Sphere Fluidics

Cyto-Mine®

Application Note 03

Selective screening and isolation of high-value single cells with assured clonality in a fully-integrated workflow

This Application Note explains how Cyto-Mine® enables rare cells to be mined from complex cell populations and subsequently cloned to microplates with proof of single-cell status via a seamless and fully automated process.

Introduction

One of the most critical steps in submitting a New Drug Application (NDA) for a biologic is evidence supporting that the molecule is produced from a clonal cell line. Conventionally, this has relied heavily on presenting statistical probability that the cell bank was cloned from a single cell progenitor. More recently, cell-in-well imagers have gained traction as a visual verification tool downstream of cloning by limiting dilution, cell sorting or colony picking. Some top pharma companies have invested large sums integrating the separate systems into a continuous workflow, while for many the screening, cloning and verification process requires multiple discrete steps with manual intervention at each stage. The major limitation of cell-in-well imagers is that only the base of each microplate well is visualised such that cells that have not or cannot settle to the bottom will fail to be detected.

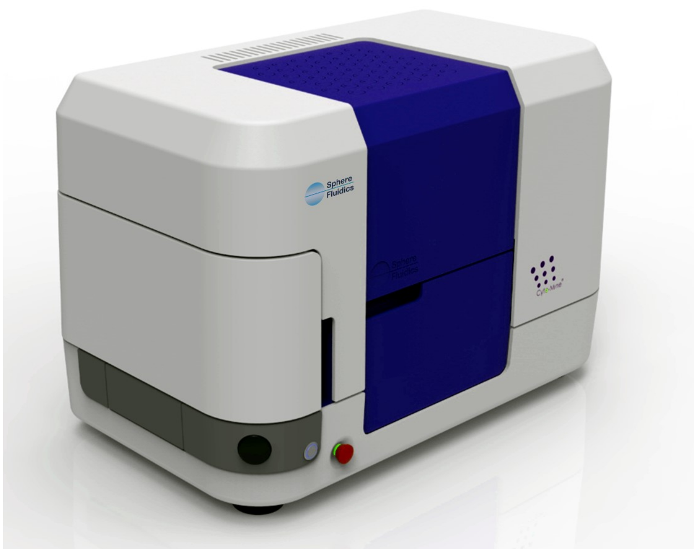


Figure 1. The benchtop Cyto-Mine® system designed for use in Class II biosafety cabinets.

The Cyto-Mine® Single Cell Analysis and Monoclonality Assurance System (**Figure 1**) overcomes the limitations of current technologies by screening hundreds of thousands of individual cells for protein production, specificity or functionality at the picoliter level. It then sorts out positive hits and gently dispenses each one individually to microplate wells. Assurance of clonality is made possible by multi-frame imaging of each cell's single status at the point of dispense. Cell encapsulation provides a protective environment offering high viability and assay sensitivity across a broad range of cell types such as B cells, hybridomas and CHO cells.

The present study illustrates the key steps through which Cyto-Mine® finds, selects and isolates single, viable, high-value cells with assured clonality.

The Cyto-Mine® Workflow

Cyto-Mine® emulates the limiting dilution and cell screening processes but in a highly efficient and fully automated manner. Its unique ability to offer high-throughput cell screening and monoclonality assurance is made possible through a series of 4 seamless steps:

1) Encapsulation of cells into picodroplets at very high dilution to maximise the number of picodroplets containing only one cell.

2) *In situ* incubation of these picoliter 'test tubes' to assay the protein secreted by every single cell.

3) A sorting phase that selectively isolates the highest value cells in picodroplets, and diverts all other picodroplets to waste.

4) High-speed, multi-frame imaging of each picodroplet immediately prior to dispense to provide visual proof of single-cell status.

Step 1: Encapsulation of cells into picodroplets

The Cyto-Mine® process is set up by preparing the target cell population to the indicated concentration in preferred culture medium, mixing with appropriate detection probes, and then pipetting into a disposable Cyto-Cartridge® (**Figure 2**), which is then loaded into Cyto-Mine®. When the run is initiated, Cyto-Mine® pumps the cell suspension gently through microfluidic structures, cross-mixing it with Cyto-Surf®, a bio-compatible surfactant, to encapsulate cells into 300pL picodroplets of culture medium (**Figure 3**). In High Fidelity Mode, the dilution of the sample is set to 0.05 cells per picodroplet such that ~95% of picodroplets are empty, ~5% contain a single cell, and the probability of 2 or more cells per picodroplet is ~0.1%.

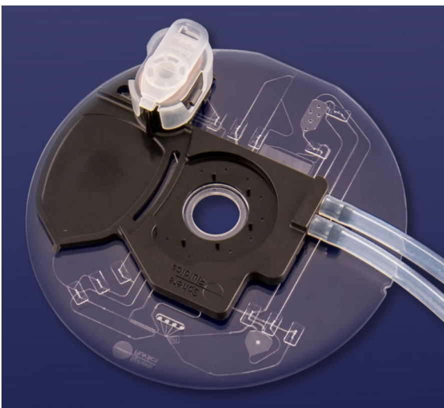


Figure 2: Single-use Cyto-Cartridge®.

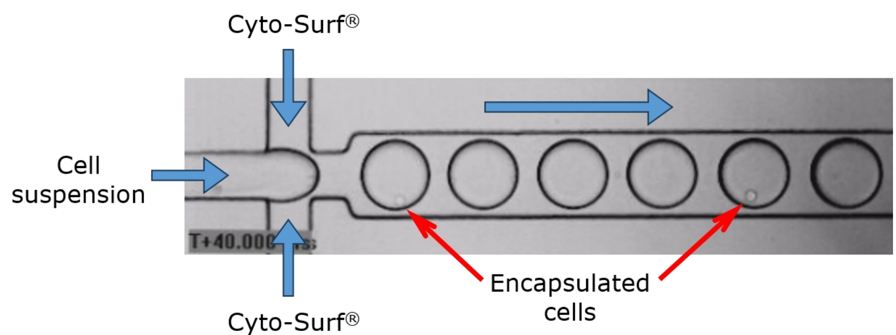


Figure 3: Cell encapsulation. Picodroplets at a uniform size of 300pL are generated such that only one in 20 contains a cell.

Step 2: Incubation and secreted protein assay

Millions of generated picodroplets are collected together into a chilled, Peltier-controlled incubation chamber (**Figure 4**), where they are incubated at up to 37°C to activate the required detection assay. The miniaturized scale means that secreted protein such as IgG from a single cell can be quantified after just 0.5 to 2 hours. Further details on the Cyto-Mine® IgG Secretion Assay and B Cell & Hybridoma Mining can be found in Application Notes 01 and 02, respectively. After incubation, the chamber is returned to its chilled state to prevent further assay reaction. The picodroplets are then channelled to the sort phase.

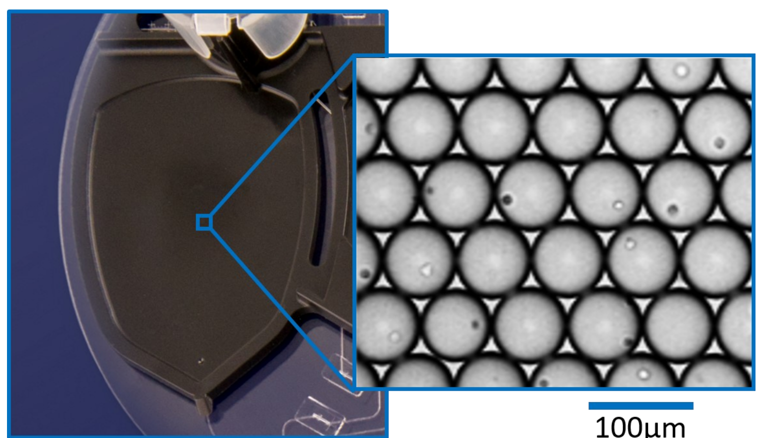
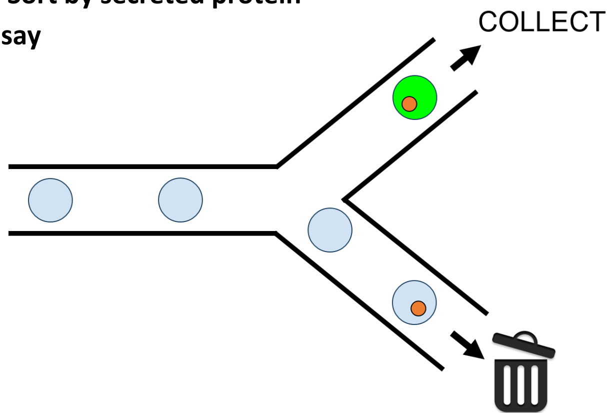


Figure 4: Picodroplets held in the Cyto-Cartridge® incubation chamber are incubated for a defined period to enable the single-cell-based assay to develop.

Step 3: Picodroplet sorting

Following incubation, Cyto-Mine® sequentially sorts all the picodroplets by fluorescence detection, with the positives being actively channelling for collection. The bulk of picodroplets, which contain low-fluorescing cells or are empty, are diverted to waste. Cyto-Mine® detects fluorescence dispersed within the picodroplet such as by a secreted protein assay, or intracellular fluorescence such as by viability stain or an intrinsic functional assay marker (**Figure 5**). The user has the option to define and adjust the population of cells for collection. Selected picodroplets are then stored in a chilled microchamber prior to dispense.

A. Sort by secreted protein assay



B. Sort by cell viability or functional assay

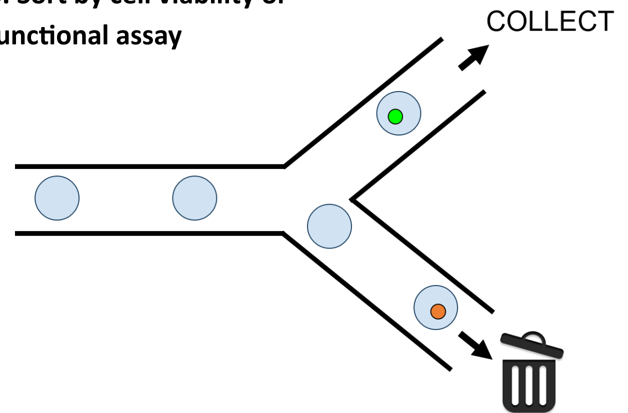


Figure 5: Options for sorting on Cyto-Mine® by detection of fluorescence either trapped in the picodroplet (**A**) or intrinsically within the cell inside the picodroplet (**B**).

Step 4: Visual verification and dispensing

After completion of the sorting phase, the selected picodroplets containing the high-value cells are spaced out, imaged and dispensed to 96- or 384-well microplates pre-filled with preferred culture medium (**Figure 6**). The imaging process uses an ultra high-speed, brightfield camera to acquire multiple frames of each picodroplet as it moves along the microfluidic channel immediately prior to dispense. Because cells within picodroplets are in constant motion, this multi-frame imaging circumvents the situation where two superimposed cells might be misdetected as one (**Figure 7**).

On-the-fly software analysis identifies and annotates each picodroplet that unambiguously contains a single cell. Any picodroplets with more than one cell, or those showing any ambiguous result, are annotated accordingly on the microplate map (**Figure 8**). When in assay mode, picodroplet fluorescence is additionally measured again and documented at the point of dispense.

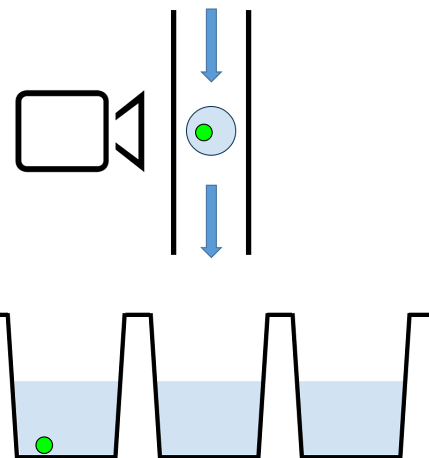


Figure 6: Cyto-Mine® visual verification and dispensing. Immediately prior to dispense, picodroplets are brightfield-imaged to identify and record the number of cells per picodroplet, and also re-measured for picodroplet fluorescence.

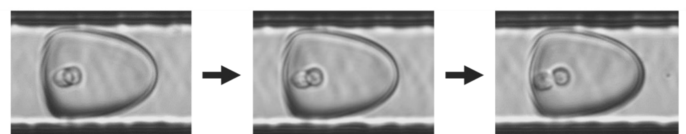


Figure 7: Brightfield multi-frame imaging avoids potential miscounting by detecting encapsulated cells at multiple timepoints as they rotate into different positions.

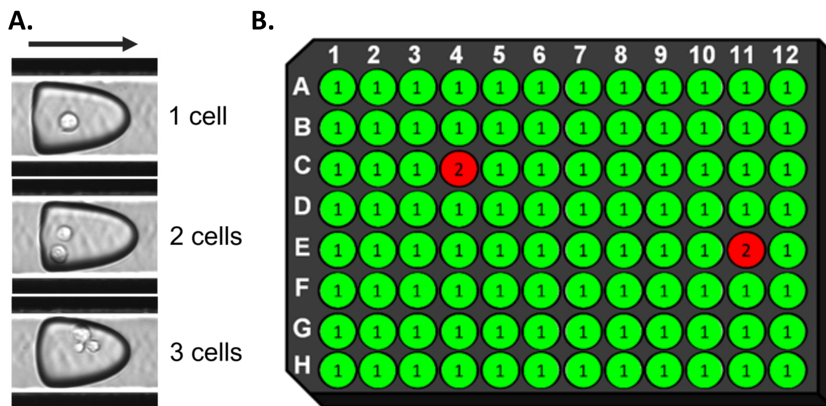


Figure 8: Cyto-Mine® data-tracking and verification of single-cell status. Software analyses and identifies the number of cells per picodroplet (**A**), and displays the results in microplate map format (**B**). Only picodroplets that that unambiguously contain a single cell are given single-cell status. Fluorescent intensity is also documented for each well (not shown).

Cyto-Drop™, the proprietary dispense mechanism of Cyto-Mine®, gently dispenses verified single cells to prefilled microplates. As the picodroplet contacts the culture medium in the microplate well, it dissociates liberating the cell into the culture medium. The plate-filling speed is rapid, taking less than 2 minutes to fill a 96-well plate and 4-6 minutes for a 384-well plate. The protective nature of the Cyto-Mine® picodroplet technology and workflow generates clones with strong outgrowth potential.

Conclusions

The 4 sequential steps within the fully integrated Cyto-Mine® workflow described here offer a unique and powerful solution to the challenge of screening large numbers of cells and then isolating the highest-value clones with the confidence that they derive from a single cell progenitor. Combined with single-use, disposable consumables and reagents, Cyto-Mine® offers the following major benefits:

Simplicity:

Seamless, fully automated process for screening and isolating verified clones on a single platform.

Quality:

GLP-compliant platform with unique ability to mine and isolate rare, single, viable and high-producing cells.

Traceability:

Storable visual proof of single-cell status at the point of dispensing provides the required documented evidence of monoclonality.

Strong outgrowth:

Gentle picodroplet encapsulation and processing ensures compatibility with a broad range of animal and human cell types with high viability of selected clones.

Speed and efficiency:

Reduces Discovery and Cell Line Development process timelines and enables multiple projects to be run in parallel. No downtime between runs.

Sterility:

Benchtop system compatible for use in Class II biosafety cabinets with end-to-end sterility and disposable AOF consumables.

This work was partly funded by the UK Government Advanced Manufacturing Supply Chain Initiative (AMSCI) as part of the BioStreamline Project which exists to develop new approaches for the more efficient development and manufacture of next generation biologics.
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