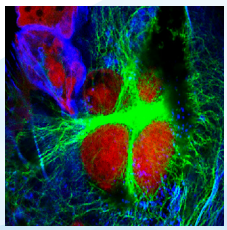
HORIBA

Fluorescence

Fluorescence Indicators for Ion Detection



Technical Note
FL TN 08-19-24

It has long been recognized that ions such as calcium, magnesium, and sodium play an important physiological role in regulating a wide variety of cellular functions. For a better understanding of that role, it is necessary to measure the ion concentrations inside living cells.

Fluorescence is the best technique for non-invasive measurements in cells. Because fluorescence is an absolute measurement, it possesses an inherent sensitivity that is among the highest of all analytical instruments. The high sensitivity of fluorescence spectroscopy for biochemically interesting molecules has led to an increasing popularity in biomedical and life science research.

Fluorescent Probes

Calcium, magnesium, sodium, and similar species, do not naturally fluoresce. They can be measured indirectly, however, by complexing them with fluorescent molecules. Molecules that bestow fluorescence properties on non-fluorescent species are called probes, indicators, or dyes.

Fluorescent probes can be designed to exhibit a high affinity for a specific ion. The typical fluorescent dye has a single excitation and emission wavelength.

Unfortunately, there are practical factors that limit the usefulness of single wavelength dyes for intracellular applications:

- The volume of the cell affects the intensity of the fluorescence emission in an unpredictable way.
- It is impossible to control the concentration of dye within the cell.

Fluorescent Probes

The problems of single wavelength dyes were overcome in 1985 with the introduction of the dual wavelength probes Fura-2 and Indo-1 for calcium. These probes gave birth to the quantitative ratio fluorescence technique. The key characteristic of these indicators is a wavelength shift that occurs in the fluorescence spectrum upon binding with an ion such as calcium[1]. The shift may occur in either the excitation or the emission spectrum.

An example of an excitation-shifted, or dual excitation, indicator is Fura-2. In the absence of calcium, the excitation maximum is found at 372 nm. When bound to calcium, the maximum shifts to 340 nm. In both cases, the fluorescence emission intensity is measured at 510 nm.

Indo-1 is an emission-shifted, or dual emission, indicator. The emission spectrum of the free dye has a maximum at 472 nm. When bound to calcium, the maximum shifts to 400 nm. The excitation wavelength for these spectra was 353 nm.

Ion Quantitation

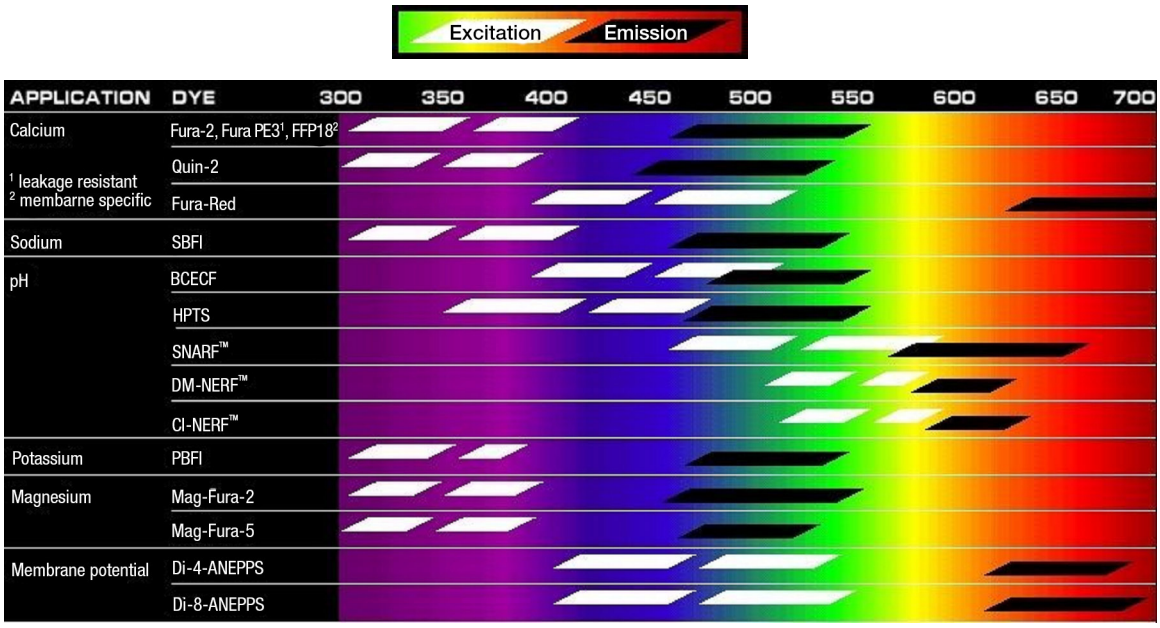
Calcium and other ions can be quantitatively measured by ratioing the fluorescence intensity of the calcium-bound dye and that of the calcium-free dye. Measuring the ratio of intensities has profound implications. The ratio measurement is independent of dye concentration and optical path length, parameters that cannot be controlled within a cell. Degradation of the dye due to prolonged exposure to the excitation source, and variations in the excitation intensity are also compensated for by the ratio technique.

The ratio measurement made possible by the dual wavelength indicators allows specific ions to be quantitated inside living cells with a high degree of confidence. In the last few years, a myriad of indicators has been developed for a broad range of ions, and more appear every day.

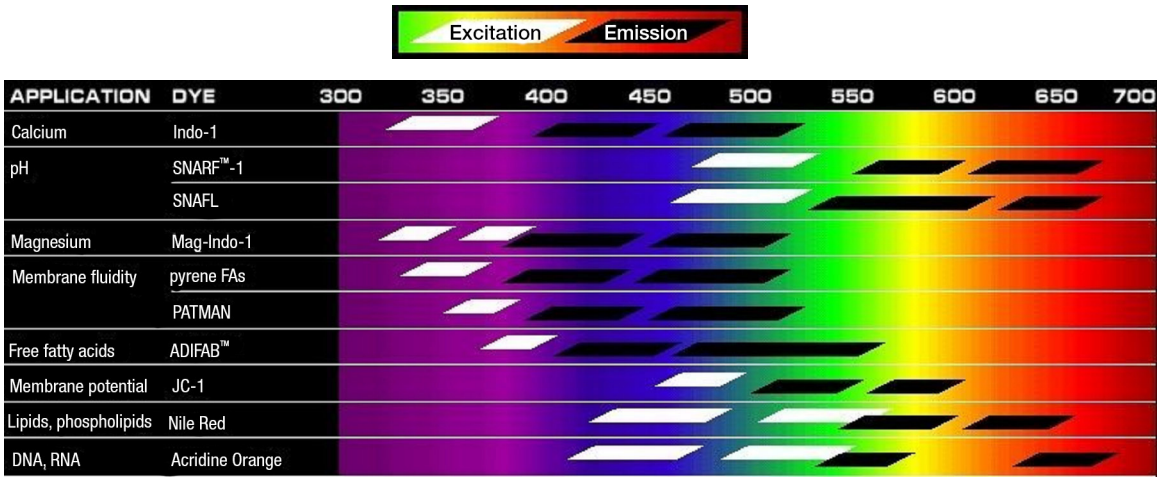
Frequently Used Fluorescent Dyes

These sub-pages present the excitation and emission spectra of frequently used fluorescent dyes. Note that the pages contain very large color images.

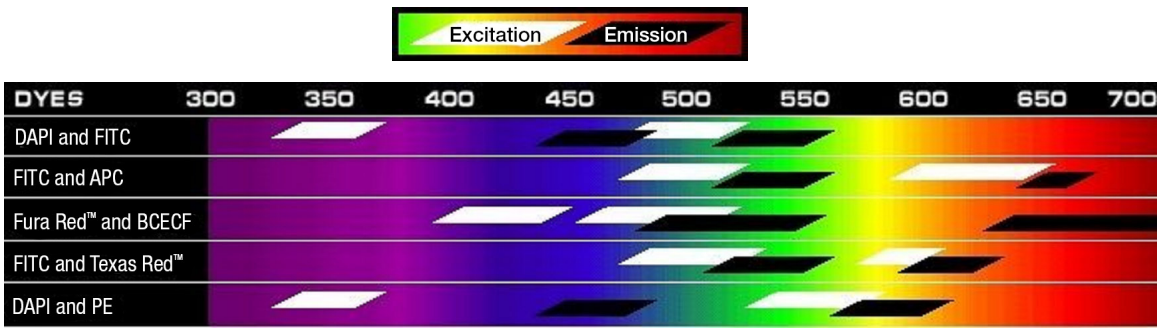
Excitation-Shifted Fluorescent Dyes



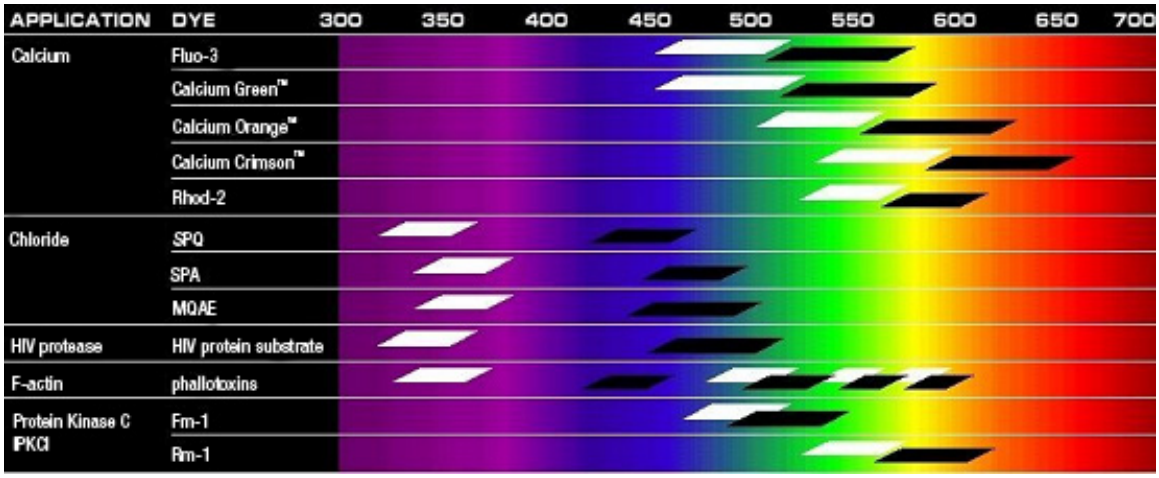
Emission-Shifted Fluorescent Dyes



Dual Fluorescent Dyes



Non-Ratiometric Fluorescent Dyes



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