Fluorescence microscopy

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Fluorescence Microscopy

Introduction

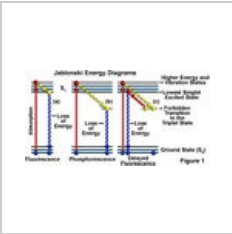
Fluorescence microscopy is used in proper understanding in different regions of the cell. The idea is very simple; to analyze a living cell we use a dye- fluorescence dye, which has a property called fluorescence nature. The cell is marked with a particular dye. This dye can receive particular wave length and emit a different color which can be measured, the green emission. By looking at it we can where exactly our specimen is present. Actin and microtubule dynamic can be seen and analyzed. It can only be used for living cell.

Discussion

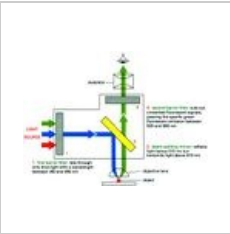
Jablonski diagram, light is looked at as a photon, and each photon has a different energy level, the photons excite the electron to a new energy state, electron is kicked to higher energy state by the photon (light source). Once the electron is in excited state, it will go down and release energy (jump from excited state to ground state).The fluorescence can be measured. Since the reaction happens very quickly, it is measured in femto second which 10-15. Some amount of energy is released as heat, and some as fluorescence. When the emission energy is high wavelength is short, and when emission energy is low wavelength is long. The difference between absorption light and the emission light is called as the stock shift.

Usual components of a fluorescence microscope are a light source (xenon arc lamp,mercury-vapor lamp; more advanced forms are high-power LEDs and lasers), the excitation filter, the dichroic mirror, and the emission filter .

The sample contains fluorescence compound that emits the fluorescence light. An Example would be GFP (which needs blue light to excite). The fluorescence compound needs to be excited by light source, but only a certain wavelength can excite the fluorescence compound. In case of GFP (green fluorescence protein) we only need the blue light and since the normal light contains a spectrum of different color , we need to filter the light so only the blue light can go through, and in order to that we need excitation filter. Once the blue light passes, it’s reflected by a mirror (Dichcroic mirror), it’s a special mirror because it can reflect particular kind of wave length and then allow a certain other kind of wavelength to pass through, in this case the green light. Once the blue light hits the sample , the fluorescence compound gets excited and emits a green light (in case of the GFP), and the dichroic mirror will let it pass. Now the green light will go through the emission filter. Emission filter is required due to the fact that the waves of green light are scattered and this filter helps arranging them in one place, it also blocks the wavelength of any other color and it only allows the green light to pass. We can measure how fluorescent lights are coming in by the quantification, and by measuring the fluorescence we can detect how many proteins of same type are present. The fluorescence light microscopy gives us good contrast and high resolution. Fluorescence microscopy has many applications in medicine, and it has helped the scientific community a great deal. One very important use its application in diagnosis of diseases and environmental monitoring, which is very well explained in an article written and submitted to WHO during the 2005 Regional Office for the Eastern Mediterranean Cairo.



Jablonski Energy Diagram



Fluorescence Microscope

Advantages

- High contrast Imaging
- Quantitative imaging
- Live cell imaging

Disadvantages

- Photo bleaching

Conclusion

Fluorescence microscopy has a bright a future in field of medicine and biology, as it has and it will always be a great help and facilitator for scientists. With more and more technological advances, scientists have been successful so far in making images with higher resolution and quality.

References [1]

1. * https://www.uni-leipzig.de/~pwm/web/?section=introduction&page=fluorescence * http://www.esric.org/education/fluorescence.html * https://en.wikipedia.org/wiki/Fluorescence_microscope * http://applications.emro.who.int/dsaf/dsa281.pdf