Application Note

CL-Quant Add-on Module

Measurement of mesenchymal stem cell numbers using "MSC Count"

＜BioStudio-T usage example＞

When cell growth is evaluated over time for the purpose of selecting a batch of serum, developing culture conditions, or examining the course of the effects of drugs over time, cell numbers are periodically counted. Cells seeded in multi-well plates are dissociated and cell numbers counted at the same time every day for several days. A cell growth curve is generated from the counted results to confirm cell proliferation. Such processes are time-consuming and labor-intensive work, are difficult to standardize, require skillful techniques, and consume valuable resources including large numbers of cells, serums and media. Furthermore, even if using the cell suspension that has been adjusted by counting the number of cells contained, there is a disparity between the calculated number of cells and the actual seeded number of cells.
By using the CL-Quant image analysis software in combination with the Add-on Module "MSC Count", it is possible to measure the number of cells from phase contrast images of MSC cells in culture.
The plate in which mesenchymal stem cells (MSCs) were seeded was placed in BioStudio-T in a CO_2 incubator for several days and images of the cells were captured over time. Cell numbers were counted from phase contrast images using the Add-on Module.

Observation device

- BioStudio-T (Nikon, BS-T04A)

Image analysis software

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

CL-Quant Add-on Module

- PC-CO04 MSC Count (Nikon, MLS30104)

Cells

- Bone marrow-derived mesenchymal stem cells hTERT, HPV E7-introduced, immortalized cell line, UE7T-13 (JCRB1154, JCRB cell bank)

Reagents and materials

- Gibco™ DMEM, low glucose, pyruvate (Thermo Fisher Scientific, 11885084; with Low Glucose, L-Glutamine, Sodium Pyruvate, without HEPES)
- Gibco™ Fetal Bovine Serum, mesenchymal stem cell-qualified, USDA-approved regions (Thermo Fisher Scientific, 12662-029)
- Gibco™ PBS (-), pH 7.4 (Thermo Fisher Scientific, 10010023)
- TrypLE™ Select (1x), no Phenol Red (Thermo Fisher Scientific, 12563011)
- StemSure® 0.1w/v% gelatin solution (Fujifilm Wako Pure Chemical Industries, 190-15805)
- Costar® 6-well Clear TC-treated Multiple Well Plates (Corning, 3516)

Methods

UE7T-13 cells were seeded in each well of a 6-well plate at cell densities of 1.0×10^4, 2.0×10^4, and 4.0×10^4 cells per well. BioStudio-T (4X model) was set in a CO_2 incubator. The cells were cultured at 37°C, in a humidified atmosphere containing 5% CO_2. 12 x 9 image fields at the center of the well were captured with phase contrast using a 4x objective lens every six hours from four hours after seeding and confirmation of cell attachment. The values of each Z-position that was manually adjusted at the center of each well were set in the grid menu, and time-lapse images were captured with the image AF setting. The captured images were imported into CL-Quant, and the number of cells in 10 x 7 image fields (which were not affected by meniscus and which were cut out from the captured images) were analyzed using the Add-on Module "MSC Count". A growth curve was created from the obtained values to confirm cell growth.

Results

The 10 x 7 image fields not affected by meniscus were cut out from the original phase contrast images after cells were cultured for six days, and analyzed using the Add-on Module. The cell number was automatically counted by CL-Quant. A representative image and analyzed image are shown in Fig. 1 and growth curves were created from the analyzed result (Fig. 2).

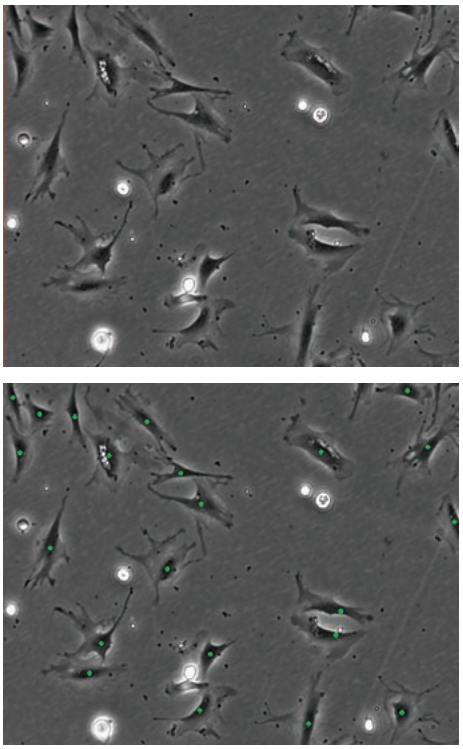

Fig.1 Phase contrast image and masked image of UE7T-13 cells

(Above) A part of a phase contrast image taken four hours after seeding UE7T-13 cells at a density of 4.0 x 10^4 cells per well. (Below) Image after application of CL-Quant's Add-on Module

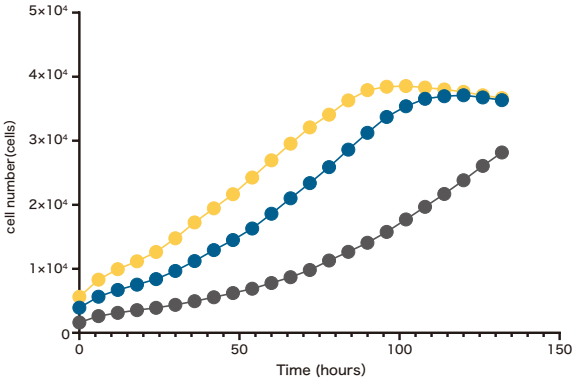

Fig. 2 Growth curve of UE7T-13 cells

UE7T-13 cells were seeded at 1.0 x 10^4 (black), 2.0 x 10^4 (blue), and 4.0 x 10^4 (yellow) cells per well. Cell numbers were digitized from 10 x 7 field images not affected by meniscus. The size of the 10 x 7 field of view is 3.575126cm^2.

Summary

- It is possible to digitize UE7T-13 cell numbers from the phase contrast image using CL-Quant without detaching or dispersing the cells during the cell culturing process.
- Cells used for evaluating cell growth can be used again in the next experiment, preventing waste.
- Since cell numbers can be digitized while the cells are being cultured, the appropriate timing for passage and experiments can be determined using the results.

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