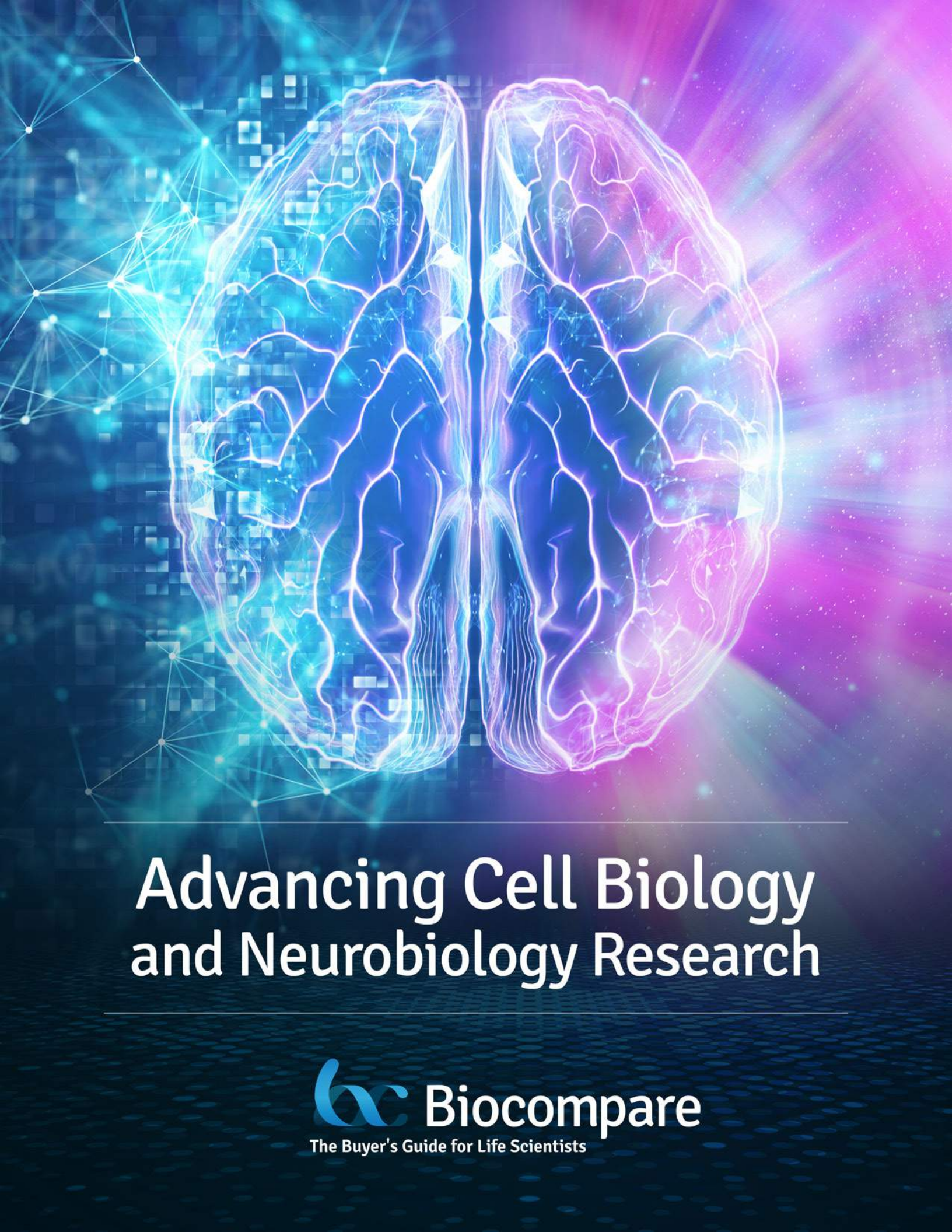

Advancing Cell Biology and Neurobiology Research

Biocompare

The Buyer's Guide for Life Scientists

Cellular Assay Using Brightfield and Fluorescence-Based Live Cell Imaging

With Celloger series to image cells in real time.

Live cell imaging makes it possible to understand and study various biological phenomena by enabling the observation of complex dynamics of live cells in real time via time-lapse microscopy. Real-time imaging of cellular phenomena, such as cell migration, development, and trafficking, serves as an important tool in various academic fields including cell biology, neuroscience, pharmacology, and developmental biology. In order to observe the cells in a live state, incubator functions are needed to control carbon dioxide, temperature, and humidity (Figure 1A). But in many cases, controlling the temperature and humidity suitable for cell growth is challenging due to difficulties in maintaining airtightness and covering a large volume.

To overcome such shortcomings, affordable and compact imaging devices that can be put into cell culture incubators are being developed. Such live cell imaging devices provide bright-field images and at times include fluorescence imaging functionalities to observe fluorophores being excited and emitted in a specific wavelength. However, live cell imaging using fluorescence staining has a limitation since making fluorescence brighter and clearer not only results in improved image quality but inevitably causes cellular phototoxicity. Thus, it is essential for time-lapse imaging systems to enable efficient fluorescence imaging even at a low light intensity. As mentioned earlier, it is a crucial aspect for the live cell imaging system to ensure image quality while maintaining temperature and humidity when processing experiments that generate significant amount of heat such as fluorescence imaging inside an incubator.

The Celloger series, live cell imaging systems developed by Curiosis are compact so that they can be placed in a general cell culture incubator (Figure 1B) and designed to endure the self-generated heat enabling long-term imaging. In addition to that, it can obtain clear bright-field images using contrast-enhanced optics and fluorescence images of live cells in real time with a minimum light intensity by optimizing fluorescence filter and light path. The systems were tested to verify applications in various cell-based research in fields such as cell biology and pharmacology. The results showed that the devices had higher bright-field image quality than other live cell imaging systems with the same functions,

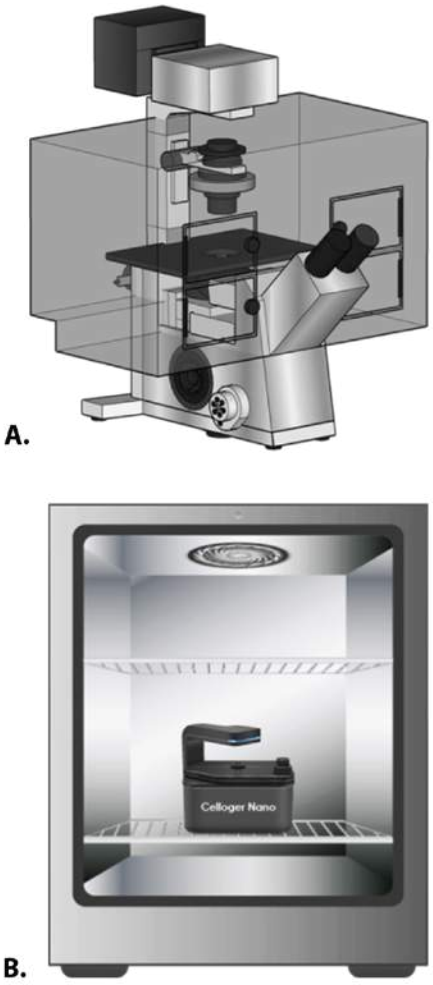

Figure 1: Live cell imaging systems.
A. Conventional microscope.
B. Celloger Nano placed in an incubator.

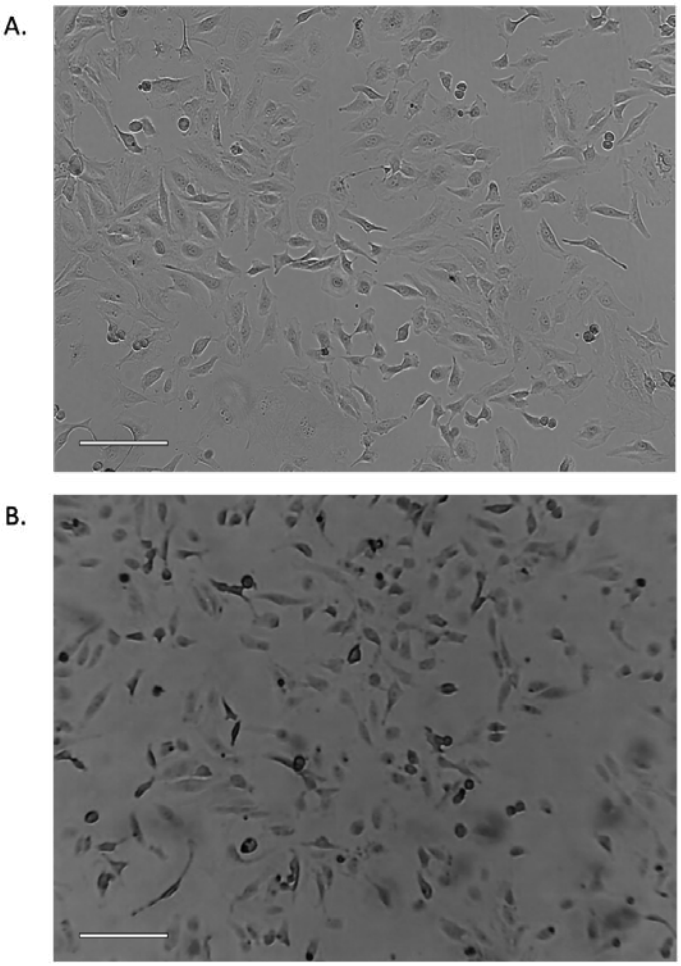

Figure 2: Hela cell bright-field imaging (Scale bar, 200μm)
A. Image taken by Celloger Nano
B. Image taken by live cell imaging device of another company

and fluorescence imaging results were comparable to the images obtained from fluorescence microscopy using cMOS cameras with specifications corresponding to that of Celloger.

Bright-field imaging application

For drug screening, it is important to obtain a clear image in the process of real-time cell monitoring while performing treatment according to the type or concentration of a drug. Celloger's bright-field imaging has increased the contrast in comparison to the existing live cell imaging equipment, making it possible to display more vivid cell contours and boundaries despite the usage of transparent cell samples (Figure 2).

Morphology monitoring and drug screening

With Celloger Mini, the designated positions of multiple points can be scanned according to a set

schedule as it has automatic motorized stages. This feature makes it possible to track the changes over time when cells are treated with different drug concentrations. Nocodazole, an anticancer drug, is known to cause mitotic arrest by inhibiting the polymerization of microtubule within cells and has different action mechanisms of efficacy depending on which cell and what concentration are used.[1,2]

Cells were treated with different concentration levels of nocodazole and observed by Celloger Mini. The results showed that most cells died and had similar confluency at the final endpoint, 20 hours after the treatment with the drug when the drug concentration is over 62.5 nM. On the contrary, there were differences in cell death and confluency depending on concentration levels of the drug in early time.

As such, it was possible to obtain important data for morphological dynamics of cells and the antitumor efficacy of drugs through real-time cell monitoring and confluency imaging using Celloger Mini. As shown in Figure 3, time-lapse images are generated

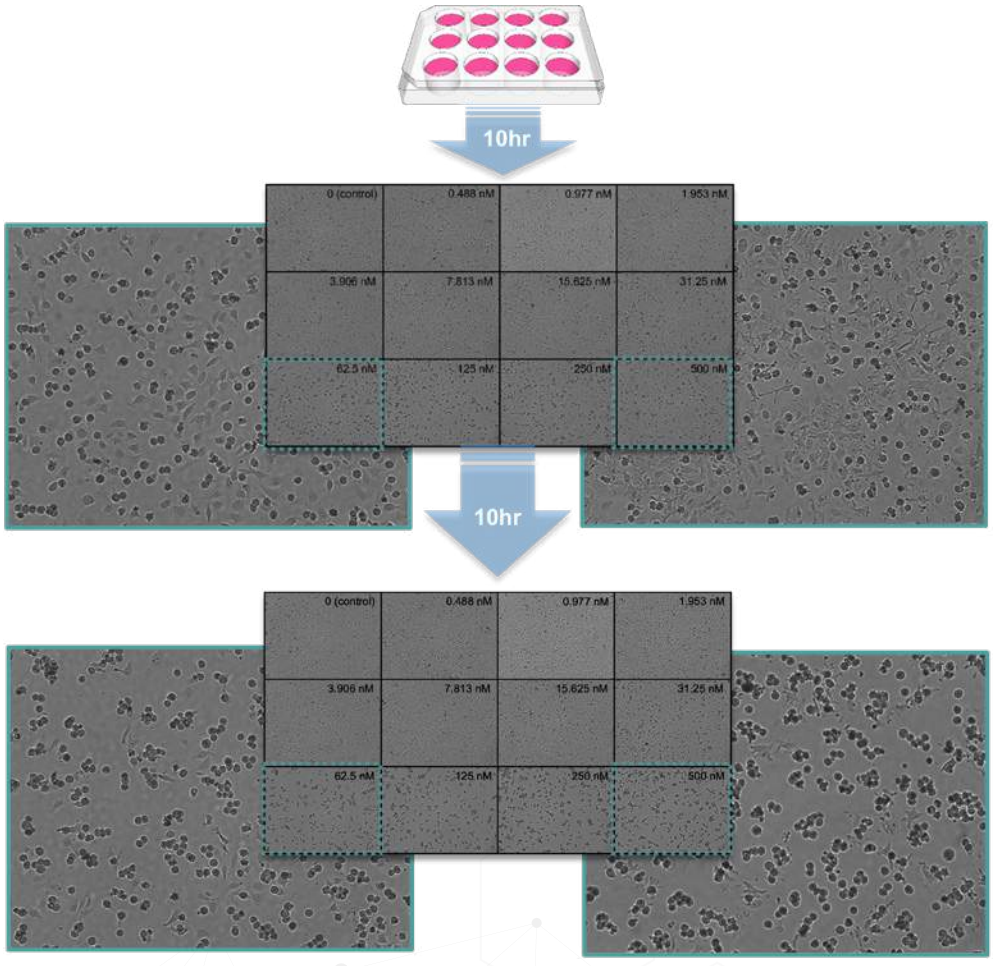

Figure 3. Image tiles for different concentration levels of nocodazole and time-lapse images generated by Celloger Mini.
**The images were collected hourly by Celloger Mini for 20 hrs.*

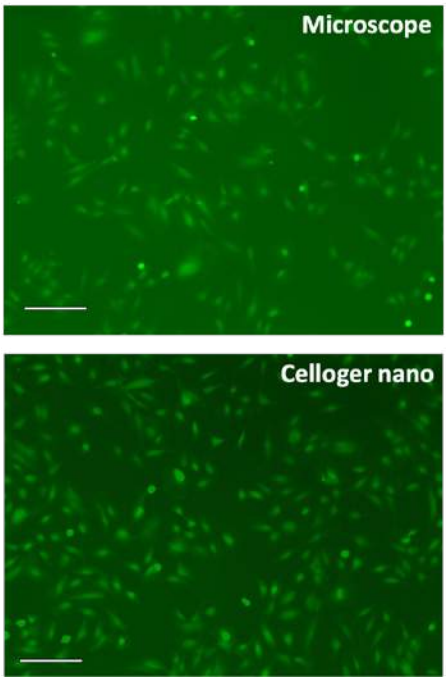

Figure 4. Fluorescence imaging of CMFDA stained cells (Scale bar, 200 μm)

in tile images, making it easy to compare the differences depending on conditions.

Fluorescence imaging application

Using live cell imaging equipment such as Celloger Nano, it is possible to visually investigate the dynamics of intracellular changes using live cell staining fluorescent dyes with specific staining properties for subcellular organelles and cell labeling. Using this characteristic, it is possible to monitor and quantify the efficacy of a drug through various mechanisms. The fluorescence optics of Celloger Nano were optimized to increase the ratio of detected fluorescence to light source intensity, resulting in improved fluorescence image quality while minimizing phototoxicity that occurs inevitably during excitation. The fluorescence images taken by Celloger Nano were compared with those taken by a fluorescence microscope equipped

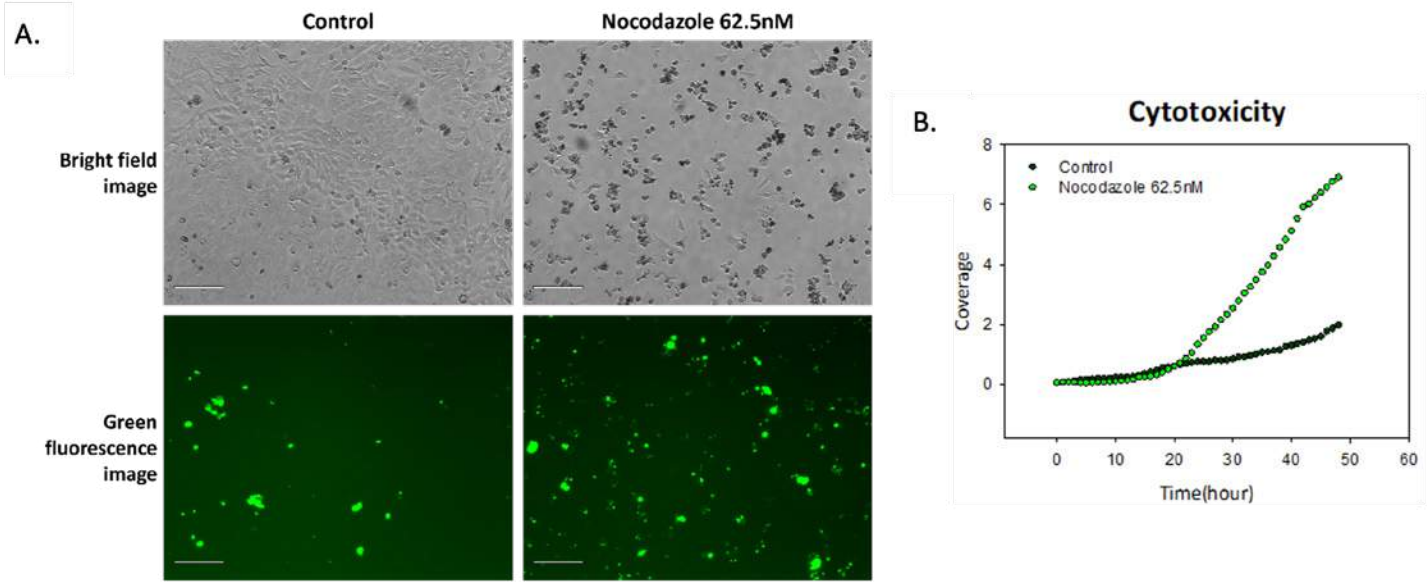

Figure 5. Cytotoxicity assay using cell-impermeant dye.
A. Cell image after 35 hours from the treatment with 62.5 nM nocodazole. (Scale bar, 200 um).
B. Fluorescence coverage by hour.

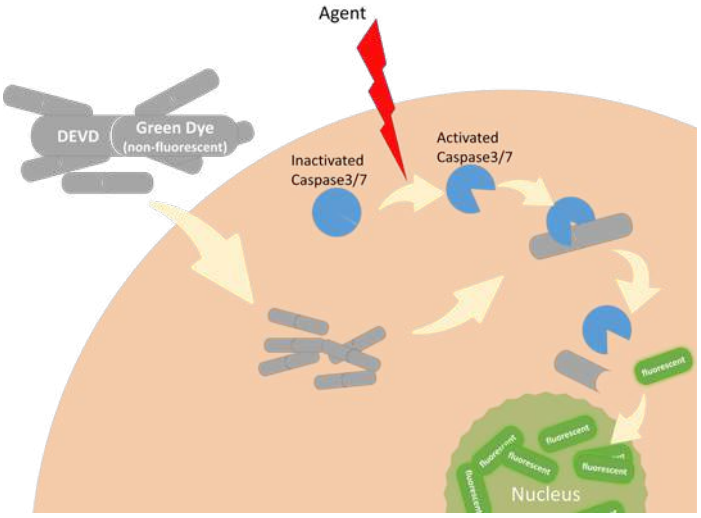

Figure 6. Illustration on the action mechanism of Caspase-3/7 Green Detection Reagent

with a ASI174MM camera (SONY IMX174 cMOS image sensor) whose specification is comparable to that of Celloger Nano to verify the quality of fluorescence images. The fluorescence image of Hela cells stained with fluorescence dye using CMFDA, a green fluorescent cell tracker, taken by Celloger Nano showed that the fluorescence intensity was comparable to that of fluorescence microscope and the image was clear since the contrast between the background and cells was high (Figure 4).

Cytotoxicity assay

Several staining reagents that measure the degree of cell death using a phenomenon in which the integrity of the cell membrane is damaged and the cell permeability is increased during the cell death are commercially available.

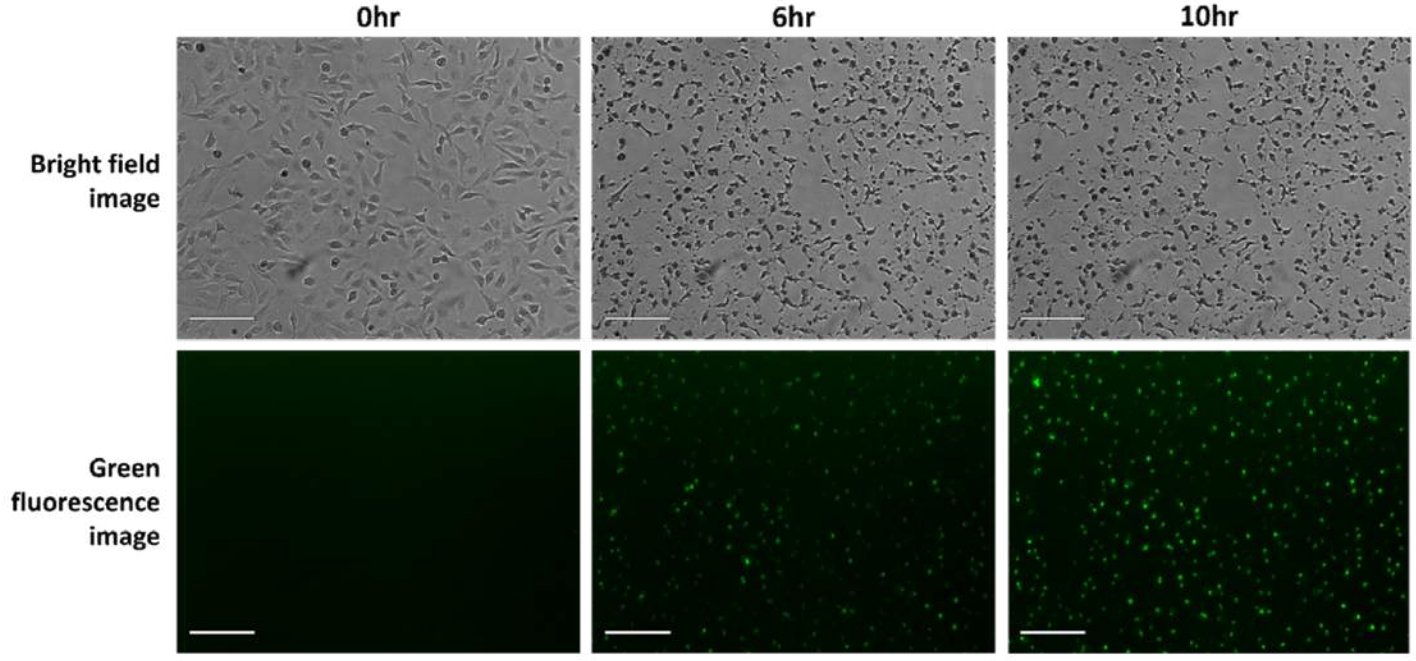

*Figure 7. Using fluorescence detection of activated caspase to quantify apoptosis of Hela cells caused by staurosporine (Scale bar, 200 μm). *The images were collected every 30 mins by Celloger Nano for 15 hrs and 30 mins*

To measure the cytotoxicity by nocodazole, dead cells were stained with green fluorescent CellTox™ dye. It was confirmed that the number of cells measured by fluorescence increased as the cell permeability increased due to cell death after 20 hours (Figure 5).

Apoptosis assay

During apoptosis, caspase is activated to mediate nuclear fragmentation. Activated Caspase3/7, one of the caspase family, specifically cleaves certain peptides known as DEVD, and fluorophores conjugated to DEVD are useful to quantify Caspase activity and apoptosis (Figure 6).

Fluorescent materials were released and detected after cleavage of DEVD caused by the treatment with staurosporine, a material known to activate caspase and cause apoptosis. The number of fluorescent materials increased with time (Figure 7).

Fluorescence coverage graph is shown to quantify apoptosis by time. The graph illustrates that fluorescence began to be detected two and a half hours after the treatment with staurosporine and reaction became saturated from 10 hours after the

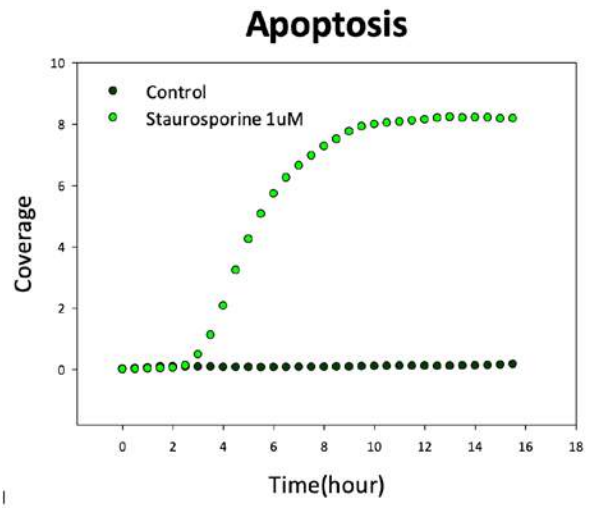

Figure 8. Fluorescence coverage graph by time

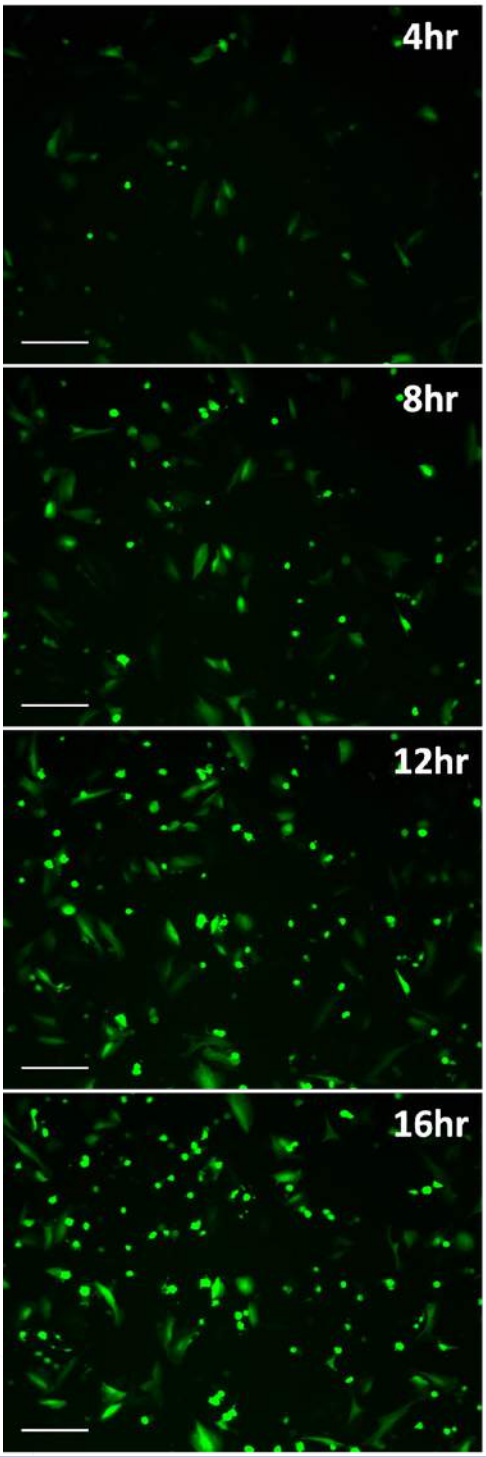

*Figure 9. Time lapse image of EGFP expression following pCMV GFP plasmid Transfection (Scale bar, 200 μm). *The images were collected every 2 hrs by Celloger Nano for 40 hrs.*

treatment, making it possible to detect fluorescence in all cells (Figure 8).

Transfection

Gene transfection is conducted for various research and therapeutic purposes. Real-time cell imaging helps quantify cell transfection efficiency and monitor the effect of transfected genes. The fluorescence with the expression of green fluorescence protein in pCMV-GFP vector transfected in a cell was observed every 2 hours through Celloger Nano, and it was confirmed that the green fluorescence protein started to be expressed 4 hours after transfection and it was maintained strongly until 16 hours after transfection (Figure 9).

Conclusion

The Celloger series improves the efficiency of fluorescence imaging by enabling imaging even at a minimum level of excitation light, which can also reduce phototoxicity caused by fluorescence staining, a priority consideration for live cell imaging. The Celloger systems that were used to carry out the applications mentioned above work perfectly inside an incubator, which makes them ideal tools for various imaging applications and experiments.

References

1. Jordan, M. A., et al. (1992). J. Cell Sci., 102(3), 401-416.
2. Blajeski, A. L., et al (2002). J. Clin. Invest., 110(1), 91-99.

Additional Resource

Cell-Based Research & Diagnostics Solutions

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Automated live cell imaging systems inside your incubator

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