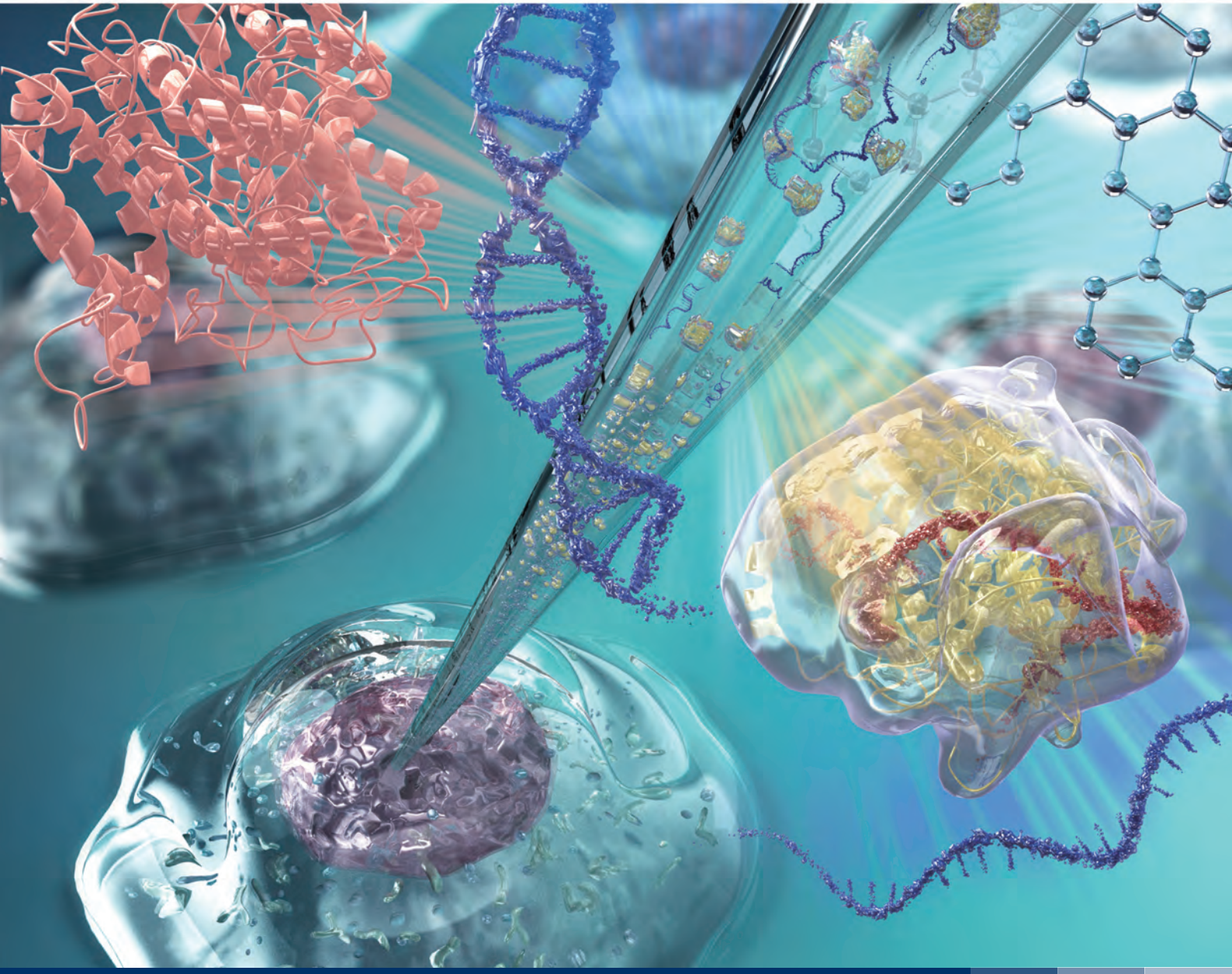
Co-innovating tomorrow™

YOKOGAWA



Single-cell Analysis Solution

Single Cellome™ Unit
SU10

Nano-point Delivery / Nano-point Sampling

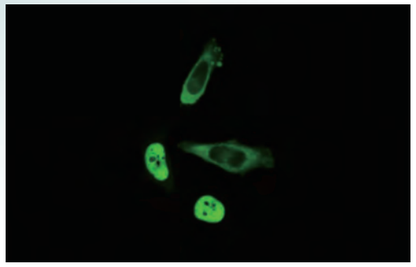
Bulletin 80S01A01-01E

Single Cellome™ Unit **SU10**

Nano-point Delivery

Advantages of the SU10

Single-cell targeting with direct delivery into the nucleus or cytoplasm



FITC-labeled dextran solution (molecular weight 70,000) was delivered into HeLa cells

Highly efficient delivery of reagents with low membrane permeability

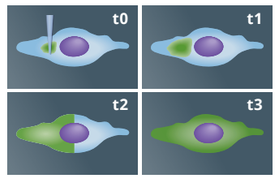
CRISPR-Cas9 RNP/protein (antibody, etc.)/other small molecule reagents, etc.

Multiple substances can be delivered at the same time

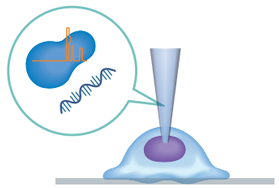
Cas9 RNP & donor DNA/molecules of interest & marker molecules (fluorescent reagents, etc.), etc.

Application examples

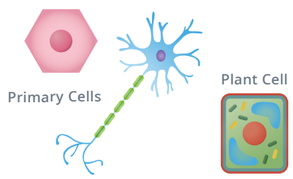
Imaging live cells immediately after substance delivery



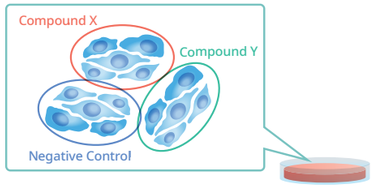
Direct delivery of genome editing tools and other tools into the cell nucleus



Delivery to cells that are difficult to transfect



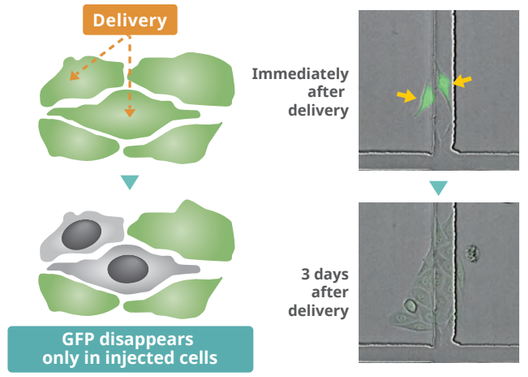
Evaluation of drug efficacy and toxicity by delivery of drug candidate molecules



Application example

Delivery of genome editing tools

Delivery of CRISPR-Cas9 RNPs targeting the GFP gene to GFP-expressing HeLa cells and evaluation of GFP gene disruption by genome editing based on the loss of GFP fluorescent signal.



*Reference: Appl. Sci. 2019, 9, 965 dol 10.3390/app9050965
Courtesy of Kamakura Laboratory, Department of Applied Biological Science, Faculty of Science and Technology, Tokyo University of Science

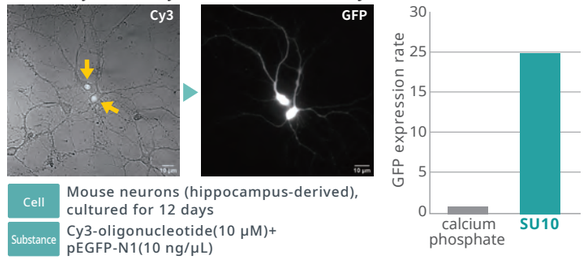
User's voice
Professor Takashi Kamakura
Tokyo University of Science

With the SU10, high genome editing efficiency was achieved, and the ability to introduce Cas RNPs into cells with high efficiency is appealing and may improve editing efficiency in cell types that have been difficult to edit genomes with conventional methods of transfection. We look forward to seeing the number of additional cell types to which the SU10 can be applied.

Application example

Delivery to primary cells and stem cells

Delivery of GFP-expressing plasmid to primary neurons

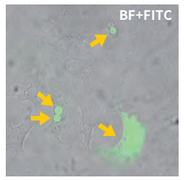


Cell: Mouse neurons (hippocampus-derived), cultured for 12 days
Substance: Cy3-oligonucleotide(10 μM)+ pEGFP-N1(10 ng/μL)

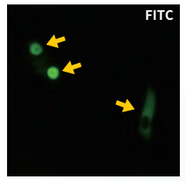
Courtesy of Ms. Kanoko Iwasaki, Yasushi Okada Lab, The University of Tokyo

Total cells for delivery	Delivery success rate	GFP expression rate	GFP expression rate
4 nanopipettes used	$Cy3^+$/total cells	$GFP^+/Cy3^+$ cells	Conventional Method
162	94.4%	24.8%	0.1-1%*

* calcium phosphate transfection



Cell: Primary mouse hepatocytes
Substance: FITC-dextran (MW70K, 10 mg/mL)



Cell: Human iPS cells (201B7, 2D culture)
Substance: FITC-dextran (MW70K, 10 mg/mL)

Courtesy of Matsuzaki Lab, RIKEN

User's voice
Ms. Kanako Iwasaki
The University of Tokyo

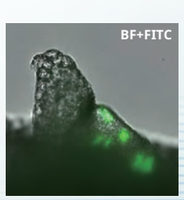
The SU10 has expanded the field of neuronal cell-based experiments and imaging. In addition to DNA, we are considering introducing mRNA and protein in the future.

Application example

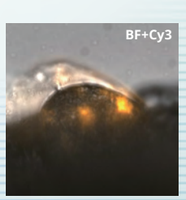
Delivery to cultured plant cells and plant tissues

Cell: Tobacco BY-2 cell
Substance: FITC-dextran (MW70K, 10 mg/mL)

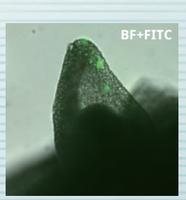
Courtesy of Dr. Daisuke Kurihara,Higashiyama Group, The Institute of Transformative Bio-Molecules, Nagoya University



Cell: Apical meristem of white radish sprouts
Substance: FITC-dextran (MW70K, 10 mg/mL)



Cell: Apical meristem of white radish sprouts
Substance: Cy3-oligonucleotide (50 μM)



Cell: Leaf primordium of maize
Substance: FITC-dextran (MW70K, 10 mg/mL)

Verified SU10 performance applications

Target cell types with successful confirmed results

Cell lines	HeLa, HEK293, MDCK, etc.
Primary cells	Neurons, hepatocytes, etc.
Stem cell lines	iPS cells, ES cells
Plant cells	Cells in tissues, such as apical meristem, BY-2 cells

Examples of materials aptly delivered

Proteins	Antibody, GFP, etc.
Genome editing tools	Cas9 protein-sgRNA complex (RNP), Cas9 RNP & Donor DNA
Nucleic acids	Oligonucleotides DNA, Plasmid vector, RNA
Other reagents with low membrane permeability	FM4-64, SYTOX, etc.

FAQ

What is the volume to deliver into a cell?

It is estimated to be tens of femtoliter(fL) per second (1fL=1x10^{-15}L). The volume can be changed by software settings.

*The delivery volume may vary depending on the solute and vehicle.

Is the nanopipette disposable?

Yes, but one nanopipette can deliver to 50 cells or more*.

*Experiment using HeLa cells by Yokogawa

What is the difference from transfection reagents?

The SU10 can deliver materials into the selected cells. The SU10 enables the direct delivery of reagents into the cytoplasm or nucleus.

What is the difference from electroporation?

In addition to the above-mentioned "difference from transfection reagents, due to automated cell surface detection, the suspension of cells is not required during the injection.

What is the difference from microinjection?

The SU10 lowers the damage to a cell with the nanopipette because its tip size is less than 1/10 of a tip used for microinjection. Automatic detection of cell surface enables a high success rate of insertion and insertion to the intended depth of a cell. The delivery operation uses an electrical mechanism rather than pneumatic or hydraulic pressure.

What is the difference from the existing methods of introducing substances into plant cells?

Difference from the Agrobacterium method

Agrobacterium is a gene delivery method, whereas the SU10 can deliver a wide range of substances, including proteins and Cas9 RNPs, in addition to genes.

Difference from Particle Gun

Particle Gun is a method of random intracellular delivery, whereas the SU10 can deliver to specific cells.

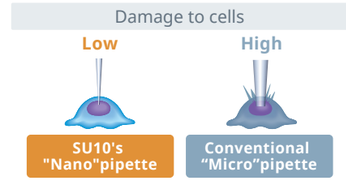
Difference from microinjection

When using micropipettes, the backflow of intracellular components into the pipette or leakage of cell contents can occur, whereas the use of SU10 (nanopipettes) may solve these issues.

Minimum damage to cells

The nanopipette is a glass pipette with a minimum tip outer diameter of several tens of nanometers

The tip of the nanopipette under an electron microscope

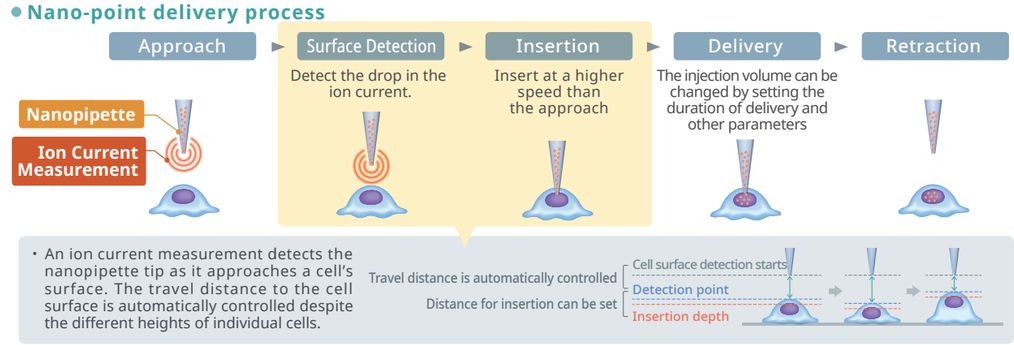


Automated, high speed, and high success rate

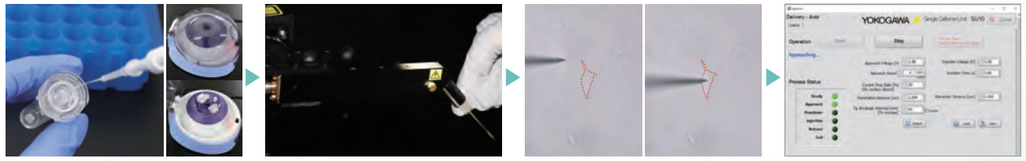
The SU10 uses automated cell surface detection, insertion, and delivery to the cell. The process takes approximately 10 seconds with a 90% success rate.*

* Experiment by Yokogawa

● **Nano-point delivery process**

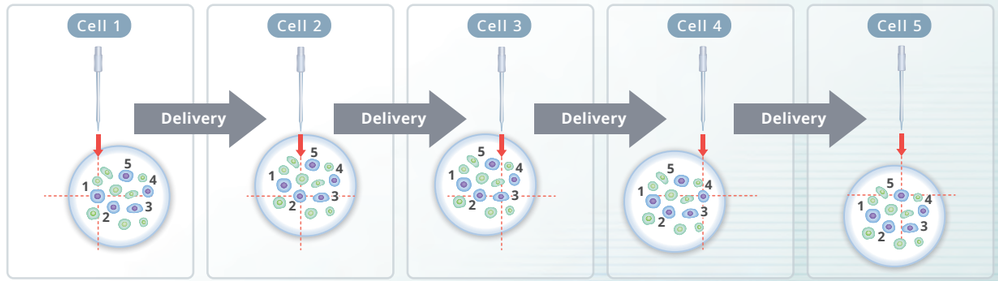


SU10 Automated nano-point delivery operation procedures



Continuous automatic nano-point delivery including XY positioning of cells

Increased efficiency and reduced operation time can be expected



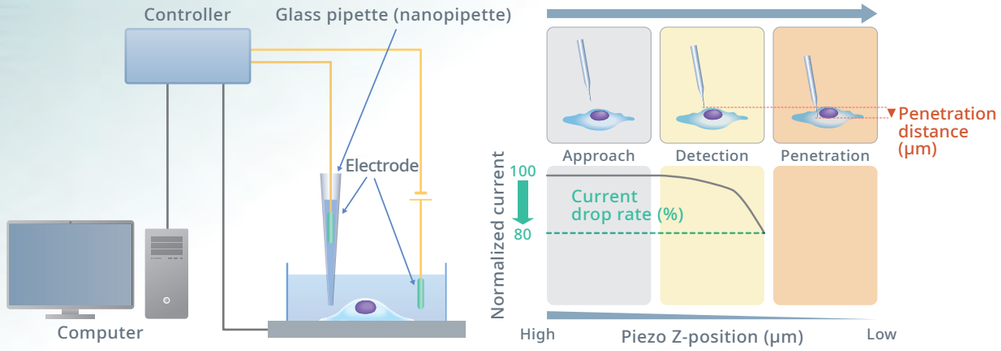
Continuous delivery to multiple cells is possible by connecting the SU10 to the motorized stage of the microscope via external trigger linkage.

STEP 1 Register cell positions in imaging software ▶ STEP 2 Set delivery conditions in SU10 software ▶ STEP 3 Start software

*Please contact us for more information, as the required items need to be purchased and set up separately.

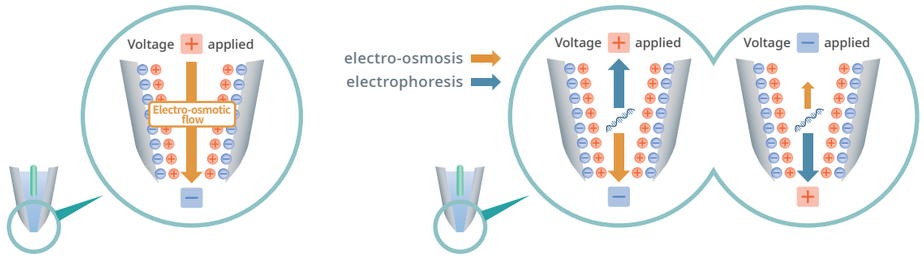
Operation principle

Automatic cell detection and penetration is a SICM* based technology



*A scanning Ion Conductance Microscope: acquires the surface 3D shape of a material by utilizing the decrease in ion current value that occurs as the distance between the probe (glass pipette) and the sample (cells, etc.) gets closer. The SU10 does not have an image acquisition function.

Delivery of solutions and substances into cells is performed by electro-osmosis and electrophoresis



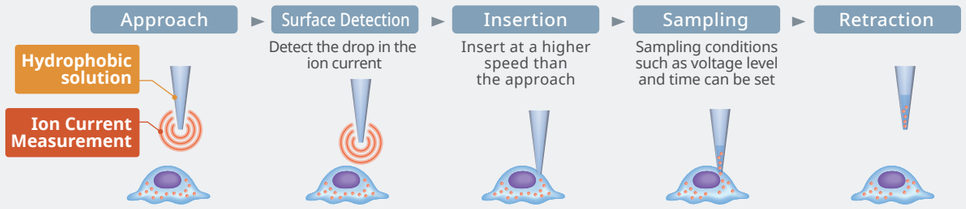
Since the inner wall of the nanopipette is negatively charged, cations and other ions in the liquid inside the nanopipette form an ionic layer. The ionic layer moves toward the tip of the nanopipette when a higher voltage is applied than at the time of approach. The movement of the ionic layer causes the liquid in the nanopipette to move (electro-osmotic flow) and is injected into the cell.

Substances that are charged strongly negatively, such as nucleic acids, can be delivered more efficiently by applying a negative voltage. This is because electrophoresis generates a force that travels in the opposite direction of electro-osmosis. Yokogawa or its representatives will support you in determining optimal conditions.

Nano-point Sampling

A very small amount can be sampled from a specific part of the cell.
Collected samples can be used for genetic analysis, etc.

Process of nano-point sampling



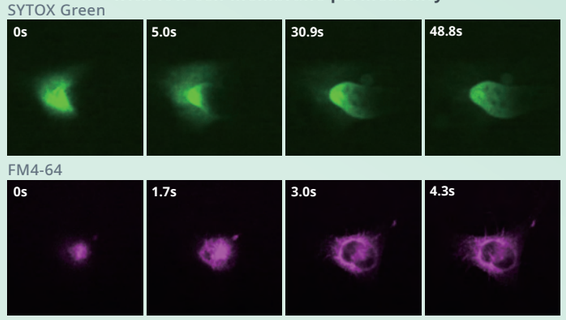
Collaboration with YOKOGAWA Life Science Products

High-speed and reduced phototoxicity Confocal Scanner Unit CSU-W1

Over 3,500 units in the series sold

- The dual-microlensed spinning disk minimizes damage to live cells and living organisms.
- Easy to upgrade a standard optical microscope to confocal.
- Additional upgrade to super-resolution live cell imaging (CSU-W1 SoRa).
- Combined with the SU10, it is possible to capture cellular changes before and after delivery and the intracellular kinetics of the delivered substance.

observation immediately after delivery of reagent with low cell membrane permeability



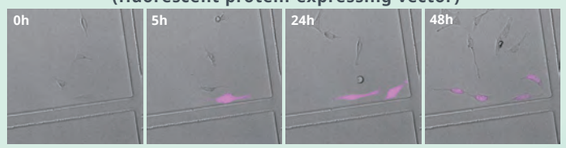
CSU-W1 SoRa
CONFOCAL SCANNER UNIT
SUPER RESOLUTION via OPTICAL Re-ASSIGNMENT

Benchtop High-Content Analysis System CellVoyager™ CQ1

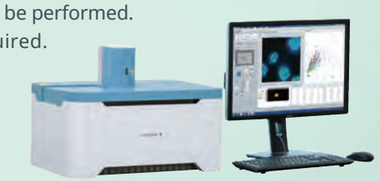
Great for 3D Live Imaging

- The CQ1's confocal scanner unit enables high-speed, high-definition 3D imaging, cell recognition, and quantification.
- In combination with the incubator option, 3D time-lapse acquisition can be performed.
- With a small benchtop footprint, no vibration isolation table is required.
- Time-lapse observation can be performed on cells that have been delivered to the target by the SU10.

time-lapse observation after delivery of plasmid (fluorescent protein-expressing vector)



0h: CQ1 observation start time

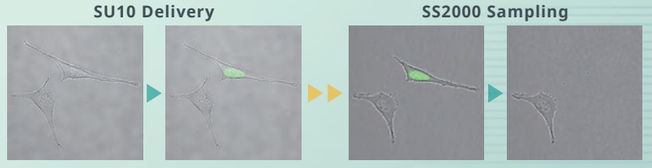


CellVoyager CQ1

Subcellular Sampling System SS2000

- Sampling with positional and morphological information based on live cell imaging technology using confocal microscopy.
- The internal incubator allows for the sampling of cells, while maintaining cell viability.
- In addition to time-lapse observation, cells injected by the SU10 can be sampled.

SU10 Delivery ▶ ▶▶ **SS2000 Sampling** ▶



Single Cellome System SS2000

Specification

Actuator Module	Coarse movement (Motor actuator)	Stroke: Approx.50mm/axis (setting resolution XYZ axis: 0.625μm)
	Fine movement (Piezo actuator)	Stroke: 100μm/axis (setting resolution XYZ axis: 10nm,at penetration and extraction: 1nm)
Measurement Module	Voltage generation range	-10V～+10V (setting resolution: 10mV)
	Current measurement range	-900nA～+900nA (setting current range: ±9V)
Power supply	Power consumption (Main controller+Piezo controller)	100VA or lower
	Supply Voltage (Main controller)	100～120V/220～240VAC (Switching not required)
	Supply voltage (Piezo controller)	100～120V/220～240VAC (model must be specified when placing an order)
	Power supply frequency (Main controller+Piezo controller)	50/60 Hz
External dimensions and weight	Main controller	260(W) x 99(H) x 280(D) mm, Approx. 2.8kg
	Piezo controller	236(W) x 88(H) x 273(D) mm, Approx. 4.6kg
	Actuator module	270*(W) x 219(H) x 245*(D) mm, Approx. 2.2kg * In case the X and Y axes move in the direction of the maximum distance
	Measurement module	85(W) x 30(H) x 43(D) mm, Approx. 0.1kg
	Joystick	100(W) x 162(H) x 144(D) mm, Approx. 1.3kg
	Safety guard	130(W) x 230(H) x 287(D) mm, Approx. 0.7kg
	Tip outer diameter of nanopipette (in case of SU10ACC-NP01)	Under 100nm (reference value)
Operation Environment	15 to 35°C, 20 to 70%RH No condensation, altitude up to 2000m	
Microscope compatibility	For use with an inverted optical microscope.* Microscope is not included with the SU10. Please contact Yokogawa to possibly install the SU10 on a different inverted microscope. Installation examples: Nikon Ti2, Olympus IX71, IX73, IX83, Zeiss Axio Observer	

Installation example

- The SU10 does not come with an optical microscope.
- Depending on the microscope, the condenser may have to be removed when using the SU10.
- Brightfield imaging, fluorescence imaging, and operation of the motorized stage are still possible.

SU10 for stereo microscope is under development

Contact us for more information and demonstration requests

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