High-Throughput Single Cell Analysis, Sorting and Dispensing using Multi-Laser Picodroplet Technology

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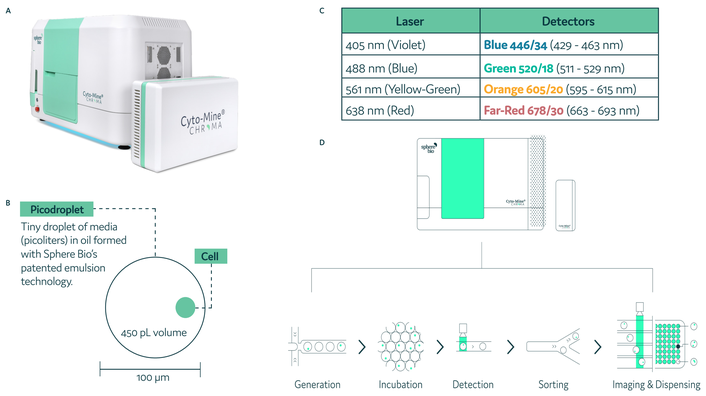
1. Introduction

Antibody therapeutics dominate drug development; however, lengthy workflows and rising costs in their discovery and development present significant challenges. Sphere Bio's Cyto-Mine® system leverages picodroplet technology, microfluidics, and optics to overcome these hurdles in Antibody Discovery (AbD) and Cell Line Development (CLD), accelerating therapeutic development. Here we introduce the next-generation Cyto-Mine® platform, Cyto-Mine® Chroma. This advanced microfluidic platform, equipped with multi-laser, single-cell analysis capabilities, enables high-throughput, multiplex functional screening of millions of cells in a single day. Our findings demonstrate that Cyto-Mine® Chroma can precisely sort and dispense cells based on fluorescence signals and sequential gating. Additionally, it achieves over 90% accuracy in isolating cells based on productivity and cellular markers from mixed populations. In an experiment simulating an AbD workflow, Cyto-Mine® Chroma successfully isolated rare cells secreting antigen-specific antibodies with high precision. Furthermore, by utilizing a viability dye alongside IgG-specific probes, we demonstrated its ability to isolate CHO cells producing human IgG4 based on both productivity and viability, without compromising cell performance. These results highlight Cyto-Mine® Chroma's robust multiplexing capabilities, making it an invaluable tool for isolating specific cell populations in both CLD and AbD workflows.

2. Materials and Methods

Instrument: Cyto-Mine® Chroma, like its predecessor, is a fully automated single cell analysis platform, capable of encapsulating, analyzing, sorting, and dispensing viable single cells (**Fig 1a**). Cyto-Mine® Chroma has been upgraded to include 4 lasers and 4 detectors (**Fig 1b**), expanding its utility, enabling multiplexing within picodroplets (**Fig 1c**). The integrated stages of the Cyto-Mine® Chroma process are summarized in **Fig 1d**. Cells and assay reagents are encapsulated into picodroplets, incubated within the incubation chamber at optimum temperature for target protein secretion. Secreted target protein accumulates inside the picodroplet, which is captured. Cells secreting the desired protein are detected in Cyto-Mine® Chroma during sorting and dispensed into tissue culture plates.

Figure 1: Cyto-Mine® Chroma, the next-generation single-cell analysis platform.



Laser	Detectors
405 nm (Violet)	**Blue 446/34** (429 - 463 nm)
488 nm (Blue)	**Green 520/18** (511 - 529 nm)
561 nm (Yellow-Green)	**Orange 605/20** (595 - 615 nm)
638 nm (Red)	**Far-Red 678/30** (663 - 693 nm)

Study 1) Accuracy of sequential gating during sorting: To determine sorting and gating accuracy, four cell samples were labelled with different cell membrane dyes (CellTracker™Green CMFDA (**Green**, CT- Green), CellTracker™ Orange CMTMR (**Orange**, CT- Orange), eBioscience™Cell Proliferation Dye eFluor™ 450 (**Blue**, eFL450), eBioscience™Cell Proliferation Dye eFluor™ 670 (**Red**, eFL670)). Stained cells were mixed and loaded onto Cyto-Mine® Chroma. Cells were gated for a single dye, excluding all others and dispensed. Accuracy of gating and sorting was determined using a fluorescence microscope. Process was repeated for all four colours (**Fig 2a, Fig 2b**).

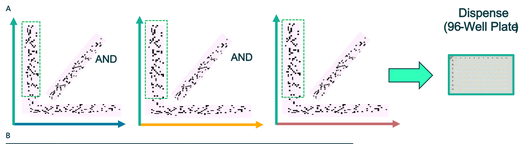
Study 2) Detecting antibody secretion: To detect cells secreting antibody, two different hybridoma cell lines were labelled with different dyes (eFL450 (**Blue**)); eFL670 (**Far Red**)) and mixed with anti-mouse IgG FRET probes (Donor- Dylight488 (DL488, **Green**) and Acceptor-Dylight594 (DL594, **Orange**)). Cells were loaded onto Cyto-Mine® Chroma and sequentially gated at sorting first by FRET+ response then by presence of **Blue** cells. Cells were dispensed using the same sequential gating used in sorting and the accuracy of gating, sorting and dispensing was determined using a fluorescence microscope (**Fig 3a, Fig 3b**).

Study 3) Detecting antibody binding to its antigen: To detect antigen-specific antibody binding, antibody-secreting hybridoma cells (labelled with eFL450 (**Blue**)) were mixed with target cells expressing specific antigen (labelled with eFL670- **Far Red**). Antibody binding to target cells was detected using a detection antibody-AF488 (**Green**). Cells were loaded onto Cyto-Mine® Chroma and sorted for droplets containing localized antibody secreting cells (ASC), target cells and detection antibody. Cells were dispensed using the sequential gating and the accuracy of gating, sorting and dispensing was determined using a fluorescence microscope (**Fig 4a, Fig 4b**).

Study 4) Detecting apoptosis and dead cells in droplets: A CHO cell line secreting human IgG4κ antibody was mixed with Cyto-Cellect® (Sphere Bio, Donor conjugated with AF488 (**Green**), and Acceptor conjugated to AF594 (**Orange**)) and a viability dye conjugated to AF670 (**Far Red**). Cells were loaded onto Cyto-Mine® Chroma. Droplets were gated for the presence of FRET+ viable cells. Droplets were dispensed and accuracy of gating and sorting was determined using Flowjo software (v10.10.0; **Fig 5a, Fig 5b**).

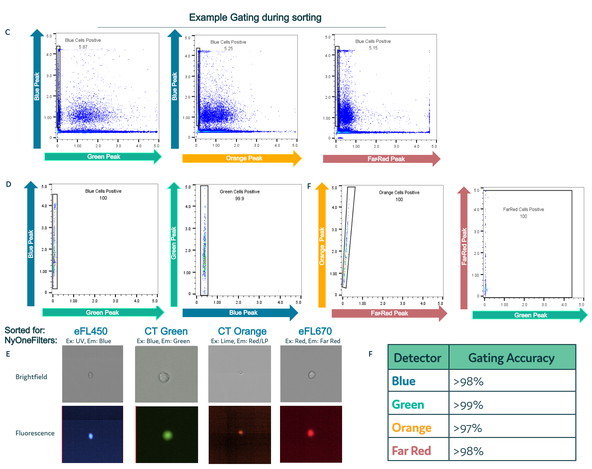
3. Results

Study 1) Accurate Sorting and Gating of Encapsulated Live Cells



B

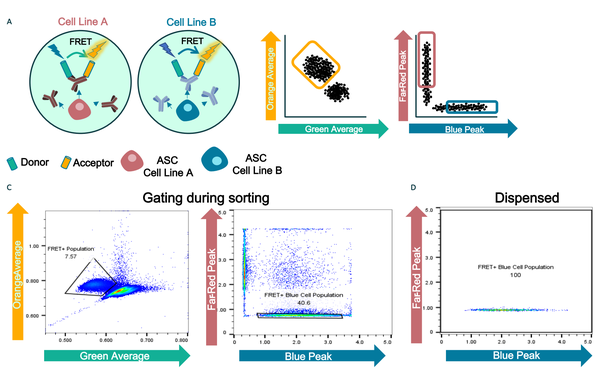
Dye	Laser	Detectors
eFL450	405 nm (Violet)	**Blue 446/34** (429 - 463 nm)
DL488	488 nm (Blue)	**Green 520/18** (511 - 529 nm)
DL594	561 nm (Yellow-Green)	**Orange 605/20** (595 - 615 nm)
eFL670	638 nm (Red)	**Far-Red 678/30** (663 - 693 nm)



Detector	Gating Accuracy
Blue	>98%
Green	>99%
Orange	>97%
Far Red	>98%

Figure 2: Accuracy in sorting and gating in Cyto-Mine® Chroma. Four cell samples, each labelled with a different membrane dye, were mixed and loaded onto Cyto-Mine® Chroma. Cells were gated during sorting for a single dye, excluding all others, followed by dispensing. (a) Schematic diagram for gating during sorting. (b) List of fluorochromes used in the assay. (c) An example of gating strategy, used for sorting of **Blue** cells and the corresponding cell population following dispensing. (d) Dot plot of dispensed population for **Blue**, **Green**, **Orange** and **Far Red** labeled cells, using the example gating strategy shown in (c). (e) Example images of dispensed cells following sorting and dispensing of specific cell population. (f) Microscopy data of dispensed cells was used to determine the accuracy of gating during sorting.

Study 2) Single Cell Detection and Isolation of Antibody-Secreting Cells



B

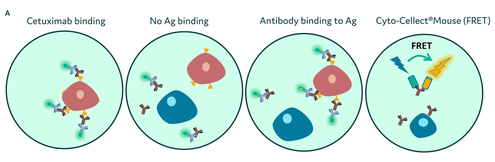
Dye	Labeled	Laser	Detectors
eFL450	Cell B	405 nm (Violet)	**Blue 446/34** (429 - 463 nm)
DL488	Donor	488 nm (Blue)	**Green 520/18** (511 - 529 nm)
DL594	Acceptor	561 nm (Yellow-Green)	**Orange 605/20** (595 - 615 nm)
eFL670	Cell A	638 nm (Red)	**Far-Red 678/30** (663 - 693 nm)

F

Sorted Population	Cell Detected
FRET+/ Blue Cells	Blue only (100%)

Figure 3: Isolation of Subpopulations of Secreting Cells on Cyto-Mine® Chroma. Two differentially labelled cell populations were pre-mixed with anti-mouse IgG FRET probes and loaded onto Cyto-Mine® Chroma. (a) Schematic diagram for assay reaction and gating strategy during sorting. (b) List of fluorochromes used in the assay. c) Cells were gated in sorting on FRET+ followed by Blue cell- only gating. d) Profile of cells dispensed. (e) Example images of dispensed cells following sorting and dispensing of specific cell population. (f) Microscopy data of dispensed cells was used to determine the accuracy of gating during sorting.

Study 3) Isolation of Antibody-Secreting Cells Through Antigen-Specific Binding



B

Dye	Labeled	Laser	Detectors
eFL450	ASC	405 nm (Violet)	**Blue 446/34** (429 - 463 nm)
AF488	Detection Ab (Fig 4c, 4d, 4f, & 4g) Donor (Fig 4e)	488 nm (Blue)	**Green 520/18** (511 - 529 nm)
AF594	Acceptor (Fig 4e)	561 nm (Yellow-Green)	**Orange 605/20** (595 - 615 nm)
eFL670	Target Cell	638 nm (Red)	**Far-Red 678/30** (663 - 693 nm)

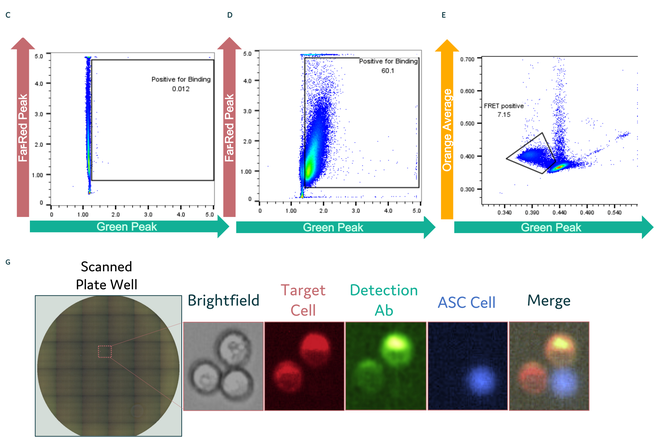
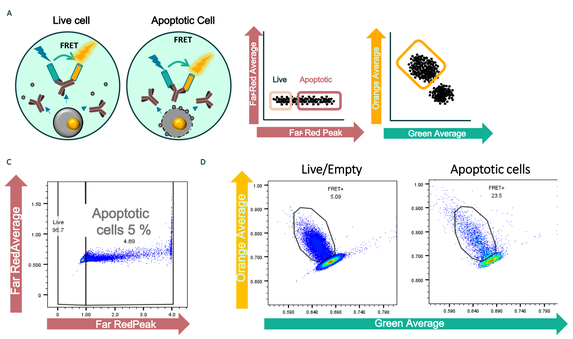


Figure 4: Isolation of ASC using surface binding assay in Cyto-Mine® Chroma. (a) Schematic diagram for assay reaction and gating strategies used during sorting. (b) List of fluorochromes used in the assay. (c-d) A431 target cells labelled with eFL670 were mixed with anti-hIgG-AF488 Ab in the absence (c) or presence (d) of Cetuximab. Cells were loaded on Cyto-Mine® Chroma and gated for Far Red & Green signals during sorting. (e) IgG secretion of ASC using Cyto-Cellect® Mouse FRET probes. (f-g) ASC labelled with eFL450 were mixed with eFL670- labelled target cells in the presence of AF488-detection antibody. Droplets were gated during sorting (f) and dispensed into 96 well plates. (g) Dispensed cells were imaged using a fluorescence microscope.

Study 4) Differentiating FRET+ Viable Cells from Apoptotic or Dead Cells



B

Dye	Labeled	Laser	Detectors
N/A	N/A	405 nm (Violet)	**Blue 446/34** (429 - 463 nm)
AF488	Donor Probe	488 nm (Blue)	**Green 520/18** (511 - 529 nm)
AF594	Acceptor Probe	561 nm (Yellow-Green)	**Orange 605/20** (595 - 615 nm)
AF 670	Viability	638 nm (Red)	**Far-Red 678/30** (663 - 693 nm)

Figure 5: Differentiating FRET+ dead/dying cells from live cells in picodroplets. CHO cells secreting hIgG4 antibody were mixed with Cyto-Cellect® probes and viability dye. Cells were loaded onto Cyto-Mine® Chroma and incubated for 1 hour. Cells were gated for apoptosis and FRET signal. (a) Schematic diagram for assay reaction and gating strategy during sorting. (b) List of fluorochromes used in the assay. (c) Gating of cells during sorting. (d) Analysis of FRET response in live versus apoptotic cell population.

4. Conclusions

- The platform reliably detects localized (antibody-antigen binding) and dispersed (IgG secretion, FRET signal) fluorescence.
- Combined with fluorescently stained cells, the platform accurately distinguishes between different cell types based on their specific staining.
- The platform demonstrated remarkable sorting accuracy and enrichment of target populations following sequential gating.
- Cyto-Mine® Chroma can reliably be used in multiplexing assays for CLD (productivity & viability) as well as AbD (productivity & antigen specificity).