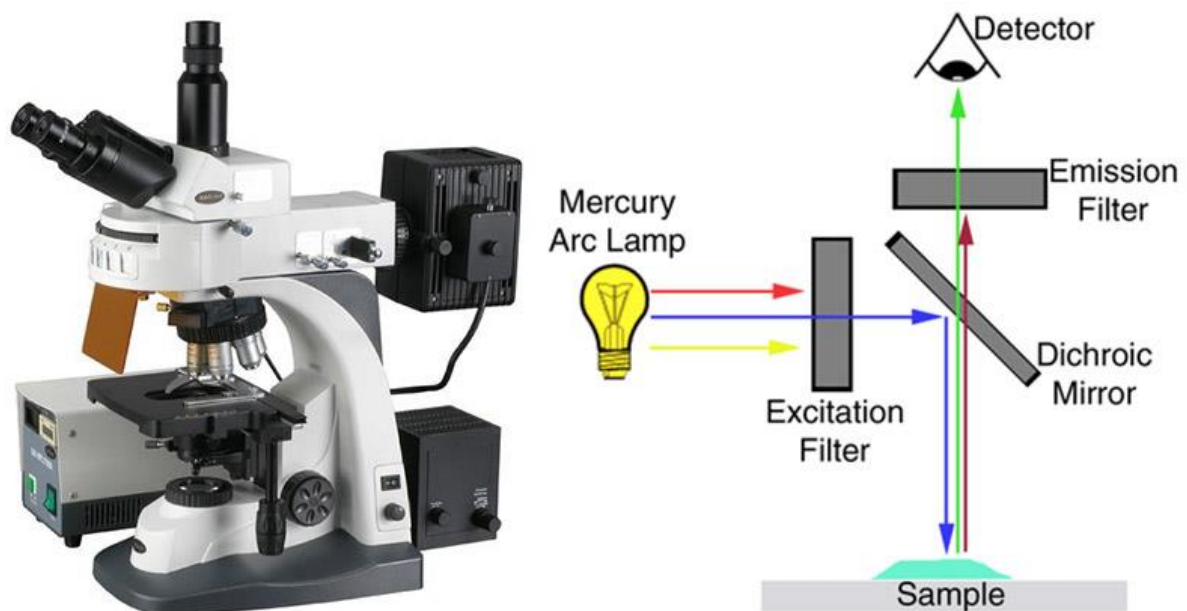


## M.Sc. Zoology Semester II, Paper-VI, Unit-I, Topic- Principle and uses of Fluorescence microscope Lecture Notes by Dr. Arjun Pratap Singh

- 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 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.

### Fluorescence Microscope



### Principle of Fluorescence Microscopy

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 uses a much higher intensity light to illuminate the sample. This light excites fluorescence species in the sample, which then emit 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).

### **Advantages of Fluorescence Microscope**

- 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 the molecule to be tracked simultaneously.
- These factors combine to give fluorescence microscopy a clear advantage over other optical imaging techniques, for both in vitro and in vivo imaging.