Label-free image analysis and its applications of human iPSC-derived intestinal organoids

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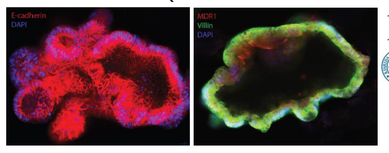
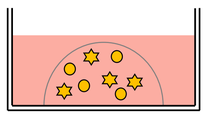
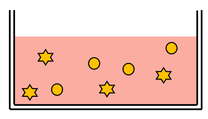
1. Abstract

In recent years, research on organoids in regenerative medicine and drug discovery research has developed rapidly. In particular, the research for producing intestinal organoids from induced pluripotent stem cells (iPS cells) and human biological tissues has been remarkably developed, and the culture conditions for each have been almost established. However, imaging and image analysis methods of organoids have not been established yet. Organoids are composed of heterogeneous cell populations, and in addition to exhibiting a non-uniform morphology for each organoid, cell populations other than the evaluation target are likely to be included in the image. For these reasons, only by using image processing with a simple parameter threshold, high-precision segmentation of organoids cannot be performed, and quantification by image analysis is difficult. In the case of image analysis using a fluorescent label, there is a possibility that the above problems can be overcome, but today, the importance of label-free analysis without using a fluorescent label is increasing. In general, a three-dimensional culture method with an extracellular matrix is used for organoid culture, which makes imaging itself and image analysis more difficult. Therefore, in this study, we attempted a bright field image analysis of human iPSC-derived intestinal organoid using deep learning technology, which utilized in various fields. Since individual organoids cultured in Matrigel are located at different heights, all-in-focus images were obtained by Z-stack imaging. By using deep learning on the acquired image, only organoids exhibiting specific morphologies were segmented from the image, and feature quantities such as organoid count, diameter and area were calculated. Cell3iMager duos (SCREEN Holdings Co., Ltd.) was used for this imaging and image analysis. In this presentation, we will discuss the label-free analysis of organoids and their applications.

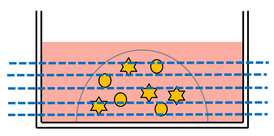
2. Methods

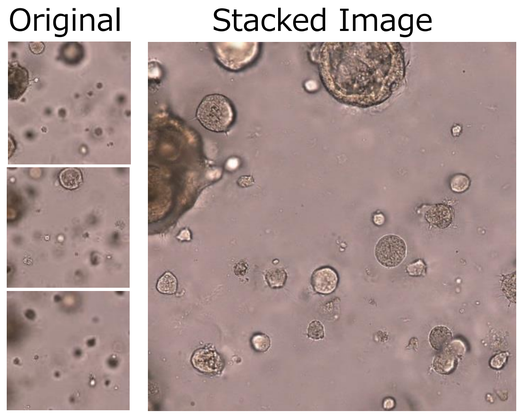
1. Organoid Culture → 2. High Throughput-Brightfield Imaging → 3. Deep Learning Analysis

Culture	Type A	Type B
Organoid	Human iPSC-derived intestinal organoid (Def-INTESTINAL WT)  DefiniGEN KISHIDA By courtesy of DefiniGEN	
Labware	24well plate	96well plate
Medium Component	・Matrigel dome 60μL ・Medium 700μL 	・Matrigel 5μL ・Medium 95μL 

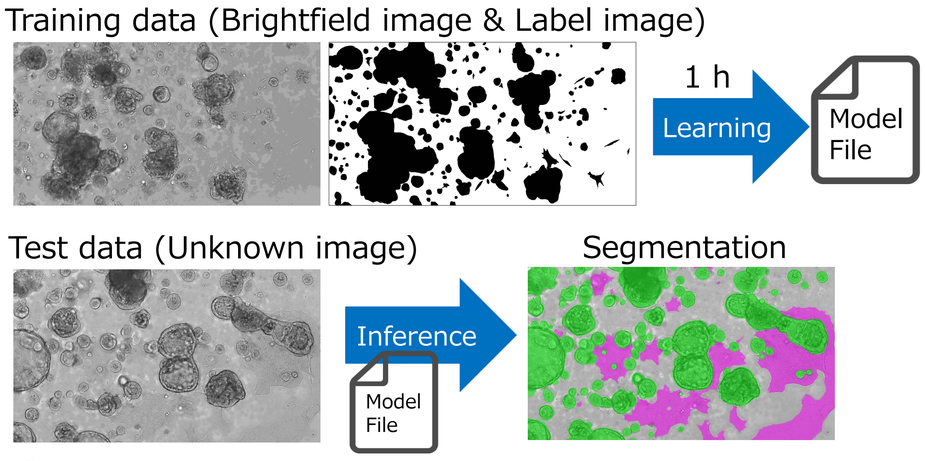
Def-INTESTINAL WT Organoid were cultured by 2 types of method.

SCREEN Cell³iMager duos 



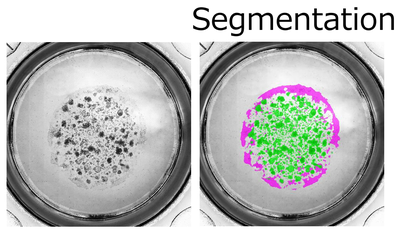


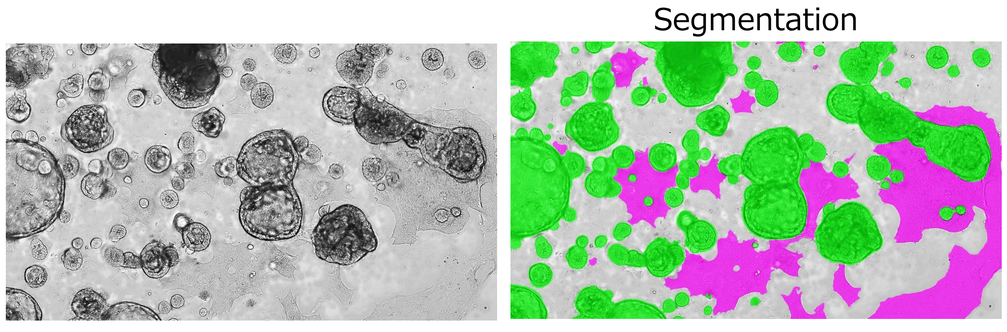
Organoids were scanned as a brightfield stacked image. Stacked image was obtained by changing focus in the Z direction (dotted blue line).



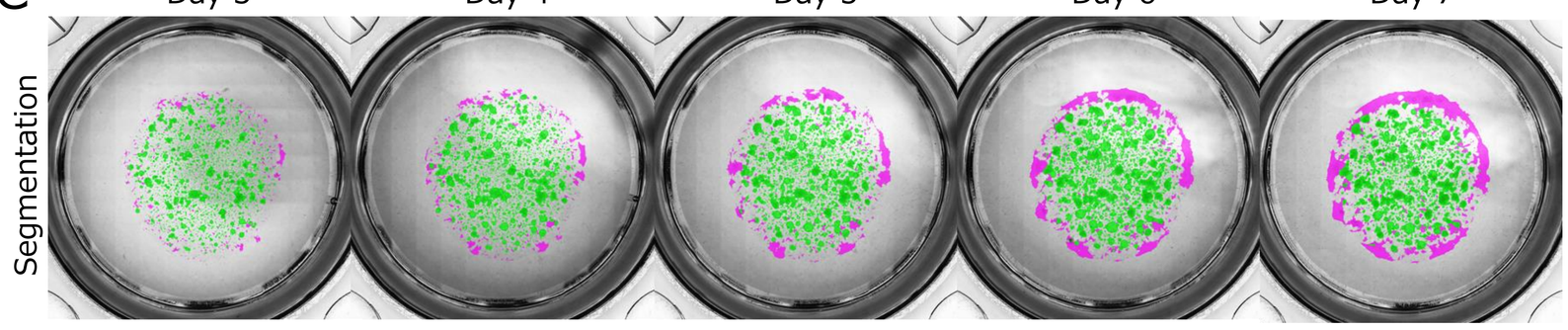
Organoids in brightfield images were segmented using the deep learning function of Cell³iMager duos.

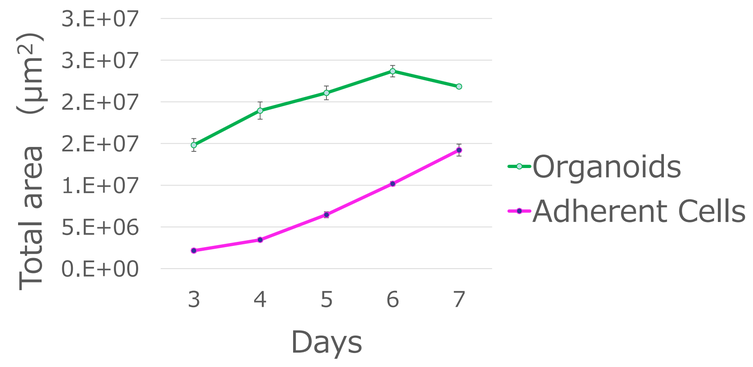
3. Result : Proliferation analysis of organoids

A 

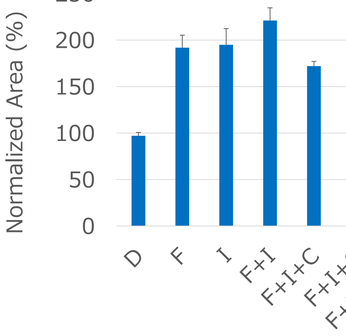
B 

Organoids cultured in type A were imaged by bright-field Z-Stack and segmented by Deep Learning. The green label shows the organoid and the pink label shows the segmentation results of adherent cells.
(A) Brightfield image and segmentation results in whole well image.
(B) Enlarged display of a part of A.
(C) Brightfield imaging and segmentation of the same sample every 24 hours. It was found that adherent cells were gathered at the edge of matrigel dome.
(D) Time course of the total area in the whole well of organoids and adherent cells. It is considered that Day 6 to Day 7 is appropriate as the passage timing of this organoid.
⇒ Organoids and adherent cells were segmented label-free (non-invasive), respectively, and their proliferation status could be analyzed.

C 

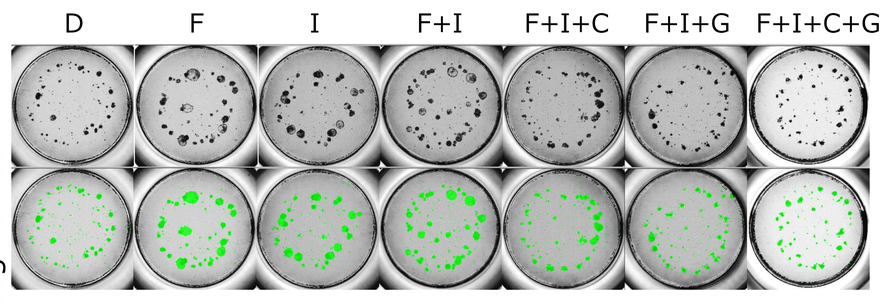
D 

4. Result : FIS assay

A 

Forskolin stimulation was added to organoids cultured in type B, and the area increase rate was quantified.
(A) Whole well images 72 h after Forskolin stimulation. Organoid swelling was observed by Forskolin stimulation.
(B) Whole well images under various stimulus and the segmentation results of organoids (green label).
(C) The area increase rate (%) from the segmentation image of B. Swelling of organoids stimulated by Forskolin or IBMX was suppressed by CFTR inhibitors.
(D) It was found that the area increase rate was different depending on the type of matrigel.
D:DMSO, F:Forskolin, I:IBMX
C:$CFTR_{inh}$-172, G:GlyH-101

⇒ The rate of increase in the area of organoids could be quantified in a label-free manner and applied to the Forskolin-Induced Swelling (FIS) assay.

B 

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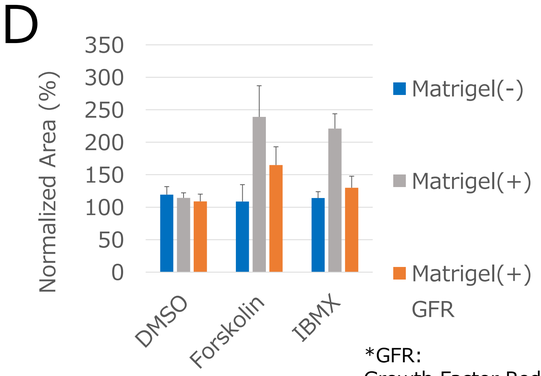
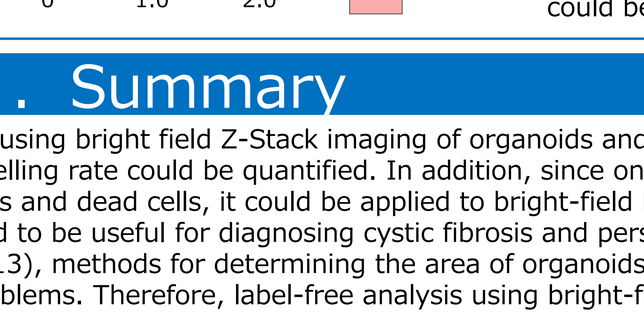
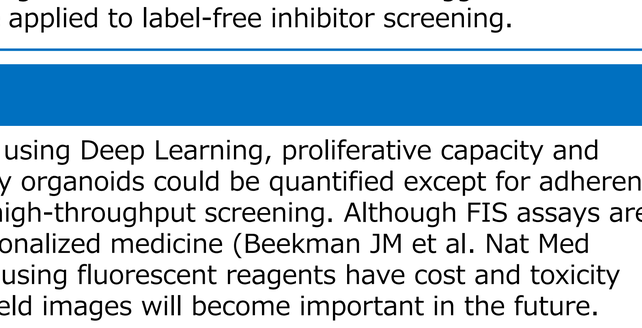
D 

*GFR: Growth Factor Reduced

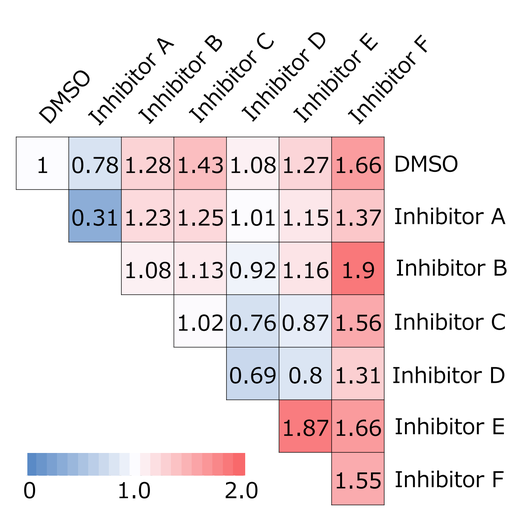
5. Result : Inhibitor screening

A 

B 

Various combinations of inhibitors were added to the organoids cultured in type B, and the rate of increase in area was quantified.
(A) Example of brightfield image of organoid and segmentation result (green label). Dead cells and debris were excluded, and only organoids could be segmented.
(B) Example of brightfield image and segmentation result (green label) of single cell. Even when cells were crushed to a single cell at the time of seeding organoid, segmentation could be performed except for dead cells and debris.
(C) Heat map of area increase rate 6 days after addition of inhibitor. Differences in the area increase rate of organoids were observed depending on the combination and concentration of the inhibitors.

⇒ Segmentation from single cells to organoids was possible, eliminating dead cells and debris. It was suggested that it could be applied to label-free inhibitor screening.

C 

6. Summary

By using bright field Z-Stack imaging of organoids and using Deep Learning, proliferative capacity and swelling rate could be quantified. In addition, since only organoids could be quantified except for adherent cells and dead cells, it could be applied to bright-field high-throughput screening. Although FIS assays are said to be useful for diagnosing cystic fibrosis and personalized medicine (Beekman JM et al. Nat Med 2013), methods for determining the area of organoids using fluorescent reagents have cost and toxicity problems. Therefore, label-free analysis using bright-field images will become important in the future.