Technical Overview

Simple Analysis of Xdrop Droplets on the Agilent NovoCyte Quanteon Flow Cytometer

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Abstract

The Samplix Xdrop instrument enables quick and easy encapsulation of cells and small molecules in double-emulsion droplets that are compatible with flow cytometry. In this techncial oveview, two types of fluorescent beads were coencapsulated in droplets and identified using the Agilent NovoCyte Quanteon flow cytometer.

Introduction

Flow cytometry is a widely used method for analyzing single cells, but it is limited to detecting fluorescently tagged molecules inside or on the surface of cells.[1–3] This limits the ability to detect critical extracellular biomarkers and cell phenotypes, such as secreted proteins and extracellular metabolites.[4] There is an increasing demand for approaches that allow flow cytometers to capture a more comprehensive range of biomarkers, including extracellular, spatial, and temporal markers for biological, industrial, and clinical significance.

Droplet microfluidics has emerged as a valuable tool, allowing the compartmentalization of individual cells within microdroplets.[5,6] Unlike conventional water- in-oil (W/O) droplets, double emulsion (DE) droplets are water-in-oil-in-water (W/O/W) and therfore compatible with flow cytometry.[7,8] This enables the complete characterization of individual cells, beyond traditional surface and intracellular biomarkers. The approach has led to multiple discoveries in enzyme evolution, drug response heterogeneity, rare cell identification in microbial communities and disease biomarker identification.[9–12]

This technical overview demonstrates how the Xdrop platform enhances the Agilent NovoCyte Quanteon flow cytometer's ability to analyze cells or particles within DE droplets. Xdrop is a droplet generator which, together with the Xdrop DE50 cartridge, generates double-emulsion droplets, referred to as DE50 droplets. Mammalian cells or biomolecules can be encapsulated in DE50s that have a volume of approximately 100 pL. Here, we encapsulate fluorescent beads instead of cells to show how DE50 droplets can be analyzed by flow cytometry. Fluorescent beads, with their uniform size, shape, and fluorescence characteristics, help refine scatter and fluorescence parameters in flow cytometry while reducing the biological variability seen with cells.

Encapsulation of fluorescent beads in droplets

DE50 droplets were prepared according to the Xdrop Manual using RPMI media (Gibco) supplemented with 10% fetal bovine serum (Sigma-Aldrich) and 100 units/mL Penicillin and 100 µg/mL Streptomycin (Gibco).[13]

Three different samples of droplets were prepared as outlined in Figure 1A. Unlabeled droplets contained only RPMI media with or without phenol red. The control droplets contained a FAM-labeled DNA probe (0.25 µM) (Integrated DNA Technologies) and media. The coencapsulation sample contained fluorescent beads (2.5 µm AlignFlow Flow Cytometry Alignment Beads, Life Technologies) for the blue laser (green beads) and red laser (red beads). The different double-emulsion droplets can be seen in Figure 1B.

A.

Sample Name	Droplet Contents	Illustrations
Unlabeled droplets	RPMI media +/- phenol red	
FAM-labeled droplets	RPMI media + FAM- labeled probe	
Coencapsulation	RPMI media + ~ 380,000 green and ~ 380,000 red beads	

B.

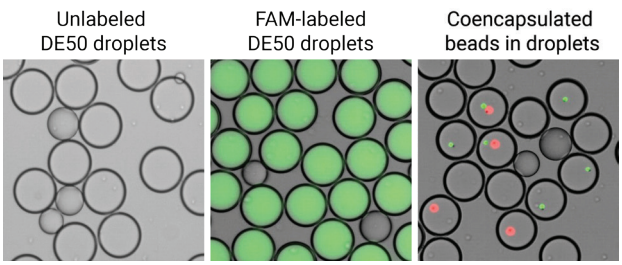

Figure 1. A. Overview of droplet types used in this study. Unlabeled DE50 droplets made with or without phenol red, droplets containing a FAM-probe, and droplets with coencapsulated fluorescent beads. B. Microscopy of DE50 droplets produced as outlined in A. 10x magnification.

Approximately 380,000 beads of each type for each fluorescent channel were used for one droplet production. The encapsulation process follows a Poisson-distributed occupancy. This number was selected to ensure that most droplets are either empty, contain a single bead, or contain two coencapsulated beads. The samples were mixed with a flow diluent consisting of 650 µL dPBS (Gibco), 250 µL DE Stabilizer D (Samplix), and 100 µL OptiPrep (Stemcell Technologies).

NovoCyte Quanteon setup

DE50 droplets were analyzed using an Agilent NovoCyte Quanteon flow cytometer to determine scatter morphology (FSC and SSC) and fluorescence using channels APC (red) and FITC (green). Instrument settings for these and other criteria are summarized in Table 1.

Table 1. Summary of the NovoCyte Quanteon parameter settings for acquisition of the droplet flow cytometry data.

NovoCyte Quanteon Parameter	Setting
FSC gain	380
SSC gain	495
B525 gain (FITC channel)	415
R667 gain (APC channel)	340
Flow rate	Fast (66 µl/min)
Trigger	FSC
Threshold	500,000

Identification of the DE50 droplet population

The DE50 droplet productions were analyzed on the Agilent NovoCyte Quanteon, see Table 1. The FAM- labeled droplets were used to identify the DE50 droplet population (Figure 2). The entire droplet production collected from the DE50 Cartridge will have a scatter profile reminiscent of the one shown in Figure 2A. The profile may vary depending on factors such as sample pressure and sample buffer, as well as size and composition of the encapsulated material. The DE50 droplet production contains a mix of double-emulsion droplets and pure carrier oil droplets. The oil droplets neither impact the assay analysis nor the performance of the flow cytometer.

We used the FAM- labeled droplets to illustrate the identification of DE50 droplets. The population of double-emulsion droplets form a distinct population discernible from the oil droplets by a higher SSC. This is clear when this population is gated out and plotted against green fluorescence (FITC) (Figure 2B). The remaining events lower in SSC are oil droplets with low background florescence (Figure 2C). In this way, the DE50 droplets are easy to separate from the carrier oil droplets.

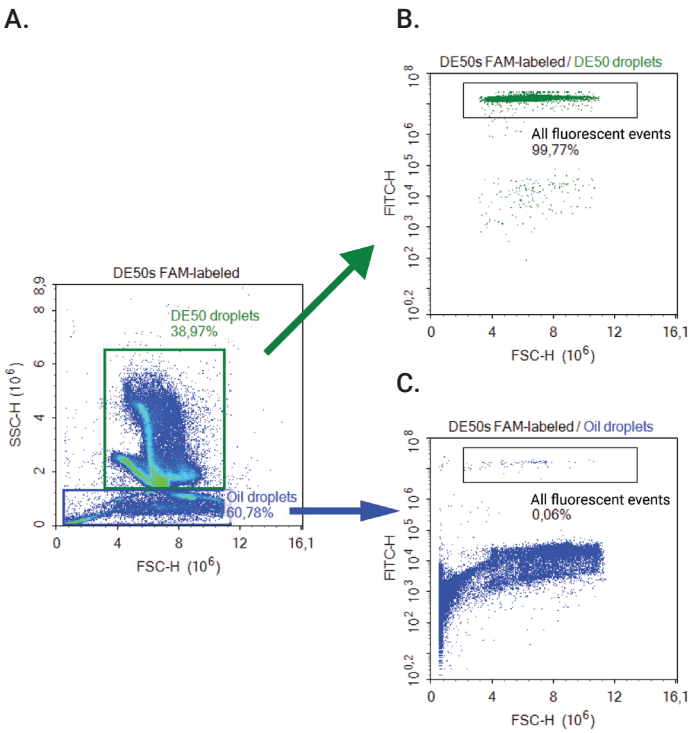

Figure 2. Using a back-gating strategy and droplets containing FAM- labeled DNA, the DE50 droplet population can be identified by flow cytometry. A. Most Xdrop droplet productions will look similar to this when plotted on a FSC-H vs. SSC-H density plot. The population with higher SSC is gated out. B. When the SSC-high population is plotted on a FSC vs. FITC plot, it is clear that all events are fluorescent, which means they contain the FAM probe and are thus DE50 droplets. C. Events with lower SSC are not fluorescent and can in that way be identified as non-DE droplets.

Efficient coencapsulation

The DE50 droplet population was identified as described above and then plotted on a FITC-H vs. APC-H plot. The empty droplets, made with media containing phenol red, exhibit a low level of autofluorescence due to phenol red in the media (Figure 3A and B). In the coencapsulation sample (Figure 3C), distinct populations of droplets were observed: (1) droplets containing only red beads (upper left quadrant), (2) droplets with coencapsulated green and red beads (upper right quadrant), (3) empty droplets without beads (lower left quadrant), and (4) droplets containing only green beads (lower right quadrant).

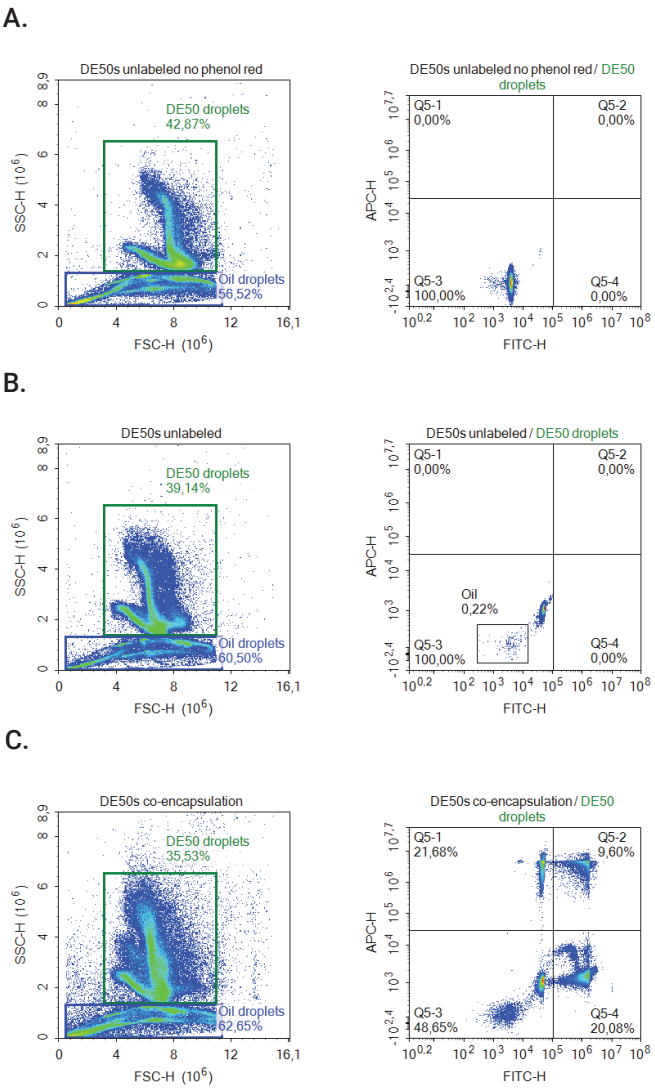

Figure 3. Identification of populations of DE50 droplets with fluorescent beads. All events were plotted on an FSC-H vs. SSC-H plot and the DE50 droplets were gated out (left column dot plots). The DE50 droplets were then set apart based on their green (FITC) and red (APC) fluorescense (right column) A. The unlabeled empty DE droplets made mith media without phenol red. B. Unlabeled empty DE droplets made mith media containing phenol red. Phenol red makes the droplets slightly autofluorescent. C. DE droplets containing (1) red beads only (upper left quadrant), (2) co-encapsulated green and red beads (upper right quadrant), (3) no beads (empty droplets – lower left quadrant), (4) green beads only (lower right quadrant). The analyses were performed on a NovoCyte Quanteon in the same manner for all samples.

Using Samplix' proprietary Cell Distribution Calculator, the theoretical percentage of droplets with beads can be calculated based on the Poisson distribution.[14] For the coencapsulation of green and red beads, the expected percentage of droplets containing both color beads is 12.4%. For droplets containing a single green or red bead, it is 18.2% each.

Conclusion

With accessible microfluidics, Xdrop simplifies the encapsulation of living cells and biological materials into double-emulsion (DE50) droplets, which can be easily detected and analyzed using flow cytometry. Here, we outline how to setup the Agilent NovoCyte Quanteon cytometer for fast and reliable identification and analysis of double-emulsion droplets containing beads. The consistency and characteristics of beads compared to living cells facilitates the set up of flow cytometry for use in cell-based assays.[15]

References

1. Picot, J.; Guerin, C. L.; Le Van Kim, C.; Boulanger, C. M. Flow cytometry: Retrospective, fundamentals and recent instrumentation. *Cytotechnology.* **2012** *64*, 109–130. DOI: 10.1007/s10616-011-9415-0
2. Cheung, M.; Campbell, J. J.; Whitby, L.; Thomas, R. J.; Braybrook, J.; Petzing, J. Current trends in flow cytometry automated data analysis software. *Cytometry Part A.* **2021**, *99*, 1007–1021. DOI: 10.1002/cyto.a.24320
3. Lazarski, C. A.; Hanley, P. J. Review of flow cytometry as a tool for cell and gene therapy. *Cytotherapy.* **2023**, *26* (2), 103-112. DOI: 10.1016/j.jcyt.2023.10.005
4. Karagiannis, G. S.; Pavlou, M. P.; Diamandis, E. P. Cancer secretomics reveal pathophysiological pathways in cancer molecular oncology. *Mol. Oncol*. **2010**, *4*, 496–510. DOI: 10.1016/j.molonc.2010.09.001
5. Joensson, H. N.; Andersson Svahn, H. Droplet microfluidics-A tool for single-cell analysis. *Angew. Chem. Int. Ed.* **2012**, *51*, 12176–12192. DOI: 10.1002/anie.201200460
6. Rakszewska, A.; Tel, J.; Chokkalingam, V.; Huck, W. T. S. S. One drop at a time: toward droplet microfluidics as a versatile tool for single-cell analysis. NPG Asia Mater. **2014**, *6*, e133. DOI: 10.1038/am.2014.86
7. Brower, K. K.; Khariton, M.; Suzuki, P. H.; Still, C.; Kim, G.; Calhoun, S. G. K.; Qi, L. S.; Wang, B.; Fordyce, P. M. Double emulsion picoreactors for high-throughput single-cell encapsulation and phenotyping via FACS. *Anal Chem.* **2020**, *92*, 13262–13270. DOI: 10.1021/acs.analchem.0c02499
8. Brower, K. K.; Carswell-Crumpton, C.; Klemm, S.; Cruz, B.; Kim, G.; Calhoun, S. G. K.; Nichols, L.; Fordyce, P. M.; Double emulsion flow cytometry with high-throughput single droplet isolation and nucleic acid recovery. Lab Chip. **2020**, *20*, 2062–2074. DOI: 10.1039/d0lc00261e
9. Tu, R.; Martinez, R.; Prodanovic, R.; Klein, M.; Schwaneberg, U. A flow cytometry-based screening system for directed evolution of proteases. *J Biomol Screen.* **2011**, *16*, 285–294. DOI: 10.1177/108705711039636
10. Chokkalingam, V.; Tel, J.; Wimmers, F.; Liu, X.; Semenov, S.; Thiele, J.; Figdor, C. G.; Huck, W. T. Probing cellular heterogeneity in cytokine-secreting immune cells using droplet-based microfluidics. *Lab Chip*. **2013**, *13*, 4740–4744. DOI: 10.1039/c3lc50945a
11. Ohan, J.; Pelle, B.; Nath, P.; Huang, J. H.; Hovde, B.; Vuyisich, M.; Dichosa, A. E.; Starkenburg, S. R. High-throughput phenotyping of cell-to-cell interactions in gel microdroplet pico-cultures. *Biotechniques*. **2019**, *66*, 218–224. DOI: 10.2144/btn-2018-0124
12. Scanlon, T. C.; Dostal, S. M.; Griswold, K. E. A high-throughput screen for antibiotic drug discovery. *Biotechnol Bioeng*. **2014**, *111*, 232–243. DOI: 10.1002/bit.25019
13. Samplix. *Samplix Xdrop Manual.* **2023**. https://samplix.com/support#manuals
14. Samplix. *Samplix Cell Distribution Calculator*. n.d. https://apps.samplix.com/app/cell-poisson
15. Jacobsen, M. J.; Petersen, D. L.; Arnasia, M.; Frosig, T. M.; Guenther, G.; Sleiman, R.; Mouritzen, P.; Mikkelsen, M. J. Rapidly identifying active natural killer cells. *Agilent Technologies Application Note*. **2023**, 5994-6863EN.

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