Corning® High Content Imaging Cyclic Olefin Copolymer (COC) Microplates Prove to be Effective Tools for High Throughput Data Capture and Analysis

Application Note

CORNING

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Introduction

High content analysis is a valuable tool for drug screening and studying the effects of drug candidates on cellular phenotype and function. In order to obtain high quality fluorescent images for analysis, it is essential that high content microplates exhibit optimal flatness and optical properties. While glass bottom microplates meet these needs, they are an expensive option that often require biological coatings to promote cell attachment. To overcome these disadvantages, Corning offers a new microplate with a film bottom that is comprised of cyclic olefin copolymer (COC). This material exhibits superior optical properties[1] and gas permeability[2] compared to polystyrene, which is often used as an alternative to glass. Additionally, since COC is amenable to oxidization, microplates made of this material can be tissue culture-treated (TCT) to enhance cell attachment.

This study explores the benefits of Corning's high content imaging COC microplates by measuring microplate flatness, by evaluating cell retention compared to glass bottom, high content microplates, and by utilizing the microplates for apoptosis and neurite outgrowth high content imaging assays.

Materials and Methods

Microplate and Well Flatness

To measure the overall flatness of Corning COC microplates compared to competitor polystyrene-based microplates, a Keyence laser system (Keyence LT-8105) was used to measure the distance to the well bottom. The difference between the maximum and minimum distance was used to generate an overall variance in microplate flatness. A Litematic drop indicator (Mitutoyo VL-50S) was used to measure the flatness within each well. The indicator was dropped in 5 locations within all wells of a 96 well microplate and 4 locations within all wells of a 384 well microplate. The difference between the maximum and minimum value was calculated to determine the variance in flatness within each well. The variance of each well was than averaged to determine overall well flatness.

Cell Retention

HEK-293 cells (ATCC Cat. No. CRL-1573) were seeded into Corning 384 well glass (Corning Cat. No. 4581) and COC (Corning Cat. No. 4681) microplates at 80,000 cells/cm^2 in 40 µL DMEM (Corning Cat. No. 10-013-CM) per well supplemented with 10% FBS (Corning Cat. No. 35-010-CV). After overnight incubation at 37°C and 5% CO_2, microplates were washed twice with HBSS (Corning Cat. No. 21-023-CM) using Molecular Devices DW4 AquaMax® microplate Washer. After washing, the cells were fixed with 4% paraformaldehyde (Boston BioProducts Cat. No. BM-155) for 15 minutes, washed again, and then stained with Hoechst 34580 at 1 µg/mL (Life Technologies Cat. No. H21486). After a final wash to remove unbound stain, nuclei were enumerated using the Thermo Scientific CellInsight™ Personal Cell Imager. This retention study was performed 3 independent times.

Apoptosis

HeLa cells (ATCC® Cat. No. CCL-2) were seeded in 384 well Corning COC high content micoplates at 32,000 cells/cm^2 in 40 µL IMDM (Corning Cat. No. 10-016-CM) per well supplemented with 10% FBS. After an overnight incubation at 37°C and 5% CO, the medium was replaced with fresh medium containing various concentrations of staurosporine (Sigma Cat. No. S6942), a kinase inhibitor that induces apoptosis in HeLa cells. The vehicle control wells (0 µM) contained 1% DMSO (Sigma Cat. No. D2650). After a 4-hour incubation, the cells were fixed with 4% paraformaldehyde, washed twice with HBSS using the DW4 AquaMax Plate Washer, and then blocked and permeabilized for 1 hour at room temperature with HBSS containing 1% BSA (Sigma Cat. No. A9205), 5% FBS, and 0.3% Triton X-100 (Integra Cat. No. T756.30.30). The cells were then stained for an apoptotic marker, FITC-conjugated cleaved caspase-3 (1:50 dilution) (Cell Signaling Technologies, Cat. No. 9669), at 4°C overnight in the blocking buffer. The next day, cells were washed twice with HBSS with a 5 minute incubation between each aspiration. Nuclei were stained with Hoechst 34580 as described previously. This study was performed 2 independent times and analyzed on the CellInsight Personal Cell Imager.

Neurite Outgrowth

For neurite outgrowth assays, 96 well half-area COC high content microplates (Corning Cat. No. 4680) were coated with 5 µg/cm^2 of Poly-D-Lysine (PDL) (Corning Cat. No. 354210 for 2 hours, followed by 2 washes with sterile water (Corning Cat. No. 25-055-CV). PC-12 cells (ATCC Cat. No. CRL-1721) were then seeded at 10,000 cells/cm^2 in 100 µL DMEM per well supplemented with 10% FBS and 5% horse serum (Corning Cat. No. 35-030-CV). After 24 hours, the medium was changed to Ham's/F12K (Corning Cat. No. 10-025-CV) supplemented with 1% horse serum and 0-400 ng/mL rat nerve growth factor (NGF) (Sigma Cat. No. N2513). The medium was changed every 2 to 3 days until day 7. On day 7, cells were fixed with 4% paraformaldehyde, washed and permeabilized with 0.1% Triton X-100 in HBSS for 10 minutes. The cells

were then washed again and blocked for 30 minutes using staining buffer (R&D Systems Cat. No. FC001). After blocking, the cells were stained for 1 hour at room temperature with β-III Tubulin (BD Biosciences Cat. No. 560338) and diluted 1:10 in staining buffer. Following 1 additional wash, the nuclei were stained as previously described.

Results and Discussion

Microplate and Well Flatness

Microplate flatness is critical for capturing detailed, high resolution fluorescent images. A microplate bottom that exhibits suboptimal flatness is likely to yield images that are out of focus, which can lead to increased scan times and/or scan errors. Corning® COC microplates have an overall microplate flatness differential within 50 µm and an intra-well flatness differential within 10 µm. To evaluate the flatness of Corning COC microplates, we compared the flatness of 3 commercially available polystyrene high content microplates. The results demonstrate that the Corning COC microplates exhibit superior flatness across the microplate and within individual wells when compared to the competitor microplates (Figs. 1 and 2).

Cell Retention

High content applications typically require multiple reagent substitutions and wash steps to perform various cell based studies. When studies are performed on glass microplates, biological coatings are required to enhance cell attachment and prevent cell loss. The coating process can be time consuming, inconsistent and can add unknown biological components to the assay. By using tissue-culture-treated COC high content microplates, we observed statistically higher HeLa cell retention when compared to traditional uncoated glass high content microplates (Fig. 3).

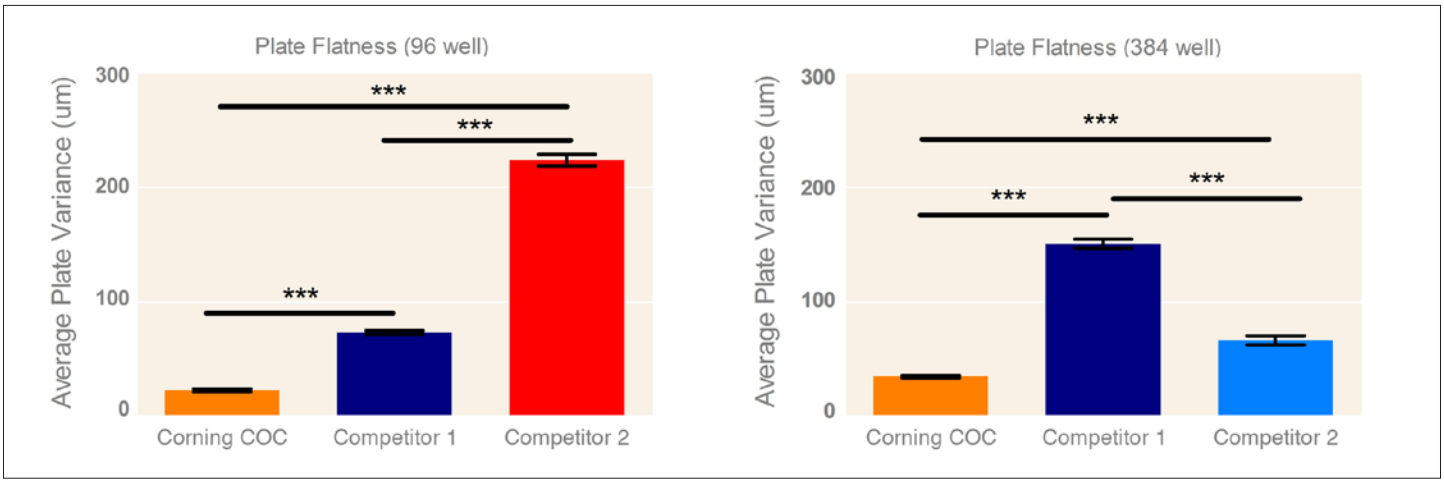

Figure 1. Analysis of Microplate Flatness. Corning COC microplates exhibit statistically less variability in flatness across the microplate compared to competitor polystyrene bottom microplates as measured by a Keyence laser system. Shown with standard errors. One-way ANOVA with Newman-Keuls Post Test ***p<0.001. n=10 microplates.

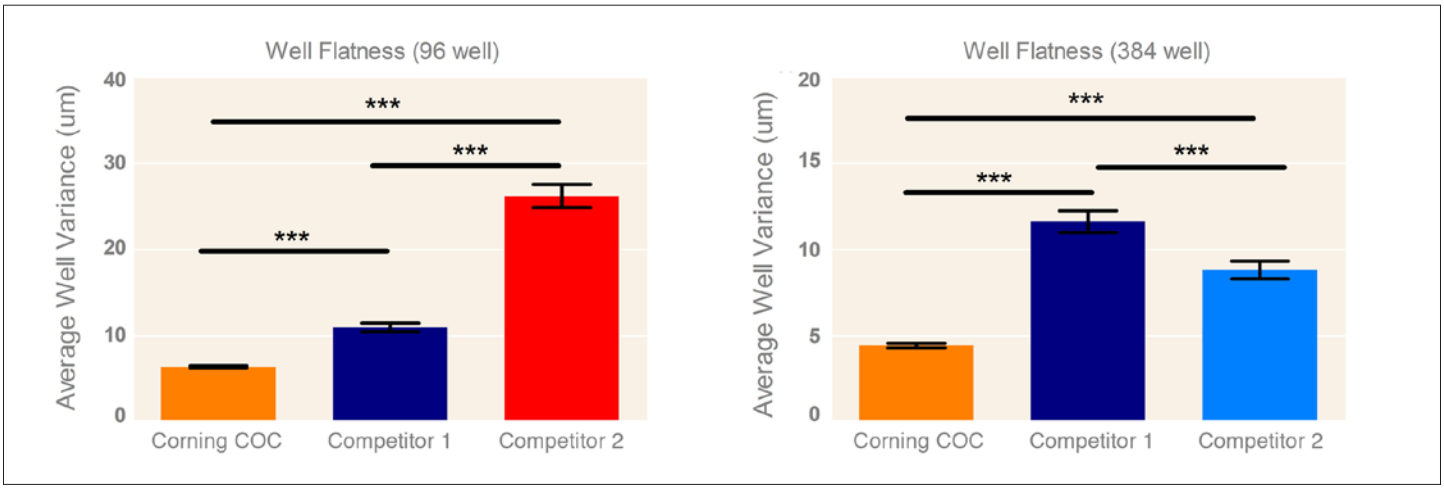

Figure 2. Analysis of Microplate Well Flatness. Corning COC microplates exhibit statistically less variability in flatness across each well compared to competitor polystyrene bottom microplates as measured by a Litematic drop indicator . Shown with standard errors. One-way ANOVA with Newman-Keuls Post Test ***p<0.001. n=192 and 768 wells for 96 and 384 well microplates, respectively.

Apoptosis

Understanding the effects of bioactive compounds on cellular physiology is critical for drug screening. For example, high content imaging can be used to assess cellular cytotoxicity (i.e., apoptosis) in response to various compounds. To address this application, a known marker for apoptosis, cleaved caspase-3, was used to measure the effects of the protein kinase inhibitor staurosporine on HeLa cells. When HeLa cells were exposed to increasing concentrations of staurosporine, we observed a dose-dependent increase in cleaved capase-3. These results indicate an increase in the number of apoptotic HeLa cells. Additionally, the EC_{50} value for staurosporine was found to correlate with reported values[3] (Figs. 4 and 5).

Neurite Outgrowth

Another common application for high content imaging is evaluating the effect of various molecules on neurite outgrowth to study neuropathy and neurodegenerative diseases. Conversely, understanding promoters of neurite outgrowth can help to better

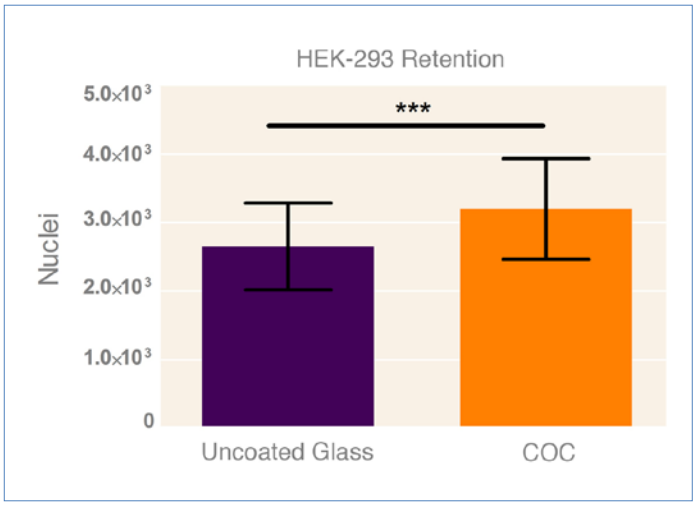

Figure 3. HEK-293 Retention on glass and COC microplates. HEK-293 cell exhibited higher retention on COC microplates compared to glass microplates after multiple washes. Shown with standard deviations. Five fields analyzed per well, 3 independent studies, n=1152 wells. Unpaired t-test,*** is P<0.001.

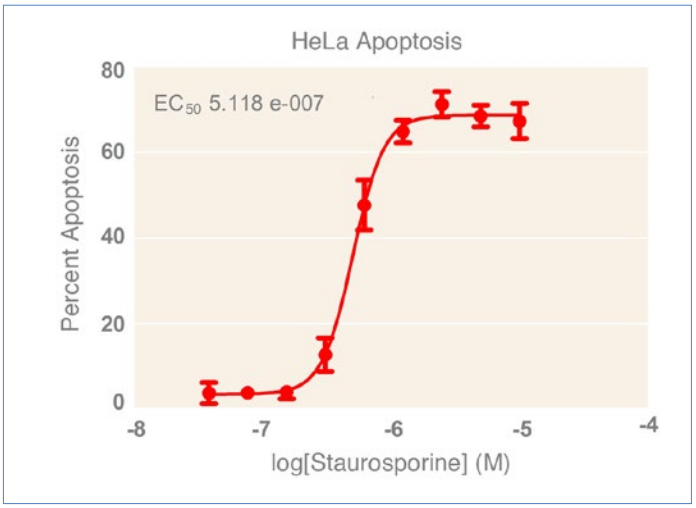

Figure 5. Analysis of HeLa Cell Apoptosis. HeLa cell apoptosis was determined by analyzing the expression of cleaved caspase-3 following exposure of cells to increasing concentrations of stauroporine. standard deviations. Five fields per well were analyzed using a 20x objective. N=16 wells per dose with standard deviations, 2 independent studies.

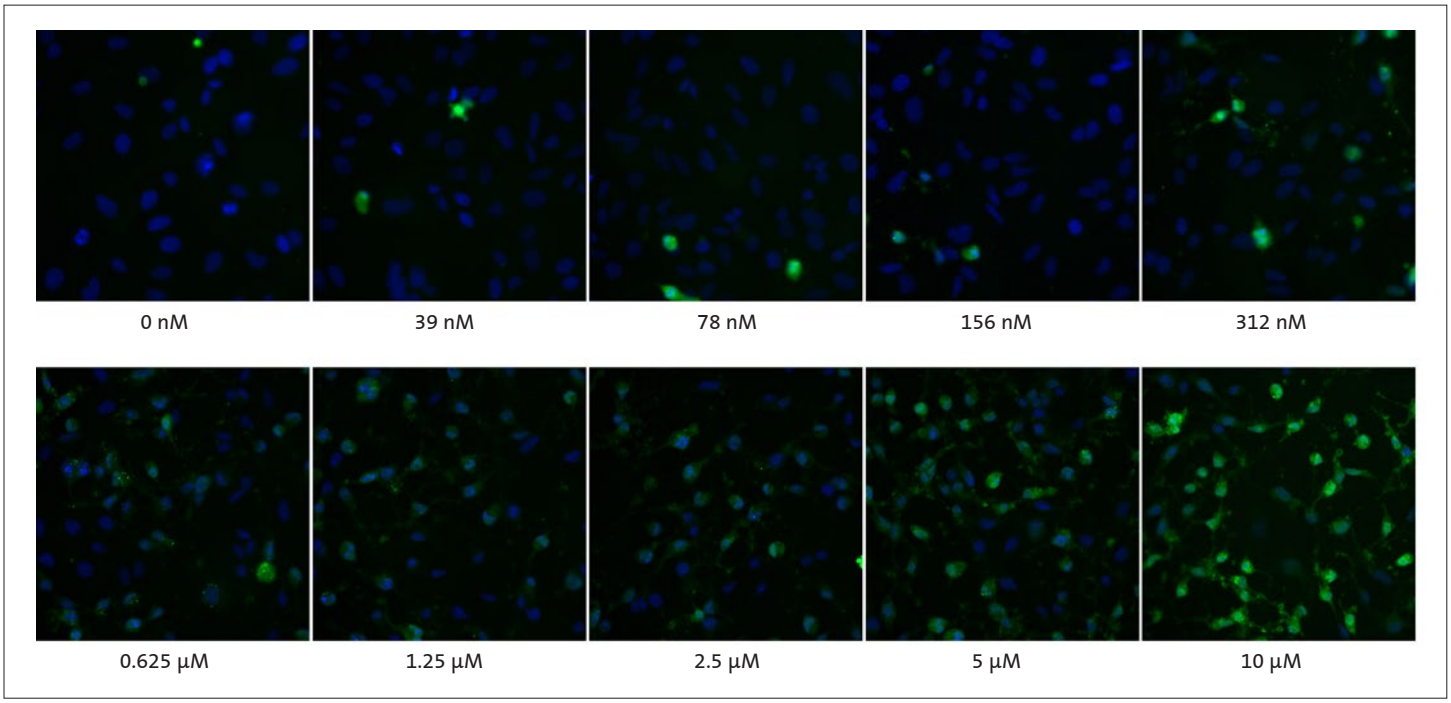

Figure 4. Expression of HeLa Cell Caspase-3. Representative micrographs of HeLa cells exposed to various concentrations of staurosporine. Green indicates cleaved caspase-3 staining (apoptosis marker) and blue indicates hoechst stained nuclei. Images from CellInsight Personal Cell Imager 20x objective.

understand potential treatments for diseases and traumas. This study evaluated whether PDL-coated COC microplates could be used for PC-12 cell attachment and differentiation when exposed to NGF. These experiments demonstrate that increasing concentrations of NGF promote a dose-dependent increase in PC-12 neurite outgrowth, with an EC_{50} value for NGF that correlates with previous reports[4] (Figs. 6 and 7).

Conclusions

- Corning® COC microplates provide an effective alternative to glass bottom microplates for high content analysis of cellular assays and fluorescence-based labeling.
- Corning COC microplates exhibit superior flatness when compared to other commercially available polystyrene high content microplates.
- Cells cultured on the tissue-culture-treated COC film bottom microplates exhibit higher cell retention after washing protocols when compared to uncoated glass bottom microplates.
- Corning COC microplates enable the acquisition of high resolution images, which provides researchers with an ideal tool for high content imaging assays.

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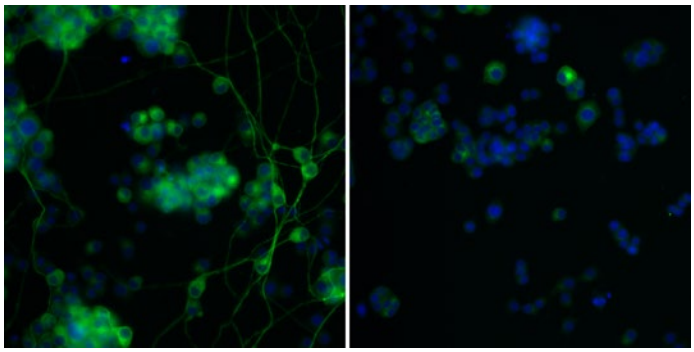

Figure 6. Analysis of Neurite Outgrowth. Representative micrographs of PC-12 cells after exposure to β-NGF for 7 days. Representative images demonstrate neurite outgrowth in the presence of 400 ng/mL (left) and 0 ng/mL (right) β-NGF. Neurites stained for β Tubulin, class III (green), and nuclei stained with hoechst (blue). 20x objective using CellInsight Personal Cell Imager.

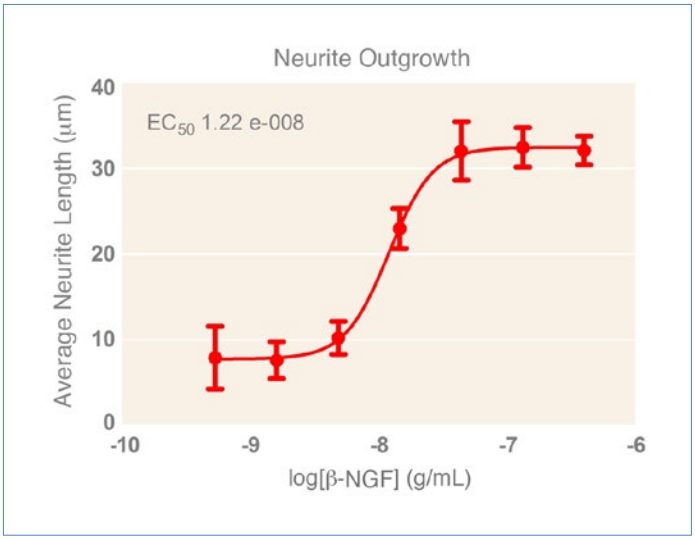

Figure 7. Analysis of PC-12 Neurite Outgrowth. Neurite length was determined in cells exposed to increasing concentrations of β-NGF. Graph plotted with standard deviations. Sixteen fields per well analyzed using a 10x objective, n=10 wells per concentration from 2 independent studies.

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