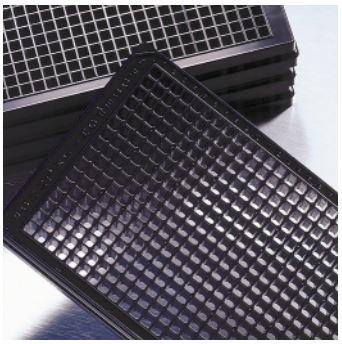
Instrument and Microplate Considerations to Improve Image Capture and Data Generation During High Content Screens

Application Note



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Abstract

Optimization of several parameters is essential during the development of a robust and informative high content screen, particularly when considering the complexity involved in cell-based assays. Moreover, accurate and timely data capture during such a screen requires that the interface between the assay reader and microplate have a high degree of precision. In this study, the impacts of instrument settings and microplate characteristics on assay robustness and data validity were investigated. We first describe how to dramatically improve image capture time by using the correct form factor for the ArrayScan® II reader when using Corning® 384 well black clear bottom Optical Imaging (Cat. No. 3985) microplates for cell growth. Image capture time is also affected by well-to-well plate flatness, and the Optical Imaging plate shows approximately 35% less across plate variation than a leading competitor plate. For the marginally adherent HEK-293 cell line, we then demonstrate an approximately two-fold improved retention after cell staining and microplate washing by using Corning CellBIND® Surface microplates (Cat. No. 3340), increasing assay robustness. Corning CellBIND Surface microplates also show superior flatness, in the same range as the Optical Imaging plate. Finally, we describe the opportunity to reduce reagent costs by showing that comparable signal is generated between normal volume (Cat. No. 3904) and half area (Cat. No. 3882) microplate formats. Together, our results can serve as a guide to significantly improve results when conducting a high content screen.

Introduction

Two major goals in developing a high throughput screen in drug discovery are to increase the robustness of the assay in question and the relevance of the data generated from that assay. To achieve these goals, the researcher must consider and optimize several assay parameters, including, but not limited to:

- the instrumentation used to set up and to read the assay,
- characteristics of the assay microplate,
- how the instrumentation interacts with the microplate and
- how the assay microplate interacts with assay components.

Advances in optics and bioinformatics tools have allowed the development of High Content Screening (HCS) technology, which has the capability to capture cellular events in an automated fashion. Using this technology, drug effects on complex cellular cascades can be measured using several parameters. Due to the increased complexity of data capture and the precision of the optical instrumentation used in an HCS screen, it is imperative to assure that the interface between reader and microplate is optimal. In this work, we demonstrate dramatically improved data quality and assay throughput in representative high content assays by assuring the accuracy of the form factor for the microplate used.

As with any cell-based assay requiring adherent cells, high content screening results can be improved by the use of a microplate surface chemistry that increases attachment and retention of cells (especially during several labeling and washing steps). Untreated polystyrene has a hydrocarbon backbone with benzene rings, resulting in a very hydrophobic surface that shows low wettability. Standard tissue culture treated (TCT) surfaces incorporate oxygen and oxygen functional groups into the surface making the polystyrene more wettable. Corning CellBIND Surface is a

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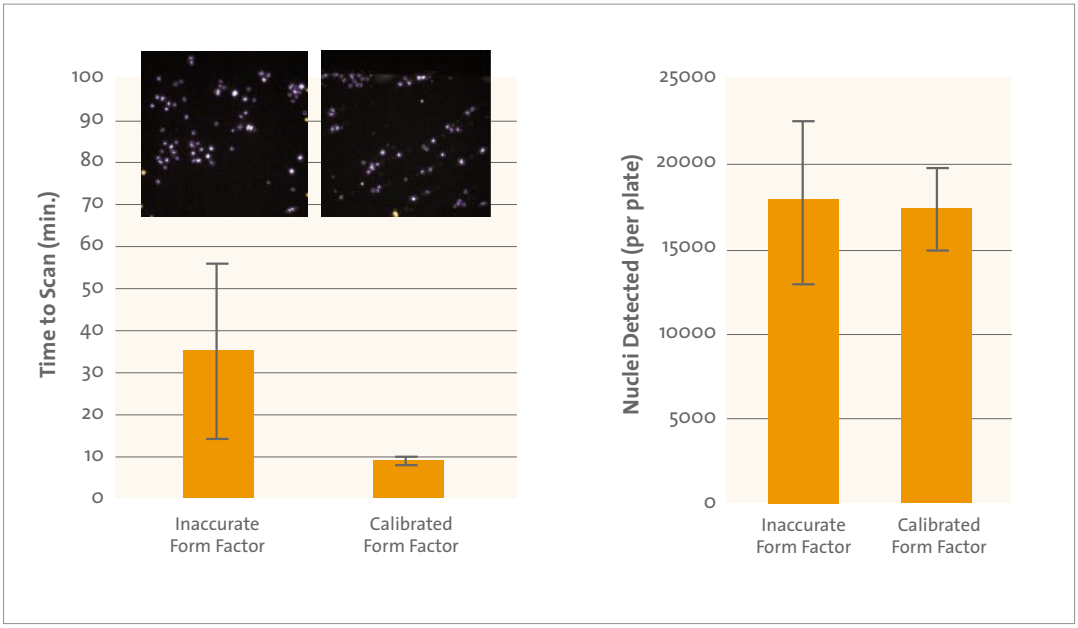

Figure 1A. Instrument Settings – Optimizing Form Factors. By entering the appropriate plate definition into the ArrayScan® microplate database, the scan time for a 384 well microplate can be reduced from >30 minutes to <10 minutes (left), without compromising data capture (nuclei detected, right).

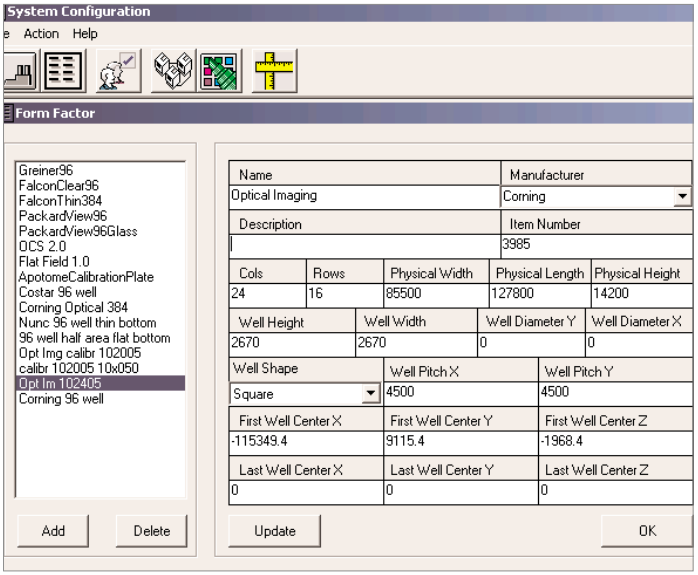

Figure 1B. Optical Imaging Plate Form Factor

patented plasma surface treatment that further improves oxygen incorporation and surface wettability, and increases attachment and recovery of selected cells. We demonstrate here that the Corning® CellBIND® surface improves retention of HEK-293 cells (by approximately two-fold) when they are subjected to Hoechst staining and subsequent washes.

Another major goal in screening efforts (or most any laboratory endeavor) is to reduce the costs associated with consumables and reagents used for a screen. To that end, we compared data generated using normal volume and half area clear bottom microplates (Cat. No. 3882) as the substrate for cell attachment and labeling. Our data indicate that similar results can be generated with either microplate well geometry, and highlight the cost benefits inherent in assay miniaturization.

Methods and Results

Improving scanning throughput – instrument settings and plate characteristics

To test the impact of the interaction between high content scanner and the chosen microplate on scan time and data generation, results with inaccurate and calibrated microplate form factors were compared (Figure 1). Neuro 2A cells (2,000 cells/well) were seeded onto 384 well Corning Optical Imaging microplates (Cat. No. 3985). The next day, cells were fixed and permeablized, and nuclei were fluorescently labeled with Hoechst 33342. Fields were captured (Figure 1A, insets in left graph) using the ArrayScan II reader and a 10X objective, with relaxed object identification parameters set within the Target Activation BioApplication (insets showing stained nuclei). One field from each of 384 wells was captured, and ten plates were scanned using a preloaded (incorrect) form factor for a standard 384 well microplate or one following plate calibration. The calibration wizard is located in c:\program files\Cellomics\ArrayScan50\Tools\Customer. Representative fields are inset. The average time to scan an entire plate was plotted (+S.D), revealing dramatic improvement in throughput with the correct form factor. The number of detected nuclei following scans using the conditions described was also plotted (Figure 1A, right graph). Using either the inaccurate (left) or the calibrated (right) form factor did not affect the overall data captured (although there was a slight improvement in reproducibility), indicating that throughput is the primary improvement when using the correct form factor. In the case of the Corning Optical Imaging plate (Cat. No. 3985), the most relevant parameter for an appropriate form factor is the "First Well Center Z" due to increased thickness of these plates. A screen capture of an appropriate form factor for the 384 well Optical Imaging plate derived from "asnsystem.exe" is shown. The program can be found in c:\program files\Cellomics\

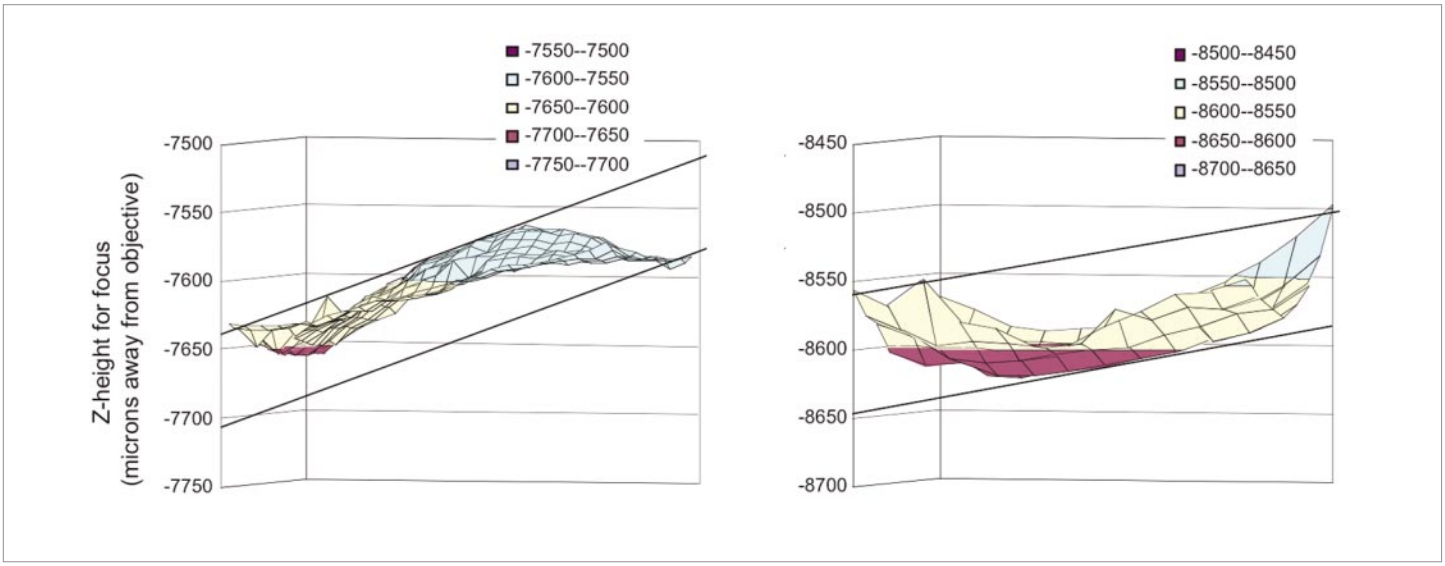

Figure 2. Microplate Characteristics – Superior Flatness of Optical Imaging Microplates. Surface plots of the across-plate Z values reported by the ArrayScan® (focus position relative to scanner optics) reveal that the Corning® Optical Imaging plate is flatter across the plate (left) than a leading polystyrene competitor (right).

ArrayScan50\, and recent ArrayScan software upgrades contain a built-in form factor wizard. To input dimensions directly into the form factor, the Corning® microplate dimension guide gives the appropriate measurements (*http://www.corning.com/Lifesciences/technical_information/techDocs/microplate_ dimensions.pdf*). The fields for First Well Center x, y and z are measurements (in micrometers) from the top right corner of the plate (A12 corner in a 96 well plate) and the bottom of the skirt to the center of the well A1.

Focusing algorithms use the microplate form factor as a baseline reference from which to begin searching for bright objects to detect, and having an incorrect baseline can dramatically reduce throughput. Across-plate flatness is another important factor affecting high content scan speeds, as wide variation from well to well increases the time required to find the correct focal plane. To assess the overall flatness of the Corning Optical Imaging plate used in Figure 1A, as well as its flatness relative to a leading competitor's polystyrene microplate, fluorescent beads were used to isolate the focal plane across microplates. Two thousand beads in 40 µL PBS were dispensed into each well of a competitor and Corning Optical Imaging microplate, and plates were scanned at 10X magnification. The ArrayScan focuses by finding the brightest signal within each well automatically and recording the distance (Z value) between the optics and the object detected in the wells. A surface plot of each well's Z value reflects the bottom flatness of the plate. Representative three-dimensional surface plots shown in Figure 2 reveal two advantages of the Corning Optical Imaging plate over a leading competitor plate: 1) the plates have 70 and 90 µm flatness variances (indicated by the parallel lines), respectively and 2) a larger proportion of the Optical Imaging plate is in the same plane. Taken together, these observations indicate that the Corning microplate allows for a narrower focal plane search range and increased scan speeds. Note to Figures 2 and 4: turning the microplates 180 degrees and rescanning revealed that the approximately 100 µm tilt in the surface plots was caused by the scanner's stage insert and not the microplates themselves (data not shown).

Effect of surface treatment on selected cell attachment/retention

Although the use of cell lines stably expressing fluorescently tagged protein markers is increasing, the majority of high content screens currently utilize subcellular stains. Such stains require several handling steps (stain addition and plate washing) that can influence cell retention within the microplate well. To assess the impact of an improved polystyrene treatment for cell attachment, HEK-293 (3,000 cells/well) cells were seeded onto either tissue culture treated (TCT, Cat. No. 3904) or Corning® CellBIND® Surface (CB, Cat. No. 3340) 96 well black clear bottom microplates. The next day, cell nuclei were stained and fields were captured as described in Figure 1, except using a 5X objective (Figure 3, inserts showing stained nuclei). Ten fields from eight wells were captured, and the experiment was repeated three times (data shown +S.D). Representative fields are inset. A plot of cell counts per well reveals that adherent HEK-293 cells are retained approximately two-fold better on Corning CellBIND Surface versus TCT microplates after staining and washing. Following the same procedure using beads described in Figure 2, the focal plane of Corning CellBIND Surface microplates was determined by capturing autofocus Z-heights. Along with improved binding and/or retention of HEK293 cells, the Corning CellBIND Surface treated microplates also demonstrated excellent flatness (Figure 4), comparable to that of the Optical Imaging plate (approximately 70 µm).

Data capture is consistent in a miniaturized assay well format

To assess whether cost savings can be realized through assay miniaturization, NIH3T3 cells were seeded onto either Corning® 96 well black clear bottom normal volume microplates (Cat. No. 3904, 10,000 cells/well) or half area microplates (Cat. No. 3882, 5,000 cells/well). Along with a 50% reduction in cell numbers seeded, the costs due to all other reagents were also reduced by half. The next day, cells were fixed and permeablized, and subcellular components (nuclei, mitochondria and actin) were fluorescently labeled following the Cellomics™ HitKit™ protocol recommendations, with Hoechst 33342 (nuclei), MitoTracker Red (mitochondria) and Alexa Fluor 488-Phalloidin (actin) as subcellular dyes. Fields were captured using the ArrayScan® II reader and a 5X objective, with object identification parameters set within the Target Activation BioApplication (inserts in blue circles showing stained nuclei). Three fields from triplicate wells were captured, and the experiment was repeated three times. Representative merged fields of labeled cells are shown (Figure 5, left). Our data demonstrate that NIH3T3 cells perform equally well in attachment (cell counts equivalent, data not shown), morphology and image detection on the normal volume and half area 96 well plates. Similar results were seen with HEK-293 and MCF-7 cells (data not shown). As noted in Figure 2, assay robustness (as measured by scan speed) is highly dependent on having the correct form factor for the microplate format and vendor being used (in particular for Z-height). Screen captures of appropriate form factors for the 96 well normal volume (top right) and half area (bottom right) derived from the asnsystem.exe are shown. As in Figure 2, Z-height is the most important parameter affecting image processing.

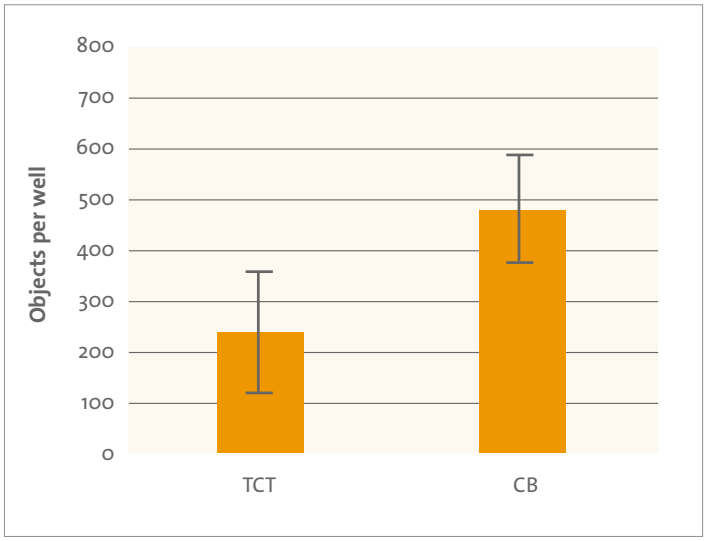

Figure 3. Microplate Characteristics – Cell Retention on Corning CellBIND® Surface Microplates. Following the addition of Hoechst nuclear stain and multiple microplate washes (by hand), HEK-293 cells demonstrated a significant ~two-fold increase in retention on Corning CellBIND Surface microplates.

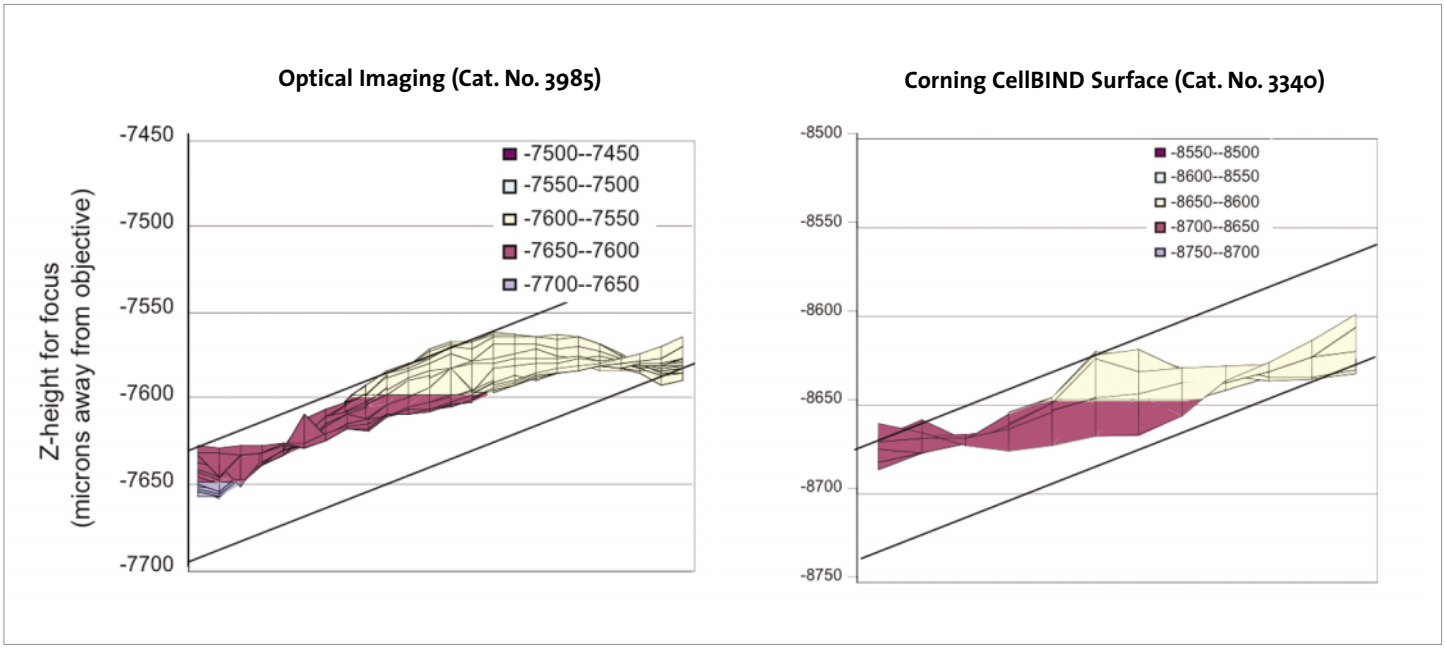

Figure 4. Microplate Characteristics – Flatness of Corning CellBIND Surface Microplates. Surface plots of the across-plate Z values reported by the ArrayScan (focus position relative to scanner optics) reveal that CellBIND Treated microplates possess superior flatness similar to the Optical Imaging microplate.

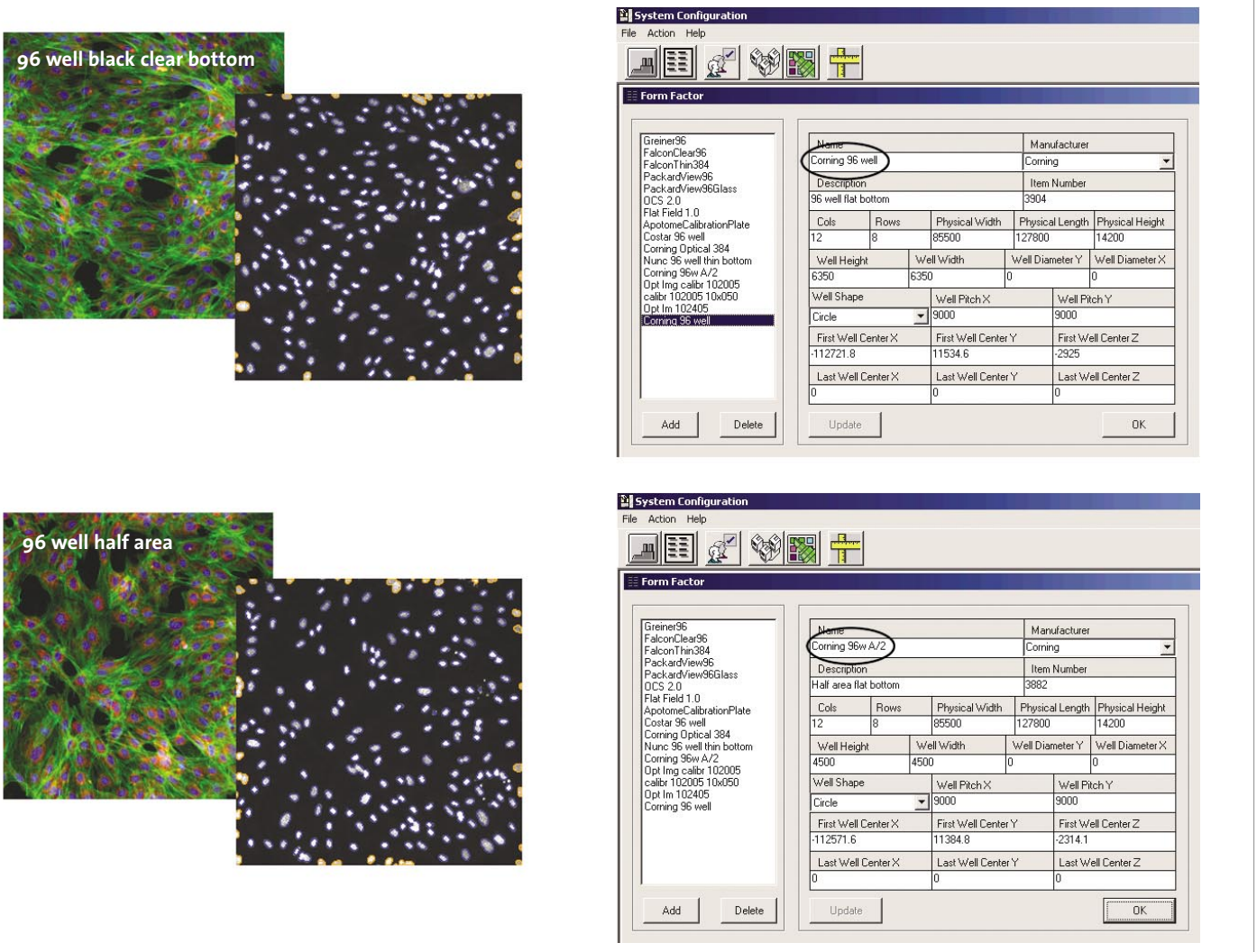

Figure 5. Miniaturization in Corning® Half Area Microplates. Comparable data (i.e., nuclei and cell features detected) were generated using either normal volume (top left) or half area (bottom left) 96 well microplates. For the latter, 50% of cells per well were seeded and reagent volumes were reduced accordingly, resulting in dramatic savings in cell culture and assay reagent usage with similar data generation.

Summary and Conclusions

- *Optimizing throughput of image capture – instrument settings* (*Figures 1 and 5*): Using the proper instrument/ microplate interface (Form Factor) dramatically improves HCS throughput.
- *Optimizing throughput of image capture – microplate flatness* (*Figures 2 and 4*): Superior microplate flatness, found in the Corning® Optical Imaging and Corning CellBIND® treated black clear bottom microplates, improves ArrayScan® autofocus speed by minimizing the range of Z heights required to sample.
- *Maximizing cell retention – microplate surface chemistry* (*Figure 3*): The Corning CellBIND surface can increase adherence of fastidious cell lines (such as HEK-293 cells used here), and therefore enable rigorous handling or automation.
- *Optimizing reagent usage and assay cost – microplate well geometry* (*Figure 5*): The use of half area (A/2) microplates allows ≥50% savings in cells, media preparations and assay reagents. Again, the proper form factor is essential for best scanner throughput.

For more technical or product information, please refer to product literature and protocols. Alternatively, you may call Technical Services at 800.492.1119 or visit **www.corning.com/lifesciences.**

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