YAMAHA Advanced approach for 3D-cell phenotypic assay

■Improving 3D cell fixation and optical clearing process

In conventional 3D image analysis, spheroids in plate wells are washed iteratively with PBS by manual operation followed by fixation and an optical clearing method (OCM). Sample loss and collapse of spheroids are frequently observed during this process (Fig. 1). In contrast, the CELL HANDLER™ transfers spheroids themselves in an automated manner using an ultra-small volume of 0.13 µl. Since the iterative washing steps are eliminated, the risk of cell damage, sample loss or contamination is greatly reduced. Furthermore, full traceability is ensured because images of the spheroids are taken before and after the cell transfer for analysis of various parameters. In the experiment described here, effects of a drug were evaluated on the micro-environment of tumor spheroids using the CELL HANDLER and FLUOVIEW FV3000 Confocal Laser Scanning Microscope (The FV3000 microscope) and Novisight™ 3D cell analysis software from Olympus. First, spheroids with a diameter of 100 µm were created in an ultra-low attachment U-bottom plate and treated with 2 nM of paclitaxel (PTX), the maximum concentration maintaining spheroid formation, or with control vehicle DMSO. After a 3-day culture, spheroids were stained with ReadyProbes™ Cell Viability Imaging Kit, transferred to a plate with fixative and then treated with OCM. The size and shape of spheroids were maintained during these steps without any sample loss as shown in CELL HANDLER images (Fig. 2). No differences were observed in area value between the two cell lines treated with and without PTX. However, the fluorescent signal showed that PTX greatly reduced the number of living cells in HT-29, but not HCT15 spheroids (Fig. 3). To analyze the effects of PTX on the tumor spheroid micro-environment, the spheroids were observed by the FV3000 microscope and image analysis was conducted using NoviSight software. PTX greatly increased the presence of dead cells (Fig. 4) and drastically reduced viability of HT29 cells throughout the spheroids, from the outside to the core, whereas no effects were observed in HCT15 spheroids (Fig. 5).

In summary, the use of CELL HANDLER in imaging analysis improves the accuracy of 3D cell experiments by reducing the risk of cell damage, sample loss, and contamination with reliable traceability.

Conventional method

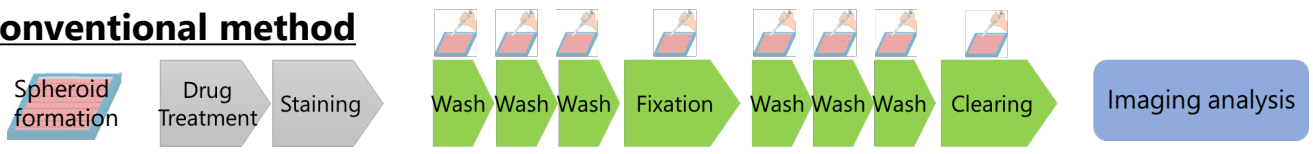
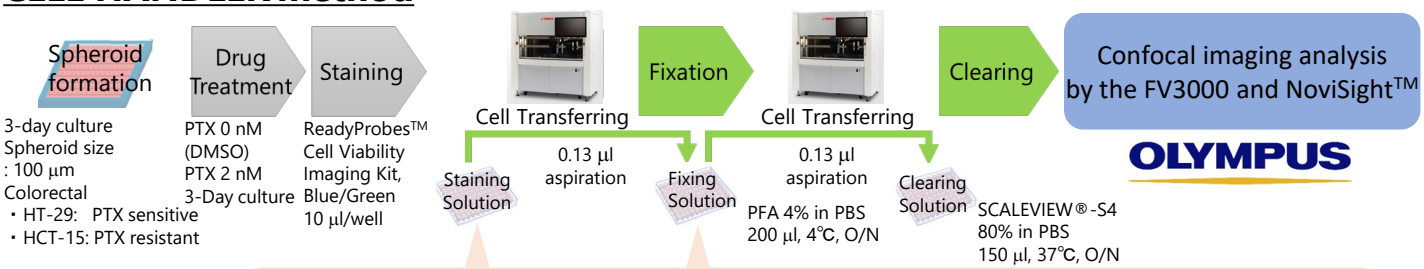


Figure 1

CELL HANDLER method



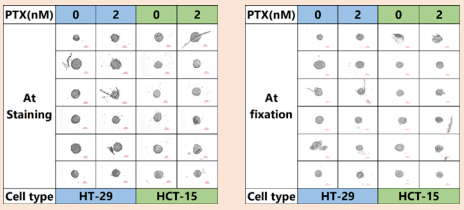
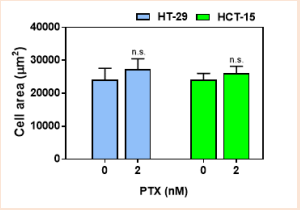


Figure 2



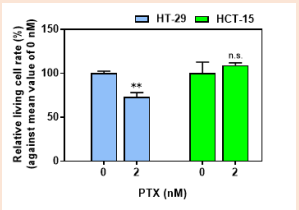


Figure 3

N=6, ** : p < 0.01, Error bars: SE

Confocal imaging analysis

Blue: All cells, Red: Dead cells

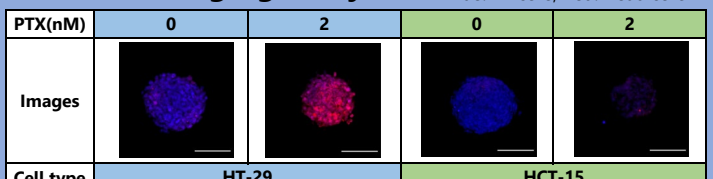
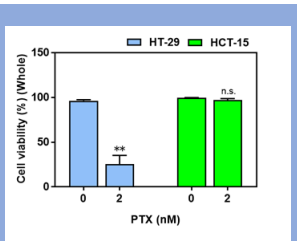


Figure 4

Imaging by the FV3000 microscope



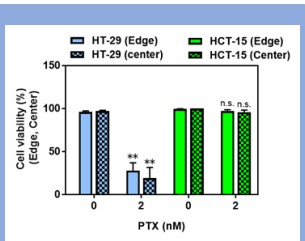


Figure 5

N=3~6, ** : p < 0.01, Error bars: SE

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