N° 18

FORUM

TECHNICAL NOTES & APPLICATIONS FOR LABORATORY WORK

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SCREENSTAR AND CELLVIEW: MICROPLATES FOR ADVANCED MICROSCOPY

1/ APPLICATIONS & FEATURES

1.1/ APPLICATIONS

- High-content screening
- Water or oil immersion objectives
- High-magnification objectives (40x and above)
- High-resolution objectives

1.2/ FEATURES

- SCREENSTAR: 190 µm cycloolefin film bottom microplate
- CELLview: 175 µm glass bottom microplate
- Recessed microplate bottom for high magnification and improved resolution
- Superior bottom quality to enable high-quality images
- Meets ANSI standards
- Universal microscope objective compatibility
- Proven cell culture surface treatment

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2/ ABSTRACT

SCREENSTAR and CELLview are microplates for advanced microscopic applications such as high-content screening with water or oil immersion objectives. The special design of the two microplates allows low working distances and combines excellent optical features with high-quality surfaces for cell culture and biochemical assays.

SCREENSTAR microplates are manufactured with an optically transparent 190 µm cycloolefin film bottom.

CELLview microplates are manufactured with a 175 µm borosilicate glass bottom.

3/ MICROSCOPY IN CELL BIOLOGY

Microscopy is a basic and highly important method in life science frequently used in research as well as medical diagnostics such as histological cancer classification. Technological progression in confocal microscopy, optical systems and emerging technologies such as total internal reflection fluorescence microscopy (TIRFM), continues to elevate microscopy as one of the most powerful tools in cell biology.

Unless intentionally cultivated as suspension or spheroidal cultures, adherent cells and tissues customarily require a surface to attach and/or grow **(Fig. 1)**.

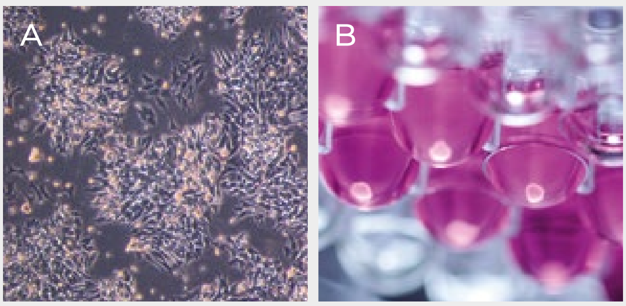


Figure 1:
(A) HEK 293 cells growing on the surface of a black clear bottom (µClear®) tissue culture treated microplate (Item No. 655090)
(B) Tumour spheroid in a well of CELLSTAR® cell-repellent U-bottom microplate (Item No. 650970)

Typical specimen supports in microscopy for cells or tissues are objective slides, flasks, dishes and microplates. For microscopic analysis, inverted microscopes are commonly used. The objective is positioned below the microscope stage that houses the substrate containing the biological specimen, causing inclusion of the biological sample support within the optical pathway **(Fig. 2)** of microscopic detection.

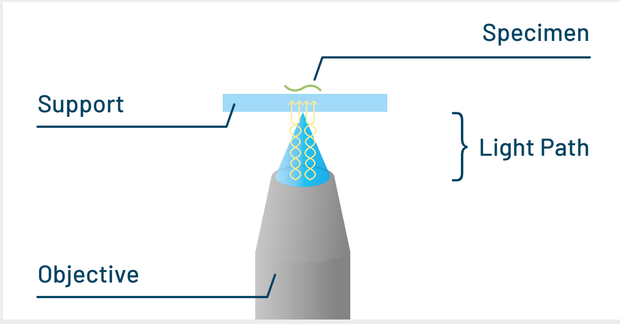


Figure 2: **Specimen support**, e.g. microscopic slide as part of the light path in an optical system

Coming from standard top microscopy, microscope objectives are typically calibrated to accommodate a 175 µm glass surface, as the standard thickness of cover slip glass. However, microscope slides, standard flasks, dishes and multiwell plates or microplates generally feature an approximate 1 mm bottom thickness **(Table 1)**. For demanding microscopic applications at higher magnifications, a 1 mm bottom thickness can lead to a major reduction in resolution due to focusing problems with standard specimen supports. This effect is exacerbated especially with oil or water immersion objectives, as the microscope objectives are typically calibrated to the refractive index of glass, which differs from air or oil **(Table 2)**, as well as adjusted to a cover slip glass thickness of 175 µm.

For sophisticated microscopic applications, specialised supports that feature a 175 µm bottom thickness can be advantageous **(Fig. 3)**. In example, for high-resolution non-invasive fluorescence microscopy of live organisms and cells, small petri dishes that contain an embedded 175 µm glass cover slip bottom such as the CELLview Dish **(Fig. 4)** can be used to perform multiposition time-lapse imaging.

Item No.	Description	Well Bottom Thickness	Distance Well Bottom Internal	Distance Well Bottom External
664160	Cell Culture Dish	1 mm	1.6 mm	0.6 mm
658175	Cell Culture Flask	1.4 mm	2.5 mm	1.1 mm
657160	6 Well Multiwell Plate	1.2 mm	2.5 mm	1.3 mm
665180	24 Well Multiwell Plate	1.2 mm	2.5 mm	1.3 mm
655180	96 Well Solid Bottom Microplate	0.95 mm	3.5 mm	2.55 mm
655090	96 Well µClear® Microplate	0.19 mm	3.5 mm	3.31 mm
655866	96 Well SCREENSTAR Microplate	0.19 mm	0.7 mm	0.51 mm
655891	96 Well CELLview Microplate	0.175 mm	0.7 mm	0.525 mm

Table 1: Well bottom thickness

Media	Refractive Index
Air	1.0
Glycerine	1.47
Water	1.33
Immersion Oil	1.52
Glass	1.52
Cycloolefine	1.53
Polystyrene	1.58

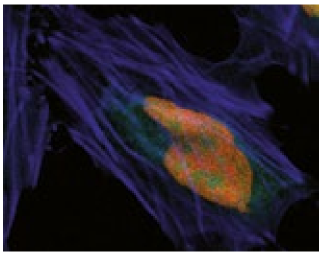
Table 2: Refractive index of different materials

Figure 4: CELLview Dish (Item No. 6278XX and 6279XX)

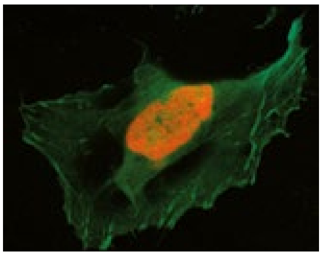
POLYSTYRENE AND CYCLOOLEFIN YIELD HIGH RESOLUTION SIMILAR TO GLASS

Fluorescence Microscopy of HELA cells, 63x (LSM 710 Zeiss)

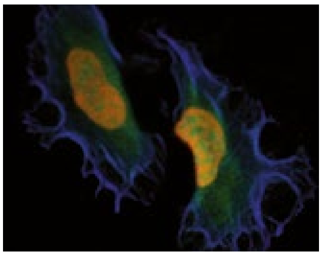
● cytoplasm ● nucleus ● cytoskeleton



Polystyrene
(µClear® bottom)
190 µm



CELLview
(glass bottom)
175 µm



SCREENSTAR
(cycloolefin bottom)
190 µm

Figure 3: Fluorescence microscopy with an inverted microcscope (LSM 710, Carl Zeiss AG, Germany, 63x with oil). Comparison of different base materials of ultra-thin bottoms: polystyrene (µClear® film), glass (CELLview) and cycloolefin (SCREENSTAR). All materials gave a very high resolution.

4/ SUBSTRATES IN MICROSCOPY

4.1/ GLASS

The typical substrate for biological samples in microscopy is glass. Glass displays excellent optical properties **(Fig. 3, 5, 6, 7)** as it is highly transparent, clean, and less susceptible to scratching and dust contamination. Glass bottom microplates such as the 96 well CELLview feature excellent intra- and inter-well flatness due to the rigidity of the glass bottom. As microscope objectives are adapted to the bottom thickness and refractive index of cover glass, generally the best optical quality with highest resolution can be achieved using glass substrates **(Fig. 7)**.

OUT-OF-FOCUS BLUR OF DIFFERENT MATERIALS

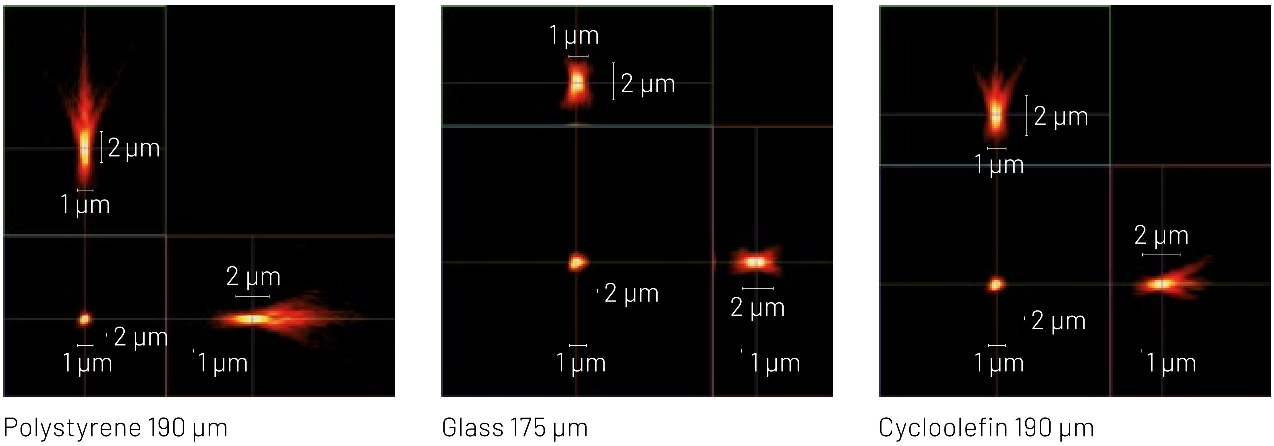


Figure 5: Confocal imaging
(LSM 710, 63x, oil immersion, Zeiss AG, Göttingen, Germany) of fluorescent beads in microplates with different film bottoms. Images display the out-of-focus blur obtained with different materials (polystyrene, glass and cycloolefin). Cycloolefin shows approximately the same low level of out-of-focus blur as glass.

AUTOFLUORESCENCE OF DIFFERENT MATERIALS

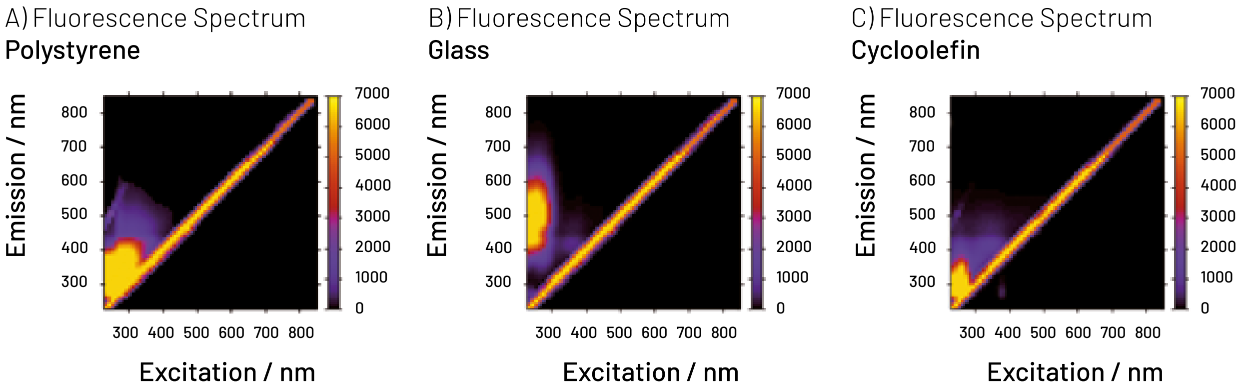
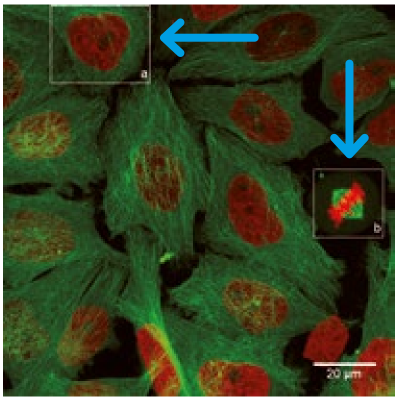
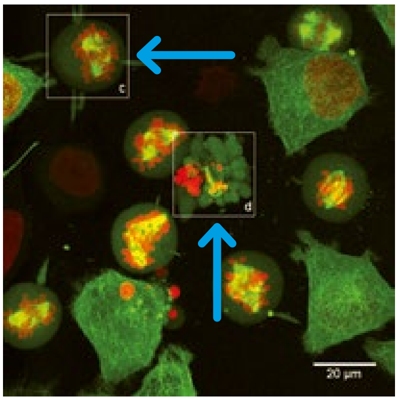


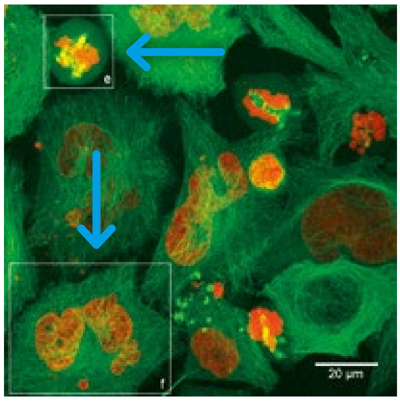
Figure 6: Autofluorescence of polystyrene (A), borosilicate glass (B) and cycloolefin (C) in the UV
The graphs display autofluorescence in a 2D diagonal plot, where the diagonal peak represents equal excitation and emission wavelengths. Due to energetic loss, emitted light generally exhibits a higher wavelength than that of excitation, therefore autofluorescence only occurs when the emission wavelength is above the excitation. The lowest autofluorescence can be detected in the cycloolefin microplates (C), followed by the glass bottom microplate (B).



Control: Cell division with correct chromosome alignment (a, b)



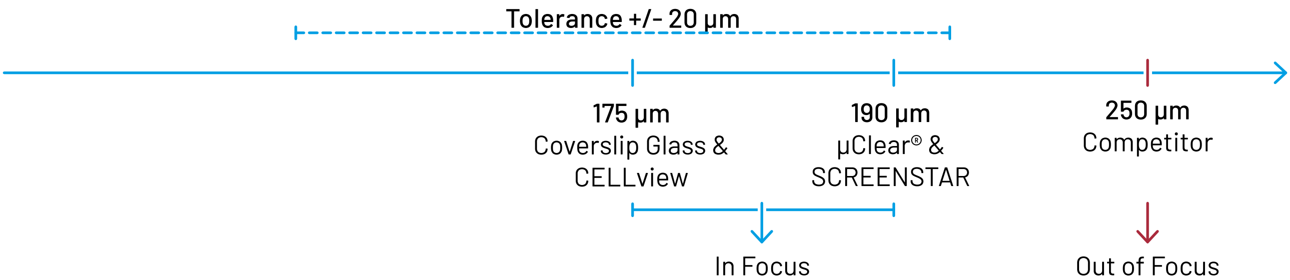
siRNA-1: Cell division with chromosome alignment problems
(c) multipolar spindle
(d) cell death



siRNA-2: Cell division with
(e) chromosome segregation problem
(f) polylobed nucleus

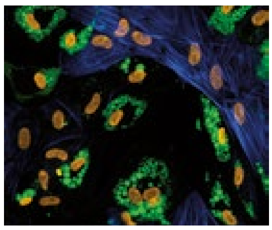
Figure 7: **Phenotypic profiling of human genes by time-lapse microscopy in glass bottom microplates**
(96 well CELLview; Item No. 655891). Maximum projection of confocal still images of HeLa cells stably expressing GFP-tubulin (green) and H2B-mCherry (red) after RNAi knockdown show phenotypes during cell division and mitotic consequences such as multipolar spindle, polylobed nucleus and cell death. Images are courtesy of Dr. Beate Neumann, Advanced Light Microscopy Facility, European Molecular Biology Laboratory, Heidelberg, Germany.

INFLUENCE OF BOTTOM THICKNESS ON RESOLUTION AND IMAGE QUALITY

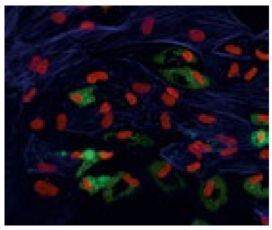


Adipogenesis of hMSC
(# R7788-115, StemPro® human ADSC, Invitrogen, Carlsbad, USA)
LSM 710, Zeiss, 20x

- Lipid Vesicles
- Nucleus
- Cytoskeleton



190 µm



250 µm

Figure 8: **Influence of bottom thickness on resolution and image quality**
The adipogenesis of human mesenchymal stem cells (hMSC) was analysed in microplates with 190 µm film bottom and competitive microplates with 250 µm film bottom. The 190 µm film bottom demonstrates exceptional performance, giving high image quality with excellent resolution due to the bottom thickness being within the tolerance range of the microscopic system (Operetta® High Content Imaging System, PerkinElmer, Waltham, Massachusetts, USA). The 250 µm film bottom of the competitive microplate yield blurry images, incapable of sharp focus and magnifications above 20x.

4.2/ POLYSTYRENE

Polystyrene is the most widely used base material for microplates. Polystyrene film bottom plates are an optimal solution for routine high-throughput and imaging applications. In general, polystyrene microplates are reasonably priced due to highly automated production processes and relatively low material costs. The 190 µm polystyrene film bottom of the Greiner Bio-One black and white µClear® microplates is perfectly suited for basic microscopic applications as it fits into the tolerance window of most microscopic objectives **(Fig. 8)** and does not require any special adaptations or corrective lenses. Although polystyrene substrates are well suited for many cell-based assays, they are not optimized for high magnifications i. e, use of high numerical aperture (NA) objectives with short working distances. This does not only refer to the standard plate geometry but also to the optical properties of the polystyrene polymer. Major advantages are the high variety of cell culture surfaces that facilitate optimal cell culture condition for various applications. CELLSTAR® surfaces for adherent cells are tissue culture treated to provide a perfect support for adherence and growth of many cultures. Moreover, polystyrene can easily undergo different modifications to generate specialised surfaces for sensitive or stressed cultures such as the Greiner Bio-One Advanced TC surface or CELLCOAT® protein coatings (Poly-D-Lysine, Collagen I, etc.), as well as modifications to inhibit cell attachment for suspension or spheroidal cell cultures such as with the Greiner Bio-One CELLSTAR® cell-repellent surface. The result of this flexibility is a large range of specialised surfaces within the polystyrene microplate product range for numerous and varied applications.

4.3/ CYCLOOLEFINS

Although polystyrene substrates are well suited for many cell-based assay and imaging applications, with more challenging microscopic analyses, polystyrene cannot achieve the image quality of glass. Glass substrates can also show limitations for use in demanding cell culture applications, e.g. with cultivation of primary cells on glass, there is an imminent risk for cells to be washed away during the harsh immunochemical staining procedures typically required in fluorescence microscopy. Additionally, the surface modification required to improve cell attachment to glass is time-consuming and expensive.

To overcome the drawbacks for use of both glass and polystyrene as cell culture substrates, cycloolefins have become increasingly popular as a base material for microplate manufactures.

Cyclic olefins have become increasingly popular for use as a base material for microplates. The material possesses excellent optical features with a high spectral transmission and a low background in the lower UV range. With a refractive index which is very close to that of glass, cyclic olefin bottom plates combine excellent optical properties with improved cell growth and cell attachment due to their organo-polymeric nature. Although cyclic olefin bottom plates typically do not achieve the planarity of glass substrates, they show a very good inter- and intra-well flatness.

RECESSED MICROPLATE BOTTOM OF SCREENSTAR AND CELLVIEW MICROPLATES

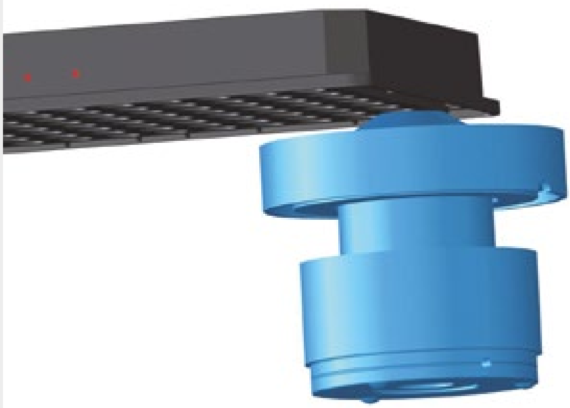


Figure 9: 40x water immersion objective for microscopic detection in a 96 well microplate.
Within the standard 96 well microplate design, the distance between well undersides and microplate skirt bottom causes interference of oil or water immersion objectives with the microplate skirt.
This is not the case with the SCREENSTAR or the CELLview microplates, as the recessed well bottoms enable a close proximity for the objectives to fully access all microplate wells.

5/ SCREENSTAR AND CELLVIEW: MICROPLATES FOR HIGH-RESOLUTION MICROSCOPY

The increasing significance of microscopic methods in drug discovery, with a trend of focusing on subcellular levels with higher resolution, leads to a demand for advanced microscopic technologies. Improved microscopic devices and sophisticated software programs for data analysis in the subcellular level has been launched in the recent decade. However, the exploitation of the technical benefits in high-content screening has been somewhat limited due to restrictions in the design and performance of the available specimen supports.

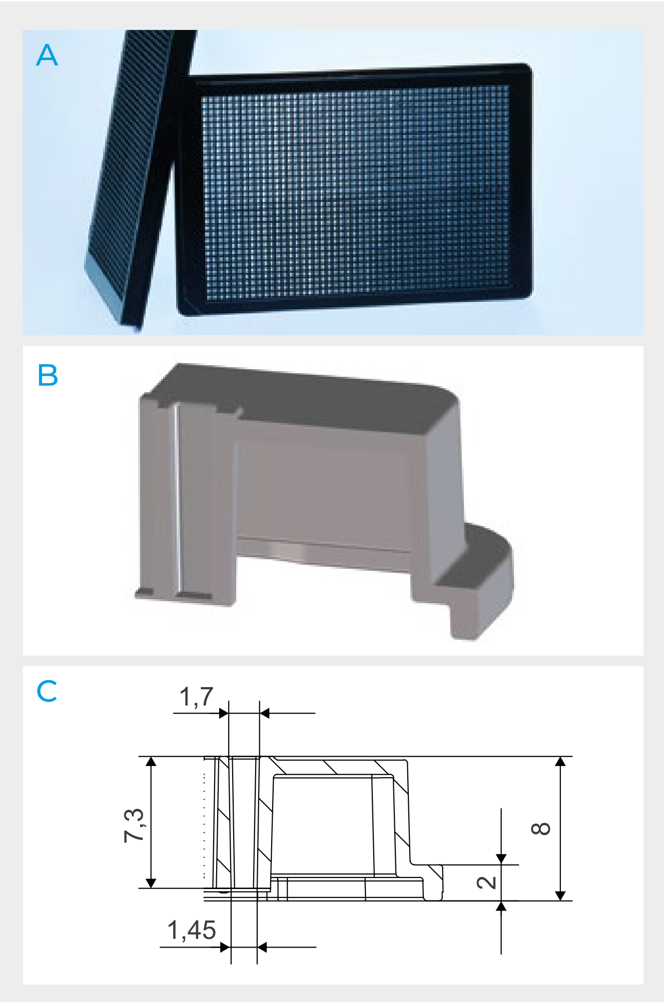


Figure 10: 1536 well SCREENSTAR Microplate
A) Black microplate with clear cycloolefin film bottom (190 µm) for high-content screening
Smooth microplate top absent of alphanumeric coding facilitates flush lid mounting for use within automated systems and improves heat sealing

B + C) Well design of a 1536 well SCREENSTAR microplate

Plate height:	8 mm
Well depth:	7.3 mm
Well diameter top:	1.7 mm
Well diameter bottom:	1.45 mm
Distance microplate rim to internal well bottom:	0.7 mm
Distance microplate rim to external well bottom:	0.51 mm
Film bottom thickness:	190 µm

The abundantly available microscope slide is not suitable for high-content screening applications where multiple samples must be analysed in parallel. Rather, microplates have become the standard substrate of choice in high-content screening, as they facilitate an easy automation and analysis.

RECESSED MICROPLATE BOTTOM

One of the major drawbacks of a classic polystyrene microplate is the distance from the microplate well undersides to the plate skirt bottom. In microscopy, a distance of greater than 1 mm can prohibit access to peripheral wells, especially when high-magnification objectives that almost touch the well undersides, separated just by a thin oil or water layer, are used. For such demanding applications, it is critical that the microplate well undersides are in very close proximity to the bottom of the microplate skirt to allow full access of the objective to all peripheral microplate wells. The CELLview and SCREENSTAR microplates provide a recessed, optically transparent bottom to allow full use of high-magnification oil or water immersion objectives with access to all microplate wells, including perimeter and corner positions **(Fig. 9)**.

5.1/ 1536 WELL SCREENSTAR MICROPLATE

As classic polystyrene microplates are not always preferred for advanced microscopic applications, Greiner Bio-One has developed cycloolefin-based microplates especially designed for microscopy. The initial microplate launched within this portfolio was the 1536 well SCREENSTAR, a black 190 µm film bottom microplate for high-throughput and high-content screening applications **(Fig. 10)**. This plate is followed by two microplates in 384 well and 96 well format.

5.2/ 96 WELL SCREENSTAR AND 96 WELL CELLVIEW MICROPLATE

5.3.1/ PLATE DIMENSIONS AND TOLERANCES

The 96 well SCREENSTAR and CELLview microplates **(Fig. 11)** meet the ANSI 1-2004 through ANSI 4-2004 standards, which define footprint, height dimensions, outer flange, and well position dimensions. The intra-well and inter-well flatness are described by the ANSI 6-2012: „Microplates - Well Bottom Elevation". Most optical devices can deal with an overall flatness (inter-well flatness) below 0.2 mm, which is specified for the SCREENSTAR and CELLview microplates.

5.3.2/ IMPROVED CELL CULTURE CONDITIONS (ENLARGED WORKING VOLUME AND PERIMETER DITCH)

Demanding cell biology applications, such as detection of subcellular reactions, require prolonged cell culture,

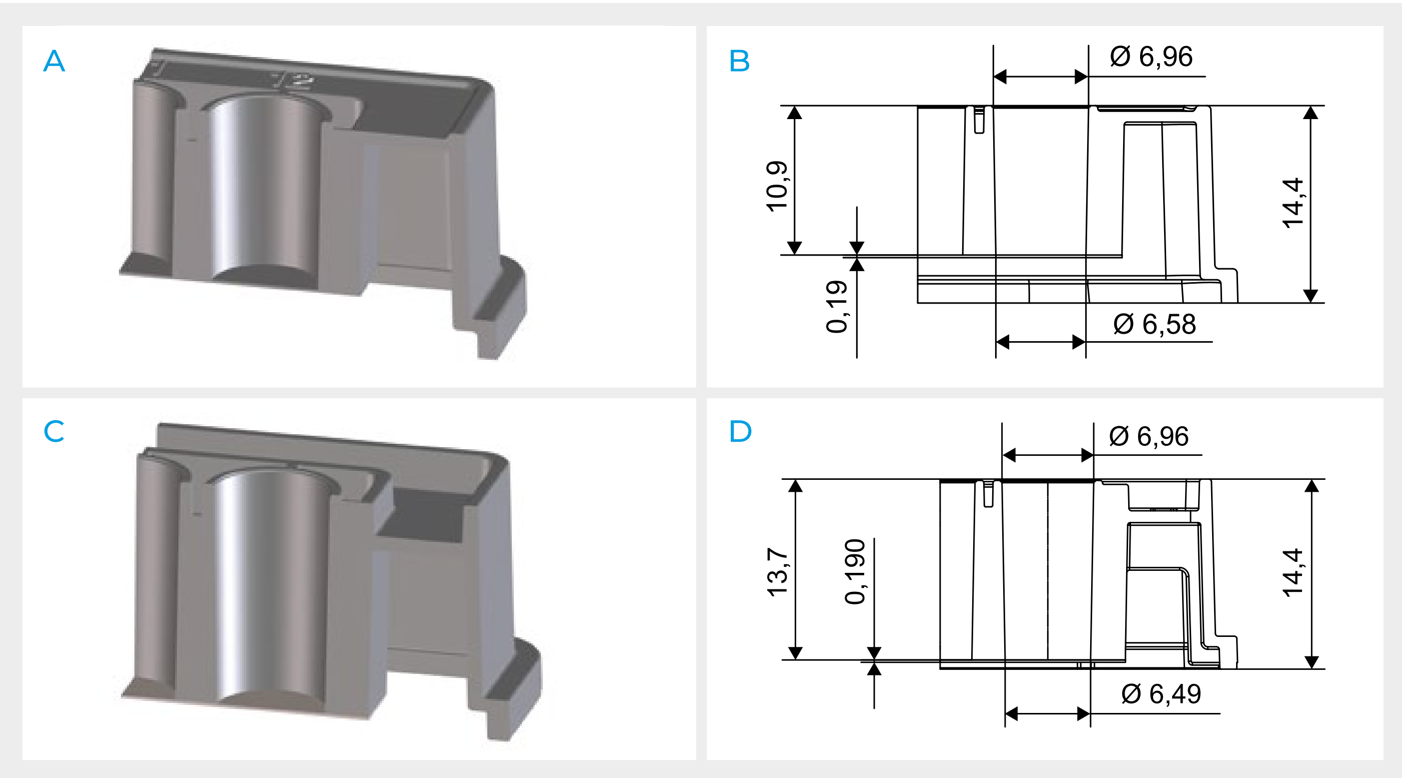


Figure 11:
A) Well bottom distance in a 96 well microplate (Item No. 655090)

C) Well bottom distance in a 96 well SCREENSTAR microplate (Item No. 655866 and 655891)

A basic requirement for the 96 well SCREENSTAR and CELLview microplate was the compatibility of high-magnification objectives such as oil and water immersion lenses. These objectives almost touch the microplate well undersides, separated just by a thin oil or water layer. For this technology, the microplate well undersides must be close to the microplate skirt bottom (Fig. 9) to easily allow interchange of microscopic objectives and complete access of peripheral wells.

B) Well design of a 96 well µClear® PS microplate (Item No. 655090)

Plate height:	14.4 mm
Well depth:	10.9 mm
Well diameter top:	6.96 mm
Well diameter bottom:	6.58 mm
Distance microplate rim to internal well bottom:	3.5 mm
Distance mictoplate rim to external well bottom:	3.31 mm
Film bottom thickness:	190 µm

D) Well design of a 96 well SCREENSTAR microplate (Item No. 655866)

Plate height:	14.4 mm
Well depth:	13.7 mm
Well diameter top:	6.96 mm
Well diameter bottom:	6.49 mm
Distance microplate rim to internalwell bottom:	0.7 mm
Distance microplate rim to external well bottom:	0.51 mm
Film bottom thickness:	190 µm

to include critical media changes. Low working volumes and high evaporation during such long-term cell culture could adversely affect the consistency and performance of a cell-based assay.

To reduce evaporation, the 96 well SCREENSTAR and CELLview microplates offer a ditch around the perimeter which can be filled with either sterile media or water **(Fig. 12)**. This can help create a saturated water vapour barrier to significantly reduce evaporation.

With a working volume of 440 µl/well in SCREENSTAR and CELLview, the volume is increased nearly 100 µl in comparison to a standard 96 well chimney well design **(Table 3)**.

The larger working volume enables use of more media to potentially enlarge time periods between media changes. The nutrition of cells can additionally be improved due to the application of a higher volume of media.

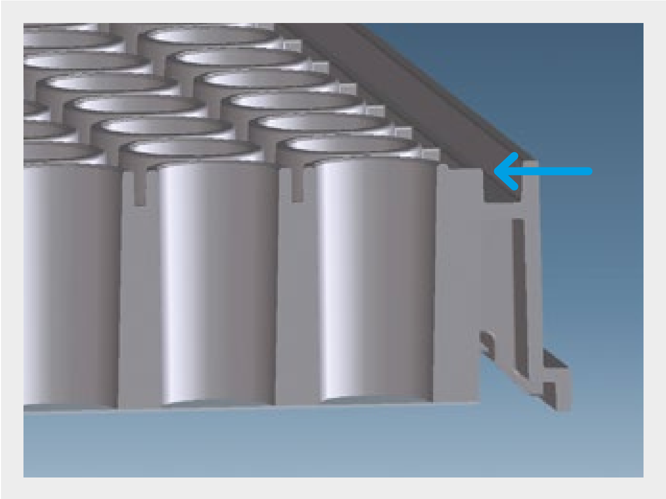


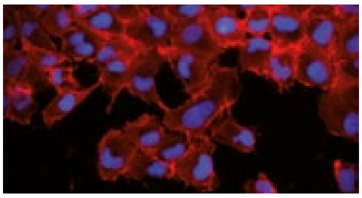
Figure 12: Detail of the 96 well SCREENSTAR microplate demonstrating the perimeter ditch which can be filled with sterile water or media
Well depth: 13.7 mm
Working volume: 25 - 440 µl
Growth area: 33 mm^2
Total volume: 483 µl
Well diameter (bottom): 6.49 mm

Description	SCREENSTAR / CELLview	Standard Chimney Well
Length / Width	127.76 x 85.48 mm	127.76 x 85.48 mm
Height	14.4 mm	14.4 mm
A1 position	11.01 x 7.87 mm	11.01 x 7.87 mm
Top	6.96 mm	6.96 mm
Bottom	6.49 mm	6.58 mm
Skirt	2.0 mm	2.0 mm
Pitch	9 mm	9 mm
Well depth	13.7 mm	10.9 mm
Total volume	483 µl	392 µl
Working volume	25 - 440 µl	25 - 340 µl
Growth area	33 mm^2	34 mm^2

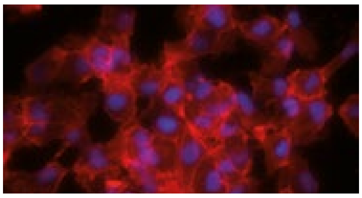
Table 3: Comparison of the major microplate and well dimensions between SCREENSTAR / CELLview and a standard 96 well chimney well microplate

FLUORESCENCE MICROSCOPY OF HELA CELLS CULTIVATED ON DIFFERENT MICROPLATE SURFACES

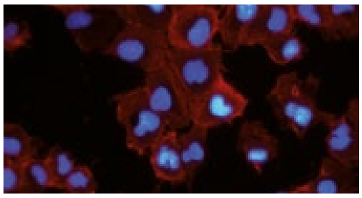
40 x Leica DM IL Fluo (Leica Microsystems GmbH, 35578 Wetzlar, Germany) ● Phalloidin ● DAPI



µClear® PS 190 µm

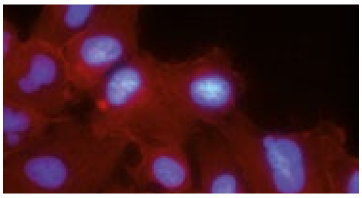


Competitor PS 250 µm

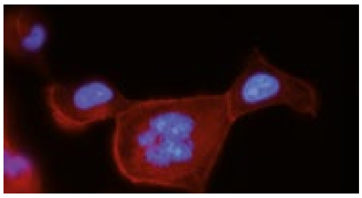


SCREENSTAR 190 µm Cycloolefin
excellent resolution & best image quality

63 x Oil Immersions (Leica Microsystems GmbH, 35578 Wetzlar, Germany) ● Phalloidin ● DAPI



µClear® PS 190 µm



SCREENSTAR 190 µm Cycloolefin
excellent resolution & best image quality

Figure 13: Optical quality of different clear bottom microplates.
SCREENSTAR microplates show excellent image quality with highest resolution.

6/ ORDERING INFORMATION

SCREENSTAR Microplates – 96 / 384 / 1536 Well

Bottom: Cycloolefin film, Raw material: COP, Surface treatment: TC

Item no.	Well format	Growth area / unit	Well profile	Product colour	Working volume (well)	Lid	Sterile	Qty. inner / outer
655866	96	33 mm^2	F-bottom / Chimney Well	● black	25 µl - 440 µl	yes	+	1 / 16
781866	384	8.1 mm^2	F-bottom	● black	10 µl - 110 µl	yes	+	8 / 32
789866	1536	2.1 mm^2	F-bottom	● black	3 µl - 15 µl	no	+	17 / 68

CELLview Plates

Well format: 96, Growth area / unit: 33 mm^2, Well profile: F-bottom / Chimney Well, Bottom: glass, Raw material: COP, Working volume (well): 25 µl - 440 µl, Lid: yes

Item no.	Surface treatment	Product colour	Sterile	Qty. inner / outer
655891	TC	● black	+	1 / 16
655981	Advanced TC	● black	+	1 / 16

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