Technical Overview

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Agilent Cell Culture and Imaging Microplates

Abstract

The Agilent Cell Culture and Imaging microplates are multifunctional plates for cell analysis, providing quality high-resolution imaging with low autofluorescence and superb optical clarity. The plates accommodate live cell imaging, fixation techniques, immunostaining, and standard fluorescence detection assays. The black plate's thin micro clear polystyrene film bottom is comparable to glass-bottom plates delivering favorable imaging characteristics in widefield and confocal. The tissue culture surface treatment enhances cell attachment and supports cell proliferation. Available in 96- and 384-well formats, the plates supply flexibility in research throughput. The plates meet ANSI/SLAS standards for compatibility with automation. The Agilent Cell Culture and Imaging microplates supply research laboratories with utility across applications.

Introduction

High content screening (HCS) is an imaging-based multiparametric approach to cell analysis that provides a quantitative analysis of complex cellular events and visualization of the relevant phenotypes. These data require consistency in plate features to enable quality image acquisition and interpretation of results. The attributes of the microplate directly affect the quality of the images and the resulting data. Often different assays are run consecutively or in parallel, and the availability of a plate that functions across multiple applications supports efficient laboratory workflows. Researchers cannot afford multiple repeats or lost data due to inconsistencies in microplates.

This study demonstrates the attributes of the Agilent Cell Culture and Imaging microplates by measuring microplate compatibility with cell adhesion, cell proliferation, imaging of live and fixed cells, widefield imaging, and confocal imaging.

Materials and methods

Imaging microplates

Agilent 96-well (204626-100) and 384-well (204628-100) cell culture and imaging microplates were used in the following experiments.

Cell maintenance

NIH3T3-GFP, HCT116-H2B-GFP, U87-GFP, and MCF7-GFP cells were maintained in Advanced DMEM (Dulbecco's Modified Eagles Medium) supplemented with 10% FBS, 2 mM glutamine, and penicillin/streptomycin antibiotics. Cell lines were maintained at 37 °C in a humidified incubator with 5% CO_2 and routinely passaged at 80% confluency. NIH3T3-GFP and MCF7-GFP lines were obtained from Cell BioLabs, Inc. (San Diego, CA). HCT116 H2B-GFP cells were a generous gift from Dr. Jason Stumpff (University of Vermont, VT, USA). U87-GFP cells were a generous gift from Dr. Sachin Katyal (University of Manitoba, Winnipeg, MB, CA). Depending on the specific assay, the cells were plated at 1,000 cells per well or 7,000 cells per well into 96-well microplates or 3,000 cells per well into 384-well microplates and incubated overnight at 37°C, 5% CO_2 in a humidified environment.

Fixation and staining

Cells were fixed in 4% paraformaldehyde at room temperature for 10 minutes, followed by a 3x wash with phosphate buffered saline (PBS). The fixed cells were permeabilized with PBS-0.2% Tween20 (PBST) containing 0.1% TritonX-100. After permeabilization, the cells were stained with the nuclear stain Hoechst 33342 (10 µM) (Sigma part number B2261) and 1:200 dilution of the actin fiber stain Texas Red-X phalloidin (Invitrogen part number T7471) for 15 minutes at room temperature. Plates were subsequently washed 3x with PBS and filled with PBS containing 0.01% sodium azide. All fluidic steps were performed using an Agilent BioTek EL406 washer dispenser.

For antibody staining, fixed cells were permeabilized with PBST with 0.1% TritonX-100 and incubated for 1 hour at room temperature in blocking buffer (1% BSA (bovine serum albumin) in PBST). After blocking, a combination of DAPI (10 µM), 1:500 dilution of phalloidin-CF633 (BioTium part number 00046), and 1:500 dilution of anti-α-tubulin primary antibody (Sigma part number T9026) in PBST were added and incubated 2 hours at room temperature. The plate was then washed 3x times with PBS and incubated overnight at 4 °C in 1:500 secondary antibody (Invitrogen part number A21422) in PBST. Following secondary antibody incubation, plates were washed 3x with PBS, and wells were filled with PBS with 0.01% sodium azide.

Imaging cells

For fully automated long-term imaging, cells were maintained in an Agilent BioTek BioSpa 8 automated incubator coupled to an Agilent BioTek Cytation 5 cell imaging multimode reader with a wide field of view camera. The BioSpa maintained cells at 37 °C, 5% CO_2, and 80% to 90% humidity throughout the 72-hour experiment. The environmental parameters were monitored and reported live through BioSpa software. At 2-hour intervals, the imaging microplate was automatically transferred from the BioSpa incubator to the Cytation 5. During the imaging procedure, other environmental controls inside the Cytation 5 maintained cells at 37 °C and 5% CO_2.

For evaluation of multiple magnifications, microplates were imaged with an Agilent BioTek Cytation C10 confocal imaging reader instrument configured with a Hamamatsu camera and 60 µm spinning disk. Images were captured using both widefield and confocal modalities in the DAPI, GFP, TRITC, and Cy5 channels. Objective correction collars were adjusted by plate type (~0.17 for glass and Agilent, ~0.5 for competitor polystyrene). Images used for comparisons of background fluorescence were captured with consistent acquisition settings across the plate types, presented raw with no processing steps, and display settings (brightness/contrast) held constant. Images used for cell counting comparisons were processed to flatten the background and analyzed for cell count determinations using Agilent BioTek Gen5 software. Z-projection images were produced in Gen5 software using either the “Focus Stacking” method for widefield images, or by using the “Maximum” method for confocal images.

Results and discussion

Cell attachment and proliferation

Adherent cells are routinely seeded into microplates and allowed to attach overnight to establish a growth cycle before conducting experiments. It is critical that the plate properties not affect the number of cells that attach and proliferate. NIH3T3-GFP cells were seeded at a constant density across multiple plates and the following day the mean cell count of each well was measured. Figure 1 shows the equivalence between the Agilent 96-well microplates and competitors in cell seeding attachment. Note that cell counts are for the area imaged and not the total cells plated in the entire well.

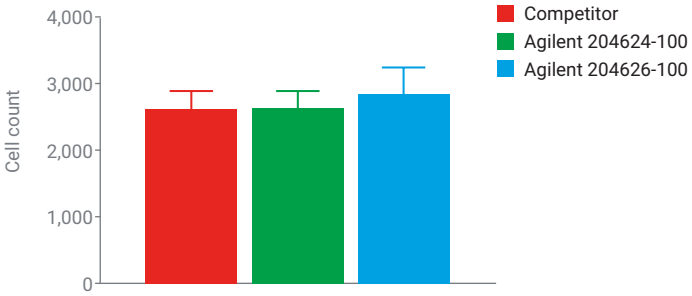


Figure 1. Comparison of NIH3T3 cell numbers after seeding and attachment to the microplate. NIH3T3 cells were seeded at 7,000 cells per well into three separate plates of each plate type. The following day, cells were fixed with paraformaldehyde, stained with Hoechst 33342, and imaged with a 4x objective. The cell number for each well was determined from a single image of the center of the well. Data reflect the mean and standard deviation of 288 determinations.

By monitoring the cell number over time, the cell proliferation rate can be determined. HCT116 cells expressing a histone H2B-GFP reporter enabled live cell counting. Figure 2 shows the fold change in HCT116 cell number seen over a 72-hour period. The proliferation rate was consistent with the published proliferation rates for HCT116 cells. The BioSpa 8 gripper arm was used to transport plates to the Cytation imager over the time course of data acquisition, showing the compatibility of plates with automation.

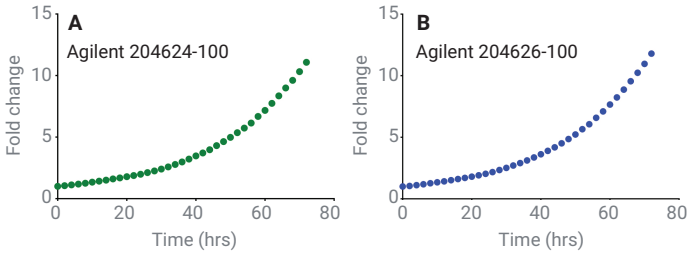


Figure 2. HCT116 cell proliferation in Agilent 96-well plates. HCT116 H2b-GFP cells were imaged every two hours for 72 hours and nuclei were counted at each timepoint using Agilent BioTek Gen5 software. Fold change in cell number is calculated relative to the first measurement. Data reflect the mean of 96 determinations at each time point.

384-Well microplate fluorescence image whole well capture

Agilent 384-well optical bottom polystyrene plates can be used for high-throughput fluorescence cell counting and proliferation applications. Combined with Agilent BioTek wide-field-of-view instrumentation, whole-well cell counts in the 384-well plate format can be achieved with a single 4x magnification fluorescence image. This technical overview compares Agilent 384-well microplate whole-well images against a competitor 384-well imaging microplate, and the results show Agilent's advantages for consistent whole well fluorescence images.

The whole-well images of an Agilent 384-well microplate and competitor 384-well microplate are shown in Figure 3. In the Agilent 384-well microplate image (left), small blue dots scattered throughout the image correspond to individual cell nuclei and this single low-magnification image is suitable for quantifying cell numbers across the entire well. Because the plate material autofluorescence is minimal, the well-edge is not visible and is instead shown by the dashed white outline overlay. In contrast, the image of the competitor's whole-well image shows elevated levels of autofluorescence from the edge of the well and plate material. This artifact requires analysis workarounds to ensure correct cell counting when using the entire image, such as restriction of the analysis to a smaller region within the well. In addition to autofluorescent plate components, the competitor plate type has structural inconsistencies at the well-bottom surface and shows well-to-well variability. Throughout the same microplate, well bottoms are seen in both centered (middle panel) and variably shifted positions (right panel). Cells can be seen growing within the main well bottom region and within the offset well bottom regions (Figure 3, right panel, arrowhead). These well bottom malformations vary in position, and size and dramatically reduce the usable image of the well. In summary, these data show the importance of consistent and high-quality plates for efficient whole-well imaging in the 384-well plate format.

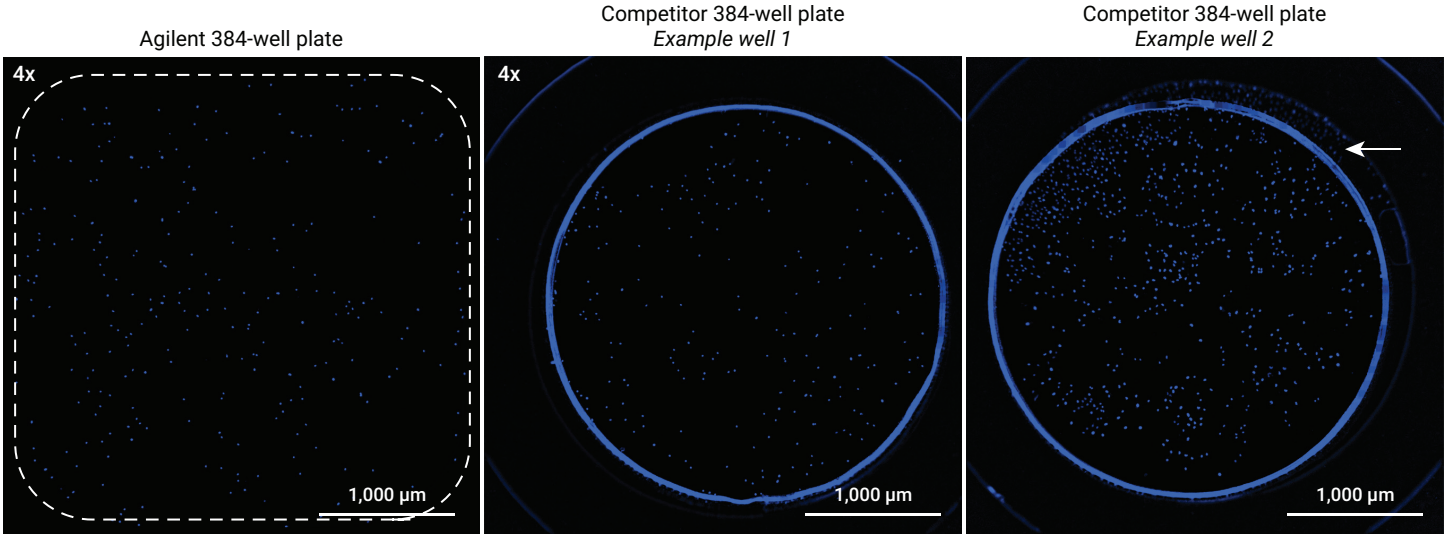


Figure 3. Whole-well fluorescence images in Agilent and competitor 384-well imaging microplates. Live NIH-3T3 cells stained with Hoechst 33342 nuclear stain are imaged at 4x magnification. In the Agilent 384-well microplate (left), well-edge materials are minimally autofluorescent and are not visible when imaging in the commonly used blue channel (DAPI). The well-edge is designated by a dashed line overlay. In competitor 384-well plate types (center and right), however, strong autofluorescence from the well-edge materials creates artifacts during whole-well image analysis, even after image processing to reduce background. In addition to the highly autofluorescent plate materials, well-bottom structural defects found throughout the plate (right image example) result in variability in the location and integrity of the well bottom edge and even allow cell growth in poorly defined subregions outside of the main well bottom area (arrowhead).

Live cell high-magnification confocal imaging

In the study of cellular function, researchers often incorporate live cell imaging applications that include the detection of fluorescent signals from key organelles and small subcellular structures. To achieve the proper resolution that is demanded, the use of confocal imaging may be required. The image quality of the Agilent thin-bottom polystyrene imaging plates were compared to the "gold standard" glass-bottom microplates and competitor polystyrene microplates to determine their ability to be used for high-magnification live cell confocal imaging. At lower magnification, images appear similar in quality, but at higher magnification, the advantages of the Agilent polystyrene microplates become particularly apparent. Figure 4 shows example images from each microplate of live MCF7 cells expressing GFP throughout the cytosol and nucleus. At 60x magnification, the clarity and contrast of the images taken in the Agilent microplate resemble that of the glass-bottom counterpart, and background fluorescence is relatively low. In contrast, the images taken in thicker-bottom polystyrene competitor microplates have higher background fluorescence, 2-fold higher than glass-bottom, and an appreciable reduction in the clarity of the MCF7 subcellular features.

Imaging fixed cells in multiple settings

While applications such as cell proliferation can be performed under low magnification, it is essential in many functional or phenotypic assays to obtain higher magnification to capture detailed images. The Cytation C10 Confocal imager was used to collect images in widefield and confocal modes. A series of Z-stacked images were captured by using both imaging methods; the z-projections produced in widefield were focus-based and in confocal maximal projection was used. The thin film bottom of the Agilent black-sided clear bottom plates (96-well or 384-well) allow for excellent imaging in either mode and clear images were obtained using all three-color channels in both widefield and confocal modalities. However, confocal imaging demonstrates the expected increase in contrast and apparent resolution of subcellular features, compared to the widefield imaging modality. Representative images from a 384-well plate comparing widefield and confocal are shown in Figure 5, and these results are consistent in the 96-well plate format.

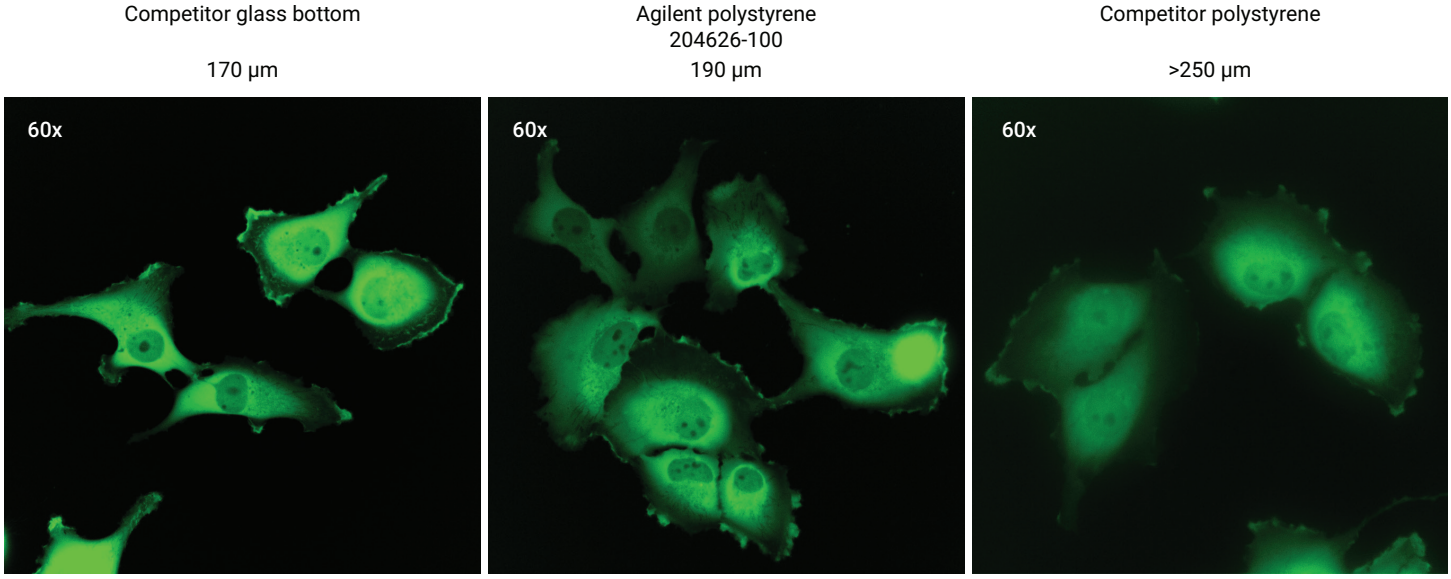


Figure 4. Comparison of MCF7-GFP live cell images in glass-bottom, Agilent thin-bottom, and competitor thicker bottom 96-well microplates. MCF7-GFP cells were initially seeded at 1,000 cells per well and imaged after 24 hours in complete growth media. In the resulting images, the background fluorescence of the Agilent plate was approximately 1.2-fold higher than the glass microplate, while the thicker polystyrene plate showed an approximately 2-fold higher background compared to the glass plate.

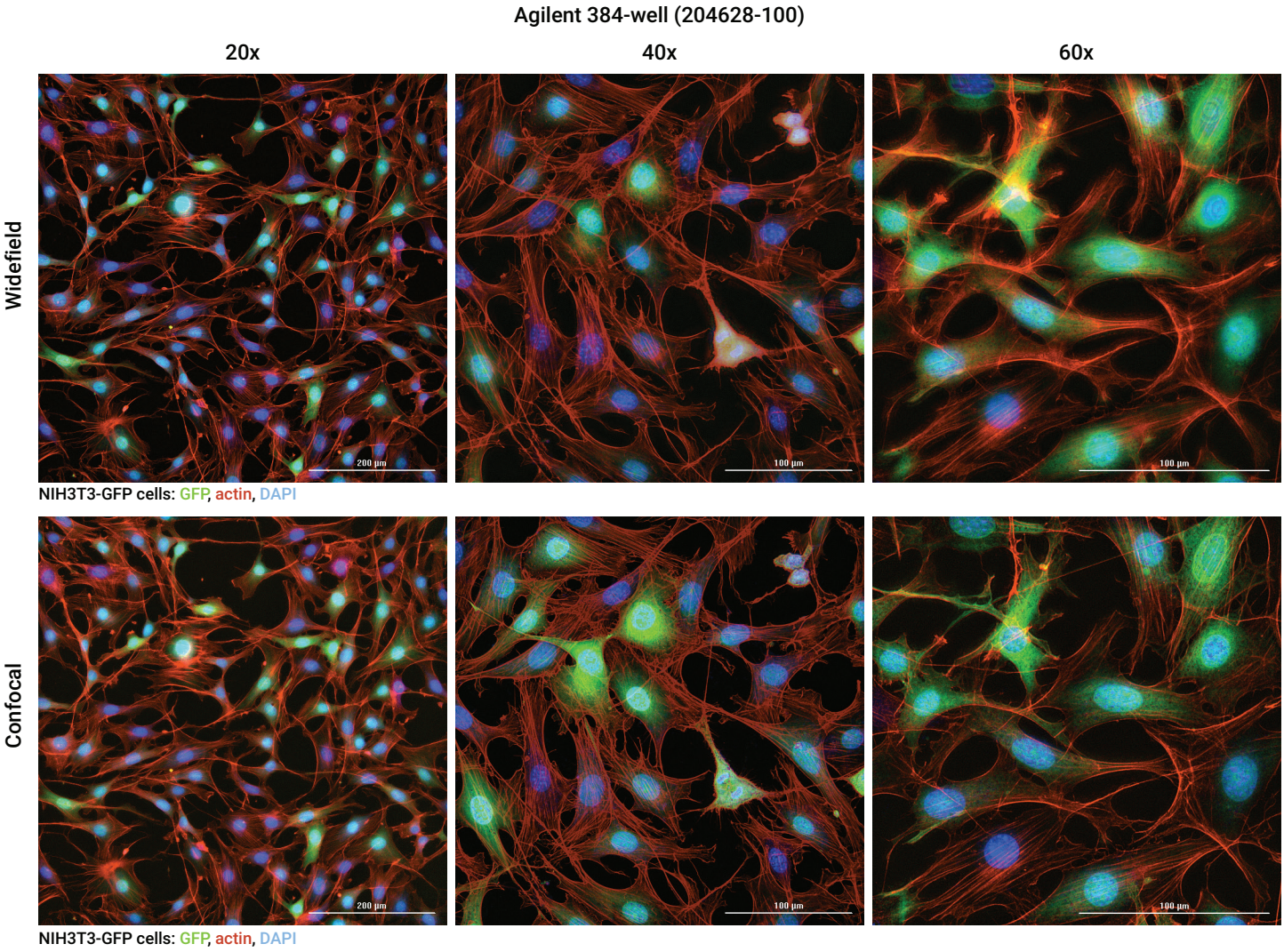


Figure 5. Comparison of widefield and confocal imaging of NIH3T3-GFP using an Agilent 384-well black-sided clear bottom plate. NIH3T3-GFP cells were fixed and stained for actin and nuclei (DAPI). A series of Z-stack images were acquired in three colors at the specified objective magnification using an Agilent BioTek Cytation C10 multimode imager. Agilent BioTek Gen5 software was used to produce the z-projected images presented for both widefield and confocal image series.

Fixed-cell high-magnification confocal imaging comparing polystyrene to glass-bottom plate

MCF7-GFP and U87-GFP cell lines with differing morphology attributes were plated, fixed, and stained for subcellular structures. A three-way comparison was undertaken to compare image quality among a competitor's glass-bottom microplate format, Agilent's thin-bottom polystyrene imaging microplate, and a competitor's polystyrene microplate.

In Figure 6A, the image of a field of MCF7 cells includes at least one cell undergoing mitosis. At 60x magnification, both the nuclear restructuring and the microtubule reorganization during cell division can be appreciated. In the glass microplate and Agilent microplate format, the DAPI staining of DNA shows clear chromosomal condensation and alignment with the mitotic spindle fibers that are stained brightly with the tubulin antibody. A similar image taken from a thicker polystyrene plate exhibits decreased contrast and a reduction in the apparent subcellular resolution compared to the Agilent microplate.

In Figure 6B, U87-GFP cells are stained with a dye-conjugated phalloidin stain, highlighting the filamentous actin component of the cytoskeleton. Actin fibers are visualized throughout U87 cells and are enriched at cell edges. Consistent with the live cell imaging results, the glass and Agilent microplates produce similar quality images, while the thicker polystyrene plate has increased background (particularly in the green channel) and decreased contrast resulting in the reduced definition of subcellular structures.

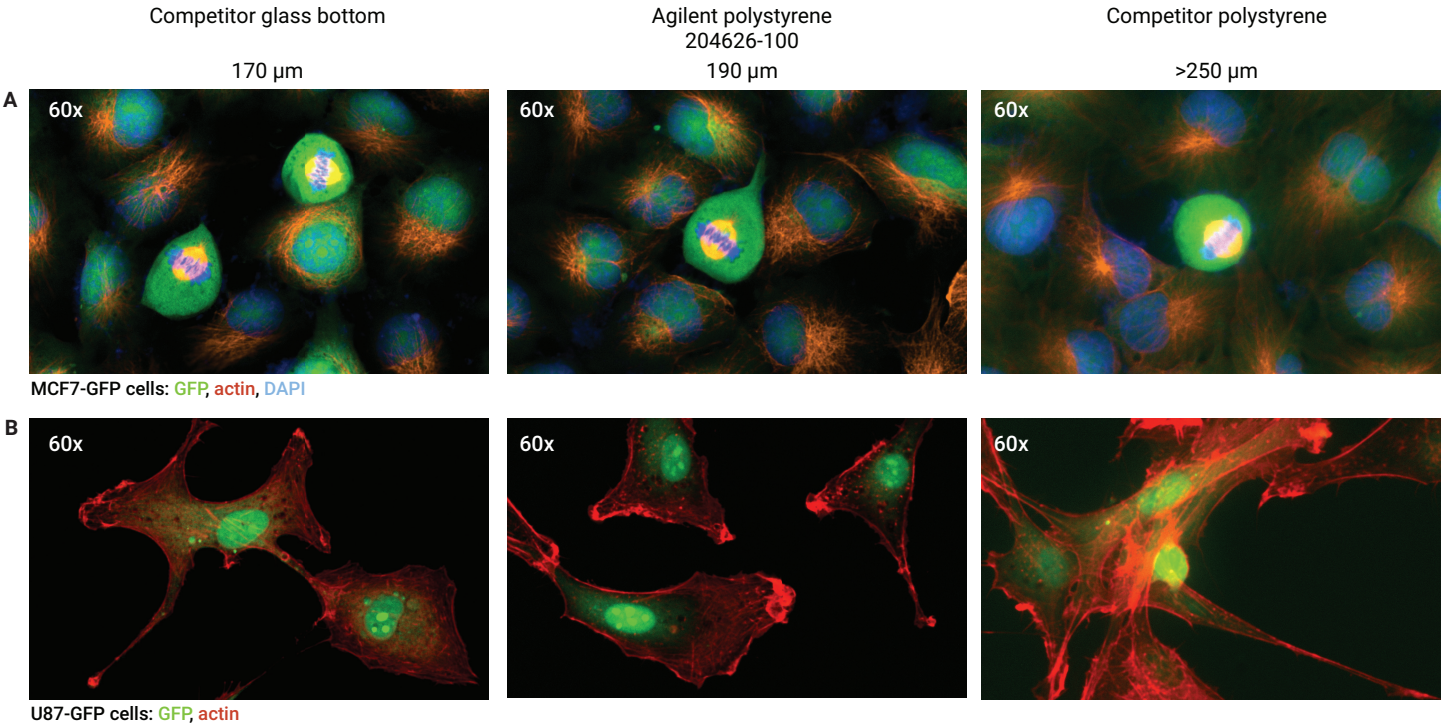
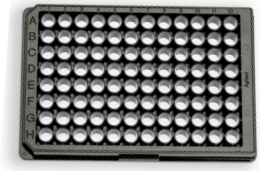


Figure 6. Comparison of cytoskeletal staining in images of cells in a competitor's glass-bottom, an Agilent, and a competitor's 96-well microplates. (A) GFP expressing MCF7 cells were fixed and stained for α-tubulin and nuclei (DAPI). One or two mitotic MCF7 cells are displayed in each image, surrounded by non-mitotic cells. Tubulin staining strongly labels mitotic spindle structures, and DAPI indicates condensed chromatin structure. Both glass-bottom and Agilent microplates produce similar high-quality images with clearly visible subcellular cytoskeletal structures, while images captured from thicker polystyrene plates have increased background and decreased clarity. (B) U87-GFP cells were stained with phalloidin to label actin filaments of the cytoskeleton. Actin filaments are visible throughout the cytoplasm and enriched at cell peripheries. Quality images are generated using the glass bottom and the Agilent microplates, but the thicker competitor polystyrene plate has a high background in the green channel with a lower resolution of subcellular compartments.

Conclusion

Imaging cells using high-resolution fluorescence in a high-throughput manner requires quality microplates. When the plate attributes are suboptimal, the resulting low-quality images can lead to poor object identification and misinterpreted conclusions. Research studies often include several assays run consecutively or in parallel. A cell microplate that can reliably function across multiple applications can streamline workflows. The data presented in this technical note illustrate the Agilent Cell Culture and Imaging plate's suitability for assays ranging from low magnification live cell fluorescence cell counting assays to higher magnification fixed-cell confocal imaging of subcellular structures. The thin optical bottom (190 µm) allows for superb optical clarity comparable to the "gold standard" glass-bottom plates. The similarity in well bottom thickness ends the need to adjust the object correction collar if also imaging glass slides or glass plates on the imaging system. The tissue culture-treated surface enables efficient cell attachment and cell proliferation. In contrast, glass-bottom plates are expensive and often require the addition of a biological coating to promote attachment. The whole-well imaging of the 384-well plate shows the consistent quality of the plate's well-bottom architecture and the low autofluorescence of the polystyrene. Automation compatibility allows easy integration into laboratory screening workflows.

Ordering information

Description		Part Number
Clear Polystyrene, Flat Bottom, 96-Well, with Lid and Tissue Culture Treatment, Irradiated		204624-100
Black Polystyrene, Clear Flat Bottom, 96-Well, with Lid and Tissue Culture Treatment, Irradiated		204626-100
Black Polystyrene, Clear Flat Bottom, 384-Well, with Lid and Tissue Culture Treatment, Irradiated		204628-100

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