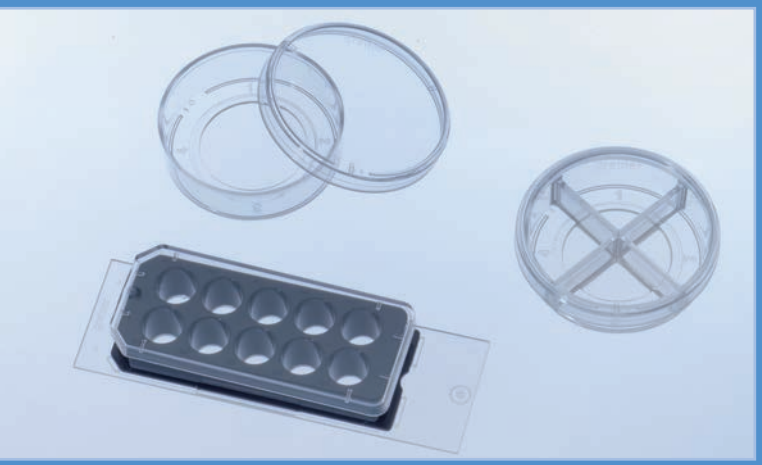
Your Power for Health

greiner bio-one



CELLview™

Ideal Cell Imaging Consumables

www.gbo.com/bioscience

Improve your Microscopic Vision

With its advantages for molecular selectivity and capability of live observation, fluorescence microscopy currently is among the most widely used approaches for high-resolution, non-invasive imaging of living cells. Depending on the complexity of live cell imaging experiments and the requirements of the corresponding microscope the requests for the utilised disposables can be as comprehensive.

Greiner Bio One's CELLview™ imaging consumables are tailored solutions to provide optimal basic settings for your microscopic experiment:

- High quality cover glass bottom
- Maximal light transmission for enhanced signal to-noise ratio
- Innovative design guarantees maximal pla narity and stable focal plane
- Reduced meniscus effect due to optimised well design
- Proprietary surface technology ensures optimal cell attachment and viability
- Subdivided versions for multiplex analysis and comparability
- Elaborate features to reduce reagent consumption

CELLview™ Quality Standard:

As for all Greiner Bio-One cell culture devices CELLview™ Cell Culture Dish and Slide are made of high grade polystyrene and are guaranteed to be sterile, non-pyrogenic, non-cytotoxic and free of detectable DNase, RNase and human DNA.

Free of detectable DNase, RNase, human DNA

non-pyrogenic

non-cytotoxic

This brochure contains:

1. CELLview™ General Information and Features

Cover glass bottom

For live cell imaging experiments and high-resolution microscopic applications high Numerical Aperture (N.A.) objectives are required. Standard glass slides with a thickness of 1 mm are much too thick to image when using N.A. objectives. Using such glass slides or thicker coverslips will lead to spherical aberration resulting in a loss of contrast and image sharpness whereas immanent thickness variations can cause axial shift, affect spatial resolution, loss of contrast as well as reduced intensity in fluorescent imaging. Generally speaking, the higher the numeric aperture of the objective, the more serious the loss in resolution is, if the wrong cover glass thickness is used. For some high-aperture objectives, a cover glass thickness variation of only a few micrometers can considerably reduce resolution. Beside that the general quality of the glass significantly influences image quality in bright field and fluorescence applications. Low quality cover glasses containing scratches and digs can cause diffraction and stray light.

The CELLview™ cover glass is of high optical quality according to DIN ISO 719 and ISO 8255-1 for microscopic applications, assuring accurate planarity and inhibiting any depolarisation of light. Both adhesives used for the assembly of the CELLview™ components are solvent-free UV- or radiation-curing medical device sealant which exhibit absolute no cytotoxic effect.

Note: Plastic slides or film bottoms can interfere with fluorescent imaging modes due to their inherent autofluorescence and strain birefringence. Therefore all CELLview™ imaging consumables contain a superior cover glass bottom in the microscopic relevant imaging area to obtain the best possible image quality.

CELLview™ cover glass bottom features:

- High transparent achromatic borosilicate glass; hydrolytic class 1 (DIN ISO 719)
- Glass thickness 0.17 mm
- Maximal spectral transmission; no autofluorescence
- Accurate planarity
- Manufactured according to ISO 8255-1
- Refractive index of 1.5255 +/- 0.0015 optimised for microscope objectives
- Abbe number of 56 +/- 2

Embedding Technology

To facilitate superior and fast microscopic analysis, the CELLview™ imaging consumables feature an innovative bottom design. The embedded coverglass guarantees a single-plane flat bottom with a consistent working distance and maximal planarity. Thus the CELLview™ Dish and Slide require no re-focussing while changing between individual compartments. Due to the stable focal plane there is no need to adapt the objective between individual imaging positions. Focus problems that are sometimes experienced when long sample distances have to be travelled by the microscope can be excluded using CELLview™ imaging consumables. The number of images which can be acquired during a specific time frame has to coincide with the temporal resolution of the cellular event. If sequential images in different positions or compartments are taken it also depends on the distance to be moved by the microscopic stage and a potential re-focussing time. The smaller the temporal resolution of the cellular event, the shorter the distances moved by the microscopic stage should be as well as re-focussing should be constrained to a minimum. Due to its specific design (Figure 1) CELLview™ disposables require no re-focussing while changing between the individual compartments and there is no need to adapt the objective between individual imaging positions.

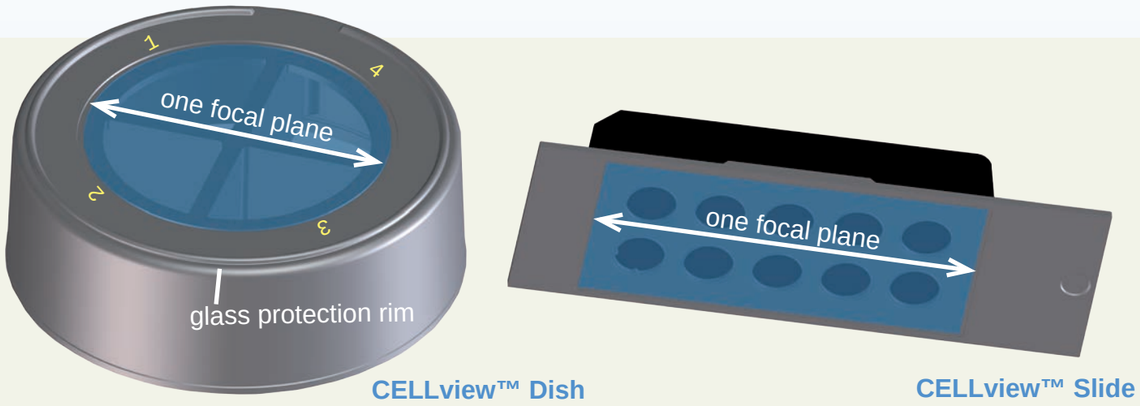


Figure 1: Technical drawing of the CELLview™ Dish and CELLview™ Slide displaying the embedded cover glass in blue. Based on this proprietary technology maximal planarity and a stable focal plane are assured for both products.

Innovativ well design

Nowadays a wide range of different colour-shifted fluorescent proteins as well as specifically designed sensors are available, enabling multi-colour time-lapse imaging and the analysis of physiological parameters. To compare several live cell experiments, the imaging conditions should be as constant as possible in order to minimise systematic and cellular deviations. Excitation light intensity and environmental conditions (temperature, CO_2 concentration, humidity) influence the physiological state of the cells as well as cell culture conditions can vary to some extend from experiment to experiment. Instead of screening one dish at a time, four experimental approaches such as various stimulations, diverse transfections or different cell lines can be monitored simultaneously using the subdivided CELLview™ Dish. If a higher number of parallel experiments is required the CELLview™ Slide facilitates analysis of up to ten individual settings. In each well or compartment a separate experiment can be performed. If the required temporal resolution permits, different positions can be sequentially imaged in each well before the first position is revisited. The numbers of images which can be acquired during one time frame strongly depend on the distance to be moved by the microscope stage and the required refocussing time (see also Embedding Technology). In accordance to these features the CELLview™ Dish with four or the CELLview™ Slide with ten wells enable multi-position imaging with minimal travel distance between the individual compartments to facilitate both analysis and identification of fast cellular events and multi-colour imaging of different fluorophores.

Detailed well specifications can be found in table 1 below.

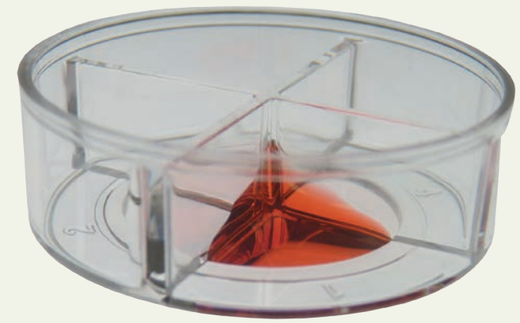


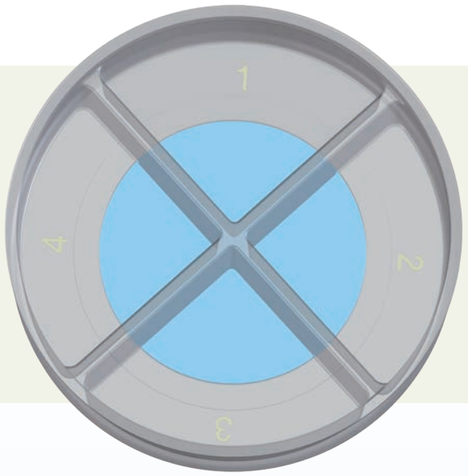
Figure 2: Subdivided CELLview™ Dish filled with a droplet of media to illustrate the meniscus effect omnipresent at rectangular walls.

Table 1: Well specifications

	CELLview™ Dish 1 compartment	CELLview™ Dish 4 compartments*[1]
Growth area total	8.7 cm^2	1.9 cm^2
Growth area glass surface	3.1 cm^2	0.5 cm^2
Total volume	10 ml	1.5 ml
Working volume total	5 ml	0.5 ml
Working volume glass surface	2 ml	0.1 ml
Cell number total*[2]	1-2x10^6	8x10^4
Cell number glass surface*[2]	5x10^6	2x10^4

	CELLview™ Slide
Number of wells	10
Growth area	34 mm^2
Total volume	340 µl
Working volume	50-100 µl
Cell number*[2]	1x10^4

*[1] Specifications per well
*[2] Average cell density of a confluent well



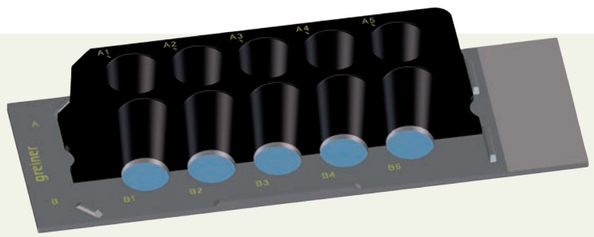


Figure 3: Technical drawing of the subdivided CELLview™ Dish and CELLview™ Slide demonstrating the optimised well design to reduce the meniscus effect.

Beside fluorescence microscopy brightfield and phase contrast images are often included before starting a time-lapse experiment to display uniform cell growth, general cell attachment and cell specific morphology. Image quality in this case can be affected by light scattering due the meniscus effect of rectangular or quadratic wells. Based on the triangular shape (Dish, Figure 2 and 3) or conical round design (Slide, Figure 3) of the CELLview™ compartments light refraction is essentially minimised in the imaging relevant glass area to ensure best possible image quality. Additionally the meniscus effect which is omnipresent in square wells also influences the distribution of cells and therefore the overall outcome of the experiment. Due to its inherent capillarty force cells are directed where the meniscus effect is most intense.

Figure 4 displays a crystal violet staining of seeded cells either in a CELLview™ Dish or a CELLview™ Slide. As mentioned above it is clearly visible that cells are accumulated in the corners of the subdivided dish where the meniscus effect is most intense.

However in the imaging relevant area (= glass bottom, blue triangle, Figure 4) cellular distribution is equably to provide optimal prerequisites for your imaging experiment. The specific round conical well design of the CELLview™ Slide prevents any unequal cell allocation and is therefore the best possible imaging consumable for superior microscopic experiments.

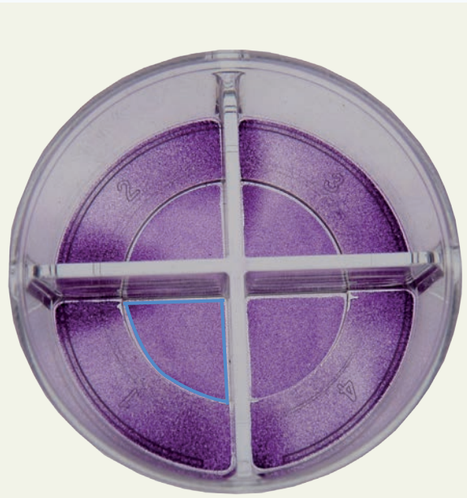


Figure 4: Crystal violet staining of a CELLview™ Dish and CELLview™ Slide verifying uniform cell distribution in the optical relevant areas.

Surface Technology

Beside visual requirements a glass bottom should also meet cell culture demands and facilitate successful cultivation of various cell types. Due to the general hydrophobicity of glass only a minor proportion of cells can adhere to it. Therefore these cells are often only loosely attached, do not display their cell specific morphology and can get lost during staining and washing steps. Furthermore live cell analysis of such ailing cells might not reveal their realistic in vivo response.

To guaranty optimal cell culture conditions during your cultivation and microscopic analysis the CELLview™ cell culture disposables are provided with two surfaces: Tissue-culture treated (TC) for propagation of standard cell lines and the Advanced TC™ surface facilitating in particular cultivation of sensitive and primary cells. The high advantage is that these surfaces are identical to the ones provided on GBO plastic disposables.

The pre-cultivated cells, coming for example from a tissue culture flask, do not face any adaption problem when transferred onto the cover glass bottom.
The non-biological surface optimisation leads to consistent and homogenous cell attachment reducing cell loss during transfection or staining procedures making previous required protein coatings of glass surfaces dispensable (Figure 5). Neither the proprietary tissue culture treatment nor the Advanced TC™ technology interferes with the spectral transmission or fluorescent analysis of stained samples. If still a cell specific protein coating is required this can be achieved using the TC treated version. As the surface is polar, proteins will bind to it equivalent to tissue culture treated plastic ware.

Note: Due to its non-polarity a protein coating on untreated glass is ineffective.

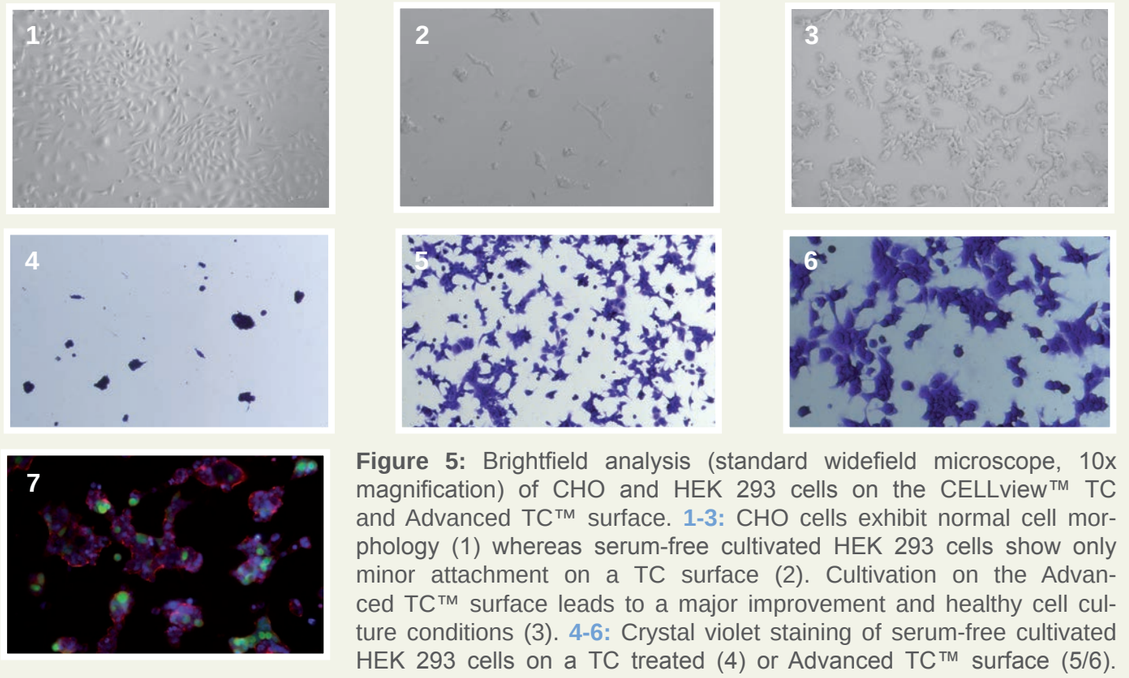


Figure 5: Brightfield analysis (standard widefield microscope, 10x magnification) of CHO and HEK 293 cells on the CELLview™ TC and Advanced TC™ surface. **1-3:** CHO cells exhibit normal cell morphology (1) whereas serum-free cultivated HEK 293 cells show only minor attachment on a TC surface (2). Cultivation on the Advanced TC™ surface leads to a major improvement and healthy cell culture conditions (3). **4-6:** Crystal violet staining of serum-free cultivated HEK 293 cells on a TC treated (4) or Advanced TC™ surface (5/6). Even after vigorous washing steps cells are strongly attached to the Advanced TC™ surface and display their cell typical morphology; clearly visible in the 20x magnification (6). **7:** Fluorescent analysis (20x magnification) of GFP-transfected, serum-free cultivated HEK 293 cells on an Advanced TC™ surface. Cultivating these cells on the Advanced TC™ surface leads to stable attachment, optimal cell survival and high transfection efficiency.

2. CELLview™ Cell Culture Dish

The CELLview™ Cell Culture Dish combines the convenience of a standard size 35 mm disposable plastic dish with the optical quality of glass, providing researchers with superior high resolution microscopic images of their in vitro cultures. The premium optical quality of the glass coverslip according to DIN ISO 719 and ISO 8255-1 for microscopic applications assures maximal spectral transmission and avoids autofluorescence or any depolarisation of light.

The innovative design of the CELLview™ Cell Culture Dishes with the embedded coverslip guarantees a single-plane flat bottom with a consistent working distance and maximal planarity. Moreover the dish bottom configuration leads to optimal thermal conductivity in heated platforms used for live cell imaging avoiding thermal variations. The subdivided version of the CELLview™ Cell Culture Dish enables simultaneous multiplex analyses facilitating the comparable completion of up to four time-lapse experiments in parallel by minimising systematic and cellular deviations. Cells can be cultivated under identical conditions during cell propagation and the consistent working environment is retained throughout the complete analysis time.

Quartering the Cell Culture Dish considerably minimises the amount of required cells and reagents per individual assay as well as reducing the time required for four different analyses. Instead of screening one dish at a time, four experimental approaches such as various stimulations, diverse transfections or different cell lines can be monitored simultaneously. As the four wells are located in the centre of the dish the required travel distances are very short to facilitate both analysis and identification of fast cellular events, multi-colour imaging and contemporaneous analysis of different fluorophores.

Based on the triangular shape of the CELLview™ compartment the meniscus effect and light refraction are essentially minimised in the imaging relevant glass area to ensure best possible image quality and uniform cellular distribution (Figure 3 and 4, page 6).

To protect the high quality glass coverslip the CELLview™ Cell Culture Dish has a glass protection rim on the bottom side of the dish avoiding for examples scratches on the glass during transport, cultivation or storage (Figure 1, page 4).

Cell seeding

Depending on your cells and application choose the appropriate surface. In case you are insecure which surface would lead to optimal experimental results we recommend to use both surfaces in a preliminary test. For this purpose sample packs are available (see ordering information, page 15).
Depending on the final cell concentration per well prepare your cell suspension. Thereafter the dish is incubated accordingly to your cell culture protocol. The glass protection rim on the bottom side will prevent scratches on the cover glass bottom during incubation and handling.
The subdivided version of the CELLview™ Cell Culture Dish enables simultaneous multiplex analysis. Quartering the Cell Culture Dish leads to four individual compartments with a growth area of approximately 1.9 cm^2 minimising the amount of required cells and reagents for each individual essay. As indicated in Table 1 (page 5), volumes and number of cells can be reduced to an even higher extend if only the glass area is used.

The edge or rim created by the embedded cover glass bottom (indicated by the arrow, Figure 6) can act as a liquid barrier facilitating the usage of small volumes for specific steps as cell seeding or staining. Using small droplets of liquids only in the imaging relevant glass area not only reduces waste of cells and reagents but also minimises the total costs of your experiment.

To seed cells only in the imaging relevant glass area a small droplet of 100 µl cell suspension is pipetted into the recession created by the upper plastic dish (Figure 6). Incubate the CELLview™ Dish for 30-60 minutes before adding the remaining amount of media (e.g. 0.5 ml in total) to ensure nutrient supply during cultivation.

Based on this procedure cells will only attach to the glass surface and not grow on the imaging irrelevant plastic surface. Equivalent to this procedure also other reagents can be reduced and constrained only to the microscopic significant glass bottom.

Comparing a single CELLview™ Dish with the glass area of a quartered version leads to a fiftyfold reagent and up to hundredfold cell number reduction.

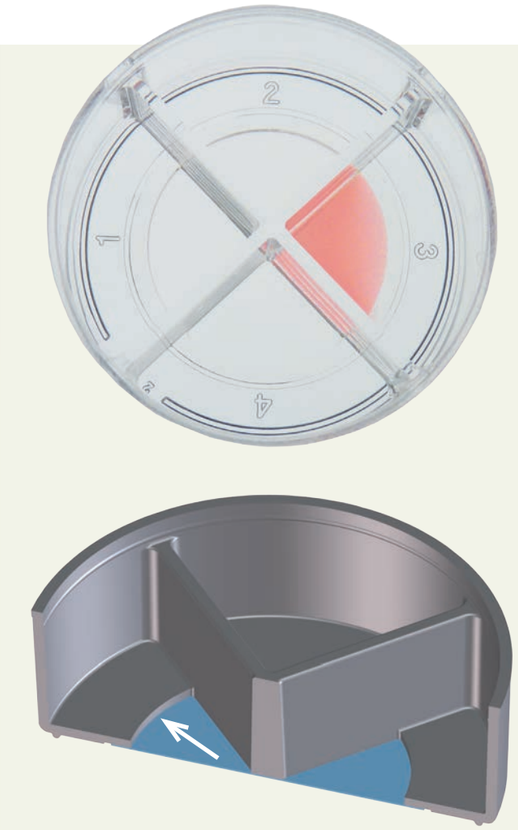


Figure 6: Picture and technical drawing of the CELLview™ Dish illustrating the recession created by the embedded cover glass (arrow) facilitating cell seeding only in the imaging relevant glass area.

Cell fixation

If cells have to be fixed, different aqueous based or organic solutions can be applied. A detailed overview on possible fixation methods is provided in Table 2 (page 13).

3. CELLview™ Cell Culture Slide

The CELLview™ Slide was developed in collaboration with the ALMF (Advanced Light Microscopy Facility) at EMBL in Heidelberg. The high quality cover glass according to DIN ISO 719 and ISO 8255-1 is embedded in a transparent plastic slide equipped with a detachable black polystyrene housing. This housing divides the slide into ten round compartments, each of which has the dimensions of a well of a standard 96 well microplate. Due to the format the usage of multichannel pipettors is possible and simplifies pipetting steps. The round well design reduces meniscus effects, which are pronounced in square wells, assuring equal cell distribution and optimal microscopic analysis (Figure 4, page 6). The compartmentalisation as well as the slide underneath contains the identical alpha-numeric well coding ensuring unmistakable sample identification (Figure 3, page 6). With the two integrates positioning pins within the A1 well an automated positioning is possible. According to these positioning pins the microscope can define the center of the well, zero in on the correct focal plane and subsequently move into each compartment without any need of re-adjustment. This simplifies and speeds up the analysis of the total slide on an automated microscope.

CELLview™ Slides are excellently suited for all microscopic applications. The embedded cover glass guarantees a complete, even focal plane which is necessary for high-speed and high-resolution microscopy. Furthermore, the black compartmentalisation reduces cross talk between adjacent wells during fluorescence microscopy; and the optical glass which has virtually no autofluorescence, allows for maximum spectral transmission without depolarisation of transmitted light.

After detaching the black compartment the CELLview™ Slide can be processed for further analysis, mounting or long time storage. Beside the TC surface CELLview™ Slide is also available with the Advanced TC™ surface for sensitive cells or complex applications. What matters is that due to the black housing, stray light cannot bleach out samples within nearby wells as it appears in transparent slides. Therefore it is guaranteed that each cell shows the maximum signal strength at the beginning of the recording and that the analysis will not be affected negatively by bleaching and phototoxic effects.

This very important reduction of crosstalk and photobleaching effects can be visualised and measured (Figure 8 and 9). Wells of a CELLview™ Slide and a common coverglass product with transparent subdivision were filled with a Phallodin-TRITC solution and primary emission was analysed with a plate reader. To analyse possible bleach out in adjacent wells 10 subsequent measurements were initiated

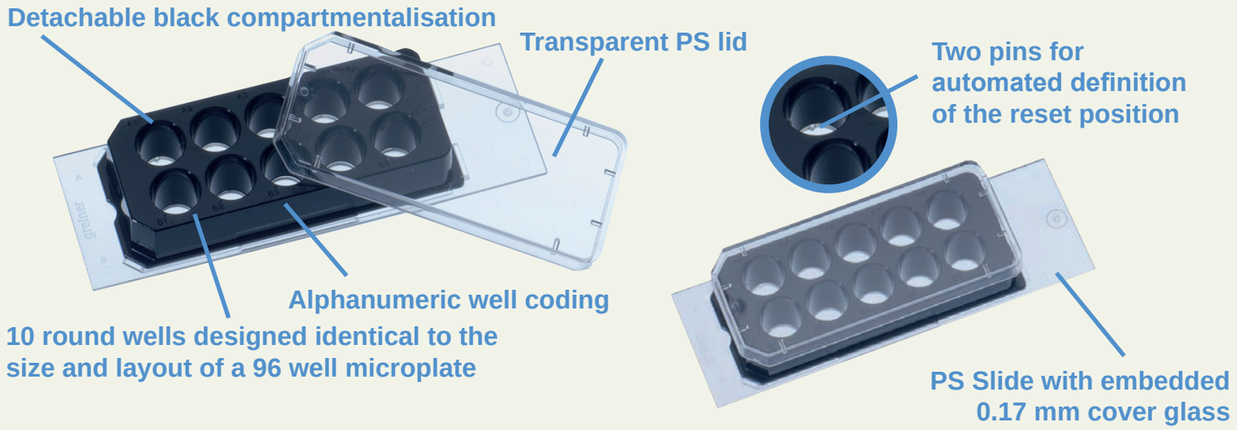


Figure 7: Innovative Features of the CELLview™ Slide.

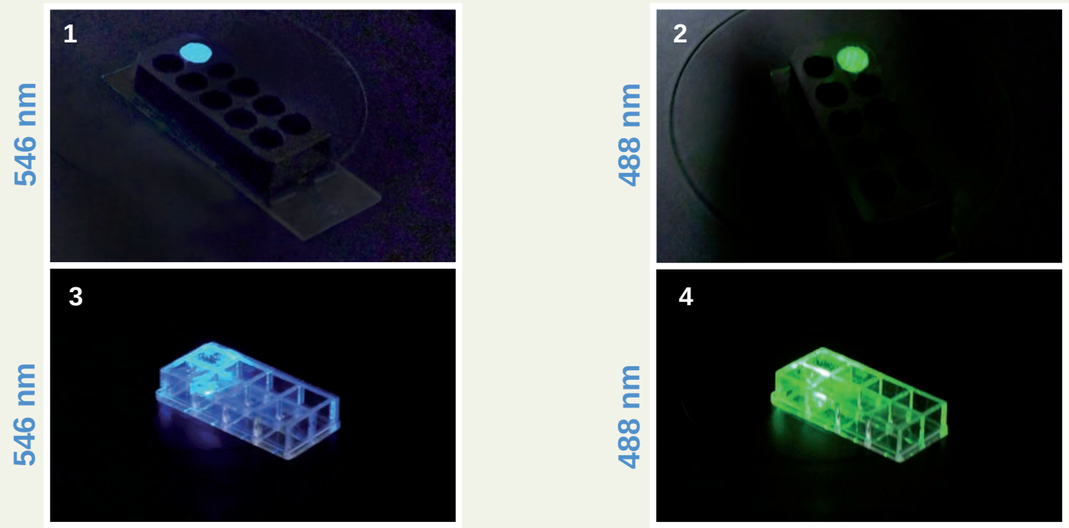


Figure 8: Excitation of fluorophores on a standard widefield microscope using either a CELLview™ Slide (1/2) or a transparent square well format with cover glass (3/4). The black compartmentalisation of the CELLview™ Slide inhibits crosstalk and bleach out in neighboring wells.

in the first well and a single measurement in the neighbouring well 2 and 3. Thereafter a two minutes excitation of the first well with a widefield fluorescent microscope was performed at the corresponding wavelength followed by a second plate reader measurement in all three wells. Figure 9 depicts comparable starting emissions values in all three wells for the CELLview™ Slide. This means that 10 subsequent measurements in well A1 did not affect the fluorescent intensities in well A2 and A3. After 2 minutes excitation with the microscope the fluorescent intensity decreases by 13 % in the first well and only between 5 and 6 % in the neighboring wells.

On the other side the disposable with transparent housing already displays a strong decrease of 25-35 % after 10 subsequent measurements in the neigbouring wells 2 and 3. That means that the fluorescent signal is already reduced, before the beginning of the recording. For these wells cells could be affected negatively through phototoxic effects. After the identical two minutes fluorescent excitation the signal decreases in the first well stronger then in well A1 of the CELLview™ Slide. With the transparent subdivision the fluorescent signal in the second and third well is diminished even though there was no intended excitation. This effect does sum up to an extensive loss of fluorescent signal in the concessive wells influencing not only the outcome of microscopic analysis but also cellular physiology facing phototoxic intermediates before the relevant analysis is started.

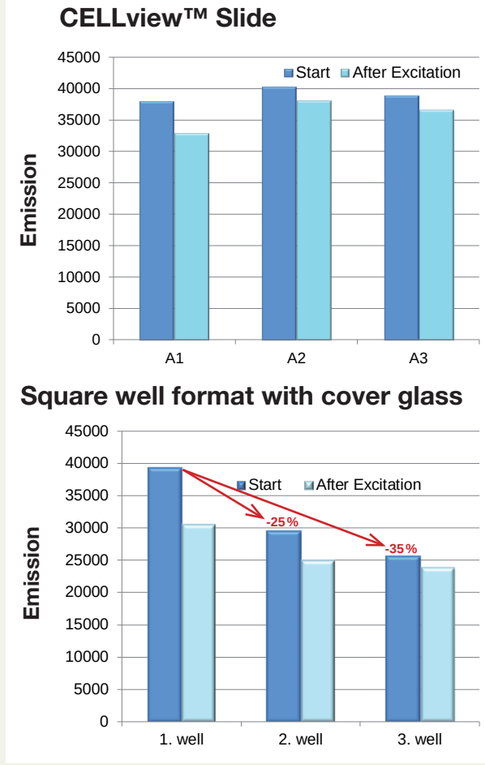


Figure 9: Measurement of fluorescent intensities before and after a 2 minutes excitation on a standard widefield microscope (Leica).

Figure 10: Convenient liquid transfer using a multichannel pipette.

Figure 11: Staining and washing of CELLview™ Slide without compartmentalisation.

Cell seeding

The well design of the CELLview™ Slide is identical to a 96 well plate with a growth area of 34 mm^2. Therefore existing cultivation protocols can be directly applied to the slide format. However for a microscopic approach cell densities should be smaller than for a standard assay. Confluent wells are difficult to analyse as an optical analysis in general requires individual cells. For more details see table 1, page 5. As mentioned in section „Surface Technology“ the CELLview™ Cell Culture Slide is provided with two surfaces: Either tissue culture treated or Advanced TC™.
Depending on your cells and application choose the appropriate surface. In case you are insecure which surface would lead to optimal experimental results we recommend to use both surfaces in a preliminary test. For this purpose sample packs are available (see ordering information, page 15).
Depending on the final cell concentration per well prepare your cell suspension. If all wells of the slide will be seeded with the same cell type and concentration you can use a multichannel pipette to simplify and speed up the seeding process. Thereafter the slide is incubated accordingly to your cell culture protocol. The tray in which the slide is delivered is a very convenient device for slide handling transport and incubation (Figure 10).

Cell fixation

If cells have to be fixed, different aqueous based or organic solutions can be used. A detailed overview on possible fixation methods is provided in Table 2 (see page 13). As the black compartmentalisation is removable a wider field of organic fixatives is applicable if only the slide is utilised. This is due to the fact that the non-removable adhesive between slide and cover glass has a higher chemical resistance. Detaching the compartmentalisation also has the advantage that reagent consumption can be reduced and general handling and washing steps can be simplified (Figure 11).
Comparable to the CELLview™ Dish the outer rim of the small well generated between the plastic slide and cover glass bottom minimises the required liquid volumes. A small droplet of 50 µl is sufficient to fill the recession. As the well design and geometry is identical to a 96 well plate multichannel pipets can be used for liquid transfer whereas washing steps can be easily performed using 50 ml tube (Figure 11).

Table 2: Fixation Methods and Chemical Resistance:

	Incubation (min)	CELLview™ Dish	CELLview™ Slide	CELLview™ Slide*
100 % Methanol -20°C	5/10	+/+	-/-	+/+
100 % Acetone -20°C	5/10	-/-	(+)/-	-/-
50 % Methanol, 50 % Acetone -20°C	5/10	+/+	-/-	+/+
95 % Ethanol, 5 % glacial acetic -20°C	5/10	+/+	+/-	+/-
10 % neutral buffered formalin RT	10/20	+/+	+/+	+/+
3-4 % paraformaldehyde / PBS RT	10/20	+/+	+/+	+/+

*Without compartmentalisation

Detaching of the black compartmentalisation

Detaching the back compartmentalisation is very convenient and does not require any tools. Hold the upper housing with one hand and bend the slide slightly with the other hand as depicted on the pictures in Figure 12. The compartmentalisation will easily come off and the removable adhesive will remain on the black part. If accidentally some glue is left on the slide it can be removed with a tweezer. The remaining slide contains 10 small micro-wells with the same design and distances of a 96 well Microplate.

That means, your cells growing on the cover glass, are located on a different plane than the slide frame. When removing the black housing you never need to worry about accidently scraping off or damaging your cells. Furthermore the micro-well facilitates drastically reduced reagent consumption and saving on e.g. antibody costs as you can choose to incubate just in the small well. For unmistakable sample identification the micro-wells have the identical alpha-numeric well coding as on the detached black compartmentalisation.

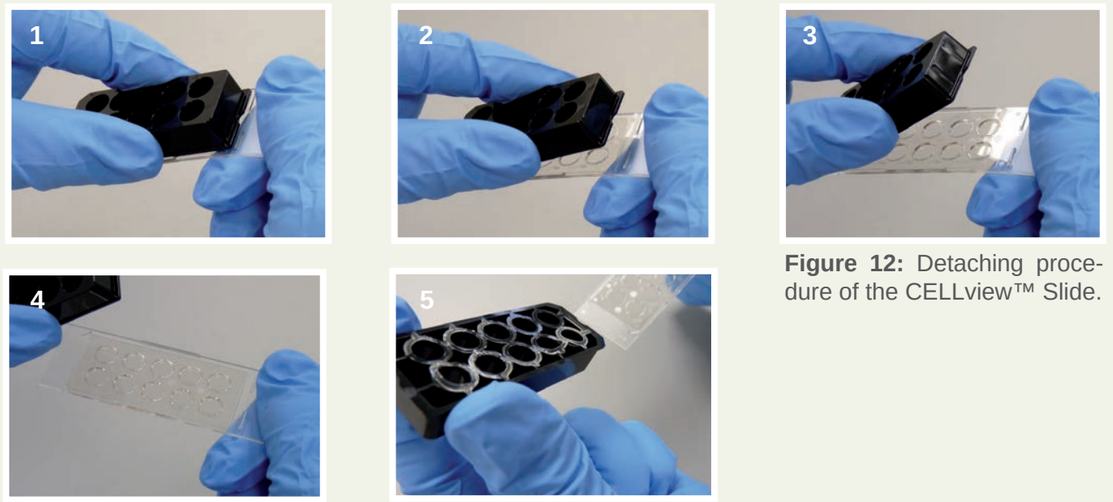


Figure 12: Detaching procedure of the CELLview™ Slide.

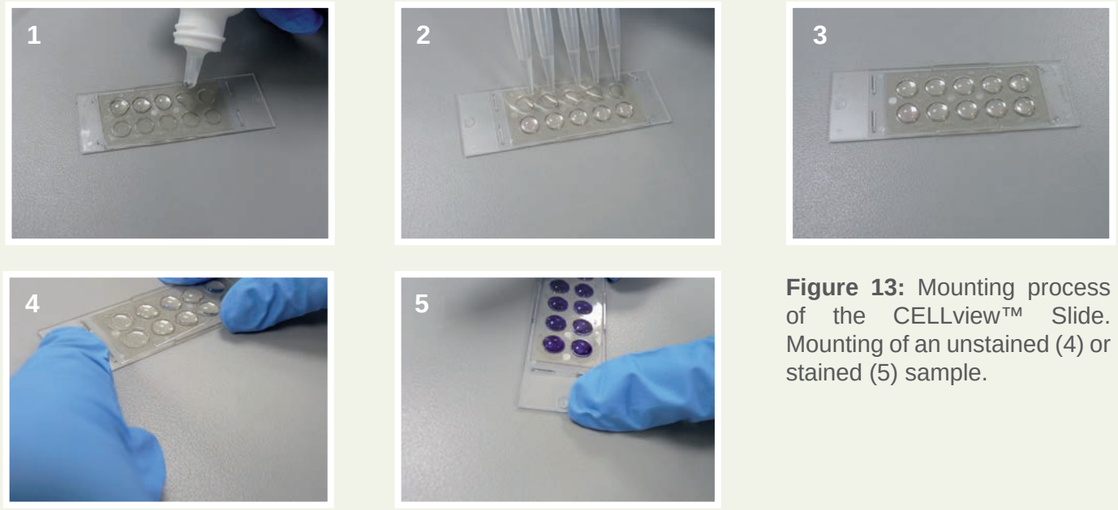


Figure 13: Mounting process of the CELLview™ Slide. Mounting of an unstained (4) or stained (5) sample.

Mounting the CELLview™ Slide

After detaching the black housing the CELLview™ Slide can be processes for further analysis or mounting for long-time storage. To achieve an optimal mounting result and avoid trapped air bubbles the well/recession should be filled with sufficient mounting media (Figure 13). Generally 40-50 µl per well is an adequate quantity but as these liquids can vary in their viscosity the correct amount should be decided on visual judgment. A sufficient amount is important to prevent air inclusions. Depending of what type of mounting media is used they can be either applied with the respective drop flask or a multichannel pipet (Figure 13). After filling the recessions the slide can be covered by a standard 22x50 mm cover glass. It is of upmost importance to use cover glasses with the indicated dimension as a larger cover glass will rest on the outer frame lobes which generally keep the black compartmentalisation in its position (Figure 14). For covering the slide lean the cover glass against the frame lobes on the small site of the slide next to well A1 and B1 in an angle of around 45° and then let it come down slowly so that possible air bubbles can be pushed outside the imaging relevant area. During this process the cover glass is guided by the frame lobes on the long side of the slide. If air bubbles are visible on the upper surface of the mounting media before applying the cover glass, they should be disploded using a pipette tip or tweezer. If in spite of all precautions air bubbles have been trapped between slide and cover glass they can be pushed outside the imaging field by pinching the cover glass at this position and moving the air bubble to the border. Thereafter let the slide dry depending on the embedding media supplier's indications. An additional possibility for an air bubble free embedding result is the usage of a so called liquid cover glass. The type of mounting media is dependent on your application. For fluorescent stainings for example embedding media containing anti-fading reagents can elongate the lifetime of your sample. Depending on the micro-well design little more mounting media is required. Therefore we would recommend to use clear mounting media rather than DAPI containing liquids.

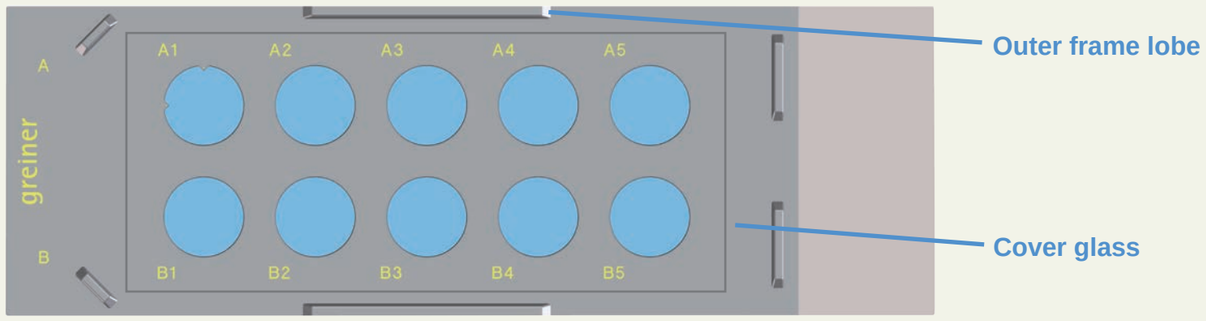
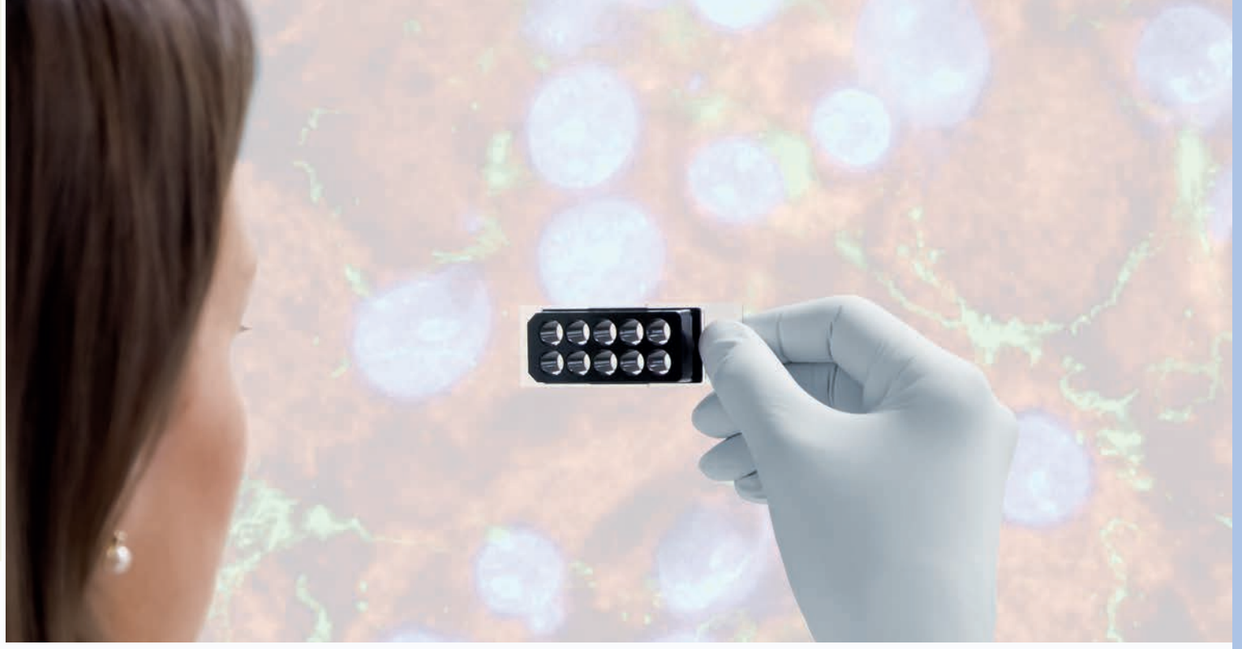


Figure 14: Technical drawing of a CELLview™ Slide displaying the optimal position of a mounted cover glass.



Depending on the respective DAPI concentration you might get a blue background.

Note: Always image closest to the cells. If you use an upright microscope flip your mounted slide upside down. Don't image through the micro-well. As long as the microscopic analysis is applied through the cover glass (being part of the slide) the amount of mounting media does not affect the imaging result. Only if the light is transmitted through the upper cover glass (used for mounting) signal intensity might be slightly reduced due to the longer light path.

4. Ordering Information

CELLview™ Dish / TC		
Item no.	Wells	Quantity bag / case
627 860	1	10 / 40
627 870	4	10 / 40
627 898	4	1
627 899	1	1
CELLview™ Dish / Advanced TC		
627 965	1	10 / 40
627 975	4	10 / 40

CELLview™ Slide / TC		
Item no.	Wells	Quantity bag / case
543 079	10	5 / 45
543 999	10	1
CELLview™ Slide / Advanced TC		
543 979	10	5 / 45
543 998	10	1

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