

Application Forum

Advantages in multiplex and live-cell imaging using the CELLview™ cell culture dish

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Introduction

To disclose cellular pathways and identify the involved signaling molecules, it is essential to monitor the localization and interactions of these proteins. The resulting need to observe these molecular events within the living cell became reality in recent decades due to the identification of green fluorescent protein (GFP) and improvements in microscopic technologies.

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 organisms and cells (1).

Several types of instrumentation and disposables have been developed to facilitate real-time observation of cellular processes. Using water-dipping objectives, cells on any kind of culture dish can be monitored, but these objectives are not so common, and depending on the numerical aperture the resolution is likely to be lower than with an oil immersion objective (2). The latter can be used in combination with glass-bottom dishes, allowing high-resolution analysis of living cells. Plastic disposables should be avoided due to their inherent autofluorescence, and their strain birefringence, interfering with fluorescent imaging modes.

Depending on the complexity of live-cell imaging experiments and of the corresponding microscope requirements for the utilized disposables can be as comparable.

Advantages of the CELLview™ cell culture dish

The new CELLview™ cell culture dish (Figure 1) meets the required high-quality standard and broadens the possible application range of glass-bottom dishes. CELLview™ cell culture dishes combine the convenience of a standard-sized 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 8255-1:1986 for microscopic applications assures maximal spectral transmission and avoids autofluorescence or any depolarization 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's bottom configuration leads to optimal thermal

conductivity in heated platforms used for live-cell imaging and avoids thermal variations.

The subdivided version of the CELLview™ cell culture dish enables simultaneous multiplex analysis facilitating the comparable completion of up to four time-lapse experiments in parallel by minimizing systematic and cellular deviations. Quartering the cell culture dish leads to four individual compartments with a growth area of approximately 1.9 cm², minimizing 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.

To sequentially image different positions within the four individual compartments, the required temporal resolution of the cellular event has to coincide with the number of images that can be acquired during a specific time frame. This strongly depends on the distance that the microscopic stage has to move and the potential re-focussing time. The smaller the temporal resolution of the cellular event, the shorter the distances moved by the microscopic stage should be, and re-focussing should be constrained to a minimum.

Because of its embedded coverslip the CELLview™ cell culture dish requires no re-focussing while changing between the four individual compartments. Due to the dish's maximal planarity and constant working distance there is no need to adapt the objective



Figure 1. CELLview™ cell culture dishes.

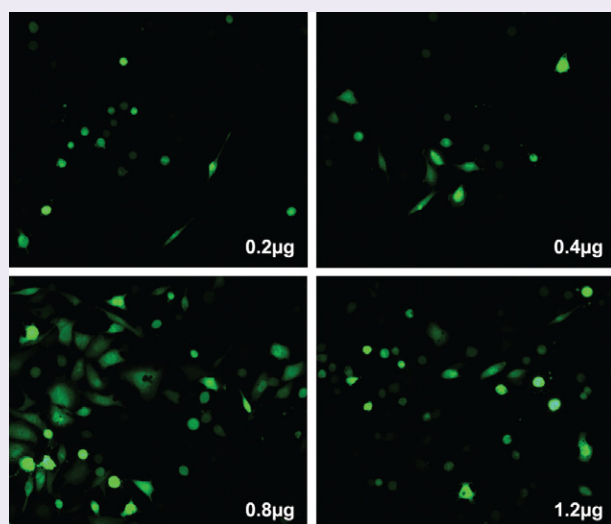


Figure 2. 20,000 cells were seeded per compartment in a subdivided CELLview™ cell culture dish and cultivated for 24 h at 37°C and 5% CO₂ prior to transfection. Enhanced GFP expression was induced by transfecting the DNA plasmid pcDNA3-EGFP using 0.2, 0.4, 0.8, or 1.2 µg DNA, respectively. Transgene expression was analyzed after overnight incubation at 37°C and 5% CO₂.

between individual imaging positions. Focus problems that are sometimes experienced when large sample distances have to be traveled by the microscope can be avoided using the CELLview™ cell culture dish.

Because the four wells are located in the center of the dish, the required travel distances are very short to facilitate both analysis and identification of fast cellular events, multi-color imaging, and contemporaneous analysis of different fluorophores.

In addition to fluorescence microscopy, brightfield and phase-contrast images are often included before starting a time-lapse experiment to display uniform cell growth, general attachment, and cell-specific morphology. Image quality in this case can be affected by light scattering due to the meniscus effect of rectangular or quadratic wells. Based on the triangular shape of the CELLview™ compartment, light refraction is essentially minimized in the imaging relevant glass area to ensure the best possible image quality.

Results

CHO cells cultivated under identical conditions using the subdivided version of the CELLview™ cell culture dish displayed different transgene expression depending on the amount of transfected DNA: 0.2 and 0.4 µg DNA led to a very low transfection efficiency, whereas 1.2 µg induced partial cell death due to the high DNA concentration (Figure 2; 20× magnification). When compared to the other three compartments, transfection with 0.8 µg DNA revealed the optimal transfection efficiency without affecting cell growth and morphology. This result was confirmed by counterstaining

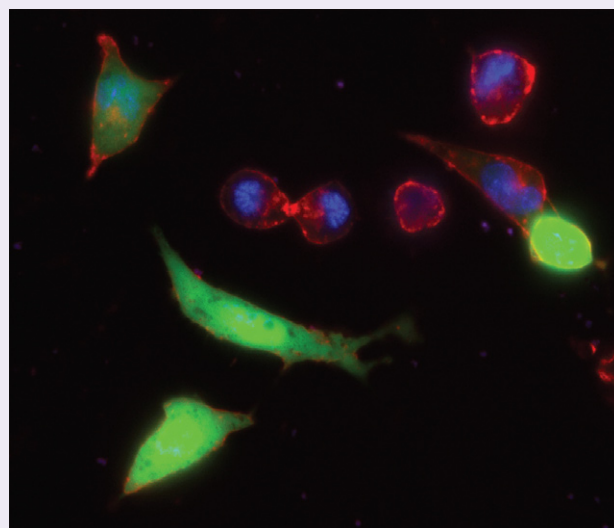


Figure 3. The proportion of transfected cells was determined by counterstaining cells with red fluorescent Alexa Fluor® 594 wheat germ agglutinin [2.5 µg/ml] for selective staining of the plasma membrane, and the blue fluorescent Hoechst 33342 dye [2.5 µg/ml] to identify the cell nucleus.

GFP and non-transfected cells with the red fluorescent Alexa Fluor® 594 wheat germ agglutinin, which selectively stains the plasma membrane, and the blue fluorescent Hoechst 33342 dye, which identifies the cell nucleus (Figure 3; 63× magnification).

Conclusion

The subdivided version of the CELLview™ cell culture dish facilitated simultaneous analysis of four different transfection approaches. The ideal DNA concentration could be identified by one single overnight experiment using all four compartments in parallel. The amount of cells and reagents could be considerably reduced compared to the quantity required in general when using undivided dishes. The high optical quality of the CELLview™ cell culture dish in combination with the special design led to high-quality pictures without the need to re-focus or re-adjust the microscopic settings while changing between the four different compartments.

Overall, the new CELLview™ cell culture dish is a high-quality platform for multiplex and live-cell imaging experiments, minimizing the amount of required cells, reagents, and the overall analysis time.

References

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