Performance Equivalence of Redesigned Falcon® Film-bottom Microplates in Cell-based Assays

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Application Note

Introduction

Microplates are a foundational platform for high-throughput cell-based assays and drug discovery. While their design may appear simple, precise dimensional tolerances, optical clarity, and seamless integration with automated liquid handling and imaging systems are critical for assay reproducibility and scalability across diverse applications. Falcon film-bottom microplates have long been favored for imaging and homogeneous cell-based assays due to their optically clear bottoms and opaque walls, enabling compatibility with absorbance, fluorescence, and luminescence detection. Additionally, these plates are offered with a range of Corning® BioCoat® extracellular matrix coatings to support optimal cell attachment and retention. To improve supply assurance and better align with user needs, Falcon film-bottom microplates have been redesigned. The new design maintains plate dimensions as well as the performance and consistency users have come to expect. In this study, we evaluated legacy and newly designed film-bottom microplates by comparing cell attachment, cellular function, and imaging quality. Based on our results, both formats exhibit equivalent optical properties and cell performance, confirming that the new design delivers comparable results to the previous generation.

Materials and Methods

Fluorescent Imaging Assay

HeLa cells (ATCC CCL-2) were maintained in Dulbecco's Modified Eagle's Medium (DMEM; Corning 10-013-CM) supplemented with 10% fetal bovine serum (FBS; Corning 35-010-CV). Upon assay set up, cells were harvested with Accutase® Cell Detachment Solution (Corning 25-058-CI) and seeded into 96-well microplates (Table 1) at a density of 40,000 cells/cm^2 in 100 µL per well of medium. Microplates were allowed to stay in the laminar flow hood for 30 minutes to reduce edge effect before being transferred to a 37°C CO_2 incubator. After 16-24 hours of incubation, 50 µL of medium containing serially diluted Staurosporine (MilliporeSigma S6942) was added to each well. Microplates were incubated again for 16-24 hours. The next day, a live/dead cytotoxicity kit (Thermo Fisher L3224) was used to stain cells to assess viability. To accomplish this, 50 µL of medium containing 8 µM Calcein AM and 16 µM Ethidium homodimer-1 (EthD) was added to each well with a 40-minute pause between plate additions to ensure imaging was conducted after the same incubation time. Microplates were imaged and analyzed using an Operetta CLS™ Imager (Revvity).

Luminescent Imaging Assay

HEK-293 cells (ATCC CRL-1573) were maintained in DMEM supplemented with 10% FBS. HEK-293 cells were harvested using Accutase and subjected to six consecutive 1:2 serial dilutions, beginning at an initial concentration of 6.8 x 10^5 cells/mL. For each microplate (Table 2), 100 µL of the appropriate cell suspension was dispensed into each well of the designated microplate row. Cell attachment occurred in the laminar flow hood as previously described prior to an overnight incubation of 16-24 hours. The next day, 100 µL CellTiter-Glo® (Promega G7571) was added to each well and incubated at room temperature for 30 minutes before reading luminescence using a Revvity EnVision™ multimode plate reader.

Table 1. Black film-bottom microplate catalog numbers.

Microplate	Old	New
TC-treated	353219	353388
Collagen	356649	356649
PDL	356663	356663

Table 2. White film-bottom microplate catalog numbers.

Microplate	Old	New
TC-treated	353377	353389
Collagen	354650	354650
PDL	354651	354651

Results and Discussion

Falcon film-bottom microplates feature an optically clear bottom designed to deliver superior imaging quality, enabling users to visualize cells while maintaining compatibility with fluorescent and luminescent assays. Both new and legacy films were evaluated, and no differences were observed in film clarity or cellular morphology, regardless of whether cells were cultured on old or new microplates (Figure 1). Similarly, imaging quality under fluorescent conditions remained consistent across both versions (Figure 2). Dose-response curves were generated following exposure of HeLa cells to varying concentrations of Staurosporine (Figure 3), and individual toxic concentration 50% (TC_{50}) values were calculated for each microplate (Table 3). A Comparison of Fits test indicated that a single curve could represent both old and new microplates (p-values TC-treated: 0.3001, Collagen: 0.8561, PDL: 0.8670), suggesting no significant difference between curves generated on new versus old microplates. Similarly, when luminescent signals were evaluated across varying concentrations of HEK-293 cells using old versus new white film-bottom microplates (Figure 4), the resulting slopes and elevations showed no statistically significant differences (Table 4).

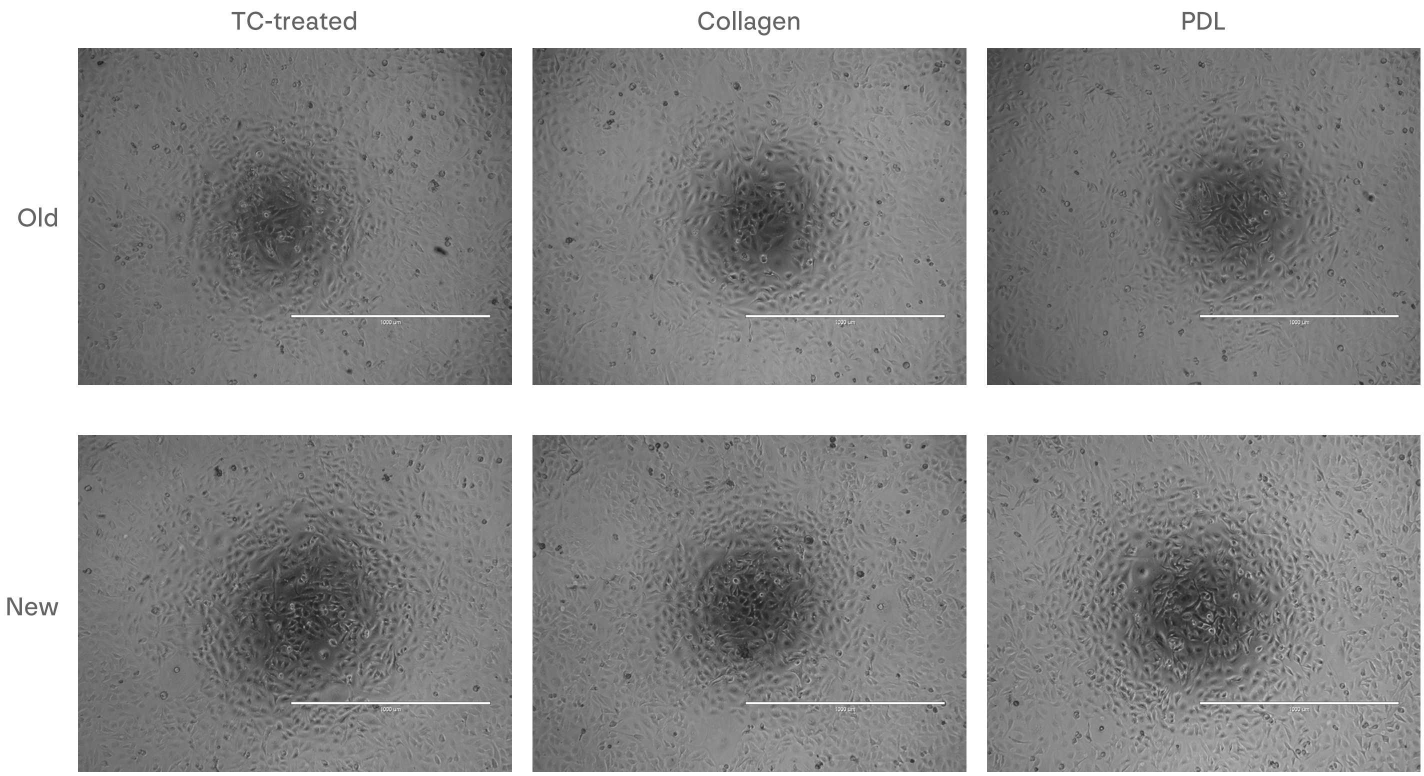

Figure 1. Film clarity and cell morphology with old versus new film-bottom microplates. HeLa cells cultured on new versus old black film-bottom microplates. Scale is 1000 µm.

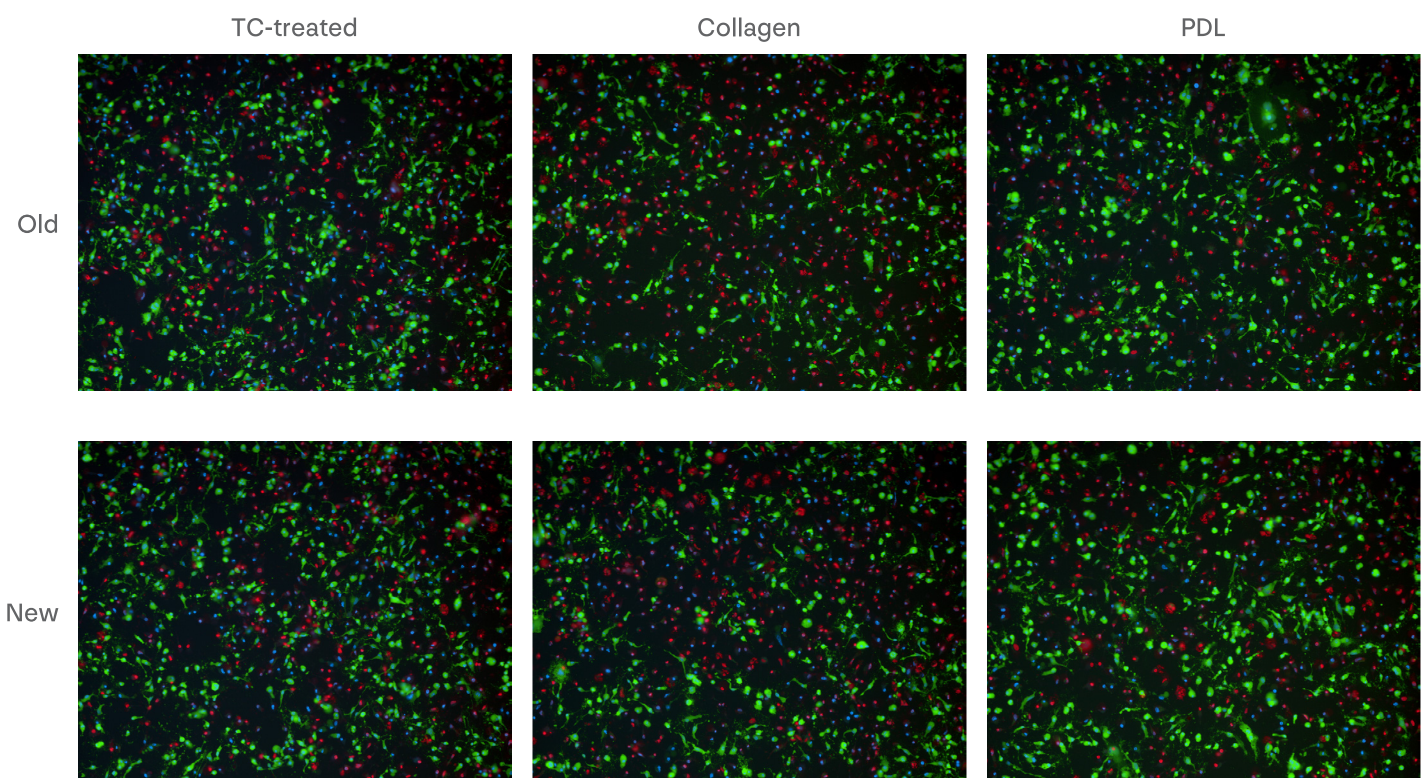

Figure 2. Fluorescence imaging quality of old versus new film-bottom microplates. HeLa cells cultured on new versus old black film-bottom microplates and stained after exposure to 0.625 µM Staurosporine. Scale is 200 µm.

Table 3. HeLa TC_{50} response. TC_{50} calculated from exposing HeLa cells to various concentrations of Staurosporine on new versus old black film-bottom microplates.

Microplate	Old	New
TC-treated	1.52 µM	1.29 µM
Collagen	0.57 µM	0.54 µM
PDL	1.01 µM	1.00 µM

Table 4. Lack of statistical differences in slope and elevation. P values associated with slope and elevation differences calculated from luminescence measurements from various HEK-293 cell densities on new versus old white film-bottom microplates.

Microplate	Slope	Elevation
TC-treated	0.81	0.53
Collagen	0.87	0.98
PDL	0.97	0.89

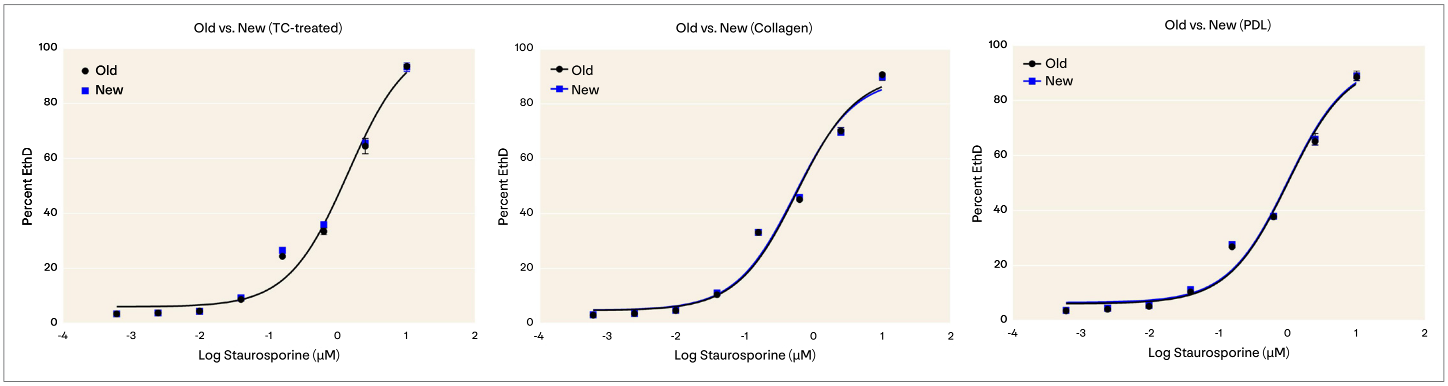

Figure 3. HeLa response to Staurosporine. Dose response curves generated from exposing HeLa cells to various concentrations of Staurosporine on new versus old black film-bottom microplates. Data are the average of 3 independent studies shown with standard error. n=36.

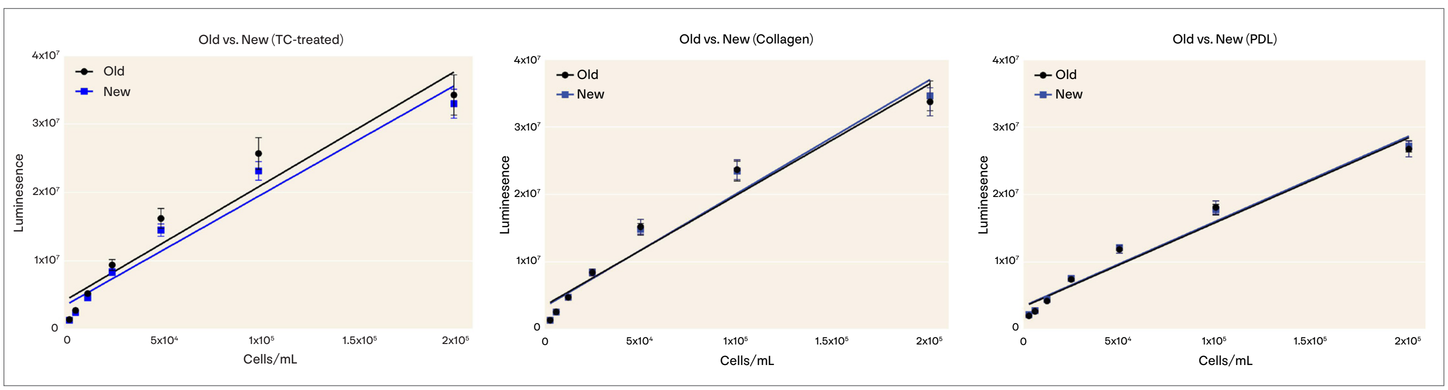

Figure 4. Dose-dependent graph of HEK-293 cell densities measured with Luminescence. Linear dose response plots generated from varying densities of HEK-293 cells on new versus old black film-bottom microplates. Data are the average of 3 independent studies shown with standard error. n=36.

Conclusions

The redesigned Falcon® film-bottom microplates maintain the high performance and reliability of the legacy design. Across fluorescent and luminescent assays, no significant differences were observed in optical clarity, imaging quality, cell morphology, or assay response between old and new microplates. Dose-response analysis and cell density evaluation confirmed equivalent functionality, regardless of plate coating. These findings demonstrate that the new Falcon film-bottom microplates deliver consistent results, ensuring seamless integration into existing workflows for high-throughput cell-based assays and imaging applications.

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The Netherlands
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United Kingdom
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t +31 (0) 206 59 60 51

LATIN AMERICA
grupoLA@corning.com
Brazil
t 55 (11) 3089-7400
Mexico
t (52-81) 8158-8400