1536 well microplates live cell imaging and analysis via high throughput 3D screening

Light-Sheet Microplate Cytometer

CYTOQUBE™

Assays using 1536 well microplates offer significant benefits in terms of throughput and cost reduction as they allow the evaluation of many samples simultaneously. However, it requires a lot of time and effort, especially in assays using live cells, the time taken to acquire images of a whole plate can lead to measurement artifacts due to temporal differences between wells.

With CYTOQUBE®, it is possible to capture 3D fluorescence images of four columns of wells simultaneously with a single-axis scan for 1536 well microplates, enabling high speed measurement and optical resolution equivalent to 10× confocal microscope in Z-axis direction. Here, we introduce an example of fluorescence imaging and analysis of live cells seeded in a 1536 well microplate using CYTOQUBE in just 16 minutes.

Key benefits

1536 well microplates high speed scan (4 columns simultaneous scan)

Same scanning time as 384 and 96 well microplate formats.

Analyze in parallel during the scan

Voxel resolution: 2.75 µm (X) × 2.75 µm (Y) × 6.215 µm (Z)

Optical resolution equivalent to 10× confocal microscope in the depth (Z-axis) direction.

Introduction

Merit of using 1536 well microplate is that being able to test many samples in one microplate and greatly contributes to the throughput and reducing the cost per well saving the reagent and cell culture medium use.

However, imaging and analyzing all 1536 well requires huge amount of time and effort for optimization, especially in the assay using live-cell may have artifact due to the timing difference between the 1st No1 well scan and the last No 1536 well scan, the time difference is so big that the cell state may have been changed. This is one of the hurdle for using 1536 well microplate.

CYTOQUBE is scanning 4 columns at a time for its 3D scanning, so it is possible to scan the whole plate in a few minutes per 1 color with the optical resolution equivalent to 10× confocal microscope for the depth Z-axis direction in high speed so that reduces the artifacts of time differences between first well and the last well.

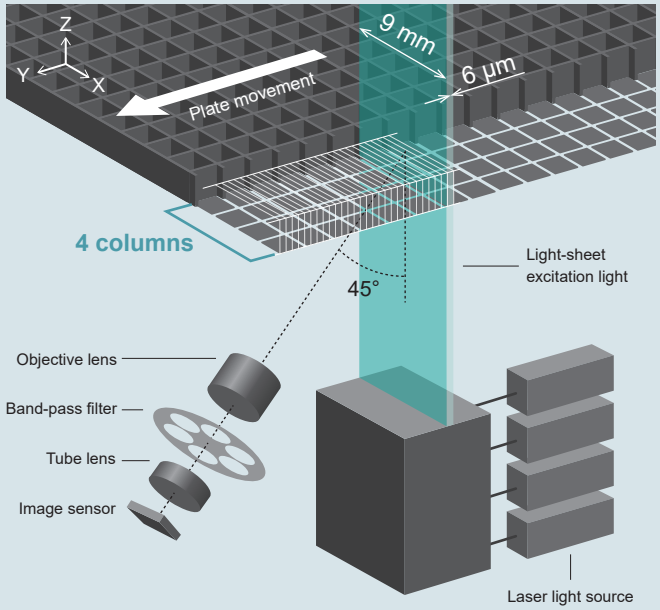
Here in this document, we would like to introduce one example of using 1536 well microplate having live cell cultured, and 3D scanned in CYTOQUBE and having them analyzed in just 16 minutes for 3 color assay.

Light-sheet enables high throughput

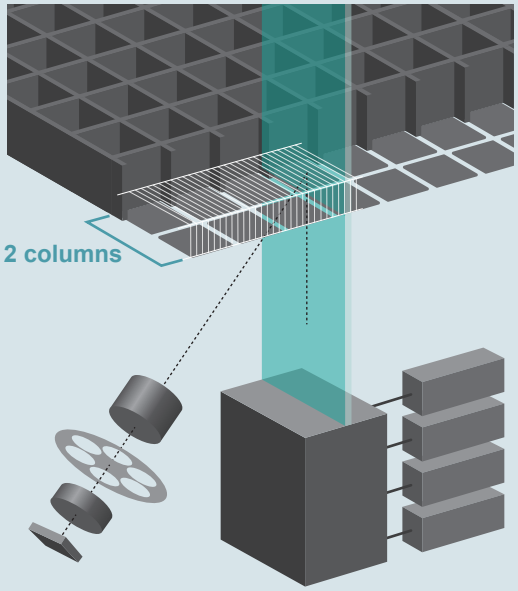
CYTOQUBE observation range

The CYTOQUBE is designed to scan SBS standard high content imaging plates, and it has a 9 mm width observation range along the X-axis that covers 4 columns in a 1536 well microplate, 2 columns in a 384 well microplate, and 1 column in a 96 well microplate. The scanning operation is the same for the 1536, 384, and 96 well microplates, which results in the same scan time for all well microplate formats.

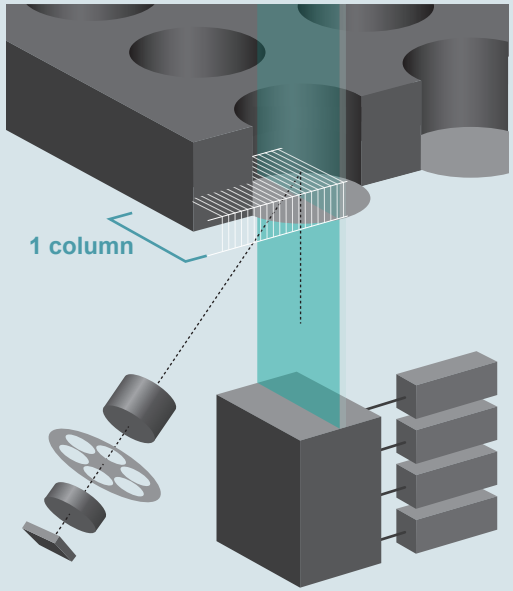
1536 well microplate



384 well microplate



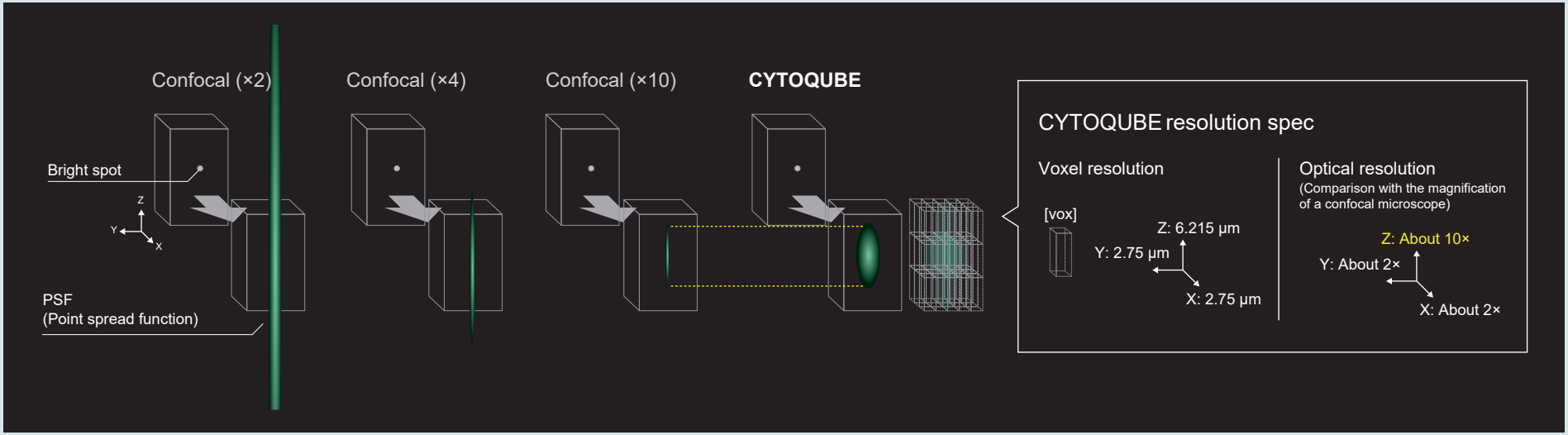
96 well microplate



CYTOQUBE optical and voxel resolution

Observing a sample using optics such as a lens generally results in an image with a blurred spread, which is expressed as the term PSF (point spread function). In a confocal microscope, the PSF is generally detailed in the XY-axis and extended in the Z-axis. The CYTOQUBE's PSF has a more spherical shape, which is different from that of a typical confocal microscope.

The CYTOQUBE's optical resolution is equivalent to a 2× magnification confocal microscope in the XY-axis and 10× magnification in the Z-axis from its PSF shape. The CYTOQUBE's voxel resolution is 2.75 µm (X) × 2.75 µm (Y) × 6.215 µm (Z). A voxel is the minimum unit that composes a 3D image, whereas a pixel is the minimum unit that composes a 2D image.



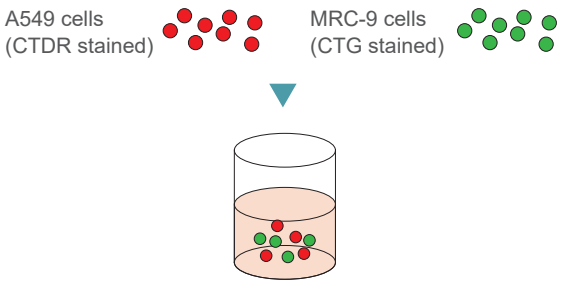
Method

Human lung cancer cell line A549 were stained with 1 µmol/L CellTracker Deep Red (CTDR), human lung fibroblast cell line MRC-9 were stained with 7 µmol/L CellTracker Green CMFDA (CTG), and both were incubated for 60 minutes at 37 °C. The cell lines were washed 3 times with PBS buffer, and then seeded in a 1536 well microplate in various mix ratios of the two cell lines following the pattern shown in the image on the right. After 48 hours, 5 µg/mL of Hoechst 33342 was added and incubated for 30 minutes at 37 °C, and then the microplate was scanned in the CYTOQUBE with no-wash (without removing the dye).

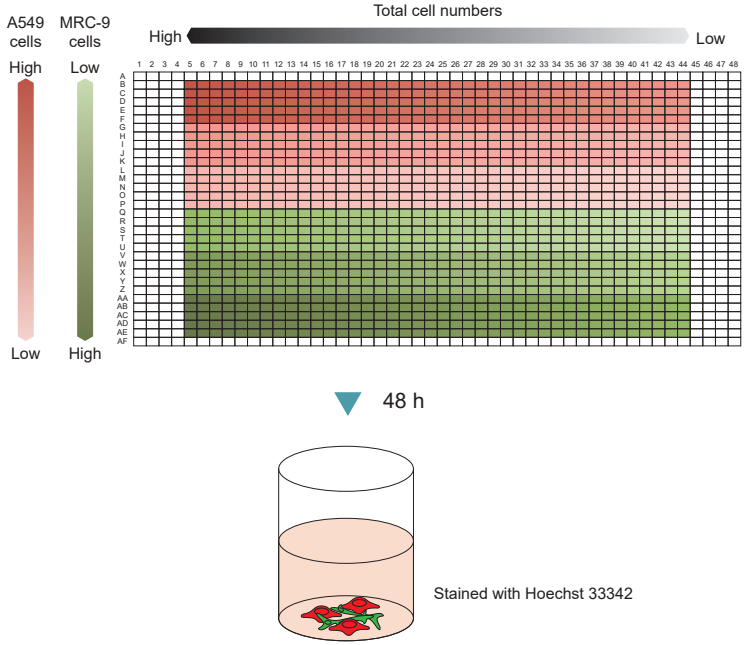
CYTOQUBE scan settings

Sample depth		100 µm
Background subtraction		ON
Sensitivity	Hoechst 33342	17
	CellTracker Deep Red	15
	CellTracker Green CMFDA	11

Approximately 16 minutes are required to scan (3 colors) and analyze 3D fluorescence images of 40 columns of a 1536 well microplate.



Seeding pattern of 2 types of cells in 1536 well microplate



Results

Acquired images

Fig. 1: 1536 well microplate fluorescence image
Maximum projection of 3D images, Blue: Hoechst 33342 (nuclei), Magenta: CTDR (A549), Green: CTG (MRC-9)

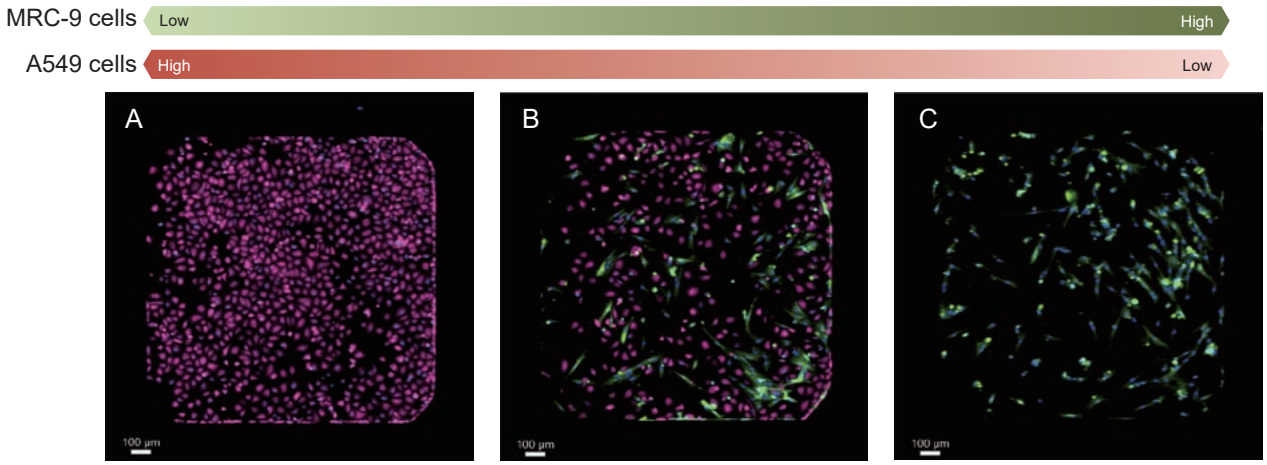


Fig. 2: Maximum projection of 3D fluorescence images for A, B, and C wells marked in Fig. 1
Blue: Hoechst 33342 (nuclei), Magenta: CTDR (A549), Green: CTG (MRC-9)

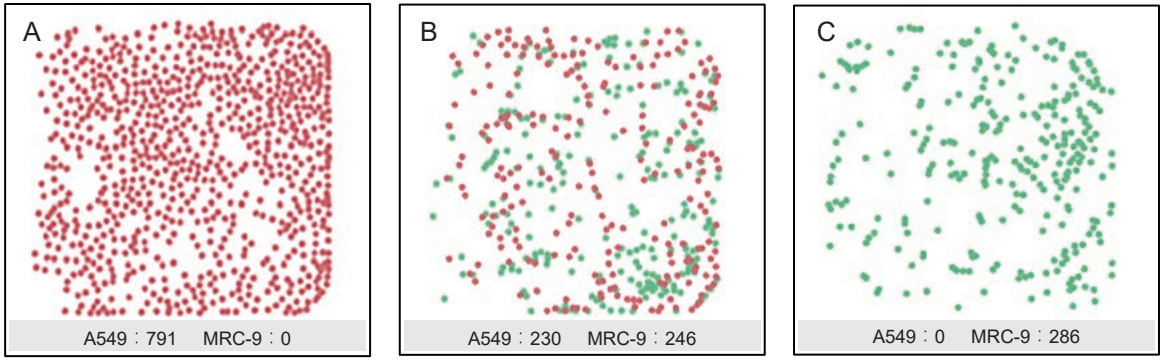


Fig. 3: Plotting XY coordinates of cells in each well and cell numbers from A, B, and C wells marked in Fig. 1

Fig. 3 shows the XY coordinates where the maximum fluorescence intensity was found for both A549 (red dot) and MRC-9 (green dot) cells, as well as the cell numbers obtained from analyzing the well images.
CTDR positive cells and CTG positive cells were counted according to the ratio in each well.

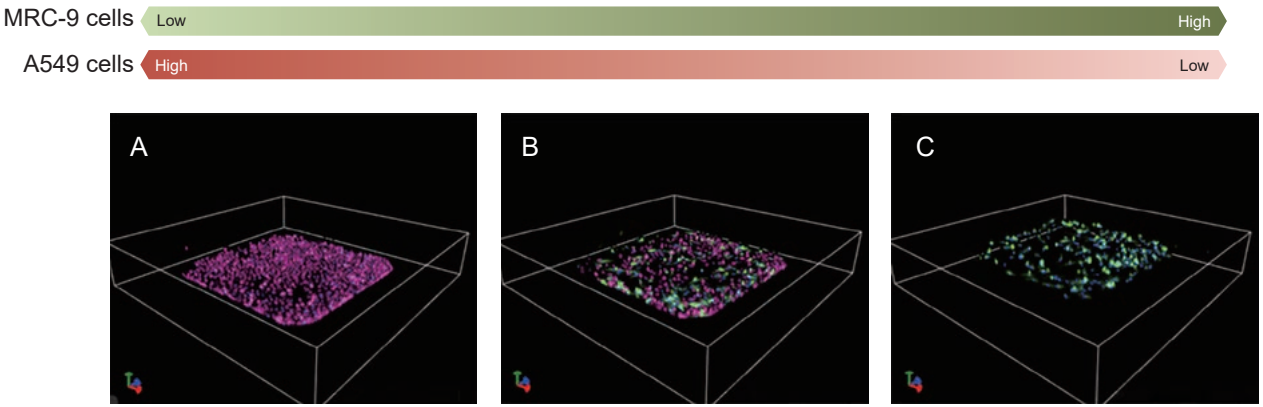


Fig. 4: 3D fluorescence images of A, B, and C wells marked in Fig. 1
Blue: Hoechst 33342 (nuclei), Magenta: CTDR (A549), Green: CTG (MRC-9)

Three-dimensional differences between A549 cells and MRC-9 cells were observed.

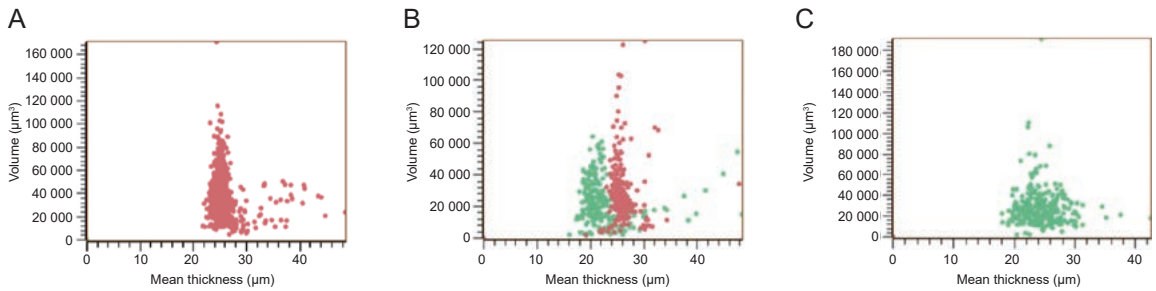


Fig. 5: Analysis examples of A, B, and C wells marked in Fig. 1

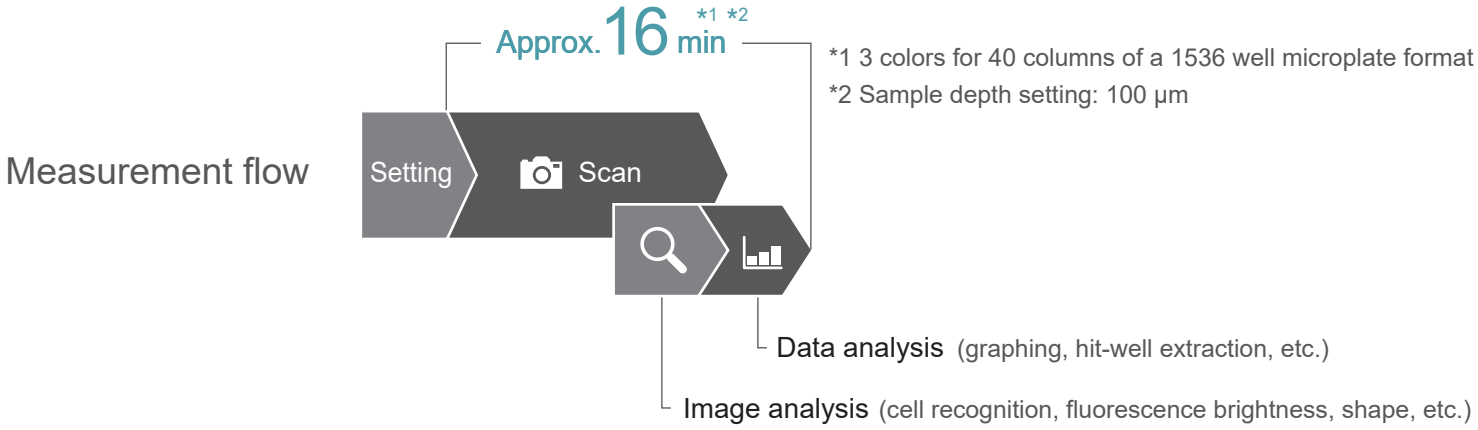
The 3D fluorescence images were analyzed, and the cell thickness and volume were plotted in the X-axis and Y-axis, respectively (Fig. 5). The height information obtained through 3D fluorescence imaging were used for analysis.

3D fluorescence images of a 1536 well microplate co-cultured with two cell types stained in different colors were acquired and analyzed using the CYTOQUBE. From the fluorescence images in the wells, both types of cells were counted, and the fluorescence intensity, volume, and thickness were analyzed. In addition, the spatial distribution of cells in the wells were observed from the 3D fluorescence images. The scan and analysis time to obtain these results was 16 minutes in total.

Conclusion

The CYTOQUBE captures 3D fluorescence images of 4 columns of wells simultaneously with a single-axis scan for 1536 well microplates. It can rapidly scan the whole plate in a few minutes per color, with an optical resolution equivalent to a 10× confocal microscope in the Z-axis (depth) direction.

In this experiment, the time to scan fluorescence images of cells seeded in 40 columns of a 1536 well microplate and analyze the cell numbers, fluorescence brightness, thickness, and volumes were 16 minutes for 3 colors. The CYTOQUBE can help accelerate cell-based assay workflows in a higher throughput using 1536 well microplates.



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