

Video Article

# Cytosolic Calcium Measurements in Renal Epithelial Cells by Flow Cytometry

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## Abstract

A variety of cellular processes, both physiological and pathophysiological, require or are governed by calcium, including exocytosis, mitochondrial function, cell death, cell metabolism and cell migration to name but a few. Cytosolic calcium is normally maintained at low nanomolar concentrations; rather it is found in high micromolar to millimolar concentrations in the endoplasmic reticulum, mitochondrial matrix and the extracellular compartment. Upon stimulation, a transient increase in cytosolic calcium serves to signal downstream events. Detecting changes in cytosolic calcium is normally performed using a live cell imaging set up with calcium binding dyes that exhibit either an increase in fluorescence intensity or a shift in the emission wavelength upon calcium binding. However, a live cell imaging set up is not freely accessible to all researchers. Alternative detection methods have been optimized for immunological cells with flow cytometry and for non-immunological adherent cells with a fluorescence microplate reader. Here, we describe an optimized, simple method for detecting changes in epithelial cells with flow cytometry using a single wavelength calcium binding dye. Adherent renal proximal tubule epithelial cells, which are normally difficult to load with dyes, were loaded with a fluorescent cell permeable calcium binding dye in the presence of probenecid, brought into suspension and calcium signals were monitored before and after addition of thapsigargin, thapsigargin and ionomycin.

## Video Link

The video component of this article can be found at <https://www.jove.com/video/51857/>

## Introduction

Calcium is an important second messenger responsible for the transmission of cellular physiological and pathophysiological signals<sup>1</sup>. Cytosolic calcium concentrations are kept low through the activity of calcium pumps and calcium exchangers. The sarcoplasmic/endoplasmic reticulum calcium ATPase (SERCA) refills the endoplasmic reticulum (ER) calcium store as part of a "pump leak" system whereas the plasma membrane calcium ATPase (PMCA) extrudes calcium to the extracellular compartment, both in ATP-dependent manners<sup>2</sup>. Physiological messengers, such as hormones or neurotransmitters, transmit their signals through G-protein coupled receptors, activation of phospholipase C, which hydrolyses phosphatidylinositol 4,5-bisphosphate (PIP2) in the plasma membrane to generate diacylglycerol and inositol triphosphate (IP3)<sup>3</sup>. While diacylglycerol remains in the plasma membrane, IP3 diffuses into the cytosol and binds to IP3 receptors (IP3Rs), which are ligand activated calcium channels, found in the ER membrane and calcium is released from the ER luminal store culminating in an increase in cytosolic calcium concentrations<sup>4</sup>. An alternative route for calcium to reach the cytosol is through calcium channels and exchangers present in the plasma membrane. Crosstalk between these two compartments have been described: calcium induced calcium release (CICR) where extracellular calcium induces calcium release from ER stores<sup>5</sup>, and store operated calcium entry (SOCE) where emptying of the ER stores is sensed by STIM and causes opening of Orai calcium channels in the plasma membrane to promote refilling of the ER stores<sup>6</sup>.

Under pathophysiological conditions, the cellular calcium response is deregulated and cytosolic calcium increases in the absence of physiological stimulators<sup>1</sup>. The calcium response may be affected in a number of ways: prolonged calcium signal, delayed calcium removal from the cytosol, depletion of calcium stores or localized changes in calcium. Furthermore, the mitochondria take on a central role in buffering and releasing calcium<sup>7</sup>. Prolonged and/or supramaximal cytosolic calcium increase leads to cell death. In fact, calcium is more often than not involved in the cellular pathophysiological response and is a key event in a wide array of diseases, such as neurodegenerative disease, heart disease, cancer, bone disease and kidney disease, which are mainly associated with cell death and loss or alteration of organ or tissue function<sup>8-10</sup>. Moreover, perturbation of calcium signals has been linked to cellular adaptation and changes in cellular functions and responses.

Traditionally, calcium signals are measured with a negatively charged fluorescent calcium indicator that is loaded into cells in a cell permeable acetoxymethyl (AM) ester form. Once cleaved by cellular esterases, the fluorescent indicators remain within the intracellular compartment and increase in fluorescence intensity when calcium is bound. The most well known and used calcium indicator is the ratiometric Fura-2 developed by Roger Tsien and colleagues<sup>11</sup>. Calcium indicators with differing affinities for calcium allow different calcium pools to be monitored. Detection methods include live cell imaging, fluorescence microplate reader and flow cytometry. The relatively fast kinetics of calcium signals (usually within a few seconds to minutes) makes live cell imaging the best method for gaining the most information about characteristics of the calcium signal. Aside from the fast kinetics of calcium signals (within milliseconds), live cell imaging is a better method for studying cellular

compartmentalization of calcium signaling within a single cell<sup>12</sup>. Absolute calcium concentrations can be calculated by determining the minimum and maximum fluorescence of the calcium indicator by adding a calcium chelator and permeabilizing the cells, respectively, as described by Grynkiewicz *et al.*<sup>11</sup>.

The use of flow cytometry to measure calcium signals was first developed in the 1980s in immunological cells where the opportunity to measure multiple parameters, such as membrane integrity and separation of cell population, and the requirement of cells in suspension combined with the development of single wavelength calcium indicators made flow cytometry an ideal and convenient detection method<sup>13-15</sup>. In adherent cells, live cell imaging provides the most information about calcium signaling, most importantly the kinetics, but requires a complicated setup comprising a fluorescence microscope, a perfusion system, maintenance of the cellular environment, such as temperature, and specialized microscope software for acquiring and analyzing data. Alternative methods such as a fluorescence microplate reader or pharmacological means through use of calcium chelators are incomparable in terms of gained information. Flow cytometry is becoming more widely used to monitor cytosolic calcium in adherent cells though, in the majority of studies, only end-point measurements are performed. Using the method initially developed by Gergely *et al.* in immunological B cells<sup>16</sup>, changes in cytosolic free calcium concentration by flow cytometry with real-time kinetics and monitoring the fluorescence intensity in individual cells from a sample population have been successfully measured in cancer epithelial cells and cancer stem cell hybrids<sup>17</sup>. This method was further adapted for use in adherent epithelial cells, which are difficult to load with calcium indicators<sup>18</sup>.

Here, using an immortalized adherent epithelial cell line derived from the S1 segment of the rat kidney proximal tubule (WKPT-0293 Cl.2)<sup>19</sup>, we describe an optimized simplified method for measurement of changes in cytosolic calcium concentration by flow cytometry. Since many epithelial cells possess a number of organic transporters for anionic compounds, loading of cells with a calcium indicator can prove to be a challenge. To prevent the efflux of calcium indicators, probenecid, the prototypical inhibitor of organic anion transporters and originally developed to decrease renal excretion of penicillin<sup>20</sup>, is applied simultaneously in the incubation medium. The cells are maintained in monolayers for calcium indicator loading, brought into suspension and calcium signals can be detected immediately after compound addition.

## Protocol

### 1. Preparation of Reagents and Solutions

1. Prepare a modified Hank's balanced salt solution (HBSS) (in mM: 136.9 NaCl, 5.37 KCl, 0.34 Na<sub>2</sub>HPO<sub>4</sub>, 0.44 KH<sub>2</sub>PO<sub>4</sub>, 4.17 NaHCO<sub>3</sub>, pH 7.2, no phenol red). Store at 4 °C.
2. Make 1.5 M CaCl<sub>2</sub> and 2 M 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) (pH 7.2) stock solutions using distilled water.
3. Prepare a 250 mM stock solution of probenecid. For the free acid form (often referred to as water insoluble), dissolve probenecid powder in 1 N NaOH, fill to three quarters of the final volume with distilled water and slowly titrate to pH 7.4 with 1 N HCl. Store at 4 °C.
4. Dissolve fluorescent membrane permeable calcium binding dye in anhydrous dimethyl sulfoxide (DMSO) to a stock concentration of 1 mM. Store at -20 °C in the dark.
5. Prior to each experiment, freshly prepare probenecid-containing HBSS flux (PHF) buffer by combining modified HBSS with (in final concentrations) 1.5 mM CaCl<sub>2</sub>, 10 mM HEPES, pH 7.2, 5% fetal bovine serum, 2 mM probenecid, and 5.55 mM glucose. Warm to 37 °C.

### 2. Cell Culture

1. Plate  $2.5 \times 10^5$  kidney proximal tubule cells (WKPT-0293 Cl.2) per well of a 6 well plate or 35 mm dish in standard culture medium and grow for 2 days reaching a confluency of approximately 70%.

### 3. Calcium Dye Loading

1. For each well or dish, mix 1 ml culture medium containing 5% fetal bovine serum, 4 µM cell permeable calcium binding dye and 2 mM probenecid.
2. Replace the culture medium with 1 ml loading dye mixture in each well and incubate at 37 °C for 30 min in a humidified incubator with 5% CO<sub>2</sub>. Cover the plate with aluminium foil to prevent bleaching of the fluorescent indicator.

### 4. Preparation of Cells for Flow Cytometry

1. Warm 0.05% trypsin-ethylenediaminetetraacetic acid (EDTA) solution to 37 °C.
2. Aspirate calcium loading dye solution from each well and add 1 ml trypsin along the wall of the well.
3. Incubate at 37 °C until all cells are detached.
4. Transfer cells to a 1.5 ml tube and pellet by centrifuging in a microcentrifuge at 10,000 g for 1 min.
5. Carefully aspirate the supernatant without disturbing the pellet.
6. Wash cells by adding 1 ml PHF buffer and resuspend by pipetting up and down.
7. Pellet cells by centrifuging at 10,000 g for 1 min.
8. Repeat steps 4.6. and 4.7. a further two times.
9. Resuspend cells in a final volume of 500 µl PHF buffer and use the cells for experimentation within 1 hr.
10. Optional: count cells to determine the cell concentration and use  $5 \times 10^4$  –  $1 \times 10^5$  cells per measurement.

### 5. Flow Cytometer Setup

1. Start the flow cytometer, open the fluidics drawer and flip the vent valve toggle switch to increase the air pressure of the sheath fluid tank.
2. "Prime" the instrument to remove potential residing air bubbles from within the inner tubes and the flow chamber.

3. In the flow cytometry software, open two dot plot windows.
  1. The first dot plot requires forward scattered light (FSC) for the X parameter and side scattered light (SSC) for the Y parameter to control for the quality of the cells within the sample through their size and their granularity, respectively.
  2. The second dot plot measures the fluorescence intensity of the calcium-bound dye. Select the appropriate fluorescence detection channel for the Y parameter using a logarithmic scale and time in seconds for the X parameter on a linear scale.
4. Set the flow rate to 'high'.

## 6. Flow Cytometry Measurement

1. Perform the measurements at RT. In a flow cytometry sample tube (11.5 x 75 mm), add 480  $\mu$ l PHF buffer.
2. Add 20  $\mu$ l cell suspension and briefly vortex to mix.
3. Move the tube support arm to the side and position the sample tube over the sample injection tube until a tight seal is formed. Return the tube support arm to its original centered position.
4. Optimize measurement by adjusting the fluorescence channel such that the mean fluorescence intensity of the sample is at approximately  $10^2$  on the y-axis. Save and use for subsequent experiments.
5. To start an experiment, repeat steps 6.1 to 6.3.
6. Record baseline fluorescence for 50 sec.
7. Pause measurement, remove the sample tube, add compound of interest, vortex briefly and return to the sample injection tube. This step should not take more than 15 sec.
8. Resume measurement and continue for a total of 204.8 sec.
9. Use a calcium ionophore, such as ionomycin (10  $\mu$ M), and a calcium chelator, such as ethylene glycol tetraacetic acid (EGTA),  $\text{MnCl}_2$  or  $\text{CoCl}_2$  (2 mM), for positive and negative controls, respectively.

## 7. Data Analysis

1. Analyze data using dedicated flow cytometry software for offline analysis, such as WinMDI (Windows Multiple Document Interface for Flow Cytometry), a free software package developed by Joe Trotter at The Scripps Institute, Flow Cytometry Core Facility.
2. Open a dot plot with SSC and FSC parameters.
3. Select the region of cells for analysis using the "Regions" tool (right-click on the image) to exclude dead cells or cell debris found in the bottom left corner from the data analysis (**Figure 1**).
4. Quadrant analysis (**Figure 2**)
  1. Open a density plot with time on the x-axis and fluorescence intensity on the y-axis. Use all events.
  2. Use the same gating region as in 7.3.
  3. Quantify the data by using the "Quadrant" tool.
  4. Adjust the quadrant separators so that the vertical line is placed at the time of compound addition and the horizontal line is placed at the center of the baseline fluorescence in the controls. Ensure that the positioning of the quadrant separators is equal in all samples.
  5. The percentage of cells in each quadrant is displayed in each corner (**Figure 2**). Compare the upper right quadrant (UR) between samples.
5. Histogram analysis (**Figure 3**)
  1. Open the control data in a histogram window with fluorescence intensity on the x-axis and events on the y-axis. Use all events.
  2. For a pictorial overview, add the test data to be compared to the control plot as overlay plots (go to File  $\rightarrow$  Open File  $\rightarrow$  Select file  $\rightarrow$  Overlay).
  3. For quantitative analysis, single histogram windows must be used. Gate the cell population using the region R1 from step 7.3.
  4. In the non-treated control, use the marker function ("Markers") to mark the area for analysis starting from the midpoint between the minimum and maximum fluorescence of the control peak and ending at the maximum fluorescence intensity. The percentage of cells in this area is calculated.
  5. Retrieve data from the statistics window. Repeat for remaining data files using the same marked area for analysis.
6. Fluorescence intensity analysis (**Figure 4**)
  1. Open a dot plot with time on the x-axis and fluorescence intensity on the y-axis. Use all events.
  2. Enable "Kinetics Mode" and "Overlay Kinetics Line". A blue line will appear in the dot plot representing the mean fluorescence intensity.
  3. Quantify the relative calcium influx by creating multiple rectangular regions. Each region should have a width of about "50", which is equivalent to "10 sec". Define up to 15 rectangular regions.
  4. Retrieve quantification from the statistics window and copy into a spreadsheet.
  5. Use the y-mean data for analysis. Calculate the mean of R2 to R6, which is equivalent to 20 to 50 sec. This is the baseline value.
  6. Choose an appropriate time interval for calcium influx analysis (depends on onset, duration and intensity).
  7. Calculate the calcium signals from each region within this time interval relative to the mean baseline value, that is, the fluorescence signal before compound addition, which is set to 100%. Determine the mean and standard deviation of the regions post compound addition; this is the mean calcium signal evoked by the compound.

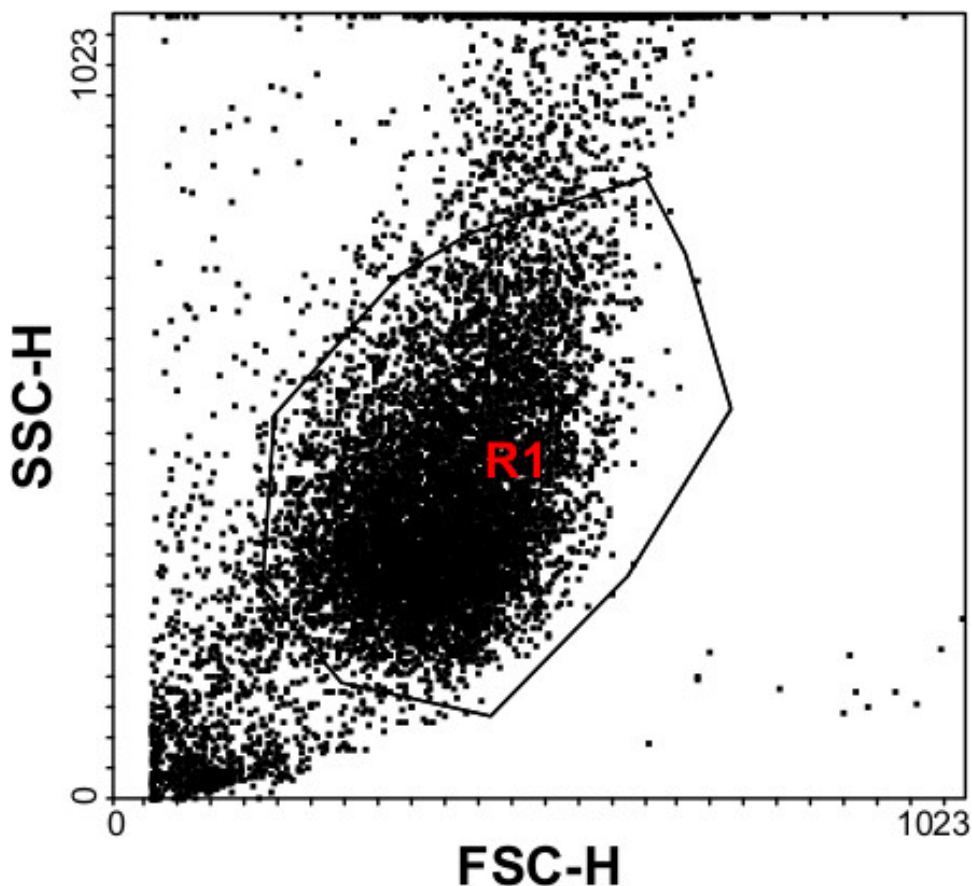
## Representative Results

To increase cytosolic calcium, two pharmacological compounds were applied to renal proximal tubule cells (WKPT-0293 Cl.2) loaded with cell permeable calcium binding dye, Fluo-3-AM. Nontreated control samples were loaded with calcium binding dye in the presence of probenecid and were mixed but without the addition of any compounds. Thapsigargin (TG) is a classical inhibitor of the SERCA pump leading to leakage

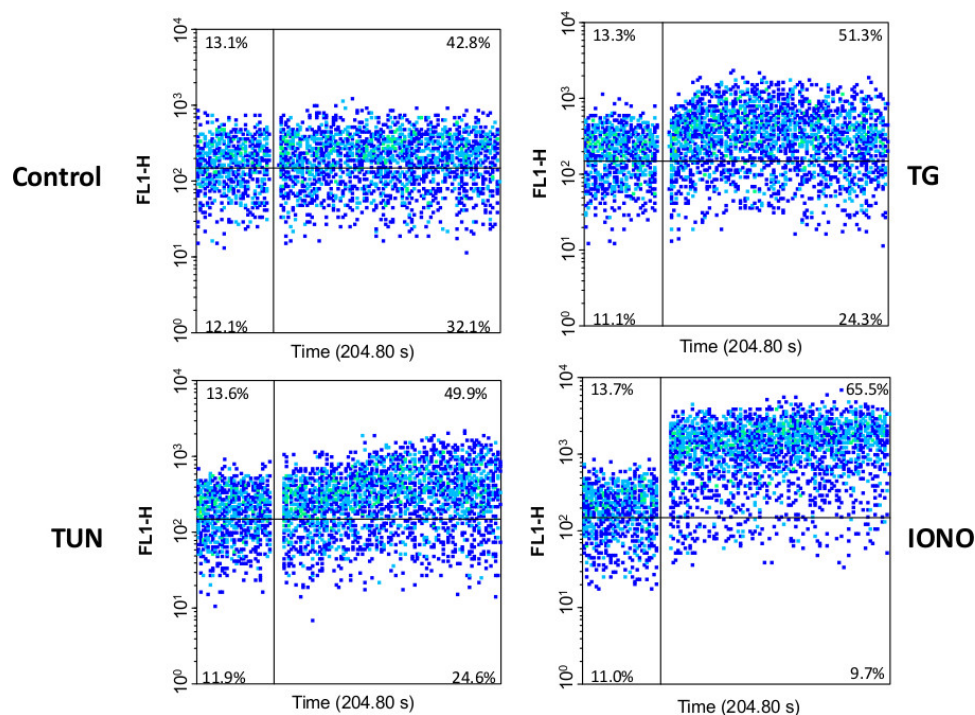
out of the ER and resulting in increased cytosolic calcium. Tunicamycin (TUN) blocks N-glycosylation of proteins leading to activation of the unfolded protein response but also increases cytosolic calcium<sup>18,21</sup>. As a positive control, ionomycin (IONO), a calcium ionophore making the plasma membrane permeable for extracellular calcium, was used. For analysis, the cell population was gated by selecting a region in the SSC/FSC dot plot (R1 in **Figure 1**). A density plot for fluorescence intensity vs. time was created for each data file. By using the quadrant function, the dot plot was separated into four areas representing Fluo-3 fluorescence intensity before and after compound addition and above and below the baseline fluorescence. Quantification as percentage of gated cell population in each quadrant allows determination of increased cytosolic calcium concentration. In this example, 3  $\mu$ M TG causes an increase in the percentage of cells in the upper right quadrant indicating that a calcium signal has been induced. The percentage of cells increases from 42.8% in control to 51.3% (1.20 fold) in TG treated cells. Similarly, 6  $\mu$ M TUN augments the percentage of cells with increased calcium fluorescence intensity to 49.9% (1.17 fold). Application of 10  $\mu$ M IONO resulted in 65.5% (1.53 fold) of cells in the upper right quadrant (**Figure 2**). The histogram analysis resulted in 1.41 fold, 1.36 fold and 2.11 fold by TG, TUN and IONO, respectively (**Figure 3**) (for statistics, see<sup>18</sup>). Finally, using the multiple region analysis mode, TG, TUN and IONO caused 1.55 fold, 1.29 fold and 4.54 fold increases in calcium signal over control, respectively (**Figure 4**).

The most sensitive analysis mode was the multiple region analysis. In contrast to the quadrant and histogram analysis modes, where the distribution of the cell population is quantified, the multiple region analysis mode quantifies the change in fluorescent signal from the total cell population. The largest discrepancy in the results from different analysis modes was for ionomycin that ranged from 1.53 fold increase over control from the quadrant analysis to 4.54 fold from the multiple region analysis. The compounds tested did not exhibit such variance. However, throughout all analysis modes, the extent of calcium signal evoked remained the same though the absolute values differed, *i.e.*, IONO had the largest effect followed by TG and then TUN.

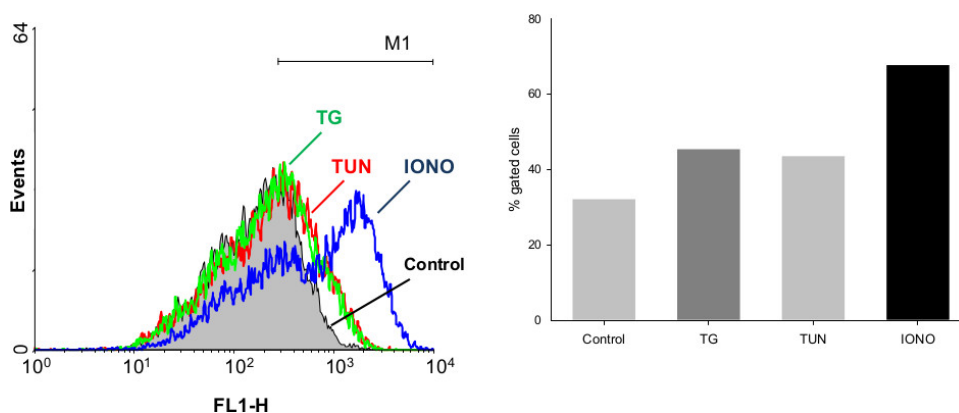
Application of TG, TUN or IONO results in permanent changes in cytosolic calcium. To determine whether physiological calcium signals, which are transient, can be measured using this method, ATP was applied to WKPT-0293 Cl.2 cells. ATP evoked a rapid and transient calcium signal, which could not be recorded in its entirety by the flow cytometry method. ATP concentrations at 20 - 100  $\mu$ M were difficult to detect therefore the concentration was reduced to "slow down" the calcium signals. As shown in **Figure 5**, a change in cytosolic calcium could be measured after addition of 5  $\mu$ M ATP. Still, the peak of fluorescence change was not recorded. Using the quadrant analysis (**Figure 5B**) and histogram analysis (**Figure 5C**) modes, no difference in calcium signals in ATP could be detected due to the transient nature of the calcium signal. However, with selected parts of the trace, the multiple rectangular region analysis mode recorded a 1.85 fold increase in calcium signal immediately after resumed measurement (**Figure 5A**).



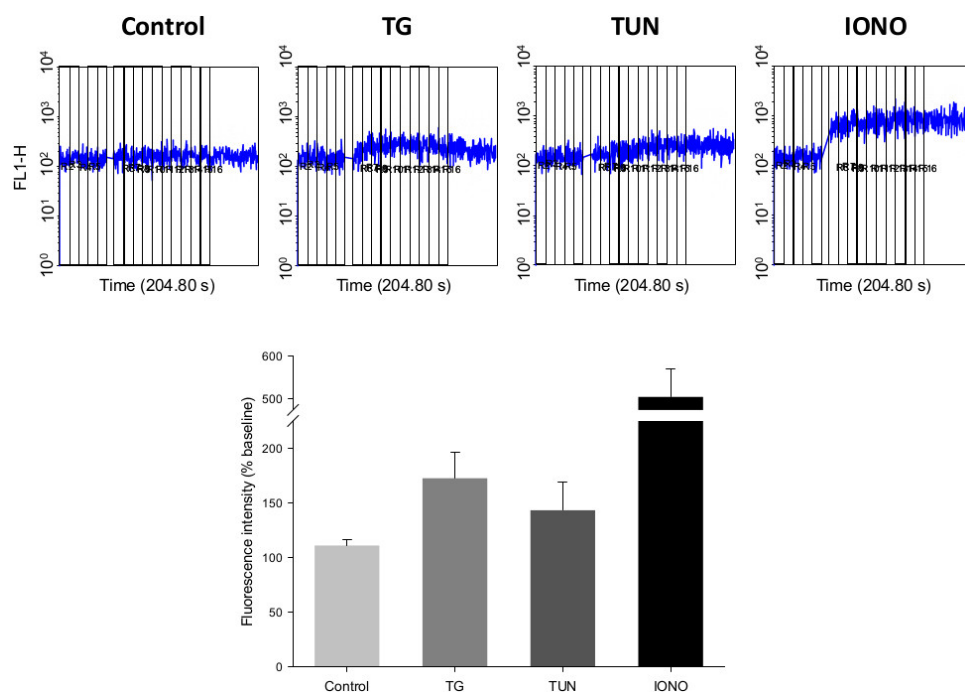
**Figure 1. SSC versus FSC dot plot.** The integrity of the cell population is assessed using side scatter (SSC) and forward scatter (FSC). A region is selected (R1) for further analysis. Dead cells and cell debris, which have reduced light scattering, are excluded. A representative dot plot is shown.



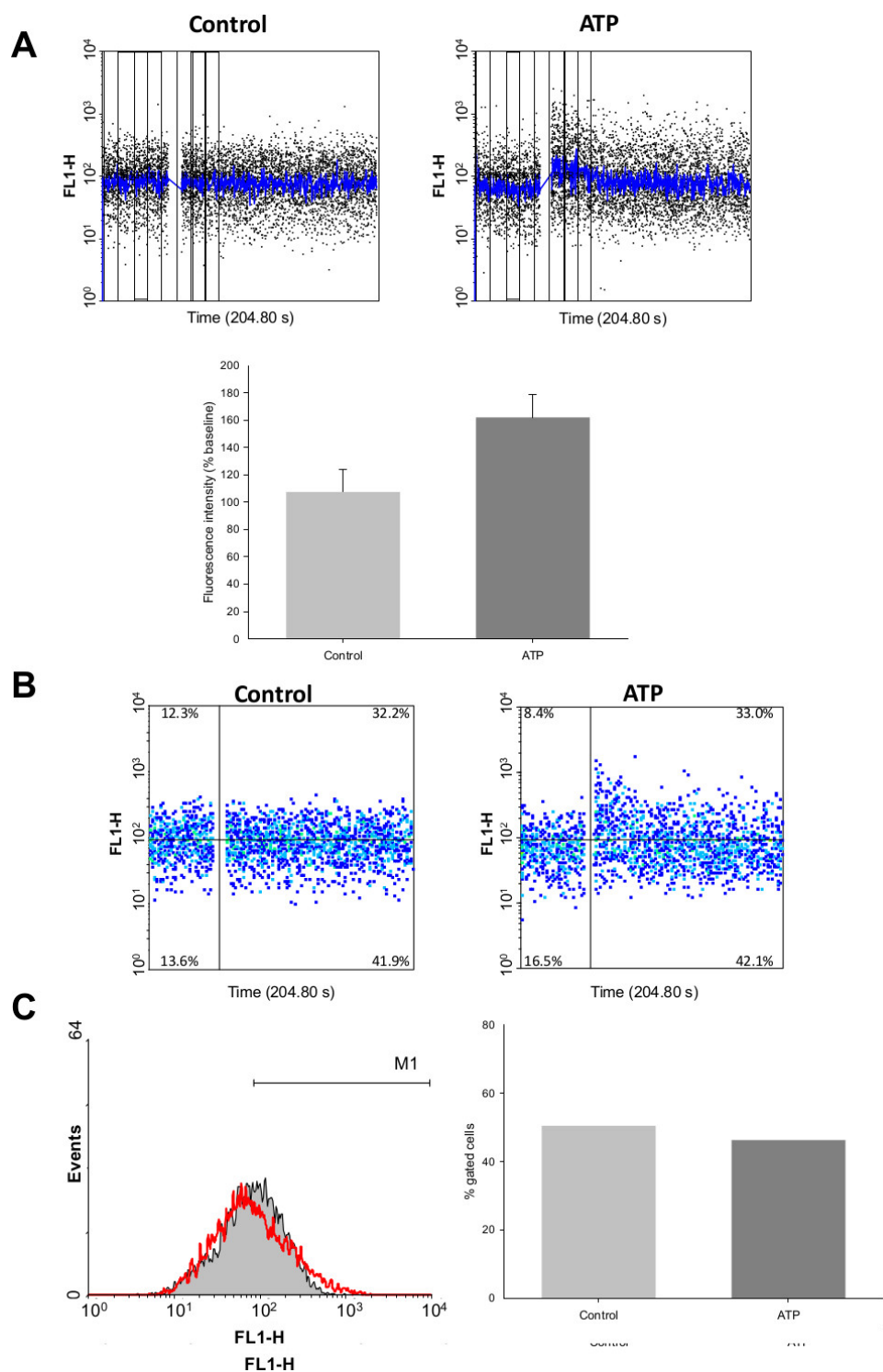
**Figure 2. Quadrant analysis.** Baseline fluorescence of calcium binding dye-loaded WKPT-0293 Cl.2 cells was measured for 50 sec. Measurement was stopped, compound or diluent was added, vortexed to mix and measurement was resumed immediately for a total of 204.8 sec. Quantification was performed using quadrant analysis. [Please click here to view a larger version of this figure.](#)



**Figure 3. Histogram analysis.** Histogram plot of calcium binding dye-loaded WKPT-0293 Cl.2 cells treated with TG, TUN or IONO. Using the marker function, the percentage of gated cells with high calcium binding dye fluorescence intensity was quantified by positioning the starting point of the marker at the peak of the control curve and ending at the maximum fluorescence. [Please click here to view a larger version of this figure.](#)



**Figure 4. Multiple region analysis.** Baseline fluorescence of calcium binding dye-loaded WKPT-0293 Cl.2 cells was measured for 50 sec. Measurement was stopped, compound or diluent was added, vortexed to mix and measurement was resumed immediately for a total of 204.8 sec. Quantification was performed using multiple rectangular region analysis. [Please click here to view a larger version of this figure.](#)



**Figure 5. Analysis of ATP-induced calcium signals.** Baseline fluorescence of calcium binding dye-loaded WKPT-0293 Cl.2 cells was measured for 50 sec. Measurement was stopped, 5  $\mu$ M ATP was added, vortexed to mix and measurement was resumed immediately for a total of 204.8 sec. Data was quantified using multiple rectangular region analysis (**A**), quadrant analysis (**B**) or histogram analysis (**C**). For the multiple region analysis, only the transient calcium signal was analyzed. Representative data or analysis from  $n = 3 - 7$  are shown. [Please click here to view a larger version of this figure.](#)

## Discussion

Calcium is a key event in multiple cellular processes acting as a second messenger signaling molecule and also as a means to communicate between the ER and mitochondria. The ability to measure changes in free cytosolic calcium concentrations is a useful technique that can apply to all areas of cell biology. There are various methods to detect cytosolic calcium signals. Classically, signals from a ratiometric calcium indicator,

such as Fura-2, are monitored in live cells using a fluorescence imaging setup and absolute calcium concentrations can be derived from determining minimum and maximum fluorescence intensities. However, this technique requires specialized equipment, such as a fluorescence microscope, a live cell imaging set up and analysis software. Here, we describe a simple method for calcium detection in the cytosol of adherent epithelial cells using flow cytometry, which is accessible to most laboratories.

Epithelia are in possession of an array of transporters responsible for the transport of metabolites, xenobiotics and drugs. Of particular importance is the SLC22A superfamily, to which organic cation and organic anion transporters belong<sup>22</sup>, and the ATP-binding cassette (ATP) superfamily, which counts the multidrug resistance P-glycoprotein ABCB1 and multidrug resistance proteins (MRPs) amongst its members<sup>23</sup>. Initial experiments using the rat kidney proximal tubule cell line WKPT-0293 Cl.2 were performed as described in the paper by Gergely *et al.*<sup>16</sup>. The cells were brought into suspension and loaded with Fluo-3 AM. However, only a modest change was observed with 10  $\mu$ M ionomycin (38.14% versus 50.95% in control by quadrant analysis or 1.78 fold by multiple region analysis). We hypothesized that epithelial transporters were responsible for the poor loading of the cells with Fluo-3 AM. To circumvent this problem, probenecid and PSC833, inhibitors of organic anion transporters and the multidrug resistance P-glycoprotein, respectively, were applied simultaneously with the calcium binding dye to cell monolayers. Probenecid improved calcium binding dye loading, as indicated by an increase in baseline fluorescence intensity, whereas PSC833 had no effect, which is in contrast to a previous report in Chinese hamster ovary cells<sup>24</sup>. This difference probably lies in the levels of ABCB1 in the different cell lines; the WKPT-0293 Cl.2 cell line harbors a low endogenous level of ABCB1, which can be induced by toxic substances, such as toxic metals<sup>25,26</sup>. The use of probenecid to improve calcium dye loading can be extended to other cell types that exhibit probenecid-sensitive transport systems, such as cells of the nervous system, blood-brain barrier and the liver, where calcium signaling may play a role in intracellular signaling pathways.

The easy detection of calcium binding to Fluo-3 lies in its requirement of only one excitation wavelength (506 nm) and one emission wavelength (525 nm). However, the data generated with single wavelength calcium binding dyes, as a non-ratiometric dye, can only be used to quantify relative increases in cytosolic calcium over non-treated controls. To determine absolute calcium concentrations, a ratiometric dye is necessary because a spectral shift occurs after calcium binding that allows for correction of unequal dye loading and bleaching. The ratiometric dye Indo-1 has been used in detection of calcium mobilization in immunological cells by flow cytometry<sup>27,28</sup> but so far, this has not been performed in epithelial cells. Using the two emission spectra of UV-excitable Indo-1 (400 nm when calcium bound and 475 nm when calcium free), absolute cytosolic calcium concentrations can be determined as described by Grynkiewicz *et al.*<sup>11</sup>.

Our example data employed pharmacological compounds that induce a permanent change in cytosolic calcium. Physiological calcium signals are of low intensity, display fast kinetics and are transient, thus the question remains as to whether the flow cytometry method is sensitive enough to detect physiological calcium signals. Experiments with ATP were performed with the WKPT-0293 Cl.2 cell line, which harbors purinergic receptors as demonstrated by increased calcium signal upon application of ATP (measured by live cell imaging). Using the flow cytometer, increased cytosolic calcium could be observed with 1 - 100  $\mu$ M ATP, but the rapid nature of the signal combined with the delay in resuming measurement after compound addition meant that only a portion of the signal could be recorded. Thus a major disadvantage of the flow cytometry method is its limited capacity to record rapid (<10 sec) and transient calcium signals; rather the method is more suitable for stimuli that give rise to slower transient signals (>10 sec) or persistent elevated calcium signals. These limitations could be circumvented by reducing stimulator concentration or by applying inhibitors for calcium reuptake mechanisms to retain the released calcium in the cytosol but caution must be taken to select an inhibitor concentration that does not increase cytosolic calcium *per se*.

The information acquired by live cell imaging and flow cytometry are quite different. With live cell imaging, the high resolution means that spatial changes in calcium can be measured by selecting intracellular regions of single cells for imaging. Furthermore, fluorescence signals are acquired nonstop every 1 - 2 sec therefore rapid calcium signals can be recorded. In contrast, flow cytometry is more suited for slower and long lasting calcium signals and measures calcium signals from a whole cell population. Only relative changes in calcium signals can be quantified with the flow cytometry method. But, the flow cytometry method has a number of advantages over conventional live cell imaging: 1) high throughput screening of compounds (stimulators and inhibitors of calcium signaling) can be performed; 2) specific imaging expertise is not required; 3) research technicians can perform the measurements; 4) clinical and non-clinical laboratories that do not have the means to perform high resolution imaging and ratiometric calcium measurements can investigate calcium signaling. Taken together, the flow cytometry method is useful if the investigator initially wishes to establish a role for calcium and could easily apply inhibitors to determine upstream and downstream signaling pathways, whereas live cell imaging would be required to characterize the calcium signal (e.g., kinetics, intensity, spatial changes) in further detail.

In summary, we describe a quick, simple and sensitive method for the detection of real-time relative changes in cytosolic calcium in difficult-to-load adherent epithelial kidney cells, which have been brought into suspension. For experimenters without access to a flow cytometry, this method can be easily applied to a spectrofluorimeter with the cell suspension in a cuvette.

## Disclosures

The authors have nothing to disclose.

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