Application Note

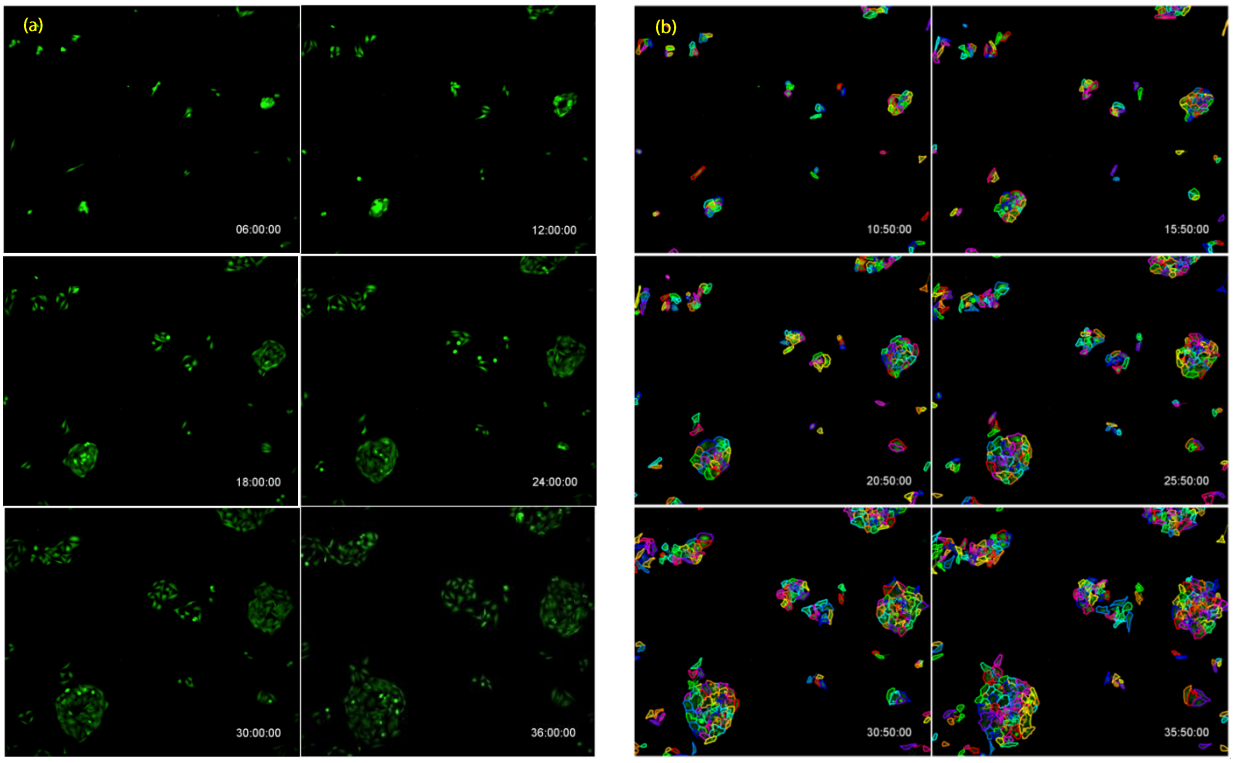
Live Cell Tracking Analysis

Introduction

Live cell observation and analysis have become increasingly important in accordance with technological progress. Since each cell's behavior is different even under the same experimental conditions, continuous tracking of a number of individual cells is required. Thus, three-day time-lapse observation was conducted while cells were being cultivated in the CV8000 stage incubator, and tracking analysis was implemented using the obtained images.

Analysis Results

The three-day time-lapse observation of the cells in the CV8000 stage incubator demonstrated steady cell division and growth (Fig. 1a). Also, recognition of individual cells using the CV8000 analysis software resulted in numerical data showing an increase in the number of cells (Fig. 1b, c). Next, tracking analysis was conducted: cell migration distance was measured, and a graph was created using Spotfire® (Fig. 2). As a result, it was possible to chronologically track and analyze the transition of cell division, indicating relationships such as in a genealogical tree. A wide variety of data are obtainable using the CV8000 analysis software, including cell area and fluorescence intensity, thus enabling multifaceted evaluation of cell behavior.



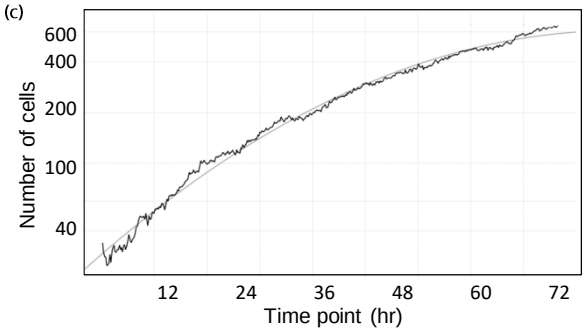
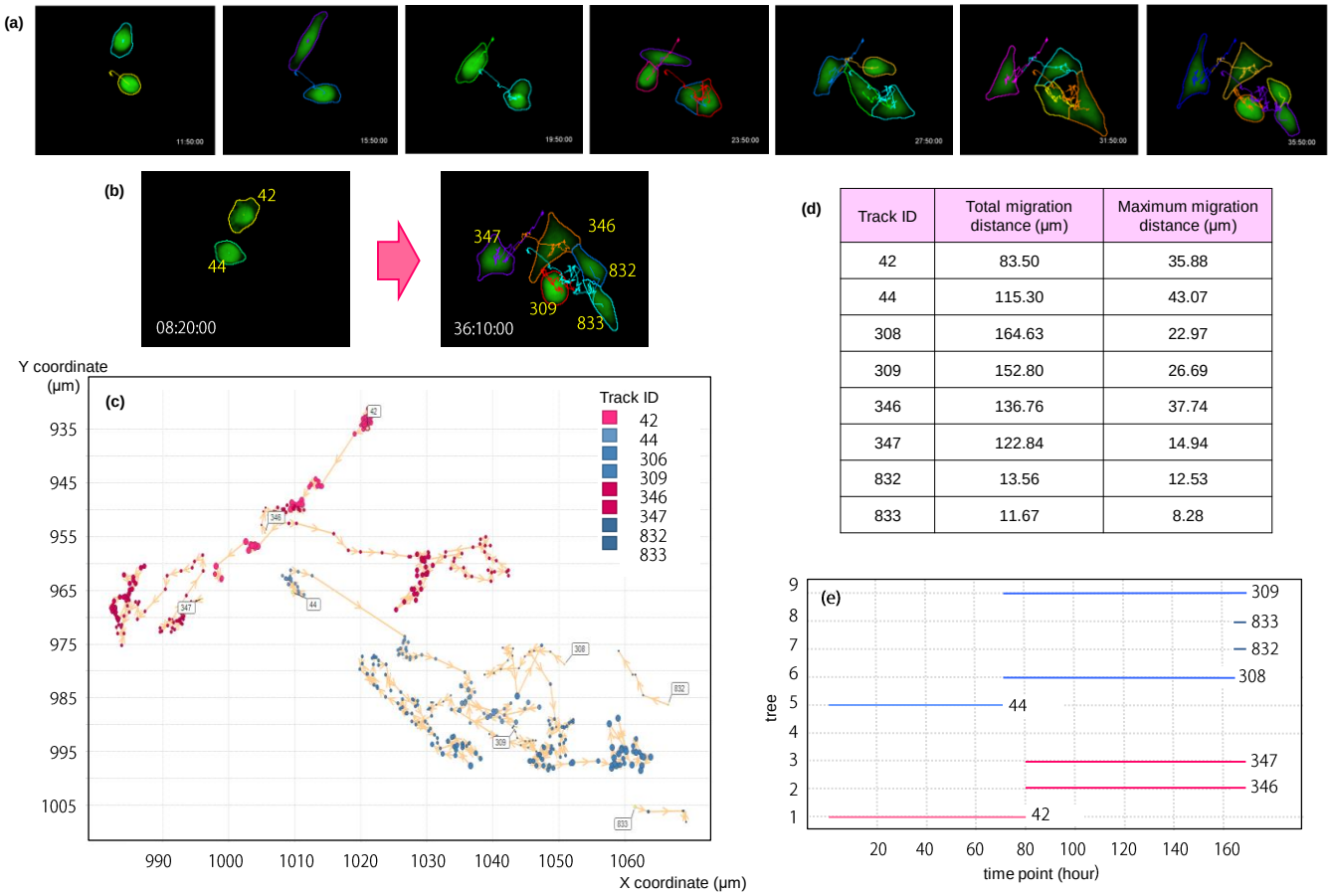


Fig. 1: Cell images and recognition results (time-lapse)
Numbers in the images indicate the time passed since the start of imaging.
Steady cell proliferation over time can be observed both from the images and numerical data.

(a) Time-lapse images (original) captured every six hours
(b) Cell recognition images captured every five hours
(c) Change in the number of cells based on the recognition result

Experiments

1. HeLa cells, in which Azami-Green was expressed, were inoculated in an uncoated half-area 96-well glass-bottom plate (Corning #4580) at the ratio of 5,000 cells/well.
2. Images were captured using the CellVoyager CV8000 under the following conditions:
   - Magnification: 10x
   - Images captured per well: 4
   - Wavelength: 488nm
   - Exposure time: 200msec
   - Imaging interval: 10 minutes (over three days)
   - Cultivation environment on the stage: 37ºC, 5%CO_2, forced humidification
3. Tracking analysis of captured images was conducted using the CV8000 analysis software; numerical data describing the number of cells, trajectory of the center of gravity of individual cells, and migration distance were obtained, and graphs were created using Spotfire®.



Track ID	Total migration distance (μm)	Maximum migration distance (μm)
42	83.50	35.88
44	115.30	43.07
308	164.63	22.97
309	152.80	26.69
346	136.76	37.74
347	122.84	14.94
832	13.56	12.53
833	11.67	8.28

Fig. 2: Calculation of cell migration distance through tracking analysis
The trajectory of the center of gravity of individual cells was identified through tracking using the obtained images (a). The thick lines indicate cell contours, and the thin lines in the center show the trajectories of the centers of gravity. A graph of the trajectories of the centers of gravity was created, and the migration distance at each time point, the total migration distance, and the maximum migration distance from the first time point were calculated (b, c, d). Each cell was given a track ID; therefore it was possible to identify cell appearance and disappearance due to cell division. Thus, it is possible to chronologically track and analyze the transition of cell division, indicating relationships such as in a genealogical tree (e).

YOKOGAWA

Yokogawa Electric Corporation Life Science Center
Kanazawa:
2-3 Hokuyodai, Kanazawa, Ishikawa 920-0177 Japan
TEL: +81-76-258-7028 FAX: +81-76-258-7029
Tokyo:
2-9-3 Nakamachi, Musashi, Tokyo 180-8750 Japan
TEL: +81-422-52-5550 FAX: +81-422-52-7300
Email: CSU@CSV.yokogawa.co.jp
Website: http://www.yokogawa.co.jp/scanner

Inquiries

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