PTI Technical Note

Improving Signal-to-Noise Ratios in pH Measurements with BCECF

The fluorescence excitation spectrum of 0.1 µM BCECF in pH 6.8, 10 mM EGTA-MOPS buffer is shown in Fig. 1.

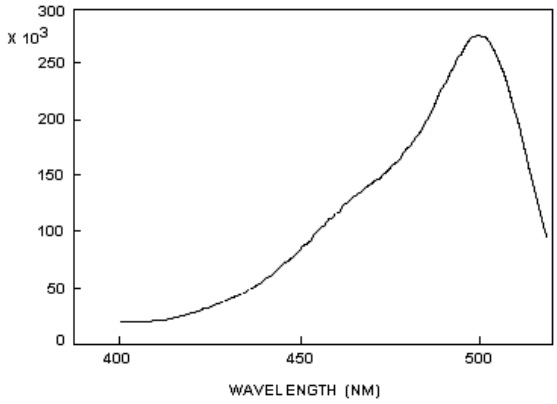

Figure 1

The fluorescence intensity at short wavelengths is much lower than at the peak. Thus, fluorescence ratios (e.g. 500/415) will have considerable noise associated with them that can be significantly reduced by employing the following simple trick:

In the DeltaScan™ dual excitation light source, increase the slit widths of the monochromator delivering 415 nm light such that the resultant fluorescence intensity increases two-, three-, or even four-fold. (Of course, the same can be done if you use 500/440 nm ratios.)

As evidenced by Figure 2, time-based measurements of 500/415 nm ratios of 0.1 µM BCECF in EGTA-MOPS buffers (pH=6.8, 7.0, 7.2, 7.4) will subsequently yield significantly improved S/N ratios.

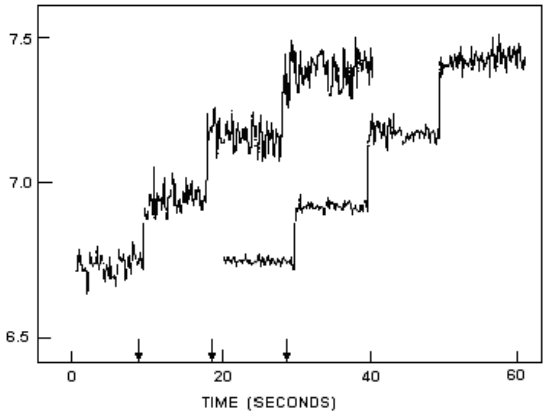

Figure 2

The two ratio traces representing a four-step titration (pH=6.8, 7.0, 7.2, 7.4) were mapped to pH and are offset in time for easy comparison. In both cases, all parameters of the data acquisition setup were identical (20 points/second) as were the four samples. The only difference was in the increased slit width of the monochromator providing 415 nm excitation during acquisition of the second set of data. The improvement in S/N was greater than two-fold, allowing better resolution of small changes in pH.

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