

Detection of Microbial Growth in Droplets Using Membrane Staining Reagents

Direct microbial staining enables the detection of microbial growth based on the microbial cell count.

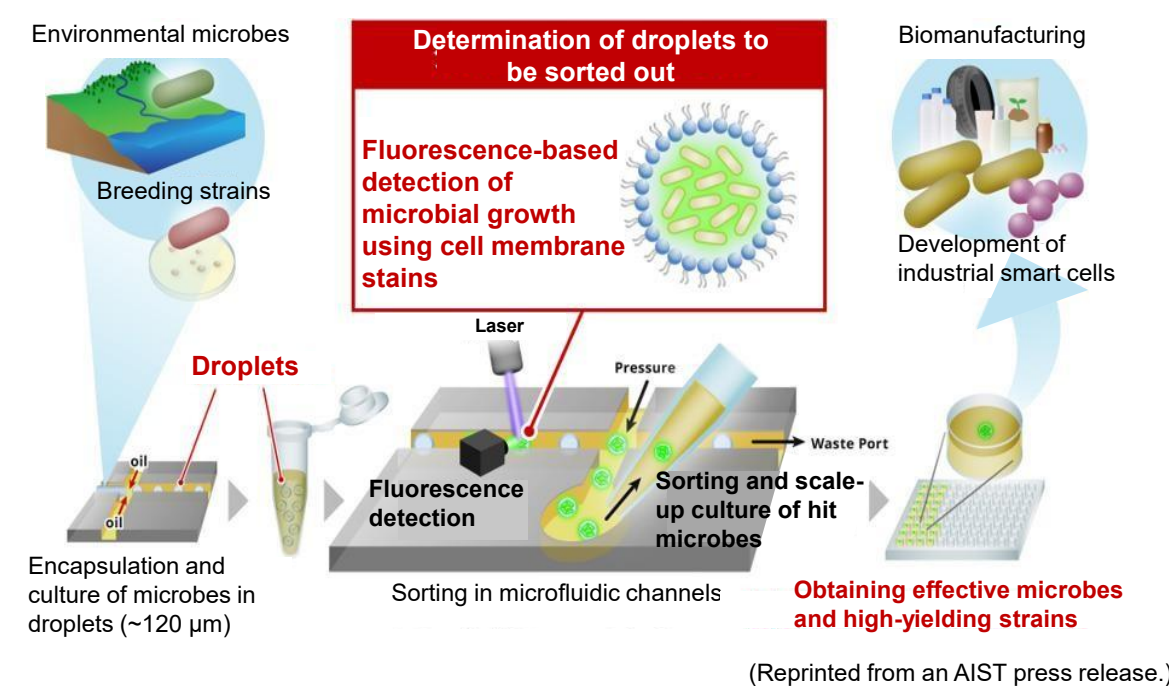
- A cell count-dependent detection system based on cell membrane staining using “turn-on” type membrane stains:
- Enables fluorescent staining of microbes growing in droplets simply by encapsulating microbes and cell membrane stains together.
 - Is applicable to microbial screening using the cell count of microbes present in droplets as an indicator.
 - Functions as a universal microbial detection system because cell membranes exist in both prokaryotes and eukaryotes.

Abstract

In the “million-scale screening” using droplets, the technology to distinguish between droplets containing microbes and those without is the basis of measurement and evaluation. In this study, we developed a novel method to determine microbial growth in water-in-oil droplets based on fluorescent staining of the cell membrane components of the microbes. Encapsulation of *E. coli* together with “turn-on” type cell membrane staining reagents (e.g. On-chip MiMe-Stain reagents) in droplets allowed us to obtain fluorescence intensity dependent on the cell count. This method also enabled the selective detection and sorting of droplets containing existing/proliferating microbes for various types of microbes, allowing for quantitative evaluation of the degree of proliferation.

Detection of microbial growth in W/O droplets

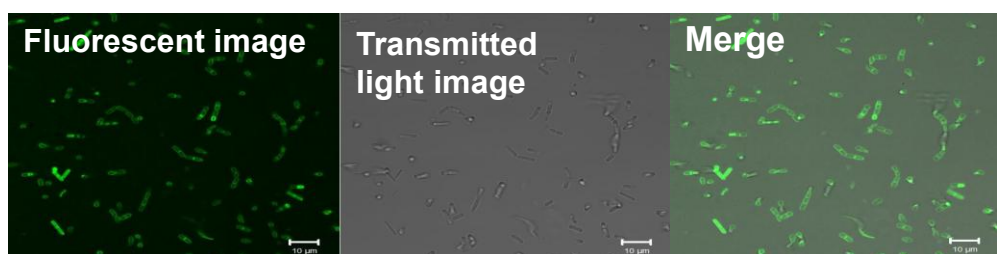
One of the strengths of droplets is that they can be used to evaluate proliferative activity at the single-cell level.



FNAP-sort: Highly sensitive but has issues with background caused by factors such as culture medium components and does not necessarily directly reflect the microbial cell count.

Features of cell membrane stains

- Fluorescent reagents that show a significant increase in fluorescence intensity when localized to cell membranes.
- Usable for any type of microbes or cells since they all have membrane components.
- Have a certain degree of specificity, leading to possible capability for excluding non-microbial substances.
- Enable the cell count-dependent detection of cells since there is a correlation between cell counts and the amounts of membrane components.
- Allow us to standardize cell counts as an indicator to study microbial activity in combination with other indicators.
- Show low cytotoxicity.

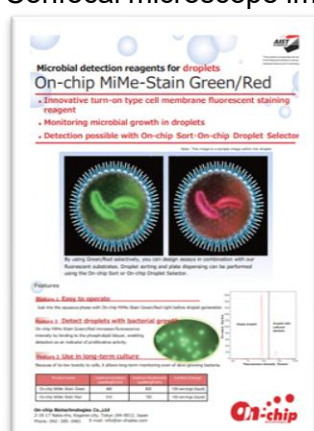


Confocal microscope images of soil microbes stained with a cell membrane stain (red)

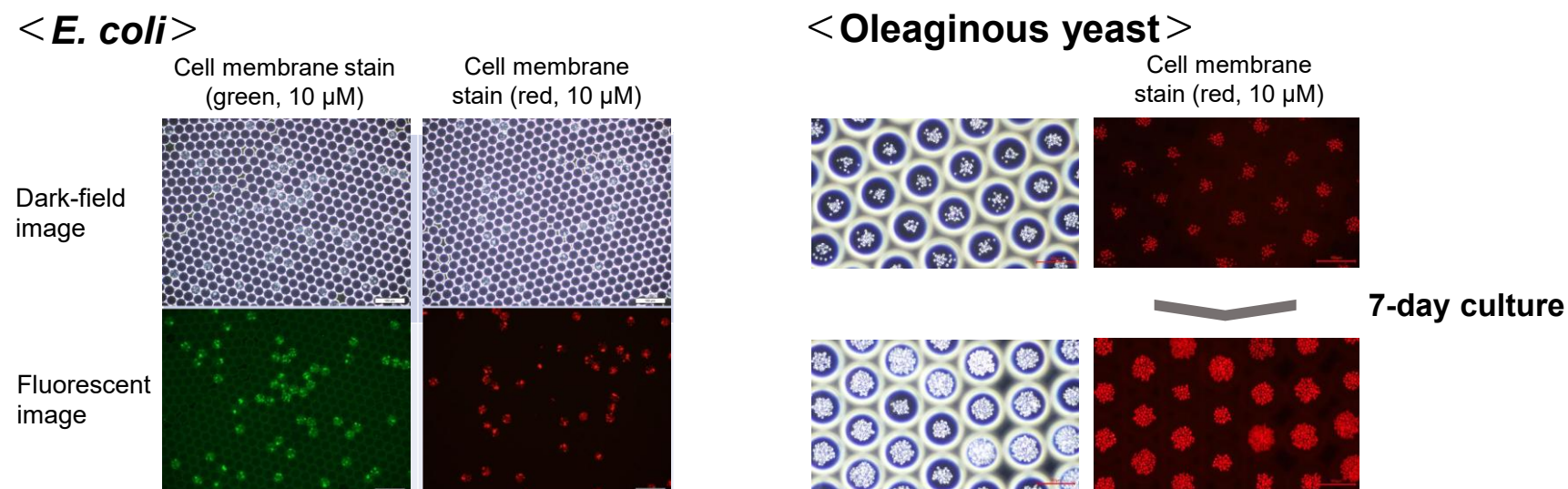
Microbial detection reagents for droplets On-chip MiMe-Stain Green/Red

Available from On-chip Biotechnologies
(from October 1, 2024).

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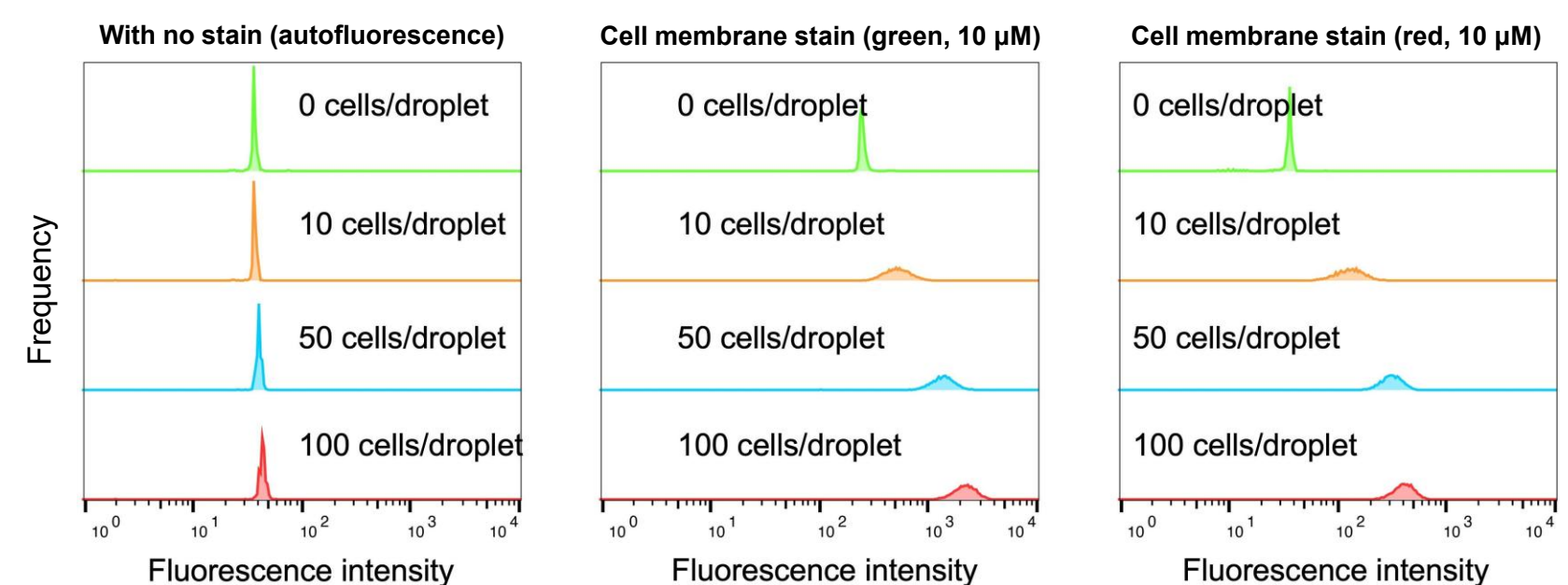


Examples of microbial staining in droplets



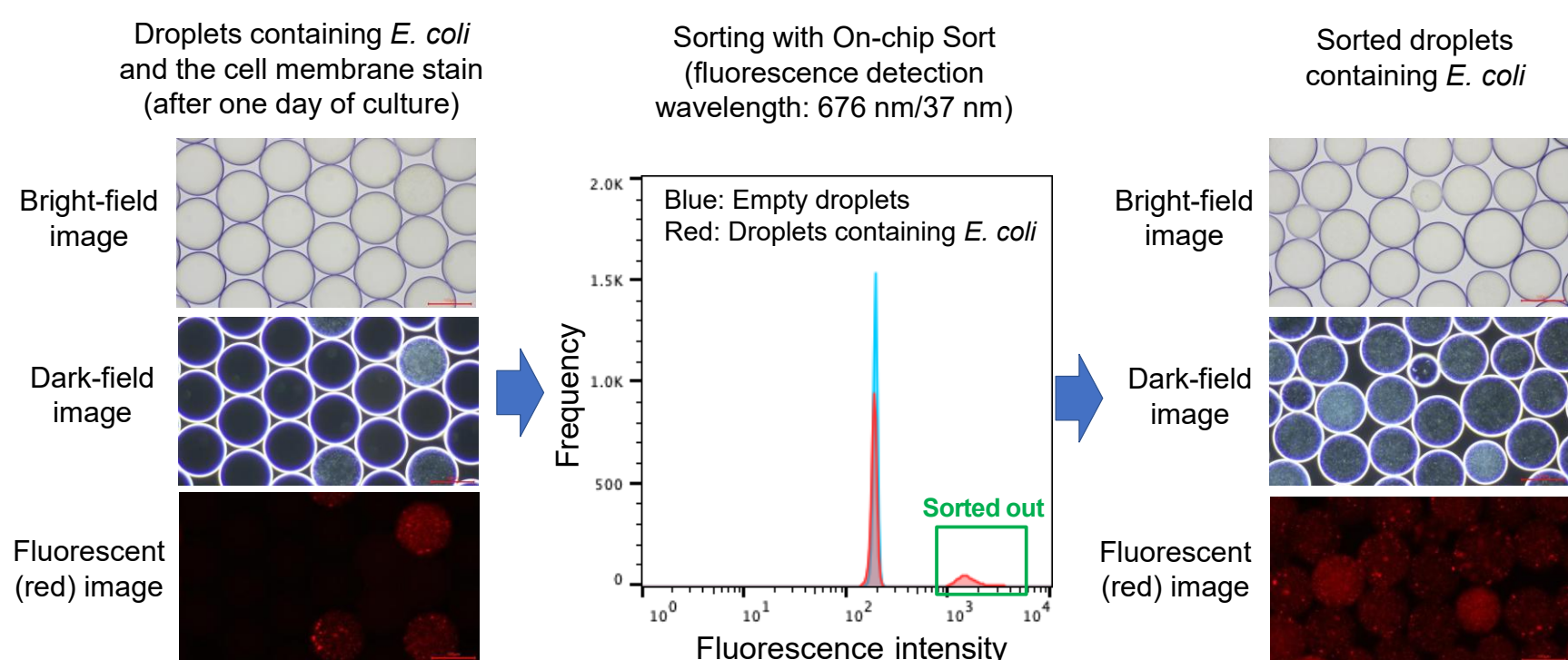
► Droplets containing proliferated microbial cells were successfully detected based on fluorescence.

Detection of cell count-dependent fluorescence intensity using the droplet sorter (for *E. coli*)



► Fluorescence intensity dependent on the cell count of *E. coli* encapsulated in droplets was shown.

Droplet sorting and isolation using cell membrane stains



Sorting results obtained after culturing *E. coli* encapsulated in droplets at $\lambda = 0.2$ for one day and microscope images of the droplets before and after the sorting (cell membrane stain: 20 μM (final conc.))

► Sorting of droplets containing proliferated microbes was possible using the fluorescence of the cell membrane stain as an indicator.

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