Application Note

CL-Quant Add-on Module

Digitizing the cell confluency of HeLa cells using "Cell Confluency"

< BioStudio-mini usage example >

Cell confluency is an important criterion for confirming cell proliferation during cell culturing. It is used as an index to evaluate cell proliferation capacity and determine passage or inoculation timing in experiments. However, estimation of cell confluency is currently performed by visual management in a qualitative manner. Improper timing of cell passage affects experiment results, reproducibility, and post-passage cell quality. When cultured cells are passaged at a confluency of, for example, 70% using visual estimation, the actual cell coverage may vary depending on who is doing the estimation.

Using CL-Quant image analysis software and the Add-on Module "Cell Confluency", the coverage area of cancer cells in culture vessels can be quantitatively monitored.

Here, to evaluate proliferation of HeLa cells, the images of HeLa cells cultured in a flask were captured by BioStudio-mini in a CO_2 incubator and analyzed by the Add-on Module "Cell Confluency".

Observation device

■ BS-M04 BioStudio-mini (Nikon, MLA21000)

Image analysis software

■ CL-Quant ver. 5.02 (Nikon, MLS21000)

CL-Quant Add-on Module

■ MA-PC-UR-AR01 Cell Confluency (Nikon, MLS30201)

Cells

■ Human cervical carcinoma cell line HeLa RCB0007 (RIKEN BRC through National BioResource Project of MEXT/AMED, Japan)

Reagents and materials

■ Minimum Essential Medium Eagle With Earle's salts, L-glutamine and sodium bicarbonate, liquid, sterile-filtered, suitable for cell culture (SIGMA, M4655)

■ Fetal Bovine Serum, qualified, USDA-approved regions (Thermo Fisher Scientific, 10437028)

■ DPBS, no calcium, no magnesium (Thermo Fisher Scientific, 14190144)

■ Trypsin-EDTA (0.25%), phenol red (Thermo Fisher Scientific, 25200072)

■ Corning® 25cm^2 Rectangular Canted Neck Cell Culture Flask with Vent Cap (Corning, 430639)

Methods

HeLa cells dissociated with Trypsin/EDTA were seeded in Minimum Essential Medium Eagle supplemented with 10% Fetal Bovine Serum into a 25cm^2 flask at a cell density of 1.8 x 10^4 cells/cm^2. The cells were cultured in humidified 5% CO_2 at 37°C, and phase contrast images of the cells were captured with BS-M04 BioStudio-mini one day after seeding.

Acquisition conditions were an exposure time of 20 milliseconds and manual focus. The captured images were imported into CL-Quant and analyzed using the Add-on Module "Cell Confluency". Cell confluency was calculated as "CellRegion Area to Image Size Ratio" (ratio of the cell occupation area to the entire visual field) by automatic calculation using CL-Quant.

Results

The phase contrast images were analyzed by CL-Quant and the Add-on Module "Cell Confluency". The analyzed masked image is shown in Fig.1. The cell area was correctly confirmed by referring to the mask image. Cell confluency was automatically quantified as 70%.

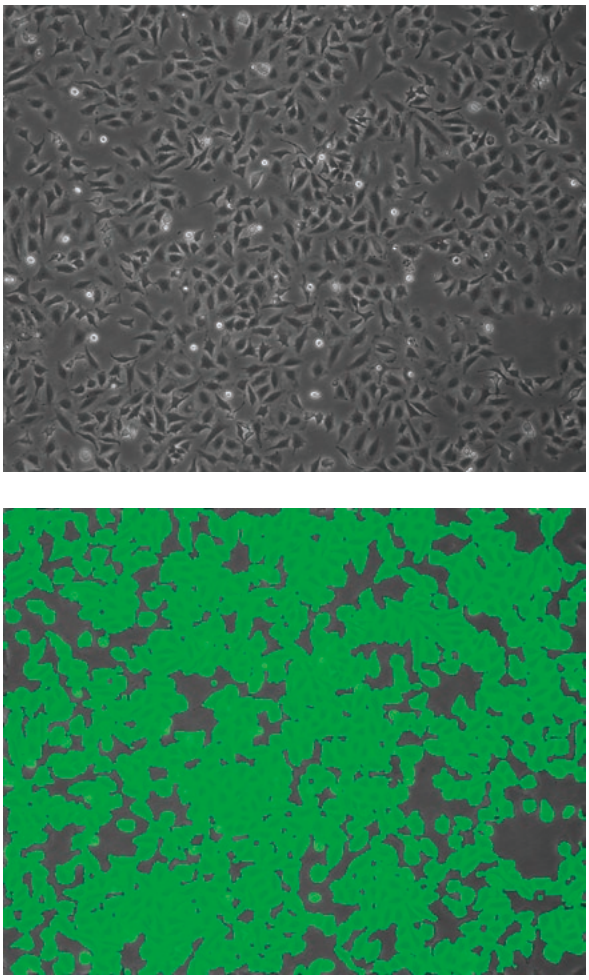

Fig.1 Digitization of cell confluency of HeLa cells

A phase contrast image of HeLa cells was captured with BioStudio-mini, and cell confluency was digitized. Above: Phase contrast image Below: Cell coverage area displayed with a green mask

Summary

- Cell confluency can be automatically digitized by analyzing a phase contrast image of HeLa cells with CL-Quant.
- Whether the coverage area of the cell has been correctly recognized can be readily confirmed by referring to the mask image.
- Cell confluency used to determine passage timing can be evaluated while monitoring the culture status during culturing.
- Introducing the CL-Quant system enables digitization of confluency and standardization of conventional visual management.

< Introducing Nikon's observation systems >

BioStudio-mini is a compact phase-contrast imaging device, featuring an exceptionally waterproof and chemical-resistant design. This easy-to-clean microscope may be sterilized using hydrogen peroxide gas and/or UV sterilization methods. The compact footprint allows for installation of the BioStudio-mini in various isolators, incubators, and biosafety cabinets.

BioStudio-mini

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