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Application Note

Detection of Calcium Concentration Changes Using the FLx800™ Fluorescence Microplate Reader

The divalent cation, calcium, serves as a mediator for a large number of intracellular biological responses. The advent of specific fluorogenic compounds that specifically bind to calcium has allowed investigators to study changes in Ca2+ under a variety of conditions. The purpose of this application note is to demonstrate the utility of the FLx800 fluorescence microplate reader to detect rapid changes in calcium ion concentration.

Introduction

Cell Biologists often need to detect and quantitate intracellular spectroscopically silent ions such as Cl-, Na+, K+, or Ca2+. Detection of these ions is most often accomplished by using sensing probes or dyes. These dyes typically consist of a fluorophore and a region that confers analyte specificity, such as azacrown ether for Na+ or K+ or a BAPTA group for Ca2+ [1].

Calcium

Intracellular Ca2+ levels modulate a number of different cellular processes. While extracellular concentrations of calcium are generally quite high, 3-4 mM, those inside the cell are tightly controlled and generally kept at or below 100 nM. Calcium can enter the general cytoplasm of cells from either the extracellular fluid or by release from intracellular stores depending on the use [2]. The calcium required for neurotransmitter release from nerve terminals comes from extracellular sources, while muscle contractions use calcium from intracellular stores. In regards to the triggering event to mobilize calcium movement there are a variety of mechanisms for calcium entry.

Ligand-gated and G-protein coupled channels are the most common means of calcium entry. These channels are a heterogeneous group of proteins, which have been distinguished into different classes based on their pharmacological and physiological properties. Other mechanisms for entry of calcium include direct receptor-operated channels and stretch receptors, which directly allow for calcium movement across the plasma membrane. Intracellular release of calcium is generally induced by the generation of Inositol triphosphate (InsP3) subsequent to interaction of the cell with hormones or neurotransmitters. Once inside the cell, calcium activates a number of protein kinases, proteases, phospholipases and nucleases, which then act to regulate activities such as gene expression, mitogenesis, metabolism, and motility. After the calcium mediated effect is no longer needed, cells then use a variety of mechanisms to restore calcium levels to the original or resting state. For small levels of calcium buffering, there are several different calcium binding proteins which respond rapidly to changes in calcium. Intracellular organelles, such as the sarcoplasmic reticulum, remove or add calcium to the cytosol via active calcium pumps. This mechanism, while slower than binding proteins, has a much greater over all capacity. Ultimately, calcium that enters the cell from extracellular sources must be removed from the cell. This is accomplished via active transport, where the cell utilizes energy to move calcium across the plasma membrane against the concentration gradient.

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Calcium Measurement

Calcium ion probes are probably the most widely used intracellular indicators. These indicators have evolved largely through the efforts of Roger Tsien and colleagues [3]. As stated previously, calcium ion detection is most often accomplished by using a dye that has a recognition portion as well as a region that confers fluorescence. The most commonly used structure for calcium specific binding is 1,2-bis(2-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid, (BAPTA) (Figure 1). The multiple anionic carboxyl groups bind the divalent calcium ion in a similar fashion as the calcium specific chelator, ethylene glycol bis(β-aminoethylether) (EGTA). Note that the remainder of the chemical structure confers the different spectral properties of the calcium specific dyes. In some cases the fluorescent moiety is incorporated into the BAPTA structure, such as Fura-2, while in other instances the fluorophore is linked to the BAPTA structure, e.g. Calcium Green-1 (Figure 1).

Table 1. Suggested Filters for Commonly Used Calcium ion Indicators

Compound	Excitation Filter(s)		Emission Filter(s)	
Dual wavelength Ratiometric indicators				
Fura-2 Excitation: 338/366 nm Emission: 505 nm	340/11 (5002043) or 340/30 (7082230)	380/20 (7082228)	508/20 (7082218)	
Indo-1 Excitation: 355 nm Emission: 405/485 nm	340/30 (7082230)		410/10 (7082242)	485/40 (7082209) or 485/20 (7082221)
Single wavelength indicators				
Calcium Green-1 Excitation: 506 nm Emission: 531 nm	500/27 (5002052) or 485/20 (7082221)		540/25 (7082249) or 528/20 (7082247)	
Calcium Orange Excitation: 549 nm Emission: 575 nm	540/25 (7082249) or 530/25 (7082223)		575/15 (7082245) or 590/35 (7082224)	
Fluo-4 Excitation: 494 nm Emission: 516 nm	485/20 (7082221)		528/20 (7082212) or 530/25 (7082223)	
Fluo-3 Excitation: 503 nm Emission: 526 nm	485/20 (7082221)		528/20 (7082247) or 530/25 (7082223)	

Calcium indicator dyes can be categorized into two groups; the first are the dyes that increase their fluorescence in the presence of calcium, while the second group are dyes that have different excitation and/or emission wavelengths in the presence of calcium than they have in its

absence. The calcium indicator dyes, calcium green-1, calcium green-2, and Fluo-4 are representative of the dyes that increase their fluorescence in the presence of calcium ion (Ca2+) without changing wavelengths. Fura-2 and Indo-1 are ratiometric Ca2+ indicators that are generally considered interchangeable in most experiments. Fura-2, upon binding Ca2+, exhibits a shift in its absorption or excitation peak from 338 nm to 366 nm [4], making Fura-2 the dye of choice for microscopy, where it is easier to change excitation wavelengths than emission. Indo-1 on the other hand has a shift in the emission from 485 nm to 405 nm in the presence of calcium (Table 1). Thus, Indo-1 has a greater utility with flow cytometry where it is easier to use a single argon-ion laser for excitation and to monitor two different emissions. Note that either dye can be measured using filter based microplate readers with equal efficacy.

There are advantages and disadvantages to each of the two groups of calcium indicators. The ratiometric dyes, Indo-1 and Fura-2, are excited by UV-light, which can result in high background fluorescence from the microplate or cause cellular photo-damage due to its higher energy. Ratiometric determinations also require rapid filter switching by the microplate reader, particularly when intracellular calcium levels are changing rapidly. Optimally both wavelengths are being measured simultaneously, but this is not always possible. Photo-bleaching of the compound itself may present a problem if only one filter set is used, but is corrected for if ratiometric determinations are being made. Additionally, ratiometric analysis can also correct for dye leakage. Direct measurement dyes have been developed for excitation in the visible wavelength range resulting in less phototoxicity. In general, direct measurement indicators have a greater fluorescence response to calcium than the ratiometric dyes. This may allow for the use of lower intracellular concentrations of indicator dye reducing any cytotoxic effects of the dye.

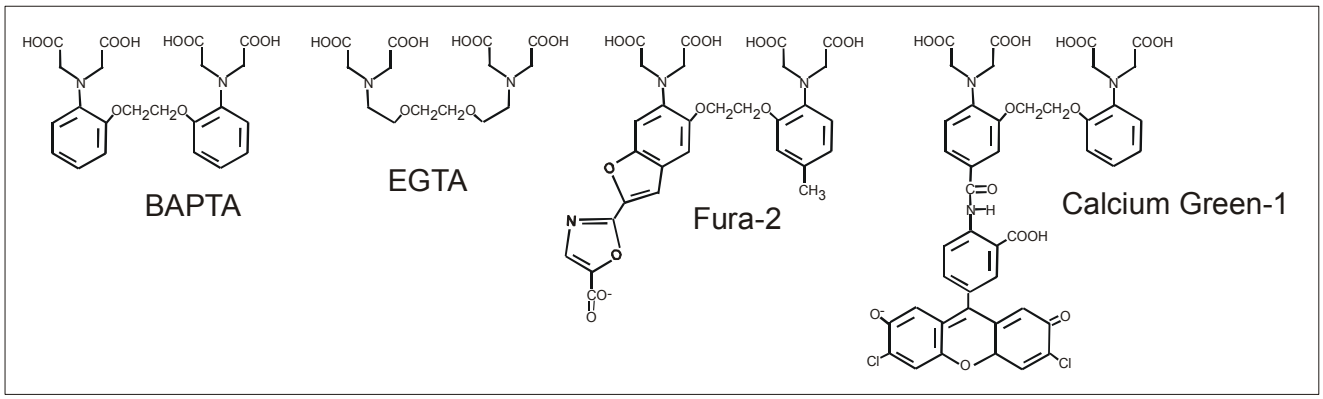

Figure 1. Chemical structure of BAPTA, EGTA, and Fura-2. Calcium ion sensitive dyes, such as Fura-2 and Calcium Green-1, are generally derived from the chelator, BAPTA (1,2-bis(2-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid). The nonfluorescent chelator of calcium, EGTA, (ethylene glycol bis(β-aminoethylether)- N,N,N',N'-tetraacetic acid) is also shown.

Cell Loading

Loading techniques can be divided into two basic groups: the treatment of individual cells with microinjection or bulk loading where large numbers of cells are treated simultaneously. There are several different bulk loading procedures applicable to large populations; the most commonly used is acetoxymethyl (AM) ester loading. This noninvasive technique uses compounds where the carboxylate groups of the indicator dyes have been derivatized as acetoxymethyl esters resulting in a nonpolar compound that is permeable to cell membranes. Once inside the cell, these compounds are hydrolyzed by intracellular esterases (Figure 2). Once acted upon by esterases, the resultant activated indicator is now a polar molecule that is no longer capable of freely diffusing through the cell membrane, essentially trapping the compound inside the cell. AM Ester cell loading, while the easiest and most straightforward method to bulk load cells, is not without problems. It is assumed that the calcium indicator dye distributes evenly throughout the cell cytosol and is equally responsive to intracellular calcium concentrations. Unfortunately, AM

esters and their hydrolysis products can accumulate in any membrane-enclosed organelle. In some instances, these molecules, particularly the polyanionic-hydrolyzed moieties, can be partitioned by active transport systems. Cells with low levels of intracellular esterase activity may not completely activate the indicator dye. Cellular esterase activity may vary greatly from one cell type to another. It must be noted that complete hydrolysis results in the formation of cytotoxic compound byproducts, such as a formaldehyde and acetic acid (Figure 2). Leakage of the active indicator dye from loaded cells can also occur. Many cell types have organic ion transport systems, such as the P-glycoprotein multidrug-transporter, which are capable of actively eliminating the indicator dye from the cell.

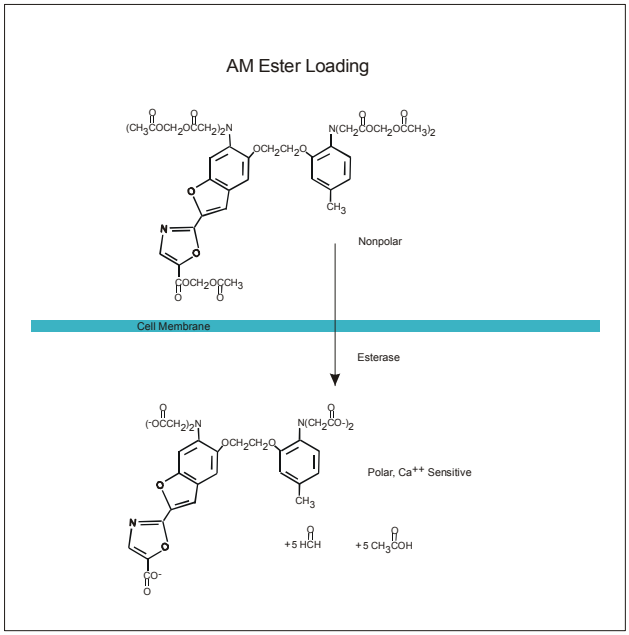

Figure 2. Diagram of Cellular Esterase activity on Fura-2 AM. Fura-2 AM ester is a nonpolar calcium insensitive compound that freely crosses the cell membrane. Once inside the cell, ubiquitous cellular esterases hydrolyze the compound to a calcium ion sensitive negatively charged moiety, Fura-2. Due to its anionic charge, Fura-2 can no longer cross the cell membrane and is essentially trapped within the cell.

Photo Bleaching

Continuous excitatory illumination can often result in the irreversible destruction or photo bleaching of fluorophores. This phenomenon is often observed as a steady decline in fluorescent signal over time with exposure to excitatory light despite a constant indicator concentration (Figure 3). Calcium ion uptake experiments are particularly prone to this due to their kinetic nature. While the mechanism by which this occurs is quite complex, there are several means by which this phenomenon can be minimized, such as shuttering the excitatory light in between measurements if possible. Or the use of low intensity light in conjunction with emission filters with as wide a bandpass as is compatible with signal isolation. Or using compounds that are more photostable whenever possible. Ratiometric compounds such as Fura-2 or Indo-1 can be used to correct for photobleaching. Photo bleaching results in a loss of fluorescence at the molecular level, thus the loss of signal is proportional regardless of the wavelength examined (Figure 3). Because calcium ion is measured as a function of the ratio between the two measurements rather than the absolute signal, any photobleaching of the fluorophore is corrected for. Fura-2 fluorescence intensity, as measured at 505 nm, increases in response to calcium ion binding when excited at 340 nm, while the fluorescence from a 380-nm excitation will decrease. The corresponding ratio between the two signals (340/380) will increase accordingly. Note however that the actual fluorescence from either 340 nm or 380 nm excitation generally decreases over time irrespective of stimuli. The decrease in signal can be the result of

photo bleaching, leakage, compartmentalization, or some other process of degradation. However, the ratio between the two signals remains a constant with differences being the result of differences in calcium ion concentration.

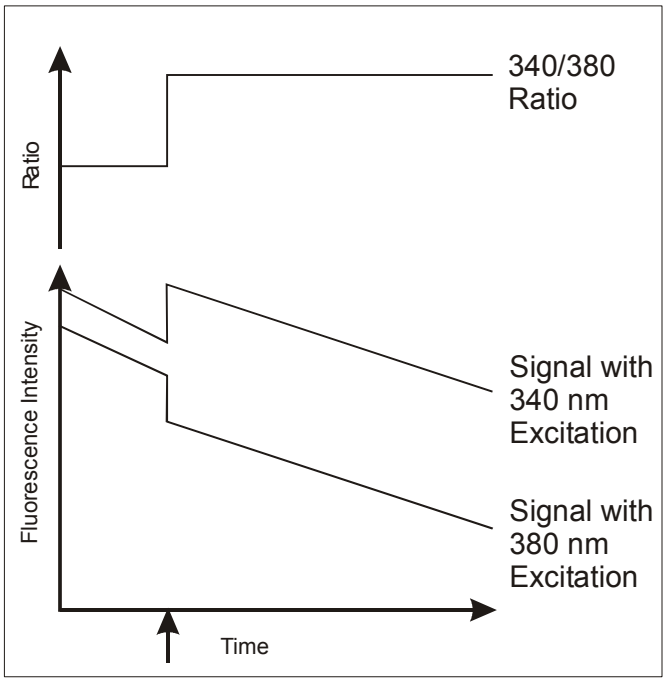

Figure 3. Hypothetical Fura-2 Fluorescence Demonstrating the Value of Ratiometric Fluorescence Calculations. The schematic demonstrates the hypothetical changes over time with the fluorescent emission at 505 nm when excited with 340 nm and 380 nm light. Note the arrow indicated the time at which the stimulus was added. Picture redrawn from schematic provided by Molecular Probes (Eugene, OR).

The purpose of this application note is to demonstrate the utility of the FLx800 Microplate fluorescence reader injector model in regards to measuring calcium ion concentration changes using various calcium specific indicator dyes. Additionally, the intent is to provide some of the necessary background to calcium ion measurements using microplate readers.

Materials and Methods

The indicator dyes, Fura-2 (F-1200); Indo-1 (I-1202); Calcium Green-1 (C-3010); Calcium Green-2 (C-3730); and Fluo-4 (F-14200) were purchased as free salts from Molecular Probes (Eugene OR). In addition a Calcium calibration kit (catalog # C-3721) was also purchased from Molecular Probes (Eugene OR). General chemical supplies including Trizma base, EGTA, and calcium chloride were obtained from Sigma-Aldrich Chemical Company (St Louis, MO). Stock solutions of all the indicator dyes were made with calcium free water to 1 mM and stored at 5°C until needed. Stock solutions of CaCl2 (100 mM), EGTA (100 mM pH 7.2), and Tris (100 mM, pH 7.2) were prepared as well.

In general, the indicator dyes were tested in two different ways. In some experiments the dye was diluted in a calcium free environment and a calcium solution was injected into the well. In other experiments the dye was diluted in a solution that contained calcium and an EGTA solution was injected. In either case, the working indicator dye solutions were diluted to a final concentration of 10 µM in a buffer containing 10 mM Tris (pH 7.2) and either 1 mM EGTA (Calcium free) or 1 mM CaCl2 (Calcium containing). Aliquots of the appropriate working indicator solutions were pipetted into wells of a microplate. As indicated, specific injection

volumes of solutions of either 100 mM CaCl2 or 100 mM EGTA were then used to elicit changes in fluorescence.

Results

In order to demonstrate the capabilities of the FLx800 with an injector in regards to cellular-calcium uptake determination, several different experiments using a variety of calcium sensitive fluorescent dyes were performed. These dyes were tested for the most part in two different ways. In some experiments the dye was diluted in a calcium-free environment and calcium solution was injected into the well. In other experiments the dye was diluted in a solution that contained calcium and an EGTA solution was injected. The compound EGTA is a calcium-chelating agent that sequesters calcium ions and effectively lowers the calcium ion concentration. The calcium indicator dyes, calcium green-1, calcium green-2, and Fluo-4 are representative of the dyes that increase their fluorescence in the presence of calcium ion (Ca+2) without changing wavelengths.

Figure 4 demonstrates the ability of the FLx800 to inject fluid that elicits a calcium specific change in fluorescence of a calcium indicator dye Calcium Green-2. Note that little or no change in fluorescence is observed without injection of calcium. Similar changes in fluorescence are observed when the related compound Calcium Green-1 is treated in the same fashion (Figure 5).

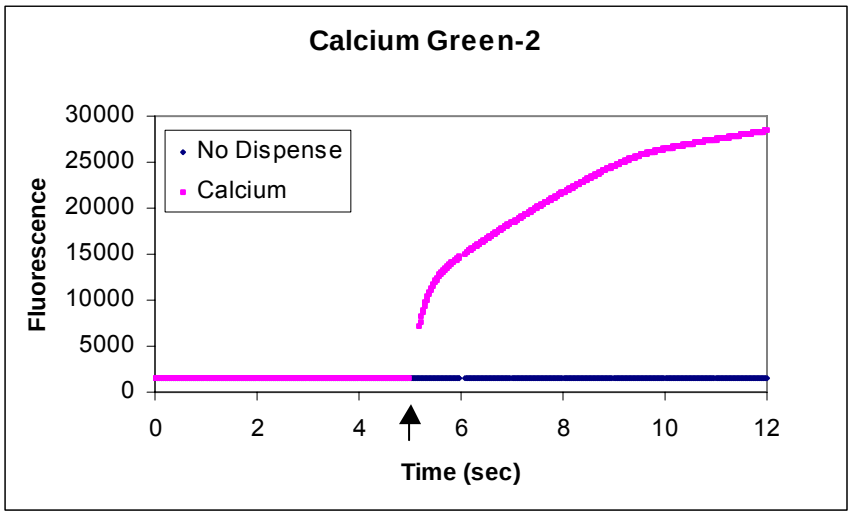

Figure 4. Fluorescence change of Calcium Green-2 in solution with calcium chloride injection. The fluorescent dye calcium green-2 was diluted to 10-µM final concentration in a buffer containing 10 mM Tris and 1 mM EGTA. 200 µl of this solution was then placed into several wells of a Costar 3603 clear bottom plate. Using an FLx800 configured with the injector above the bottom detector, 20 µl of a 100 mM CaCl2 solution was injected. Fluorescence detection was from the bottom using a 485/20-excitation and a 530/25-emission filter, with reading taking place every 20 milliseconds. Note that the CaCl2 solution was injected 5 seconds after the beginning of the reading as indicated by the arrow.

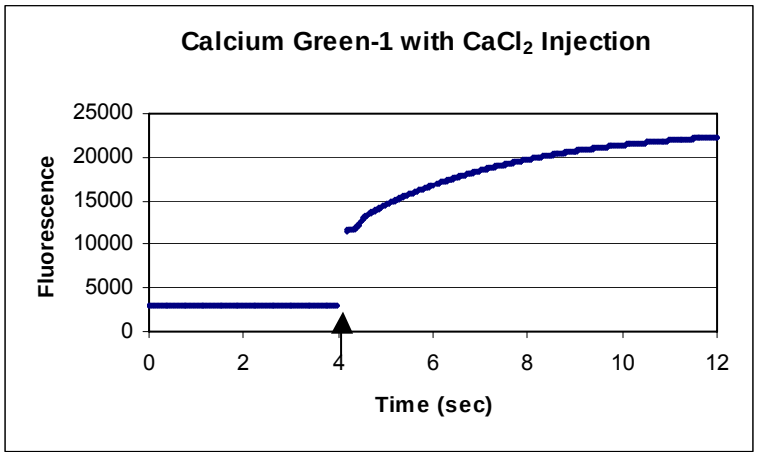

Figure 5. Fluorescence change of Calcium Green-1 in solution with calcium chloride injection. The fluorescent dye Calcium Green-1 was diluted to a 10-µM final concentration in a buffer containing 10 mM Tris and 1 mM EGTA. 200 µl of this solution was then placed into several wells of a Costar 3603 clear bottom plate. Using an FLx800 configured with the injector above the bottom detector, 20 µl of a 100 mM CaCl2 solution was injected. Fluorescence detection was from the bottom using a 485/20-excitation and a 530/25-emission filter, with reading taking place every 20 milliseconds. Note that the CaCl2 solution was injected 4 seconds after the beginning of the reading as indicated by the arrow.

When the fluorescence of serial dilutions of Calcium Green-1 in the presence of a constant free calcium ion concentration is measured a linear relationship between indicator dye concentration and fluorescence is observed (Figure 6). This suggests that one can titrate the amount of indicator dye to the sensitivity requirements needed for the experiment. The use of lower concentrations of indicator dye minimizes the cytotoxicity associated with the conversion of the AM ester to the active compound, while still allowing for adequate sensitivity.

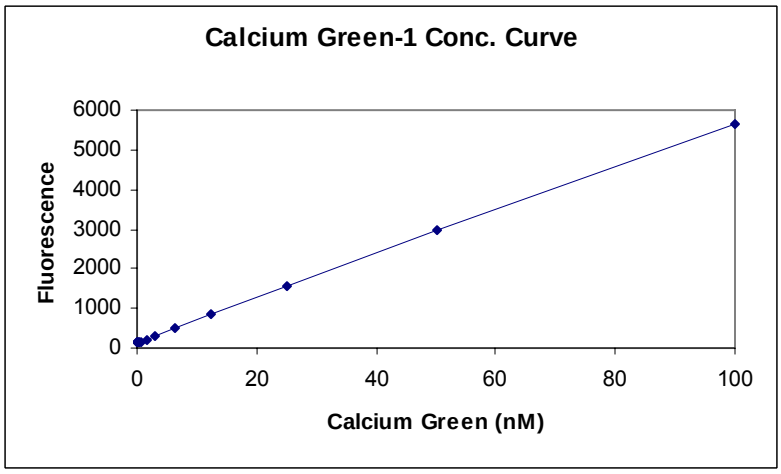

Figure 6. Calcium Green-1 Calibration Curve. Serial dilutions of Calcium Green-1 indicator dye were made using a working solution of 10 mM Tris, 10 mM CaCl2 as the diluent. 200 µl aliquots of each concentration were pipetted into Costar 3603 microplates in replicates of eight. Measurements were made using an FLx800 from the bottom using a 485/20 excitation and a 530/25 emission filter at a sensitivity of 40.

As demonstrated in Figure 7 the FLx800 microplate reader can distinguish different calcium concentrations with a calcium responsive indicator dye. Using a constant concentration of the indicator dye, Fluo-4, various amounts of a 100 mM CaCl2 solution were injected into different wells of a microplate. The change in fluorescence with larger injection volumes (i.e. more Ca2+) was more rapid, as well as being of a greater extent than the smaller volumes.

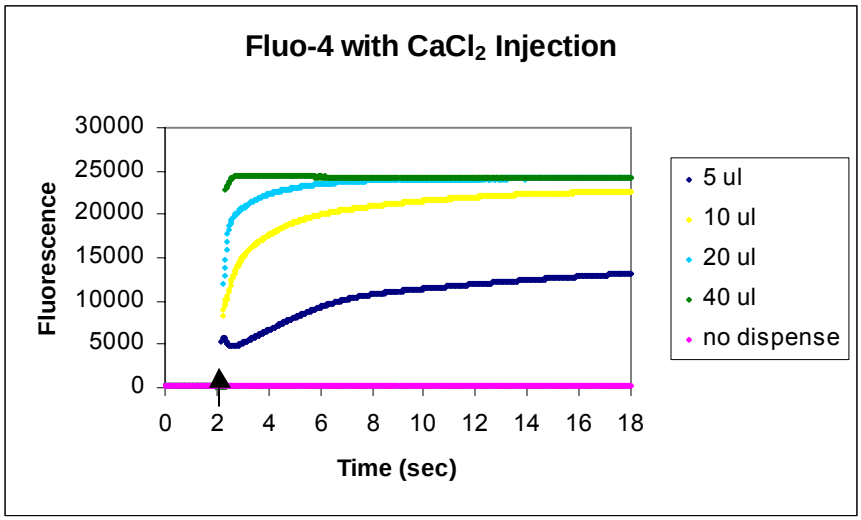

Figure 7. Fluorescence change of Fluo-4 in solution with calcium chloride injection. The fluorescent dye Fluo-4 was diluted to 10-µM final concentration in a buffer containing 10 mM Tris and 1 mM EGTA. 200 µl of this solution was then placed into several wells of a Costar 3603 clear bottom plate. Using an FLx800 configured with the injector above the bottom detector, various volumes of a 100 mM CaCl2 solution were injected to several different wells. Fluorescence detection was from the bottom using a 485/20 excitation and a 530/25-emission filter, with reading taking place every 30 milliseconds. Note that the CaCl2 solution was injected 2 seconds after the beginning of the reading as indicated by the arrow.

When constant amounts of indicator dye are incubated with increasing amounts of free Ca2+, an increase in fluorescent signal is observed. Free calcium can be tightly controlled in experimental situations using K2EGTA/CaEGTA buffering system, which utilizes the dissociation constant (Kd) of CaEGTA as a means to provide free calcium ion. As depicted in Figure 8, when the free calcium ion concentration increases from 0 to approximately 0.6 µM, the increase in fluorescent signal from Fluo-4 is virtually linear. Levels of free calcium above 0.6 µM result in greater fluorescence, but the increase is no longer linear (Figure 8).

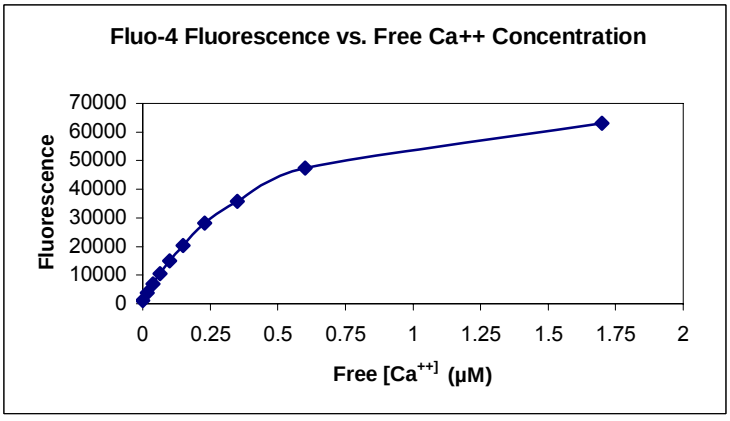

Figure 8. Free Calcium Concentration Curve. Using a Calcium Calibration kit from Molecular Probes, various concentrations of free Calcium ion are reacted with a constant concentration of Flo-4 indicator dye.

The fluorescence was measured from the top using an FLx800 fluorescence reader with a 485/20 Excitation and a 530/25 Emission filters.

Experiments using the indicator dye Indo-1 were used to demonstrate several hardware features, in addition to the ability to measure another calcium indicator dye. The dye Indo-1 has the characteristic that its emission wavelength is different in the presence of free Ca2+ than in the absence. Figure 9 demonstrates the ability to read two filter sets every 250 milliseconds, such that the excitation filter is not changed, but the emission filter changes between two adjacent filters. Also demonstrated in Figure 9 are the changes in the ratio between the two filter sets when Indo-1 is in the presence or absence of calcium. When Indo-1 is in a calcium free environment (e.g. in the presence of EGTA) the ratio of filter set 1 (360/40 excitation, 410/10 emission) to filter set 2 (360/40 excitation, 485/20 emission) is much lower than the ratio after calcium ions are injected. Conversely, the ratio of the two filter sets is relatively high in the presence of calcium and rapidly falls when the calcium chelator, EGTA, is added.

Indo-1 measurements were also used to compare bottom and top detection with the use of the injector. The reader has been designed so that the injector can be positioned either above the bottom probe or adjacent to the top probe. In either case the injector is positioned so that it dispenses into the well being measured. Figure 10 demonstrates that injection with either top or bottom detection returns a similar response in terms of time and magnitude.

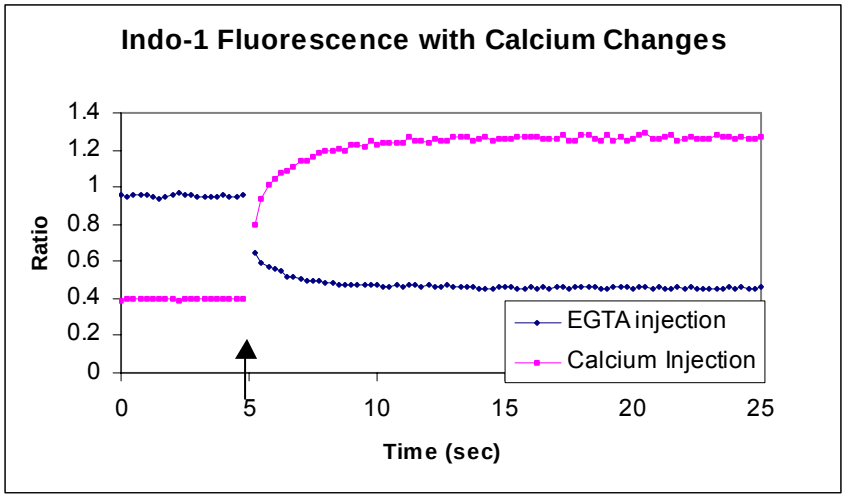

Figure 9. Ratiometric changes with the addition of calcium or EGTA to Indo-1 dye. The fluorescent dye Indo-1 was diluted to 10 µM final concentration in a buffer containing 10 mM Tris and either 1 mM EGTA or 1 mM CaCl2 and 200 µl was then placed into several wells of a Nunc opaque black plate. Using an FLx800 configured with the injector adjacent to the top detector, 20 µl of a 100 mM CaCl2 solution were injected into the wells containing dye and EGTA (calcium injection) or 20 µl of a 100 mM EGTA solution was injected into wells containing dye and calcium (EGTA injection). Fluorescence detection was from the top using two filter sets. Filter set 1 used a 360/40-excitation and a 410/10-emission filter, while filter 2 used a 360/40-excitation and a 485/20-emission filter, with reading taking place every 250 milliseconds. Note that the CaCl2 or EGTA solutions were injected 5 seconds after the beginning of the reading as indicated by the arrow.

Comparison of top and bottom dispense using Indo-1 demonstrates that both detector positions are adequate for fluorescence determinations with injections. As demonstrated in Figure 10 calcium injection with either top or bottom read results in a marked increase in the ratio between the 410 nm emission and the 485 nm emission. Because of differences in signal output the raw data was first normalized to the pre-injection measurements. The resultant ratio values are very similar top or bottom. Much like endpoint determinations both detector positions offer

advantages dependant upon what is being measured. Therefore, the ability to easily switch between the two positions is important.

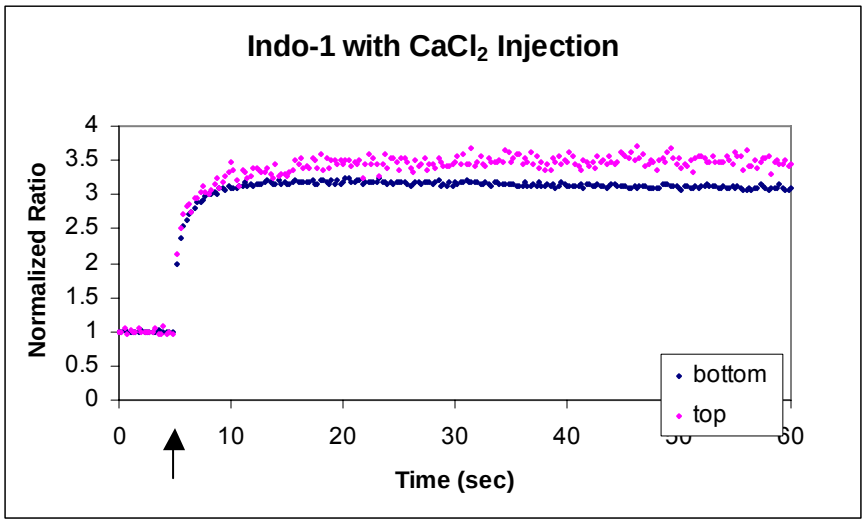

Figure 10. Comparison of injection with top and bottom probe detection. The calcium responsive fluorescent dye Indo-1 was diluted to 10-µM final concentration in a buffer containing 10 mM Tris and 1 mM EGTA and 200 µl was then placed into several wells of a Costar 3603 clear bottom black sided plate. Using an FLx800 configured with the injector adjacent to the top detector or above the bottom detector, 20 µl of a 100 mM CaCl2 solution was injected.

Fluorescence detection was made using two filter sets. Filter set 1 used a 360/40-excitation and a 410/10-emission filter, while filter 2 used a 360/40-excitation and a 485/20-emission filter, with reading taking place every 250 milliseconds for 60 seconds. Note that the CaCl2 solutions were injected 5 seconds after the beginning of the reading as indicated by the arrow. Raw fluorescence values were first normalized to the values before injection, then the ratio between filter set 1 and filter set 2 were calculated and plotted.

The ability to switch excitation filters in a rapid manner in order to perform ratiometric determinations using Fura-2 was tested. The peak in excitation for Fura-2 changes from 366 nm to 338 nm when in the presence of free Ca2+ ions. As demonstrated in Figure 6, the fluorescence output with a 380/20 excitation and a 508/20-emission filter set falls quite dramatically when calcium is injected, while the output from a 340/11 excitation 508/20 emission filter set increases (Figure 11). As with Indo-1, the ratio of these values can be calculated and plotted, as seen in Figure 12. Note that injection of fluid alone is not sufficient to alter the ratio, as injection of Tris buffer results in no ratio change.

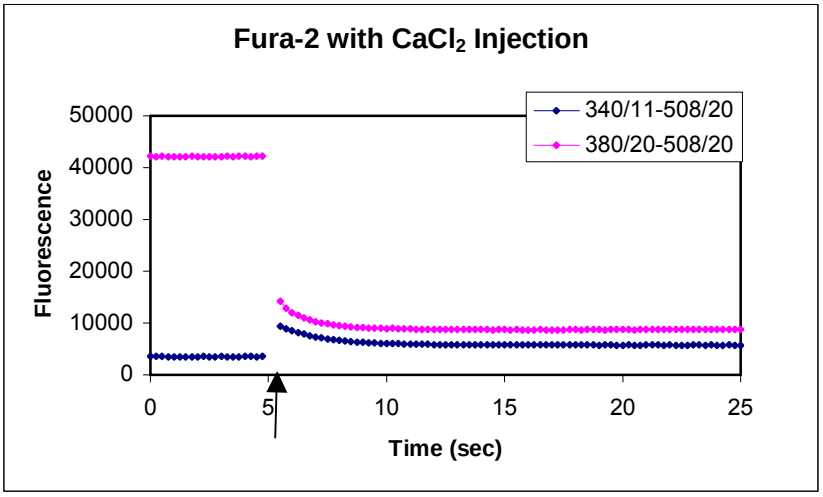

Figure 11. Fluorescence changes of Fura-2 with the addition of calcium ions. The fluorescent dye Fura-2 was diluted to 10-µM final concentration in a buffer containing 10 mM Tris and 1 mM EGTA. 200 µl of this solution was then placed into several wells of a Nunc solid black plate. Using an FLx800 configured with the injector adjacent to the top detector, 40 µl of a 100 mM CaCl2 solution was injected to several different wells. Fluorescence detection was from the top switching between two excitation filter sets, a 340/11 or a 380/20, with a 508/20-emission filter, with reading taking place every 250 milliseconds for 25 seconds. Note that the CaCl2 solution was injected 5 seconds after the beginning of the reading as indicated by the arrow.

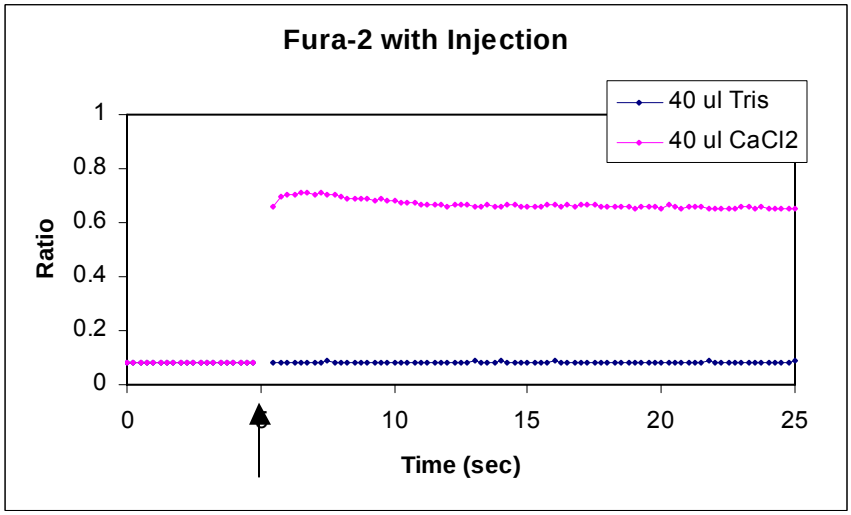

Figure 12. Comparison of Calcium and Tris buffer injections. The fluorescent dye Fura-2 was diluted to a 10-µM final concentration in a buffer containing 10 mM Tris and 1 mM EGTA. 200 µl of this solution was then placed into several wells of a Nunc solid black plate. Using an FLx800 configured with the injector adjacent to the top detector, 40 µl of either a 100 mM CaCl2 solution or a 10 mM Tris solution was injected to several different wells. Fluorescence detection was from the top switching between two excitation filter sets, a 340/11 and a 380/20, and a 508/20-emission filter, with reading taking place every 250 milliseconds for 25 seconds. Data represents the ratio between the fluorescence signal of filter set 1 (340/11, 508/20) and filter set 2 (380/20, 508/20). Note that the CaCl2 solution was injected 5 seconds after the beginning of the reading as indicated by the arrow.

Discussion

These data demonstrate the ability of the FLx800 to dispense fluid into microplate wells and measure changes in free Ca2+ in solution using fluorescence. However, several issues have to be taken into account when measuring cellular calcium concentration. As with any fluorescence application the use of appropriate filters is paramount to obtaining good quality data. Table 1

provides filter recommendations for several fluorescent calcium indicators. Setting the PMT sensitivity setting is generally accomplished empirically. Rapid filter switching precludes switching sensitivity levels between filter sets when measuring ratiometric dyes such as Indo-1 or Fura-2. In these experiments, a compromise between the two different filter sets must be empirically determined prior to cellular analysis. This is accomplished by measuring equivalent concentrations of dye with both filter sets in the presence and absence of calcium ion using an endpoint determination. Injection volumes that are small relative to the total well volume will exhibit problems associated with mixing. Mixing of the injected fluid with the contents of the well is entirely dependent on the inertia of the dispensed fluid. Very small volumes do not have the necessary mass to completely agitate the existing fluid. The result is often a delayed or extended fluorescence response that occurs as the solutions mix. Dispense volumes that are roughly 50% by volume or greater provide rapid mixing. When measuring intracellular levels of calcium, one needs to be aware that variability of dye loading, dye leakage, compartmentalization, and the presence of other ions can affect the fluorescent signal.

The FLx800 has several features that enable the user to get optimal results from their experiments. With an excitation range of 300 nm to 650 nm and an emission range from 340 nm to 800 nm, the FLx800 is capable of measuring all of the commonly used calcium specific indicators. Temperature control from ambient to 50°C allows cell cultures to be maintained at the appropriate temperature during the experiment. The external dispenser module contains a high precision syringe designed to deliver reagents directly into the wells of a 6-, 12-, 24-, 48-, or 96-well microplate. For ratiometric analysis, the FLx800 has the ability to rapidly switch two filter sets. Single filter switching and measurement can take place in approximately 125 msec. Filter pairs that have either two different excitation filters and a common emission filter or two different emission filters and a common excitation filter can be read with a repeat read interval of 250 msec. Filter switching that requires both excitation and emission filters to be different can be read every 500 msec. If the interval between readings is sufficient, the FLx800 can be programmed to automatically shutter off the light source to the sample, reducing deleterious photobleaching as much as possible, while maintaining lamp stability.

The software and injector allow for full functionality in regards to its use. The injector position can be optimized for detection from the top or the bottom of the microplate for increased application flexibility. Dispense-volume, rate and timing can be controlled via software. Because the syringe unit has a switching solenoid valve, reagent priming and purge functions can be utilized. In addition, a small volume tip prime can be programmed at the beginning of a plate or before dispensing to each well.

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