TECHNICAL NOTE

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Efficient cell capture in droplets with Xdrop®

Summary

- With accessible microfluidics, Xdrop® encapsulates living cells into double-emulsion droplets.
- Cell encapsulation follows the Poisson distribution, and droplets with different number of cells provide powerful and convenient integrated controls.
- Mammalian cells are encapsulated in double-emulsion droplets at an efficiency > 90%.

Introduction

The analysis of single cells and their interactions presents a promising avenue for innovative research [1, 2]. Xdrop is a droplet generator instrument, which in conjunction with Xdrop DE50 cartridge, produces double-emulsion droplets (referred to as DE50 droplets), each with a volume of approximately 100 pl. The simultaneous production of 8 times 750,000 double-emulsion droplets is accomplished in minutes. These droplets serve as ideal vessels for encapsulating mammalian cells, facilitating single cell studies.

The encapsulation of cells into DE droplets, enables high-throughput analysis of single cells and their environment using well-established methods such as flow cytometry. However, the success of such studies depends on the ability to estimate the number of cells encapsulated within each droplet.

The Poisson distribution is a statistical distribution model, that predicts the probability that a given number of events occurs in a fixed interval of time or space. In the cell encapsulation context, the Poisson model predicts the theoretical distribution of cells into a given number of droplets.

Here, we demonstrate that encapsulation of mammalian cells with Xdrop follows the Poisson distribution, enabling the prediction of the number of droplets with encapsulated cells. The encapsulation efficiency is above 90% for optimal cell concentrations and encapsulation into and incubation in DE50 droplets does not impact cell viability.

The percentage of droplets with encapsulated cells follows Poisson distribution

Cells to be encapsulated were cultured in RPMI-1640 media (K-562 lymphoblasts, Ramos B lymphocytes and Jurkat T lymphocytes) or in F12 media (CHO epithelial ovary cells) supplemented with 10% FBS and 1% antibiotics. At the onset of the experiment, cells were harvested and stained with CellTrace® CFSE dye (Thermo Fisher). The cells were resuspended at different concentrations in fresh media containing a Cy5-conjugated DNA probe for droplet staining, along with 10% OptiPrep™. Suspensions of stained cells were used for droplet production with Xdrop and the Xdrop DE50 cartridge as described in the Xdrop manual[3]. Droplets were then analyzed on the Sony® SH800S flow cytometer to count the total number of cells and droplets.

The expected number of cells distributed in droplets is calculated using the Cell Distribution Calculator[4] following the Poisson formula where the concentration of loaded cells is the variable parameter, and the droplet volume is the constant. The total number of droplets (all DE droplets marked with Cy5) and of encapsulated cells (DE droplets (Cy5) + cell marker (CFSE)) were measured by flow cytometry to calculate the experimental distribution.

Figure 1 shows that the predicted and measured number of droplets containing cells matches significantly at various cell concentrations (from 130,000 to 17 million cells/ml) and cell types.

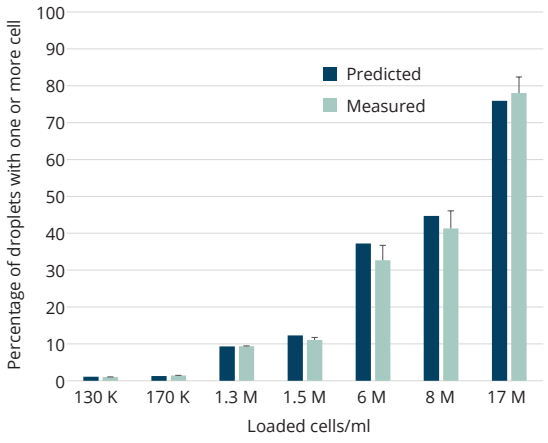

Figure 1. Comparison between the theoretical percentage of droplets containing cells as given by the Cell Distribution Calculator[4] (dark blue bars) and the measured percentage (light blue bars). The two sets of numbers are not significantly different. Results for K-562 lymphoblasts and Ramos B lymphocytes.

Calculate the Poisson distribution of your cells in droplets using the Cell Distribution Calculator

To increase the likelihood of encapsulating a single cell per droplet, a lower initial cell concentration is required. The relationship between cell concentration and droplet occupancy aligns with the principles of Poisson distribution. The Samplix Cell Distribution Calculator[4] predicts the distribution of cells in droplets.

Figure 2 shows two samples with 100,000 and 1 million cells that were encapsulated in DE50 droplets. For traceability, every droplet contained a Cy-5 conjugated probe (red), and the cells were stained with a CFSE-labelled cell marker (green). A clear difference in the number of encapsulated cells is noticeable in the resulting droplets (Figure 2, right column). Empty droplets may conveniently serve as internal negative controls for cellular assays.

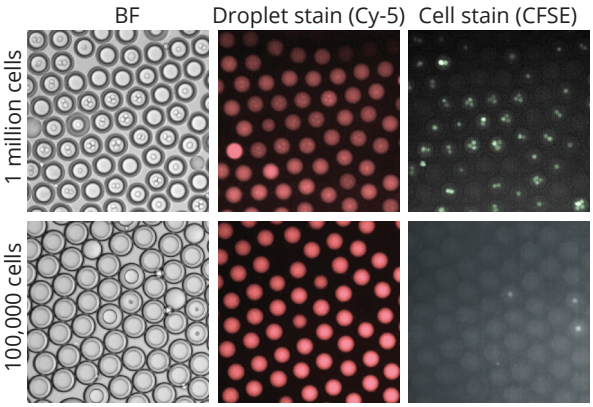

Figure 2. Microscopy images of DE droplets with encapsulated cells. Upper row: Droplets where 1 million cells were loaded into a DE50 cartridge. Bottom row: 100,000 cells loaded. Bright field images, red FL (Cy-5) for droplet marker and green FL (CFSE) for cell marker. 10x magnification.

Cell encapsulation efficiency is above 90% for optimal cell concentrations

For the investigation of the encapsulation efficiency of living mammalian cells into DE50 droplets, the four cell lines mentioned above were stained as previously described. For viability studies, the cells were in addition stained with propidium iodide (PI) (Thermo Fisher, 50 µg/ml). Stained cells were diluted to different concentrations and encapsulated into DE50 droplets following the Xdrop user manual[3]. Before and after encapsulation, the cells as well as droplets were counted by flow cytometry.

As Figure 3 shows, encapsulation efficiency is high, 80-90% of the processed sample. The outcome is independent of cell type (3 suspension cell lines and 1 adherent cell line were tested) (data not shown). Cells are efficiently encapsulated over a broad range of cell concentrations (from 170,000 to 17 million cells /ml) with an optimum range as shown in the graph (Figure 3).

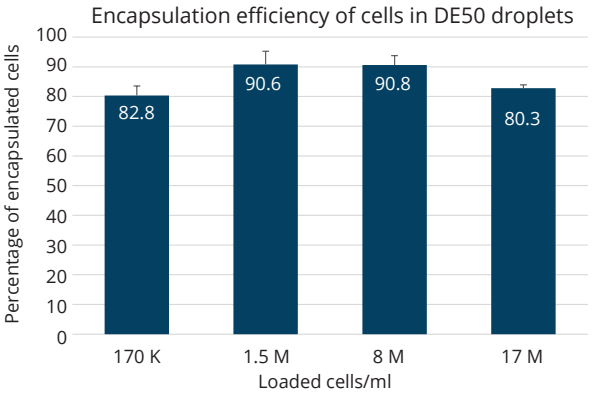

Figure 3. Encapsulation efficiency of mammalian cells in DE50 droplets. Results from four concentrations of loaded cells and two different cell lines (K-562 lymphoblast and Jurkat T lymphocytes are shown here).

For the investigation of the cell viability, encapsulated cells were analyzed with NovoCyte® Quanteon (Agilent) flow cytometer. Cells encapsulated for 3 hours in DE50 droplets retain the same viability as the same cells before encapsulation (data not shown). Thus, encapsulation in DE50 droplets or incubation inside droplets in a standard CO_2 cell incubator does not impact viability of the cells .

Conclusion

Xdrop and the Xdrop DE50 cartridge facilitate the encapsulation of one or more cells into double-emulsion droplets. Encapsulation follows the Poisson distribution, and the number of cells per droplet can be regulated by adjusting the concentration of cells loaded into the cartridge. Empty droplets generated during encapsulation can serve as built-in negative controls, enhancing the reliability and reproducibility of experimental results. Xdrop thus simplifies and democratizes single-cell studies in microfluidic droplets.

For more information about Xdrop products and applications, visit samplix.com.

References

1. Santra, T. S. & Tseng, F. G. Single-Cell Analysis. Cells vol. 9 (2020).
2. Bode, D., Cull, A. H., Rubio-Lara, J. A. & Kent, D. G. Exploiting Single-Cell Tools in Gene and Cell Therapy. Front Immunol 12, (2021).
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4. Samplix Cell Distribution Calculator https://apps.samplix.com/app/cell-poisson