Announcements 4/29/10

PS 13 due next Weds but if you turn it in early I will give you the solutions early also

Information about final exam posted on web site (handouts area) under "Final exam", review assignment coming

I will have office hours 1:30 – 3:15 every weekday between now and the exam, review session Monday 5/10.

J.J.

4/29/10

Today

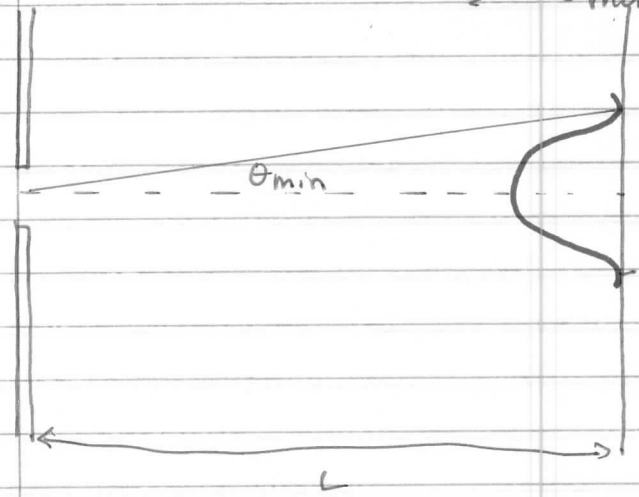
1. Wrap diffraction through single slit
2. Diffraction through circular aperture
   - Now aperture is small in all directions → diffraction in all directions
   - Limits on resolution of images with lenses: focused spot is at least θ_{min}!
3. How confocal microscopy allows getting 3D images of (mostly transp.) objects with maximum detail
   - Fluorescence allows eliminating interference from reflections
   - Illuminate object one tiny spot at a time: allows 3D imaging by eliminating ~~background especially~~ light from out-of-focus planes

Diffraction through slit:

light spreads to form pattern w/ central maximum

width of central max determined by angles to first minima:

$$\sin\theta_{min} = \frac{\lambda}{a}$$



y_{min} from trig

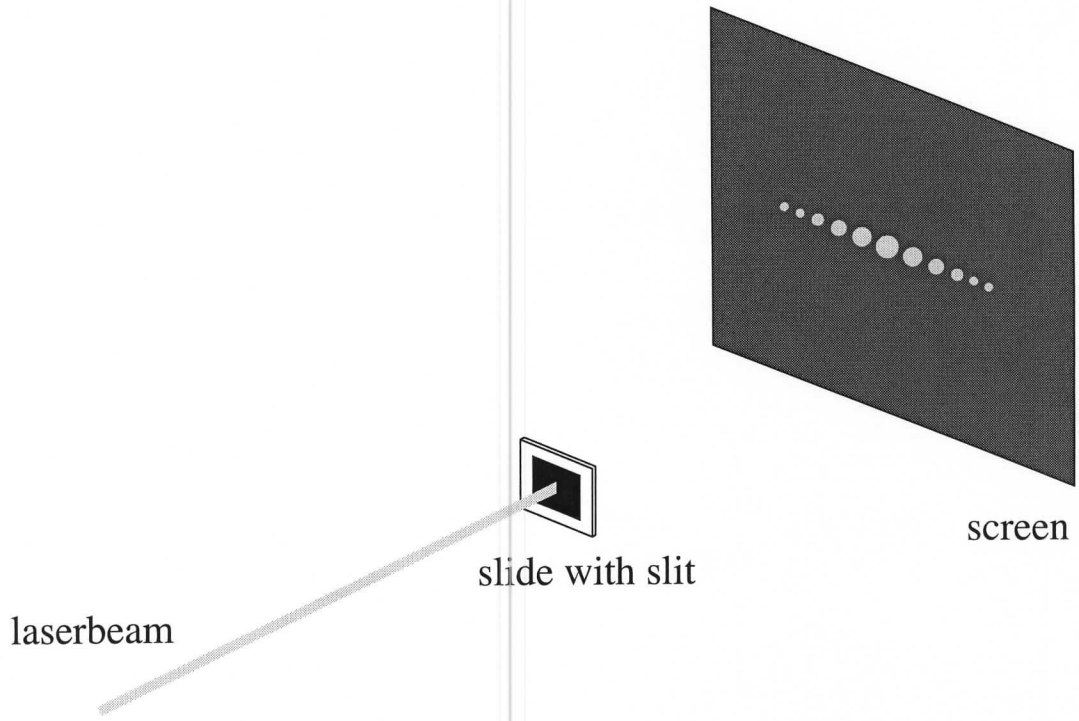
$$\sin\theta_{min} = \frac{y_{min}}{\sqrt{L^2+y_{min}^2}} \approx \frac{y_{min}}{L}$$

$$\Rightarrow \frac{y_{min}}{L} \approx \frac{\lambda}{a}$$

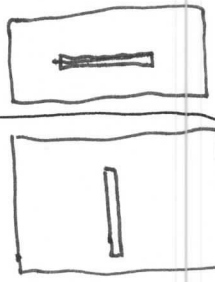
two minima are distance

$$2y_{min} = \frac{2\lambda L}{a} \text{ apart}$$

The pattern on the screen is due to a narrow slit that is



1. horizontal.
2. vertical.



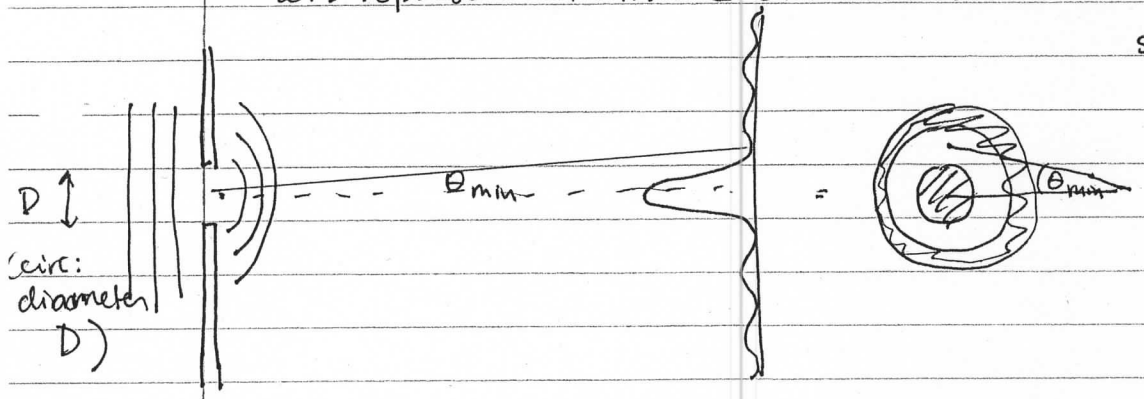
Diffraction through slit is spread out in just one direction
b/c slit is narrow in just one direction

CT Spreading is in same direction as narrow dimension of slit: narrow dimension → point sources that spread spherically

What if slit is narrow both ways? Get diffraction both ways
Square → cross pattern
Most useful shape: circle

(many of you have seen this in microscopes - dust grains!)

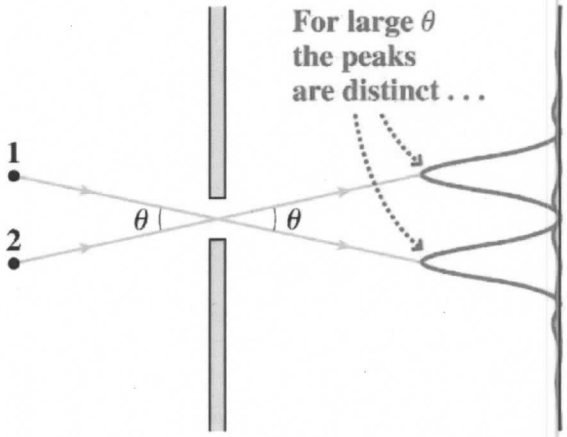
DEMO circ aperture: you have almost all seen this in lab now
let's represent it in the overhead view:



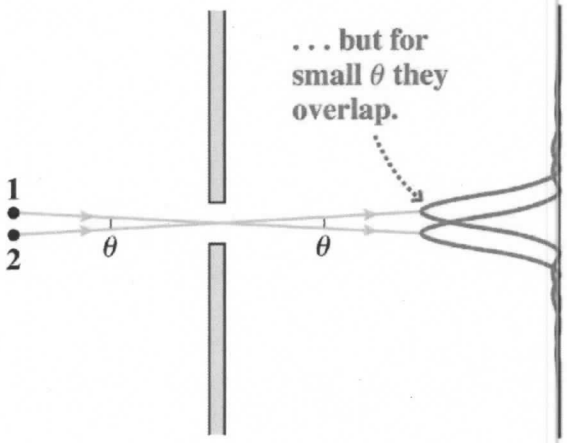
$$\sin\theta_{min} = \frac{1.22\lambda}{D}$$

Why 1.22? more spreading than a slit of width D b/c aperture is narrower than D above/below mid

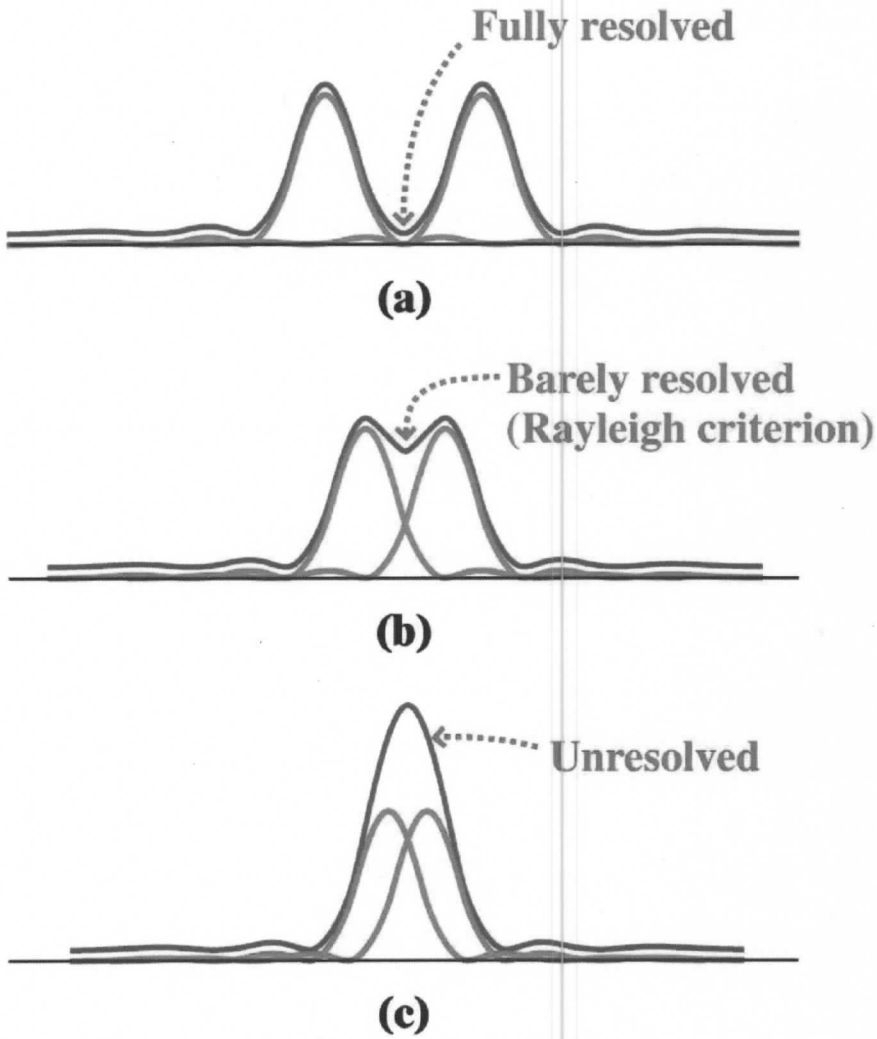
This diffraction limits the size of details in an image formed by a lens

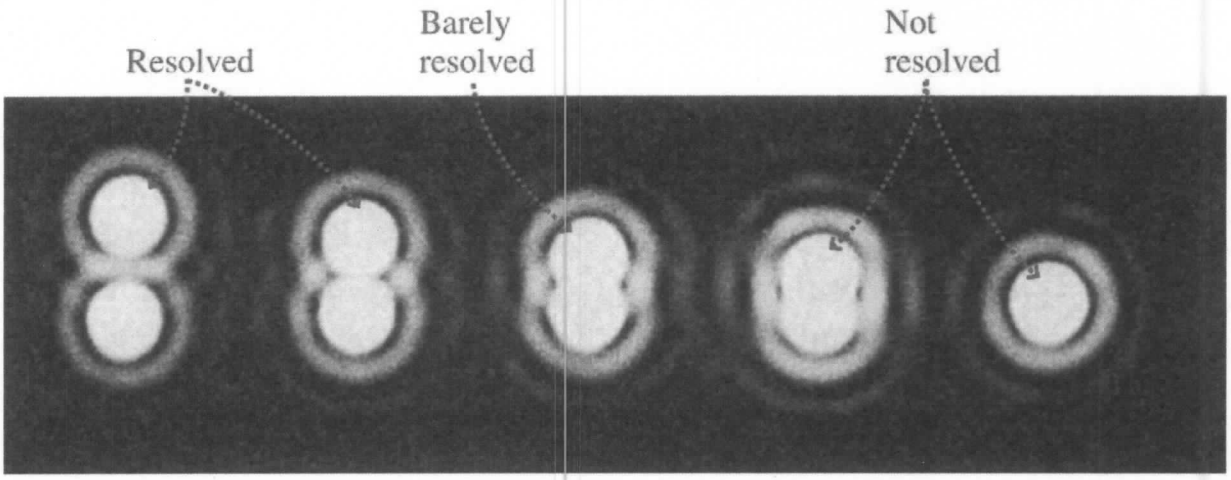


(a)



(b)



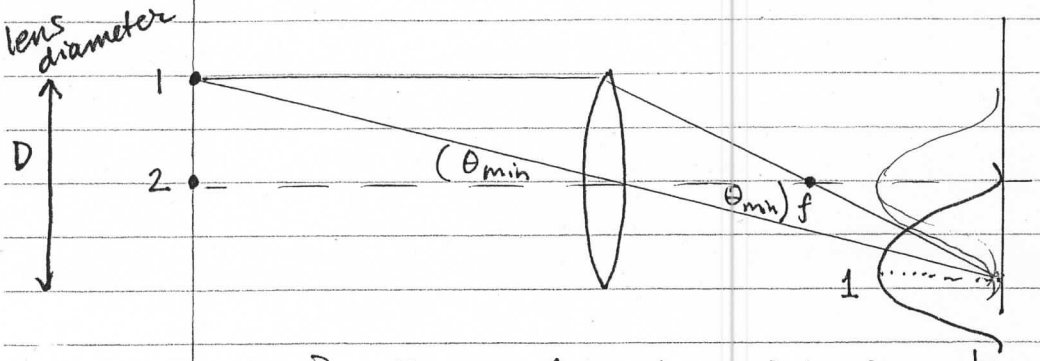


Our critical question:

Suppose there are two tiny organelles in a cell that we want to see in a microscope image.

What determines whether they can be distinguished in the image — i.e. "resolved"?

Fundamentally: the angular separation determines this



Why? Image of each point is not a point but a diffraction pattern — location of the point in the image = center of bright spot

Can tell where the peaks are if no closer than having max of 1 at same place as min of 2

How big are the diff patterns?

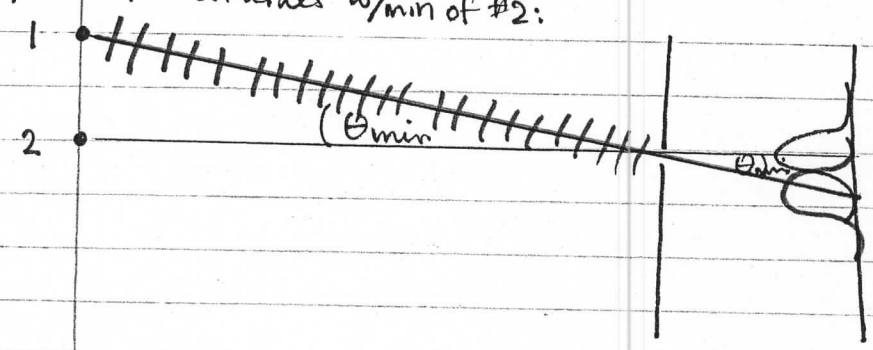
Let's consider what we'd get if we had two distant (aka point sources) → light from them = plane waves

Light just comes through hole, no lens

If 1 & 2 are θ_{min} apart, max of #1 coincides w/min of #2:

2 comes straight through → max at center, 1st min at

$$\sin\theta_{min} = \frac{1.22\lambda}{D}$$

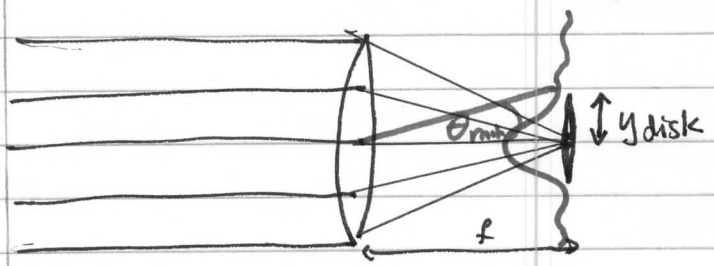


1 comes down @ angle θ - its max is where light goes its min is angle θ_{min} away

Turns out that angular size of focused spot from a lens is still affected by diffraction in the same way

Consider source #2 focused by a lens of diameter D:

(handout)



Geo optics says these rays all meet at a point
Diffraction through lens says the point must be a bull's-eye
center spot:
~~base~~ angular size θ_{min} where

$$\sin\theta_{min} = \frac{1.22\lambda}{D_{lens}}$$

How large the spot is depends on f – bigger f ⇒ bigger y_{disk}
Fundamentally its size is θ_{min}
But sometimes we want to know y_{disk}:

$$\sin\theta_{min} = \frac{y_{disk}}{\sqrt{f^2 + y_{disk}^2}} \approx \frac{y_{disk}}{f} \quad \text{for small } \theta_{min}$$

$$\Rightarrow y_{disk} \approx \frac{1.22\lambda}{D_{lens}} f$$

Problem:

$$y_{disk} = \frac{1.22(550\times10^{-9}\,m)(0.25\,m)}{(0.10\,m)} = 1.68\times10^{-6}\,m\ !!$$

Ratio of areas:

$$S = \frac{\text{power}}{\text{area}} \quad \text{so} \quad \frac{S_{spot}}{S_{sunlight}} = \frac{\text{power}/A_{spot}}{\text{power}/A_{lens}} = \frac{A_{lens}}{A_{spot}}$$

$$= \frac{\pi D_{lens}^2/4}{\pi D_{spot}^2/4} = 8.7\times10^{8}\ !!$$

You are trying to burn a hole in a piece of paper by focusing sunlight with a magnifying glass. The lens of the magnifying glass is 10 cm in diameter and has a focal length of 25 cm.

(a) How big is the spot of focused sunlight? Choose a reasonable value for the "wavelength" of sunlight (sunlight includes the entire visible spectrum but we'll just consider the focusing of a single wavelength).
(b) By what factor has the intensity of light in the spot increased, compared to the intensity of the sunlight on the lens?
(c) Now considering the full range of wavelengths in sunlight: what color of the visible spectrum would be on the outside of the focused spot?

largest $y \rightarrow$ largest $\lambda \rightarrow$ red

So minimum angular size of a focused spot is θ_{min}.
How can we shrink this? Only 2 parameters:

smaller λ

bigger D_{lens}

We'll get back to D_{lens} later – other constraints

Key way that λ can be shrunk

- change color

ask CT

- focus in material with $n > n_{air}$: water or oil

$\Rightarrow$ smaller $\lambda = \frac{\lambda_{vac}}{n}$!

The same limits apply to forming focused images of point sources – the image of a point will also have angular size θ_{min}

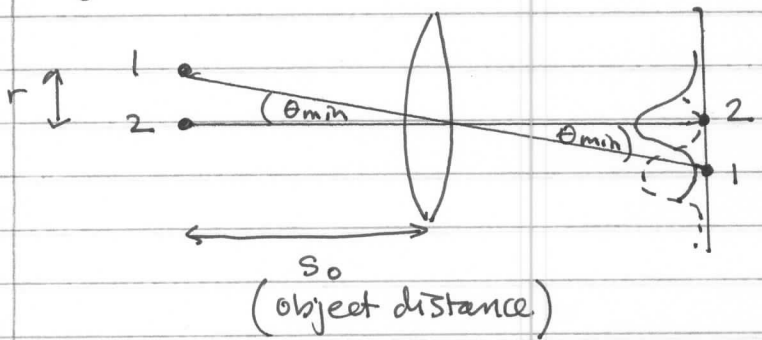
For a simple thin lens we still have that

$$\sin\theta_{min} = \frac{1.22\lambda}{D_{lens}} = \frac{1.22\,\lambda_{vacuum}}{n D_{lens}}$$

where n = index of refraction of medium surrounding sample & lens

What does this dictate for the linear distance between distinguishable details in a sample?

Again the two points must be separated by θ_{min}:



Let's call the smallest distinguishable distance r for "resolution"

$$\sin\theta_{min} = \frac{r}{\sqrt{s_o^2 + r^2}} \approx \frac{r}{s_o} \text{ for small } \theta_{min}$$

$\Rightarrow$ Smallest resolvable distance r between details in sample is dictated by

$$\frac{r}{s_o} = \sin\theta_{min} = \frac{1.22\lambda_{vac}}{n D_{lens}} \Rightarrow r = \frac{1.22\lambda_{vac}}{n D_{lens}} s_o$$

How does the angular size θ_{min} of the central diffraction peak formed in water ($n_{\text{water}} = 1.33$) compare to that formed in air ($n_{\text{air}} = 1.00$)?

1. Bigger in water than in air.
2. Bigger in air than in water.
3. The size of the disk does not depend on whether the light is focused in water or air.

Angular size is smaller:

$$\sin\theta_{min} = \frac{\lambda}{D_{lens}}$$

In water $\lambda = \frac{\lambda_{vacuum}}{n_{water}} = \frac{\lambda_{vacuum}}{1.33}$

In air $\lambda = \frac{\lambda_{vacuum}}{n_{air}} = \lambda_{vacuum}$ b/c $n_{air} = 1$

Microscope limits of resolution

Microscope objectives are much more complex – now we have strongly focused light, sources very near the lens, etc

Diffraction is still the limit to resolution!
Solve much more complex diff eqs to find how diff pattern forms but underlying principle is same

$\Rightarrow r = \frac{1.22 \lambda_{med} s_o}{n D_{lens}}$ becomes $r = \frac{1.22 \lambda_{vac}}{2NA} = 0.61 \frac{\lambda_{vac}}{NA}$

with NA "numerical aperture"
analogous to $\frac{n R_{lens}}{s_o}$ $(NA = n \sin\alpha)$

Tells you r depends on λ and NA
- NA must be less than 1 for lenses in air, but immersion lens has $n > 1 \Rightarrow$ NA up to n
- Still true that shorter $\lambda \Rightarrow$ better resolution

This is why electron microscopes → even finer detail!

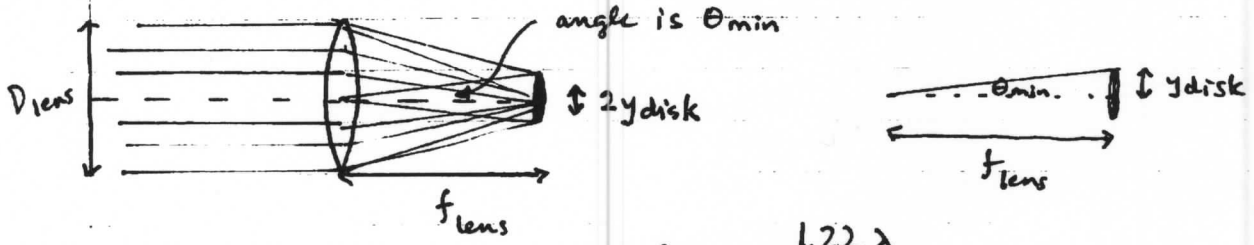
So, big picture result is:

- smallest resolvable distance in something you are looking at is set by λ_{vac} of the light forming the image and the numerical aperture of the microscope objective
- shorter λ → better resolution
  immersion in oil or water → better resolution

Can't do better than $\sim \frac{1}{2}\lambda$ because NA is only up to about n

Circular aperture diffraction

Parallel rays focus to a spot of radius y_{disk}:

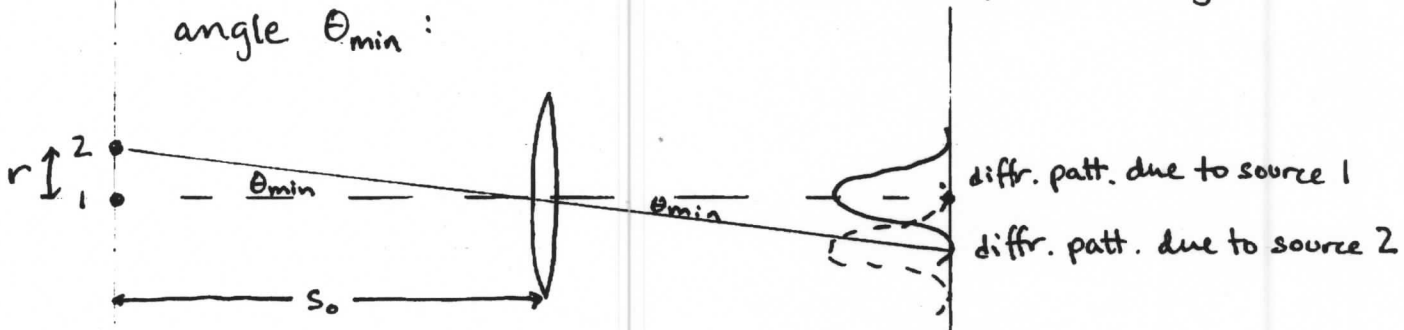


$$\sin\theta_{min} = \frac{1.22\lambda}{D_{lens}}$$

Small θ_{min}: $\sin\theta_{min} \approx \tan\theta_{min} = \frac{y_{disk}}{f_{lens}}$

$$\Rightarrow \quad y_{disk} = \frac{1.22\lambda f_{lens}}{D_{lens}}$$

Two resolvable point sources must be separated by angle θ_{min}:



Again $\sin\theta_{min} = \frac{\lambda\, 1.22}{D_{lens}} = \frac{1.22\lambda_{vacuum}}{n D_{lens}}$

$$\sin\theta_{min} \approx \frac{r}{s_0} = \tan\theta_{min} \quad \Rightarrow \quad \frac{r}{s_0} = \frac{1.22\lambda_{vac}}{nD_{lens}} \Rightarrow r = \frac{1.22\lambda_{vac} s_0}{nD_{lens}}$$

For a microscope: numerical aperture (NA) is analogous to $\frac{nR_{lens}}{s_0}$ $\Rightarrow$ $r = \frac{1.22\lambda_{vac}}{2NA} = \frac{0.61\lambda_{vac}}{NA}$