CYTO-MINE®: AN INTEGRATED PICODROPLET SYSTEM FOR SINGLE CELL ANALYSIS AND MONOCLONALITY ASSURANCE

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INTRODUCTION:

A number of different techniques are routinely used in the biopharmaceutical discovery and development workflows. These include single cell analysis, sorting, imaging and dispensing into individual wells of microtiter plates (MTPs). Traditionally, different instruments would be required for each technique; which is costly, time-consuming and requires extensive lab space and manual handling that increases the risk of sample contamination. Picodroplet techniques allow for sophisticated and sensitive manipulation of cells at the single cell level. Cyto-Mine® technology is the first integrated device to automatically perform all of these crucial techniques in a single compact system. This high-throughput instrument uses picodroplet technology and microfluidics to process up to 10 million heterogeneous mammalian cells in a few hours. Each cell is encapsulated in a picodroplet containing cell culture growth media, which acts as a bioreactor to compartmentalise the cell and let it grow; eventually trapping secreted molecules such as antibodies (Abs). The system also allows selection and deposition of single cells into individual wells of 96- and 384-well MTPs - ensuring high fidelity monoclonality assurance (required for FDA-approval of all monoclonal Abs).

CHALLENGES IN ANTIBODY DISCOVERY:

- → Low efficiency in hybridoma technology.
- → Single cell screening is needed for deep repertoire interrogation.
- → Single cell assays are required to detect surface bond and/or **secreted antibodies.**
- → Good screening specificity is desirable to reduce downstream costs.
- → Large number of cells, *e.g.* 1 to 20 million, need to be screened.
- → Cell friendly processing is desirable to maximize transcript recovery.

CHALLENGES IN CELL LINE DEVELOPMENT:

- → Larger scale cloning is desirable to maximize the chance of wining.
- → Random cloning results in costly post cloning screenings.
- → Lack of assays reliably ranking and selection based single cell productivity.
- → Cell friendly processing is desirable to allow cell out-growth.
- → Evidence of monoclonality is a regulatory requirement.

CYTO-MINE® KEY FEATURES

SCREENING	CLONING	OTHERS
❑ Quantitative single cell secretion assay	❑ Cell friendly dispensing	❑ Sterile
❑ Customizable assay designs	❑ Monoclonality assurance	❑ Animal origin free
❑ Cell friendly sorting	❑ Database compatible	❑ User friendly
		❑ Automation compatible

CYTO-CARTRIDGE®

- ❖ Fully integrated microfluidic functional modules.
- ❖ Mass manufactured.
- ❖ Sterilized.
- ❖ Single-use.
- ❖ Animal origin free.

CYTO-MINE® INSTRUMENT

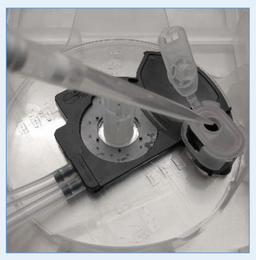
- ❖ Fully integrated control modules.
- ❖ User-friendly GUI.
- ❖ Compact design.
- ❖ Single use consumables.
- ❖ Animal origin free.
- ❖ Robotic arm accessible.

CYTO-SURF®

- ❖ Proprietary formulation.
- ❖ Biocompatible.
- ❖ Sterilize.
- ❖ Animal origin free.

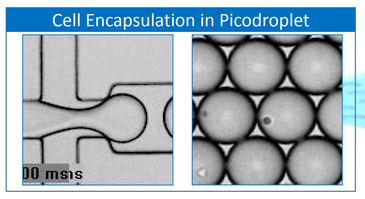
Dimensions	L × H × W = 860 × 566 × 463 mm
Weight	85 Kg
Detection mode	Laser induced fluorescence
Excitation Laser wavelength	λ_{ex}=488 nm
Detection colours	Two
Picodroplet size	300 pL
Screening capacity	Up to 2,000,000 picodroplets/run
Screening throughput	10,000-10,000,000 cells in 2-7 hours
Speed of dispensing	1 picodroplet per second
Max. no. of dispensings	10,000
Consumables	Cyto-Cartridge®
	Cyto-Surf®
Compatible cell types	CHO, B Cells, Hybridoma

CYTO-MINE® WORKFLOW:



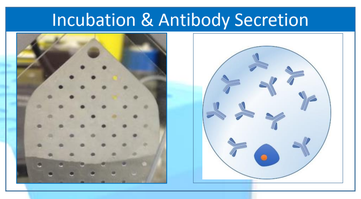
1. User's cells and assay reagents are pipetted into the Cyto-Cartridge®.

Cell Encapsulation in Picodroplet



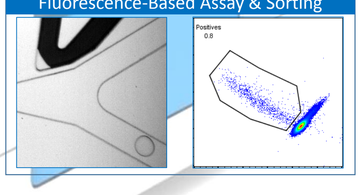
2. Cells and assay reagents are encapsulated in picolitre volume picodroplets, together with assay reagents. The distribution of cells in picodroplets follows a Poisson distribution. Hundreds of thousands to several million cells can be encapsulated and processed in a single run.

Incubation & Antibody Secretion



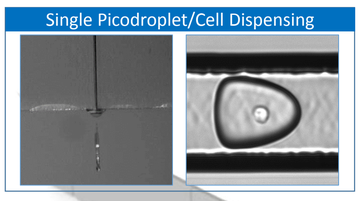
3. Picodroplets containing cells are incubated at 37°C for up to several hours. The cells secrete antibody molecules during incubation. Due to the small volume of the picodroplets, the antibodies produced by a single cell can reach a detectable concentration in a relatively short time window.

Fluorescence-Based Assay & Sorting

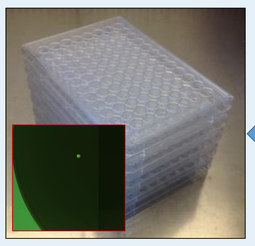


4. The antibodies in picodroplets secreted by single cells can be detected using a FRET-based quantitative fluorescent assay. Positive cells in picodroplets can be sorted based on the gate settings defined by the user.

Single Picodroplet/Cell Dispensing



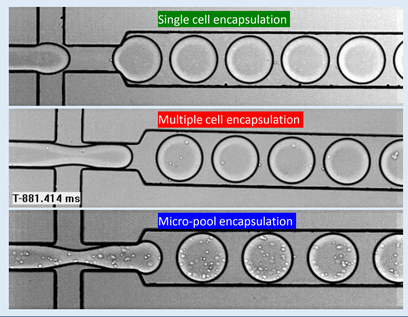
5. Sorted positive picodroplets containing single cells are dispensed one by one into individual wells of 96- or 384- well plates. Monoclonality is assured by recording multiple images of each picodroplet before they are dispensed.



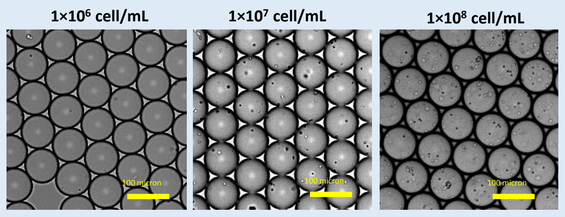
6. Individual cells dispensed in the wells can be directly processed for antibody cDNA retrieval or further cultured to grow into colonies.

CELL ENCAPSULATION :

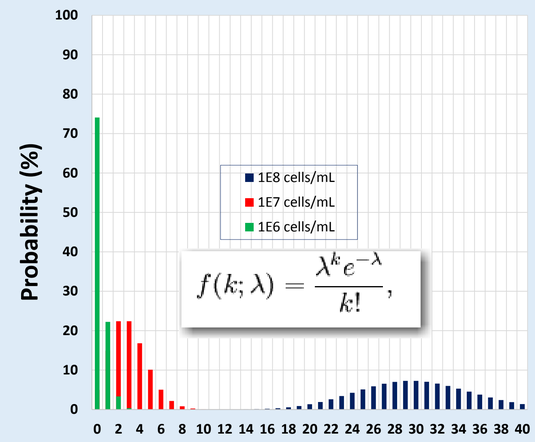
CELL ENCAPSULATION AT DIFFERENT INPUT CONCENTRATIONS



ENCAPSULATED CELLS IN PICODROPLETS

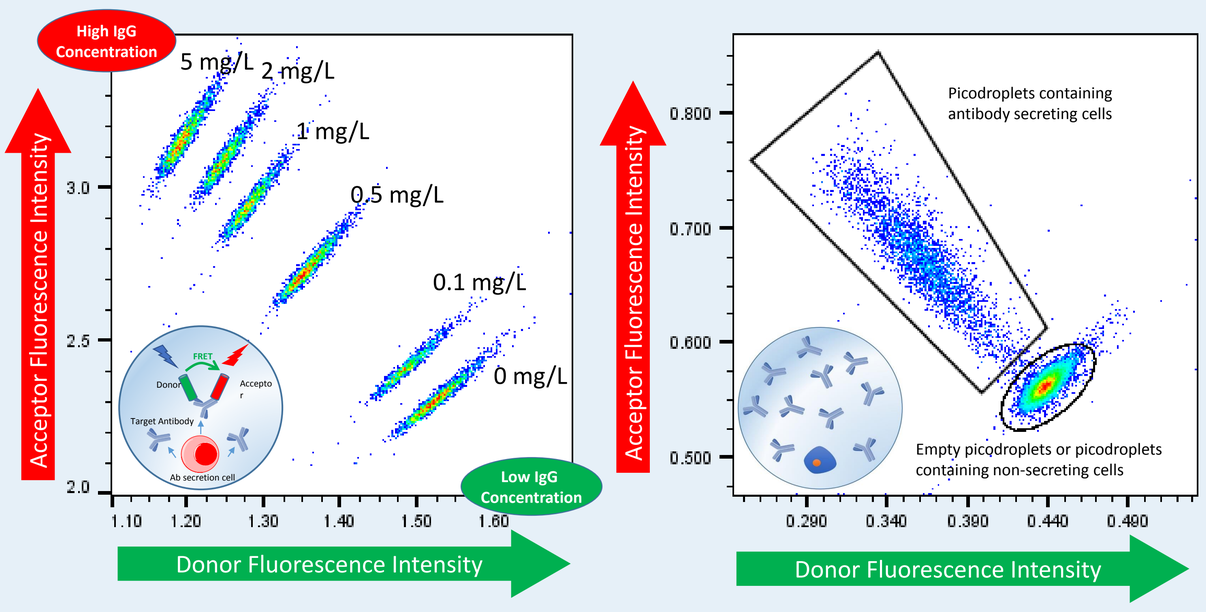


POISSON DISTRIBUTION OF CELLS IN PICODROPLETS



The cell number distribution in picodroplets is a factor which needs to be controlled and optimized by the user. High-quality cell encapsulation is achieved by: 1) accurately counting and adjusting the cell concentration in the medium and 2) minimizing cell sedimentation during the encapsulation process. In an ideal case scenario, the cell number in picodroplets will follow a Poisson distribution.

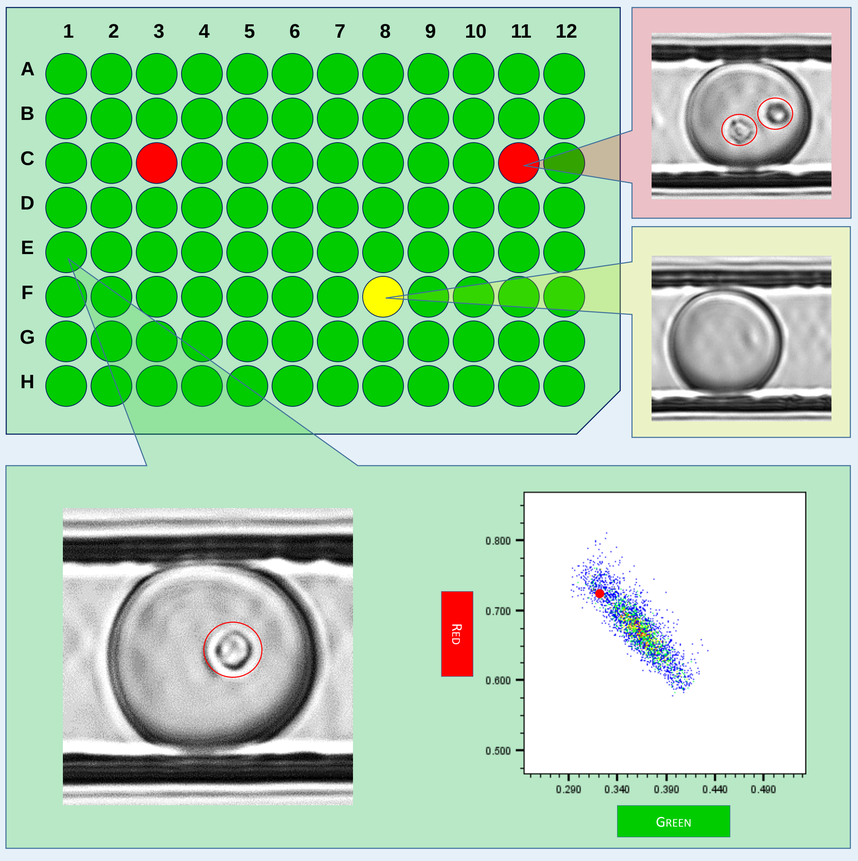
ASSAY AND SCREENING:



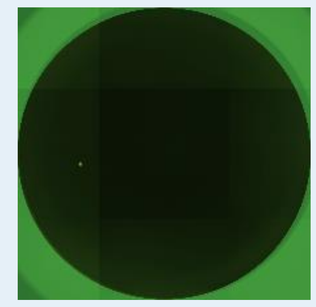
Encapsulating single cells in picodroplets enables detection of molecules secreted by the cells (rather than cell surface-bound molecules). Sphere Fluidics has developed a panel of homogeneous FRET assays which allows detection of IgG from various species inside picodroplets. The homogeneous assays can provide information on the endpoint 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, and 5 mg/mL target IgG were generated separately, then mixed at a equal ratio.

The size of the picodroplets generated in Cyto-Mine® is only several hundreds of picolitres in volume, about 5-6 orders of magnitude lower than the 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 conventional assay. In Cyto-Mine®, it only takes 0.5-4 hours incubation time before the system can detect antibody secretion from each of the encapsulated cells. 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. From 10,000 up to 2,000,000 picodroplets can be analysed in a single Cyto-Mine® experiment run.

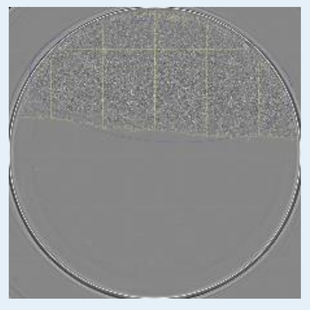
SINGLE CELL DISPENSING:



A SINGLE CELL DISPENSED IN A WELL OF A 96-WELL PLATE



A COLONY GROWN FROM A SINGLE CELL DISPENSED BY CYTO-MINE®



Cyto-Mine® uses a patented technology to dispense each picodroplet containing a cell into an individual well of a 96 or 384-well microtiter plates, after the screening step. Just prior to the picodroplet being dispensed, the system takes several images of the picodroplet and uses an object recognition algorithm in the software to identify the number of cells inside this picodroplet. A second fluorescent reading on each picodroplet is also carried out at dispensing step. The movement of micro-titre well plate is synchronized with each dispensing event and the location of the dispensed picodroplet/cell in the well will be recorded by the system as well. After each experimental run, the system will provide 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. Cyto-Mine® work flow is proven to be bio-friendly. No reduction in cell viability is observed after the Cyto-Mine® process, the CHO cells dispensed into wells are still alive and proliferate into colonies.

CONCLUSIONS:

By encapsulating single cells in picolitre volume water-in-oil 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 productivity. The system also enables monoclonality assurance thus providing a comprehensive solution for accelerated biotherapeutic discovery and development. The entire systems is also animal origin free, ISO 9001 and GLP-compliant.

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