Smart Fluorescence and Dual Live Cell Imaging

NanoEnTek

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Compact Real-Time Cell Analyzer Specialized For Fluorescence-Expressing Cell

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Numerous cells are widely used and being cultured to test the efficacy and toxicity of drugs in cancer therapies and regenerative medicines in combination with drug delivery, tissue engineering and theragnostic investigations. In particular, analyzing cell behaviors and morphological changes from the drug administration would be critical for the comprehensive understanding of their mechanisms. Many high-performance microscopes have been developed and utilized in monitoring the cell behaviors, such as cell growth and death, but still continuous monitoring of live cells has been difficult due to the inevitable disturbance and conditional changes to the cells in their environmental conditions. Although several interrupting factors could hinder the optimal experimental conditions, such as temperature change and contaminations, no microscope was available to be placed and operational in a thermo-hygrostat, like inside of an incubator.

NanoEnTek's JuLI™ FL seemed to fit above needs, where it was compatible with most incubators due to its compact scope size, and it could easily monitor the live-cells and obtain live images in cell growing environment. Furthermore, the dual microscopes, which could be controlled by an external main station, were able to capture time-lapse cell images in bright, fluorescence and merge modes.With the versatile modalities of dual JuLI™ FL, we were able to conduct continuously real-time toxicity experiments with U-2 OS (transfected osteosarcoma cell with green or red fluorescence protein (GFP, RFP) genes) and anti-tumor drug, Doxorubicin (DOX).

Drug efficacy testing

Doxorubicin (DOX) is one of the widely used anti-tumor agents for various cancer chemotherapies, which can slow down the growth rate of cancer cells. However, many side effects of DOX, such as myelosuppression and cardiotoxicity, were reported. Since it can be intercalated into DNA and hinder the DNA replication of cancer cells, as well as of normal cells, we are planning to develop natural protein based DOX delivery system to reduce unintended toxicity by carrier-free DOX administration and to increase the drug efficiency through site-specific and targeted delivery. As the first step of developing DOX delivery system, the time-lapse images of live cell proliferation were captured with GFP- expressing U-2 OS. And, the efficacy of carrier-free DOX was examined with RFP-expressing U-2 OS.

Simply, the GFP-expressing U-2 OS cells were cultured for 36 hours in 12 well plates, and their morphological changes and confluences were detected at 15 minute intervals. Figure 1 and Figure 2 showed the captured images and growth curve from the confluences.

On the other hand, the RFP-expressing U-2 OS cells were incubated for 12 hours in 12 well plates with and without DOX (10 μg /mL), and images of their cell behavior and confluences were recorded. Beneficially, the images were captured and saved in bright, fluorescence and merge mode at the same time, enabling multi-comparisons (Figure 3). In addition, the cell growth curves were automatically obtained from cell confluence results (Figure 4). Unfortunately, the backgrounds of DOX- treated group were showing red fog since DOX also emitted the red fluorescence.

Through these easy and simple experiments, the cell death by DOX was confirmed through gradual cell shrinkage and decreasing confluences by cell detachment from the culture plate. When DOX-exposed cells were dying, the red fluorescence intensity of the dying cells were increased by carrier-free DOX in cell culture media, which might have entered in the cells and been accumulated in their cytoplasm.

The growth and death of U-2 OS expressing GFP or RFP were easily monitored by JuLI™ FL fluorescence live cell movie analyzer. The compact size JuLI™ FL only occupied approximately one-third of incubator space (Figure 5) and it seemed to be able to fit inner compartment of most incubators. Furthermore, user can utilize JuLI™ FL's various live cell performances, such as live cell imaging, counting, and GFP/RFP expression level measurements.

Conclusion

The flexible JuLI™ FL can be used with ease in monitoring the cell growth and death, which can be compared automatically between experimental and control groups using its dual scope system. Moreover, the time-lapse live cell imaging in bright, fluorescence and merge modes provided accurate data for multiple comparisons at a given time points or durations.

Figure 1

Proliferation images of GFP-expressing U-2 OS cells: the cells presented continuous growth and increasing confluence from 38.71 % to 72.55 %.

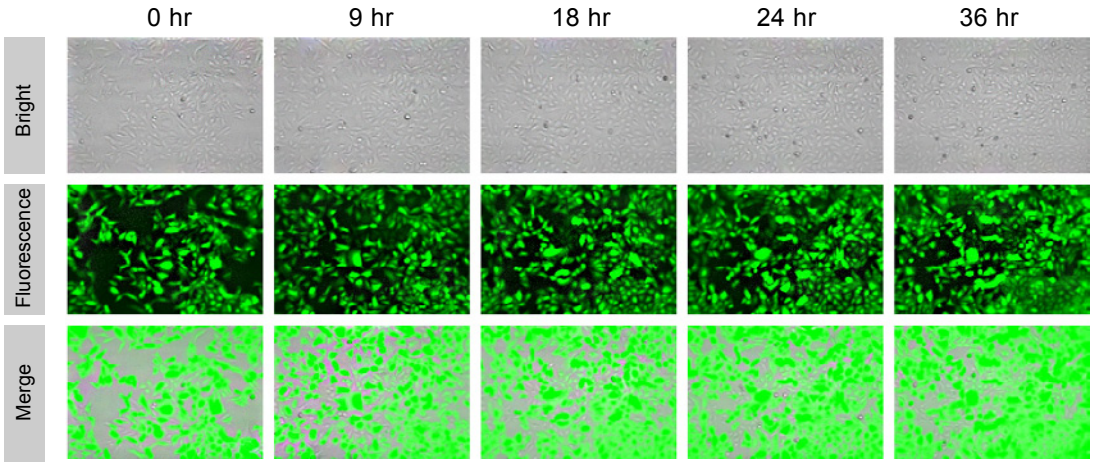


Figure 2

Cell confluence of GFP-expressing U-2 OS cells.

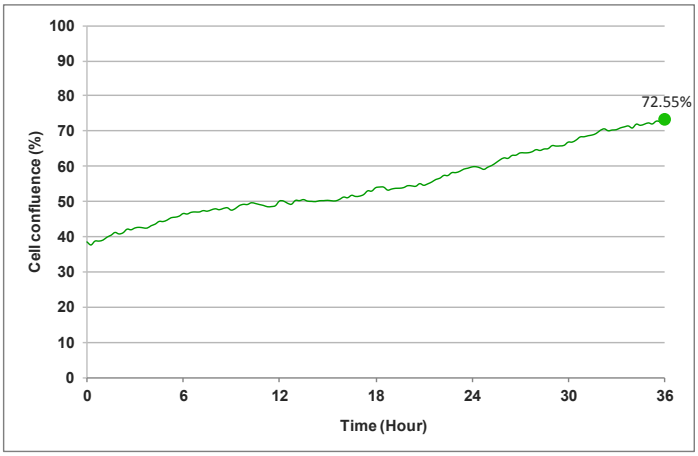


Figure 3

Recording of RFP-expressing U-2 OS cells: DOX-treated cells showed the cell death and decreasing confluence from 52.59 % to 30.63 %, while the DOX-untreated cells presented progressive cell growth with the confluence from 47.57 % to 66.62 %. (A) Bright, (B) fluorescence, (C) merge images.

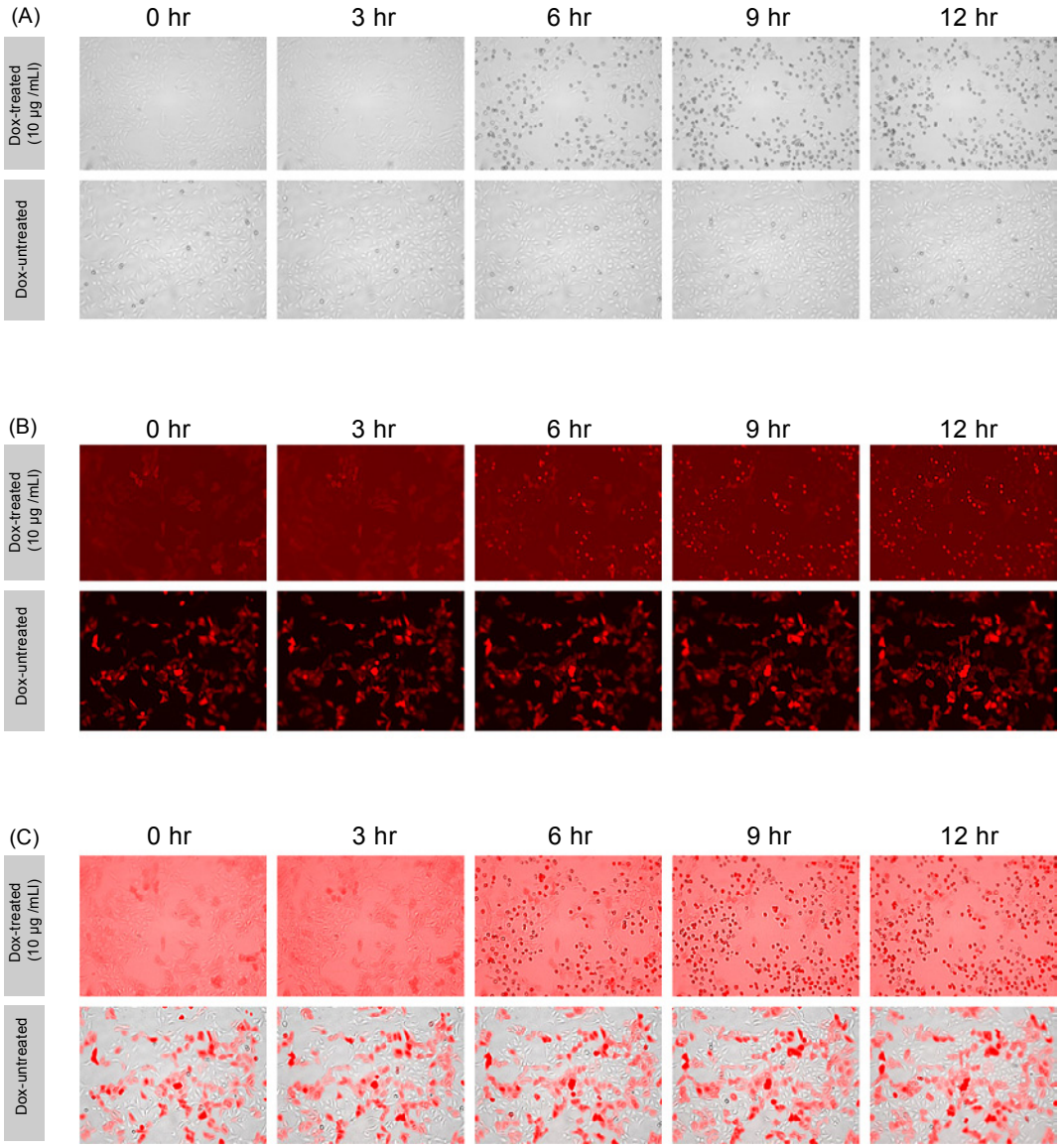


Figure 4

Recording ofRFP-expressing U-2 OS cells: The growth curves comparing each confluence of DOX-treated and untreated cells.

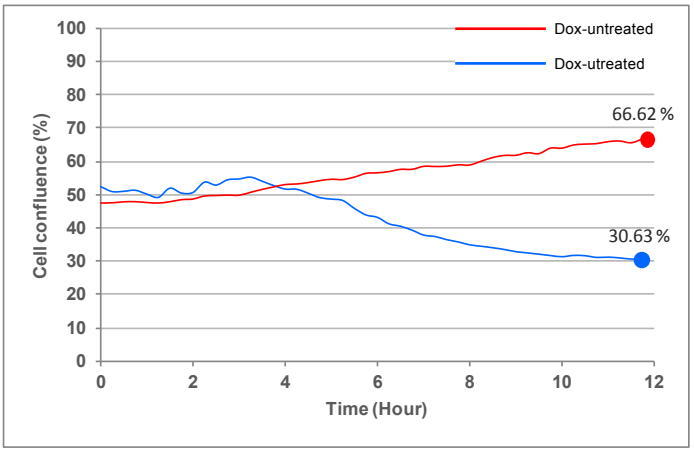
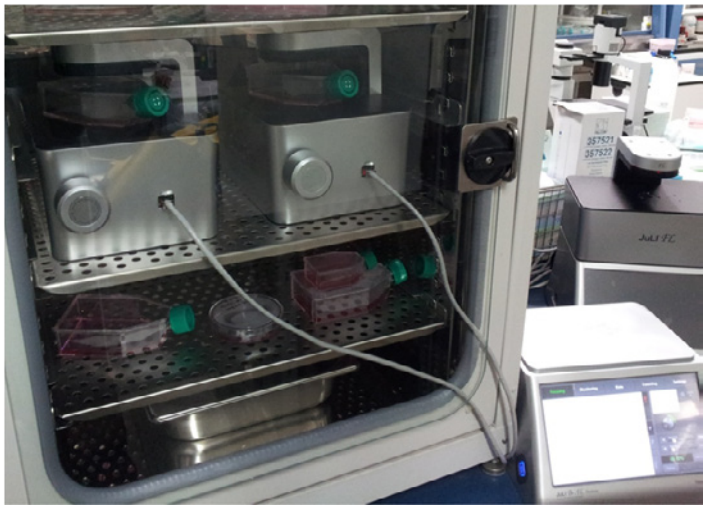


Figure 5

JuLI™ FL installation in a CO2 incubator (dual mode): Internal two compact scopes can be well operated at a thermohygrostat environment, and an external main station can control the monitoring conditions such as magnification,detector exposure and brightness.



JuLI™ FL

Fluorescence live cell movie analyzer

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