Fluorescence on Small or Solid Samples

Sensitivity to small samples

Samples can be valuable. Either you don't want to waste them, or you may not have very much starting material. Biological proteins and enzymes, for example, are often obtained in small volumes and may be expensive. In a materials-science application, you may have access only to a single fine crystal or a minute slice of a polymer.

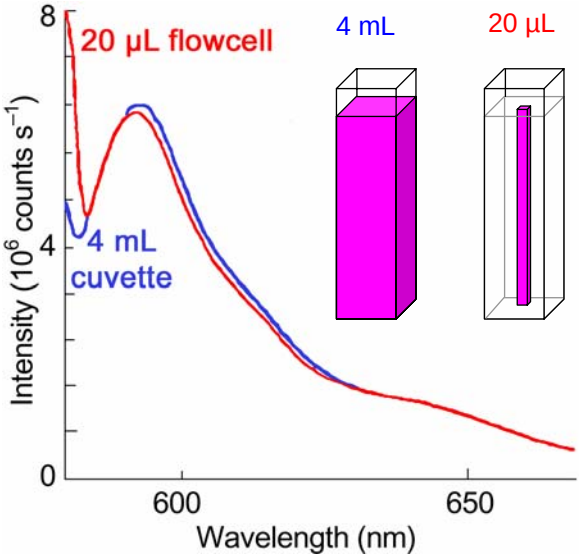

Fig. 1. *Emission spectra of 20 nM resorufin using the flowcell and cuvette. Signal-level is maintained no matter what volume of sample is used. Inset is a comparative sketch of the 4 mL cuvette and 20 µL flowcell.*

That's when the fine imaging quality and photon-counting sensitivity of the FluoroMax® and Fluorolog® spectrofluorometers are essential. In Figure 1, the signal level is maintained with either the 4-mL (# 1920 or 1925) or the 20-µL (# 1955) cell containing indentical samples. A variety of reduced-volume cells (20 µL, 50 µL, 250 µL, and 1 mL) are available for all your fluorescence needs.

Front-face fluorescence detection

For highly concentrated, opaque, or solid samples such as blood, paint, optical brighteners, living cells, polymer films, or phosphors, front-face fluorescence detection offers many advantages for quantitative and qualitative fluorescence measurements. Concentrated and opaque liquids typically suffer from self-absorption and complete attenuation of the beam. When measuring fluorescence at 90°, intensity measurements may be unreproducible or undetectable, and the excitation or emission spectra may appear distorted. Front-face detection solves these problems (see Fig. 2).

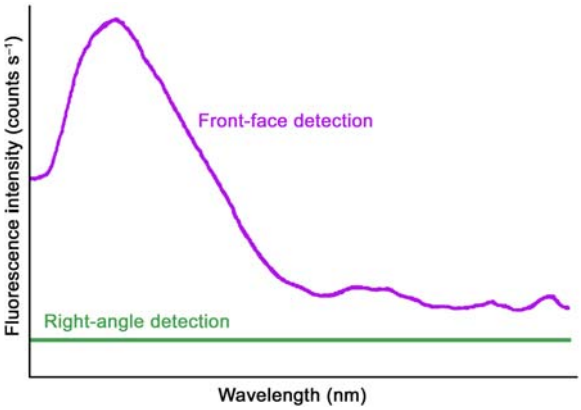

Fig. 2. *Comparison of fluorescence emission from sickle-cell hemoglobin using right-angle detection and front-face detection. The β-37 tryptophan is primarily responsible for this fluorescence.*

Front-face detection for opaque liquids and solids can be an important analytical tool for characterizing fluorescence of various sample types (See Fig. 3). In the front-face technique, the excitation light is focused to the front surface of the samples and then fluorescence emission is collected from the same re-

gion at an angle that minimizes reflected and scattered light.

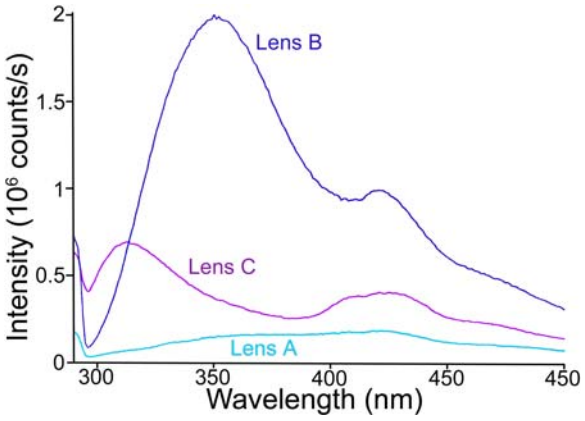

Fig. 3. *Comparison of fluorescence emission signals from three contact-lens samples. These spectra can be used to determine if protein from tears adhered to the lens surface. Contact-lens manufacturers must verify that enzymatic cleaners actually remove these proteins. (A) Tyrosine; (B) tryptophan; (C) enzymatically cleaned lens. This verifies that the solution actually cleaned the lens.*

For our Fluorolog® modular spectrofluorometer, use the 22.5° collection-path for liquid and solid samples. For our bench-top FluoroMax® spectrofluorometer, use the 1933 Solid-Sample Holder (Fig. 4) at a 30° or 60° angle for solids. For liquids, use the 1967 Front-Face Thermostatted Cell Holder with magnetic stirrer accessory.

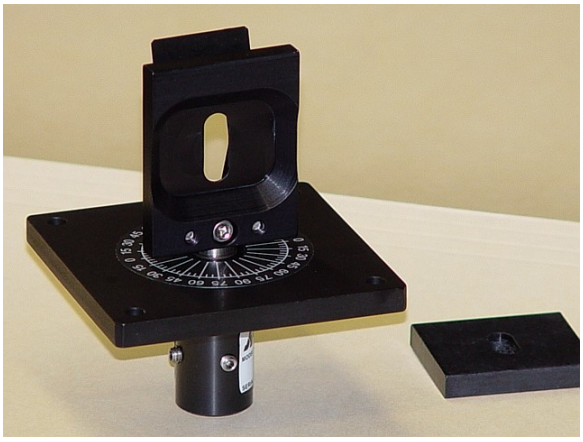

Fig. 4. *1933 Solid-Sample Holder accessory with sample block.*