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Department of Chemistry

Project Title : Fluorescence Microscopy

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Certificate

This is to certify that the project is titled Fluorescence Microscopy. This project is submitted by Mr. Amol Sukhdan . He had successfully completed his chemistry project. Under the Guidance of Miss. V.R. Bondre

Miss. V.R. Bondre

What is a Fluorescence Microscope?

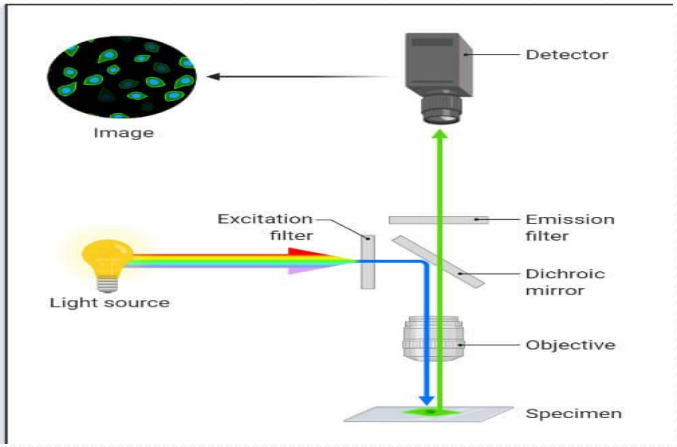
A **fluorescence microscope** is an optical microscope that uses fluorescence and phosphorescence instead of, or in addition to, reflection and absorption to study the properties of organic or inorganic substances. Fluorescence is the emission of light by a substance that has absorbed light or other electromagnetic radiation while phosphorescence is a specific type of photoluminescence related to fluorescence. Unlike fluorescence, a phosphorescent material does not immediately re-emit the radiation it absorbs. The fluorescence microscope was devised in the early part of the twentieth century by August Köhler, Carl Reichert, and Heinrich Lehmann, among others.

Principle of Fluorescence Microscope

Most cellular components are colorless and cannot be clearly distinguished under a microscope. The basic premise of fluorescence microscopy is to stain the components with dyes. Fluorescent dyes, also known as fluorophores or fluorochromes, are molecules that absorb excitation light at a given wavelength (generally UV), and after a short delay emit light at a longer wavelength. The delay between absorption and emission is negligible, generally on the order of nanoseconds.

The emission light can then be filtered from the excitation light to reveal the location of the fluorophores.

Fluorescence Microscopy



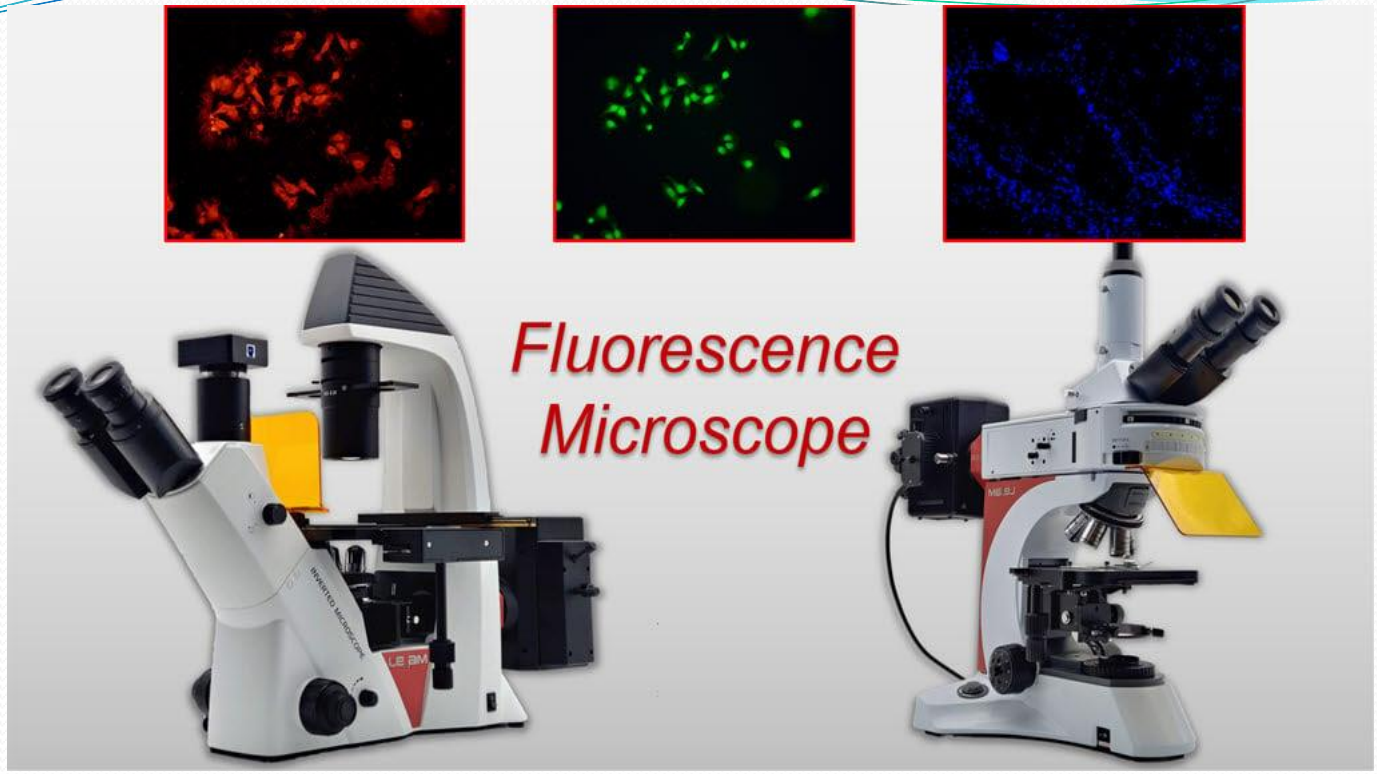
- Fluorescence microscopy uses a much higher intensity light to illuminate the sample. This light excites fluorescence species in the sample, which then emits light of a longer wavelength.
- The image produced is based on the second light source or the emission wavelength of the fluorescent species rather than from the light originally used to illuminate, and excite, the sample.

Working

Light of the excitation wavelength is focused on the specimen through the objective lens. The fluorescence emitted by the specimen is focused on the detector by the objective. Since most of the excitation light is transmitted through the specimen, only reflected excitatory light reaches the objective together with the emitted light.

Forms

The “fluorescence microscope” refers to any microscope that uses fluorescence to generate an image, whether it is a more simple set up like an epifluorescence microscope, or a more complicated design such as a confocal microscope, which uses optical sectioning to get better resolution of the fluorescent image. Most fluorescence microscopes in use are epifluorescence microscopes, where excitation of the fluorophore and detection of the fluorescence are done through the same light path (i.e. through the objective).


Fluorescence
Microscope

Fluorescent dyes (Fluorophore)

A fluorophore is a fluorescent chemical compound that can re-emit light upon light excitation.

Fluorophores typically contain several combined aromatic groups, or plane or cyclic molecules with several π bonds.

Many fluorescent stains have been designed for a range of biological molecules.

Some of these are small molecules that are intrinsically fluorescent and bind a biological molecule of interest. Major examples of these are nucleic acid stains like DAPI and Hoechst, phalloidin which is used to stain actin fibers in mammalian cells.

Applications of Fluorescence Microscope

To identify structures in fixed and live biological samples. Fluorescence microscopy is a common tool for today's life science research because it allows the use of multicolor staining, labeling of structures within cells, and the measurement of the physiological state of a cell.

Thank You!