

Automated Measurement of Agonist / Antagonist Mediated Intracellular Ca^{2+} Changes at the Single Cell Level Using the Cellular Imaging Workstation

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- Fully Automated CO_2 incubation and delivery of cell assay plates to the Thermo Scientific Cellomics® ArrayScan VTI

- On Board Liquid Handling Module with “read while dispense” capability

- On board Live Cell Chamber in Reader

- Automated computation of rate constants

- Multiple targets capability

Introduction

Changes in intracellular Ca^{2+} levels play an important role in several physiological processes including gene regulation, cell proliferation, neuronal transmission, muscular contractions and apoptosis (1, 2). Typically, changes in intracellular Ca^{2+} happen within a few seconds after the event that acts as the trigger—such as the binding of an agonist to its receptor. Thus, quantitating Ca^{2+} changes in cells require reagents and instruments that allow instantaneous measurements on cells following agonist addition.

Fluorescent dyes that change their excitation and/or emission characteristics upon Ca^{2+} binding are the reagents of choice for such measurements (3). The combination of such dyes used in fluorescent imaging systems with an automated liquid handling device that allow rapid dispensing and read while dispensing capability, allow for successful quantitation of Ca^{2+} flux in cells. These types of systems allow for set up of rapid screening systems to screen for agonists and antagonists

of Ca^{2+} responses (4). Unfortunately, the output from these HTS systems is a mixture of responses from the cell population. In order to better understand compound pharmacology, quantifying the individual responses of cells would be an advantage. Here we show the use of the Thermo Scientific Cellular Imaging Workstation (CIW) together with the Liquid Handling and Live Cell Modules on the Thermo Scientific Cellomics ArrayScan VTI to measure carbachol mediated Ca^{2+} changes in cells over expressing the rat M1 muscarinic acetylcholine receptor. This benchtop system is designed to provide the capacity to complete confirmatory and secondary screening of active compounds from HTS screening campaigns.

Materials and Methods

- M1WT3 cells (ATCC CRL -1985) were plated at 5,000 cells per well in 96 well plates and cultured overnight.
- Load plate into Thermo Scientific Cytomat® C2 incubator.

- Thermo Scientific CataLyst Express robot removes plates from Cytomat, de-lids plates, and loads plates into ArrayScan VTI Live Cell Module.
- Liquid Handling Module aspirates and discards media and delivers 100 μL /well of Fluo-4NW (5) with a 25 μL air gap and then 50 μL of antagonist (100 μM Pirenzepine) or control treatment.
- CataLyst Express re-lids and returns plates to Cytomat for 30 minute incubation at 37°C.
- CataLyst Express removes plates from Cytomat, de-lids, and reloads into the ArrayScan.
- Acquire baseline images before adding 50 μL of agonist (0.1 μM Carbachol).
- Use the read through dispense feature to image each well for a total of 1 minute, with approximately two images acquired every second.
- Analyze images ‘on-the-fly’ with the Target Activation.V3 BioApplication.
- Visualize data using the Thermo Scientific Cellomics View application.

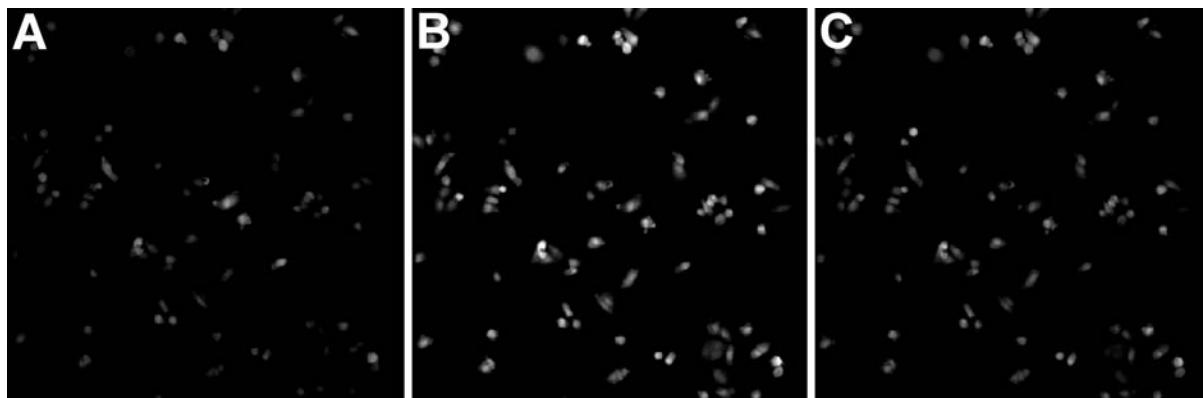
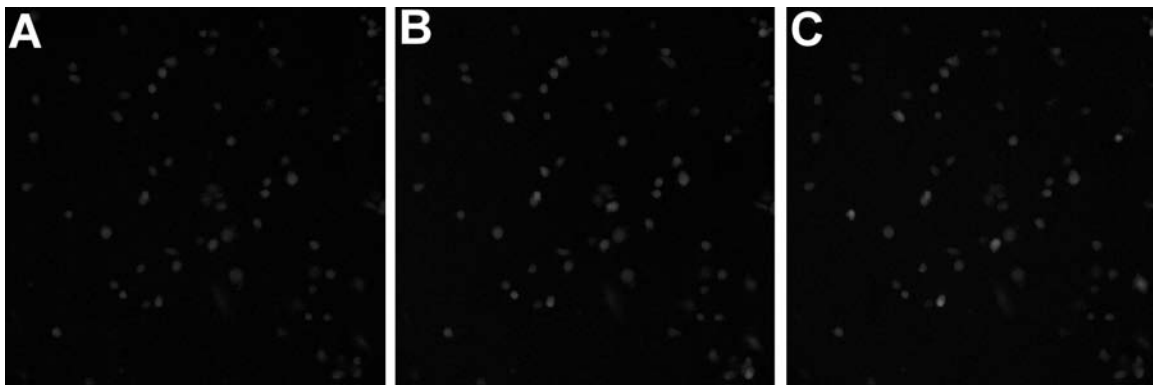


Figure 1. M1WT3 cells pre-incubated with HBSS only and treated with 0.1 μM carbachol: M1WT3 cells were imaged as described above in Materials and Methods. Panels A, B and C are images of cells just before (A), 6 sec (B) and about 30 sec (C) after addition of media into a well in a 96 well micro-plate using the CIW. Images were captured using a 10x objective on an ArrayScan VTI Live Cell Module.



Results

- The Cellular Imaging Workstation allows for automated compound addition, incubation and stimulation with agonist along with read through during agonist addition for measuring Ca^{2+} mobilization.
- Liquid Handling Module allows for automated delivery of antagonists or agonists to wells. Also, we could image and analyze the data as the agonist was being dispensed into the wells.
- Read through dispense allows faster image acquisition and data analysis providing valuable early kinetics data.
- The Live Cell Module keeps cells in a physiological environment and maintains cell viability for experimental duration.

Conclusions

- The CIW provides a fully automated method for compound addition, incubation and assaying capability for Ca^{2+} mobilization assays.
- Pirenzepine mutes the response of Ca^{2+} mobilization.

Figure 2. M1WT3 cells pre-incubated with 100 μM pirenzepine for 30 min and then stimulated with 0.1 μM carbachol. M1WT3 cells were imaged as described above in Materials and Methods. Panels A, B and C are images of cells just before (A), about 6 sec (B) and about 30 sec (C) after addition of 100 μM carbachol into a well in a 96 well micro-plate by using the CIW.

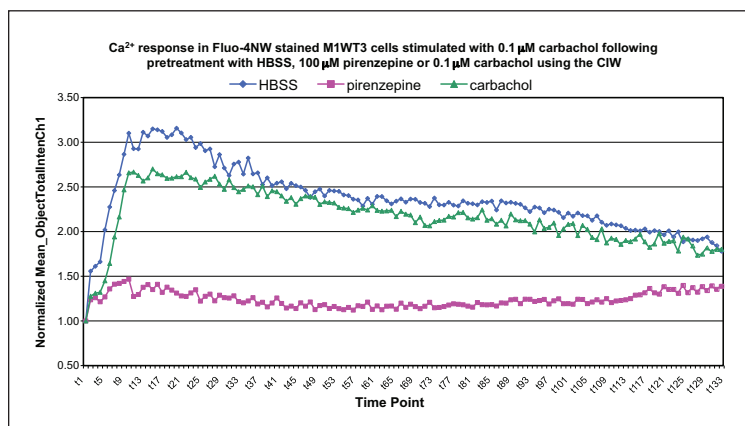


Figure 3. Ca^{2+} mobilization in M1WT3 cells pre-incubated for 30 min with either HBSS, 10 μM carbachol or 100 μM pirenzepine. The graph represents changes in Fluo-4 intensity as a function of time of individual cells in a well stimulated with carbachol. All 3 wells were dosed with 0.1 μM carbachol following 30 minutes of pretreatment.

- Pre-treatment of cells with carbachol (agonist) alleviates the Ca^{2+} response on later challenge with carbachol.
- Read through dispense enables quantitation of early rapid kinetic features on-the-fly in receptor mediated Ca^{2+} mobilization.
- Individual cell level response is also quantitated in the Live Cell Module. This could allow sub-population analysis of Ca^{2+} responses.
- With the CIW, fully automated measurement of Ca^{2+} mobilization is possible, post cell plating in 96 well microplates.

References

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