JoJo Life Science

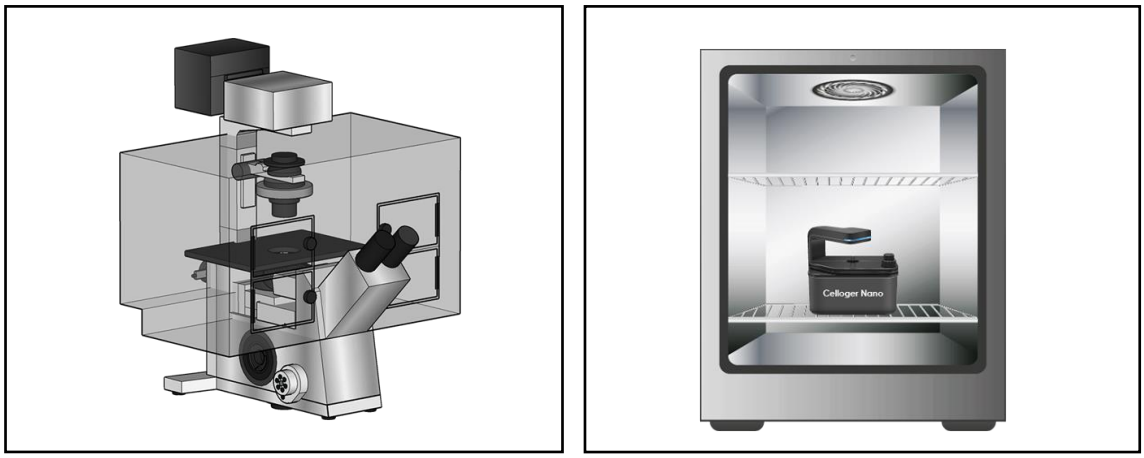
APPLICATION NOTE

Cellular assay using brightfield and fluorescence-based live cell imaging

Using Celloger series to image cells in real time

Live cell imaging technique makes it possible to understand and study various biological phenomena by enabling the observation of complex dynamics of live cells in real time using time-lapse microscopy. Real-time imaging of cellular phenomena such as cell migration, development and trafficking serves as an important means for research in various academic fields including cell biology, neuroscience, pharmacology and developmental biology. In order to observe the cells in a live state, incubator function is added to cover the microscope 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 a cell culture incubator are being developed. Such live cell imaging devices basically provide bright-field images and at times come with fluorescence imaging functionality 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 the time-lapse imaging system 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 the image quality while maintaining temperature and humidity when processing experiments that generate significant amount of heat such as fluorescence imaging inside an incubator.

Celloger series, live cell imaging systems developed by Curiosis, are made in a compact size so that they can be placed in a general cell culture incubator (Figure 1B) and designed to endure the self-generated heat enabling the 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 the applications in various cell-based research on the fields such as cell biology and pharmacology. The results showed that the devices had higher bright-field image quality than other live cell imaging system with the same functions and fluorescence imaging results were comparable to the images obtained from fluorescence microscopy using cMOS cameras with specifications corresponding to that of **Celloger.**



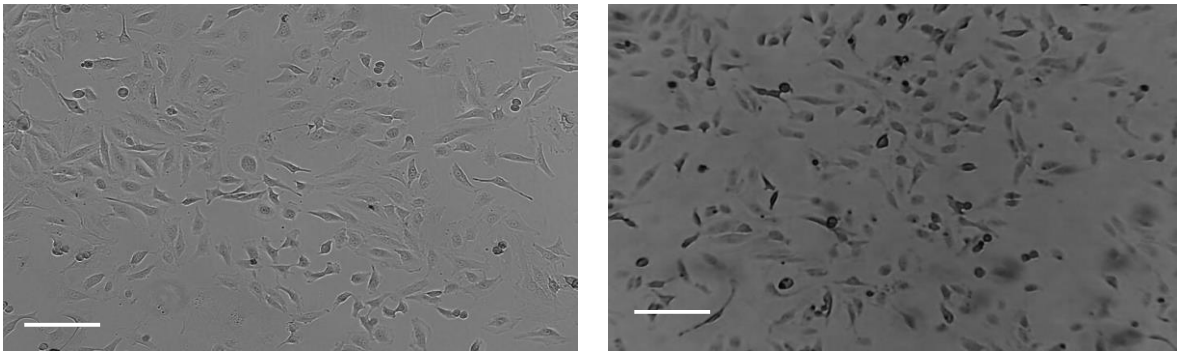
A. Conventional microscope

B. Celloger Nano placed in an incubator

Figure 1. Live cell imaging systems

1. Bright-field imaging application

Drug screening is a very important and essential process for the development of drugs including anticancer drugs. 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).

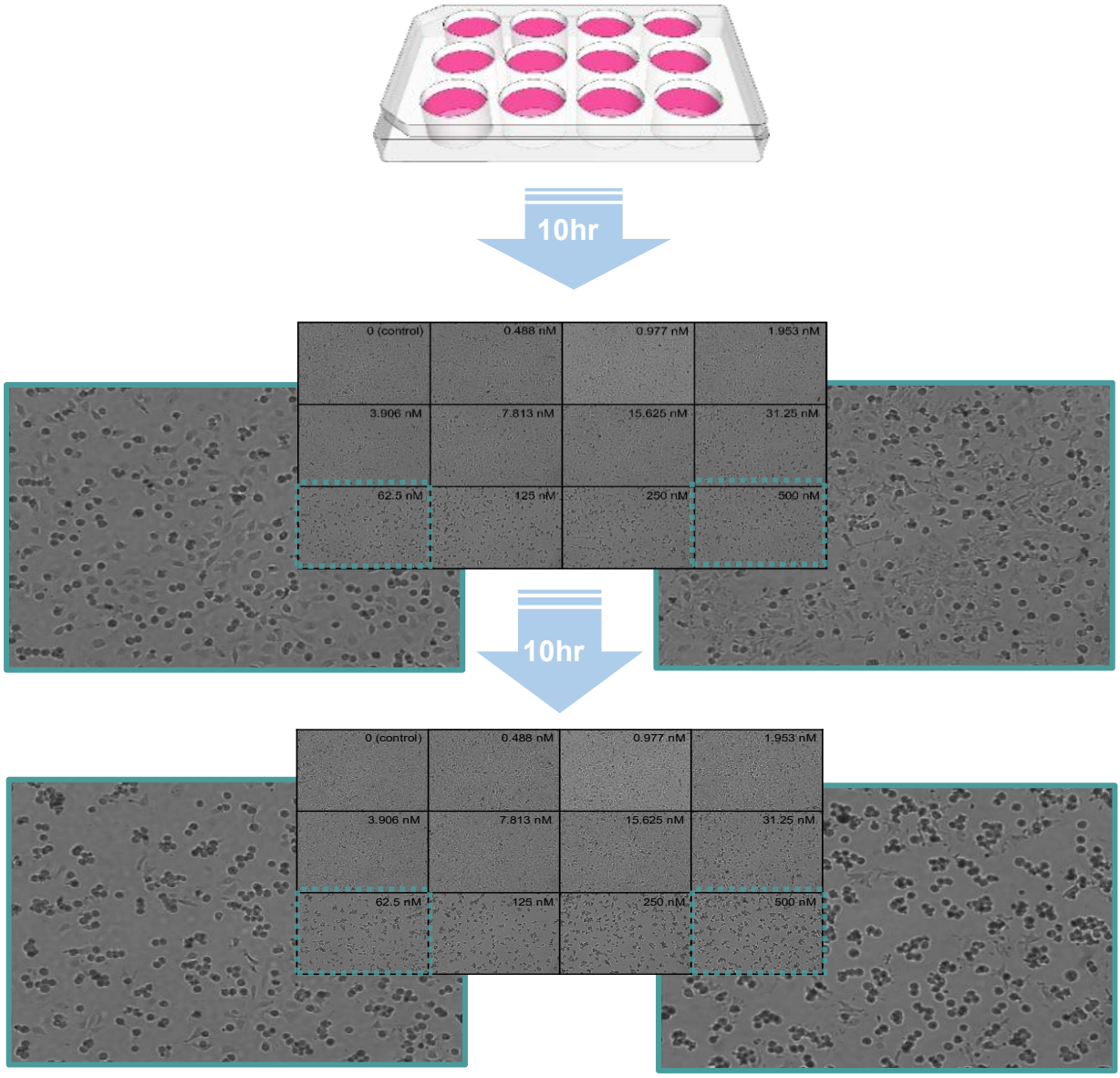


A. Image taken by Celloger Nano

B. Image taken by live cell imaging device of another company

Figure 2. Hela cell bright-field imaging (Scale bar, 200μm)

1.1. Morphology monitoring and drug screening



*The images were collected hourly by Celloger Mini for 20hrs.

Figure 3. Image tiles for different concentration levels of nocodazole and timelapse images generated by Celloger Mini.

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, one of the anticancer drugs, is known to cause mitotic arrest by inhibiting the polymerization which cell and what concentration it is used[1,2]. Cells were treated with different concentration levels of nocodazole and observed by **Celloger Mini**. The results showed that most cells died had similar confluency at the final endpoint, 20 hours after the treatment with the drug when the drug concentration is over 62.5nM. On the contrary, there was difference 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 antitumor efficacy of drug through real-time cell monitoring and confluency imaging using **Celloger Mini.** As shown in Figure 3, time-lapse images are generated in tile images, making it easy to compare the differences depending on conditions.

2. Fluorescence imaging application

Using live cell imaging equipment such as **Celloger Nano**, it becomes possible to visually investigate the dynamics of intracellular changes using the live cell staining fluorescent dyes with specific staining properties for subcellular organelles and cell labelling. Using this characteristic, it is possible to monitor and quantify the efficacy of a drug through various mechanisms. 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 phototoxicitythat occurs inevitably during excitation. The fluorescence images taken by **Celloger Nano** were compared with those taken by a fluorescence microscope equipped 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).

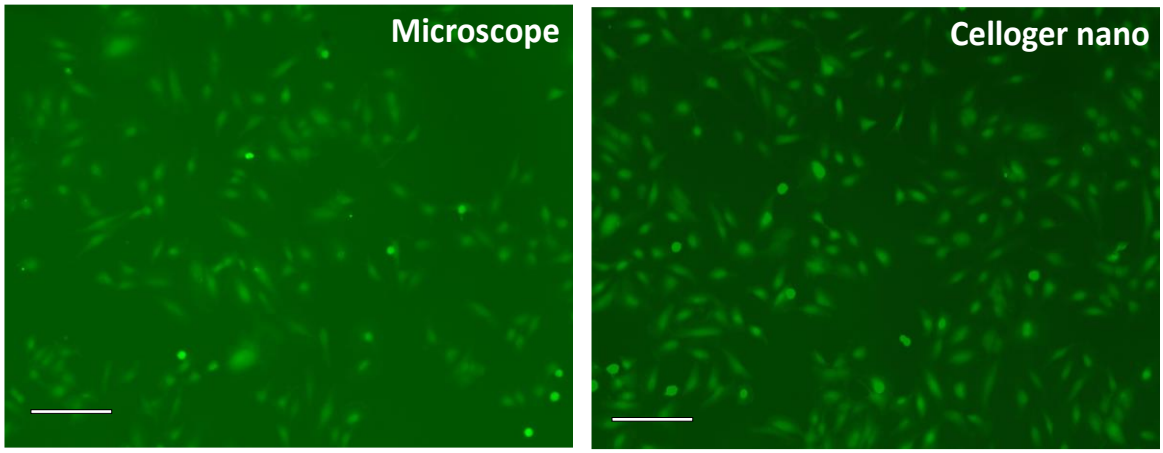
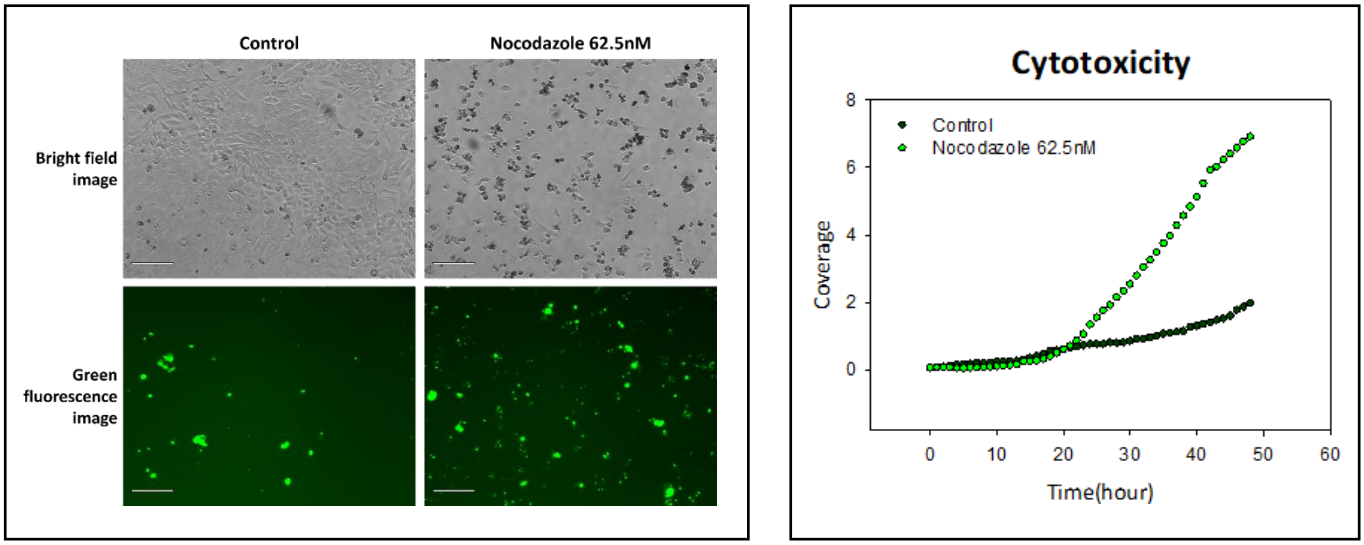


Figure 4. Fluorescence imaging of CMFDA stained cells (Scale bar, 200μm)

2.1. 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. 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 increases due to cell death after 20 hours (Figure 5).



A. Cell image after 35 hours from the treatment with 62.5nM nocodazole. (Scale bar, 200um)

B. Fluorescence coverage by hour

Figure 5. Cytotoxicity assay using cell-impermeant dye

2.2 Apoptosis assay

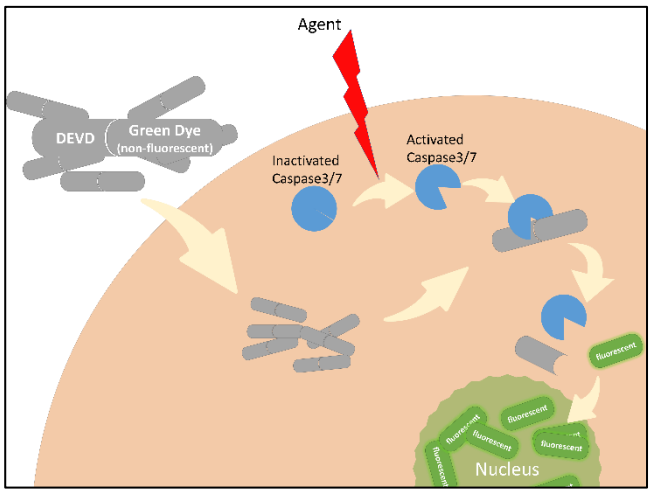
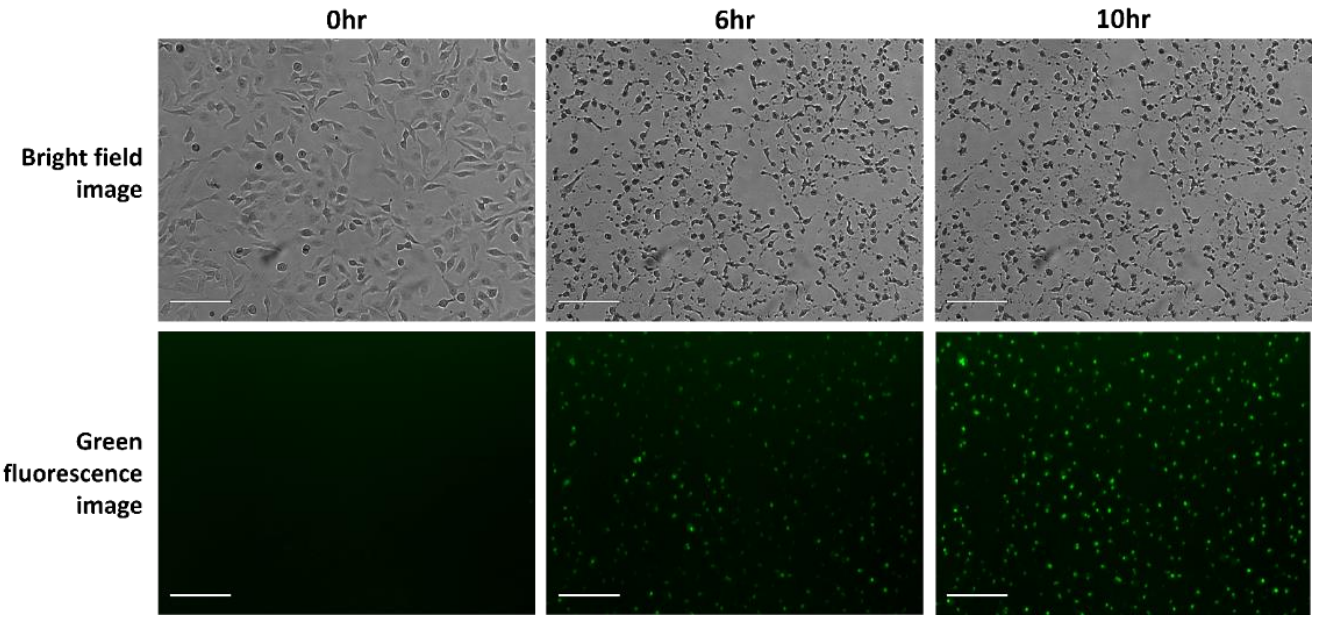


Figure 6. Illustration on the action mechanism of Caspase-3/7 Green Detection Reagent

Apoptosis is the process of programmed cell death where processes such as membrane blebbing, cell shrinkage and nuclear fragmentation occur. In this process, the enzyme called caspase is activated to mediate this reaction in the cell. Activated Caspase3/7, one of the caspase family, specifically cleave certain peptide known as DEVD, and fluorophores conjugated DEVD is useful to quantify Caspase activity and apoptosis (Figure 6).

It was found that 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 amount of fluorescent materials increased with time (Figure 7).



* The images were collected every 30min by Celloger Nano for 15hrs and 30mins.

Figure 7. Using fluorescence detection of activated caspase to quantify apoptosis of Hela cells caused by staurosporine (Scale bar, 200μm)

Fluorescence coverage graph is shown to quantify the apoptosis by time. The graph illustrates that fluorescence began to be detected from two and a half hours after the treatment with staurosporine and reaction became saturated from 10 hours after the treatment, making it possible to detect fluorescence in all cells (Figure 8).

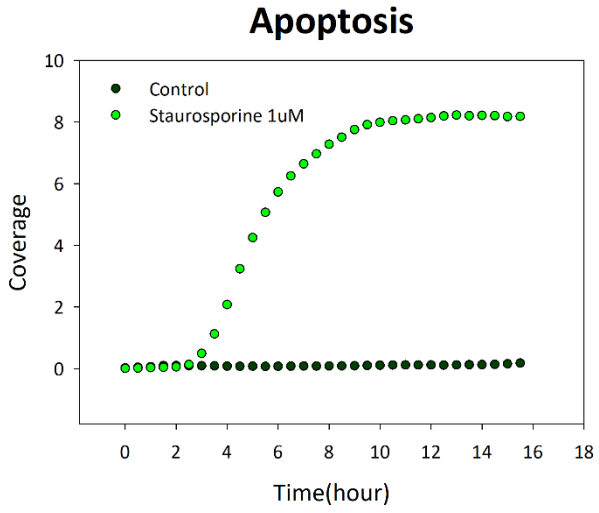
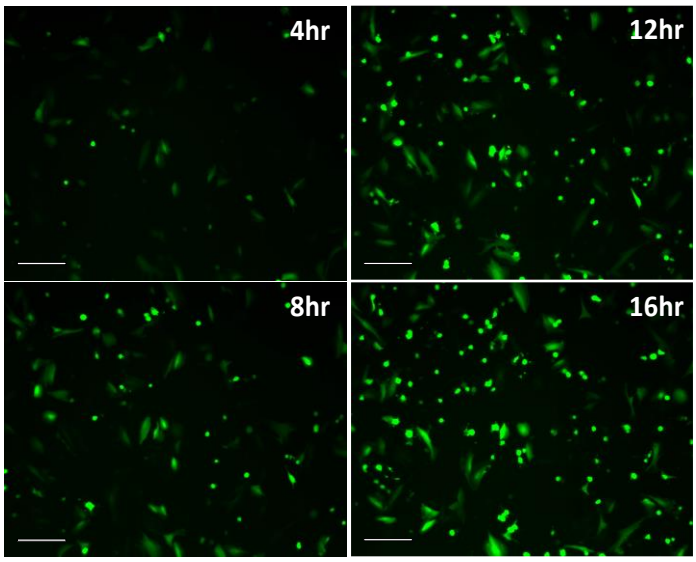


Figure 8. Fluorescence coverage graph by time

2.3 Transfection



* The images were collected every 2hrs by Celloger Nano for 40hrs.

Figure 9. Time lapse image of EGFP expression following pCMV GFP plasmid Transfection (Scale bar, 200μm)

Gene transfection is conducted for various research and therapeutic purposes. Real-time cell imaging is considered to be used well in various applications for quantifying cell transfection efficiency or monitoring 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 from 4 hours after transfection and it was maintained strongly until 16 hours after transfection (Figure 9).

3. 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.

4. Reference

1. Jordan, M. A., Thrower, D., & Wilson, L. (1992). Effects of vinblastine, podophyllotoxin and nocodazole on mitotic spindles. Implications for the role of microtubule dynamics in mitosis. *Journal of cell science*, *102* (1), 401-416.

2. Blajeski, A. L., Phan, V. A., Kottke, T. J., & Kaufmann, S. H. (2002). G 1 and G 2 cell-cycle arrest following microtubule depolymerization in human breast cancer cells. *The Journal of clinical investigation*, *110* (1), 91-99.