

Lecture four

Questions

Quiz

- Define Numerical Aperture
- Define diffraction
- Define radiated energy that is said to be coherent
- What is the process called when a person is taking photographs using a light microscope?

OPTICS & objectives

- Magnification
 - 10x lens = 16mm fl
- *4x, 10x, 20x, 40x, etc*

Relationship of NA & Magnification

4x - .1

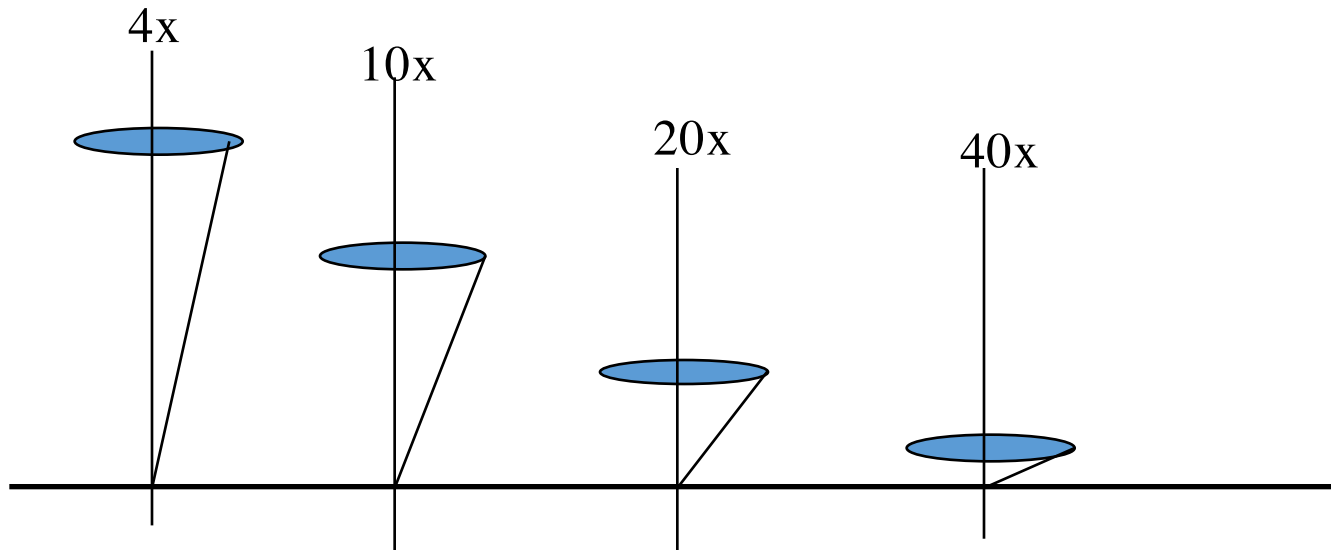
10x - .25

20x - .35

40x - .45

100x - 1.25

Working distance Achromatic objectives



$$NA = n \sin \varnothing$$

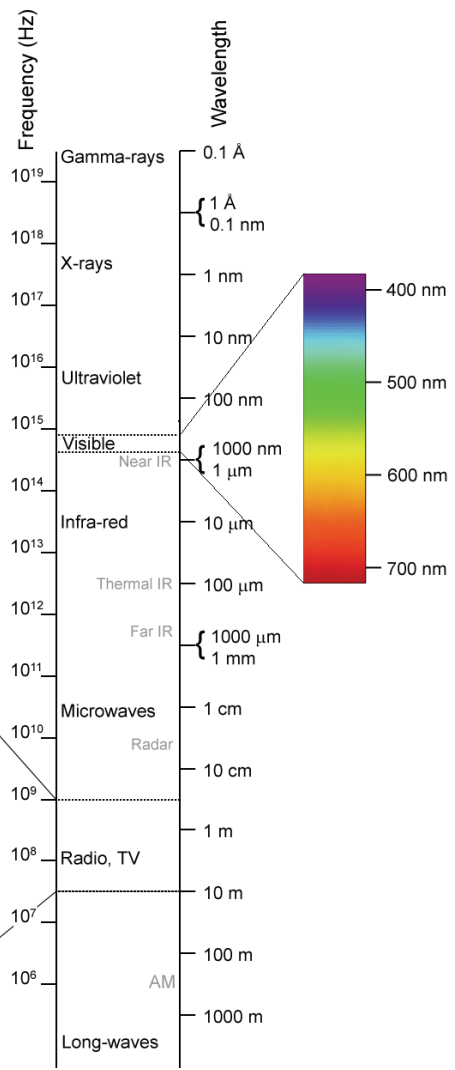
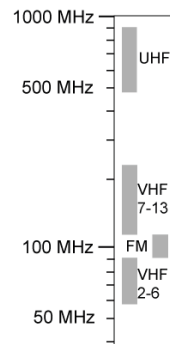
NA influences many
aspects of image formation

$1000 \times \text{NA} = \text{empty magnification}$

Illumination and Spectrums

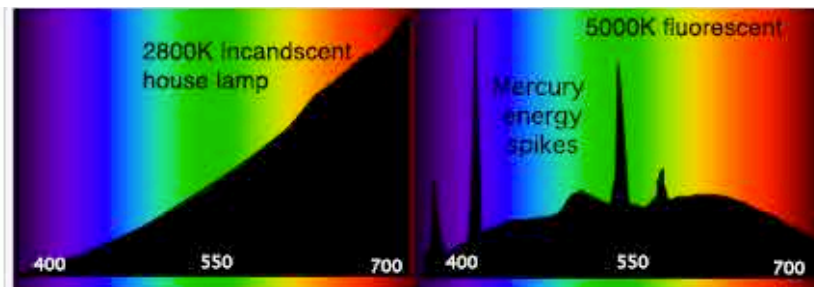
- Tungsten halogen
 - LED
- Metal halide
 - OLED

Electromagnetic Spectrum



Illuminants & Color Temperature

Described in degrees Kelvin, e.g. 3200K or 5500K



Continuous source and discontinuous source

Metal Halide Lamp Spectrum

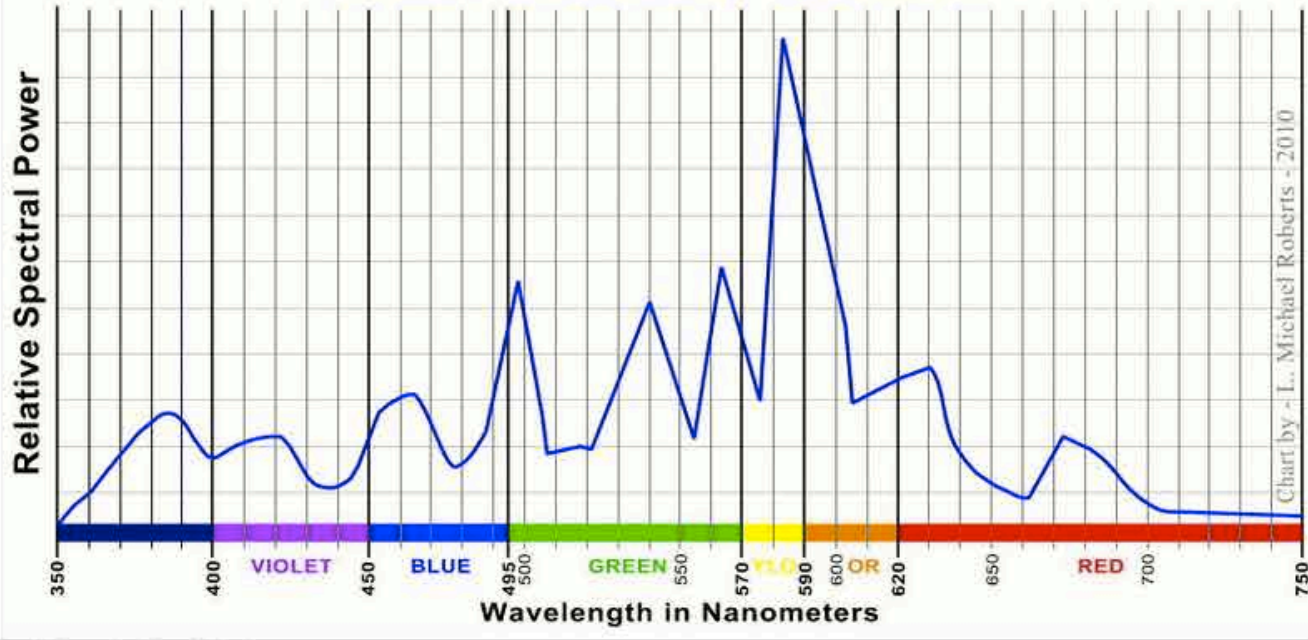
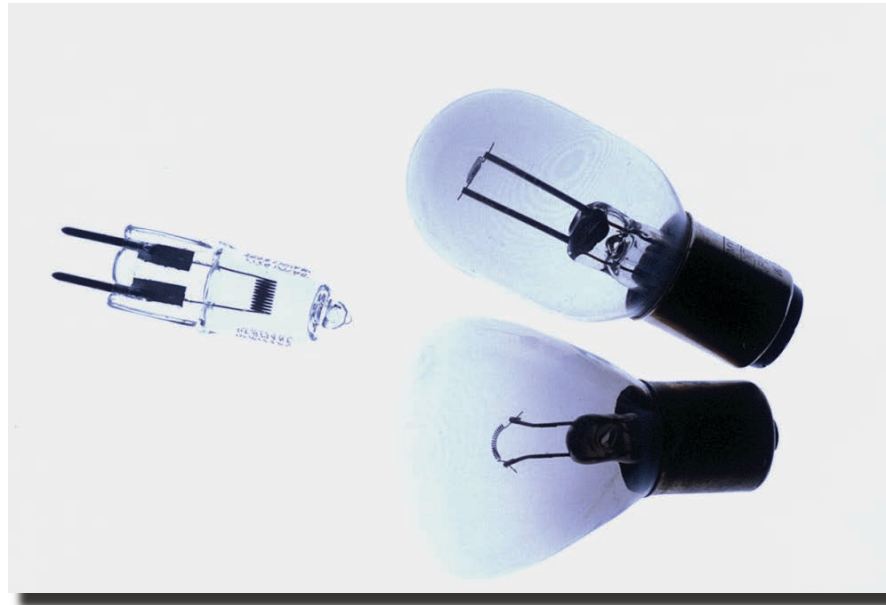


Chart by: L. Michael Roberts - 2010

Tungsten Lamps



Eye pieces

- Viewing
- Photo/video
 - Indexed
- Magnification

Corrected or NOT
Setting and using

Prism head or beam splitter

- 100%

- 80%

- 50%

Filters

- ND
- Light Balancing
 - Diffusion
 - Fluorescence
- Heat absorbing

Substage Condensers

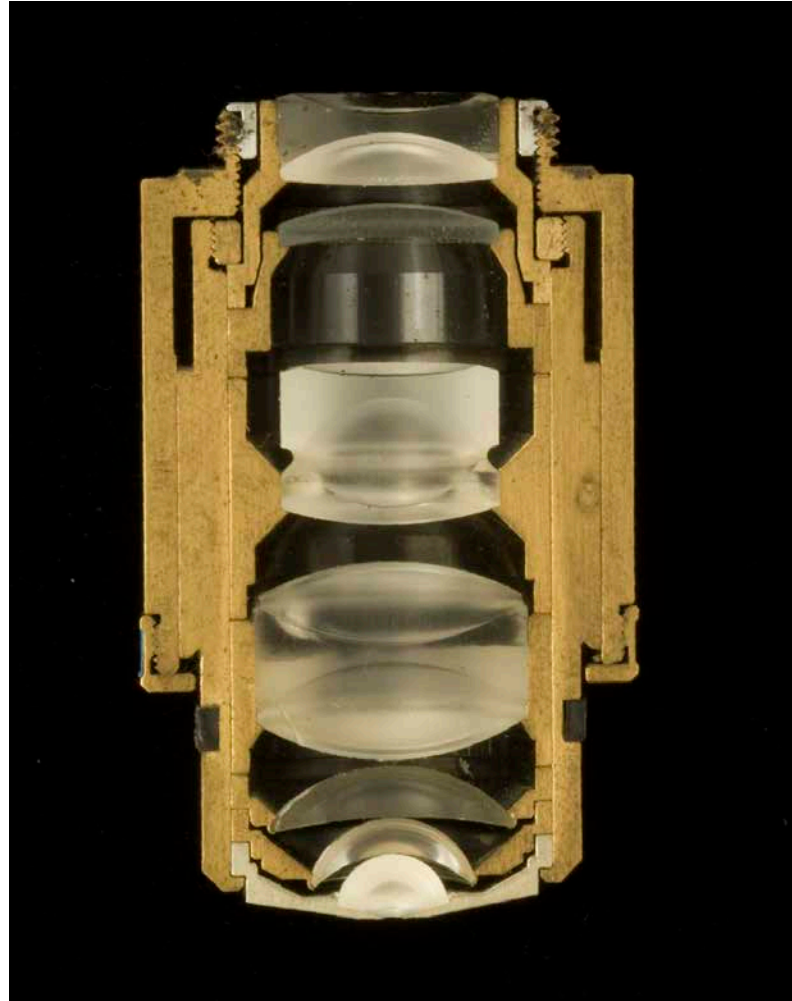


Substage condensers

- Abbe inexpensive NA 1.25
 - Swing out NA .90
- Apo Aplanic NA 1.40
 - Universal or Phase

OBJECTIVES





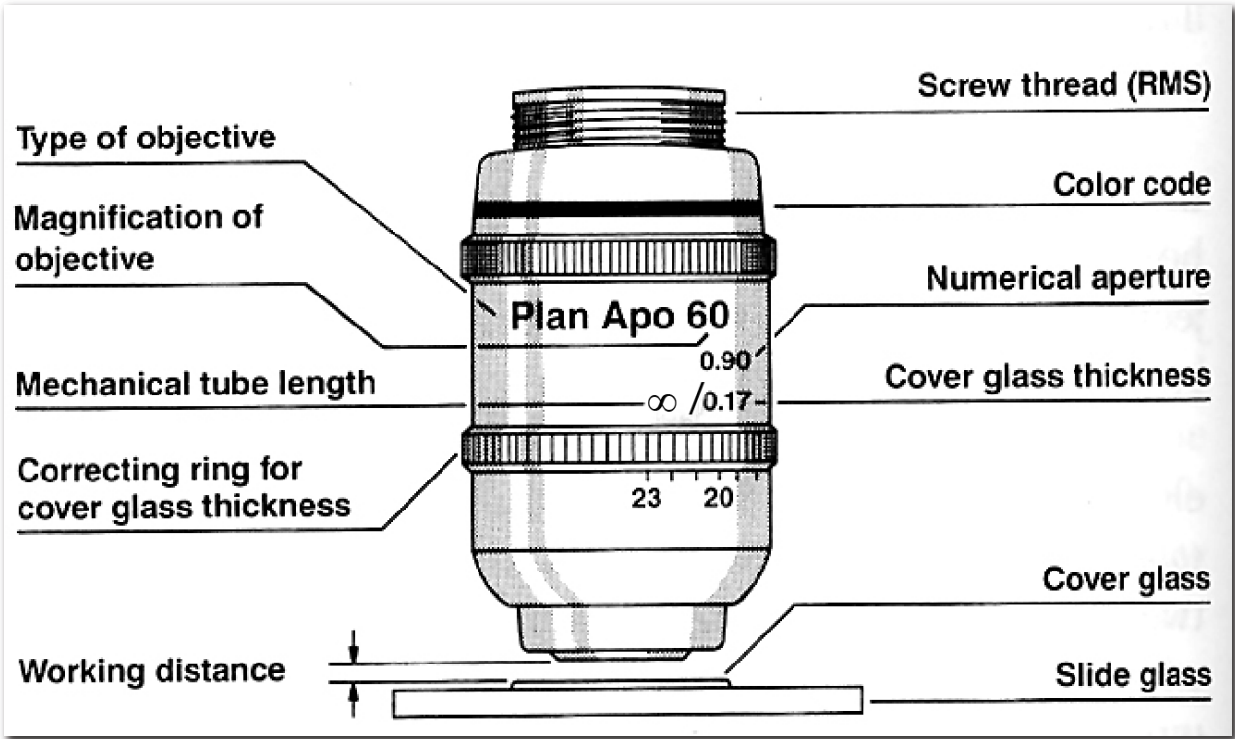
Classifications

Degree of Correction

Achro

FI

APO



Objectives

- Numerical aperture
- PLAN
- Tube length
 - OLD Instruments - 160mm
 - NEW - infinity/ ∞
- Cover slip



Numerical Aperture - NA

1000 x NA = empty mag

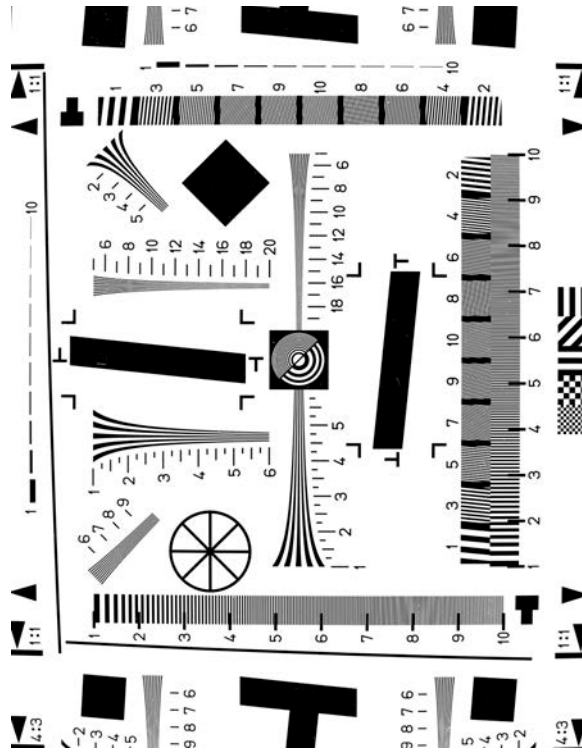
$$d(\text{res}) = \frac{\lambda}{\text{NA}_{\text{objective}} + \text{NA}_{\text{condenser}}}$$

Numerical Aperture

in Air - 1.00

in Oil - 1.53

Optical Resolution



Aperture diaphragm

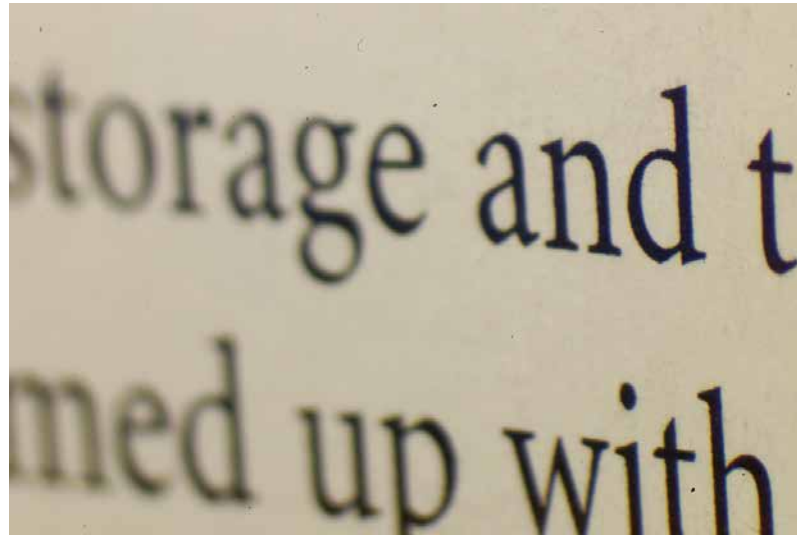
Observed in the body tube
at the Exit Pupil of the Objective

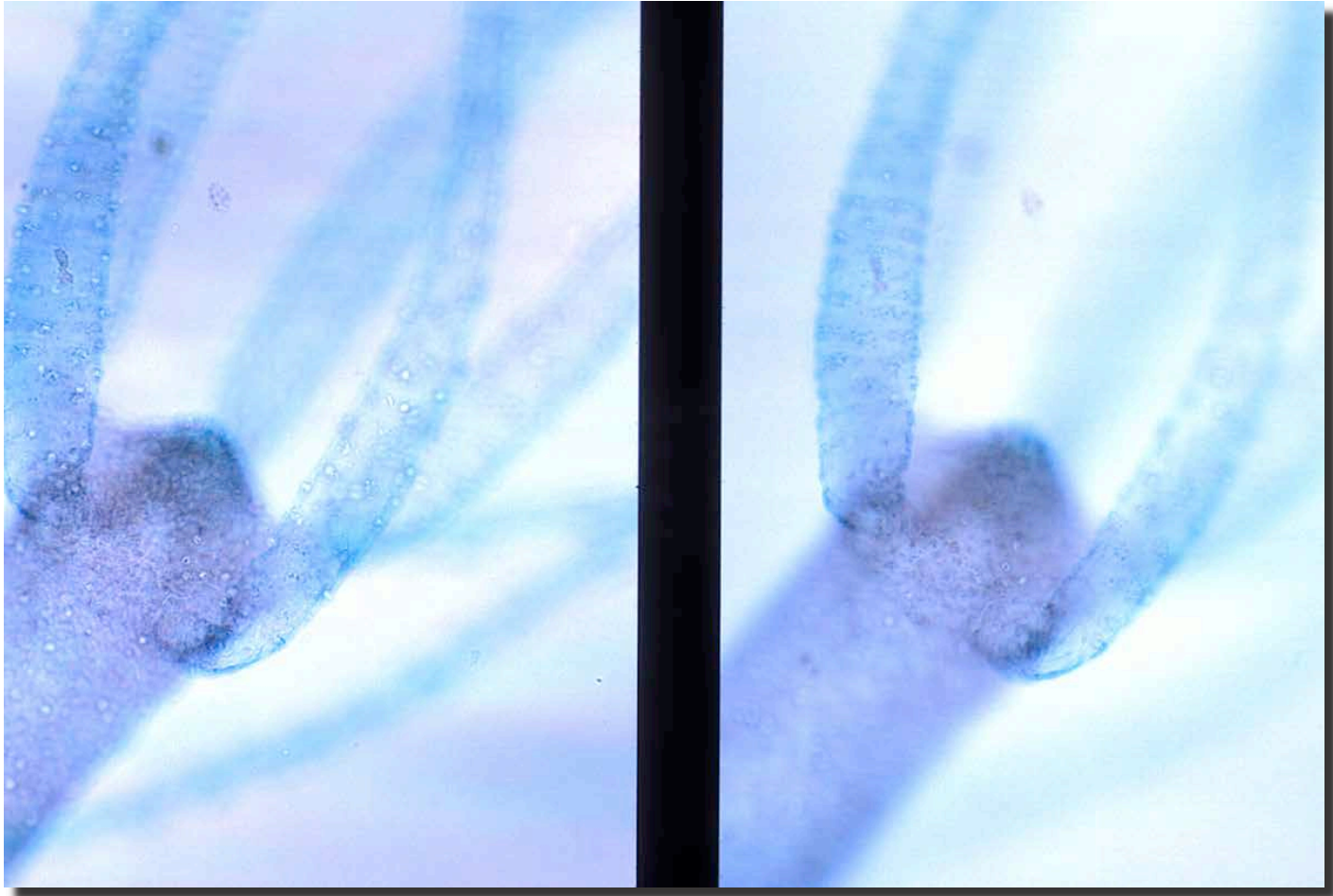
Aperture Diaphragm

- Intensity
- Depth of field
- Resolution
- Contrast

Depth of Field of your image

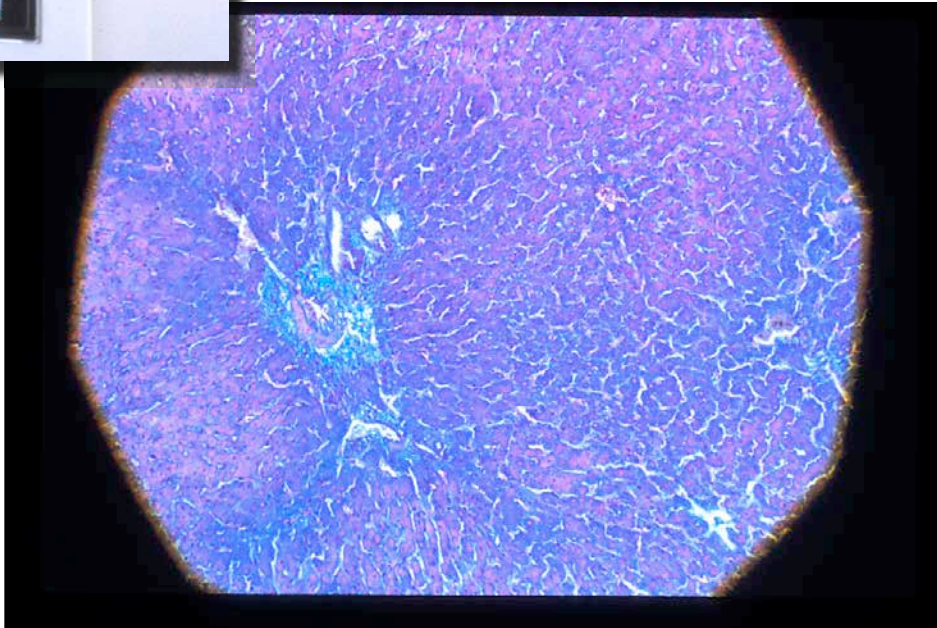
- Influenced by magnification
- Aperture choice



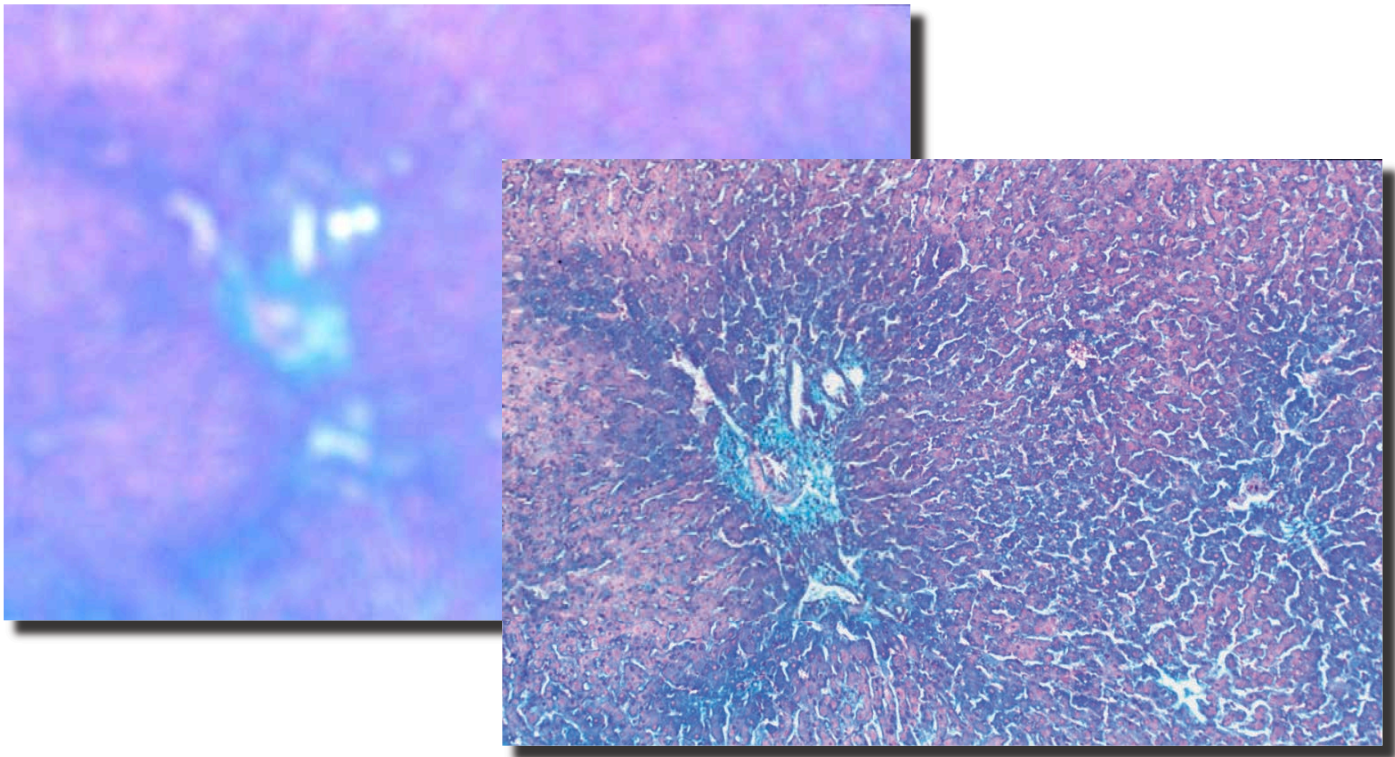


Field diaphragm

Diameter of the
illumination of the beam



focusing

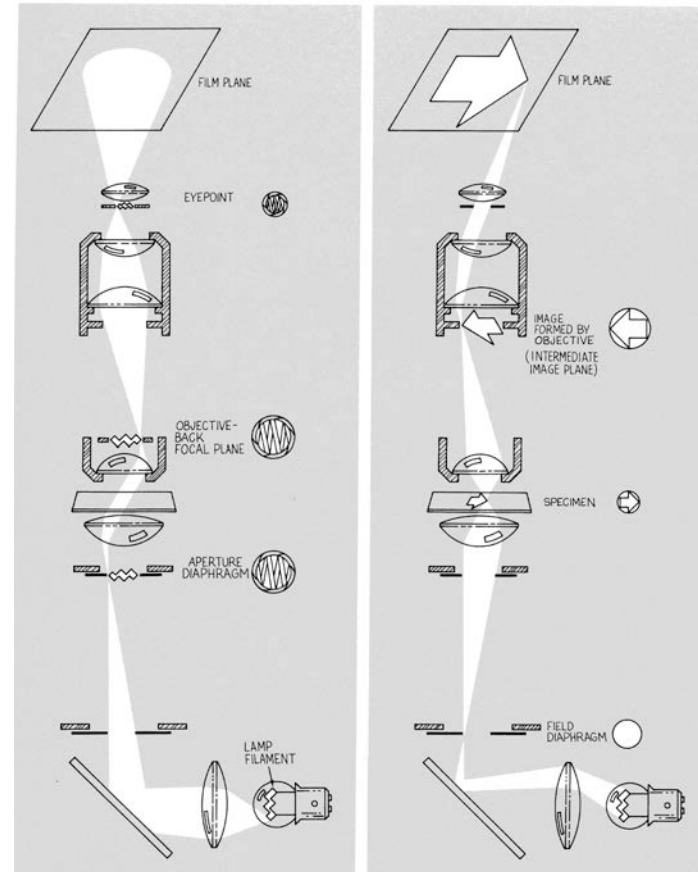


Kohler Illumination



August Kohler
“Kohlering”

- Imaging pathway
- Illumination pathway



IMAGING pathway

- Film plane
- Intermediate image plane
- Specimen
- Field diaphragm

Illumination pathway

- Filament
- Aperture diaphragm
- Exit pupil of the objective
- Eye point

Aperture Diaphragm

