

### 3.3: Instrumentation

#### What would constitute the basic instrumental design of a fluorescence spectrophotometer?

In many ways the design of a fluorescence spectrophotometer is similar to an UV/VIS absorption spectrophotometer. We need a source of radiation and a monochromator to select out the desired wavelength of light. The device needs a sample holder and a detector to measure the intensity of the radiation.

Just like UV/VIS absorption spectroscopy, radiation is used to excite the sample. Unlike absorption spectroscopy, a fluorescent sample emits radiation, and the emission goes from the  $S_1$  level to either the  $S_0$  level or higher vibrational states of the  $S_0$  level. Since fluorescence involves an excitation and emission process, and the wavelengths that these two processes occur at will almost always be different, a fluorescence spectrophotometer requires an excitation and emission monochromator. Also, since the emitted radiation leaves the sample in all directions, the detector does not need to be at  $180^\circ$  relative to the source as in an absorption instrument. Usually the detector is set at  $90^\circ$  to the incident beam and mirrors are placed around the sample cell  $180^\circ$  to the source and  $180^\circ$  to the detector to reflect the source beam back through the sample and to reflect emitted radiation toward the detector. A diagram of the components of a fluorescence spectrophotometer is shown in Figure 3.3.1.

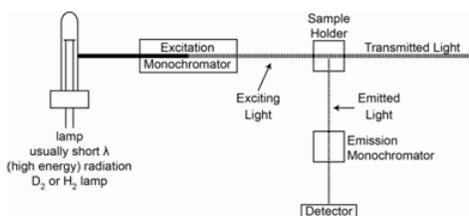


Figure 3.3.1: Diagram of the components of a fluorescence spectrophotometer.

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