



Improvements in High Content Cell-Based assays using the Caliper Sciclone ALH 3000 and various Corning Microplates

Abstract

Improvements in optical imaging instrumentation and microplate performance have made the use of high content screening (HCS) programs in drug discovery more feasible. Importantly, these more physiologically predictive assays can rule out bad leads earlier in the pipeline. However, cell-based assays typically involve more liquid-handling steps and the results are more variable. In this application note, we show improvements in cell-based assay results when using the Caliper Sciclone ALH 3000. We also discuss the nature of the cells used and the impact of microplate characteristics. We first assess the performance of batch frozen cells in cell retention following microplate manipulation, noting that batch frozen cells can demonstrate more sensitivity to handling shortly after plating from the freezer. Such sensitivity can be alleviated by choosing microplate chemistries with higher affinity for cell attachment. The use of automation is compared to manual liquid handling and shows improved consistency and throughput. Together, our results can serve as a guide to improve results during high content screening.

Introduction

In drug discovery screening, two major goals in developing a high content cell-based assay are to increase the robustness of the assay in question, and to decrease the amount of hands-on time an operator is required to run the assay. To achieve these goals, several assay parameters must be considered and optimized, including, but not limited to:

- The ability to treat cells like reagents (i.e., using frozen batch cultures).
- The effects of surface chemistries on cell retention and morphology.
- The instrumentation used to set up and to read the assay.
- The coordination of multiple pieces of equipment in an assay.

In this note, we demonstrate that improvements can be made in overall data quality and assay throughput in representative high content assays by investigating each of these parameters.

For automated systems, the ability to treat cells like reagents is important for throughput. Using frozen batch cultures is one way to meet these throughput demands. In order to assess the use of frozen batch cultures, we studied the effects of plating cells 1-3 days after thawing. We show that the surface chemistry of the plate as well as the type of cell has an affect on assay performance.

Untreated polystyrene is a very hydrophobic surface that shows low wettability. Standard tissue culture treatment (TCT) oxygenates the surface making the polystyrene more wettable. Corning® CellBIND® (CB) surface is a patented plasma surface treatment that further improves oxygen incorporation and surface wettability, increasing attachment and recovery of certain cells. Poly-D-Lysine (PDL), finally, has long been used to capture and retain poorly adhesive cells or cells subjected to particularly aggressive handling, owing to the dense charge on coated substrates.

We show that microplate surface chemistries that increase the attachment and retention of cells (especially during several labeling and washing steps) can improve assay results. In particular, PDL and, to a lesser extent the CellBIND surface, improve the retention of thawed LNCaP cells that are subjected to Hoechst staining and subsequent washes.

Improved retentions were not observed with actively cultured C166-GFP cells, indicating that cell type as well as surface chemistry must be considered. However, these cells did demonstrate differential morphology after 24h of incubation, suggesting potential differences in growth and metabolism dependent on surface chemistry.

Assay reproducibility and throughput were both improved by incorporating and optimizing automation into these high content assays.

Materials

- Batch frozen LNCaP cells (ATCC)
- Continuously culturing C166-GFP cells (ATCC)
- Corning 96-well Black Clear Bottom plates:
 - Tissue Culture Treated (Cat# 3904)
 - CellBIND (Cat# 3340)
 - Poly-D-Lysine (Cat#3372)
- Cellomics ArrayScan II
- Caliper Sciclone ALH 3000

Methods and Results

To study the sensitivity of frozen LNCaP cells to microplate surface chemistry, LNCaP cells, frozen in 90% FBS, 10% DMSO, were thawed at 37 °C, and brought to 105 cells/mL in RPMI+FBS. These cells were then seeded into Corning 96-well Black Clear Bottom Tissue Culture Treated (Cat# 3904), CellBIND (Cat# 3340), or Poly-D-Lysine (Cat#3372) microplates at 10,000 cells/well in 100 μ L. After 1, 2 or 3 days, cell nuclei were stained with Hoechst, cells were formaldehyde-fixed and plates were washed by hand 2X with PBS. Plates were then scanned with a Cellomics ArrayScan II (one channel, 10X objective), and the number of nuclei detected per field vs. days of incubation were plotted, relative to the number detected on PDL, which was set at 100%. On the first day after thaw, ~30% fewer cells were retained on TCT versus PDL plates ($p < 0.01$); CellBIND was nearly indistinguishable from PDL. By days 2 and 3, no significant differences were observed (see Figure 1).

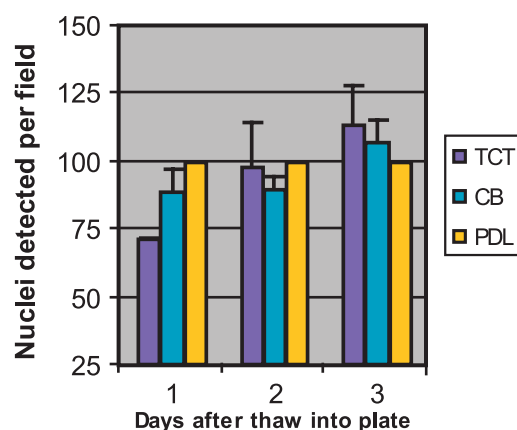


Figure 1. Nuclei detected for LNCaP cells plated 1, 2, and 3 days after thawing on 3 different plate types. Three plates were run of each surface chemistry type for each day, and the experiments were repeated twice. The error bars represent Standard Error between experiments. Surface chemistry types include: Tissue Culture Treated (TCT), CellBIND (CB) and Poly-D-Lysine (PDL).

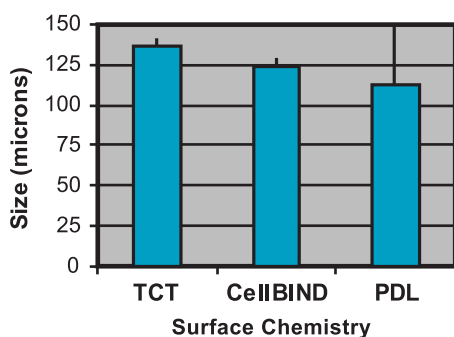


Figure 2. Nuclear size for C166-GFP cells plated on TCT, CellBIND, and PDL plates. Three plates were run of each surface chemistry type, and the experiments were repeated twice. The error bars represent Standard Error between experiments. Surface chemistry types include: Tissue Culture Treated (TCT), CellBIND and Poly-D-Lysine (PDL).

To study the effect of surface treatment on C166-GFP cell morphology, C166-GFP cells (murine endothelial cells which constitutively express a GFP construct) were seeded (4,000 cells/well) onto 96-well black clear bottom TCT, CellBIND, or PDL plates in IMDM + FBS. After 24h, cells were stained with Hoechst and fixed, and plates were washed as in Figure 1. Plates were then scanned on the ArrayScan II (two channels, 20X objective) and both nucleus number and nuclear size were assessed. While there was no significant difference in the number of C166-GFP nuclei retained in this experiment (data not shown), nuclear size (Figure 2) and cell spreading (Figure 3) varied considerably among the three surface chemistries tested. Nuclear area was somewhat larger on CellBIND than on TCT, and a corresponding increase in cell spreading and overall cell size was also observed. Likely due to the tight capture of cells by the PDL chemistry, nuclear size, cell spreading, and cell size were considerably smaller on these plates than on TCT or CellBIND. Interestingly, unlike the frozen LNCaP cells from Fig. 1, these continuously cultured C166-GFP cells did not appear to show significant differences in attachment or retention among the three different surfaces tested (Figure 4). Follow-up experiments suggest that cell type, frozen vs. culturing status and wash regimen all contribute to observed attachment, and these data will be discussed in a separate application note.

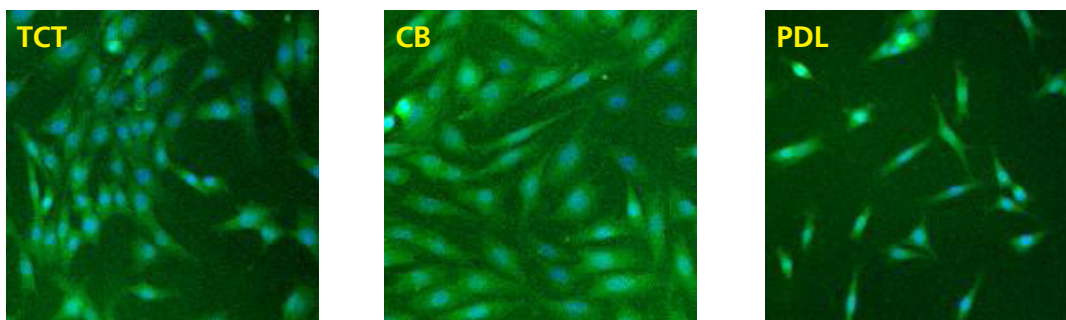


Figure 3. Cell spreading for C166-GFP cells plated on TCT, CellBIND (CB) and PDL plates.

To study improvements in scanning reproducibility via automation, C166-GFP murine epithelial cells were suspended in IMDM+FBS at a concentration of 4×10^6 cells/mL, and 100 μ L per well were seeded onto 96-well black clear bottom CellBIND plates, either by hand using a multichannel pipettor or using the 96-well manifold (HVH) of a Caliper Sciclone ALH 3000 Workstation (see Figure 6). Plates were incubated for 24h and processed as for in Figure 1. Reproducibility of the assay readouts objects per field, GFP median intensity, and nuclear area were assessed and are shown in Figure 5. While no significant differences in assay results were observed among these three readouts when comparing seeding by hand or by the Sciclone (data not shown), in all cases, the Sciclone generated dramatic improvements in %CV. The use of the Sciclone ALH 3000 workstation to seed cells improved reproducibility as much as 50% compared to seeding by hand.

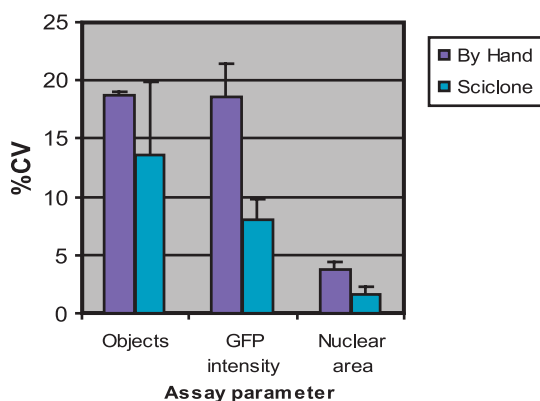


Figure 5. Comparison of manual versus automated pipetting on the precision of various assay parameters. Three plates were run for each technique, and the experiments were repeated twice. Error bars represent Standard Error between experiments.

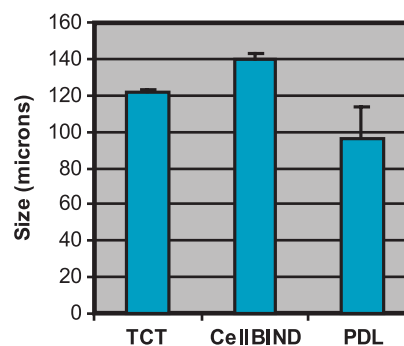


Figure 4. Nuclei detected for C166-GFP cells plated on 3 different plate types. Three plates were run of each surface chemistry type, and the experiments were repeated twice. The error bars represent Standard Error between experiments. Surface chemistry types include: Tissue Culture Treated (TCT), CellBIND (CB) and Poly-D-Lysine (PDL).



Figure 6. Sciclone ALH 3000 workstation.

Conclusions

In our study to maximize cell retention by comparing various microplate surface chemistries, we found that LNCaP cells thawed from frozen stock and seeded directly into microplates were retained better on CellBIND and PDL than on TCT. This effect is time dependent and evident at 24h, but not 48h or 72h, after cells have had sufficient time to attach and proliferate. This differential retention was cell type and handling specific, as continuously cultured C166-GFP cells did not vary in retention to the three surfaces.

In studying the effects of surface chemistry on cell morphology, we found that C166-GFP cells demonstrated differential morphology when grown for 24h on TCT, CellBIND and PDL microplates. While cells on the CellBIND surface appeared more spread out, this was perhaps due to the increased oxygenation and lower contact angle of the surface. Cells on PDL appeared more compact and isolated, perhaps due to the strong interaction of PDL with cells.

In comparing manual liquid handling to the use of the Sciclone ALH 3000 workstation, we found that automated assays had as much as 50% greater precision when compared to the manual results.



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