Analysis of GPCR Signaling Events Using HCS Technology

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Abstract

We have developed GPCR software modules for the Discovery-1™ and ImageXpress™ HCS platforms for analysis of GPCR activation. The Discovery-1™ software module uses Adaptive Background Correction™ (ABC) technology to segment cells from the background and handle uneven staining of samples. Recently, Norak Biosciences introduced the Transfluor® Assay, a universal assay for GPCR desensitization using cells expressing GFP-tagged β-arrestin. Upon GPCR activation, β-arrestin translocates from the cytosol to the plasma membrane, binding the receptor and targeting it for internalization into coated pits and endosomal vesicles. The Discovery-1 and ImageXpress GPCR analysis modules successfully detect β-arrestin translocation as a function of GPCR activation. The rapid acquisition and analysis capabilities of the Discovery-1 and ImageXpress systems combined with the universality of the Norak Transfluor® Assay provide a uniquely powerful approach for screening GPCR modulators.

Introduction

G-protein coupled receptors form the majority of pharmaceutical targets, and as a result, assays for detecting GPCR receptor agonists/ antagonists play a prominent role in screening operations. Here we have demonstrated the capabilities of the Discovery-1 and ImageXpress® GPCR analysis modules using the Norak Transfluor Assay. Norak pit-forming or vesicle-forming cells were treated with varying concentrations of isoproterenol, or with varying concentrations of propranolol in the presence of isoproterenol stimulation. Images were acquired and analyzed by the Discovery-1 and ImageXpress systems. Images acquired with the ImageXpress system were analyzed by both modules for comparison.

Materials and Method

1. Norak cells were plated on Corning Costar 384-well plastic imaging plates and incubated overnight at 37° C, 5% CO_2.
2. **Agonist Dose Response**: Varying concentrations of isoproterenol were added to one set of wells (6-well replicates).
3. **Antagonist Dose Response**: Varying concentrations of propranolol were added to another set of wells in the presence of 50 nM isoproterenol (6-well replicates).
4. Treated cells were incubated at 37° C, 5% CO_2 for 40-45 minutes (vesicle formers) or 30 minutes (pit formers).
5. Cells were fixed in 2% formaldehyde (45 min, RT), washed, stained with 0.05 mg/ml DAPI (15 min, RT), washed.
6. Images were acquired and analyzed with the ImageXpress 5000A system or the Discovery-1 system as indicated.

Figure 1: Norak Assay

Figure 1. Role of β-arrestin in model of GPCR desensitization and resensitization. (1) Agonist-activated GPCR's are phosphorylated by GRK's on their Carboxyl-terminal tails. (2) Arrestin (Arr) translocates to and binds the agonist occupied, GRK-phosphorylated receptors at the plasma membrane. (3) Arrestin targets the desensitized receptors to clathrin coated pits for endocytosis. (4a) Some receptors bind arrestin with low affinity and dissociate from arrestin at or near the membrane. (5) These receptors internalize without arrestin into endocytic vesicles and recycle rapidly. (4b) Other receptors bind arrestin with high affinity and remain associated with arrestin such that the receptor-arrestin complex internalizes as a unit into endocytic vehicles. (6) These receptors recycle slowly.

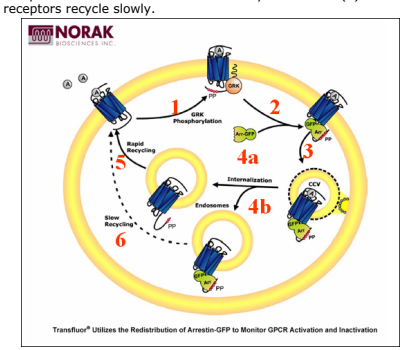


Figure 2: Images of the Norak Assay

Figure 2a. Cells imaged using Discovery-1. Norak cells were stimulated with isoproterenol. Top left: control, top right: pits, bottom: vesicles.

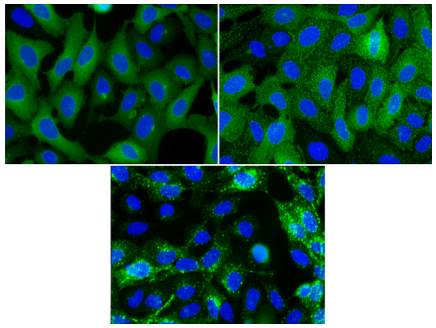


Figure 2b. Cells imaged using ImageXpress. Norak cells were stimulated with isoproterenol. Top left: control, top right: pits, bottom: vesicles.

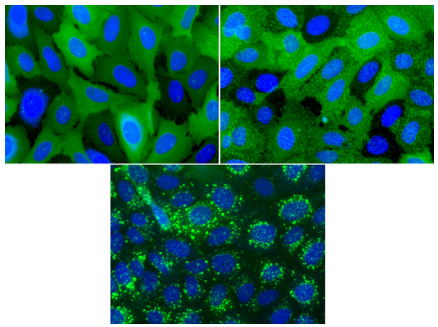


Figure 3: GPCR Module in Discovery-1

Figure 3. Interface of the GPCR module in Discovery-1.

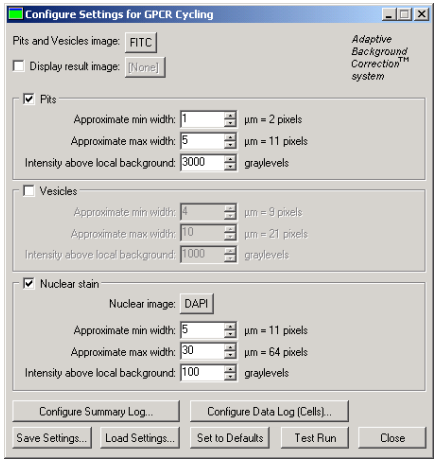


Figure 4: Analysis Overlay

Figure 4a. Pits top left: 0.026 nM, top right: 3.2 nM, bottom left: 16 nM, bottom right: 50000 nM (concentrations refer to isoproterenol). Green: detected nuclei, white: detected pits.

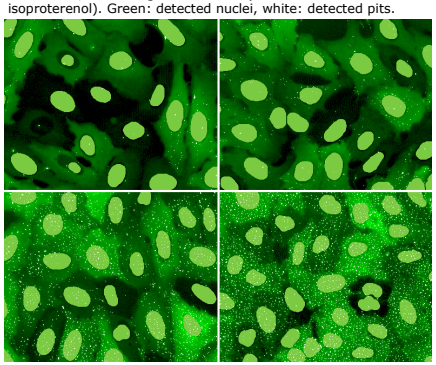


Figure 4b. Vesicles top left: 0.026 nM, top right: 3.2 nM, bottom left: 16 nM, bottom right: 50000 nM (concentrations refer to isoproterenol). Green: detected nuclei, red: detected vesicles.

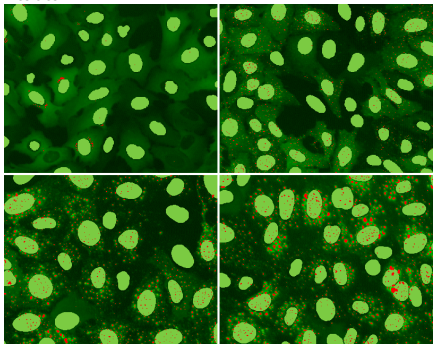


Table 1: Summary of Isoproterenol EC_{50} (nM)

	ImageXpress	Discovery-1
Pit	12.0 ± 7.1	13.6 ± 7.2
Vesicle	2.3 ± 1.0	6.5 ± 2.6

Table 2: Summary of Propranolol IC_{50} (nM)

	ImageXpress	Discovery-1
Pit	3.9 ± 1.1	5.8 ± 3.0
Vesicle	11.0 ± 3.1	4.7 ± 0.8

Table 1 and 2. Results from Discovery-1 and ImageXpress (3 independent experiments for pits, 4 independent experiments for vesicles) with standard error.

Figure 5: Dose Response Curves

Figure 5a. Pit dose response to isoproterenol. Error bars show standard error.

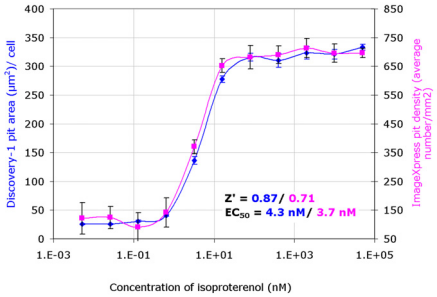
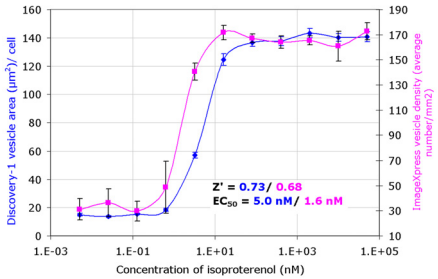


Figure 5b. Vesicle dose response to isoproterenol. Error bars show standard error.



Conclusion

The Discovery-1 and the ImageXpress 5000A high-content screening systems rapidly acquire high quality images of GPCR-activated cells. Norak Transfluor® assay images acquired and analyzed by the Discovery-1 and ImageXpress systems provide dose-response data consistent with each other and with results reported by Norak Biosciences. Both systems produced robust Z' values for agonist and antagonist dose-response curves.

The Discovery-1 and ImageXpress GPCR analysis modules provide quantitative analysis of vesicle- and pit-formation as demonstrated by the Norak Transfluor® assay. Both systems provide a rapid, robust solution for screening for GPCR receptor agonists and antagonists.

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