

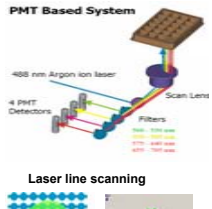
Abstract

The Acumen Explorer, a laser-based fluorescent microplate cytometer, is a key tool in the development and execution of high content screening assays. This instrument can monitor up to four fluorescent signals from objects contained in microplates with well densities as high as 1536/plate. The instrument can measure changes in fluorescent protein expression/distribution as well as gross cell morphology parameters in live or fixed cells. As such, this technology can address diverse areas of biology using either fluorescent proteins or dyes for detection. We describe here three assay types to illustrate the process of developing laser-scanning microplate cytometry assays. The first is a functional assay for the beta-adrenergic receptor that employs a protein fragment complementation assay (PCA) utilizing a bifurcated YFP as the reporter (Odyssey Thera). The second is an assay to measure proteasome activity using a ubiquitinated GFP reporter (Biomeq). The third is an assay employing *Drosophila* cells where the accumulation of lipid droplets is measured upon staining the cells with fluorescent dyes. Each of these assays were optimized for 1536-well screening using the Acumen Explorer for detection and then tested using the NCGC screening approach where compounds are screened at multiple concentrations (qHTS). The beta-adrenergic receptor PCA-based assay underwent a qHTS against the LOPAC collection where known adrenergic receptor agonist and antagonists were identified, thus validating that this system can identify pharmacological relevant compounds. A qHTS of 70K compounds was achieved for the proteasome assay that involved the Kalypsys robotic screening system. Finally, the assay for lipid droplet accumulation, we used both a fluorescence lipid droplet stain (BODIPY 493/503) and a fluorescent cytoplasmic stain (Invitrogen, CellTracker) to develop a live cell assay for lipid formation following feeding with oleic acid. Triacsin C, a potent inhibitor of long-chain fatty acyl CoA synthetase, was used as an antagonist reference compound for this last assay.

Acumen Explorer: Plate-based subpopulation distribution analysis for HTS

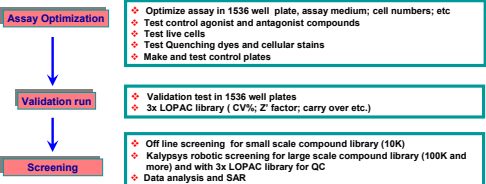
Key Features

The Acumen Explorer collects data by focusing a laser excitation beam on the bottom of the microtiter plate and collecting the resulting epifluorescence using photo multiplier tubes (PMT) for specific wavelength ranges.



The amount of image data saved is determined by the need of the process. For screening, HTS mode is used to collect only the critical assay parameters and saved as a .csv file. The laser can be set to read at high resolution in an assay development mode and the resulting images can be seen on the right. As plate data file size is kept small, microplate cytometers facilitate HTS of large chemical libraries against various cell-based assays as large-scale storage of image files is not required.

Basic steps in 1536-well assay optimization and screening



Summary

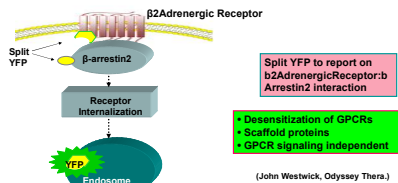
Optimal assay performance with the Acumen Explorer requires reducing background fluorescence, maximizing signal, and choosing the proper cell density and object characteristics. For PCA format, we found that the fluorescence-absorbing dye naphthol blue black, can reduce background fluorescence. This provides an alternative method to reduce background fluorescence when weakly adherent cells are used and cell washing is not possible.

Microtiter plate laser scanning cytometry is a powerful technology enabling HT cell population analysis in 1536-well plates. In the lipid droplet subproteome assay, we found two sets of fluorescence dyes, BODIPY 493/503 and CellTracker™ Red CMTPX, aided in the analysis subcellular fluorescent objects where both the relevant biology as well as cell health and total cell numbers are simultaneously measured.

Acknowledgements

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Protein Fragment Complementation (PCA) Assay Principle



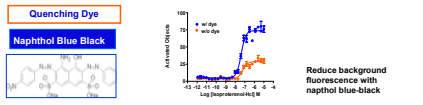
PCA with Acumen: β 2 adrenergic receptor: β Arrestin2 interaction

Stimulation Protocols for Screening (1536 format)

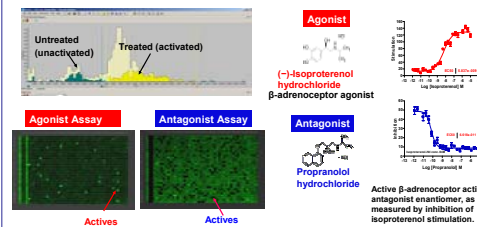
Sequence	Parameter	Value	Description
1	Reagent	Sul (700 cells/well)	HEK 293T/ARRB2:beta2AR
2	Time	overnight	37°C, 5% CO2
3	Screening compounds	20nM	40uM-0.5nM
4	Control compound	20nM	Agonist (AC100)
5	Time	1.5hr	37°C, 5% CO2
6	Reagent	1ul	200uM black blue
7	Detector	GFP intensity	Acumen

Inhibition Protocols for Screening (1536 format)

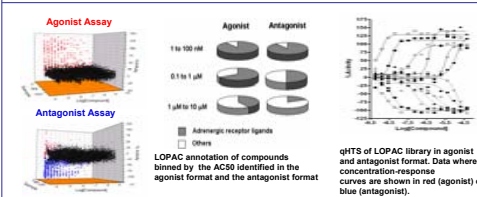
Sequence	Parameter	Value	Description
1	Reagent	Sul (700 cells/well)	HEK 293T/ARRB2:beta2AR
2	Time	overnight	37°C, 5% CO2
3	Screening compounds	20nM	40uM-0.5nM
4	Control compound	20nM	agonist (AC50), antagonist (AC100)
5	Time	1.5hr	37°C, 5% CO2
6	Reagent	1ul	200uM black blue
7	Detector	GFP intensity	Acumen



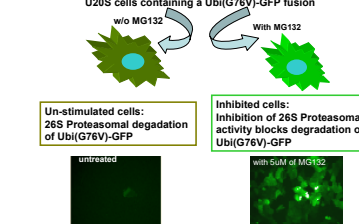
Acumen analysis: Two populations in YFP Peak Intensity



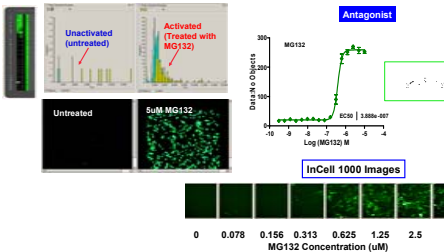
Results for qHTS of LOPAC library using PCA (bARR: b2AR)



Proteasome Assay Principle

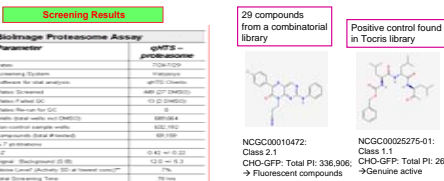


Acumen Data and Population Analysis

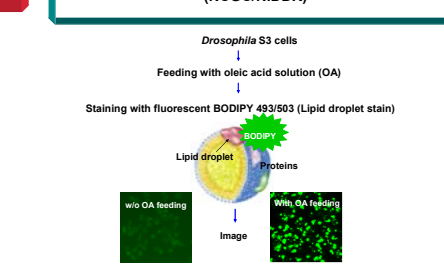


Assay protocols for Screening (1536 format)

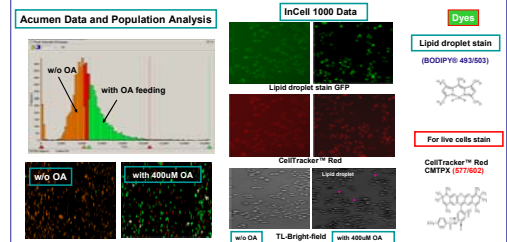
Sequence	Parameter	Value	Description
1	Cells	Sul (700 cells/well)	U2OS ps 2042 Cells
2	Incubation	overnight	37°C, 5% CO2
3	Screening compounds	20nM	40uM-0.5nM
4	Control compound	20nM	MG132, antagonist
5	Incubation	4 hr	37°C, 5% CO2
6	Detector	GFP intensity	Acumen



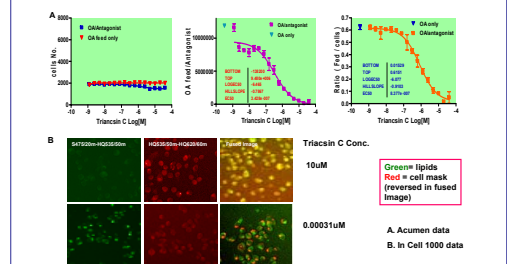
Lipid Droplet Subproteome Assay Principle (NCGC/NIDDK)



Oleic Acid Feeding of Drosophila S3 Cells with BODIPY/Red CMTPX Staining



Triacsin C Inhibition of OA induced lipid droplets in Drosophila S3 Cells



Results for qHTS of LOPAC library using lipid droplets Assay in Drosophila S3 Cells

Sequence	Parameter	Value	Description
1	Reagent	4ul (5,000 cells/well)	Drosophila S3 cells
2	Test compound	20nM	40uM-0.5nM
3	Control compound	20nM	Triacsin C
4	Reagent	1ul	Oleic acid solution (400uM)
5	Time	O/N	24°C incubation
6	Reagent	4ul	dyes (1:1,000)
7	Time	2hrs	24°C incubation
8	Detector	Ratio (GFPintensity/cellintensity)	Acumen

Assay Protocols for Screening (1536 format)



Active Antagonist Compounds in LOPAC library: AC50 0.1uM-20uM

