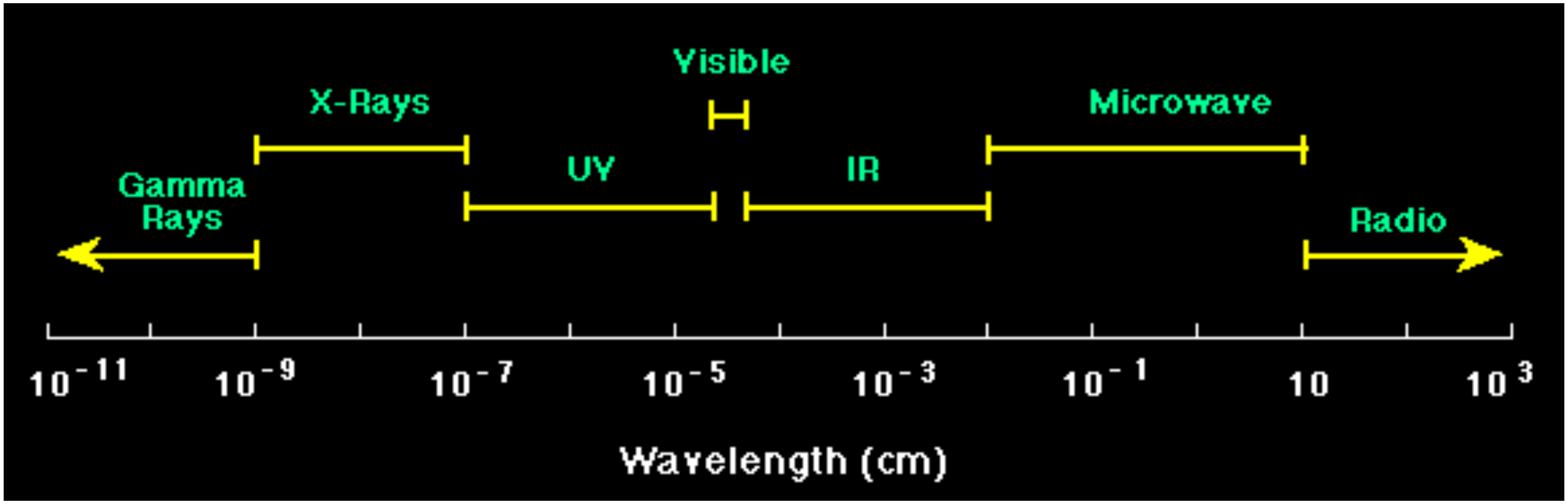
Protein Structure Determination '20

Lecture 2:

The scattering of Xrays by electrons

Wave physics

the electromagnetic spectrum

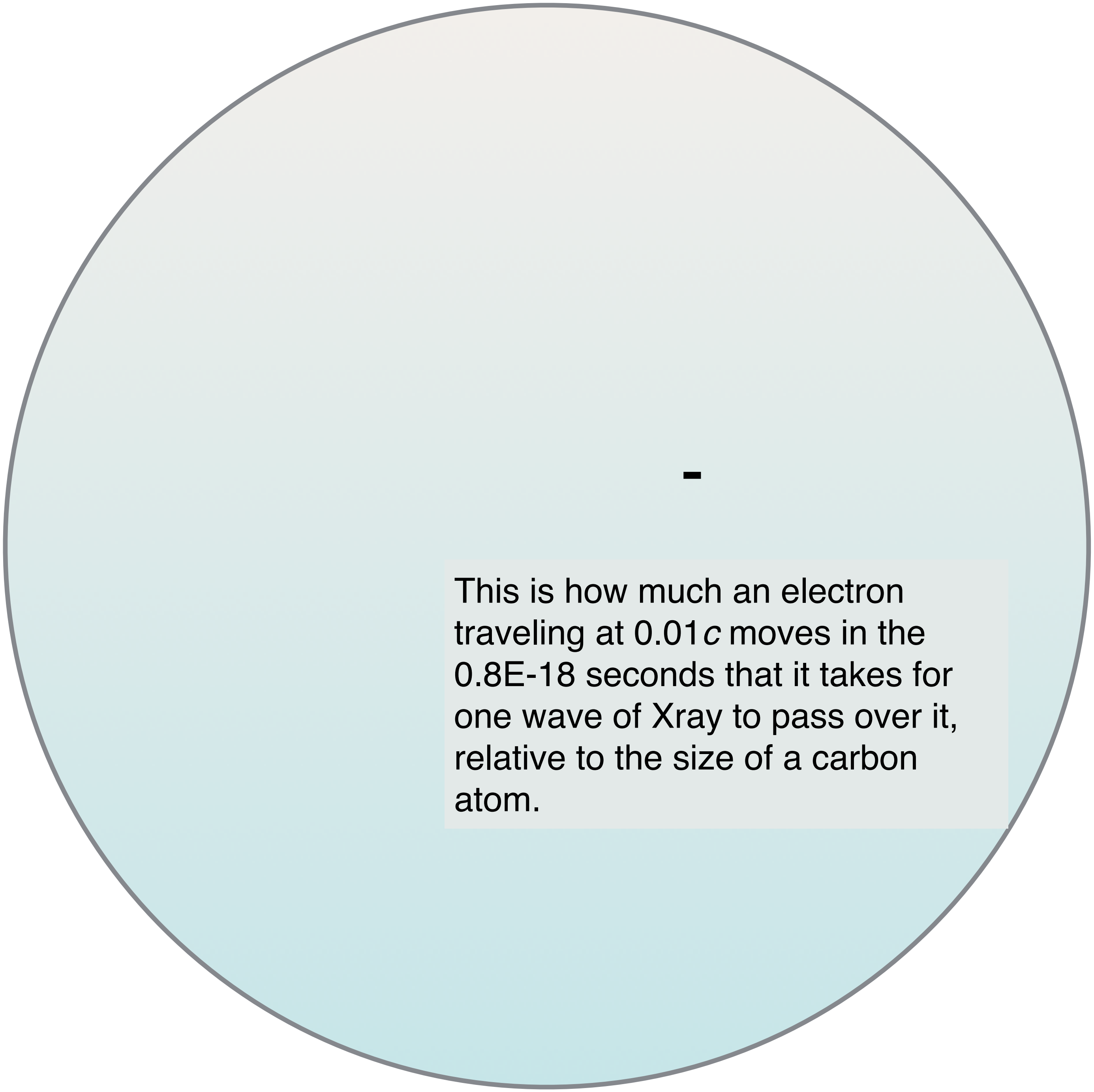


Wavelength of X-rays used in crystallography: 1Å - 3Å
(Å = 10^{-10}m) most commonly 1.54Å (Cu)

Frequency of oscillation of the electric field = c/λ

$$=(3\text{x}10^{8}\text{m/s}) / (1.54\text{x}10^{-10}\text{m}) \approx 2\text{x}10^{18}\ \text{s}^{-1}$$

Much faster than electron motion around the nucleus.

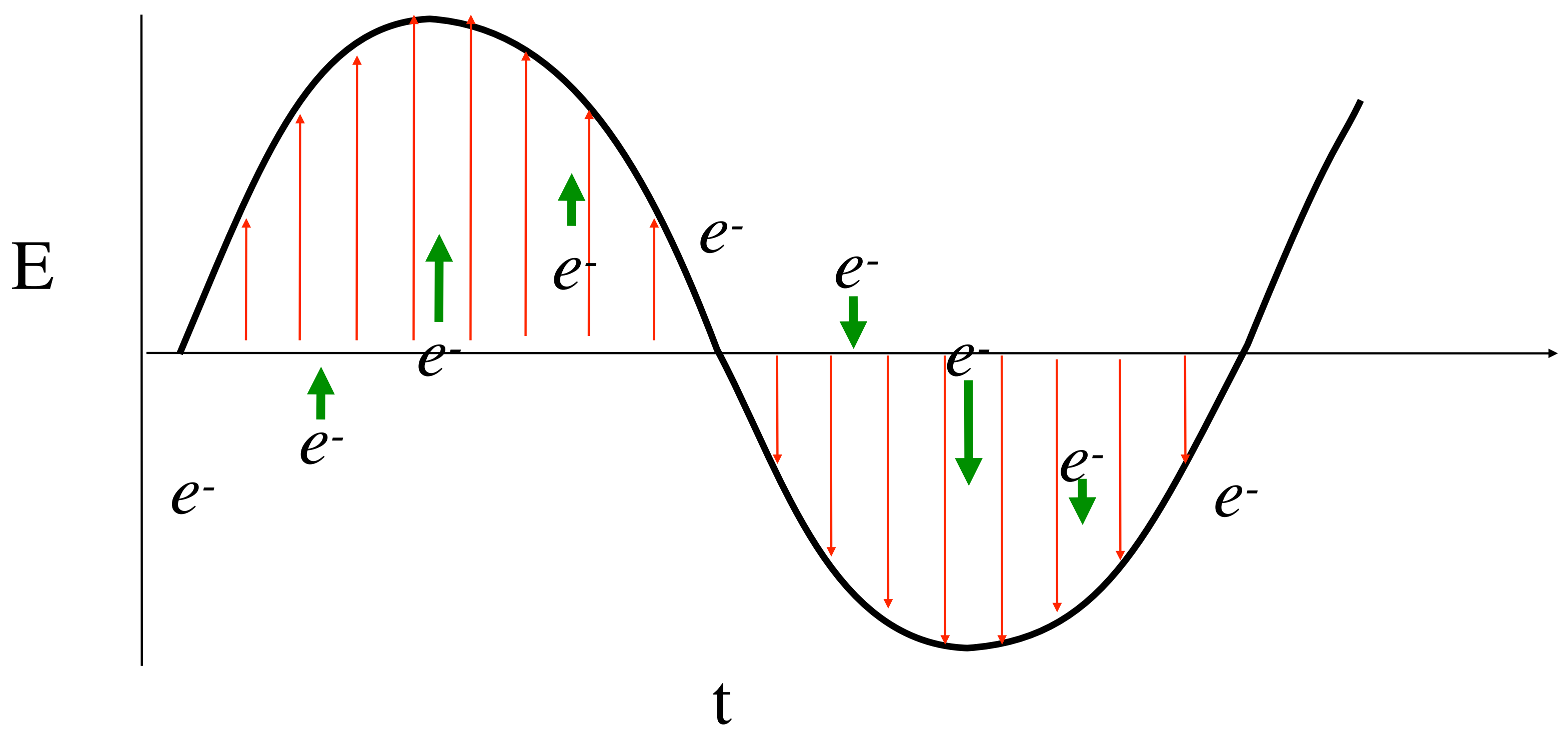

This is how much an electron traveling at 0.01*c* moves in the 0.8E-18 seconds that it takes for one wave of Xray to pass over it, relative to the size of a carbon atom.

What happens to an electron e^- when it oscillates in an electric field?

• *e-* oscillation is the same frequency as the X-rays

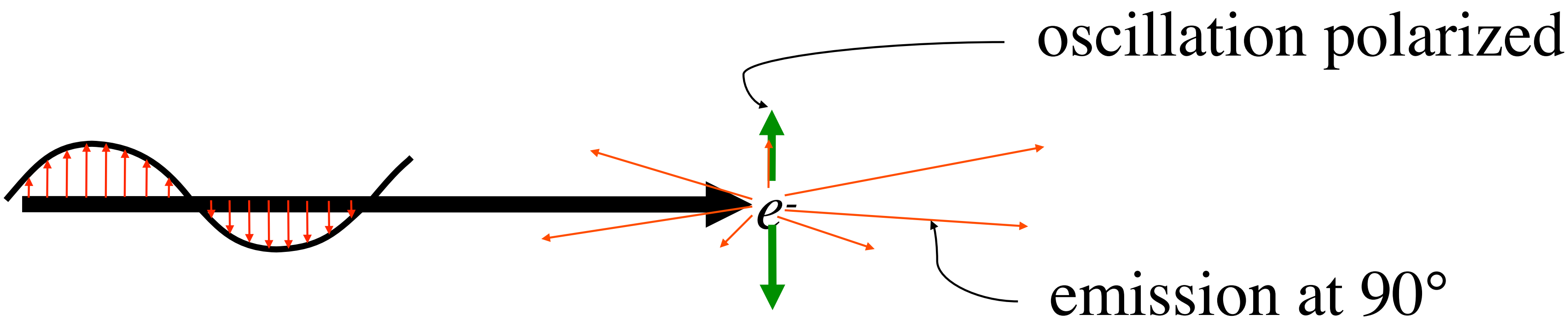
• *e-* oscillation is much faster that orbiting motion.

• The amplitude of the *e-* oscillation is large because the mass of an e^- is <u>small</u>. Atomic nuclei don't oscillate much.

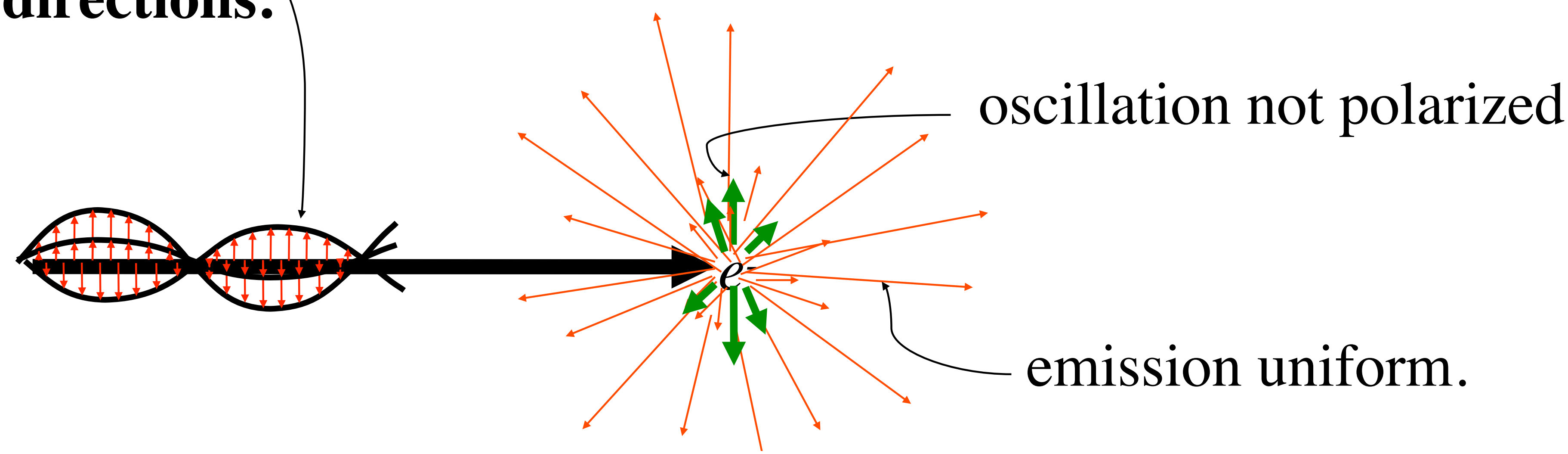


An oscillating charge emits light

⊥ to the direction of oscillation.



But since X-rays are not polarized, emission goes in <u>all</u> directions.

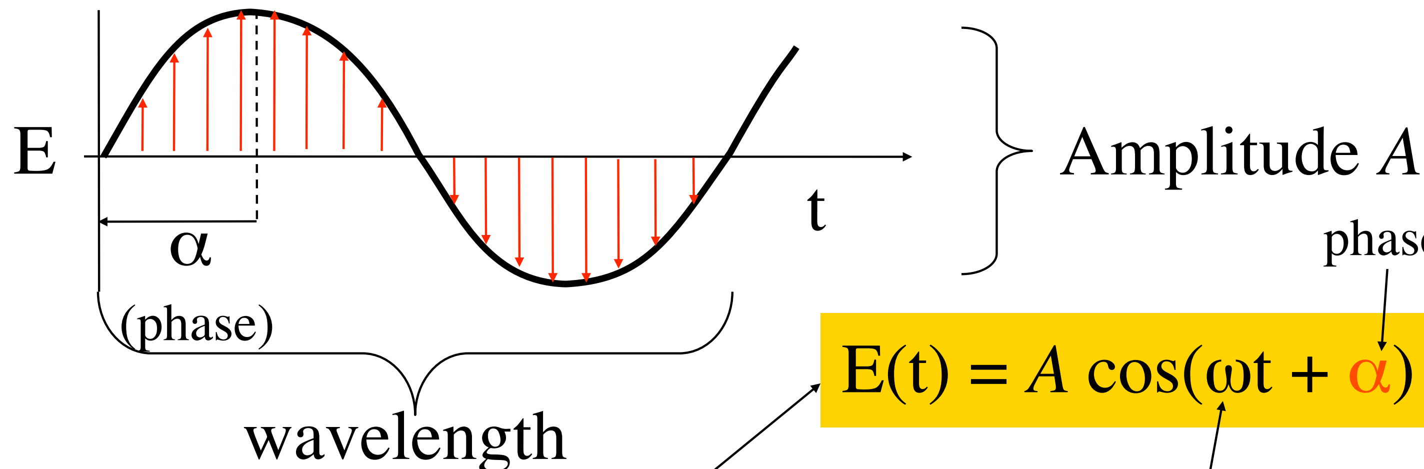


Review of *e*- scattering

- X-rays are waves of oscillating electric field.
- Charged particles are oscillated by X-rays.
- Oscillating charged particles emit light.
- Electrons oscillate with a much higher amplitude than nuclei, so they scatter more.
- The frequency of oscillation is roughly $2 \times 10^{18}\ s^{-1}$, much faster than the speed of travel of e^- around the nucleus.
- So…. X-rays scatter from electrons <u>like they are standing still</u>.
- (No Doppler effect!)

equation for a wave

Remember:
Photons are oscillating electric fields*.



phase

$$E(t) = A\cos(\omega t + \alpha)$$

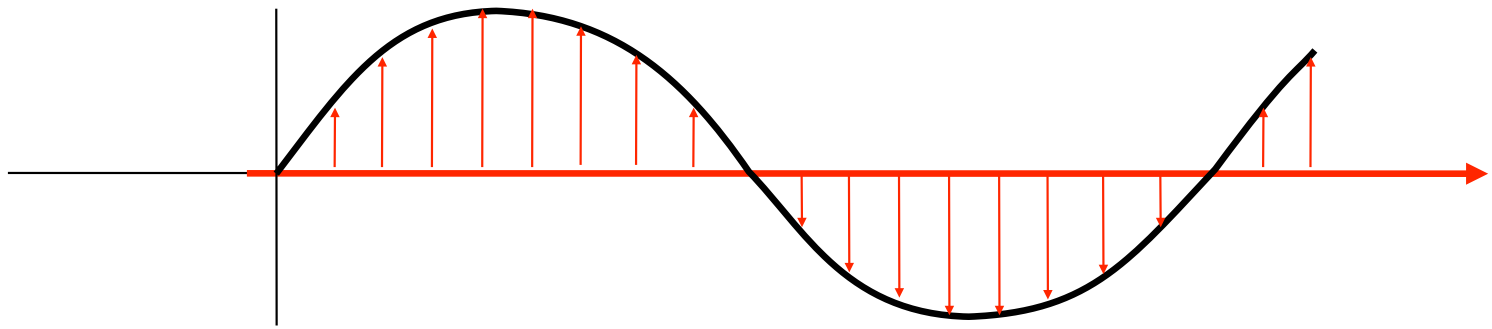
The instantaneous electric field at time **t**

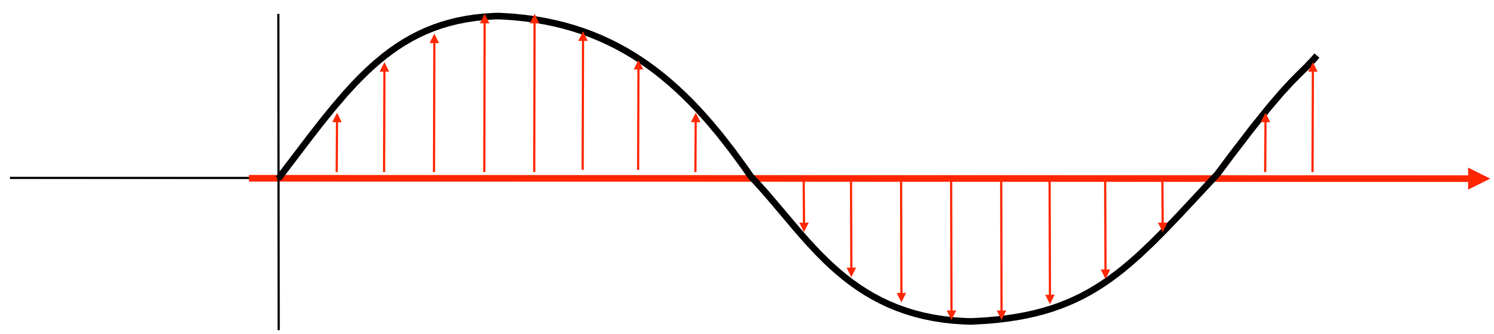
$\omega = 2\pi c/\lambda$

oscillation rate in cycles/second

*also has an oscillating magnetic field of the same frequency, 90 degrees out of phase. We ignore this.

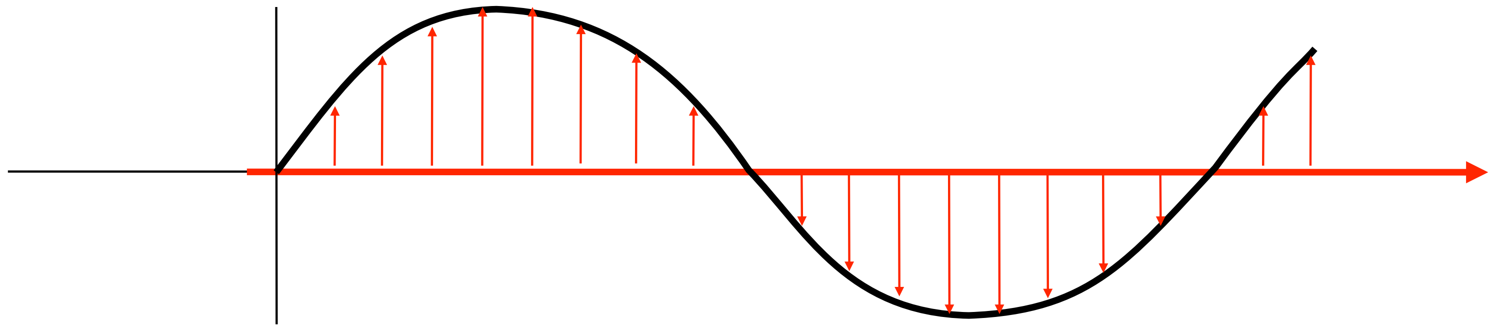
Constructive interference





+

Destructive interference



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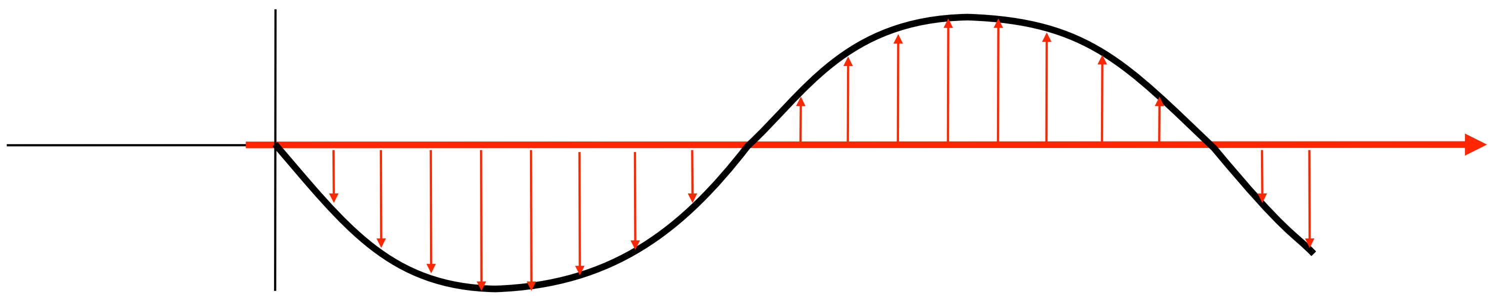
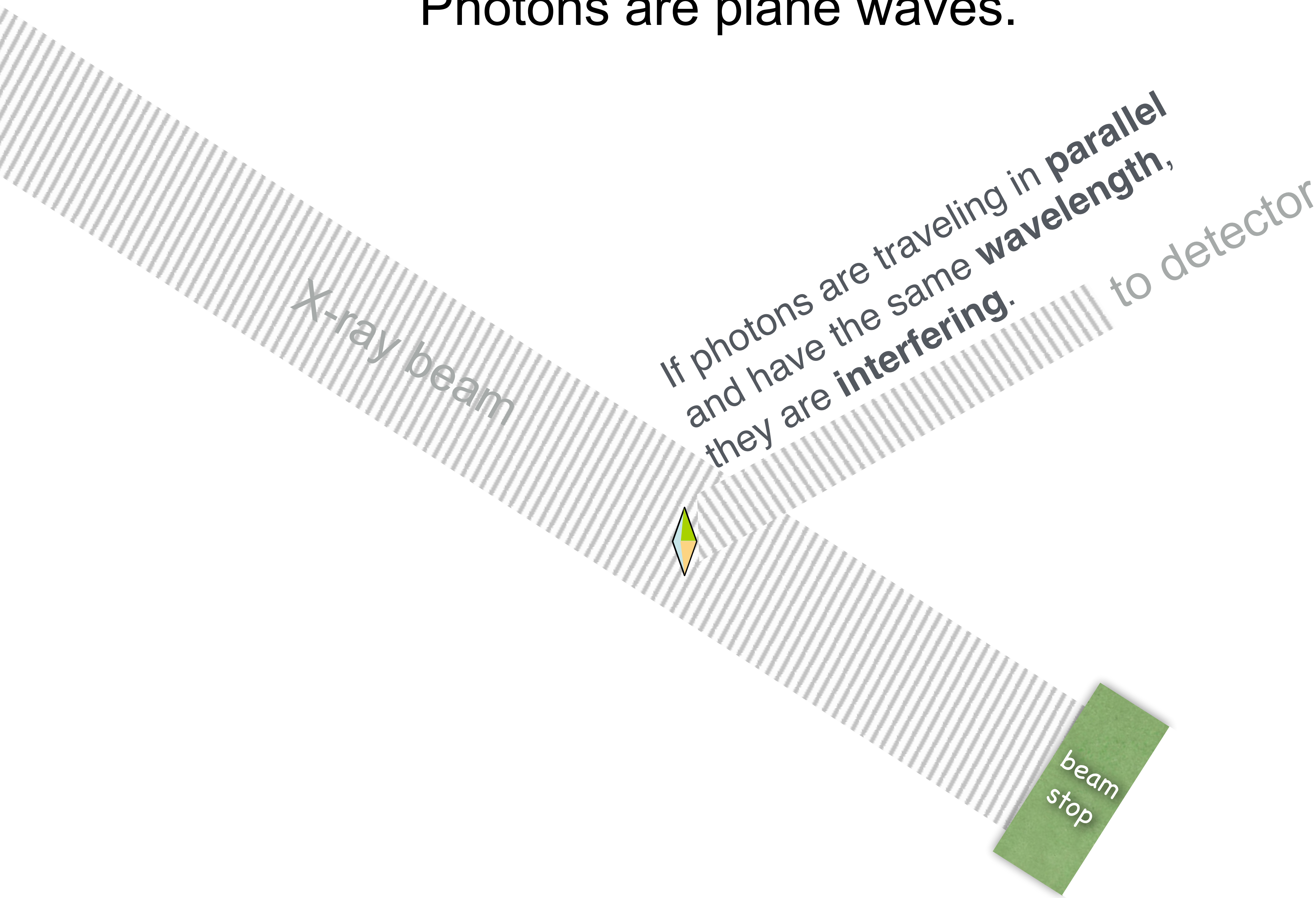
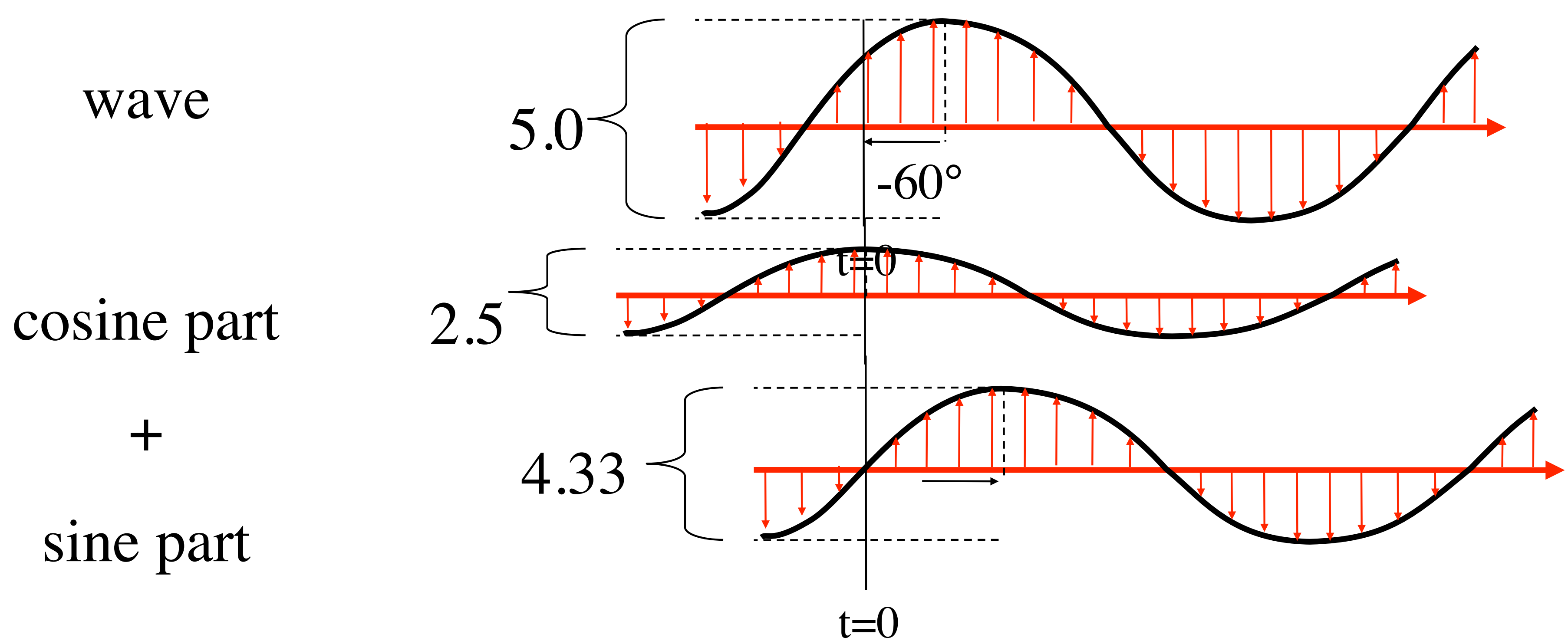


Photons are plane waves.



sneak peak: diffraction is interference cause by crystals

Waves can be decomposed.



Decomposing the oscillator equation

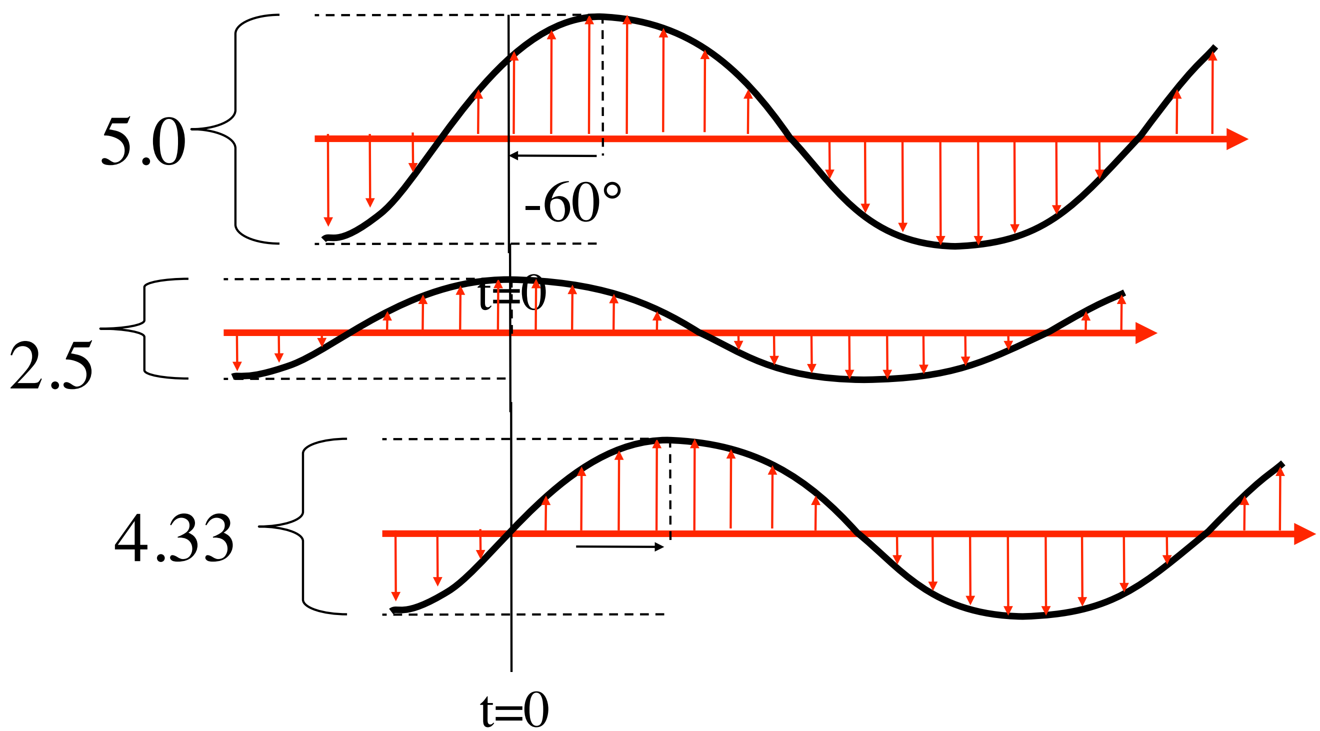
$5.0 \cos(\omega t - \pi/3)$

$=$

$2.5 \cos \omega t$

$+$

$4.33 \sin \omega t$



$5.0 \cos(\omega t - \pi/3) =$ …using the Sum of Angles rule…

$5.0 \cos(-\pi/3) \cos \omega t - 5.0 \sin(-\pi/3) \sin \omega t =$

$2.5 \cos \omega t + 4.33 \sin \omega t$ — decomposed wave

Details on decomposing a wave

amplitude

oscillator

The general wave equation,

$$E(t) = A\cos(\omega t + \alpha)$$

phase

Using the sum of angles rule, becomes

$$A\cos(\omega t + \alpha) = \underline{A\cos\alpha}\ \underline{\cos\omega t} - \underline{A\sin\alpha}\ \underline{\sin\omega t}$$

amplitude of cosine part

A unit cosine wave oscillator. We can call this the x-axis

amplitude of sine part

A unit sine wave oscillator. We can call this the y-axis

Which corresponds to a point in a 2-D orthogonal coordinate system.

The sum of angles rule

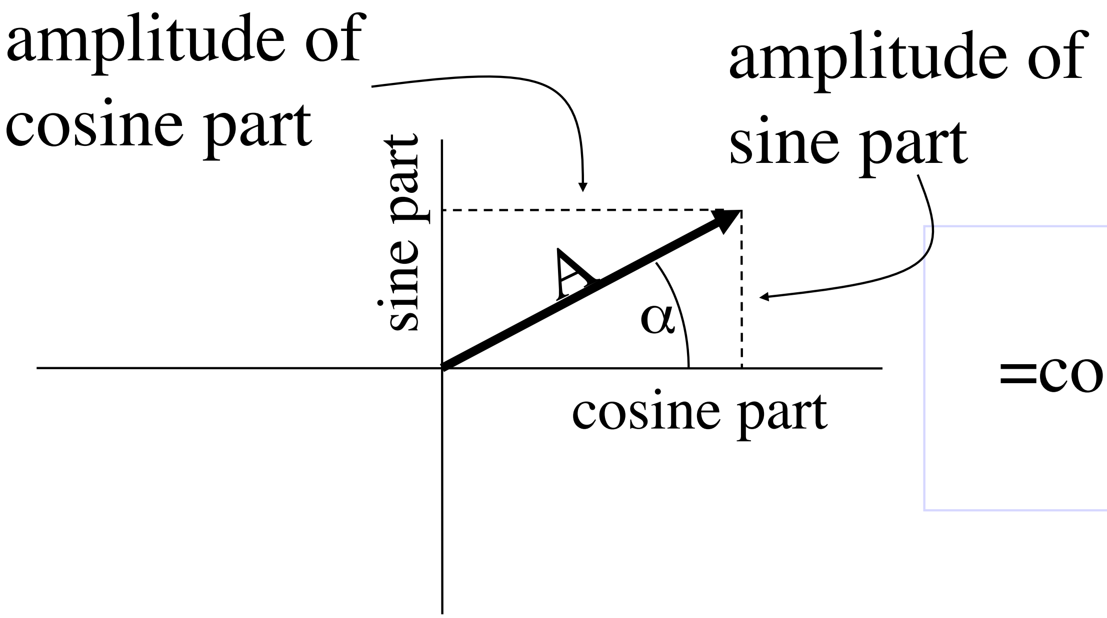
$$\cos(\alpha + \beta) = \cos \alpha \cos \beta - \sin \alpha \sin \beta$$

REVIEW of waves, so far

- Light is oscillating electrostatic potential.
- Light (photon) has a wavelength, an amplitude and a phase.
- Waves of the same wavelength interfere.
- Waves are summed by decomposing them into cosine and sine parts.

got it?

2-D wave space



x-axis
=cosine oscillator
= cosωt

y-axis
=sine oscillator = -sinωt

Length of wave vector is Pythagorian

proof

$$A = \sqrt{A^2\cos^2\alpha + A^2\sin^2\alpha}$$

Addition of wave vectors is Cartesian

proof

$$A_1 \cos(\omega t - \alpha_1) + A_2 \cos(\omega t - \alpha_2) =$$

$$A_1 \cos(\alpha_1) \cos \omega t - A_1 \sin(\alpha_1) \sin \omega t + A_2 \cos(\alpha_2) \cos \omega t - A_2 \sin(\alpha_2) \sin \omega t =$$

$$(A_1 \cos(\alpha_1) + A_2 \cos(\alpha_2)) \cos \omega t - (A_1 \sin(\alpha_1) + A_2 \sin(\alpha_2)) \sin \omega t$$

For mathematical convenience, a **wave** can be represented as a **complex number**.

Euler's Theorem: $$e^{i\alpha} = \cos\alpha + i\sin\alpha$$

Proof: write cos and *i* sin as Taylor series and sum them. You get the Taylor series for $e^{i\alpha}$.

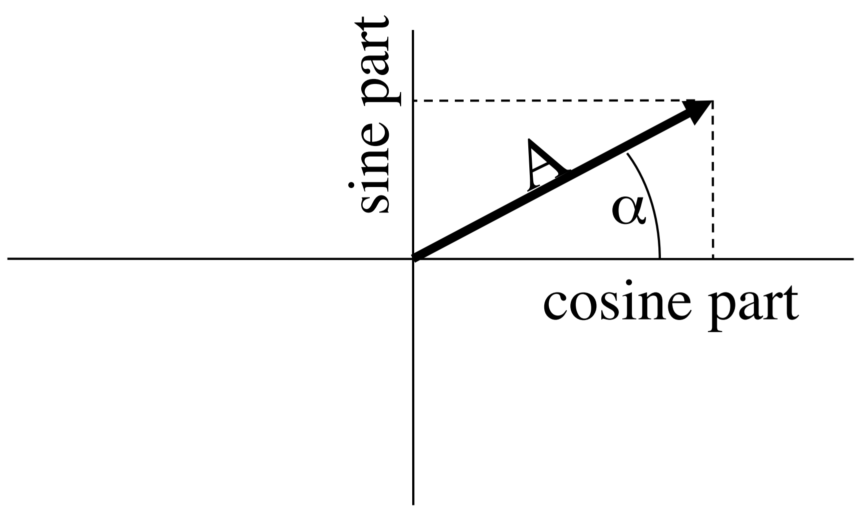
$$\cos\alpha = 1 - \alpha^2/2! + \alpha^4/4! - \alpha^6/6! - \cdots$$

$$i\sin\alpha = i\alpha - i\alpha^3/3! + i\alpha^5/5! - i\alpha^7/7! + \cdots$$

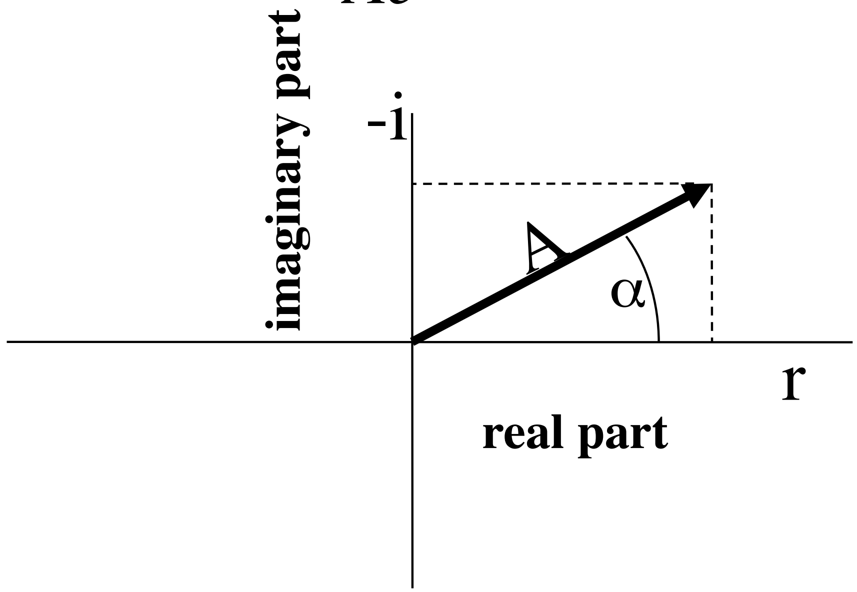
$$e^{i\alpha} = 1 + i\alpha - \alpha^2/2! - i\alpha^3/3! + \alpha^4/4! + i\alpha^5/5! - \cdots$$

Wave vector space = Argand space

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



$Ae^{i\alpha}$



Therefore, we may conveniently use complex numbers* for waves:

*Complex polar coordinates?

Euler notation (complex exponentials) is simply a convenient way to express a wave in the fewest keystrokes.

$$Ae^{i\alpha}$$

this is a wave of amplitude A and phase α

Summing complex numbers is mathematically equivalent to summing sines and cosines.

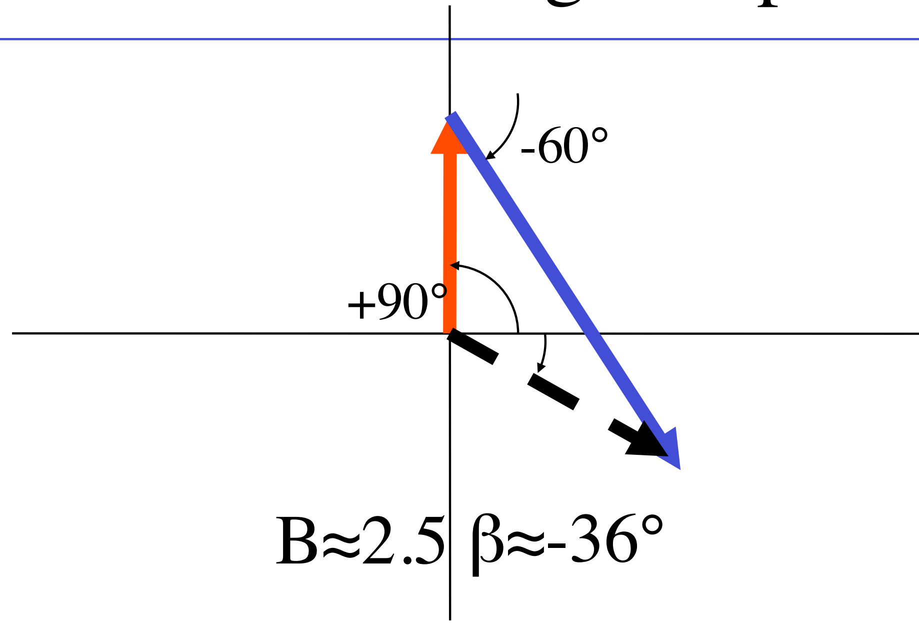
mathematical equivalents

	Cartesian		Polar	
$A\cos(\omega t + \alpha)$	$A\cos\alpha\cos\omega t$	$-A\sin\alpha\sin\omega t$	A	α
$(1,1) \equiv (\cos\omega t, -\sin\omega t)$	x	y	$\mathrm{sqrt}(x^2+y^2)$	$\tan^{-1}(y/x)$
$Ae^{i\alpha}$	$\mathrm{real}(Ae^{i\alpha})$ $=A\cos\alpha$	$\mathrm{imag}(Ae^{i\alpha})$ $=A\sin\alpha$	$\lvert Ae^{i\alpha}\rvert = A$	$\tan^{-1}(\mathrm{imag}(Ae^{i\alpha})/$ $\mathrm{real}(Ae^{i\alpha})) = \alpha$

for review

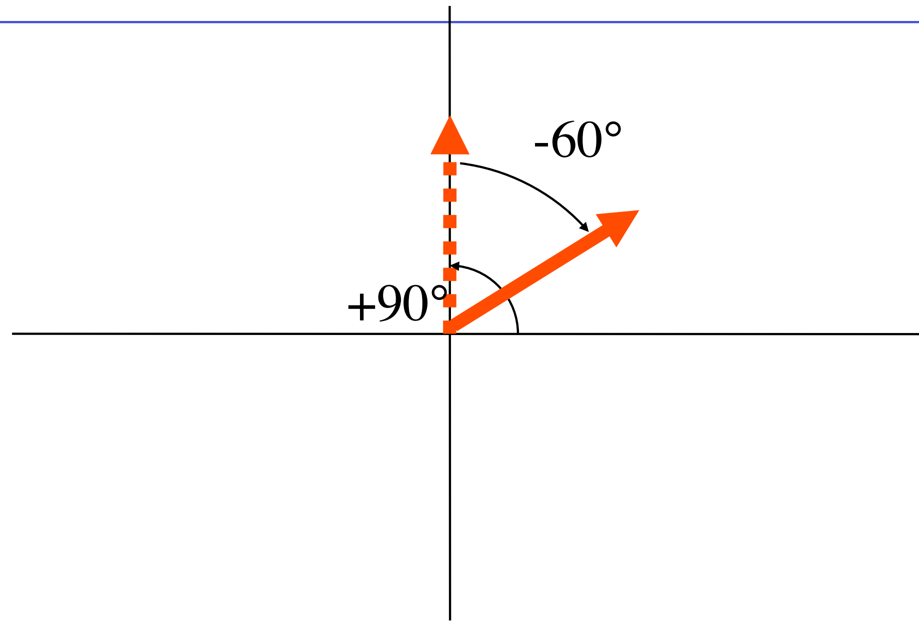
Wave addition is vector addition in Argand space

$A_1 e^{i\alpha 1} + A_2 e^{i\alpha 2}$



Multiplying complex exponentials = *phase shift*

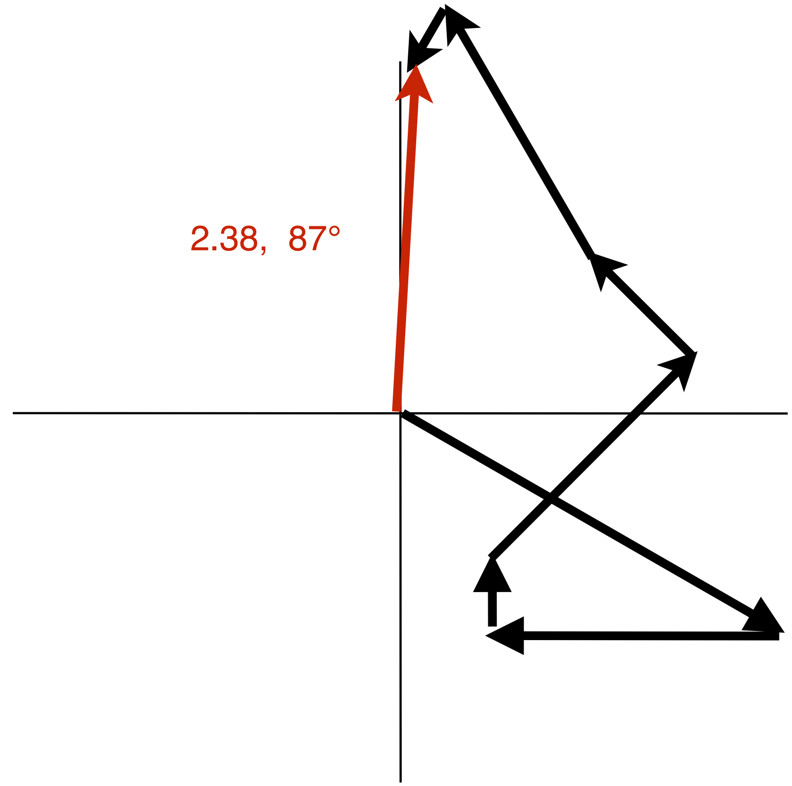
$A_1 e^{i\alpha 1}\, e^{i\alpha 2} = A_1 e^{i(\alpha 1 + \alpha 2)}$

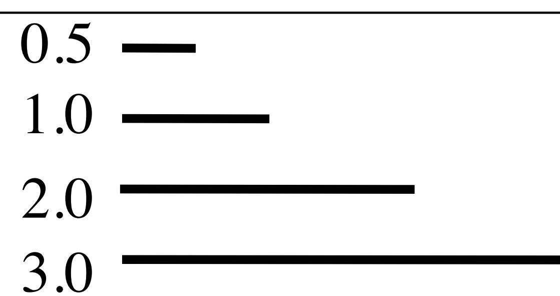


Try it: sum waves in Argand space

Start at the origin. Add head to tail.

3.0, -30°
2.0, 180°
0.5, +90°
2.0, +45°
1.0, +135°
2.0, +120°
0.5, -120°





Use these lengths.

more review

- Wave summation is equivalent to complex number summation.
- Complex numbers live in Argand space.
- Euler's theorem: $e^{i\alpha} = \cos \alpha + i \sin \alpha$
- $e^{i\alpha}$ is a "unit wave" with phase α and amplitude 1.
- $Ae^{i\alpha}$ is a wave with phase α and amplitude A,

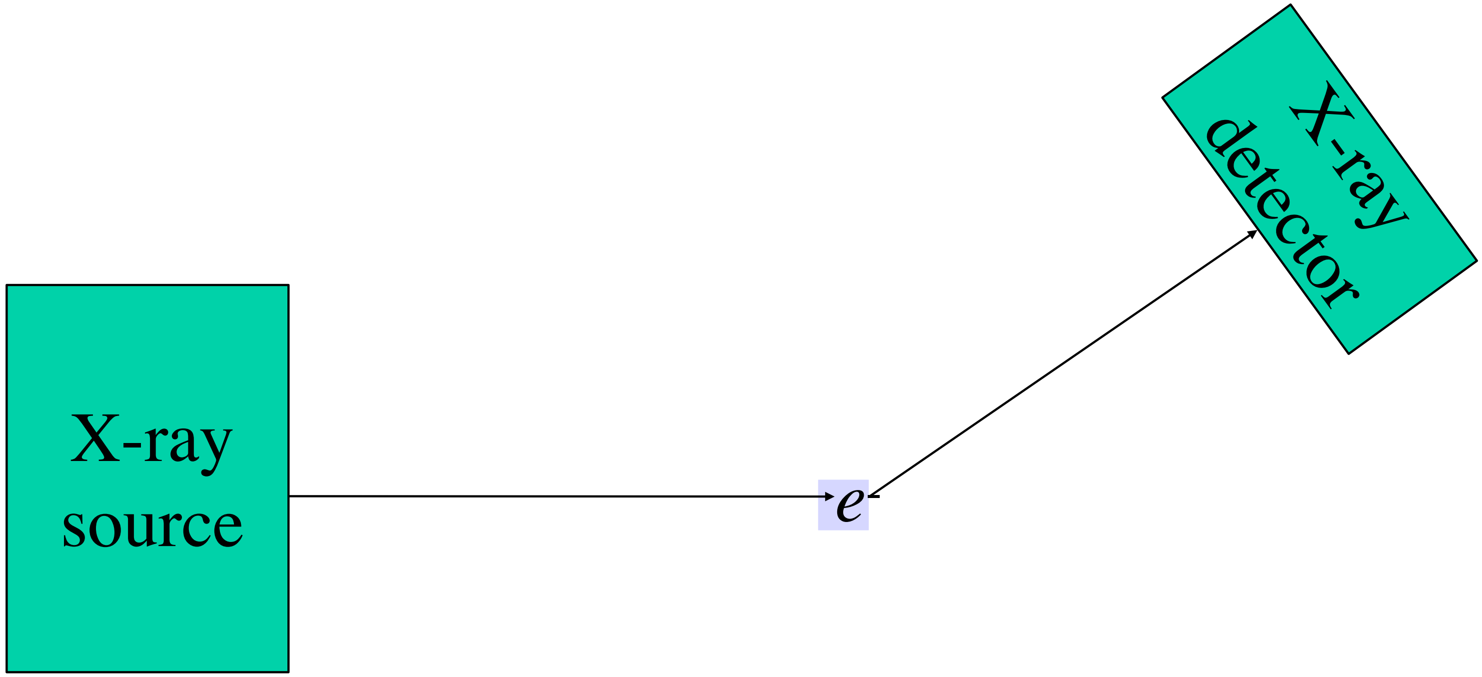
Seriously, knowing this stuff makes it easier.

Next topic……

Every electron has a location in the crystal relative to the origin. The location determines the *phase* of the scattered wave.

proof to follow...

the phase

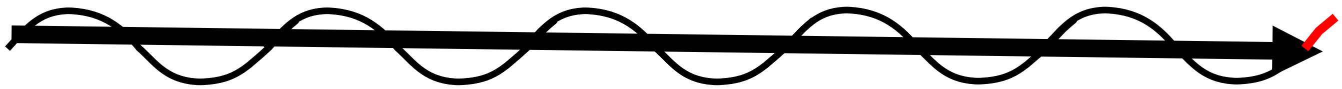


length/λ = the number of oscillations completed when hitting the plane of the detector

The phase is the non-integer part times 2π radians

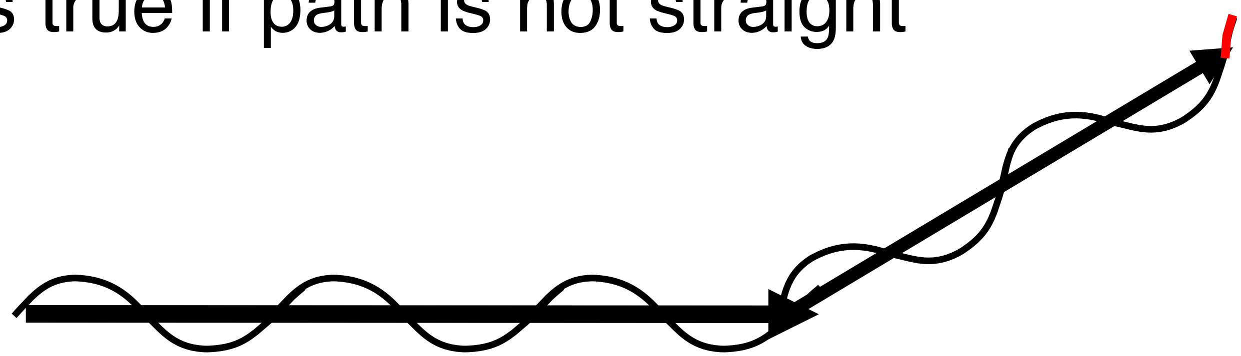
Exactly where the light turns the corner determines the phase.

Phase depends on the distance traveled



Phase = D/ λ – nearest integer(D/λ)

Same is true if path is not straight



Phase = D/ λ – nearest integer(D/λ)

Useful math review: the dot product

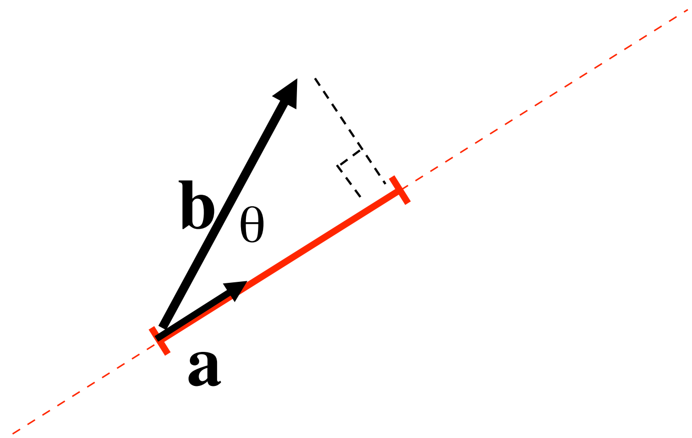
if you don't remember…

two equations: $\mathbf{a}\bullet\mathbf{b} = |\mathbf{a}||\mathbf{b}|\cos(\theta)$ $\mathbf{a}\bullet\mathbf{b} = a_x b_x + a_y b_y + a_z b_z$

where $\mathbf{a} = (a_x, a_y, a_z)$

a•b = Length of *projection* of **b** on the line containing **a**, times the lengh of **a**:

= Length of *projection* of **a** on the line containing **b**, times the lengh of **b**:



- If **a** is a unit vector, then **a•b** is the *length of the projection of* ***b*** *on* ***a*** (as shown above)
- If **b** is a unit vector, then **a•b** is the *length of the projection of* ***a*** *on* ***b***.
- **a•b = b•a**
- If **a** and **b** are both unit vectors, then **a•b** equals cos(θ).
- If **a** and **b** are orthogonal, then **a•b** equals zero.

Vector names used in these slides

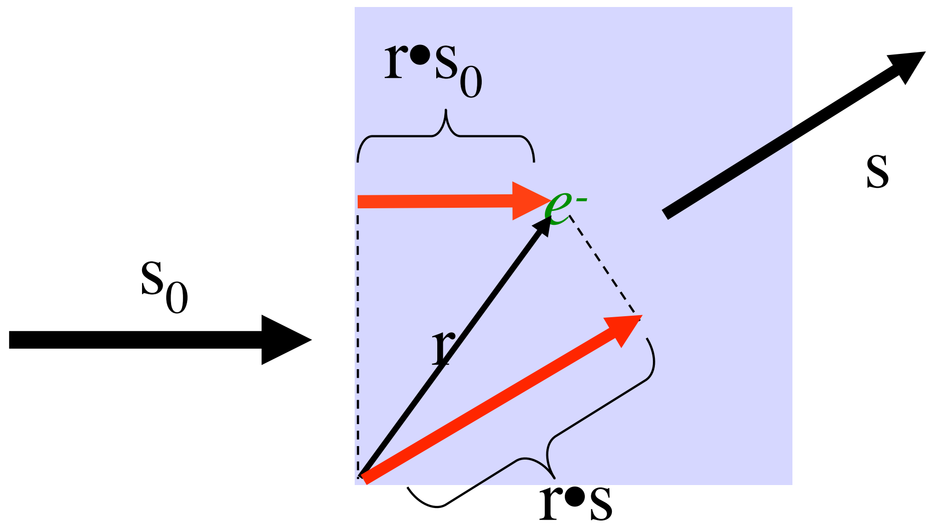
$\mathbf{s}$ bold lowercase s = a unit vector in the direction of the scattered Xray

$\mathbf{s}_0$ bold lowercase s, subscript zero = a unit vector in the direction of the incident Xrays

$\mathbf{S}$ bold uppercase **S** = the difference ($\mathbf{s} - \mathbf{s}_0$) divided by the wavelength λ.

Sorry about the confusion, but these are the names most textbook use.

The pathlengths for position r relative to the origin is the difference between their dot products

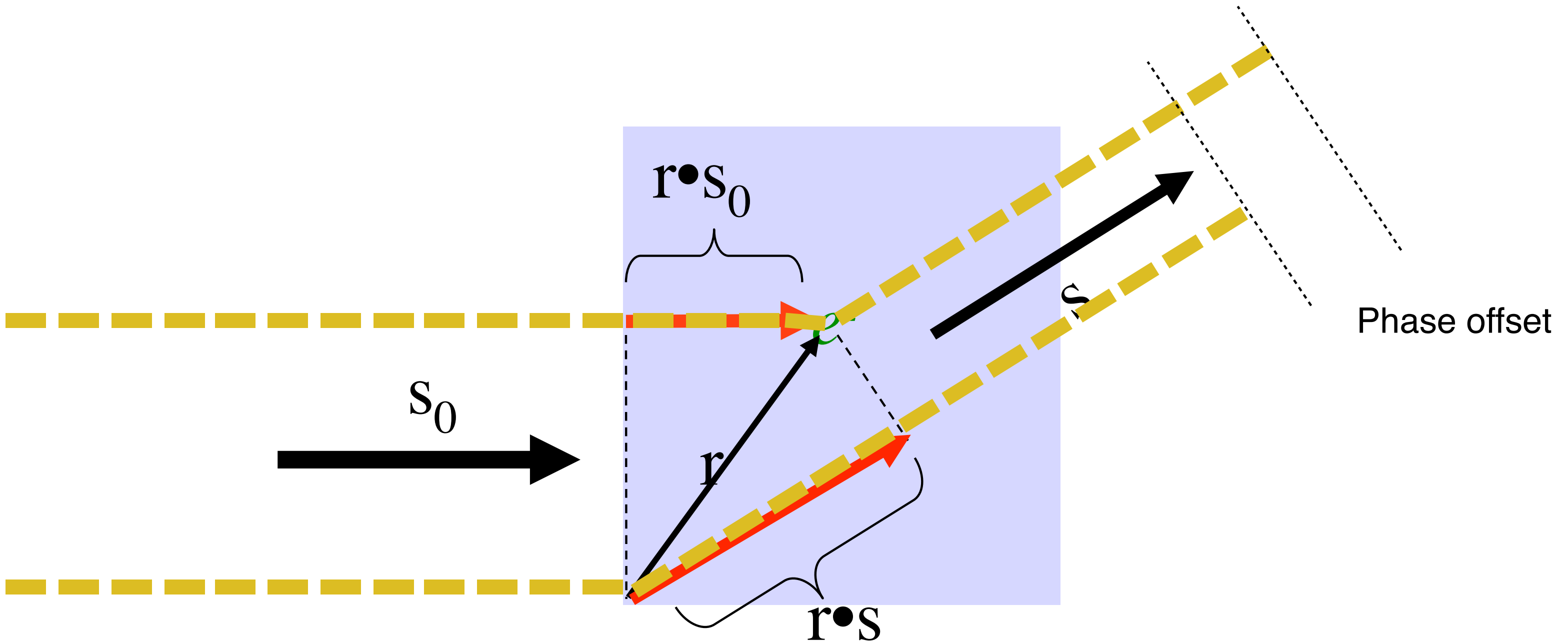


Difference in pathlength = $r \cdot s - r \cdot s_0$

phase at origin: zero by definition.

phase at r: $\alpha = 2\pi(r \cdot s - r \cdot s_0)/\lambda$

don’t believe me? watch the animation on the next slide.



imagine a marching band.

Definition of scattering vector *S*, *a vector in "reciprocal space"*

phase at *r* *(see last slides)*

$$\alpha = 2\pi(r \bullet s - r \bullet s_0)/\lambda$$

factoring

$$(r_1 \bullet s - r_1 \bullet s_0)/\lambda = r_1 \bullet (s - s_0)/\lambda$$

definition

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

substituting:

$$\alpha = 2\pi\, S \bullet r$$

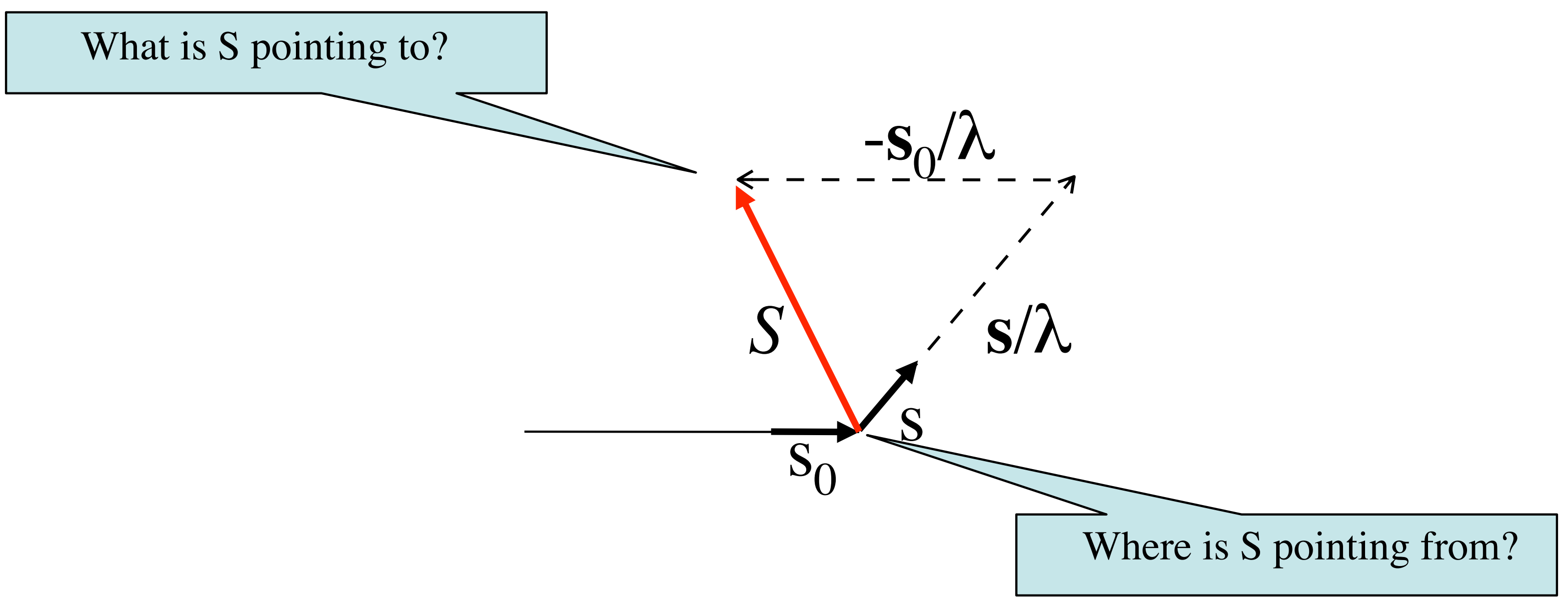
Units cancel.

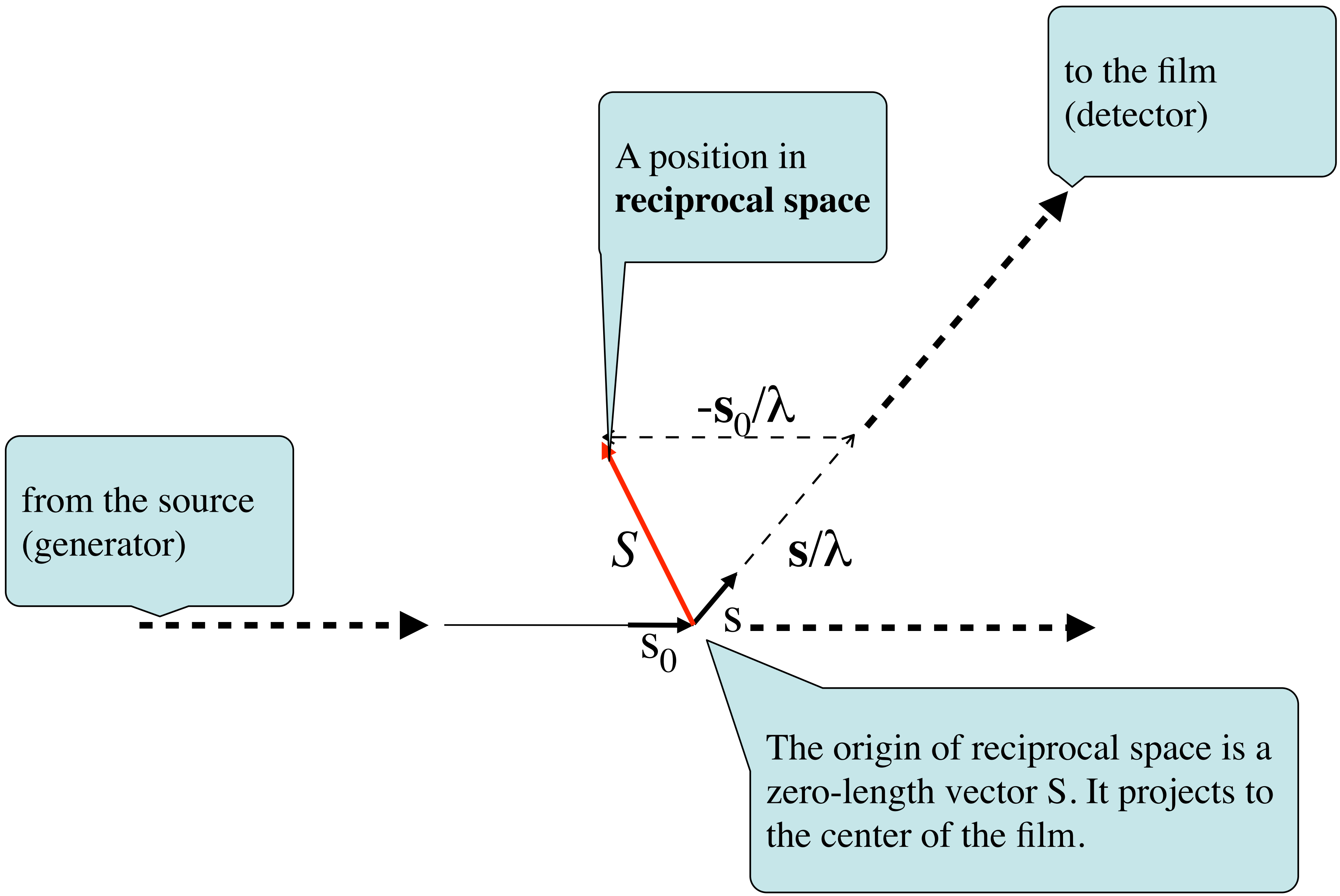
Angles without units are in radians.

Note the $Å^{-1}$ units.

S points to something, but what?

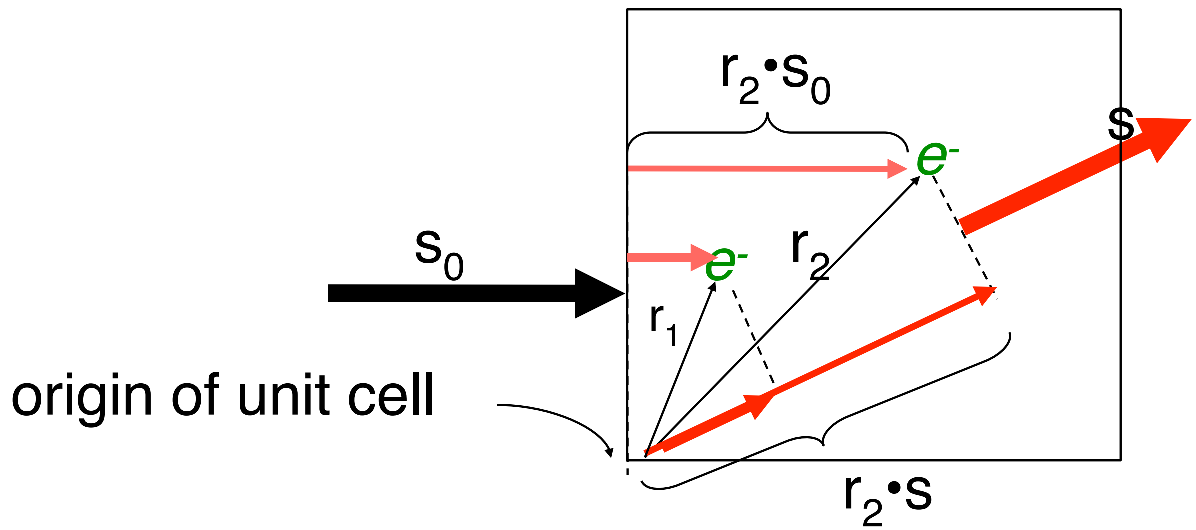
$$S \equiv (s - s_0)/\lambda$$




to the film
(detector)
A position in
reciprocal space
-$\mathbf{s}_0/\lambda$
from the source
(generator)
S
$\mathbf{s}/\lambda$
s
s_0
The origin of reciprocal space is a zero-length vector S. It projects to the center of the film.

Scattering factor for two or more regions of e^- density.

Pick any two locations in space, $\boldsymbol{r}_1$ and $\boldsymbol{r}_2$, and a direction of scatter $\boldsymbol{s}$ (a unit vector). **What is the amplitude and phase of the scattered X-rays?**



$$\alpha_1 = 2\pi\, S \bullet r_1$$

$$\alpha_2 = 2\pi\, S \bullet r_2$$

$$F(S) = A_1 e^{i\alpha_1} + A_2 e^{i\alpha_2}$$

Now we can generalize it. If we sum over <u>all points</u> k,

$$F(S) = \sum_k A_k e^{i2\pi S \bullet r_k}$$

Amplitude of scatter from a point is proportional to its electron density.

The amplitude of scatter from each infinitesimal volume unit **d*r*** *is* proportional to the number of electrons, which is the electron density at the point times the volume unit **d*r.***

$$A_k = \rho\left(r_k\right)$$

so the total wave summation can be written as

$$F(S) = \sum_k \rho(r_k) e^{i2\pi S \cdot r_k}$$

summed over all r_k the unit cell.

Fourier transform is the sum waves from *all* points in the crystal to S

The amplitude of scatter from each volume unit **d*r*** *is* proportional to the electron density at the point, **ρ*(r)*,** times the volume unit **d*r*,** and the phase is 2πS•r. This is summed over all ***dr*,** so the total wave summation can be written as

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

Please note: this is really a triple integral: *dr* is *dx dy dz*

more review

- When a wave turns a corner (scatters from), its phase depends on where it turned the corner.
- We arbitrarily choose the origin (scatter from the origin) to have phase = 0.
- The phase for a wave scattered from incident unit vector s_0 to scattered unit vector s, turning at r is
  $\alpha = 2\pi(r \cdot s - r \cdot s_0)/\lambda$
- The Scattering vector **S** (capital S) is defined as
  $(s - s_0)/\lambda$
- S is a vector in “reciprocal space” the inverse of real space where the units are reciprocal distances.
- The amplitude of scatter from point r is proportional to the number of electrons at r.

pop quiz

- How are protein crystals grown?
- What is symmetry?
- What are X-rays?
- Why do electrons scatter X-rays?
- What is the phase of a wave?
- Why can waves be expressed as vectors?
- How does the position of an electron relative to the origin determine its phase?
- In what units is the Fourier transform of 3D space?

Exercise 2 — adding waves -- due Mon. Oct 26

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

part 1

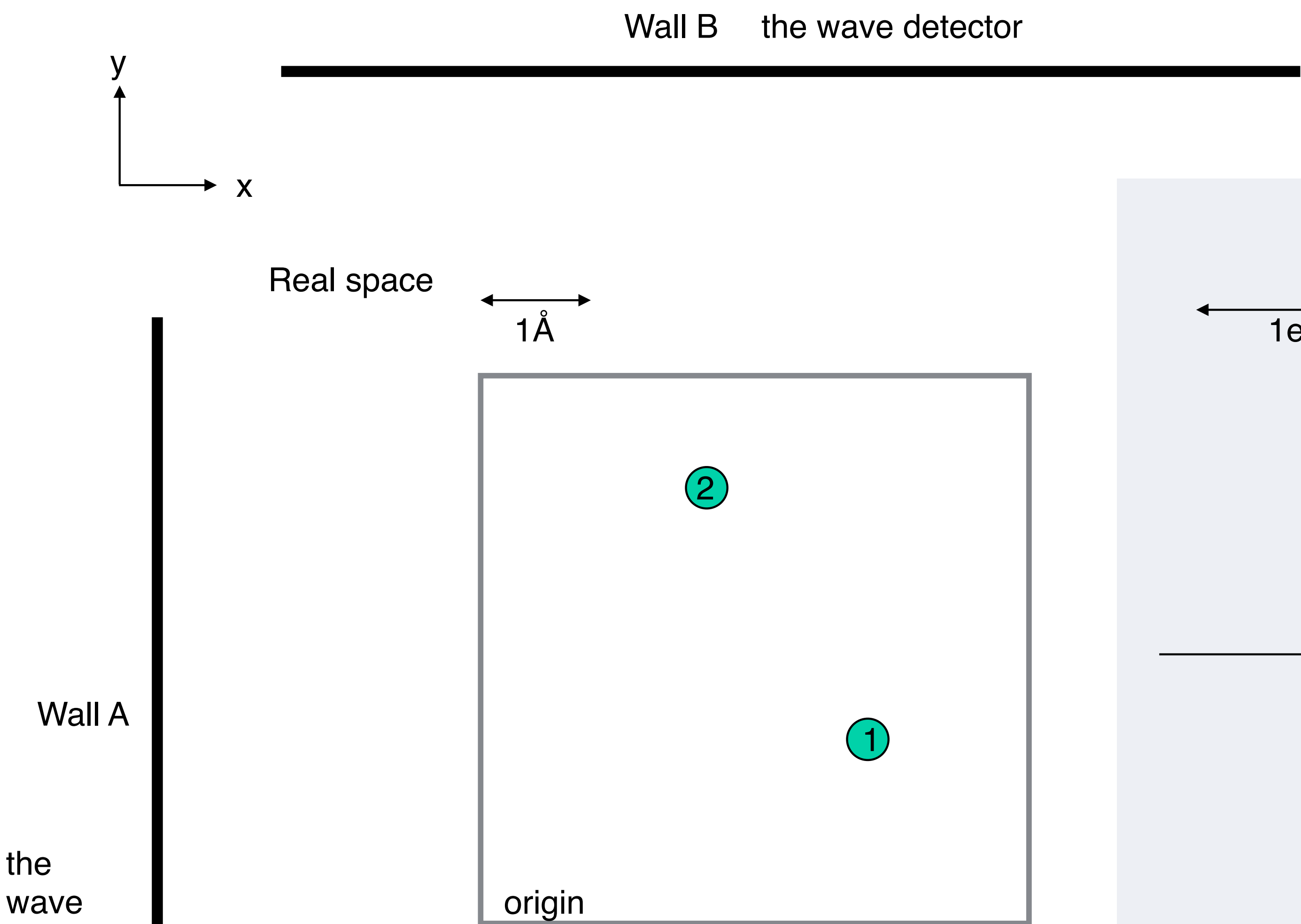
(1) Look at the setup on the next page, a square unit cell of width 5.00Å, with 2 hydrogen atoms in it. Xrays come in from the left, scatter at $2\theta=90°$.

(2) Measure the distance traveled from Wall A to Atom 1 (r_1) to Wall B, traveling along beam direction $\mathbf{s_0}= (1, 0, 0)$ and scattered wave $\mathbf{s}= (0, 1, 0)$, respectively. Divide by the wavelength. Multiply by 2π (or 360) to get the phase in radians (or degrees).

(3) Do the same for Atom 2 (r_2). Fill in Table 1.

(4) Add the two waves in Argand space (slide 20 of this lecture). Measure the resulting length (amplitude A) and phase (α).

Exercise 2 — copy this page and draw on it — due Mon. Oct 26



add the two scattered waves in

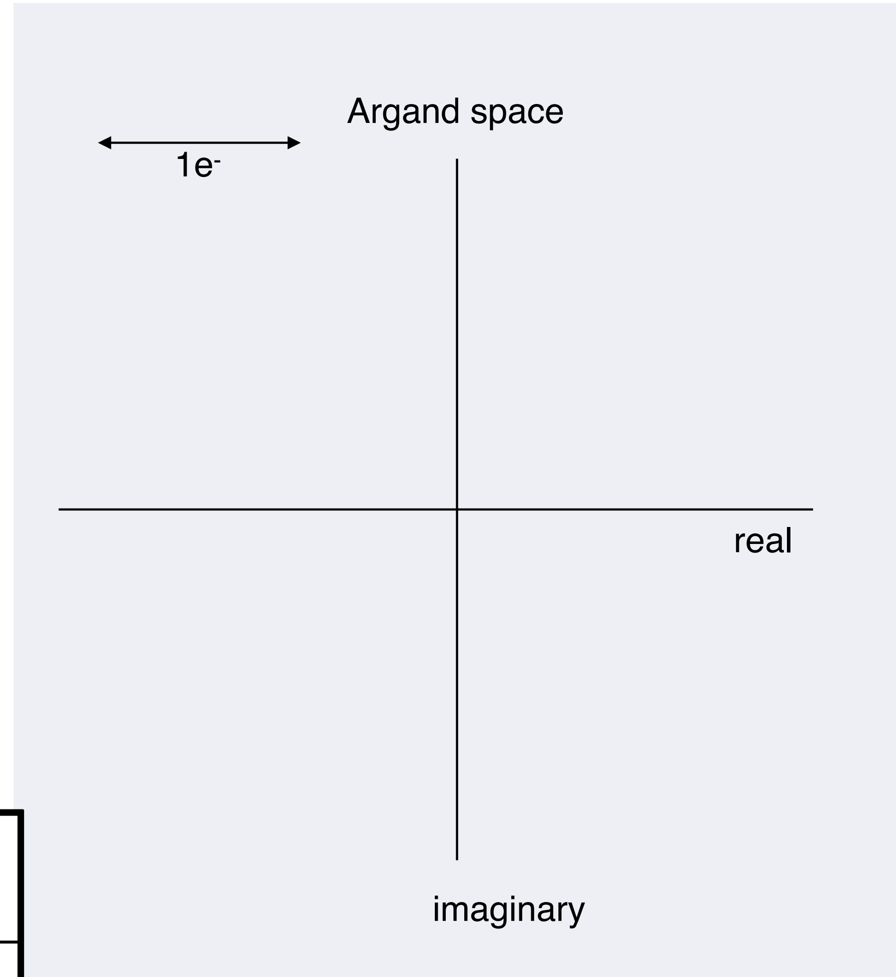


Table 1	Distance traveled	subtract origin distance traveled	phase (°) if wavelength = 1.54Å
origin			
r_1			
r_2			

Amplitude (A)	
phase (α)	

Exercise 2 — part 2

Calculate the wave sum using the Fourier transform

$$F(S) = \sum_k \varrho(r_k) e^{i2\pi S \bullet r_k}$$

$\lambda = 1.54Å$

$\mathbf{s_0} = (1, 0, 0)$

$\mathbf{s} = (0, 1, 0)$

$\mathbf{S} = (\mathbf{s} - \mathbf{s_0})/\lambda$ = (___ ___ ___)

Table 2	Measure Å coordinates of r_k relative to origin from previous page.	$A_k = \varrho(r_k)$	$\alpha_k = 2\pi\ S \bullet r_k$	$A_k \cos(\alpha_k)$	$i\ A_k \sin(\alpha_k)$
k=1					
k=2					

sum		
Amplitude (A) = | (imag, real) |		
phase (α) = $\tan^{-1}$(imag/real) (in degrees)		