Application Note

## CL-Quant Add-on Module

# Evaluation of neuronal cells by measuring neurite length using "Neurite Length" and "Neuronal Cell/Cluster Number"

**< BioStation CT usage example >**

## Benefits

- Using the CL-Quant image analysis software with CL-Quant Add-on Modules "Neurite Length" and "Neuronal Cell/Cluster Count" make it possible to measure the neuronal cell and/or cluster number and neurite length.
- To monitor the numbers of neuronal cell and/or cluster number and neurite length it is important to characterize neural function and to evaluate cell differentiation. Further, measurement of neurite length is widely used to evaluate the drug efficacy and drug toxicity of candidate compounds.
- This Add-on Module makes it possible to measure neurite length from phase contrast images without performing invasive treatment such as staining cells. While continuing the culture, it is possible to measure the length of neurites, which enables the evaluation of cultured neurons and the evaluation of drug efficacy and drug toxicity.

## Observation device

- BioStation CT (Nikon, MLA10000)

## Software

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

## CL-Quant Add-on Module

- MA-PC-10X-LE01 Neurite Length (Nikon, MLS30301)
- MA-PC-10X-CO02 Neuronal Cell/Cluster Count (Nikon, MLS30102)

## 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)
- 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 following cell density, $0.5\times10^5$, $1.0\times10^5$, and $1.5\times10^5$ cells/well, in accordance with the manufacturer's instructions. The cells were cultured in a BioStation CT at 37˚C in a humidified atmosphere of 5% $CO_2$.

Phase contrast images of 2×2 (approx. 1.6 mm×1.6 mm) fields at the center of a well were captured with a 10× objective lens every 6 hours from 24 hours after seeding, with custom focus setting.

The obtained image data were analyzed using CL-Quant with the Add-on Module "Neurite Length", and automatically calculated with "Ph-Mask Line Length (μm)" (values of total lengths of neurites in the field). Then, based on the whole field area information, the neurite length per area was calculated.

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 number of neuronal cell clusters were also counted when they exist as clusters.

When independent cells and clusters are mixed, the measurement is performed without distinction between the two. Based on the total length of neurite length, neuronal cells and neuronal cell clusters, it is possible to calculate the neurite length (average value) per neuronal cell/cluster.

## 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 recognized in phase contrast images. Figure 1 shows representative images of cells at 6 days after seeding in the well at a cell density of $1.0\times10^5$ cells/well. The lengths of neurites were processed by the Add-on Module and displayed with masking in pink (Fig. 1B). The areas of neuronal cells and/or clusters were processed by the Add-on Module "Neuronal Cell/Cluster Count," and displayed with masking in green (Fig. 1C).

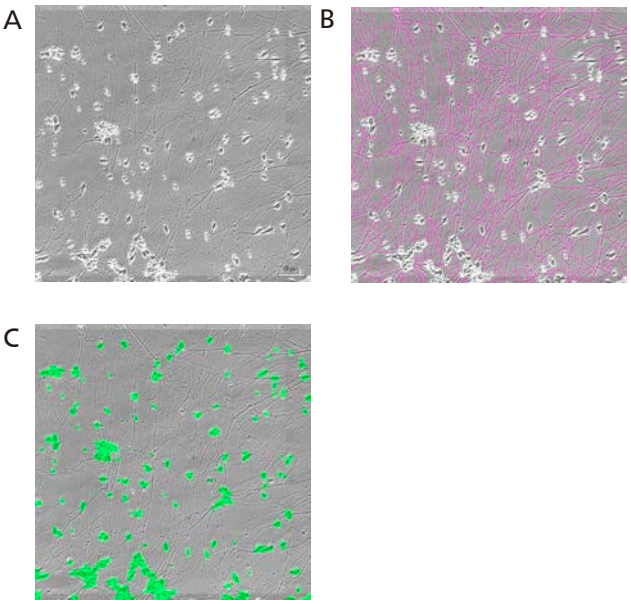


**Figure 1. Phase contrast image and analyzed images**
A representative phase contrast image of the hiPSC-derived GABAergic neurons (A) was proceeded to mask neurite areas (B; magenta), and cell body and cell clamp areas (C; green) at 6 days after seeding at a density of $1.0\times10^5$ cells/well.

The time course of neurite length over the 5-day culturing from 24 hours after seeding in wells seeded at different densities of cells is shown in Figure 2. Using this Add-on Module, the time course of neurite length per unit area or per cell seeding concentration can be displayed (Fig. 2). This function is useful to characterize neuronal cells and to detect drug response.

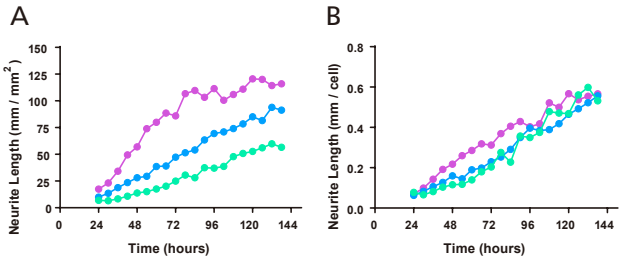


**Figure 2. Time course of neurite length**
hiPSC-derived GABAergic Neuron seeded at the cell density of $0.5\times10^5$ (green), $1.0\times10^5$ (blue), $1.5\times10^5$ (purple) cells/well were cultured for 6 days. Time course of total neurite length per 1 $mm^2$ of field (A) and neurite length per single neuronal cell/cluster (B) were automatically obtained.

## Summary

- Using CL-Quant with the Add-on Module, it automatically calculates both neurite length and number of neuronal cells/clusters by analyzing the phase contrast image of iPSC derived GABAergic Neurons without any invasive treatment. The results can be confirmed on CL-Quant on the operation screen.
- It is possible to calculate the neurite length per neuronal cell/cluster by combining these two Add-on Modules.
- The obtained numeric measured values can be output in Microsoft Excel® format.




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