New Light-sheet Microplate Cytometer for 2D and 3D Cell Cultures

HAMAMATSU
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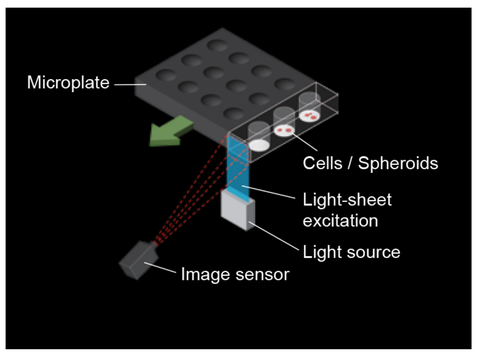
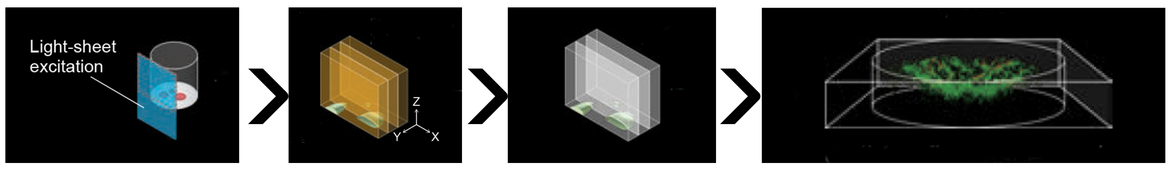
PRINCIPLE

A series of xz-plane tomographic fluorescence images are obtained by moving a plate along the y-axis while light-sheet in the xz-plane is irradiated, and a 3D fluorescent image (~400 mm thickness) of a whole well is constructed from these xz-plane images.

In the process of image construction, background fluorescence is completely separated from cell fluorescence. The scanning time for a whole 96/384/1536-well plate is several to ten minutes for one wavelength measurement, depending on fluorescent intensities of cells.





Fluorescence raw image (single xz-plane)

3D fluorescence image of cells including background

Fluorescence image of cells after background separation

3D fluorescenceimage of cells

FEATURES

1 High-speed fluorescence cytometry for 2D monolayer heterogeneous cell cultures

- 4 min/color whole well scan for any all 96/384/1536 plate format
- Focus-free (fixed focus) scan with Hamaamatsu Light Sheet Optics
- Phenotypic Screening using per cell fluorescence intensity and morphology information

2 Multiple spheroids analyses using depth information

- Whole well fluorescence scan with multiple spheroids inside in all plate format 96/384/1536
- Focus-free (fixed focus) scan with Hamamatsu Light Sheet Optics
- Phenotypic Analysis using per spheroid fluorescence intensity and morphology information

3 Fluorescence images in 3D view

4 In medium/No dye washout measurement

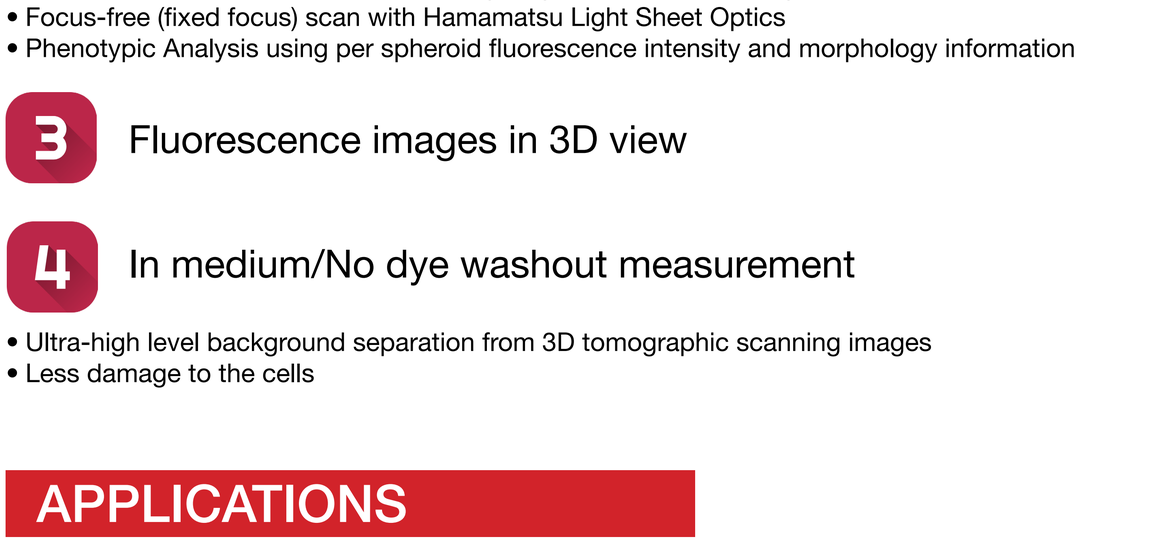
- Ultra-high level background separation from 3D tomographic scanning images
- Less damage to the cells

APPLICATIONS

Oxidative stress and Apoptosis assay in cancer cells in 2D monolayer

A549 cells were cultured in DMEM/10 % FBS in a 384-well plate. After incubation with menadione, the cells were stained with three fluorescent dyes: Hoechst 33342 for nucleus, Annexin V-Alexa Fluor 488 for apoptosis, and MitoSox Red for oxidative stress. The wells in the right half of the plate were washed with HBSS and those in the left half were kept in the medium (non-wash). The plate was then scanned three times sequentially for each fluorescent dye for a whole plate. In the fluorescent images after a background subtraction, the ratio of cells stained with Annexin V or MitoSox Red was estimated in each well, and the dose dependence curves for menadione were obtained.

Fluorescence image of a 384-well plate



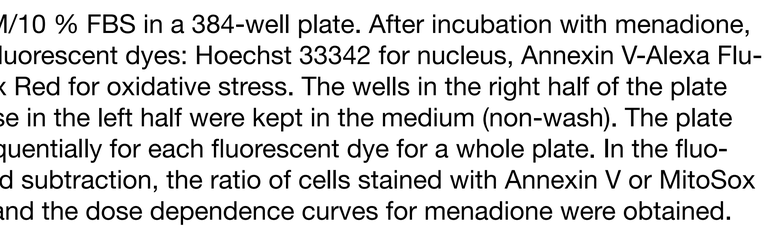
Heat map showing the ratio of cells stained with Annexin V in each well

Maximum projection image for z-axis, merging of three fluorescent dyes

Data Analysis

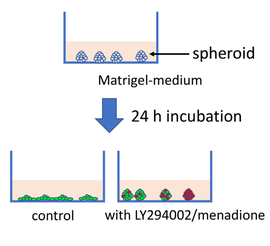
Dose dependence curves of menadione

There was no significant difference in the dose-dependence curves for oxidative stress (MitoSox Red) and apoptosis (Annexin V) between measurements in the medium (non-wash) and those in HBSS after washing out fluorescent dyes.



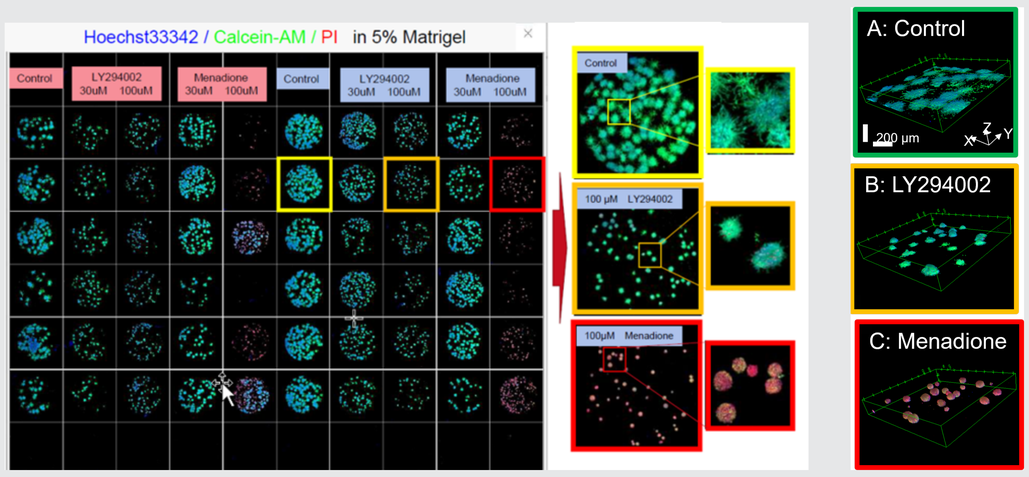
Multiple spheroids analysis using depth information

U87 Glioblastoma cells spheroid morphology and cell viability ratio

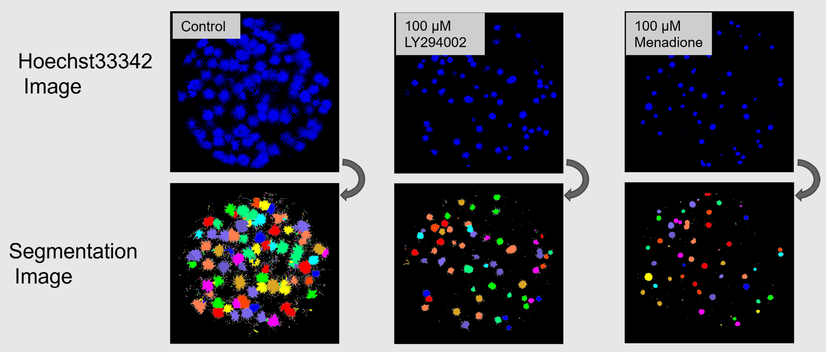


PROTOCOL
There were 40-70 spheroids with around 200µm of diameter of cancer cells (U87 MG) in each well of a 96-well plate. After incubation for 24h with or without LY294002 or menadione, the cells were stained with Hoechst 33342 for nucleus, Cacein AM for live cells, and Propidium Iodide for dead cells. The plate was scanned for each fluorescent dye sequentially in non-wash condition (800 seconds for three wavelengths for a whole plate).

3D Fluorescence imaging & analysis of cancer cell spheroids

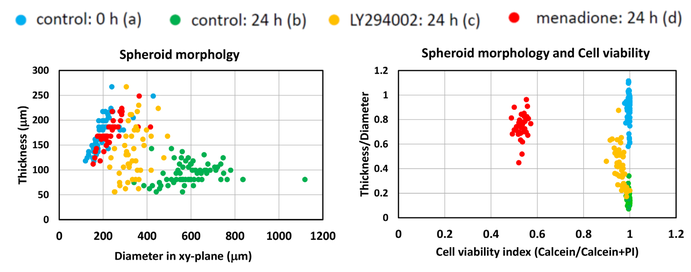


Segmentation



Recognition and identification of individual spheroids by Hoechst33342

Analysis of morphology and cell viability of individual spheroids



The diameter and thickness of individual spheroids and the cell viability in a spheroid were estimated in the Hoechst image and Calcein-and PI-images, respectively.
Each dot represents a spheroid.
The results of one well for each sample are shown.

Spheroids flattened after 24h of incubation, due to the proliferation and locomotion of cancer cells in the Matrigel-medium (b), which was inhibited partly by LY294002 (c) and completely by menadione (d). Menadione had cytotoxicity, resulting in death of the half of cells in a spheroid.

SUMMARY

We have developed a novel scanning and image processing technology in which 3D fluorescence images of multiple 3D cellular objects in a well are obtained by a single focus-free scan.

This technology provides a separation of cellular fluorescence from background fluorescence at an ultra high-level in fluorescence images constructed. We showed that the non-wash measurement can be performed in an oxidative stress and apoptosis assay in cancer cells in 2D cytometry.

We demonstrated that 3D fluorescence images of multiple spheroids of cancer cells in a well were obtained by a single scan. Morphology and cell viability of individual spheroids were analyzed.