Cellular Informatics Analysis of High Content Screening Data

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Abstract

The AcuityXpress™ cellular informatics software platform provides data visualization and statistical tools for analyzing high content assay data. Here, we assessed the utility of this platform for the analysis of screening data. Specifically, the LOPAC library (Library of Pharmacologically-Active Compounds, Sigma) was used to screen for agonists or antagonists of the beta-2-adrenergic receptor using the Transfluor® high content assay for GPCR activation. Images were collected and analyzed using MetaXpress™ image acquisition and analysis software. Here, we show how the informatics tools in AcuityXpress™ were used to assess the quality of the dataset and identify hits, outliers and false positives. The hits identified with AcuityXpress were consistent with the expected biology of the beta-2-adrenergic receptor.

Introduction

High-content screening provides detailed, multi-parametric cellular data, enabling the use of many new cell-based assays in drug discovery. However, the size and nature of high-content data require new paradigms in data management and data mining. In this study, we investigated whether database-driven statistical data-mining methods could aid in identifying hits and trends in compound library screens. The Transfluor high-content assay for GPCR activation was used to identify agonists and antagonists for the beta-2-adrenergic receptor from the LOPAC compound library. An additional module was used to analyze the same images for cell cycle-specific effects, demonstrating the utility of multiplexing assays for both QC and research purposes.

Materials and Methods

Day 1: Plated β2AR (WT)-expressing Transfluor-enabled pit-forming U2OS cells.

Day 2:

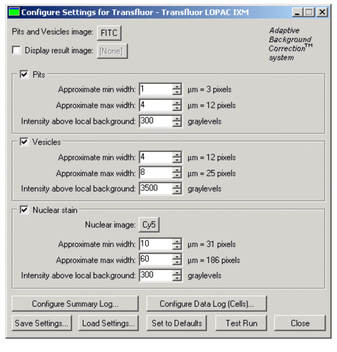
Agonist screen
- Added test compounds for 30 minutes

Antagonist screen
- Added test compounds for 30 minutes
- Added isoproterenol for 30 minutes

All screens
- Fixed cells and added DRAQ5 nuclear stain
- Imaged plates at 20X on the ImageXpress^{MICRO} imaging system
- Analyzed images in MetaXpress with the Transfluor and Cell Cycle application modules
- Performed statistical analyses in AcuityXpress

Figure 1: Image analysis with the Transfluor Module

Figure 1. Configuring Settings with the Transfluor module in MetaXpress for detection and measurement of pits, vesicles and nuclei.



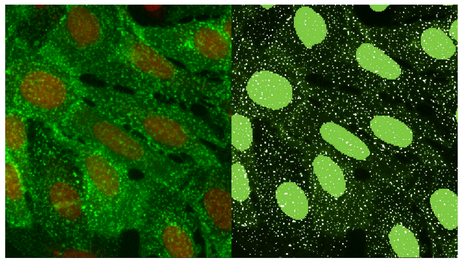


Figure 2: Plate Heat Maps

Figure 2a. AcuityXpress plate view of pit counts per cell in representative plate from β2AR agonist screen (controls in columns 23-24).

Figure 2b. AcuityXpress plate view of pit counts per cell in representative plate from β2AR antagonist screen (controls in columns 23-24).

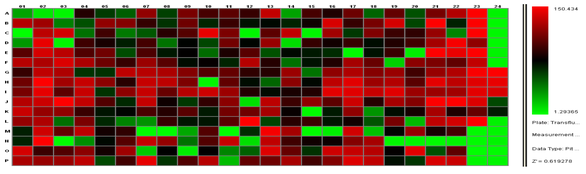


Figure 3: Analysis of Agonist Screen

Figure 3a. Self-Organizing Map analysis in AcuityXpress identifies clusters of compounds by phenotypic profile. The highlighted cluster represents hits in the β2AR agonist screen. Results were confirmed by reviewing original images. Other profiles represent compounds which are inactive or demonstrate alternative phenotypes (significant morphological effects which may be interpreted as vesicle formation)

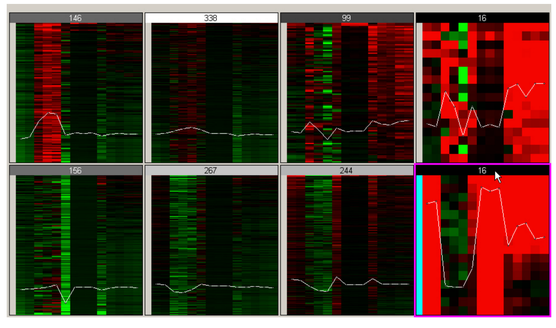


Figure 3b. Principal Component Analysis (6 components) identifies outliers. Positive hits in the β2AR agonist screen are highlighted in blue. Results were confirmed by reviewing original images.

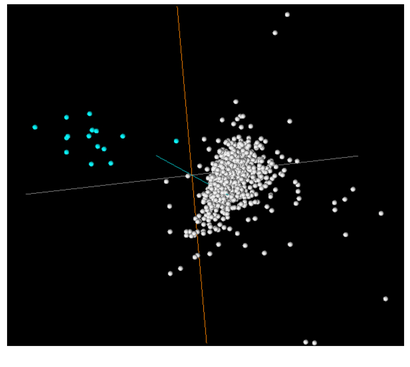


Figure 4: Analysis of Antagonist Screen

Figure 4a. Self-Organizing Map analysis in AcuityXpress identifies clusters of compounds by phenotypic profile. The highlighted cluster represents hits in the β2AR antagonist screen. Results were confirmed by reviewing original images. Other profiles represent compounds which are agonists, inactive or demonstrate alternative phenotypes.

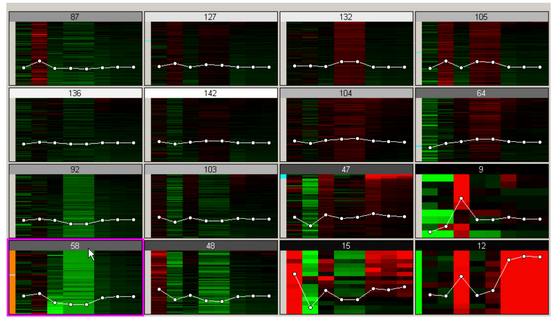


Figure 4b. Principal Component Analysis (6 components) identifies outliers. Positive hits in the β2AR antagonist screen are highlighted in orange. Compounds that enhanced GPCR signaling resulting in vesicle formation are highlighted in green. Results were confirmed by reviewing original images.

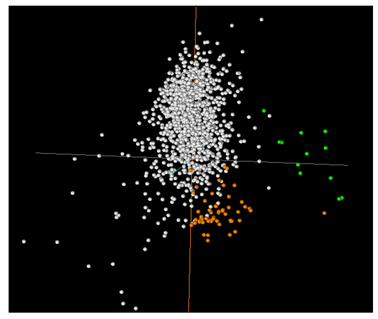


Table 1: Hits from β2AR Agonist Screen

Table 1. The top hits and associated compound descriptions identified by AcuityXpress from the β2AR agonist screen. Compounds which also demonstrated an unexpected activation phenotype in the antagonist screen (vesicle formation in a pit-forming cell line when in the presence of 50 nM isoproterenol) are shown in red.

(-)-alpha-Methylnorepinephrine	Active enantiomer; adrenoceptor agonist; vasoconstrictor; antihypertensive
(-)-Epinephrine bitartrate	Endogenous hormone and neurotransmitter
(-)-Isoproterenol hydrochloride	beta-Adrenoceptor agonist; increases cytosolic cAMP
(±)-Epinephrine hydrochloride	Adrenoceptor agonist
(±)-Isoproterenol hydrochloride	Sympathomimetic amine acting almost exclusively on beta adrenoceptors; bronchodilator
(±)-Norepinephrine (+)bitartrate	Adrenergic neurotransmitter
E-64	Irreversible inhibitor of cysteine proteases
Fenoterol hydrobromide	Beta2-adrenoceptor agonist; bronchodilator
Formoterol	beta2-Adrenoceptor agonist
Hemicholinium-3	Potent and selective choline uptake blocker
Isoxtharine mesylate	beta-Adrenoceptor agonist; bronchodilator
L(-)-Norepinephrine bitartrate	Adrenergic neurotransmitter; vasoconstrictor
Metaproterenol hemisulfate	beta2-Adrenoceptor agonist
N-Methyldopamine hydrochloride	Dopamine receptor agonist
R(-)-Isoproterenol (+)-bitartrate	Sympathomimetic amine acting almost exclusively on beta adrenoceptors; bronchodilator; active enantiomer of Isoproterenol
Terbutaline hemisulfate	beta-Adrenoceptor agonist; bronchodilator

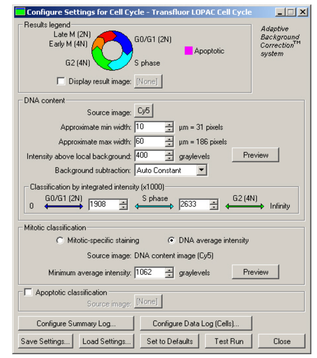
Table 2: Hits from β2AR Antagonist Screen

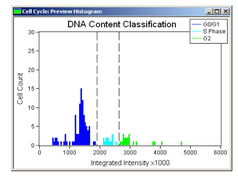
Table 2. The top hits and associated compound descriptions identified by AcuityXpress from the β2AR antagonist screen.

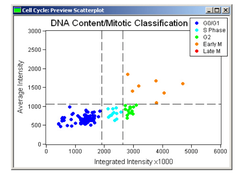
Xamoterol hemifumarate	Specific beta1-Adrenoceptor specific partial agonist
(R)-(-)-DOI hydrochloride	Selective 5-HT2 serotonin receptor agonist
(±)-CGP-12177A hydrochloride	Mixed beta adrenoceptor agonist/antagonist
1,3-dihydro-2H-benzimidazol-2-one hydrochloride	
(±)-Propranolol hydrochloride	beta Adrenoceptor antagonist; cardiac depressant (anti-arrhythmic)
(±)-Sotalol hydrochloride	Potent beta-adrenoceptor antagonist, a class III antiarrythmic; prolongs the action potential and increases the refractory period
(S)-(-)-propafenone hydrochloride	beta-Adrenoceptor blocker; class 1C antiarrythmic agent
(S)-Propranolol hydrochloride	Active beta-adrenoceptor receptor blocking enantiomer; 5-HT1 serotonin receptor antagonist
1-(1-Naphthyl)piperazine hydrochloride	5-HT2 serotonin receptor antagonist
3-alpha,21-Dihydroxy-5-alpha-pregnan-20-one	Positive allosteric modulator of GABA-A receptors
6-Nitroso-1,2-benzopyrone	Poly(ADP-ribose) polymerase (PARP) ligand which preferentially destabilizes one of the two zinc-fingers inactivating the enzyme
Alprenolol hydrochloride	beta Adrenoceptor antagonist
Ammonium pyrrolidinedithiocarbamate	Prevents induction of nitric oxide synthase (NOS) by inhibiting translation of NOS mRNA
Basal	
beta-Lapachone	Induces apoptosis in HL-60 cells; anticancer agent
Betaxolol hydrochloride	Selective beta1 adrenoceptor antagonist

Figure 5: Multiplexing: Cell Cycle Analysis

Figure 5a. Configuring Settings with the Cell Cycle module in MetaXpress for identifying the cell cycle phase of individual cells based on DNA content.







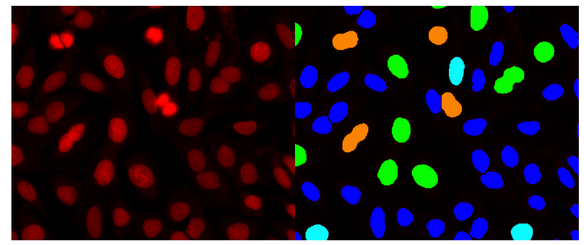
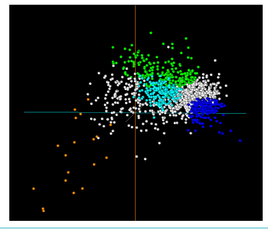
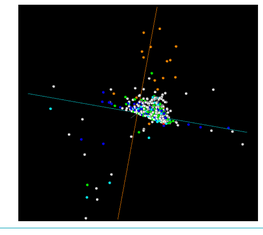


Figure 5b. The Cell Cycle application module measured profiles based on cell cycle status. Compounds associated with a higher percentage of cells in G1 (dark blue), S (light blue), G2 (green) or M (orange) were colored appropriately. Left, principal component analysis of the cell cycle profiles from the β2AR agonist screen. Right, principal component analysis of pit and vesicle phenotypes from the β2AR agonist screen. The M-phase specific compounds appear to show higher vesicle formation, but review of the original images show that these particular cells demonstrate specific morphological changes which were interpreted as vesicle formation.

Cell Cycle phenotype



Pit/Vesicle phenotype



Conclusion

AcuityXpress informatics software was used to analyze high-content data from two compound library Transfluor screens run on ImageXpress^{MICRO}.

AcuityXpress easily identified the expected hits from both an agonist and antagonist screen. In addition, it provided tools to QC the data and also identified additional phenotypes induced by certain compounds. For example, certain agonists were found to have a synergistic effect on vesicle formation when combined with the reference agonist isoproterenol.

The nuclear staining was used for additional, multiplexed analysis with the Cell Cycle module. Principal component analysis demonstrated that multiplexing helps identify false positives (compound-induced morphological changes) while also showing that there was no significant correlation between cell cycle status and the receptor-stimulated pit phenotype measured in the Transfluor assay.

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