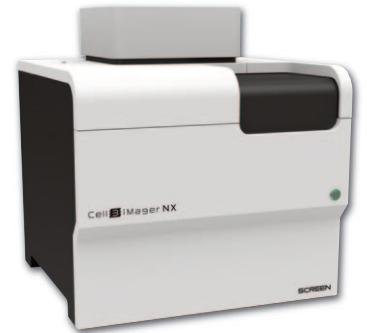


Killing/ADCC Assay by NK cells

Introduction

In recent years, NK cells, which play a major role in eliminating tumors and virus-infected cells and in antibody-dependent cell-mediated cytotoxicity (ADCC), have attracted attention. Fluorescent staining are commonly utilized in the assays evaluating the activities of NK Cells, including killing assay and ADCC assay to measure the cytotoxic activity and ADCC activity, respectively. However, the staining reagents may result in more problems such as increased costs and cell damage. In this experiment, we used Cell3iMager NX and deep learning functions to perform label-free Killing/ADCC assay by NK cells against breast cancer cells.



Materials & Methods

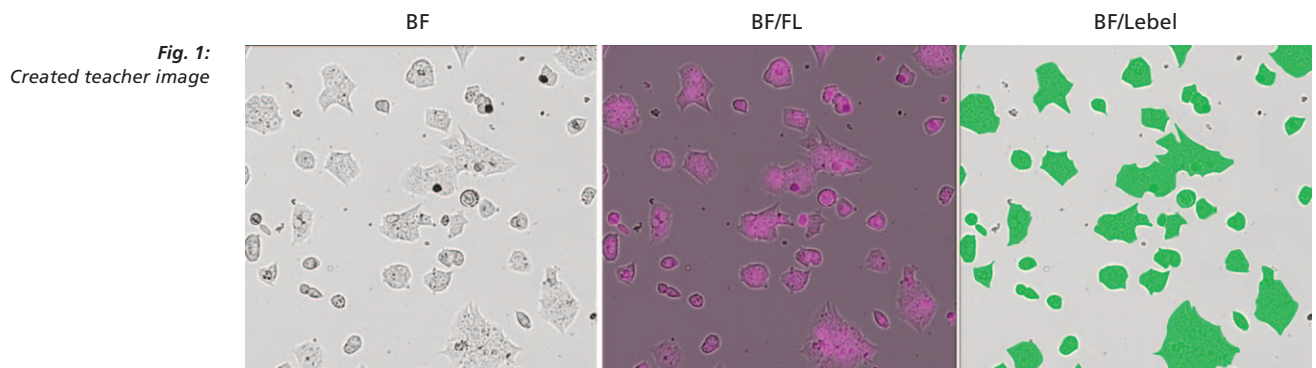
Product Used: Cell3iMager NX

Samples & Reagents: Primary NK cell (Biotherapy Institute of Japan)
 MCF7 cell line (RIKEN)
 BT474 cell line (ATCC)
 NK cell dedicated medium (Biotherapy Institute of Japan)
 DMEM (Nacalai tesque)
 RPMI (Nacalai tesque)
 FBS (Biosera)
 96 well plate Flat bottom (SUMILON)
 96 well plate U-shape bottom (SUMILON)
 CellTrace Far Red (Thermo)
 Trastuzumab (anti-HER-2-ab)

Methods

HER-2-negative MCF7 cells and HER-2-positive BT474 cells fluorescently stained with CellTrace Far Red. The cells were cultured in a 2D/3D culture method for 72 hours, then co-cultured with NK cells and Trastuzumab was added. After 96 hours, Killing/ADCC assay were performed by using Cell3iMager NX. Next, to create a teacher image for the deep learning function, we labeled the cancer cell area in the bright-field image. By referring to the fluorescence image when labeling the bright-field image, it becomes easier to distinguish between cancer cells and NK cells in the bright-field image (Fig. 1).

A deep learning model was created by learning the teacher dataset (bright-field image and teacher image). By applying the deep learning model to an unknown image, the cancer cells in the bright-field image were segmented and the feature values were measured.



Results

2D Killing/ADCC Assay

Through segmenting and quantifying the cancer cell area in the bright-field image, it is revealed that the decrease in the cancer cell area is correlated with the number of NK cells.

We found that Trastuzumab had no effect on HER-2-negative MCF7 cells (Fig. 2, Fig. 3), but decreased the area of HER-2-positive BT474 cells. It suggests that Trastuzumab enhanced NK cell cytotoxicity (Fig. 4, Fig. 5).

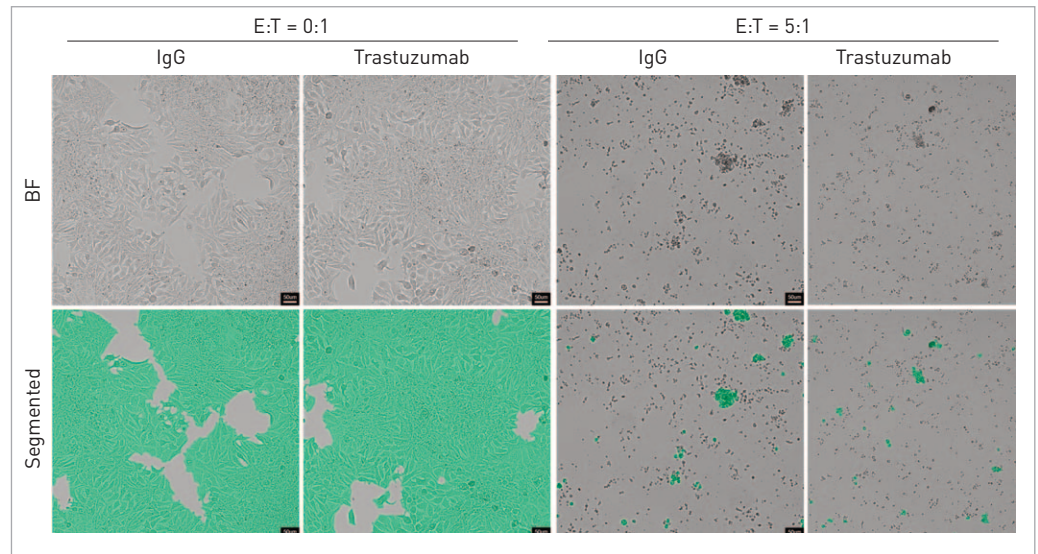


Fig. 2: Images of MCF7/NK cells (2D)

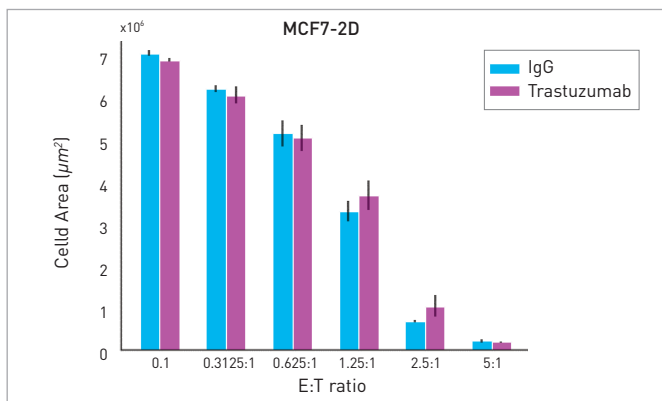


Fig. 3: Result of MCF7/NK cells (2D)

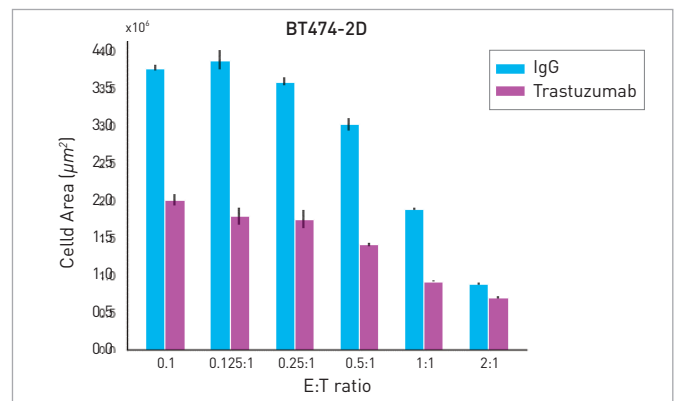


Fig. 4: Result of BT474/NK cells (2D)

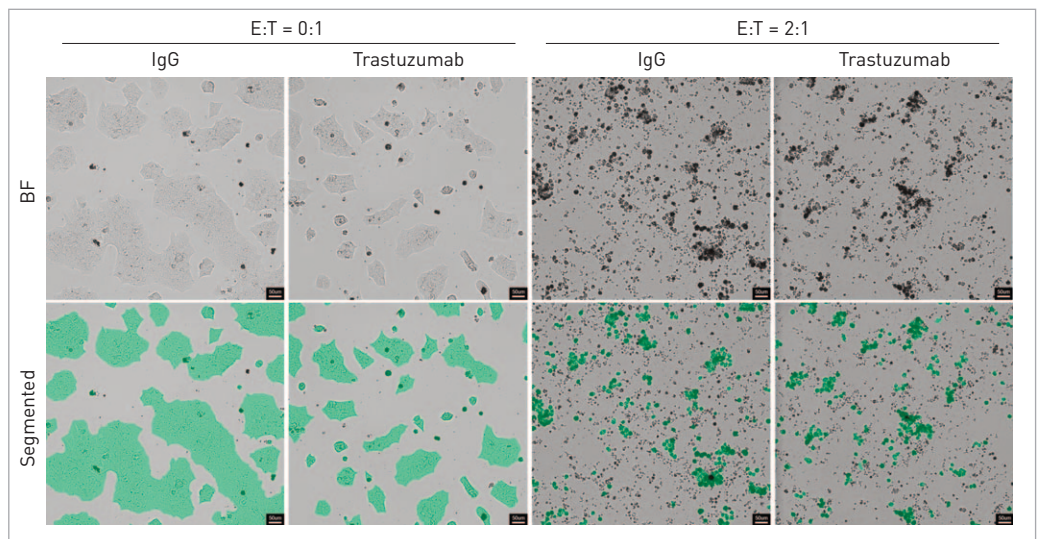


Fig. 5: Images of BT474/NK cells (2D)

3D Killing/ADCC Assay

Through segmenting and quantifying the cancer spheroid area in the bright-field image, it is revealed that the decrease in the cancer spheroids is correlated with the number of NK cells. We found that Trastuzumab had no effect on HER-2-negative MCF7 cells (Fig. 6, Fig. 7), but decreased the area of HER-2-positive BT474 cells. It suggests that Trastuzumab enhanced NK cell cytotoxicity (Fig. 8, Fig. 9).

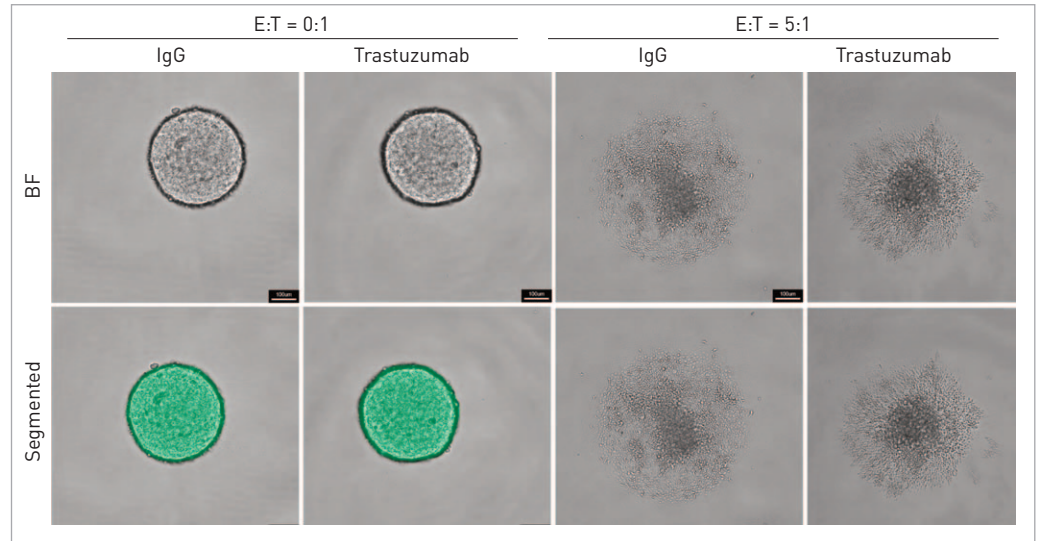


Fig. 6: Images of MCF7/NK cells (3D)

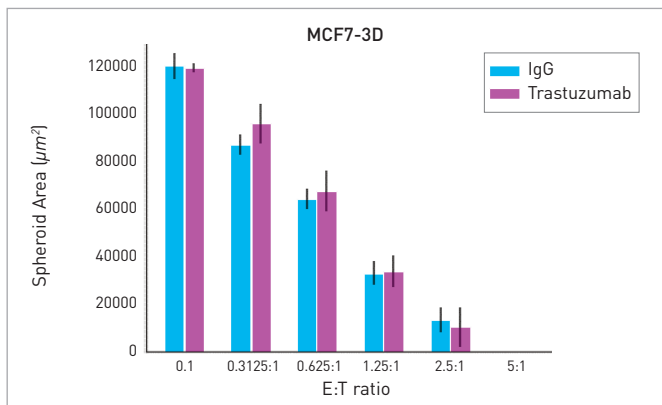


Fig. 7: Result of MCF7/NK cells (3D)

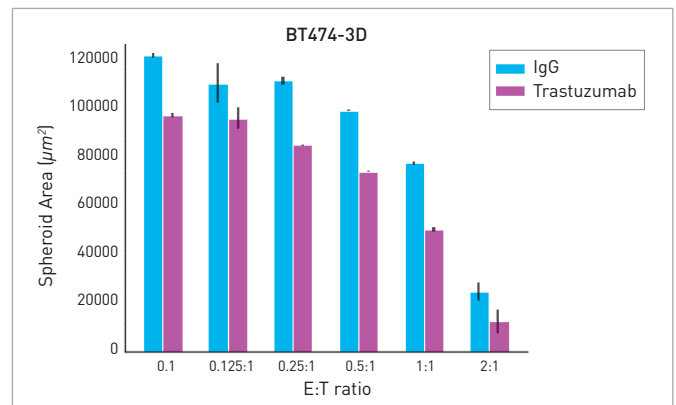


Fig. 8: Images of BT474/NK cells (3D)

Conclusion

Cell3iMager NX supports the 2D/3D bright-field and fluorescence imaging of co-cultured cancer cells and NK cells. In addition, the deep learning function of Cell3iMager NX enables cancer cell-focused segmentation and quantification in bright-field images. Cell3iMager NX is suitable for all levels of users, empowering the researchers who are not familiar with image processing and machine learning to easily perform label-free Killing/ADCC assay analysis.

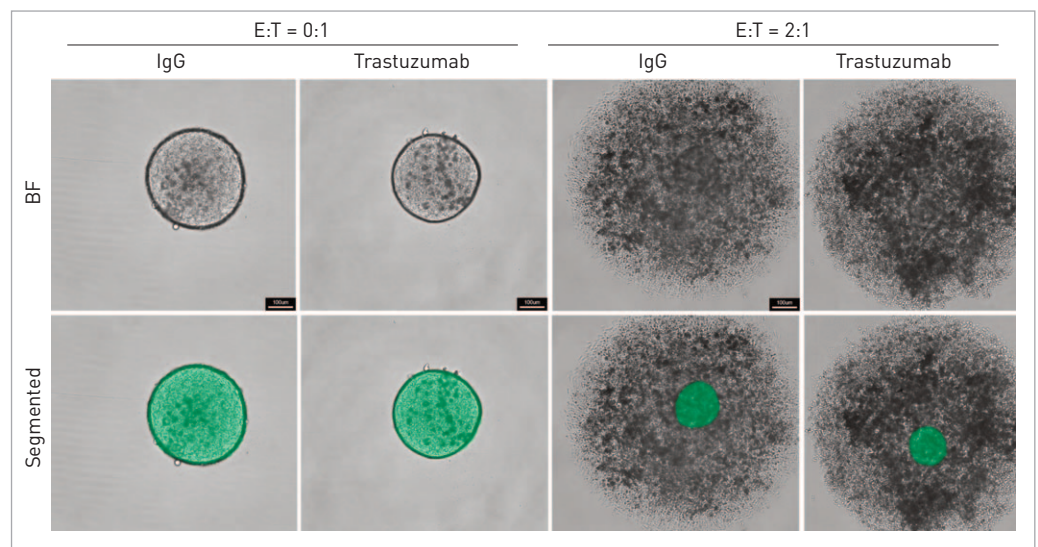


Fig. 9: Images of BT474/NK cells (3D)