# Introduction to fluorescence

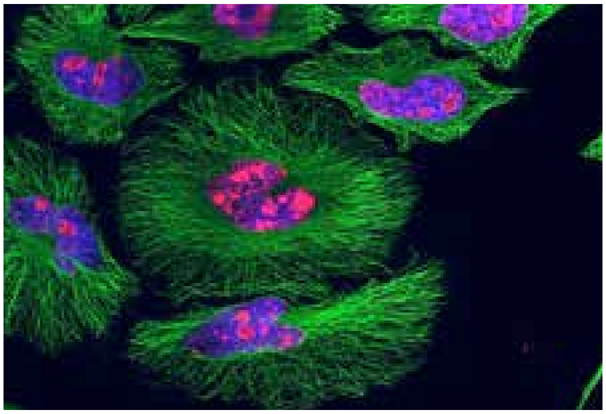
## Fluorescence and biotechnology

When you bring to mind something that is fluorescent, you probably think of a color so bright that it practically glows. In fact, things that are truly fluorescent actually do emit light. And light being a form of energy, that energy must be supplied from somewhere. Fluorescence is a phenomenon where energy is absorbed from one part of the electromagnetic spectrum and is then released as a different part of the electromagnetic spectrum—visible light. If you have ever seen things glow under a blacklight, you are familiar with the phenomenon of fluorescence. The blacklight emits ultraviolet light, a form of light that your eye cannot see. The fluorescent colors absorb that ultraviolet light and release it back out as brilliant greens, oranges, and other colors.

Different fluorescent solutions illuminated using UV light. Photo: Maxim Bilovitskiy *Creative Commons license CC 4.0)*

Many organisms have evolved to use fluorescent molecules, especially marine organisms living relatively close to the surface of the water. People also regularly use them in everyday life. Highlighters use fluorescent molecules to make important words stand out to you. Fluorescent lightbulbs save energy and light up our buildings. Fluorescent dyes help plumbers find leaks.

In the biology laboratory, fluorescence is an incredibly important tool because it has afforded scientists a way to visualize structures that were previously invisible or indistinguishable. Fluorescent proteins, such as the green fluorescent protein (GFP) and red fluorescent protein (RFP) are regularly used to track happenings inside the cell by making otherwise invisible structures light up. Fluorescent molecules can also be attached to antibodies which will then bind to specific structures in the cell showing us their location, shape and structure. And because different fluorescent molecules absorb and release unique wavelengths of light, often several of these chemicals can be observed in the cell at the same time, leading to some of the incredibly bright and colorful pictures of cells that you may have seen before.

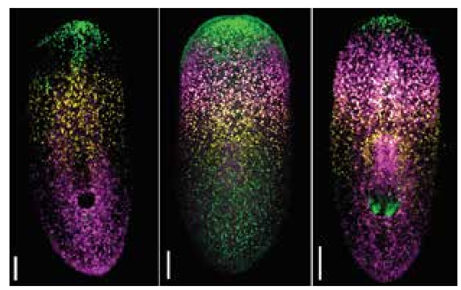


Cells grown in culture stained with three different fluorescent stains. Photo: Gerry Shaw *Creative Commons license CC 4.0)*

Different fluorescent dyes have been developed that bind to DNA. The most common use of such dyes is to see DNA on an agarose gel, but fluorescent dyes can also be used to quantify DNA. For example, in a process called quantitative PCR (qPCR), DNA molecules are copied and as each new copy is made, a fluorescent dye binds to the DNA. A light beam and optical sensor then record the relative brightness of the dye and tell you how much DNA you have made.

Fluorescence has the added benefits that it makes images that are often quite striking to look at and that it is generally safe to use. Previous to using fluorescence, the most common way of tagging molecules was using radioactivity. While radioactive probes are still sometimes used today, they are generally much more dangerous to handle and not nearly as fun to look at.

## What is fluorescence?



Fluorescent *in situ* hybridization (FISH) image of planaria

Fluorescence is emitted electromagnetic radiation that results from the release of energy after a substance is struck with electromagnetic radiation of a different wavelength.  When a photon (particle of light) strikes an electron, it transfers energy to the electron, causing the electron to jump to an excited (higher energy) state. This excited state is unstable, so the electron eventually falls back to its lower energy state, known as the ground state. But the energy that the electron absorbed from the light must go somewhere. Some of this energy is distributed as heat, and then some may be emitted as electromagnetic radiation in the visible range-light. Because some electromagnetic radiation was lost as heat, the emitted radiation has less energy than when it was initially absorbed, which means the photon emitted will have a longer wavelength than the photon that was absorbed; this is called *Stokes shift.* Therefore, fluorescent substances give off light of a different color than they absorb.

Fluorescent molecules only absorb specific wavelengths of light, and then only let off (emit) specific wavelengths of light. This is what allows scientists to use multiple fluorescent molecules at the same time. For example, most green fluorescent proteins absorb blue light (480 nanometers, nm), and emit green light (509 nm), while a red fluorescent protein may absorb yellow light (580 nm) and emit reddish/orange light (610 nm). To make GFP fluoresce, the scientist must use a blue light and the appropriate filter. But under blue light the scientist will see a diminished response from the RFP. To see RFP fluoresce, the scientist must use a yellow light and appropriate filter, which will get a dimmer response from the GFP.