



# On-chip® Droplet Selector

Emulsion droplet sorting and dispensing



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

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**ONCHIP-04B-U005**



Realizing innovation in a variety of fields  
from research to industry

# On-chip Droplet Selector



On-chip Biotechnologies will contribute to the development of science and technology in the fields of medicine, food, and the environment by providing innovations in culture/screening technologies.

By promoting our devices, On-chip Biotechnologies is committed to innovation in the fields shown below and to the realization of a sustainable society.

## SUSTAINABLE DEVELOPMENT GOALS

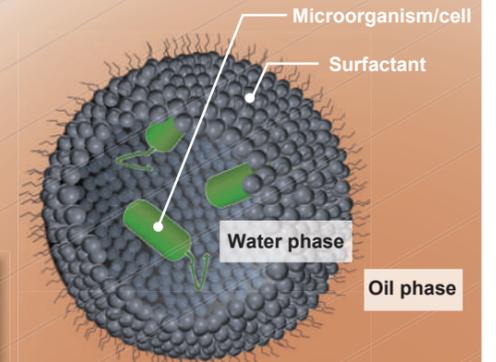
<p>Low-environmental-impact pesticides, soil improvement,</p>  <p><b>2</b> ZERO HUNGER</p>	<p><b>6</b> CLEAN WATER AND SANITATION</p>  <p>Microbial environmental purification methods</p>
<p>Biofuels</p>  <p><b>7</b> AFFORDABLE AND CLEAN ENERGY</p>	<p><b>9</b> INDUSTRY, INNOVATION AND INFRASTRUCTURE</p>  <p>Cutting-edge diagnostic technology and cell research</p>

## A Game Changer in Screening

Droplet technology is attracting attention as a next-generation method that can dramatically improve the efficiency of conventional screening. It has already being used for a wide range of applications, including cultivation of microbial dark matter, drug screening, and acquisition of mutant strains. It is expected to become more widespread in the future.

### Droplet — water-in-oil emulsions —

Microscopic water droplets dispersed in oil and stabilized by surfactants, enabling cells and microorganisms to be encapsulated and cultured one by one.



#### High throughput

Over one million droplets can be prepared in 10 min.

#### Screening

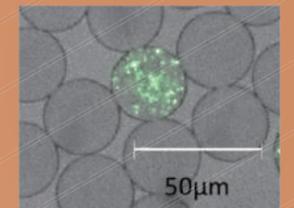
Isolation of target microorganisms from diverse environments such as soil and intestines.

#### Single-cell analysis

Easy compartmentalization of hundreds of thousands of cells to the single cell level.

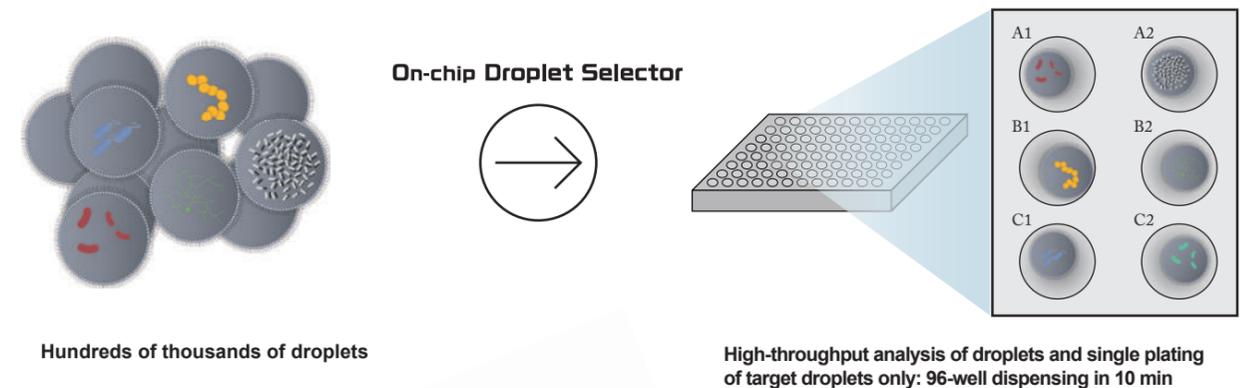
#### Small culture environment

Scarce reagents can be saved in picoliter-sized droplets.



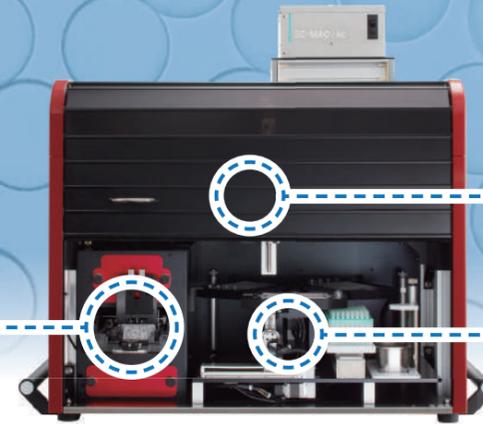
Droplet containing culture of *E. coli* (expressing GFP)

On-chip® Droplet Selector is a device that combines the sorting of desired droplets with our microfluidic chip technology and dispensing of single sorted droplets into a 96- or 384-well plate. Droplets can be dispensed without breakage, with accuracy of over 90%.



With conventional cell sorters, handling droplets in oil is not possible due to the difficulty in using oil as the sheath fluid and the droplets' lack of resistance to physical shock. This makes secondary analysis and isolation of droplets containing cells/microorganisms difficult.

With the use of On-chip<sup>®</sup> Droplet Selector, the time and effort of droplet screening and dispensing can be greatly reduced. On-chip<sup>®</sup> Droplet Selector is useful in applications including investigation of novel microorganisms in the environment, establishment of high-producing strains and screening of antibody-producing cells.



Clean work environment

- HEPA filter keeps the air inside the device clean.
- Use of disposable microfluidic chips eliminates the risk of cross-contamination.
- Can fit inside an anaerobic chamber.



Easy operation and maintenance free

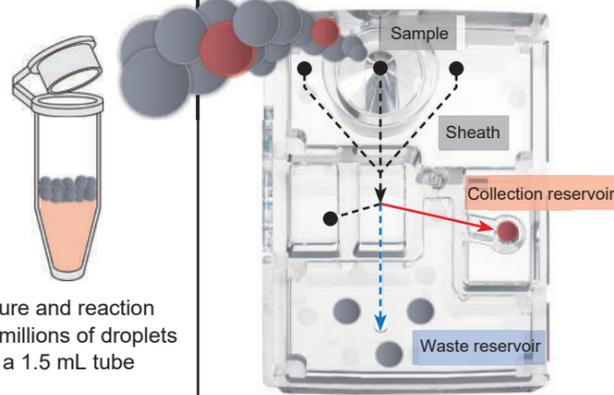
- Analysis and sorting can be started in less than 5 min after start-up.
- No maintenance required: no need for complicated cleaning of instrument.



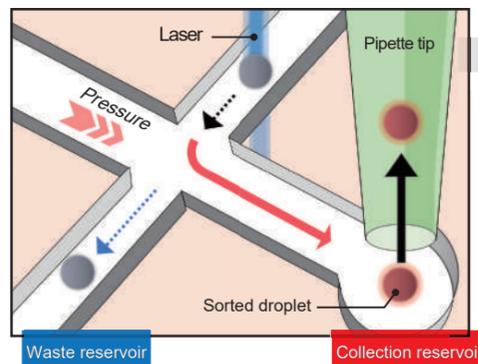
Detection and separation in a microfluidic channel

- Acquisition of forward and side scattered light (FSC/SSC) and fluorescence information of all droplets using the principle of flow cytometry.
- Target droplets are separated one by one by pneumatic control (patent Nos. US10101261, US10222317, US10724938, and US10648899).
- Not only droplets, but also cells and gel micro-drops (GMDs) can be analyzed and sorted

Filling the microfluidic chip with sample

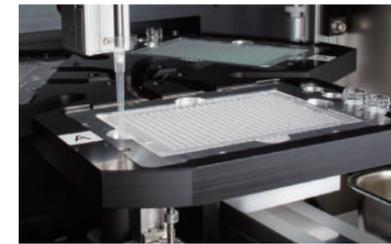


2D Chip-SD1000 (microfluidic chip)



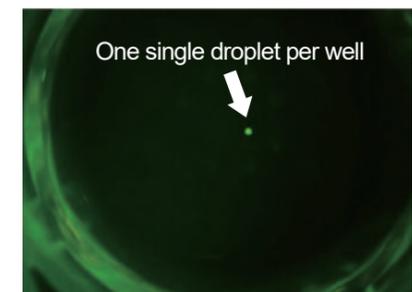
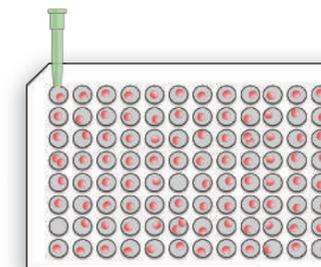
Sorting area of the microfluidic chip

Suctioned droplets are transferred to a well plate.



Dispensing one by one into a well plate

- Droplets collected on the microfluidic chip are dispensed.
- Dispensing on to a 96-well plate can be completed in 10 min.
- Up to three 96-/384-well plates can be placed.
- Culture can be carried out immediately by dispensing medium in advance.



Isolation of targets in 0.1% of one million samples



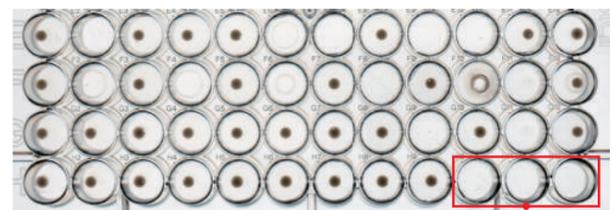
Expanding research areas with On-chip<sup>®</sup> Droplet Selector

- Search for novel microorganisms in the environment
- Rapid establishment of high-producing strains
- Screening of antibody-producing cells

\*Droplets can be prepared using On-chip Droplet Generator.

### Single plating of droplets containing rhizobia

Rhizobia were encapsulated in approximately 5% of the total droplet population and cultured and droplets containing the grown rhizobia were dispensed into 96-well plates using On-chip® Droplet Selector. After incubation in the well plate, growth was confirmed in 32 out of 45 wells where rhizobia were dispensed.



Control (not dispensed)

Well plate culture, 14 days later

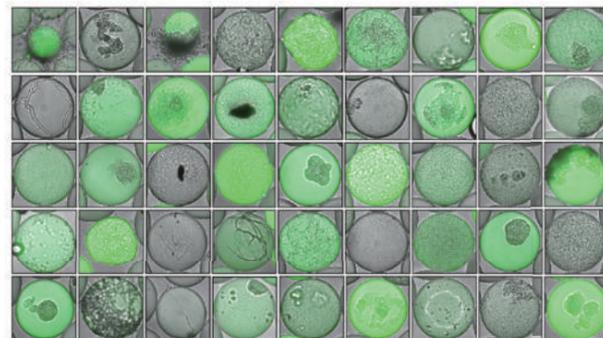
Number of wells with dispensed rhizobia : 45  
 Number of wells with rhizobial growth : 32  
 Growth rate : 71.1%

► Demonstrates that droplets containing microorganisms can be dispensed with high precision and the microorganisms can be

### Screening of enzyme-producing microorganisms from the soil environment

Microorganisms collected from soil were enclosed and cultured in droplets with substrates that react with enzymes (peptidases). As a result, various growth forms and enzyme activities of the microorganisms were observed.

Droplets showing the target enzyme activity can be isolated at high throughput using On-chip® Droplet Selector, which is expected to lead to the discovery of new microorganisms.



Droplet culture of environmental microorganisms and evaluation of fluorescence intensity

Collaboration with Prof. Ogasawara, Nagaoka University of Technology

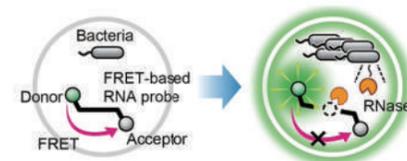
► Enables targeted screening of target activity from a variety of environments

### Detection of microorganisms grown in droplets

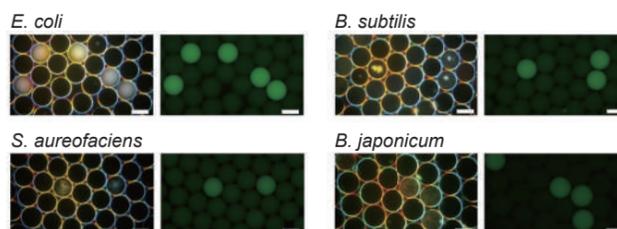
FNAP-sort (fluorescent nucleic acid probe in droplets for sorting bacteria) is a system that enables the detection of microorganisms grown in droplets.

In the cases where Escherichia coli, Bacillus subtilis, Streptomyces, and Bradyrhizobium japonicum were cultured in droplets, it was confirmed that growth activity could be easily detected as an increase in fluorescence intensity.

This system can be used for applications such as screening and culturing of environmental micro-



The principle of FNAP-sort: the fluorescence intensity increases when RNase produced by microorganisms cleaves the probe.



Detection of proliferated microorganisms (bright-field image on the left, fluorescent image on the right)

► Development of a simple method for detecting growth activity in droplet culture

Collaboration with Research Group Leader Noda, AIST, Biomedical Research Institute Ota, Y., Saito, K. et al. PLoS ONE 14(4): e0214533. Under the licence of Attribution 4.0 International (CC BY 4.0) (<https://creativecommons.org/licenses/by/4.0/>).

### Device specifications

Laser	Three lasers can be selected (405 nm, 488 nm, 561 nm, 638 nm)
Laser class	Class 1 (IEC 60825-1:2014)
Measurement parameters	Forward-scattered light (FSC), side-scattered light (SSC), and 6 PMT
Detection wavelength	FL1: 445/20 nm, FL2: 543/22 nm, FL3: 591.5/43 nm (607/36 nm when 561 nm laser is used), FL4: 676/37 nm, FL5: 716/40 nm, FL6: 775/46 nm
Sample size for analysis	0.5 – 125 µm
Sorting mode	1,000 events/sec
Dispensing speed	96 well/10 min (dependent on the target ratio)
Dispensing accuracy	> 90% (depends on the samples)
Time taken to start operation	5 min
Shutdown	10 sec (no need for cleaning)
Size (W × D × H)	29.9 × 19.7 × 31.5 in (760 × 500 × 800 mm)
Weight	165 lb (75 kg)
Control	Laptop PC (Windows 10)
Power input (main unit)	AC 100 – 240 V, 50/60 Hz
Power consumption (main unit)	240 VA
Power input (HEPA filter)	AC 100 V, 50/60 Hz
Power consumption (HEPA filter)	35 W

### 【On-chip® Droplet Selector】

Product No.	Product name	Specifications	Laser	Detectors
362DS001	On-chip® Droplet Selector HS	Laser 3, FS, SS, FL (6 colors)	488 nm, 638 nm, 405 nm	FL1, FL2, FL3, FL4, FL5, FL6
362DS001G	On-chip® Droplet Selector HSG	Laser 3, FS, SS, FL (6 colors)	488 nm, 561 nm, 405 nm	FL1, FL2, FL3, FL4, FL5, FL6
362DS001GR	On-chip® Droplet Selector HSGR	Laser 3, FS, SS, FL (6 colors)	488 nm, 638 nm, 561 nm	FL1, FL2, FL3, FL4, FL5, FL6

### 【Consumables】



#### Microfluidic chip

Product No.	Product name	Material	Channel size	Packing unit
1004001	2D Chip-SD1000	COP	80 × 80 µm	10 chips/ box
1004002	2D Chip-SD1000-w150	COP	150 × 150 µm	10 chips/ box

#### Gasket & Tip

Product No.	Product name	Packing unit
80030	Silicon Gasket for Droplet Selector	1 piece/box
1007011SF	DS Dispensing Tip 96SF	96 tips/rack

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