

Leveraging Celloomics® iQ for Rapid Assay Development in Neuronal Function and Colocalization Biologies

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Abstract

One of the fundamental decisions in developing a High Content Screening (HCS) assay is determining the proper number of cells to acquire and analyze to obtain results that are statistically relevant. Different biological processes require a different number of cells to be analyzed to achieve this statistical significance. Too many cells results in time wasted and image storage issues, while too few cells analyzed results in poor assay windows. In this poster we discuss and demonstrate, using two common assays, an automated method to help achieve statistically robust and biologically relevant results. We will show that this method, the Celloomics iQ, or intelligent acquisition approach, provides a rapid, automated, more intelligent way to develop robust HCS assays.

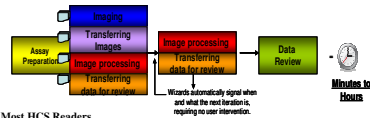
Introduction

Determining the number of required cells for a robust assay becomes tedious and expensive. Over-sampling increases scan time and storage requirements. Under-sampling affects assay performance. That is why the Celloomics iQ approach is the quickest, most effective way to develop an HCS assay. The Celloomics approach to HCS workflow combines highly flexible and advanced analysis tools along with on-the-fly analysis. This permits users to set up unattended runs so that assay development becomes automated, requiring less time, no manual intervention and more robust results (Figure 1).

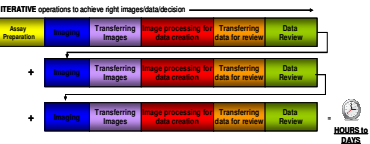
Figure 1. HCS Workflow, Iterative versus Parallel Processing.

Celloomics ArrayScan V^{HT} HCS Reader

PARALLEL operations intelligent processing get to the right decision faster and easier.



Most HCS Readers



Celloomics Plate ID/Plate Protocol Wizards

The Celloomics software permits advanced scheduling and protocol processing of both real and virtual plates via the integrated Plate ID/Plate Protocol Wizards. These wizards permit:

- The capability of setting up multiple protocols to assess assay performance.
- Flexible assay design and processing
- Walk-a-way operation

Three Simple Steps for Setting up iQ

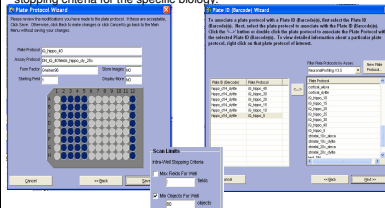
1. Setting up a Plate Protocols

Choose the assay type(s) and/or protocol(s) you want to use. Pick a decreasing number of field(s) or object(s) to analyze in each protocol and choose the scan area of the plate. (Figure 2, Left).

2. Associate Plate Protocol and Physical/virtual plates

Choose the plate ID (or Barcodes) of interest and associate these with the plate protocols (Figure 2, Right).

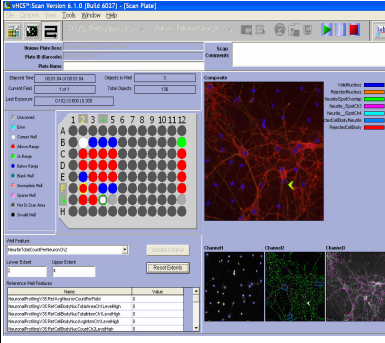
Figure 2. Steps for Setting up iQ. (Left) Setting up Plate Protocols with the Plate Protocol Wizard (Right) Using the Plate ID Wizard to associate a plate ID with plate protocols (Bottom) Scan Limits that are entered into an assay protocol once iQ has determined the optimal stopping criteria for the specific biology.



3. Execute the run

Based on the plate ID or barcode, the software will process the plate with the associated plate protocol (Figure 3).

Figure 3. Executing a run. Images are analyzed on-the-fly with the BioApplications that are associated with the protocol.



Example 1: Neuronal Profiling

Materials and Methods

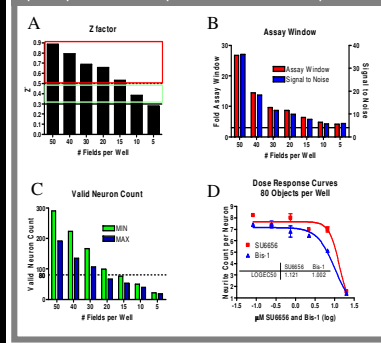
Primary rat brain hippocampal cells (Lonza, Allendale, NJ), were used to determine the number of neurites per neuron using Celloomics Neuronal Profiling V3.5 BioApplication and the Plate ID and Plate Protocol Wizards.

The rat primary hippocampal cells were cultured for 4 days *in vitro* then treated for 14 days, replacing compound and/or media every 3-4 days (Figure 5). Images were acquired on the Thermo Scientific Celloomics ArrayScan V^{HT} HCS Reader at 20x magnification with two fluorescent channels acquired per field. An exaggerated number of fields (50) per well were acquired from the minimum (12 µM of Bis-1) and maximum (media containing NSF-1) conditions. Performance of the assay was monitored by the calculation of the Z' factor¹, coefficient of variation, signal/noise ratio and assay window. The original plate images were automatically rescanned and analyzed using the Plate ID/Plate Protocol Wizards (Figure 2). The number of fields analyzed were reduced incrementally (and thus the number of cells analyzed) down to 5 fields per well. A dose response experiment was also performed with the kinase inhibitor, SU6656 and the PKC inhibitor, Bis-1 and analyzed using the optimal assay protocol conditions.

Results

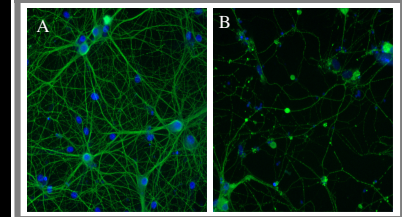
Using the Plate Protocol and Plate ID Wizards, the data in Figure 4 shows the effects of the decrementing scans and their results on determining the number of cells required for a robust, screenable assay. We compared the Z' factor, assay window, signal to noise ratio and coefficient of variation using the Neurite Count per Neuron measurement, which directly measures the compound's effect on neurite outgrowth. We found that 15 fields per well met all of our criteria, including a Z' factor of 0.53 for an excellent assay (Figure 4A), an assay window greater than 6.4 fold (Figure 4B) and a percent Coefficient of Variance (CV) of 11%. The percent CV data is not shown. Based on these results, the optimal assay required 15 fields per well which resulted in 80 neurites per well (Figure 4C).

Figure 4. Results of the decrementing scans from the original acquisition of 50 fields. The data from the dose response curve is derived from eight replicates per condition at the optimal scan limits of 80 neurites per well.



Once the optimal number of fields per well and the optimal number of neurons was determined, the scan criteria were applied to SU6656 and Bis-1 dose response experiments using the definable, intra-well stopping criteria in the vHCS™-Scan software. (Figure 2, Bottom and Figure 4D).

Figure 5. Images captured with the Neuronal Profiling v3.5 BioApplication. Shown are composite 20x images of the Primary Rat Hippocampal neuronal cells with nuclei stained with Hoechst (blue) and indirect immunofluorescence against neuronal cells (green). (A) Untreated cells and (B) 30 ng/ml Bis-1.



Example 2: Colocalization

Materials and Methods

HeLa cells (ATCC) were used to determine the percent overlap area of cytochrome-c colocalized with mitochondria via the iQ approach using Celloomics Colocalization BioApplication. The HeLa cells were treated with varying doses of FCCP (Sigma Aldrich, St. Louis, MO, USA) which induces apoptosis and incubated for 17-18 hrs at 37°C with 5% CO₂ (Figure 6).

Ten fields per well were acquired from the minimum (100 µM FCCP) and maximum (untreated) conditions. Performance of the assay was monitored using the same criteria in example number one. The original plate images were automatically rescanned and analyzed using the Plate ID/Plate Protocol Wizards (Figure 2). The number of fields analyzed were reduced incrementally (and thus the number of cells analyzed) down to 1 field per well. A dose response experiment was also performed with FCCP and analyzed using the optimal assay protocol conditions.

Results

Using the Plate Protocol and Plate ID Wizards, the data in Figure 7 shows the effects of the decrementing scans and their results on determining the number of cells required for a robust, screenable assay. In this example we analyzed the percent overlap area measurement, which measures the percent of cells that the mitochondrial staining colocalized with the cyt-c staining in a user defined region. We found that 5 fields per well met all of our criteria, including a Z' factor of 0.32 for a screenable assay (Figure 7A), an assay window of 4.4 fold (Figure 7B) and a 5% CV. We then determined the number of selected cells per well for 5 fields which was 117 cells per well (Figure 7C). Once the optimal number of fields and the optimal number of selected objects per well were determined, the scan criteria were applied to a FCCP dose response experiment using the definable, intra-well stopping criteria. (Figure 7D). These studies indicate that although mitochondria potential is affected by FCCP, some of the cytochrome-c is still retained in the mitochondria, suggesting that FCCP at the doses tested do not induce complete translocation of cytochrome-c from mitochondria to the cytoplasm.

The blue line in the dose response curve represents the number of fields required to obtain a minimum of 117 cells per well, note that at higher concentrations of FCCP, more fields are needed to obtain the required significant number of cells (Figure 7D).

Figure 6: Colocalization BioApplication Automatically Quantifies Colocalization of Targets Proteins with Markers. HeLa cells were stained with MitoTracker Orange and Hoechst-33342 followed by fixation. The cells were then permeabilized, blocked and incubated with anti-cytochrome-c followed by secondary staining. Images were acquired on the Thermo Scientific Celloomics ArrayScan V^{HT} HCS Reader at 20x magnification with three fluorescent channels acquired per field.

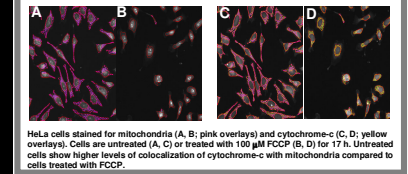
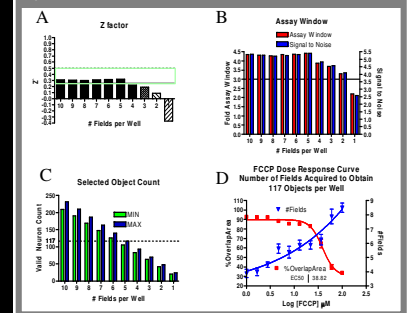


Figure 7. Results of the decrementing scans from the original acquisition of 10 fields. The data from the dose response curve is derived from eight replicates per condition at the optimal scan limits of 117 selected objects per well.



Conclusions

Using the Celloomics iQ approach image analysis parameters were determined during assay development to obtain a robust statistically relevant assay in an automated process.

•In the Neuronal Function assay, 80 neurites per well were required for a Neurite outgrowth measurement to achieve a Z' factor of 0.53.

•In the Colocalization assay, 117 selected cells per well were required for a determination of the percent of cells that Mitochondria and Cytochrome-c colocalized in a defined area of the cell to achieve a Z' factor of 0.32.

References

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2. J. L. Scarlett and M. P. Murphy. *FEBS Lett.* 1997, **418**, 282-286.
3. Thoenen, H. 1991. *Trends Neurosci.* 14:165-170