

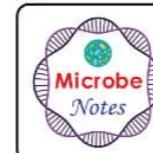
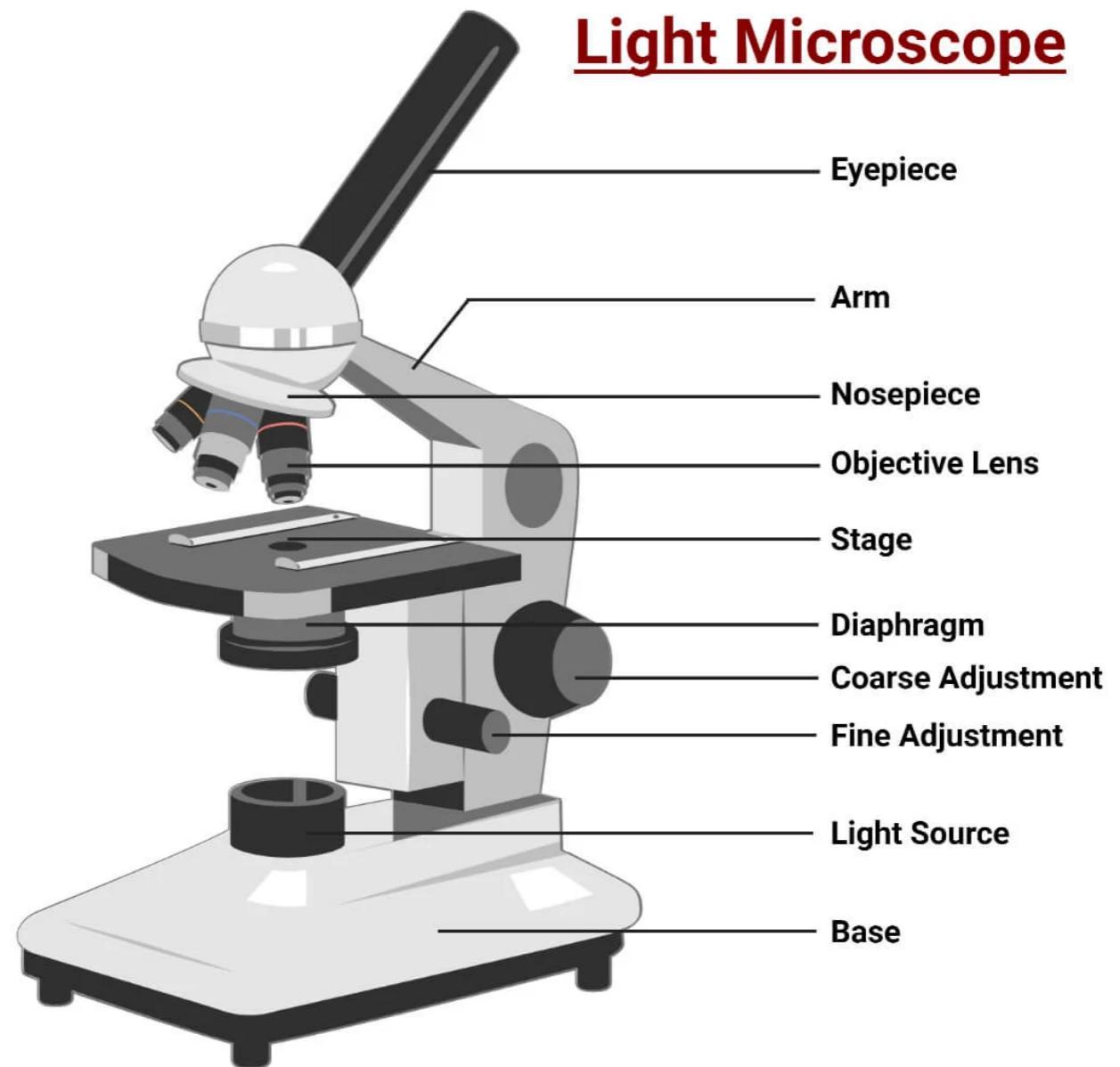
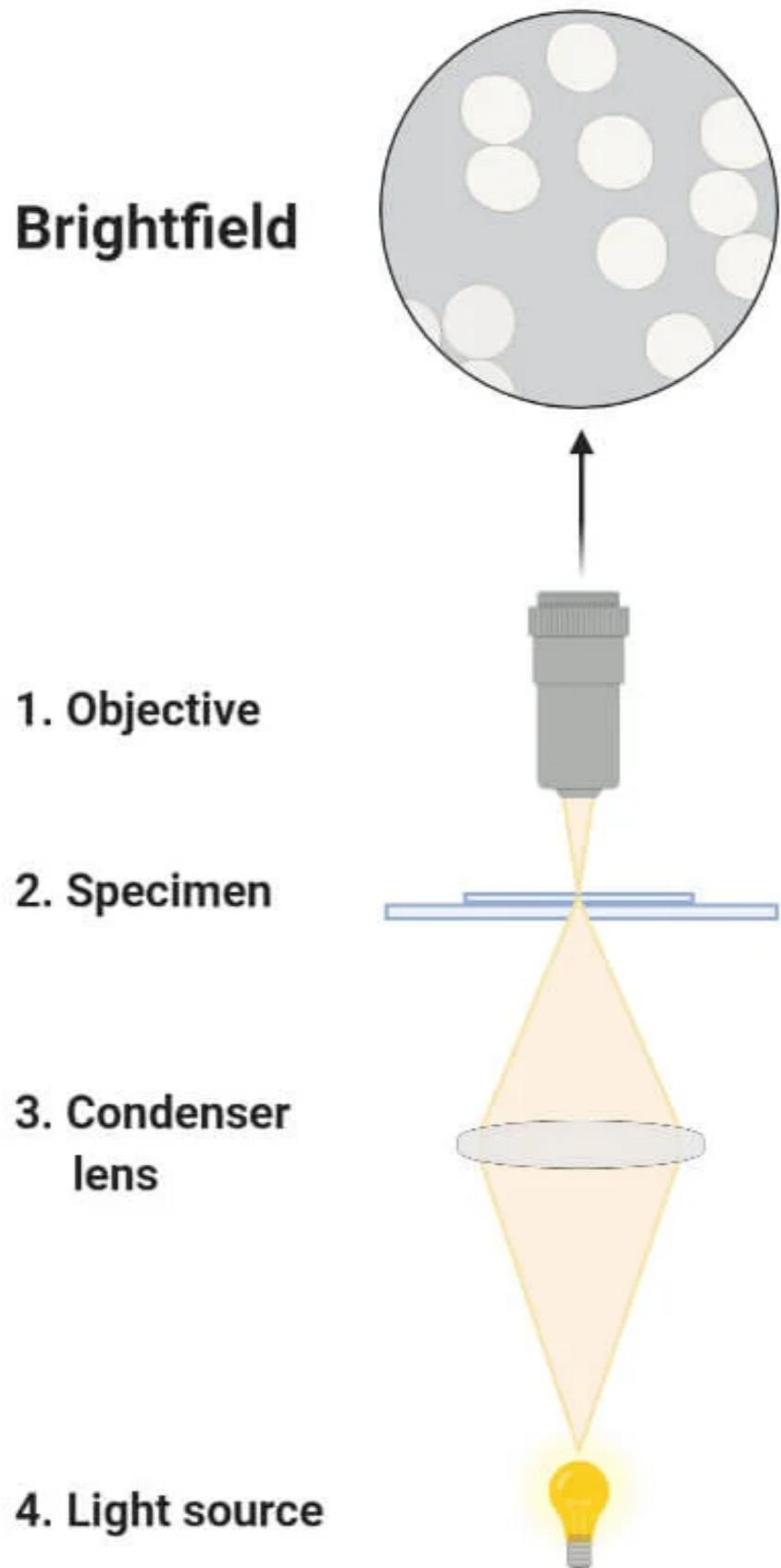
# Today's class:

## Lasers and Light Microscopy part 2

*This lecture follows the materials from the following books*

- *Prescott's Principles of Microbiology, McGraw-Hill 2009*

# Principles of light microscopy



The  
Biology  
Notes

The  
Chemistry  
Notes

Created with  
**bio**  
RENDER  
Templates

# Types of light microscopy

The modern types of light microscope include

Bright field light microscopy

Phase contrast light microscopy

Dark field light microscopy

Fluorescence microscopy

# Bright field light microscopy

**Most basic type of microscopy - ‘naked microscopy’**  
**Magnification achieved by combining an objective and an eyepiece lenses**



Yeast cells under bright field microscope

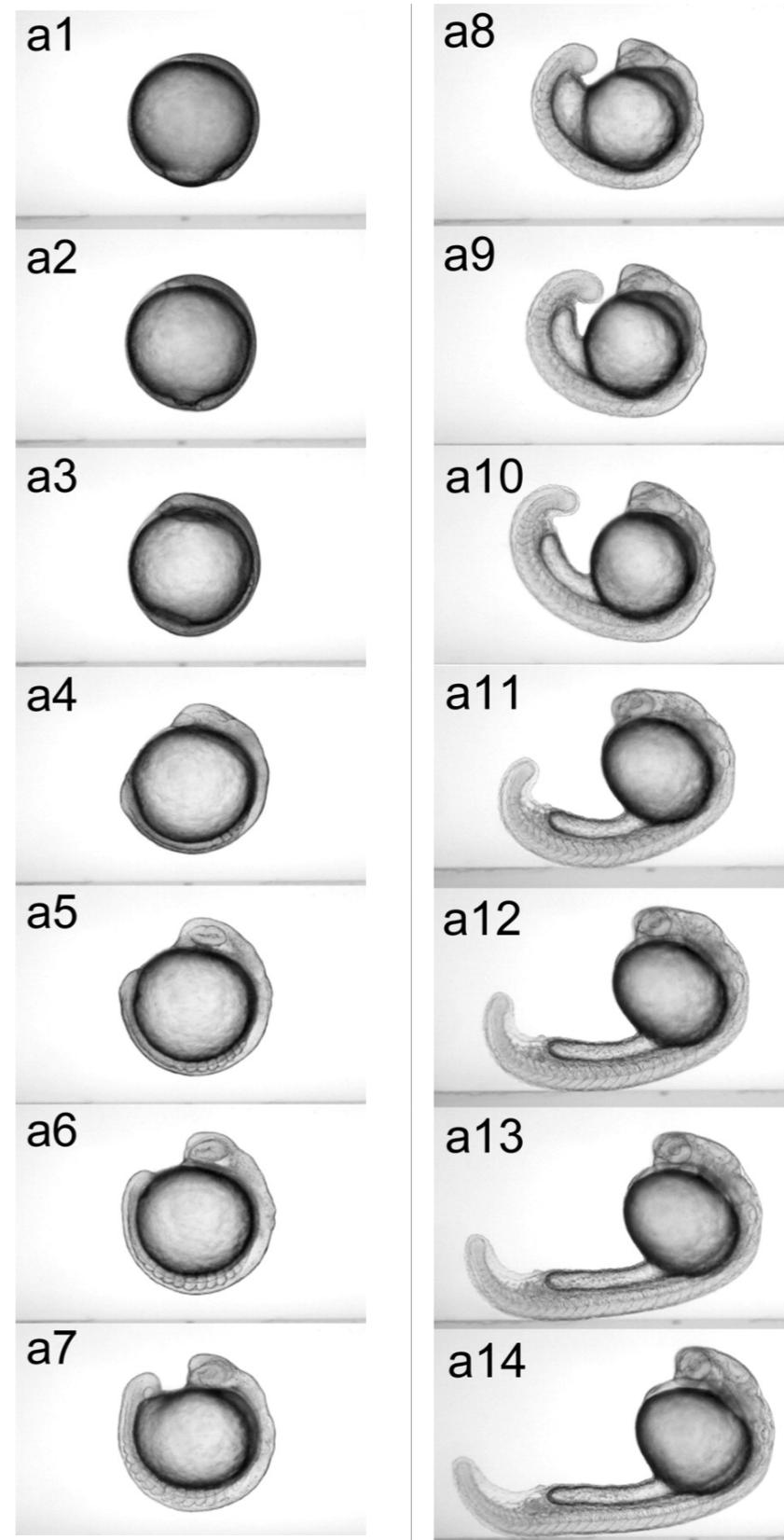
## Advantages

- Inexpensive
- Little to no manipulation of the sample

## Disadvantages

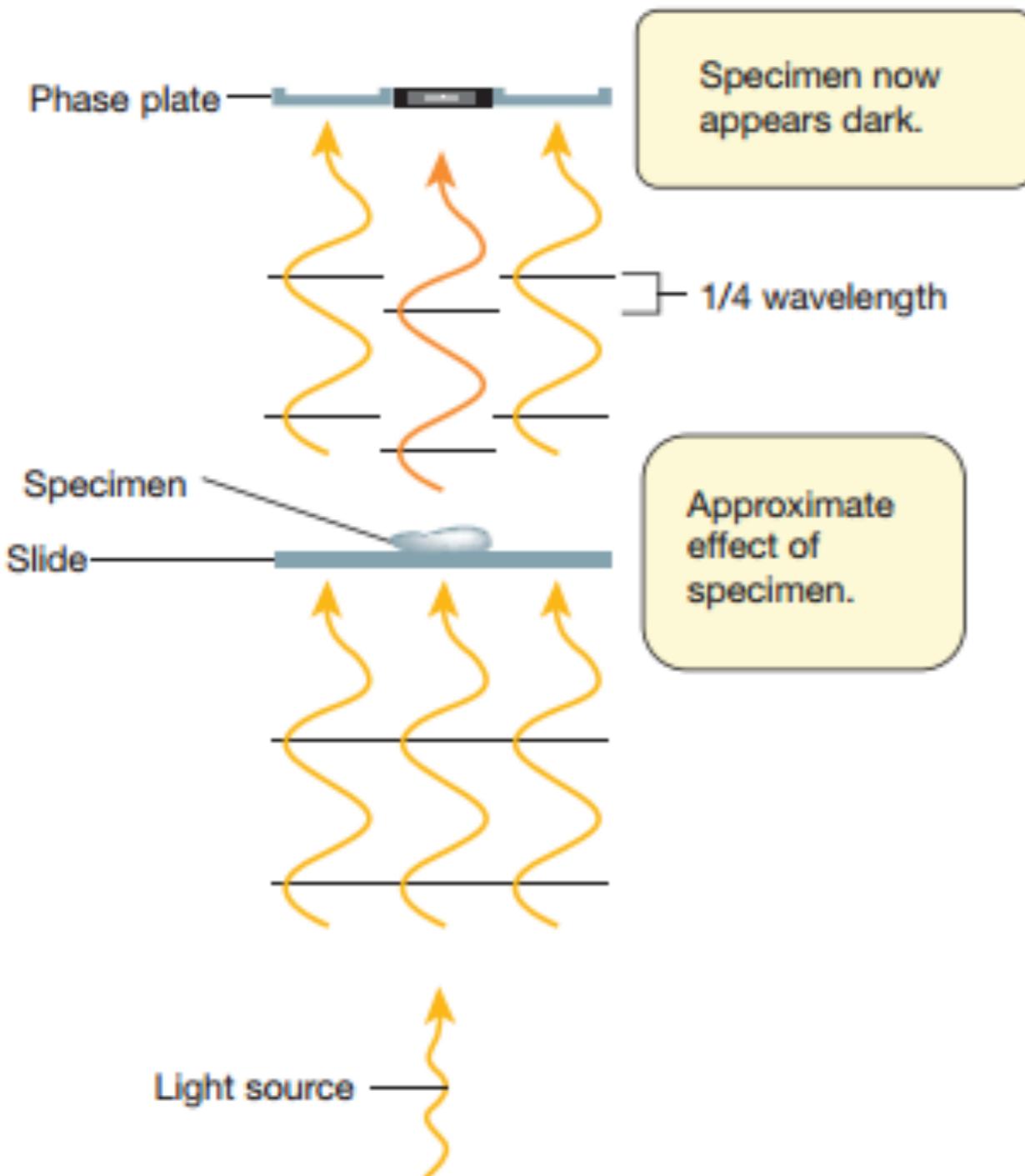
- Limited in resolution
- Low magnification power
- Transparent samples hard to resolve
- Staining often need for contrast and fixing (killing) the cells

Bright field images of a developing zebrafish



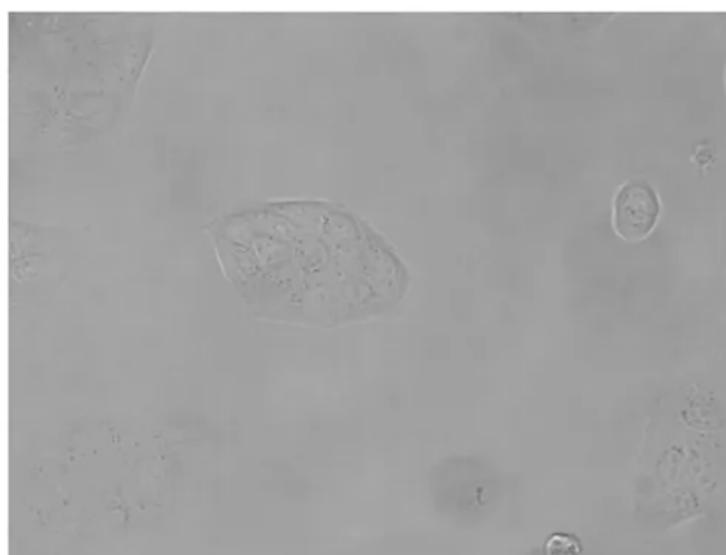
# Phase contrast light microscopy

The principle: to transform a specimen into an amplitude image



- Light refracted through sample undergo phase shifts because of heterogeneity in refractive indices in the sample
- PCM combines these phase shifted lights to create a high contrast image
- The shifts that occur during light penetration, become converted to changes in amplitude which causes the image contrast.
- Thus PCM can be used to **view unstained cells in their natural state, in high contrast and efficient clarity.** This is an advantage over bright field light microscopy.

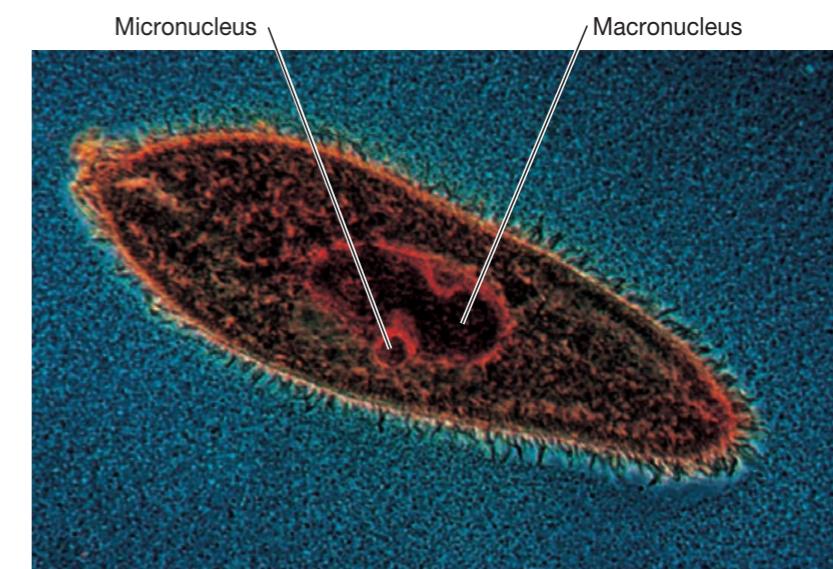
# Phase contrast light microscopy



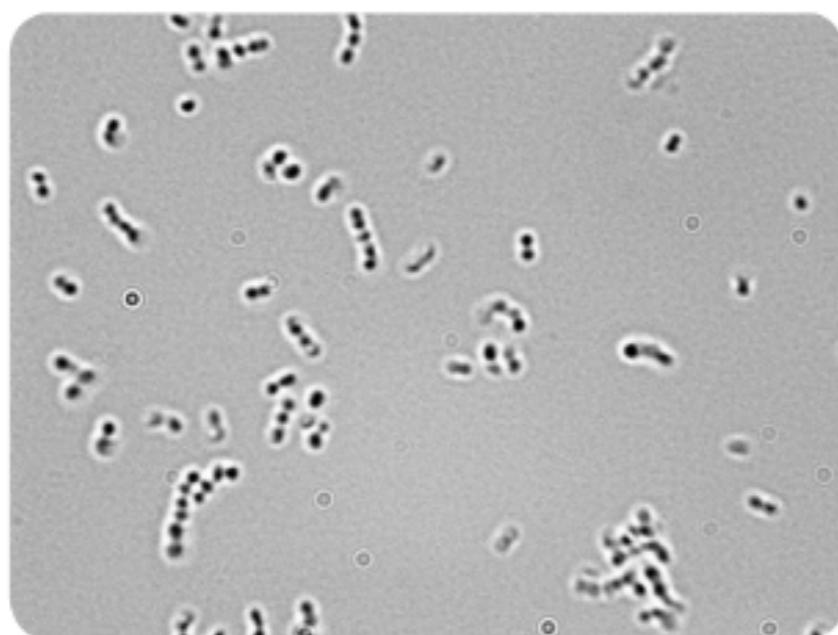
MDCK Cell, Bright Field



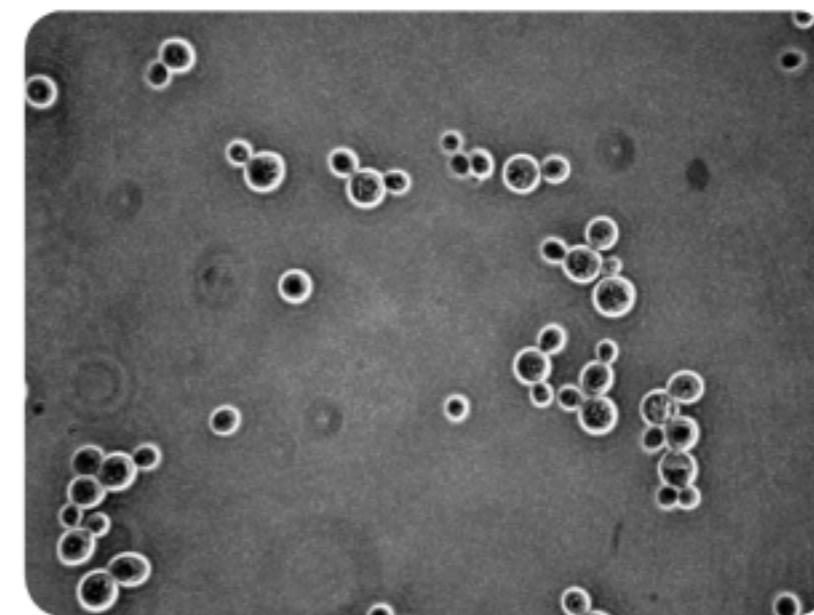
MDCK Cell, Phase Contrast



(e) *Paramecium*: phase-contrast microscopy with stained specimen

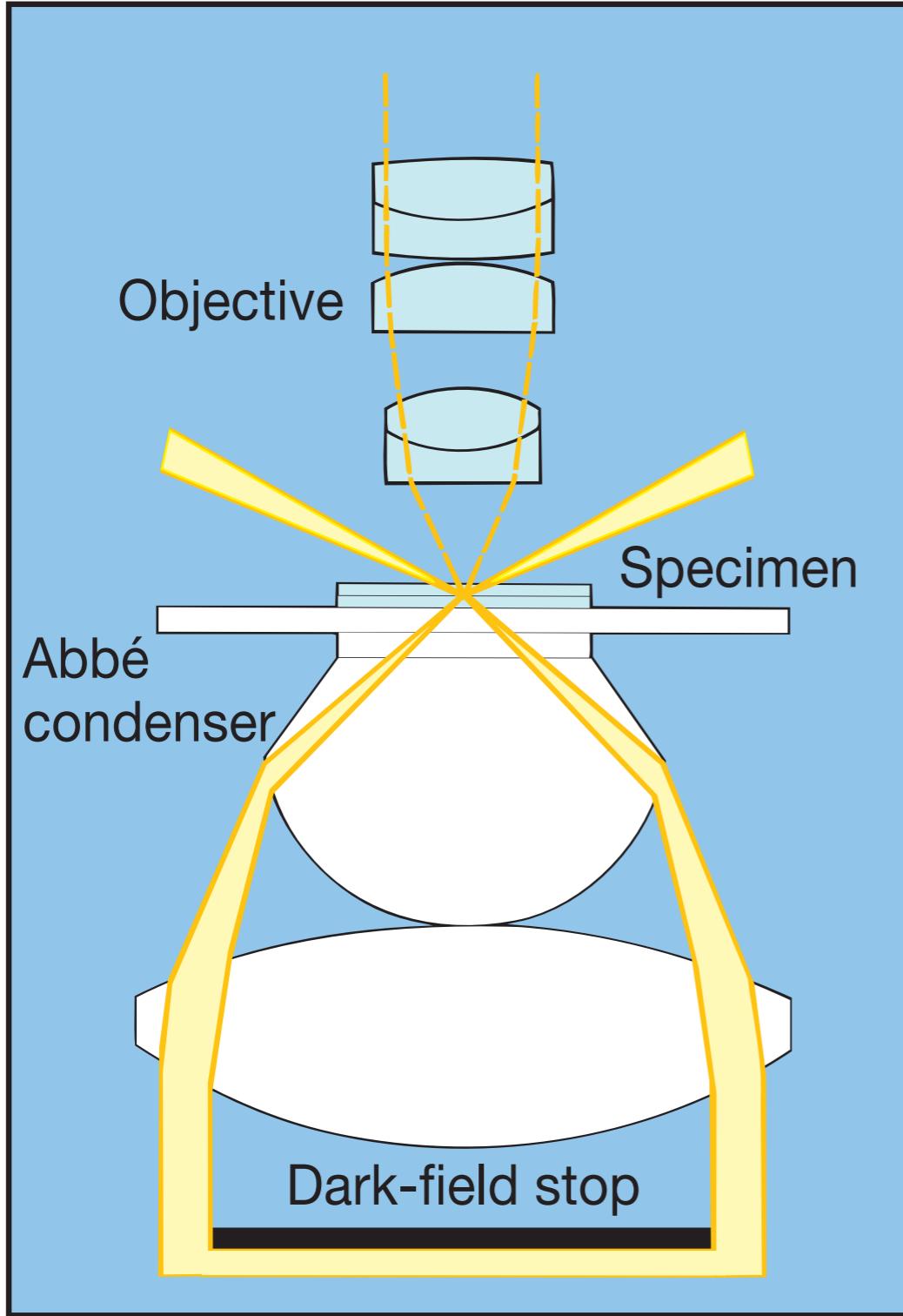


(a) *Saccharomyces* under bright field microscope



(b) *Saccharomyces* under phase contrast microscope

# Dark field light microscopy

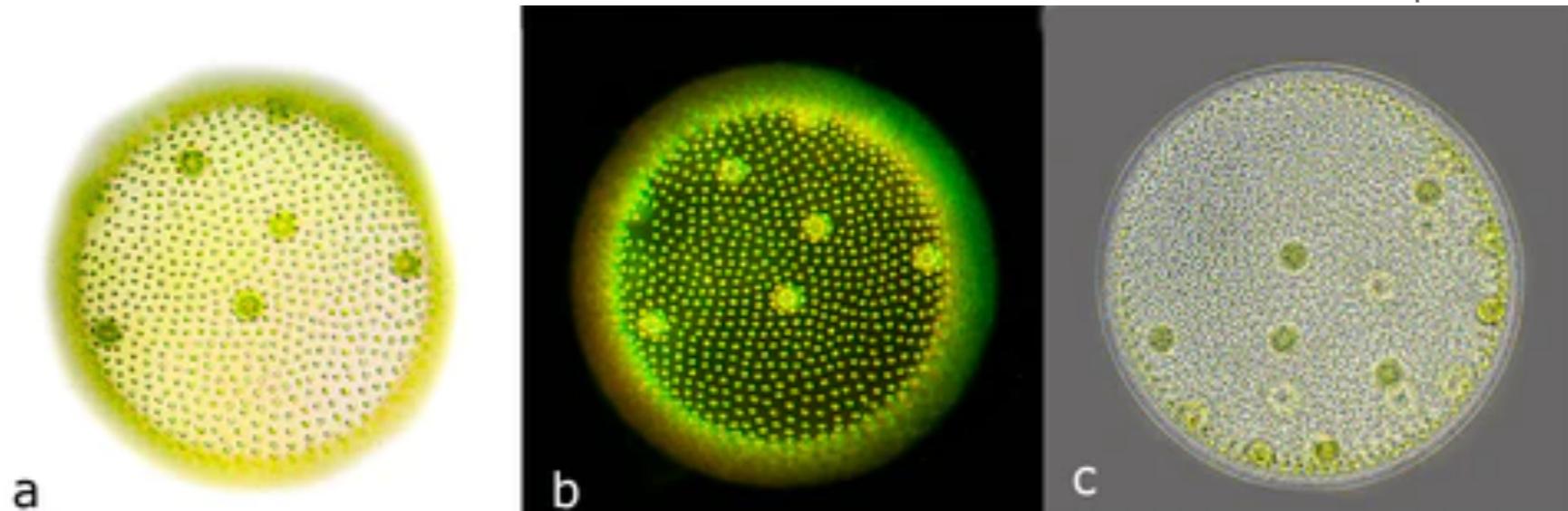


Here the dark field stop is inserted below the condenser to create a hollow cone of light going to the specimen

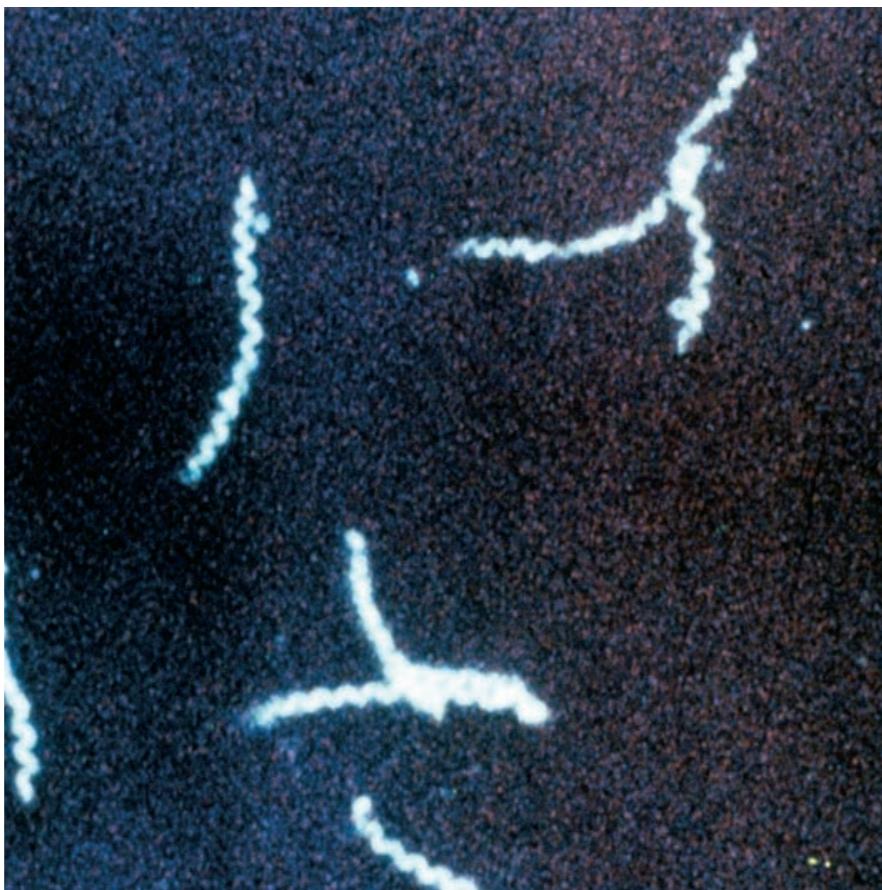
So any light entering the objective now comes from specimen only and the background appears dark

# Dark field light microscopy examples

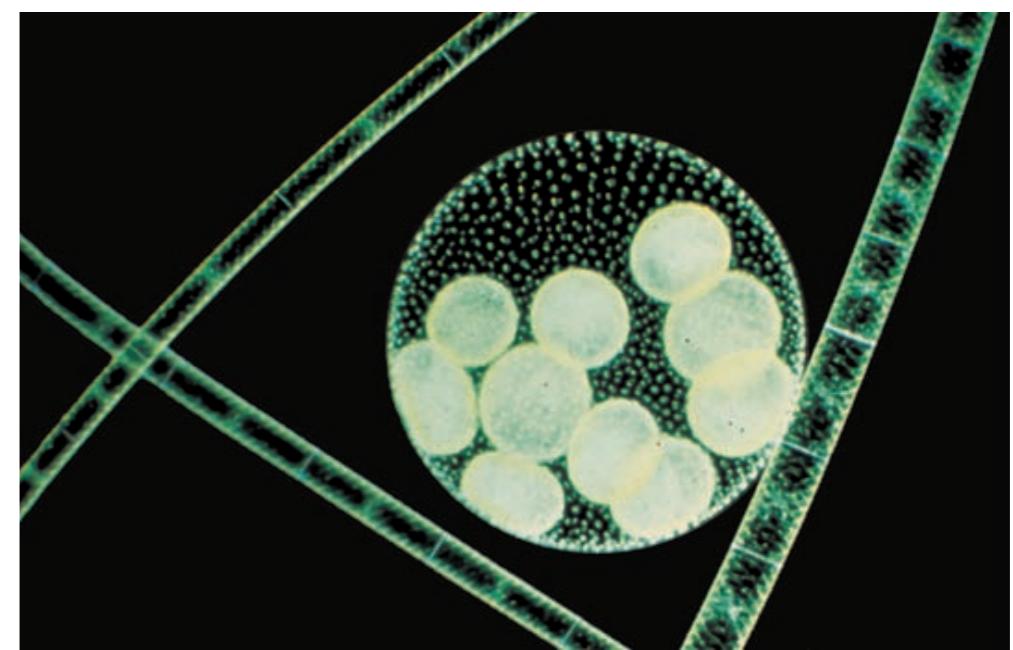
[moticmicroscopes.com](http://moticmicroscopes.com)



Bright field vs dark field vs phase contrast for *Volvox aureus*



(a) *T. pallidum*: dark-field microscopy

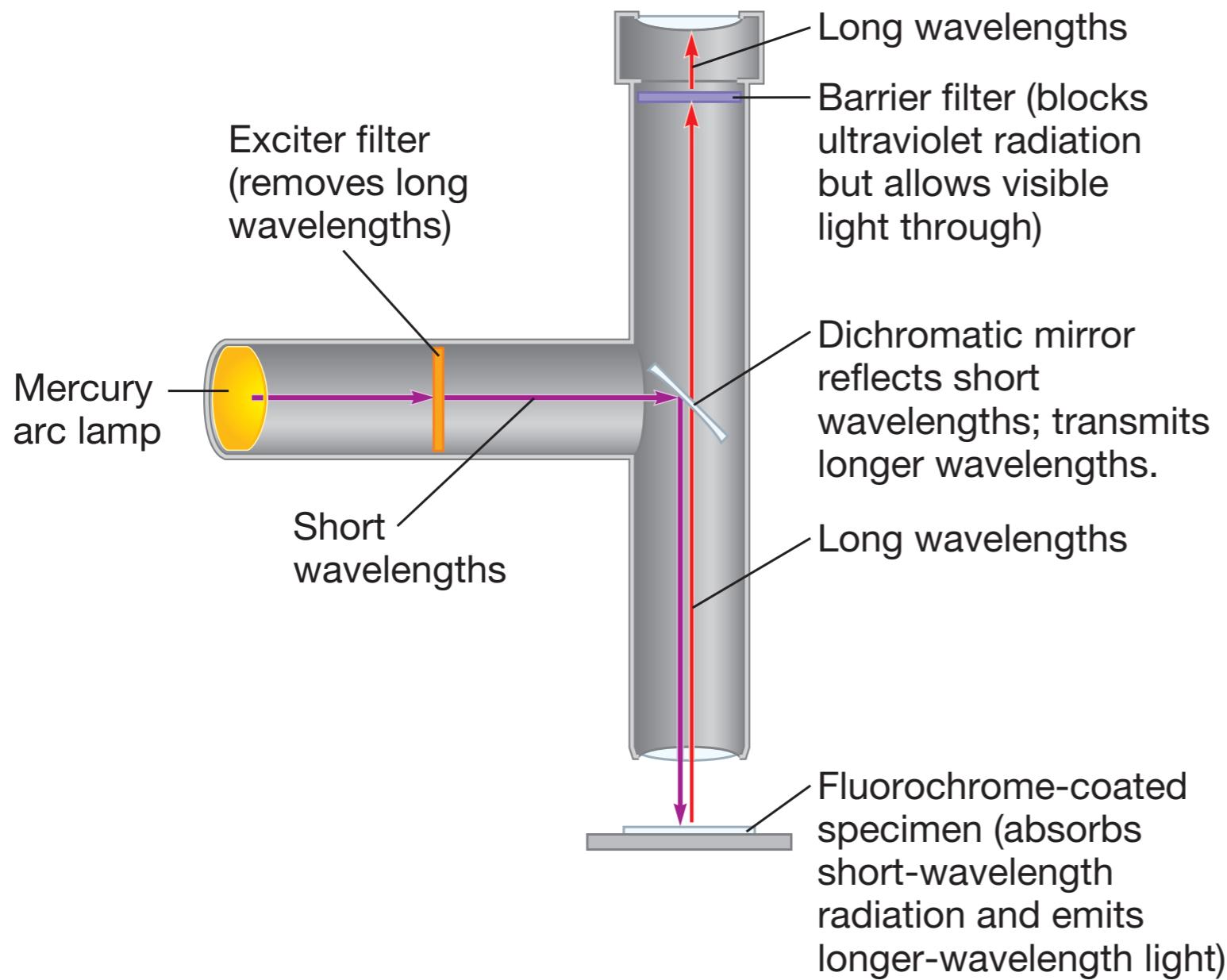


(b) *Volvox* and *Spirogyra*: dark-field microscopy

# Fluorescence microscopy

Using fluorescence to visualize a specimen

Most common form: epifluorescence microscopy  
or incident light or reflected light fluorescence microscopy



Similarly, detector of fluorescence emission in spectrophotometers always is placed at a 90 degree angle w.r.t the light source

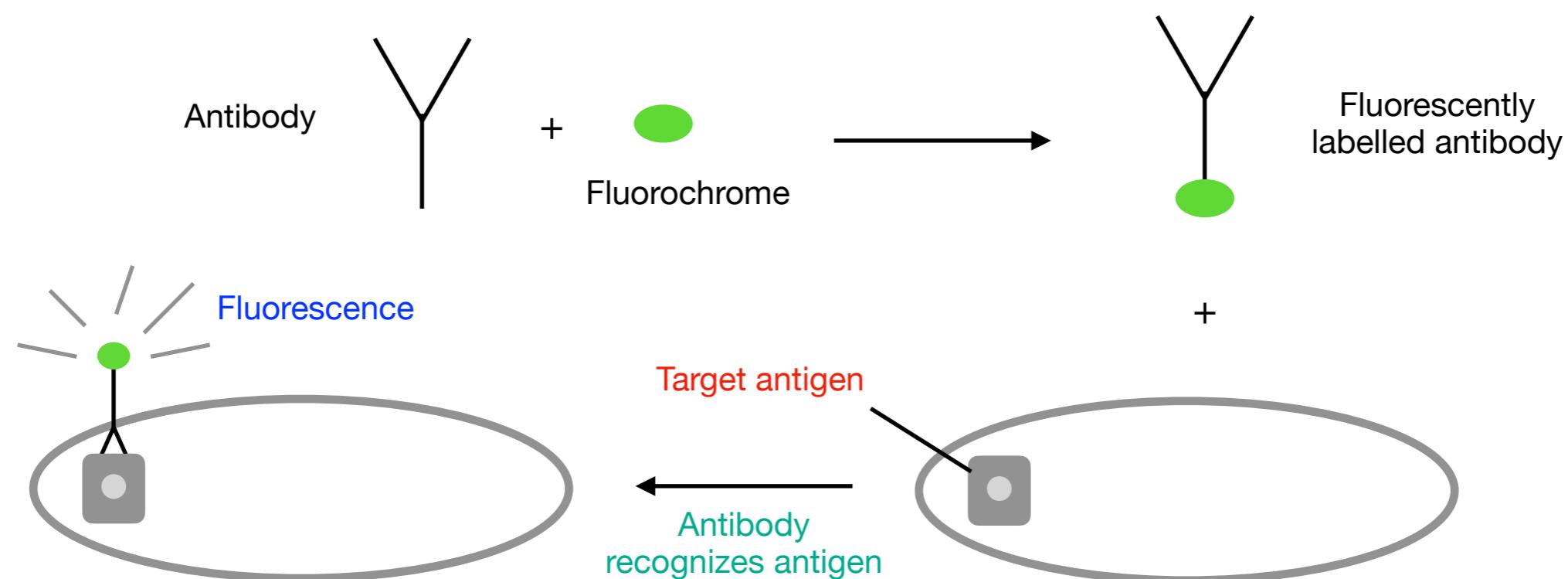
# Fluorescence labelling by fluorochromes and immunofluorescence

Table 2.3

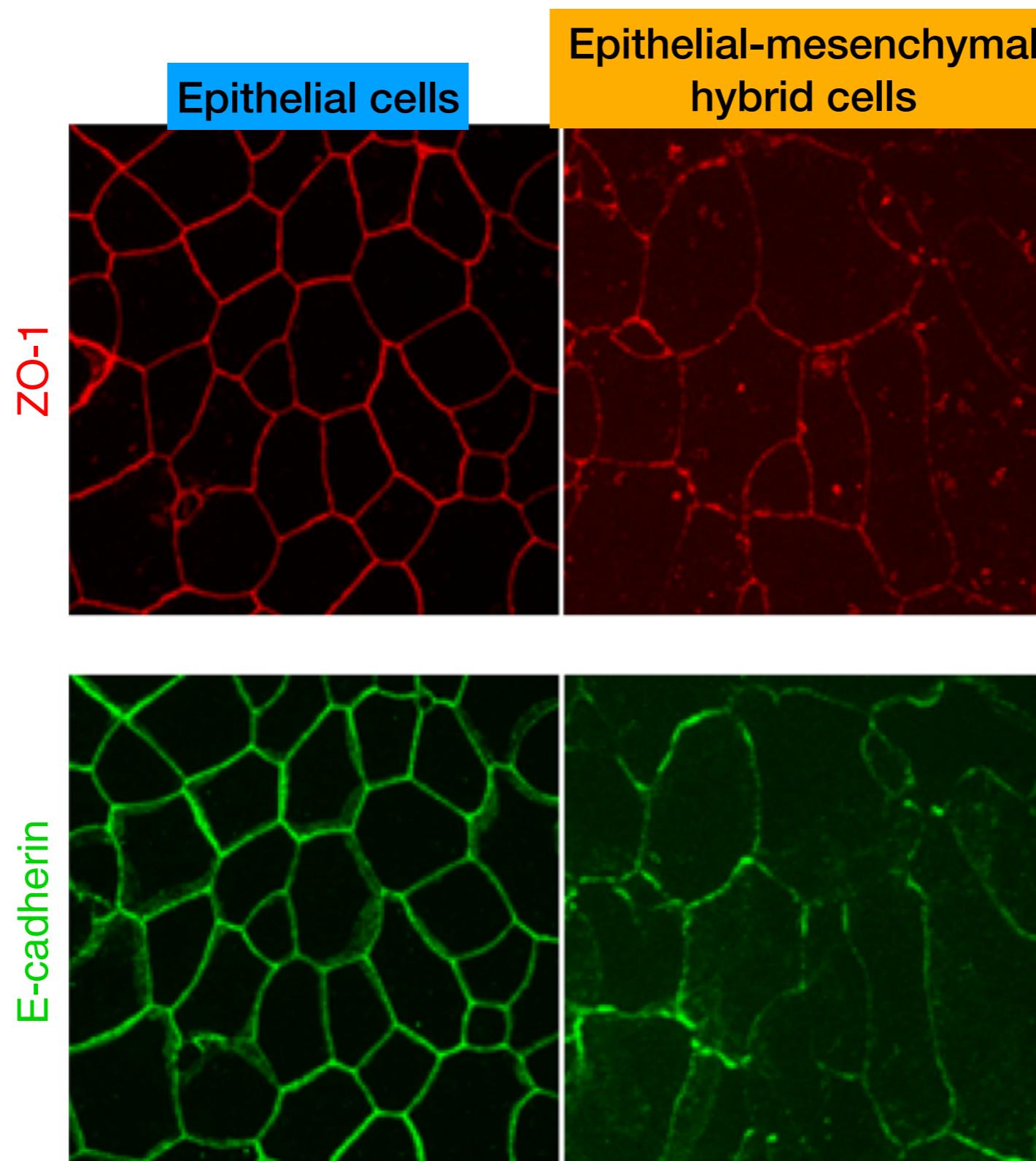
Commonly Used Fluorochromes

Fluorochrome	Uses
Acridine orange	Stains DNA; fluoresces orange
Diamidino-2-phenyl indole (DAPI)	Stains DNA; fluoresces green
Fluorescein isothiocyanate (FITC)	Often attached to antibodies that bind specific cellular components or to DNA probes; fluoresces green
Tetramethyl rhodamine isothiocyanate (TRITC or rhodamine)	Often attached to antibodies that bind specific cellular components; fluoresces red

## Immunofluorescence staining



# Immunofluorescence staining can capture cell phenotype changes



# Beating the optical resolution barrier: super-resolution microscopy

**STED microscopy = STimulated Emission Depletion microscopy**



Photo: Matt Staley/HHMI

**Eric Betzig**

Prize share: 1/3

UC Berkeley Physics



© Bernd Schuller, Max-Planck-Institut

**Stefan W. Hell**

Prize share: 1/3

Max Planck Institute  
Biophysical Chemistry  
Göttingen



Photo: K. Lowder via  
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**William E. Moerner**

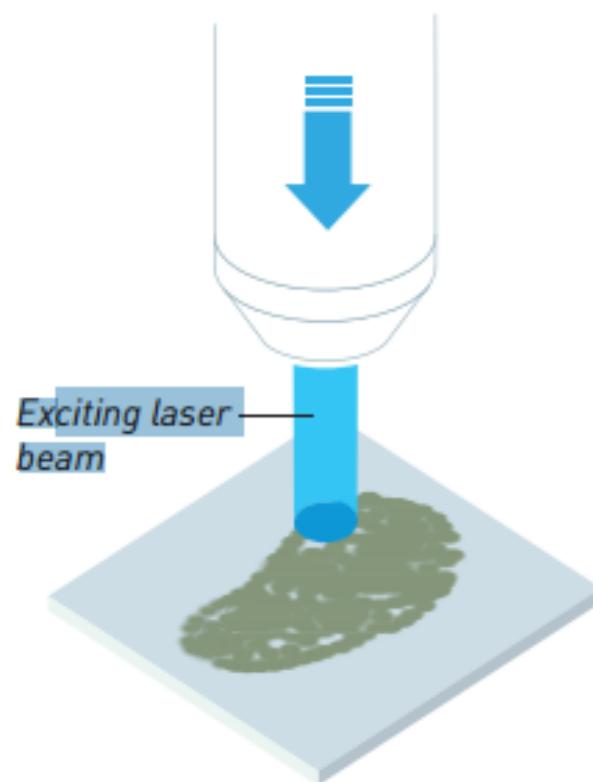
Prize share: 1/3

Stanford Chemistry

Nobel prize in Chemistry in 2014

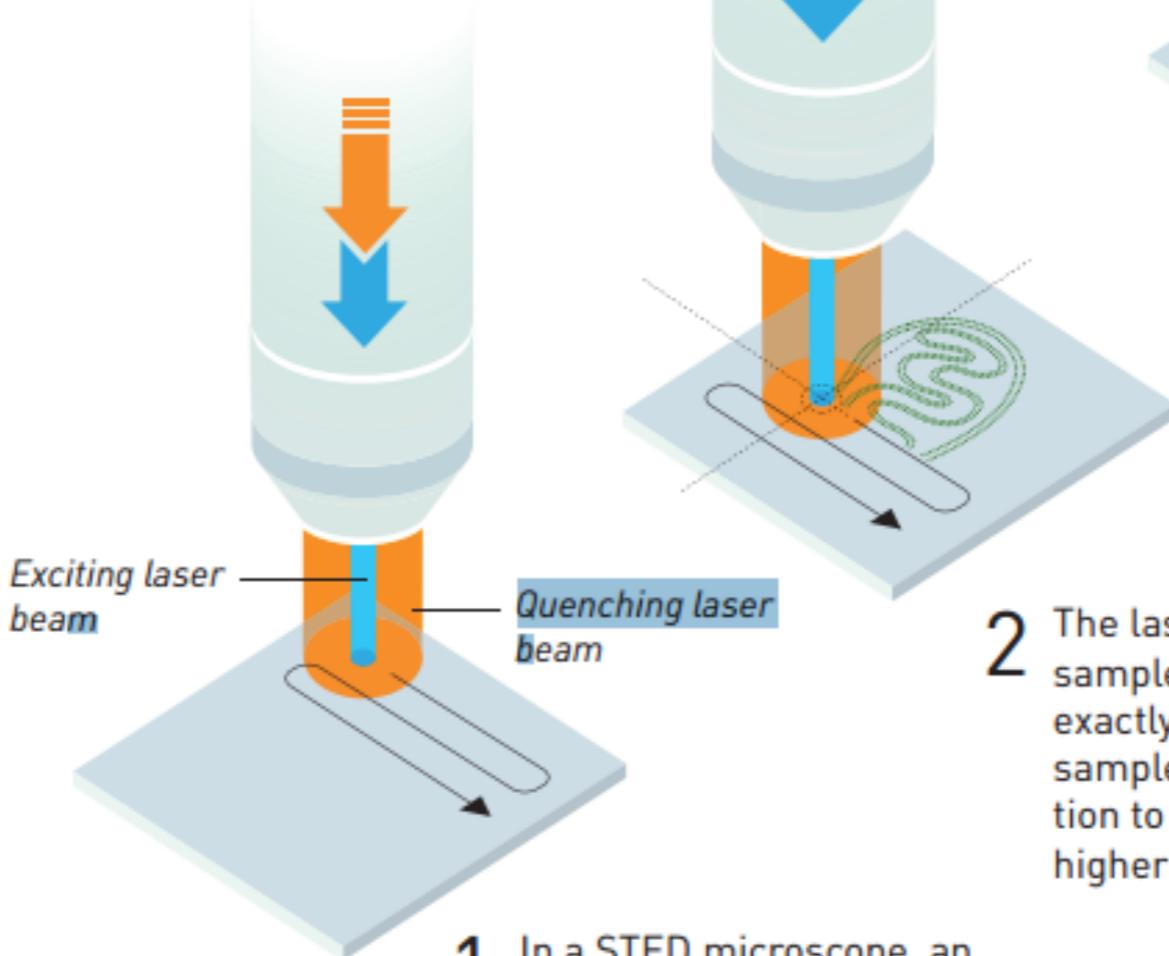
# The principle of STED microscopy

Regular optical microscope

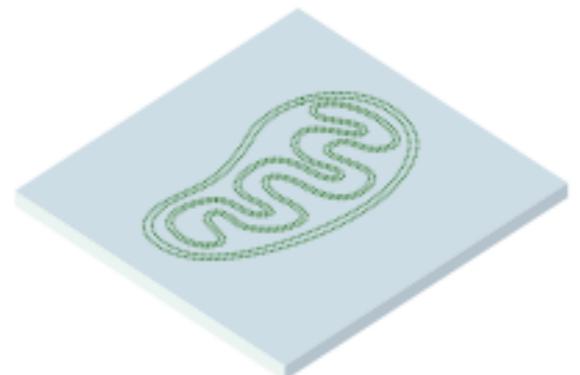


In a regular optical microscope, the contours of a mitochondrion can be distinguished, but the resolution can never get better than 0.2 micrometres.

STED microscope



- 1 In a STED microscope, an annular laser beam quenches all fluorescence except that in a nanometre-sized volume.



- 2 The laser beams scan over the sample. Since scientists know exactly where the beam hits the sample, they can use that information to render the image at a much higher resolution.
- 3 The final image gets a resolution that is much better than 0.2 micrometre.

# Examples of STED microscopy images

