FLUORESCENCE

Fluorescence is the phenomenon that light with a relative short wave length (high energy) will be absorbed by a molecule inducing an ‘exited’ state. After a short time in which some energy is lost the molecule will fall back to the ground state and send out a short light pulse with a longer wave length (lower energy). If for example a solution with green fluorescent molecules is exited with blue light than green light is emitted. This makes it possible to study fluorescently labeled molecules. The big advantages of fluorescence microscopy are the high contrast due to the dark background and the use of different colors.

GFP

Green fluorescent protein (GFP) can be used to make proteins in cell or organisms visible. Therefore the gene that has the code for that protein will be linked to the gene that has the code for GFP. The fusion gene that is created can be put back into cells (and animals) after which the cell start to produce the protein, but now with a fluorescent part attached to it. The discovery of GFP and the development of colour variants was awarded with a Noble price for Chemistry in 2008 to Shimomura, Chalfie and Tsien.

CONFOCAL MICROSCOPY

In the confocal microscopy the object of study is excited point by point with a focused scanning laserbeam. Fluorescent molecules in the focused spot, but also molecules above and below the focus plane will send out fluorescent light. By placing a so called pinhole before a sensitive detector, only fluorescent light coming from the focal plane will be detected. In this is way a typical confocal image will represent an optical slice of about one µm (0.001 mm) thickness. By scanning on different heights in a sample a 3D image can be reconstructed. Because of the scanning character images can only be made visible by a computer.