



## CL-Quant Add-on Module

# Measuring individual hPSC colony areas using “hPSC Colony Area Package”

< BioStation CT usage example >

### Benefits

- Using the “CL-Quant” image analysis software with CL-Quant Add-on Module “hPSC colony area package” make it possible to recognize individual human pluripotent stem cell (hPSC) colonies, and measure their area and number of colonies, as well as the average, total size and number of colonies.
- When hPSCs are properly dissociated at passage, they will grow as colonies of almost uniform size. If the dissociation method is inadequate, the size of the colonies varies and will affect the quality after the next passage. Also, when transformed colonies appear, rapidly growing colonies appear. Using this Add-on Module, you can check for variations in the area of colonies from the images, and also compare to the previous passage. In this way, it is possible to evaluate the quality of hPSCs, the evaluation of dissociation process at passage, and the culture skill between workers.

### Observation device

- BioStation CT (Nikon, MLA10000)

### Software

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

### CL-Quant Add-on Module

- AP-PC-4X-AR03 hPSC Colony Area Package (Nikon, MLS30203)

### Cells

- Human induced pluripotent stem (iPS) cell line, Tic-FX (JCRB Cell Bank, JCRB1331.01, MRC-5-derived, Substrain of Tic iPS cell (JCRB1331) adapted to feeder-free culture)

### Materials

- TeSR™-E8™ Kit for hESC/hiPSC Maintenance (STEMCELL Technologies, 05990)
- Fibronectin from bovine plasma (SIGMAALDRICH, F1141)
- StemPro™ EZPassage™ Disposable Stem Cell Passaging Tool (Thermo Fisher Scientific, 23181010)
- Cell Scraper S (Sumitomo Bakelite, MS-93100)
- Costar® 6-well Clear TC-treated Multiple Well Plates (Corning, 3516)

### Methods

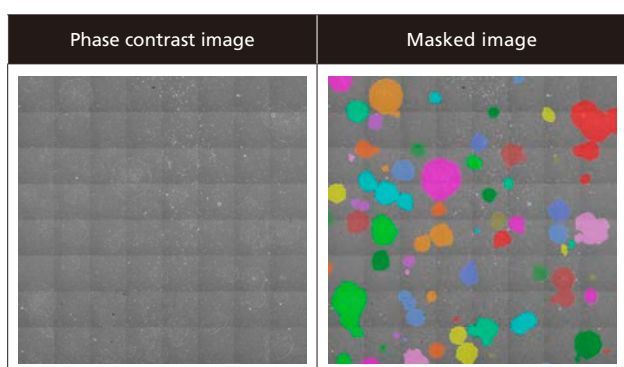
Human iPS cells, Tic-FX cells which were maintained in TeSR™-E8™ medium on a fibronectin-coated 25cm<sup>2</sup> flask without feeder cells were inoculated using Ezpassage™ and cell scraper. The cell clumps were seeded into a 6-well plate coated with Fibronectin and were cultured for 3 days in a BioStation CT at 37°C in a humidified atmosphere of 5% CO<sub>2</sub>.

Phase contrast images of 8×8 fields (approx. 16.0 mm×16.0 mm) at the center of a well were automatically captured every 12 hours with a 4x objective lens, in a BioStation CT every 12 hours from 3 days after seeding. Auto-focusing was adjusted on the first field of view for a well and then images were automatically taken using that auto-focusing setting within that well.

The obtained image data were analyzed using CL-Quant and the Add-on Module “hPSC Colony Area Package”, then automatically measured with the values of “Count”, “Ph-Mask\_Area (μm<sup>2</sup>)”, “Mean of Ph-Mask Area (μm<sup>2</sup>)”, and “Total of Ph-Mask Area (μm<sup>2</sup>)”. In the operation screen, each human iPS cell colony was recognized in random colors and the measured values were confirmed. The obtained values can be output in Microsoft Excel® format, and the distribution of colony size was graphed.

## Results

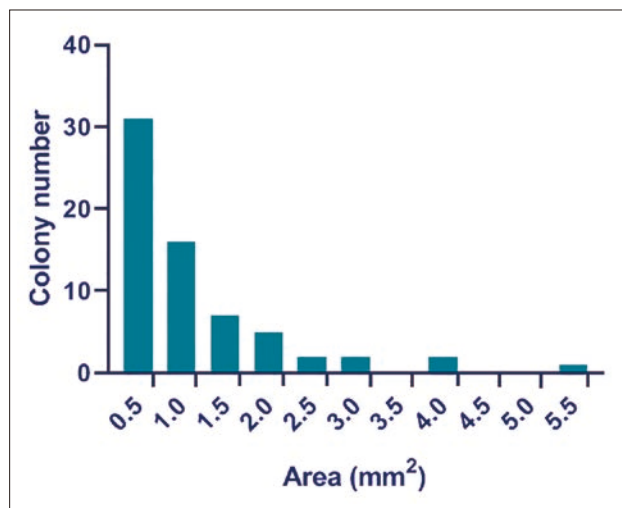
The analysis results using CL-Quant with the Add-on Module "hPSC Colony Area Package" show the colony area of Tic-FX cells cultured in TeSR™-E8™ in random coloring. Figure 1 shows an unprocessed phase contrast image of Tic-FX and its masked image after processing using CL-Quant with the Add-on Module "hPSC Colony Area Package". By displaying the areas recognized as colonies in random colors, it was possible to confirm the areas of individual iPS cell colonies. Further, individual colony areas and colony numbers were obtained by automatic measurement.



**Figure 1. Phase contrast and masked images of hPSC colonies**

Phase contrast image of Tic-FX human iPS cells (left) was proceeded to mask colony areas with random colors (right).

Figure 2 shows the distribution of the areas of individual colonies, showing that there is variation in colony areas and their proportion. By using this analysis to determine the variations in colony area and number, it would be possible to evaluate cell quality, cell passage process and cell passaging skills.



**Figure 2. Distribution of hPSC colony areas**

The obtained image data of Tic-FX human iPS cells were analyzed using CL-Quant with the Add-on Module "hPSC Colony Area Package". The obtained "Ph-Mask\_Area (μm<sup>2</sup>)" values were converted to the mm<sup>2</sup> unit and graphed.

## Summary

- Using the combination of CL-Quant and the Add-on Module "hPSC Colony Area Package", individual hPSC colonies are confirmed and the area of individual colonies is automatically measured. The obtained values are displayed on the operation screen.
- Monitoring the cell status during culturing hPSCs, and obtaining the information of the area, average, and total size of cells can be used to assess the quality of hPSCs and to help determine passage timing.
- The numeric measured values obtained can be output in Microsoft Excel® format.

## < Introducing Nikon's observation systems >

The BioStation CT is equipped with an incubator for long-term monitoring of cells via microscope, and the BioStudio-T allows capturing without moving the stage. Both reduce stress on the cells and allow time-lapse photography of changes over time. Using Nikon's live cell imaging equipment and unique image analysis technology enables observation and real-time analysis of cell characteristics over time.



BioStation CT



BioStudio-T

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