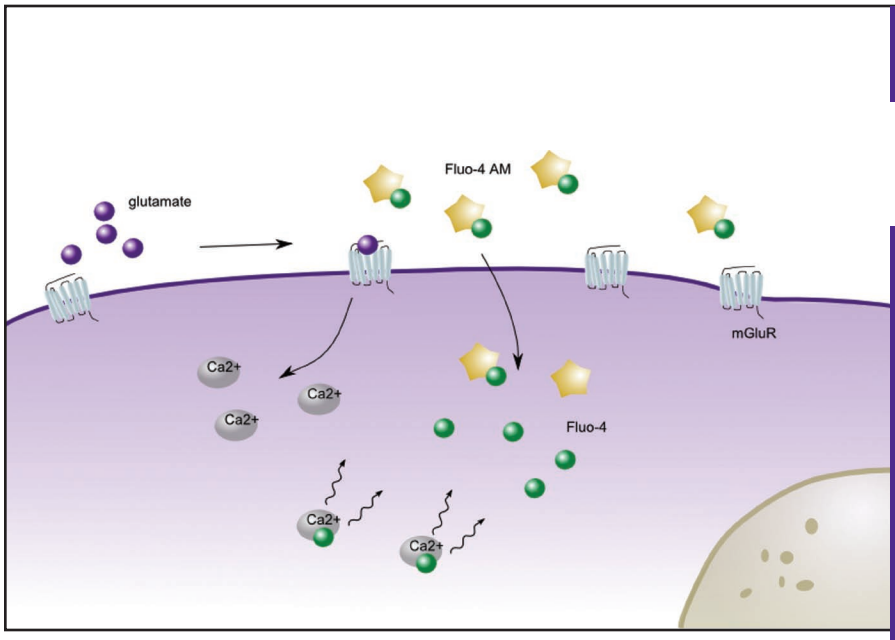

APPLICATION NOTE

Image-based Quantification of Calcium Flux using the Opera

Key Features

- Automated confocal image acquisition of live cells using the Opera™ High Content Screening System
- Tracking fluorescence intensity changes of individual cells in a time series of images
- Image analysis using the versatile Acapella™ **Kinetic Intensity Analysis** script

Activation of GPCR, Calcium Release

Background

G protein-coupled receptors (GPCRs) comprise one of the largest protein families encoded by the human genome and are a target for approximately 40% of all approved drugs [Eglen *et al*., 2007]. GPCRs sense molecules outside the cell and activate intracellular signal transduction pathways which ultimately lead to different cellular responses, most of which are linked to calcium signaling. Consequently, there are a number of cell-based, high throughput screening (HTS) assays used for drug discovery that assess changes in the intracellular Ca^{2+} as a functional readout of GPCR activation [Filmore, 2004].

Metabotropic glutamate receptors (mGluRs) are GPCRs that bind to glutamate, an amino acid that functions as an excitatory neurotransmitter, and are targets for the treatment of neurological disorders [Byrnes *et al*., 2009].

Most GPCRs have been cloned and can therefore be routinely expressed in immortalized cell lines. For the application described here, a stable CHO-K1 cell line expressing an mGluR under the control of an inducible promoter was used for HTS calcium flux studies to identify compounds that modulate mGluR activity.

Application

A CHO-K1 cell line stably expressing an mGluR under the control of an inducible promoter was used in this cell-based calcium flux assay. The intracellular calcium mobilization due to glutamate-induced GPCR activation was detected using the Ca^{2+} -responsive dye, Fluo-4. The Fluo-4 calcium indicator is essentially non-fluorescent in the absence of Ca^{2+} and shows a very large fluorescence intensity increase upon binding to Ca^{2+}. The specificity of this signal was verified by adding the receptor-specific antagonist 6-methyl-2-(phenyl-ethynyl)-pyridine (MPEP) to the assay system. Cells were

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seeded at a density of 2.5 x 10^4 cells / well in 384 well CellCarrier™ plates, diluted in growth medium containing 1 µM dexamethasone to induce receptor expression, and were incubated overnight. Cells were then incubated with 2 µM Fluo-4 and with varying concentrations of MPEP. The nuclei were stained with 16.23 µM Hoechst 33342. For image acquisition we used the Opera QEHS equipped with a dispenser unit and the 20x water immersion objective. To study calcium flux kinetics, 25 images were captured within 60 sec. Directly after the first exposure, 20 µl glutamate (final concentration of 17 µM) was dispensed to initiate receptor activation (Figure 1). The images were analyzed with the Acapella **Kinetic Intensity Analysis** script. This is designed for tracking fluorescence intensity changes in a time series of images, analyzing intensity changes over time in individual cells, determining subpopulations of cells e.g. "active" vs. "inactive" cells (Figure 2) and monitoring or rather correcting image shifts in the time series.

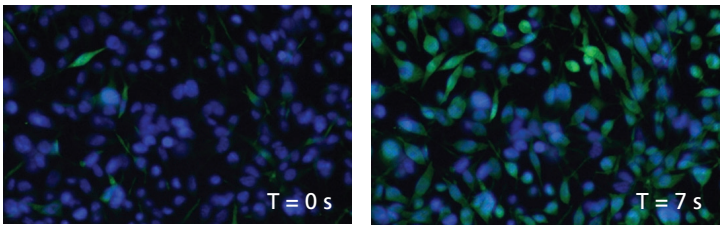

Figure 1. False color overlay of Hoechst 33342 nuclear stain (λ_{EX} = 365 nm, blue) and Fluo-4 Ca^{2+} indicator (λ_{EX} = 488 nm, green) at time zero (left panel) and 7 sec after dispensing glutamate (right panel) when incubated without MPEP. The Fluo-4 fluorescence intensity of most of the cells can be seen to clearly increase after 7 sec.

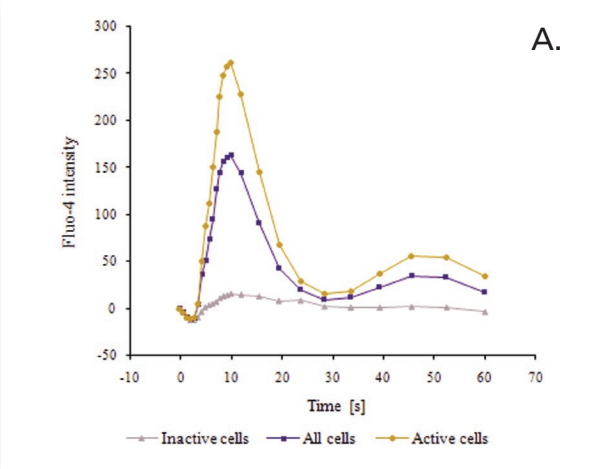

Figure 2. A | Time course of Fluo-4 mean fluorescence intensity for the whole population (purple) and for the active (yellow) and inactive (gray) subpopulations incubated without MPEP. The intensity was averaged for each time point, over all the cells in each group (231 cells in total). Classification of cells into the two subpopulations was achieved by introducing a peak intensity threshold at 150. B | An example dose response curve generated from 3 replicate measurements. A significant decrease in the percentage of active cells is observed with increasing concentrations of MPEP.

Conclusions

GPCRs are among the most heavily investigated drug targets in the pharmaceutical industry. The High Content Analysis application presented here is a useful and powerful tool for an image-based quantification of drug induced GPCR activation. To analyze fluorescence intensity changes in individual cells in a time series of images we have used the versatile **Kinetic Intensity Analysis** script. This Acapella script is capable of determining subpopulations of cells based on their calcium response. In pharmacological analysis this approach will decrease the number of false positives identified compared to systems that average the response across the entire population within a well.

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