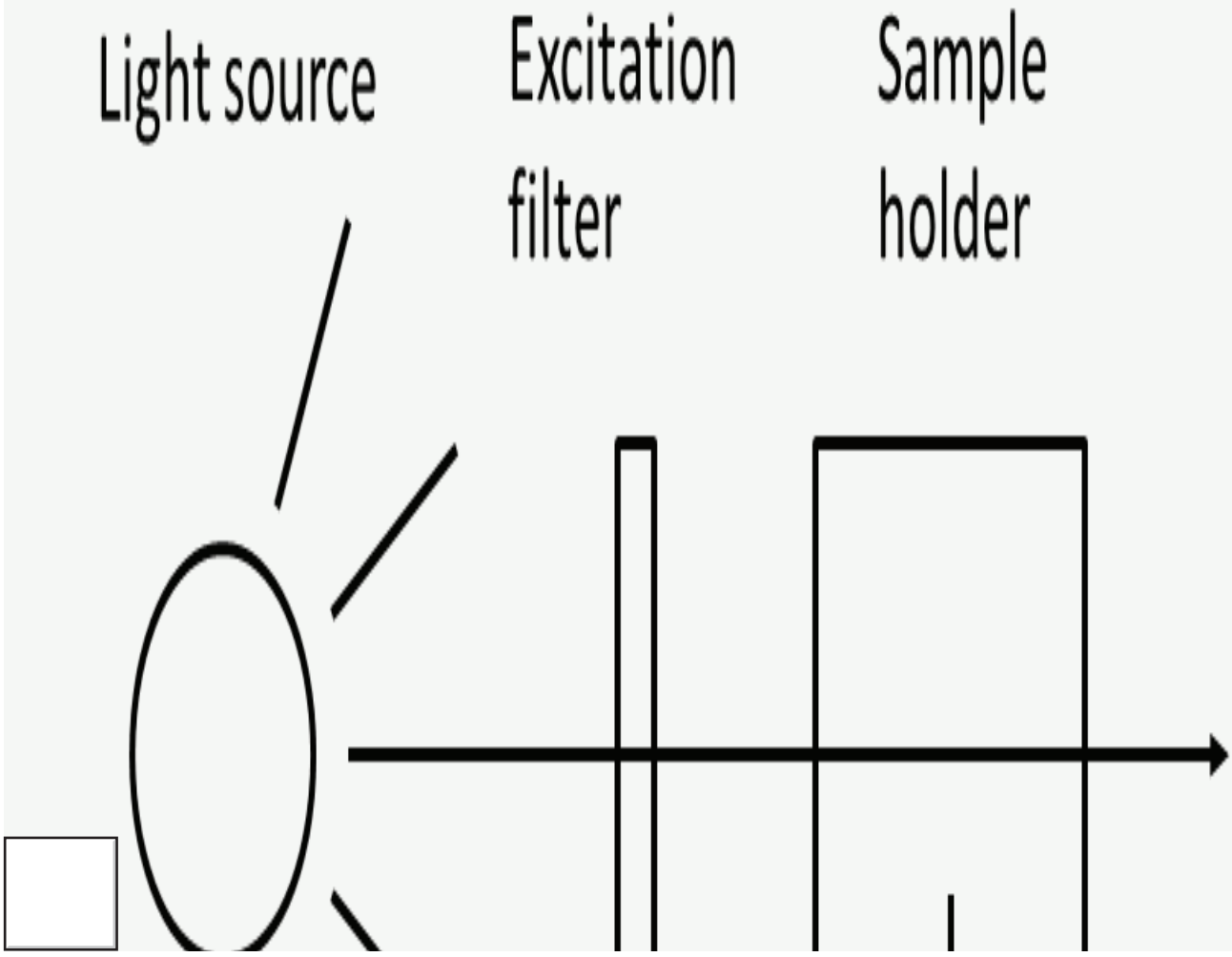
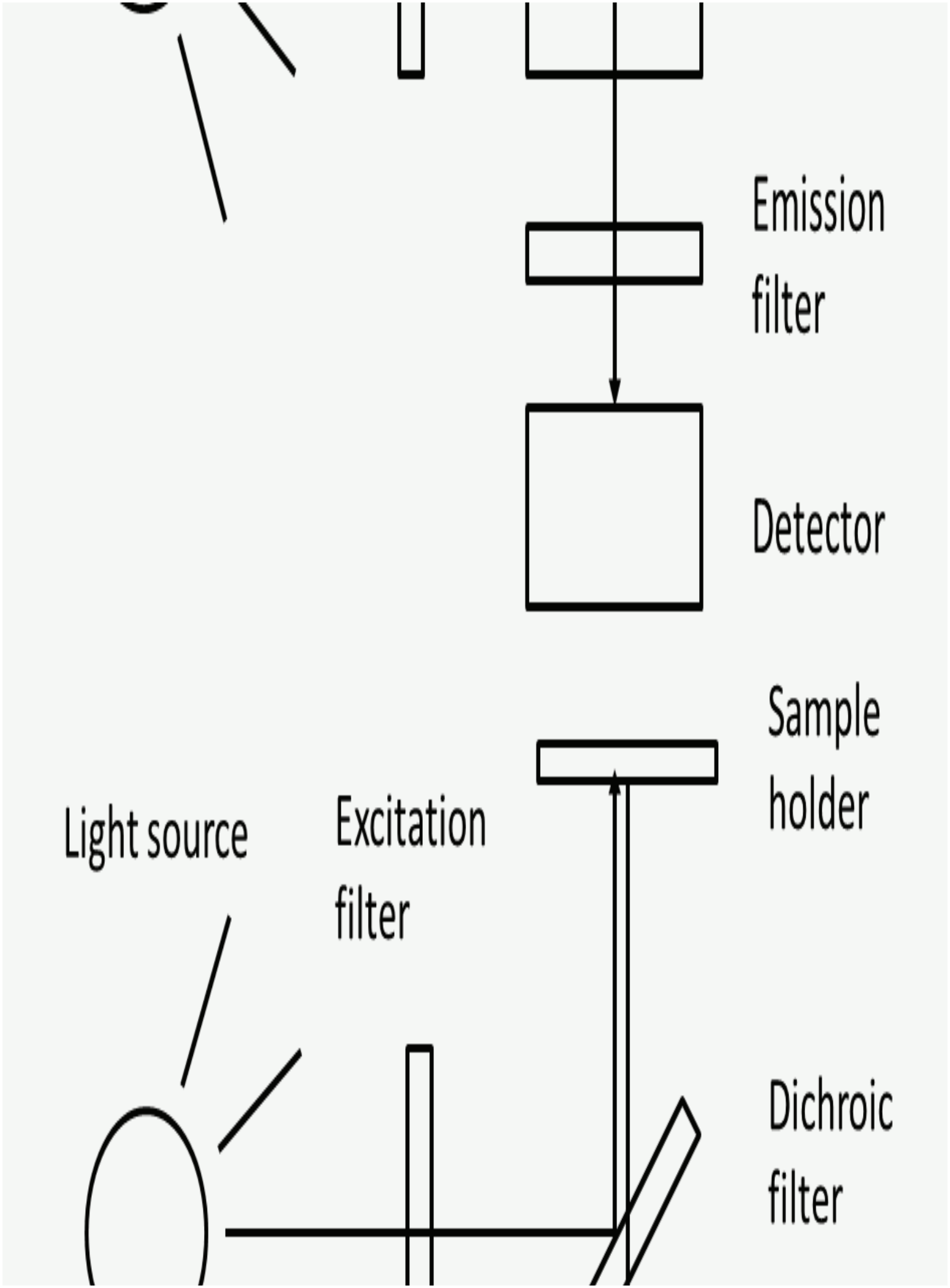
Fluorescence Instrumentation

This guide will provide some high level guidelines for how to choose the right components for your fluorescence spectroscopy instrument.

There are basically two types of fluorescence spectroscopy instrument configurations – transmissive and reflective as shown on the figures below. The only real difference between the two is that in the reflective version a dichroic mirror is used to direct the excitation light to the sample and collect the emission light from the sample whereas in the transmission configuration the sample is excited directly from the light source and the emission light is collected at an angle of 90 degree measured from the excitation light.




Emission
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Detector
Sample
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Light source
Excitation
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Dichroic
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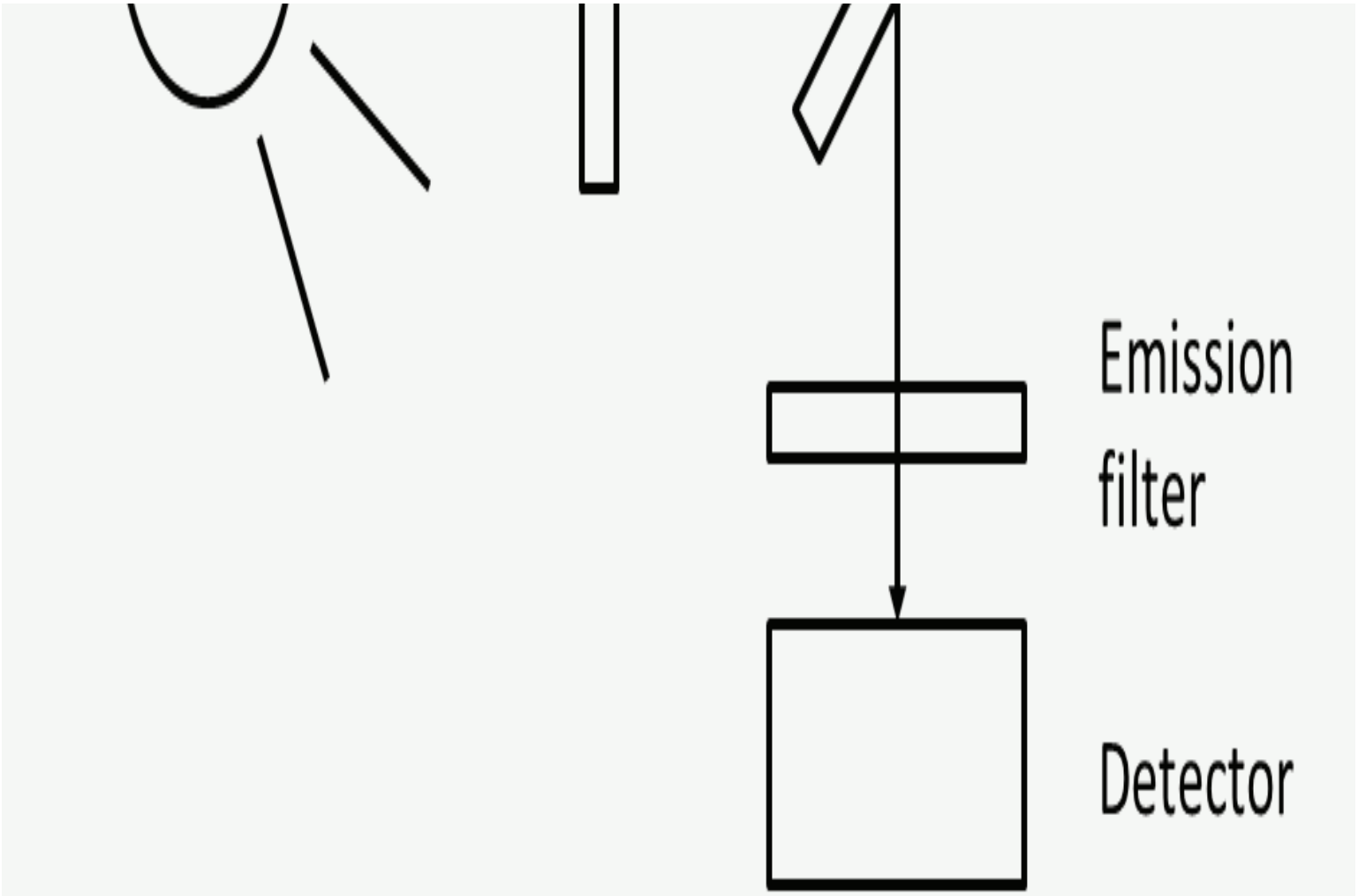
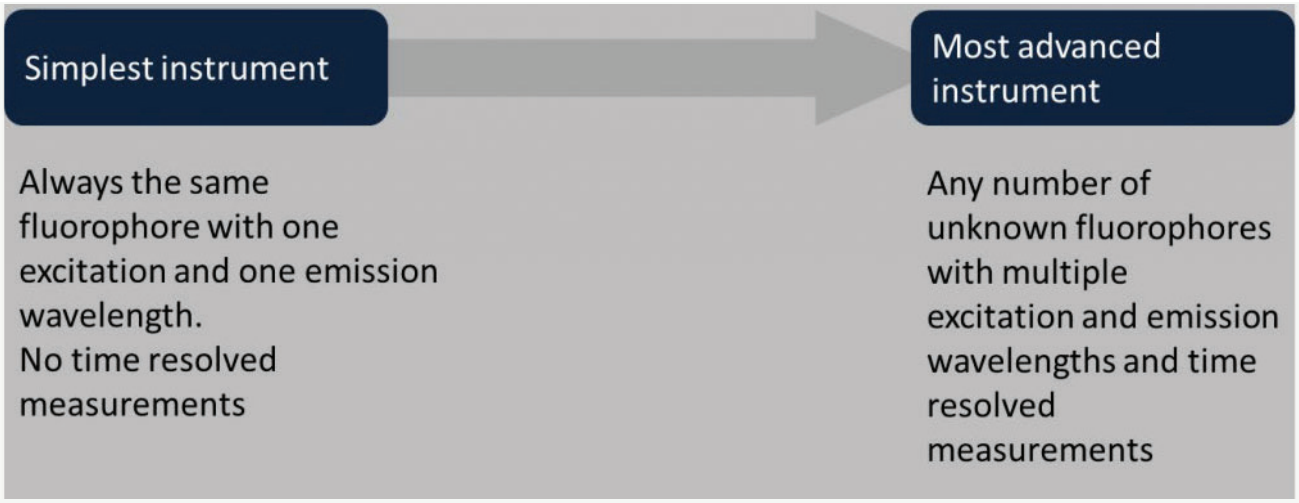


A fluorescence instrument consists of the following basic components:

- Light source
- Excitation filter(s)
- Sample holder
- Dichroic mirror (in reflective configuration)
- Emission filter(s)
- Detector(s)

There are many choices for each type of component and these choices depend first of all on the fluorophores your instrument should be able to analyze.

Below is a graphic showing the two extremes. At one end you have a very simple instrument intended to only analyze one single fluorophore with one excitation and one emission wavelength and no time resolved experiments. In contrast, the most advanced and complicated instrument is at the other end of the scale where your instrument needs to have the flexibility to analyze any (unknown) number of fluorophores with multiple excitation and emission wavelengths and with the option to perform time resolved experiments. However, for most application the instrument need to be somewhere in between these two extremes.



In the following the components inside the fluorescence instrument is described in more details such that you can better judge what will work for your specific application.

Light Sources

Almost any type of light source can be used for fluorescence spectroscopy so the best choice depends on the actual requirements to spectral wavelength coverage, intensity, size, cost, efficiency and whether the light source needs to pulsed.

Type	Typical application	Spectral		Power level	Size	Cost	Pulsed
		Band	Options				
Table top Laser	Time resolved flourescence	Narrow < 1 nm	A limited number of options for laser wavelengths are possible	High peak power	Large (meter sized)	High	Yes, down to fs
Diode laser	Flow Cytometry	Narrow < 1 nm	A limited number of options for laser wavelengths are possible	Medium	Small (mm-sized)	Medium	Yes, down to µs
LED	Compact, Low cost instruments	Medium ~ 10 – 50 nm	Many wavelength options exist in VIS, fewer in UV and NIR	Medium	Small (mm-sized)	Low	Yes, down to µs
Gas Lamp	Accurate excitation wavelength required	Narrow << 1 nm	Each type of gas has many discrete wavelengths to choose from	Low	Medium (cm-sized)	Medium	No
Xe-flash lamp	Selectable excitation wavelength required	Broad ~2000 nm	Any wavelength can be selected	Medium	Medium (cm-sized)	Medium	Yes, down to µs
Halogen lamp	Selectable excitation wavelength required	Broad ~5000 nm	Any wavelength can be selected	Medium	Medium (cm-sized)	Medium	No

Click the image to enlarge

The table above lists the most common types of light sources and the key characteristics of these. In general, LEDs are a great choice if you want to build a low cost, compact fluorescence instrument for analyzing a limited number of known fluorophores. Pulsed table top lasers are often the preferred choice if you need very accurate time resolved measurements with nanosecond timing. And broadband sources are the best choice if you need the flexibility to analyze a large set of unknown fluorophores and thus need to select almost any excitation wavelength.

Excitation filters

The excitation filters fall into two categories:

- Fixed low pass/band pass filters
- Variable band pass filters

Variable optical band pass filters are mostly realized as scanning grating mono-chromators and are used together with broadband light sources to select the right excitation wavelength. The main benefit is of course the flexibility to choose any wavelength but the drawback is a high cost, large size, need for electronics control and stability issues due to the moving parts inside the mono-chromator. Therefore, variable bandpass filters are mostly used in versatile large laboratory instruments. The fixed filter's main benefits are that they are small, simple, relatively low cost and very stable. However, the price you pay is a limited flexibility in the choice of excitation wavelength. Some instruments come with the option to select between a set of filters with different center wavelength. For this reason fixed filters are mostly used in handheld/portable lower cost fluorescence instruments for dedicated applications.

Sample holder

The choice of sample holder really depends on the application. The main thing to consider is that in the case a transmissive cuvette or flow cell you must ensure that the sample holder material is transparent for both your excitation and emission wavelength. This is especially important for UV wavelengths where most glasses absorb light so special types of materials needs to be used.

Dichroic filter

The dichroic filter is used in a 45 degree configuration. The function of the dichroic filter is to reflect the excitation light (shorter wavelengths) and transmit the emission light (longer wavelengths). For really simple systems the dichroic filter can actually function as both the excitation and emission filter.

Emission filters

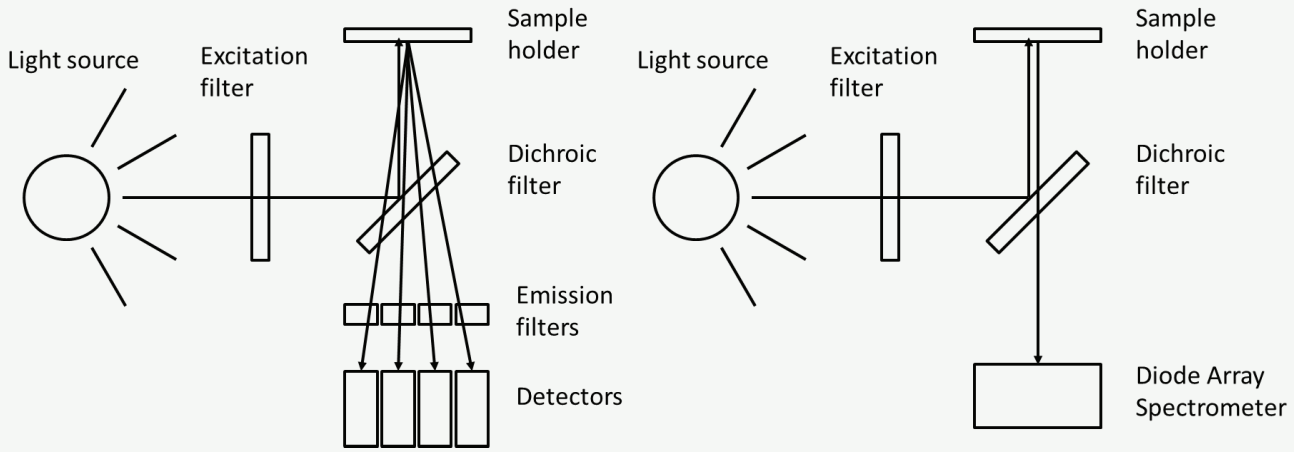
The emission filters falls into three categories:

- Fixed low band pass filters
- Variable band pass filters
- Full spectrum diode array spectrophotometers

Variable optical band pass filters are mostly realized as scanning grating mono-chromators and are used to select the right peak emission wavelength. The main benefit is off course the flexibility to choose any wavelength but the drawback is a high cost, large size, need for electronics control and stability issues due to the moving parts inside the mono-chromator. Therefore, variable bandpass filters are mostly used in versatile large laboratory instruments. The fixed filters main benefit is that they are small, simple, relatively low cost and very stable. However, the price you pay is a limited flexibility in the choice of emission wavelength. You may design your fluorescent instrument with multiple filters but, when you need more than 3 – 4 filter this very quickly becomes bulky and expensive. Full spectrum diode array spectrophotometers will – as the name says – collect the full spectrum. This means you have all emission peaks and details about their shape and the back-ground level. For this reason diode array spectrophotometers are a good choice when you are going to measure multiple fluorophores and/or more complex spectra.

Detector

In fluorescence spectroscopy it is common to use Photo Multiplying Tubes (PMT) as detectors due to the high sensitivity and fast response of these detectors. However, Silicon-based solid-state detectors can also be used.



The number of detectors needed depends on the system configuration as shown on the figure above. If you are using a variable band pass filter (like a mono-chromator) you only need one detector. If you are using fixed bandpass filters, you need one detector per filter. If you are going to analyze multiple emission wavelengths this however, very quickly becomes bulky and expensive and a diode array spectrometer will be the preferred option. The diode array spectrometer will typically include hundreds of detectors. Using a ultra compact diode array spectrometer like our PEBBLE VIS will record the full spectrum of all peaks sampled at several hundred wavelengths.

More diode array spectrophotometers could be our Compact FREEDOM VIS spectrometer being cost-efficient with high performance and our High Throughput ROCK VIS spectrometers for their un-compromised performance.

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Sign up for our monthly newsletter and access the recording of our latest webinar How to Select the Right Spectrometer

Name *

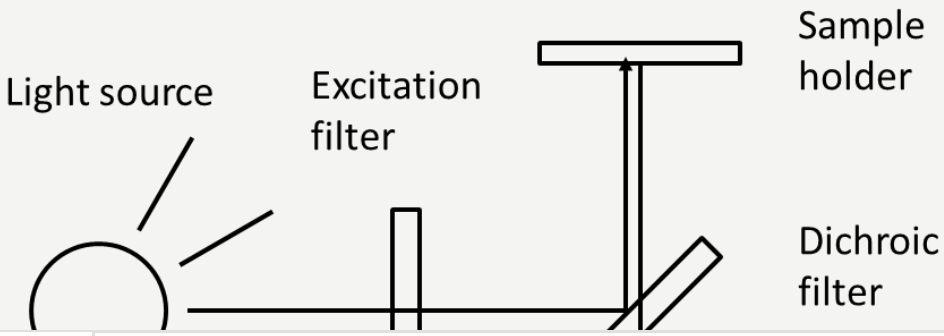
How to Build a Fluorometer

A fluorometer, also known as a spectrofluorometer, is an instrument that is capable of measuring the optical fluorescence from one or more fluorophores. On this page, we illustrate the basics of a fluorometer design.

You can learn more about the basics of fluorescence spectroscopy by clicking here or watch our video tutorial here.

There are many types and names for instruments capable of measuring fluorescence, like fluorometer, fluorimeter, fluorescence spectrophotometer, and micro plate reader. You can get a more detailed description here. A fluorometer is generally used to describe an instrument with a dedicated application in mind such as chorophyll measurements.

The function of a fluorometer is to illuminate the sample with light at a specific wavelength and measure the emitted fluorescent light from the sample at one or more wavelengths. The figure below shows the basic construction of a fluorometer.



- Excitation filter(s)
- Sample holder
- Dichroic mirror (in reflective configuration)
- Emission filter(s)
- Detector(s)

Below, the various components are described in more detail.

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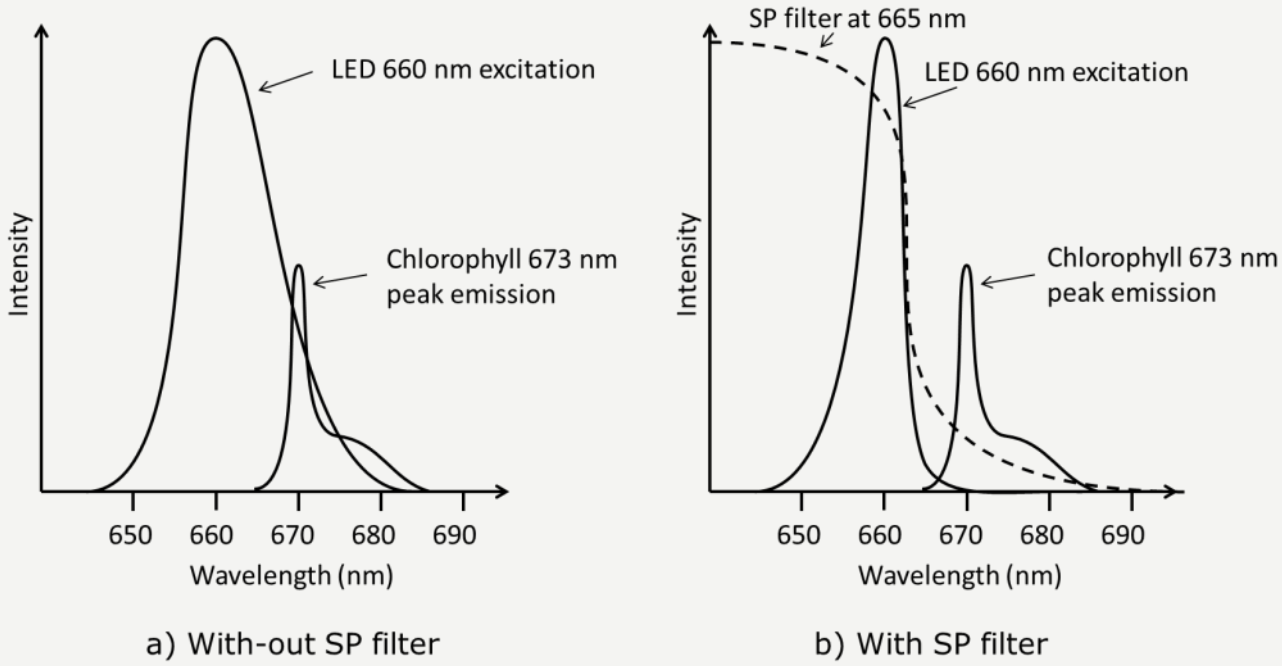
Light Sources

The light sources commonly used for fluorometers are Light Emitting Diodes (LEDs) due to their compact size, relatively low cost and good conversion efficiency. LEDs are available at many different center wavelengths and typically covers a bandwidth around the center wavelength of 10 – 50 nm. The choice LED depends on the excitation wavelength you need for your fluorophore. As an example chlorophyll absorbs light in both the blue (465 nm) and red (665 nm) range. So you may want to use a white LED which covers the full visible spectrum as your excitation source (see an example here. Alternatively you can choose to use only a blue LED (for instance a 465 nm LED like the one here. The LED will in general emit light in a cone around the main direction of light and thus it can often be beneficial to incorporate a collimating lens in front of the LED.

Excitation filters

In order to limit the amount of direct light from the LED that ends up on the detector where it can disturb the much weaker fluorescence signal a short pass optical filter is normally used as excitation filter. A good example is Chlorophyll, which has an absorption peak at 665 nm and an emission peak at 673 nm. If we use a LED at 660 nm it will also emit significant amounts of light at in the 670 – 680 nm range so, it can be difficult to separate this light from the fluorescence signal of Chlorophyll as shown on a) on the figure below. When a short pass filter with the edge at 665 nm is used it becomes much easier to measure the fluorescence as shown on figure b).



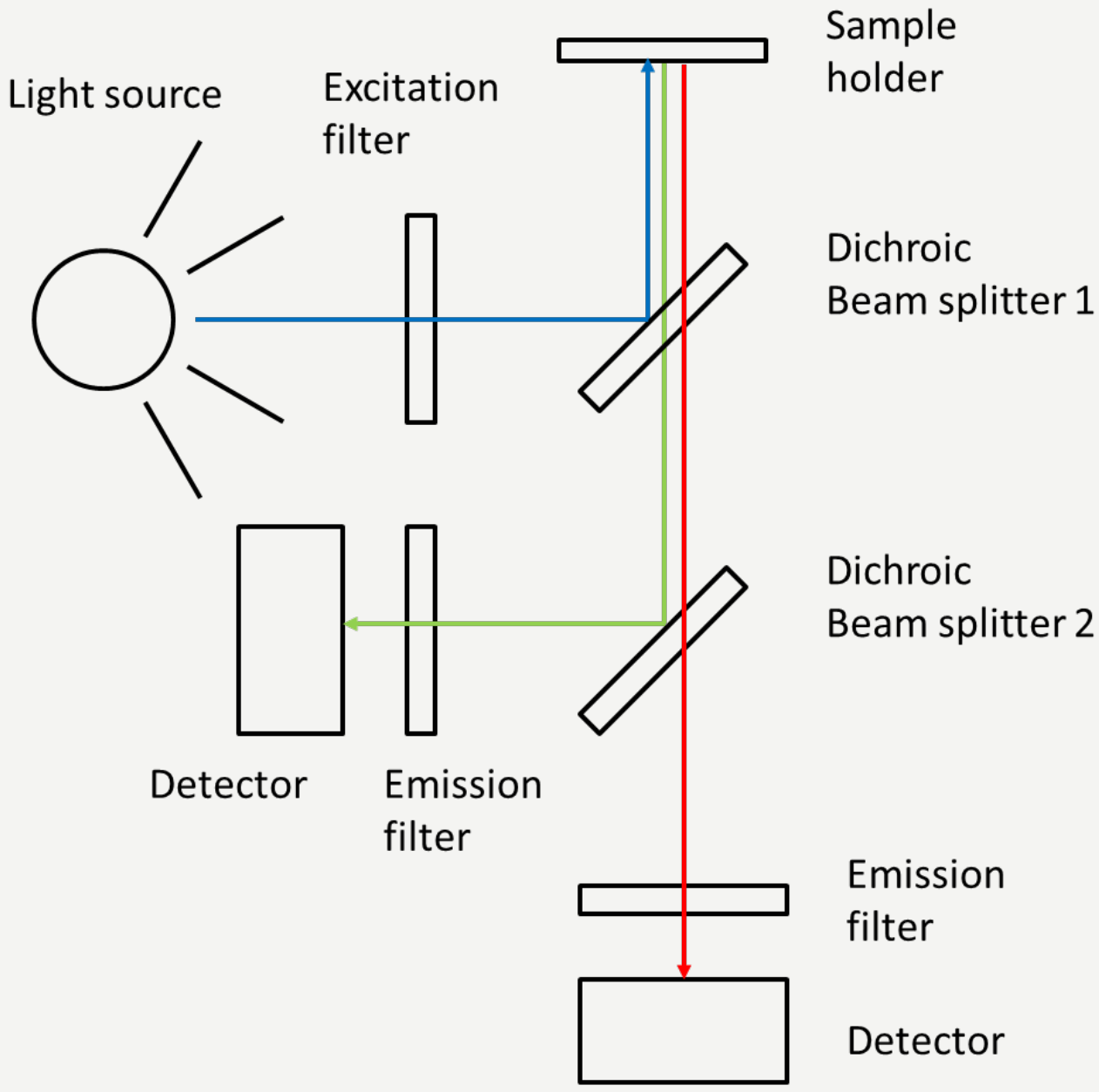
Sample holder

The choice of sample holder really depends on the application. The main thing to consider is that in the case a transmissive cuvette or flow cell you must ensure that the sample holder material is transparent for both your excitation and emission wavelength. This is especially important for UV wavelengths where most glasses absorb light so special types of materials needs to be used.

Dichroic beam splitter

The function of the dichroic beam splitter is to reflect the shorter wavelengths and transmit the longer wavelengths. In this way the excitation light from the LED gets directed to the sample whereas the collected fluorescent emission is directed to the detector. An example of a dichroic beamsplitter for 650 nm can be seen here.

In some cases where multiple emission wavelengths are to be measured simultaneously, more than one dichroic beam splitter might be used as shown in the picture below.



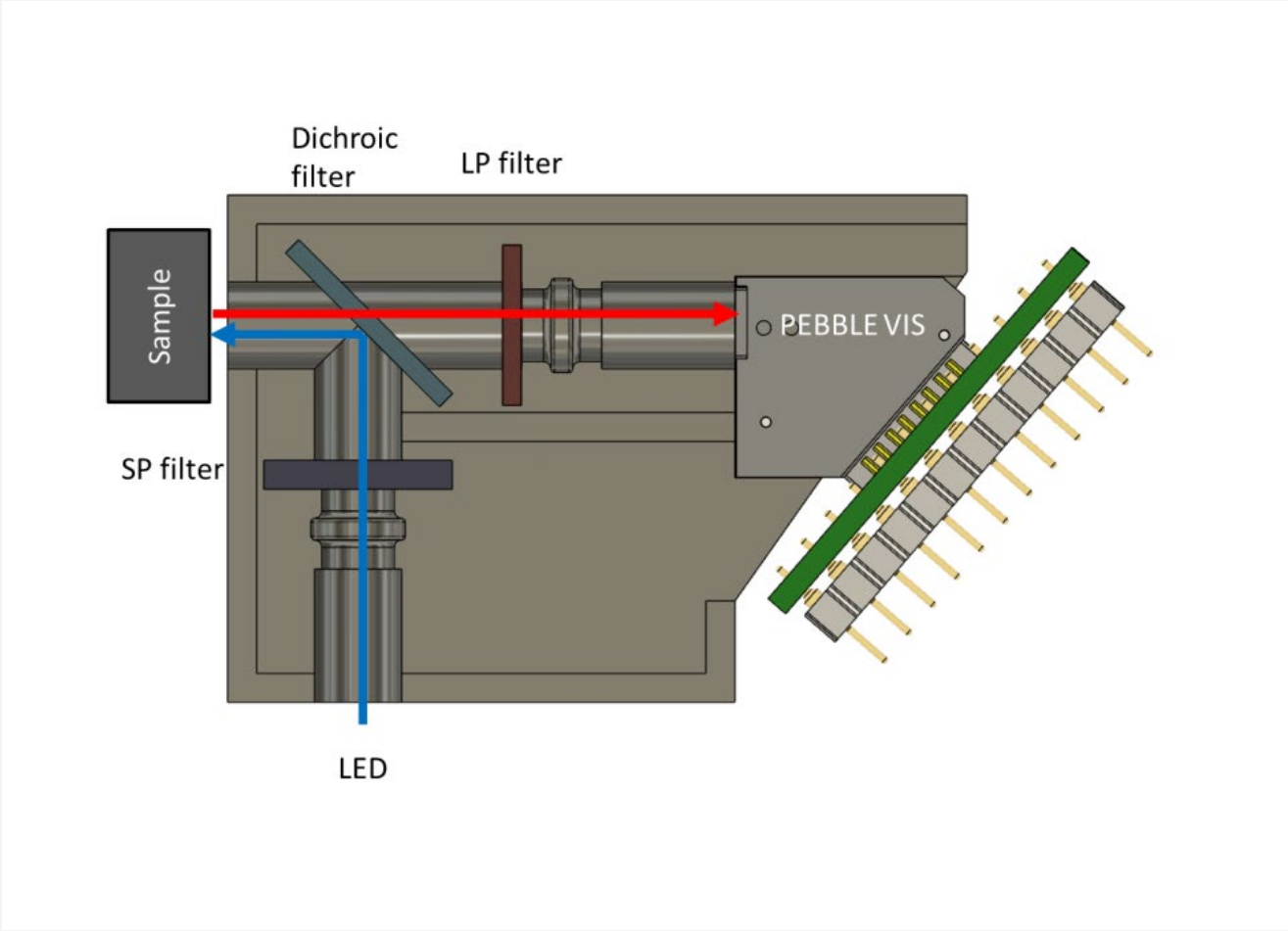
Emission filters

After the dichroic beam splitter it is common to place a long pass filter to further enhance the separation between the strong excitation light

(short wavelengths) and the weak fluorescence (longer wavelengths).

Detector

The final part of the fluorometer is the detector. The detector converts light (photons) into an electronic signal in the form of electronic charge, current or voltage. It is common to use single Photo-Multiplier Tubes or Silicon photo-detectors. However, if you need to analyze multiple fluorophores and peaks it may be more beneficial to use a diode array spectrometer like our PEBBLE VIS. Such a device will record the full spectrum of all peaks sampled at several hundred wavelengths. A design example is shown on the figure below.



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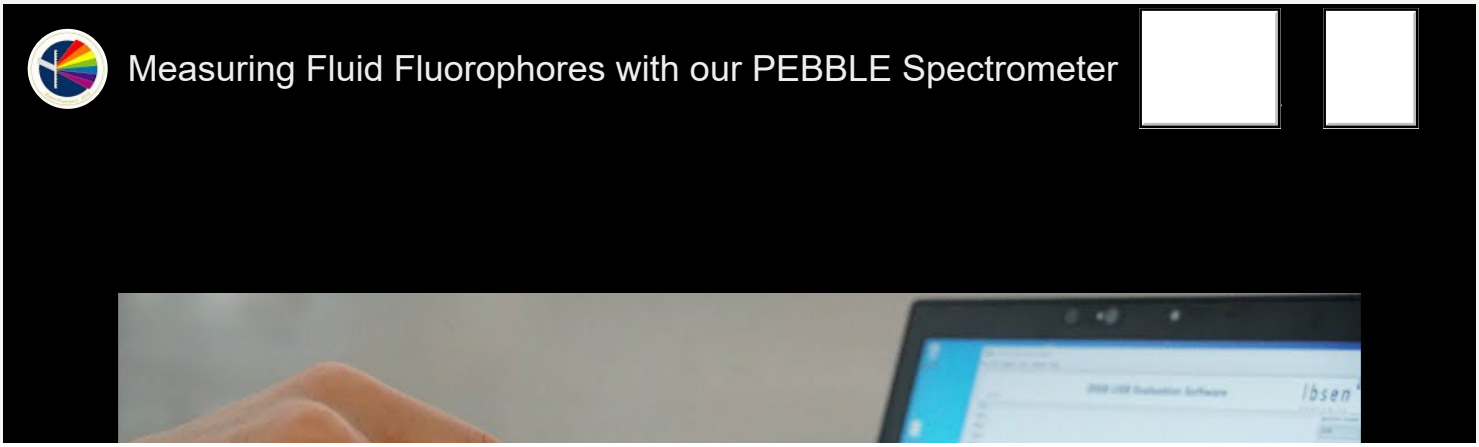
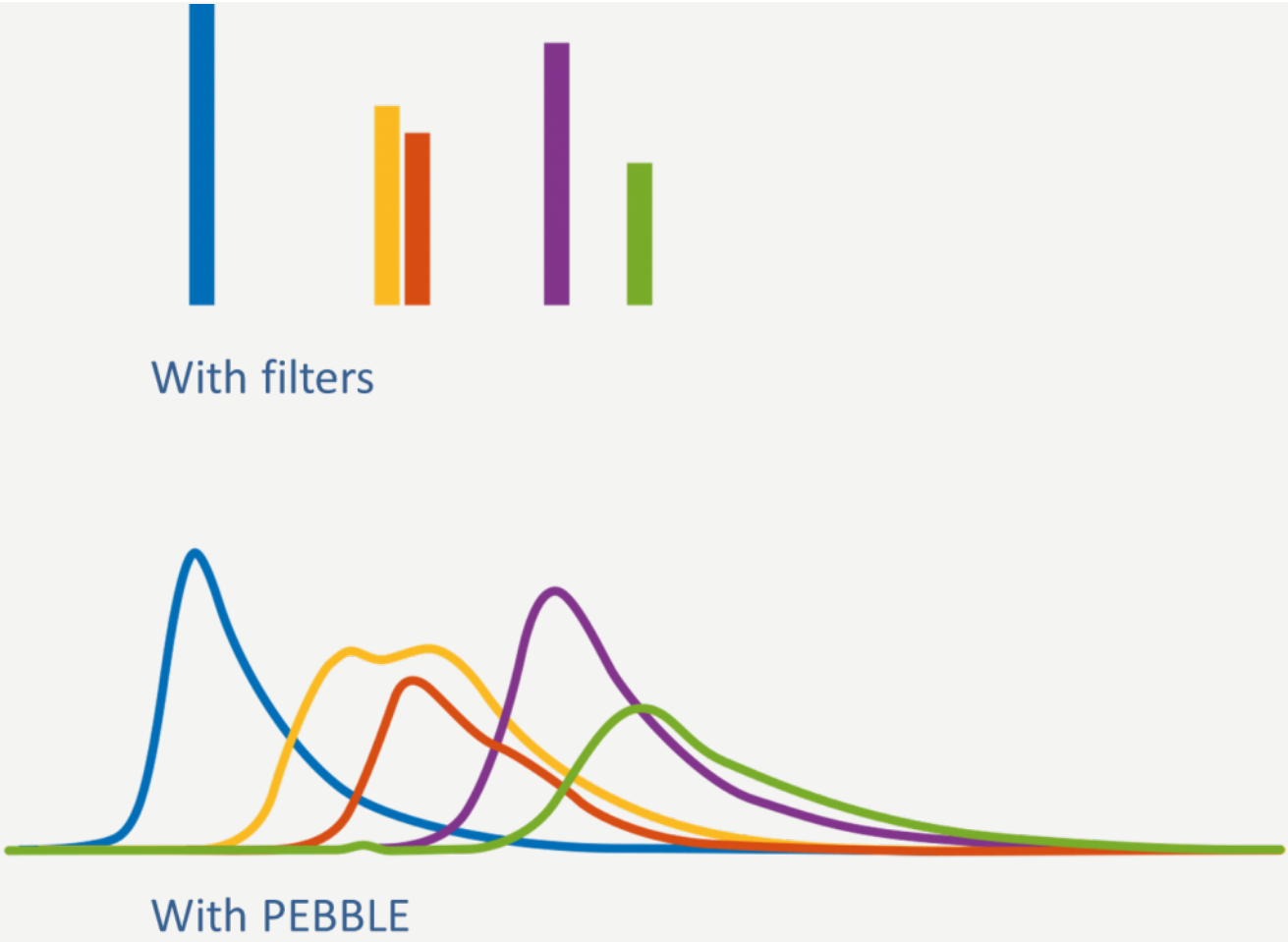
Measuring Fluid Fluorophores with our PEBBLE Spectrometer



Diode array versus filter-based spectrometers

Diode array spectrometers are typically lower cost and less complex if you need 3 or more filters in your photometer.

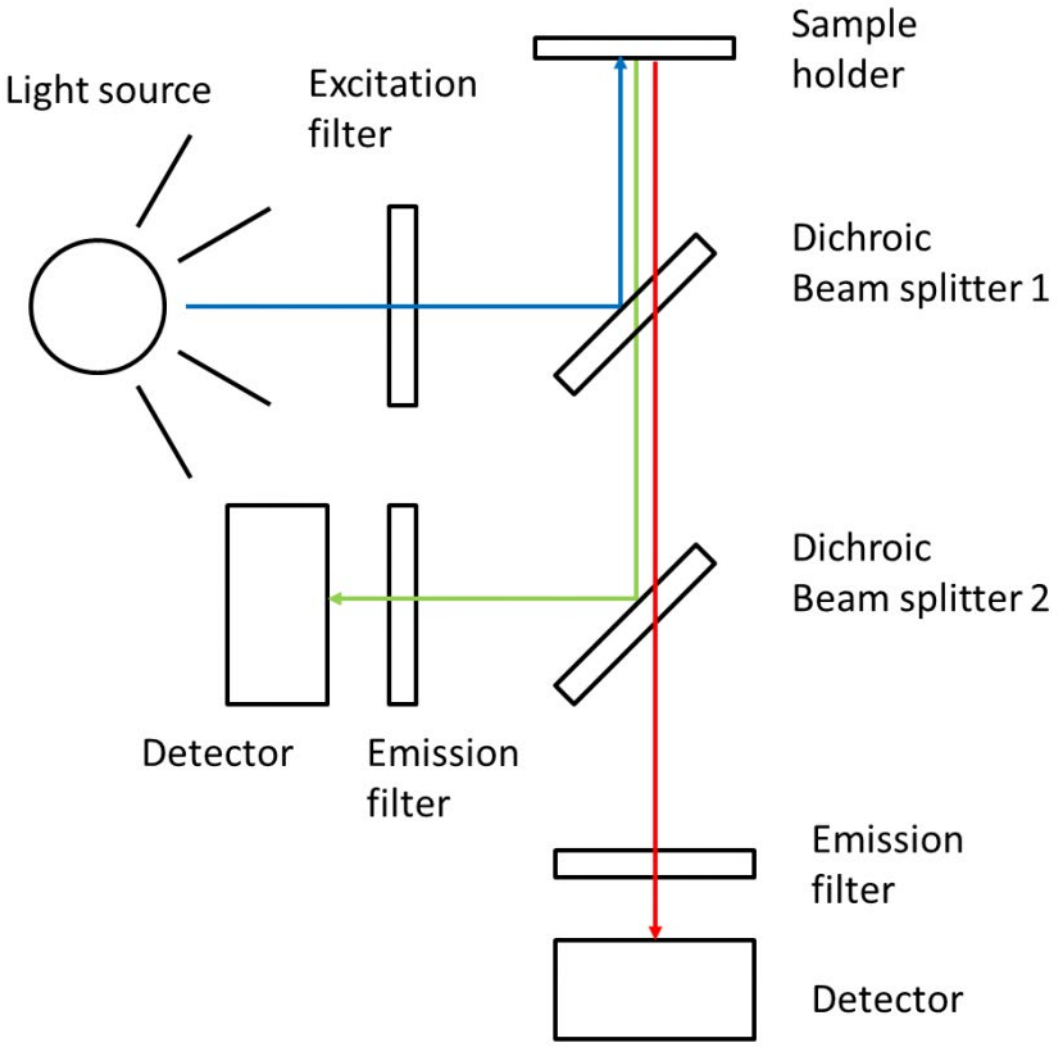
If you are building a spectral sensor for fluorescence spectroscopy, you are likely considering whether you should use either filter-based photometers or diode array spectrometers. The former will measure light intensity at one or a few specific wavelengths and the latter will measure the full spectrum as illustrated in the figure below.



On this page, you can find a general overview of how filter-based and diode array-based spectrometers are constructed. Also, we will list the key benefits and drawbacks of the two technologies such that you are better equipped to make the right choice for your project.

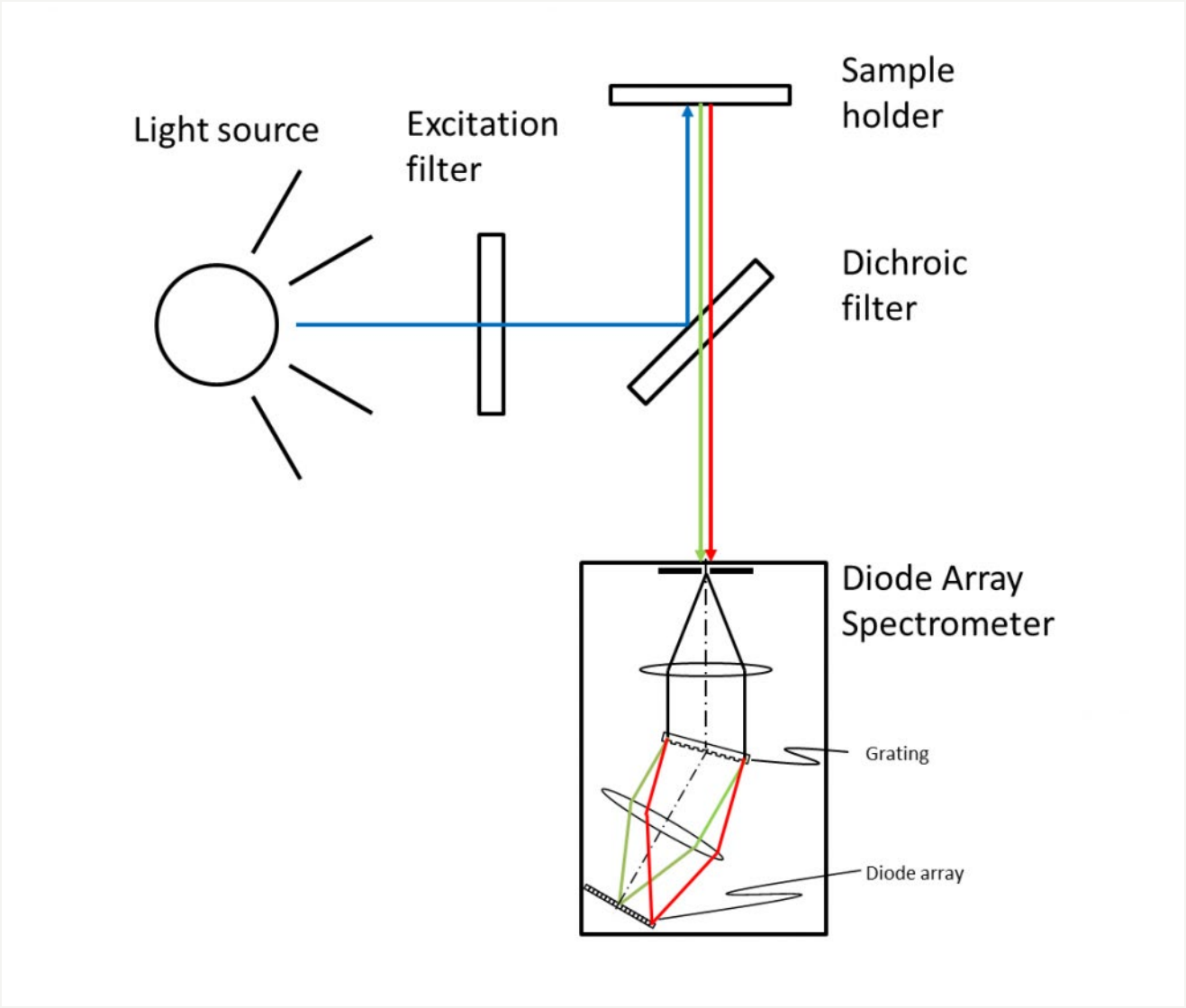
Filter based photometers

In a filter-based photometer, the wavelength selective components are optical filters and behind each filter, you need a photodiode detector. You need one filter-detector pair for each wavelength you want to measure as illustrated on the picture below for a 2 wavelength photometer. As you might imagine, the cost and complexity of such an instrument scale with the number of wavelengths. However, if you are only interested in 1 – 2 wavelengths it is a very simple and efficient method.



Diode array spectrometers

The wavelength selective element in a diode array spectrometer is a diffraction grating. And in this case, the detector is an array of photodetectors as illustrated in the picture below. The diode array detector will measure all wavelengths simultaneously so, the cost and complexity remain the same whether you want to measure one or 1000 wavelengths.

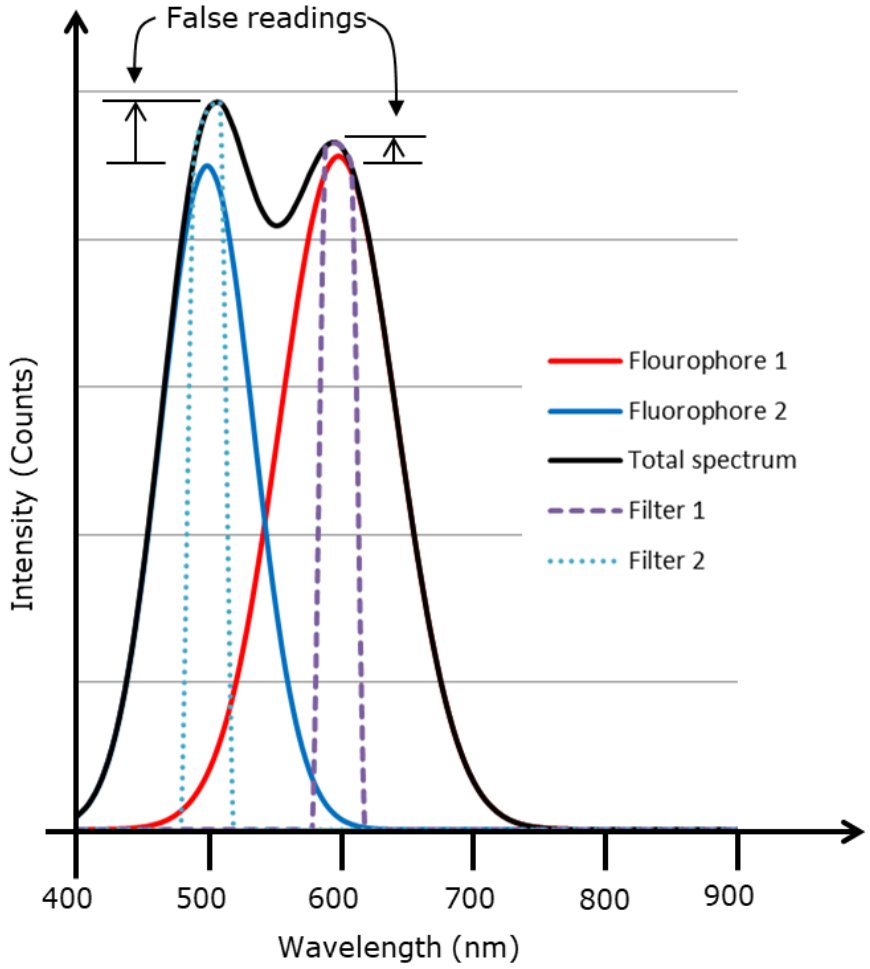


Cost and complexity

The key benefits of the filter-based photometer are its simplicity and low cost when you need to analyze only 1 or 2 wavelengths. However, it is generally agreed that once you move to 3 or more wavelengths a diode array solution like the PEBBLE VIS can be both lower cost and far less complicated.

Spectral deconvolution

In many cases, your spectrum from a sample might consist of several overlapping spectra. For instance, in case you have two fluorophores with overlapping emission spectra, you will get a false higher intensity reading at the two peak wavelengths as illustrated below.



In case you use a diode array spectrometer you will get hundreds of measurement points that you can use to decompose the spectrum into the spectra of the individual fluorophores.

In a similar manner you can use the extra information in the full spectrum to estimate and subtract things like background and Raman scattering in fluorescence spectra.

Especially for Raman and NIR spectroscopy multi-variate analysis methods (often denoted chemometrics) can be used to extra concentration information from complex spectra recorded with diode array spectrometers.

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