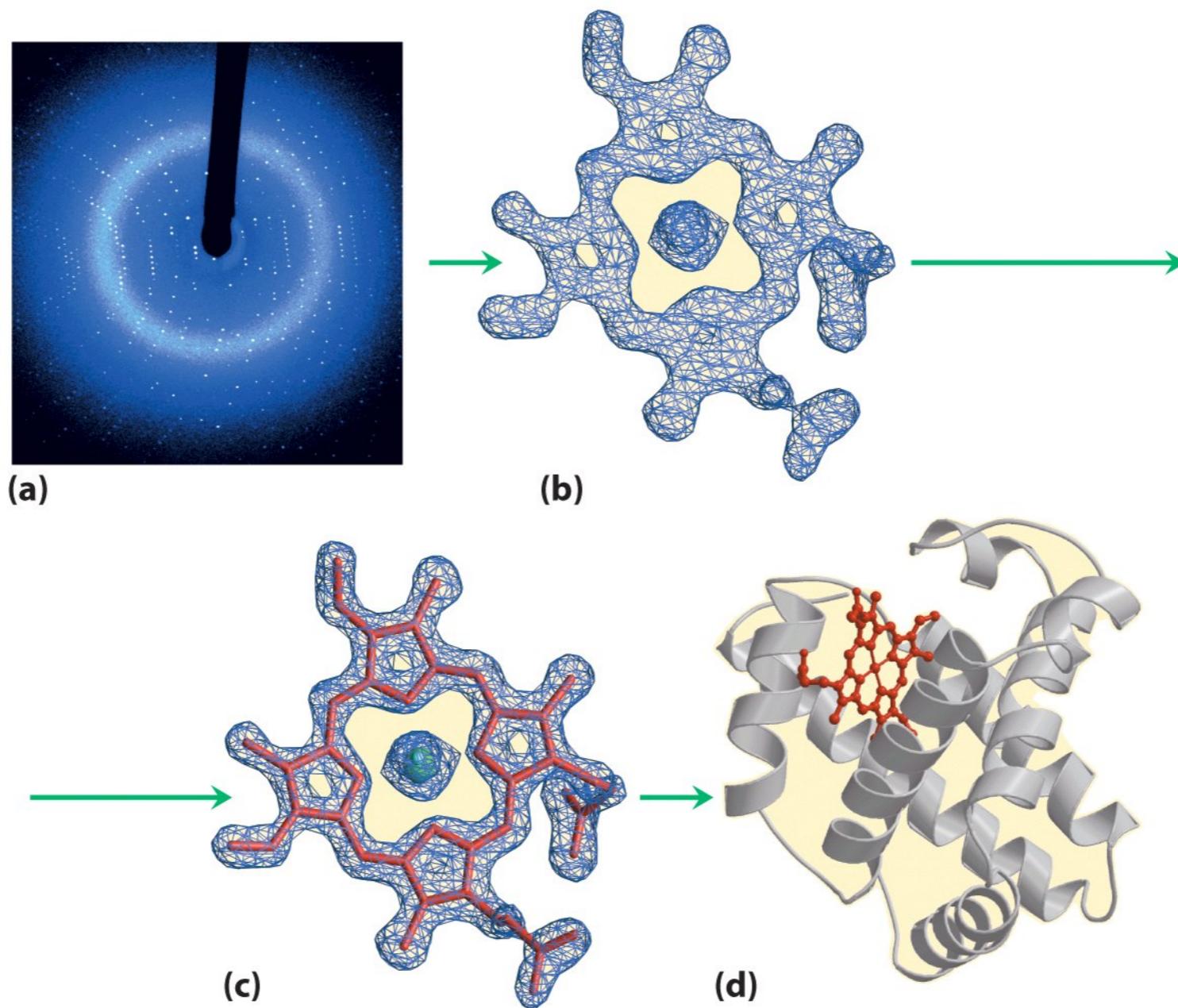


structure determination of proteins with x-rays

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



structure determination of proteins with x-rays

x-ray crystallography

crystals

x-rays

scattering of electrons, 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 photoreceptor

structure determination of proteins with x-rays

x-ray crystallography

crystals

x-rays

crystals

asymmetric unit



30 Å

crystals

asymmetric unit



30 Å

symmetry mates
screw axis



crystals

asymmetric unit

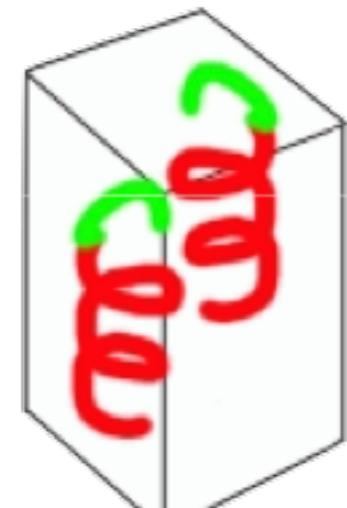


30 Å

symmetry mates
screw axis



unitcell



100 Å

crystals

asymmetric unit

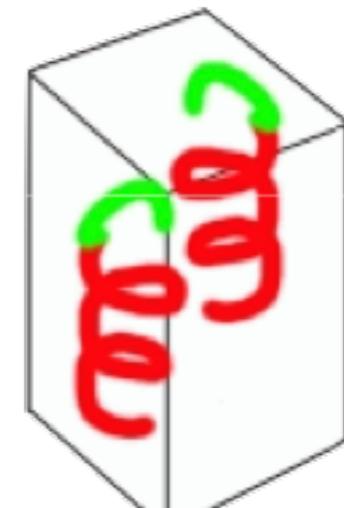


30 Å

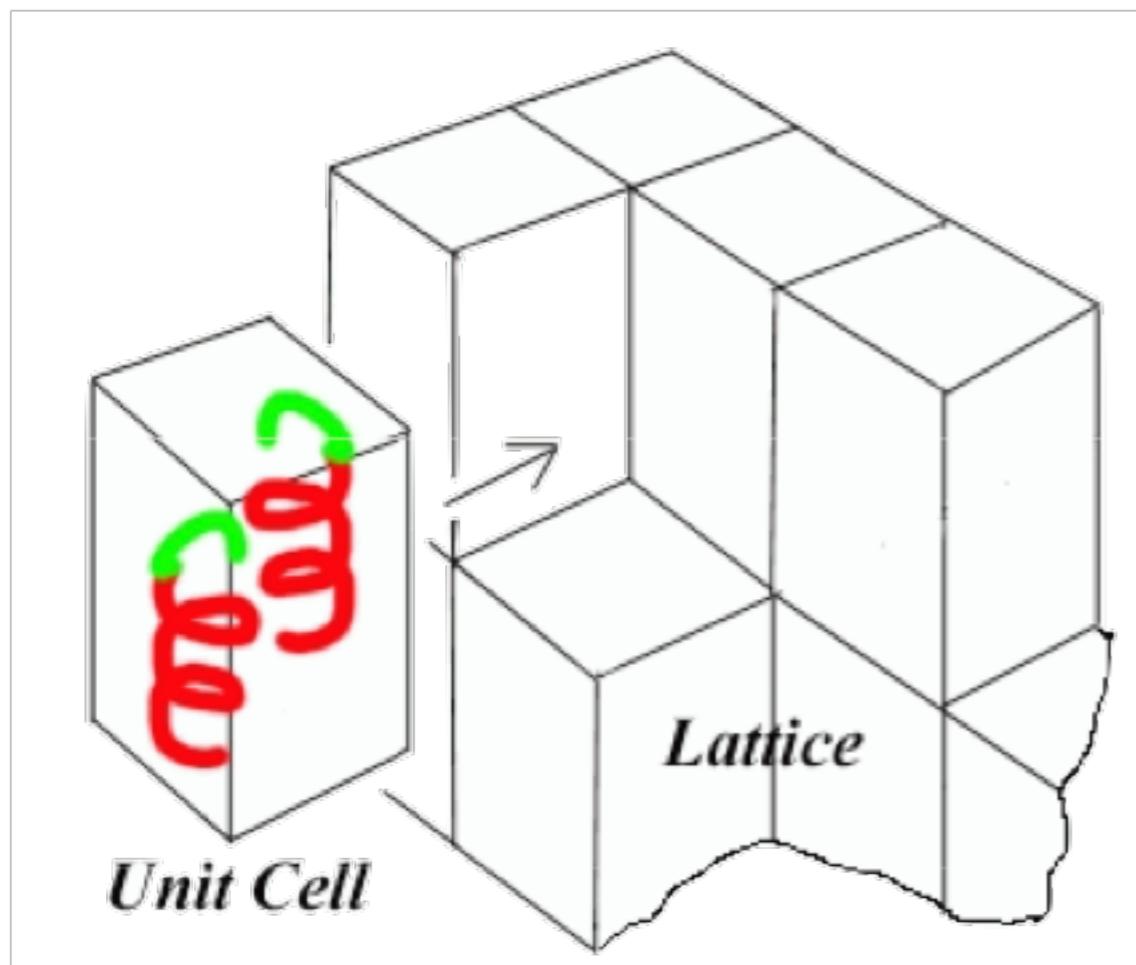
symmetry mates
screw axis



unitcell



100 Å



crystals

asymmetric unit

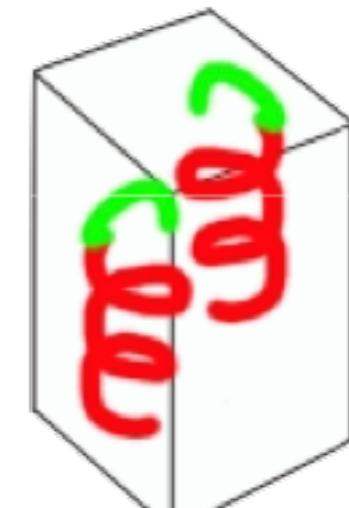


30 Å

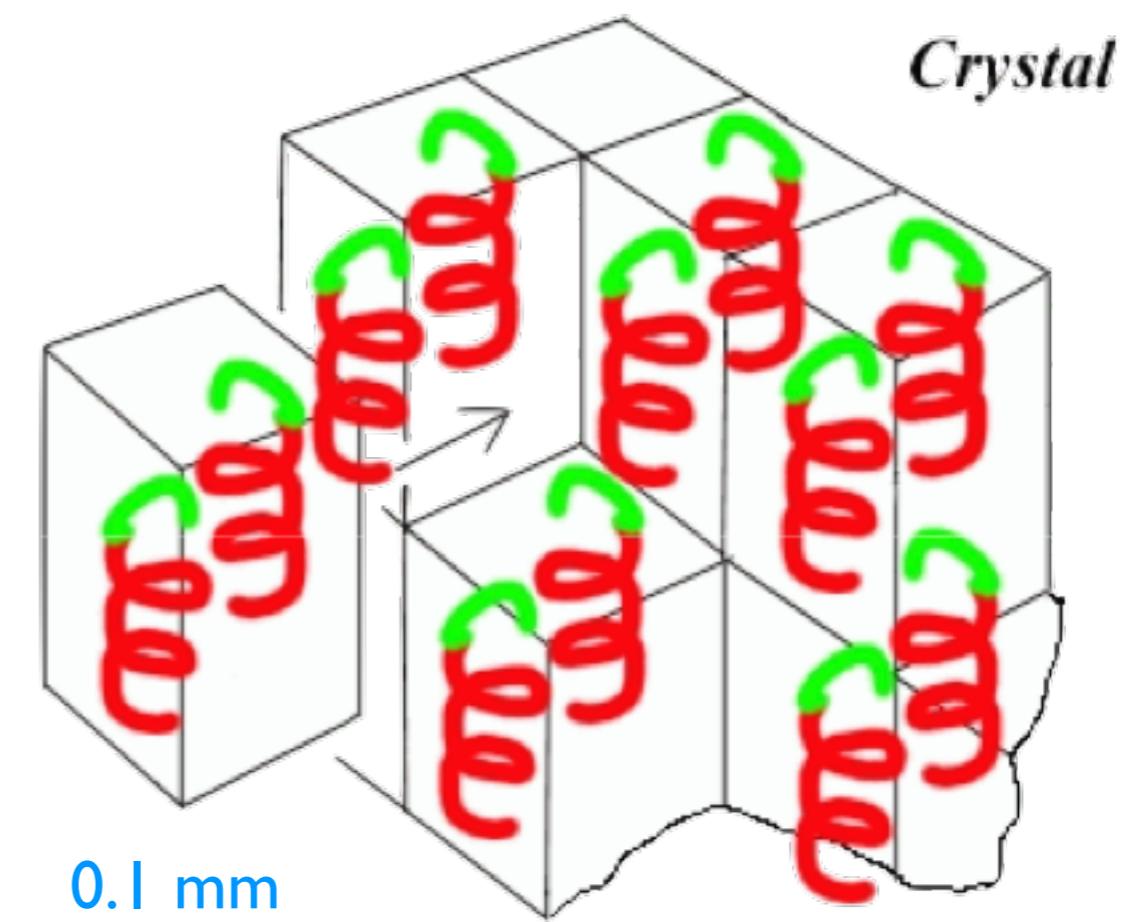
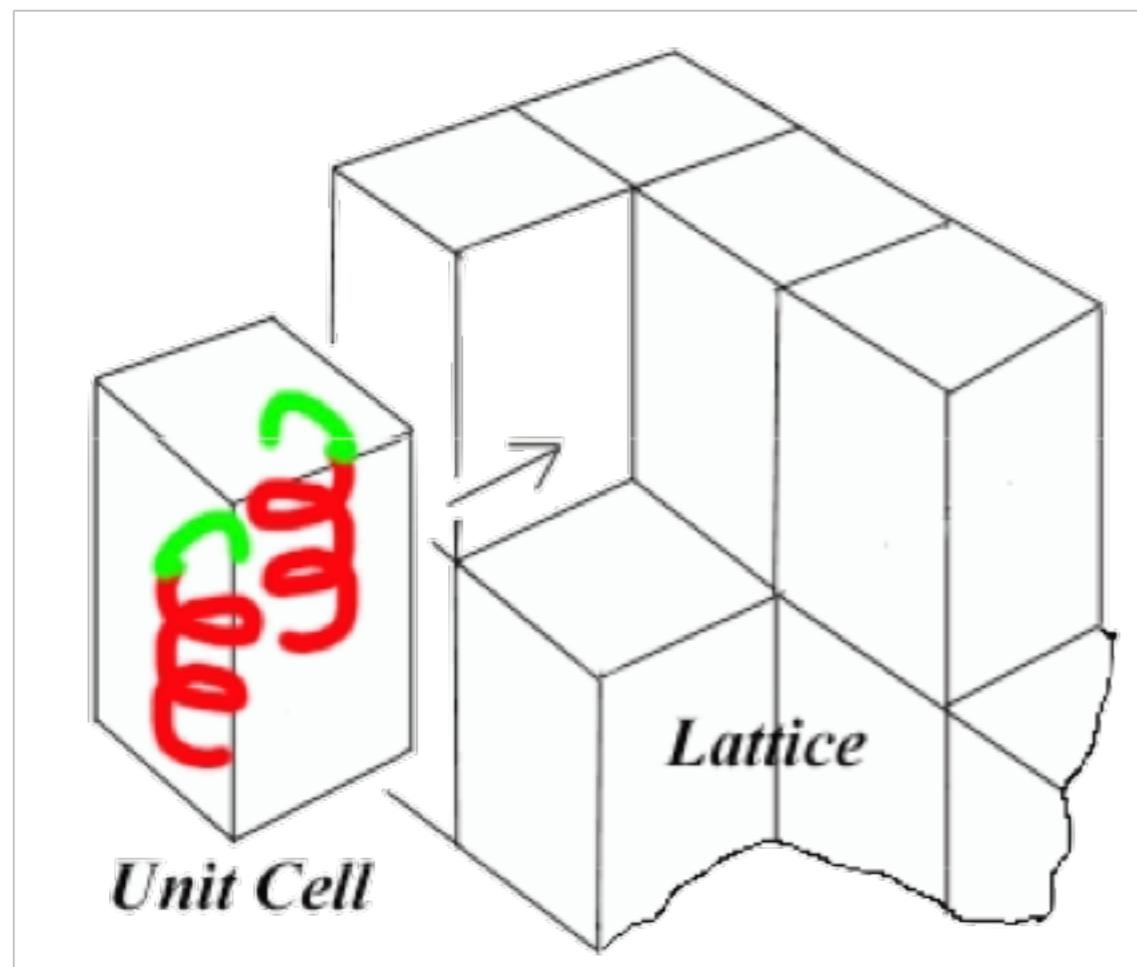
symmetry mates
screw axis



unitcell



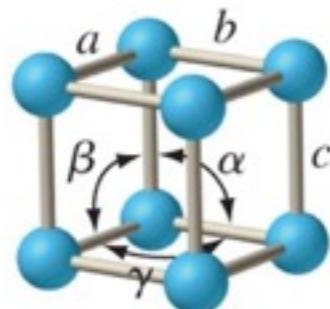
100 Å



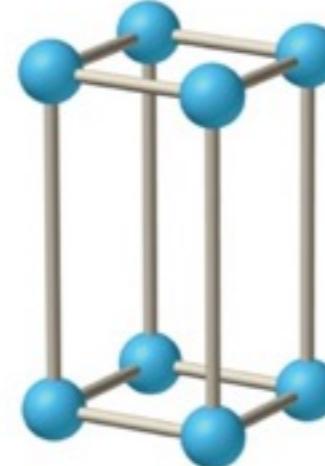
crystals

seven types of crystal lattices

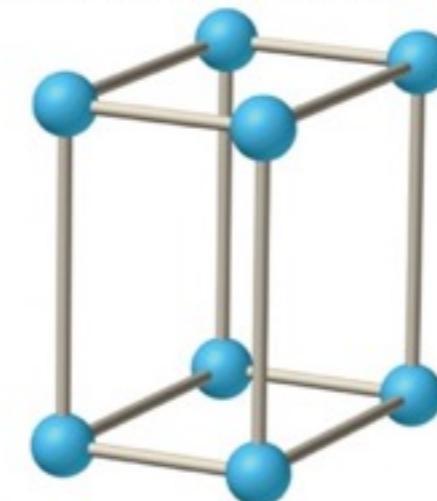
Bravais lattices



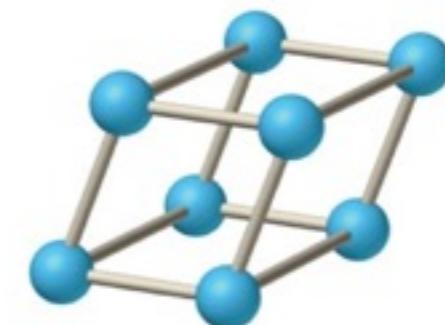
Simple cubic
 $a = b = c$
 $\alpha = \beta = \gamma = 90^\circ$



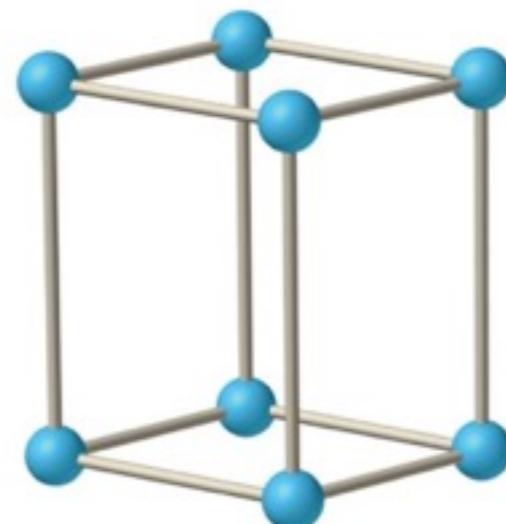
Tetragonal
 $a = b \neq c$
 $\alpha = \beta = \gamma = 90^\circ$



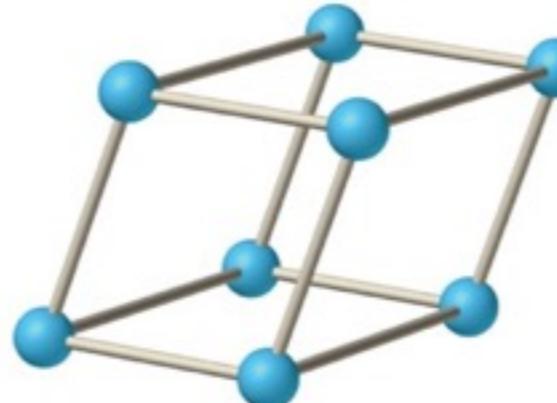
Orthorhombic
 $a \neq b \neq c$
 $\alpha = \beta = \gamma = 90^\circ$



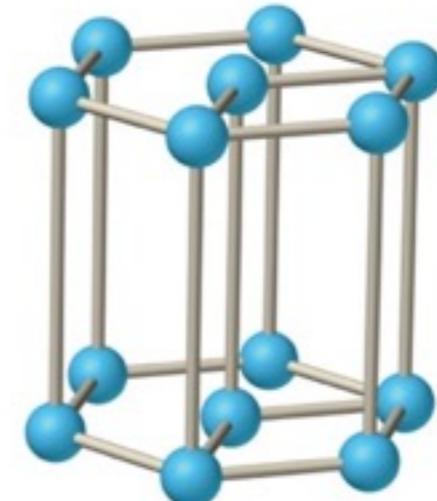
Rhombohedral
 $a = b = c$
 $\alpha = \beta = \gamma \neq 90^\circ$



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



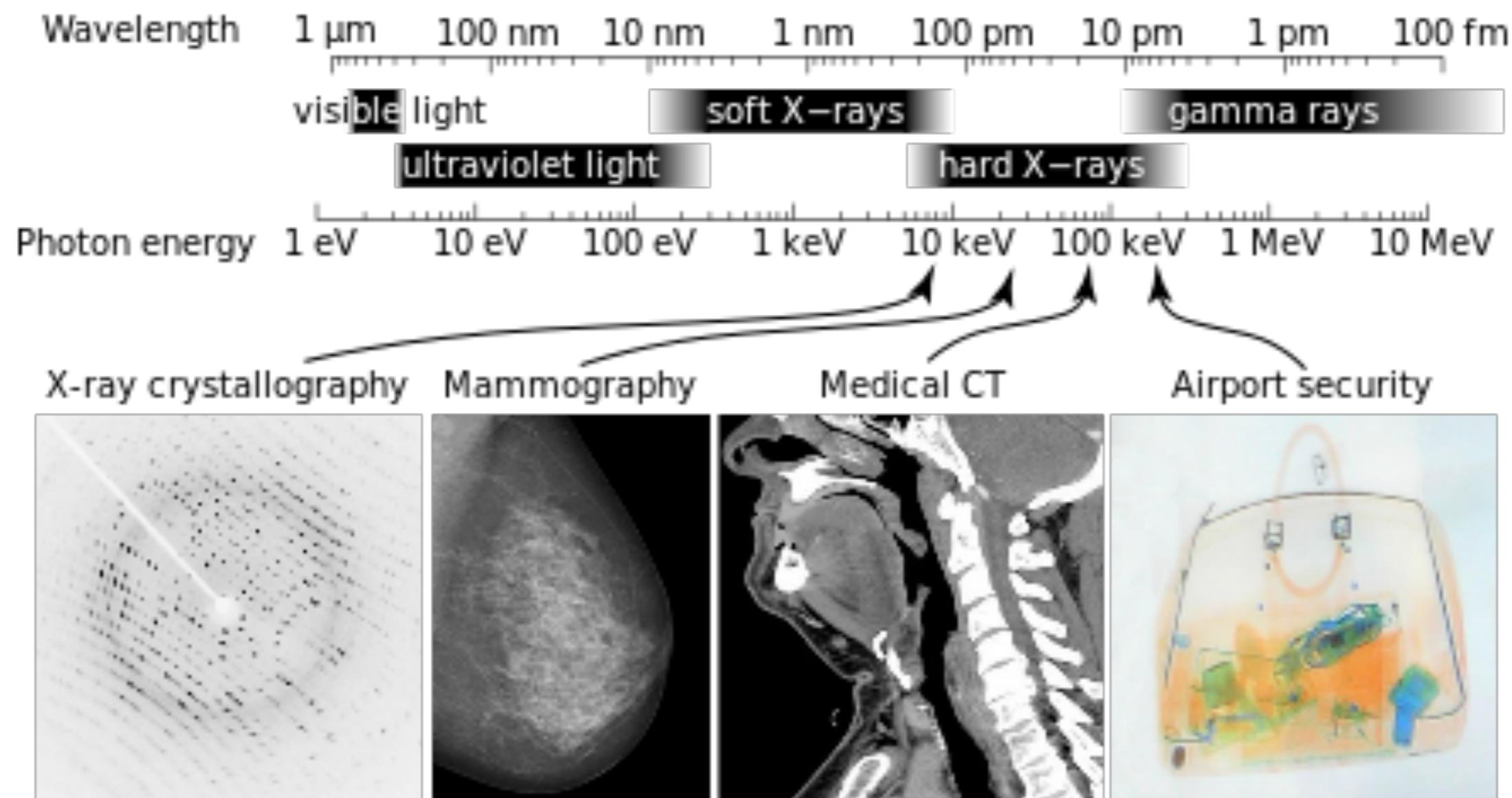
Triclinic
 $a \neq b \neq c$
 $\alpha \neq \beta \neq \gamma \neq 90^\circ$



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

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 $\sim 1.5 \text{ \AA}$

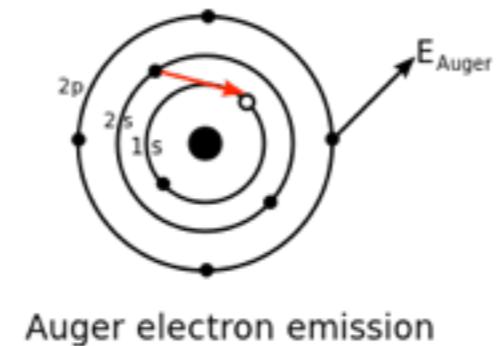
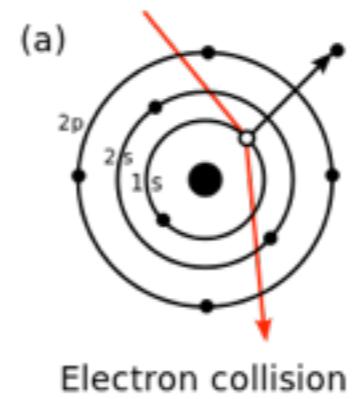
photon energy

10 keV

1s electron of C: 284 eV

inelastic scattering

(core) ionization, Auger decay, radiation damage



X-rays

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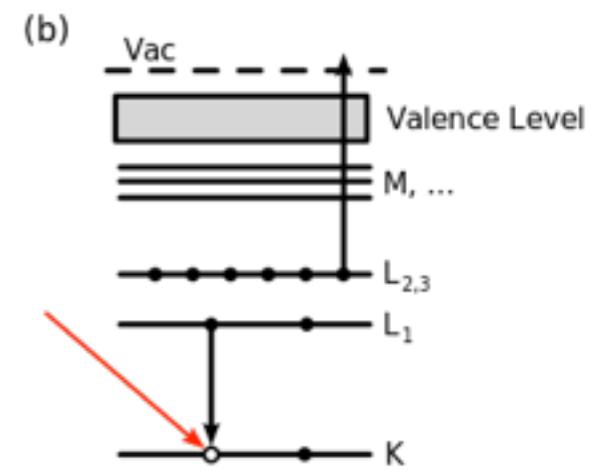
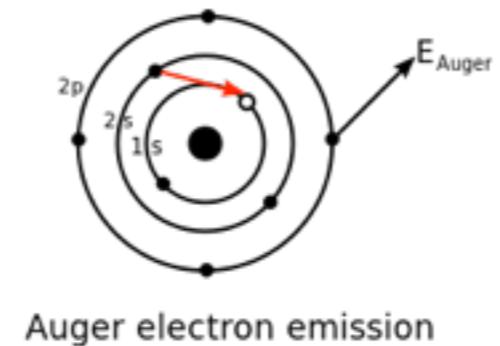
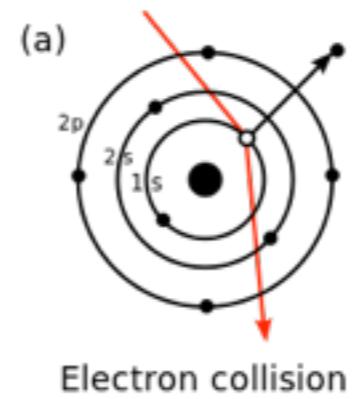
10 keV

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(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

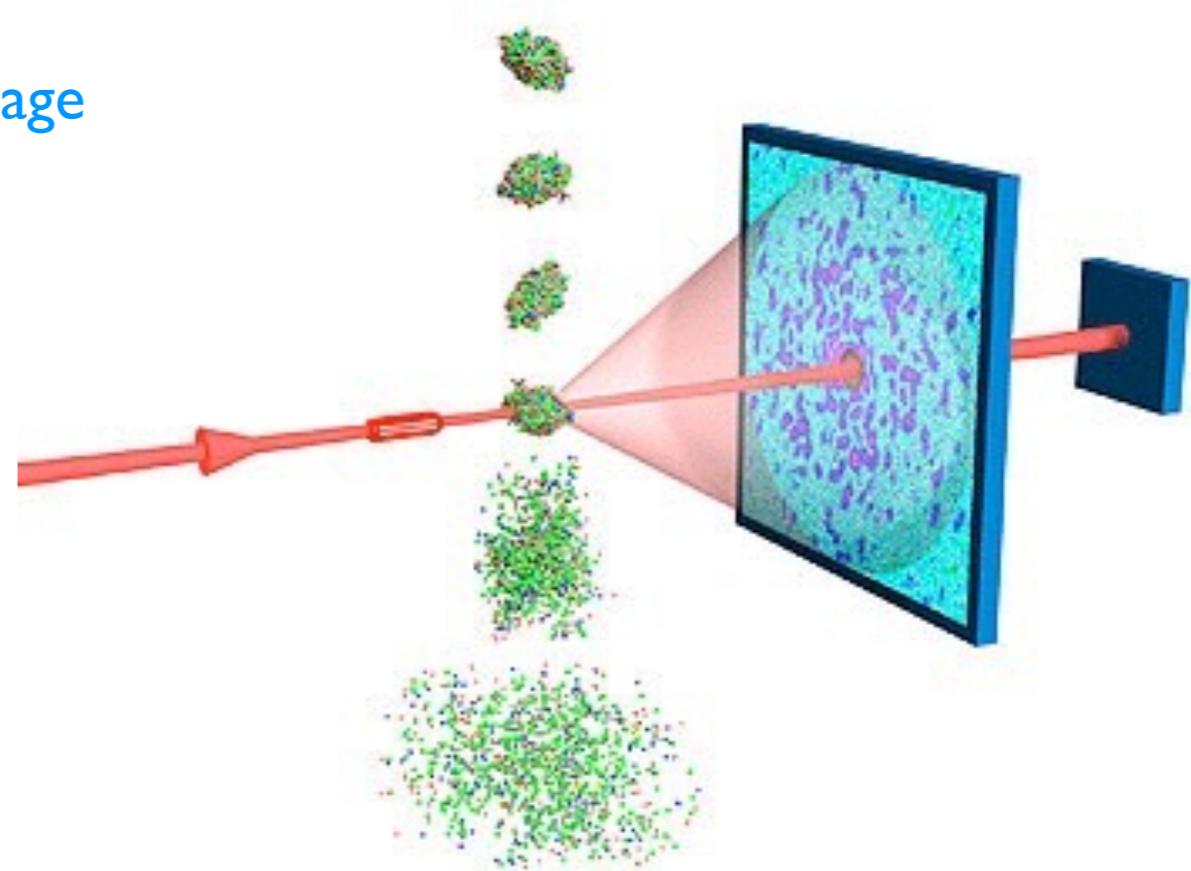
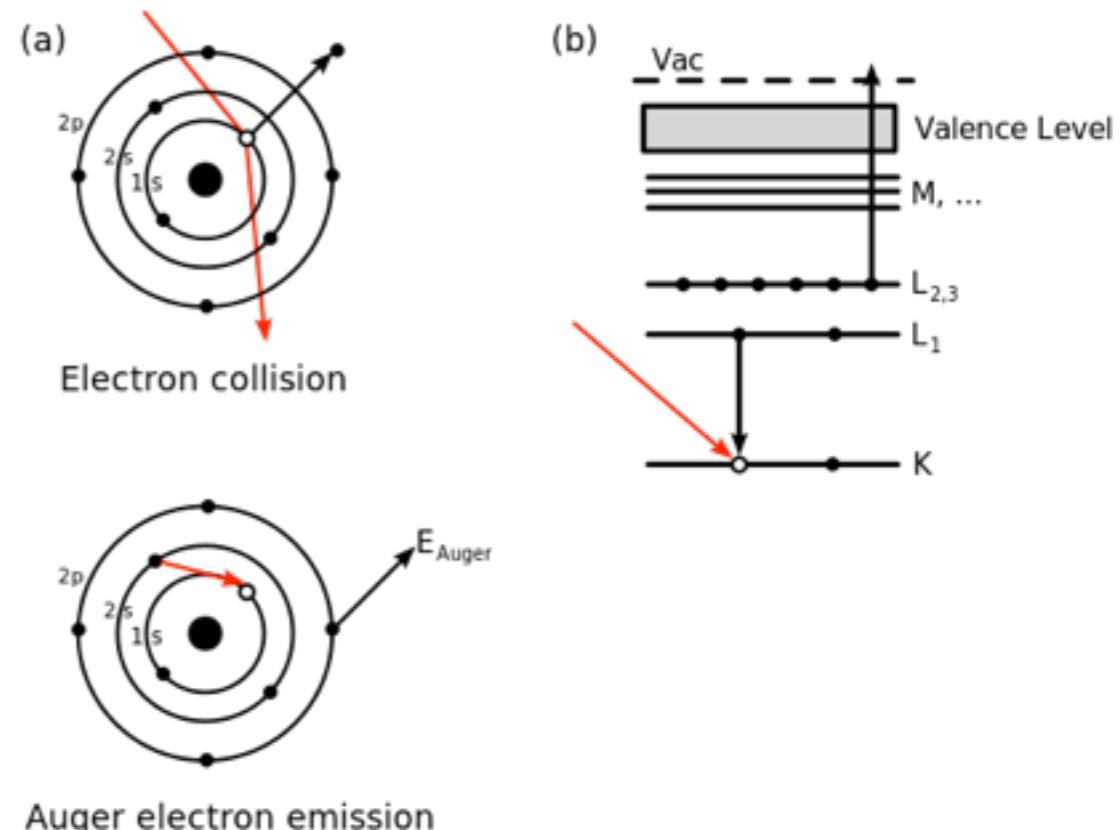
1s electron of C: 284 eV

inelastic scattering

(core) ionization, Auger decay, radiation damage

avoid damage: low temperature (77 K)

outrun damage: diffract & destroy



X-rays

some characteristics

wavelength

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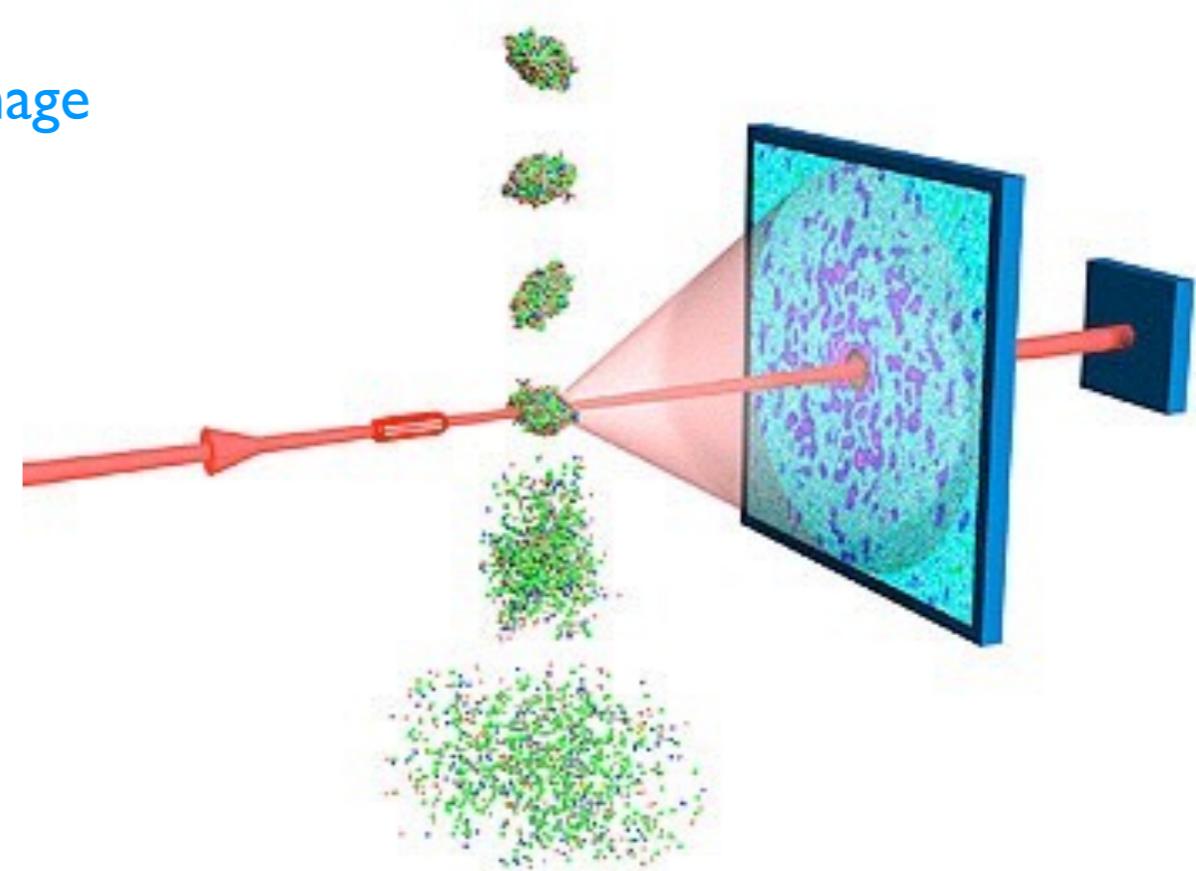
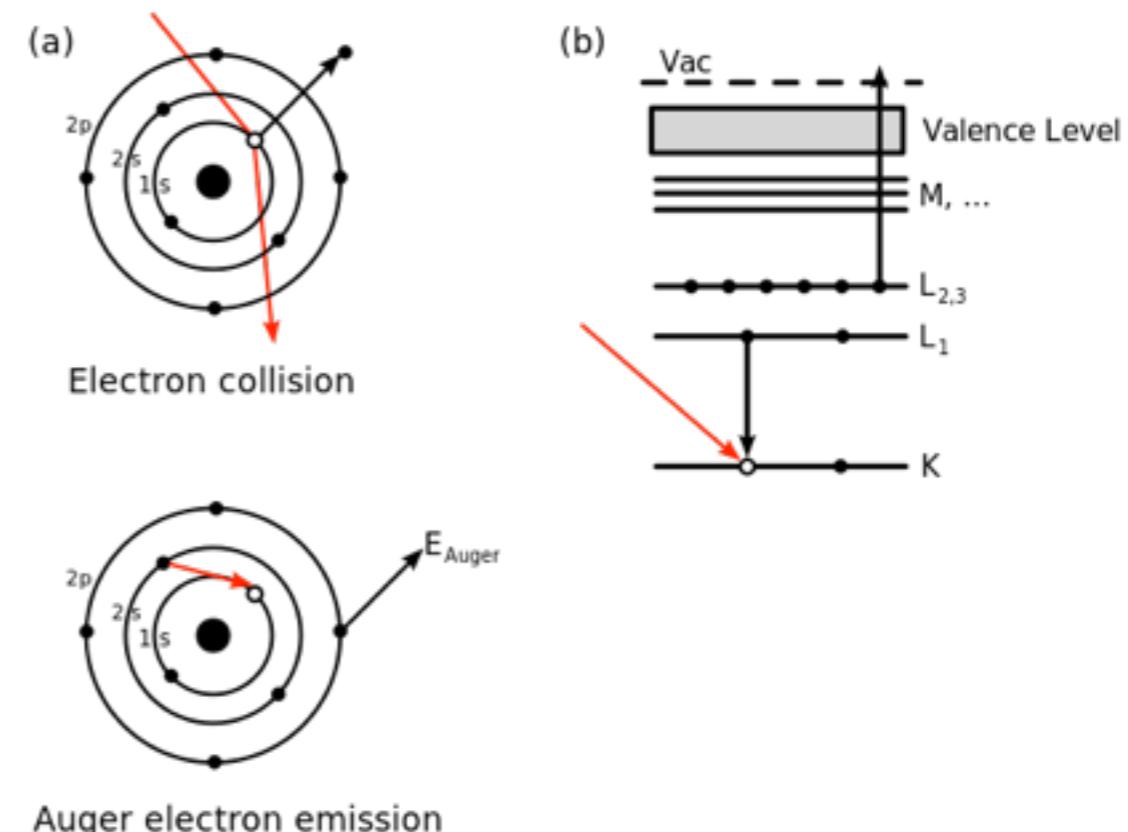
outrun damage: diffract & destroy

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

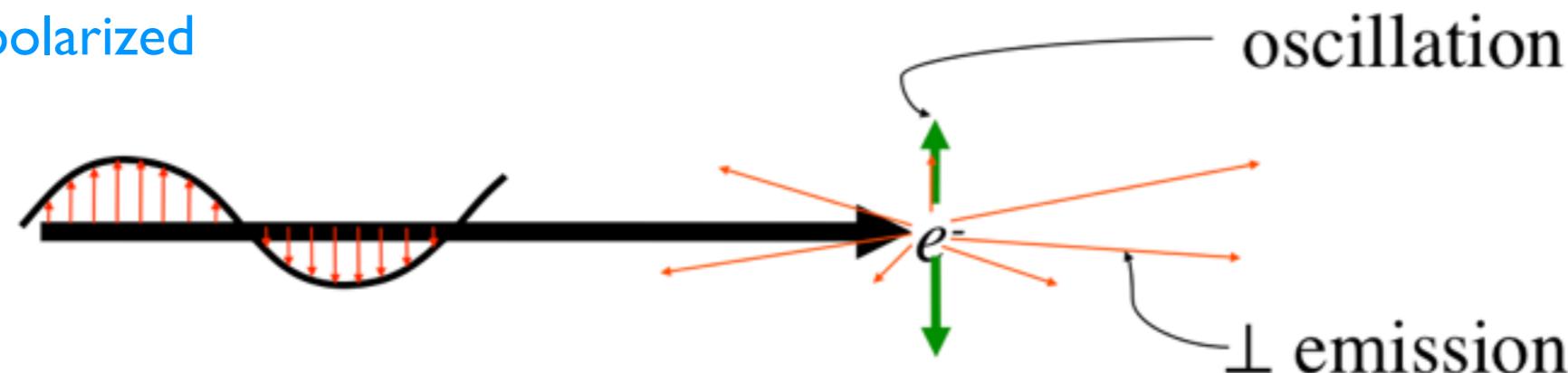
x-rays think electron stands still

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

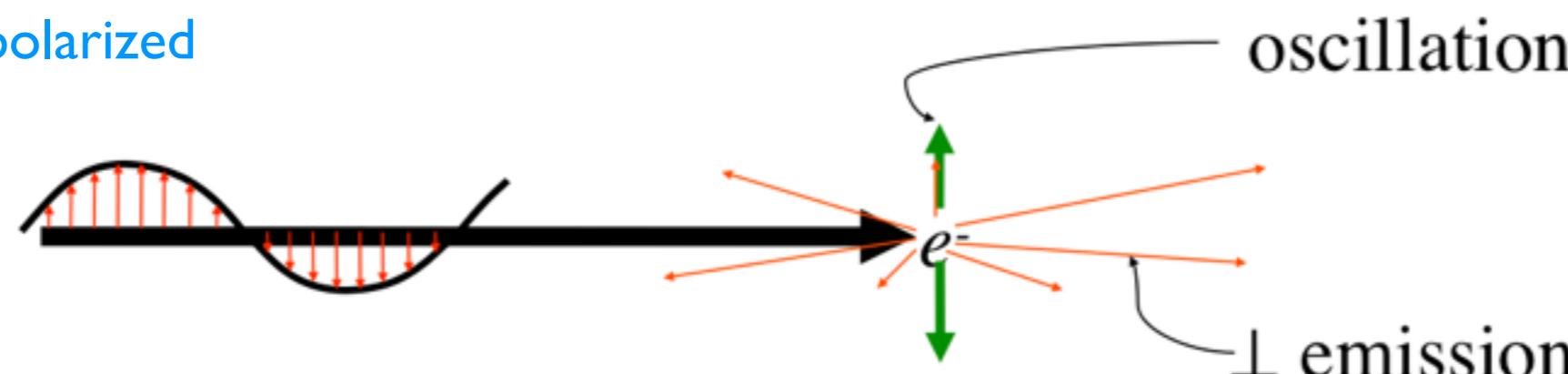
x-rays think electron stands still

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light emission (Maxwell)

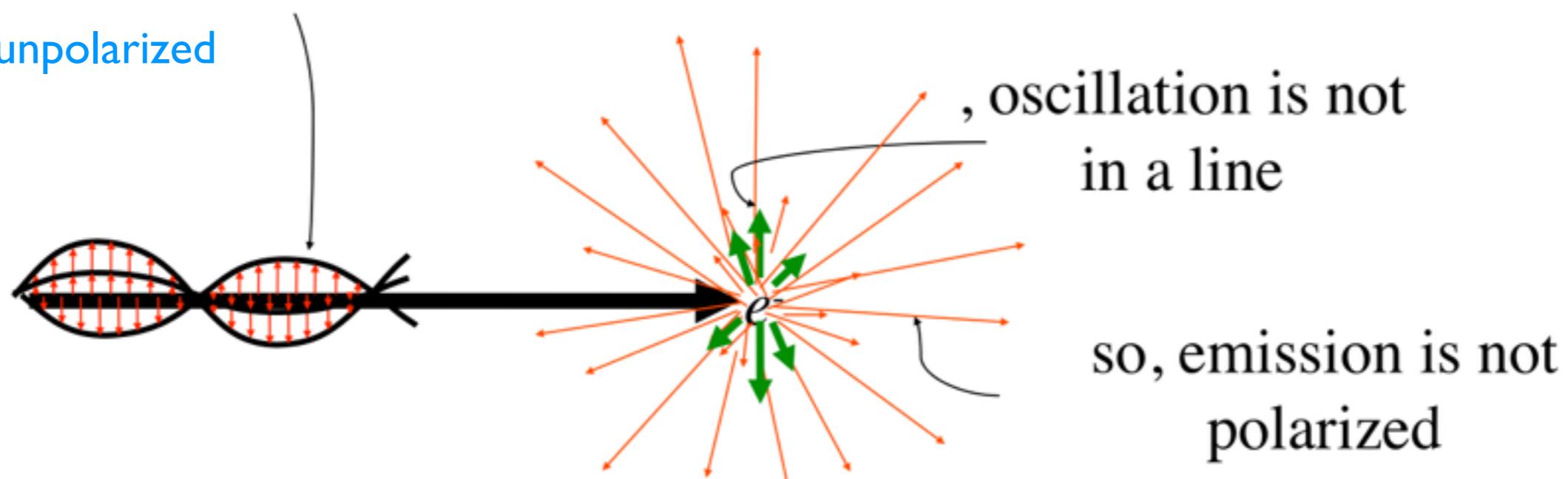
polarized



oscillation

\perp emission

unpolarized



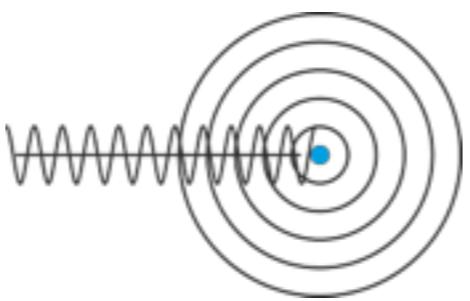
, oscillation is not
in a line

so, emission is not
polarized

scattering with crystals

one electron in the unitcell

vacuum

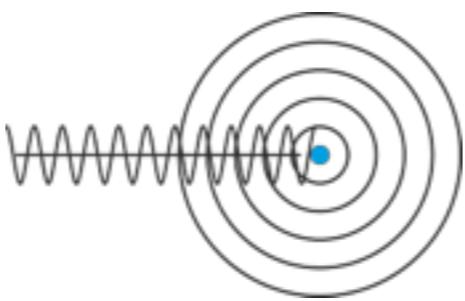


far away detector (not to scale)

scattering with crystals

one electron in the unitcell

vacuum

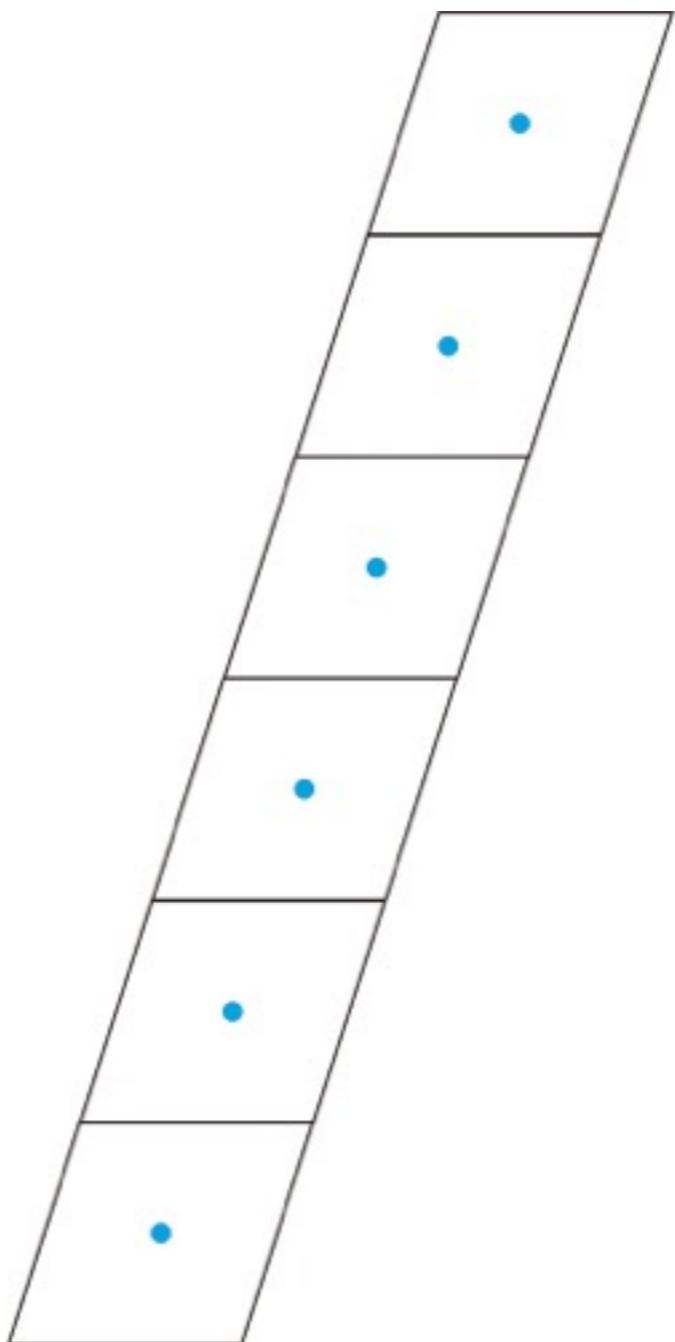


far away detector (not to scale)

scattering with crystals

one electron in the unitcell

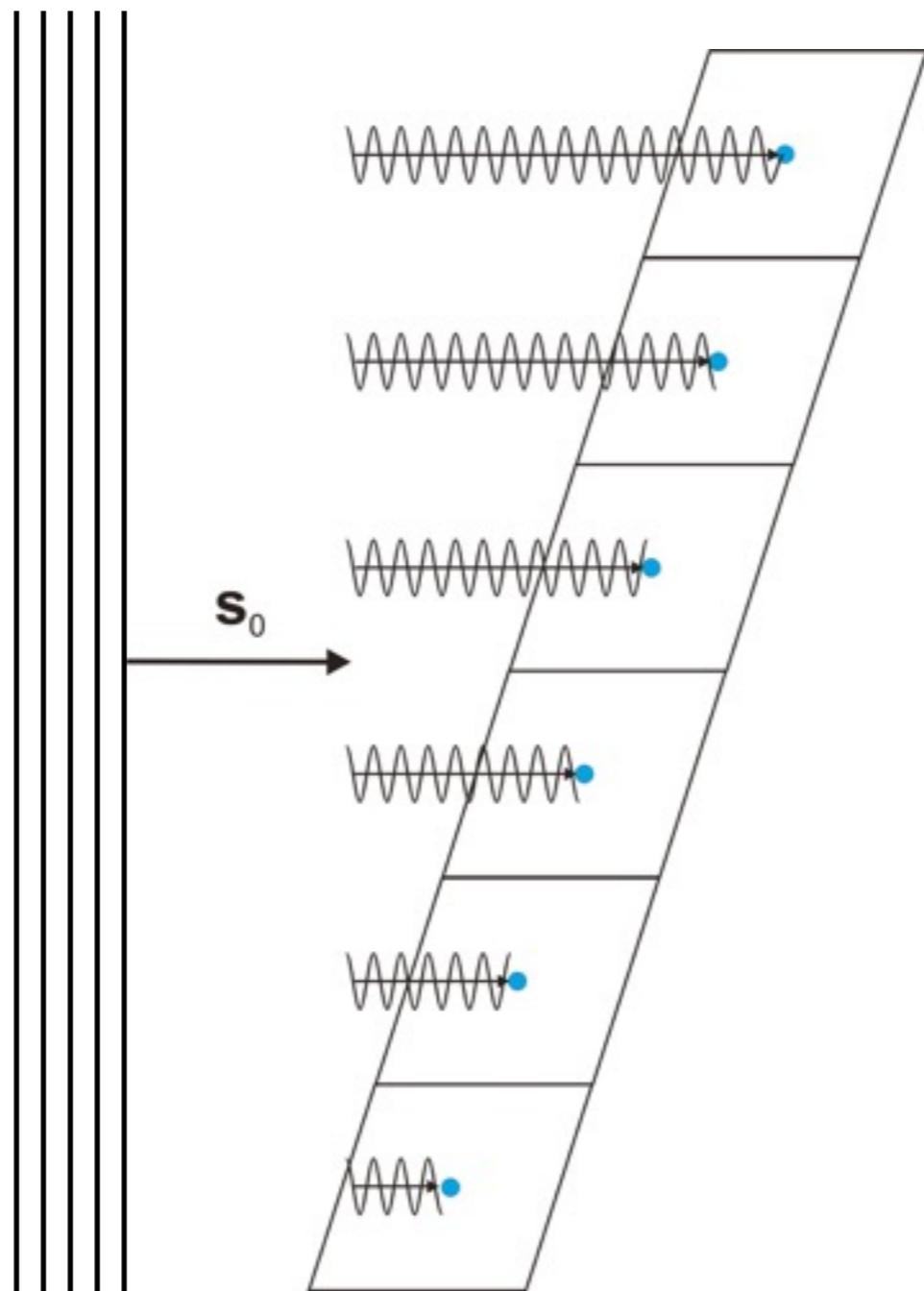
crystal (i.e lattice of unitcells)



scattering with crystals

one electron in the unitcell

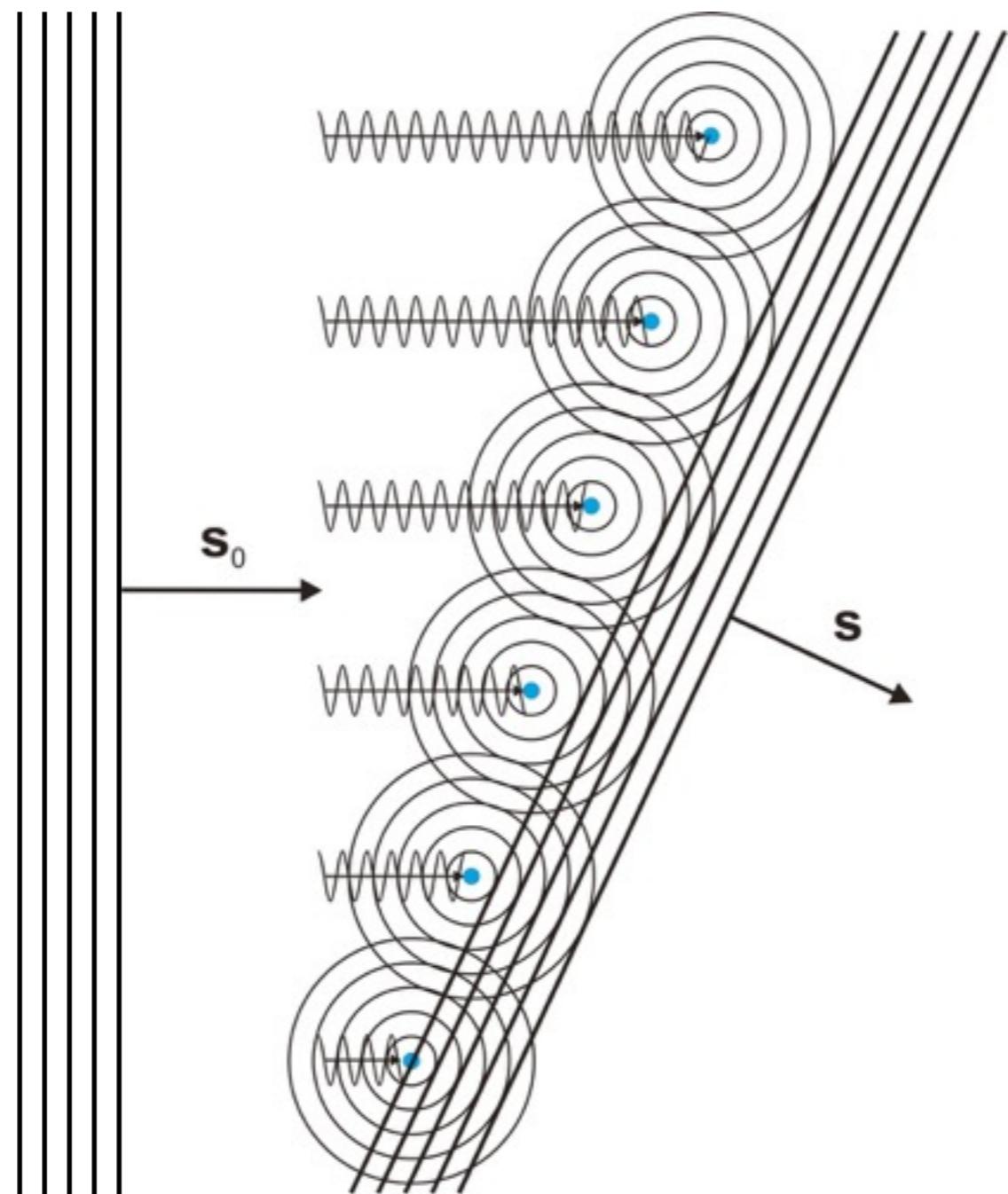
crystal (i.e lattice of unitcells)



scattering with crystals

one electron in the unitcell

crystal (i.e lattice of unitcells)

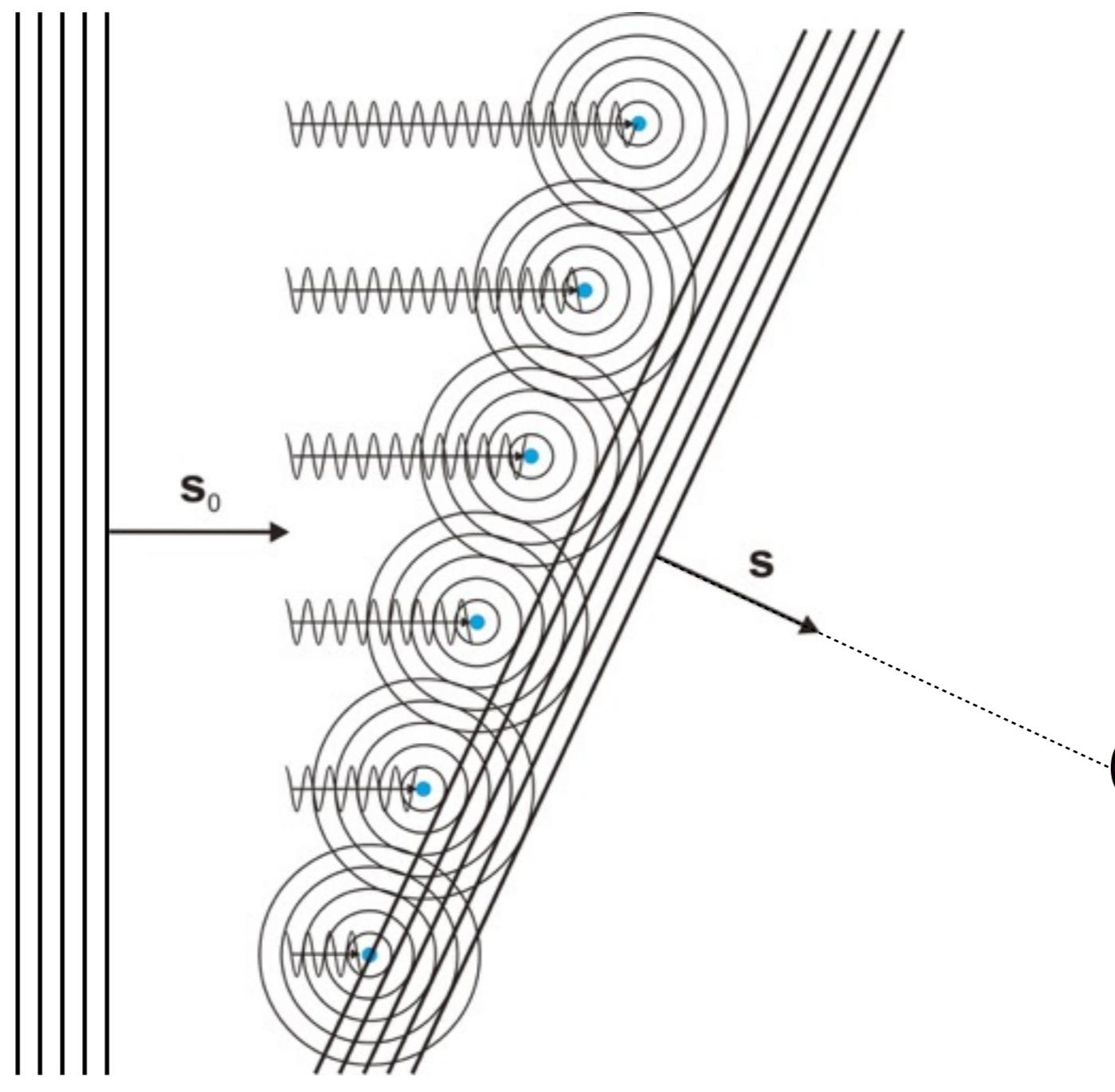


$$|\mathbf{s}_0| = |\mathbf{s}| = \frac{1}{\lambda}$$

scattering with crystals

one electron in the unitcell

crystal (i.e lattice of unitcells)



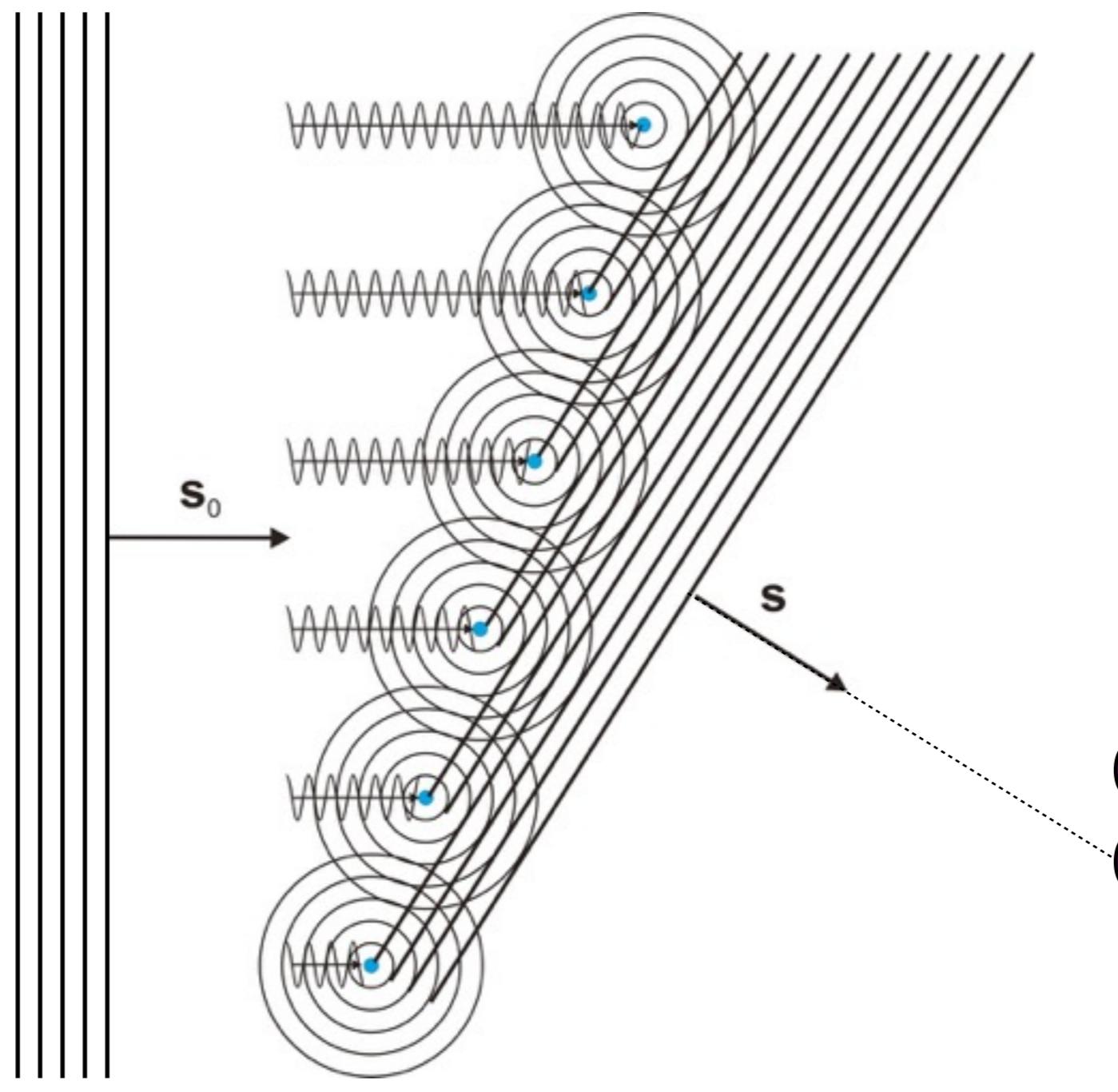
far away detector (not to scale)

$$|\mathbf{s}_0| = |\mathbf{s}| = \frac{1}{\lambda}$$

scattering with crystals

one electron in the unitcell

crystal (i.e lattice of unitcells)



far away detector (not to scale)

$$|\mathbf{s}_0| = |\mathbf{s}| = \frac{1}{\lambda}$$

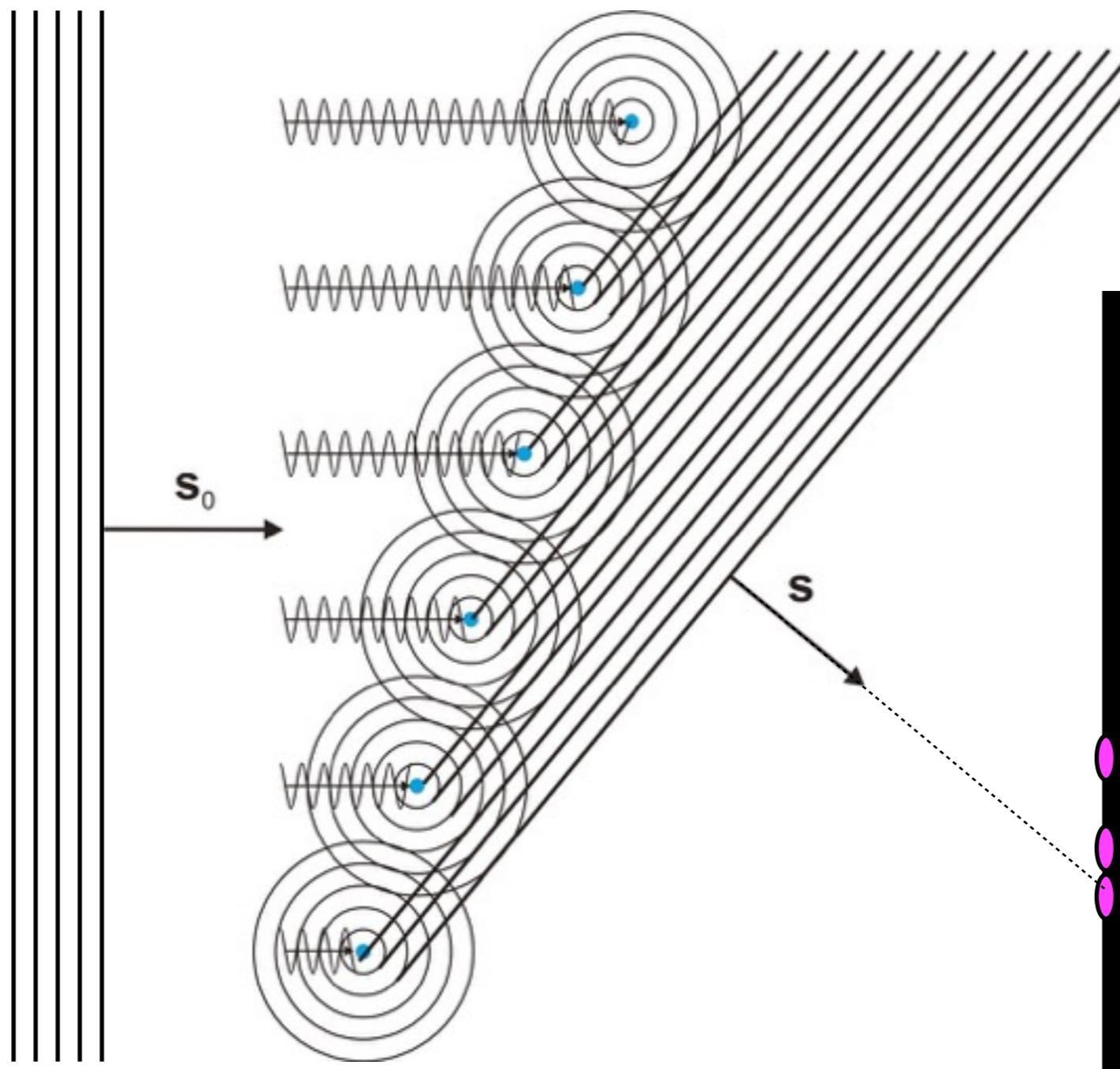
scattering with crystals

one electron in the unitcell

crystal (i.e lattice of unitcells)

discrete scattering spots: diffraction

reflections



$$|\mathbf{s}_0| = |\mathbf{s}| = \frac{1}{\lambda}$$

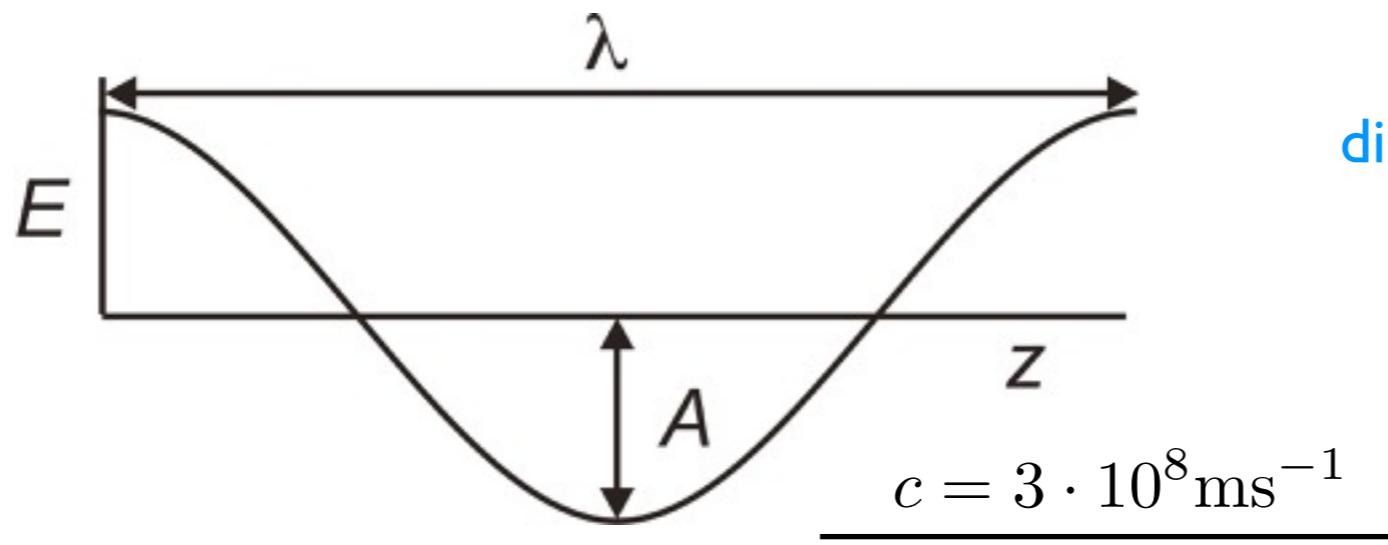
far away detector (not to scale)

addition of waves

electromagnetic wave

addition of waves

~~electromagnetic~~ wave



distance travelled by wave

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

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

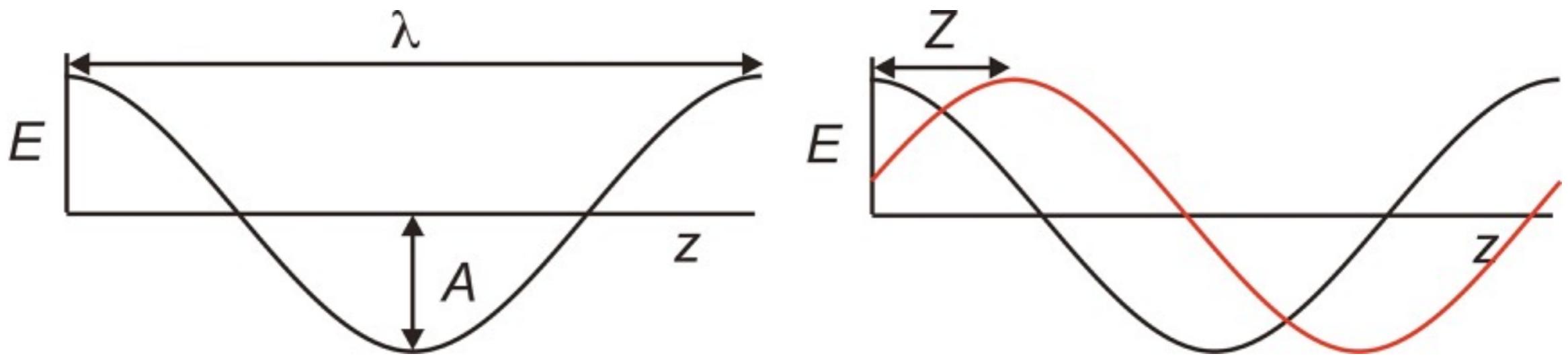
$$E(t; z) = A \cos(2\pi \frac{1}{\lambda}(z - t\lambda\nu))$$

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

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

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

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

addition of waves

new wave

phase

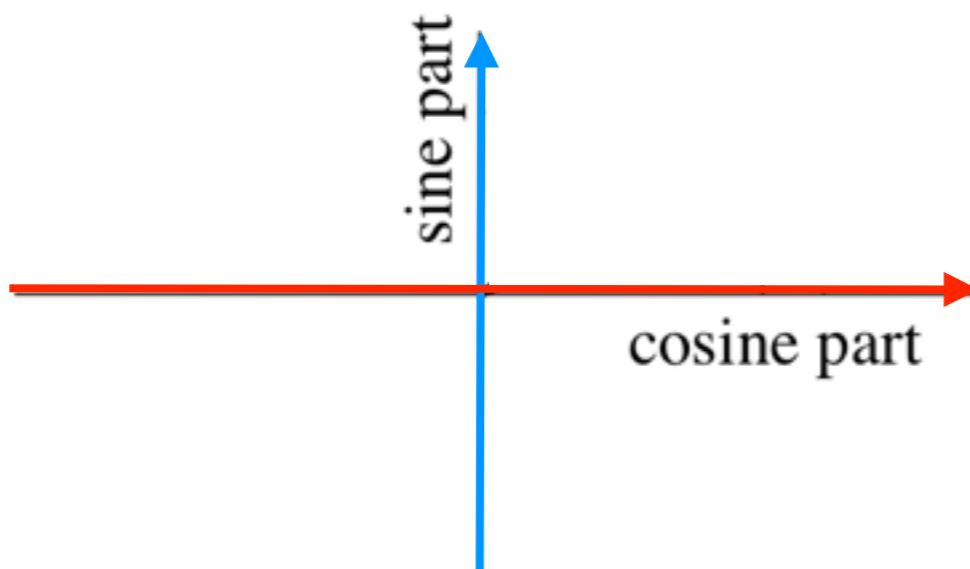
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real imaginary

Argand diagrams

real & imaginary axis

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



addition of waves

new wave

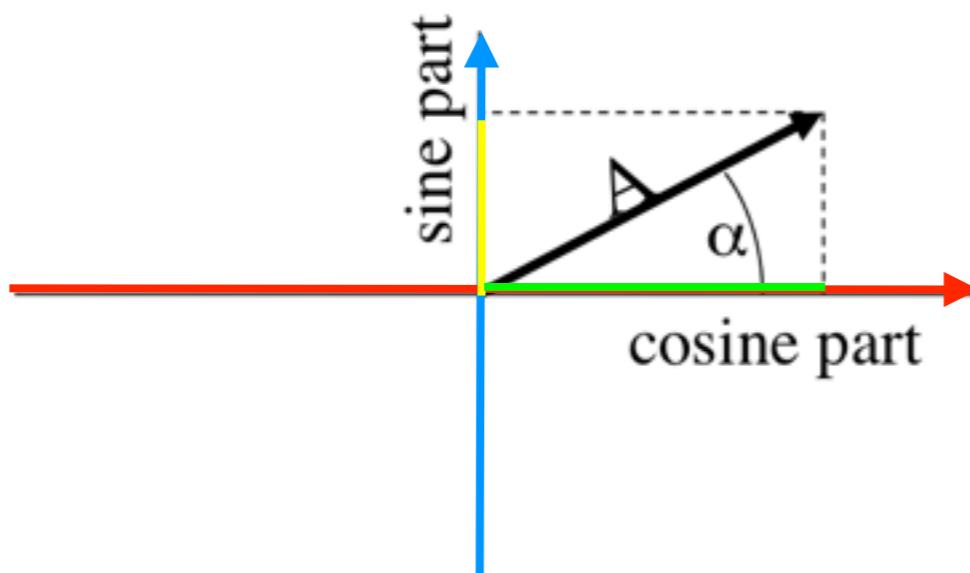
phase

$$\begin{aligned} A \cos(\omega t + \alpha) &= A \cos(\alpha) \cos(\omega t) - A \sin(\alpha) \sin(\omega t) \\ &= \underbrace{A \cos(\alpha) \cos(\omega t)}_{\text{wave 1, real}} + \underbrace{A \sin(\alpha) \cos(\omega t + \pi/2)}_{\text{wave 2, imaginary}} \end{aligned}$$

Argand diagrams

real & imaginary axis

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



addition of waves

new wave

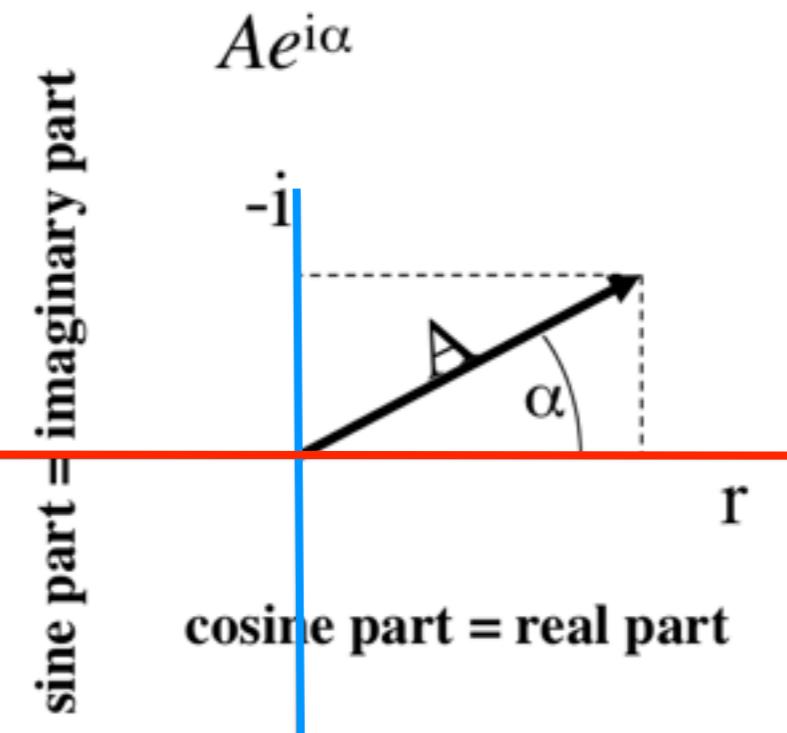
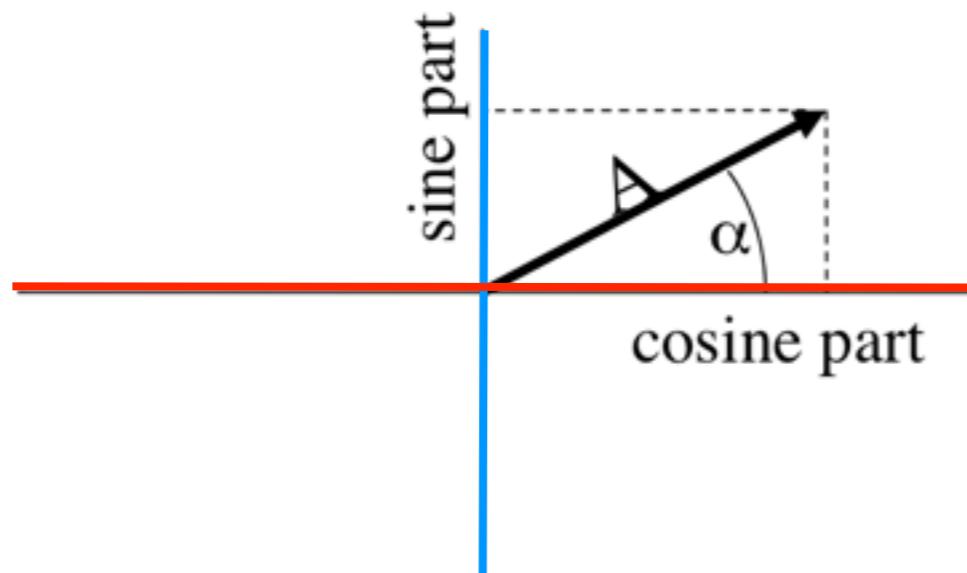
phase

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

Argand diagrams

real & imaginary axis

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



vector addition

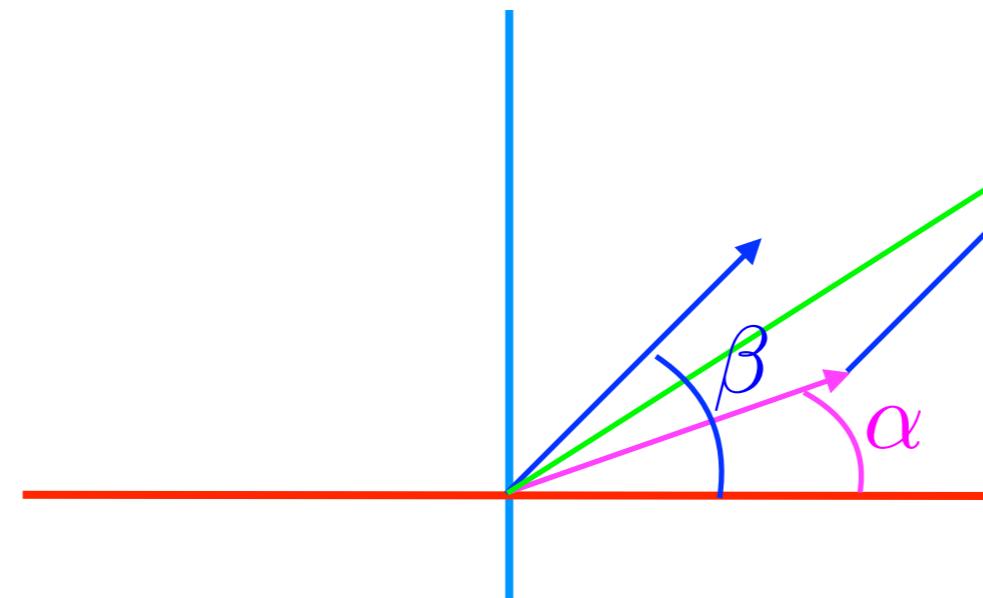
addition of waves with same wave length

vector addition in Argand diagram

example

$$A \cos(\omega t + \alpha) + B \cos(\omega t + \beta)$$

$$[(\underline{A \cos(\alpha)} + \underline{B \cos(\beta)}) + i(\underline{A \sin(\alpha)} + \underline{B \sin(\beta)})] \cos(\omega t)$$



or more conveniently for later:

$$A \cos(\alpha) + i A \sin(\alpha) = A \exp[i\alpha]$$

$$C \cos(\omega t) = (A \exp[i\alpha] + B \exp[i\beta]) \cos(\omega t)$$

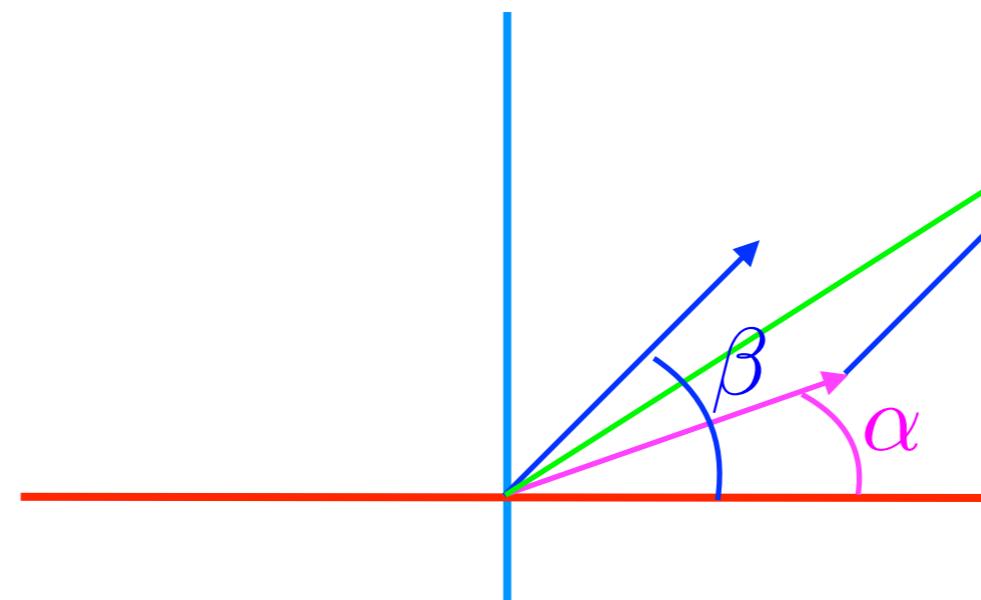
addition of waves with same wave length

vector addition in Argand diagram

example

$$A \cos(\omega t + \alpha) + B \cos(\omega t + \beta)$$

$$[(\underline{A \cos(\alpha)} + \underline{B \cos(\beta)}) + i(\underline{A \sin(\alpha)} + \underline{B \sin(\beta)})] \cos(\omega t)$$

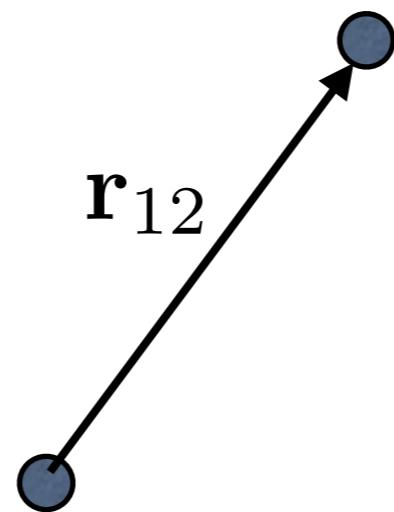


or more conveniently for later:

$$A \cos(\alpha) + iA \sin(\alpha) = A \exp[i\alpha]$$

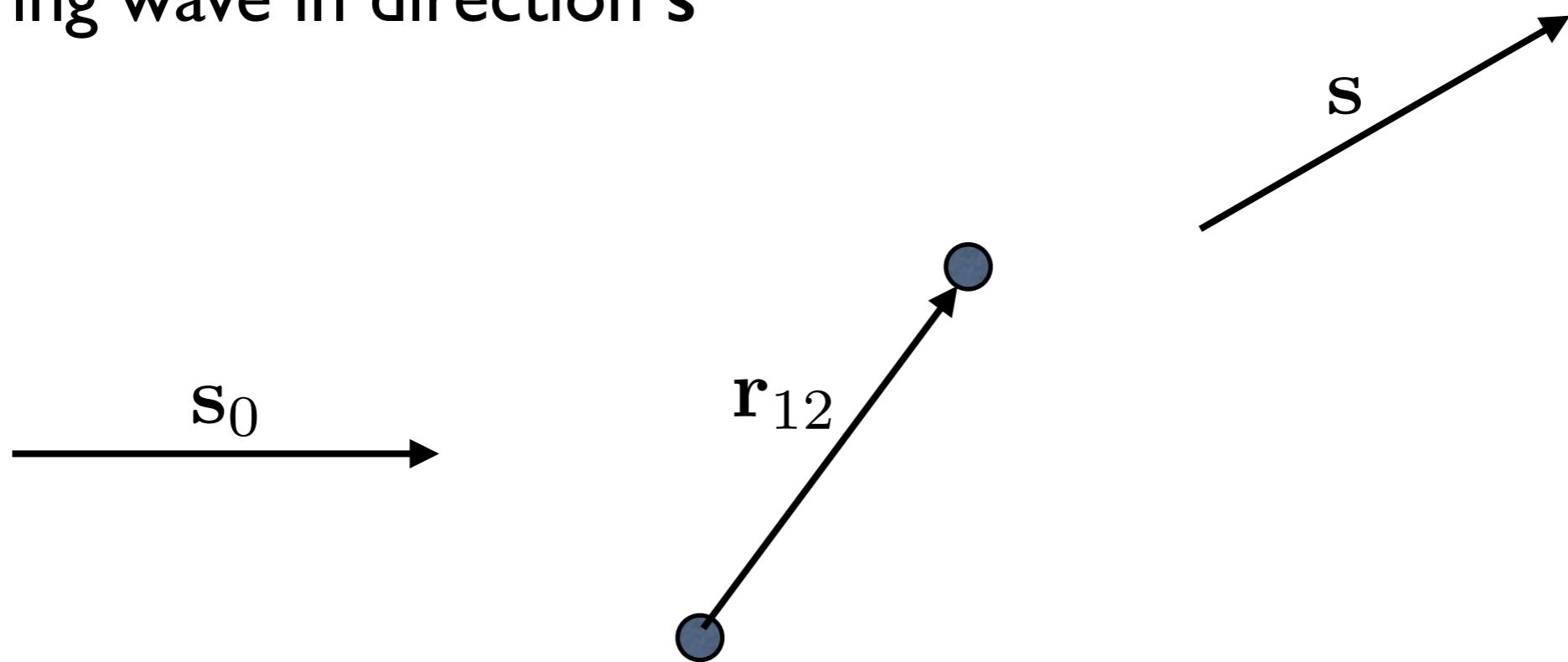
$$A^{\text{tot}} = \sum_n^N A_n \exp[i\alpha_n]$$

scattering with two electrons, no periodicity



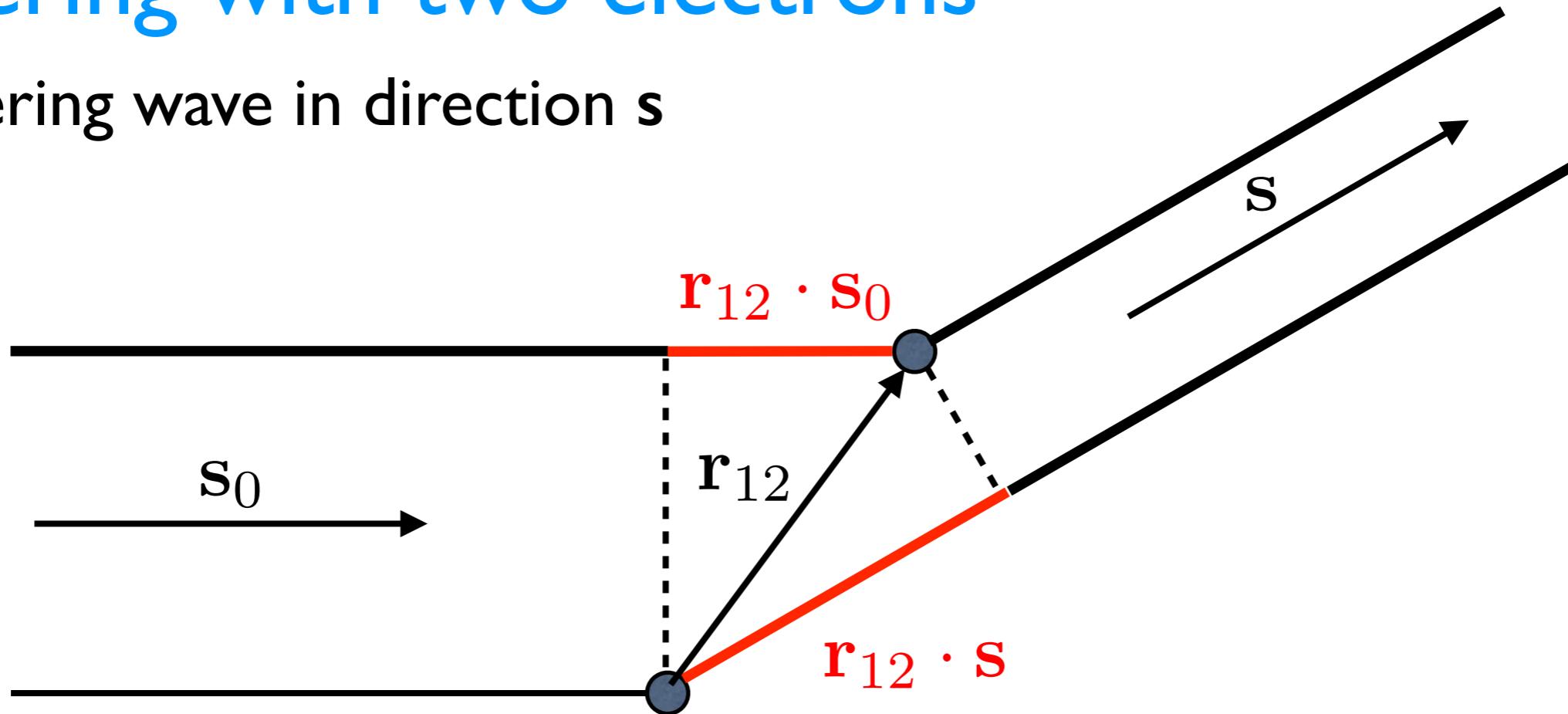
scattering with two electrons

scattering wave in direction s



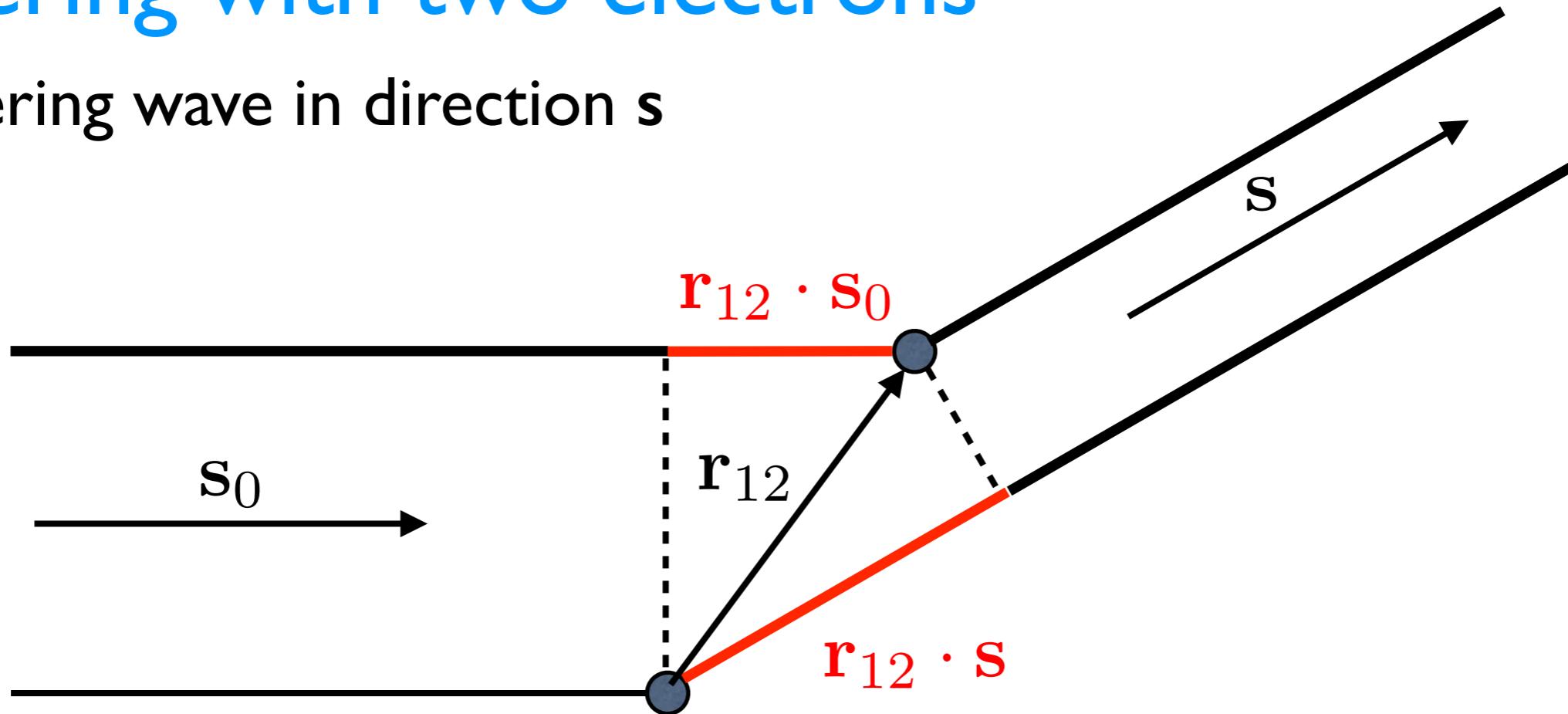
scattering with two electrons

scattering wave in direction s



scattering with two electrons

scattering wave in direction s

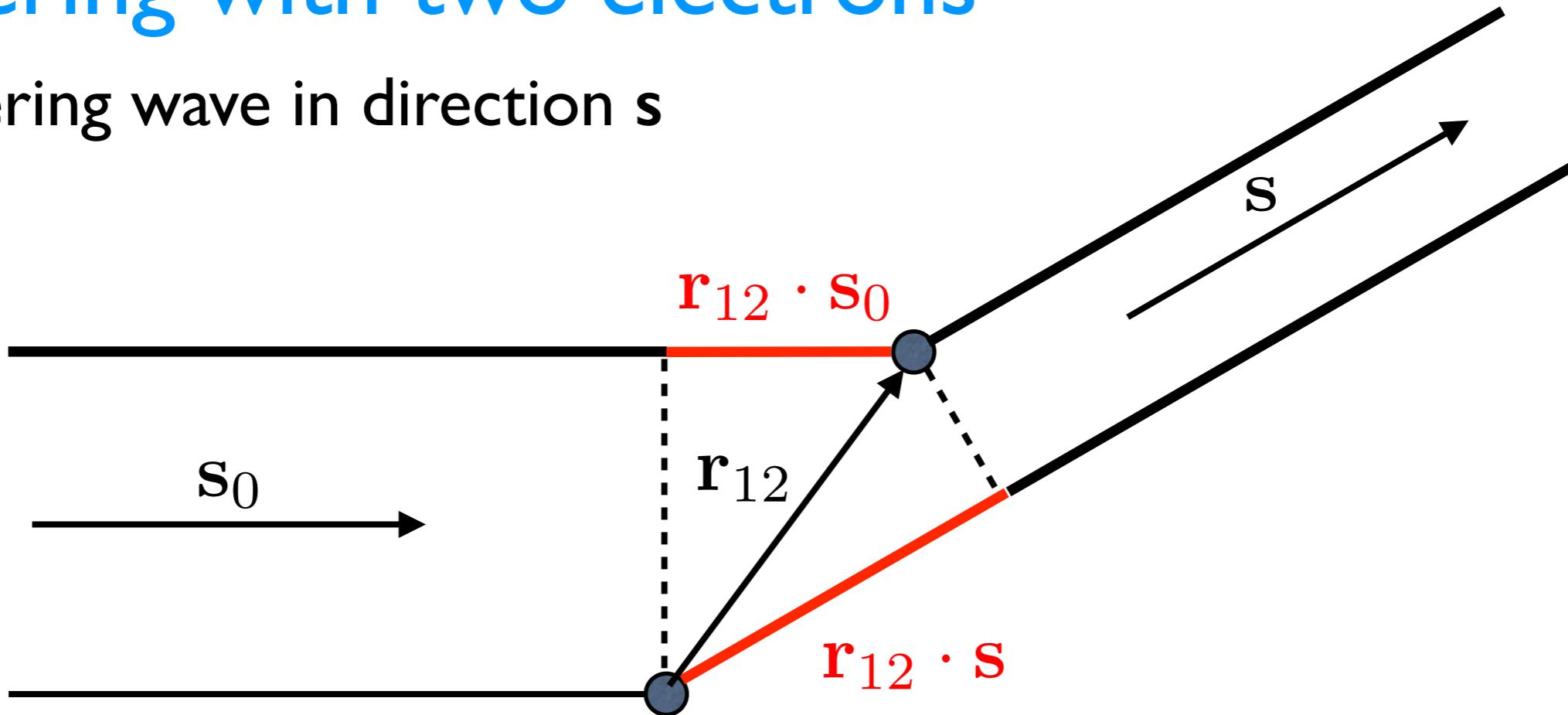


path length difference

$$\Delta = r_{12} \cdot s - r_{12} \cdot s_0 = r_{12} \cdot (s - s_0)$$

scattering with two electrons

scattering wave in direction s



path length difference

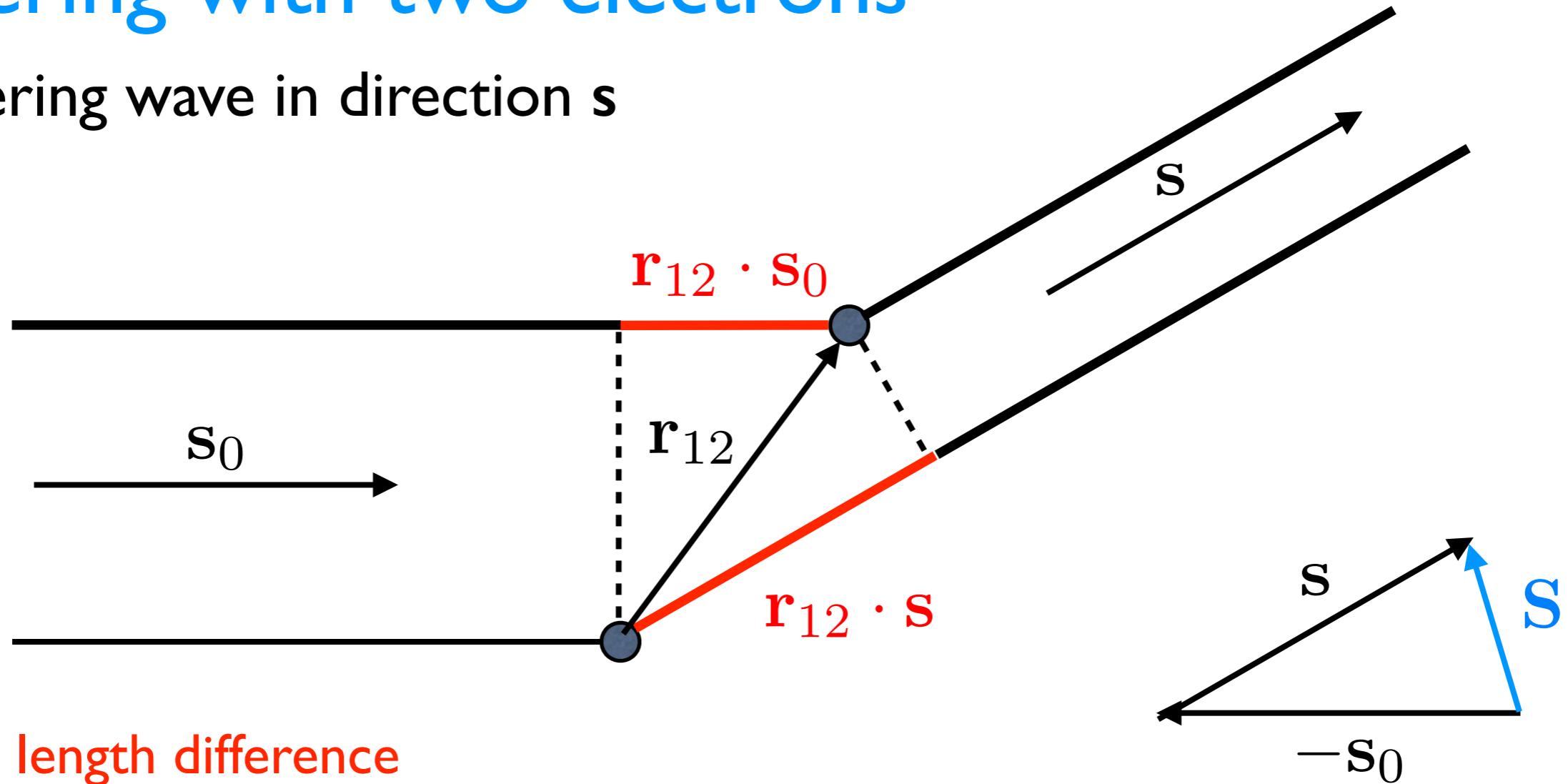
$$\Delta = \mathbf{r}_{12} \cdot \mathbf{s} - \mathbf{r}_{12} \cdot \mathbf{s}_0 = \mathbf{r}_{12} \cdot (\mathbf{s} - \mathbf{s}_0)$$

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 s



path length difference

$$\Delta = r_{12} \cdot s - r_{12} \cdot s_0 = r_{12} \cdot (s - s_0)$$

phase difference

$$\alpha = \frac{2\pi}{\lambda} (r_{12} \cdot s - r_{12} \cdot s_0) = 2\pi r_{12} \cdot S$$

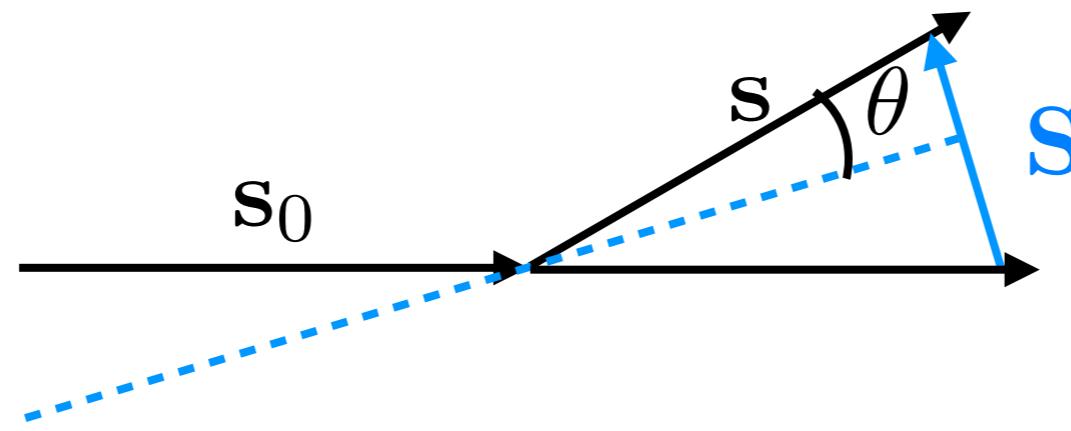
scattering vector
 $S \equiv (s - s_0)/\lambda$

reflection plane

scattering with two electrons

“reflecting” plane

imaginary, but useful later

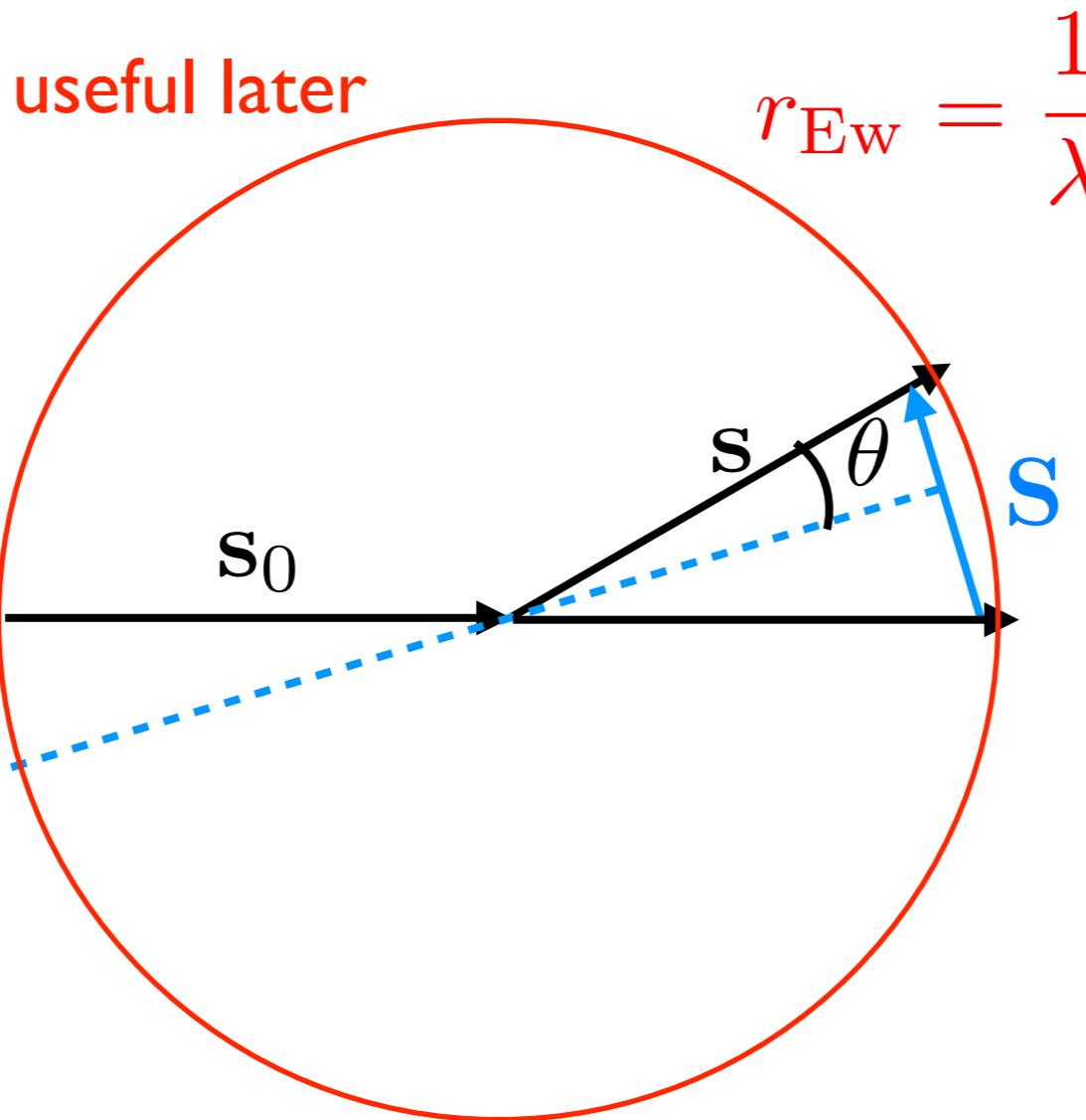


$$|\mathbf{S}| = \frac{2 \sin(\theta)}{\lambda}$$

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

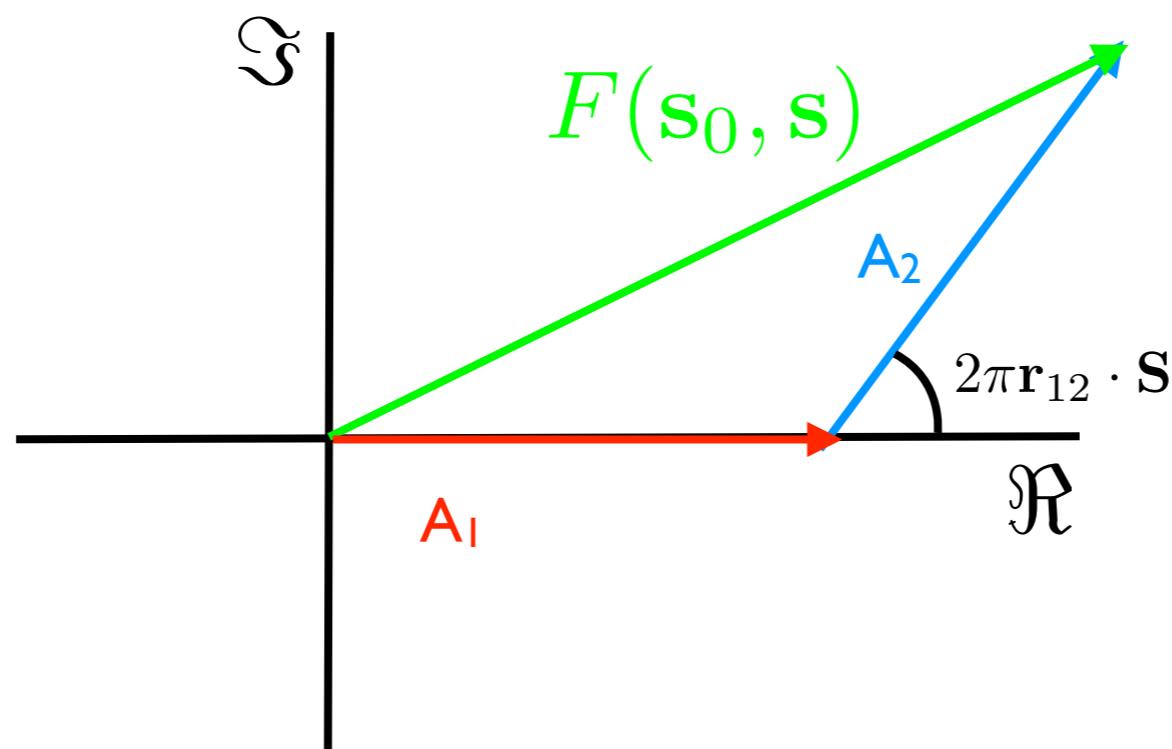
$$\alpha = 2\pi \mathbf{r}_{12} \cdot \mathbf{S}$$

scattered wave in direction S

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

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

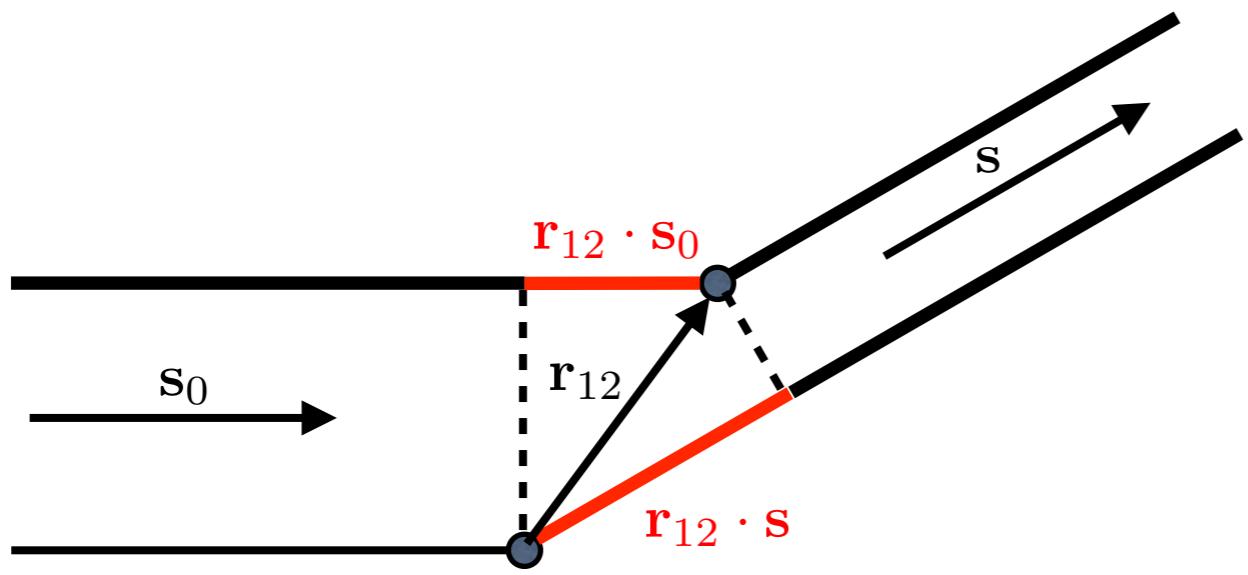
Argand diagram



scattering with two electrons

scattering wave in direction s

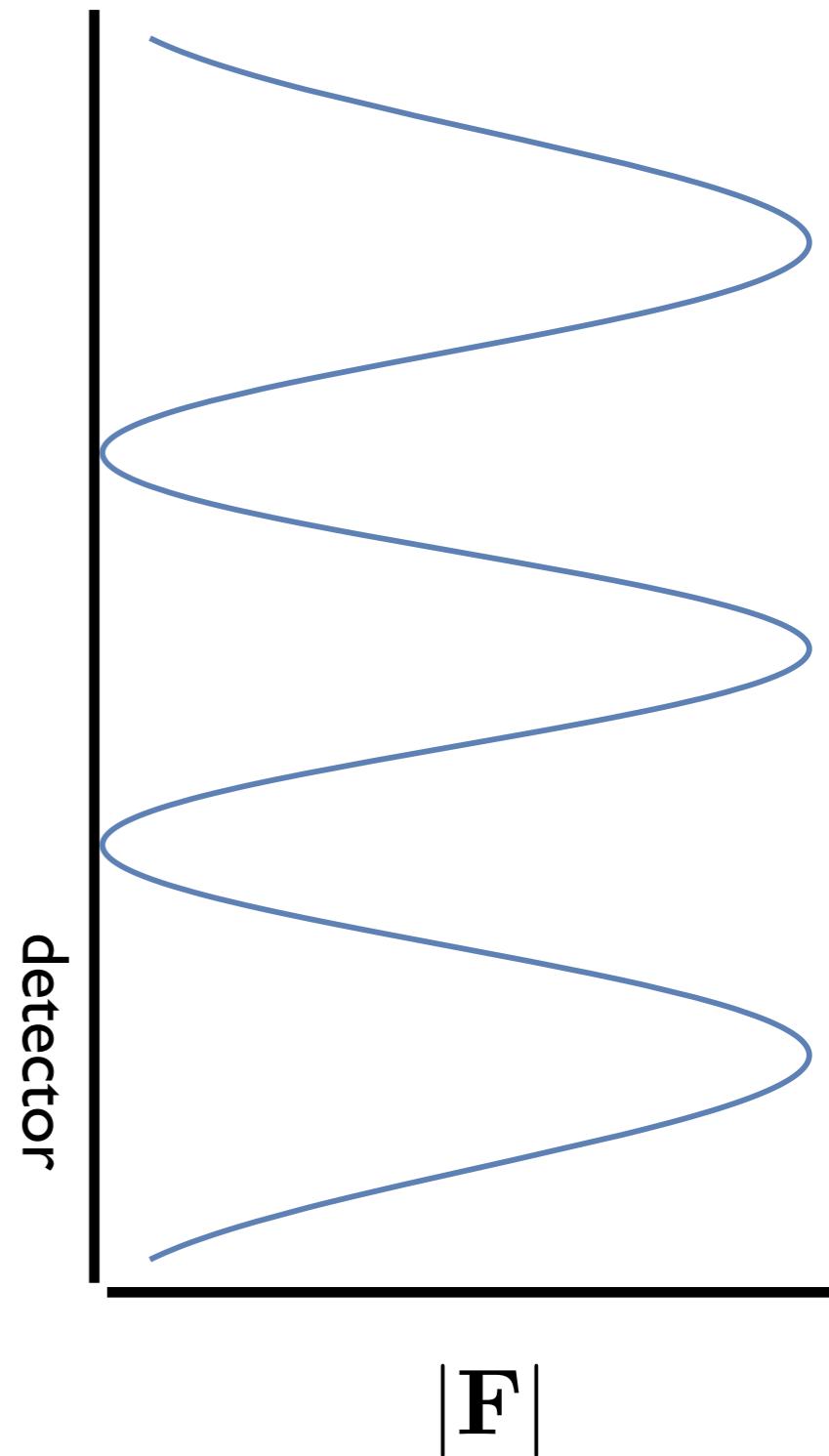
$$F(s_0, s) = A_1 + A_2 \exp[i\alpha]$$



scattering, no diffraction (yet)

little phase shift still gives signal

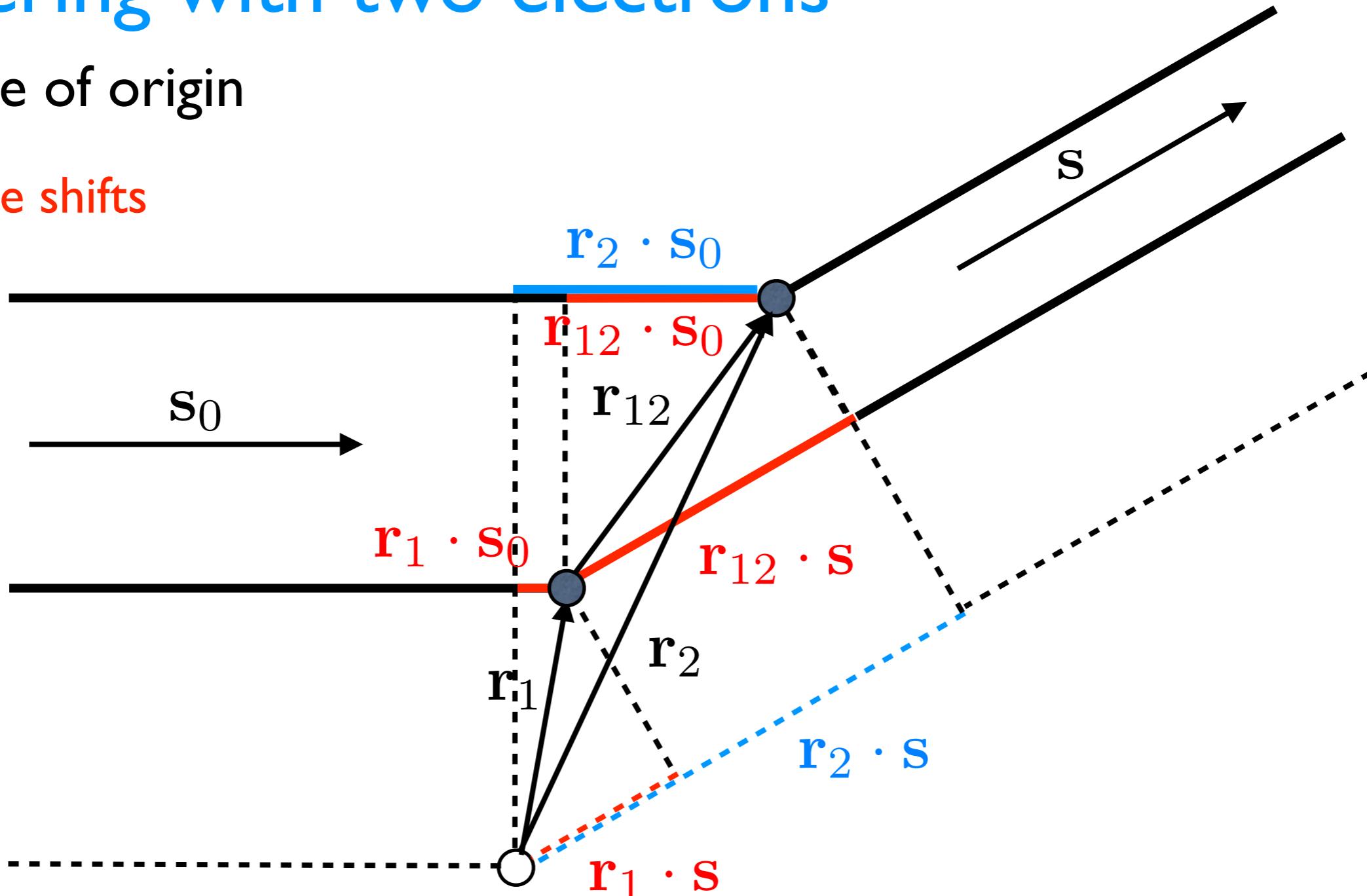
no Bragg peaks, continuous readout



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 many electrons

scattering of unitcell with N electrons

scattering factor

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

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

scattering with many electrons

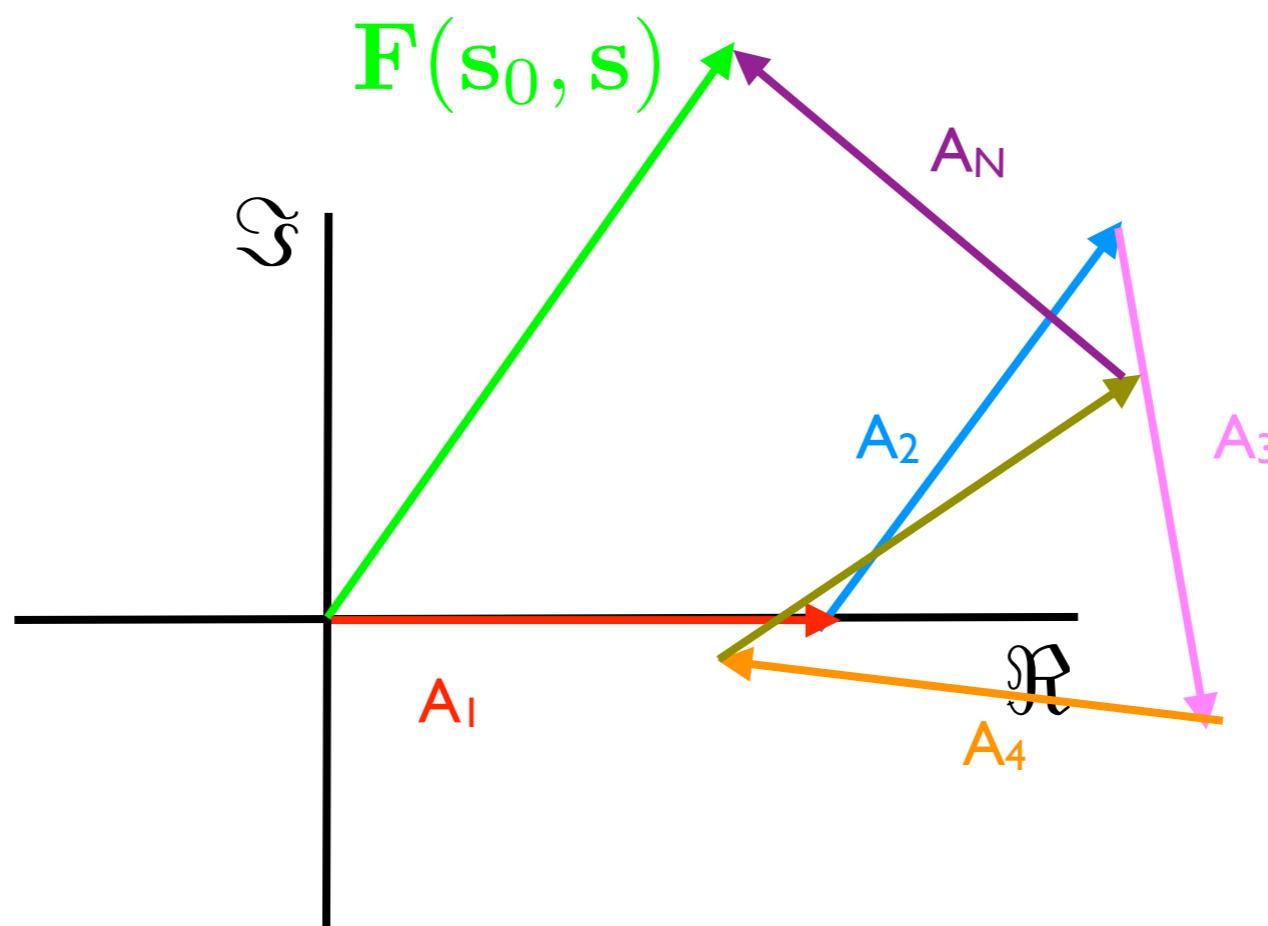
scattering of system with N electrons

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

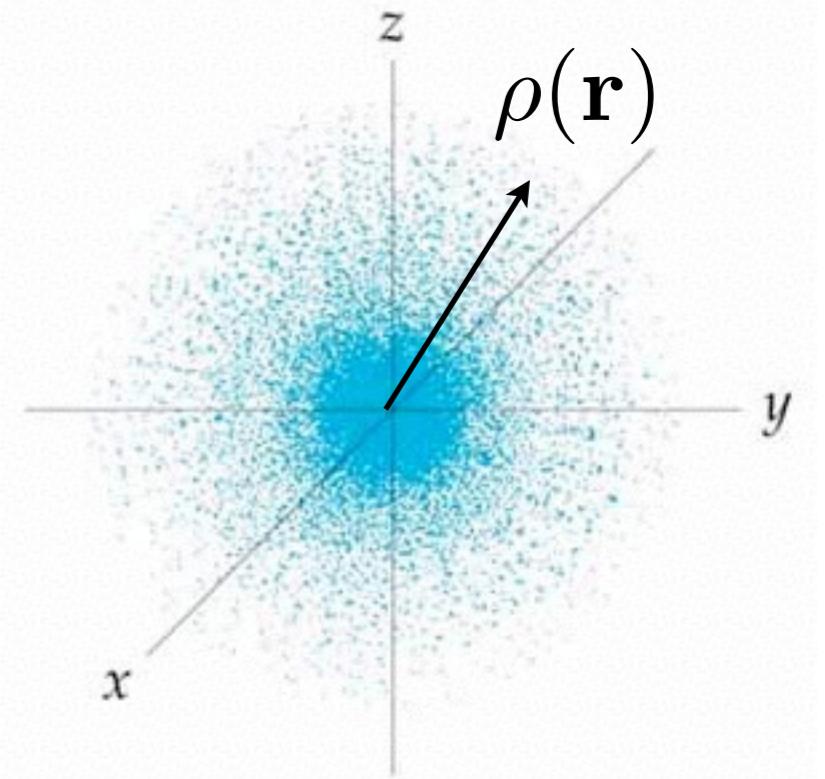


scattering with atoms

scattering of single atom

electrons are ‘free’

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



scattering with atoms

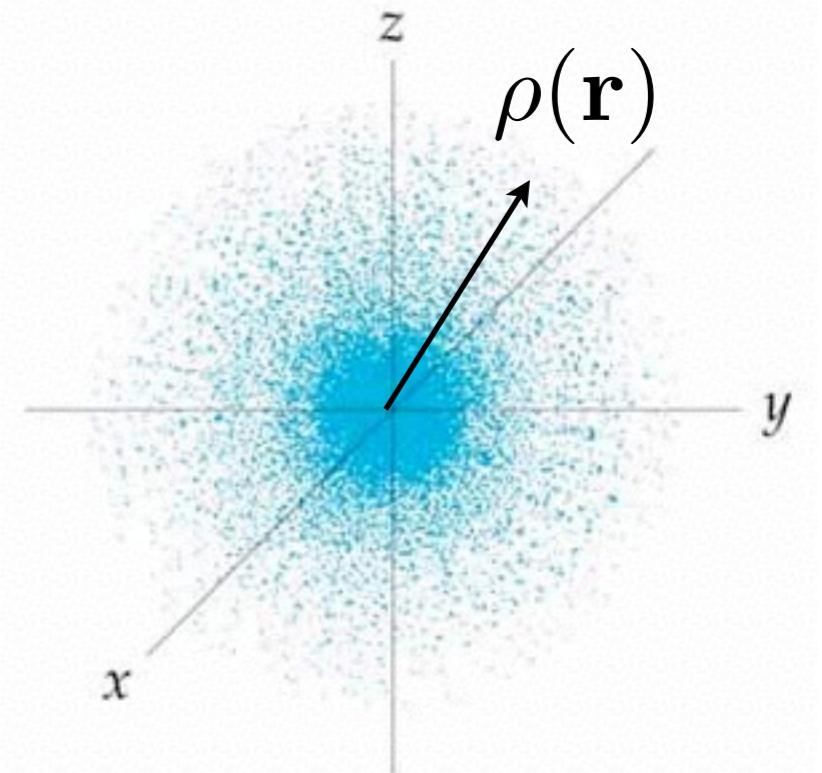
scattering of single atom

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’

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

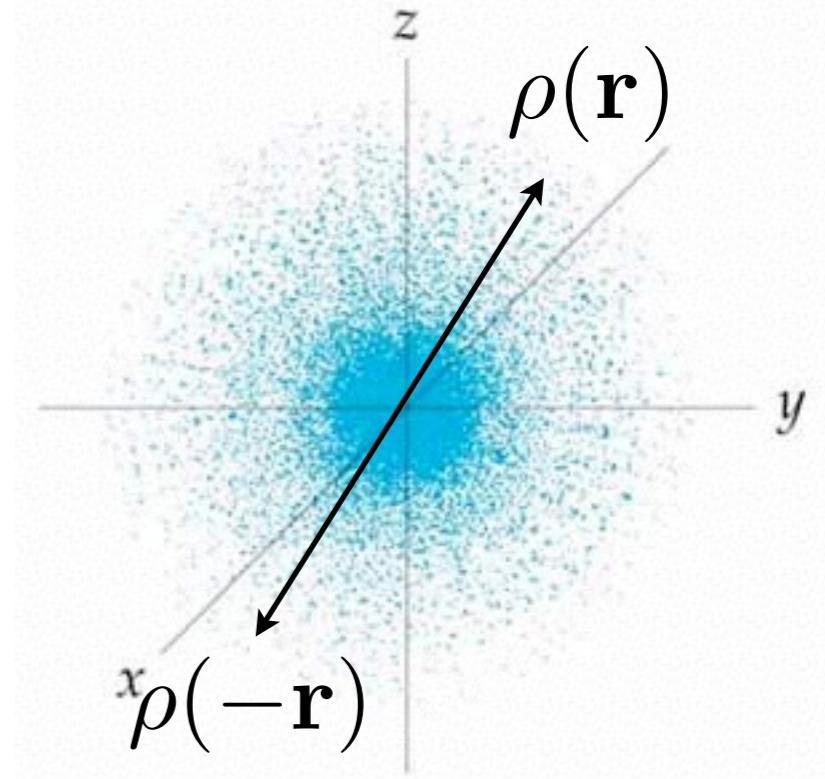
structure factor

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

spherically symmetric electron cloud

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

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



scattering with atoms

scattering of single atom

electron are free electrons

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

structure factor

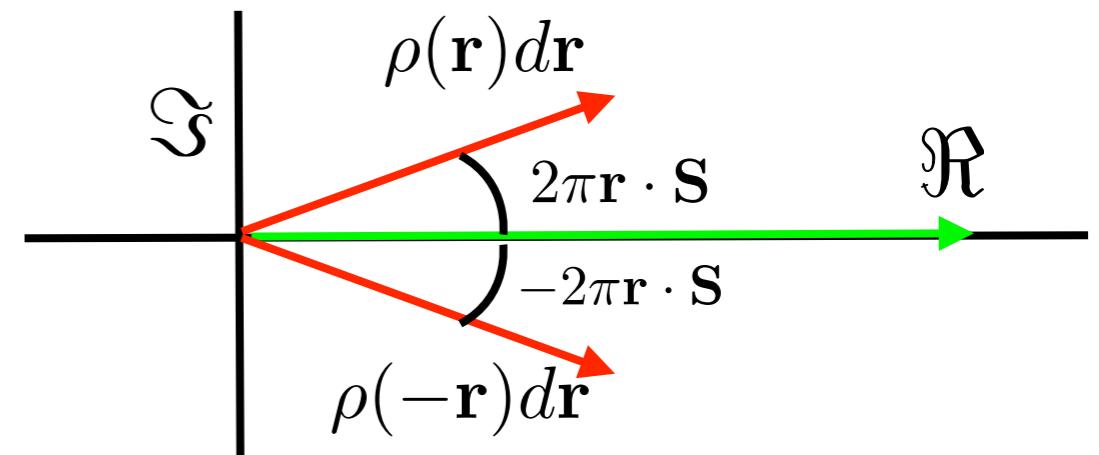
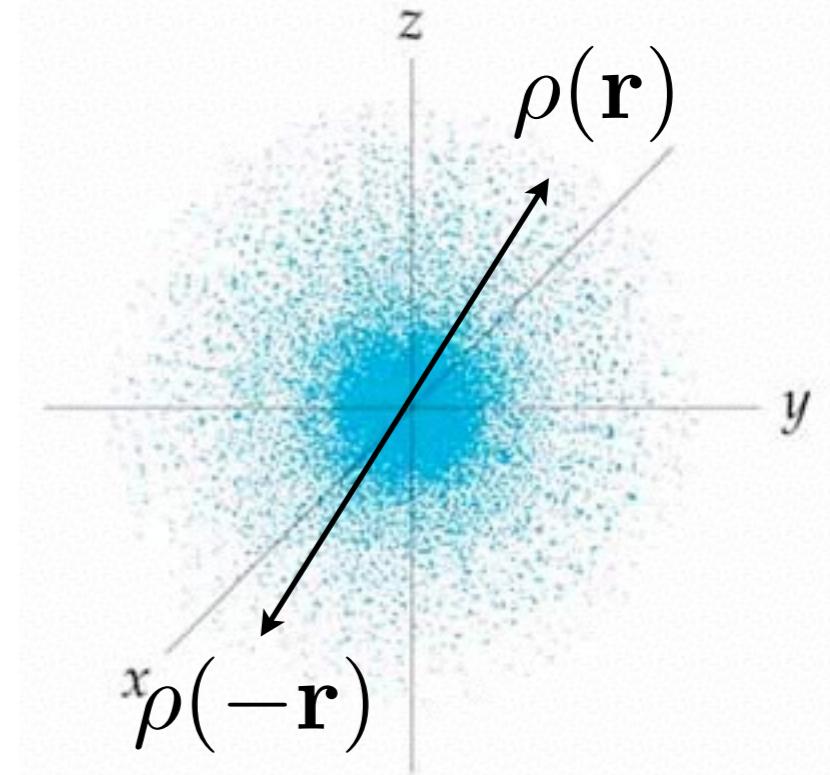
$$f = \int_{-\infty}^{\infty} \rho(\mathbf{r}) \exp[2\pi i \mathbf{r} \cdot \mathbf{S}] d\mathbf{r}$$

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

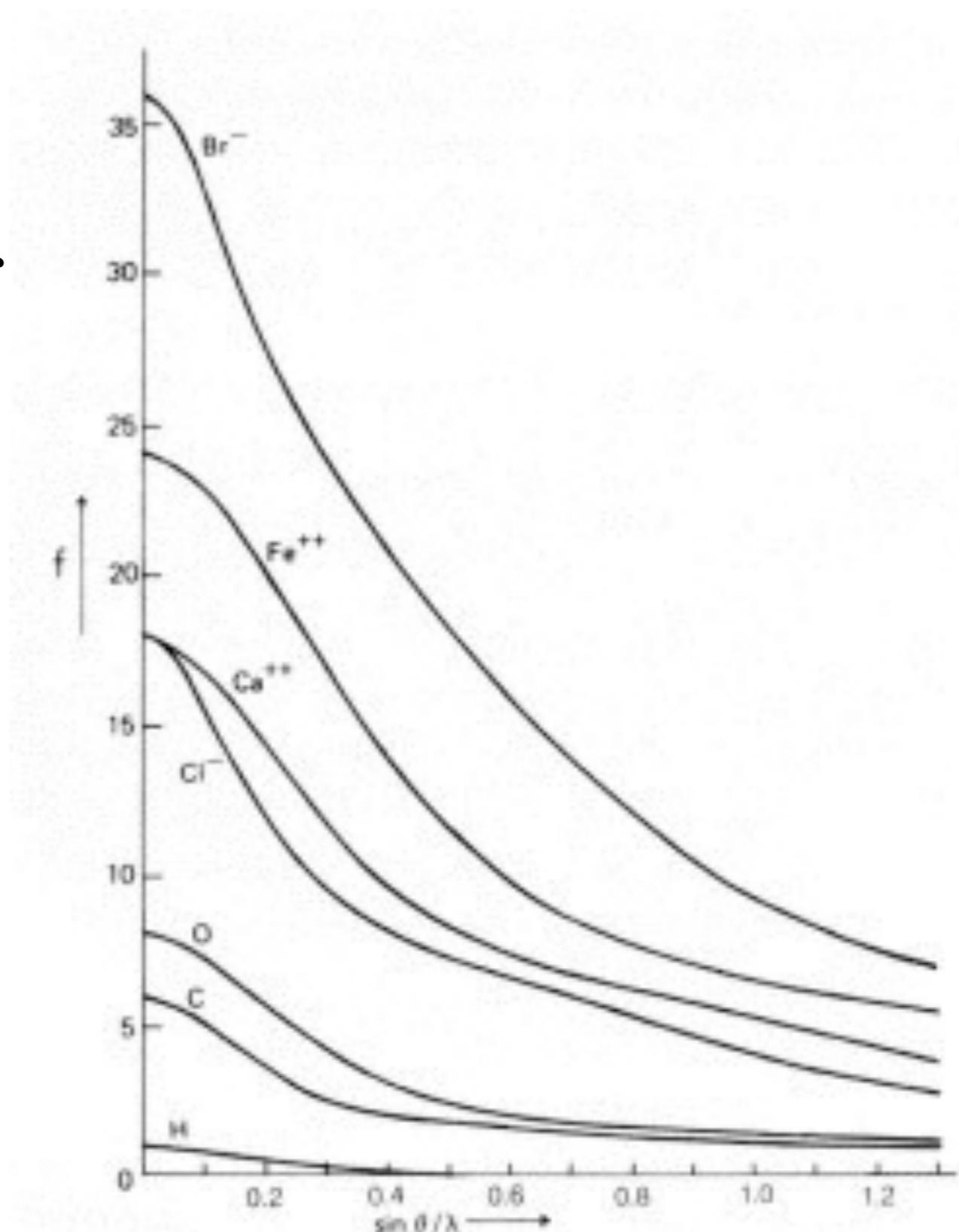
scattering of single atom

structure factor

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

angle dependence

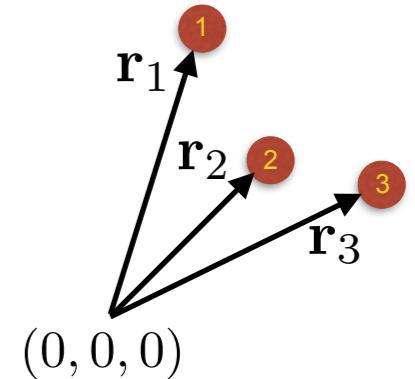
$$|\mathbf{S}| = \frac{2 \sin(\theta)}{\lambda}$$



scattering with single molecules

scattering of atoms in the molecule

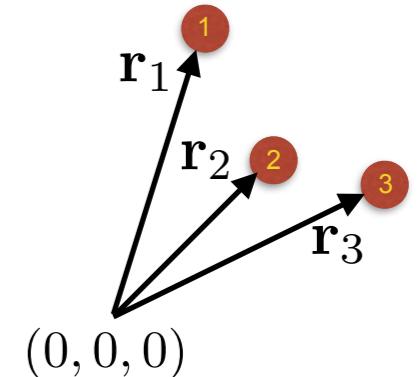
$$\mathbf{F}(\mathbf{S}) = \sum_k^N f_k \exp[2\pi i \mathbf{r}_k \cdot \mathbf{S}]$$



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

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

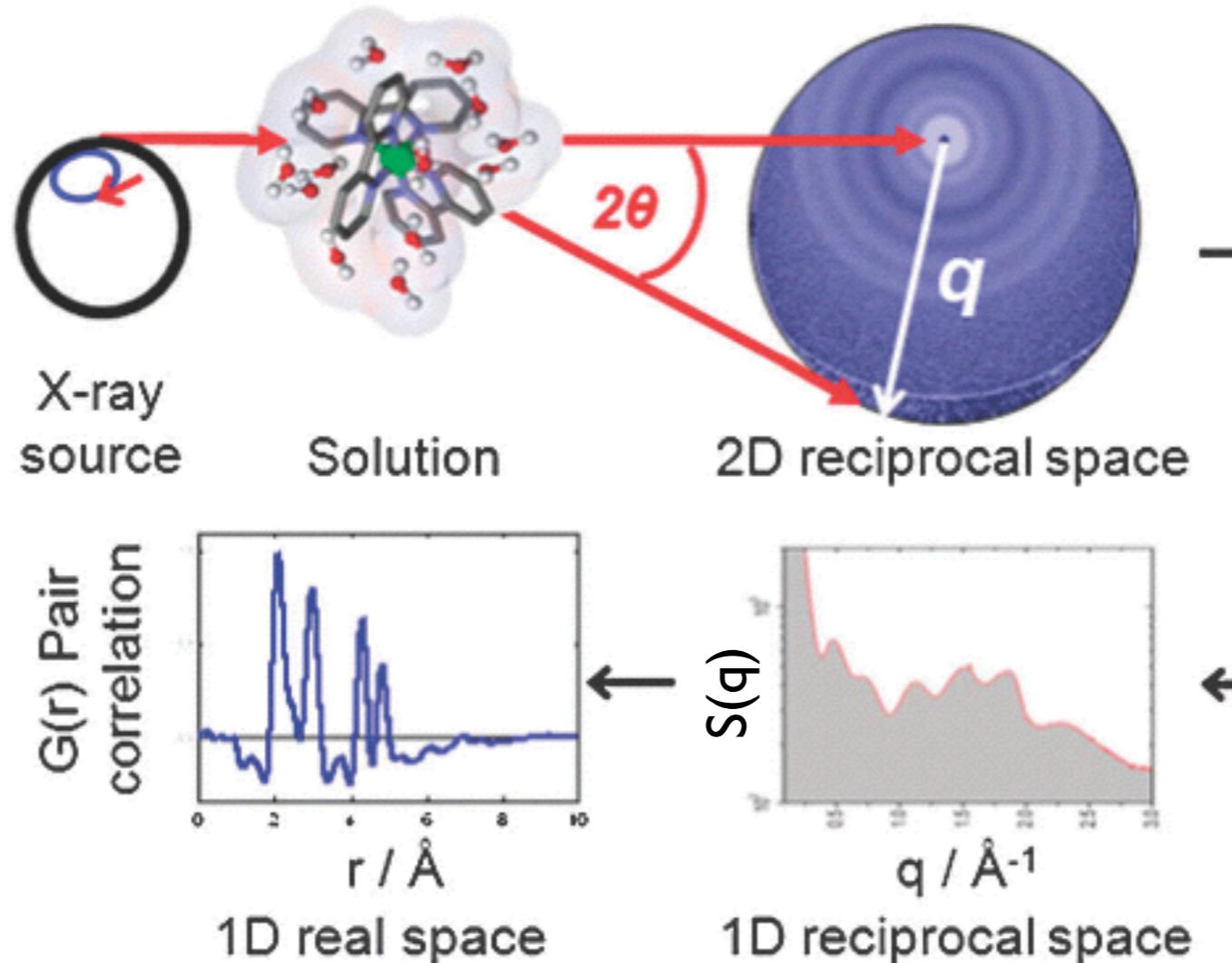
$$= \sum_j^N \sum_k^N f_j f_k \exp[-2\pi i \mathbf{r}_j \cdot \mathbf{S}] \exp[2\pi i \mathbf{r}_k \cdot \mathbf{S}]$$

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

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

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

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

scattering with crystals

scattering with one atom in the unitcell

unitcell alone

scattering vector

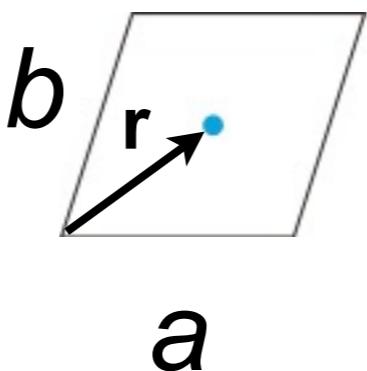
$$F(\mathbf{S}) = f \exp[2\pi i \mathbf{r} \cdot \mathbf{S}] \quad \mathbf{S} \equiv (\mathbf{s} - \mathbf{s}_0)/\lambda$$

position of the single atom with respect to origin

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

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

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

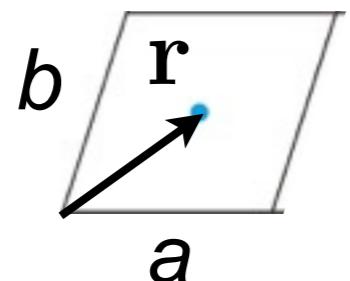


scattering with crystals

scattering with one atom in the unitcell

unitcell with lattice point at origin

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



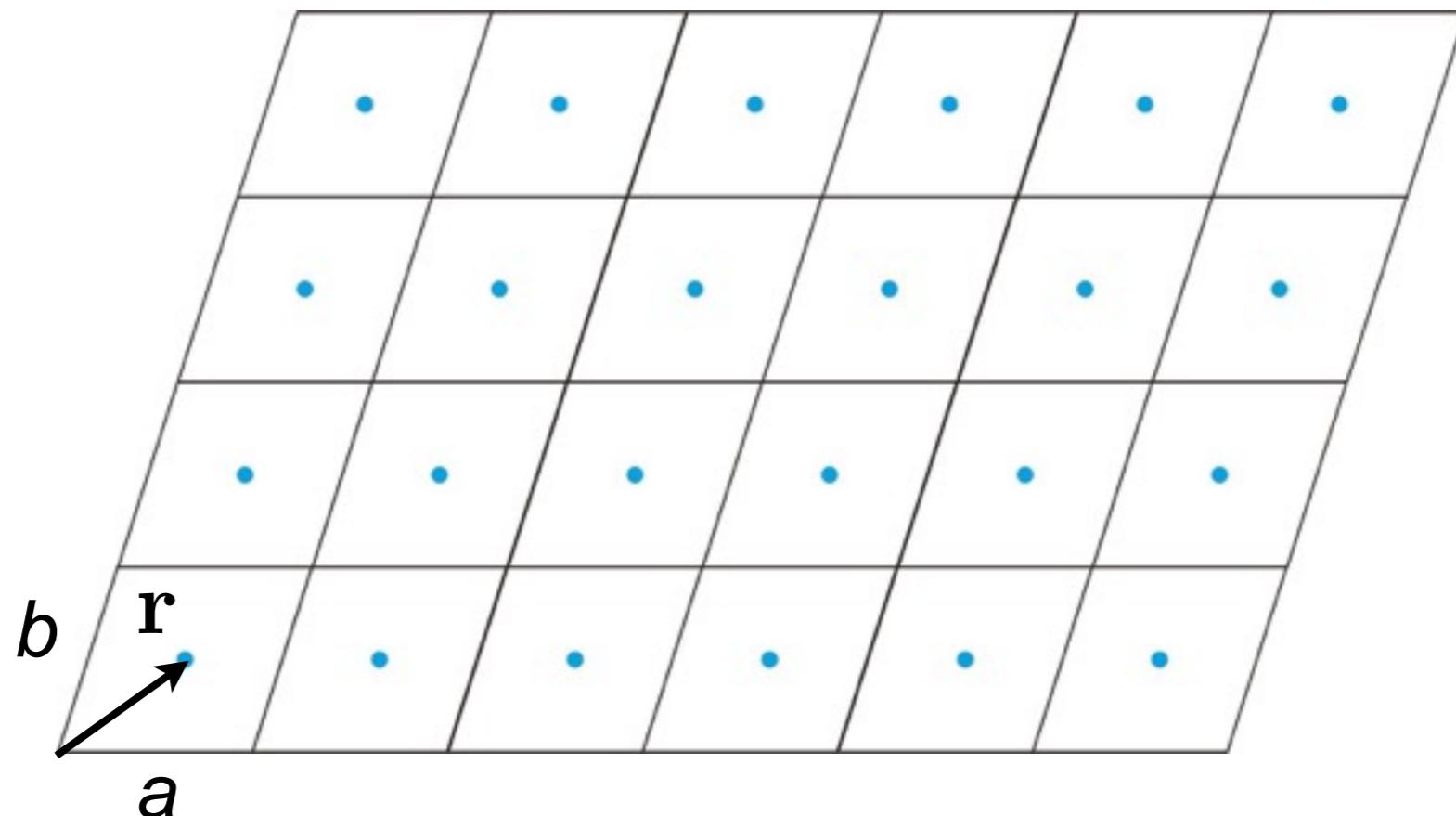
scattering with crystals

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

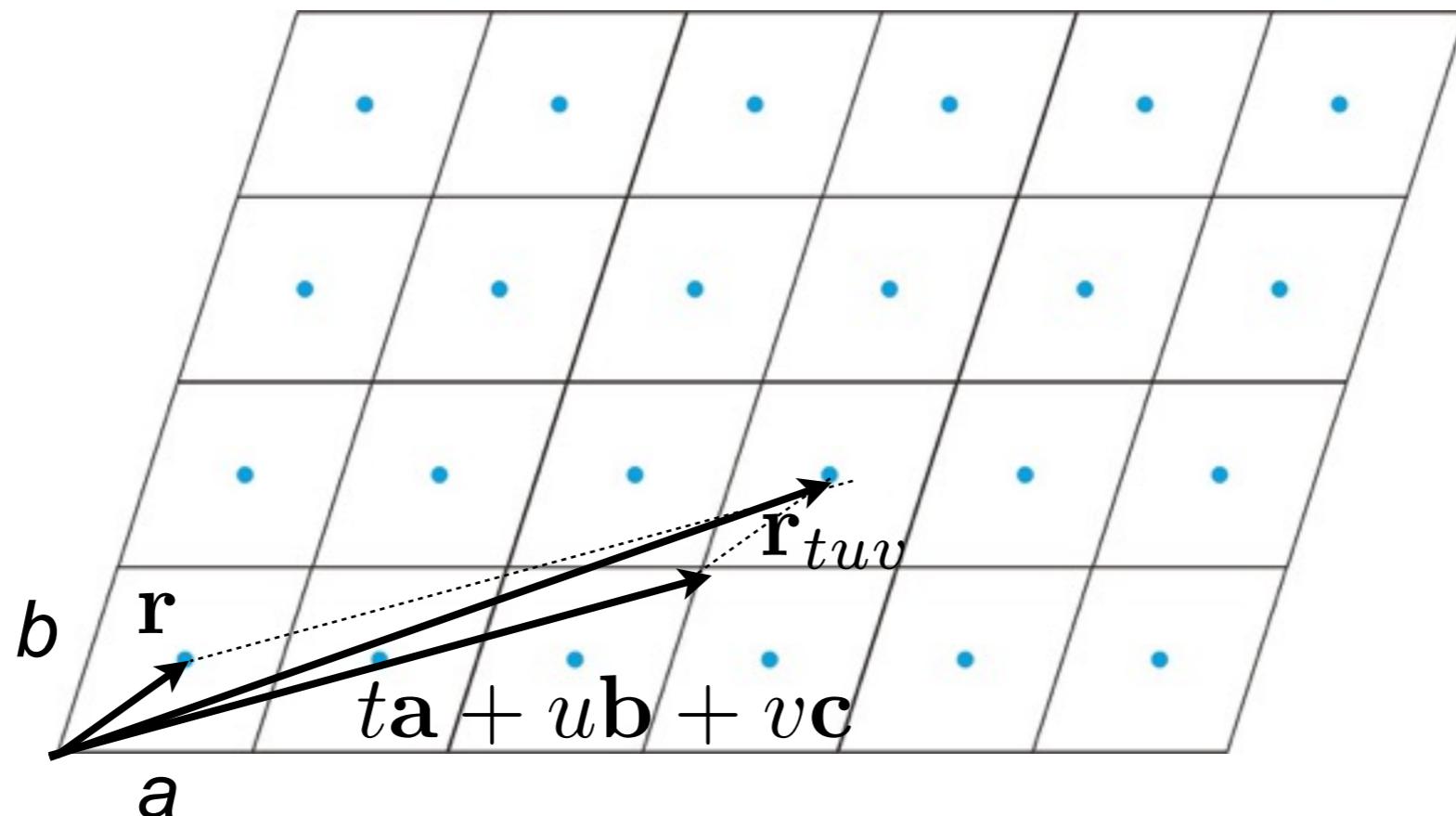
scattering with one atom in the unitcell

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

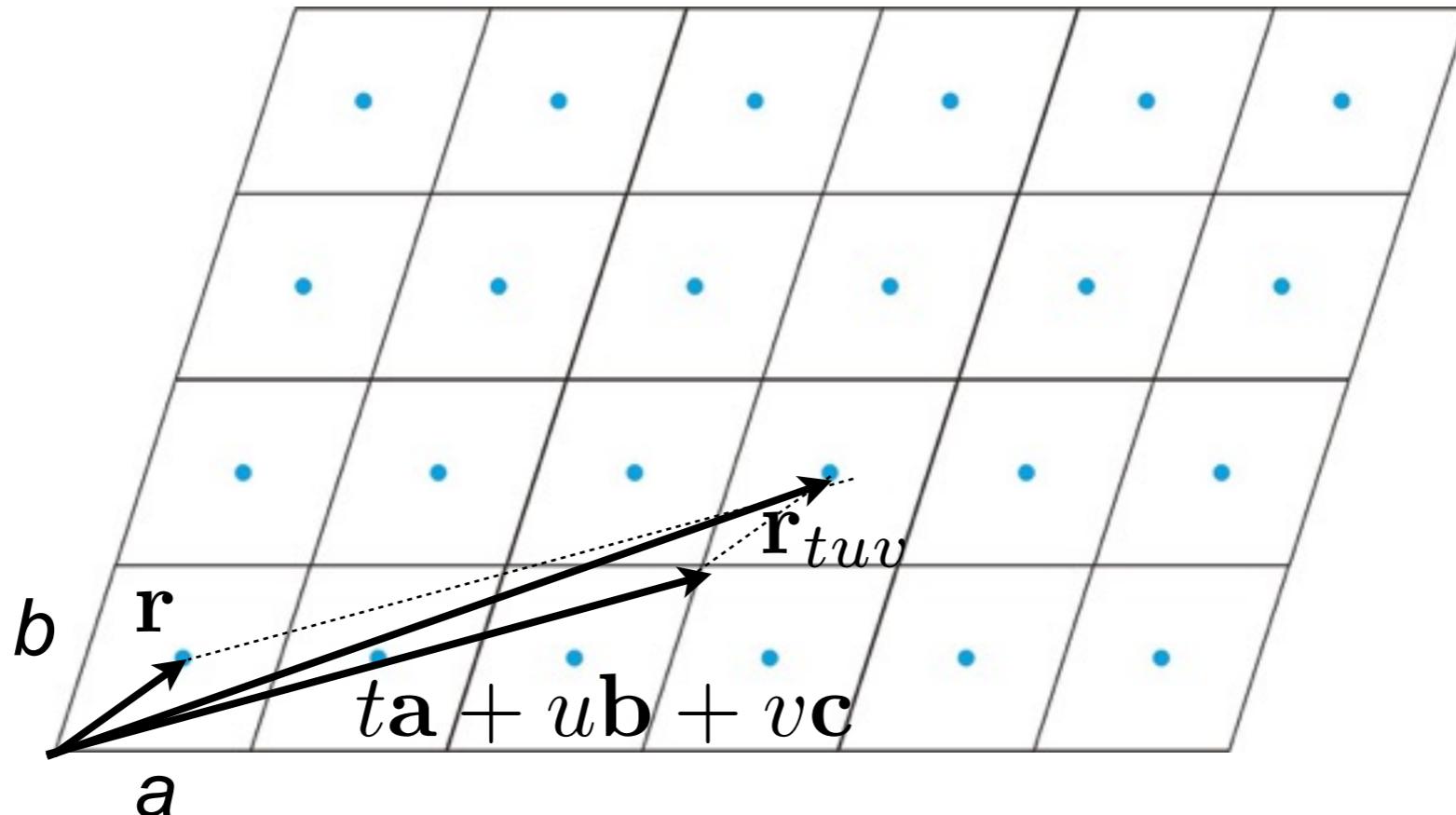
$$\mathbf{F}(\mathbf{S}) = f \exp[2\pi i \mathbf{r} \cdot \mathbf{S}]$$

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

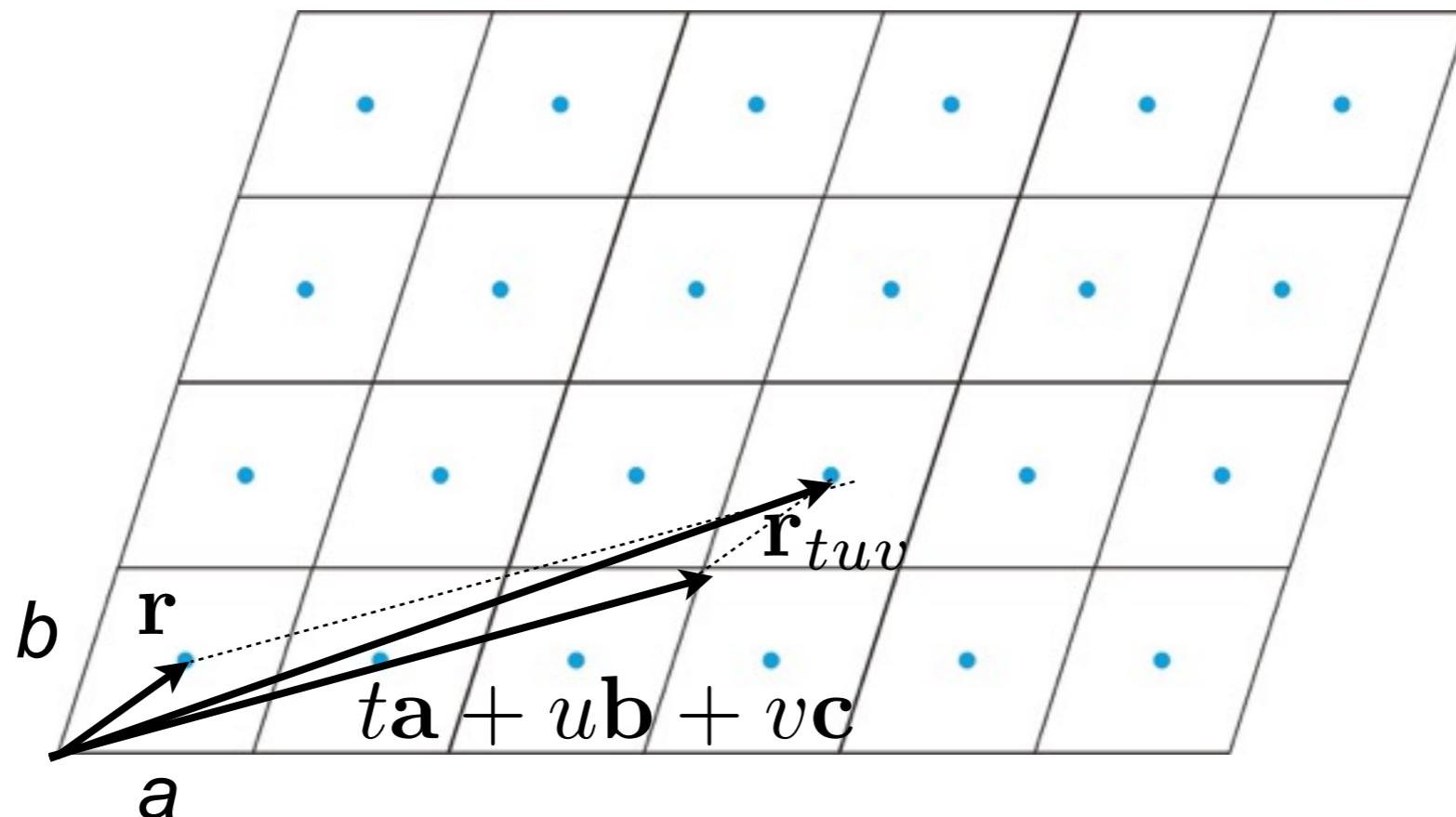
scattering with one atom in the unitcell

total scattering

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

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

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



scattering with crystals

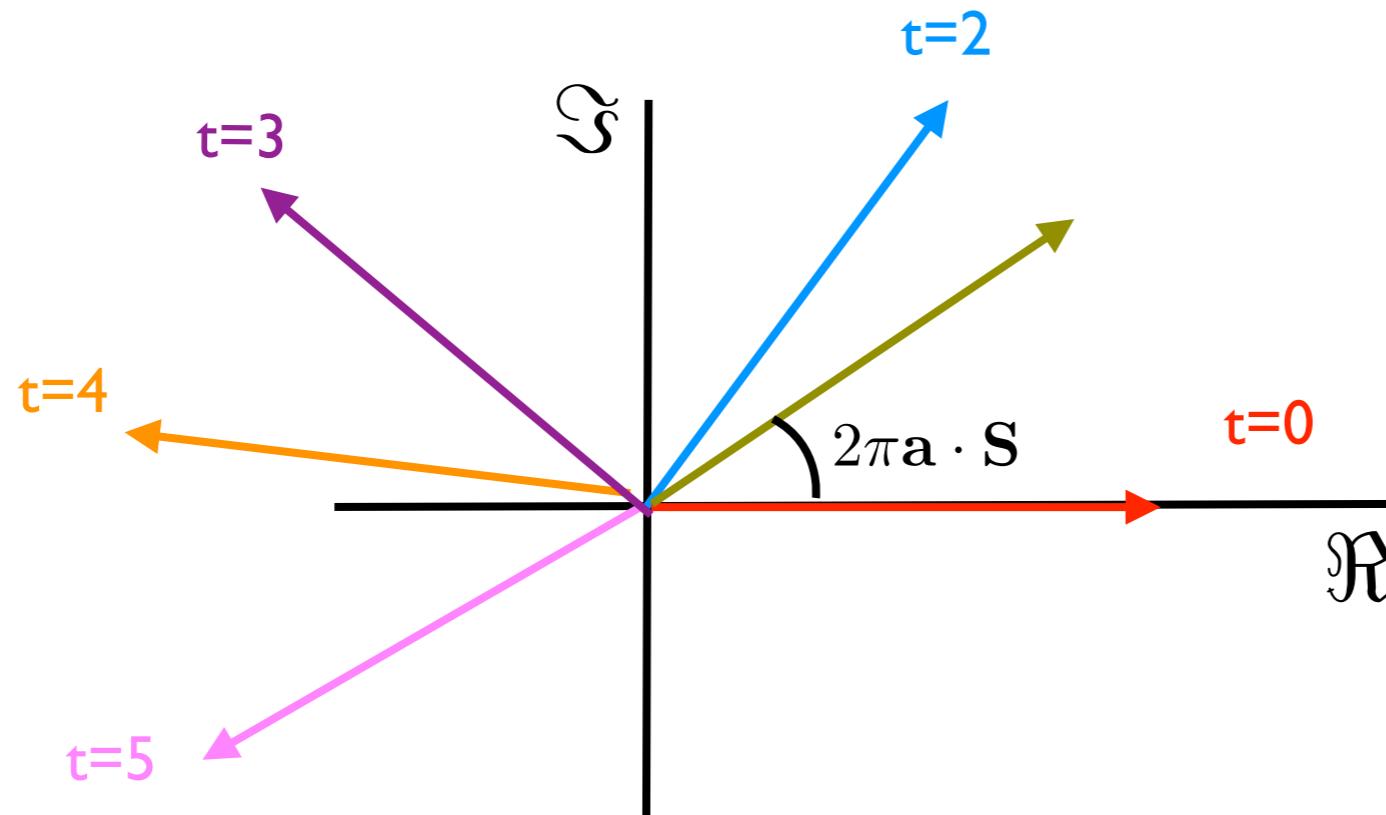
scattering of all unitcells

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

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

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

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

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

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

$$= hx + ky + lz$$

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

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

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

$$= hx + ky + lz$$

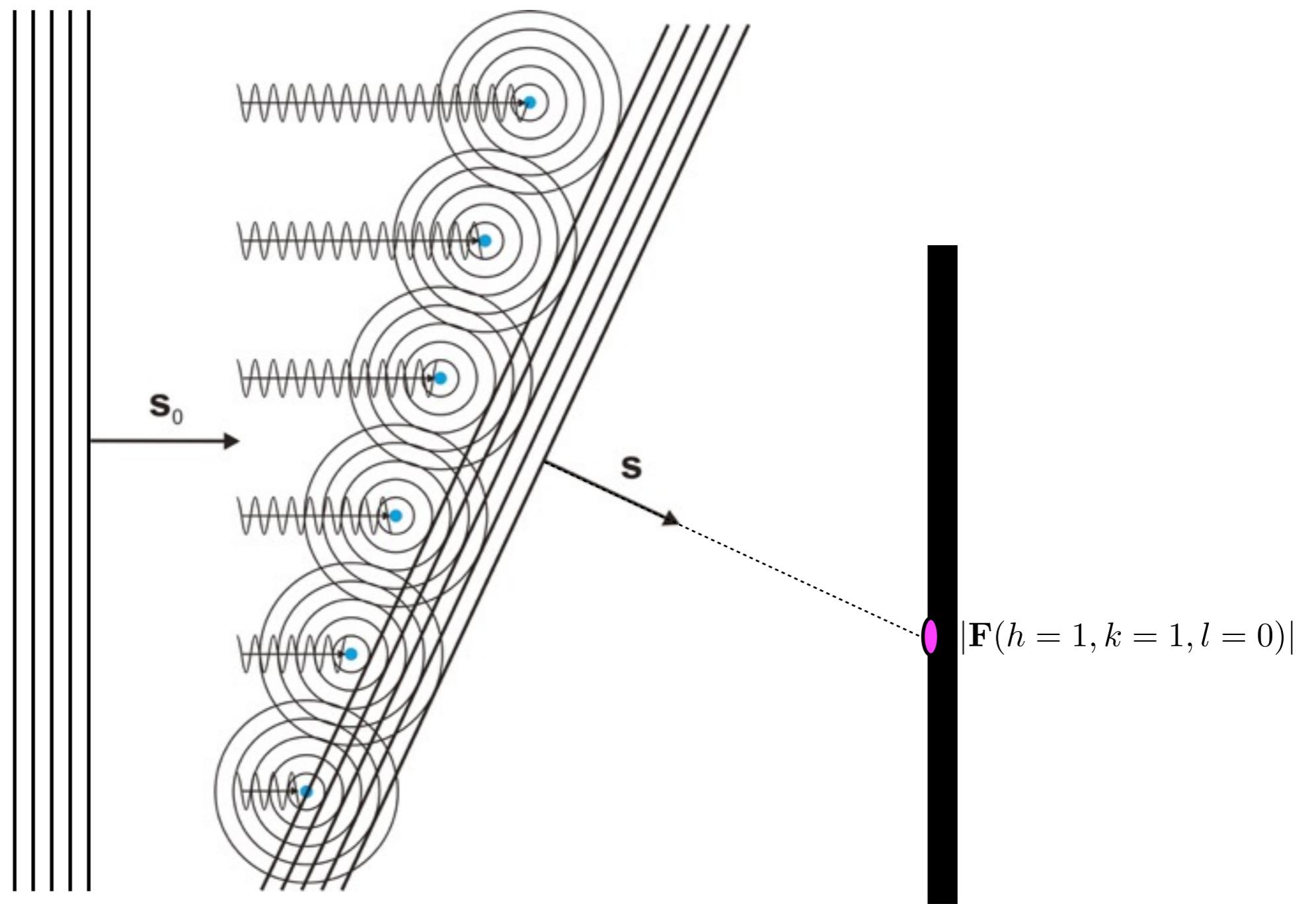
discrete Bragg peaks

$$\mathbf{F}(h, k, l) = f \exp[2\pi i(hx + ky + lz)]$$

scattering with crystals

scattering with one atom in the unitcell

crystal (i.e lattice of unitcells)

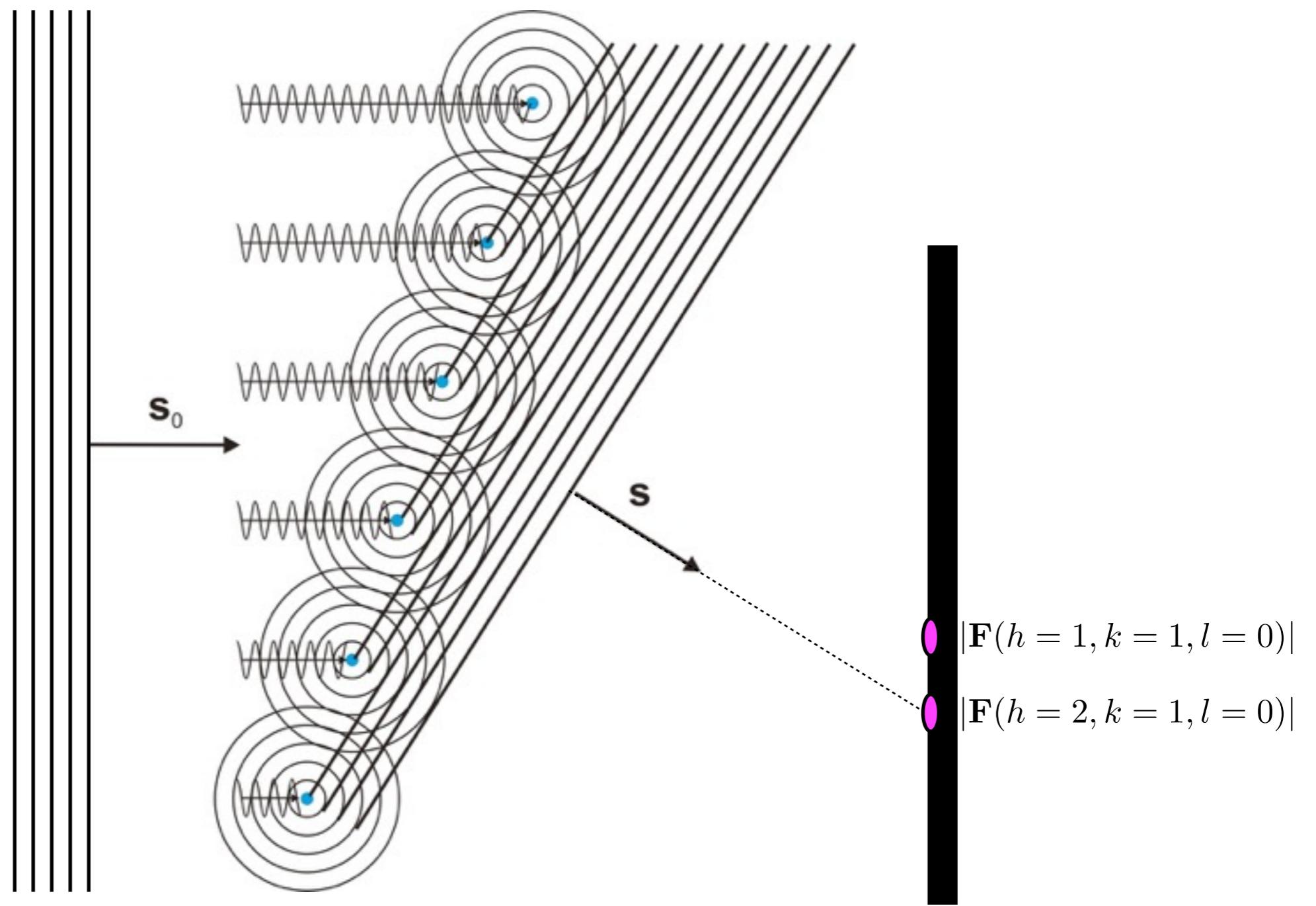


$$|\mathbf{s}_0| = |\mathbf{s}| = \frac{1}{\lambda}$$

scattering with crystals

scattering with one atom in the unitcell

crystal (i.e lattice of unitcells)



$$|\mathbf{s}_0| = |\mathbf{s}| = \frac{1}{\lambda}$$

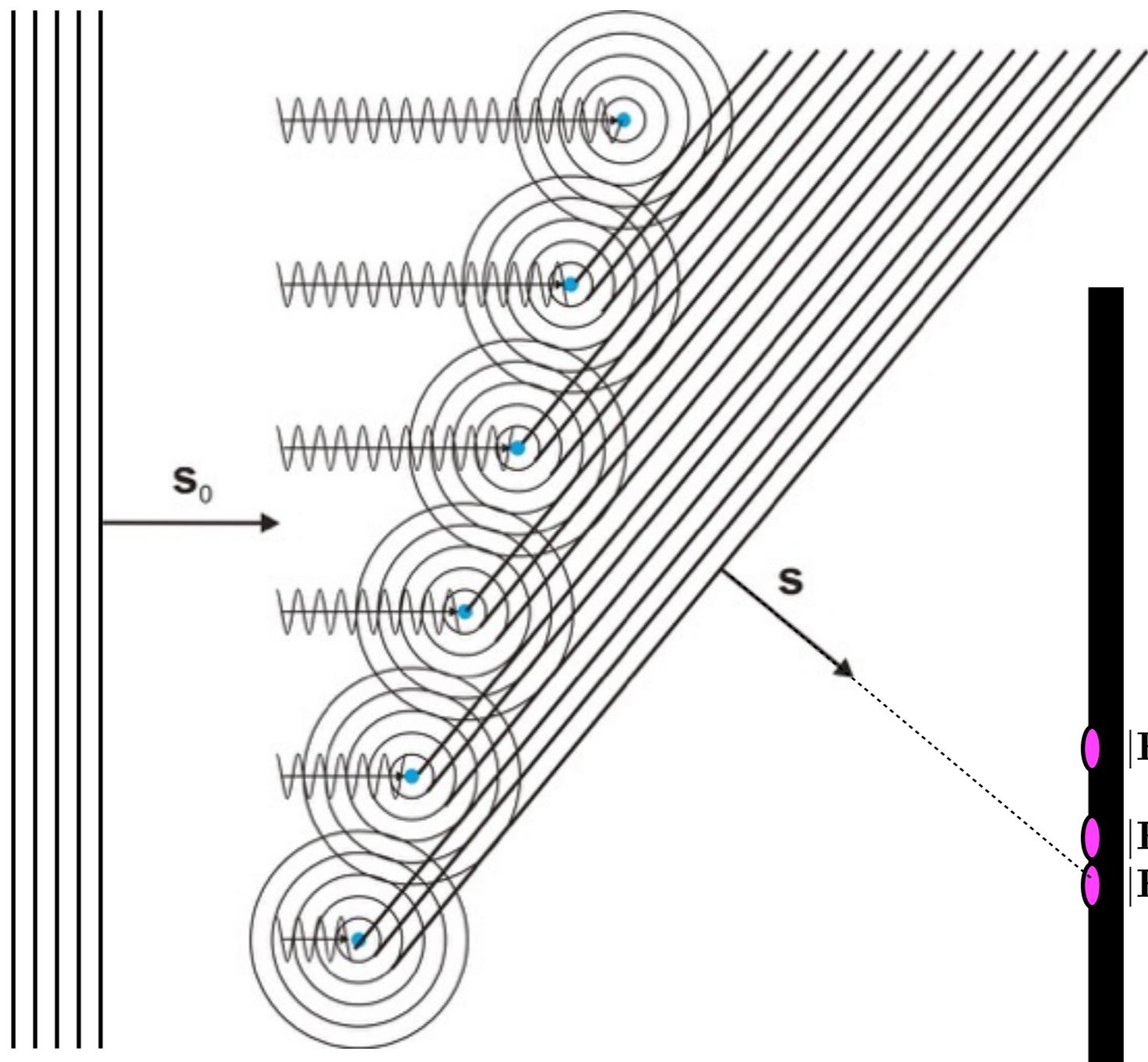
scattering with crystals

scattering with one atom in the unitcell

crystal (i.e lattice of unitcells)

discrete scattering spots: diffraction

reflections



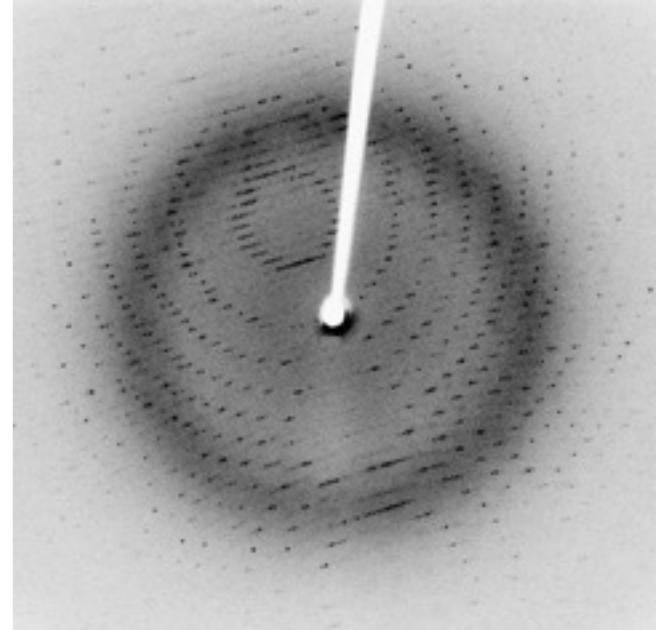
$$|\mathbf{s}_0| = |\mathbf{s}| = \frac{1}{\lambda}$$

scattering with crystals

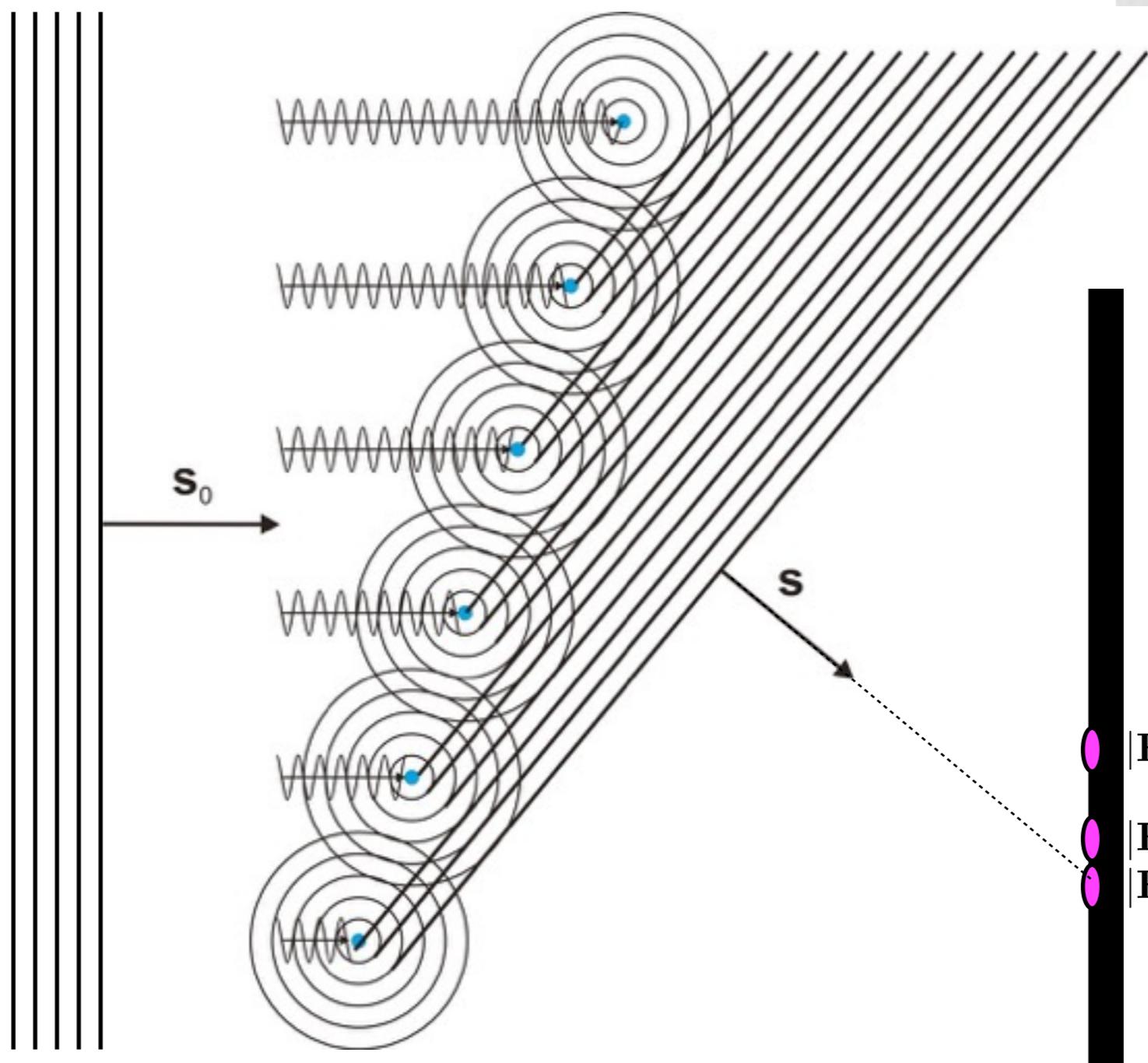
scattering with one atom in the unitcell

crystal (i.e lattice of unitcells)

discrete scattering spots: diffraction



reflections



$|\mathbf{F}(h = 1, k = 1, l = 0)|$
 $|\mathbf{F}(h = 2, k = 1, l = 0)|$
 $|\mathbf{F}(h = 2, k = 2, l = 0)|$

Diffraction

calculating the electron density of a crystal of molecules

structure factor summed over atoms in unit cell

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

Diffraction

calculating the electron density of a crystal of molecules

structure factor summed over atoms in unit cell

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

assumption: atoms have spherical electron clouds

Diffraction

calculating the electron density of a crystal of molecules

structure factor summed over atoms in unit cell

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

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 of a crystal of molecules

structure factor associated with reflection hkl

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

Diffraction

calculating the electron density of a crystal of molecules

structure factor associated with reflection hkl

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

however, we want the density, not the structure factor....

Diffraction

calculating the electron density

structure factor associated with reflection hkl

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

however, we want the density, not the structure factor....

inverse Fourier transformation

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

Diffraction

calculating the electron density

structure factor associated with reflection hkl

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

however, we want the density, not the structure factor....

inverse Fourier transformation

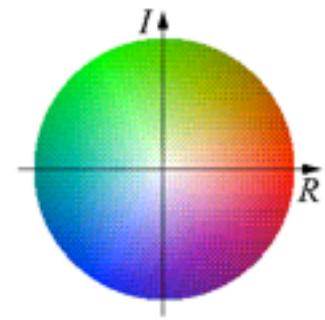
$$\rho(x, y, z) = \frac{1}{V} \sum_h \sum_k \sum_l \mathbf{F}(h, k, l) \exp[-2\pi i(hx + ky + lz)]$$

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

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

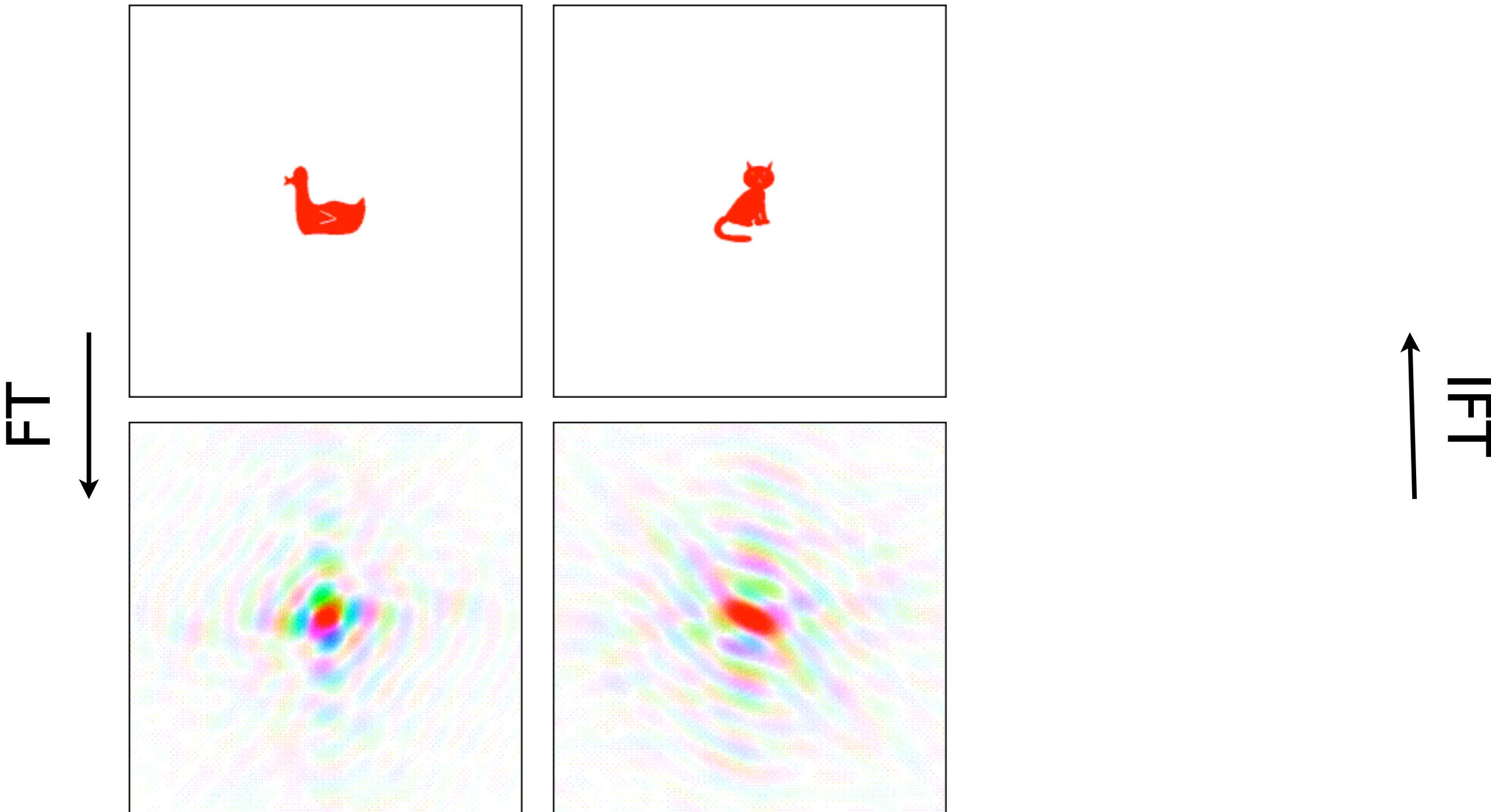
$$\rho(x, y, z) = \frac{1}{V} \sum_h \sum_k \sum_l |F(h, k, l)| \exp[-2\pi i(hx + ky + lz) + i\alpha(h, k, l)]$$

Diffraction

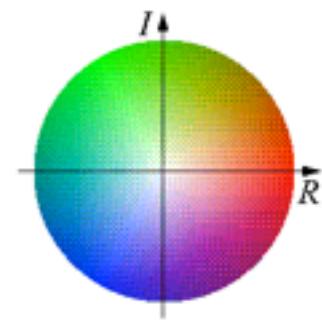


phase contains structural information

example Kevin Cowtan's Fourier Cat and Fourier Duck

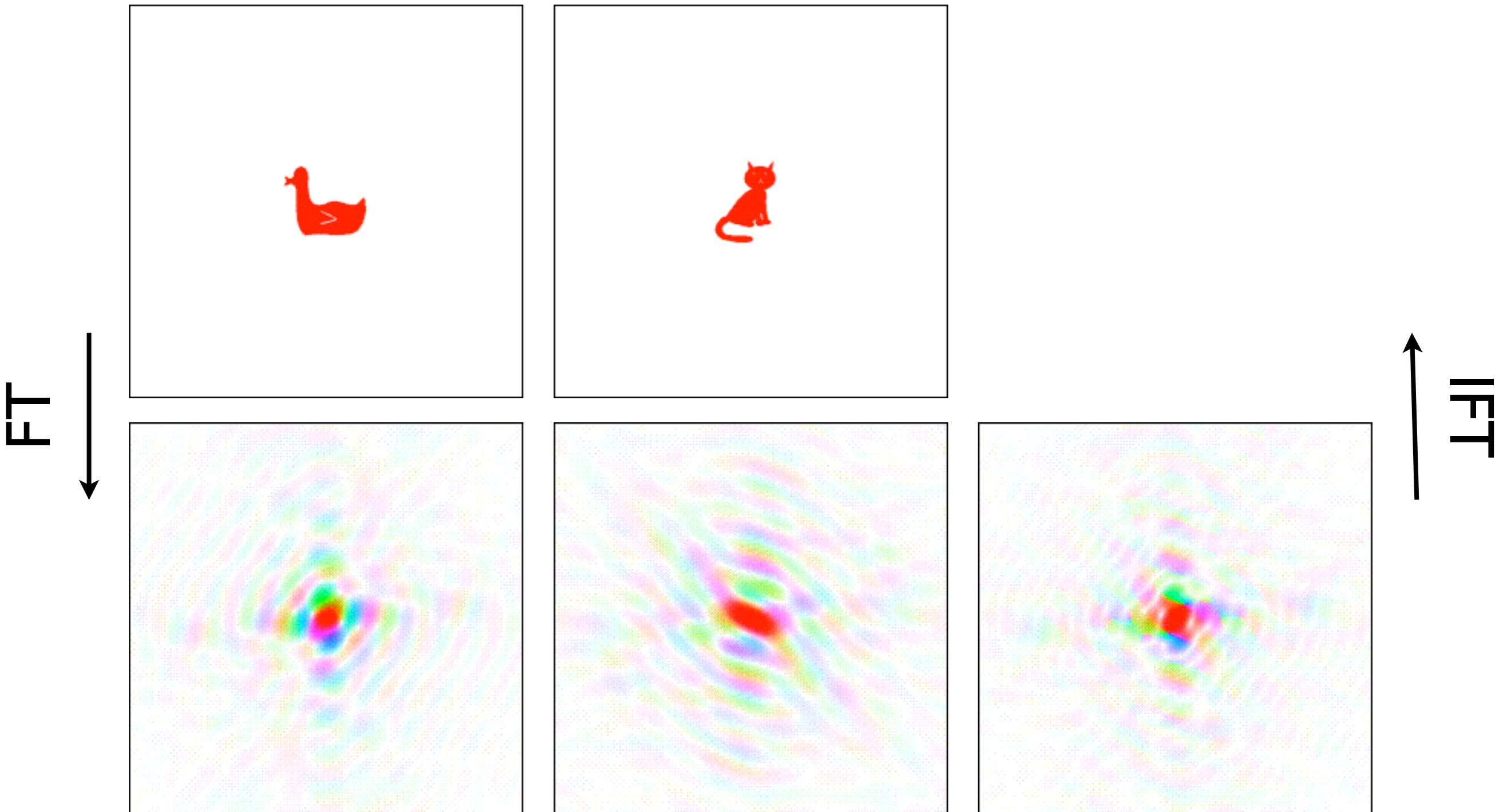


Diffraction



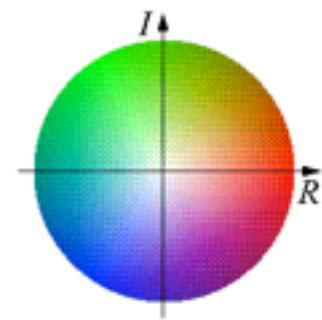
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example Kevin Cowtan's Fourier Cat and Fourier Duck



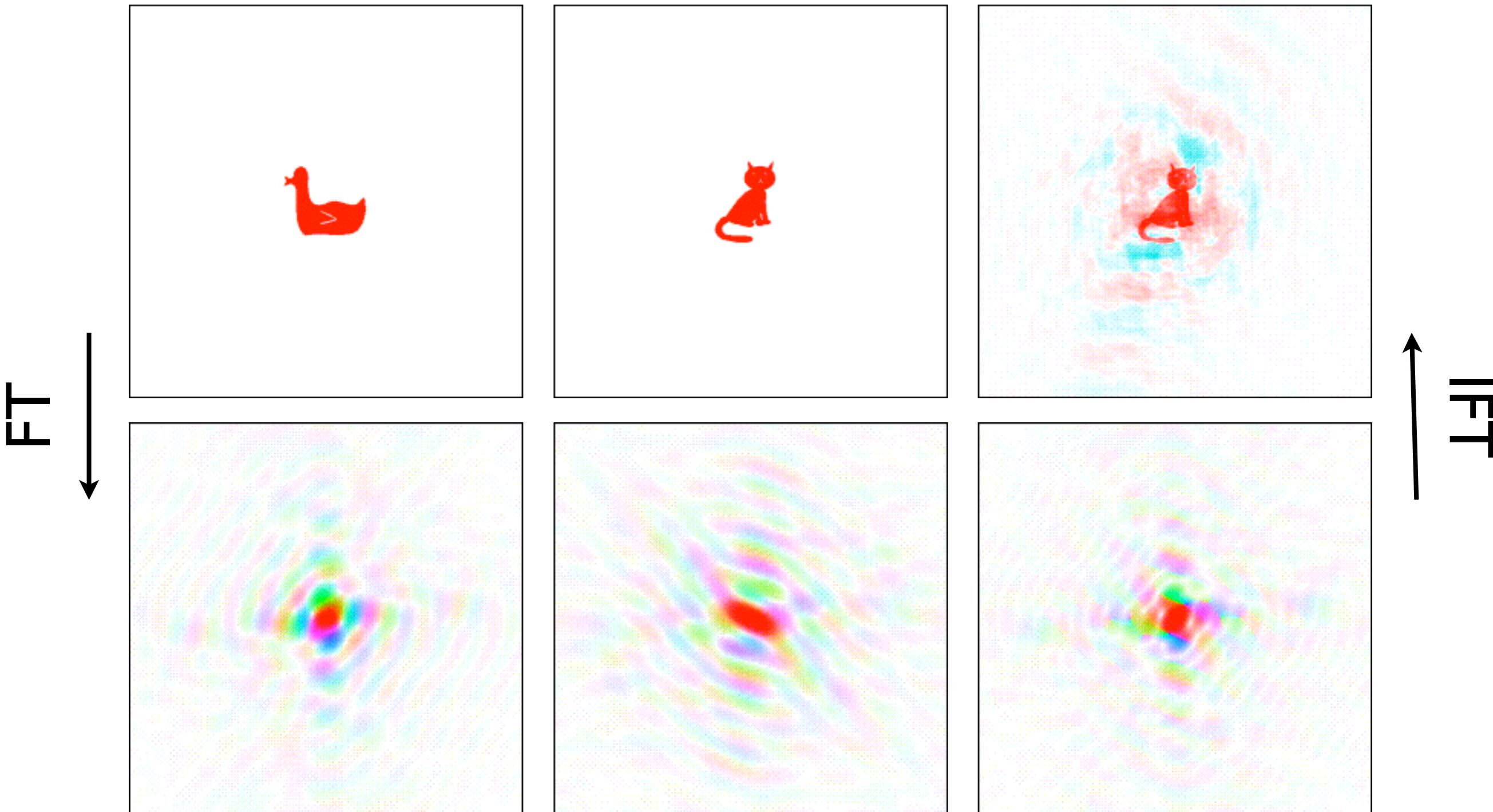
$I_{\text{duck}}, \alpha_{\text{cat}}$

Diffraction



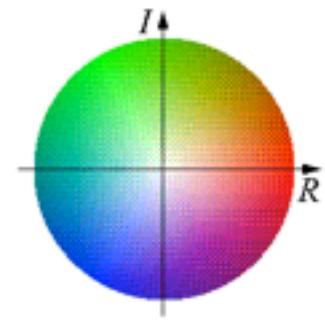
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example Kevin Cowtan's Fourier Cat and Fourier Duck



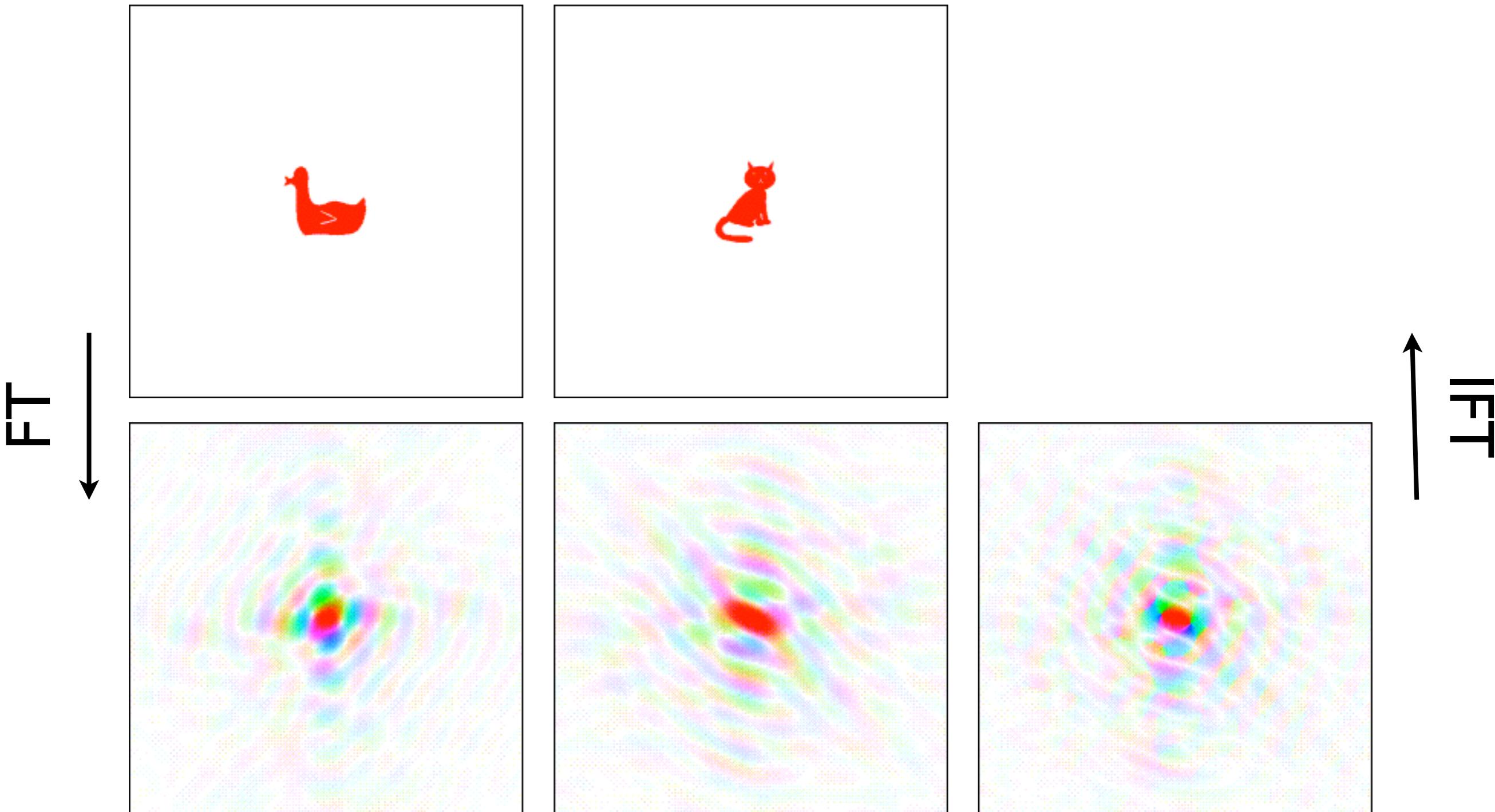
$I_{\text{duck}}, \alpha_{\text{cat}}$

Diffraction



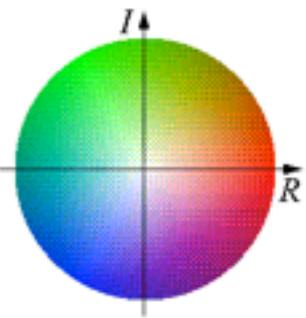
phase contains structural information

example Kevin Cowtan's Fourier Cat and Fourier Duck



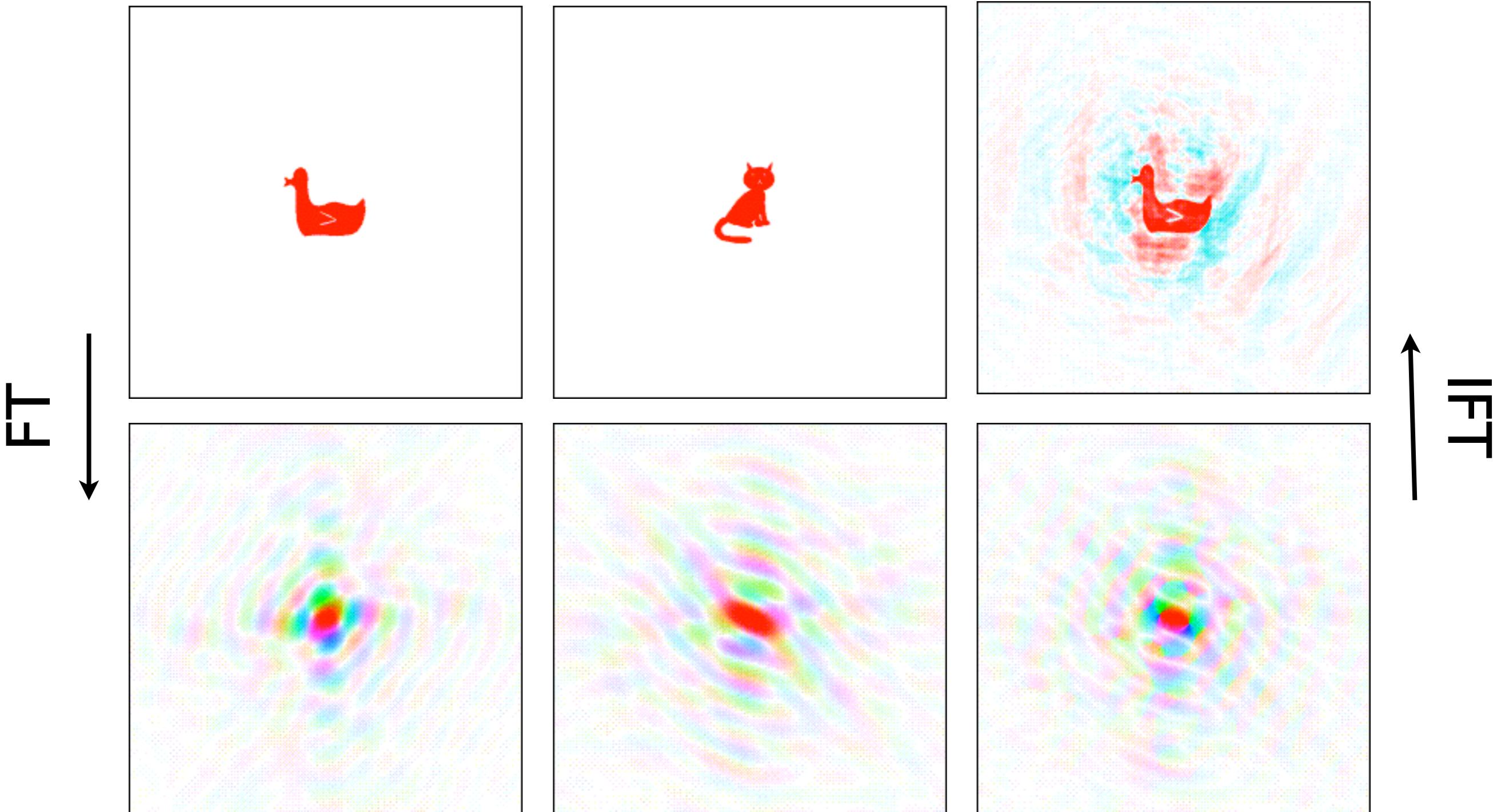
$$I_{\text{cat}}, \alpha_{\text{duck}}$$

Diffraction



phase contains structural information

example Kevin Cowtan's Fourier Cat and Fourier Duck



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

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!

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

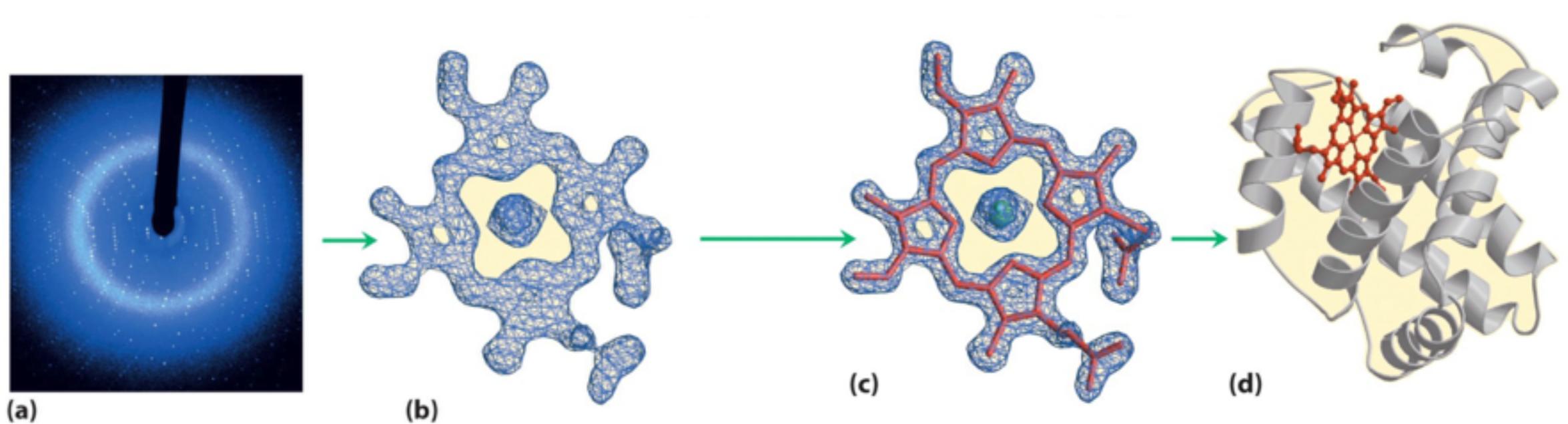
building atomic model

I fit atoms into the density

information on sequence

information on bond lengths, angles, torsions

force field



building atomic model

I fit atoms into the density

information on sequence

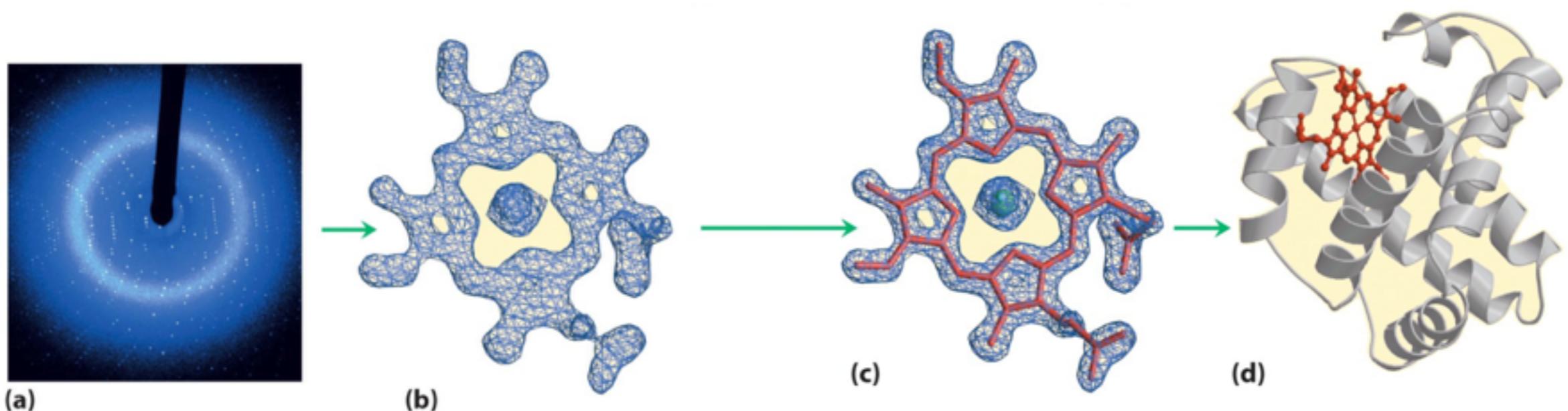
information on bond lengths, angles, torsions

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

$$R = 0.59$$

validation of model

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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)

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

validation of model

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Ramachandran

stereochemistry