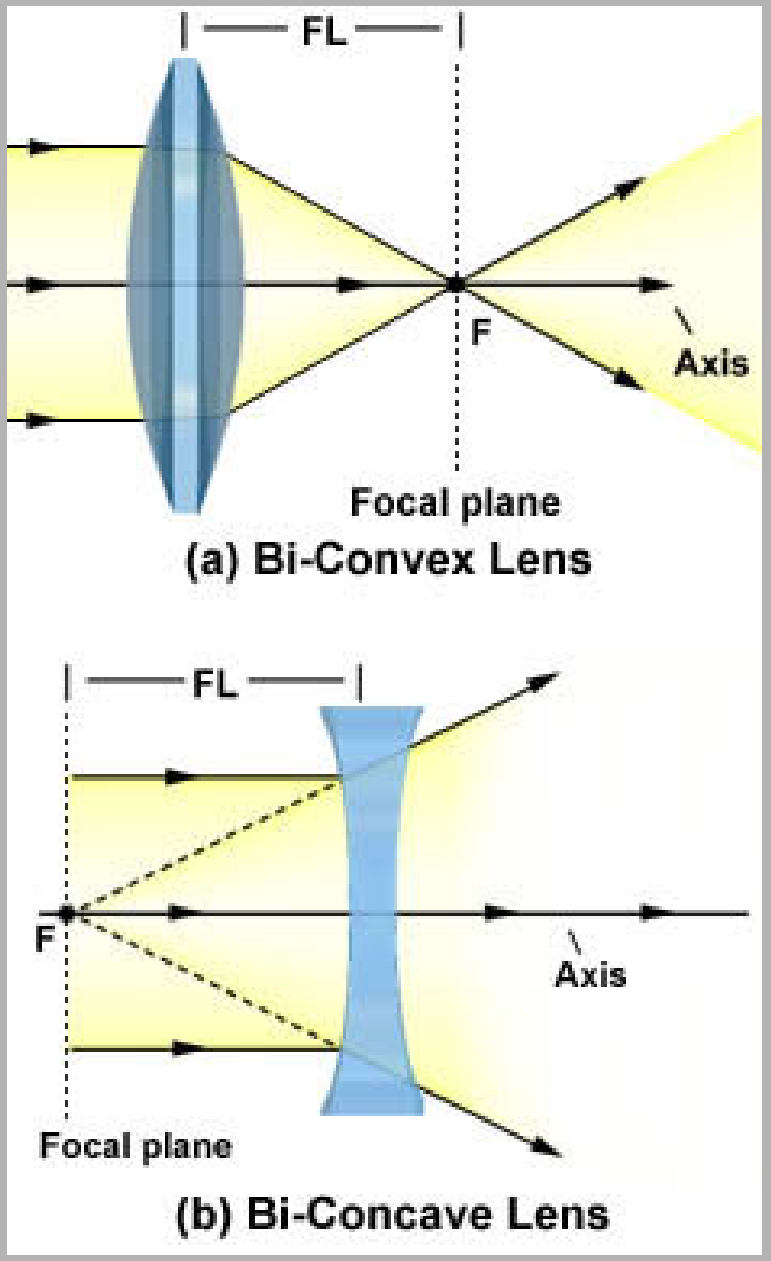
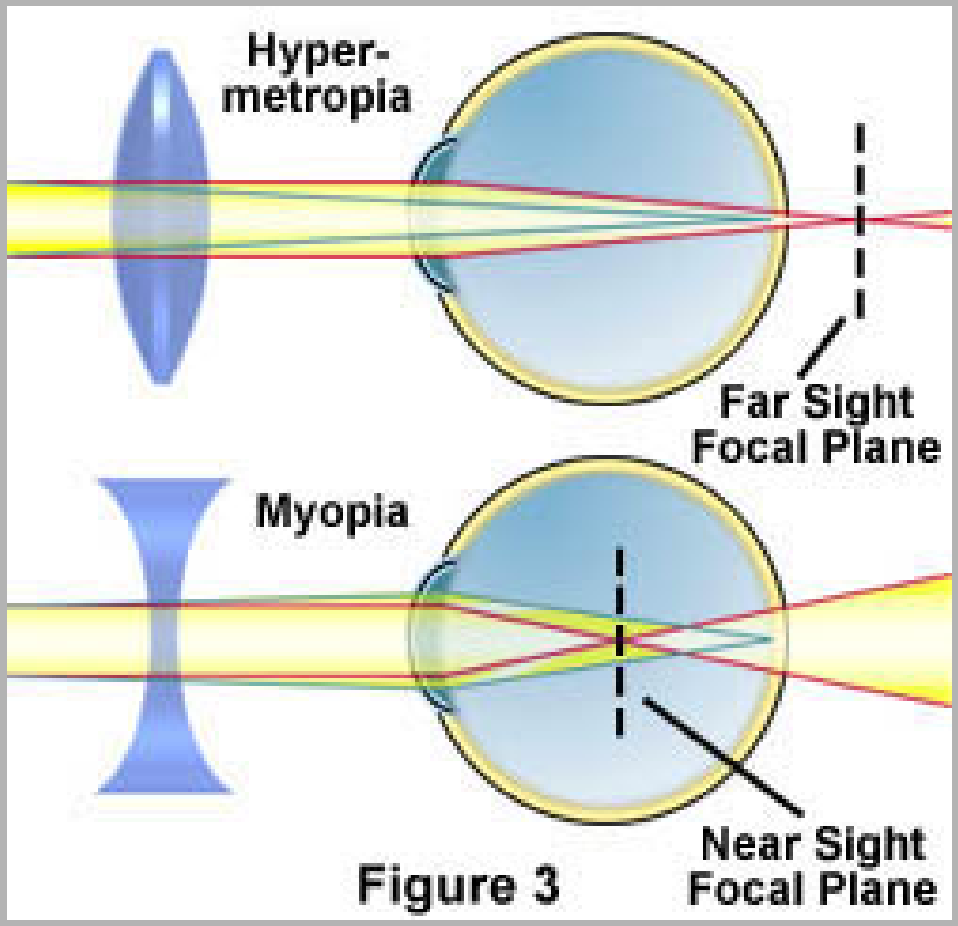


Real image
- Can project
- Upside down

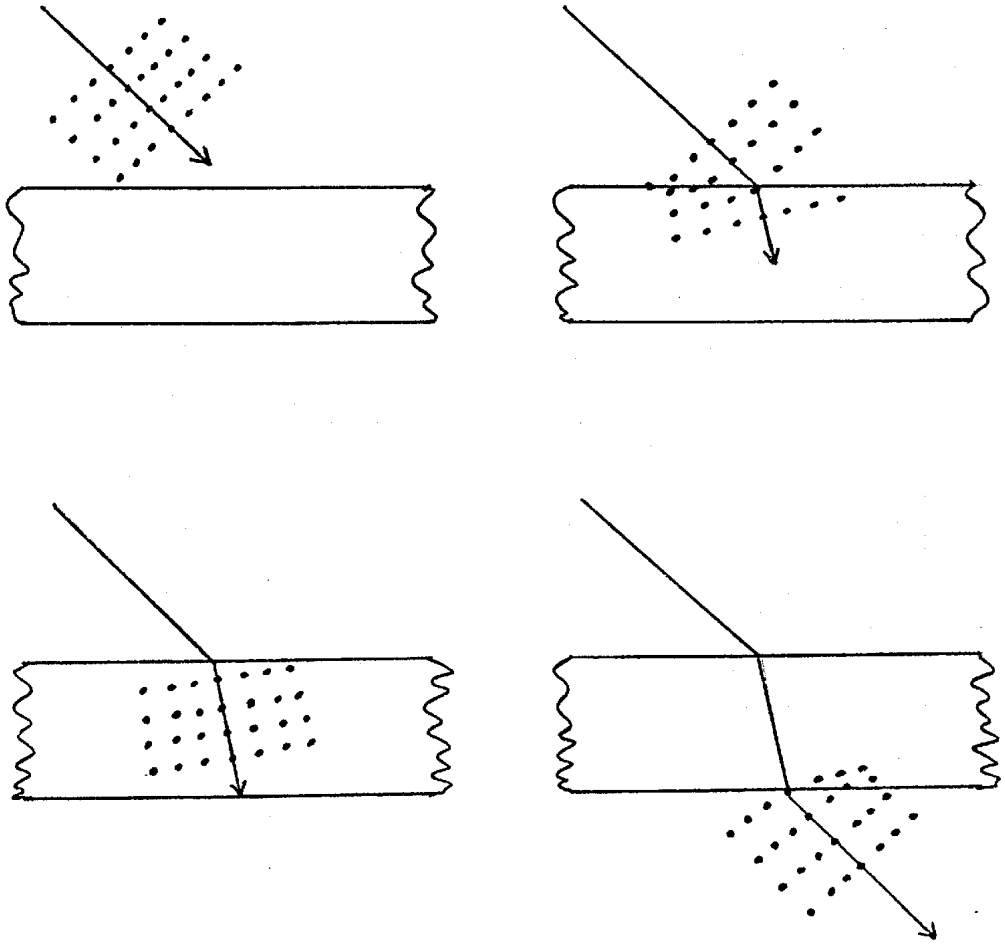
Virtual image
- Can't project
- Rightside up

Can look at both real and virtual image (basis of corrective eyeglasses)

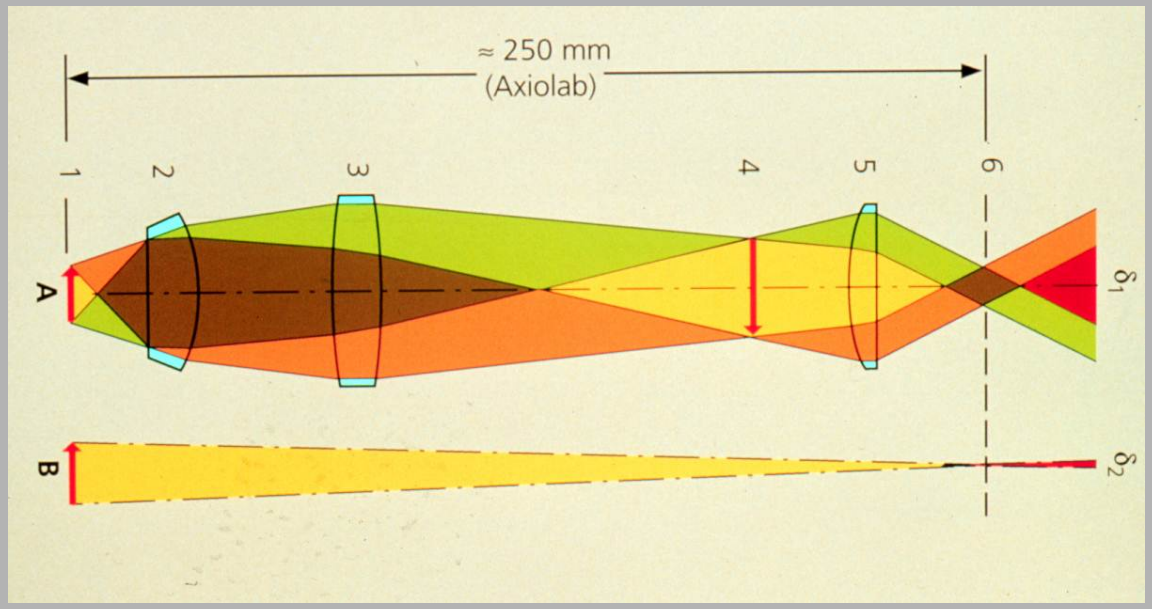


Reminder that our eyes are the last component of an optical microscope design

Refraction (Marching Band Analogy)



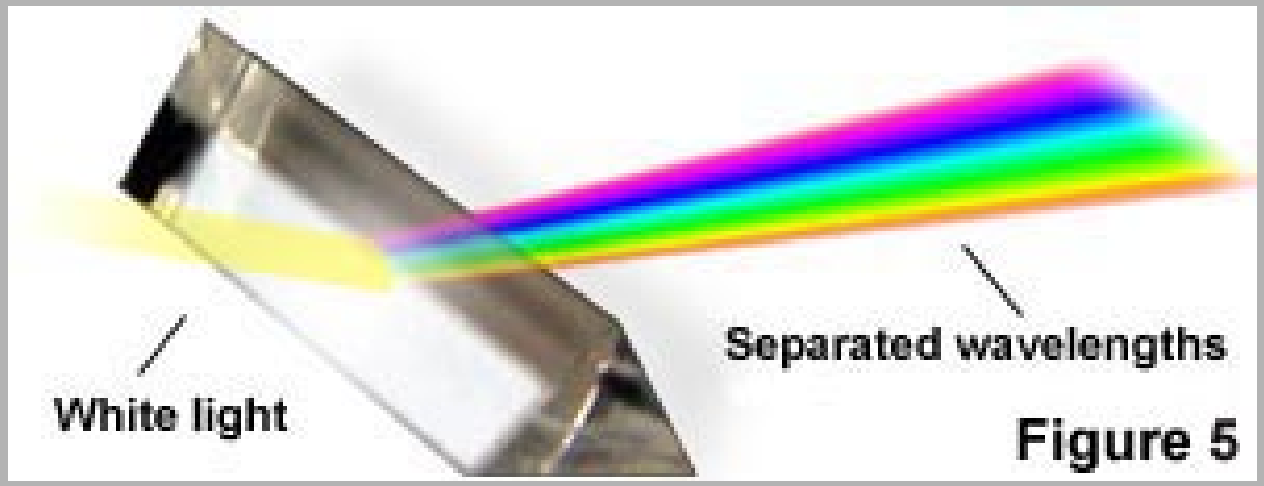
Object viewed through microscope vs the unaided eye (250 mm from eye)



Compound microscope
Large image on retina

1x view
Small image on retina

Dispersion of glass: disperses the different wavelengths of white light



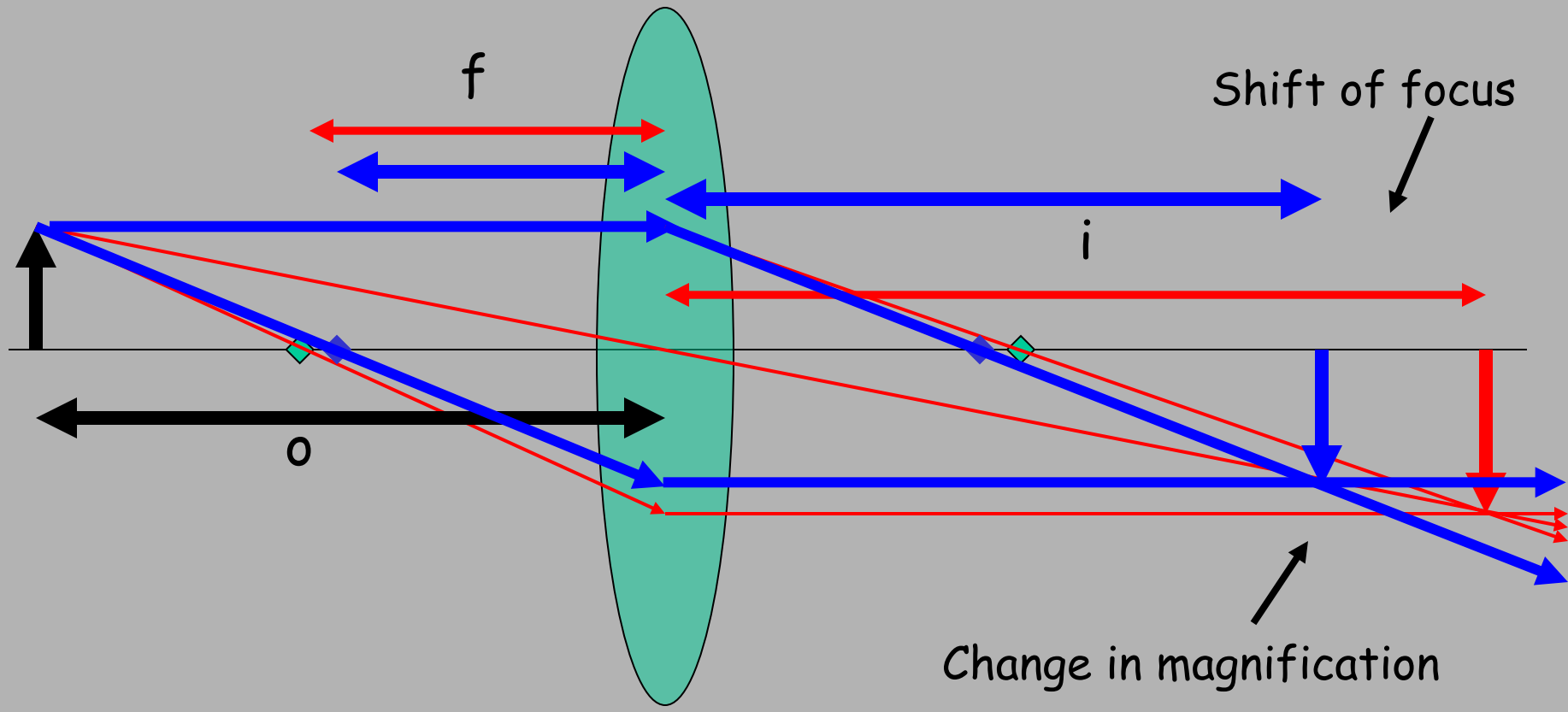
Material	**Blue** (486nm)	**Yellow** (589nm)	**Red** (656nm)
Crown Glass	1.524	1.517	1.515
Flint Glass	1.639	1.627	1.622
Water	1.337	1.333	1.331
Cargille Oil	1.530	1.520	1.516

Higher index of refraction results in shorter f

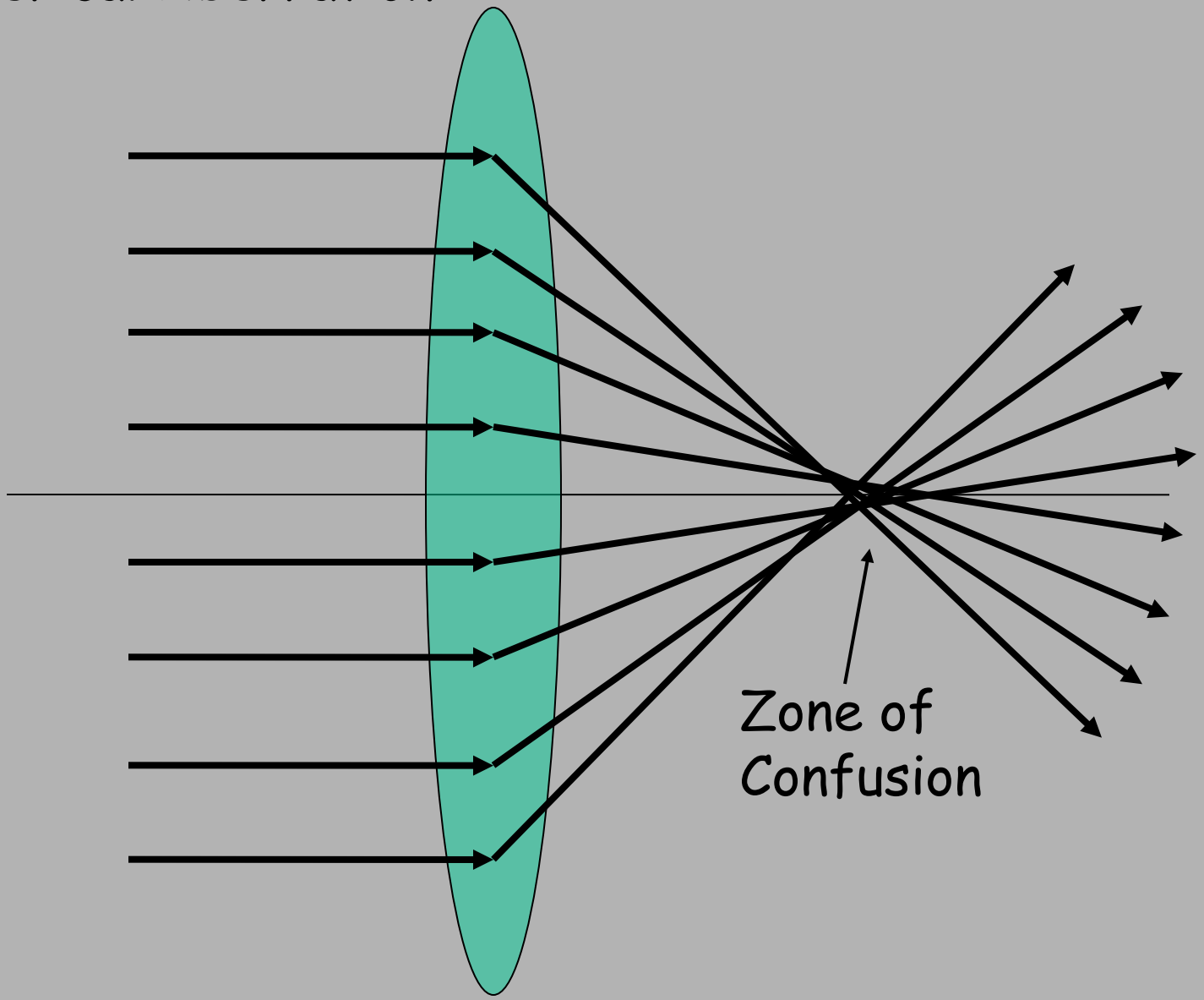
→ Chromatic Aberration

Lateral (magnification)

Axial (focus shift)

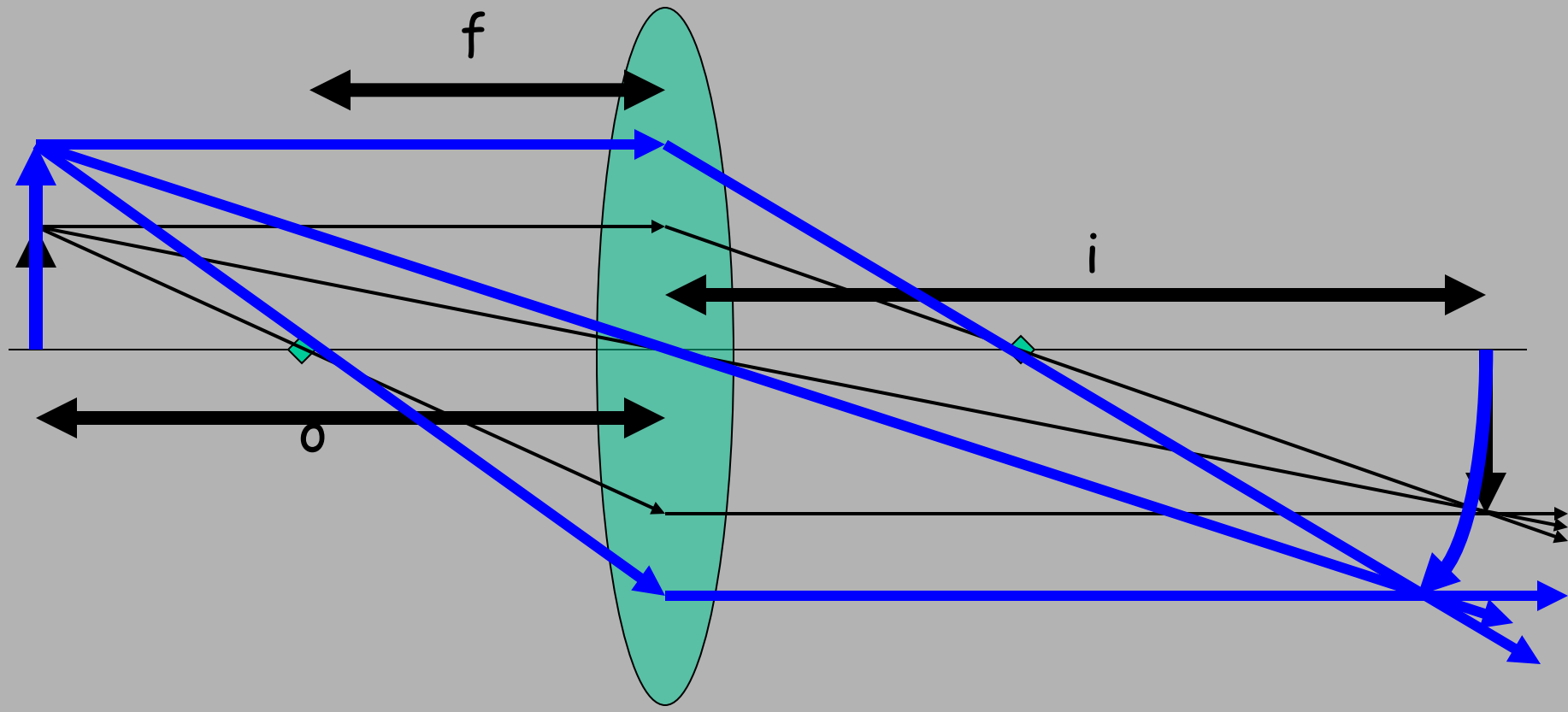


Spherical Aberration

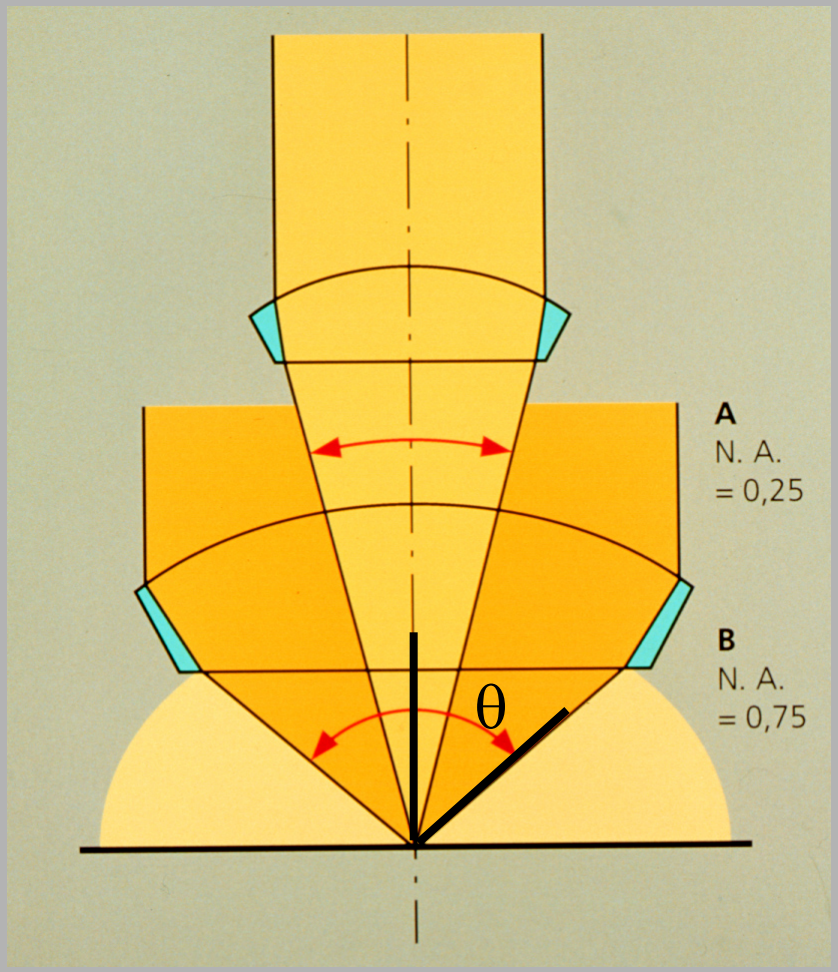


Curvature of field: Flat object does not project a flat image

(Problem: Cameras and Film are flat)



Larger Aperture collects more light

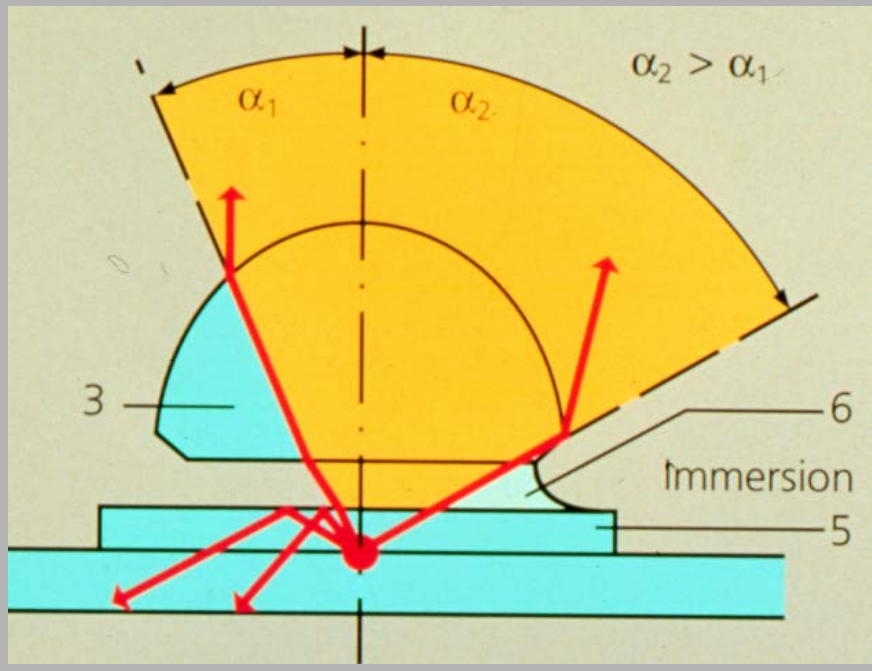


N.A. = $\eta \sin \theta$

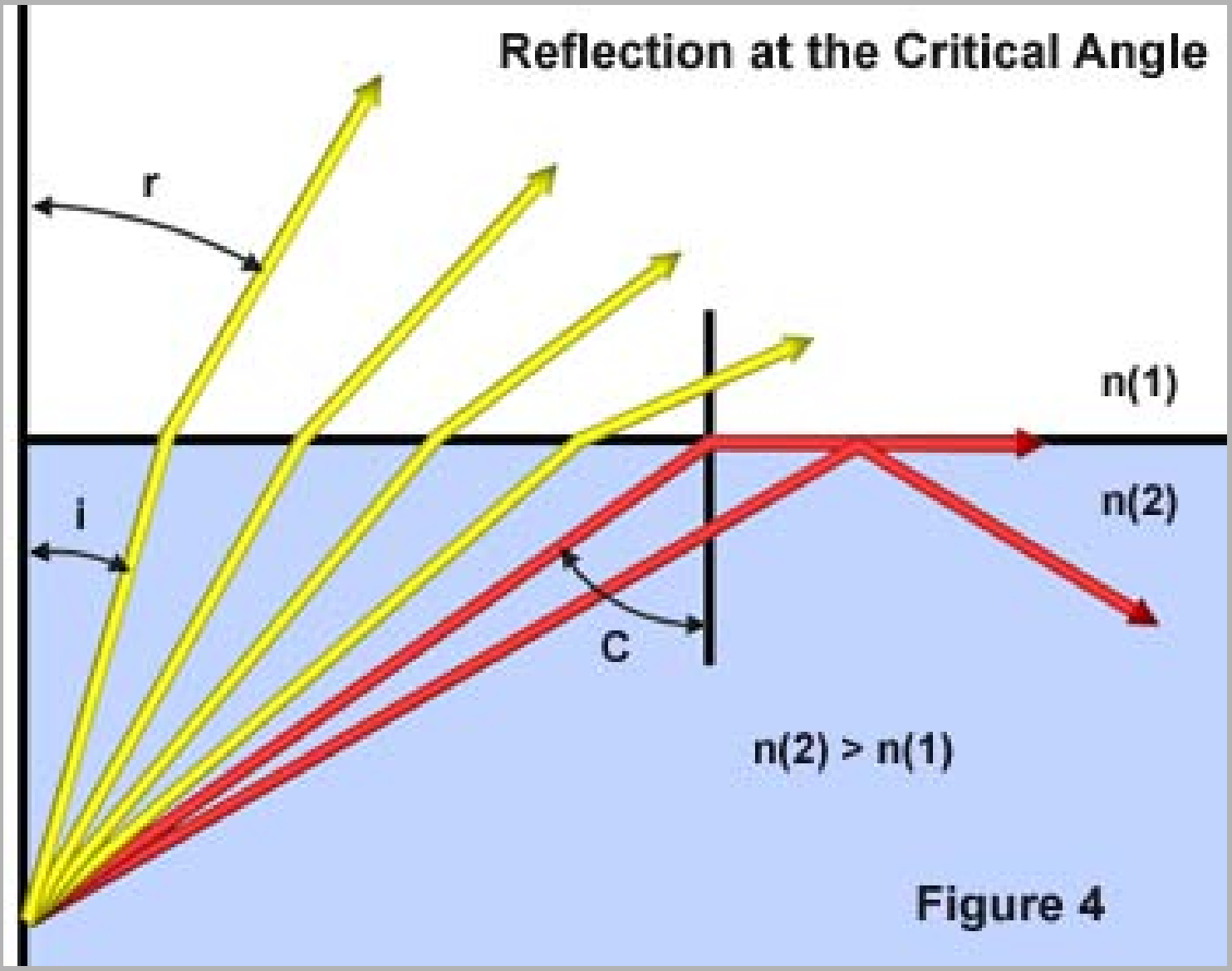
N.A. = $\eta \sin \theta$

η = index of refraction

Material	Refractive Index
Air	1.0003
Water	1.33
Glycerin	1.47
Immersion Oil	1.515



Note: $\sin \theta \leq 1$, therefore N.A. $\leq \eta$



$$\sin \theta_{\text{critical}} = \eta_1 / \eta_2$$

N.A. has a major effect on image brightness

Transmitted light

Brightness = fn (NA^2 / $magnification^2$)

10x 0.5 NA is 3 times brighter than 10x 0.3NA

Epifluorescence

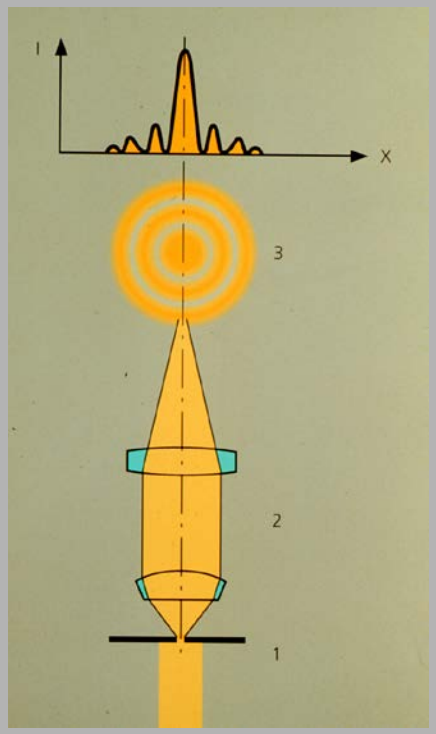
Brightness = fn (NA^4 / $magnification^2$)

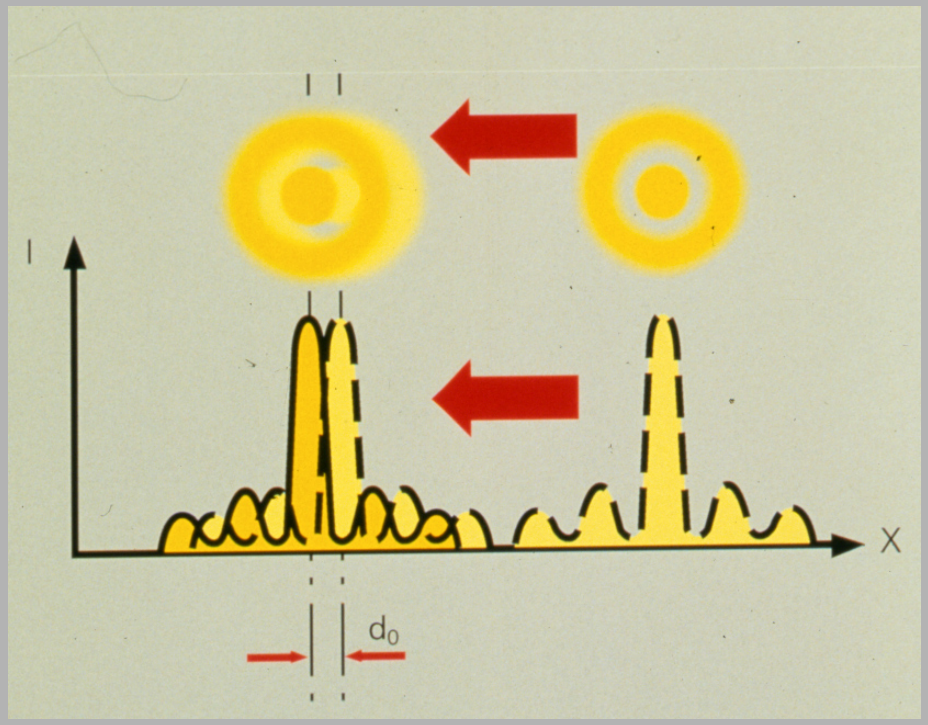
10x 0.5 NA is 8 times brighter than 10x 0.3NA

N.A. has a major effect on image resolution

Minimum resolvable distance

$$d_{min} = 1.22\ \lambda / (NA_{\ objective} + NA_{\ condenser})$$



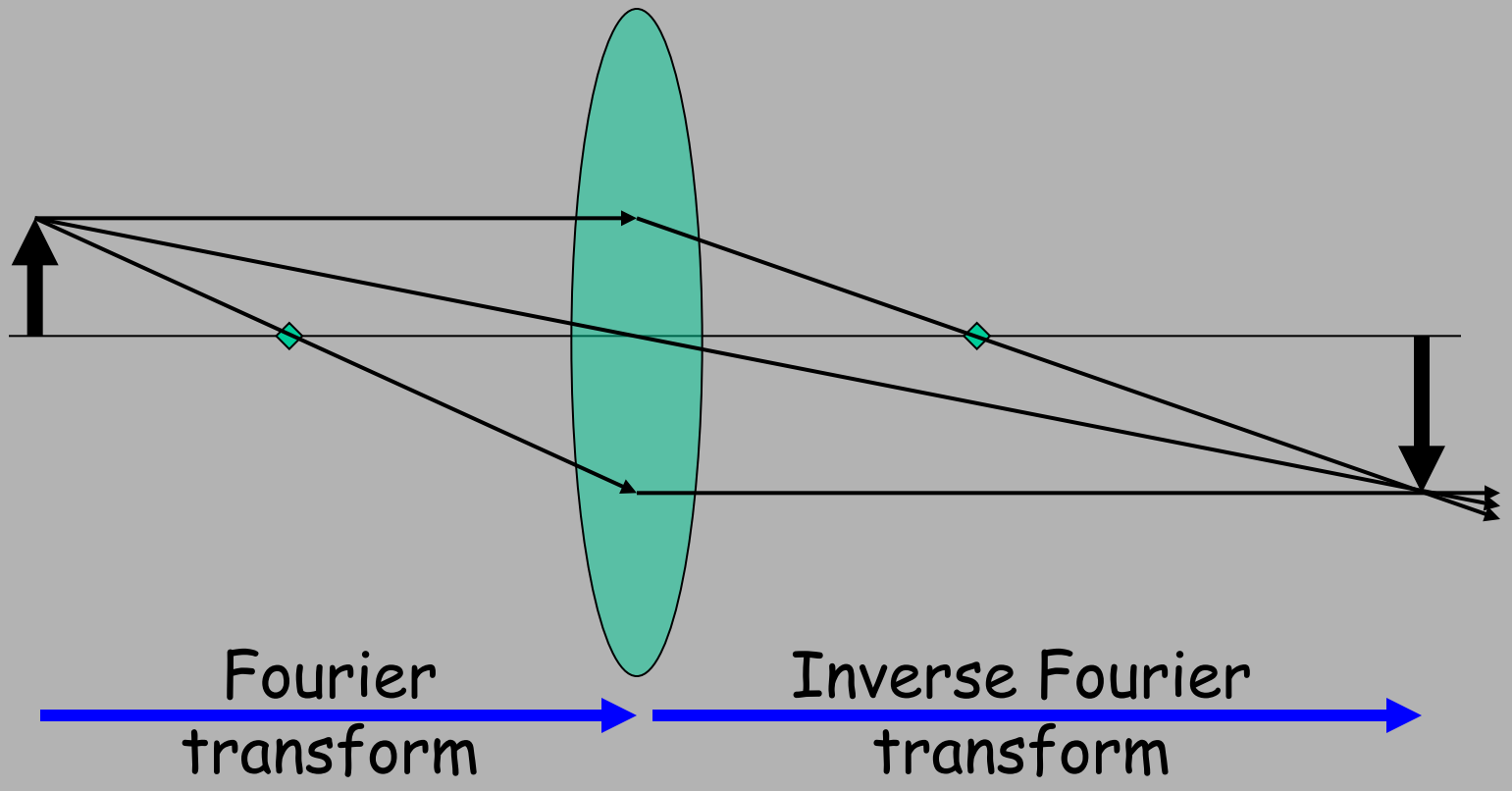


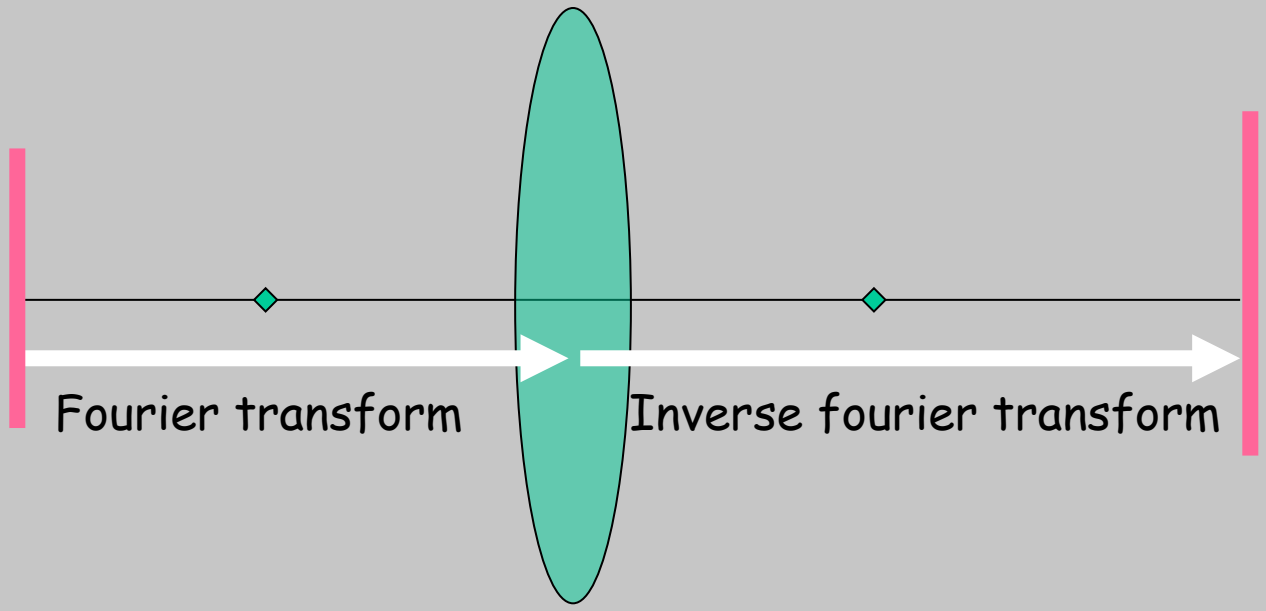
Objective names and corrections

	chromatic	spherical	
Achromat	2λ	-	
Apochromat	3λ	2λ	
PlanApochromat	4λ	4λ	flat field
Fluor or Fluar	fewλ	fewλ	max. light
Neo Fluar	2-3λ	2-3λ	

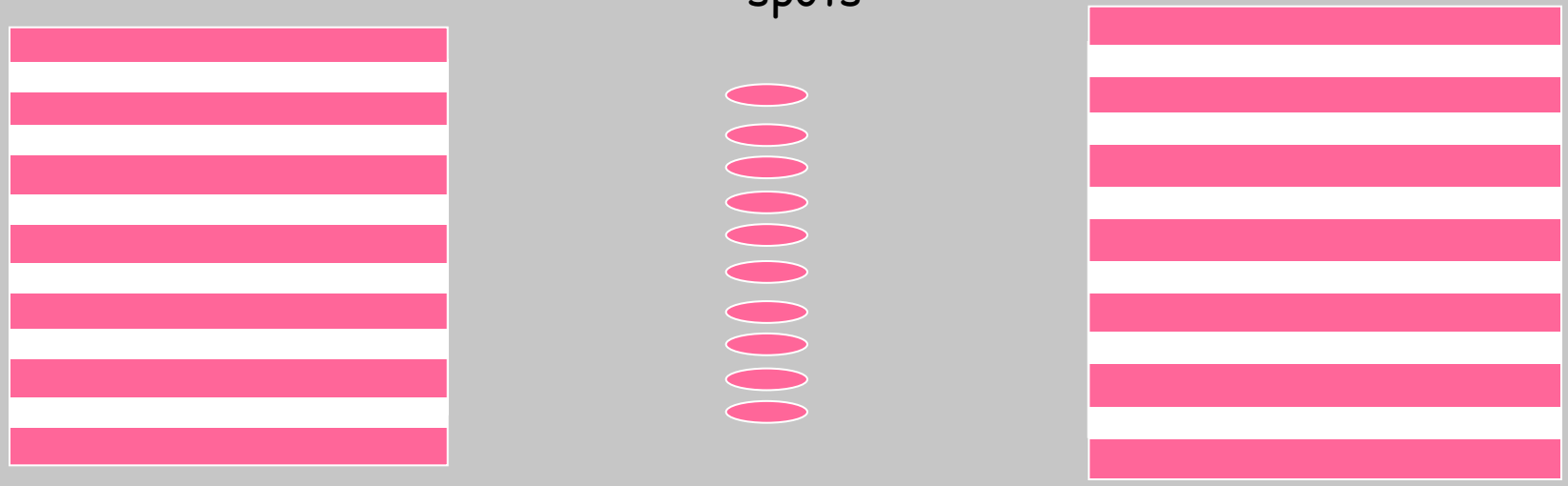
Why does larger NA give better resolution?

Abbe: it is all a problem of diffraction





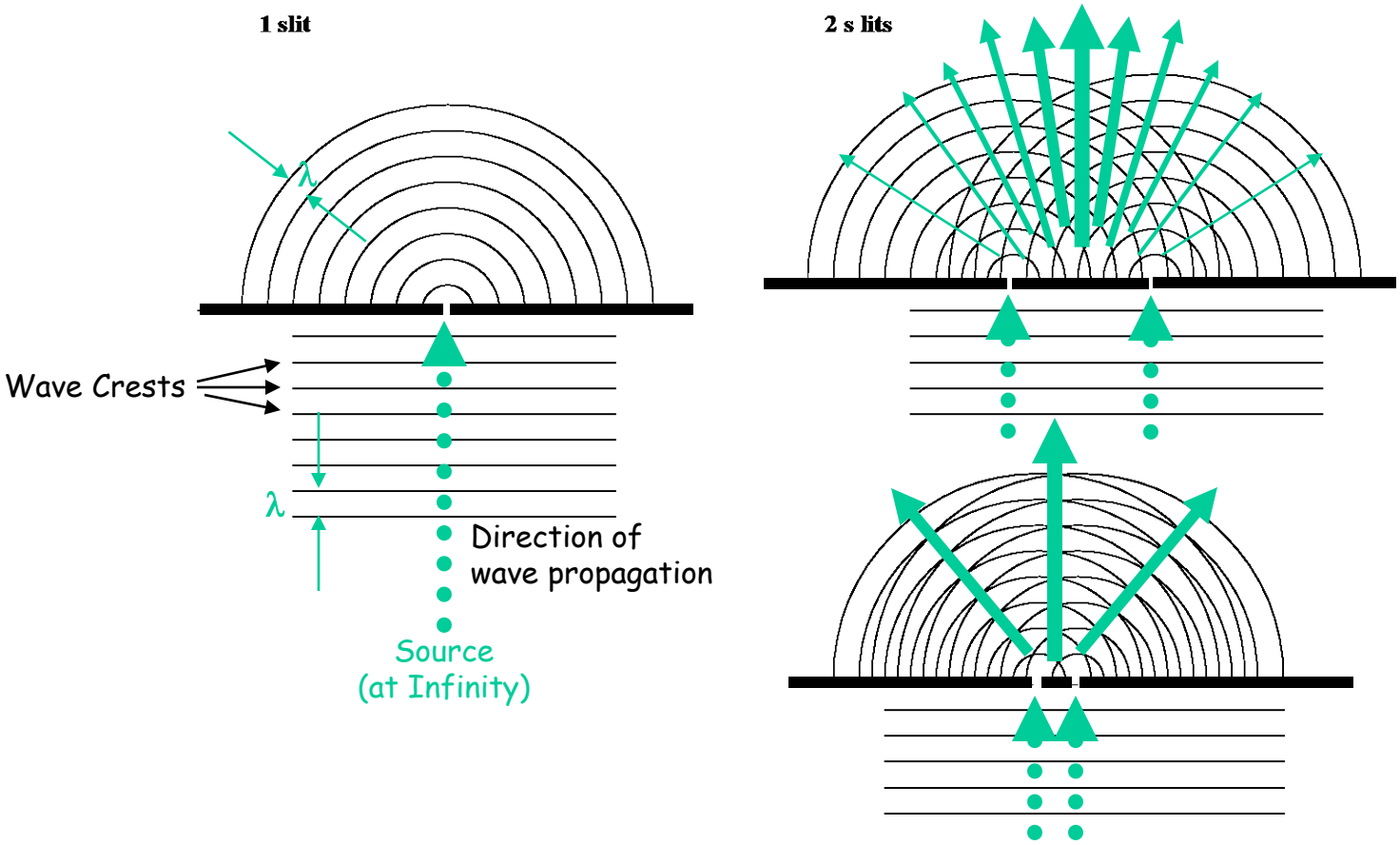
The image results from the number, position and orientation of the diffracted spots



What would happen if blocked some of the spots?

Interference of Coherent Waves

1) Different spacing, same wavelength

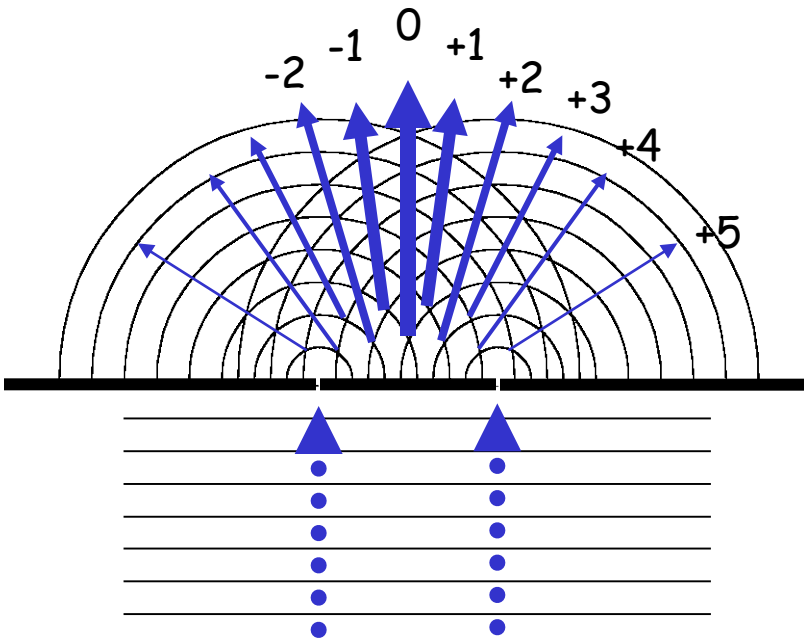


Solid Arrows show Directions of Constructive Interference

Interference of Coherent Waves

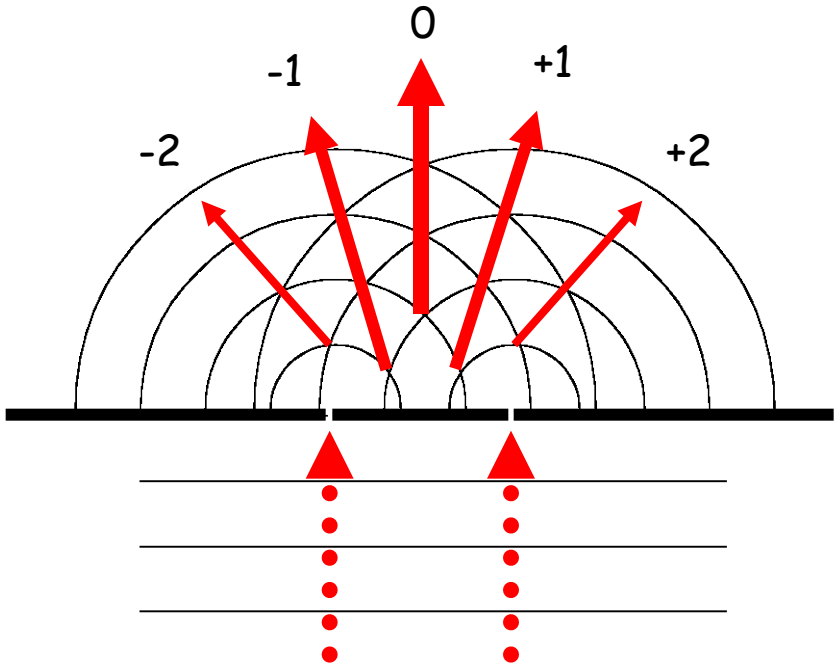
2) Same spacing, different wavelength

Short wavelength



Blue “light”

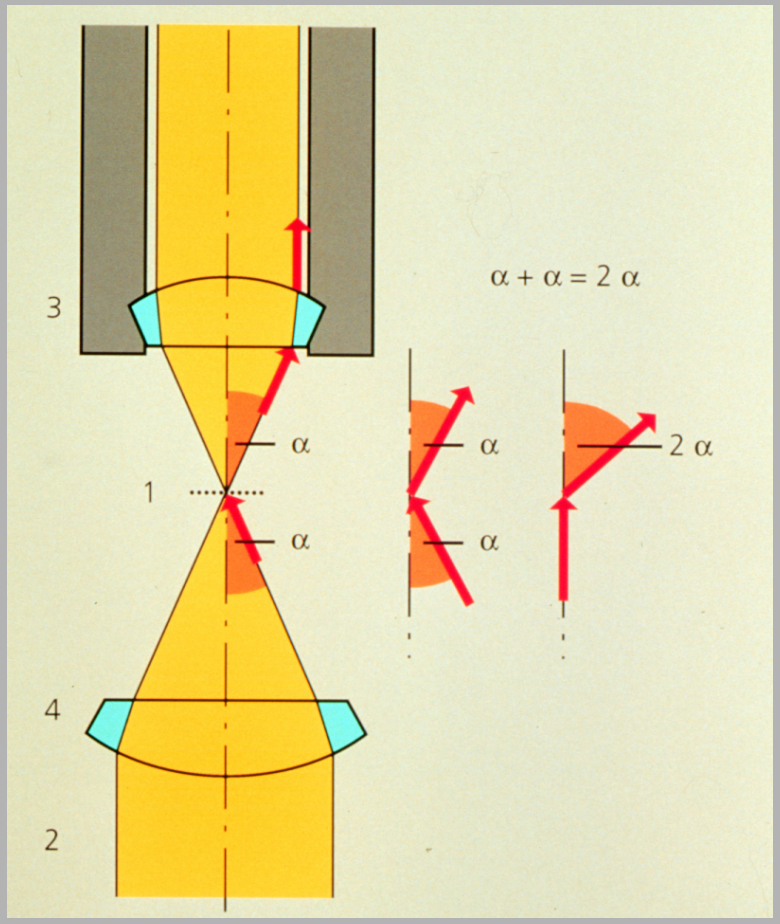
Long wavelength



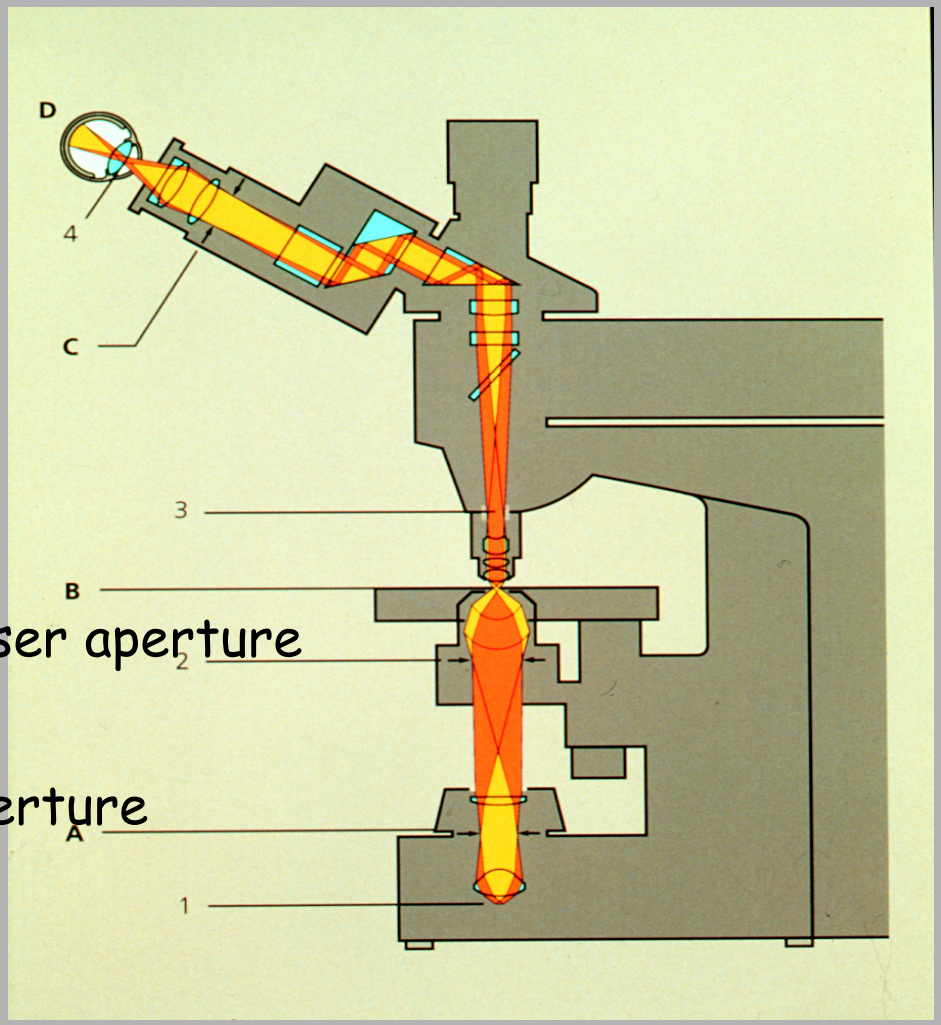
Red “light”

Condenser maximizes resolution

$$d_{min} = 1.22\ \lambda / (NA_{objective} + NA_{condenser})$$



Kohler Illumination: Condenser and objective focused at the same plane



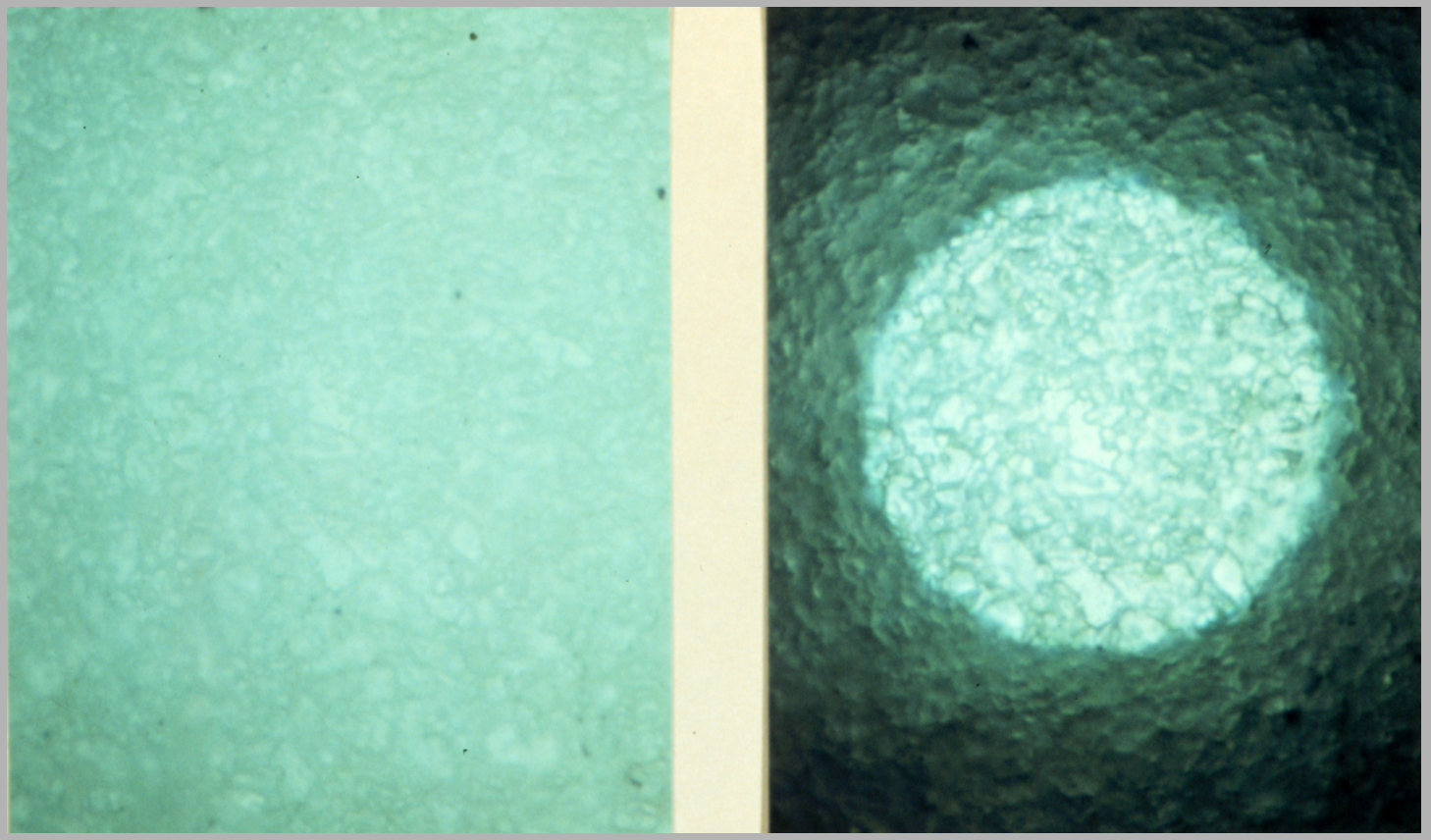
Condenser aperture

Field aperture

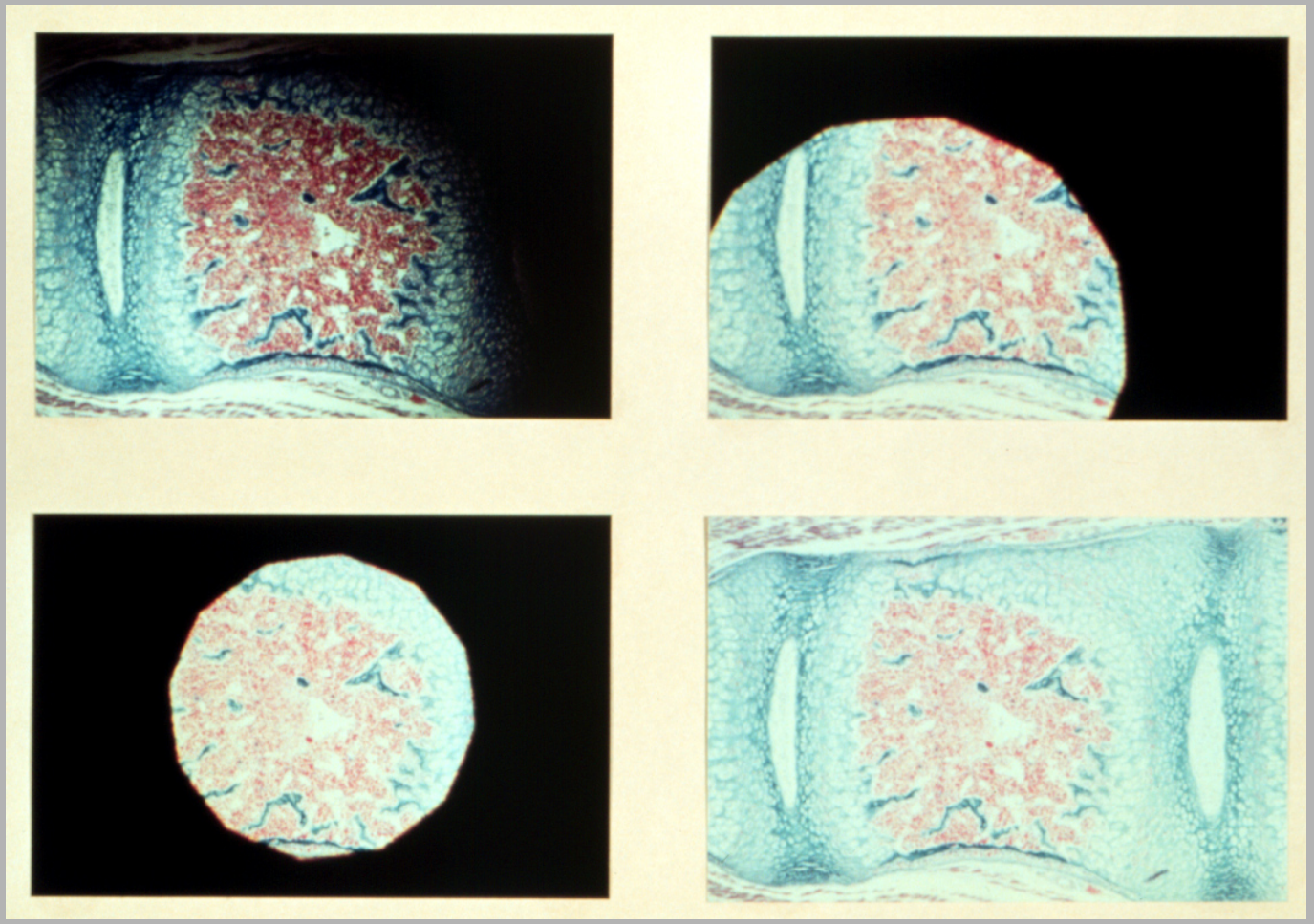
Condenser Aperture controls N.A. of condenser

Field Aperture controls region of specimen illuminated

Kohler Step 1: Close field aperture
Move condenser up-down to focus image of the field aperture

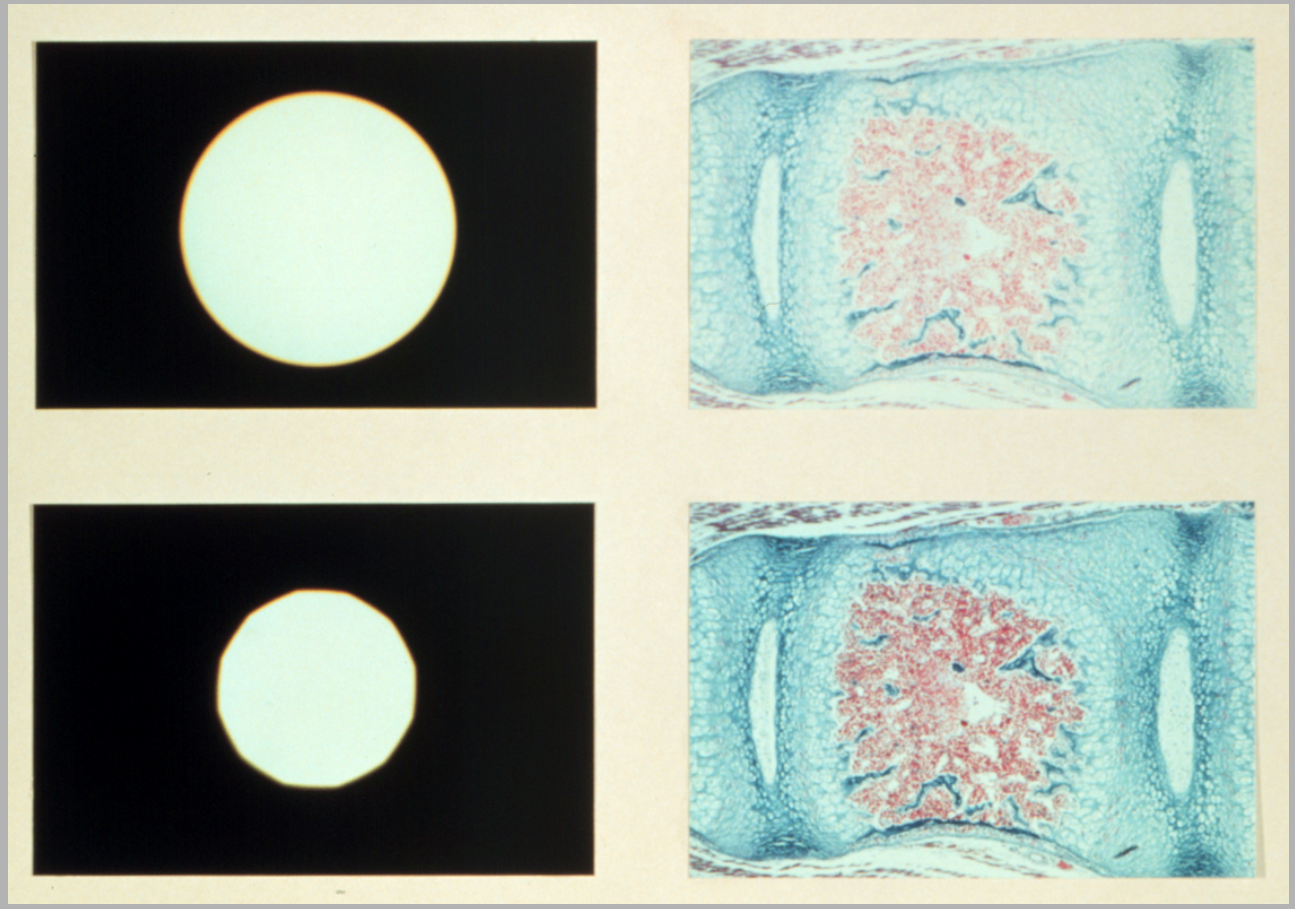


Kohler Step 2: Center image of field aperture
Move condenser adjustment



centered

Kohler Illumination gives best resolution



Set Condenser aperture so

$$NA_{condenser} = 0.9 \times NA_{objective}$$

Open field aperture to fill view

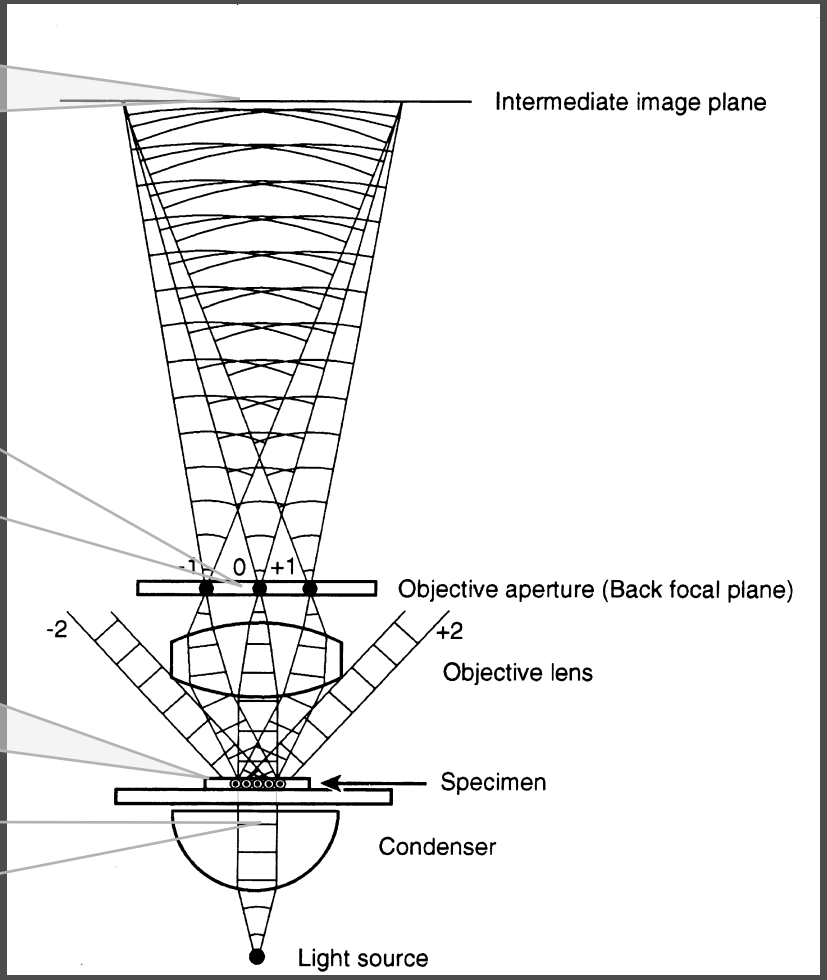
Imaging a linear grating

Intermediate Image: Formed by interfering waves from –1, 0, +1 orders

Back Focal Plane: Diffraction pattern, formed by objective (multiple images of the source as a result of line spacing)

Specimen: Slide with periodic lines. Spacing determines diffraction angles.

Condenser: Produces parallel wave front at 0° (aperture is closed down to a pinhole).



"Illumination Path"