

Under Development

Specification and appearance are subject to change without notice

Application
note
Vol.4

CELLNETTA MZM1 SERIES

Single-cell purification

Background

The extraction of single-cells from cell suspensions is very common in cell processing. In these situations, the percentage of single cells (purity) and cell damage affect the outcome of subsequent processing. For this reason, the careful purification of high-purity single-cell suspensions is an important step in cell processing.

Here, we will introduce a case study of single-cell purification of a cell aggregate suspension using CELLNETTA, conducted by Professor Masahiro Inoue of the Department of Clinical Bio-Resource Research and Development at the Graduate School of Medicine of Kyoto University.

Implementation method

- (1) Add Trypsin EDTA to the KUC6 cell mass suspension (Figure 1) and carefully pipette 10 times.
- (2) Incubate for 10 minutes, then carefully pipette 100 times.
- (3) Add 10 µg/ml of DNase I and allow to stand for one minute.
- (4) Apply hydrophilic treatment to the CELLNETTA. *
- (5) Pour the cell suspend through a 10-µm mesh CELLNETTA (Figure 2, Operation 1).
- (6) Pour the flow-through through a 6-µm mesh CELLNETTA and recover the single-cell suspension (Figure 2, Operation 2).

* For more information, please refer to the "Hydrophilic Treatment Manual" in the CELLNETTA User Guide.

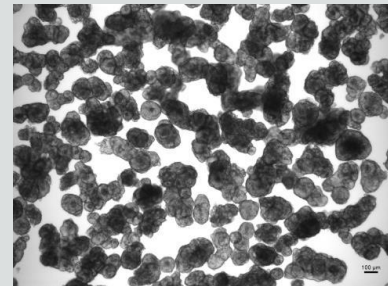
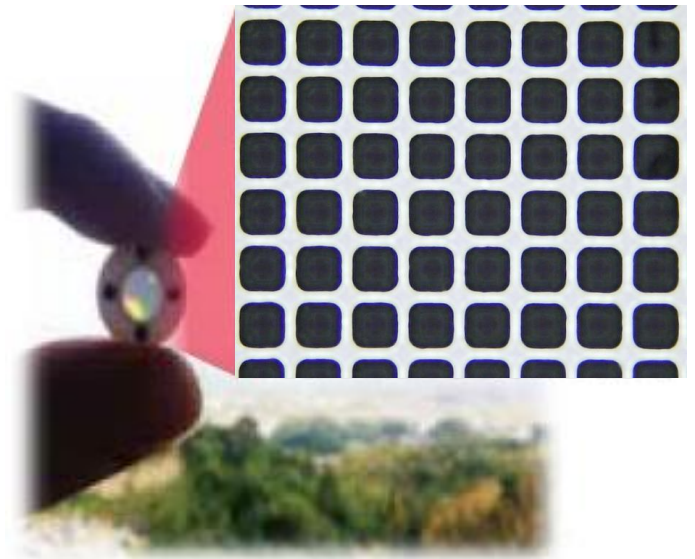


Figure 1: Micrograph of the KUC6 cell mass suspension. Cell masses larger than 40 µm in diameter can be observed. The scale bar is 100 µm.

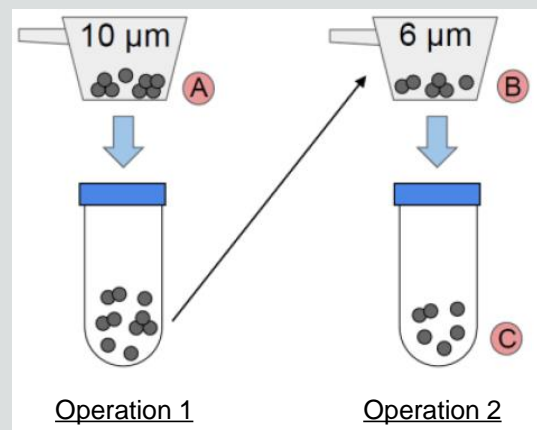


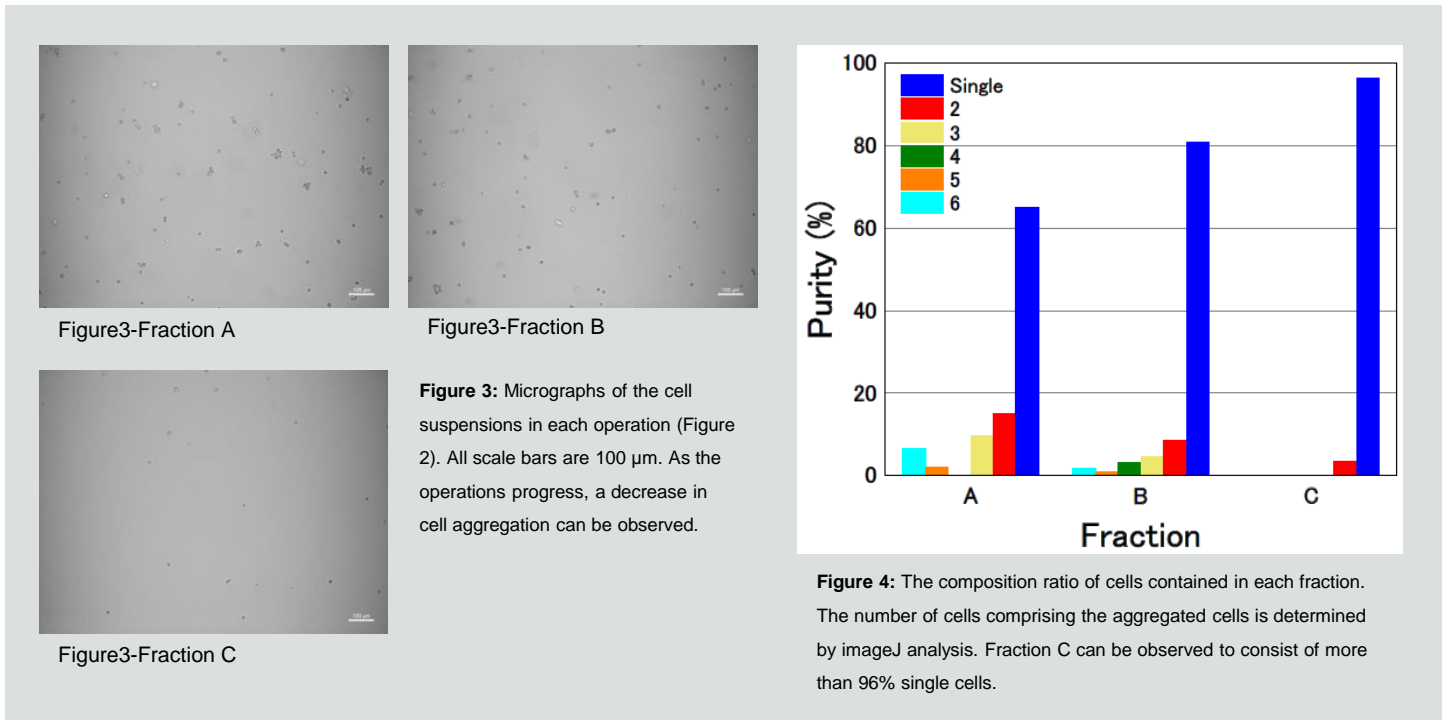
Figure 2: Operation diagram of single-cell purification. Large aggregated cells are removed in Operation 1, and the single-cell suspension is recovered in Operation 2.

Results

As shown in the micrographs (Figure 3) for each fraction (A to C) in Figure 2, solely performing the usual pipetting after the addition of Trypsin leaves a considerable amount of aggregated cells. However, as operations progress using CELLNETTA, the number of aggregated cells decreases and the number of single cells increases.

Figure 4 shows the results of the ImageJ analysis of micrographs images in order to clarify the composition of each fraction. Each colored bar chart indicates the number of cells that comprise the aggregated cells. The vertical axis indicates

The ratio of aggregated cells relative to the total number of aggregated cells in each fraction. As shown in Figure 4, it was confirmed that single-cell suspensions with a purity of 96% or higher could be recovered in Fraction C by using CELLNETTA. **These results indicate that operations using CELLNETTA can fractionate single cells and aggregated cells with high accuracy.**



Product used in this application note

Notes

Pore size	Gamma Irradiation	Product number (P/N)
6 μm (custom-made)	Gamma Irradiated	Please contact us.
	Non-Gamma Irradiated	Please contact us.
10 μm	Gamma Irradiated	MZM1B040B50G
	Non-Gamma Irradiated	MZM1B040B50N

- This product is not a medical device.
- This product is a sample for evaluation purpose.
- Please do not ship out your completed product with the sample.
- We shall not be liable for any claims on the sample in case it is shipped out to the market.

