

# Spectrofluorimetry

## Lecture 6

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### Spectrofluorimetry

#### Instrumentation

The basic instrument for measuring fluorescence is the spectrofluorometer and consists of following components:

- *Light source*
- *Monochromator*
- *A sample holder*
- *Detector*

The instrumentation is similar to that for spectrophotometer used in absorption spectrum, however, there are two significant exceptions.

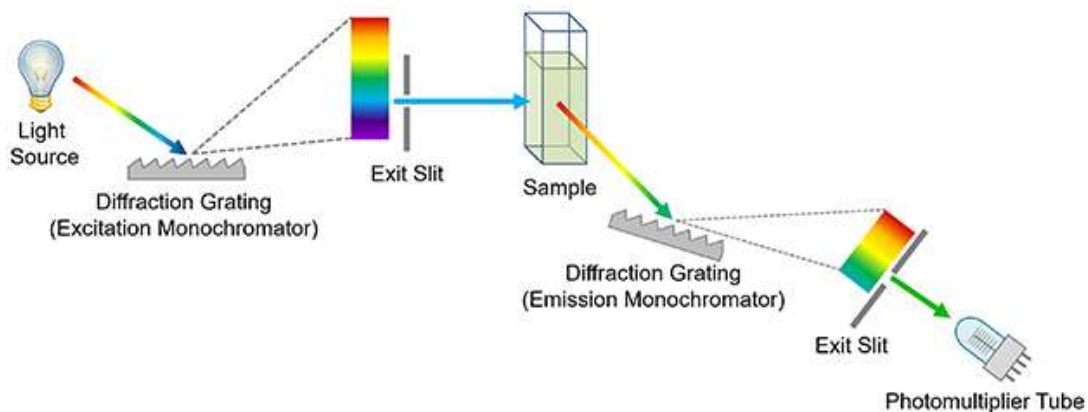
- ✓ *There are two monochromator*
  - ❖ *One for selection of the excitation wavelength and*
  - ❖ *Another for wavelength analysis of the emitted light.*
- ✓ *The detector is at an angle (usually) to the excitation beam. This is to eliminate interference by the light that is transmitted through the sample. Upon excitation of the sample molecules, the fluorescence is emitted in all directions and is detected by a photocell at right angles to the excitation light beam.*
- ✚ The lamp source used in most instruments is a xenon arc lamp that emits radiation in the
  - ❖ Ultraviolet
  - ❖ Visible and
  - ❖ Near-infrared regions (200 to 1400 nm)
- ✚ The light is directed by an optical system to the excitation monochromator, which allows either preselection of a wavelength or scanning of a certain wavelength range.
- ✚ The exciting light then passes into the sample chamber, which contains a fluorescence cuvette with dissolved sample. Because of the geometry of the optical system, a typical fused absorption cuvette with two opaque sides cannot be used; instead, special fluorescence cuvettes with four translucent quartz or glass sides must be used.
- ✚ When the excitation light beam impinges on the sample cell, molecules in the solution are excited and some will emit light. Light emitted at right angles to the incoming beam is analyzed by the emission monochromator.

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- ✚ In most cases, the wavelength analysis of emitted light is carried out by measuring the intensity of fluorescence at a preselected wavelength (usually the wavelength of emission maximum).
- ✚ The analyzer monochromator directs emitted light of only the preselected wavelength toward the detector.
- ✚ A photomultiplier tube serves as a detector to measure the intensity of the light.
- ✚ The output current from the photomultiplier is fed to some measuring device that indicates the extent of fluorescence.
- ✚ The final readout is not in terms of  $Q$ , but in units of the photomultiplier tube current (microamperes) or in relative units of percent of full scale.
- ✚ Therefore, the scale must be standardized with a known.
- ✚ Some newer instruments provide, as output, the ratio of emitted light to incident light intensity.



*Fig. Instrumentation of spectrofluorometer*