TECHNICAL NOTE

PhenoPlate 384-well dedicated design for high content screening.

Authors

Heidi Morgan
Stefan Letzsch
Rob van Beurden

Revvity, Inc.

Background

High content screening (HCS) and high content analysis (HCA) are imaging-based multi-parametric approaches of cell analysis that can be used for a broad range of applications in life science research, including target identification, primary and secondary screening, safety assessment, and systems biology. Imaging cultured cells using high resolution fluorescence and/or brightfield microscopy in a high-throughput manner with a high-content screening system, requires the use of high quality microplates. The better quality of the plate leads to a better quality image, thus more predictive of true biology and clinical disease research.

Features and benefits:

- Ultra-low plate bottom (200 µm)
- All well access when using water immersion and high NA objectives
- Excellent plate bottom flatness
- Optimal clarity and fast autofocusing
- Superior image quality with bottom made of cyclic olefin
- Reduced evaporation due to special design, low profile plate lid
- Unique corner spacers avoid damaging the imaging surface when stacking without lid
- Suitable for various applications
- Available with different coatings

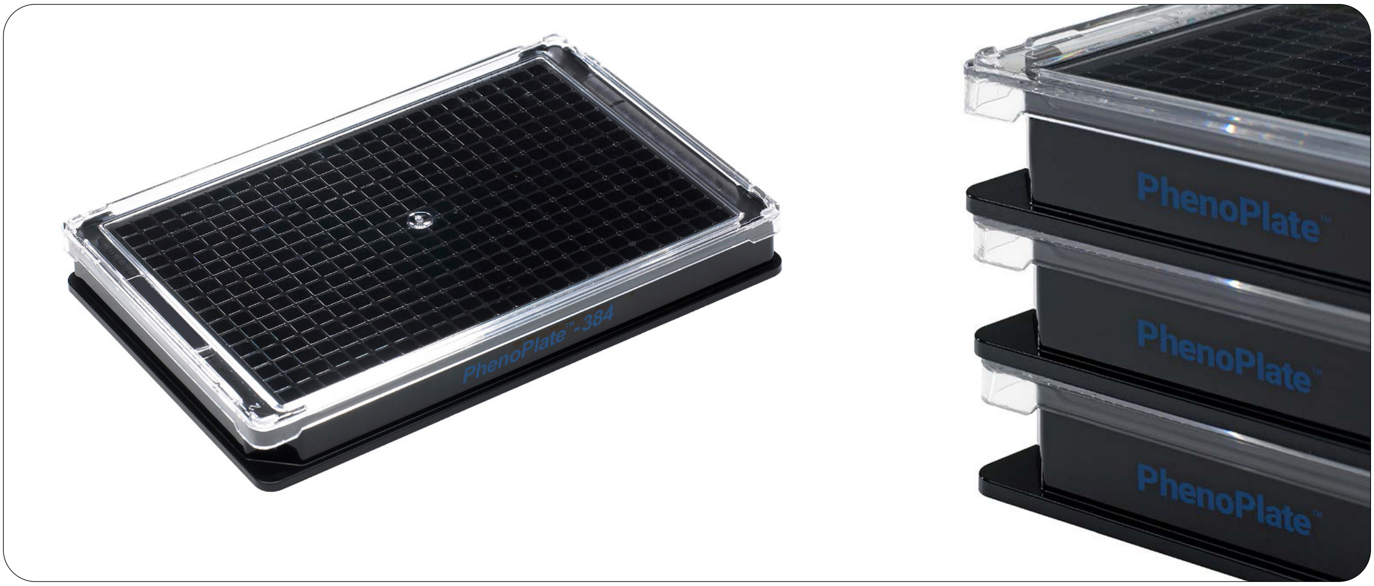

Figure 1: PhenoPlate 384-well features an ultra-low plate bottom, reduced evaporation rate with low profile lid, and unique corner spacers for ease of stacking plates with or without lid.

Based on many years of applications development in high content screening and an in-depth understanding of the requirements needed for improved data quality, Revvity's expert team has developed and validated the PhenoPlate 384-well for optimal performance in high content imaging applications. These unique plates are equipped with optimal clarity and fast autofocusing, due to excellent flatness of the plate bottom. Superior images are obtained due to high optical quality of the cyclic olefin (CO) foil imaging surface, which is very similar to glass, offering better transparency in the near UV range when compared to polystyrene, and cells adhere nicely to CO. Other features include improved well access when using water immersion and high NA objectives with the ultra-low plate bottom (200 µm), reduced evaporation proven with special design, low profile plate lid, and uniquely developed corner spacers avoid damaging the imaging surface when stacking several plates (See Figure 1). In addition, these plates are tissue culture treated, irradiated, and are available with custom coatings (poly-D-lysine, collagen, gelatin) to suit your research workflow, specific cellular adherence requirements, and application of interest.

Excellent plate bottom flatness

Excellent plate flatness, along with image clarity and superior optical quality are important features of plates used for HCS applications. Polystyrene does not provide the best clarity and can bend, while glass provides the best image, it is quite expensive. Using the PhenoPlate 384-well with cyclic olefin (CO) bottom material, Figure 2 shows sharp and focused cellular images from the Opera Phenix® HCS System captured in the entire range of magnification, from 10x air (panel A), to 20x water immersion (panel B) and to 63x water immersion (panel C). As shown in the images, there are no limitations in magnification, as all cells are in focus throughout the entire field of view with any objective used.

Figure 3 compares flatness properties of two commonly used 384-well plate types compared to PhenoPlates in a 3D graphical format. Panel A shows a standard polystyrene (PS) bottom plate, panel B shows a standard glass bottom (GB) plate, and panel C shows a CO bottom PhenoPlate 384-well. Panel D represents how the 3D graphical data was gathered. First, measurement of bottom height in positions (1) to (4) for each well was determined. The difference between the maximal and minimal value for each well was calculated which is shown in the graphs. Values higher than 10 microns (red area in panel A) can result in unfocused regions in images and reduce image quality which is the case for the standard PS bottom plate. The bottom height variation within the well reached values up to 15.6 µm per well leading to unfocused images. In contrast, the height variation within the well of the PhenoPlate 384-well was maximal 6.2 µm which is comparable to the standard glass bottom plate with a maximal height variation of 5.9 µm. These data show that the PhenoPlate 384-well is very comparable to a superior quality GB plate in terms of inner well flatness, resulting in higher image quality. The newly designed thin and flat CO bottom of the plate allows generation of better images in combination with the superior optical clarity of the CO material used, and is comparable to that of glass and is better than standard polystyrene.

Ultra-low bottom height

For high content applications, high NA objectives are preferred as they provide superior resolution images and allow for water immersion. Since most high NA objectives have a low working distance, a microplate with a thin plate bottom and low bottom height is required to fully utilize the advantages of these objectives across the whole plate. Combining high bottomed plates (bottom height >300 µm) with high NA objectives will lead to imaging measurement restrictions. The outermost wells cannot be read as the objective would collide with the plate rim or the plate table of the device. The unique design of PhenoPlates solves these issues by reducing plate bottom height to a very low level. The ultra-low plate bottom enables usage of all high NA and water immersion objectives right at the edge of the plate, and most importantly allows better accessibility to all of the wells in the plate. Figure 4 shows the ultra-low plate bottom of PhenoPlates in detail. The diagram in panel A represents the side view of a standard microplate with a high plate bottom and depicts how the objective could collide with the bottom of the plate. The sketch in panel B displays the side view of a PhenoPlate 384-well and the optimized ultra-low plate bottom. This new design reduces the bottom height to an ultra-low level (200 µm). This shows that with the enhanced ultra-low bottom design, more images can be acquired and more data generated at high resolution, because there are no limitations with high NA objectives on all HCS systems.

Reduced evaporation

Evaporation rates can drastically change the results of HCS data, particularly in longer experiments such as live cell assays. The low profile plate lid of the PhenoPlates prevents evaporation in edge wells, which allows every well to be used as samples for replicates. Figure 5 shows 3D graphical representations of evaporation, comparing a standard plate with the PhenoPlate. The plates were filled with 40 µL water per well and incubated for 24 hours at 37 °C, 5% CO_2.

The graph in panel A illustrates the standard 384-well plate with a regular lid and the graph in panel B illustrates a PhenoPlate 384-well with the newly designed low profile lid. The low profile lid shows significantly decreased volume reduction in outer wells. This clearly demonstrates that with the PhenoPlate lid, evaporation rates are lowered which results in less variability between replicates, more accurate results, and allows for usage of the entire 384-well plate for added data, and in turn saving the researcher time and money in the long run.

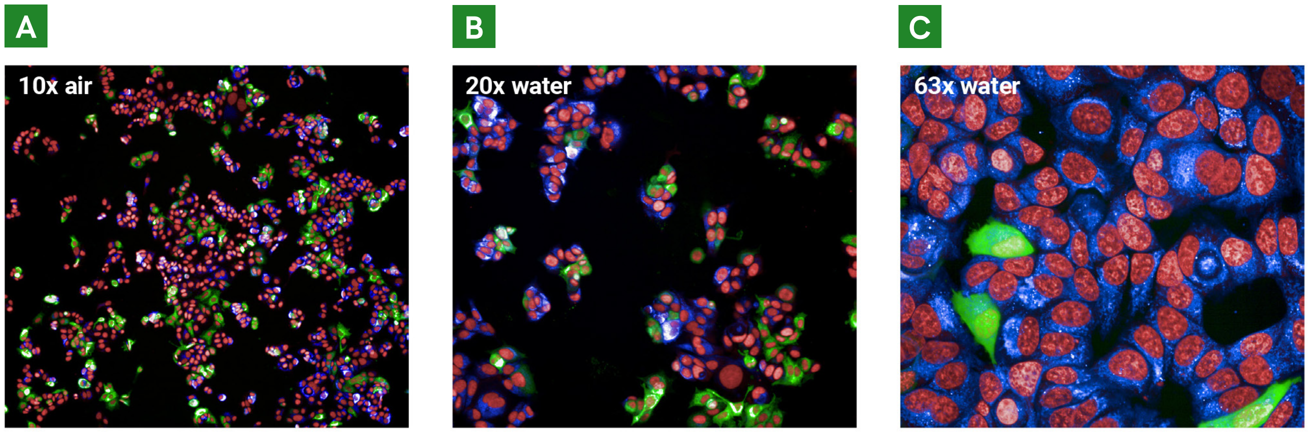

Figure 2: Cellular images from the Opera Phenix high content screening system. The cells are in focus throughout the entire field of view for each magnification due a combination of the imaging system and the excellent bottom flatness of the cyclic olefin surface in PhenoPlate 384-well.

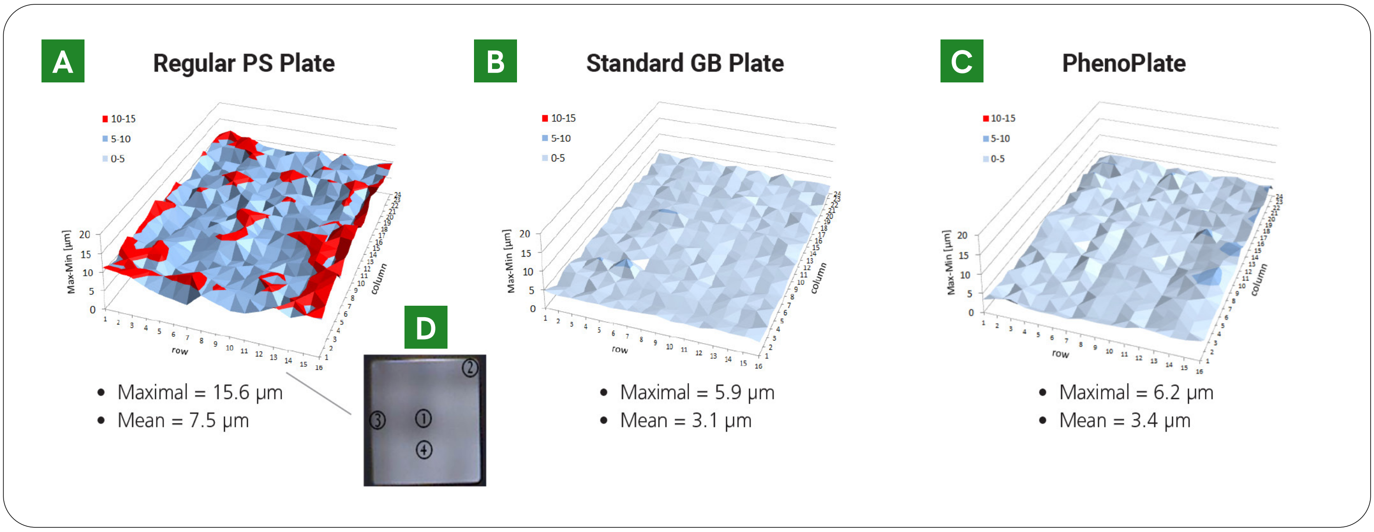

Figure 3: Flatness of plate bottom. Panel A shows a 384-well plate with a standard polystyrene bottom (PS), panel B shows a 384-well plate with a standard glass bottom (GB), and panel C shows a PhenoPlate 384-well with a cyclic-olefin bottom (CO). Panel D represents measurement of bottom height in positions 1 to 4 for each well. The difference value between the maximal and minimal bottom height in each well was calculated and plotted for each well and plate type. Values higher than 10 microns (red area) can result in unfocused regions in images for many wells of the standard PS plate. In contrast, the height variation within the well of the PhenoPlate is maximal at 6.2 µm which is comparable to the standard glass bottom plate, with a maximal variation of 5.9 µm. The PhenoPlate 384-well is very comparable to a glass bottom plate in terms of bottom flatness.

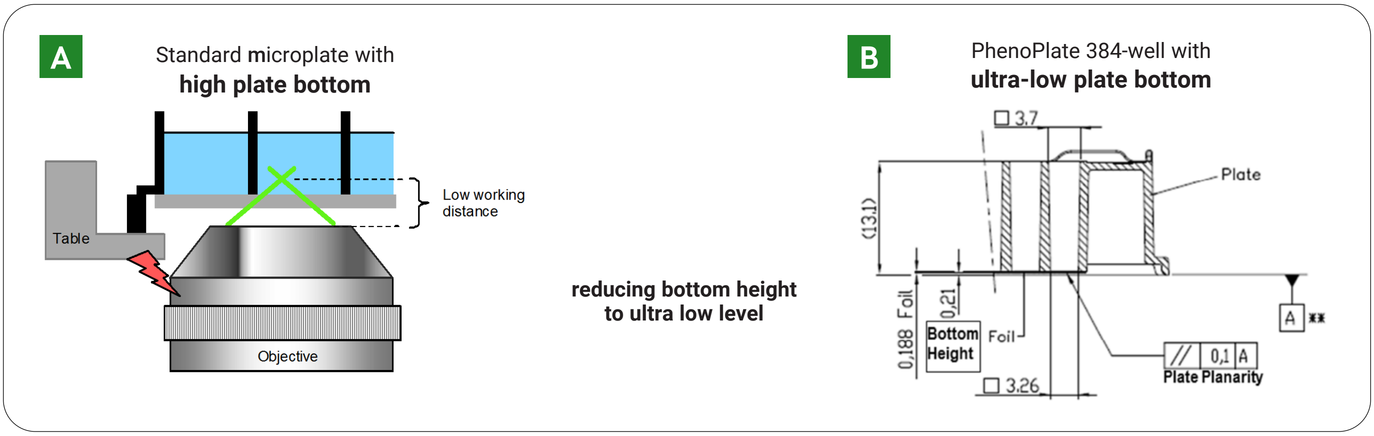

Figure 4: Ultra-low plate bottom allows use of high NA objectives in outermost wells. The illustration in panel A represents the side view of a standard microplate with a high plate bottom where the objective collides with the plate table or the bottom of the plate. The drawing in panel B represents the side view of a PhenoPlate 384-well with the optimized ultra-low plate bottom. By reducing the bottom height to an ultra-low level (200 µm), measurement restrictions are minimized and all wells of the plate can be read on HCS systems.

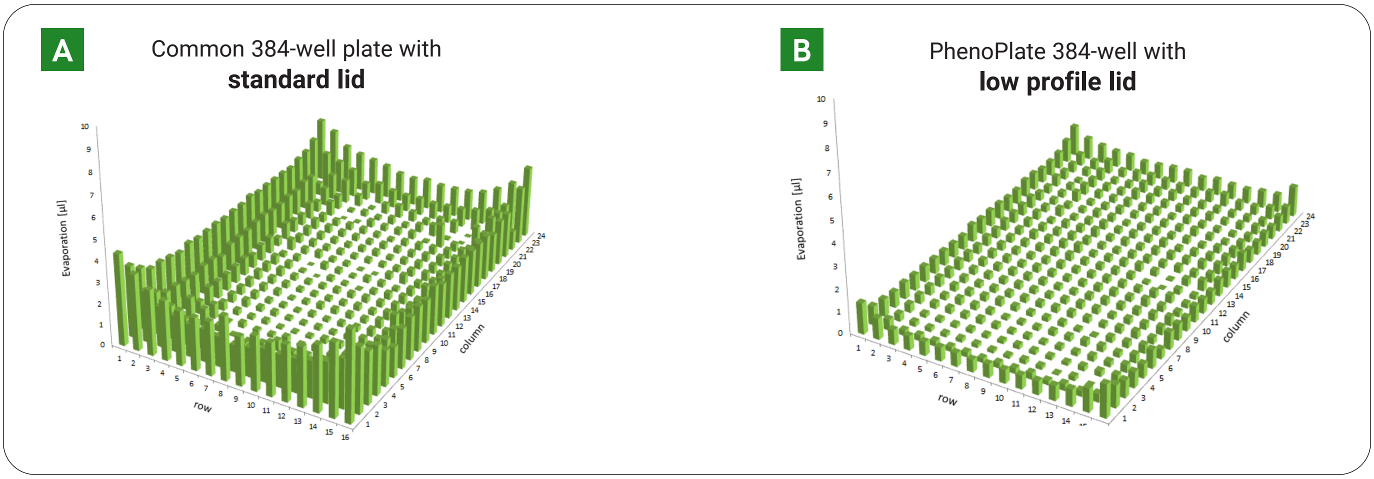

Figure 5: Evaporation measured over 24 hour incubation period (37 °C, 5% CO_2) comparing a standard plate with the PhenoPlate 384-well filled with 40 µL water. The graph in panel A illustrates the evaporation in a standard 384-well plate with a regular lid and the graph in panel B illustrates a PhenoPlate 384-well with the newly designed low profile lid. The low profile lid shows significantly decreased volume reduction in outer wells.

Conclusions

In this technical note, we have shown that 384-well PhenoPlates are suitable for high content imaging applications, including live cell assays, and offer a range of advantages:

- Optimal clarity and fast autofocusing from excellent flatness of the plate bottom
- Superior image quality from high optical quality of cyclic olefin imaging surface
- Better well access when using water immersion and high NA objectives with ultra-low plate bottom
- Reduced evaporation from new design, low profile polystyrene lid
- Specially designed corner spacers for easy stacking with or without lid
- Automation friendly and manual compatible
- Multiple reads in one platform

The 384-well PhenoPlates are part of Revvity's range of microplates for high content applications, and a complete solution for high content imaging alongside the Operetta® CLS™ and Opera Phenix® Plus high-content imaging systems, software and automation. Additionally, the PhenoPlates can be read on the EnSight™ multimode reader equipped with cell imaging capabilities.

References

1. Operetta Application Guide, 2nd Edition (2010): Example RMS, Migration-Confluency, p281.
2. Cyclic Olefin Polymers: Innovative Materials for High-Density Multiwell Plates, Walter D. Niles and Peter J. Coassin.
3. Chemical Structure and Physical Properties of Cyclic Olefin Copolymers (IUPAC Technical Report). Ju Young Shin, Ji Yong Park, Chenyang Liu, Jiasong He, and Sung Chul Kim. Pure and Applied Chemistry. Vol. 77, No. 5, pp. 801–814 (2005).

Revvity, Inc.
940 Winter Street
Waltham, MA 02451 USA
(800) 762-4000
www.revvity.com