Application Note

CL-Quant Add-on Module

Measuring neurite length in fluorescence images using “Neurite Length” and “Neuronal Cell/Cluster Count”

< BioStation CT usage example >

Benefits

- Using the CL-Quant image analysis software with CL-Quant Add-on Module “Neurite Length” and “Neuronal Cell/Cluster Count” make it possible to measure the neuronal cell and/or cluster number and neurite length.
- Measurement of neurite length is widely used in the fluorescence imaging enabling evaluation of drug efficacy and drug toxicity of candidate compounds. This Add-on Module that measures neurite length using fluorescence images helps to easily carry out generally used evaluation methods.

Observation device

- BioStation CT (Nikon, MLA10000, MLY10013)

Software

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

CL-Quant Add-on Module

- MA-FL-10X-LE02 Neurite Length (Nikon, MLS30302)
- MA-FL-10X-CO03 Neuronal Cell/Cluster Count (Nikon, MLS30103)

Cells

- Human iPS cell-derived neuron: hiPSC-derived GABAergic Neurons from Healthy Donor (Elixirgen Scientific, EXGS-QNGSVF-CW50065-1M

Materials

- DMEM/F12, no glutamine (Thermo Fisher Scientific, 21331-020)
- Neurobasal™ Medium (Thermo Fisher Scientific, 21103049)
- GlutaMAX™ Supplement (ThermoFisher Scientific, 35050061)
- Penicillin-Streptomycin, Liquid (Thermo Fisher Scientific, 15140-122)
- iMatrix-511 silk (Nippi, EXGS-NI511S)
- Y-27632 2HCl (Selleck Chemicals, s1049)
- Quick-Neuron™ GABAergic Maintenance Medium (Elixirgen Scientific, EXGS-QNGM)
- -Cellstain®- Calcein-AM solution (Dojindo Laboratories, C396)
- Coster® 24-well TC-treated Multiple Well Plates (Corning, 3526)

Methods

The cells of hiPSC-derived GABAergic neurons were thawed and plated into an iMatrix-511-coated 24-well plate at the cell density of 0.5×10^5 and 1.0×10^5 cells/well. The cells were cultured for 20 days at 37˚C in a humidified atmosphere of 5% CO_2. And then, cells were incubated with 0.1 µg/ml of Calcein-AM in the medium for 30 minutes at 37°C in a humidified atmosphere of 5% CO_2, and were rinsed twice with the medium.

Immediately after staining, both phase contrast and fluorescence images of 10 fields (1 field is approx. 0.8 mm ×0.8 mm) near the center of a well were captured with a 10× objective lens in a BioStation CT. Acquisition conditions were as follows: Exposure time (fluorescence 475 nm), 150 milliseconds; LED power, 50%; Focus setting, Custom focus.

The obtained image data were analyzed using CL-Quant with the Add-on Module “Neurite Length,” and values of total length of neurites in the field were obtained as “Ch2-Mask Line Length (µm)” by automatic calculation. Next, the neurite length per area was calculated based on both the total length of neurites and the whole field area information. The same image data were used for automatic measurement of the number of neuronal cells and cell clusters, using CL-Quant and the Add-on Module “Neuronal Cell/Cluster Count”.

*The numbers of neuronal cells were counted when neuronal cells exist independently, and the numbers of neuronal cell clusters were counted when they exist as clusters.

When independent cells and clusters are mixed, the measurement is performed without distinction between them. The count result was obtained as a value of “Ch2-Mask Component Count.”

Results

The analysis results using CL-Quant with the Add-on Module "Neurite Length" and "Neuronal Cell/Cluster Count" demonstrate that neurite and neuronal cells and/or cluster of hiPSC-derived GABAergic neurons were determined in fluorescence images. A phase contrast image (Fig. 1A) and a fluorescence image stained with Calcein-AM (Fig. 1B) of the cells at 20 days after seeding in the well seeded with 1.0×10^5 cells/well. The image was processed by the Add-on Module "Neurite Length" to display areas of neurites (Fig. 1C). The image was processed by the Add-on Module "Neuronal Cell/Cluster Count" to display areas of neuronal cells and/or clusters (Fig. 1D).

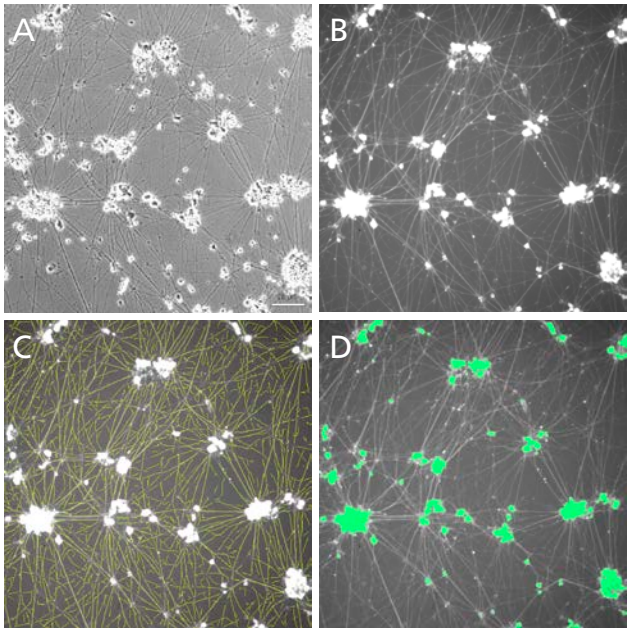

Figure 1. Phase contrast image, fluorescence image stained with Calcein-AM, and the masked images
A representative phase contrast (A) and fluorescence image (B) of hiPSC-derived GABAergic Neuron of cells at 20 days after seeding in the well seeded with 1.0×10^5 cells/well were shown. The cells stained with Calcein-AM (B) was proceeded to mask neurite (C; yellow) and neuronal cell and/or cluster area (D; green).

The neurite length in the wells seeded at different densities of cells is shown in Figure 2. Using this Add-on Module, the neurite length per single cell and the neurite length per unit area can be displayed (Fig. 2). This function is useful to characterize neuronal cells and to detect drug response.

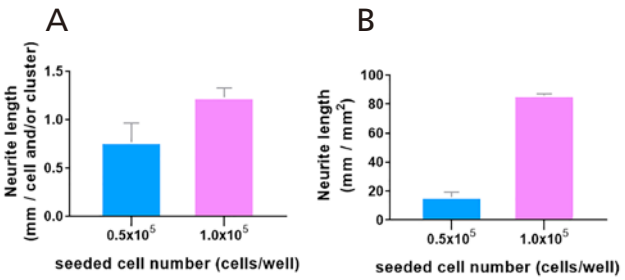

Figure 2. Comparison of Neurite Length
hiPSC-derived GABAergic Neuron seeded at the cell density of 0.5×10^5 (blue) and 1.0×10^5 (pink) cells/well were cultured for 20 days. Total neurite length per single cell/cluster (A) and total neurite length per 1 mm^2 of field (B) were automatically obtained and graphed. Error bars indicate standard errors of 10 fields of view.

Summary

- Using CL-Quant with the Add-on Module, automatic calculation of neurite length is possible by analyzing the fluorescence images of hiPSC-derived GABAergic neurons after long-term culture.
- It is possible to calculate the neurite length per neuronal cell/cluster by combining these two Add-on Modules.
- The numeric measured values obtained can be output in Microsoft Excel® format.

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