Light Done Right!

How to convert your basic light microscope into a fluorescence microscope (Utilization of Tunable Light Source)

Suppose you have a basic light microscope consisting of white light, a series of objective lenses (4X, 10X, 20X, 40X, etc.), and a charge coupled device (CCD). This microscope only gives you bright-field images. However, you would like to conduct fluorescence experiments with mammalian cells or fluorescent molecules.

What you need is Spectrolight's FWS-Poly. If you use our FWS-Poly, you can convert a light microscope into an excellent fluorescence microscope without drastic modification of the existing one. Only a few optical parts are required to become a fluorescence microscope. Once you construct a fluorescence microscope with our FWS-Poly, you can do various experiments since FWS-Poly has wide applications.

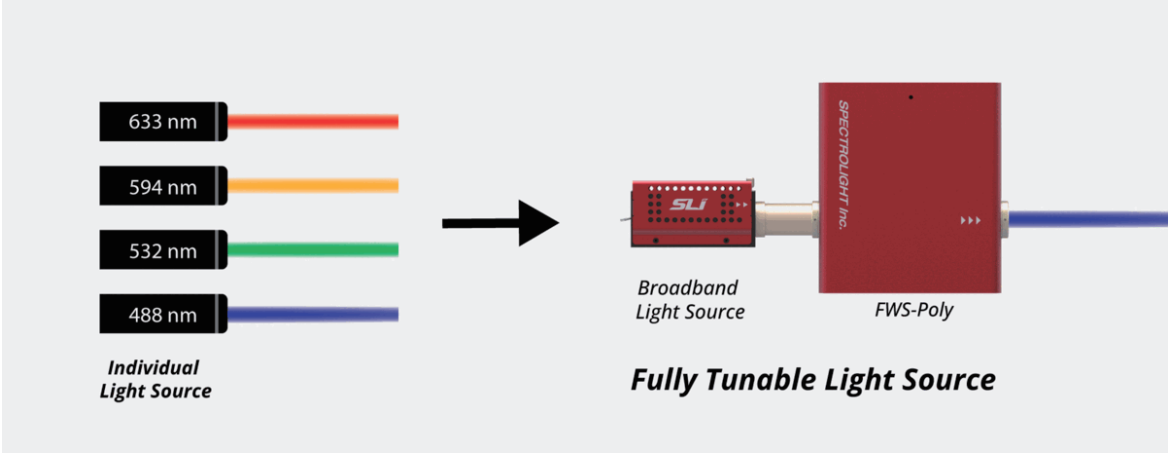
A commercial fluorescence microscope uses a tungsten lamp, mercury lamp, or LED as a light source, and it is connected to the microscope through a light guide or fixed directly to the body. In the case of a confocal microscope, lasers in the visible range are usually included, such as 405 nm, 488 nm, 561 nm, and 640 nm, and as the number of lasers increases, the size of the entire microscope system and the price increases.

In a fluorescence microscope using a lamp, light is selected from a filter cube in a turret. Excitation filters are needed to select the excitation light of the desired wavelength from the light source from all areas of visible light and part of IR. Dichroic beam splitters are required to separate the excitation light reflected or scattered along with the fluorescence from the sample, and emission filters are required to select only the desired fluorescence range and send it to CCD.

To obtain a clearer fluorescence image, an additional filter wheel is installed in front of the CCD. The fluorescent filter system has the advantage of being inexpensive and having a relatively simple configuration. However, since the spectral range is quite wide due to the intrinsic properties of the filter, the number of filters that can be used in the visible range is limited, so the available fluorophores are also limited. In addition, there is a high risk of damage to the filter if a strong excitation light source is used due to the damage threshold problem.

Spectrolight's FWS-Poly is an innovative wavelength selector that can be used for both excitation and emission wavelength selection. With this product, you can convert a very basic light microscope into an excellent fluorescence microscope.

All light sources in general, fluorescence microscopes can be used as light sources. If FWS-Poly (385 ~ 1015 nm) or FWS-Poly-SWIR (1015 ~ 1650 nm) is connected, any wavelength can be selected and used as excitation light. FWS-Poly products have a minimum bandwidth of 3 nm and can be widened to a maximum of 15 nm. This conversion is entirely automatic and can quickly adjust wavelength and bandwidth in real-time through the software provided.



To use entire range of visible light (or sometimes near IR), users have to use a strong light source with a few W outputs. To change wavelength for various fluorophores, the filters and dichroic beam splitters in the filter turret should be replaced every time, or another laser line should be installed. This is cumbersome and cost-ineffective. However, a tunable light source clearly solves this problem. Generally, a tunable light source is a unit that can manually or automatically tune wavelength and bandwidth. Recently, many fully-automated products have been developed, enabling fast, accurate, and precise adjustment. As a result, one can use any kind of fluorophores without replacing filter cubes and spectral overlap.

Spectrolight Inc. provides two types of tunable light sources Tunable Laser System (TLS) and Tunable Mighty Light (TML). The difference between TLS and TML is that TLS uses a pulsed laser, and TML uses a continuous wave (CW) light source. As a result, the output beam from TLS is coherent, whereas that from TML is incoherent. Thus, users can choose light sources according to their applications. If a supercontinuum laser is used, a pulse width of 100 ps can be obtained, which can be used for time-correlated single photon counting (TCSPC), enabling fluorescence lifetime imaging (FLIM).

Since the light passing through the FWS-poly reaches the sample by a light guide, it can be used without the need for an excitation filter. Similarly, the fluorescence from the sample and the reflected excitation light passes through another FWS-Poly, and only the fluorescence in the required range can be selected and sent to the CCD, no emission filter or filter wheel is required. If there is a lens with a short focal length in the path between the microscope body and the CCD, you can install an extender and guide fluorescence to FWS-Poly. The picture below is an example of installing FWS-Poly in our basic microscope's excitation and emission part.

Using FWS-Poly widens the pool of the available fluorophore and gives much flexibility in adjusting the wavelength range and bandwidth. In addition, it is possible to make a higher-performance fluorescence microscope on a much lower budget than when purchasing a commercial fluorescence microscope. If you are hesitating to purchase a fluorescence microscope due to a limited budget, the FWS-Poly system from Spectrolight Inc. can be an excellent choice.