MATERIALS SCIENCE AND TECHNOLOGY-1

THE FLUORESCENCE MICROSCOPE

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HISTORY

The termofluorescence was coined by Sir George Gabriel Stokes (1819–1903). In 1843, he described the luminescence in calcium fluoride as fluorescence. In 1904, August Köhler working in the Jena Zeiss factory invented the ultraviolet absorption microscope that preceded the fluorescence microscope. Together with Siedentopf, they made a fluorescence microscope. Several later developments improved the fluorescence microscope.

WHAT IS A FLUORESCENCE MICROSCOPE?

- A fluorescence microscope is basically a conventional light microscope with added features and components that extend its capabilities.
- Fluorescence microscopy is the most popular method for studying the dynamic behavior exhibited in live cell imaging.
- Different molecules can now be stained with different colors, allowing multiple types of molecule to be tracked simultaneously.

WHAT IS A FLUORESCENCE MICROSCOPE?

- A conventional microscope uses light to illuminate the sample and produce a magnified image of the sample.

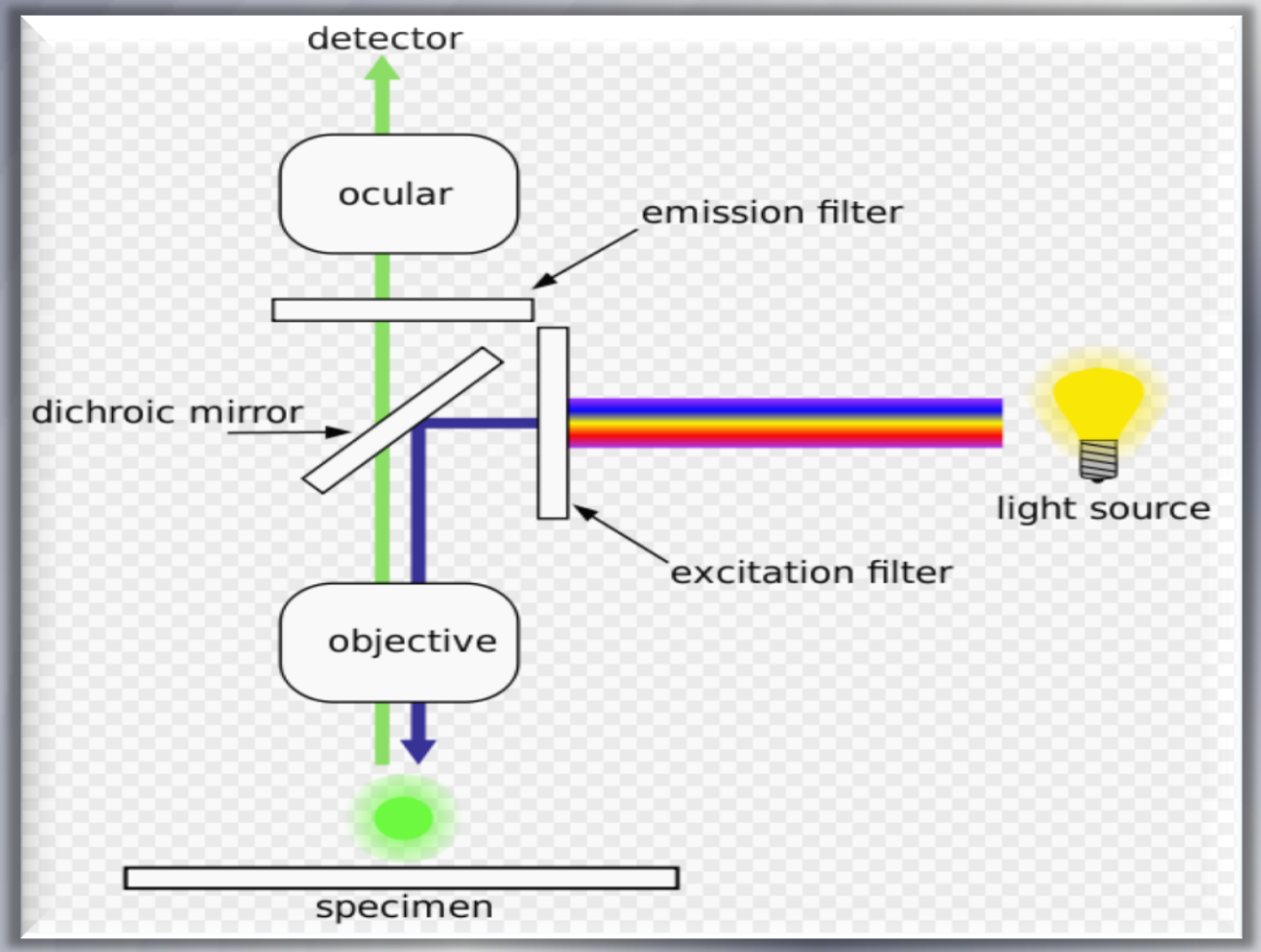
- A fluorescence microscope uses a much higher intensity light to illuminate the sample. This light excites fluorescence species in the sample, which then emit light of a longer wavelength.

BASIC REQUIREMENTS OF FLUORESCENCE MICROSCOPE OPTICS

Nearly all fluorescence microscopes use the objective lens to perform two functions:

- Focus the illumination (excitation) light on the sample.
- Collect the emitted fluorescence.

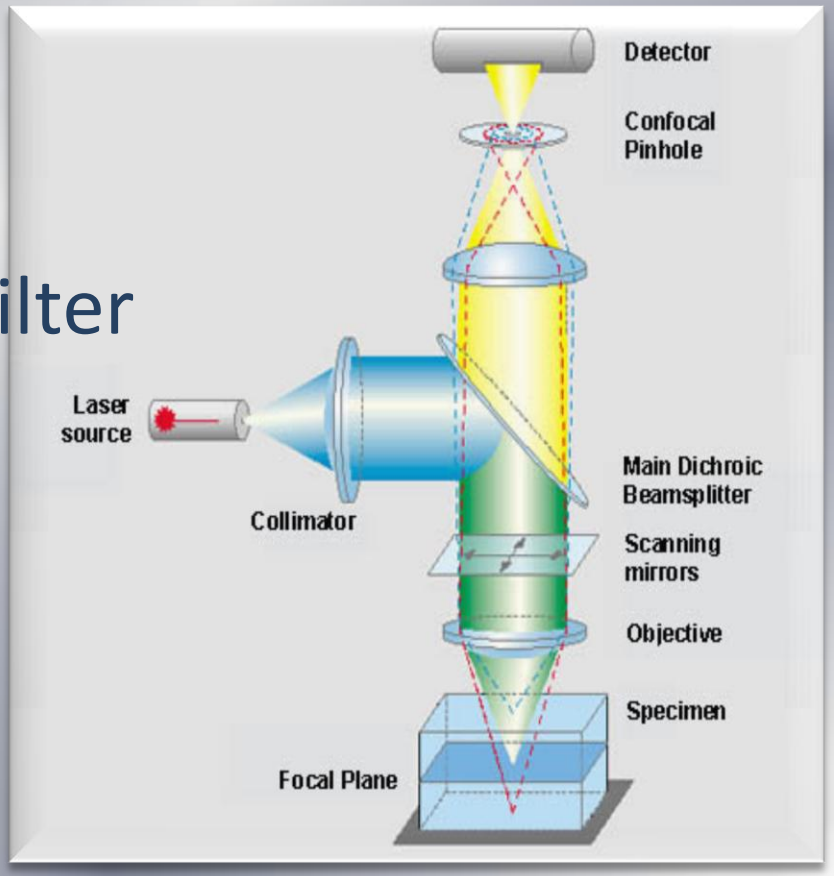
PARTS OF FLUORESCENCE MICROSCOPE



PARTS OF FLUORESCENCE MICROSCOPE

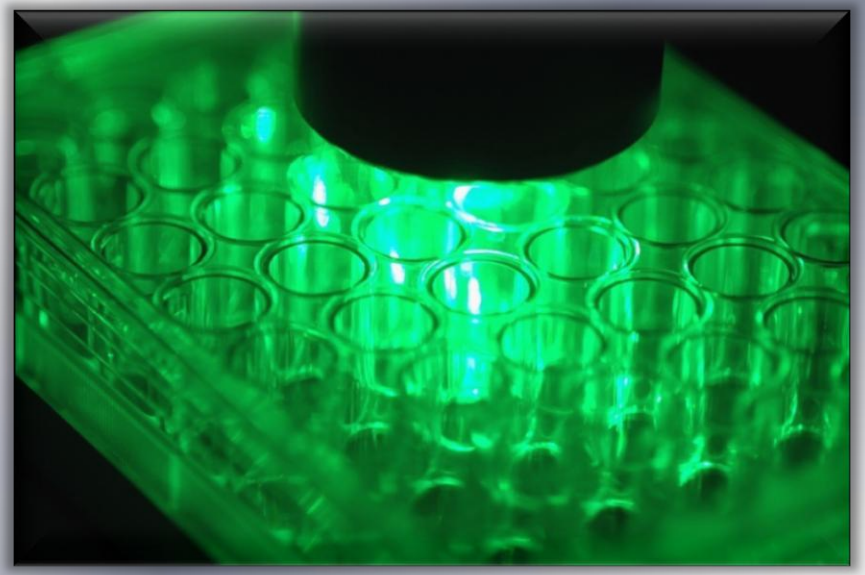
Main parts of a fluoresence microscobe are;

- ✓ dichroic mirror,
- ✓ emission and excitation filter
- ✓ detector
- ✓ the light source.



Light Source

- Direct chemical is being used as light source.
- The conventional microscope uses visible light (400-700 nanometers)
- A fluorescence microscope uses a much higher intensity light source which excites a fluorescent species in a sample of interest.



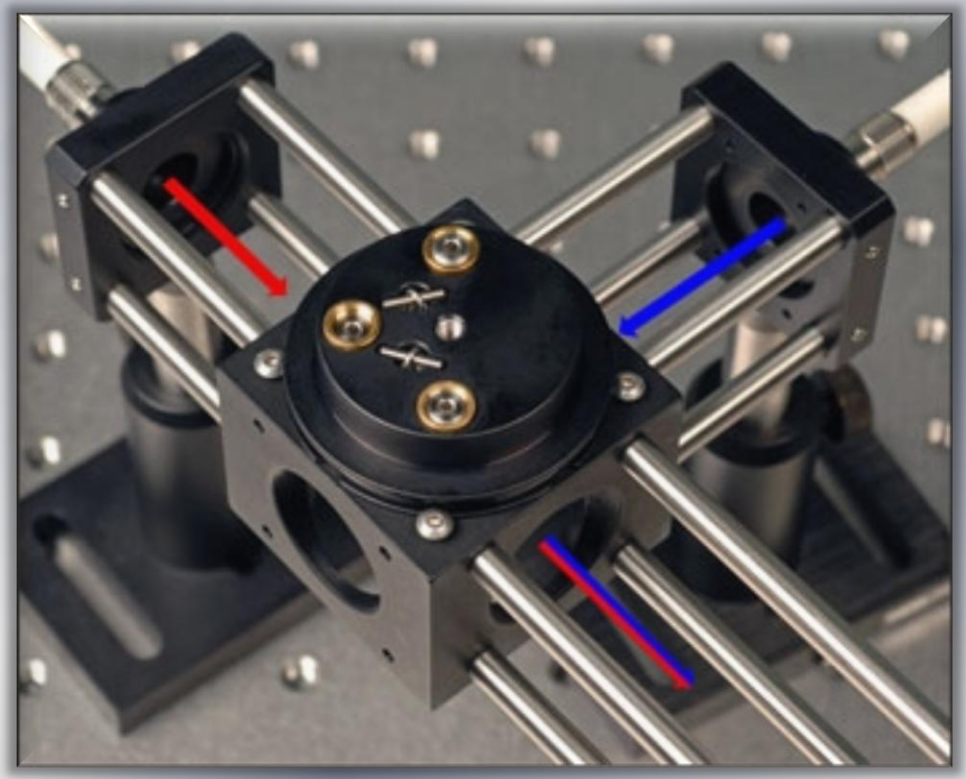
The Dichroic Mirror

In a fluorescence microscope, the dichroic mirror separates the light paths.

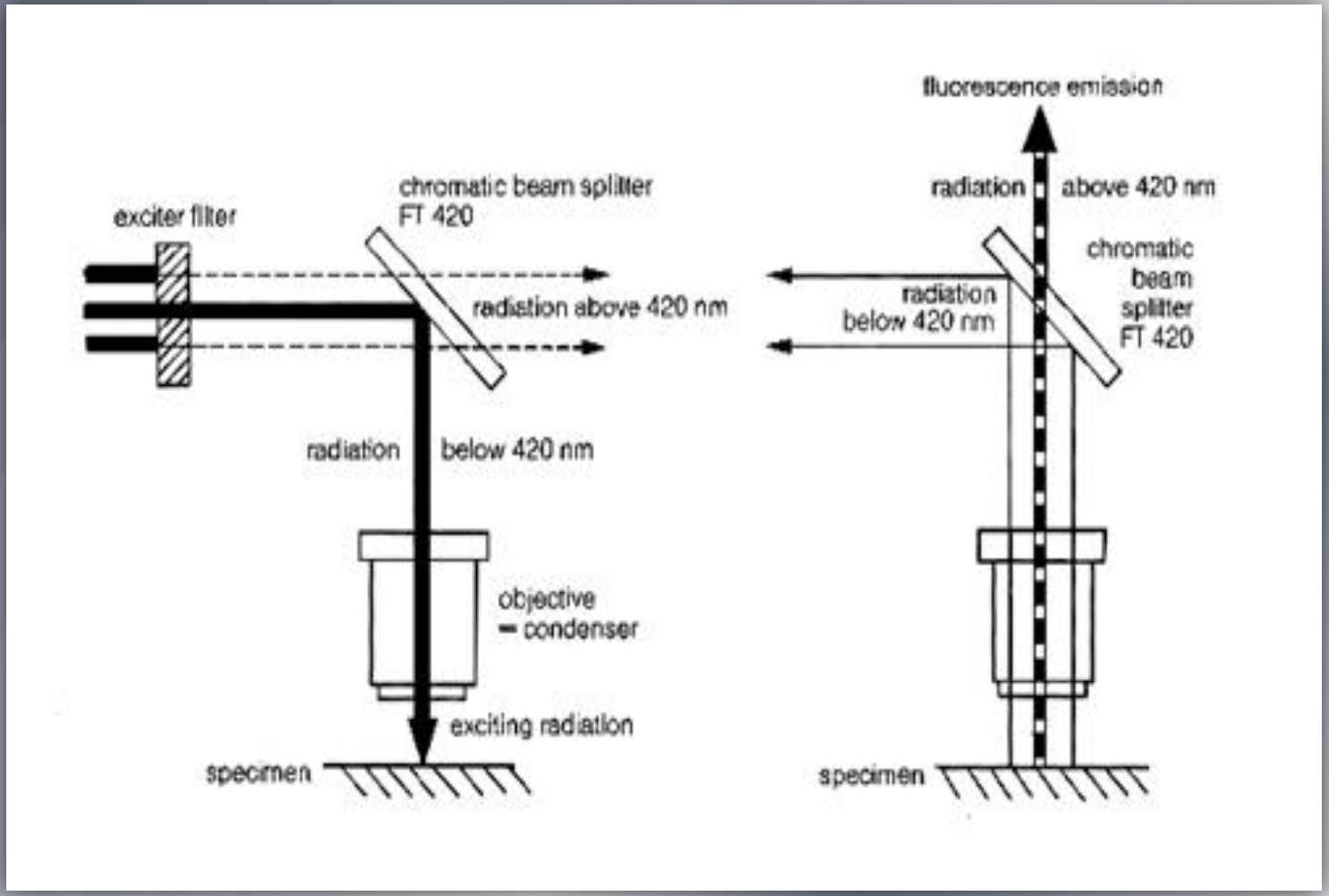
- The excitation light reflects off the surface of the dichroic mirror into the objective.
- The fluorescence emission passes through the dichroic to the eyepiece or detection system.

The Dichroic Mirror

The mirror reflects wavelengths of light below thetransition wavelength value and transmits wavelengths above this value. This property accounts for the name given to this mirror.



Excitation and Emission Filters

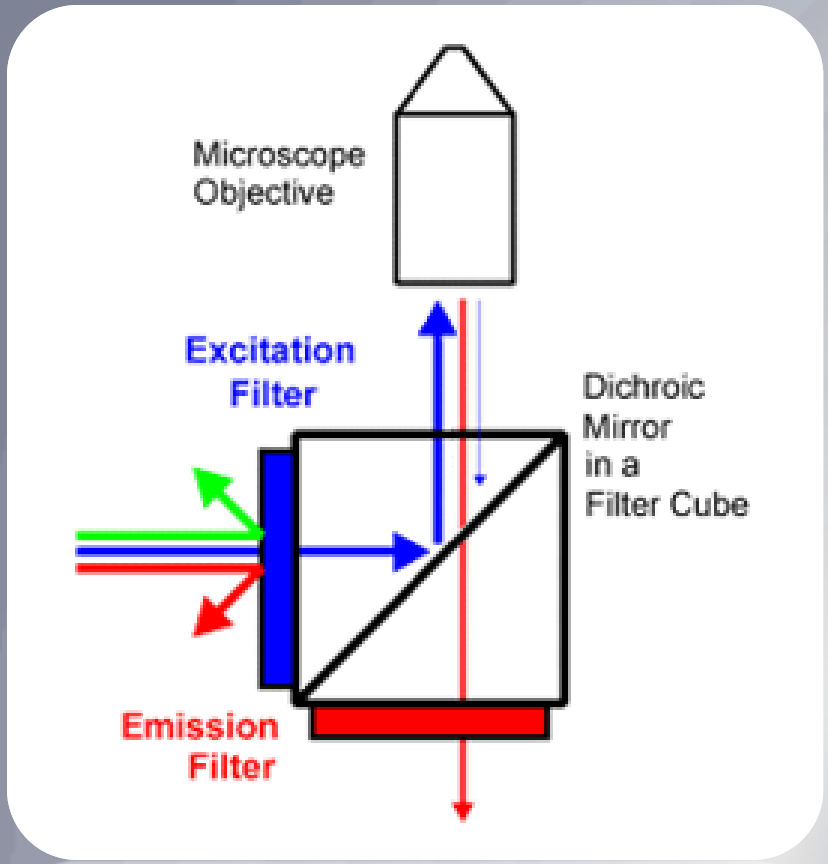


Excitation and Emission Filters

- **Excitation filter** :In order to select the excitation wavelength, an excitation filter is placed in the excitation path just prior to the dichroic mirror.

- **Emission filter**: An emission filter is placed beneath the dichroic mirror. In this position, the filter functions to both select the emission wavelength and to eliminate any trace of the wavelengths used for excitation.

The Filter Cube



✓The dichroic mirror is mounted on an optical block commonly referred to as a filter cube.

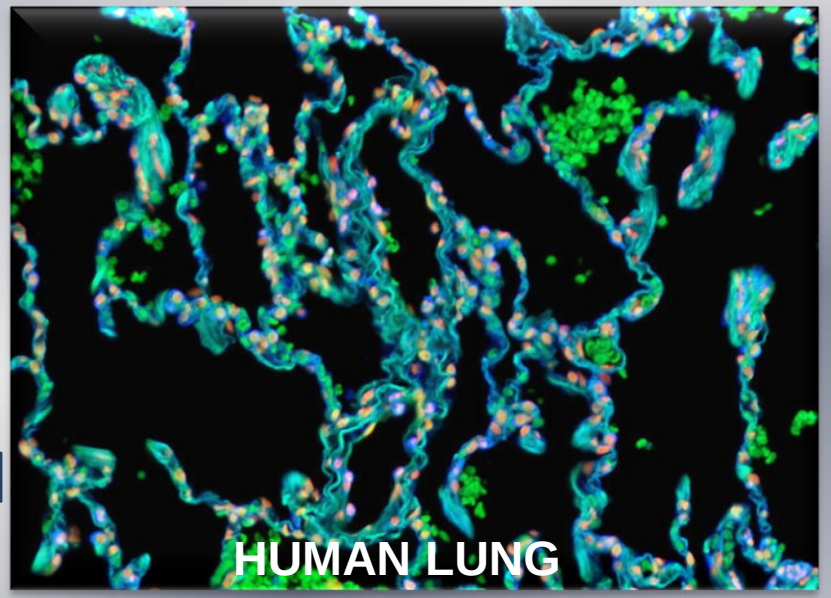
✓This cube provides a convenient means to change the dichroic mirror without direct handling of either the mirror or filters.

Detector

- The fluorescence detector is used almost exclusively in liquid chromatography and is a specific detector that senses only those substances that fluoresce.

- A flow cell is used as the sensor through which the excitation light passes axially.

WHAT IS FLUORESCENCE MICROSCOPY USED FOR?



- It is often used to image specific features of small specimens such as microbes.
- And also used to visually enhance 3-D features at small scales.
- This is achieved by using powerful light sources, such as lasers, that can be focused to a pinpoint.

HOW DOES FLUORESCENCE MICROSCOPE WORK?

- The microscope has a filter that only lets through radiation with the specific wavelength .
- The radiation collides with the atoms in the specimen.
- Electrons are excited to a higher energy level.
- When they relax to a lower level, they emit light.
- To become detectable, the fluorescence emitted from the sample is separated from the much brighter excitation light in a second filter.

ADVANTAGES OF FLUORESCENCE MICROSCOPY

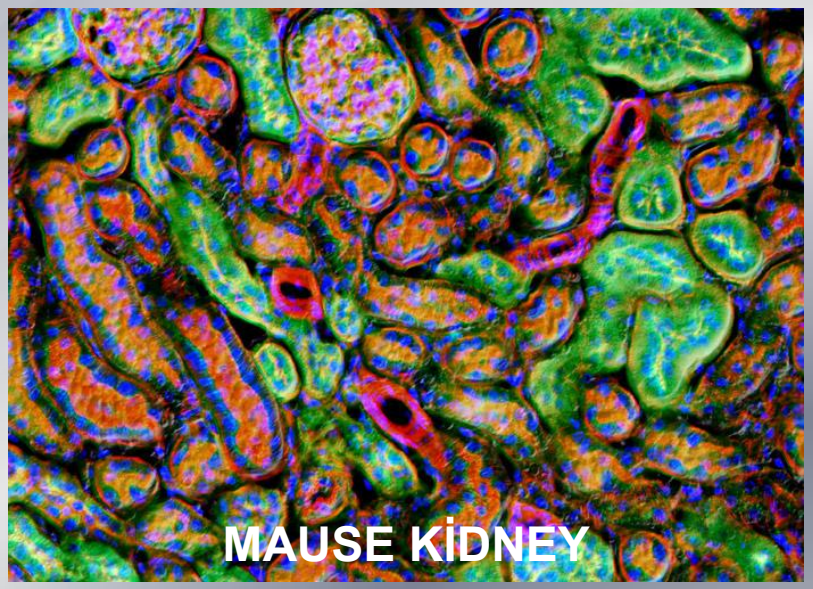
- ✓ Image clarity is higher.
- ✓ The modern fluorescence microscope combines the power of high performance optical components to achieve a level of sophistication that far exceeds that of simple observation by the human eye.
- ✓ Microscopy now depends heavily on electronic imaging to rapidly acquire information at low light levels or at visually undetectable wavelengths.

DISADVANTAGES OF FLUORESCENCE MICROSCOPY

- ✓ The relatively high costs of a microscopy unit.
- ✓ The fluorescence microscope is less robust than conventional instruments.
- ✓ Component parts,particularly bulbs, have to be replace from time to time,and may be expensive and difficult to procure;repairs are occasionally necessary.
- ✓ A continuous supply of standard electrical power with minimal voltage fluctuations is needed and it s difficult to meet these requirements in developing countries.

APPLICATIONS

A fluorescence microscope is used to study properties of organicor inorganic substances using the phenomena of



fluorescence and phosphorescence instead of, or in addition to, reflection and absorption.

These microscopes are often used for;

- Imaging structural components of small specimens, such as cells
- Conducting viability studies on cell populations (are they alive or dead?)
- Imaging the genetic material within a cell (DNA and RNA)
- Viewing specific cells within a larger population with many techniques.

SUMMARY

- The modern fluorescence microscope has emerged as an important tool in the life sciences.
- The concomitant developments in instrumentation, specifically light sources and detectors, as well as probe development have resulted in fluorescence with unprecedented sensitivity and specificity.
- Modern fluorescence microscopes have the capability of single-molecule imaging, subdiffraction limited resolution and the specificity of imaging single proteins in living cells and organisms.

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