

Label-Free Cell-Based Pharmaceutical Screening Assay Cholinergic Receptor Agonist

Summary

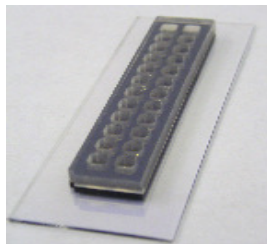
Label-free kinetic analysis of dose-response to drug challenge with carbachol, a cholinergic receptor agonist, was demonstrated in adherent hamster cells. Concomitantly, LFIRE enables real-time imaging of the localized morphological changes during drug exposure.

Background

Cell based screening assays are used to study receptor signaling, cytotoxicity, adhesion, proliferation, migration, and motility. G-Coupled Protein Receptors (GPCRs) constitute 30-50% of pharmaceutical targets, and in the US alone, cell-based screening for them comprises a \$170 million dollar reagent business. LFIRE quantitatively measures and images the “footprint” of cells, which changes dynamically in response to environmental stimuli, thereby providing a simple, sensitive, and economical high-content assay system for these critical and growing applications.

Method and Protocol

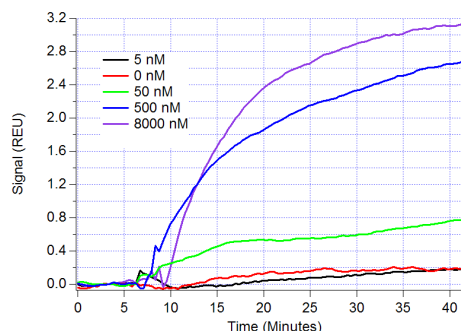
Chinese Hamster Ovary (CHO)-M1 cells, which bear an engineered g-protein-coupled receptor (GPCR) for muscarinic acetylcholine agonists, were grown 24-48 hours, to 80% confluence¹. Growth buffer was replaced with a serum-free assay buffer and cells equilibrated one hour at room temperature. Images of the cell footprint were taken at 30 second intervals, 1x magnification, 7.5 microns per pixel, for a 5 minute baseline. Cells were dosed with carbachol and observed for thirty minutes. 14-bit cellular footprint images were recorded and regions of interest were extracted for statistical analysis.



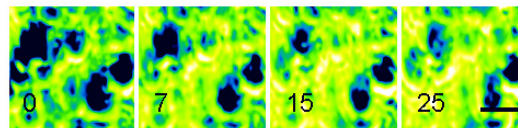
Proprietary sterile 24 micro-well slide-based disposable cell culture vessel. 96-well microplates in development.

¹ Cell culture services courtesy of Stewart Lebrun.

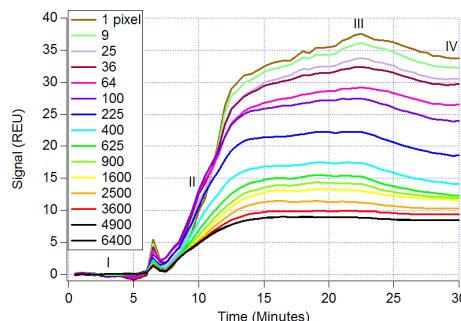
Results



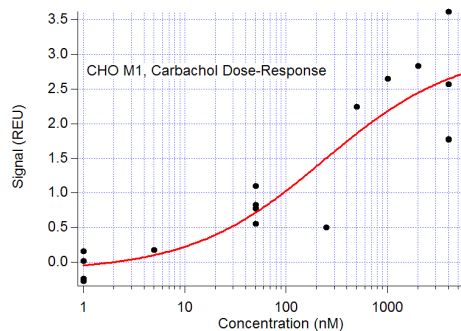
Time-resolved CHO-M1 dose response to carbachol treatment. Differential kinetic data for 0--8000 nanomolar, at 1x magnification, 7.5 microns per pixel, single 625 pixel region of analysis per well.



Cell footprint images at 5x, at 0, 7, 15, and 25 minutes in response to 500nM carbachol. Bright regions represent cell-surface contact. Scale bar 20 microns.



Time-resolved analysis of different-sized areas in a single well. Inhomogeneous cell footprint coverage and averaging effect of increased area.



Dose-Response. Calculated EC₅₀ 400 nM.