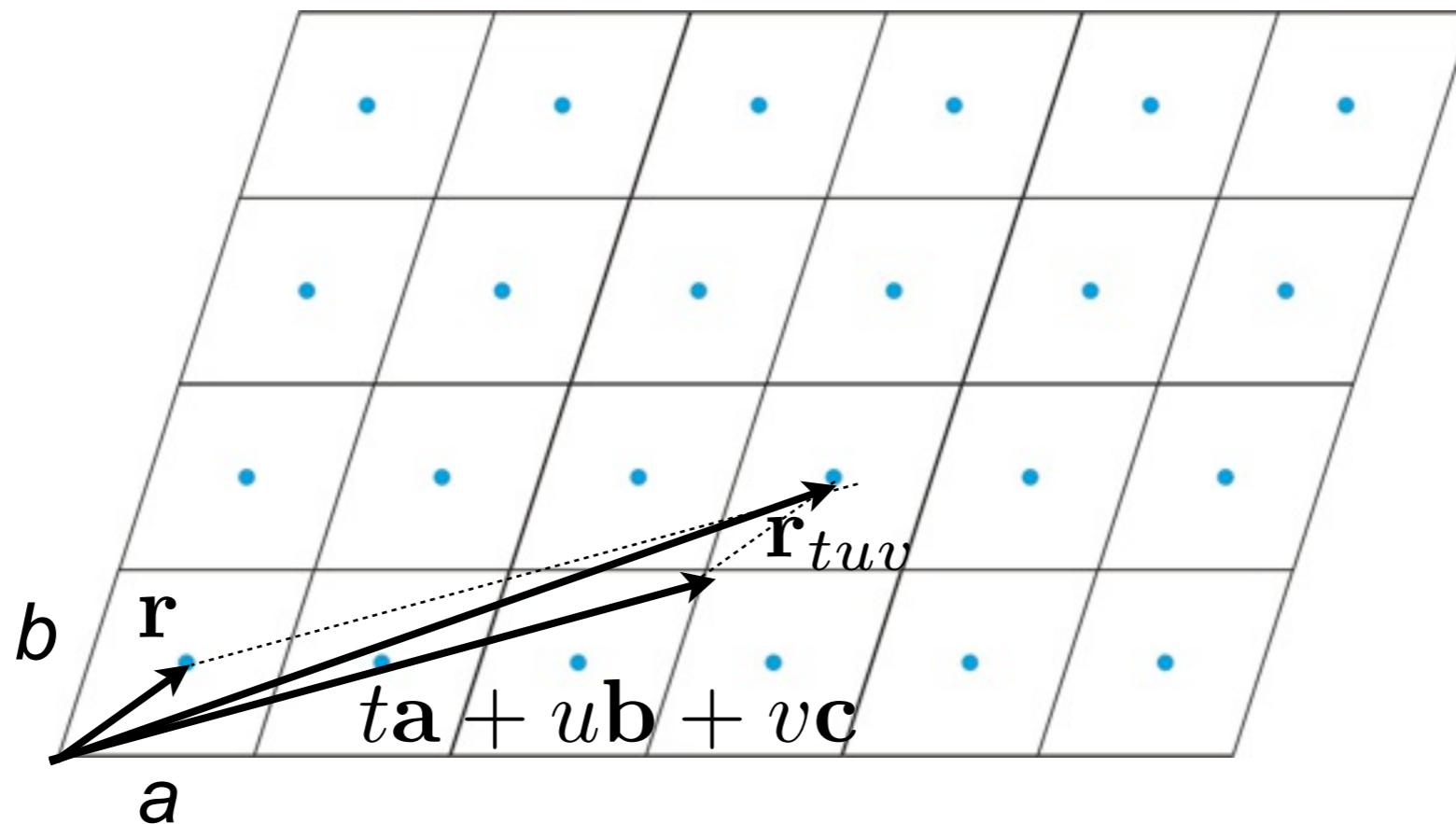
scattering with one atom in the unitcell

unitcell with lattice point at origin

$$\mathbf{F}(\mathbf{S}) = f \exp[2\pi i \mathbf{r} \cdot \mathbf{S}]$$

crystal (i.e lattice of unitcells)

$$\mathbf{r}_{tuv} = (x + t)\mathbf{a} + (y + u)\mathbf{b} + (z + v)\mathbf{c} \quad u, t, v \in N$$



scattering with crystals

scattering with one atom in the unitcell

unitcell with lattice point at origin

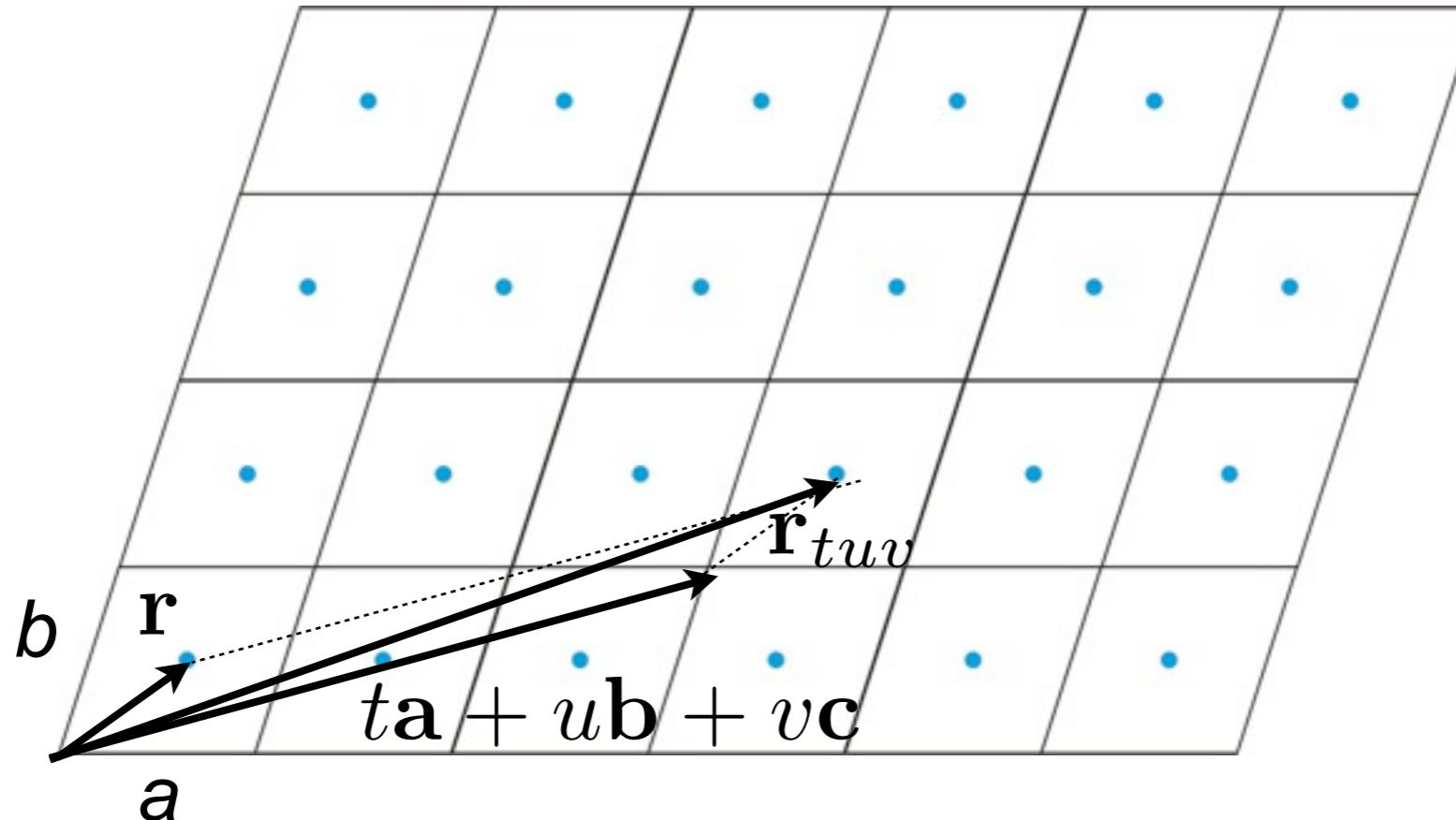
$$\mathbf{F}(\mathbf{S}) = f \exp[2\pi i \mathbf{r} \cdot \mathbf{S}]$$

crystal (i.e lattice of unitcells)

$$\mathbf{r}_{tuv} = (x + t)\mathbf{a} + (y + u)\mathbf{b} + (z + v)\mathbf{c} \quad u, t, v \in N$$

scattering of each single cell in lattice (phase shift)

$$\mathbf{F}_{tuv}(\mathbf{S}) = \mathbf{F}(\mathbf{S}) \times \exp[2\pi it\mathbf{a} \cdot \mathbf{S}] \times \exp[2\pi iy\mathbf{b} \cdot \mathbf{S}] \times \exp[2\pi iz\mathbf{c} \cdot \mathbf{S}]$$



scattering with crystals

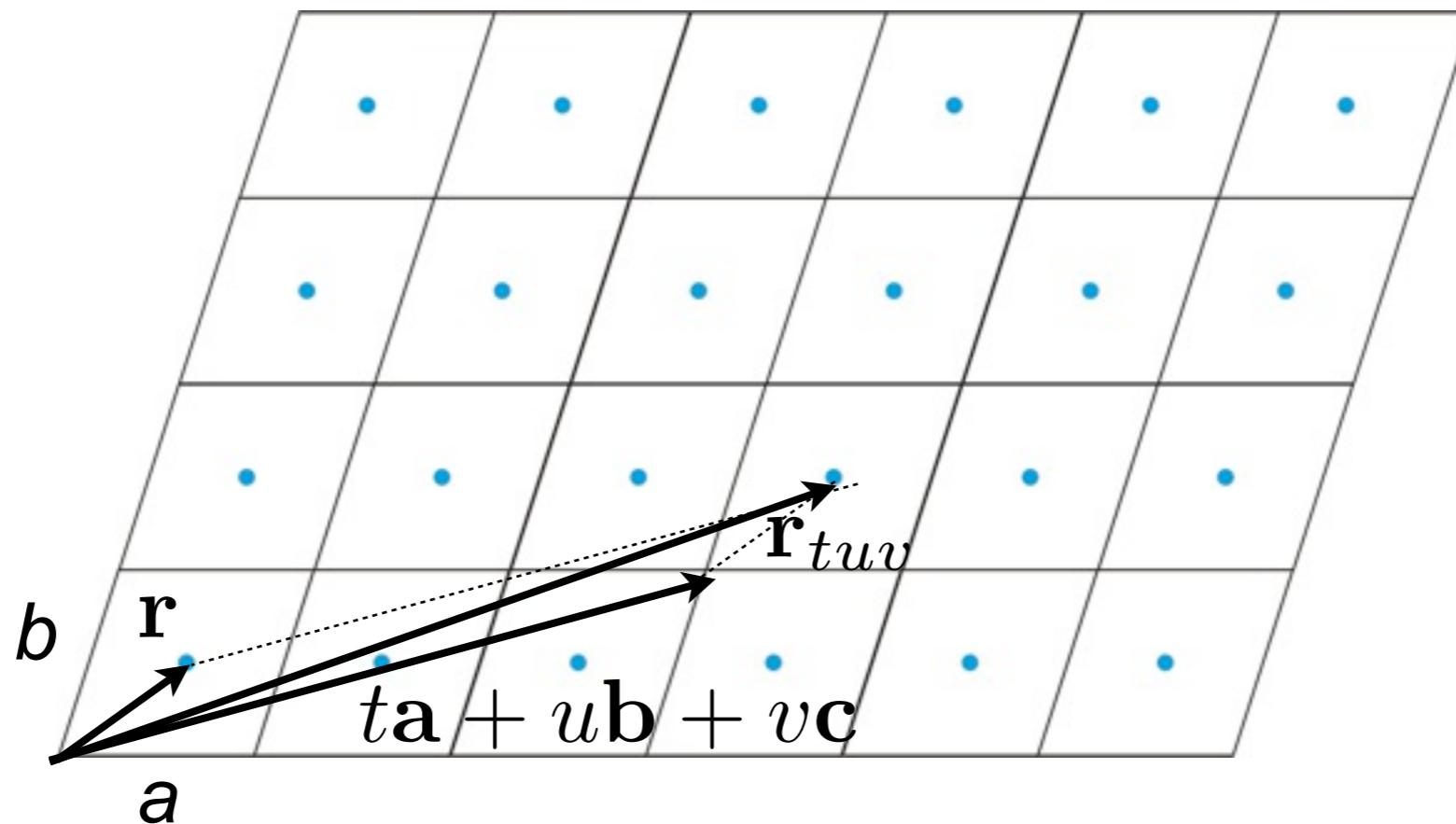
scattering with one atom in the unitcell

total scattering

$$\mathbf{K}(\mathbf{S}) = \mathbf{F}(\mathbf{S}) \times \sum_{t=0}^{N_1} \exp[2\pi it\mathbf{a} \cdot \mathbf{S}]$$

$$\times \sum_{u=0}^{N_2} \exp[2\pi iu\mathbf{b} \cdot \mathbf{S}]$$

$$\times \sum_{v=0}^{N_3} \exp[2\pi iv\mathbf{c} \cdot \mathbf{S}]$$



scattering with crystals

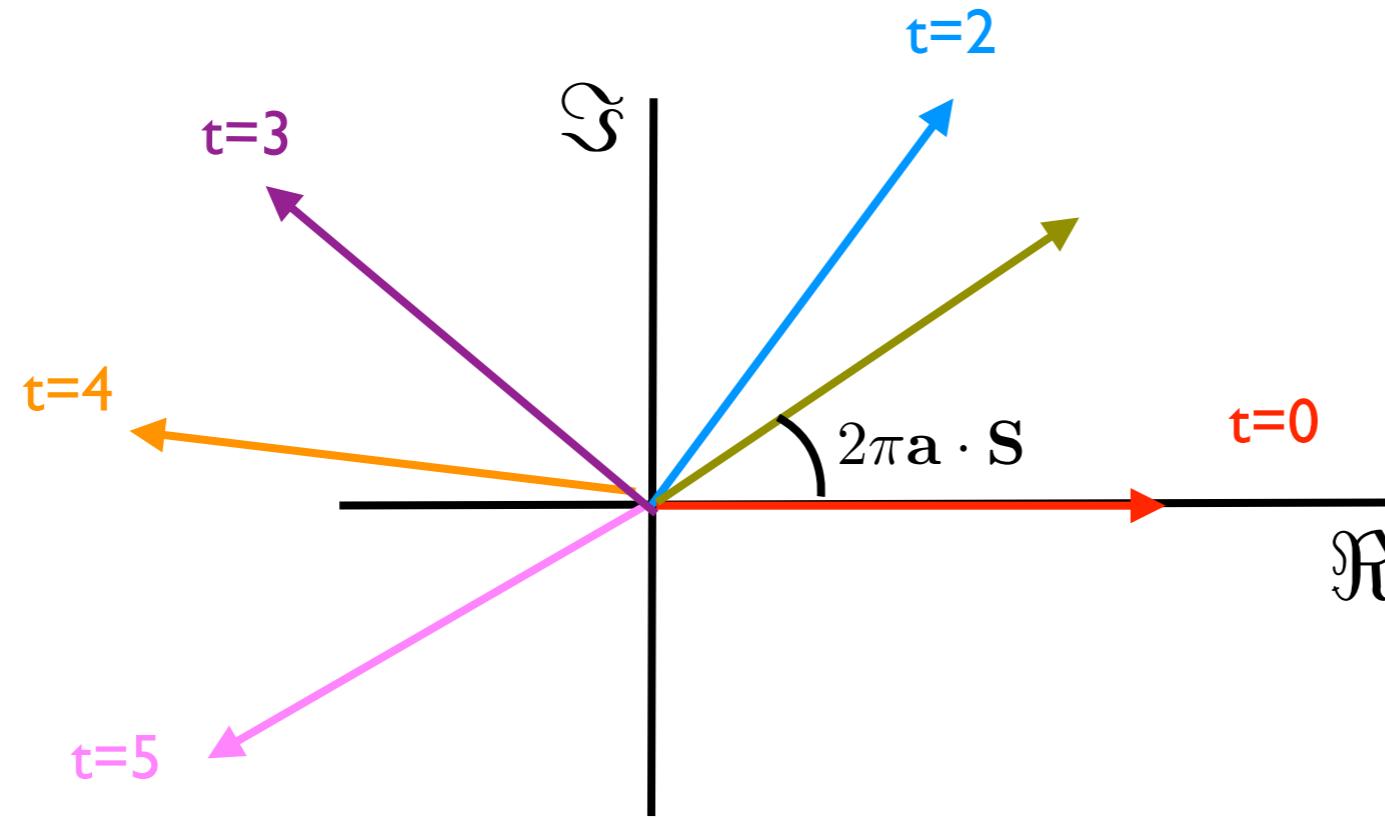
scattering with one atom in unitcell

$$\mathbf{K}(\mathbf{S}) = \mathbf{F}(\mathbf{S}) \times \sum_{t=0}^{N_1} \exp[2\pi it \mathbf{a} \cdot \mathbf{S}]$$

$$\times \sum_{u=0}^{N_2} \exp[2\pi i u \mathbf{b} \cdot \mathbf{S}]$$

$$\times \sum_{v=0}^{N_3} \exp[2\pi i v \mathbf{c} \cdot \mathbf{S}]$$

Argand diagram



scattering with crystals

scattering of all unitcells

$$\mathbf{K}(\mathbf{S}) = \mathbf{F}(\mathbf{S}) \times \sum_{t=0}^{N_1} \exp[2\pi it\mathbf{a} \cdot \mathbf{S}]$$

$$\times \sum_{u=0}^{N_2} \exp[2\pi iu\mathbf{b} \cdot \mathbf{S}]$$

$$\times \sum_{v=0}^{N_3} \exp[2\pi iv\mathbf{c} \cdot \mathbf{S}]$$

Laue Conditions

no scattering unless

$$\mathbf{a} \cdot \mathbf{S} = h \quad h \in N$$

$$\mathbf{b} \cdot \mathbf{S} = k \quad k \in N$$

$$\mathbf{c} \cdot \mathbf{S} = l \quad l \in N$$

constructive interference: diffraction

huge amplification of scattering because there are so many unitcells

scattering with crystals

scattering from unitcell

$$\mathbf{F}(\mathbf{S}) = f \exp[2\pi i \mathbf{r} \cdot \mathbf{S}] \quad \mathbf{r} = \mathbf{a}x + \mathbf{b}y + \mathbf{c}z$$

Laue conditions

$$\mathbf{a} \cdot \mathbf{S} = h \quad h \in N$$

$$\mathbf{b} \cdot \mathbf{S} = k \quad k \in N$$

$$\mathbf{c} \cdot \mathbf{S} = l \quad l \in N$$

scattering with crystals

scattering from unitcell

$$\mathbf{F}(\mathbf{S}) = f \exp[2\pi i \mathbf{r} \cdot \mathbf{S}] \quad \mathbf{r} = \mathbf{a}x + \mathbf{b}y + \mathbf{c}z$$

Laue conditions

$$\mathbf{a} \cdot \mathbf{S} = h \quad h \in N$$

$$\mathbf{b} \cdot \mathbf{S} = k \quad k \in N$$

$$\mathbf{c} \cdot \mathbf{S} = l \quad l \in N$$

phase

$$\begin{aligned}\mathbf{r} \cdot \mathbf{S} &= (\mathbf{a}x + \mathbf{b}y + \mathbf{c}z) \cdot \mathbf{S} \\ &= \mathbf{a} \cdot \mathbf{S}x + \mathbf{b} \cdot \mathbf{S}y + \mathbf{c} \cdot \mathbf{S}z \\ &= hx + ky + lz\end{aligned}$$

scattering with crystals

scattering from unitcell

$$\mathbf{F}(\mathbf{S}) = f \exp[2\pi i \mathbf{r} \cdot \mathbf{S}] \quad \mathbf{r} = \mathbf{a}x + \mathbf{b}y + \mathbf{c}z$$

Laue conditions

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$$\begin{aligned}\mathbf{r} \cdot \mathbf{S} &= (\mathbf{a}x + \mathbf{b}y + \mathbf{c}z) \cdot \mathbf{S} \\ &= \mathbf{a} \cdot \mathbf{S}x + \mathbf{b} \cdot \mathbf{S}y + \mathbf{c} \cdot \mathbf{S}z \\ &= hx + ky + lz\end{aligned}$$

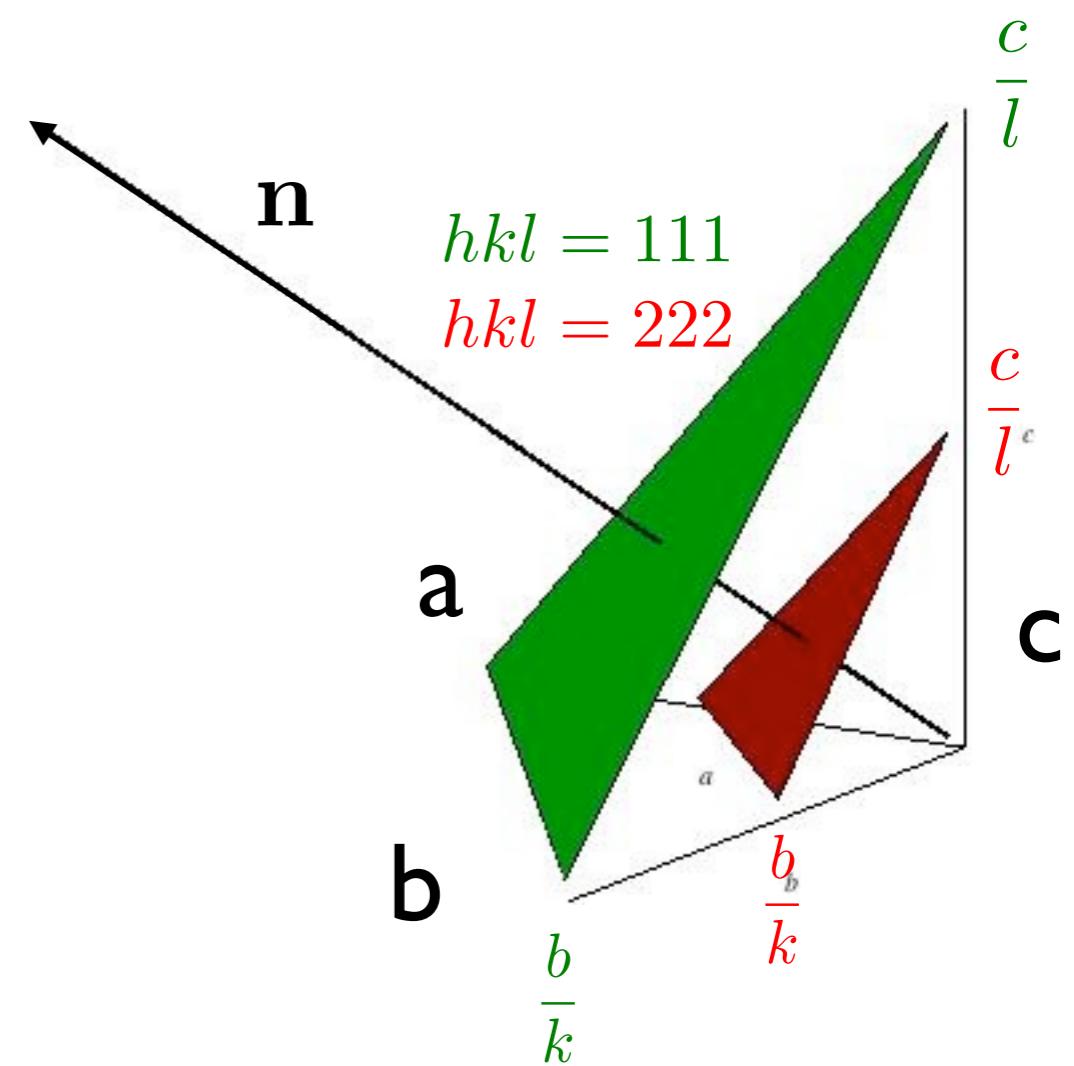
constructive interference (i.e. phase multiple of 2π) if

$$\mathbf{r} \cdot \mathbf{S} = 0, 1, 2, 3\dots$$

scattering with crystals

equation for a plane

$$hx + ky + lz = 1 \quad h = 1, 2, 3, \dots \quad k = 1, 2, 3, \dots \quad l = 1, 2, 3, \dots$$



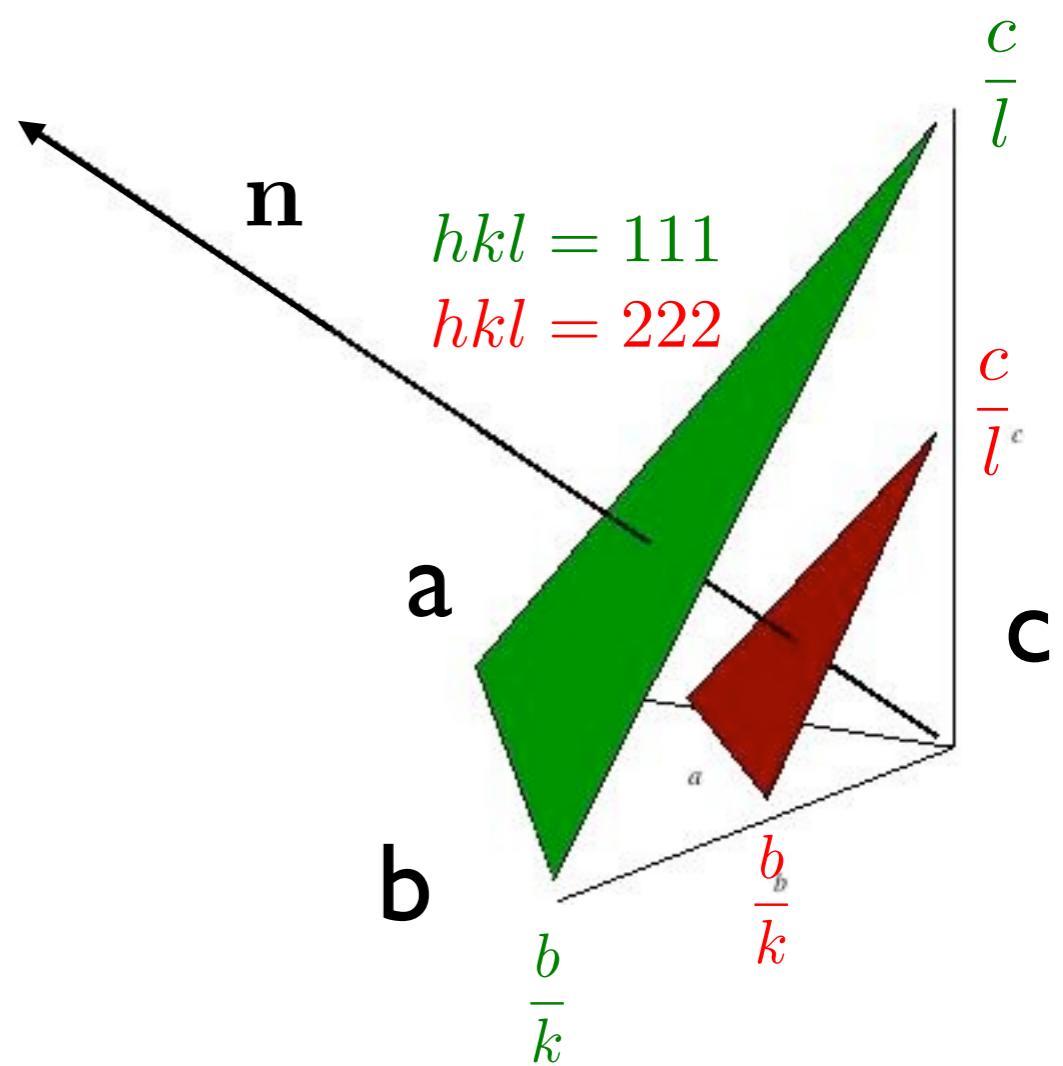
scattering with crystals

equation for a plane

$$hx + ky + lz = 1 \quad h = 1, 2, 3, \dots \quad k = 1, 2, 3, \dots \quad l = 1, 2, 3, \dots$$

intersections with unitcell axes

$$\frac{a}{h} \quad \frac{b}{k} \quad \frac{c}{l}$$



scattering with crystals

equation for a plane

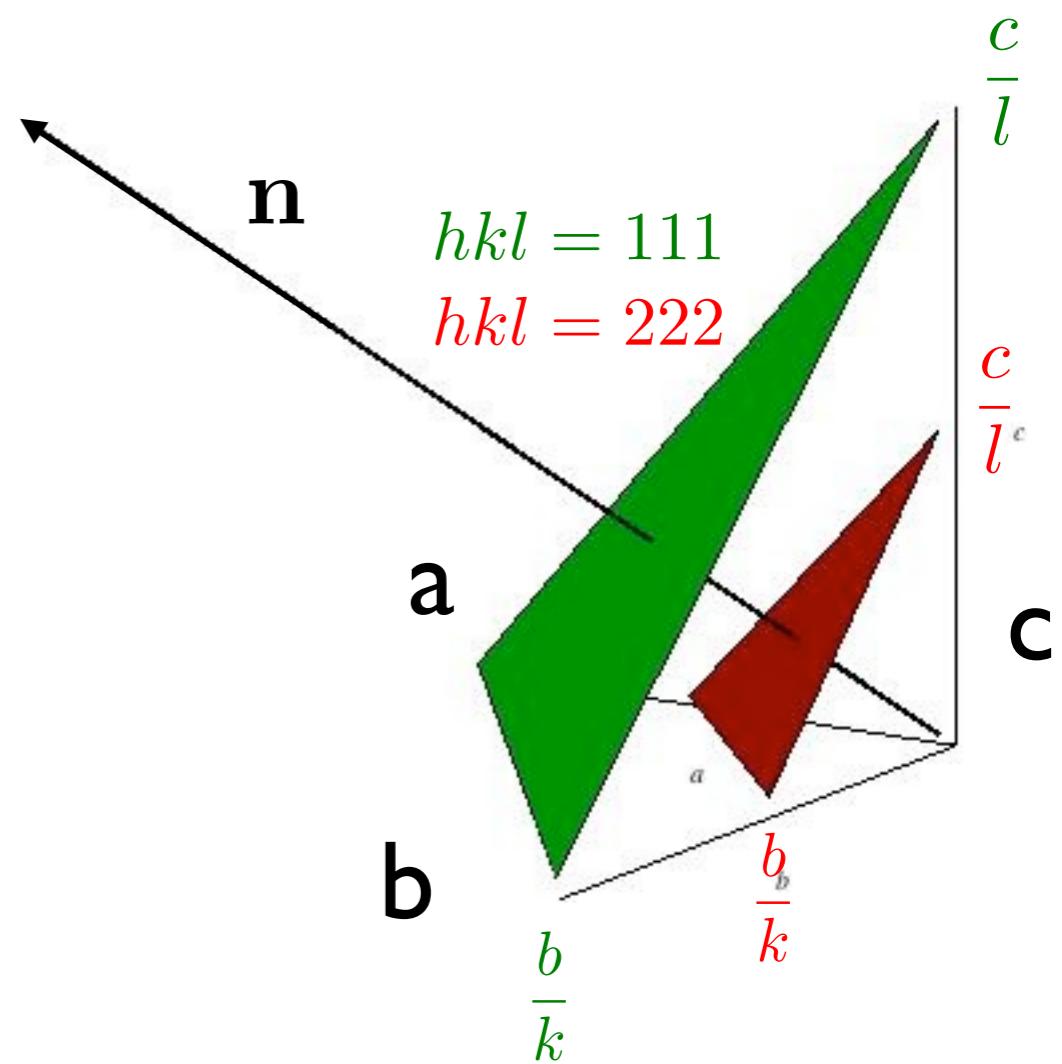
$$hx + ky + lz = 1 \quad h = 1, 2, 3, \dots \quad k = 1, 2, 3, \dots \quad l = 1, 2, 3, \dots$$

intersections with unitcell axes

$$\frac{\mathbf{a}}{h} \quad \frac{\mathbf{b}}{k} \quad \frac{\mathbf{c}}{l}$$

(unnormalized) normal vector

$$\mathbf{n}_{hkl} = h\mathbf{a} + k\mathbf{b} + l\mathbf{c} \parallel \mathbf{S}$$



scattering with crystals

equation for a plane

$$hx + ky + lz = 1 \quad h = 1, 2, 3, \dots \quad k = 1, 2, 3, \dots \quad l = 1, 2, 3, \dots$$

intersections with unitcell axes

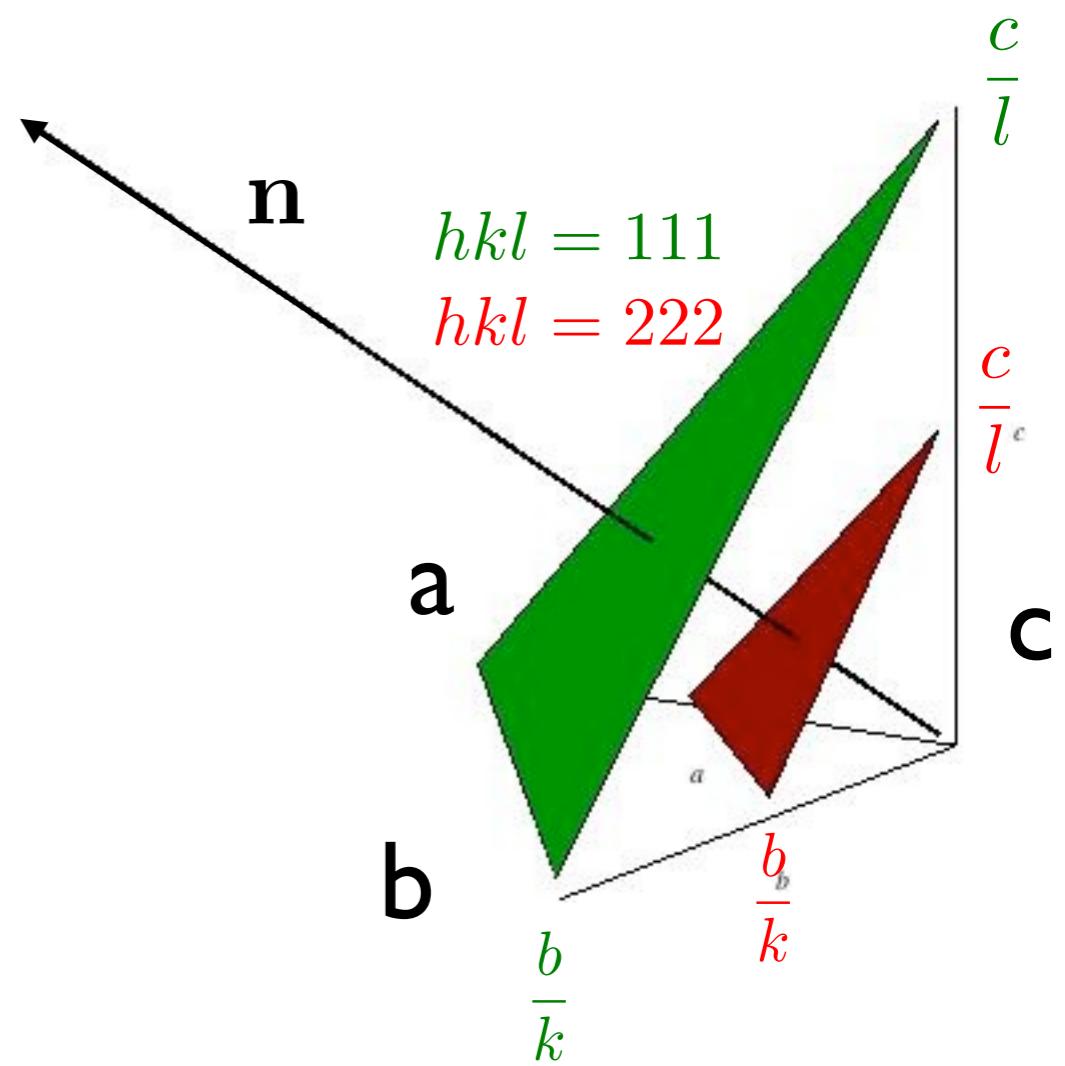
$$\frac{\mathbf{a}}{h} \quad \frac{\mathbf{b}}{k} \quad \frac{\mathbf{c}}{l}$$

(unnormalized) normal vector

$$\mathbf{n}_{hkl} = h\mathbf{a} + k\mathbf{b} + l\mathbf{c} \parallel \mathbf{S}$$

distance between planes

$$\frac{1}{d_{hkl}^2} = \frac{h^2}{a^2} + \frac{k^2}{b^2} + \frac{l^2}{c^2}$$



scattering with crystals

equation for a plane

$$hx + ky + lz = 1 \quad h = 1, 2, 3, \dots \quad k = 1, 2, 3, \dots \quad l = 1, 2, 3, \dots$$

intersections with unitcell axes

$$\frac{\mathbf{a}}{h} \quad \frac{\mathbf{b}}{k} \quad \frac{\mathbf{c}}{l}$$

(unnormalized) normal vector

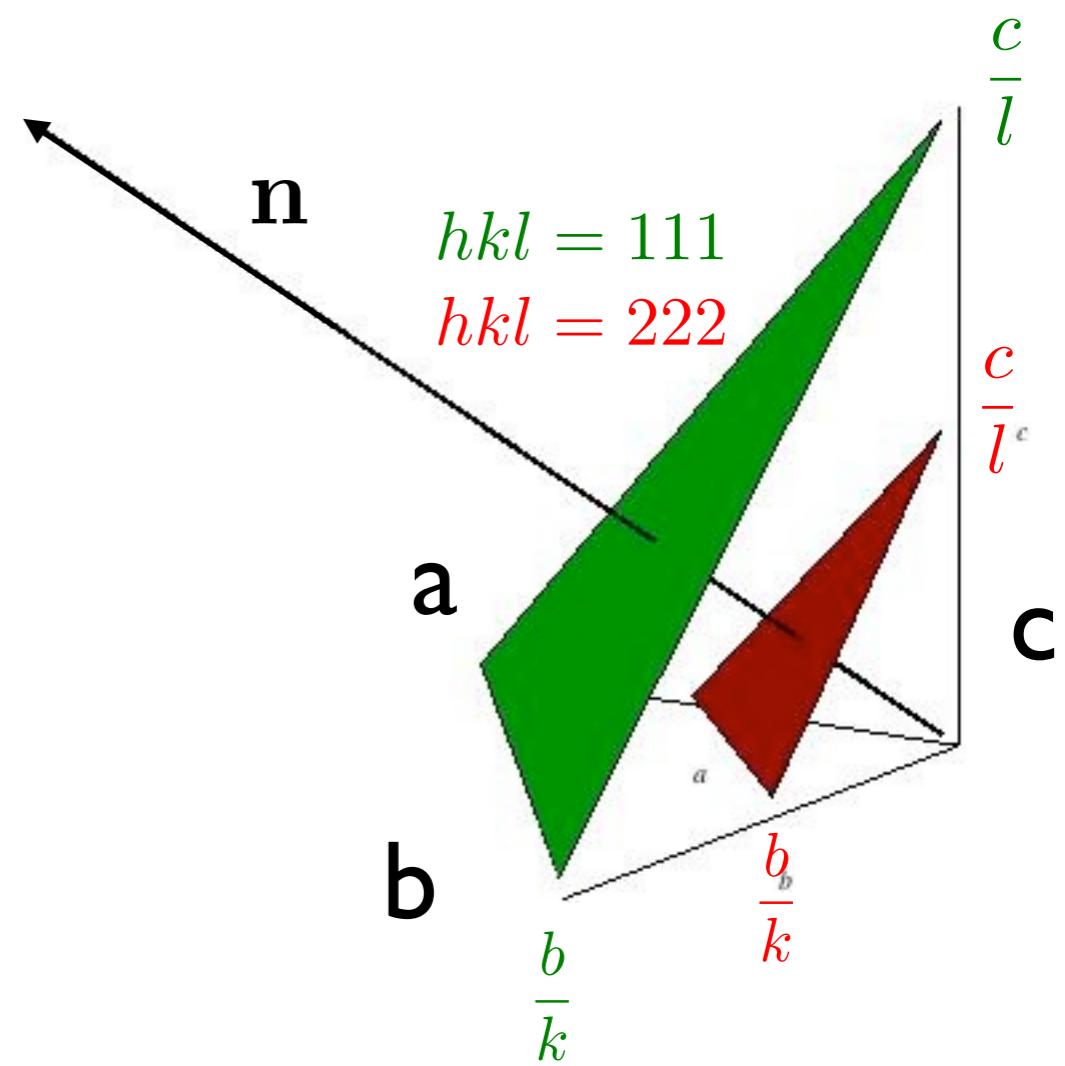
$$\mathbf{n}_{hkl} = h\mathbf{a} + k\mathbf{b} + l\mathbf{c} \parallel \mathbf{S}$$

distance between planes

$$\frac{1}{d_{hkl}^2} = \frac{h^2}{a^2} + \frac{k^2}{b^2} + \frac{l^2}{c^2}$$

lattice planes!

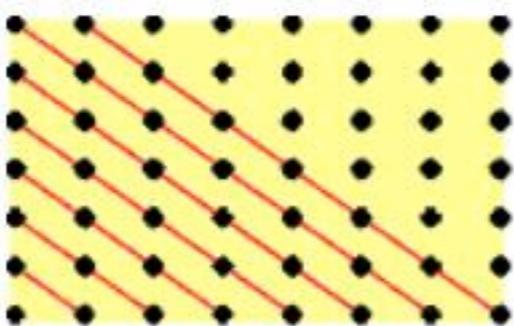
Miller indices hkl



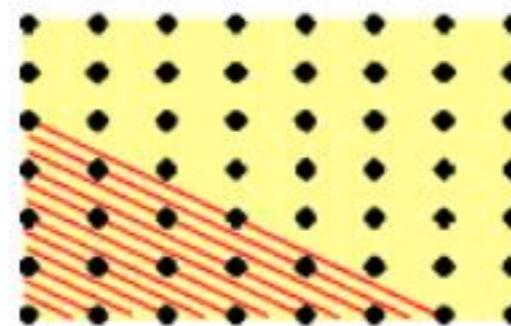
crystals

lattice planes

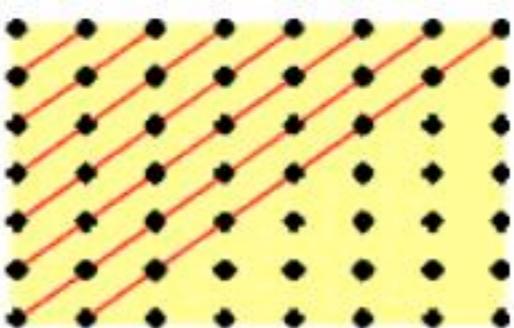
Miller indices (hkl)



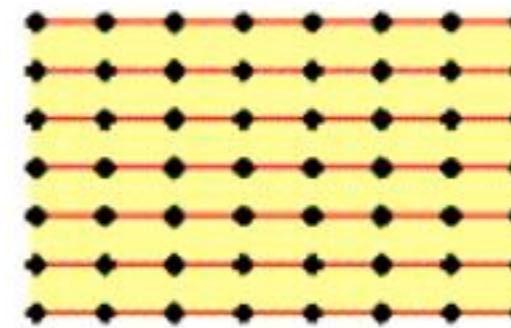
$$(hkl) = (110)$$



$$(hkl) = (230)$$



$$(hkl) = (\bar{1}10)$$



$$(hkl) = (010)$$

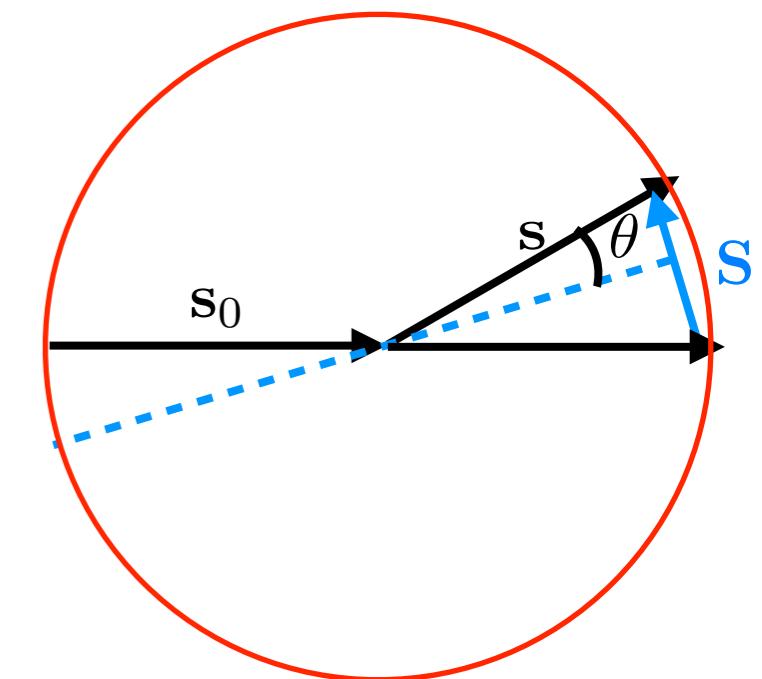
separation of planes

$$\frac{1}{d_{hkl}^2} = \frac{h^2}{a^2} + \frac{k^2}{b^2} + \frac{l^2}{c^2}$$

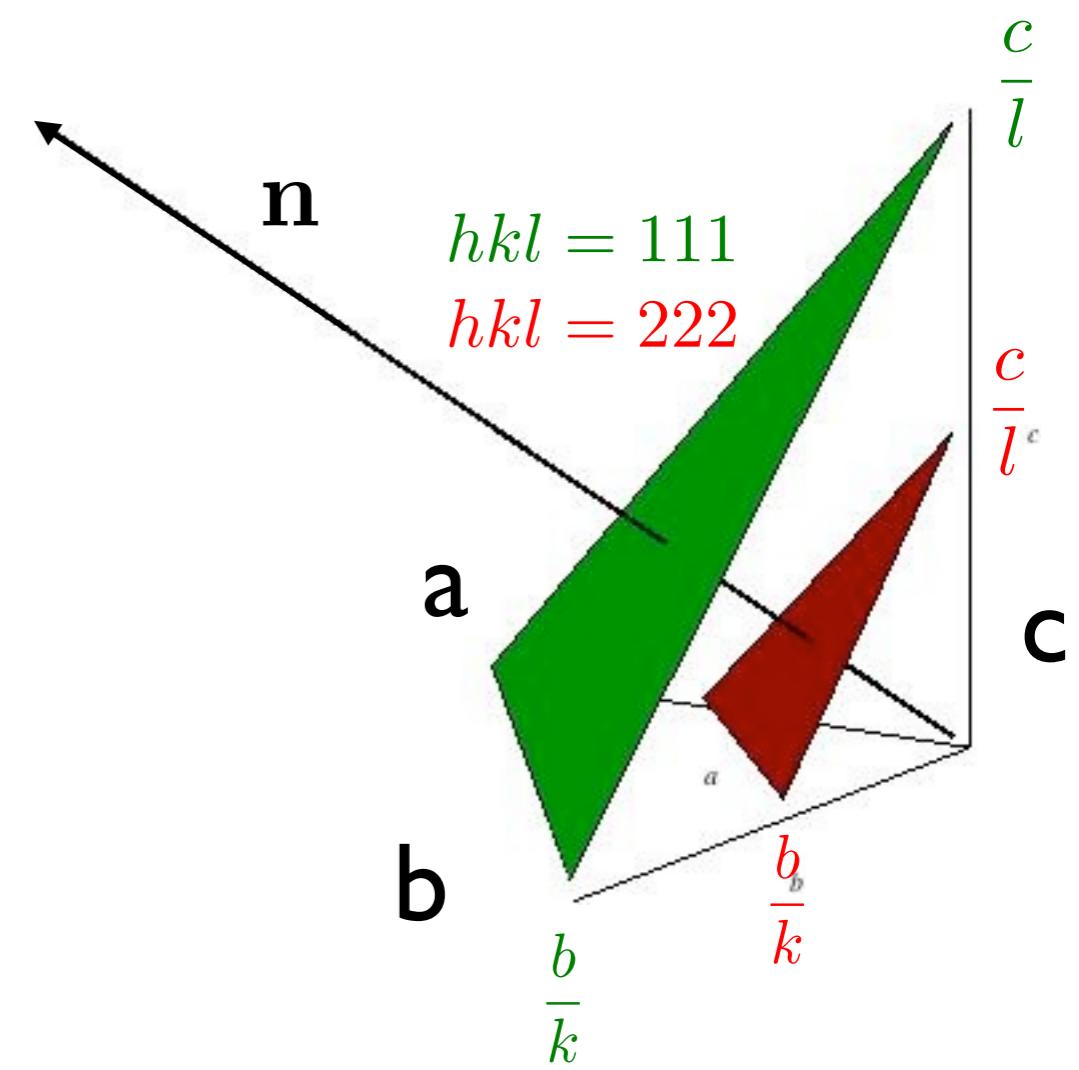
scattering with crystals

lattice planes

diffraction or “reflection” planes



$$|\mathbf{S}| = \frac{2 \sin(\theta)}{\lambda}$$



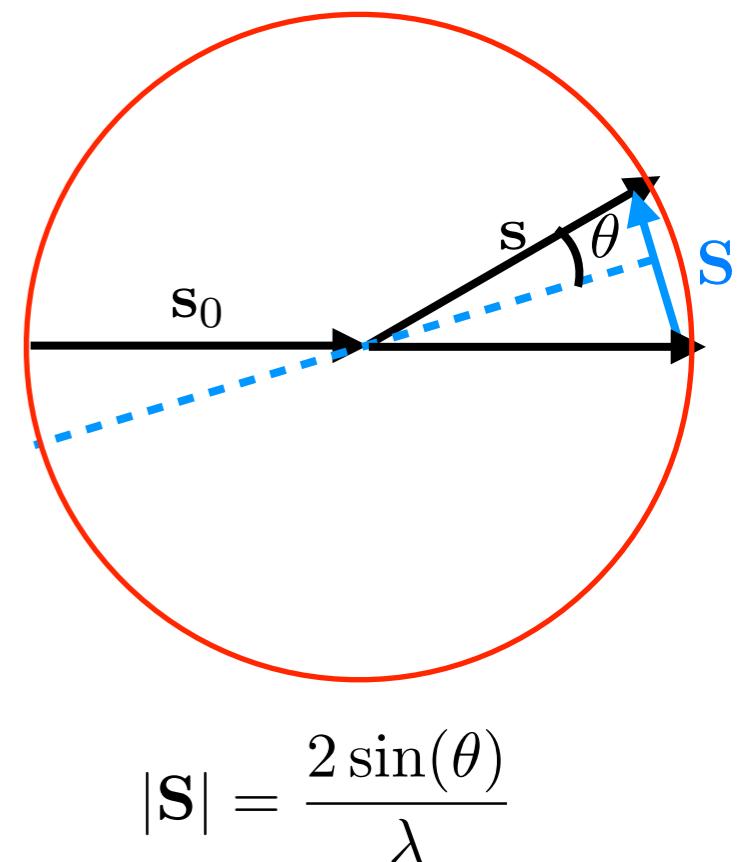
scattering with crystals

lattice planes

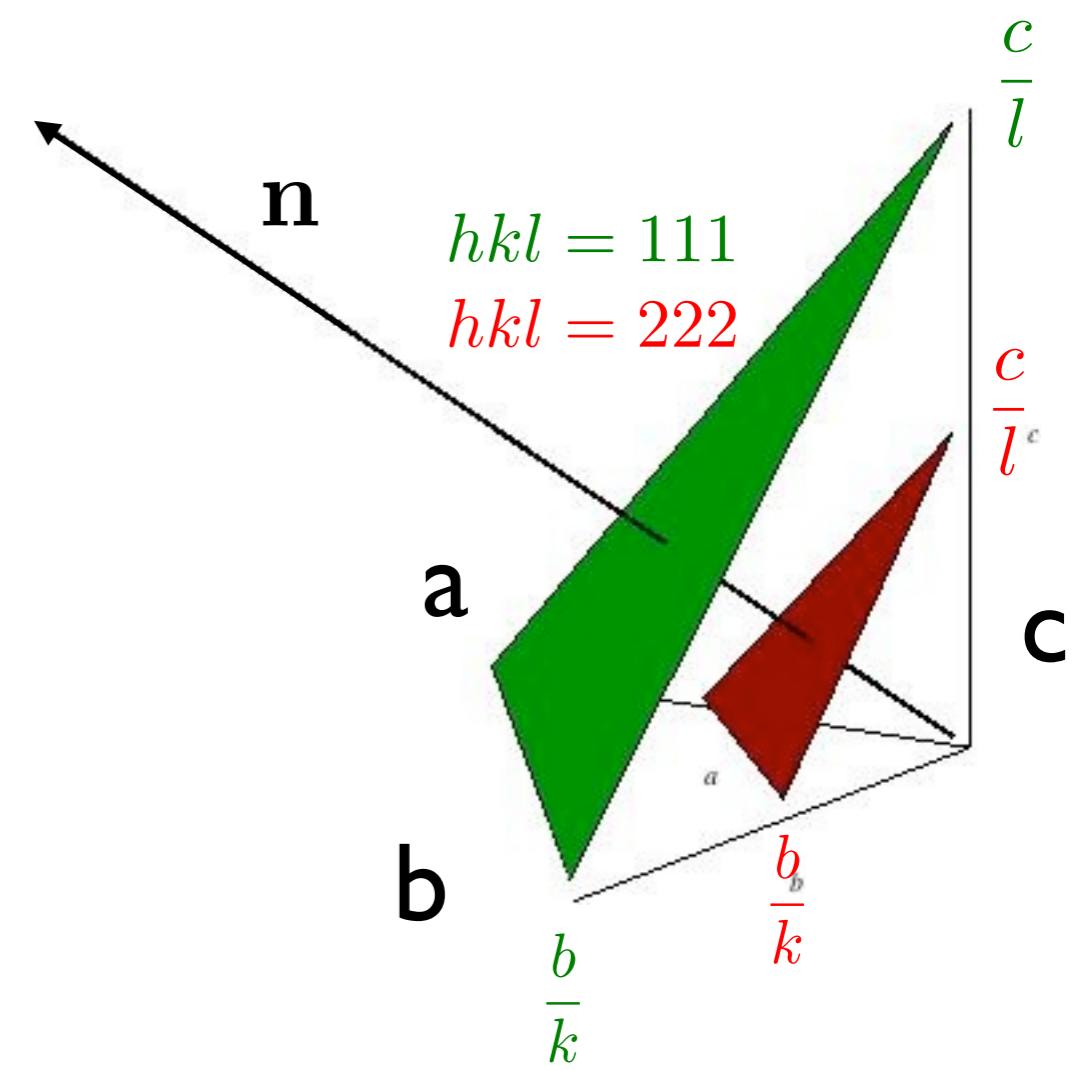
diffraction or “reflection” planes

distance between hkl planes (Miller)

$$\frac{1}{d_{hkl}^2} = \frac{h^2}{a^2} + \frac{k^2}{b^2} + \frac{l^2}{c^2}$$



$$|\mathbf{S}| = \frac{2 \sin(\theta)}{\lambda}$$



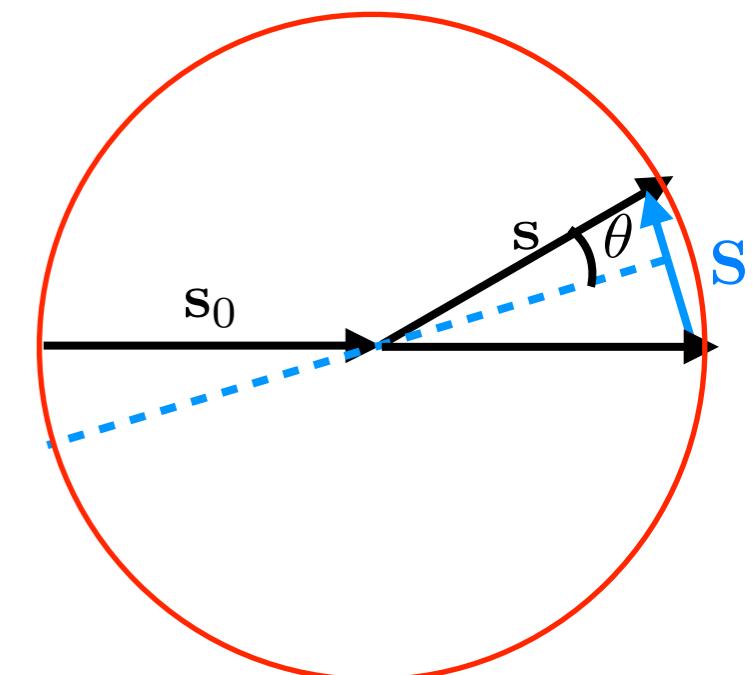
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$$\frac{1}{d_{hkl}^2} = \frac{h^2}{a^2} + \frac{k^2}{b^2} + \frac{l^2}{c^2}$$



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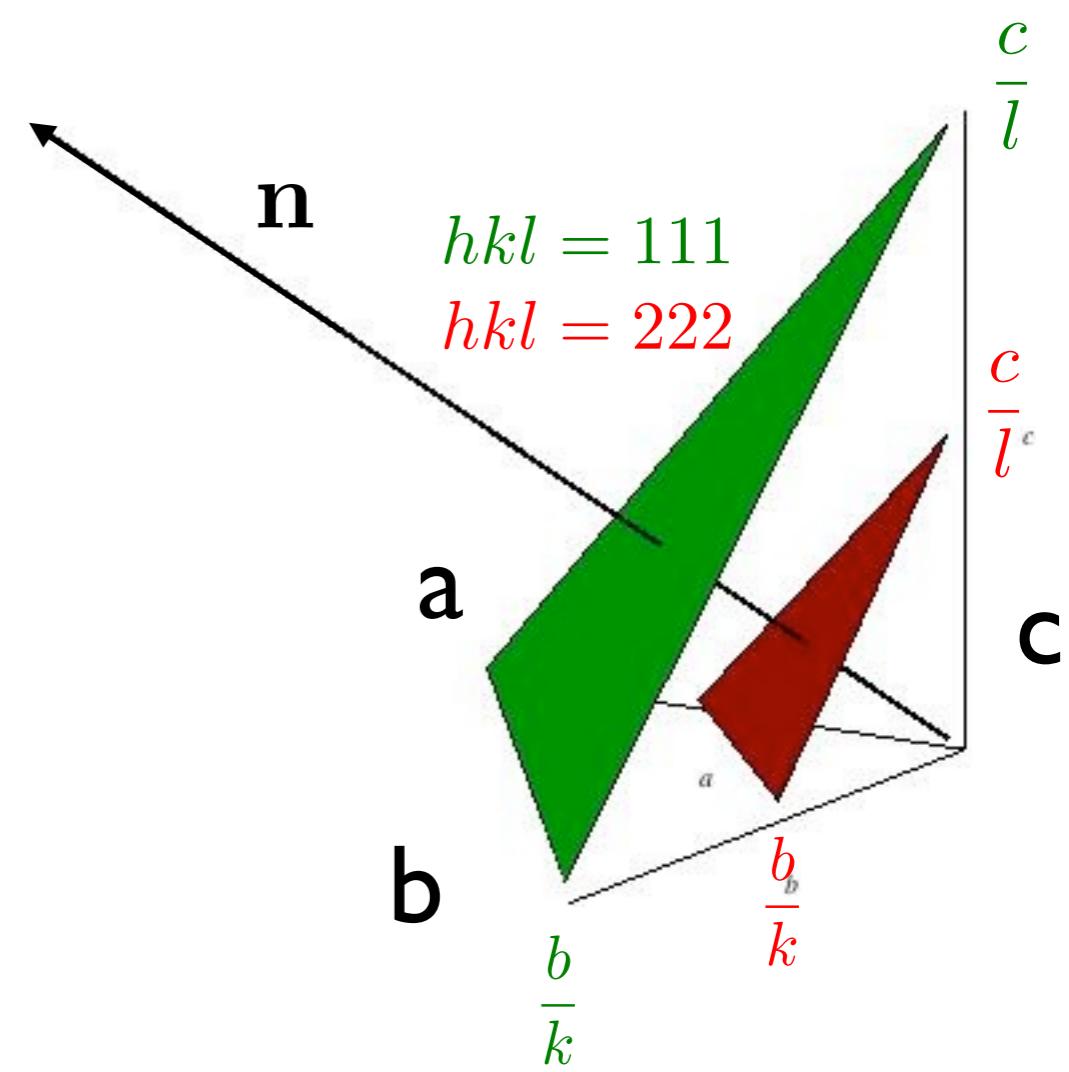
distance between hkl planes (Laue)

for all atoms in a lattice plane (no phase)

$$\mathbf{r} \cdot \mathbf{S} = 0, 1, 2, 3\dots$$

so that

$$d_{hkl} = |\mathbf{r}_{\min}| = \frac{n}{|\mathbf{S}|}$$



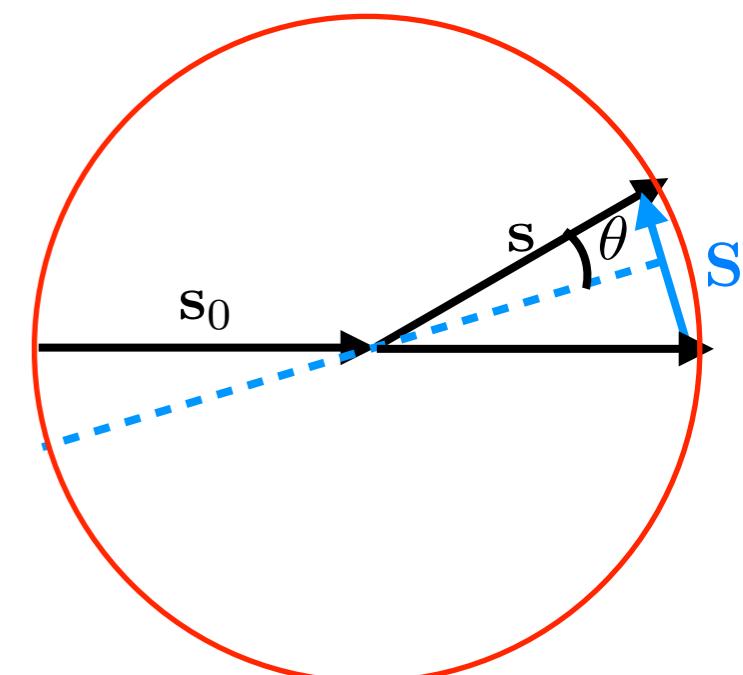
scattering with crystals

lattice planes

diffraction or “reflection” planes

distance between hkl planes (Miller)

$$\frac{1}{d_{hkl}^2} = \frac{h^2}{a^2} + \frac{k^2}{b^2} + \frac{l^2}{c^2}$$



$$|\mathbf{S}| = \frac{2 \sin(\theta)}{\lambda}$$

distance between hkl planes (Laue)

for all atoms in a lattice plane (no phase)

$$\mathbf{r} \cdot \mathbf{S} = 0, 1, 2, 3\dots$$

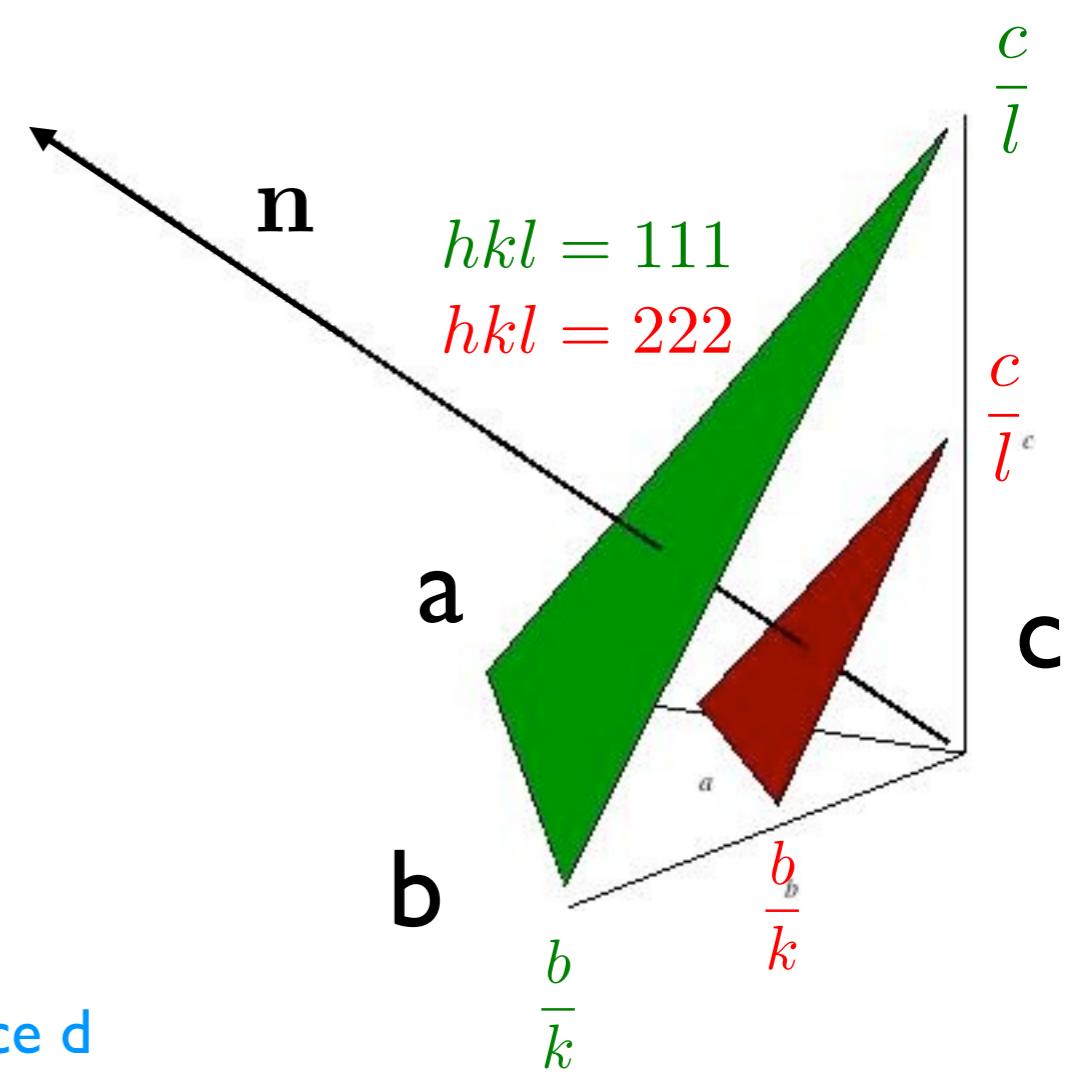
so that

$$d_{hkl} = |\mathbf{r}_{\min}| = \frac{n}{|\mathbf{S}|}$$

Bragg's law

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

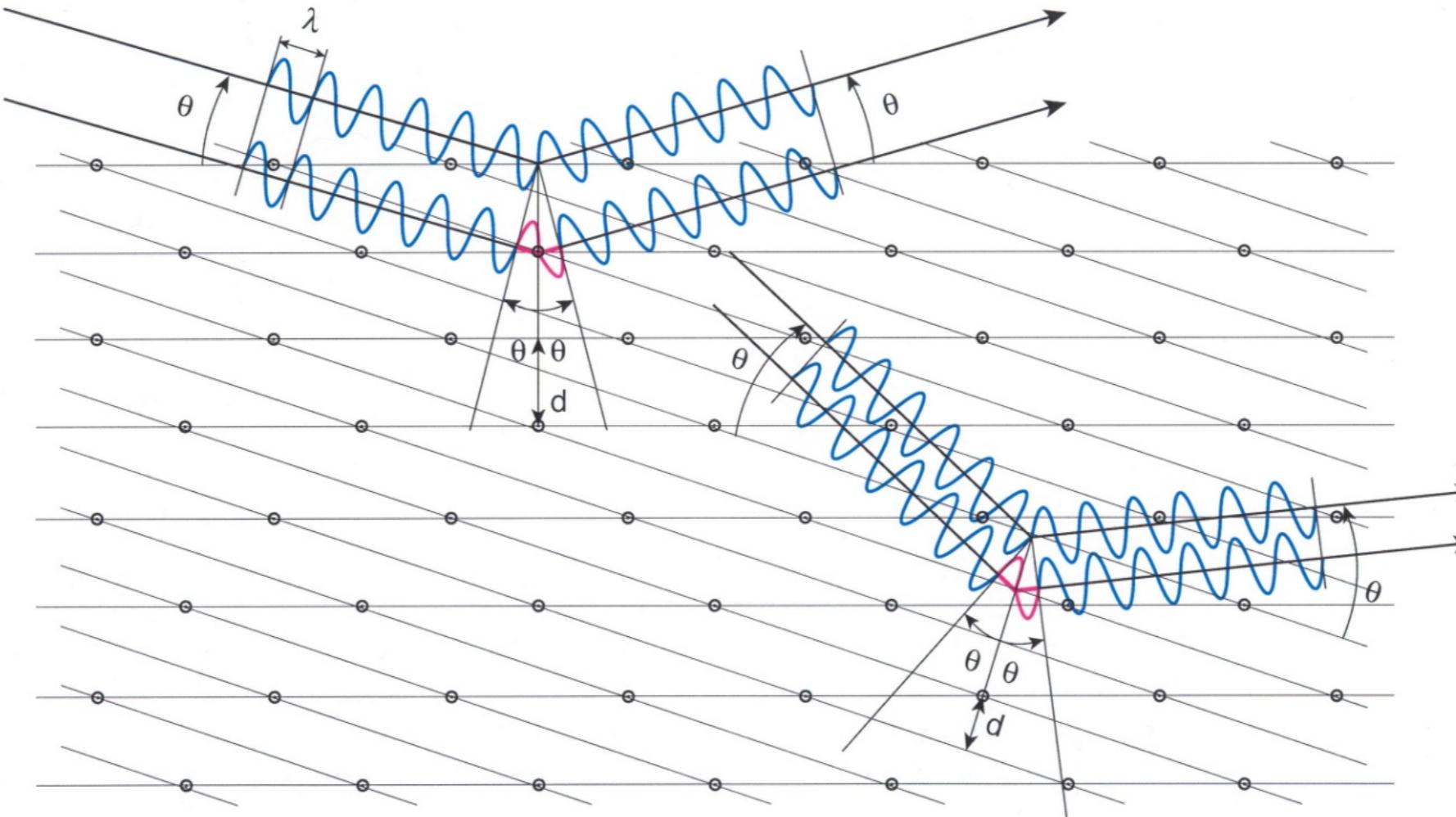
conditions for diffraction from hkl planes with distance d



Bragg's law

conventional derivation

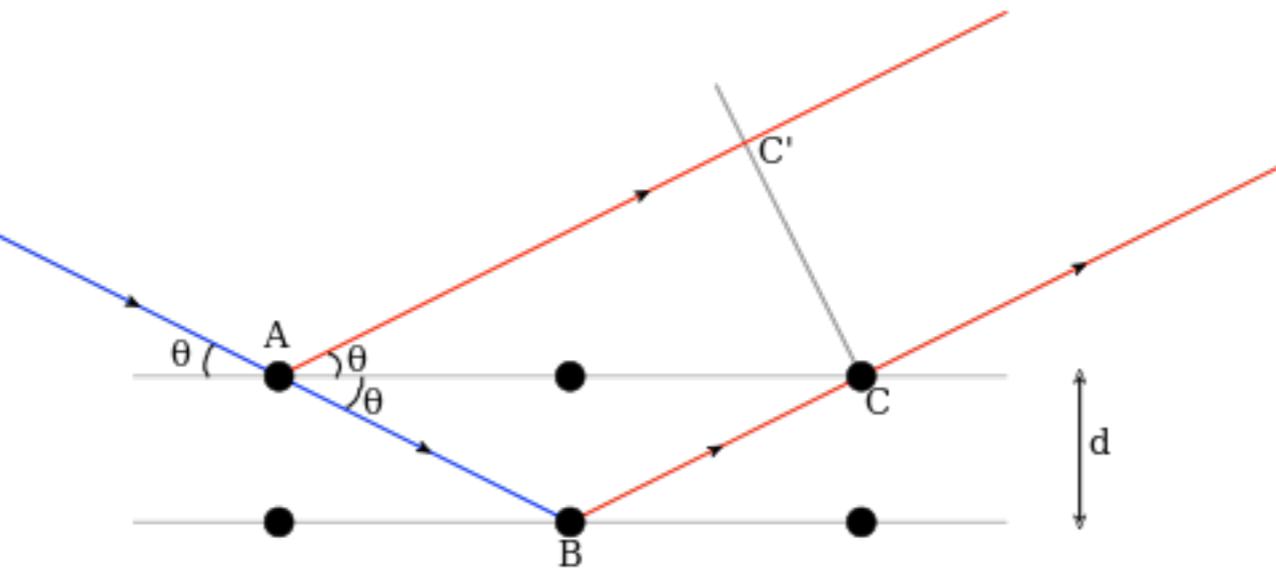
lattice planes (hkl)



Bragg's law

conventional derivation

lattice planes (hkl)



$$\mathbf{AB} + \mathbf{BC} = 2d \sin \theta$$

$$\mathbf{AB} + \mathbf{BC} = n\lambda$$

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

reflections (hkl)

scattering with crystals

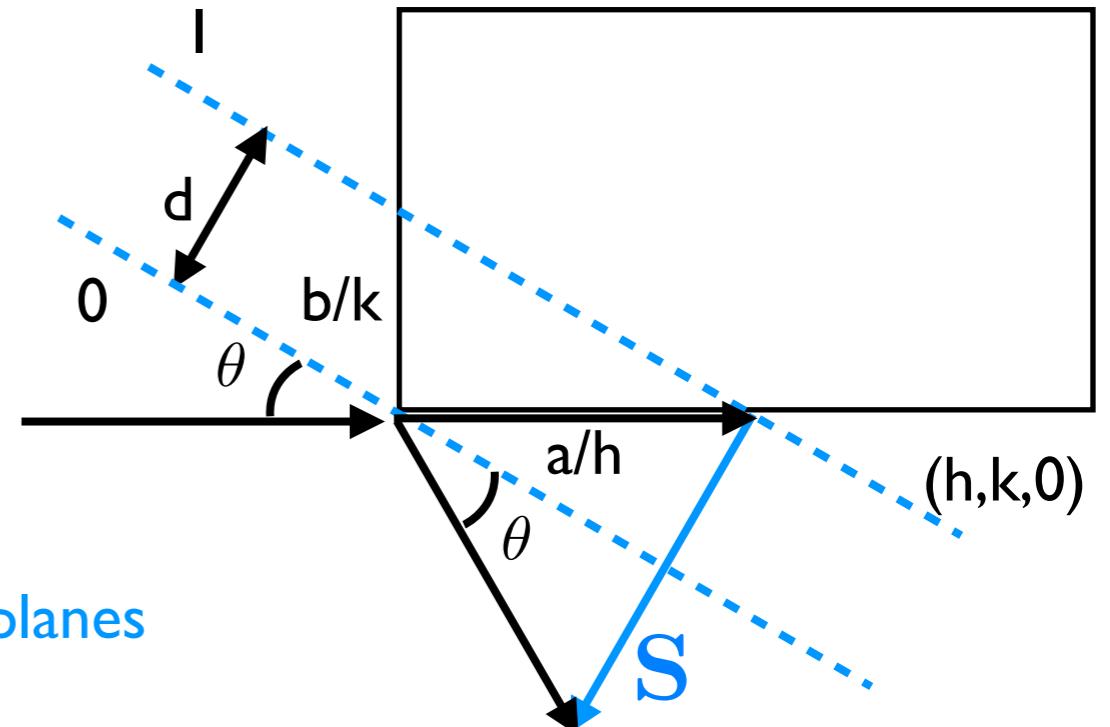
one atom per unitcell

Laue conditions or Bragg's law

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

diffraction under discrete angles: reflections of hkl planes

resolution



scattering with crystals

one atom per unitcell

Laue conditions or Bragg's law

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

diffraction under discrete angles: reflections of hkl planes

resolution

reflection planes are a theoretical concept

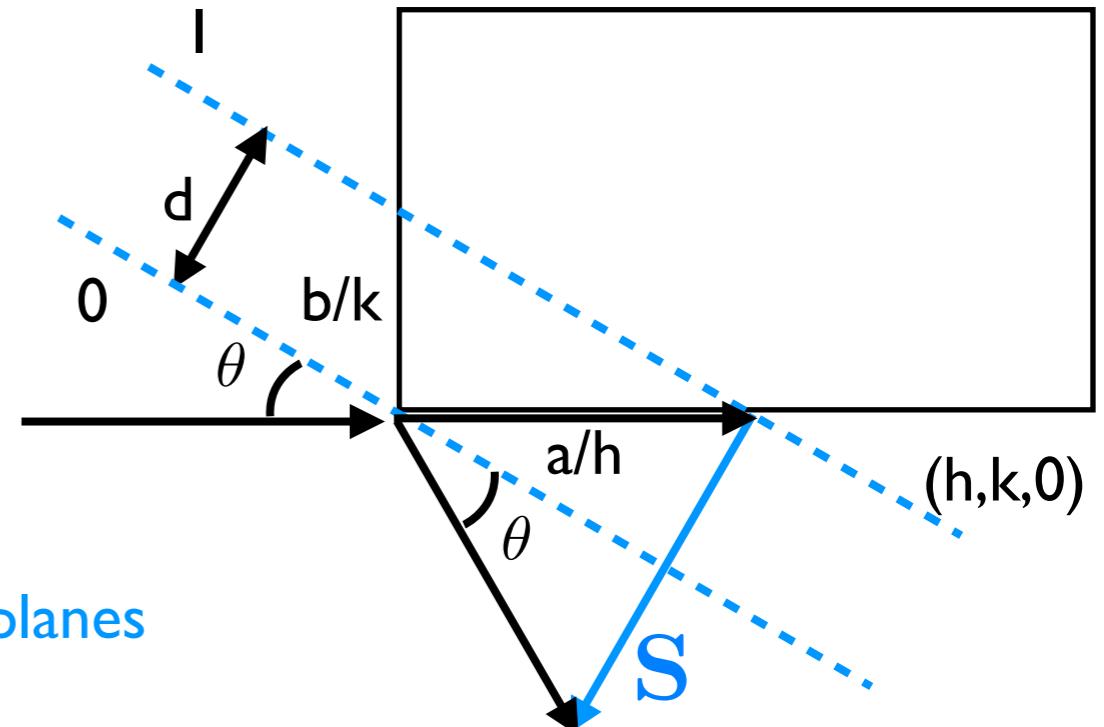
atoms do not need to be on lattice (reflection) planes!!!

scattering from atoms on hkl place has no phase

$$\exp[2\pi i n] = \cos[2n\pi] - i \sin[2n\pi] = 1 \quad n = 0, 1, 2, 3, \dots$$

scattering from atoms not on hkl plane attains a phase

$$\exp[2\pi i \mathbf{r} \cdot \mathbf{S}] \neq 1$$



scattering with crystals

one atom per unitcell

Laue conditions or Bragg's law

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

diffraction under discrete angles: reflections of hkl planes

resolution

reflection planes are a theoretical concept

atoms do not need to be on lattice (reflection) planes!!!

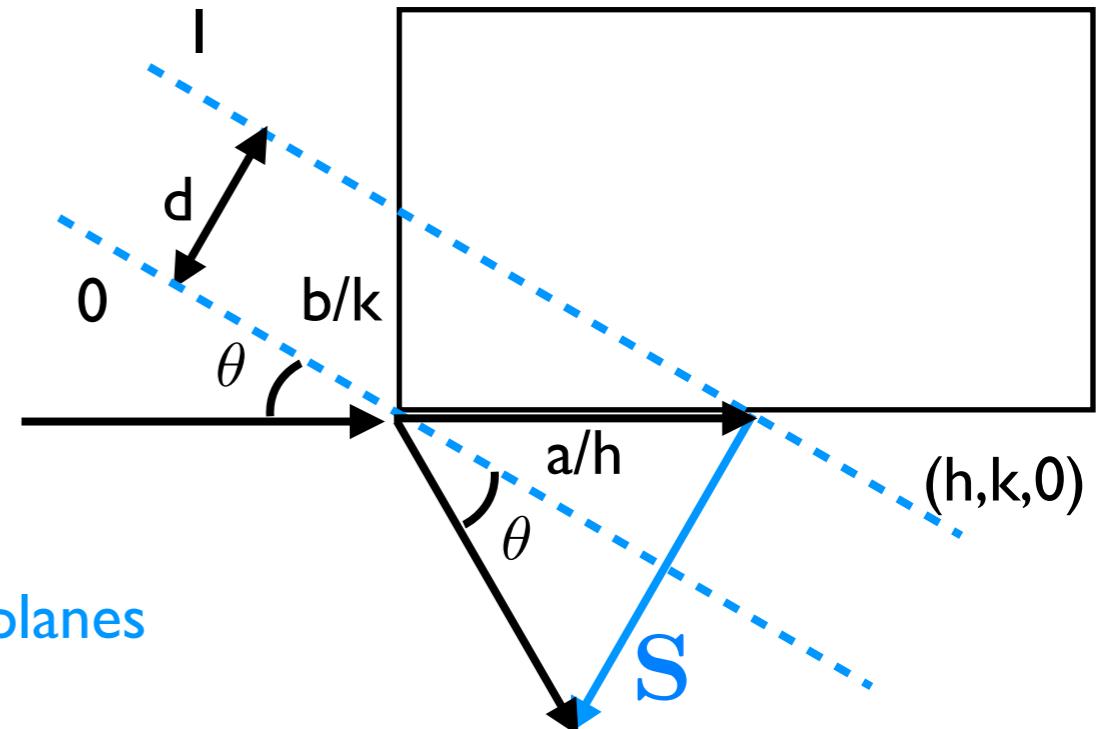
scattering from atoms on hkl place has no phase

$$\exp[2\pi i n] = \cos[2n\pi] - i \sin[2n\pi] = 1 \quad n = 0, 1, 2, 3, \dots$$

scattering from atoms not on hkl plane attains a phase

$$\exp[2\pi i \mathbf{r} \cdot \mathbf{S}] \neq 1$$

next: many atoms per unitcell



scattering with crystals

scattering of multiple atoms in the unitcell

$$\mathbf{F}(\mathbf{S}) = \sum_k^N f_k \exp[2\pi i \mathbf{r}_k \cdot \mathbf{S}]$$

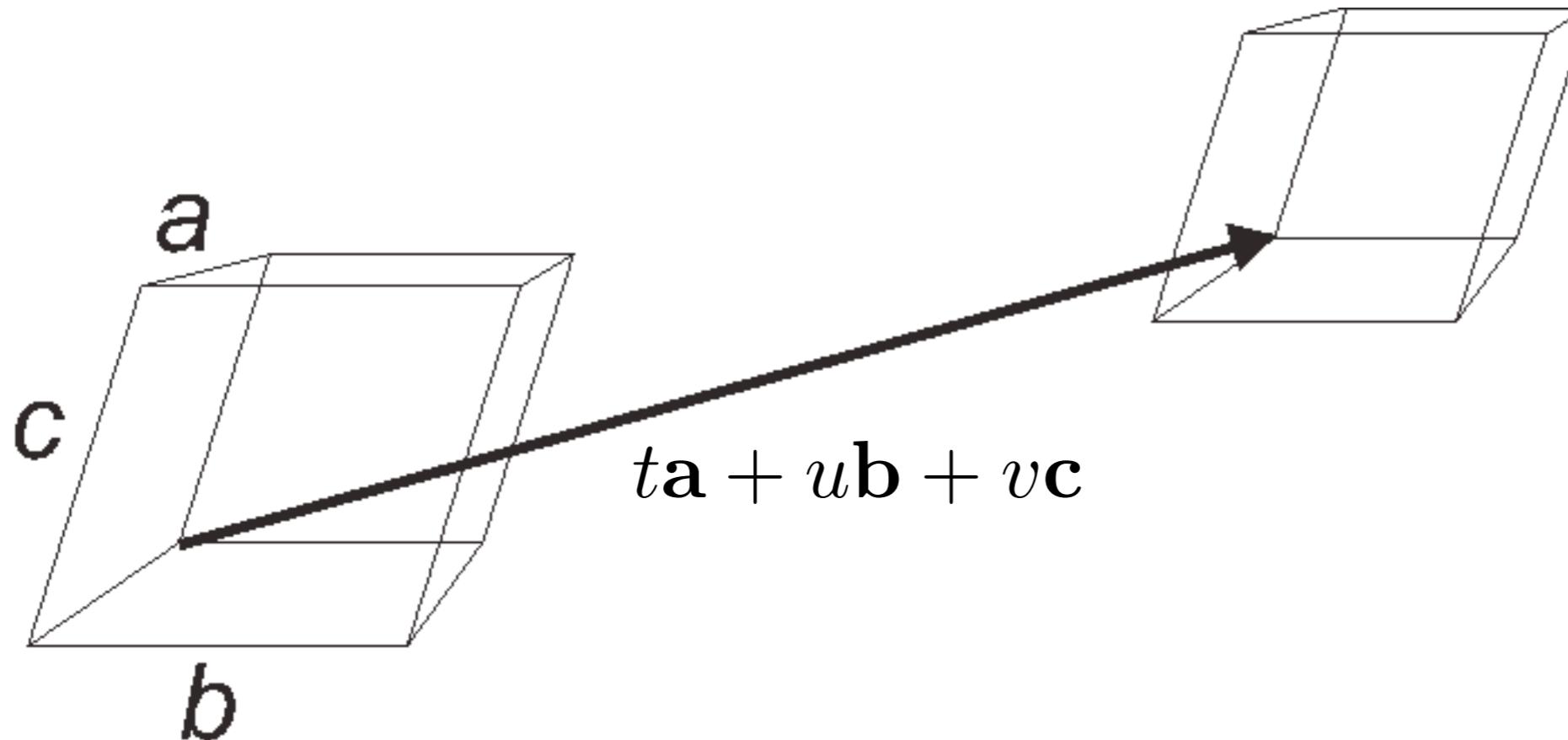
scattering with crystals

scattering of multiple atoms in the unitcell

$$\mathbf{F}(\mathbf{S}) = \sum_k^N f_k \exp[2\pi i \mathbf{r}_k \cdot \mathbf{S}]$$

scattering of unitcells in the crystal

scattering of a distant unitcell at position $t\mathbf{a} + u\mathbf{b} + v\mathbf{c}$ wrt origin



$$\mathbf{F}_{tuv}(\mathbf{S}) = \mathbf{F}(\mathbf{S}) \times \exp[2\pi it\mathbf{a} \cdot \mathbf{S}] \times \exp[2\pi iu\mathbf{b} \cdot \mathbf{S}] \times \exp[2\pi iv\mathbf{c} \cdot \mathbf{S}]$$

scattering with crystals molecules

scattering of all unitcells together

$$\mathbf{K}(\mathbf{S}) = \mathbf{F}(\mathbf{S}) \times \sum_{t=0}^{N_1} \exp[2\pi it\mathbf{a} \cdot \mathbf{S}]$$

$$\times \sum_{u=0}^{N_2} \exp[2\pi i u\mathbf{b} \cdot \mathbf{S}]$$

$$\times \sum_{v=0}^{N_3} \exp[2\pi i v\mathbf{c} \cdot \mathbf{S}]$$

scattering with crystals molecules

scattering of all unitcells together

$$\mathbf{K}(\mathbf{S}) = \mathbf{F}(\mathbf{S}) \times \sum_{t=0}^{N_1} \exp[2\pi it \mathbf{a} \cdot \mathbf{S}]$$

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$$\times \sum_{v=0}^{N_3} \exp[2\pi i v \mathbf{c} \cdot \mathbf{S}]$$

Laue Conditions (again, obviously)

hkl reflections if and only if

$$\mathbf{a} \cdot \mathbf{S} = h \quad h \in N$$

$$\mathbf{b} \cdot \mathbf{S} = k \quad k \in N$$

$$\mathbf{c} \cdot \mathbf{S} = l \quad l \in N$$

Diffraction

measuring reflections from all hkl planes

not all spots may fall on detector

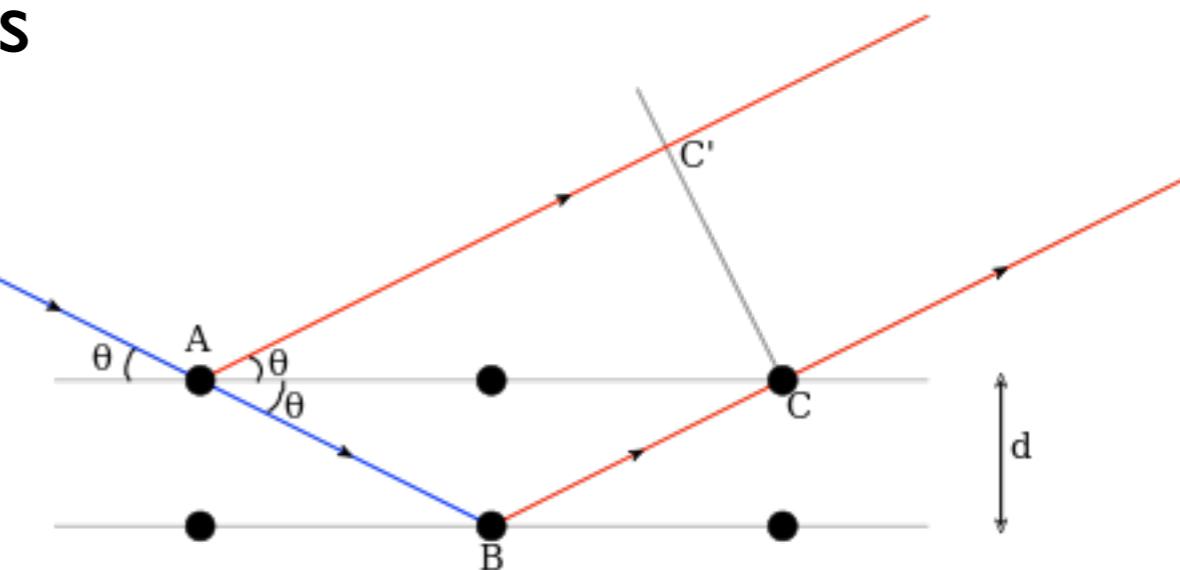
powder (Debye-Scherer approach)

rotating crystal

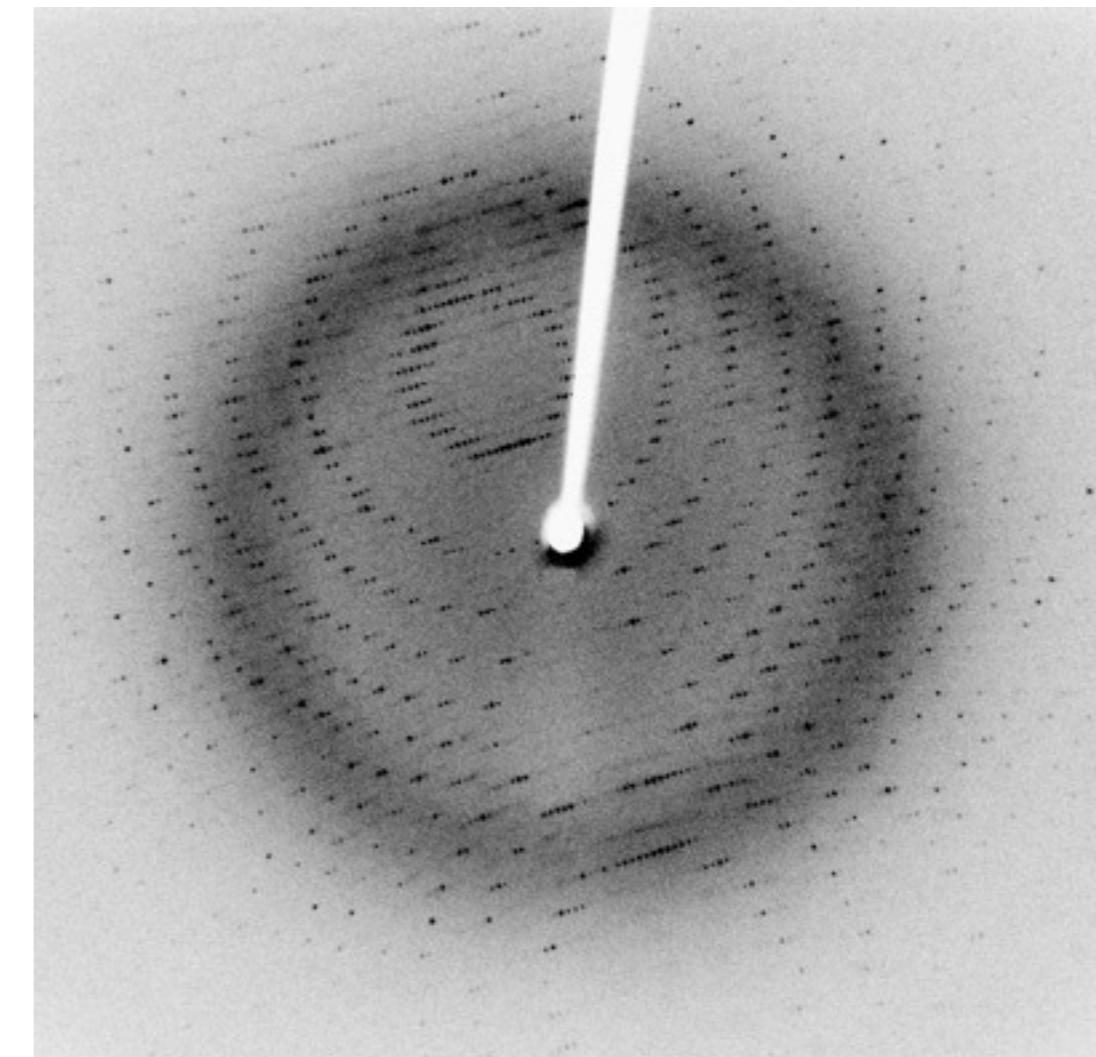
Laue

broad band x-ray

even time resolved



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



Diffraction

measuring reflections from all hkl planes

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even time resolved

indexing reflections

identify unitcell from patterns

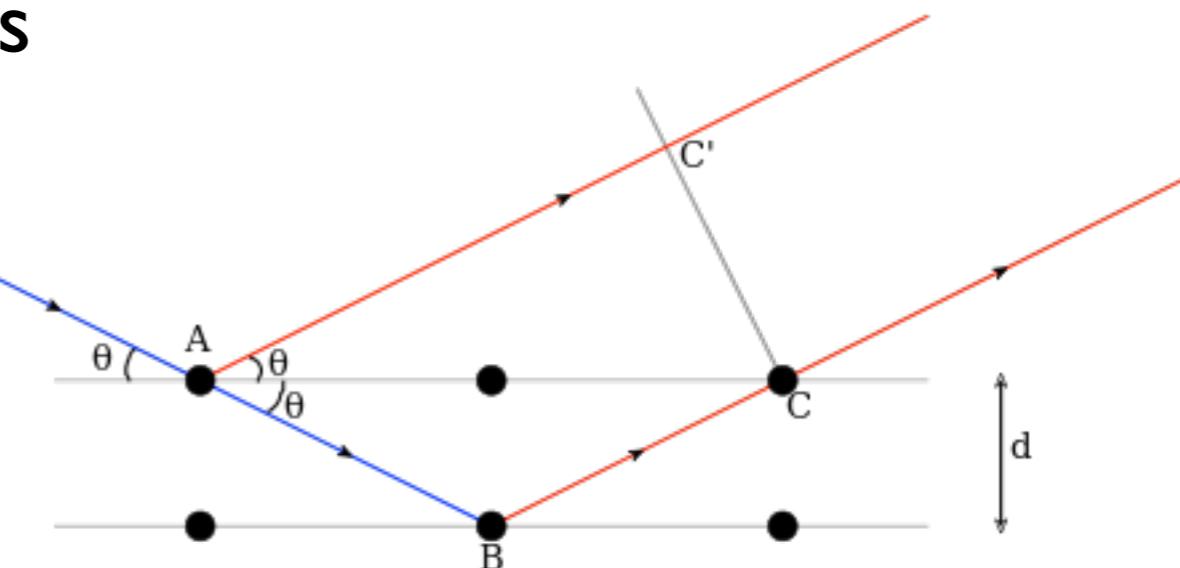
assign Miller index to each diffraction spot

reciprocal lattice and Ewald sphere

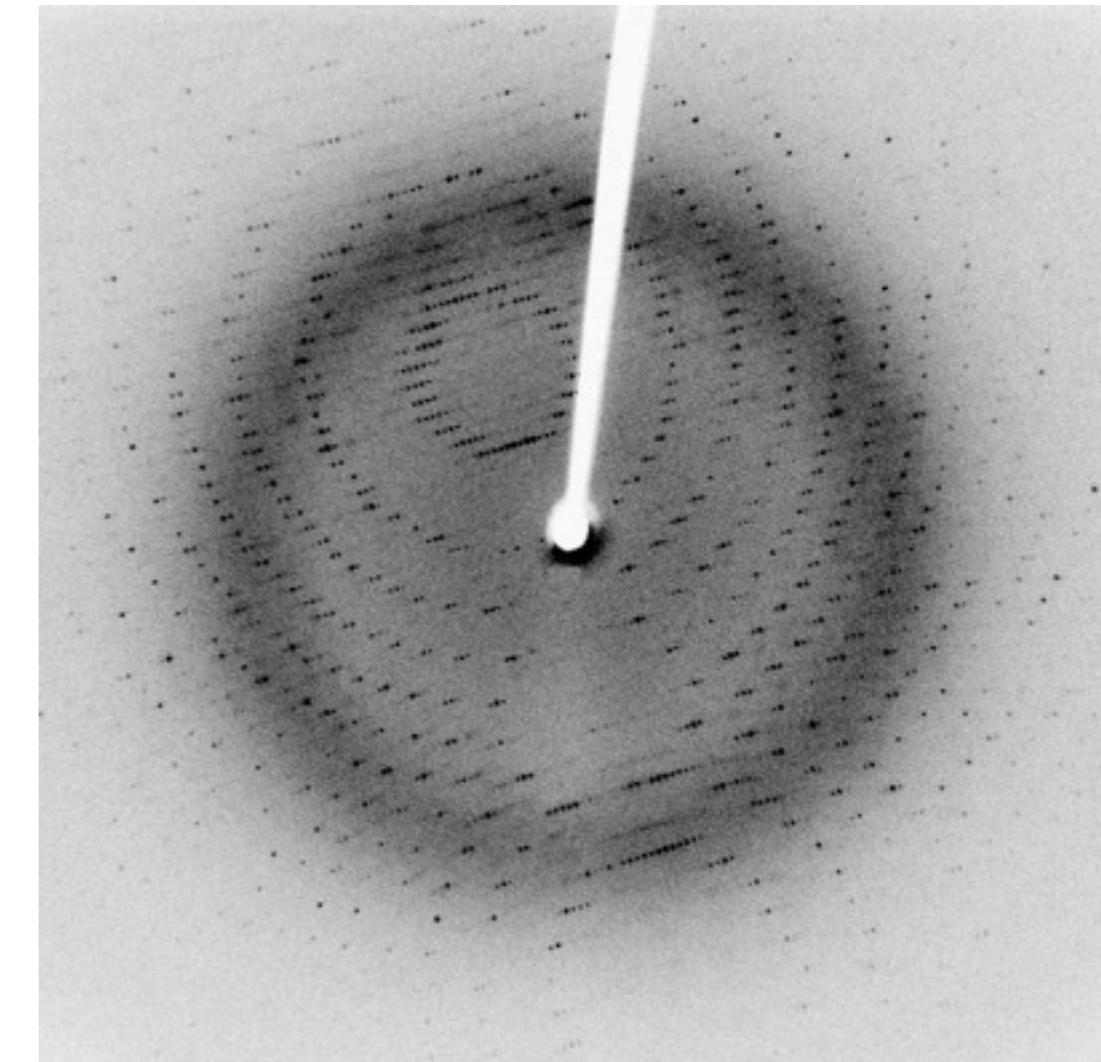
derive unitcell dimensions

e.g. for cubic unitcell

$$\sin \theta_{hkl} = (h^2 + k^2 + l^2) \frac{\lambda}{2a}$$



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



Diffraction

Note:

all atoms in the unitcell contribute to all reflections from all hkl planes !

thus, not only atoms in the hkl plane, but also atoms in between!

Diffraction

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atoms in hkl planes (no phase)

$$\mathbf{r}_a \cdot \mathbf{S} = 0, 1, 2, 3, \dots$$

$$\mathbf{F}(\mathbf{S}) = \sum_a^N f_a \exp[2\pi i \mathbf{r}_a \cdot \mathbf{S}]$$

$$= \sum_a^N f_a$$

$$= \rho_{hkl}$$

total number of electrons in the plane

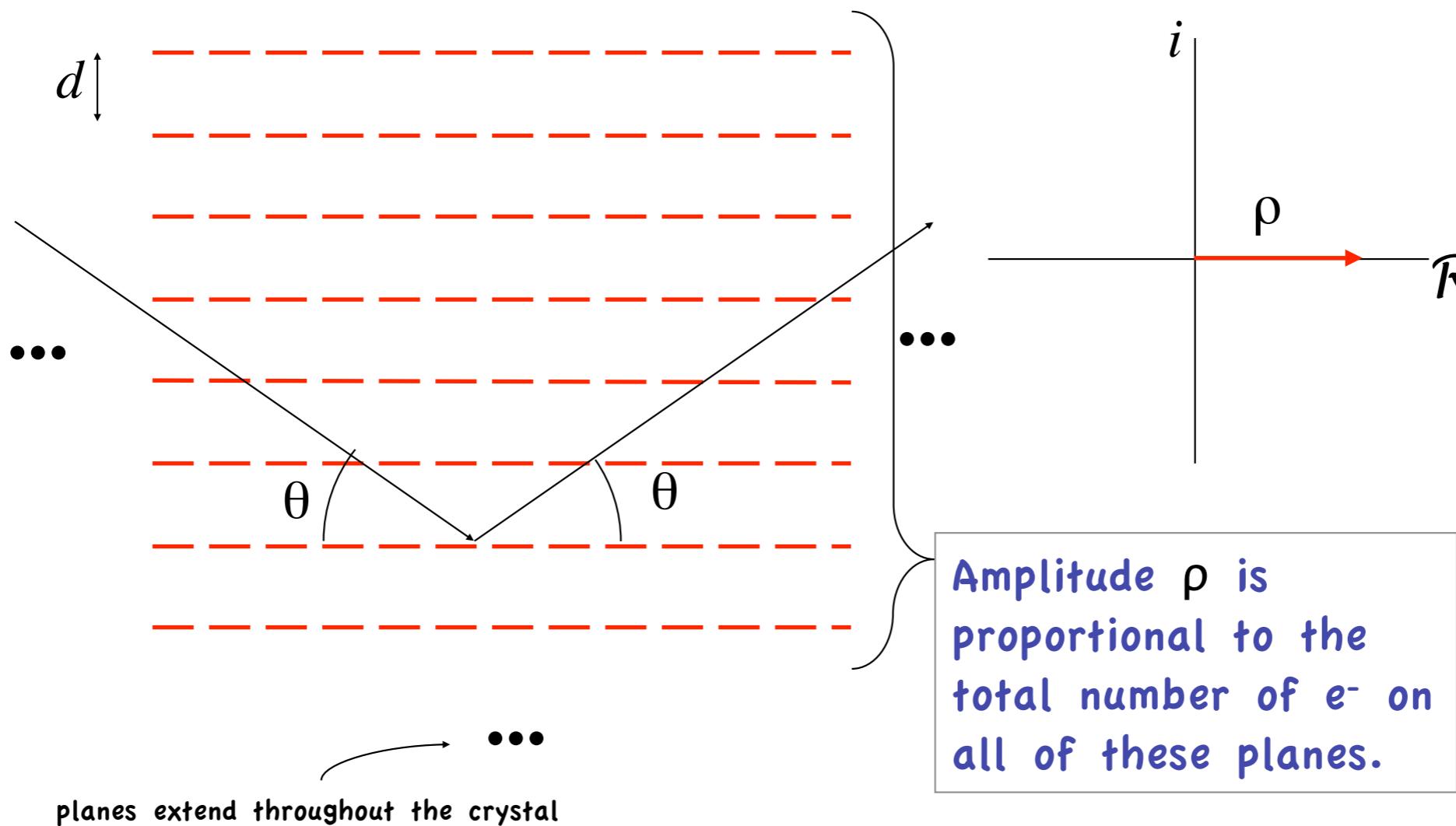
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atoms in hkl planes



Diffraction

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atoms **not** in hkl planes

$$\mathbf{F}(\mathbf{S}) = \sum_a^N f_a \exp[2\pi i \mathbf{r}_a \cdot \mathbf{S}]$$

shift planes by $\delta \mathbf{r}$ such that atoms fall on it

remember: shifting origin implies shifting phase

$$\alpha = \delta \mathbf{r} \cdot \mathbf{S}$$

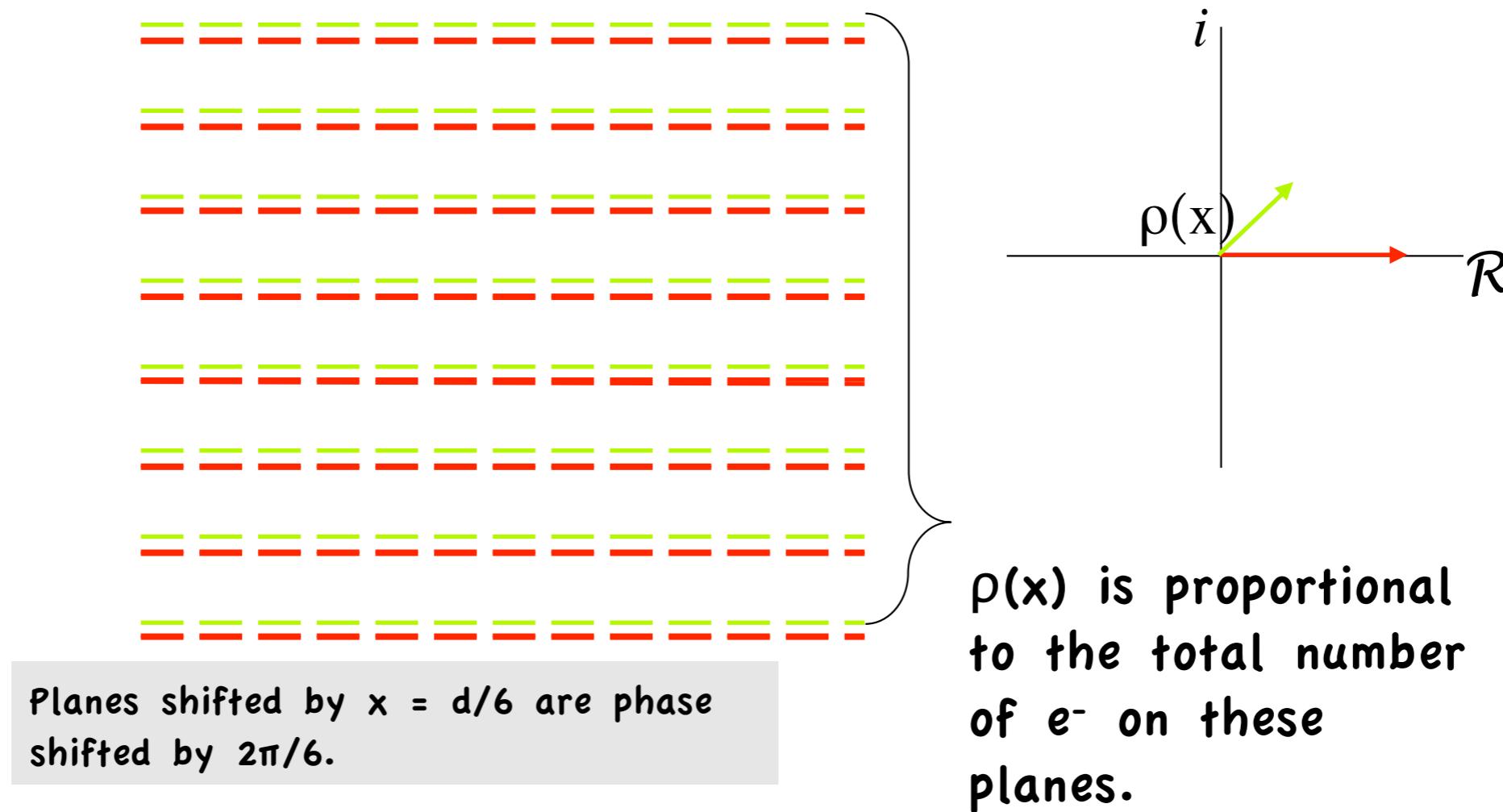
Diffraction

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



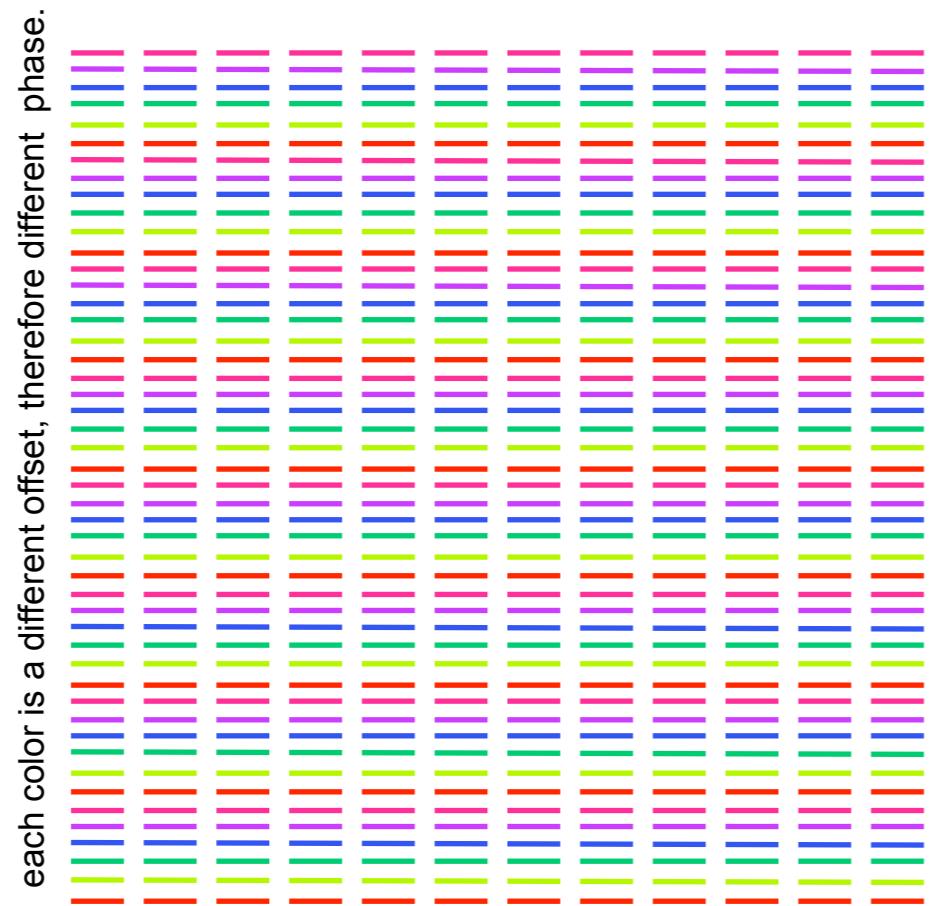
Diffraction

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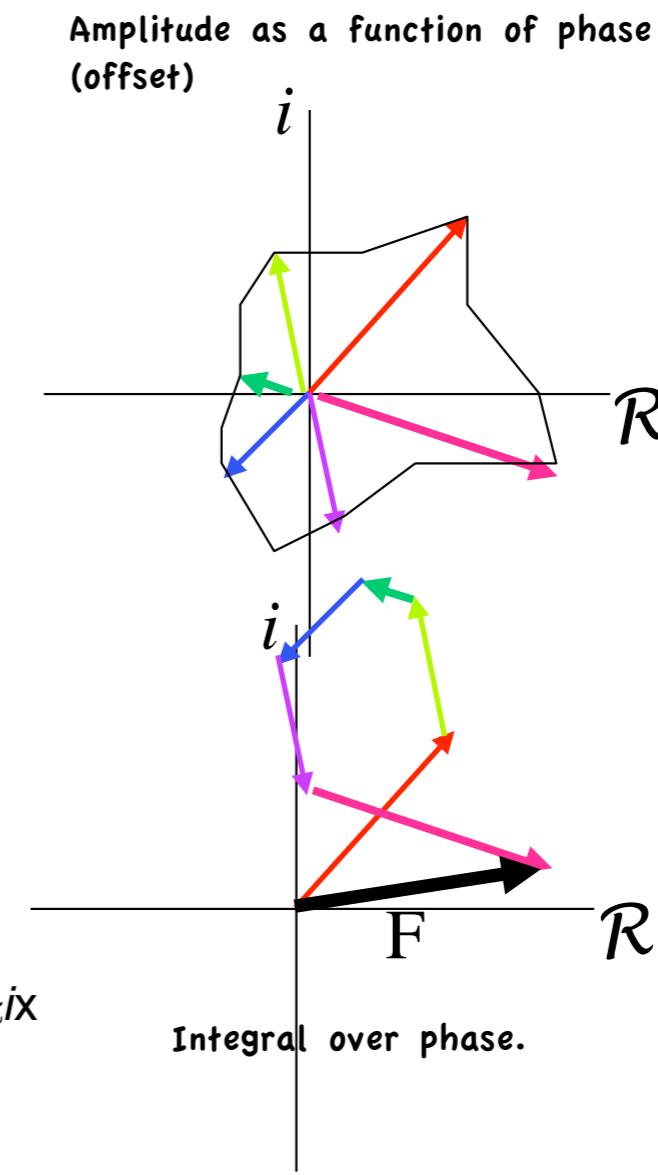
thus, not only atoms on the plane, but also atoms in between

Integrating offset Bragg planes from $x=0$ to 1



The total F is the wave sum over all offsets, x .

$$F = \sum_{x=0,1} \rho(x) e^{2\pi i x}$$



Diffraction

calculating the electron density

structure factor summed over atoms in unit cell

$$\mathbf{F}(\mathbf{S}) = \sum_k^N f_k \exp[2\pi i \mathbf{r}_k \cdot \mathbf{S}]$$

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assumption: atoms have spherical electron clouds

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assumption: atoms have spherical electron clouds

structure factor integrated over electrons in unit cell

$$\mathbf{F}(\mathbf{S}) = \int_{\text{cell}} \rho(\mathbf{r}) \exp[2\pi i \mathbf{r} \cdot \mathbf{S}] d\mathbf{v} \quad d\mathbf{v} = V dx dy dz$$

$$\mathbf{r} \cdot \mathbf{S} = (\mathbf{a}x + \mathbf{b}y + \mathbf{c}z) \cdot \mathbf{S}$$

$$= \mathbf{a} \cdot \mathbf{S}x + \mathbf{b} \cdot \mathbf{S}y + \mathbf{c} \cdot \mathbf{S}z$$

$$= h x + k y + l z$$

Diffraction

calculating the electron density

structure factor associated with reflection hkl

$$\mathbf{F}(h, k, l) = V \int_{x=0}^1 \int_{y=0}^1 \int_{z=0}^1 \rho(x, y, z) \exp[2\pi i(hx + ky + lz)] dx dy dz$$

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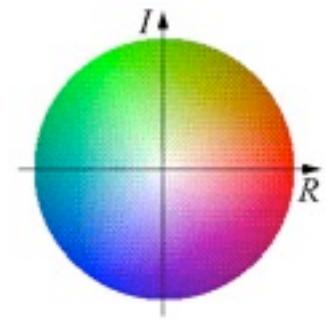
$$\rho(x, y, z) = \frac{1}{V} \sum_h \sum_k \sum_l \mathbf{F}(h, k, l) \exp[-2\pi i(hx + ky + lz)]$$

however, we can't measure the phase of the structure factor...

$$\mathbf{F}(h, k, l) = |F| \exp[i\alpha]$$

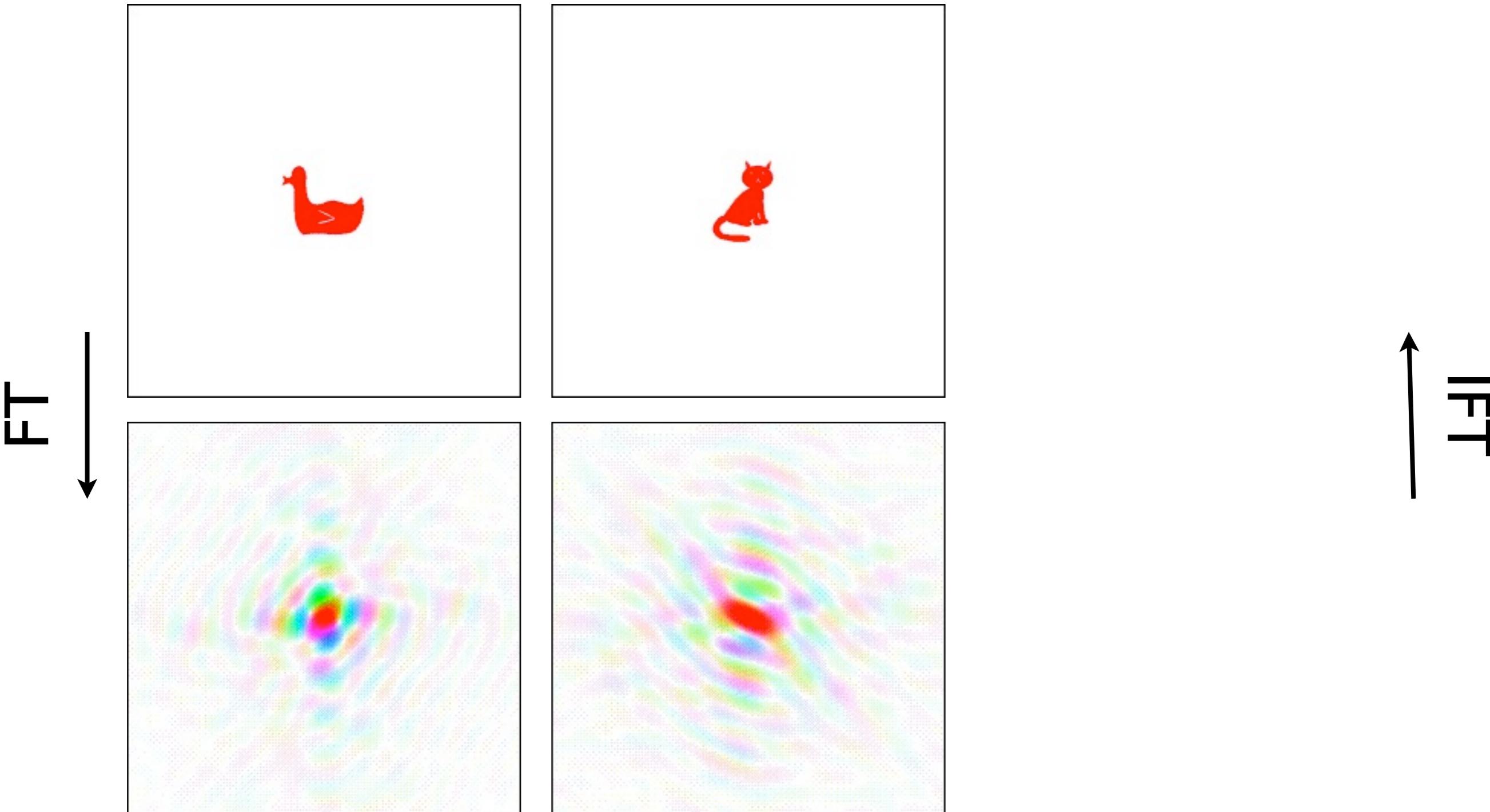
$$\rho(x, y, z) = \frac{1}{V} \sum_h \sum_k \sum_l |F(h, k, l)| \exp[-2\pi i(hx + ky + lz) + i\alpha(h, k, l)]$$

Diffraction



phase contains structural information

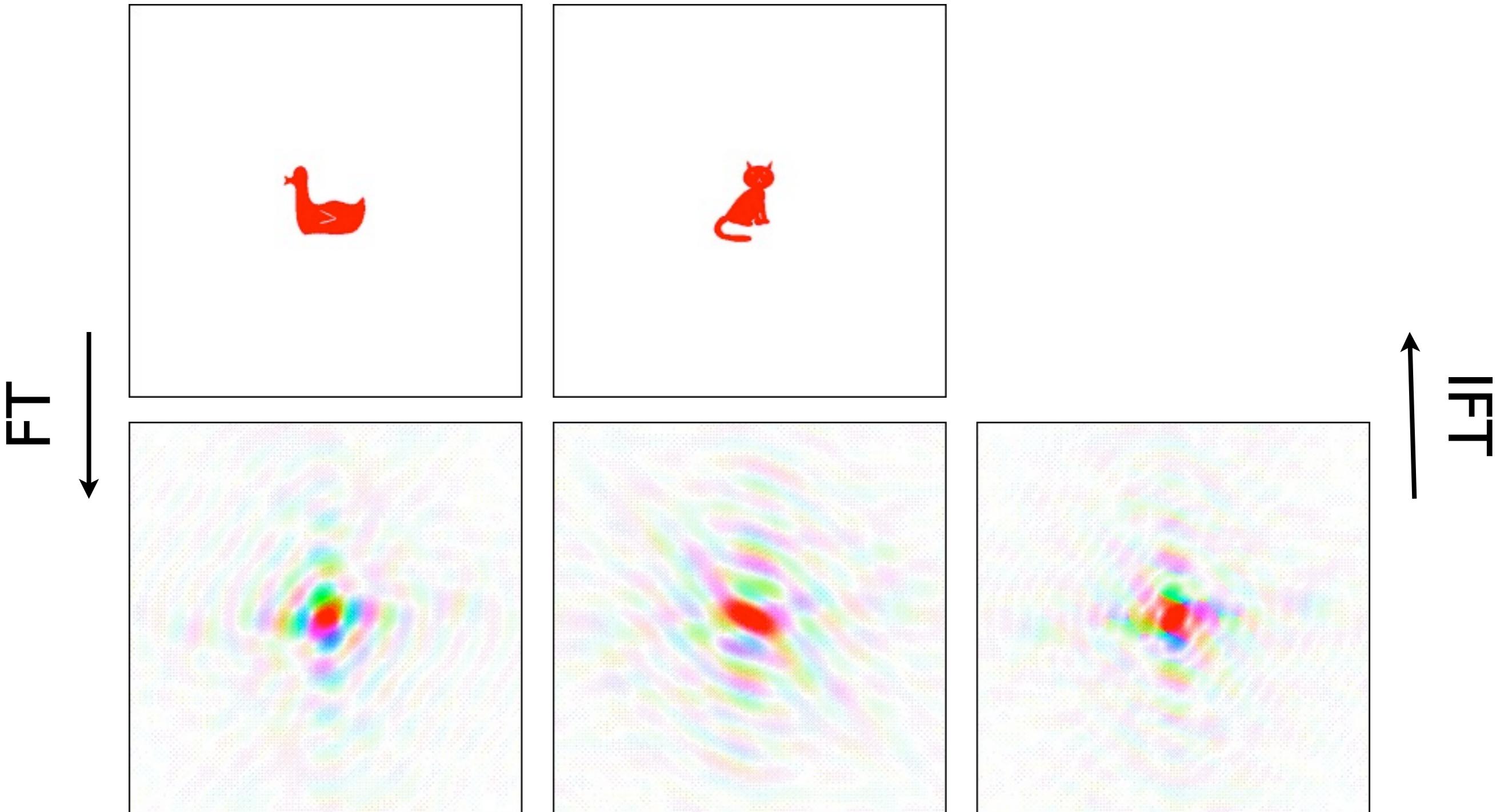
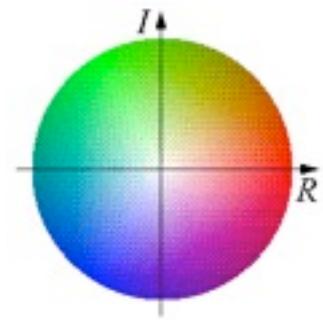
example Kevin Cowtan's Fourier Cat and Fourier Duck



Diffraction

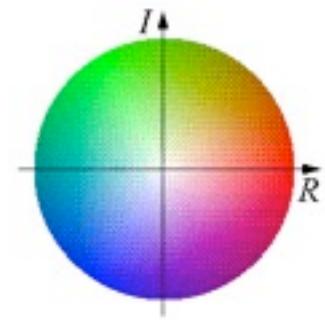
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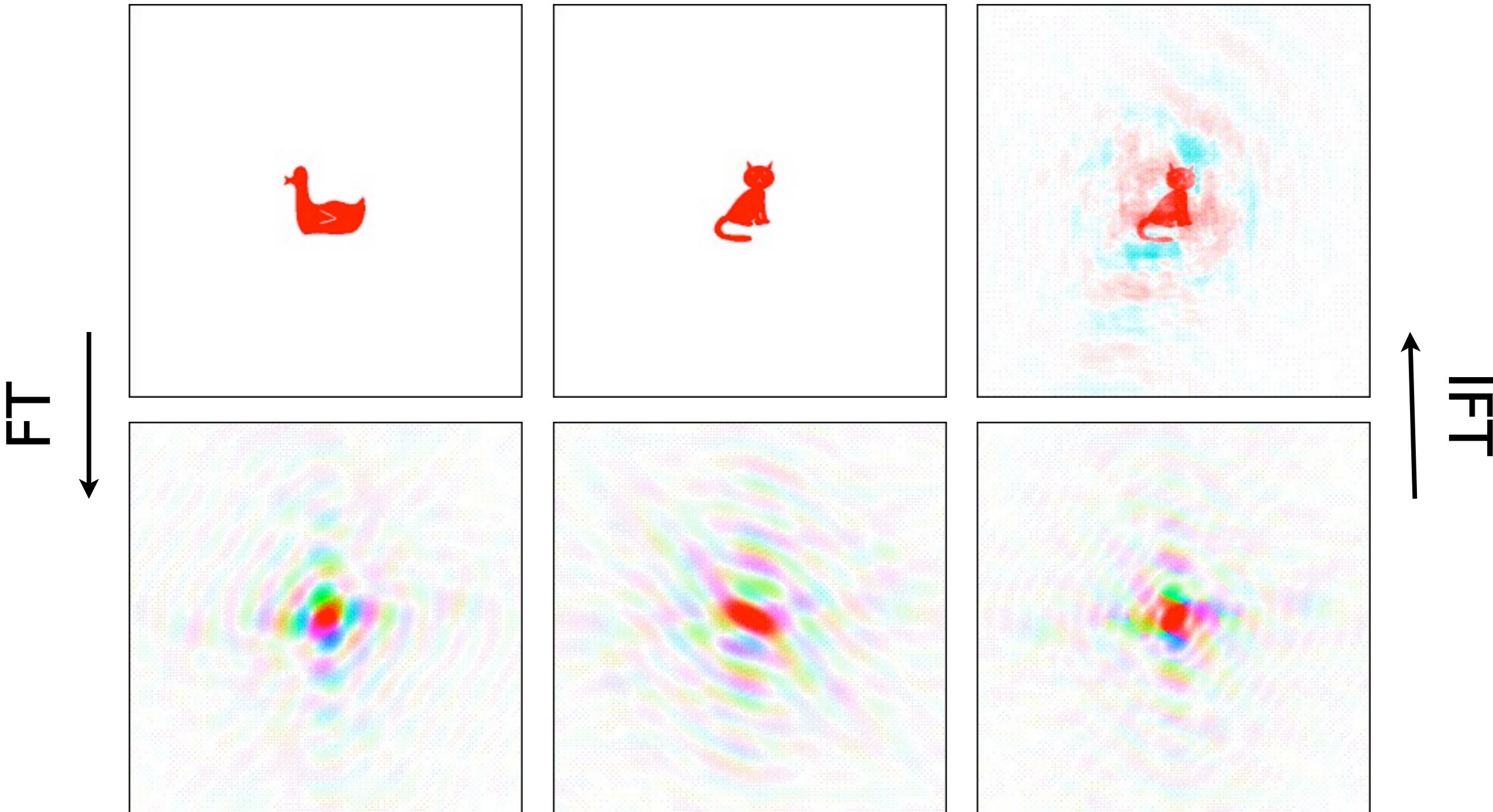
$$I_{\text{duck}}, \alpha_{\text{cat}}$$

Diffraction



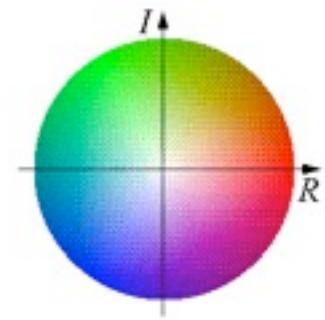
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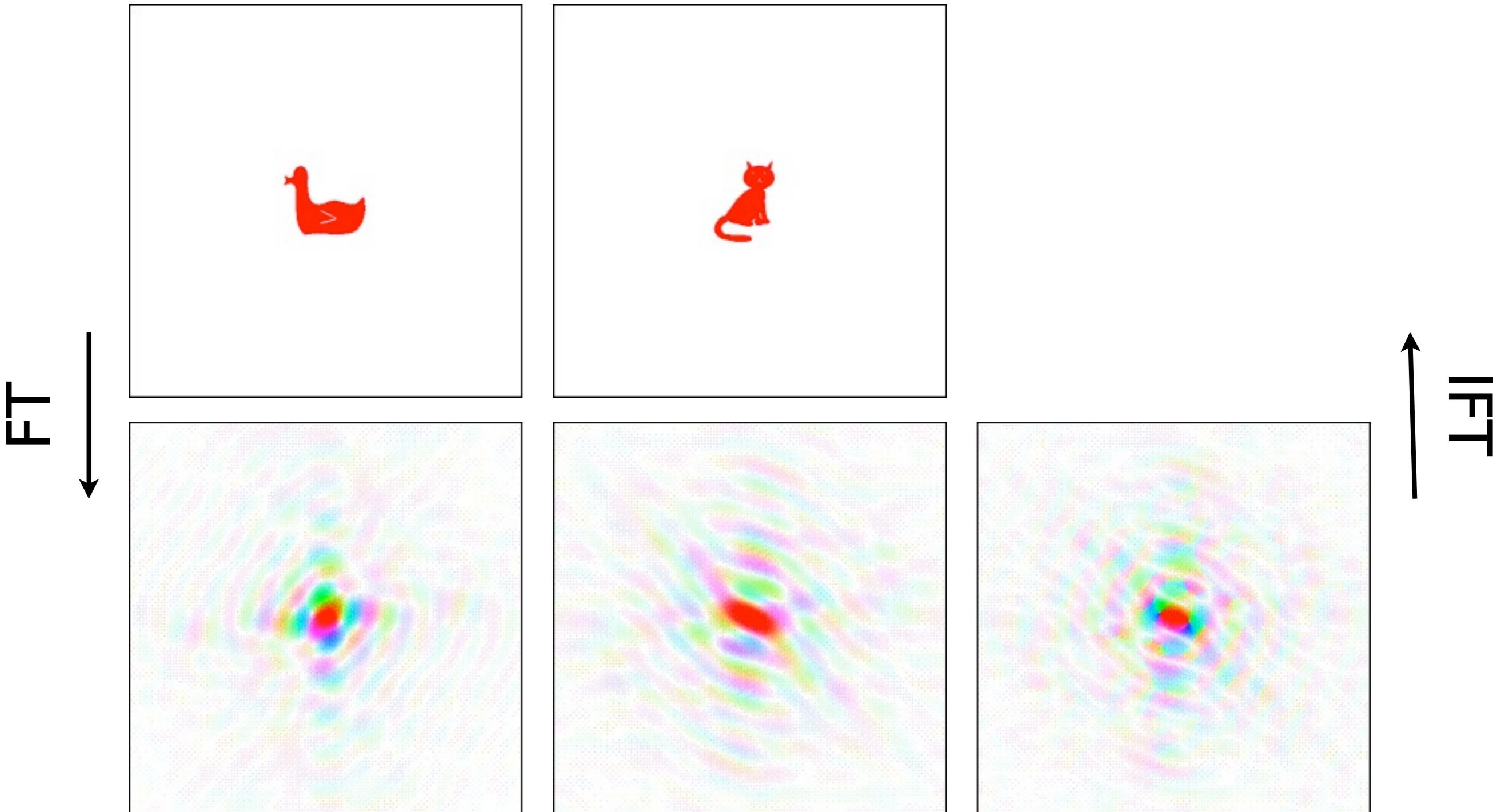
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Diffraction



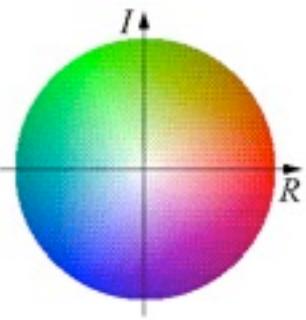
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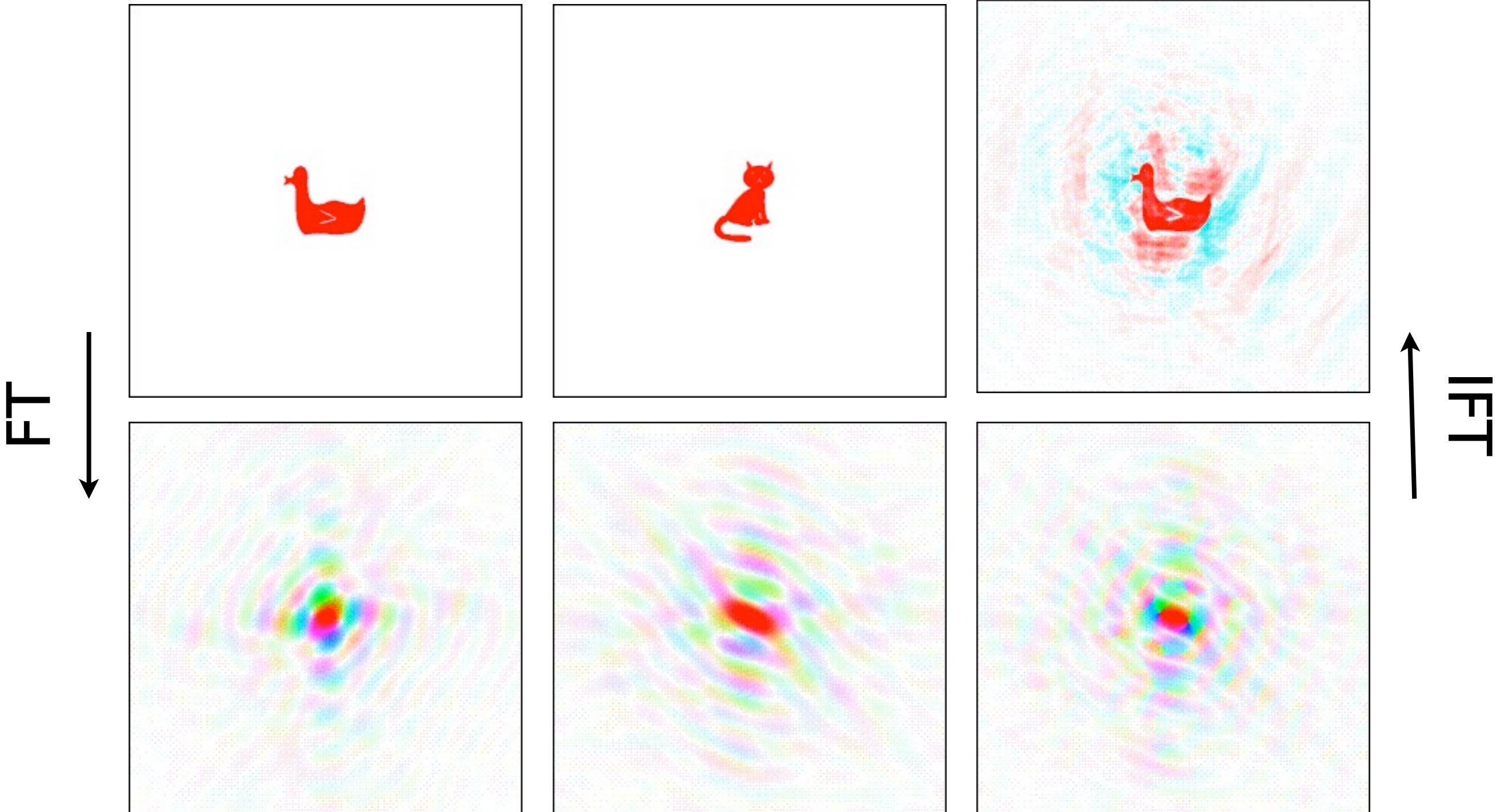
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$I_{\text{cat}}, \alpha_{\text{duck}}$

Diffraction

calculating the electron density

inverse Fourier transformation with unknown phases

$$\rho(x, y, z) = \frac{1}{V} \sum_h \sum_k \sum_l |F(h, k, l)| \exp[-2\pi i(hx + ky + lz) + i\alpha(h, k, l)]$$

solve the phase problem

hence, x-ray structure are solved!

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isomorphous replacement

electron rich elements w/o anomalous scattering

Patterson map

solve the position of the heavy atoms and use their phases

direct

only for small systems

molecular replacement

calculate phases for a homologous protein, of which 3D structure is known

use these phases for the inverse transform

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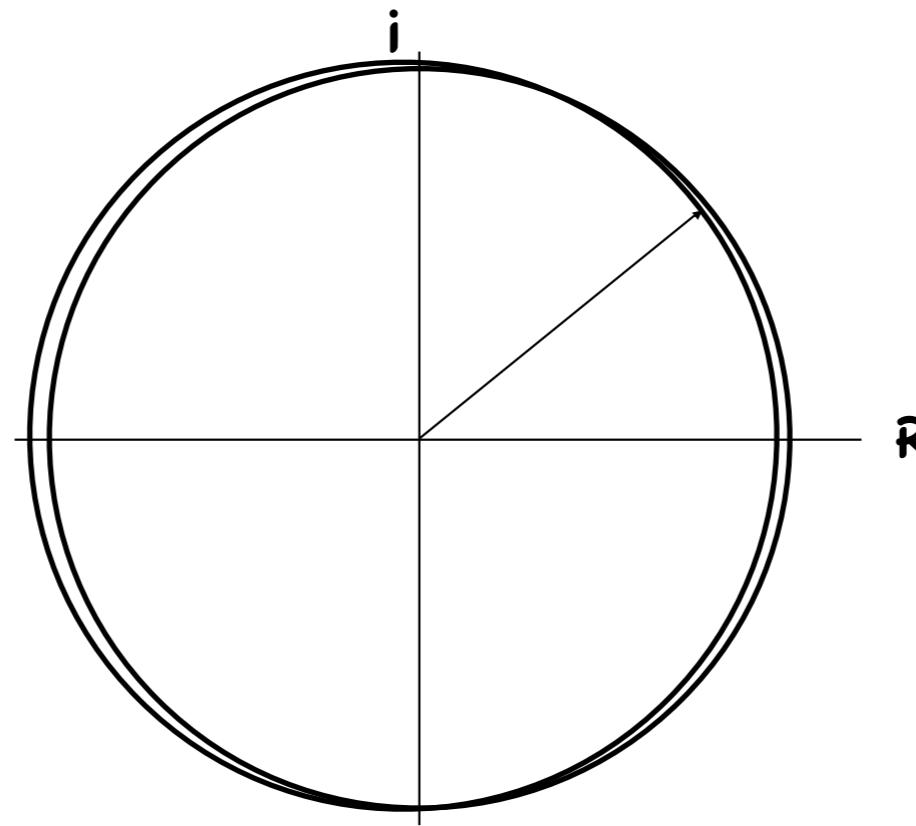
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Isomorphous replacement

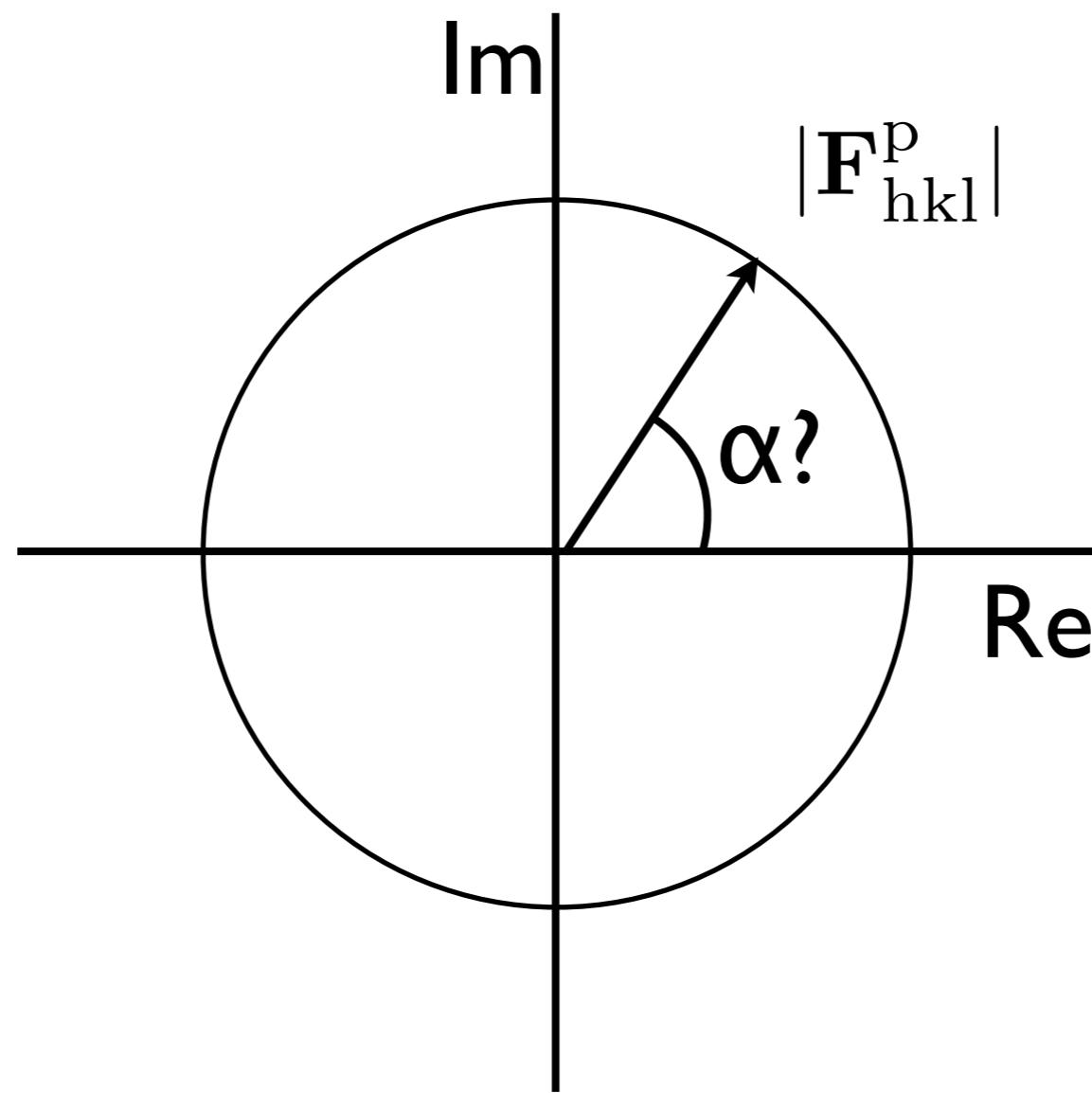
We can represent a structure factor of known amplitude and unknown phase as a circle in Argand space.



Radius of the circle is the amplitude. The true f lies somewhere on the circle.

Isomorphous replacement

structure factor (hkl) of protein with unknown fase



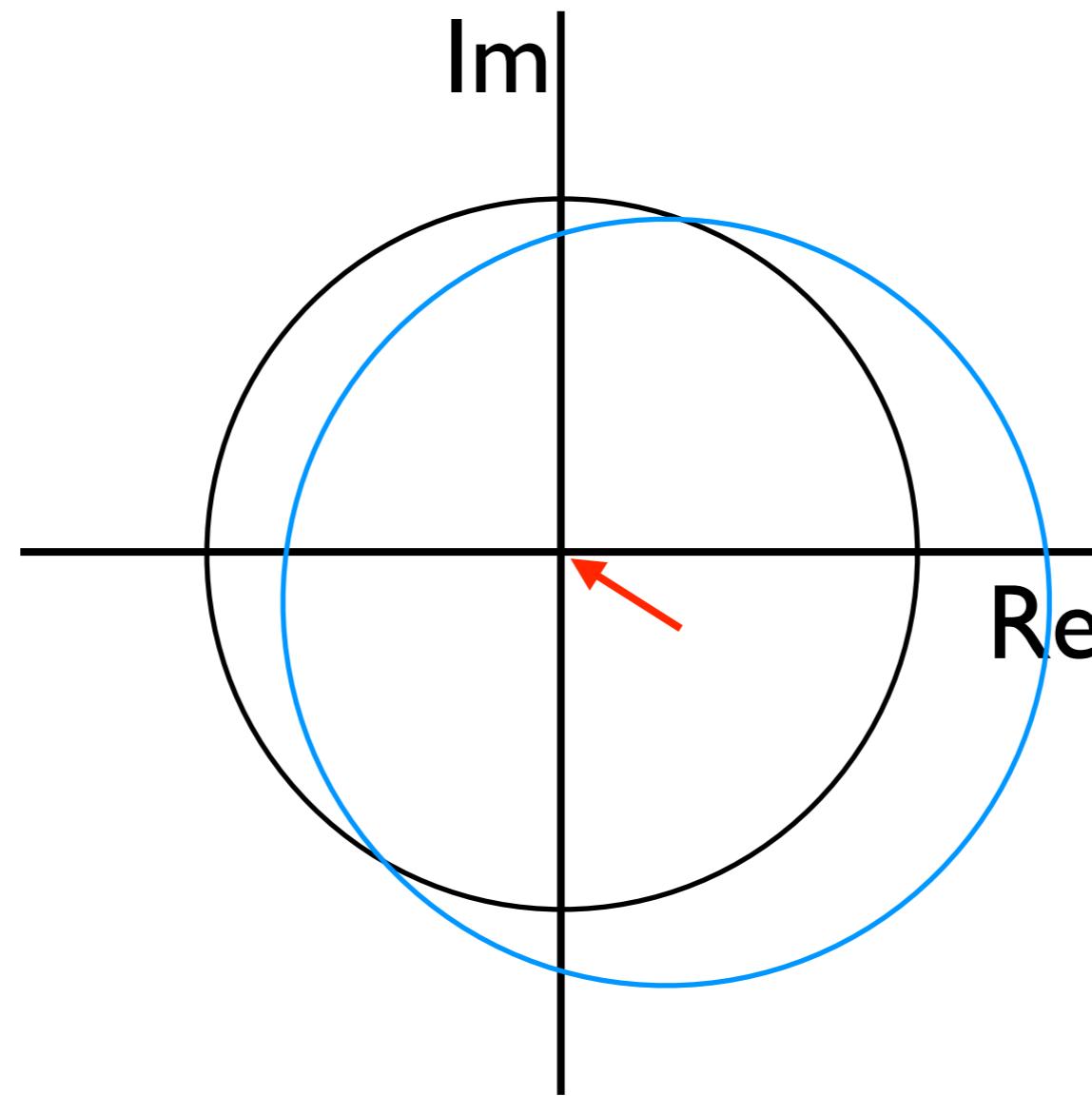
Isomorphous replacement

structure factor (hkl) of protein with unknown fase

structure factor (hkl) of protein with heavy atoms with unknown fase

structure factor (hkl) of heavy atoms with known positions

$$\underline{F_{hkl}^{PH}} = \underline{F_{hkl}^P} + \underline{F_{hkl}^H}$$



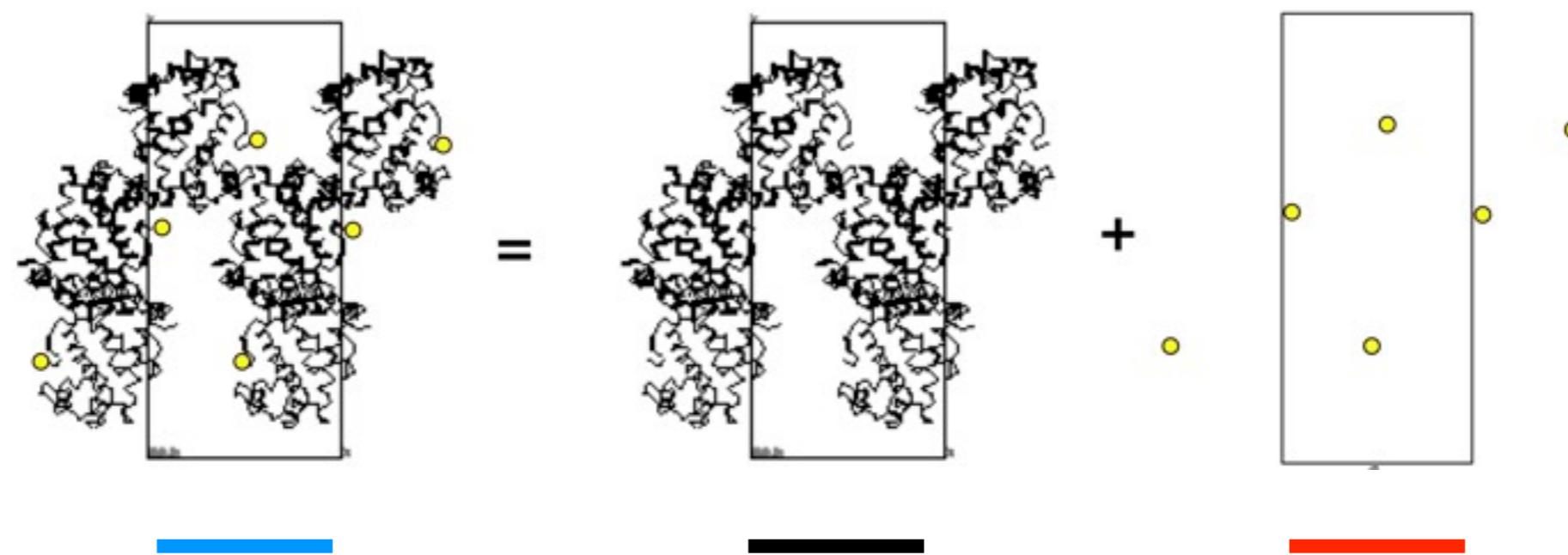
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structure factor (hkl) of protein with unknown fase

structure factor (hkl) of protein with heavy atoms with unknown fase

structure factor (hkl) of heavy atoms with known position and fase

$$\underline{\mathbf{F}}_{hkl}^{PH} = \underline{\mathbf{F}}_{hkl}^P + \underline{\mathbf{F}}_{hkl}^H$$



$$\underline{\mathbf{F}}_{hkl}^H = \sum_m^{N_{\text{heavy}}} f_m^{\text{heavy}} \exp[2\pi i(hx_m + ky_m + lz_m)]$$

Isomorphous replacement

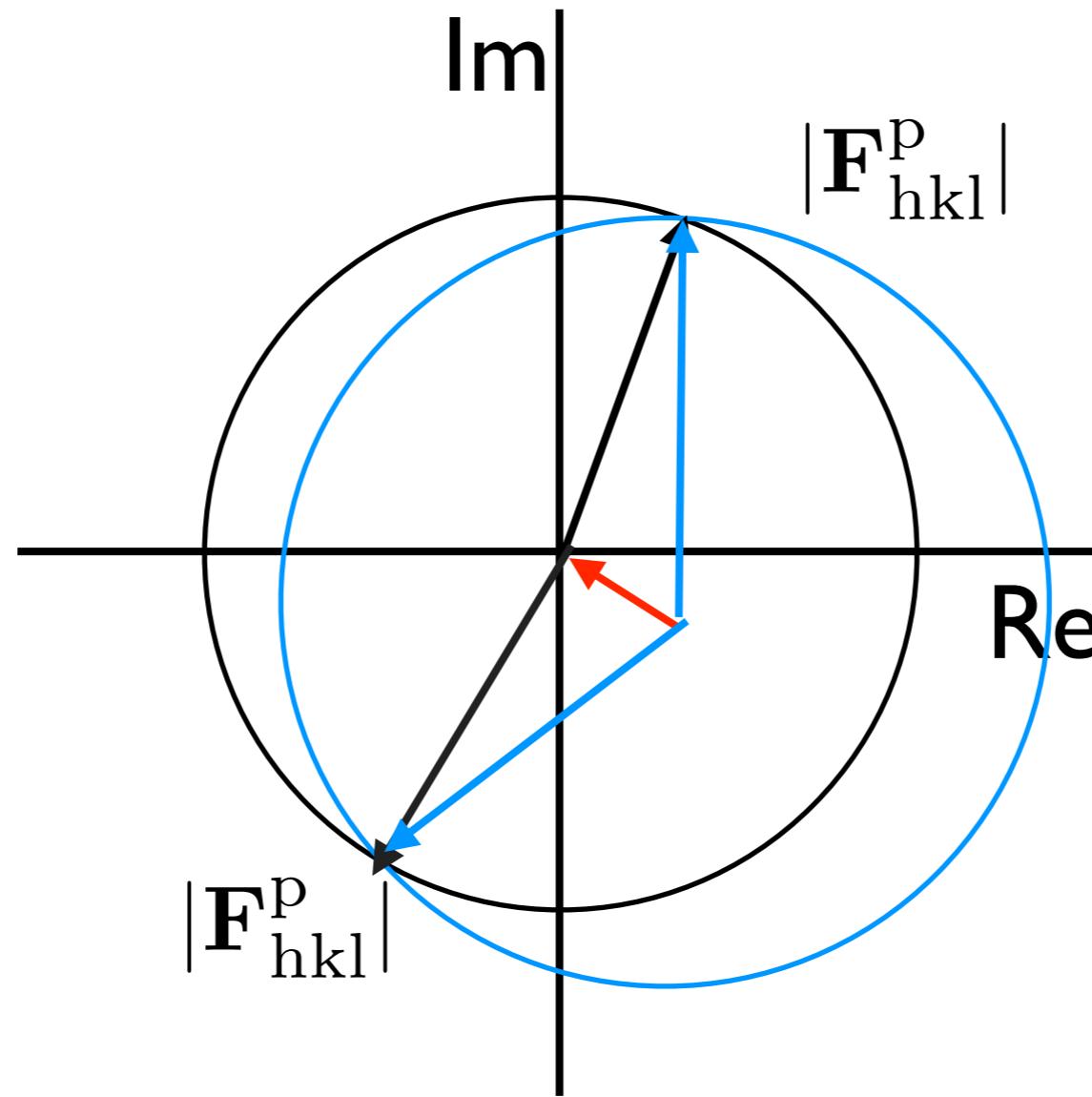
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structure factor (hkl) of heavy atoms with known fase

$$\underline{\mathbf{F}}_{hkl}^{PH} = \underline{\mathbf{F}}_{hkl}^P + \underline{\mathbf{F}}_{hkl}^H$$

two possibilities



Isomorphous replacement

structure factor (hkl) of protein with unknown fase

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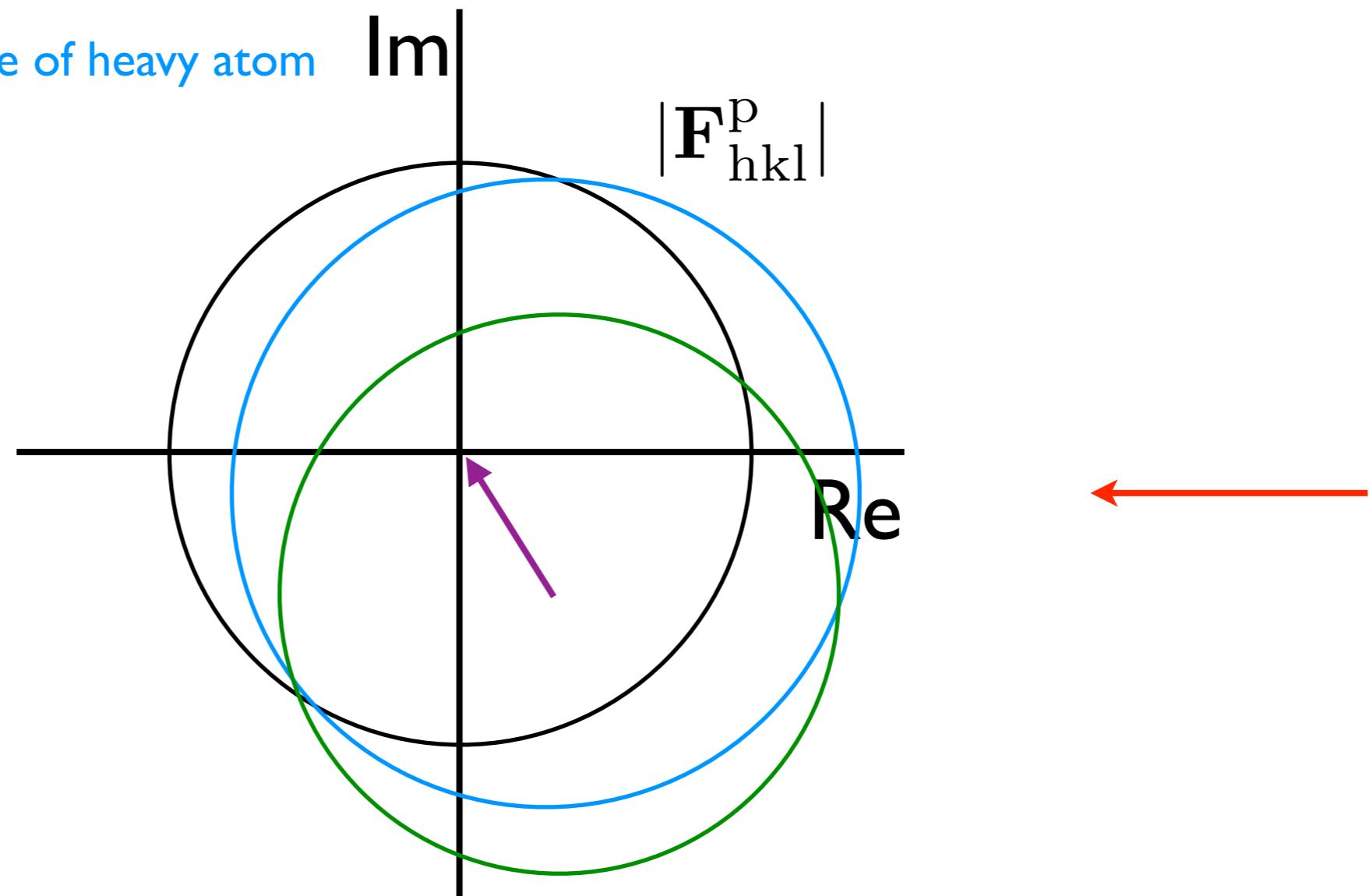
structure factor (hkl) of heavy atoms with known fase

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two possibilities

use a second type of heavy atom



Isomorphous replacement

structure factor (hkl) of protein with unknown fase

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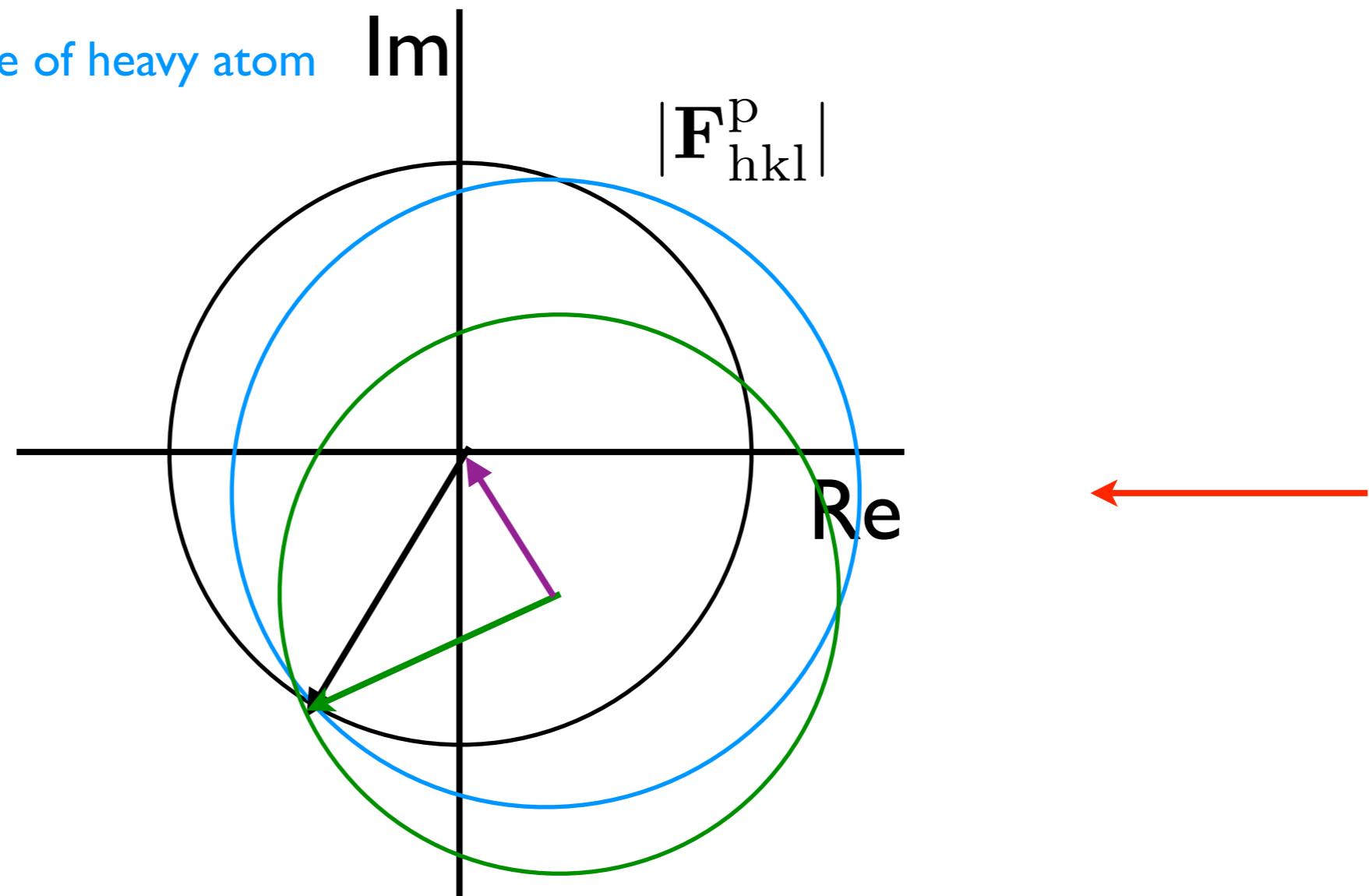
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Isomorphous replacement

locating heavy atoms with Patterson maps

inverse Fourier transformation with unknown phases

$$\rho(x, y, z) = \frac{1}{V} \sum_h \sum_k \sum_l |F(h, k, l)| \exp[-2\pi i(hx + ky + lz) + i\alpha(h, k, l)]$$

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inverse Fourier transformation with intensities but no phases

$$P(u, v, w) = \frac{1}{V} \sum_h \sum_k \sum_l |F(h, k, l)|^2 \cos[2\pi(hu + kv + lw)]$$

autocorrelation function of electron density

$$P(\mathbf{u}) = \int \rho(\mathbf{r})\rho(\mathbf{r} + \mathbf{u})d\mathbf{u}$$

Isomorphous replacement

Patterson map

autocorrelation of electron density

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example 1D Patterson map

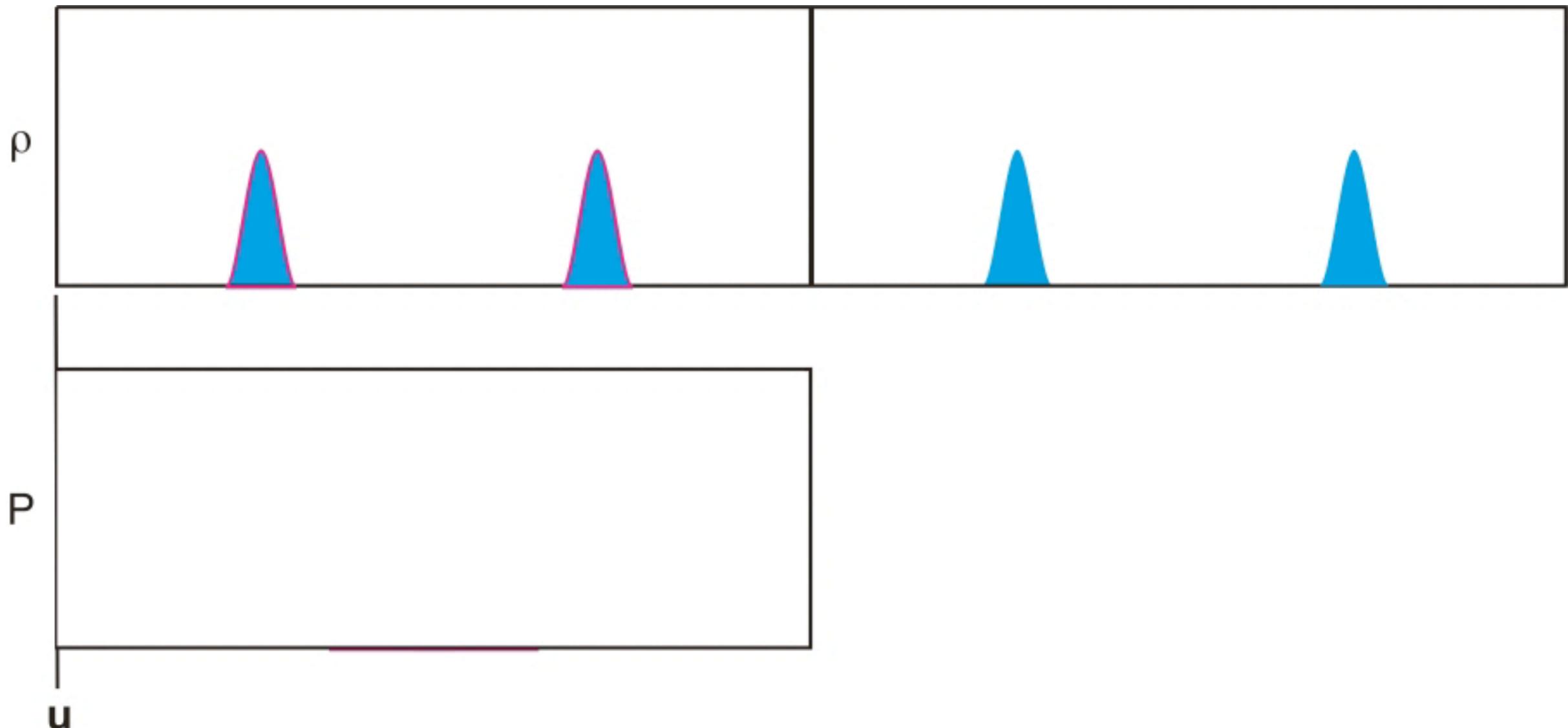
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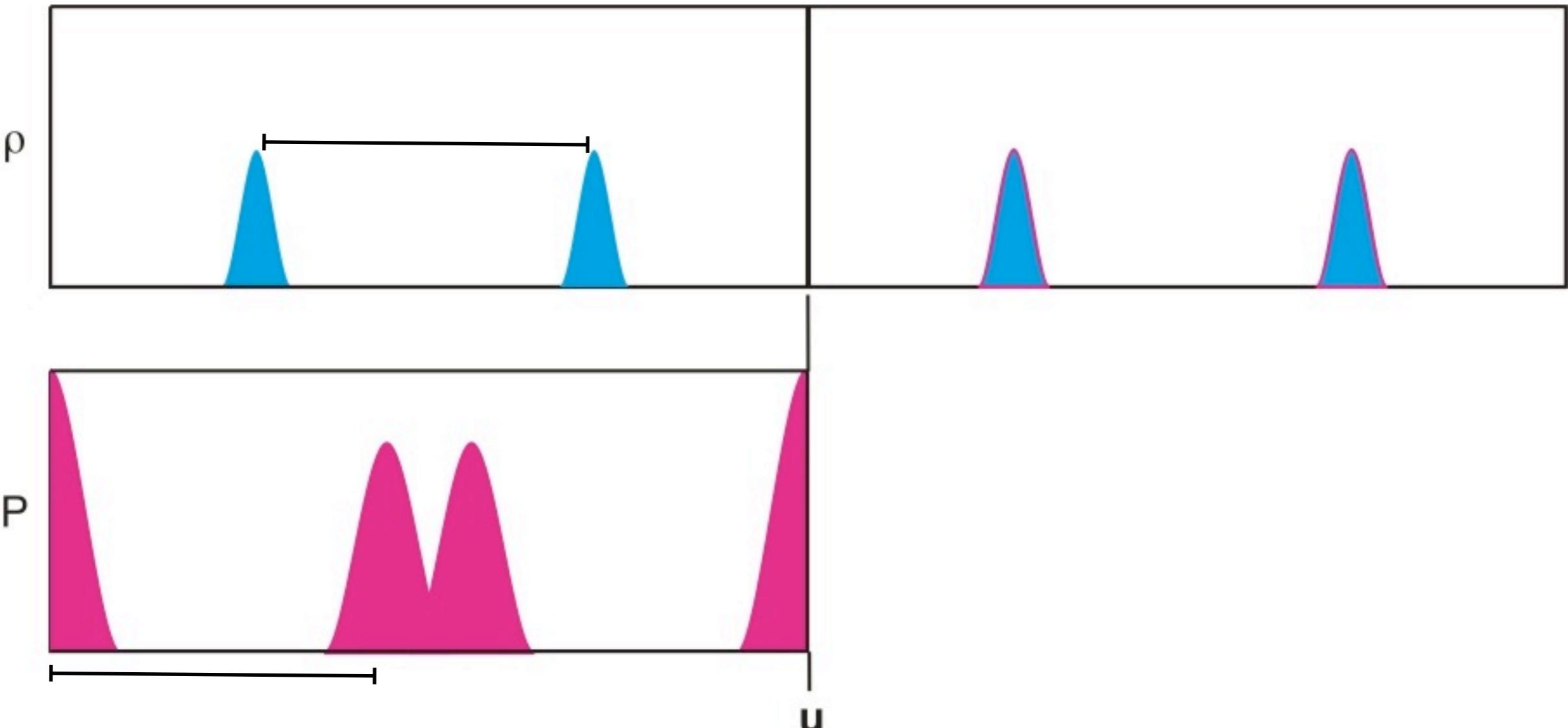
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example 1D Patterson map

peaks in Patterson map are the $N(N-1)$ interatomic distance vectors



Isomorphous replacement

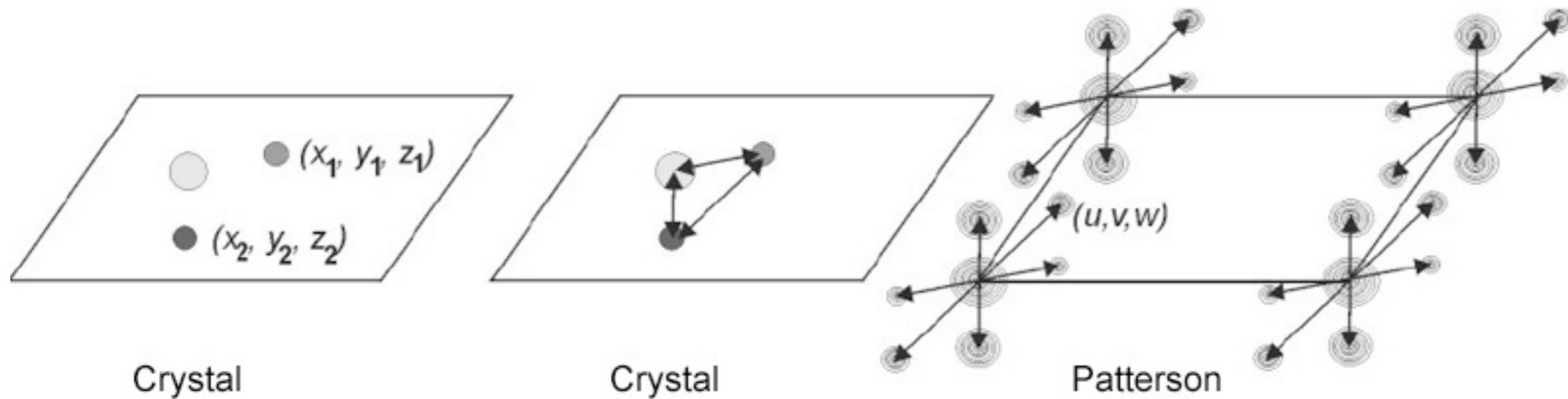
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Isomorphous replacement

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peaks in Patterson map are the $N(N-1)$ interatomic distances

Isomorphous replacement with heavy atoms

subtract the diffraction patterns and obtain the diffraction due to heavy atoms only

reconstruct positions of heavy atoms from Patterson map

compute phase F_H

solve phase F_P from F_H

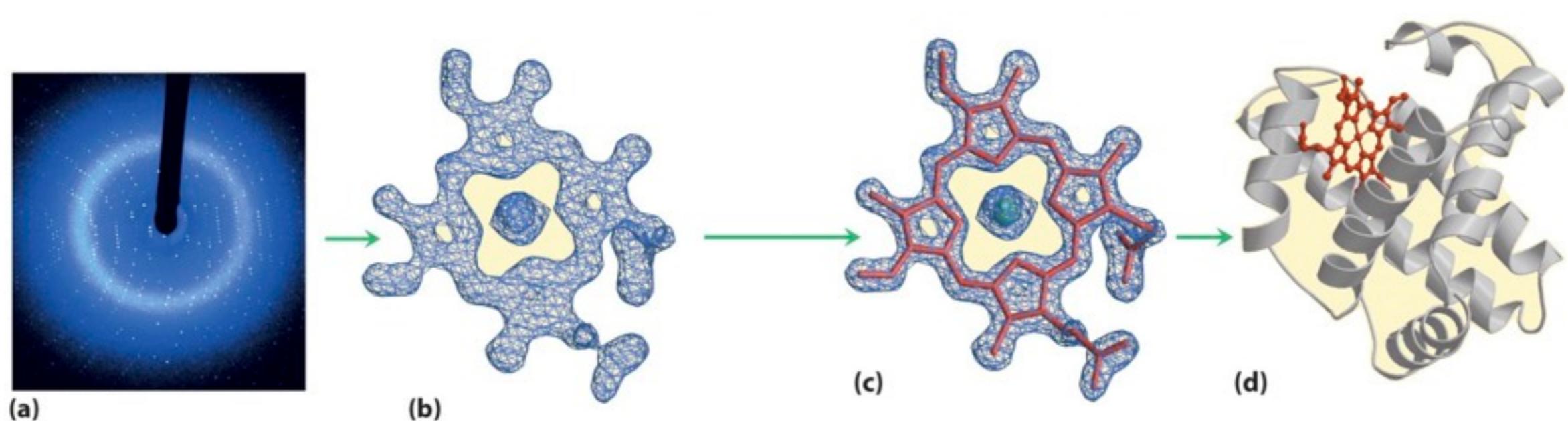
building atomic model

I fit atoms into the density

information on sequence

information on bond lengths, angles, torsions

force field



building atomic model

1 fit atoms into the density

information on sequence

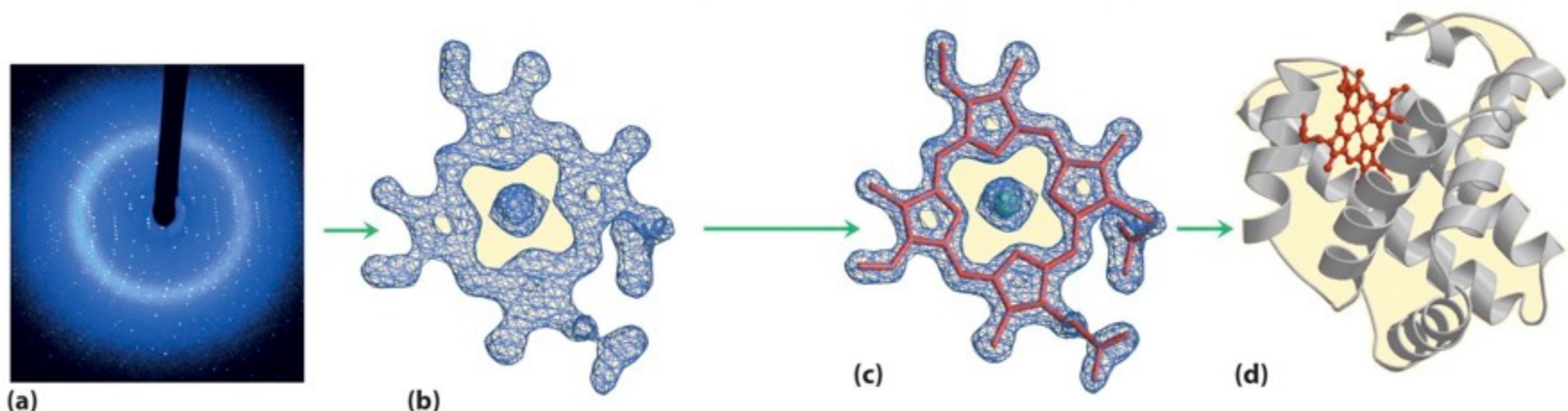
information on bond lengths, angles, torsions

force field

2 (re)compute phases of model

new density

repeat 1-2 until you're happy



validation of model

R-factors

accuracy of the model

$$R = \frac{\sum_{hkl} |F_{\text{obs}}| - k|F_{\text{calc}}| |}{\sum_{hkl} |F_{\text{obs}}|}$$

random positions of atoms in unit cell

$$R = 0.59$$

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avoid over-fitting of the model

work set, used to optimize model ($W \sim 90\%$ of reflections)

test set, not used for creating model ($T \sim 10\%$ of reflections)

$$R^{\text{free}} = \frac{\sum_{hkl \in T} |F_{\text{obs}}| - k|F_{\text{calc}}|}{\sum_{hkl \in T} |F_{\text{obs}}|}$$

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Ramachandran

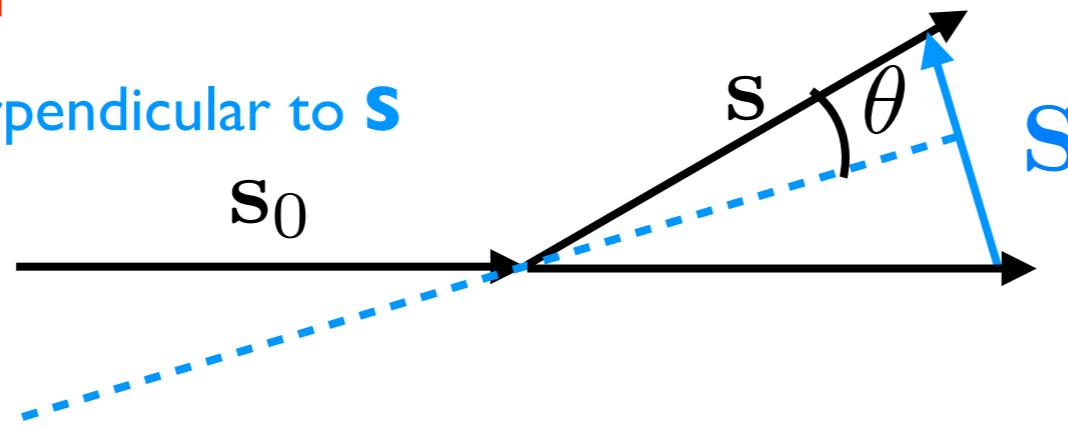
stereochemistry

scattering with crystals molecules

Bragg's law

recall “reflection planes”

reflection plane perpendicular to \mathbf{S}



$$|\mathbf{S}| = \frac{2 \sin(\theta)}{\lambda}$$

Laue conditions

all atoms on hkl plane through origin

$$\alpha_0 = 2\pi \mathbf{r}_0 \cdot \mathbf{S} = 0$$

all atoms on the next plane

$$\alpha_1 = 2\pi \mathbf{r}_1 \cdot \mathbf{S} = 2\pi$$

distance between planes

$$d = \frac{n}{|\mathbf{S}|}$$

Bragg's law:

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

resolution

