Light Microscopy

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Light Microscopy

A light microscope is an optical instrument that uses visible light and a system of lenses to magnify objects, typically allowing for the observation of small structures like cells, tissues, and microorganisms. It works by passing light through or reflecting it off the specimen, which is then magnified to make details visible to the human eye.

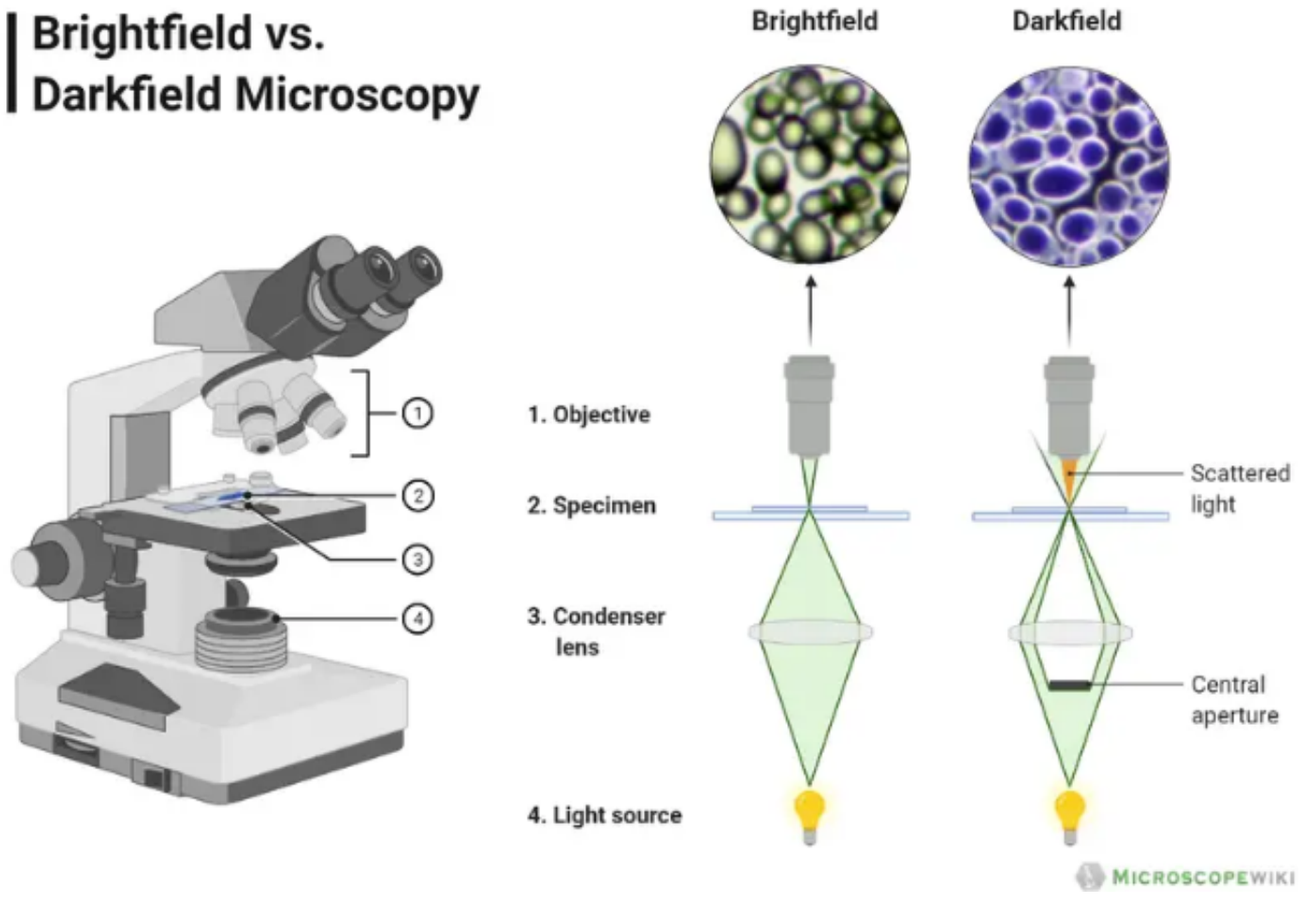
Types of light microscopes

Conventional bright-field microscopy

Fluorescence M

Phase-contrast M

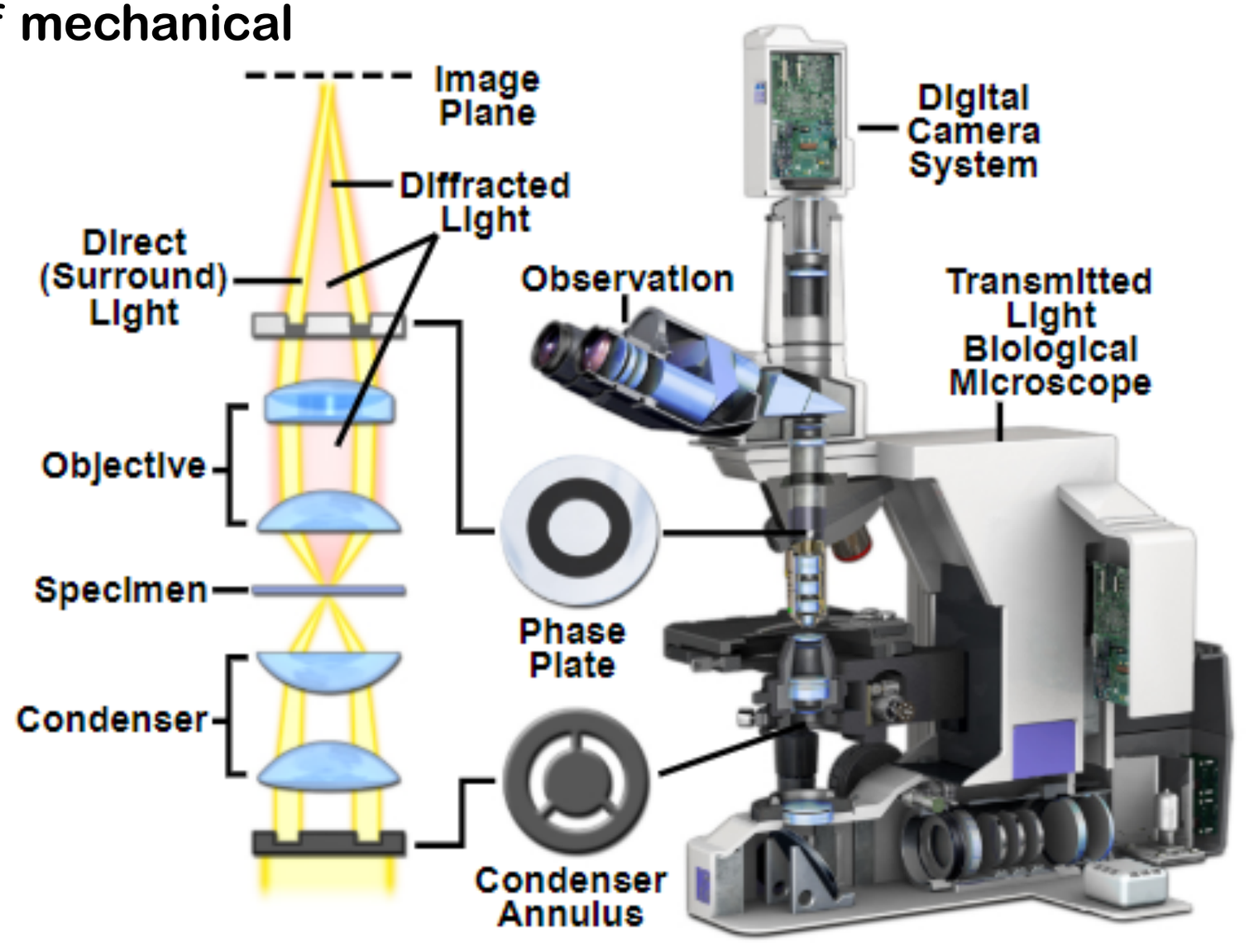
Confocal M



Bright-Field Microscopy

With the bright-field microscope (e.g. Compound Microscope), widely used by students of histology, stained preparations are examined by means of ordinary light that passes through the specimen.

The microscope is composed of mechanical and optical parts



The total magnification = magnifying power of the objective X magnifying power of the ocular lenses.

**The critical factor in obtaining a detailed image with a light microscope is its resolving power (the smallest distance between two particles at which they can be seen as separate objects).

**The maximal resolving power of the light microscope is approximately 0.2um; this power permits good images magnified 1000–1500 times.Objects smaller or thinner than 0.2um (such as a ribosome, a membrane, or a filament of actin) cannot be distinguished with this instrument

Two objects such as mitochondria will be seen as only one object if they are separated by less than 0.2 um

The optical components consist of three systems of lenses.

The **condenser** collects and focuses light, producing a cone of light that illuminates the object to be observed.

The **objective** lenses enlarge and project the illuminated image of the object in the direction of the eyepiece.

The **eyepiece** or ocular lens further magnifies this image and projects it onto the viewer's retina, photographic film, or (to obtain a digital image) a detector such as a charge-coupled device (CCD) camera

The quality of the image (its clarity and richness of detail) depends on the microscope's resolving power. The magnification is of value only when accompanied by high resolution.

The resolving power of a microscope depends mainly on the quality of its objective lens

The eyepiece lens enlarges only the image obtained by the objective it does not improve resolution. when comparing objectives of different magnifications, those that provide higher magnification also have higher resolving power.

Video cameras highly sensitive to light enhance the power of the bright-field and other light microscopes and allow the capture of digitized images suitable for computerized image analysis and printing.With digital cameras and image-enhancement programs (to enhance contrast, for example), objects that may not be visible when viewed directly through the ocular may be made visible in the video screen.

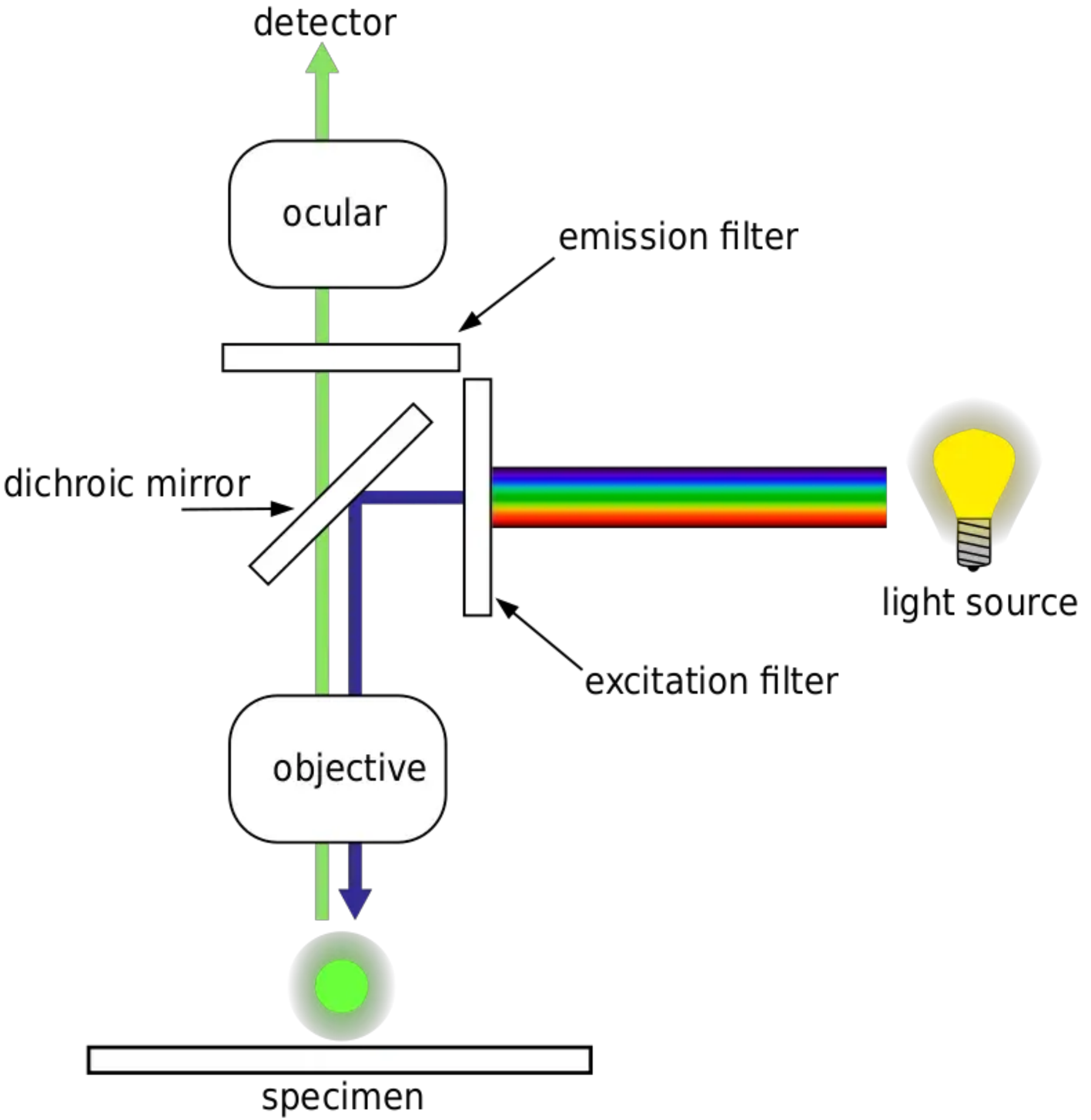
video systems are useful for studying living cells for long periods of time, because they use low-intensity light and thus avoid the cellular damage from heat that can result from intense illumination.

software for image analysis allows rapid measurements and quantitative study of microscopic structures

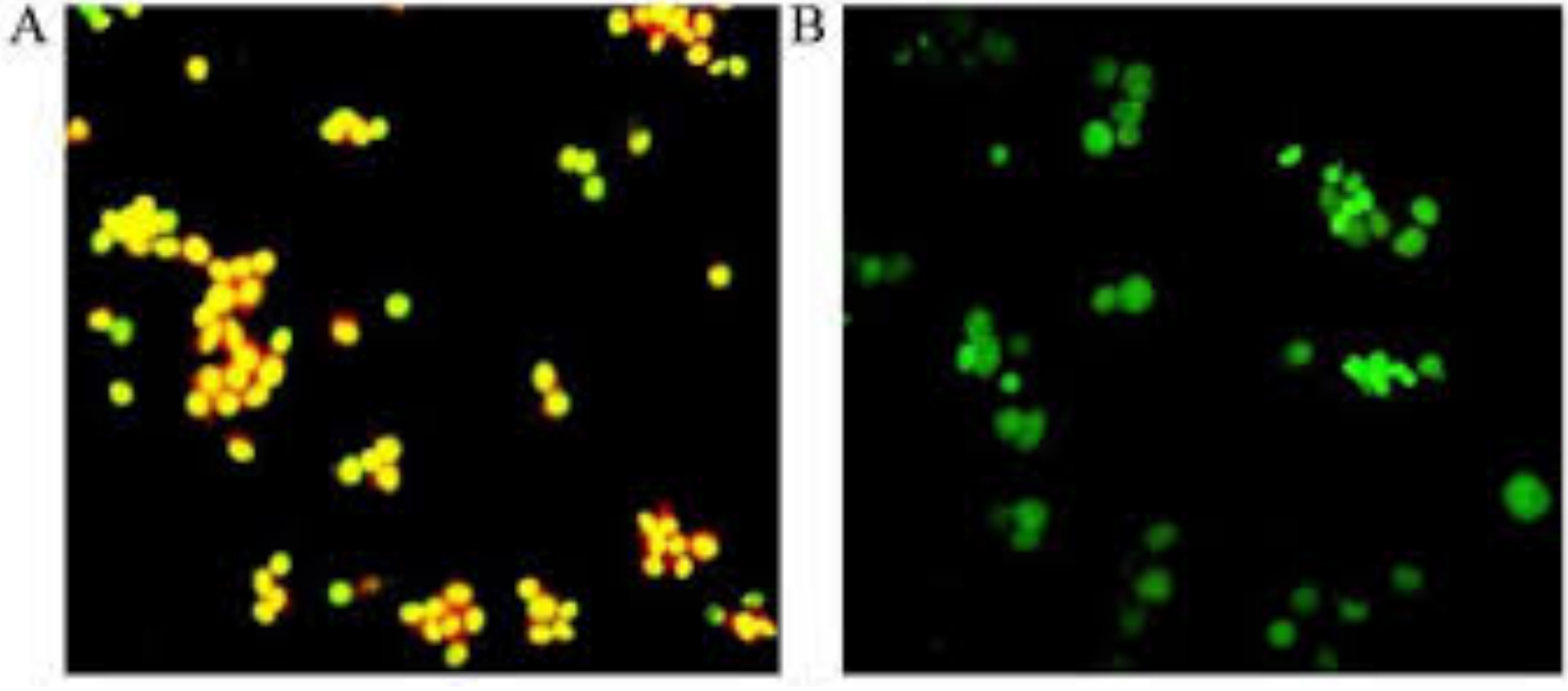
Fluorescence Microscopy

When certain substances are irradiated by light of a proper wavelength, they emit light with a longer wavelength. This phenomenon is called fluorescence. In fluorescence microscopy, tissue sections are usually irradiated with ultraviolet (UV) light and the emission is in the visible portion of the spectrum. The fluorescent substances appear brilliant on a dark background. For this method, the microscope has a strong UV light source and special filters that select rays of different wavelengths emitted by the substances.

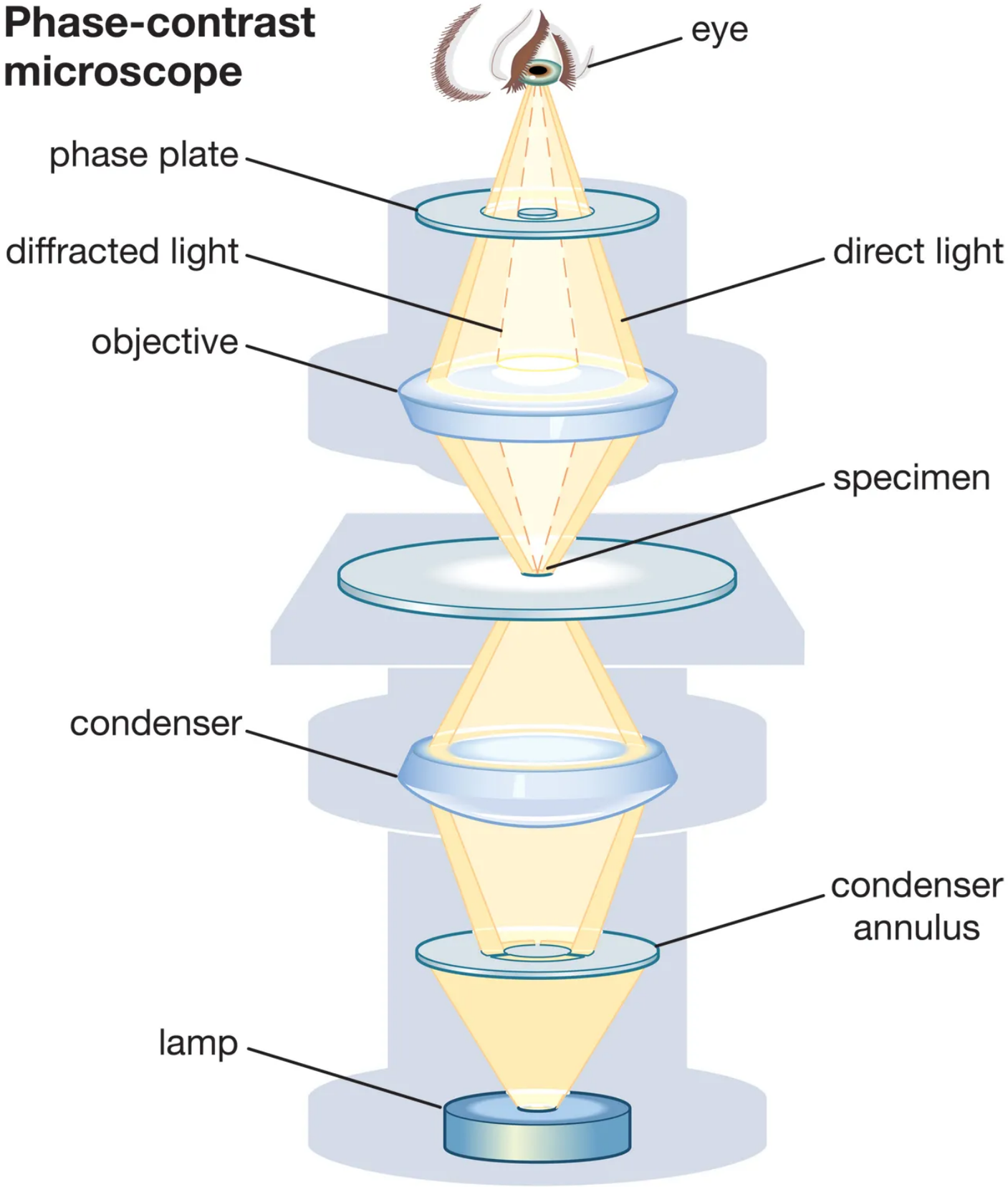
Fluorescent compounds with affinity for specific cell macromolecules may be used as fluorescent stains. Acridine orange, which binds both DNA and RNA, is an example. When observed in the fluorescence microscope,


detector
ocular
emission filter
dichroic mirror
light source
excitation filter
objective
specimen

these nucleic acids emit slightly different fluorescence, allowing them to be
localized separately in cells .Other compounds such as Hoechst stain and DAPI specifically bind DNA and are used to stain cell nuclei, emitting a characteristic blue fluorescence under UV.
Another important application of fluorescence microscopy is achieved by coupling fluorescent compounds to molecules that will specifically bind
to certain cellular components and thus allow the identification of these structures under the microscope . Antibodies labeled with fluorescent compounds are extremely important in immunohistological staining.

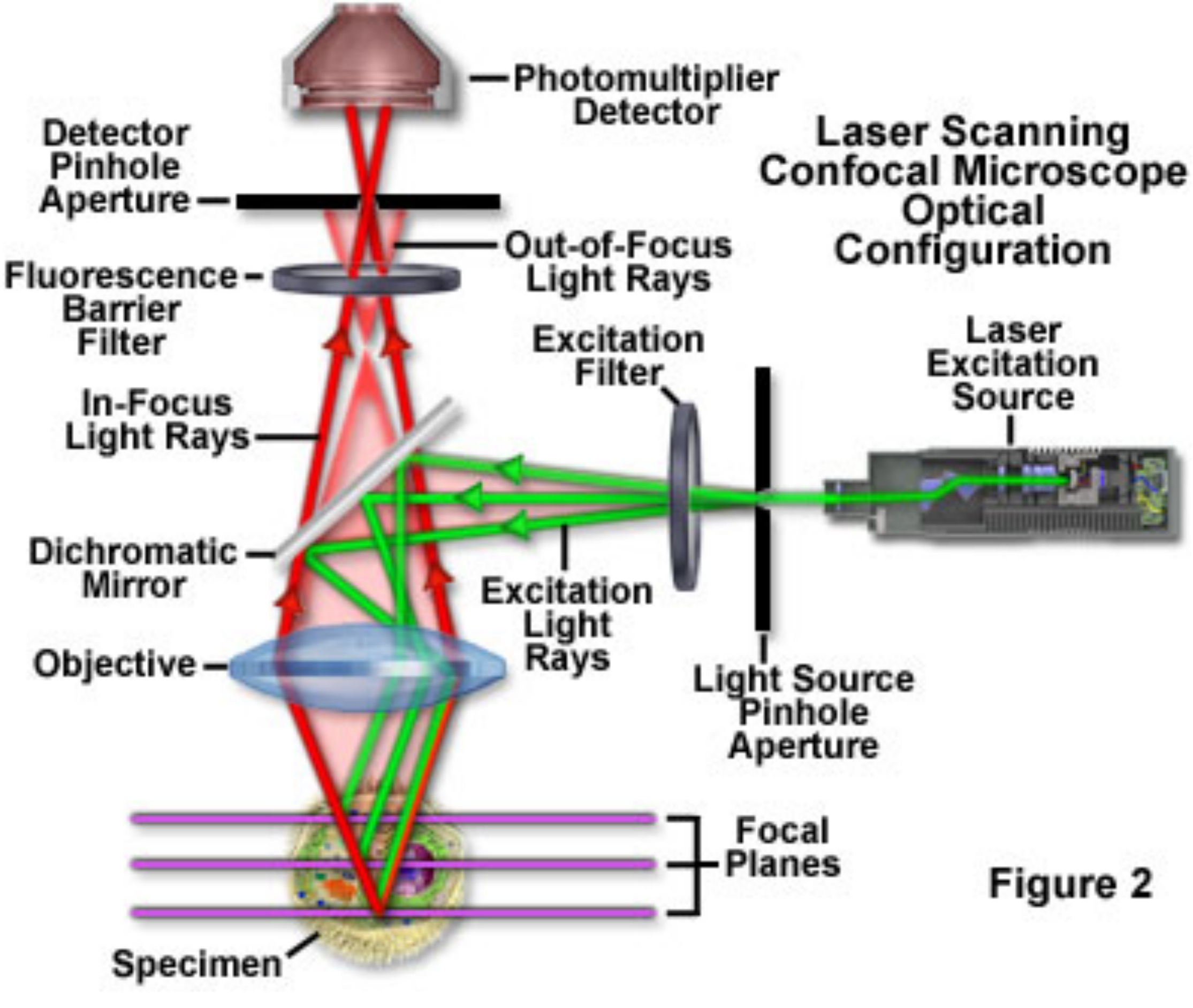


Some optical arrangements allow the observation of unstained cells and tissue sections. Unstained biological specimens are usually transparent and difficult to view in detail, because all parts of the specimen have almost the same optical density. Phase-contrast microscopy, however, uses a lens system that produces visible images from transparent objects Phase-contrast microscopy is based on the principle that light changes its speed when passing through cellular and extracellular structures with different refractive indices. These changes are used by the phase-contrast system to cause the structures to appear lighter or darker in relation to each other. Because it does not require fixation or staining, phase-contrast microscopy allows observation of living cells and tissue cultures, and such microscopes are prominent tools in all cell culture labs.



Confocal Microscopy

With a regular bright-field microscope, the beam of light is relatively large and fills the specimen. Stray light reduces contrast within the image and compromises the resolving power of the objective lens. Confocal microscopy
avoids stray light and achieves greater resolution by using: (1) A small point
of high-intensity light provided by a laser and (2) A plate with a pinhole aperture in front of the image detector. The point light source, the focal point
of the lens, and the detector's pinpoint aperture are all optically conjugated
or aligned to each other in the focal plane (confocal) and unfocused light does
not pass through the pinhole. This greatly improves resolution of the object in focus and allows the localization of specimen components with much greater precision than with the bright-field microscope.
Most confocal microscopes include a computer-driven mirror system (the beam splitter) to move the point of illumination across the specimen automatically and rapidly. Digital images captured at many individual spots in a very thin plane-of-focus are used to produce an "optical section" of that
plane. Moreover, creating optical sections at a
series of focal planes through the specimen
allows them to be digitally reconstructed into a
three-dimensional image



Laser Scanning Confocal Microscope Optical Configuration

Figure 2


Thank
you!