Find CHO Cells Of Choice: Selective Cloning In Cell Line Development

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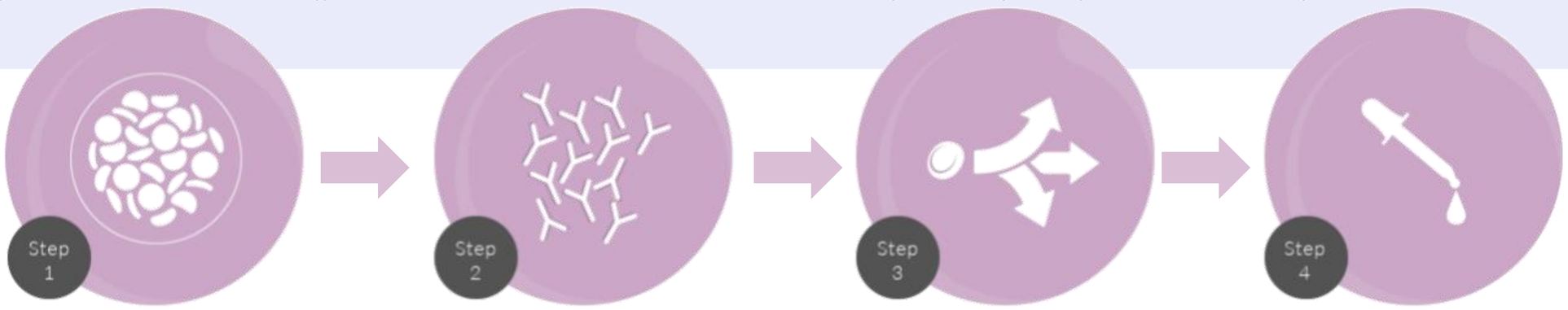
Introduction

One of the greatest challenges faced during Cell Line Development is being able to efficiently screen large cell populations for productivity and isolate high producers of interest, while ensuring monoclonality. Current methods create a bottleneck in the development of novel biotherapeutic drugs as many devices are required to isolate single cells, analyze, sort, image and dispense those 'hit' cells - resulting in resource-intensive, time-consuming and expensive workflows. This study demonstrates how Cyto-Mine®, a novel single cell analysis platform, provides a robust and efficient solution for high-throughput selective screening of single CHO cells. A large population of proprietary CHO cells, stably expressing human IgG was mixed with Cyto-Mine® human IgG-specific animal origin free detection reagent. The Cyto-Mine® platform was used to encapsulate single cells into pL volume droplets (picodroplets), where they were incubated for 2 hours. The cells were analysed using a Cyto-Mine® IgG secretion assay and high producers were selected. Prior to dispensing, the high producing cells were imaged for clonality and measured again for productivity. The data was analysed using the Cyto-Mine® Software Studio Suite.

Here we present data on the simultaneous method for the identification of high producing clones and monoclonality verification. Cyto-Mine® was used to selectively clone single CHO cells based on their antibody secretion rate. Cyto-Mine® was able to identify high producing cells from a large (approx. 100,000) starting population. The fully integrated system streamlines the cell development workflow and significantly improves the time from host cell transfection to cell bank generation. This powerful technology is poised to revolutionize cell line development with the integration of high-throughput selective screening, cell isolation, and assurance of monoclonality in one platform.

Cyto-Mine® Workflow



Encapsulate
Encapsulate up to 200,000 single cells

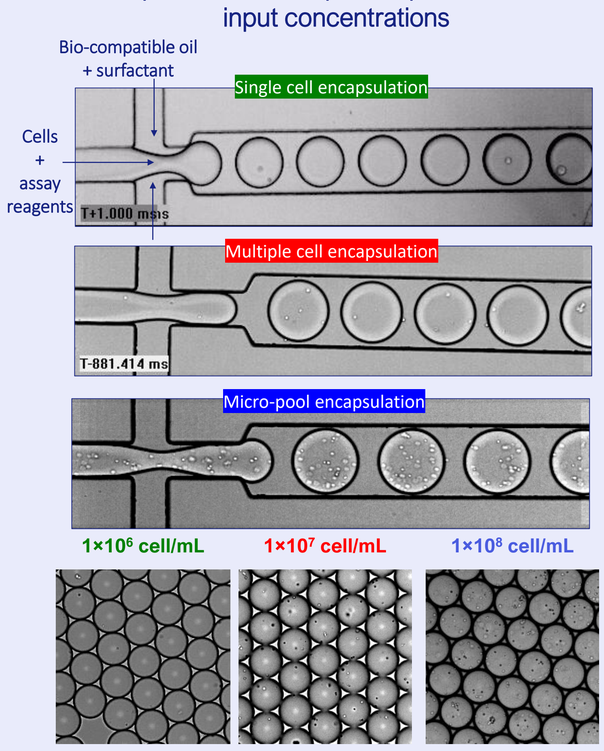
Assay
Screen cell populations by immunoglobulin productivity

Sort
Selection based on fluorescence intensity to find highest producing clones

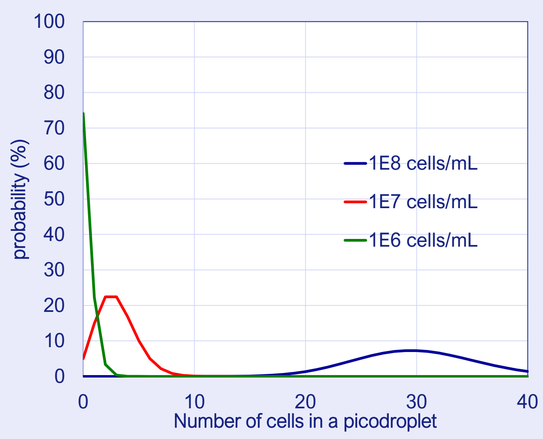
Image & Dispense cells
Image single cells during dispensing into 96- or 384- well plates to record monoclonality

Encapsulation

Encapsulated cells in picodroplets with varied input concentrations



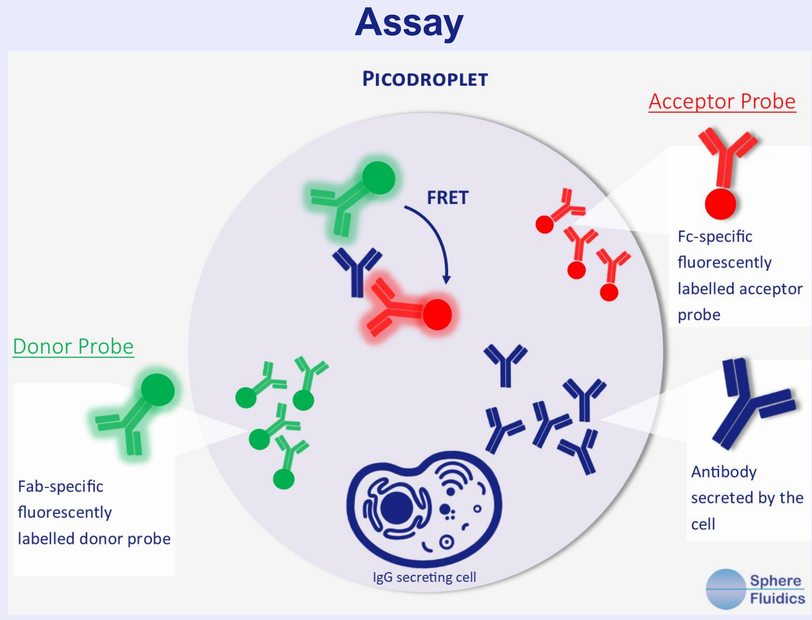
Theoretical cell number distribution in picodroplets



Single Cell Encapsulation: Cyto-Mine® is able to encapsulate up to 200,000 single cells in one experiment. Isolation of a single cell can be achieved by controlling the input concentration of cells, using Poisson distribution statistics. Cells are prepared in preferred culture medium at a dilution concentration to ensure a high probability of monoclonality during encapsulation into 300 pL picodroplets.

Multi Cell Encapsulation: Other scenarios where vast populations need to be screened, up to 40 million cells can be prepared in high concentrations to enable encapsulation of multiple or micro-pools of cells.

Assay

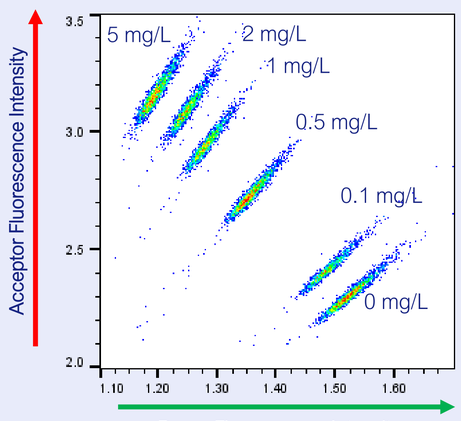


One key feature of Cyto-Mine® is its ability to measure secreted proteins from single cells enabling the specific immunoglobulin (IgG) production rate to be measured from every single cell. The starting cell population requires no prior modification or enrichment and is simply mixed with the appropriate animal-origin free (AOF) detection reagent which includes fluorescently labelled donor and acceptor probes, prior to loading on to Cyto-Mine®. Once cells are encapsulated into picodroplets they undergo *in-situ* incubation. At this point IgG, secreted by the cell, accumulates within the picodroplet. The detection probes bind to the secreted IgG inducing a FRET-mediated shift in fluorescence. Cyto-Mine® then measures the fluorescent signal generated by the acceptor probe, resulting in a quantitative measurement of IgG concentration.

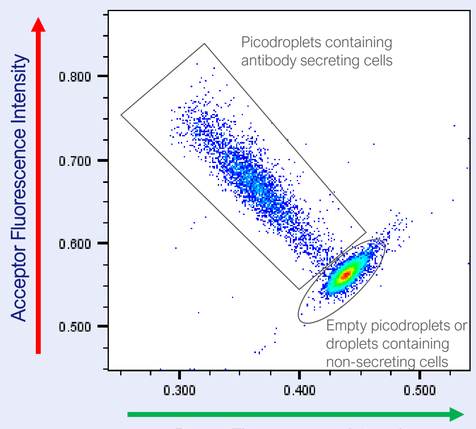
Sorting

Single Cell Selection Based on Antibody Secretion

Cyto-Mine® is able to screen large panels of clones for high expressors as well as reliably ranking candidate cells based on single cell productivity.



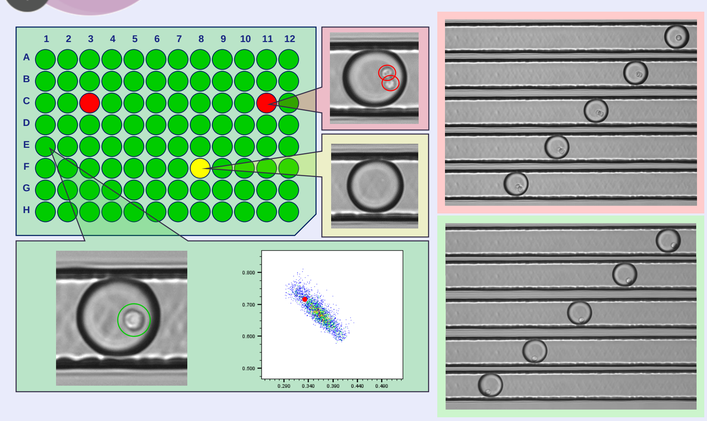
Encapsulating single cells in picodroplets enables detection of molecules secreted by the cells (rather than cell surface-bound molecules). Sphere Fluidics has developed a panel of homogeneous FRET assays which allows detection of IgG from various species inside picodroplets. The homogeneous assays can provide information on the end point concentration of the antibodies in the picodroplets, essentially, enabling single cell sorting and cloning based on ranking their productivities. The above scatter plot shows results of detection of a library of picodroplets containing different concentrations of target IgG. Picodroplets containing assay reagents and 0, 0.1, 0.5, 1, 2, 5 mg/mL target IgG were generated separately, then mixed at an equal ratio and analyzed by measuring laser-induced fluorescence.



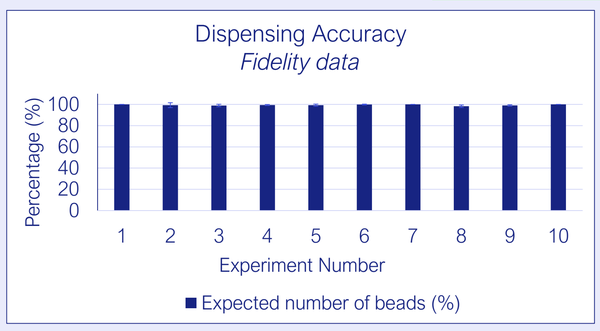
Picodroplets generated in Cyto-Mine® are just several hundreds pL in volume, about 5-6 orders of magnitude smaller than volumes in conventional assays. This means, given the sample incubation time, the concentration of secreted antibodies from a single cell in picodroplets is 5-6 orders of magnitude higher than in a conventional assay. In Cyto-Mine®, it only takes 0.5-4 hours' incubation time, before the system can detect antibody secretion from each encapsulated cell. In a Cyto-Mine® instrument run, the user can gate and sort a (sub-)population of picodroplets by manually drawing a region of interest on the scatter plot.

Single Cell Imaging and Dispensing

Monoclonality Assurance



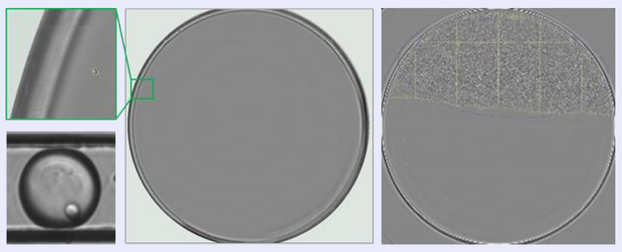
Prior to dispensing, Cyto-Mine® takes several images of each cell within a picodroplet, these images are recorded and can be retrieved as evidence of monoclonality after the experimental run. A second fluorescence reading from each picodroplet occurs at dispensing. Cyto-Mine® dispenses each selected picodroplet into 96- or 384-well microtiter plates. The movement of the microtiter well plate is synchronized with each dispensing event and the location of the dispensed picodroplets or cell in the well will be recorded by the system. After each experiment, the system provides a data pack of 1) a map of monoclonality in the wells, 2) images of each picodroplet prior to dispensing and 3) fluorescence intensity reading for that picodroplet.



Dispensing Accuracy: We carried out a longitudinal study to investigate the dispensing accuracy of Cyto-Mine®. Over 5 weeks and 10 individual experiments Fluoresbrite® YG Microsphere beads (Polysciences, Inc) were dispensed into 10560 wells of 96-well microtiter plates. Each experiment dispensed into 10 96-well microtiter plates.

The resulting data revealed that, on average, Cyto-Mine® correctly dispensed and verified the expected number of beads with over 99% accuracy.

Cell Viability: CHO cell viability is maintained during and after Cyto-Mine® selection. When single CHO cells are dispensed into a well of a 96-well microtiter plate they are able to successfully grow out into colonies.



Conclusion: By encapsulating single cells in picodroplets, Cyto-Mine® enables rapid detection of molecules (e.g. IgG) secreted by individual cells, followed by selective cloning of single cells based on their productivities. The system also enables monoclonality assurance thus providing a comprehensive solution for accelerated biotherapeutic discovery and development. The entire system is animal origin free, ISO 9001- and GLP-compliant. Cyto-Mine® significantly reduces the time from host cell transfection to a cell bank by several weeks, streamlining workflows from multiple resource intensive instruments into one easy-to-use high-throughput system.