Application Notes

YOKOGAWA
Co-innovating tomorrow™

Single Cellome™ System SS2000
Examples of Sampling for Various Cell Types

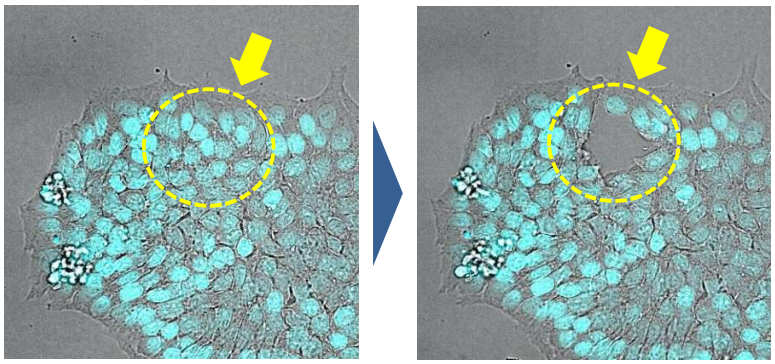
In recent years, single-cell studies have become increasingly popular as analytical techniques have become more sensitive and widespread.
The SS2000 is a revolutionary device that can sample intracellular components and single cells using glass capillary tips with a diameter of several micrometers while performing confocal microscope imaging. These application notes introduce examples of sampling for various cell types.

Stem Cell Sampling

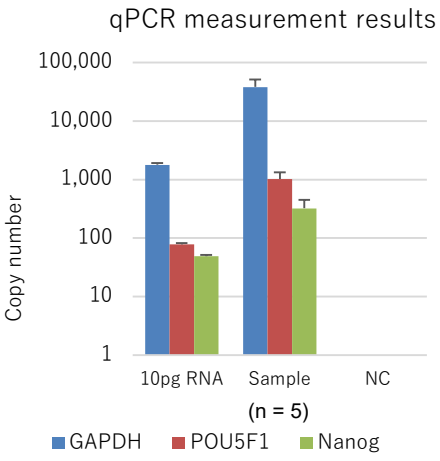
We sampled a set of cells as a batch from human iPS cell colonies cultured in two-dimensional conditions. We synthesized cDNA from the collected sample by RT-RamDA and measured the expression levels of GAPDH (a housekeeping gene), POU5F1, and Nanog (pluripotency genes) by qPCR. Copy numbers equivalent to several cells were then detected for each gene (calculated as 10 pg RNA = amount for 1 cell). The image data also confirmed that 7 to 8 cells were sampled, which was consistent with the qPCR results. It was confirmed that sampling from stem cells such as human iPS cells while maintaining pluripotency was possible.

Before sampling

After sampling



Blue: Nucleus



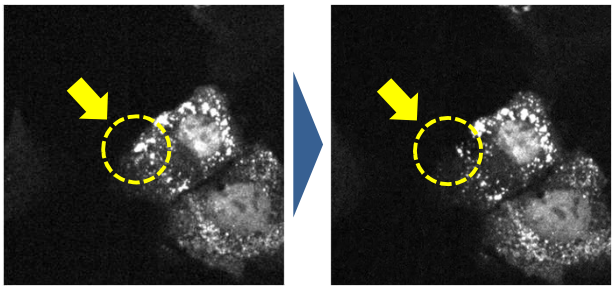
Experimental Conditions

Cells:	Human iPS cells (PBMC-derived)
Labeling reagents:	Hoechst
Sampling:	Sampling of whole cells of a portion of a colony with a glass tip (inner diameter: 10 μm)
cDNA synthesis method:	RT-RamDA method
Primer info:	GAPDH Human Housekeeping Gene Primer Set (Takara Bio) POU5F1 Oct4 (POU5F1) Human qPCR Primer Pair (OriGene Technologies) Nanog NANOG Human qPCR Primer Pair (OriGene Technologies)
Real-time PCR:	SYBR Green method

Intracellular Granule Sampling

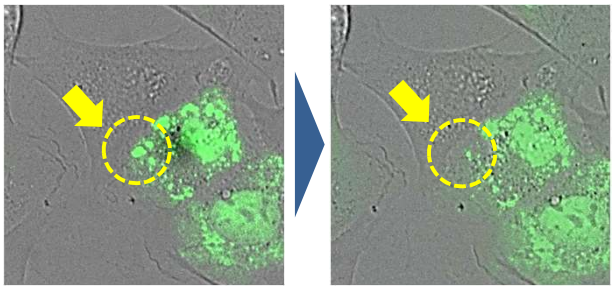
We expressed GFP-expressing intracellular granules in NIH/3T3 cells by gene transfer, sampled the granules from multiple cells, and collected them in the same well. We purified RNA from the collected samples, confirming the feasibility of library preparation. The selective sampling of specific intracellular components suggested that RNA-seq is feasible.

Before sampling | After sampling



Fluorescence image (Em: 488 nm, Ex: 525 nm)

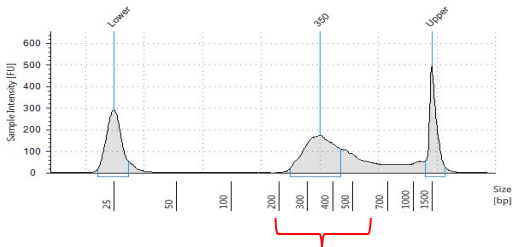
Before sampling | After sampling



Merged bright-field and fluorescence images

Image data can be acquired as bright-field images, fluorescence images, or merged bright-field and fluorescence images.

Library electropherogram



Confirmed library amplification

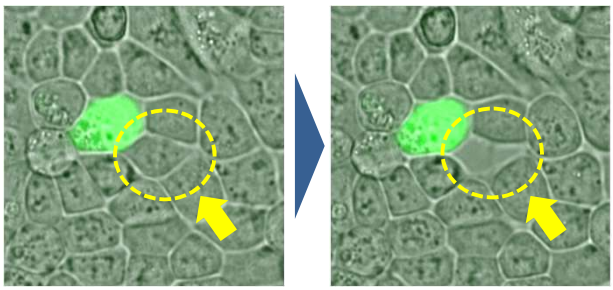
Experimental Conditions

Cells:	NIH/3T3 cells
Sampling:	Used glass tips (inner diameter: 3 µm)
RNA purification:	Purified by TRIzol
Library preparation:	SMART-Seq Stranded Kit (Takara Bio)
Library pherogram:	TapeStation (Agilent)

Sampling While Preserving Location Information

After co-culturing normal MDCK cells and green-fluorescent-labeled abnormal MDCK cells at a ratio of 50:1, we sampled normal cells that were adjacent to the abnormal cells exhibiting fluorescent signals (using glass tips with an inner diameter of 10 µm). It is thus possible, for example, to separately collect cells adjacent to cancer cells and cells located further away.

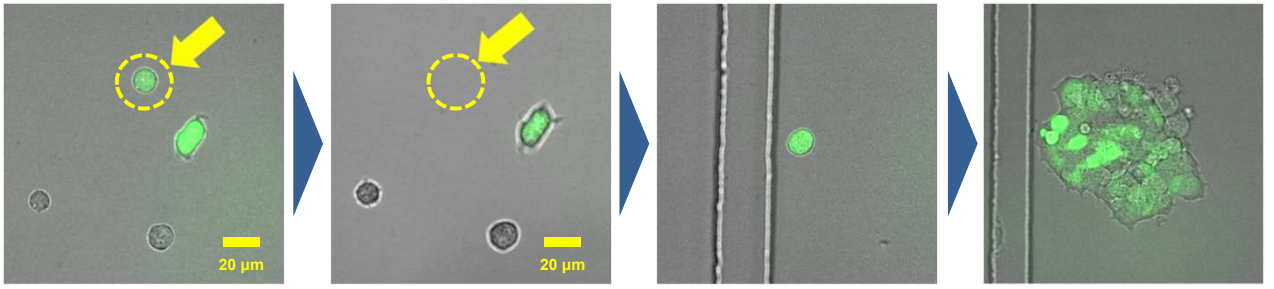
Before sampling | After sampling



Single-cell Cloning

We co-cultured GFP-expressing HEK293 cells and normal HEK293 cells and then sampled single GFP-expressing HEK293 cells (using glass tips with an inner diameter of 10 µm). After dispensing the cells into another culture vessel and culturing, we confirmed that only GFP-expressing HEK293 cells proliferated. It is thus possible to perform single cell cloning from specific cells, such as cells that show specific behavior or morphological changes under the microscope.

Before sampling | After sampling | Immediately after dispensing | After 120 hours of culturing

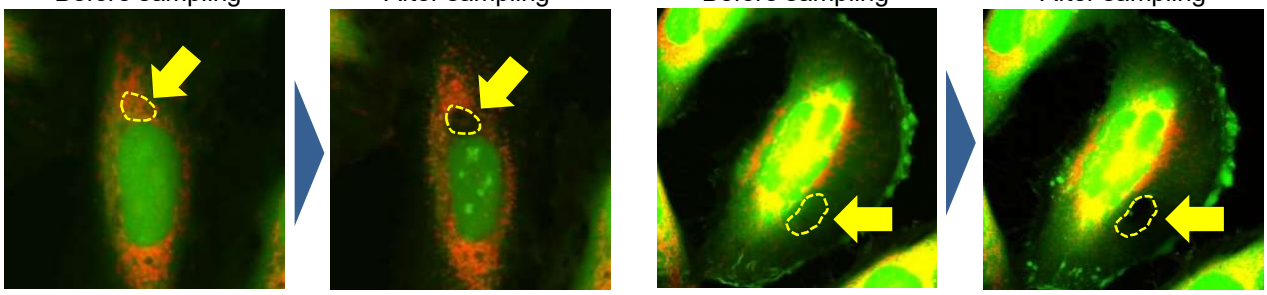


(Note: Some cells may be difficult to culture after sampling and dispensing.)

Mitochondrial Sampling

We sampled mitochondria-containing and mitochondria-free regions from HeLa cells. Selectively sampling specific organelles enables a more detailed analysis of their function. It is also possible to avoid certain organelles while sampling to make control samples or exclude organelles that affect analysis.

Before sampling　After sampling　Before sampling　After sampling



Sampling of mitochondria-containing regions
(Using glass tip with an inner diameter of 3 µm)

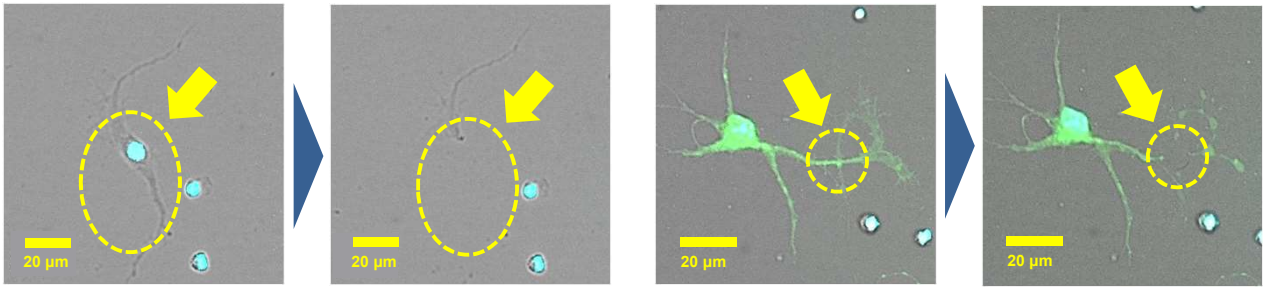
Sampling of mitochondria-free regions
(Using glass tip with an inner diameter of 3 µm)

Red: Mitochondria Green: Whole cell

Specific Cell Site Sampling

We sampled neurites and cell bodies (including nuclei) of separate sites from cultured primary neurons. This enables the selective analysis of specific sites or regions of cells.

Before sampling　After sampling　Before sampling　After sampling



Sampling of cell bodies including nucleus
(Using glass tip with an inner diameter of 10 µm)

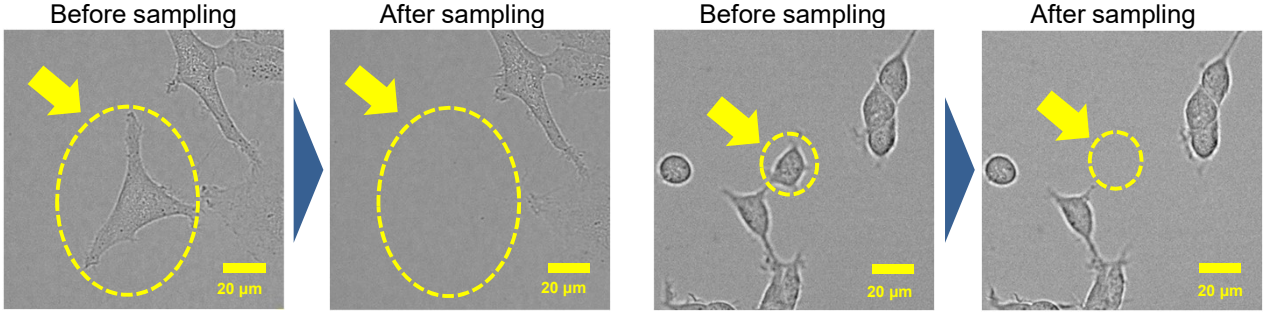
Sampling of neurites
(Using glass tip with an inner diameter of 3 µm)

Blue: Nucleus Green: Whole cell

Sampling of Cells of Various Sizes

We sampled HeLa and HEK293 cells and confirmed that SS2000 can sample whole cells of various sizes, including HeLa cells larger than 20 µm.

Before sampling　After sampling　Before sampling　After sampling



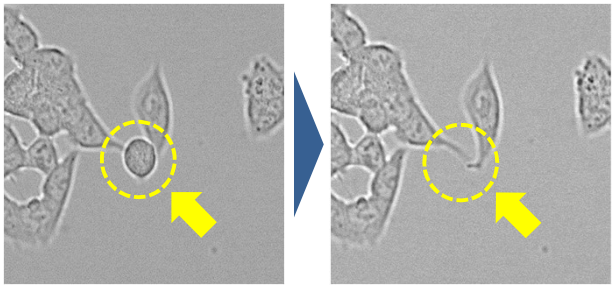
HeLa cells
(Using glass tip with an inner diameter of 10 µm)

HEK293 cells
(Using glass tip with an inner diameter of 10 µm)

Successive Sampling of the Same Culture Vessel

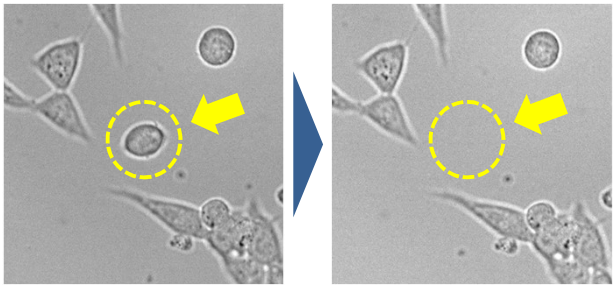
We conducted successive sampling of cells in the process of differentiating from mouse ES cells into primitive ectoderm cells (PrE cells) using glass tips with an inner diameter of 10 µm each time. Since there is no need to suspend cultured cells when sampling, they can be sampled repeatedly from the same dish at different time points. When analyzing cellular changes over time, this eliminates the need to prepare as many dishes as the number of time points sampled as well as the need to correct for dish-to-dish variation during post-analysis.

Before sampling | After sampling



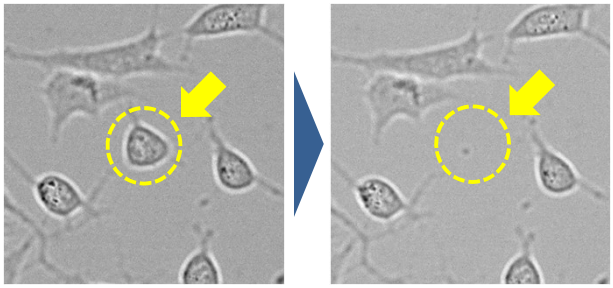
Before inducing differentiation (0 hrs.)

Before sampling | After sampling



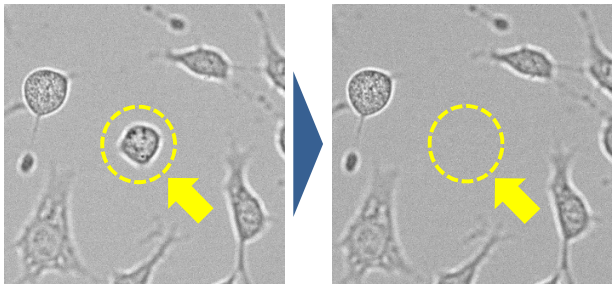
24 hours after inducing differentiation

Before sampling | After sampling



48 hours after inducing differentiation

Before sampling | After sampling

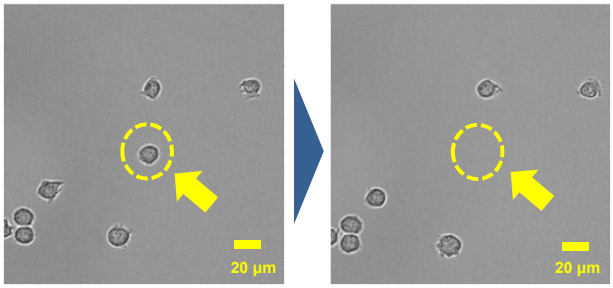


72 hours after inducing differentiation

Non-adherent Cell Sampling

We confirmed that even non-adherent cells such as Jurkat cells can also be sampled by attachment to dish coatings. In this example, we used a glass tip with an inner diameter of 10 um.

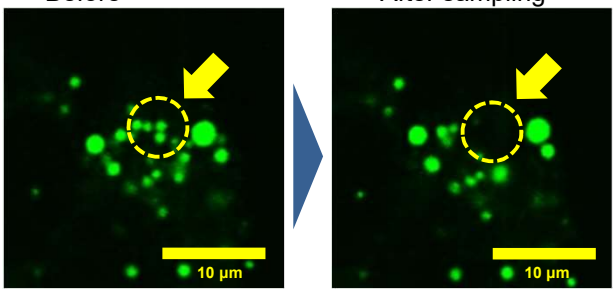
Before sampling | After sampling



Lipid Droplet Sampling

We sampled lipid droplets aggregated upon adding amiodarone to HepG2 cells using a glass tip with an inner diameter of 3 µm. This enables analysis of the intracellular localization and level of metabolism of drugs.

Before | After sampling



Green: Lipid droplets

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Printed in Japan, 310 [Ed:01]

AN 80S01B04-01EN