CHRISTIAN EMINENT COLLEGE, INDORE

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e-Content

On

"Fluorescence Microscopy"

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FLUORESCENCE MICROSCOPY

"The method of optical microscopy which comprises a fluorescent substance for the imagination, called Florescent Microscopy". Eric Betzig, William Moerner and Stefan Hell got Nobel Prize for the development of super-resolved fluorescence microscopy in 2014. A **fluorescent microscope** is an optical **microscope** that uses **Fluorescence** and phosphorescence instead of, or in addition to, reflection and absorption to study properties of organic or inorganic substances for the clear imagination.

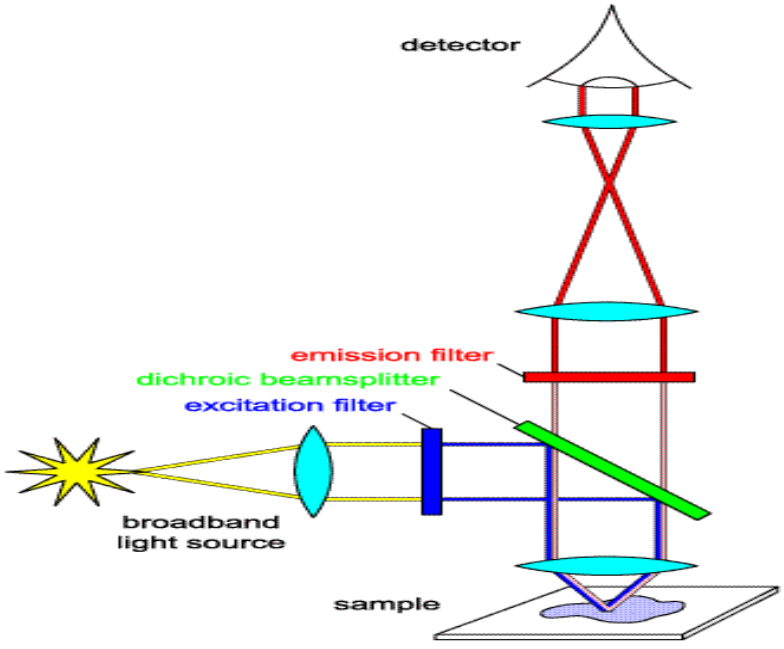
Principle:

The specimen is illuminated with light of a specific wavelength (or wavelengths) which is absorbed by the fluorophores, causing them to emit light of longer wavelengths. The illumination light is separated from the much weaker emitted fluorescence through the use of a spectral emission filter. Typical components of a fluorescence microscope are a light source (xenon arc lamp or mercury-vapor lamp are common; more advanced forms are high-power LEDs and lasers), excitation filter, the dichroic mirror , and the emission filter. 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.

SAMPLE PREPARATIONS:

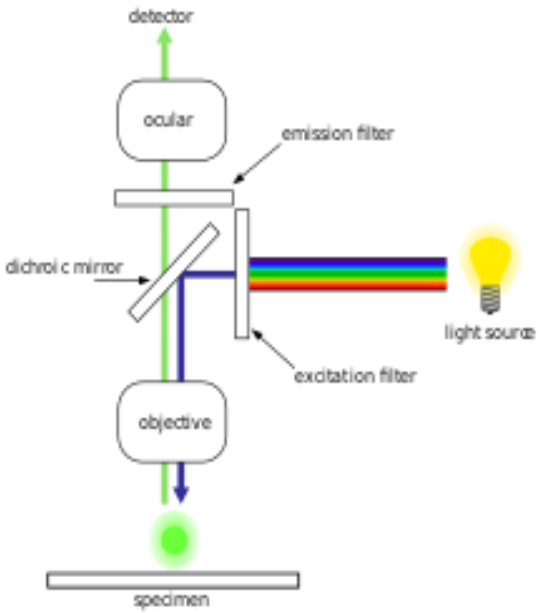
There are several methods of creating a fluorescent sample; the main techniques are labelling with fluorescent stains or, in the case of biological samples, expression of a fluorescent protein.

Alternatively the intrinsic fluorescence of a sample can be used. In the life sciences fluorescence microscopy is a powerful tool which allows the specific and sensitive staining of a specimen in order to detect the distribution of Proteins or other molecules of interest. Many fluorescent stains have been designed for a range of biological molecules. Some of these are small molecules which are intrinsically fluorescent and bind a biological molecule of interest. Major examples of these are nucleic acid stains like DAPI and Hoechst (excited by UV wavelength light) and **DRAQ5 and DRAQ7** (optimally excited by red light) which all bind the minor groove of DNA, thus labeling the nuclei of cells. Others are drugs or toxins which bind specific cellular structures and have been discovered such as: **Phalloidin** which is used to stain actin fibres in mammalian cells. There are many fluorescent molecules called fluorophores or fluorochromes such as **Fluorescein, Alexa Fluors** or **DyLight 488**, which can be chemically linked to a different molecule which binds the target of interest within the samples.



FLUORESCENCE MICROSCOPY:

The majority of fluorescence microscopes, especially those used in the life sciences, are of the epifluorescence design shown in the diagram. Light of the excitation wavelength illuminates the specimen through the objective lens. The fluorescence emitted by the specimen is focused to the detector by the same objective that is used for the excitation which for greater resolution will need objective lens with higher numerical aperture. Since most of the excitation light is transmitted through the specimen, only reflected excitatory light reaches the objective together with the emitted light and the epifluorescence method therefore gives a high signal-to-noise ratio.



APPLICATIONS:

These microscopes are widely used in biological study, immunology, nuclear DNA etc.

LIMITATIONS:

The measure limitation of such microscopy is found Photobleaching occurs as the fluorescent molecules accumulate chemical damage from the electrons excited during fluorescence which may lost physical and chemical properties of the specimen.