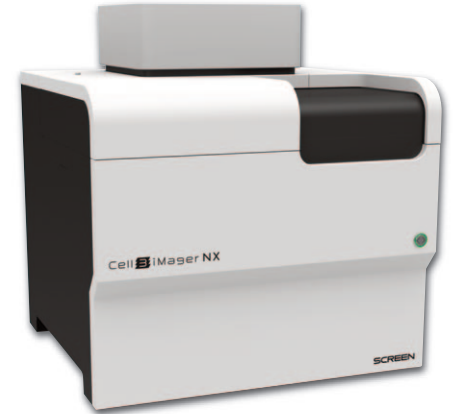


Label-free imaging and analysis of organoids

Introduction

In recent years, research on organoids for regenerative medicine and drug R&D fields has developed rapidly. Quantitative analysis of organoids by imaging is difficult due to the morphological heterogeneity as well as the typical three-dimensional culture method. Image analysis becomes easier when fluorescent labeling is used, yet this labeling may lead to more problems such as damage to cells and an increase in cost.

By using Cell3iMager NX, image analysis of organoids in bright-field images can be performed with high throughput screening. In this experiment, we performed monitoring of organoid culture process and analyzed Forskolin-induced swelling (FIS) assay by label-free manner.



Materials & Methods

Product Used: Cell3iMager NX

Samples & Reagents: Mouse intestinal organoids and dedicated medium (Stem Cell Technologies)
 Mouse hepatic organoids and dedicated medium (Stem Cell Technologies)
 24 well plate (CORNING)
 96 well plate (CORNING)
 Forskolin (R&D Systems)

Methods

Intestinal organoids and hepatic organoids were embedded in Matrigel dome and cultured at 37°C and 5%-CO₂. By periodically Z-Stack imaging with Cell3iMager NX, all-in-focus images were obtained (Fig. 1).

Next, using the deep learning function, the organoid regions in the bright-field image were labeled to create teacher image (Fig. 2). A deep learning model was created by performing two hours of learning using the teacher dataset (bright-field image and teacher image). By using the model to an unknown image, the organoid region in the bright-field image were segmented, and then measured the feature quantity.

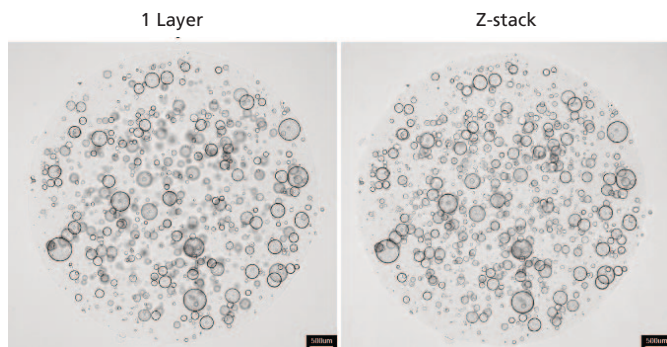


Fig. 1: All-in-focus image by Z-Stack Scan

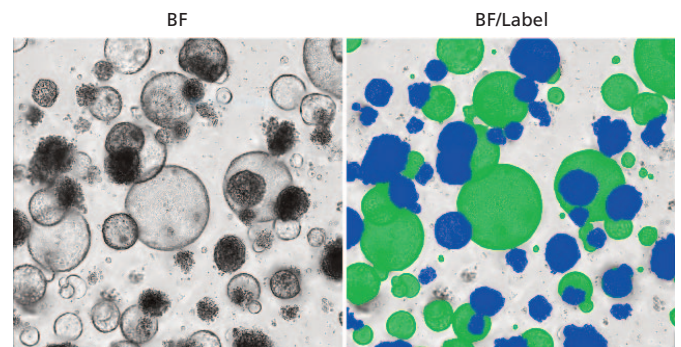


Fig. 2: Created teacher image

Results

Monitoring of organoid growth

Intestinal organoids were cultured in a 24-well plate and analyzed with Cell3iMager NX. Based on the result and the analysis, it was found that organoids with developed crypt structures increased over time (Fig. 3, Fig. 4)

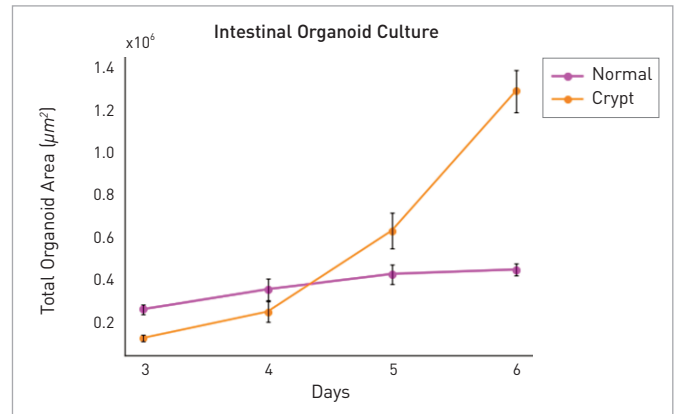
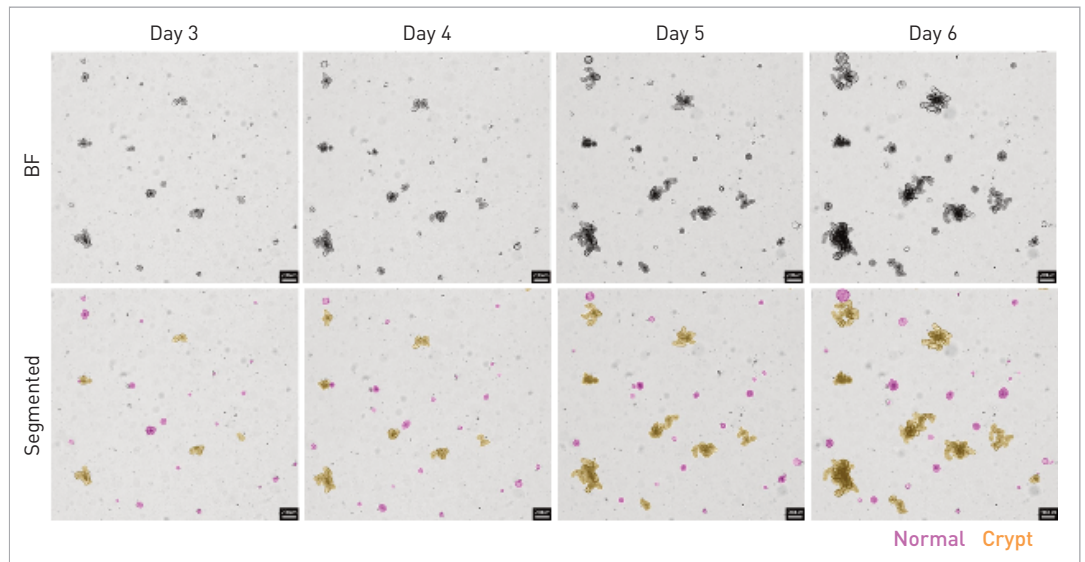


Fig. 4: Result of intestinal organoid growth

Fig. 3: Images of intestinal organoids



Similarly, the result and the analysis on culturing hepatic organoids revealed that the number of dark/collapsed organoids increased over time (Fig. 5, Fig. 6)

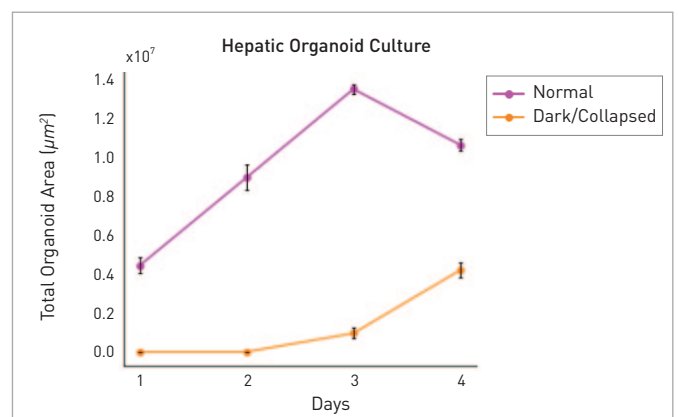
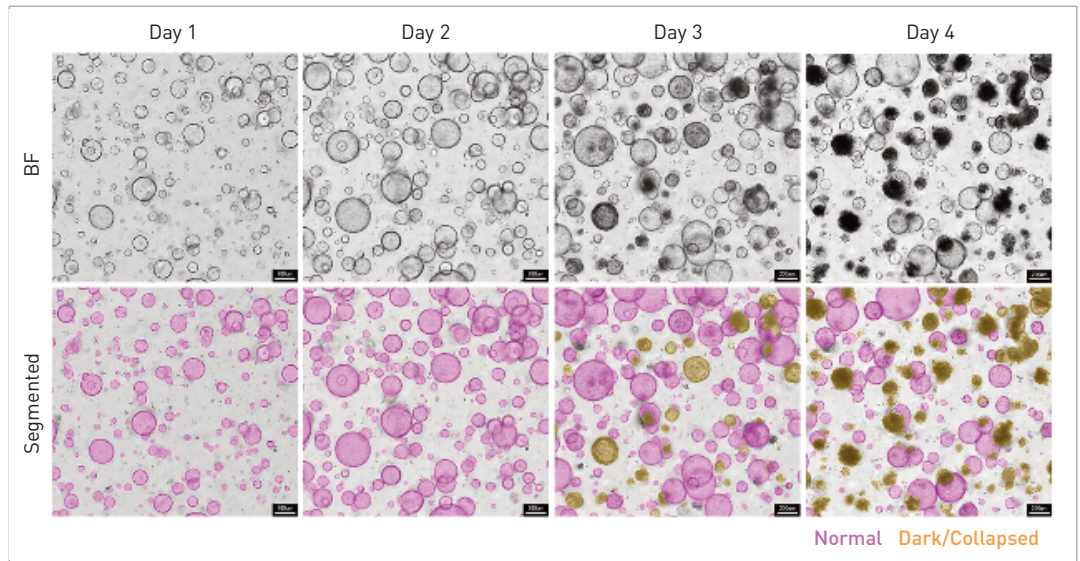


Fig. 5: Result of hepatic organoid growth

Fig. 6:
Images of hepatic organoids



FIS Assay

Intestinal organoids were cultured in a Matrigel dome in a 96-well plate, and it was confirmed that the organoids swelling due to Forskolin stimulation (Fig. 7, Fig. 8)

Conclusion

Cell3iMager NX enables imaging of three-dimensionally cultured organoids. In addition, by using deep learning, label-free analysis can be performed for each organoid morphology in bright-field images. There is a possibility that label-free analysis can replace the monitoring of organoid growth processes and high-throughput drug screening that have been performed by microscopic observation so far.

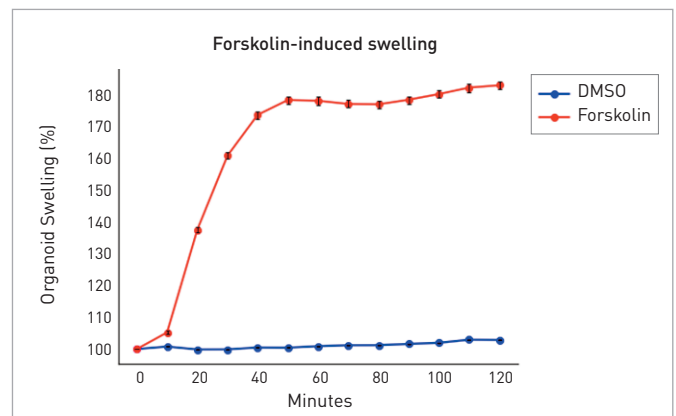


Fig. 8: Result of FIS assay

Fig. 7:
Images of FIS assay

