YOKOGAWA

Application Note

Analysis of intracellular Calcium Response Depending on Ionomycin Concentration

Introduction

Changes in intracellular Ca^{2+} concentration are an important analysis target in the study of intracellular signal transduction related to physiological phenomenon, such as muscle cell contraction. The following are the results of imaging and analysis of the increase of intracellular Ca^{2+} concentration in A10 cells, using the CellVoyager, by treatment of ionomycin, a Ca^{2+} ionophore.

Analysis Results

Using Ca^{2+} probe, Fluo4, images were captured using the CellVoyager (Fig. 1a~1c), and temporal changes in intracellular Ca^{2+} concentration were analyzed with its analysis software. The analysis software allows for the recognition of individual cells, thus enabling data acquisition for temporal changes in individual cells (Fig. 2a). Therefore, it is possible to exclude cells with abnormal reactions and analyze cells with pathognomonic reactions only. In addition, by varying the type and concentration of reagents for each well, variation in reactions can be analyzed (Fig. 2b, 2c).

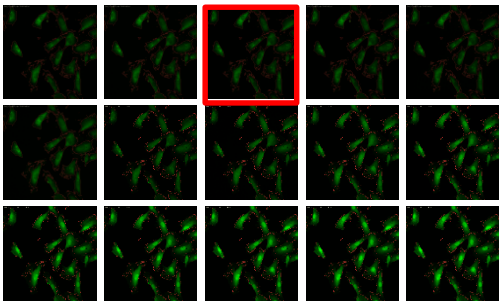


Fig. 1a:Images taken at 0.2sec intervals, from 2.6sec to 5.4sec after the start of imaging. Ionomycin stimulation was started 3.0sec after the start of imaging (the image in red frame).

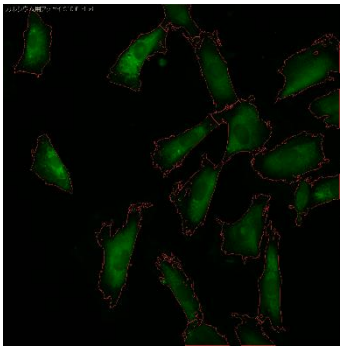


Fig. 1b: Before ionomycin treatment

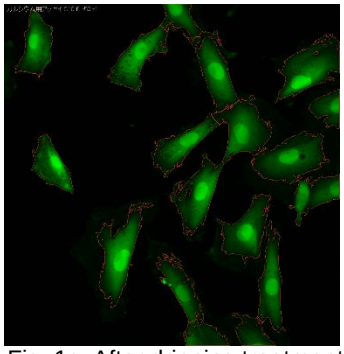


Fig. 1c: After dripping treatment

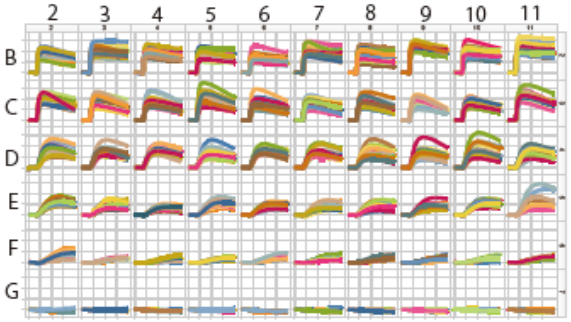


Fig. 2a: Kinetic analysis of Fluo4 bright intensities of individual cells in each well

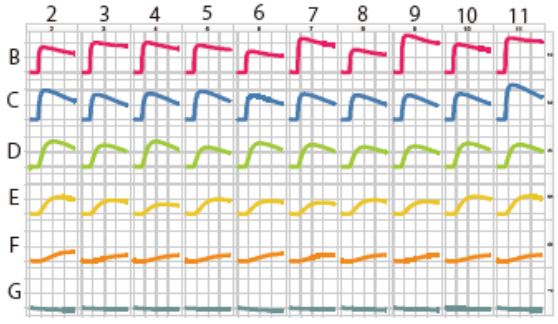


Fig. 2b: Kinetic analysis of average Fluo4 bright intensities in each well

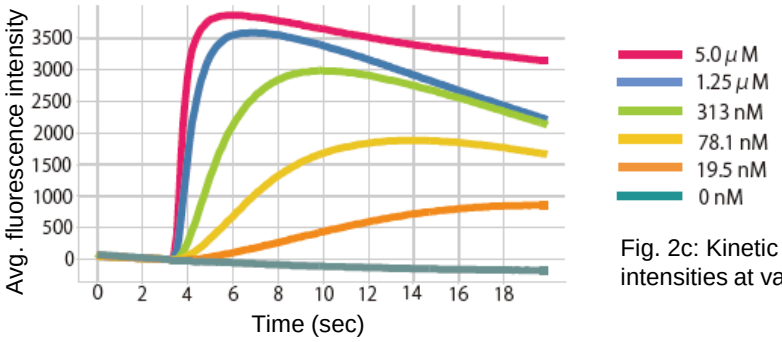


Fig. 2c: Kinetic analysis of average Fluo4 bright intensities at various ionomycin concentrations

* For all graphs above, the fluorescence intensity at 2.9sec after the start of imaging is converted to zero as a reference.

Experiment procedure / Analysis method

- Preparation of reagents

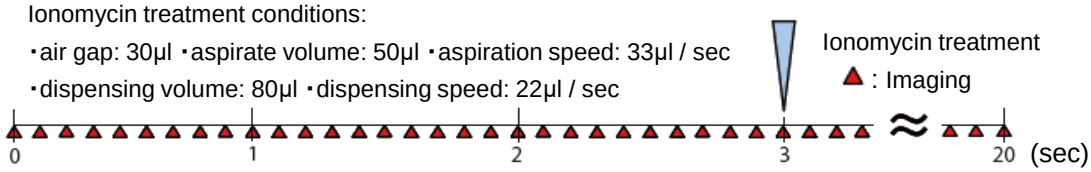
  A10 cells were seeded on 96-well plates at the ratio of 10,000 cells/well and cultured for 24 hours, then a CO_2 indicator, Fluo4, was added (Final concentration 1μM, reaction time: 20min), to monitor intercellular Ca^{2+}-influx.

- Imaging

  Time lapse images were captured using the CellVoyager CV6000 under the following conditions. Ionomycin was added to the cells using the built-in pipettor.

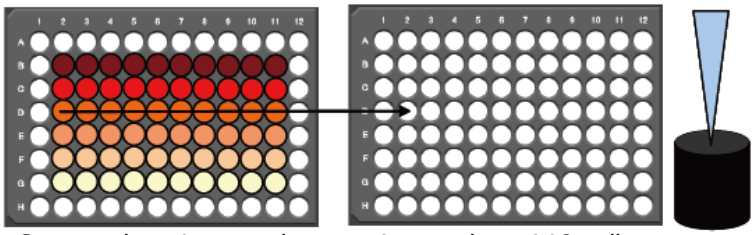
  - •Magnification: 20x objective lens
  - •Exposure time: 100msec
  - •Imaging interval: 100msec over a period of 20sec
  - •Ionomycin dripping: 50μM 3sec after the start of imaging
  - •Ionomycin final concentration: 0 - 5μM
  - •Images captured per well: 1 Field(well center)

Ionomycin treatment conditions:
・air gap: 30μl ・aspirate volume: 50μl ・aspiration speed: 33μl / sec
・dispensing volume: 80μl ・dispensing speed: 22μl / sec



- Built-in pipettor setting (Kinetics Module)

  Ionomycin was dispensed from each well of the source plate into each well of the assay plate (A10 cells) in one to one correspondence.



Ionomycin was injected from the highest position of the wells.

Source plate: Ionomycin　　Assay plate: A10 cells

- Image Analysis

  The images were analyzed using the analysis software under the following conditions.

  - •Graphs were created using Spotfire®.
  - •Binarization : Sigma Threshold Object Diameter [25] Sigma [100]
  - •Binary Transform : Opening Circle - Circle Radius [7]
  - •Labeling : Divide Each Region Watershed Threshold [50]
    Select Region For Area MinArea [640] MaxArea [999999]
  - •Logical Operating : Region Operator Region1 [After Labeling] Operator Type [EXPAND] Region2 [Cytoplasm]

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