YOKOGAWA ◆

Application Note

Spheroid Analysis

Introduction

The structures and functions of cells produced through spheroid formation are known to be more similar to live bodies than monolayer 2D organisms. Therefore, various formation methods and data accumulation for spheroids is progressing. We imaged spheroids using CellVoyager CV7000, and analyzed the images. Specifically, multiple images were captured along the Z-axis, reorganized into 2D image data*, and evaluated with regard to the maximum spheroid diameter and number of contained cells. In this way, combination of the CV7000 high-speed 3D imaging and projection functions facilitates the efficient analysis of 3D samples. Furthermore, the method can be applied to live cells to establish new experimental methods such as the time series evaluation of cells.

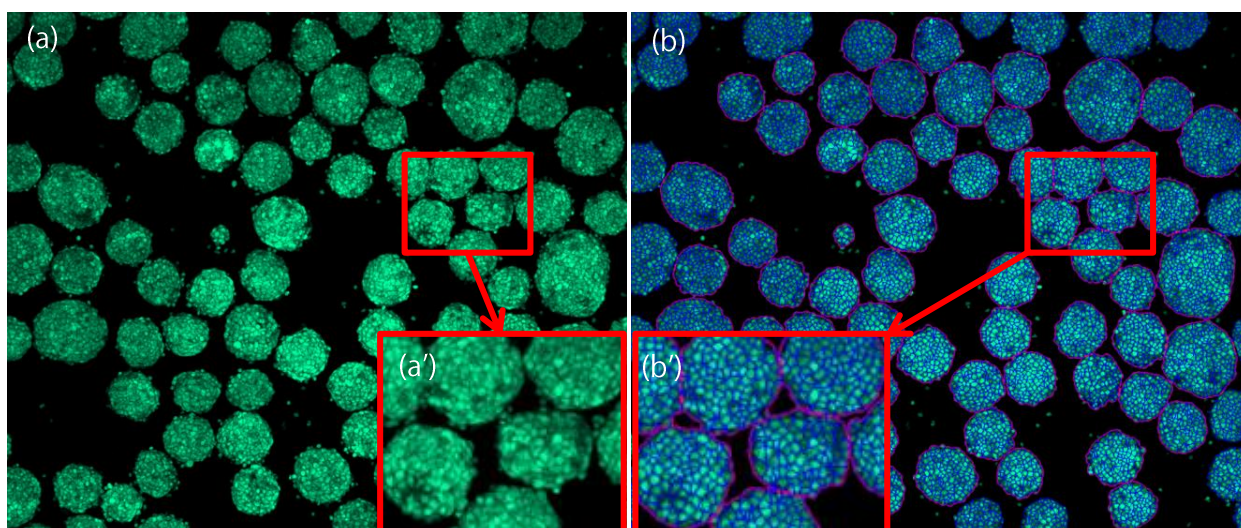※Reorganized using the maximum intensity projection (MIP) function.

Fig. 1 (a): Original image
12 images were taken at 10 μm intervals in the Z-axis direction and displayed using MIP
(a') is the magnified section.

Fig. 1 (b): Recognition image
Spheroid contour (purple) and contained cells (blue) in image (a) recognized using analysis software
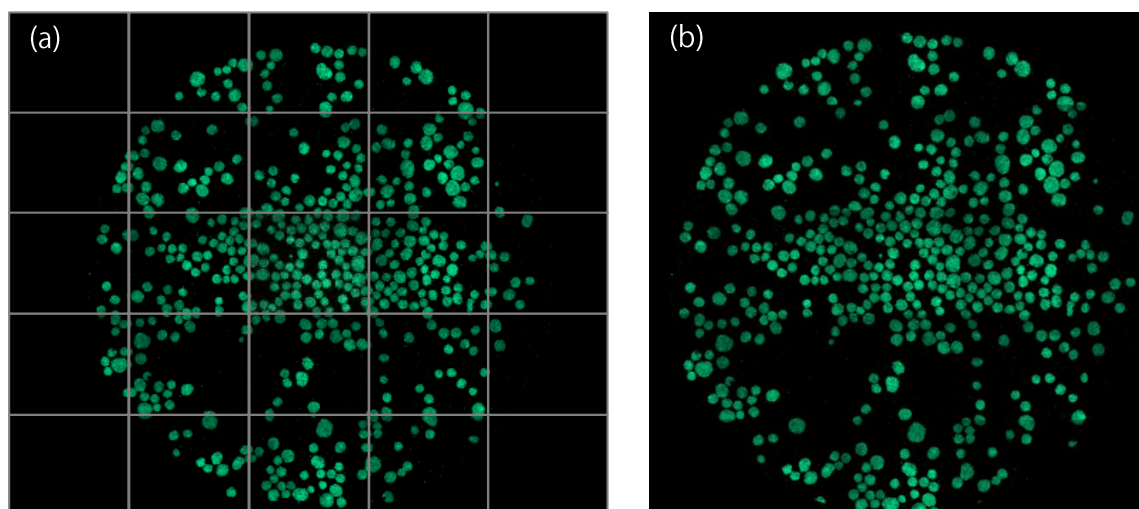(b') is the magnified section.

Fig. 2: Tile image
(a) Tile image (25 tiles) of the entire well including 50-pixel overlapping regions between adjacent tiles in the XY direction
(b) Image created by combining the tile images in (a)

Experiments

1. HeLa cells, in which Azami-Green was expressed, were inoculated in spheroid formation dishes (EZSPHERE®, AGC Techno Glass Co., Ltd.) (5 × 105 cells/dish) and cultured for seven days.
2. Spheroids were collected, fixed, and inoculated again on 96-well plates. Images were captured using the CV7000 under the following conditions:
   - Magnification: 10x (dry)
   - Wavelength: 488nm
   - Exposure time: 250msec
   - 12 images taken at 10µm intervals in the Z-axis direction over a distance of 110µm
   - Images captured per plate: 25
     (The entire well was captured using the tile function; time required per well was approx. 1.5 minutes.)
3. The maximum spheroid diameters and the number of contained cells in the MIP image were measured using the analysis software, and graphs were created using Spotfire®.
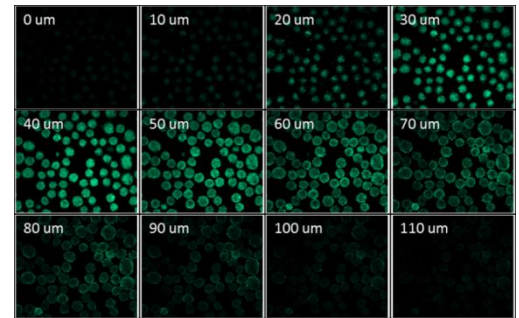

Fig. 3: Images sliced per 10µm in the Z-axis direction

Results and Conclusion

EZSPHERE® has fine pores on the cultivation surface, which is coated with a special polymer that is non-adhesive for cells. Inoculated cells, therefore, form uniform spheroids in the wells. Six types of dishes with wells of different diameters and depths (4,000-900, -901, -902, -903, -904, -905) were used to form spheroids, and images were captured and analyzed using the CV7000. As a result, the data obtained for the maximum spheroid diameters and the number of contained cells correlated with the sizes of spheroids predicted from the diameters and depths of the wells. In this way, the CV7000 allows for efficient evaluation even for 3D samples such as spheroids.
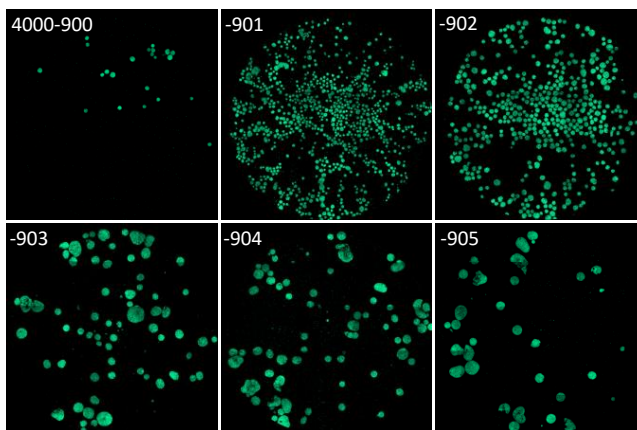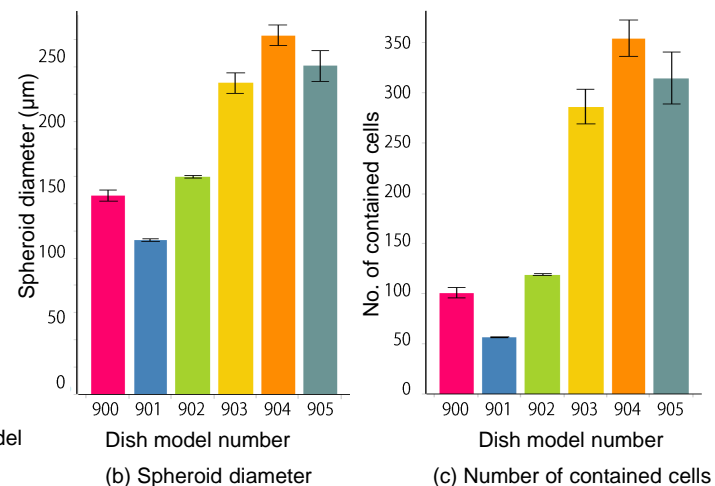

Fig. 4 (a): Tile image of each well (numbers in the images are the model numbers of used dishes.)
900 (diameter: 500µm/ depth: 100µm), 901 (diameter: 200µm/ depth: 100µm)
902 (diameter: 500µm/ depth: 200µm), 903 (diameter: 800µm/ depth: 300µm)
904 (diameter: 800µm/ depth: 400µm), 905 (diameter: 1,400µm/ depth: 600µm)

(b) Spheroid diameter

(c) Number of contained cells

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CellVoyager is a registered trademark of Yokogawa Electric Corporation.

Inquiries