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Microscope Imaging

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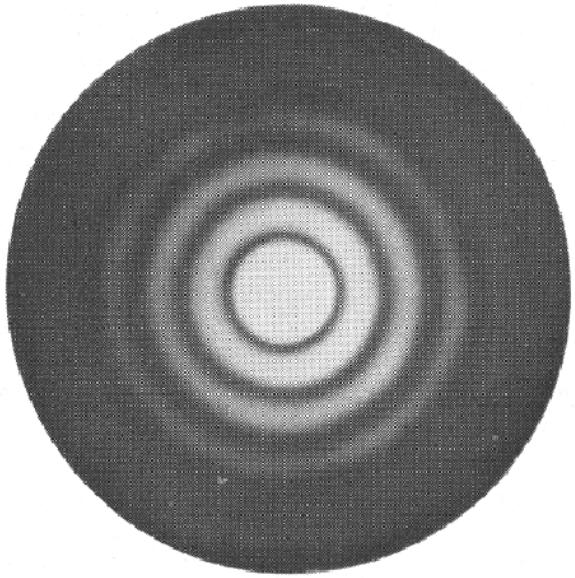
Optical microscope

- Objective lens
  - Numerical aperture ($n \sin \alpha$)
  - Air / oil immersion / water immersion
  - Corrected for cover slip (No. $1\ ^1/_2$ = 0.17mm) or not
  - Corrected for infinity or not
  - e.g 100X 1.4NA Oil 0.17/∞
- Eyepiece
- Illumination system
  - Condenser
  - Aperture stop (diaphragm)
  - Field stop

Airy disc



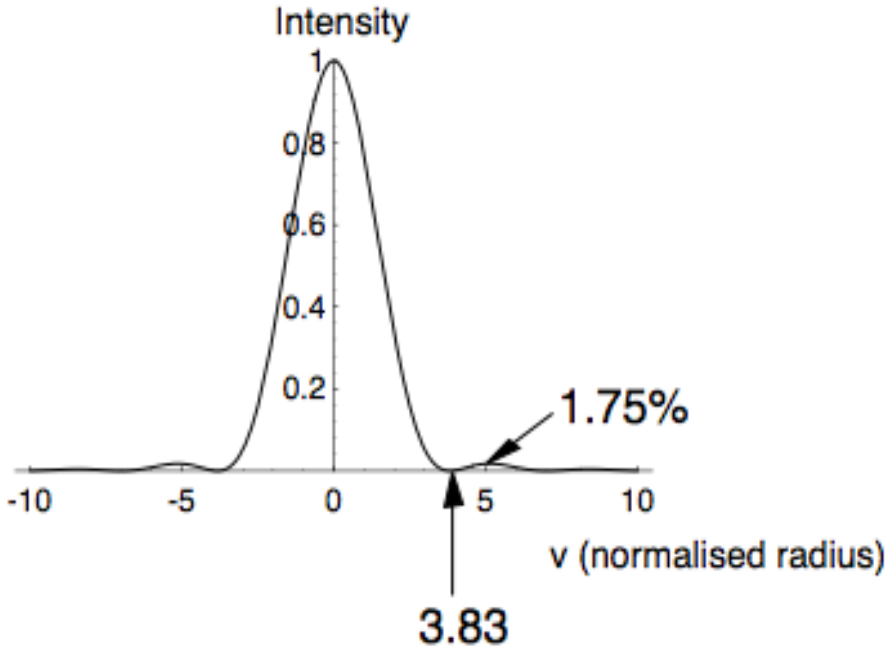


Fig. 8.12. FRAUNHOFER diffraction pattern of a circular aperture (the AIRY pattern) 6 mm in diameter, magnification 50×, mercury yellow light $\lambda = 5790$ Å. To show the existence of the weak subsidiary maxima, the central portion was overexposed. (After H. LIPSON, C. A. TAYLOR, and B. J. THOMPSON.)

$$\frac{2J_1(v)}{v} \to 1 \text{ for } v \to 0$$

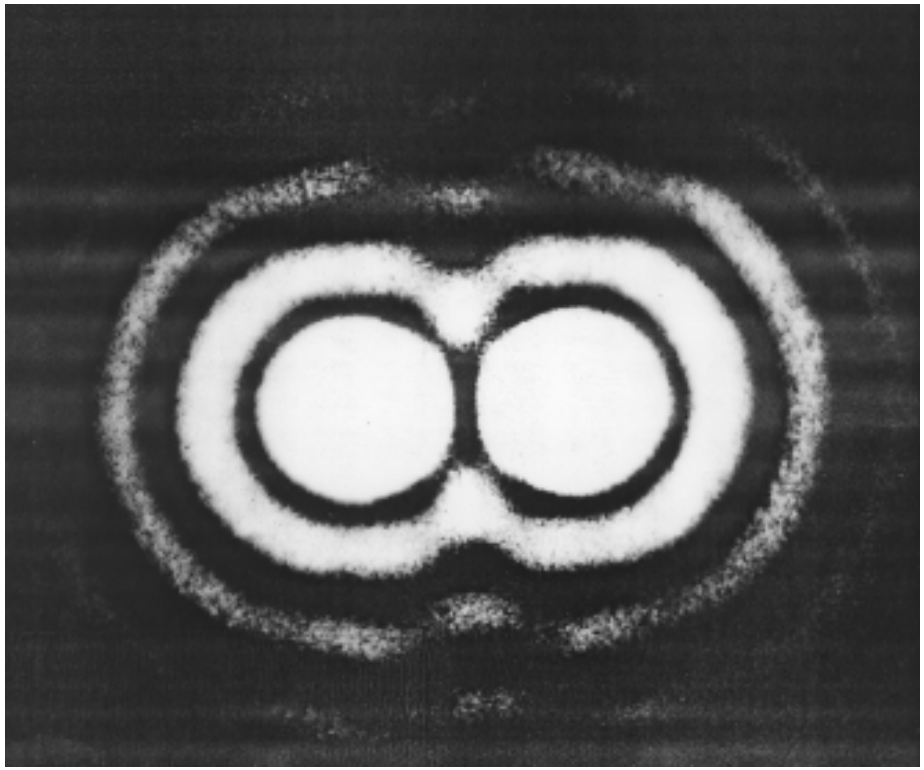
Born & Wolf

J_1 is a Bessel function

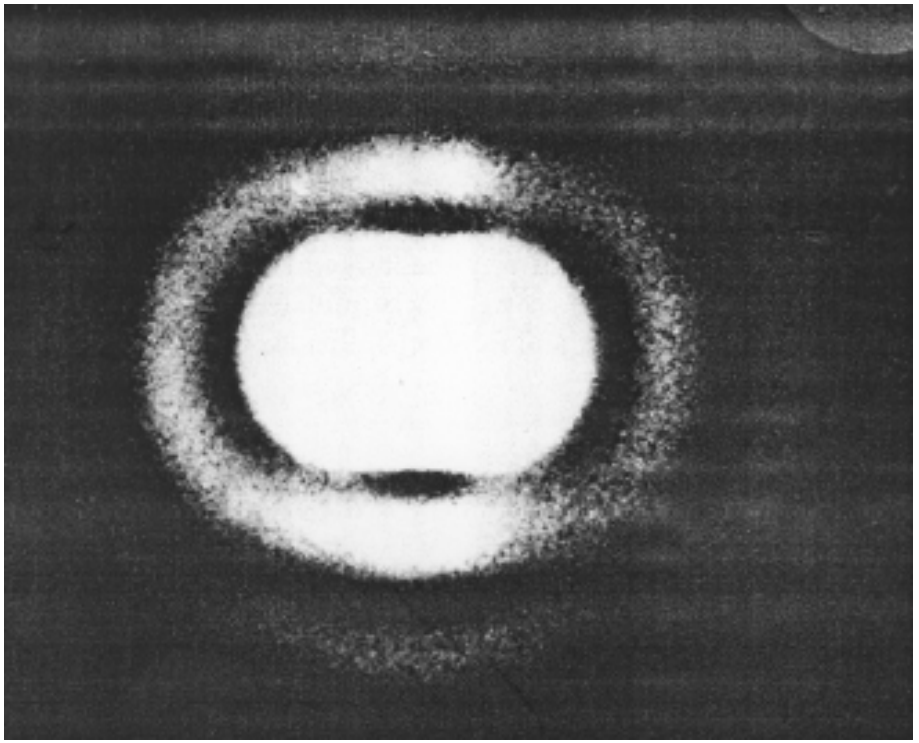
$v = (2\pi/\lambda)\, n \sin\alpha$

v is a normalized dimensionless optical coordinate

Rayleigh criterion: resolution of two points



Resolved



Not resolved

Bradbury, An introduction to the optical microscope

Rayleigh two-point resolution

- 2 points are just resolved if the second point is placed on the first dark ring of the first.

- Separation is $r_0 = 0.61\ \lambda / (n \sin \alpha)$
  Or separation is $2v_0 = 3.84$

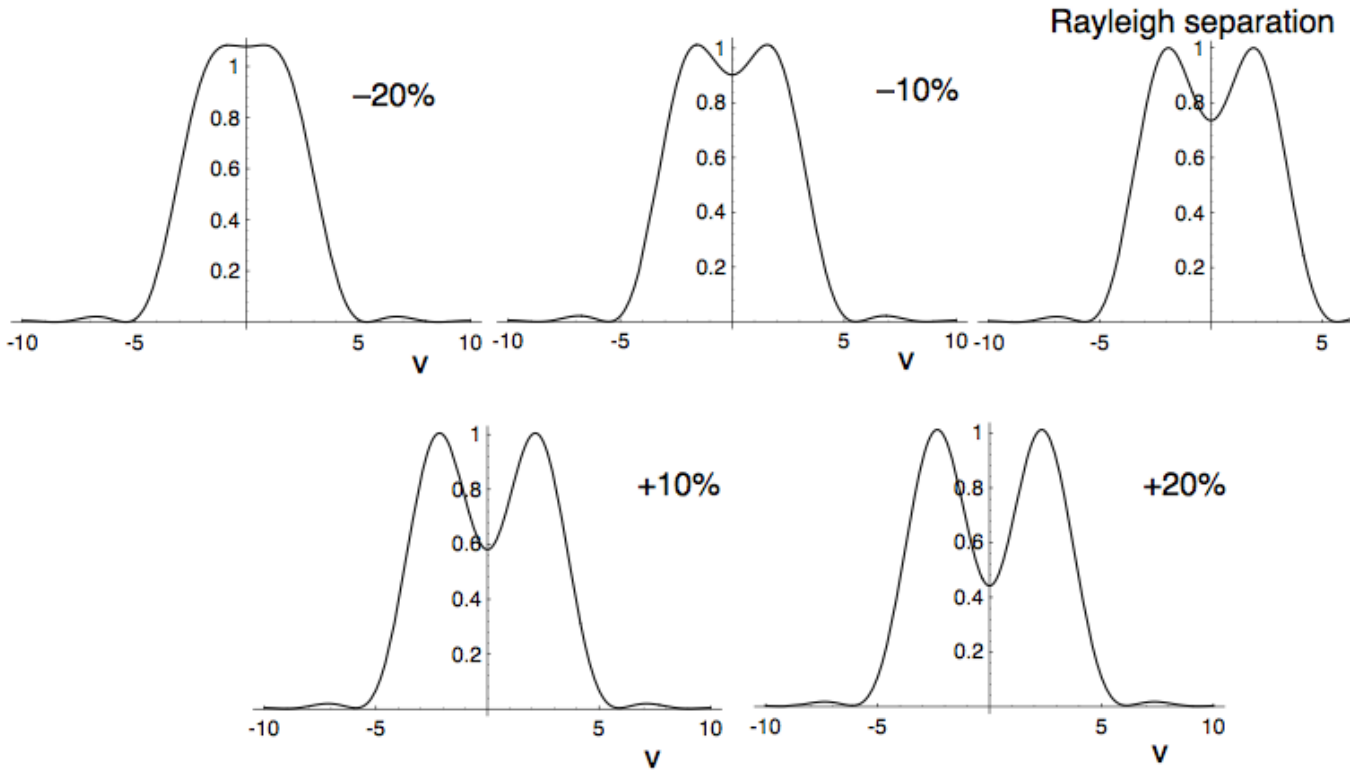
  $v = (2\pi/\lambda)\ n \sin \alpha$

  v is a normalized dimensionless optical coordinate

- Then the ratio of the intensity midway to that at the points is 0.735

"This rule is convenient on account of its simplicity and it is sufficiently accurate in view of the necessary uncertainty as to what exactly is meant by resolution."

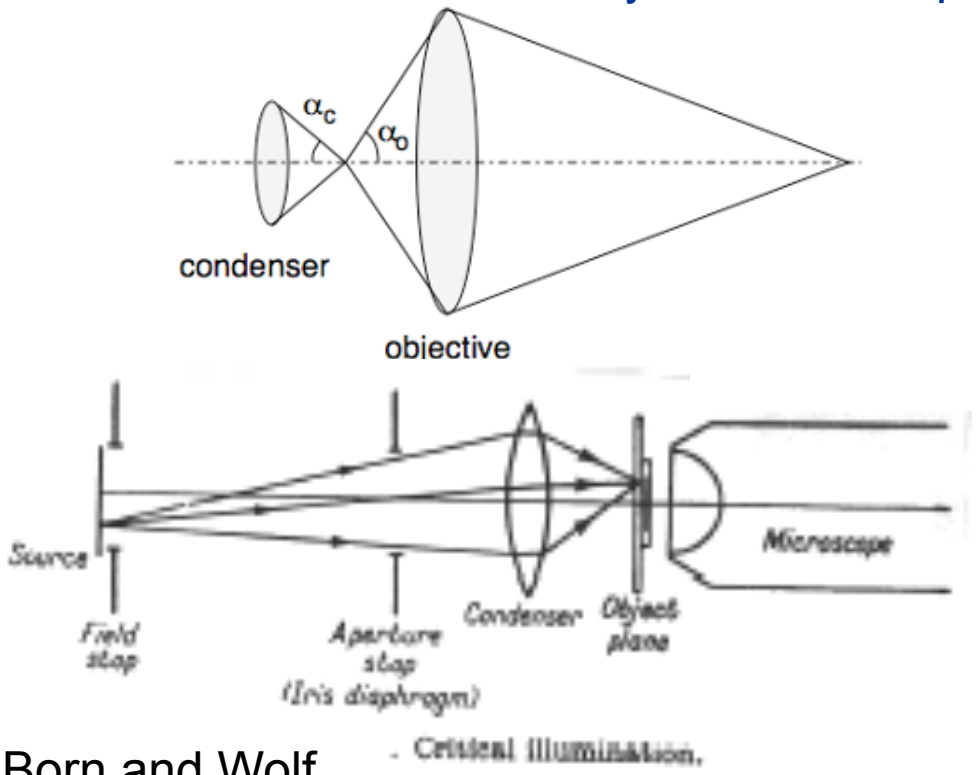
Two-point resolution



Dip changes quickly with separation

Resolution depends on coherence

Vary condenser aperture (diaphragm)



Born and Wolf

$S = 0$, coherent illumination
$S = 1$, full, matched or complete illumination
$S \rightarrow \infty$, incoherent illumination

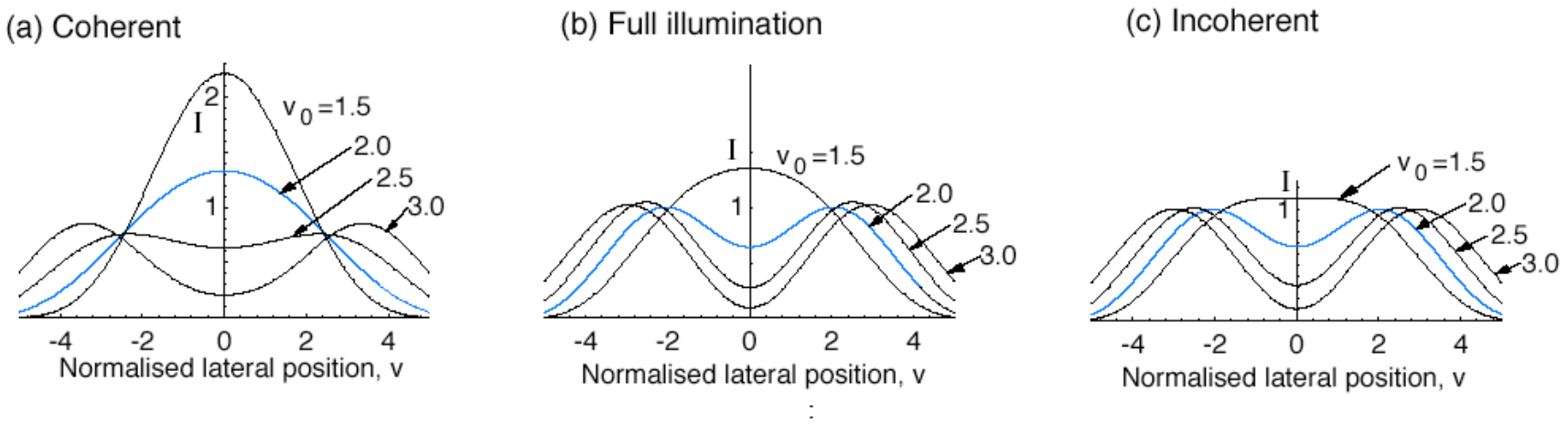
Fluorescence behaves as incoherent imaging

Resolution depends on coherence

Coherence ratio $S = \dfrac{n_c \sin \alpha_c}{n_o \sin \alpha_o}$

Images of two points

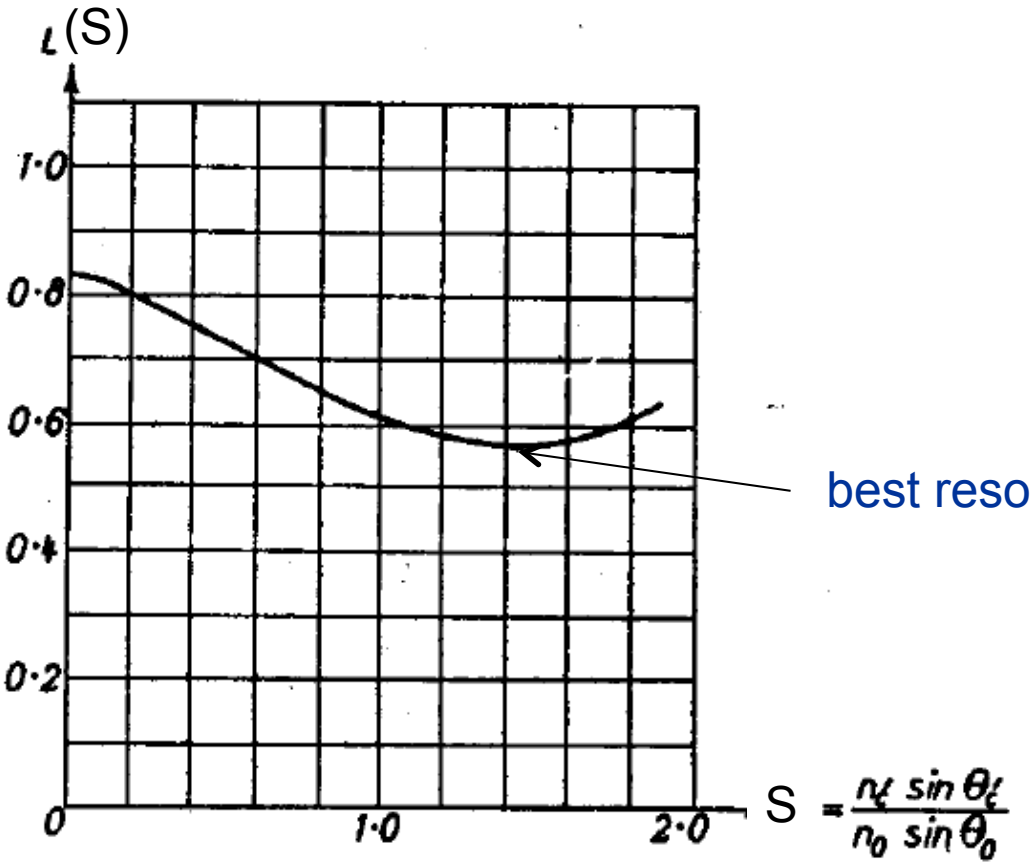
small condenser | equal apertures | large condenser



v_0 = 2.0 is close to the Rayleigh resolution for the incoherent case

Two-point resolution

$L = v_0/\pi$



best resolution (S~1.42)

Fig. 10.13. Effect of the condenser aperture on the resolution of two pinholes of equal brightness. (After H. H. Hopkins and P. M. Barham, *Proc. Phys. Soc.*, 63 (1950), 72.)

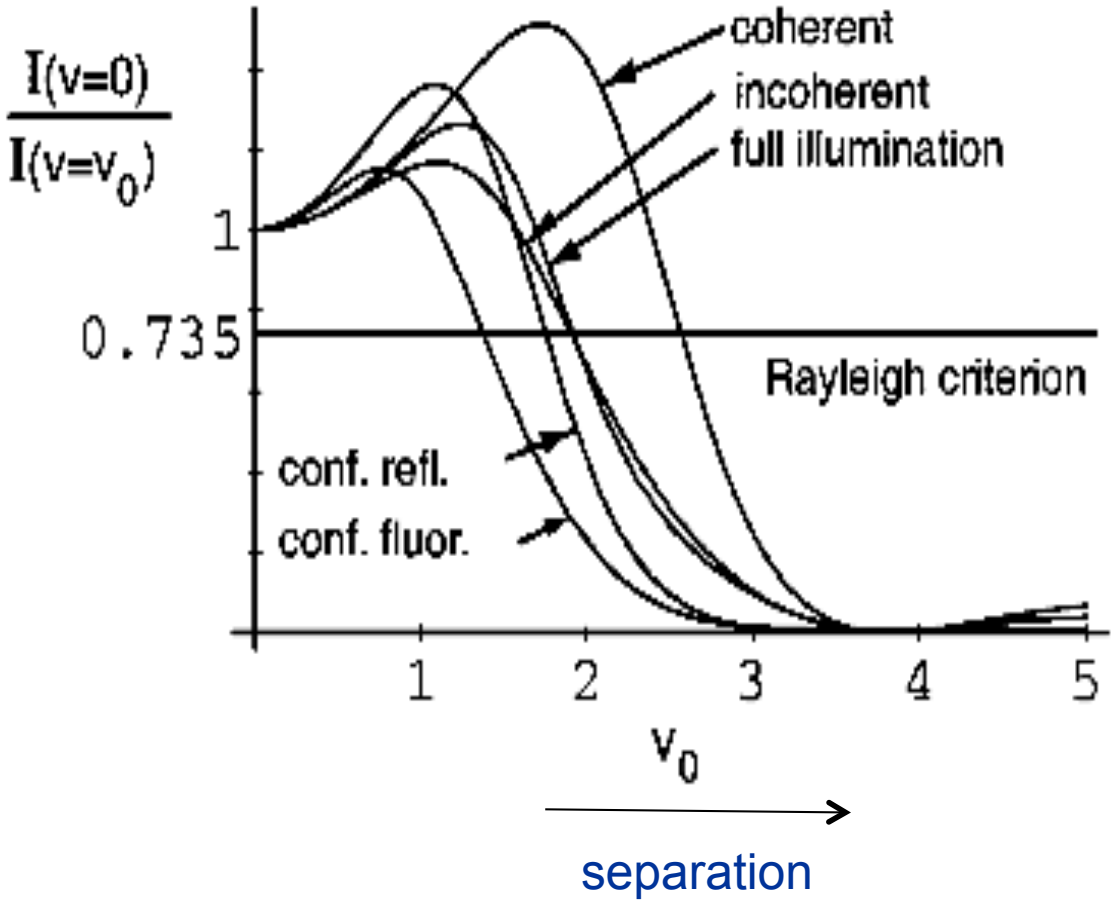
Born & Wolf

Generalized Rayleigh two-point resolution

Points resolved when
$I(0) / I(v = v_0) = 0.735$

Intensity midway

Intensity at the points,
NOT intensity of the maxima



Generalized Rayleigh criterion

- Defined for intensity **at the points**
- Actually, intensity of the **maxima** may be preferable because if we do not know the magnification exactly we do not know where the points are! (Modified Rayleigh criterion)
- FWHM is called Houston criterion
- Sparrow criterion: no minimum at centre
- Kino's interpretation of Sparrow criterion, ratio = 1.

Chen *et al.* Vol. 29, No. 6 / June 2012 / J. Opt. Soc. Am. A 1059

Resolution of aplanatic solid immersion lens based microscopy

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Perfect imaging

Object

$$t(x,y) = a(x,y)e^{i\phi(x,y)}$$

$a(x,y)$ is modulus (amplitude), real

$\phi(x,y)$ is phase, real

Perfect image

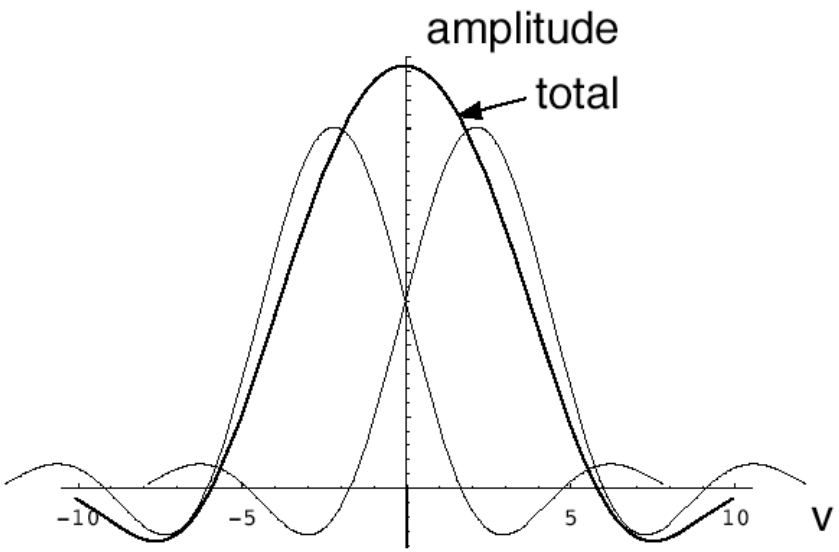
$$I(x,y) = \left|a(x,y)e^{i\phi(x,y)}\right|^2 = a^2(x,y)$$

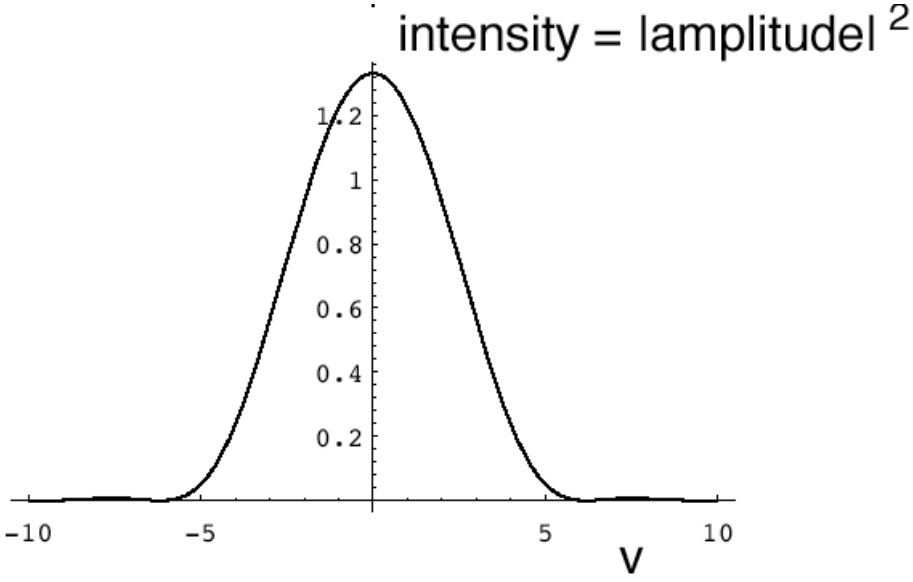
- No phase information in perfect image

Image formation (coherent case)

Add **amplitudes** of different parts of object.

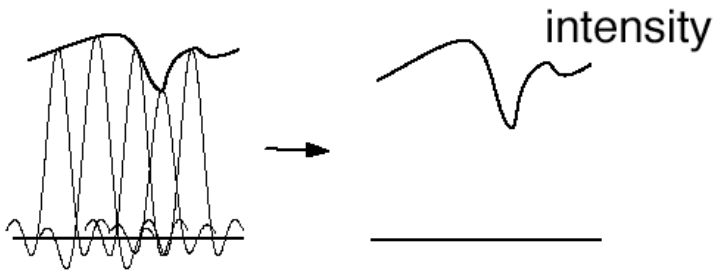
e.g. 2 points:





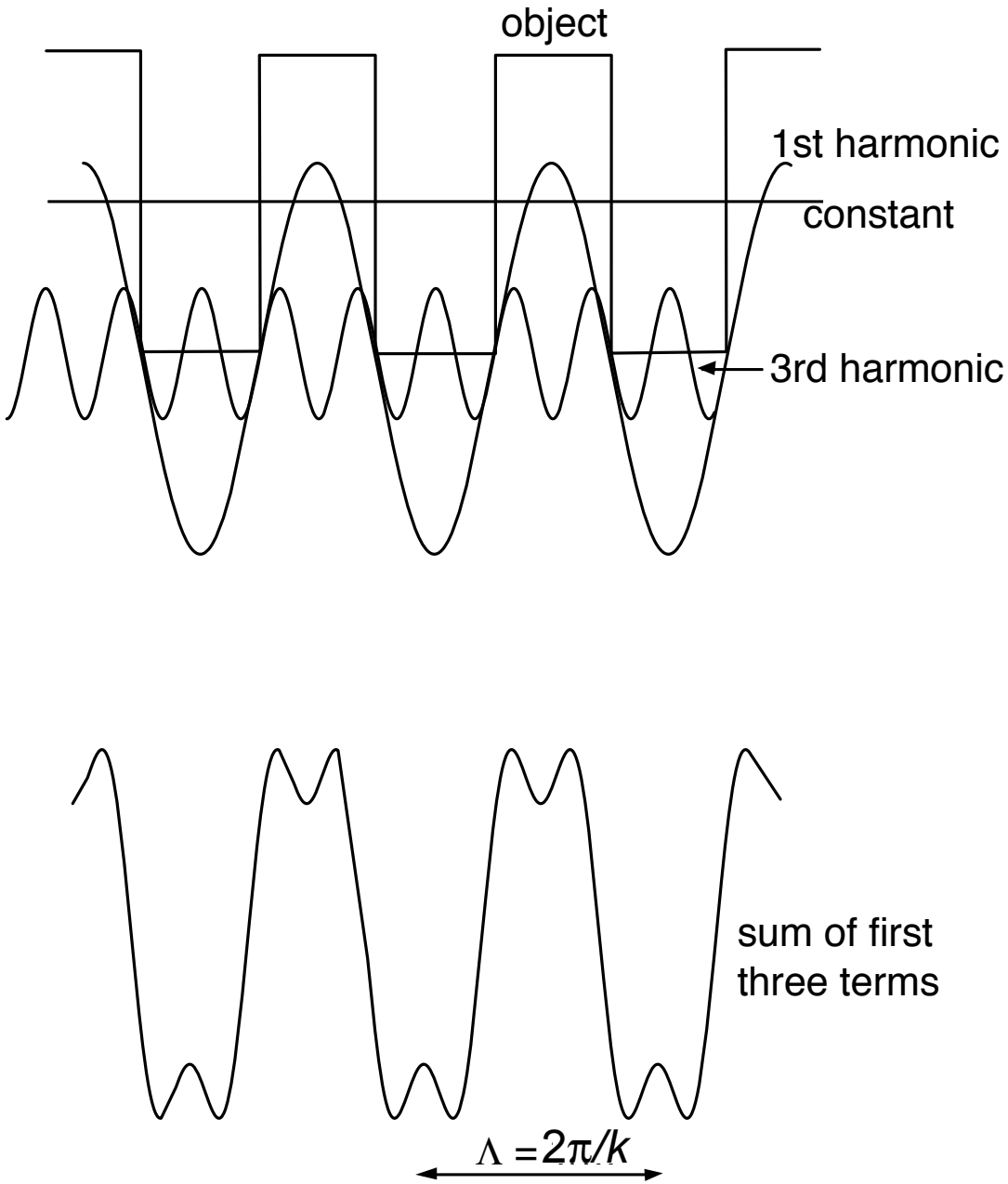
Coherent imaging

Many points:



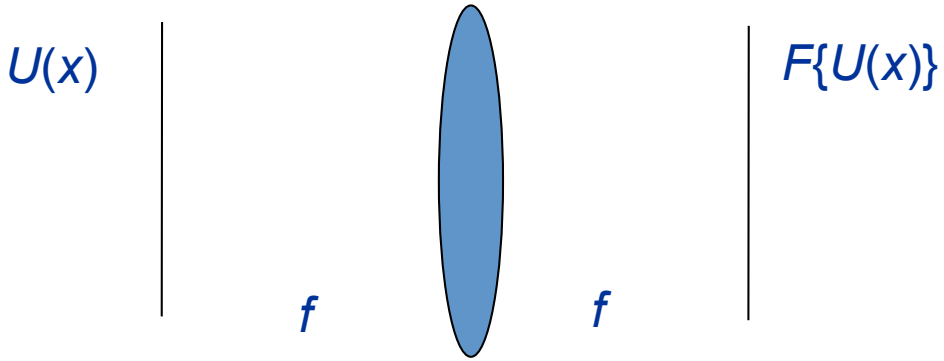
$$I = |h \otimes t|^2$$

- h is **amplitude** point spread function
- t is object amplitude
- $\otimes$ is convolution

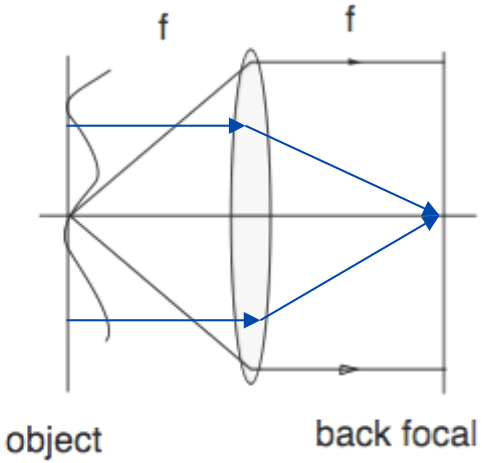


Fourier series for periodic function

Fourier transforming property of a lens

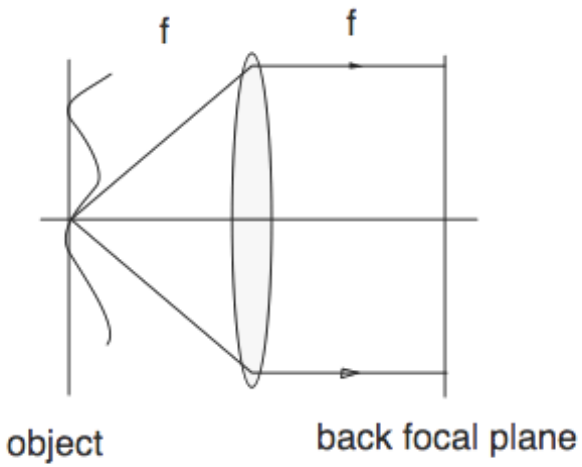


Position, slope



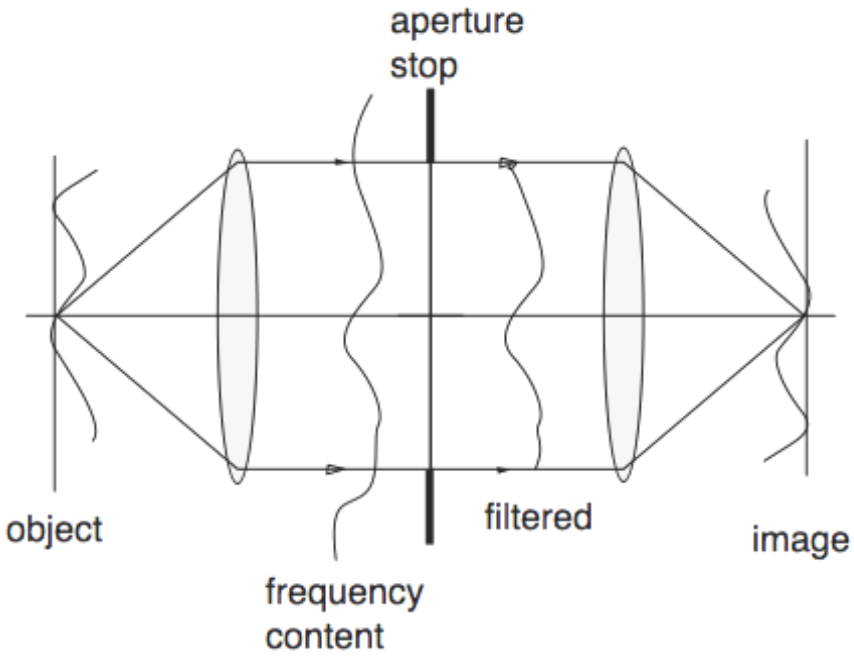
Slope, position

Abbe theory (coherent imaging)



Grating orders in back focal plane.

Fraunhofer diffraction is Fourier transform of object



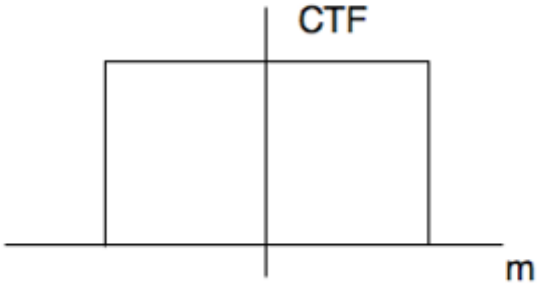
•system behaves as a low-pass filter

Abbe theory

Introduce object spectrum

$$T(m,n) = \int_{-\infty}^{\infty}\int_{-\infty}^{\infty} t(x,y)\exp\left[-i2\pi(mx+ny)\right]dxdy$$

Coherent transfer function

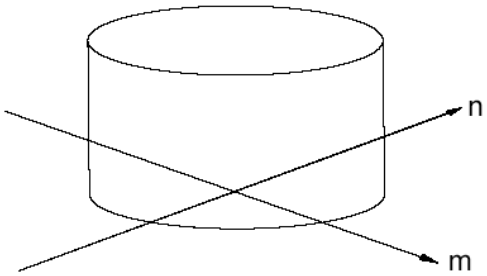


cut-off

$m = \frac{\sin \alpha}{\lambda}$

•CTF is scaled pupil function

CTF is Fourier transform of *h*



coherent transfer function (CTF)

Introduce object spectrum:

$$T(m,n) = \int_{-\infty}^{\infty}\int_{-\infty}^{\infty} t(x,y)\exp\left[-i2\pi(mx+ny)\right]dxdy$$

$$I(x,y) = \left|\iint c(m,n)T(m,n)\exp[2\pi i(mx+ny)]]dm\,dn\right|^2$$

spatial frequencies are filtered

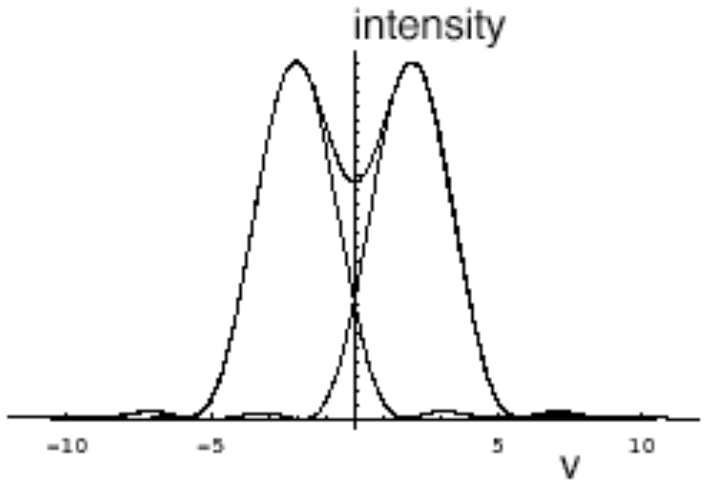
$$= \int\int\int\int c(m,n)c^*(p,q)T(m,n)T^*(p,q)\exp\{2\pi i[(m-p)x+(n-q)y]\}dmdndpdq$$

For partially coherent system *C*(*m*, *n*; *p*, *q*) does not separate (complicated!)

Incoherent imaging

Add **intensities**

2 points:



many points:

$|h|^2$= **intensity** point spread function

$|t|^2$= object intensity transmission

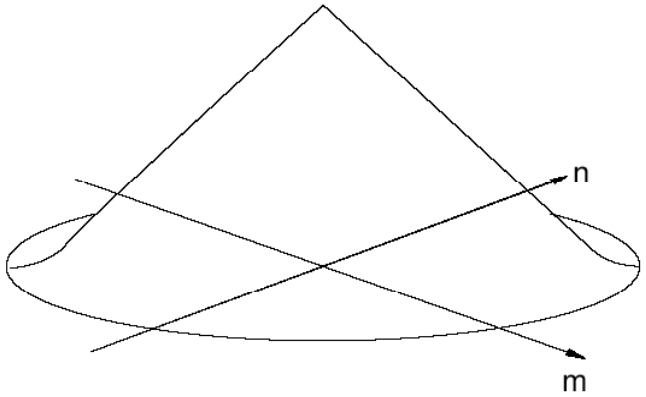
$I = |h|^2 \otimes |t|^2$

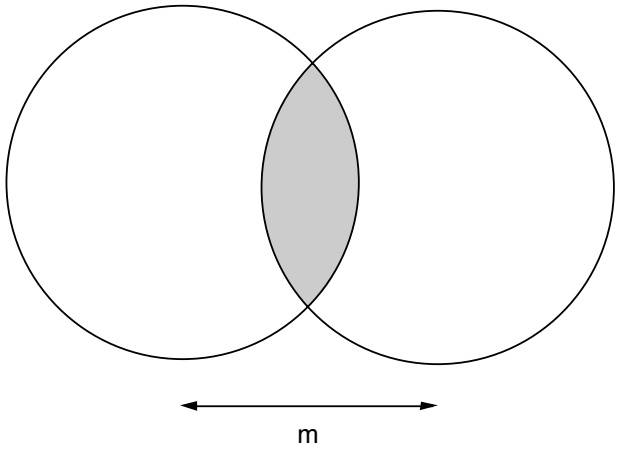
OTF for circular aperture

•We can show that the OTF is the area of overlap of two circles (convolution), which is

$$\frac{2}{\pi}\left[\arccos(m) - m\sqrt{1-m^2}\right]$$

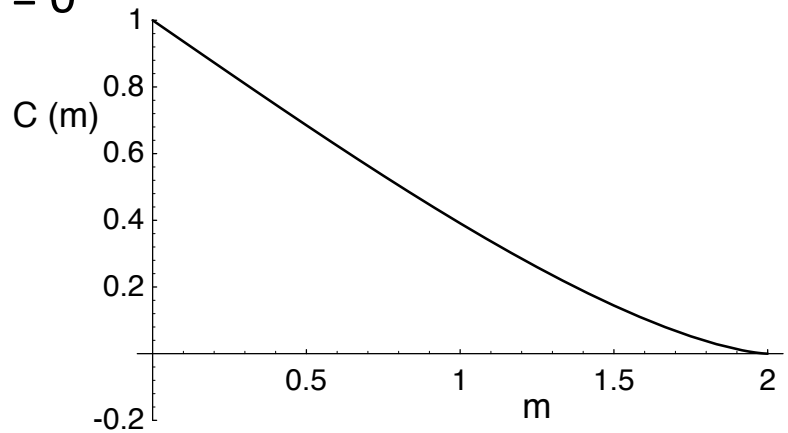
•This looks like (Chinese hat):



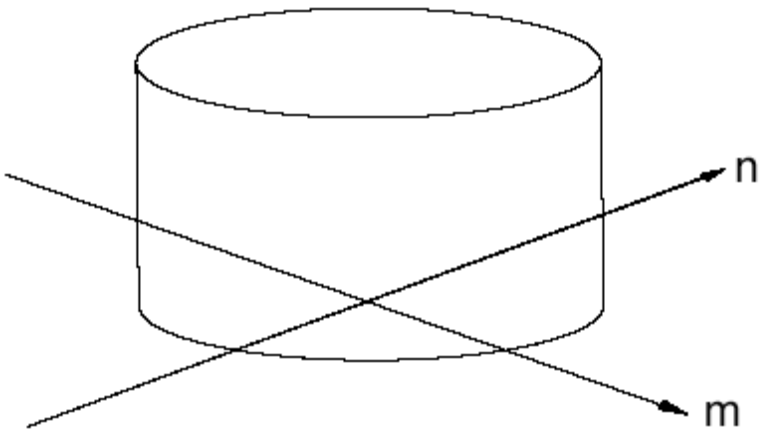


•The cut-off frequency is twice that for a coherent system

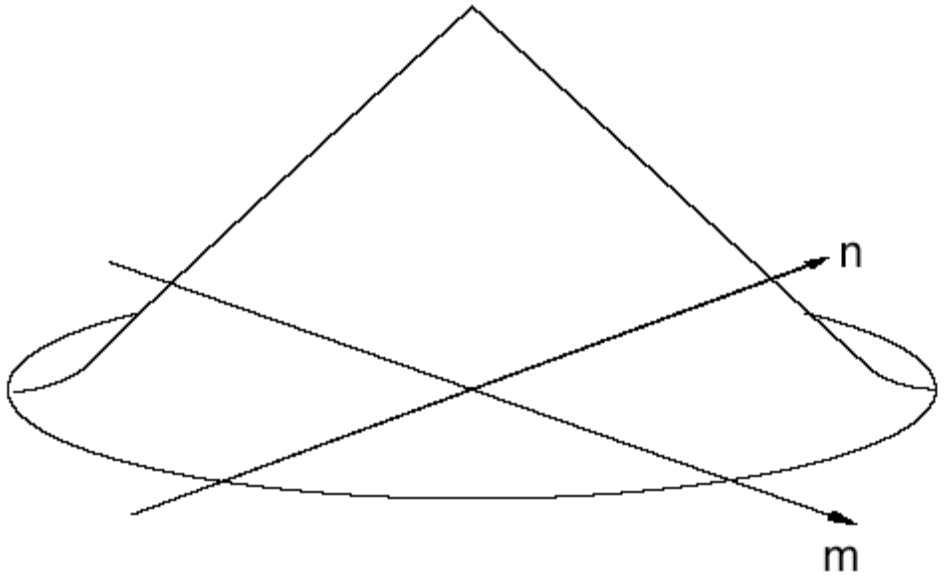
•For an object which is only a function of *x*, i.e. *n* = 0



2-D transfer functions



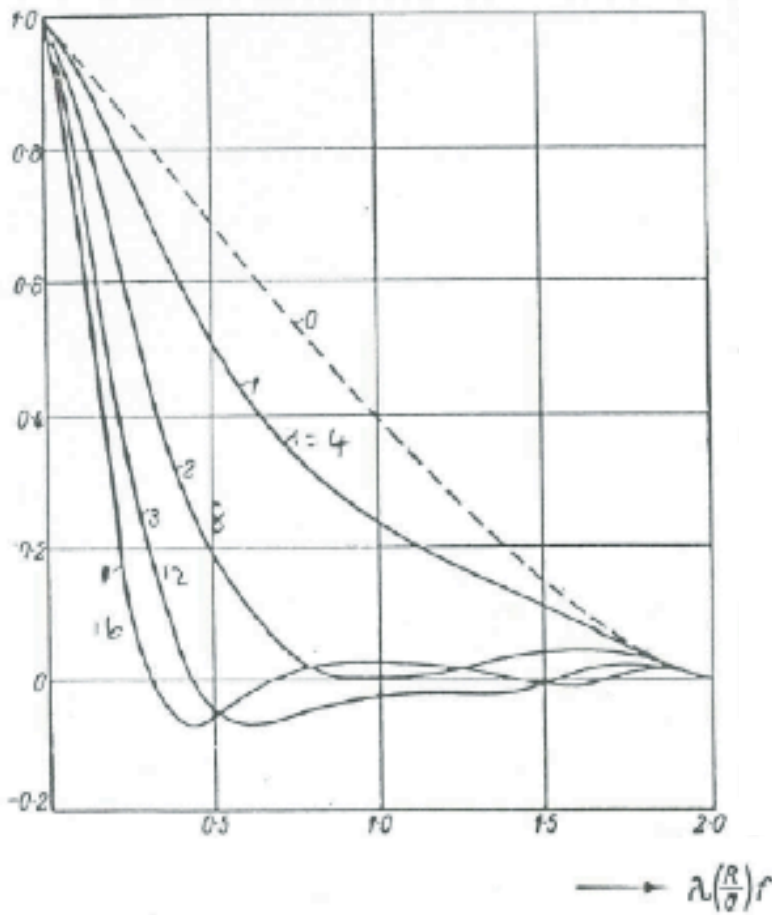
coherent transfer function (CTF)



optical transfer function (OTF)

Effect of defocus

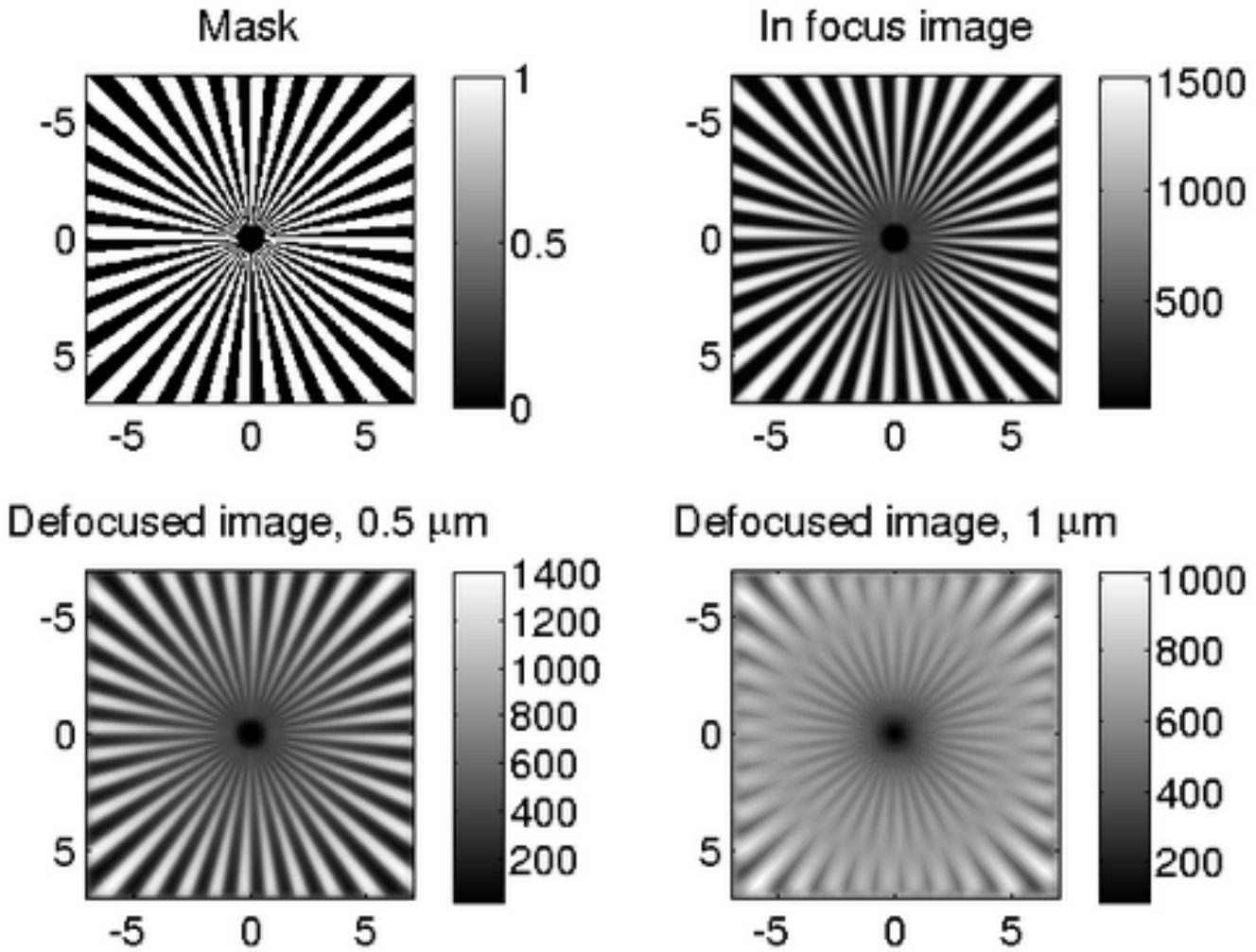
- If system is defocused, integrate over the area of overlap, taking into account the phase of the pupil (cannot be done analytically).
- Response drops off with defocus, i.e. imaging of higher spatial frequency components is worse. It is the mid-spatial frequencies which are most strongly affected, resulting in poorer imaging.
- OTF can go negative with defocus.
- OTF must always be purely real for a radially symmetric pupil (not for coma!)
- Some spatial frequency components have their contrast reversed. This results in optical artifacts, which means that you can see something that is not really there.



HH Hopkins, *Proc.R. Soc. Lond.A* **231** 98 (1955)

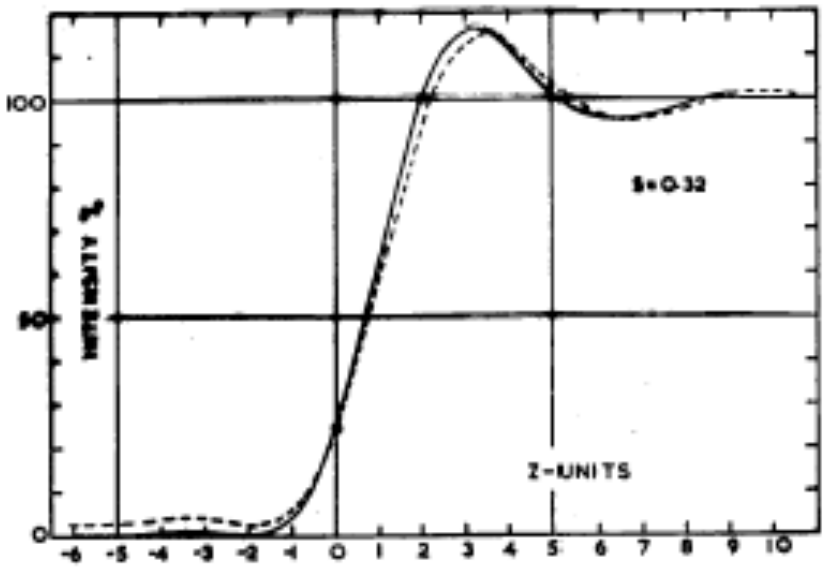
Born & Wolf

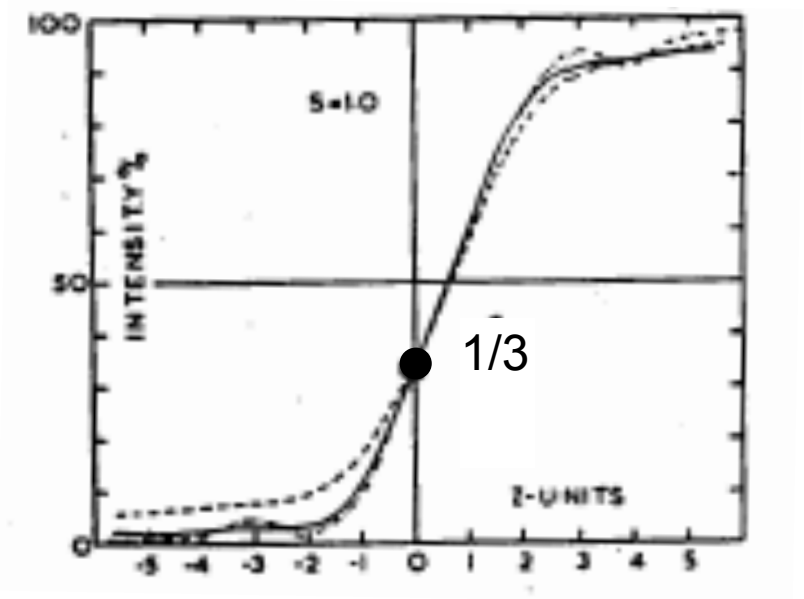
Siemens star, $S = 1$



S. Mehta and R. Oldenbourg, "Image simulation for biological microscopy: microlith," Biomedical Optics Express **5**, 1822-1838 (2014).

Image of a straight edge





S = 0.32 (nearly coherent)

S = 1 (full illumination)

B. M. Watrasiewicz, "Theoretical calculations of images of straight edges in partially coherent illumination,"
Optica Acta **12**, 391-400 (1965).

Straight edge

- Image depends on coherence

  Slope is greater for small S, so greater precision for measurement

  - $S = 0$, slope $= 1/\pi = 0.318$
  - $S = 1$, slope $= 0.270$
  - $S \rightarrow \infty$, slope $= 0.270$

- Intensity at edge is
  - $S = 0$, 1/4
  - $S = 1$, 1/3
  - $S \rightarrow \infty$, 1/2
- Important for measuring (edge appears to be at 1/2)

- Fringes for small S