

## FLUORESCENT MICROSCOPY

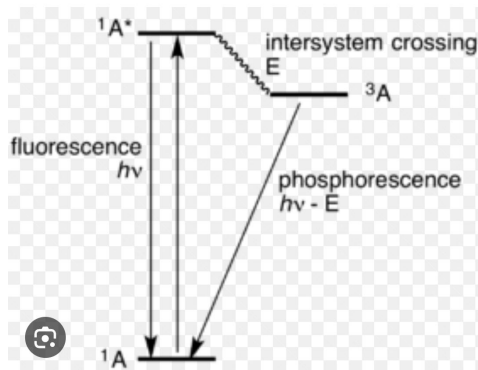
A fluorescence microscope is an optical microscope that uses fluorescence and Phosphorescence instead of, or in addition to reflection and absorption to study Properties of organic or inorganic substances.

### FLUORESCENCE:

Fluorescence is the emission of light by a substance that has absorbed light or other electromagnetic radiation.

### PHOSPHORESCENCE:

Phosphorescence is a specific type of Photoluminescence is related to fluorescent



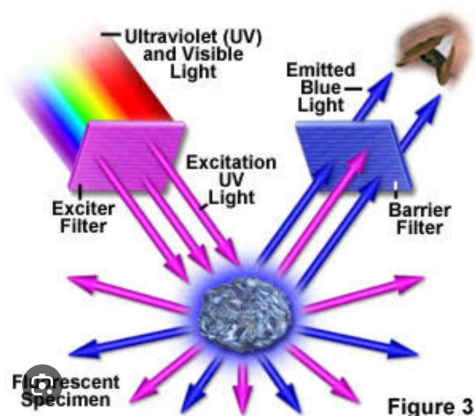
## FLUORESCENT MICROSCOPY



### PRINCIPAL:

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

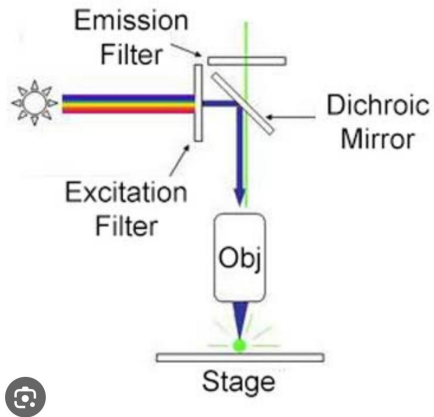
### Principle of Excitation and Emission



## INSTRUMENTATION:

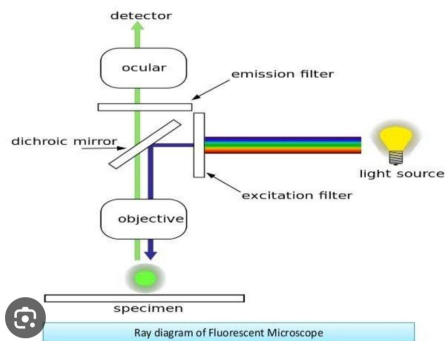
- Fluorescence dyes (Fluorophores)
- Light source
- Excitation filter
- Dichroic mirror
- Emission filter

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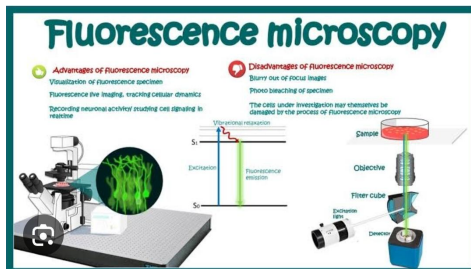
## APPLICATION:

- 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 uses of multicolour staining, labeling of structure within cells, and the measurement of the physiological state of a cell.



## ADVANTAGES:

- Fluorescence microscopy is the most popular method for studying the dynamic behavior exhibited in live cell imaging.
- This stems from its ability to isolate individual proteins with a high degree of specificity amidst non fluorescing material.
- The sensitivity is high enough to detect as few as 50 molecules per cubic micrometer.
- Different molecules can now be stained with different colors, allowing multiple types of molecules to be tracked simultaneously



## WORKING:

- Light of the excitation wavelength is focused on the specimen through the objective lens.
- The fluorescence emitted by the specimen is focused to 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

