光學顯微鏡的原理與實作

AI-MAT Summer 2022
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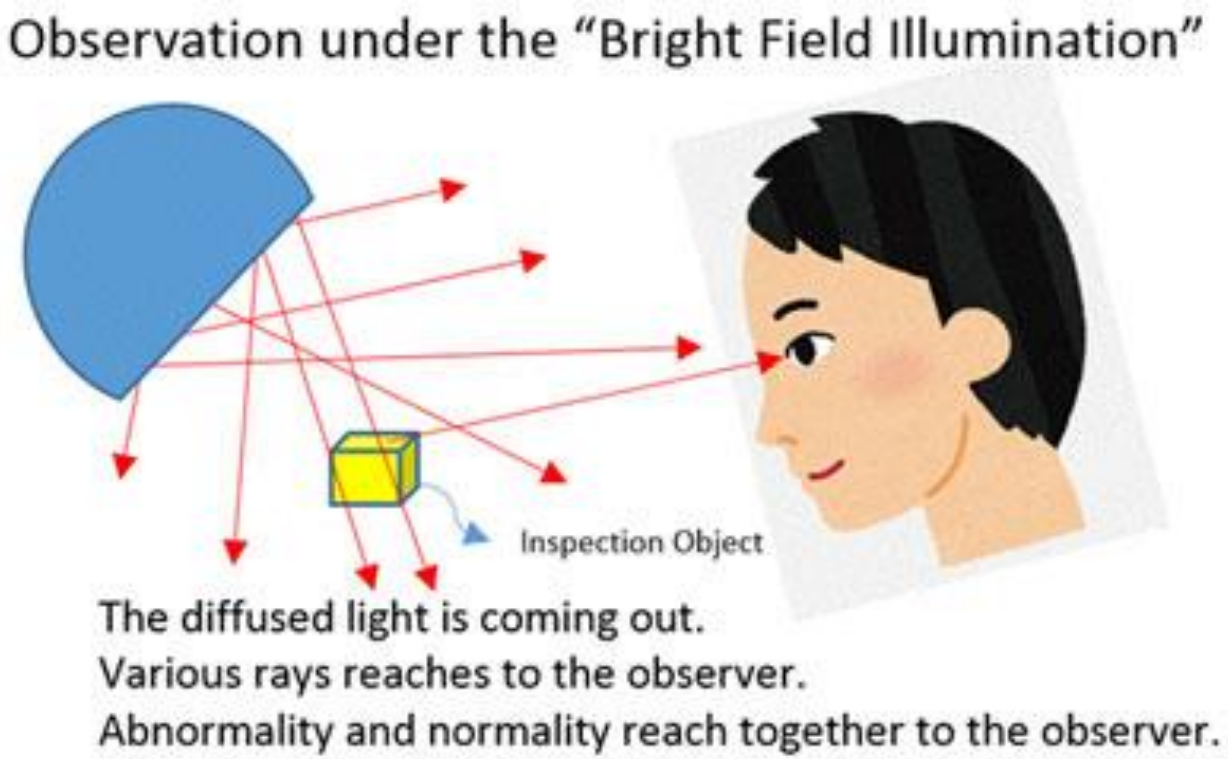
Outline

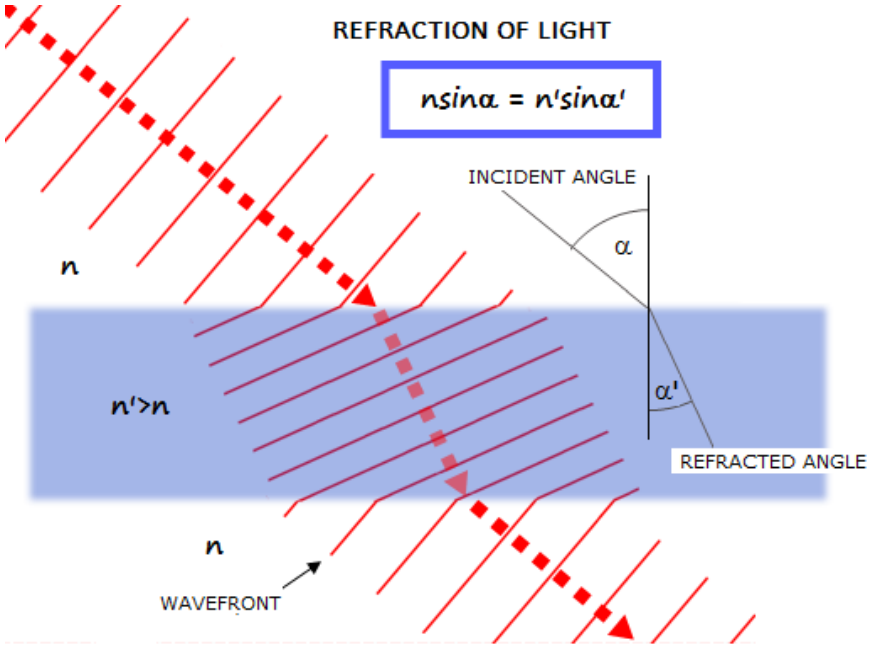
- Image formation principles
  - Ray diagram
  - How lenses magnify objects
  - Compound microscope
- Components of a (bright field) microscope
  - Optical components: objectives, ocular
  - Illuminations: transmission, reflection
  - Mechanical components: stage, nose piece
- Objective specs and resolution limit
- Other Contrast Mechanisms

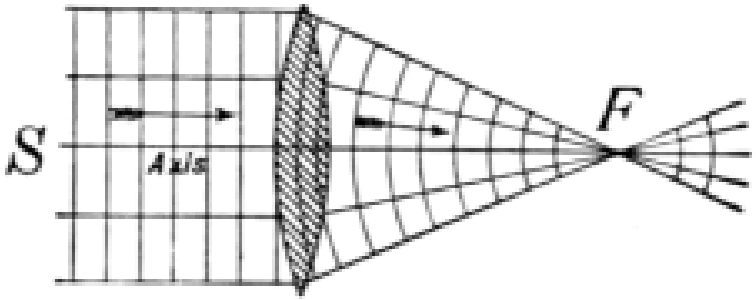
Bright Field Illumination



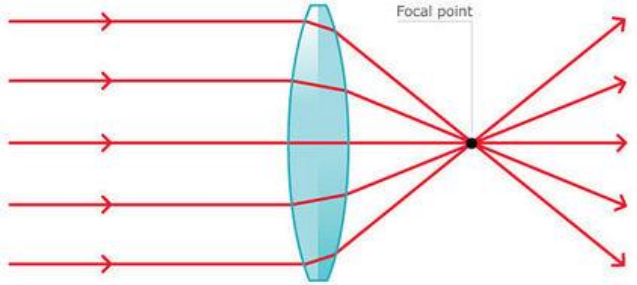
- How we see under light illumination
- If the rays can be made to come from different location, it will change the apparent position of the object
- Image source: https://www.visiononline.org/vision-resources-details.cfm/vision-resources/Visual-Inspection-method-using-dark-field-collimating-illumination/content_id/6972

Bending Light Rays



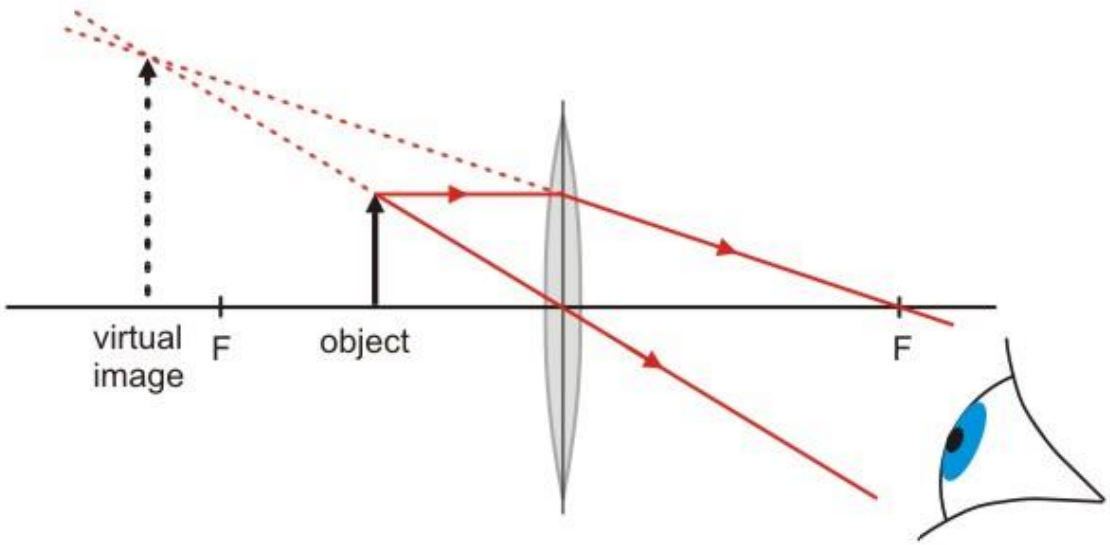


Refraction of light through a converging lens



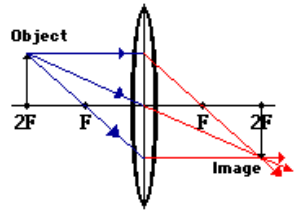
- Image sources:
  - https://www.telescope-optics.net/reflection.htm
  - https://www.cours-et-exercices.com/2018/01/light.html
  - https://www.quora.com/If-light-is-massless-then-why-is-it-attracted-by-a-black-hole

How Lens Changes Apparent Size of Object

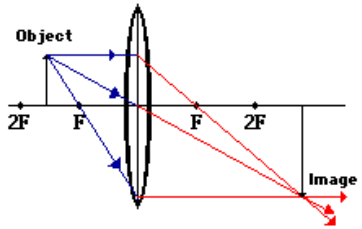


- Lenses can change the apparent size, shape, distance of an object
- Principle rays:
  - Ray parallel to principle axis gets bent to go through the focal point
  - Ray that passes through the center does not bend
  - Ray that goes through the focal point gets bent to be parallel with the principle axis (reverse of the first principle ray)
- Image Source: https://www.saburchill.com/physics/chapters3/0010.html

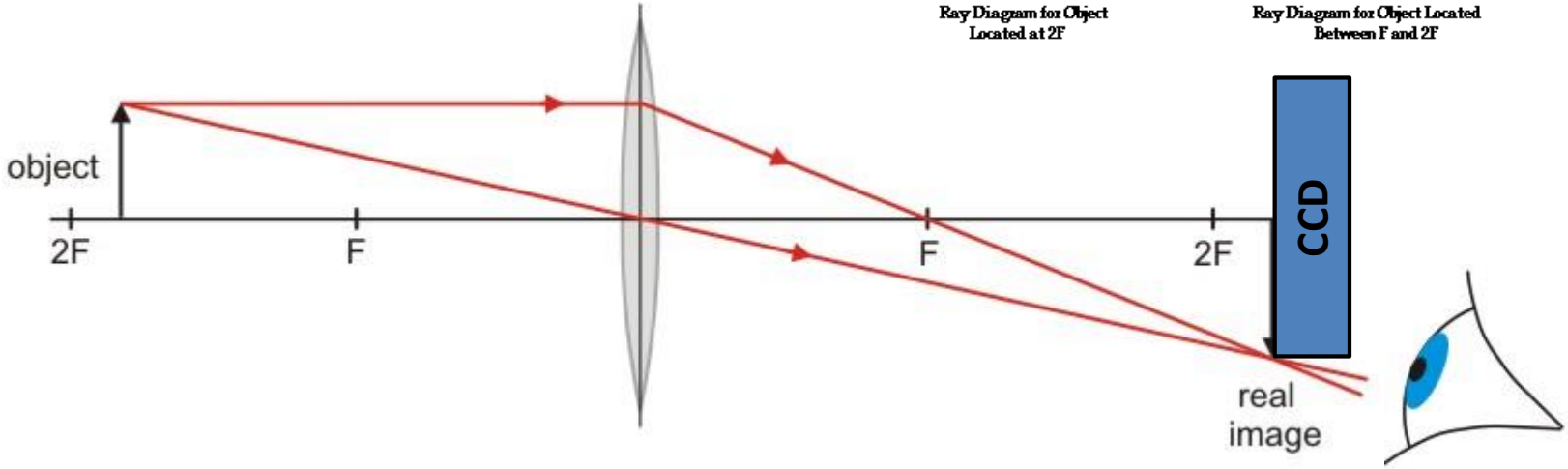
Image Formation (on CCD)



Ray Diagram for Object Located at 2F

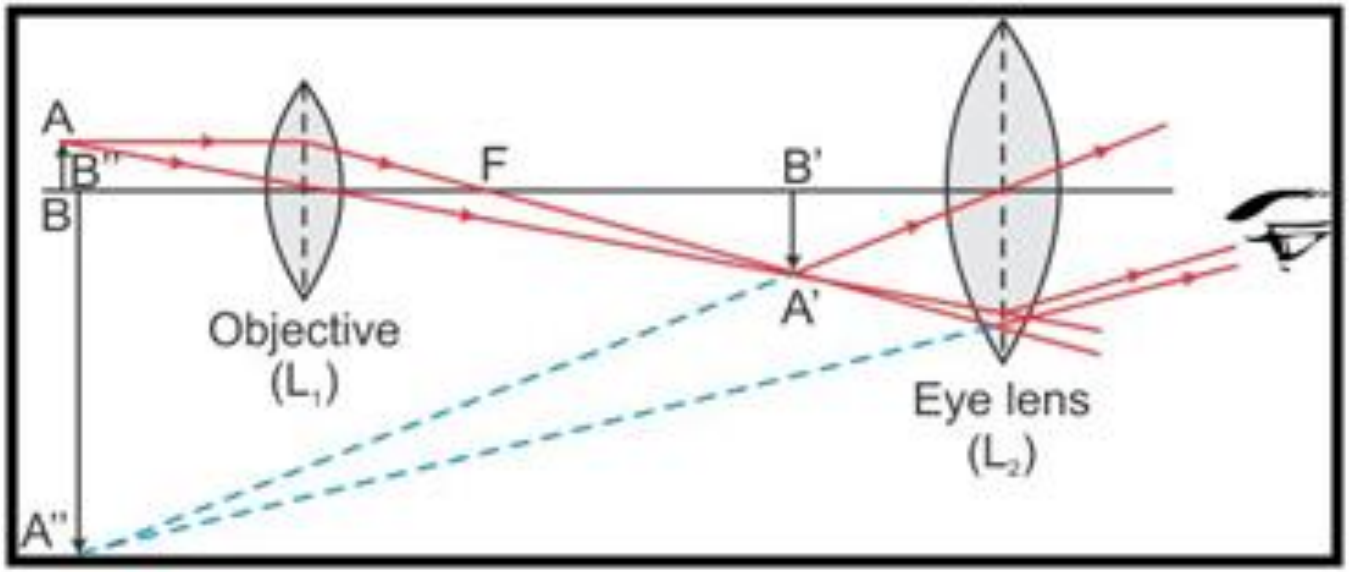


Ray Diagram for Object Located Between F and 2F



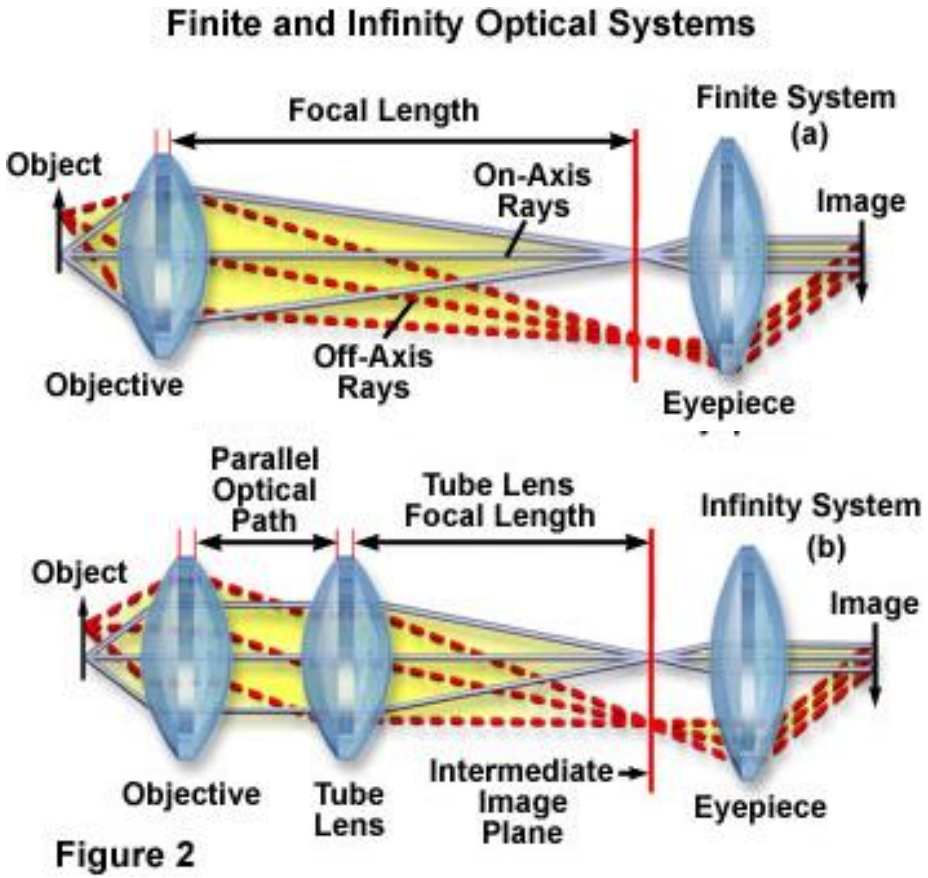
- Move object beyond the focal point leads to formation of a real image
- We can place a CCD at the position of the image to capture the image
- If lens and CCD positions are fixed, focus is achieved by moving the object position
- For short focal length, image can be greatly magnified –> a simple microscope!

Compound Microscope



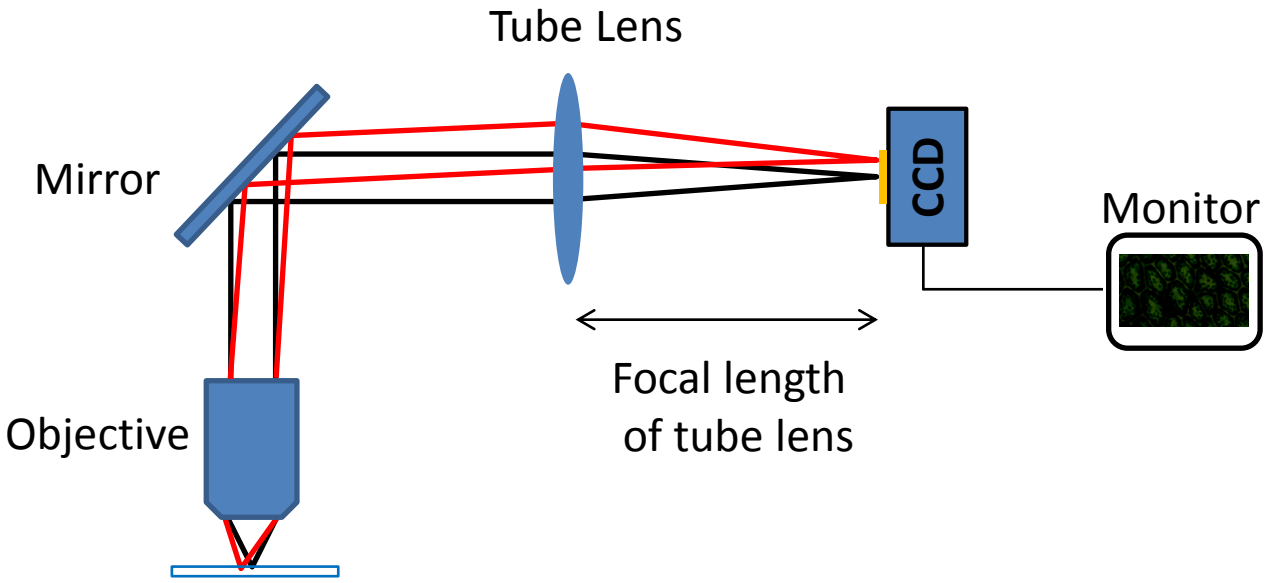
- The Eye lens (ocular) further magnify the image formed by the objective
- Source: https://www.topperlearning.com/answer/draw-a-ray-diagram-to-show-the-image-formation-by-a-compound-microscope/t819jrevv

Infinity-corrected Microscopes



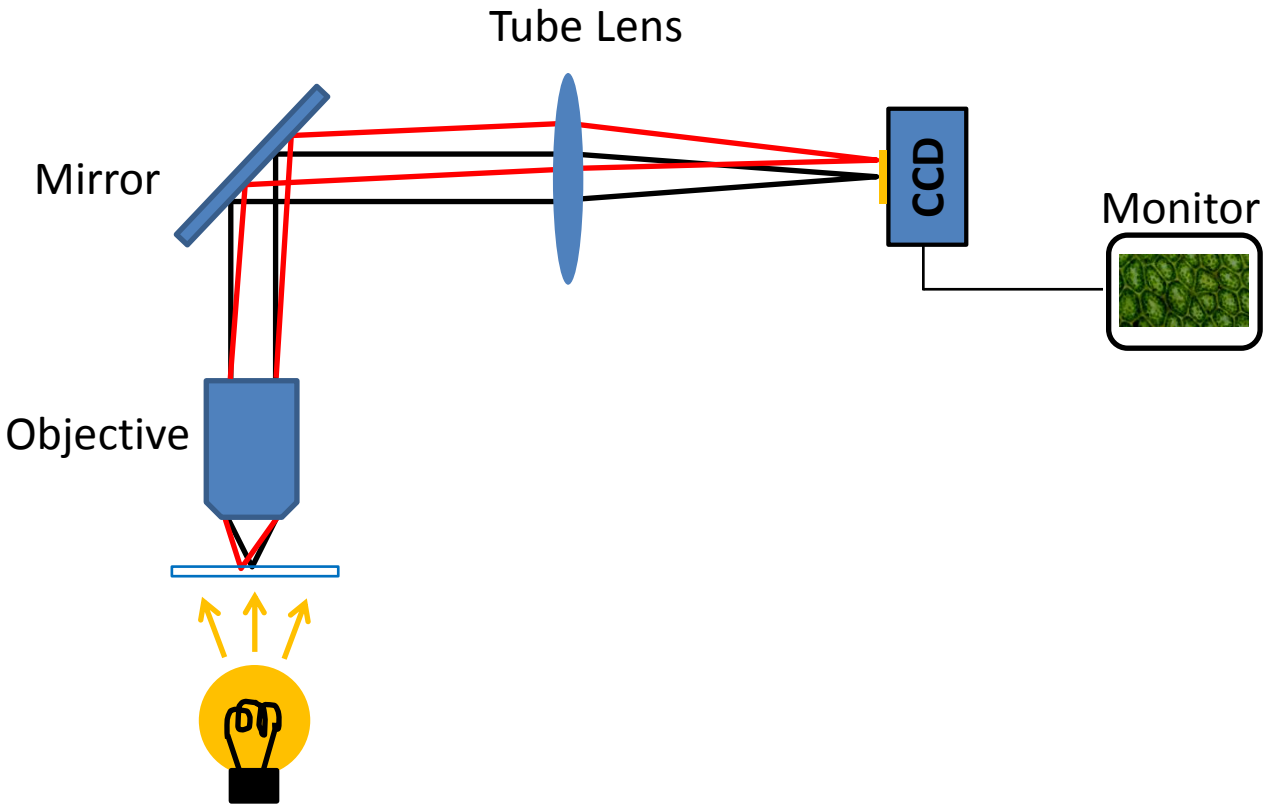
- For infinity-corrected optics, the **objective + tube lens = finite optics objective**
- Image source: https://www.olympus-lifescience.com/zh/microscope-resource/primer/anatomy/infinityintro/

A Simple CCD based Microscope



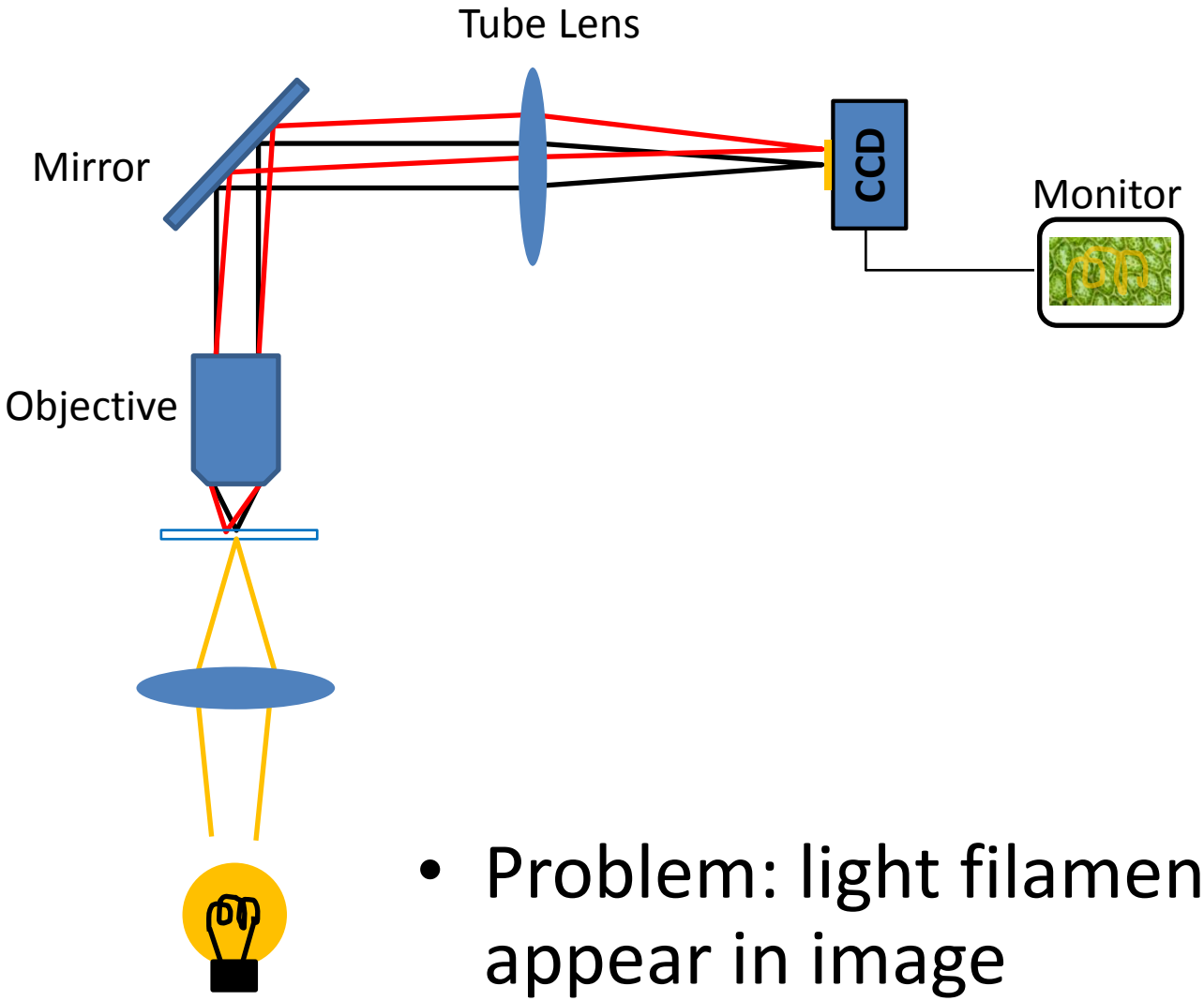
- Focusing involves either moving sample or objective
- Need illumination

Illumination - transmission



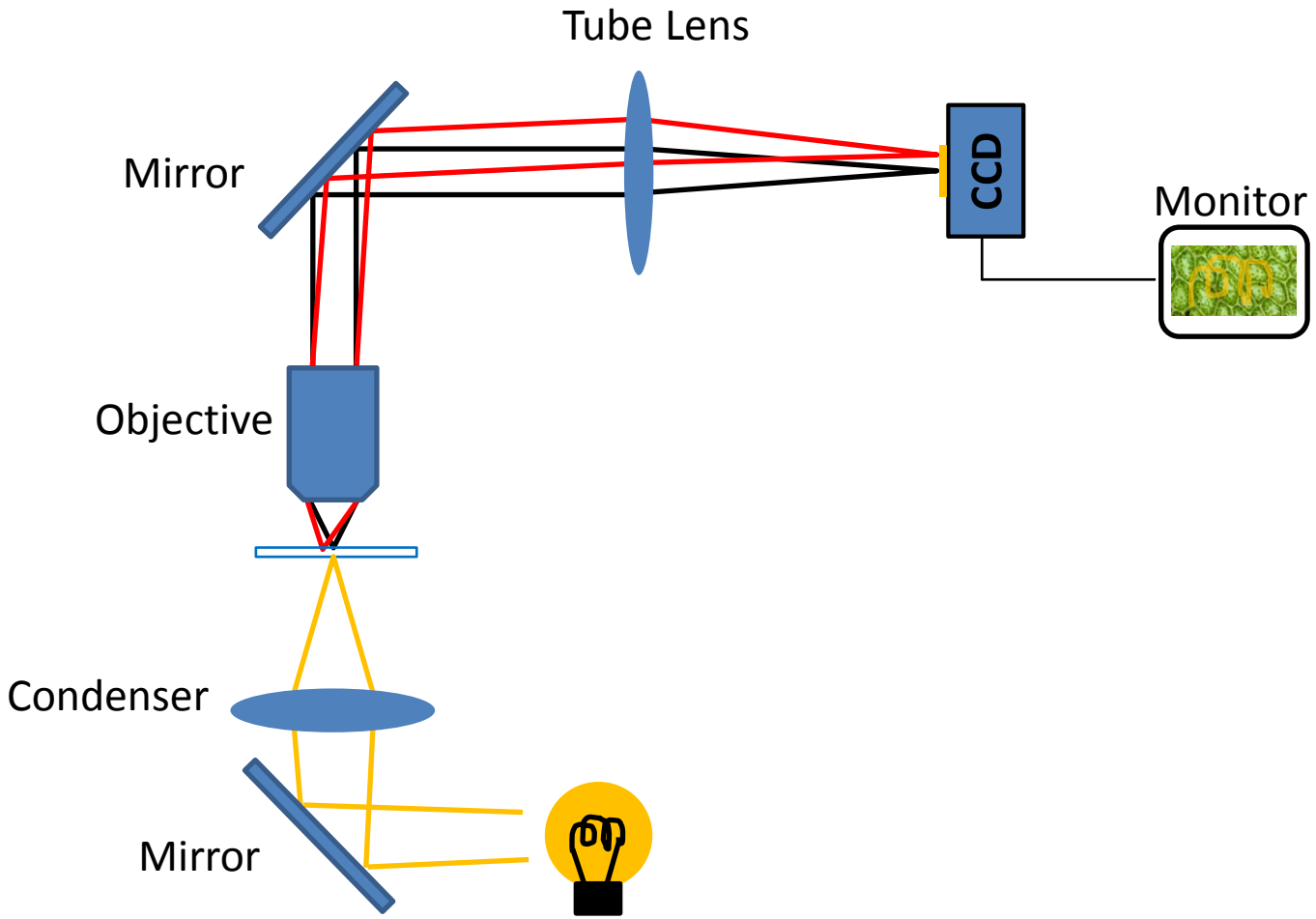
- Problem: Low intensity

Illumination - transmission



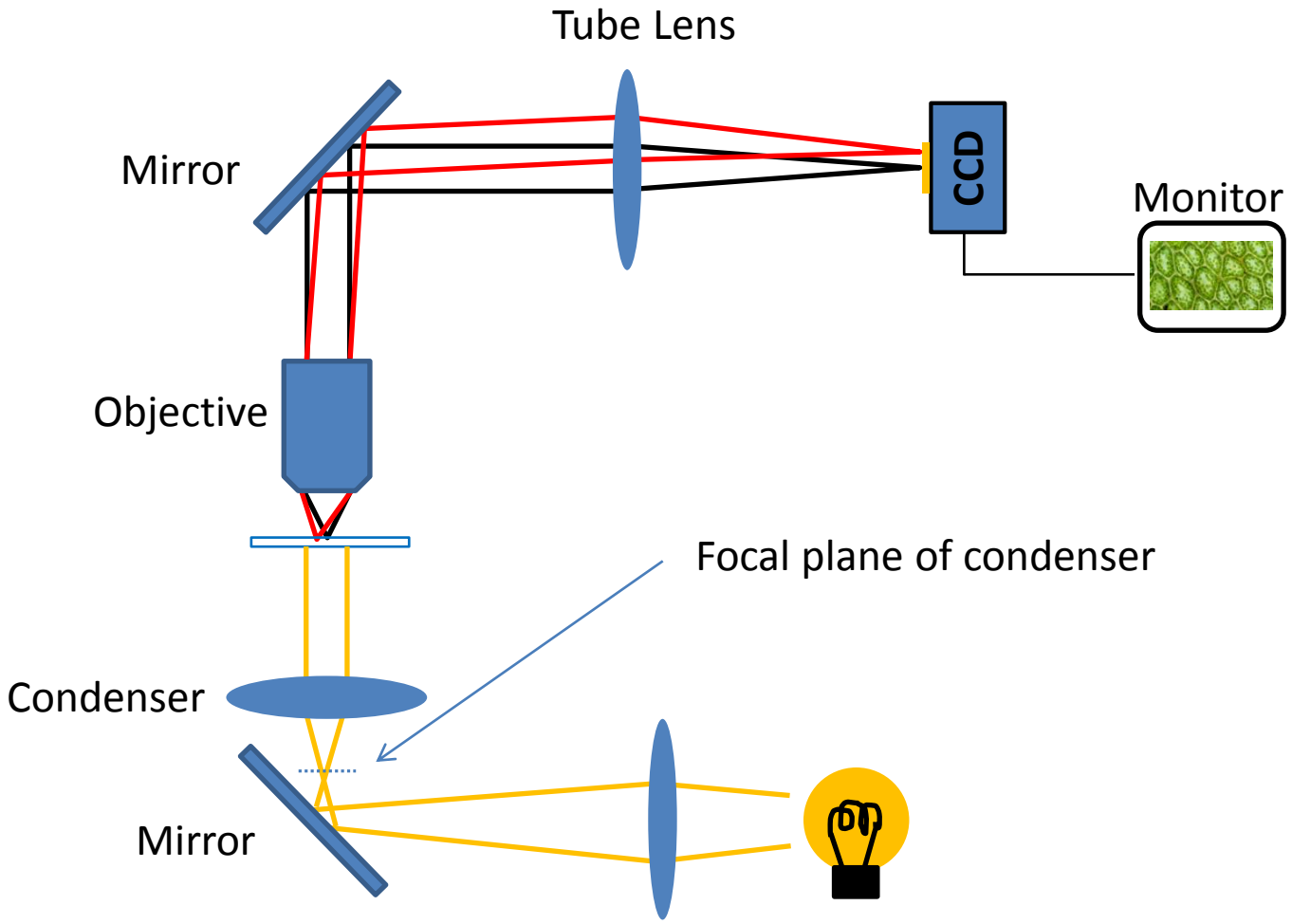
- Problem: light filament appear in image

Illumination - transmission



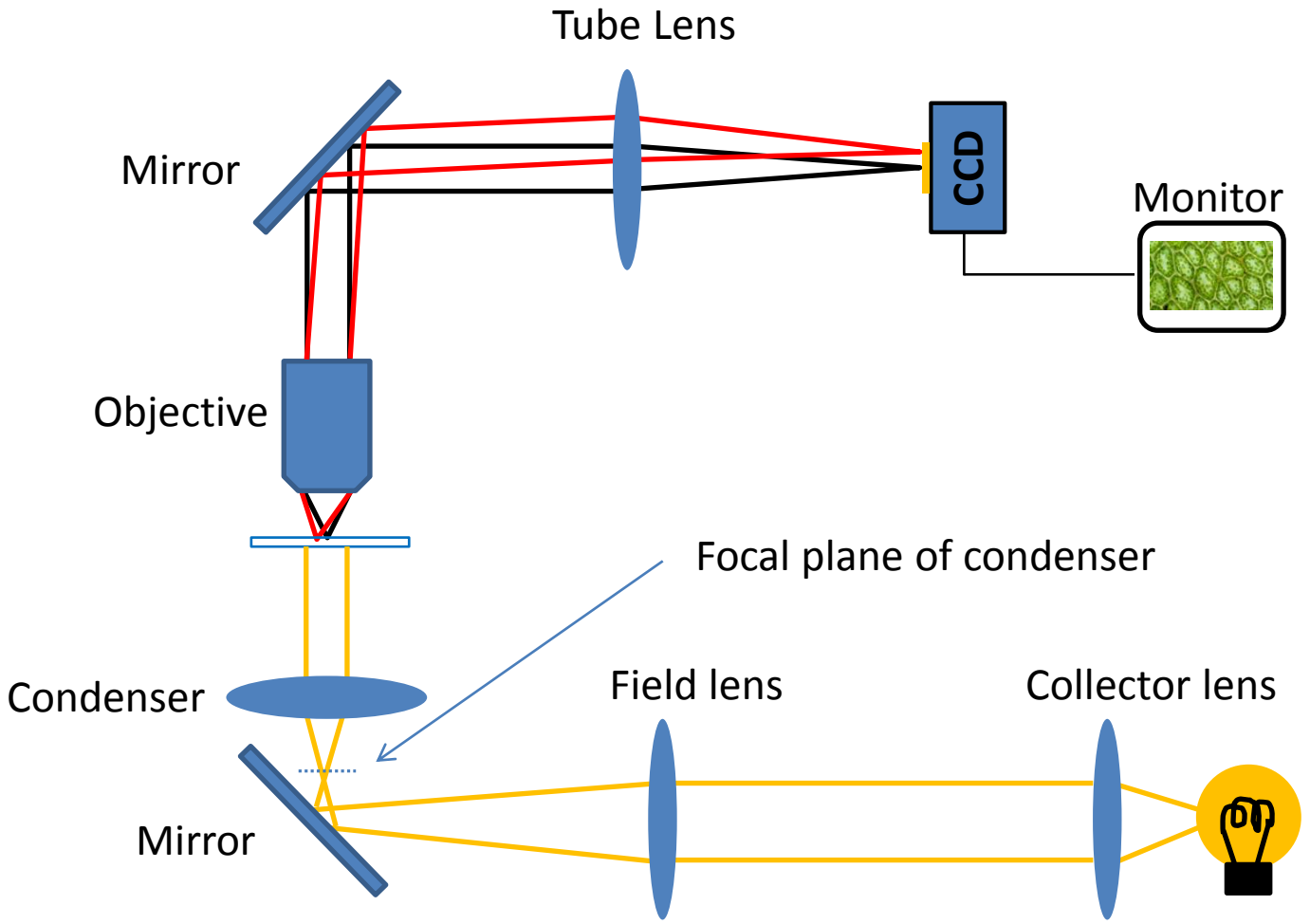
- Add mirror only changes direction

Illumination - transmission



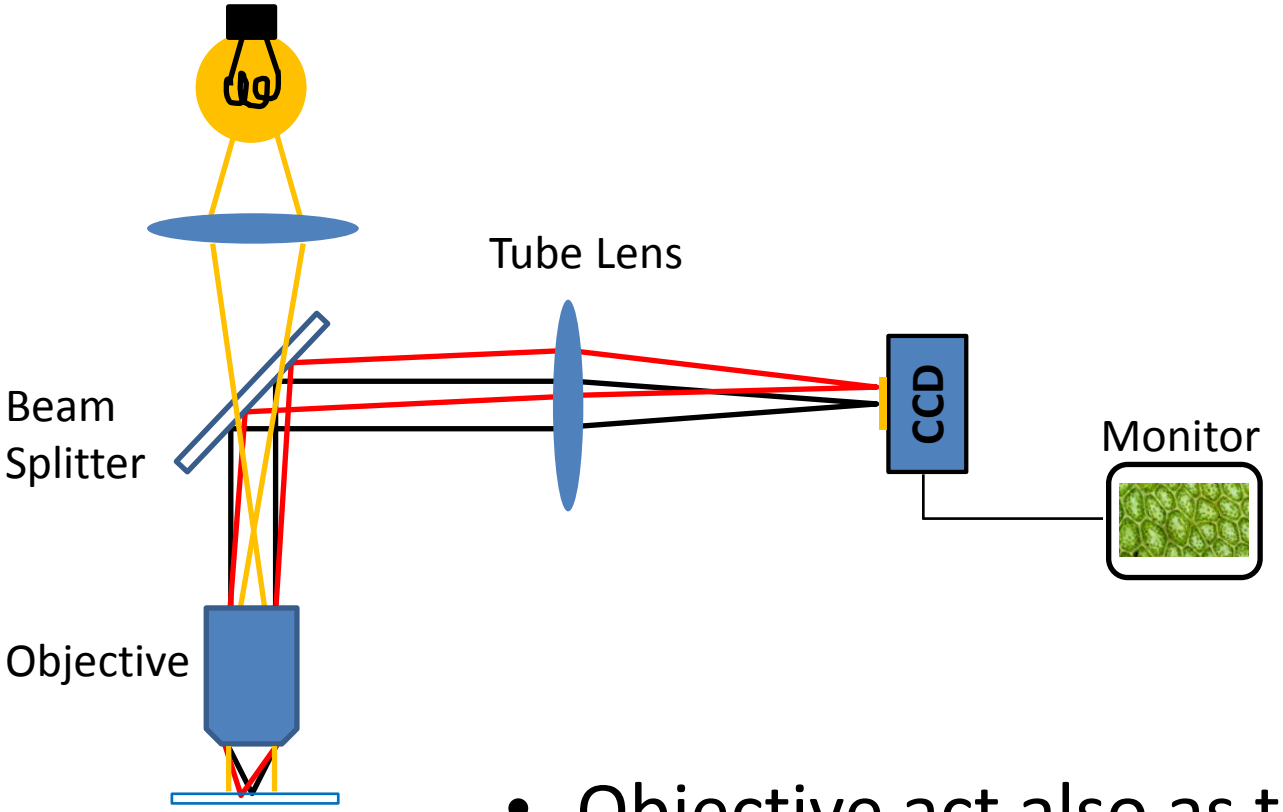
- Kohler illumination: Bright and uniform illumination

Illumination - transmission



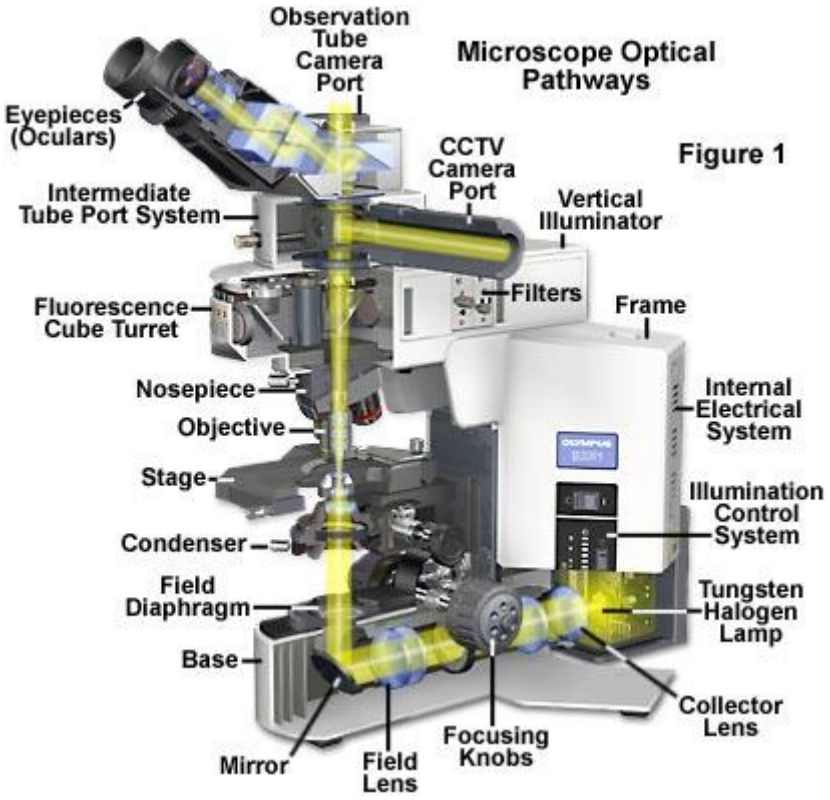
- Collector lens gather more light
- What if the sample is not transparent?

Kohler Illumination - reflection



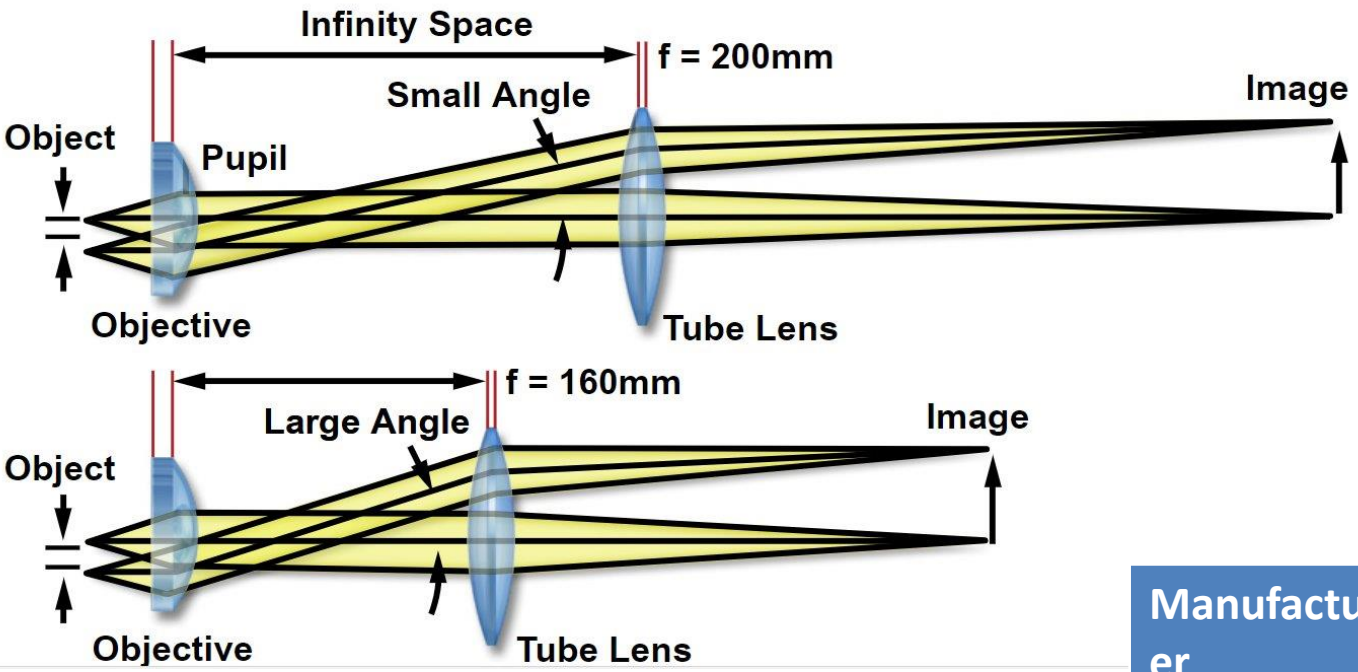
- Objective act also as the condenser lens

Microscope Example: Olympus BX51



- Both transmission and reflective illumination available
- Nosepiece allows mounting of multiple objectives
- Stage allows fine movement of sample
- Port Selector allows changing between eye viewing and CCD

Magnification for Infinity Optics

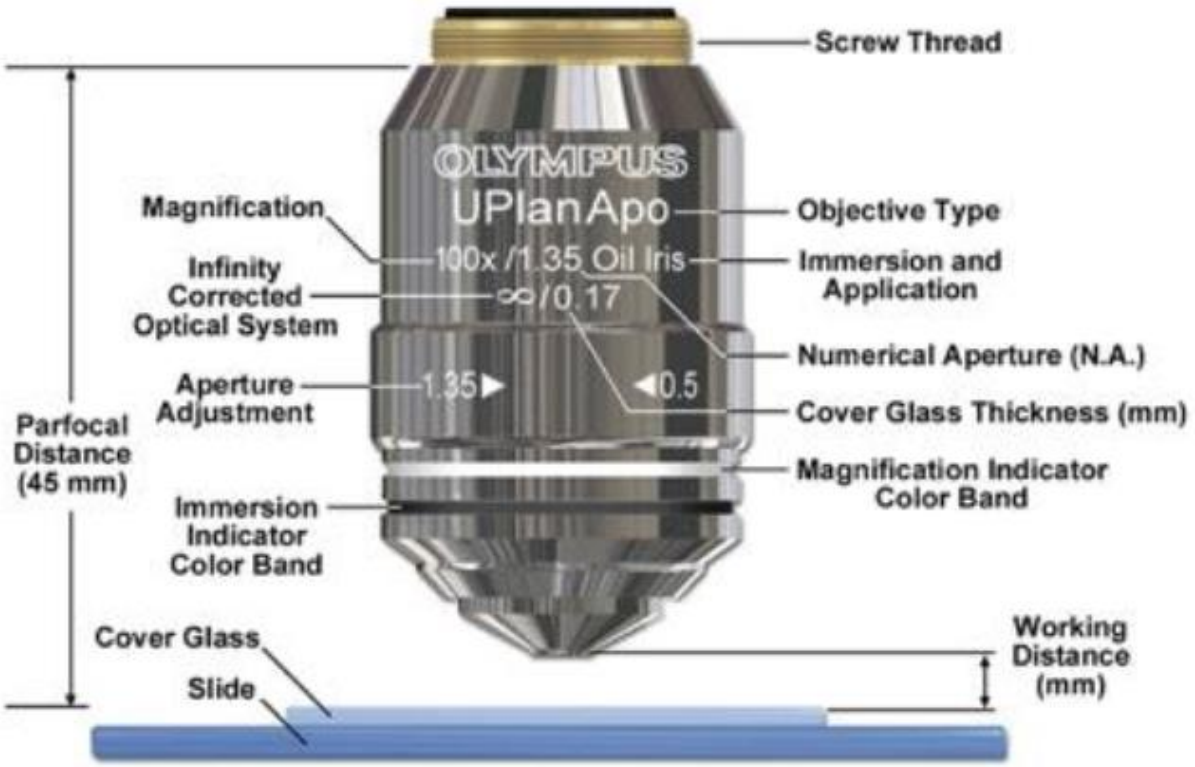


Manufacturer	Tube Lens Focal Length (mm)
Leica	200
Nikon	200
Olympus	180
Zeiss	165

- **Magnification =f_{tube}/f_{obj}** depends on tube lens focal length
- Objective and tube lens distance does not change magnification
- Tube lens focal length differs for different manufacturer
- What's the magnification when using 20x Nikon objective on an Olympus microscope?

20x(180/200) = 18x

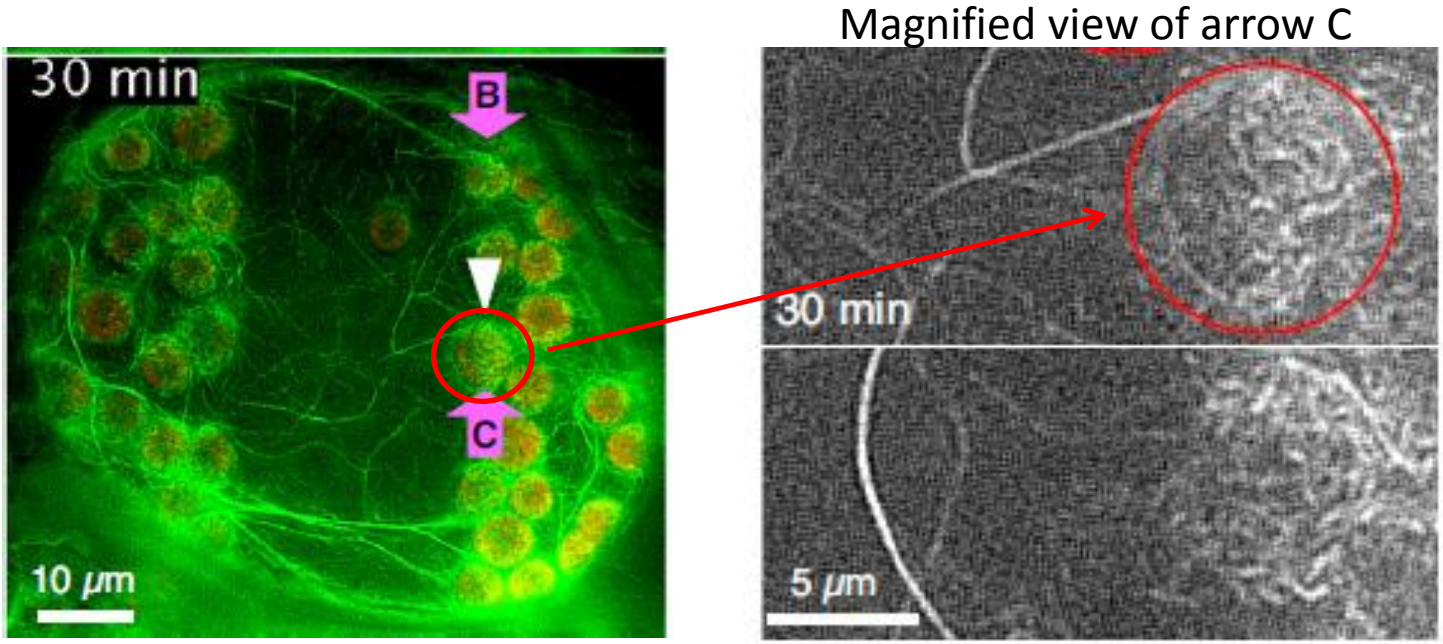
Microscope Objective Specs



Mag.	1×	2×	4×	10×	20×	40×	50×	60×	100×
Code	Black	Gray	Red	Yellow	Green	Light Blue		Dark Blue	White
Imm. Med.	Oil		Water		Glycerin		Oil/Water/Glycerin		
Code	Black		White		Orange		Red		

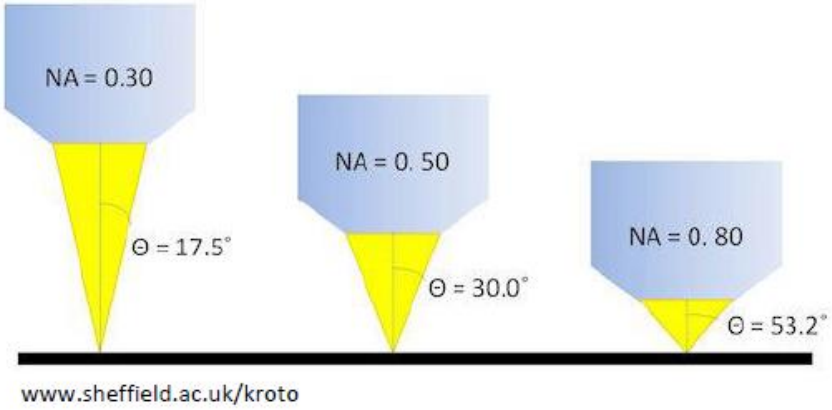
Image source: https://warwick.ac.uk/fac/sci/med/research/biomedical/facilities/camdu/training/introduction_to_light_microscopy.pdf

Magnification and Resolution

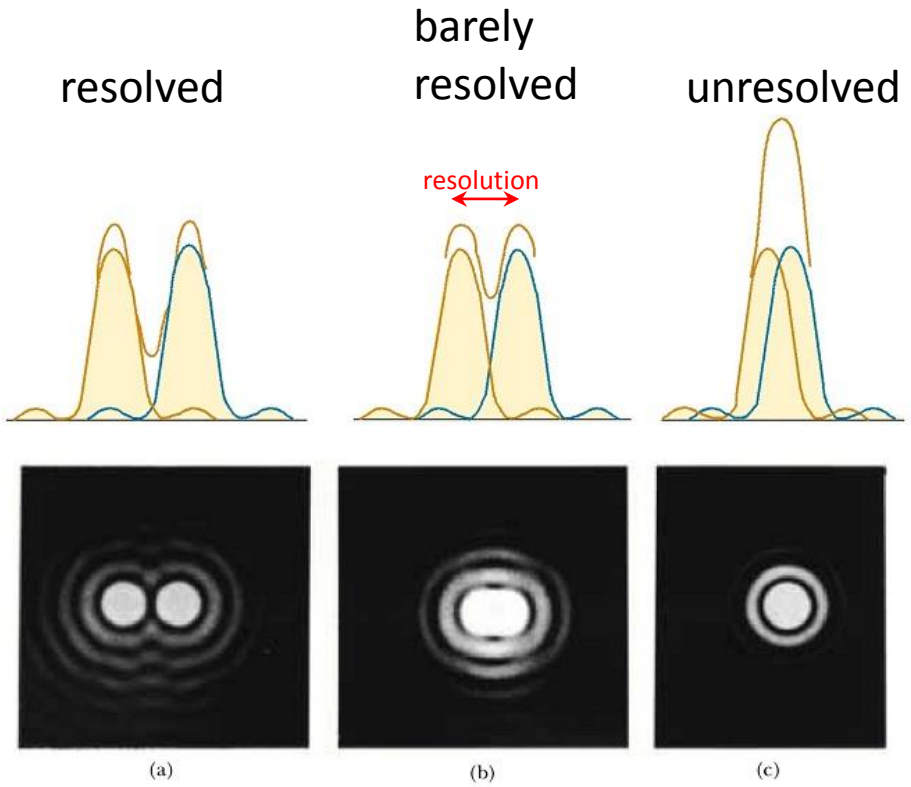


- Image of chloroplast (葉綠體)
- Objective: 63x oil immersion objective, NA1.4 (resolution ~ 240 nm)
- Magnified view shows resolution limitation
- Image Source: PNAS 2009, **106**, 13106-13111

NA and Resolution

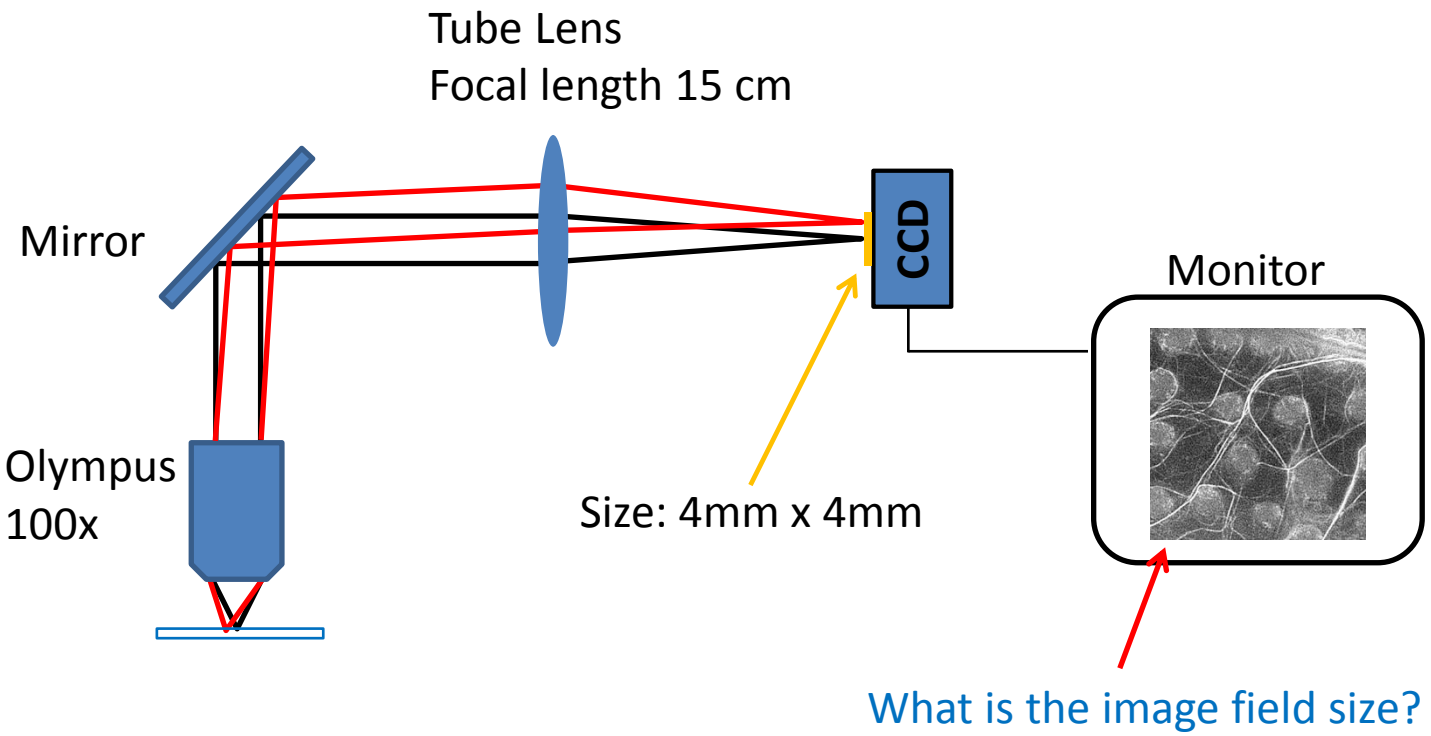


$$NA = n \sin\theta$$



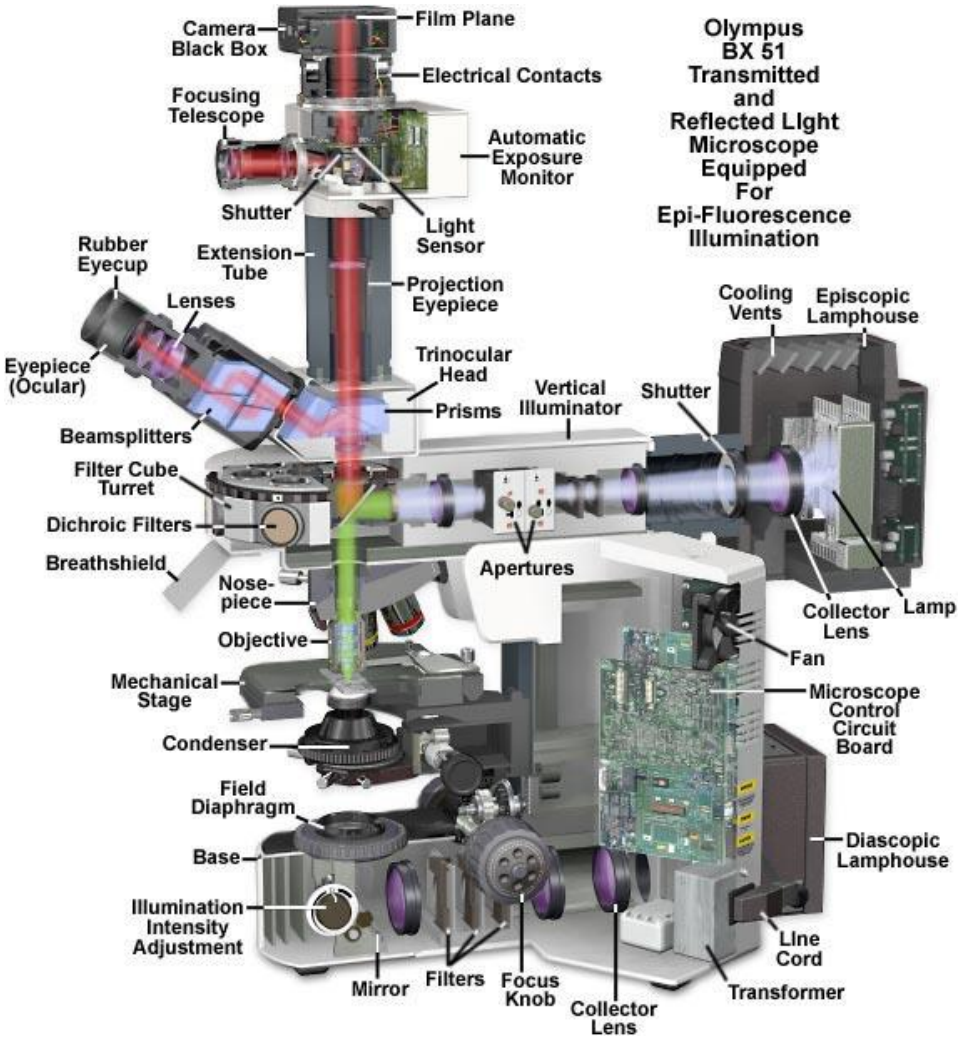
- **Resolution (Rayleigh) = 0.61λ/ NA**
- Higher NA → Higher resolution
- Higher NA objectives usually have shorter working distance, focal length, and larger magnification. Why?
- For NA=0.9, λ = 550 nm, Resolution ~ 370 nm

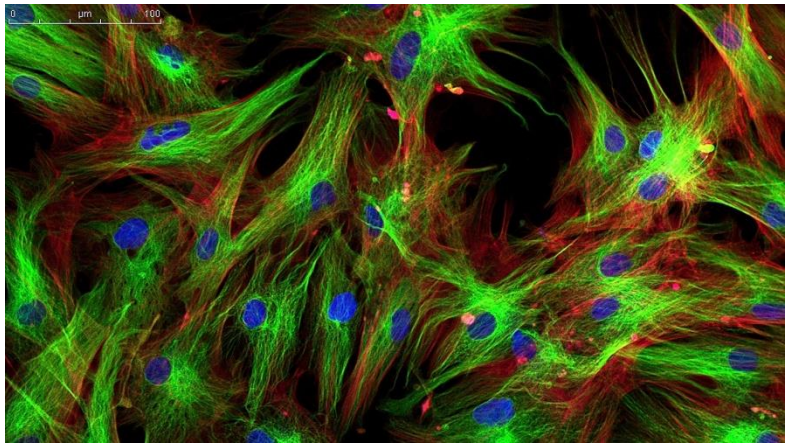
Image Field Size

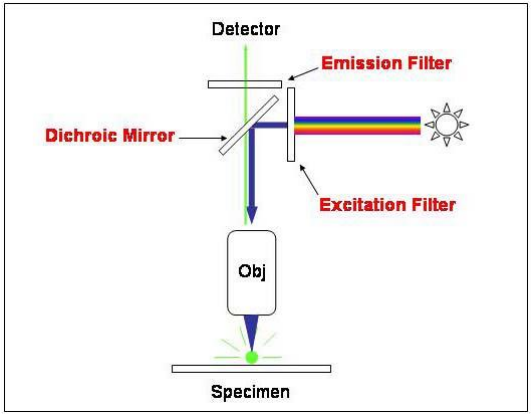


- Magnification = 100x(15/18) = 83.3
- Field Size = 4mm / 83.3 = 48 μm

Fluorescent Microscopy







- Image source: https://www.visiononline.org/vision-resources-details.cfm/vision-resources/Visual-Inspection-method-using-dark-field-collimating-illumination/content_id/6972

Summary

- Understand how microscope magnify objects
- Identify the components of a microscope
- Understand different contrast mechanisms
- Select suitable microscope objectives

- Hands On:
  - Construct a simple microscope
  - Learn system alignment
  - Calculate CCD field size and compare with measurement
  - Calculate resolution and compare with measurement