# MICROSCOPY

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# KEY CONCEPTS

- Introduction
- Basic Principle of Light microscopy
- Instrumentation
- Types and Applications

# Microscopy-Introduction

- Microscopy is defined as the use of a microscope to magnify and study the small objects that are too small to be visualized with the naked eye.
- Basically 3 main types: **optical or light** microscopy, **scanning** probe microscopy, and **electron** microscopy.
    - Naked eye $\simeq$ 0.1 mm
    - Light microscope $\simeq$ 0.1 μm
    - Electron microscope $\simeq$ 2.5 nm

# Microscopy – Basic Principle

- Light passes through specimen through a single or a series of magnifying lenses to allow a magnified view of the sample.
- Important factors in light microscope include:
  - Magnification
  - Resolution
  - Contrast

# Microscopy – Basic Principle

- A microscope is an array of two lenses.
  - Lenses include ocular lens and objective lens.
- Lenses combine to enlarge the objects.
- **Magnification:**

  Total magnification (M) achieved is the product of the magnification power of the lenses used.

  - M (Microscope) = M (Objective lens) X M (Ocular lens)
- Simple microscopes, have a magnification power of 1000-1500 whereas **electron microscopes** have **magnification power greater than 250,000.**

# Microscopy – Basic Principle

- **Resolution:**
  - Resolving power is the ability of a lens to separate or distinguish small objects that are close together.
  - Depends on the quality of lens and the wavelength of illuminating light.
    - shorter wavelength $\Rightarrow$ greater resolution
- **Contrast:**
  - Reflects the number of visible shades in the specimen.
  - Is needed to make objects stand out from the background.
  - Achieved through various staining techniques.
  - Microorganisms are essentially transparent and must be stained for bright-field microscopy.

# Microscope Types

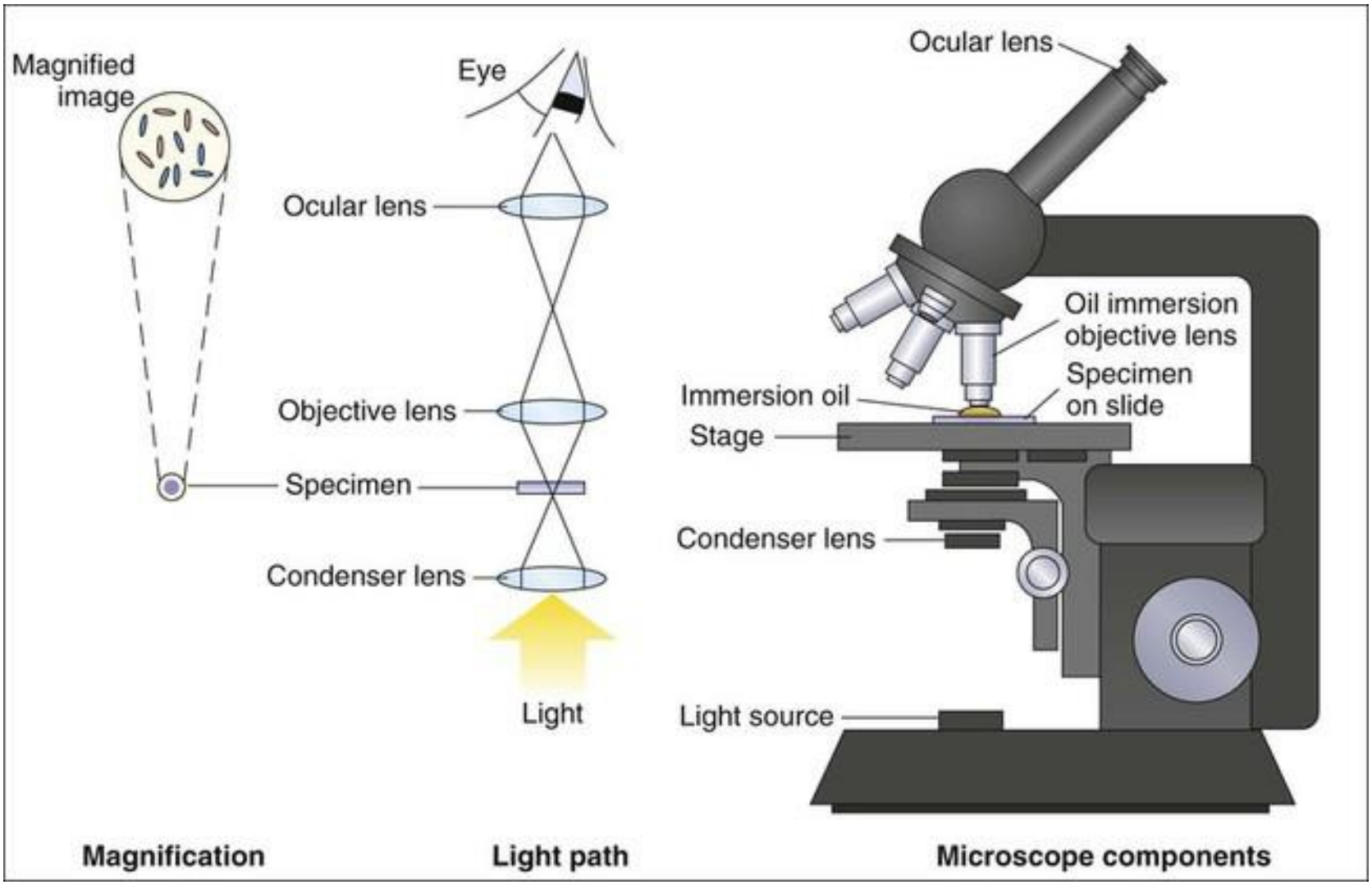
## Light microscope

- Compound microscope
- Bright-field microscope
- Dark-field microscope
- Phase-contrast microscope
- Fluorescence microscope

## Electron microscope

- Transmission electron microscope (TEM)
- Scanning electron microscope (SEM)

# Compound Light Microscopy




(Atlas RM: *Principles of microbiology,* St Louis, 2006, Mosby.)

# Compound Light Microscopy

- The light microscopes use visible light or ultraviolet rays to illuminate specimens.
- Two lenses in the compound microscope:
    - Ocular lens (10x)
    - Objective lens (4x, 10x, 20x, 40x, 100x)
- Resolution and contrast are controlled by condenser and iris diaphragm.
- The condenser functions to focus the light source on the specimen and provides an uniform illumination.
- An iris diaphragm controls the amount of light reaching the specimen.

# Applications

- ✓ A **compound microscope** is used for viewing small specimens.
- ✓ Used to visualize different samples at high magnification (40 – 1000x).
- ✓ Used to observe both prokaryotic and eukaryotic cells.
- ✓ To study cells and cell structures.
- ✓ Plays a pivotal role in the Clinical laboratory.
  - To detect the microorganisms directly in clinical specimens.
  - To characterize the growth of the organism in culture

# The Dark-Field Microscopy

- Produce a **bright image** against a **dark background**.
- **Dark-field** microscopy removes the unscattered beam from the image. Only light that has been reflected or refracted by the specimen forms the image.
- This is accomplished through the use of an annular aperture that will produce a hollow cone of light that does not enter the objective lens.

**APPLICATIONS:**

✓ Used to observe living, unstained samples.
✓ Used for visualizing different types of algae.
✓ Used for viewing blood cells and bacteria. (dark-field microscope, combined with phase contrast)

# The Bright-Field Microscopy

- **Bright field** microscopy is the conventional technique.
- Produce a **dark image** against a **brighter background**.
- Bright-field microscopy uses light from the lamp source to illuminate the specimen.
- This light is gathered in the condenser, then shaped into a cone where the apex is focused on the plane of the specimen.

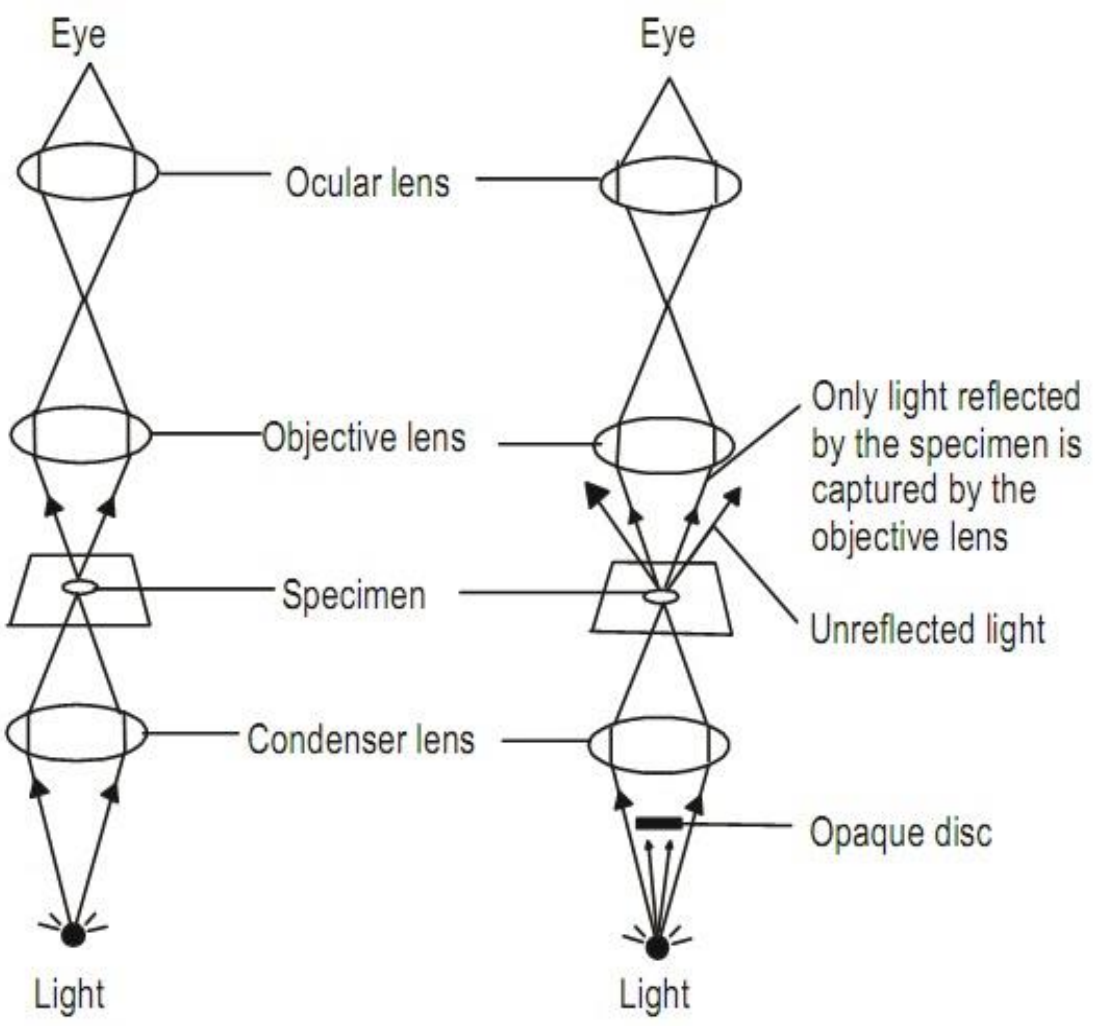
**APPLICATIONS:**

✓ Used for observing stained or naturally pigmented or highly contrasted specimens.

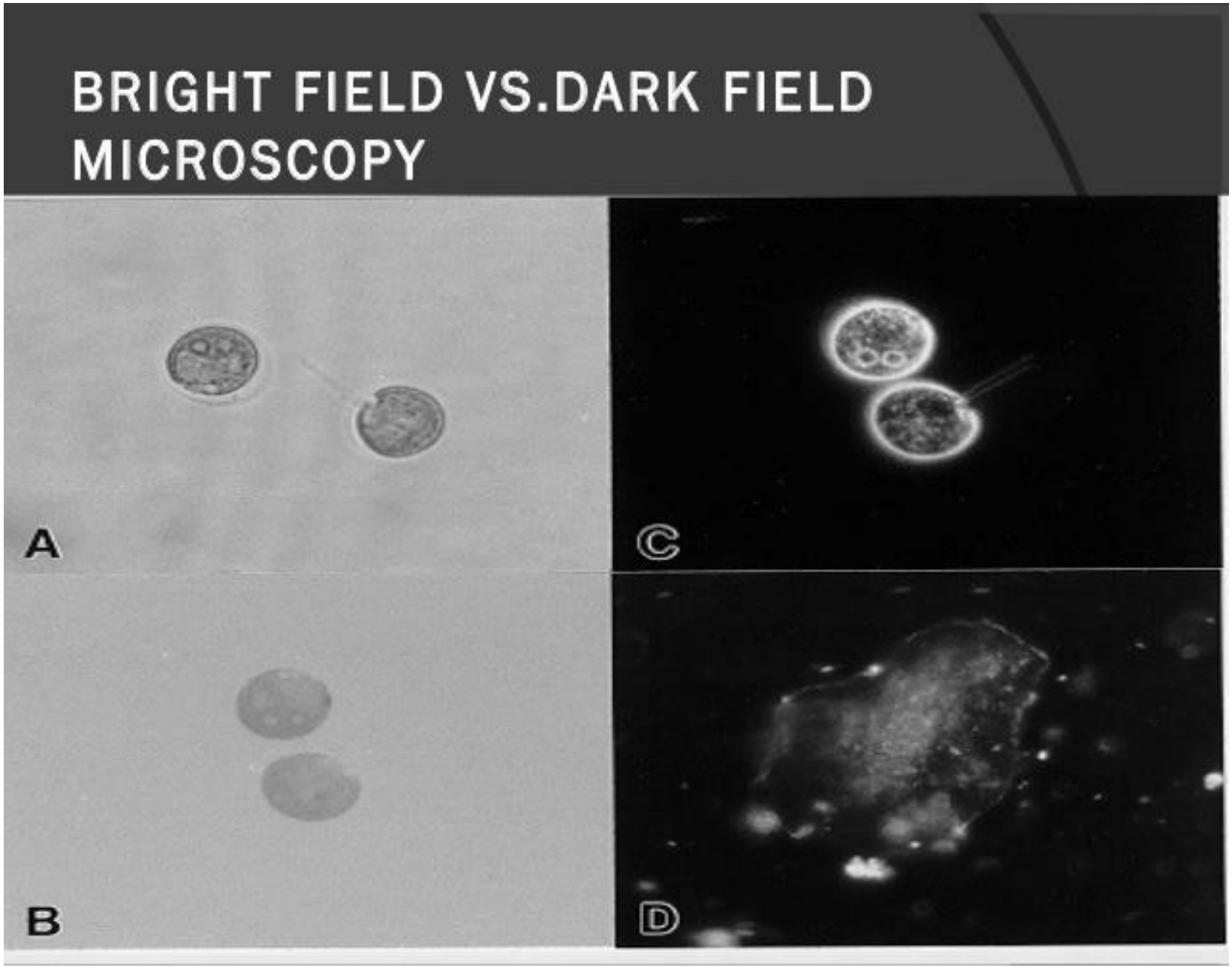
✓ Generally used with compound microscopes.

✓ Used for visualizing different types of bacteria and cell structures.

## *Comparison of Light Pathways of bright field and dark field Microscopy*




http://www.tutorsglobe.com/homework-help/microbiology/dark-field-microscope-72650.aspx



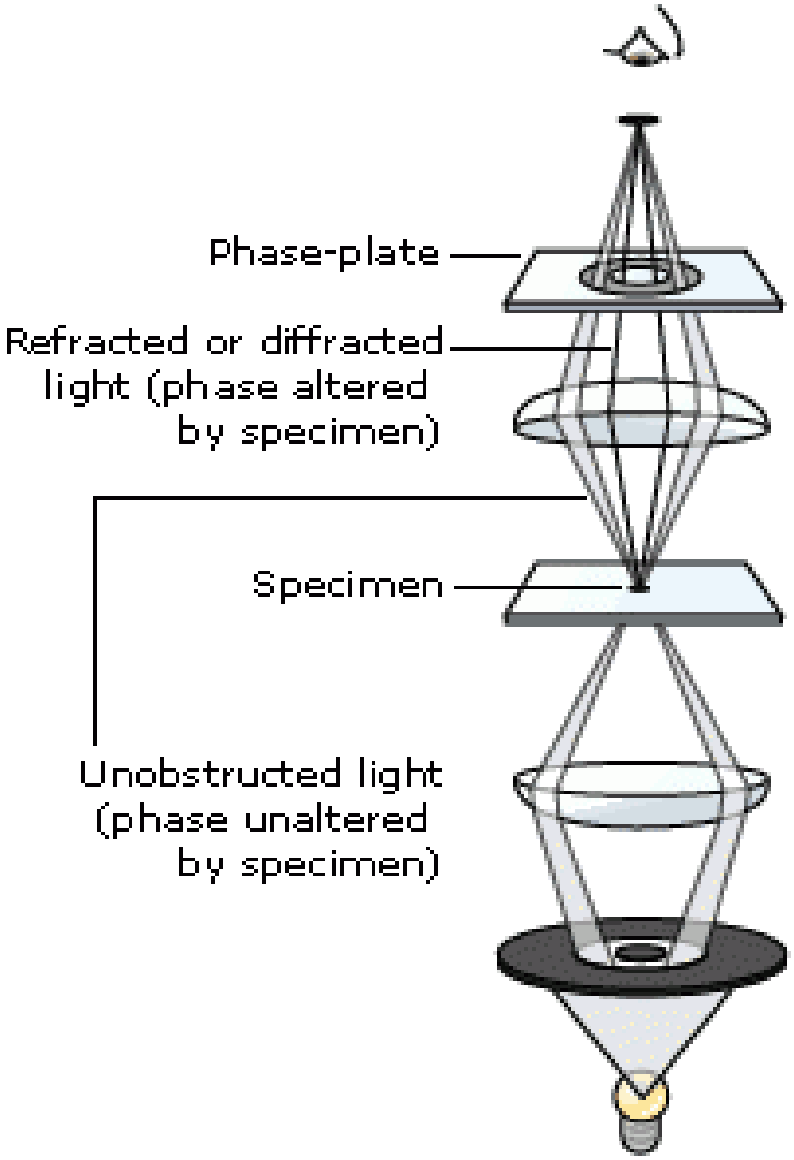

https://www.slideshare.net/shamawan/dark-field-microscopy-1-by-ehtisham-ul-haq

# The Phase-Contrast Microscopy

- Light is also an oscillation and the phase changes, when passing through an object, between the light that has passed through (diffracted light) and the remaining light (direct light).
- Even if the object is colorless and transparent, there is still a change in phase when light pass through it.
- This phase contrast is converted into brightness differences to observe specimens.
- A phase plate is located at the focal point of light between the objective lens and the image surface so that only the phase of the direct light changes. This generates contrast on the image surface.
- In phase-contrast microscope the annular ring in the objective lens and the condenser separate light.

# The Phase-Contrast Microscopy

- Used to study prokaryote and eukaryote cells.
- Used to observe living cells, bacterial components such as endospores, inclusion bodies.
- Transparent cells can be observed without staining them.
- Because it is not necessary to stain cells, cell division and other processes can be observed in a living state.




Path of light for a phase contrast microscope setup. (Courtesy of http://www.nobelprize.org/)

# The Fluorescence Microscopy

- Many substances absorbing light of a particular wavelength and energy, emit light of a longer wavelength and lesser energy.
- Application of this phenomenon is the basis of **fluorescence microscope**.
- Specimen is exposed to high energy, short wavelength (**ultraviolet, violet, or blue light**) to excite the electrons within certain molecules inside a specimen.
- When the excited electrons return to their original energy levels they emit light of a **longer lower energy wavelength,** in the visible spectrum which forms the image of the specimen.
- Usually specimens are stained with **fluorochrome or fluorophore** that fluorescents brightly upon exposure to light of specific wavelength.
- Barrier filter removes the remaining blue light and shows a bright image of the object resulting from the fluorescent light emitted by the specimen.

# The Fluorescence Microscopy

**Often Used for:**

- Imaging structural components of cells.
- Conducting viability studies on cell populations.
- Imaging the genetic material within a cell (DNA and RNA).
- Viewing specific cells within a larger population with techniques such as FISH

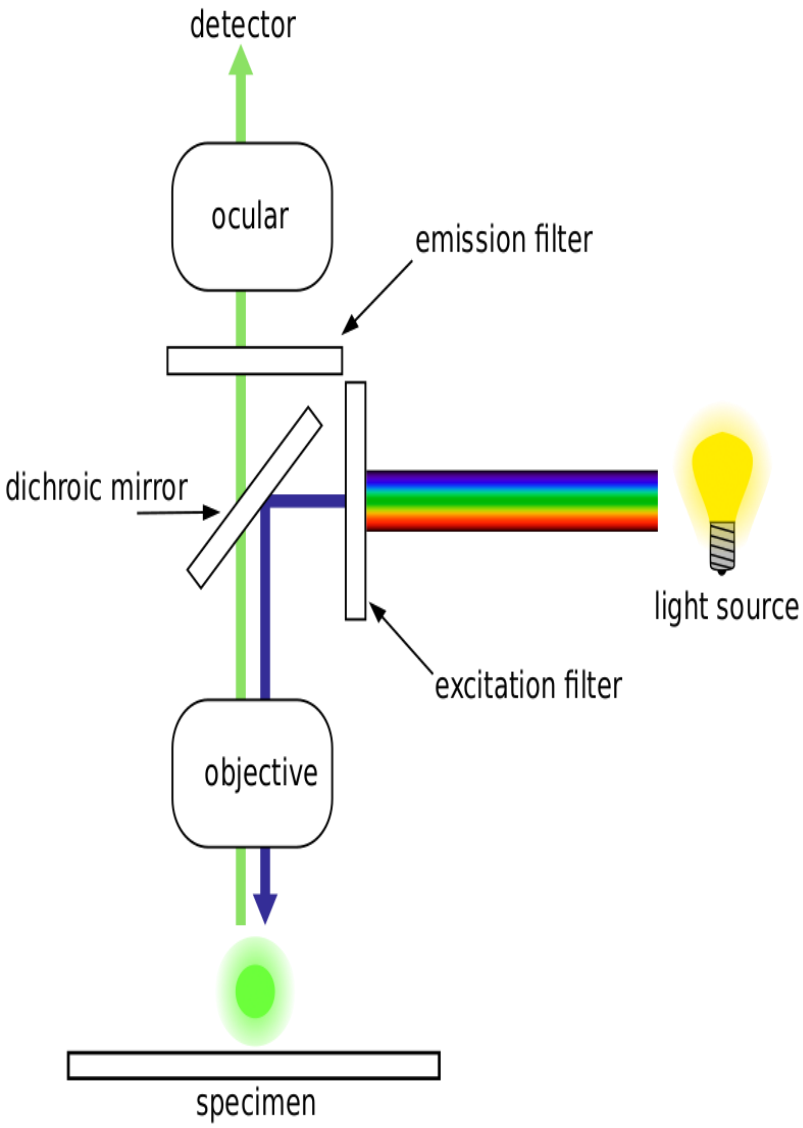



Figure showing the filters and mirror in a fluorescent microscope from Wikipedia