Microfluidic chip-based cell sorter

On-chip® Sort

The world's first microfluidic cell sorter
—for sorting cells and droplets

Awarded the Excellence Prize at the Tokyo Metropolitan Venture Technology Awards

Received the Venture Award for two consecutive years at the Japan Healthcare Venture Summit (JHVS) 2019 & 2020

On-chip Biotechnologies Co., Ltd.
2-16-17 Naka-cho, Koganei-shi, Tokyo 184-0012, Japan
TEL.+81-42-385-0461 FAX.+81-42-385-0462

ONCHIP-01B-U004

Realizing innovations in a variety of fields from research to industry

On-chip® Sort

On-chip® Sort is the world's first microfluidic chip-based cell sorter, providing value through the use of disposable microfluidic technology not only to cutting-edge life science research but also to a variety of industries.

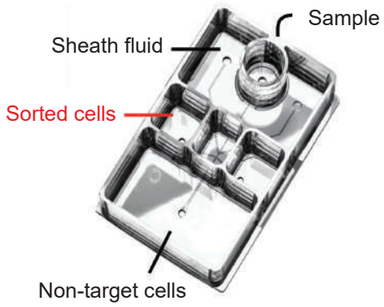
Through promotion of our innovative technology, On-chip Biotechnologies is committed to innovations in the fields shown below and to the realization of a sustainable society.

SUSTAINABLE DEVELOPMENT GOALS

The following developments are underway using our products:

- Low-environmental-impact pesticides, soil improvement, etc.
- Microbial environmental purification methods
- Biofuels
- Cutting-edge diagnostic technology and cell research

On-chip® Sort is an epoch-making cell sorter

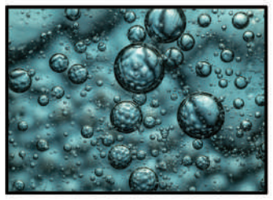


Integrating the processes of sample detection, separation, and collection within a microfluidic chip has realized a small footprint and easy-to-use cell sorter. This system contributes to the analysis and sorting of a wide range of samples including fragile cells, cell clusters, microorganisms, and emulsion droplets. Our microfluidic technology allows for sorting of variety of sample types including those that were impossible to sort using conventional cell sorters. On-chip® Sort will expand the range of sample analysis and contribute to many areas of science research and to a variety of industries.

Microfluidic chip

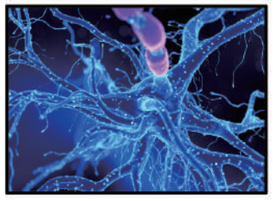
As a search engine for microorganisms and cells

Screening of environmental microorganisms, antibody-producing cells, and mutant strains using droplets



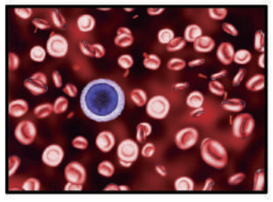
Expanding research field with damage-free sorting

Isolation of cells and particles vulnerable to stress



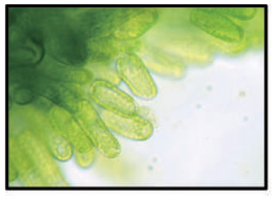
Uncovering rare cells in a short period of time

Ensuring the enrichment of rare cells— even one cell in 10^7 cells



Free from the constraints of sample size and specified sheath fluid

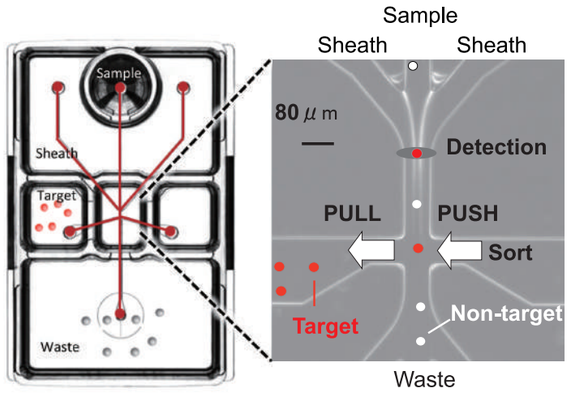
Analysis of cells and clusters up to 130 μm / Freedom of sheath fluid of your choice



Feature of On-chip® Sort

All-in-one processing of analysis and sorting with a microfluidic chip

- The On-chip® Sort system integrates the entire process from sample analysis to sorting on a small microfluidic chip with measurements of 5.5 cm × 4.0 cm.
- In contrast to Jet-in-air sorting methods, Flow Shift method of On-chip® Sort analyzes sample by gently pressurization of air, and sorts target sample into collection reservoir with damage-free (patent Nos. US10101261, US10222317, US10724938, and US10648899).



Easy, maintenance-free operation

- On-chip® Sort's workflow requires no waiting time and operation is easy and user-friendly.
- Maintenance-free operation without the need for complicated equipment cleaning.

Chip preparation → Setting in the holder → Reagent filling → Installation → Sorting/assay → All done in 5 mins

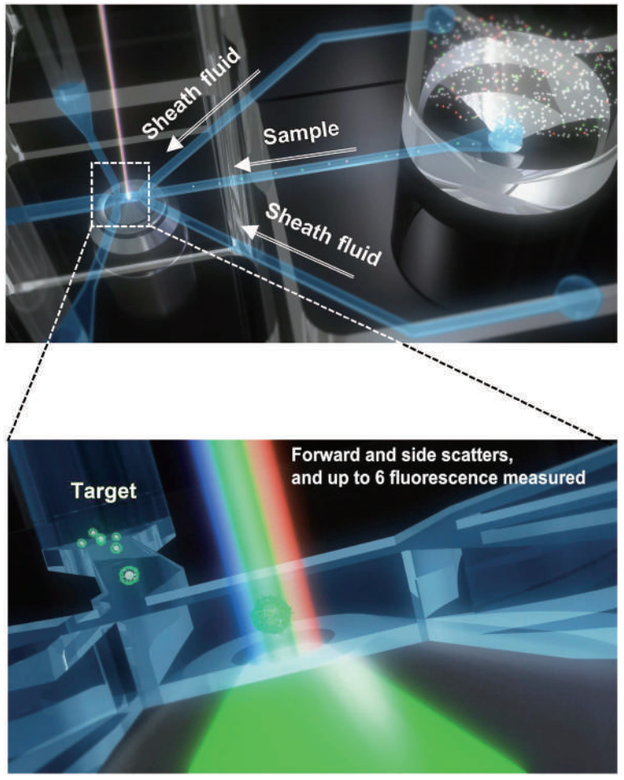
Compact and contamination-free

- Compact enough to be installed in a biosafety cabinet.
- Sample analysis and sorting are contamination-free due to the use of disposable microfluidic chips.

Precise fluid control and a wide selection of samples and sheath fluids

- As cells are sorted using regulation of liquid flow inside the microchannels, hence a wide range of liquids can be used as sample and sheath fluids, including culture medium and oil.

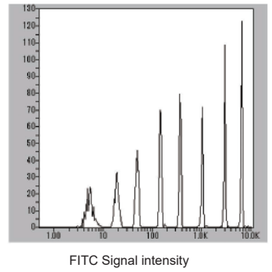
Sample examples	Sheath fluid examples
• Animal cells	• Culture medium
• Plant cells	• Isotonic solutions
• Protists	• Seawater/freshwater
• Bacteria/fungi	• Oil
• Droplets	• Highly viscous culture medium



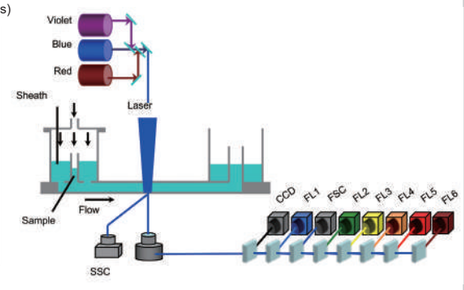
Optical technology

- The sample flowing through the microfluidic channel is irradiated with up to three separate lasers with from the top of the microfluidic chip.
- Fluorescence is detected with high sensitivity in a wide range by up to six separate detectors (FL).
- Forward-scattered light (FSC), an indicator of the size of the sample particles, and side-scattered light (SSC), an indicator of the complexity of the internal structure, are acquired.

Sample: Rainbow calibration particles (8 peaks)



Fluorescence sensitivity: FITC<200 MESF

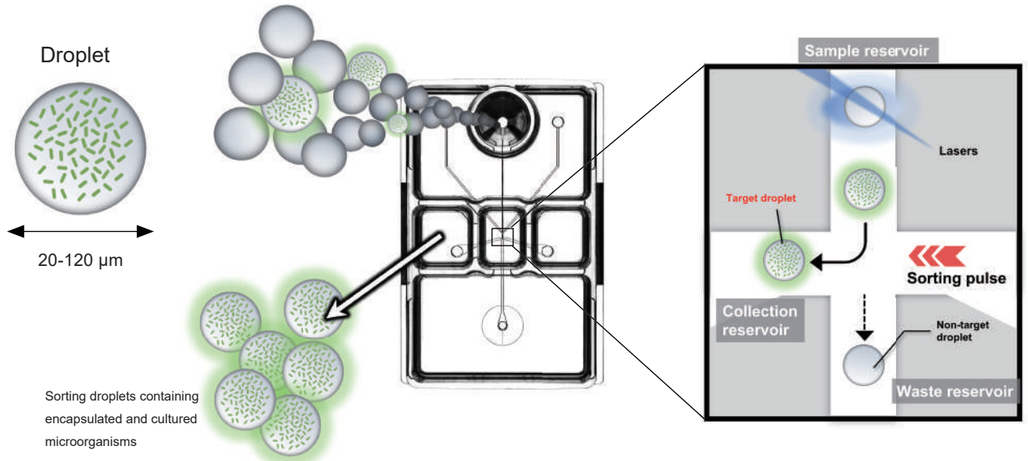


Detection

- FL1: 445/20 nm
- FL2: 543/22 nm
- FL3: 591.5/43 nm (607/36 nm when using a green laser)
- FL4: 676/37 nm
- FL5: 716/40 nm
- FL6: 775/46 nm
- FSC: Forward-scattered light
- SSC: Side-scattered light

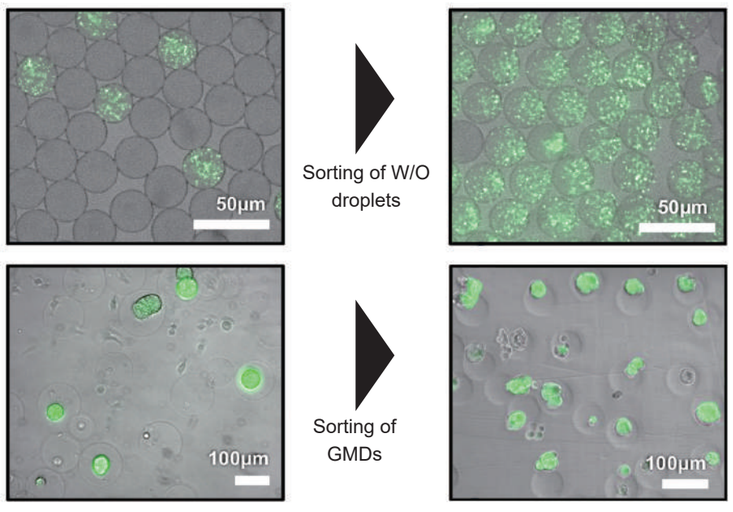
Application #1: Sorting of droplets with encapsulated cells

■ On-chip® Sort is capable of sorting water-in-oil (W/O) emulsion droplets and gel microdrops (GMDs). W/O droplets are liquid droplets dispersed in oil and are stabilized by surfactant. GMDs are droplets solidified using gels. Both W/O droplets and GMDs can be produced by our droplet generator, On-chip Droplet Generator, for single cell analysis and microorganism/cell culturing.



Droplet sorting examples

Fluorescence intensity-based sorting following the cultivation of a single cell encapsulated inside droplets allows selective sorting of only the droplets with proliferated cells.

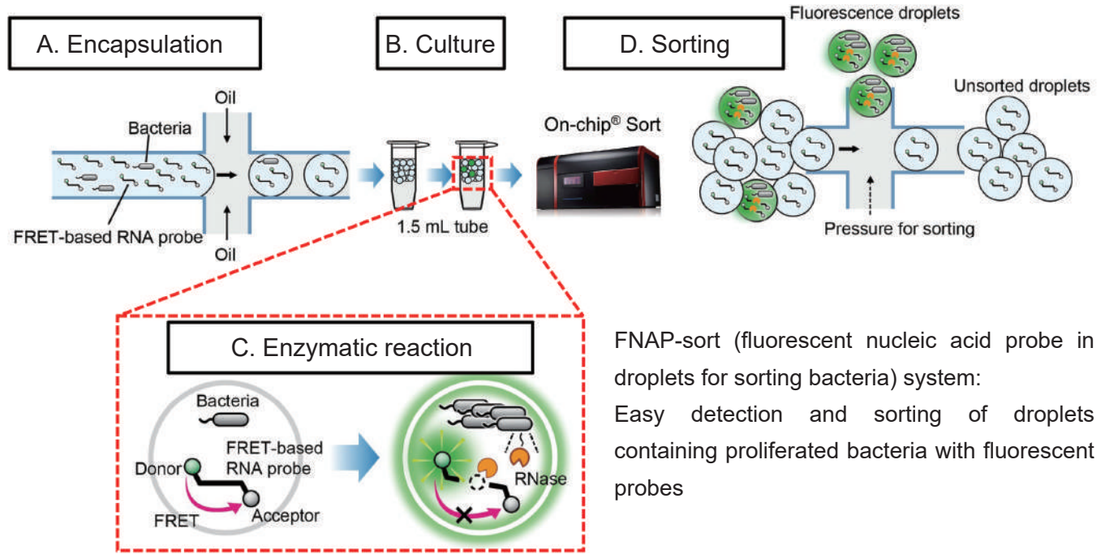


- Cell type: *E. coli* (expressing GFP)
- Composition of droplets: Liquid culture medium
- Composition of external liquid: Fluorinated oil
- Cell type: Lung cancer-derived cell line (expressing GFP)
- Composition of droplets: Medium with gelatin
- Composition of external liquid: Culture medium

Screening of environmental bacteria using fluorescent nucleic acid probes

High-throughput analysis and sorting of droplets using On-chip® Sort enables the construction of a screening system for target enzyme-producing environmental bacteria, microbial dark matter, and artificially mutated strains.

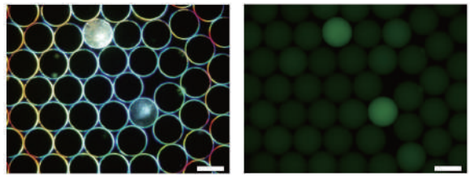
Environmental bacteria are encapsulated together with fluorescent probes in W/O droplets (one cell per droplet) and cultivated. The individual compartmentalization using many droplets for a variety of bacteria with differing growth rates enables each microorganism to be cultivated for a long period of time without being eliminated, and to be screened using fluorescent probes as indicators.



FNAP-sort (fluorescent nucleic acid probe in droplets for sorting bacteria) system:

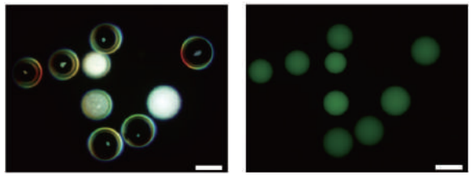
Easy detection and sorting of droplets containing proliferated bacteria with fluorescent probes

Soil environmental bacteria after seven days of cultivation



Dark-field observation Fluorescence observation

Sorting only proliferating bacteria

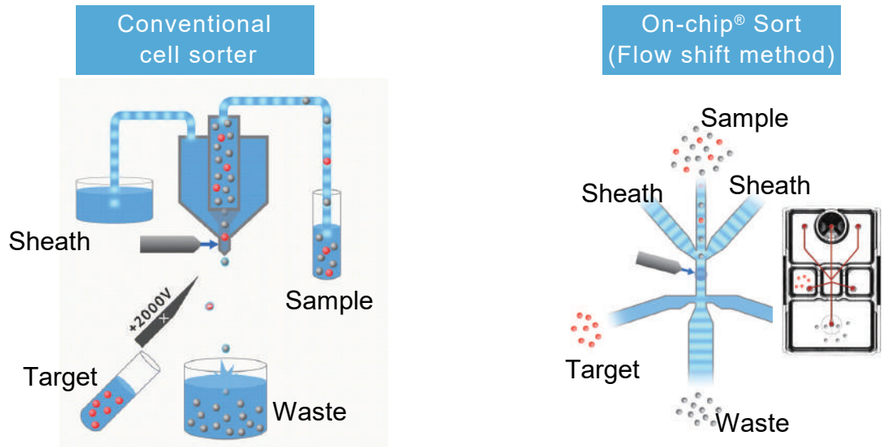


Dark-field observation Fluorescence observation

Collaboration with Research Group Leader Noda, AIST, Biomedical Research Institute

Application #2: Sorting of cells damage-free

■ Compared to sorting with conventional cell sorters, sorting using On-chip® Sort has been proven to be less damaging to cells, as it has less effect on cell proliferation, morphological changes, and gene expression. This is because On-chip® Sort employs a unique cell sorting mechanism to eliminate all the damaging steps in cell sorting on conventional sorters.

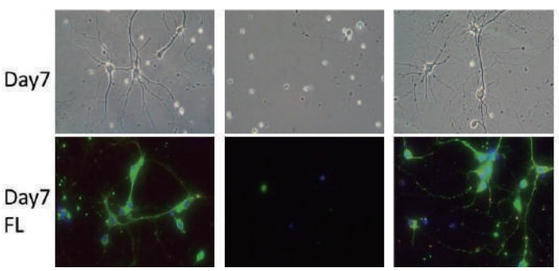


Damage factors		
Specified ×	Sheath fluid	✓ Free to choose
High (5–100 psi) ×	Pressure	✓ Low (1.3 psi)
Applied during droplet preparation ×	Ultrasonic wave	✓ None
High voltage (cells are charged) ×	Electric charge	✓ None
Collision to reservoir ×	Collision	✓ None

Effects of sorting methods on phenotype

【Effect on cell growth】

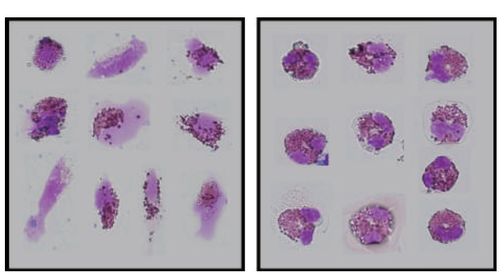
Sorting and cultivation of stress-sensitive hippocampal neurons



Without sorting | Conventional cell sorter ↓ Cell death | On-chip® Sort ↓ ✓ Cell growth

【Effect on cell morphology】

Observation of eosinophils in peripheral blood after sorting



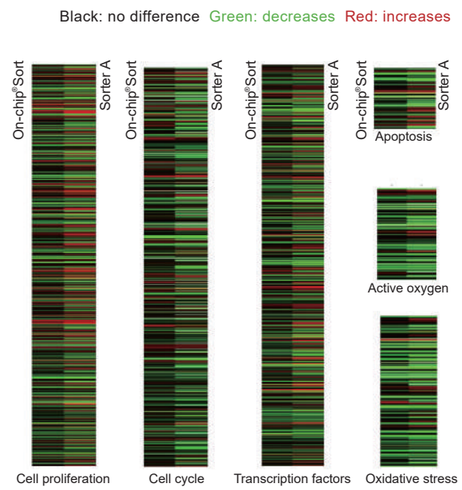
Conventional cell sorter ↓ Cell rupture | On-chip® Sort ↓ ✓Retention of cell morphology

Effects of sorting on gene expression

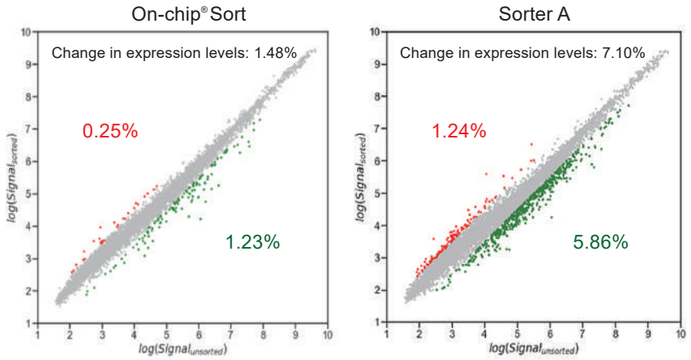
The changes in gene expression levels were investigated after sorting using On-chip® Sort and a conventional cell sorter. For genes related to cell proliferation and apoptosis, which affect cell growth, it was found that the amount of genetic change was smaller when sorting was performed using On-chip® Sort.

In addition, for all the data analyzed, the number of genes whose expression patterns changed significantly was only about one-fifth of that sorted using a conventional cell sorter.

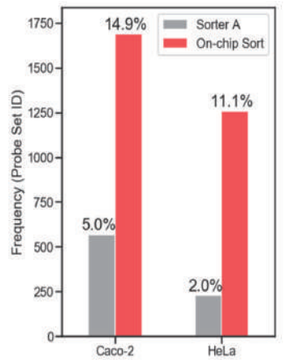
These results suggest that the genetic change induced by sorting on On-chip® Sort is less than that on conventional sorters.



The changes in gene expression levels related to cell growth are lower when using On-chip® Sort, as compared to those after sorting with conventional sorter.



Comparison of gene expression changes for all data, compared to those without sorting.



The percentages of genes with no change in expression level

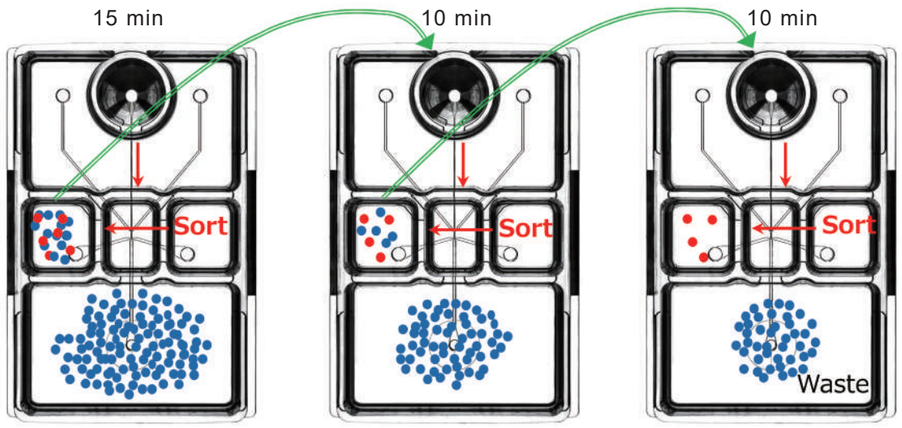
Collaboration with Mr. Oda, Sysmex Corporation & Ms. Yasuda Cell innovator Co., Ltd

Application #3: Sorting of rare cells

- On-chip® Sort enables rapid and accurate recovery of rare cells by repeated sorting of samples. Repeated sorting has always been difficult with conventional cell sorters due to the damage it causes to cells and the inability to analyze small sample volumes, but the use of On-chip® Sort's damage-free sorting technology and microfluidic chip makes multistep sorting of samples possible.

Multistep sorting technology

Multistep sorting is a novel way to isolate very rare target cells present in a highly concentrated bulk sample. At the first sort, liquid pulses created by air pressure deflect the target cell and its surrounding non-target cells into the collection reservoir. The collected cells are re-sorted further to reduce the number of non-target cells and enrich the rare target cells. Each sort takes about 10 min.

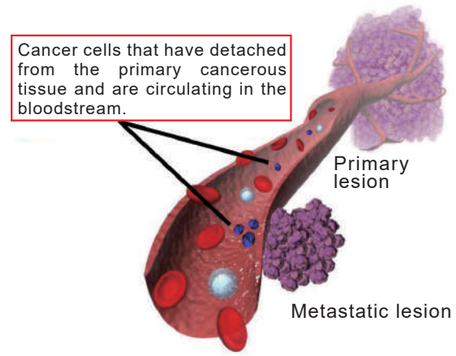


1. Sorting of targets from high-concentration samples
2. Re-sorting of the recovered sample to remove non-targets (purification)
3. Enrichment of only the targets (made possible by the damage-free process)

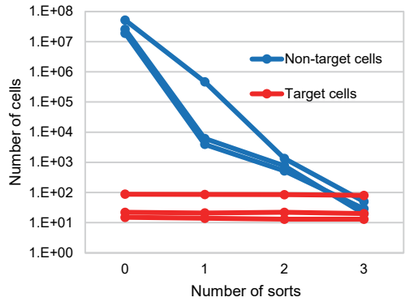
Non-target cells
Rare cells

High-purity recovery of CTCs by Multistep Sorting

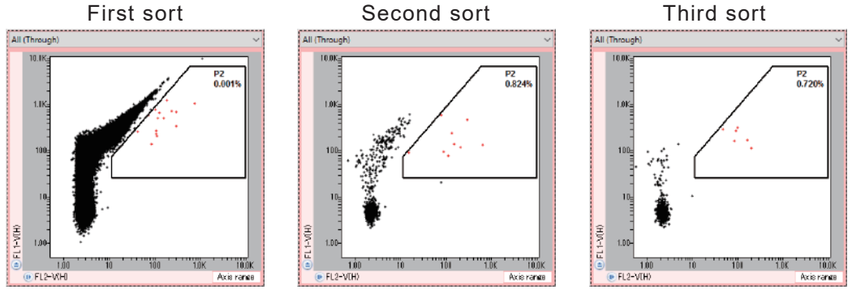
On-chip® Sort allows rapid and high purity recovery of extremely rare cells, such as circulating tumor cells (CTCs). CTCs are cancer cells that have detached from the primary cancerous tissue and are circulating in the bloodstream. The analysis of highly purified CTCs is expected to be used for clinical trials such as cancer diagnosis.

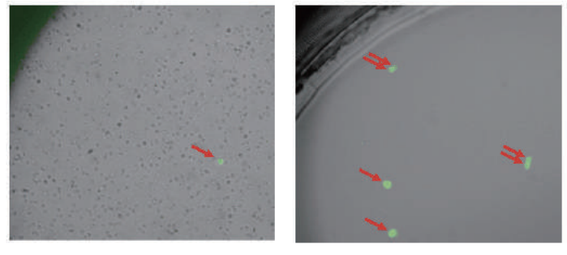


Multistep sorting demonstrated that a few dozen target cells could be recovered with minimal loss from a sample containing 10^7–10^8 cells.



A very small number of target cells (CTCs) present among cells in lysed blood were specifically stained, and multistep sorting was performed to enrich the CTCs. Microscopic observation after three repeated sorts confirmed that CTCs were recovered with high purity.



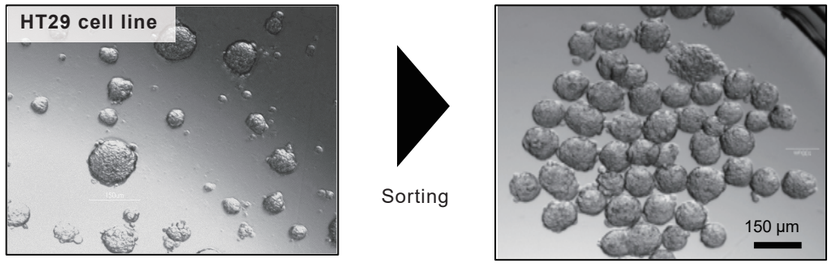


Application #4: Large cell sorting and the freedom of sheath fluid

■ Cell clusters and large particles up to 130 μm in diameter, which conventional cell sorters cannot sort, can be sorted using On-chip® Sort.

Sorting of spheroids (cell clusters)

The use of spheroids (cell clusters) to assess drug sensitivity is important for evaluating drug efficacy in cancer therapy. On-chip® Sort can sort spheroids of a particular size from a spheroid culture or clinical materials for the purpose of preparing a monodisperse spheroid sample.

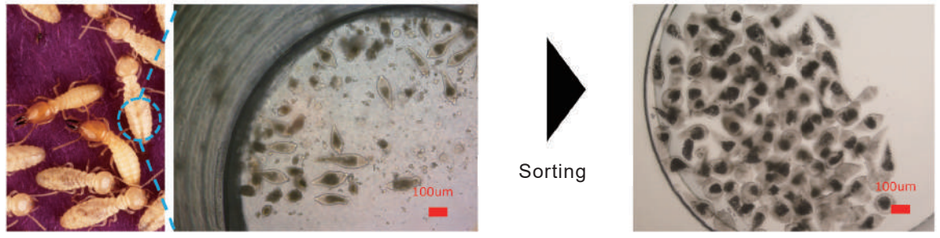


Collaboration with SCIVAX Life Sciences Co., Ltd.

■ On-chip® Sort allows the use of any sheath fluid of your choice, including saline, culture medium, and oil, in order to maintain the samples at their physiological condition and minimize the cell damage during sorting.

Freedom of sheath fluid of your choice

Using a conventional cell sorter to sort those protists remains a challenge, because some protists are large and a specific solution has to be used as sheath fluid to prevent osmotic pressure-induced cell damages during sorting. On-chip® Sort showed to successfully sort out large protozoa.



Formosan termite intestinal protists (about three species)

Collaboration with Dr. Yuki and Dr. Okuma, RIKEN BRC

On-chip® Sort analysis sample examples

Application #1: Sorting of droplets containing encapsulated cells

Microorganisms
- Escherichia coli
- Mycorrhizal fungi
- Bacillus subtilis
- Actinobacteria
- Aspergillus
- Mold
- Yeast
- Oil-producing algae
- Tetrahymena

Environmental samples
- Soil bacteria
- Microorganisms in sea
- Gut bacteria
- Microplankton

Screening (HTS)
- CHO cells
- Hybridoma
- Cell-free translation system
- GPCR reaction system

Genes
- Digital PCR

Non-living materials
- Beads
- PEG
- Gelatin
- Low melting point agarose
- Alginic acid
- Collagen

etc.

Application #2: Damage-free sorting

Cellular tissue
- Hepatocytes
- Muscle cells
- Chondrocytes
- Alveolar epithelial cells
- Purkinje cells
- Retinal ganglion cells
- Cardiomyocytes
- Neurons
- Neuronal nucleus
- Microglia
- Cone cells
- Adipocytes
- Gingival epithelium progenitors
- Fibroblasts
- Vascular endothelial cells
- Cancer cells

Stem cells
- Hematopoietic stem cells
- iPS cells
- Cancer stem cells
- Tooth root cells (Muse)
- Mesenchymal cells

Germ cells
- Spermatozoa
- Ovary cells
- Fertilized ova

Cultured cell lines
- Cloning cells
- Genome-edited cells
- HeLa cells

Blood cells
- T cells
- B cells
- NK cells
- Neutrophils
- Platelets
- Macrophages

Other cells
- Chironomid cells
- Rat neurons
- Fish primordial germ cells
- Drosophila cells
- Mouse tissue cells
- Mouse egg cells
- Zebrafish neurons

etc.

Application #3: Sorting of rare cells

Cancer cells
- Circulating tumor cells (CTCs)

Stem cells
- Removal of undifferentiated iPS cells

etc.

Application #4: Large cell sorting and the freedom of sheath fluid

Protists
- Nematodes (L1 stage)
- Euglena
- Termite intestinal protists
- Nematode eggs

Cell masses
- Spheroids
- Organoids
- Clumps of cancer stem cells
- Bone marrow cell clusters

Plant cells
- Pollens
- Stoma cells
- Mesophyll cells
- Protoplasts

etc.

As of 2022

Main specifications of the instruments

Optical system and detection sensitivity	
Lasers	Up to three lasers (405 nm, 488 nm, 561 nm, and 638 nm; customization available)
Laser class	Class 1 (IEC 60825-1:2014)
Measurement parameters	Forward-Scattered light (FSC), Side-Scattered light (SSC), and 6 PMTs
Size detection sensitivity	FSC: < 0.5 µm, SSC: < 1.0 µm
Fluorescence sensitivity	< 200 MESF FITC
Data analysis capability	Four decades, 18-bit
Pulse analysis	Height, Area, Width
Detection wavelenght	FL1 (445/20 nm), FL2 (543/22 nm), FL3 (591.5/43 nm) FL4 (676/37 nm), FL5 (716/40 nm), FL6(775/46 nm)
Fluid channel system	
Flow cell	Disposable microfluidic chip
Chip material	COP
Channel size	80 µm × 80 µm, 150 µm × 150 µm
Flow rate	≥ 500 mm/sec
Sheath fluid	Any liquid can be used as long as COP is not dissolved. Please consult us.
Sample volume	10 - 1000 µl
Sheath fluid volume	1 - 9 mL
Analysis and Sorting	
Sorting method	Flow Shift method in the microfluidic system
Purity	> 95% (depends on concentration)
Yield	> 90% (depends on conditions)
Cell damage	None
Cross contamination free	Yes, because of the disposable chip
Addressing biohazards	Yes
Pressure	0.3-3 psi
Maximum detection speed	4,000 events/sec
Maximum sorting speed	1,000 targets/sec
Start-up	5 min
Shutdown	10 sec (no cleaning necessary)
Safety	
Aerosol generation	None
Size and weight	
Size (W × D × H)	24.4 × 15.4 × 13.0 in (620 × 390 × 330 mm)
Weight	99 lb (45 kg)
Control PC and control speed	
PC	Laptop PC
OS	Windows 10, 64 bit
Data format	Own format and FCS3.0
Power supply	
Power requirement	AC100-240V, 50/60Hz
Power consumption	240 VA

Information about instruments and consumables

Instrument (On-chip® Sort)

Product number	Product name	Specification	Lasers	Detectors
362S3001	On-chip® Sort HS	Laser 3, FS, SS, FL(6 colors)	488 nm & 638 nm & 405 nm	FL1 FL2 FL3 FL4 FL5 FL6
362S3001G	On-chip® Sort HSG	Laser 3, FS, SS, FL(6 colors)	488 nm & 561 nm & 405 nm	FL1 FL2 FL3 FL4 FL5 FL6
362S3001GR	On-chip® Sort HSGR	Laser 3, FS, SS, FL(6 colors)	488 nm & 561 nm & 638 nm	FL1 FL2 FL3 FL4 FL5 FL6
262S3001	On-chip® Sort MS6	Laser 2, FS, SS, FL(6 colors)	488 nm & 405 nm	FL1 FL2 FL3 FL4 FL5 FL6
252S3001	On-chip® Sort MS5	Laser 2, FS, SS, FL(5 colors)	488 nm & 638 nm	FL2 FL3 FL4 FL5 FL6
252S3001G	On-chip® Sort MS5G	Laser 2, FS, SS, FL(5 colors)	488 nm & 561 nm	FL2 FL3 FL4 FL5 FL6
152S3001	On-chip® Sort LS5	Laser 1, FS, SS, FL(5 colors)	488 nm	FL2 FL3 FL4 FL5 FL6

Lasers: Up to three types including BLUE (488 nm) as standard and two selected from VIOLET (405 nm), YELLOW GREEN (561 nm), or RED (638 nm) can be used.

Detectors: Up to six fluorescence detectors FL1 to FL6 can be installed.
FL1: 445/20 nm, FL2: 543/22 nm, FL3: 591.5/43 nm (607/36 nm when using the green laser)
FL4: 676/37 nm, FL5: 716/40 nm, FL6: 775/46 nm

Consumables (microfluidic chip)

Product number	Product name	Material	Usage	Microchannel size	Packaging unit
1002004	2D Chip-Z1001	COP	Sorting of standard samples	80 x 80 µm	10 chips/box
1002004S	2D Chip-Z1001S	COP	Sorting of standard samples in sterilized condition	80 x 80 µm	10 chips/box
1002005	2D Chip-Z1000-w150	COP	Sorting of cell clusters or large particles	150 x 150 µm	10 chips/box
1002005S	2D Chip-Z1000-w150S	COP	Sorting of cell clusters or large particles in sterilized condition	150 x 150 µm	10 chips/box

Microchannel size;
80 µm × 80 µm

2D Chip-Z1001

Microchannel size;
150 µm × 150 µm

2D Chip-Z1000-w150

For inquiries:

On-chip Biotechnologies Co., Ltd.

Phone: +81-42-385-0461 Fax: +81-42-385-0462
E-mail: info@on-chipbio.com Home page: https://www.on-chipbio.com