Application Note 06

Reliable Single Cell Cloning with Advanced Microfluidic Dispensing

Background

Within biopharmaceutical discovery and development, there is an inherent need for single cells in the production of biologics. Ensuring that biotherapeutics are derived from a single clonal source has been stipulated by the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) in order to ensure consistency and quality of production and to reduce population heterogeneity. The Cyto-Mine® Single Cell Analysis System has been specifically designed to ensure monoclonality via accurate and reliable single cell dispensing.

Cyto-Mine® is a fully integrated device that streamlines cell isolation, productivity measurement, sorting, imaging and dispensing into one automated process which reduces timelines, increases screening capabilities and assures monoclonality (Figure 1). Dispensing is one of the key functionalities of Cyto-Mine®. Reliable and efficient dispensing is a critical performance criterion to prove the feasibility of Cyto-Mine® as a tool for cloning cells.
To demonstrate the fidelity and reliability of cell dispensing of Cyto-Mine®, a series of experiments were conducted to show that the images taken of encapsulated cells before dispensing corresponded to the number of cells dispensed into each well.



Figure 1 The Cyto-Mine® Single Cell Analysis System.

sphere bio
work small, think big.

Aims and objectives

- This study examined fidelity and reliability of cell dispensing by Cyto-Mine®
- Both fluorescent beads and cells were analyzed to test accuracy
- This study demonstrates that Cyto-Mine® provides a reliable, high-throughput platform for selective single cell cloning

Methods and results

Cyto-Mine® Workflow

Cyto-Mine® harnesses picodroplet technology to encapsulate single cells into an individual picodroplet along with assay reagents; the picodroplet is then incubated at 37°C to allow cell secretion and a productivity assay to develop. The productivity assay uses a pair of fluorescently-labelled affinity probes (e.g. antibodies), both binding to the target IgG molecule to generate a fluorescent signal which is detected by Cyto-Mine®.

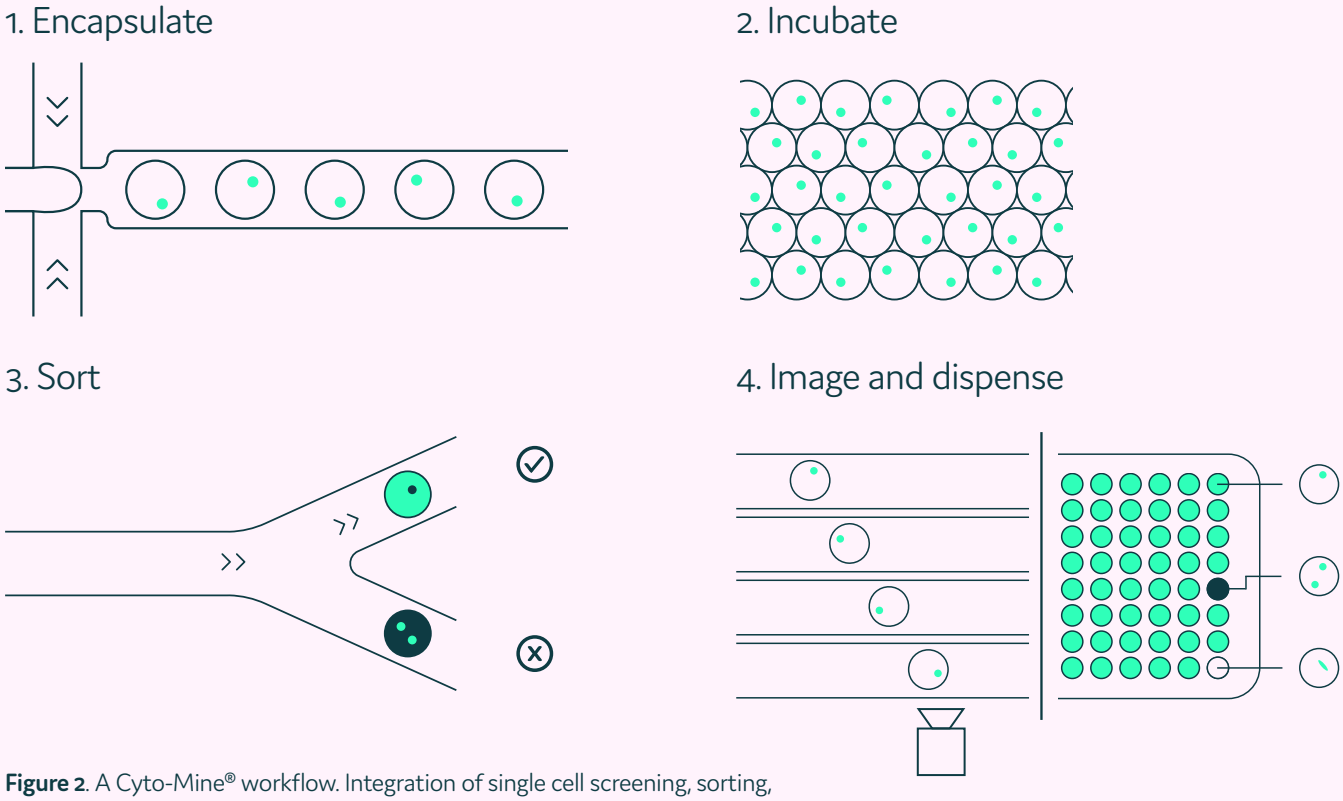

Figure 2. A Cyto-Mine® workflow. Integration of single cell screening, sorting, isolation, and verification using a fully integrated microfluidic process.

Continued

Cyto-Mine® can then sort each picodroplet based on the assay results and dispense the 'hit' cells into multi-well microtiter plates. Before dispensing the Cyto-Mine® images each picodroplet five times, providing clonality assurance, (Figure 2 and 3). This study examines the correlation of the cells in these images with the cells in the dispensed microtiter plate.

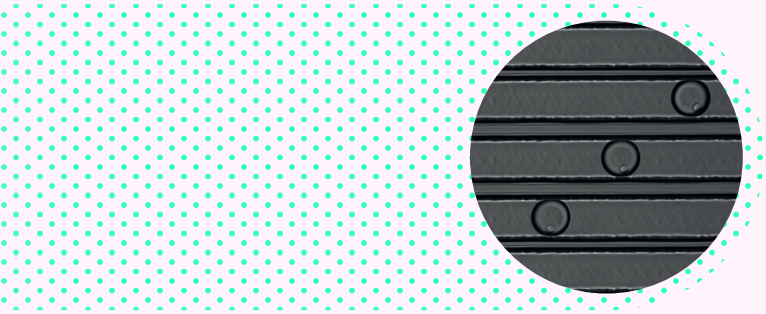

Figure 3. Assurance of monoclonality. Cyto-Mine® provides five sequential images of each picodroplet before dispensing. As the picodroplet travels through the microfluidic channels it rotates changing the position and optical view of the cell inside it.

Assessing Dispensing Fidelity

In this study we examined the fidelity of dispensing both fluorescent beads and cells. The initial experiments were carried out using fluorescent beads, to remove any biological variability and minimize user-related errors; this was then followed by analysis of an IgG-secreting CHO cell line (kindly provided by FUJIFILM Diosynth Biotechnologies, UK).
In order to evaluate the accuracy of Cyto-Mine® dispensing, the number of beads or cells in each dispensed picodroplet were manually counted from their images taken by Cyto-Mine® prior to dispensing (Cnt_{drop}) and from the corresponding wells (Cnt_{well}) (Figure 4).

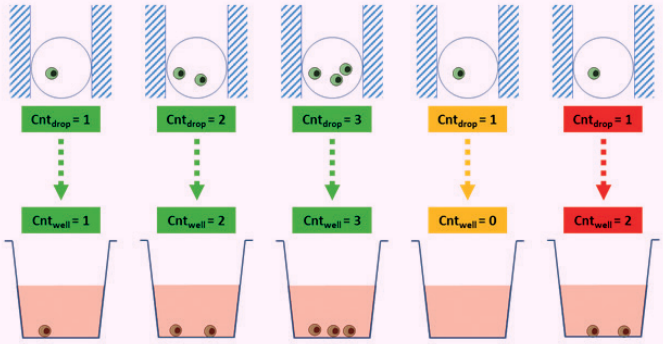

Figure 4. Principle of Cyto-Mine® Dispensing Fidelity Test. This image shows the different potential outcomes of the dispensing fidelity test for each picodroplet dispensed. Green wells represent accurate dispensing, i.e. correlation of the image and the well count; yellow represents a discrepancy where the number of cells in the well is lower than in the cells counted in the image; and lastly, red represents a discrepancy where the number of cells in the well is higher than the cells counted in the image.

Analysis of Fluorescent Beads

Over five weeks, eleven separate experiments were carried out to assess the dispensing fidelity of fluorescent beads. A high input concentration ($3x10^6$/mL) was used to maximize the diversity of occupancy of fluorescent beads in picodroplets and wells, so that any discrepancies in bead counts between the picodroplet and the well could be easily spotted.

Figure 5 shows an example data set obtained from dispensing fluorescent beads into a 96-well microtiter plate. In each square, the number at the top is the bead count in the picodroplet, and the number at the bottom is the bead count in the corresponding well. On average, Cyto-Mine® correctly dispensed the same number of beads as imaged with an accuracy of 99.3% (Table 1).

	1	2	3	4	5	6	7	8	9	10	11	12	
A	3 3	2 2	2 2	1 1	1 1	1 1	1 1	1 1	5 5	1 1	1 1	1 1	Cnt_{drop} Cnt_{well}
B	3 3	4 3	1 1	1 1	2 2	1 1	2 2	1 1	2 2	1 1	5 5	5 5	Cnt_{drop} Cnt_{well}
C	3 3	6 6	1 1	1 1	2 2	2 2	6 6	1 1	1 1	1 1	3 3	1 1	Cnt_{drop} Cnt_{well}
D	1 1	1 1	1 1	2 2	1 1	1 1	2 2	1 1	3 3	3 3	2 2	2 2	Cnt_{drop} Cnt_{well}
E	1 1	3 3	1 1	3 3	2 2	1 1	1 1	1 1	1 1	2 2	1 1	4 4	Cnt_{drop} Cnt_{well}
F	2 2	1 1	1 1	3 3	1 1	1 1	1 1	1 1	6 7	4 4	1 1	1 1	Cnt_{drop} Cnt_{well}
G	3 3	1 1	1 1	2 2	2 2	1 1	4 4	1 1	1 1	1 1	2 2	1 1	Cnt_{drop} Cnt_{well}
H		1 1	2 2	2 2	2 2	1 1	3 3	1 1	1 1	1 1	2 2	8 8	Cnt_{drop} Cnt_{well}

Figure 5. Example data for one 96-well microtiter plate dispensed with 20 µm fluorescent beads.

Table 1. Dispensing Accuracy of Beads. This table shows the number of wells where the beads counted in each well either corresponded, were fewer than, or were more than the image reported by Cyto-Mine®. Fluorescent beads were dispensed into 10,560 wells (110 96-well microtiter plates). In each of the eleven experiments ten 96-well microtiter plates were dispensed (960 wells were processed per experiment).

Experiment number	Beads counted corresponded with image ($Cnt_{well} = Cnt_{drop}$)	Fewer beads ($Cnt_{well} < Cnt_{drop}$)	More beads ($Cnt_{well} > Cnt_{drop}$)
1	940	10	10
2	960	0	0
3	957	3	0
4	950	7	3
5	958	0	2
6	953	0	7
7	959	1	0
8	960	0	0
9	943	7	10
10	951	2	7
11	960	0	0
Total	10,491	30	39
Percentage despensing accuracy	99.34%	0.28%	0.36%
CV (%) of dispensing accuracy	0.7%	/	/

Analysis of CHO Cells

After the confirmation of accurate dispensing of fluorescent beads, a CHO cell line was used to assess the cell dispensing accuracy of Cyto-Mine®. IgG-secreting CHO cells were prepared in culture medium with an IgG detection reagent and loaded into a Cyto-Cartridge®. The CHO cells were then analyzed on the Cyto-Mine® Single Cell Analysis System and sorted based on fluorescence. Fidelity was assessed over five independent experiments with CHO cells dispensed into three 96-well microtiter plates per experiment (Table 2). The results showed that Cyto-Mine® dispensed the expected number of cells with 98.9% accuracy.

Table 2. Dispensing Accuracy of Cells. This table shows the number of wells where the cells counted in each well either corresponded, were less than, or were more than the image reported by Cyto-Mine®. CHO cells were dispensed into 1,373 wells (fourteen 96-well microtiter plates, some wells were used as controls and some wells were not able to be counted). In each of the five experiments two or three 96-well microtiter plates were dispensed.

Experiment number	Cells counted corresponded with image ($Cnt_{well} = Cnt_{drop}$)	Fewer cells ($Cnt_{well} < Cnt_{drop}$)	More cells ($Cnt_{well} > Cnt_{drop}$)
1	266	5	0
2	282	2	1
3	285	0	0
4	186	3	1
5	281	2	0
Total # wells	1300	12	2
Percentage despensing accuracy	98.9%	0.91%	0.15%
CV (%) of dispensing accuracy	1.3%	/	/

Conclusions

In conclusion, Cyto-Mine® was able to dispense and report clonality of both fluorescent beads and CHO cells with accuracy greater than or equal to 98.9%. The fully integrated and automated workflow of Cyto-Mine® provides a very high fidelity of dispensing and offers a reliable and high-throughput platform for selective single cell cloning. Sphere Bio has patented and is now implementing new software developments using machine learning to further improve image recognition and enhance fidelity.

The sequential images taken before dispensing combined with the high fidelity of dispensing provides important support for the submission to various international regulatory bodies of cell lines for the production of monoclonal antibodies and other biologics.

Notes

sphere bio

work small, think big

sales@fluidic.com
spherebio.com

The trademarks used herein are the property of Fluidic Sciences Ltd or their respective owners.

For research and development purposes only.
Product specifications subject to change without notice.
Content ©Fluidic Sciences Ltd.

Fluidic Sciences