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A new approach to analyze cell-based fluorescence assay data with imaging-based microplate reader
- A proposal to analyze the data in a well of camera-based microplate readers in more detail -

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Introduction

In cell-based assays in drug discovery and screening, use of primary cultured cells/iPSC-derived disease-phenotypic cells and/or co-culture of different types of cells are rapidly expanding. In many cases of these assays, the cell samples have heterogeneous states in a well and/or well by well.

In general, microplate reader provides only an average value of fluorescence/luminescence intensity of a small or a whole region of each well. These values are not always “correct answers” to compare the results of a lot of number of wells due to the non-homogeneity of cell samples well by well. Microscope-based High-Content Analysis/Screening (HCA/HCS) instruments would provide accurate information. However, the throughput of the measurements is low.

We have been developing another approach with a camera-based microplate reader, which has been widely used for cell-based kinetics assays such as Ca^{2+} and membrane potential measurements. It acquires a fluorescence/luminescence image of entire microplate. To analyze inside of each well, we developed a novel algorism, “pixel analysis” (patent pending). To examine potential use of this method, we prepared “heterogeneous cell samples”, mixture oft wo kinds of cells, one of which is only responsive for a stimulus (chemical compound). We measured intracellular Ca^{2+} concentration changes with calcium-sensitive fluorescent dyes and performed some analysis of the data using the novel algorism.

Materials & Methods

Hamamatsu camera-based microplate reader: FDSS/µCELL

FDSS/µCELL

The FDSS/µCELL is an imaging-based plate reader equipping an integrated dispending head and a camera as an imaging detector, which has been widely used for cell-based kinetics assays such as Ca^{2+} and membrane potential measurements.

In this experiment, we attached Hamamatsu scientific CMOS camera, ORCA-Flash 4.0, to the FDSS/µCELL as an imaging detector. It provides a 2.5 times finer resolution with 6.5 x 6.5 µm pixel size and a 2.6 x larger field of view than that of a standard EM-CCD camera, with high sensitivity (over 70 % of quantum yield at 600 nm) and low noise (1.3 electrons at 100 frames/s). The higher resolution provides analysis of inside of each well.

Cell samples

Mixtures of wild-type CHO-K1 cells and CHO-K1 cells expressing VR-1 were cultures in 96-well microplates (Coster). The VR-1 expressing CHO-K1 cells are stimulated to transient increase of intracellular Ca^{2+} concentration by adding capsaicin (Sigma-Aldrich).

In various mixed ratios of wild-type CHO-K1 cells and VR-1 expressing CHO cells, we measured intracellular Ca^{2+} concentration changes with calcium-sensitive fluorescent dye, Fluo-8 (AAT Bioquest), by adding various concentrations of ATP or capsaicin. To load Fluo-8 into cells, the cells were incubated with 5 mM Fluo-8/AM, 1.25 mM probenecid (Sigma-Aldrich), and 0.05 % pluronic F-127 (Dojindo laboratories) for 1 h at 37˚C in 5% CO_2. After the culture medium containing dyes was replaced with Hanks’ HEPES Buffer (Dojindo laboratories), the fluorescence images of entire microplate were taken every 3.0 s (exposure time of one image is 3.0 s).

Analysis

In “pixel analysis” (patent pending), 400 pixels per one well were randomly picked up and fluorescence intensity of each pixel was estimated. Data were analyzed on AQUACOSMOS Version 2.6.4.3 software (Hamamatsu Photonics K.K.).

Results: Analysis of each pixel in a well in intracellular Ca^{2+} measurements in CHO cells

Figure 1

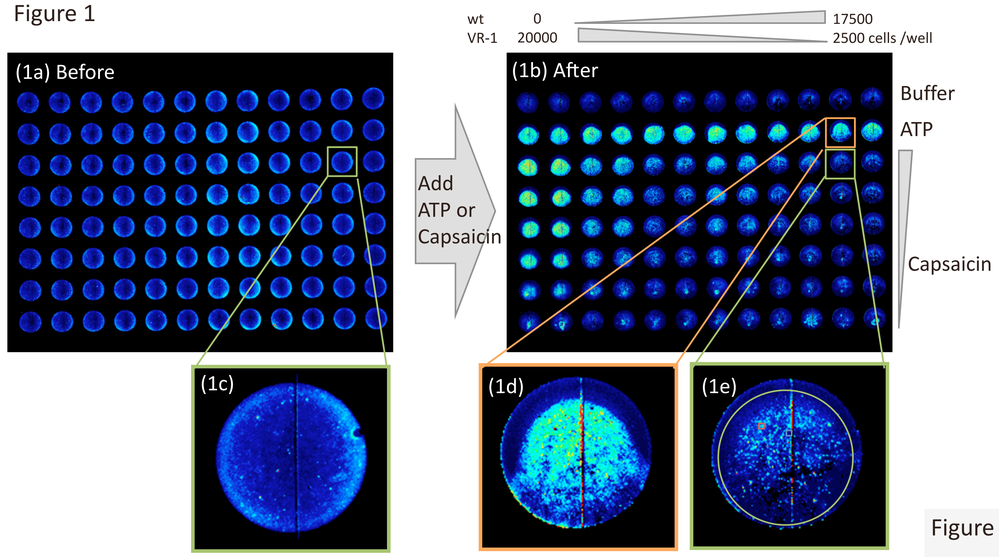
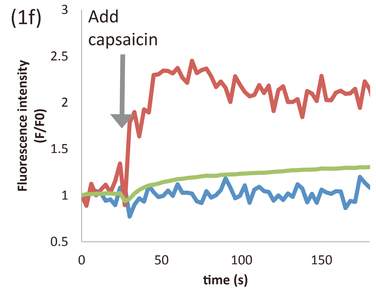


Figure 1f: pixel analysis
In this experiment, each well consists of 10563 pixels.

Figure 1f shows the time courses of fluorescence intensity changes in the well added capsaicin (Figure 1e). The green line shows the average of whole well (all pixels of the well). The red and blue lines show fluorescence intensity changes of one pixel. The red line pixel is thought to be a pixel that contains VR-1 CHO cells, and the blue line pixel not.



We measured intracellular Ca^{2+} concentration changes in various mixed ratios of wild-type CHO cells and VR-1 expressing CHO cells (total cell number, 20000cells/well) after adding 1 µM ATP or various concentrations of capsaicin. Figure 1a and 1b show the fluorescence images of entire microplate before (1a) and at 18 s after adding ATP or capsaicin (1b), respectively. Figure 1c, 1d, and 1e show the magnification image of one well; (1c): before adding capsaicin, (1d): after adding 1 µM ATP, (1e) after adding 10 µM capsaicin, in the mixture of 2500 VR-1 CHO cells and 17500 CHO cells. In an ATP-added well (1d), in which both wild-type CHO cells and VR-1 expressing CHO cells are stimulated to transient increase of intracellular Ca^{2+} concentration, increase of fluorescence intensity was observed in almost all regions in the well. In contrast, in capsaicin-added well (1e), in which only VR-1 cells (12.5 % oft otal cells) are stimulated, increase of fluorescence intensity occurred only in some regions sparsely distributed through whole well. The pixels showing increase of fluorescence intensity are thoughtt o contain some VR-1 CHO cells.

Figure 2

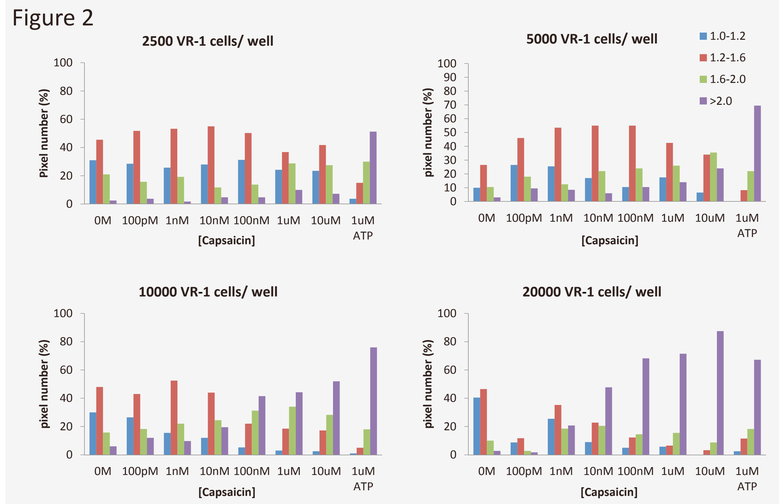


Figure 2 shows an example of analysis of acquired experimental data. In this graphs, pixels in a well were subdivided into four classes according to amount of fluorescence intensity increase. In the wells added 1 µM ATP, most of pixels showed sufficienti ncrease off luorescence intensity (> 2.0). In contrast, the wells added capsaicin, amount off luorescence intensity increase varied pixel by pixel, depending on concentration of capsaicin and ratio of capsaicin-stimulated cells (VR-1 expressing CHO cells).

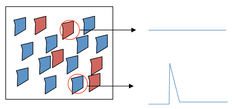
Summary

We have been developing a novel approach to analyze cell-based fluorescence/luminescence assay data of a microplate reader, which aims to provide more accurate information in the cases that the cell sample has heterogeneous states in a well and/or well by well.

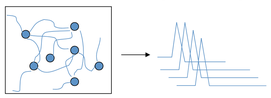
In our approach, fluorescence/luminescence images of entire microplate are acquired with a high-resolution/high sensitivity camera equipped with a microplate reader, and each pixel in a well is analyzed. This approach, if sufficient spatial resolution of inside of wells is acquired, would be able to provide accurate data to compare a lot of number of wells, even in the cases that the cell samples have non-homogeneous states in a well and/or well by well.

Also, it would be useful in using…..

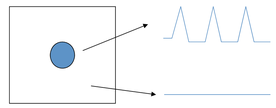
Heterogeneous cell populations



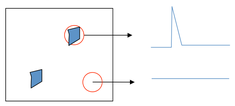
Cell populations with intercellular communication capability



Cell clumps



A small number of cell populations



We have been trying to improve spatial resolution of inside of wells.

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