CYTO-MINE® - AN INTEGRATED PLATFORM FOR RAPID THERAPEUTIC DISCOVERY FROM SINGLE CELLS

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Introduction:

A number of different techniques are routinely used in the biopharmaceutical discovery and development workflow. These include single cell analysis, sorting, imaging and dispensing into individual wells of microtitre plates (MTPs). Traditionally, different instruments would be required for each technique; which is costly, time-consuming and requires extensive lab space that increases the risk of sample contamination. Picodroplet encapsulation 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 5 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 well of 96- and 384-well MTPs - ensuring high fidelity monoclonality assurance (required for FDA-approval of all monoclonal Abs).

CHALLENGES IN ANTIBODY DISCOVERY

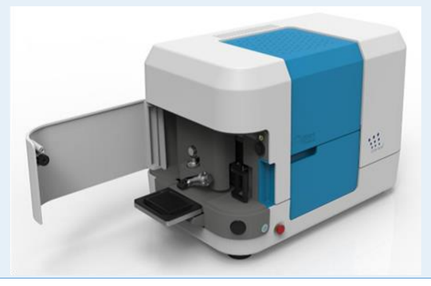
- Low efficiency in hybridoma technology
- Single cell screening is needed for deep repertoire interrogation
- Single cell assays are required to detect surface bond and/or **secreted antibodies**
- Good screening specificity is desirable to reduce downstream costs
- Large number of cells, *e.g.* 20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

CHALLENGES IN CELL LINE DEVELOPMENT

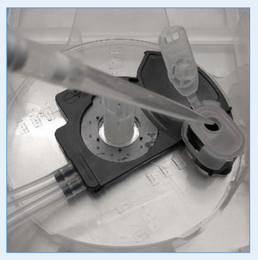
- Larger scale cloning is desirable to find the best producers
- Random cloning results in costly post cloning screening
- Lack of assays to reliably rank and select for single cell productivity
- Cell friendly processing is desirable to allow cell out-growth
- Evidence of monoclonality is a regulatory requirement

CYTO-MINE® KEY FEATURES

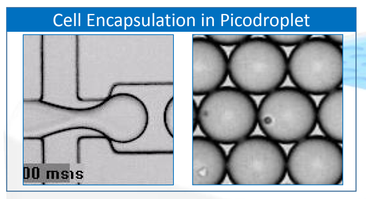
Screening	Cloning	Others
❑ Quantitative single cell secretion assay	❑ Cell friendly dispensing	❑ Sterile
❑ Customizable assay designs	❑ Monoclonality assurance	❑ Animal component free
❑ Cell friendly sorting	❑ Database compatible	❑ East to use
		❑ Automation compatible



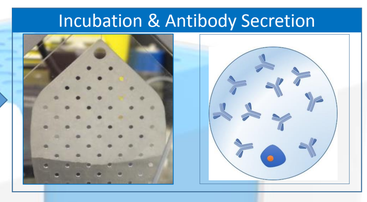
Cyto-Mine® Workflow:



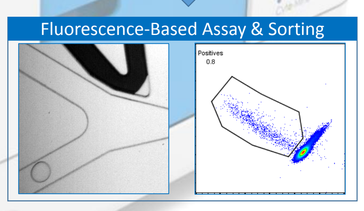
1. User load cells and assay reagents are pipetted into the Cyto-Cartridge®.



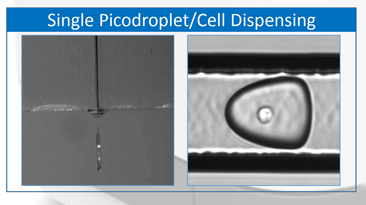
2. Cells and assay reagents are encapsulated in picolitre volume picodroplets, together with assay reagents. The distribution of cells in picodroplets follows a Poisson distribution. Hundreds of thousand to several million cells can be encapsulated and processed in a single run.



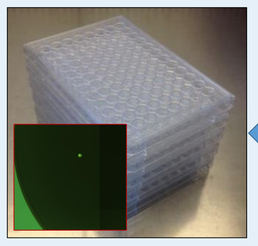
3. Picodroplets containing cells are incubated at 37ºC for up to several hours. The cells secrete antibody molecules during incubation. Due to the small volume of the picodroplets, the antibodies produced by a single cell can reach a detectable concentration in a relatively short time window.



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.



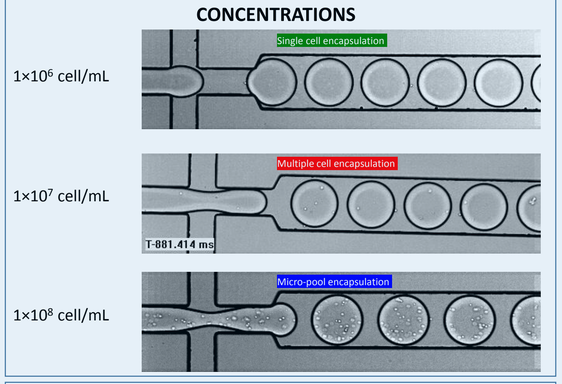
5. Sorted positive picodroplets containing single cells are dispensed one by one into individual wells of 96- or 384 well-plates. Monoclonality is assured by recording multiple images of each picodroplet before they are dispensed.



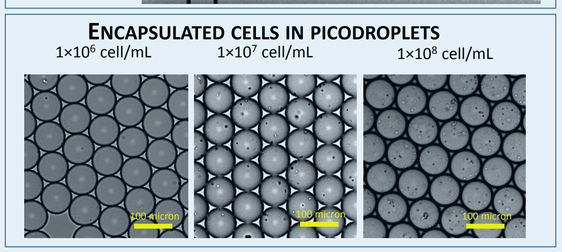
6. Individual cells dispensed in the wells can be directly processed for antibody cDNA retrieval or further cultured to grow into colonies.

Cell Encapsulation:

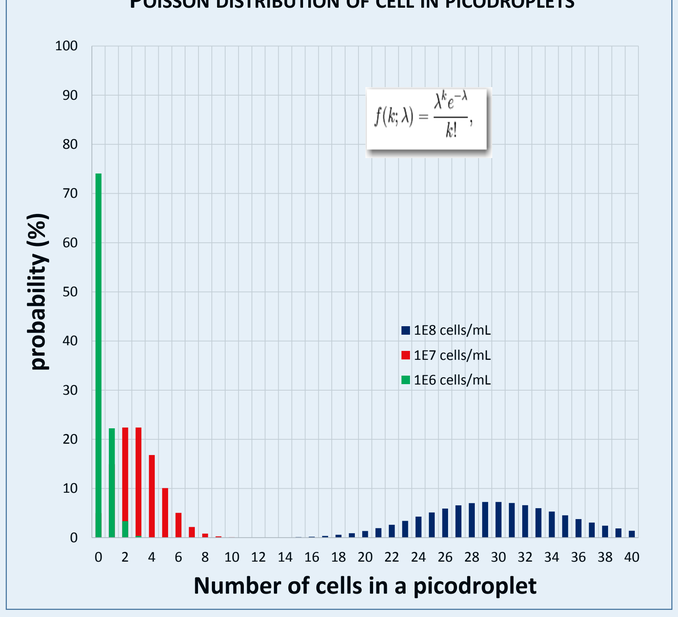
CELL ENCAPSULATION AT DIFFERENT INPUT CELL CONCENTRATIONS



ENCAPSULATED CELLS IN PICODROPLETS



POISSON DISTRIBUTION OF CELL 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 ideal case scenarios, the cell number distribution should follow Poisson statistics, as shown in the formula below:

$$f(k;\lambda) = \frac{\lambda^k e^{-\lambda}}{k!}$$

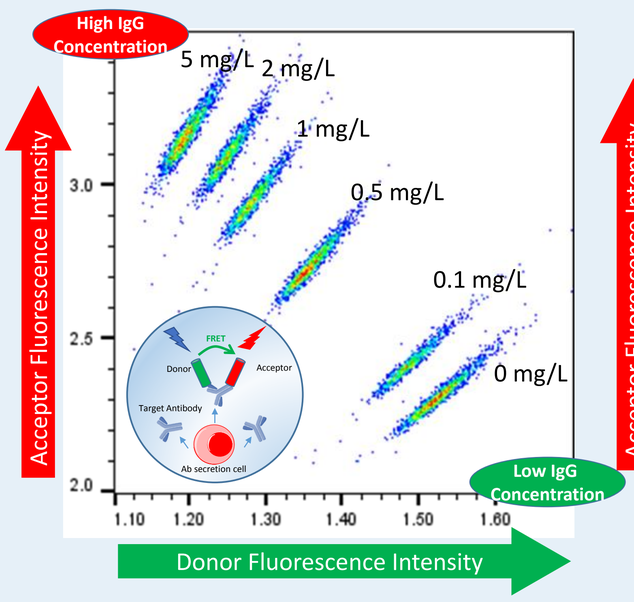
f - Frequency of occurrence

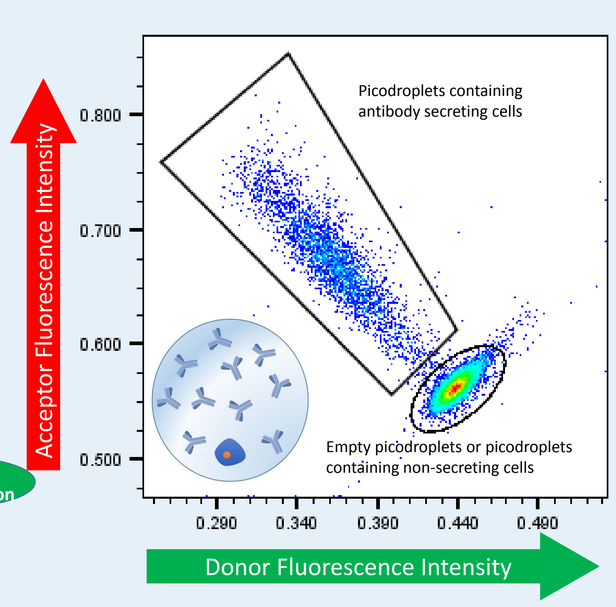
λ – Average number of cells (or particles) over number of picodroplets

κ – Number of cells in picodroplets

In the context of picodroplet experiments, Poisson statistics predicts, with a given λ value, what the expected frequencies of occurrence (f) are for a picodroplet containing 0, 1, 2 or 3 cells (κ = 0, 1, 2 or 3). The λ value can be calculated by multiplying cell concentration of the sample by the volume of picodroplets. For example, for a cell sample containing 1×10^6 cells/mL encapsulated in 300 pL picodroplets, λ= (1×10^6/1,000,000,000) × 300 or 0.3.

Assay and Screening:

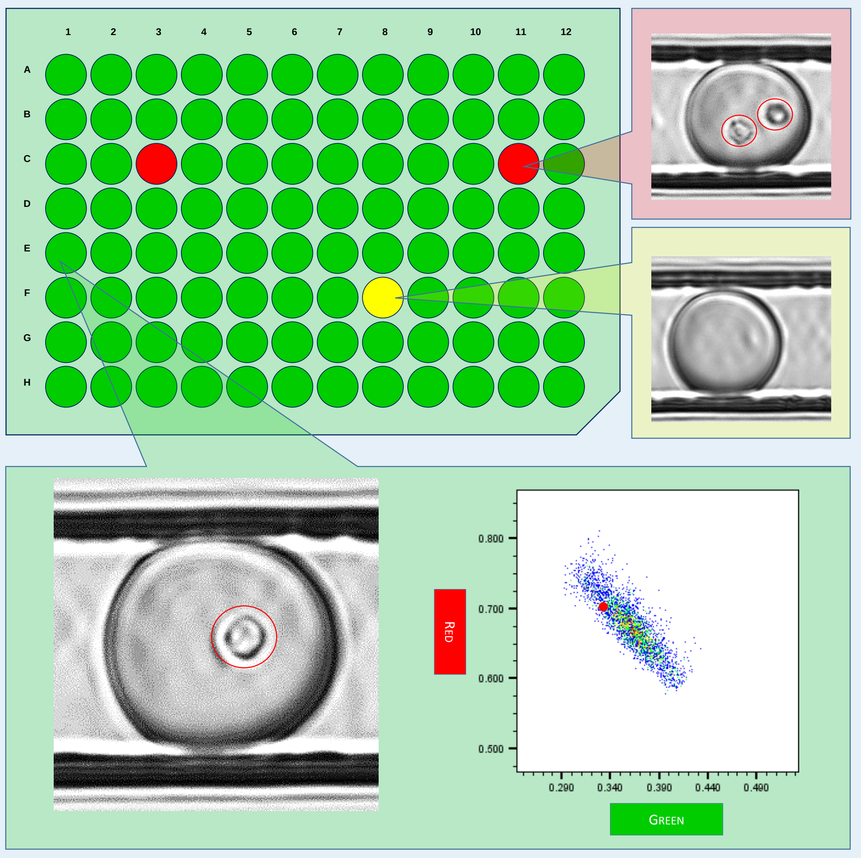




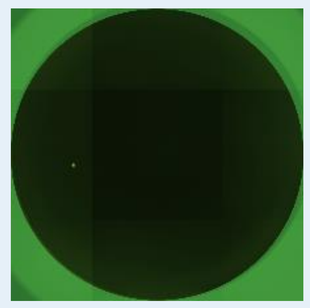
Encapsulating single cells in picodroplets enables detection of molecules secreted by the cells (rather than cell surface-bound molecules). Sphere Fluidics has developed a panel of homogeneous FRET assays which allows detection of IgG from various species inside picodroplets. The homogeneous assays can provide information on the endpoint concentration of the antibodies in the picodroplets, essentially, enabling single cell sorting and cloning based on ranking their productivities. 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 a equal ratio.

The size of picodroplets generated in Cyto-Mine® are only several hundred picolitres in volume, about 5-6 orders of magnitude lower 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 to 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. From 10,000 up to 5,000,000 picodroplets can be analysed in a single Cyto-Mine® experiment run.

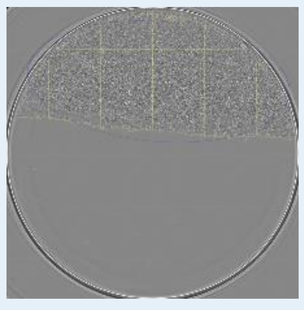
Single Cell Dispensing:



SINGLE CELL DISPENSED IN A WELL OF 96-WEILL PLATE



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



Cyto-Mine® uses a proprietary mechanism to dispense each cell-containing picodroplet into 96 or 384-well microtiter plates, after a secondary screening step. 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 that picodroplet. A second fluorescent reading on each picodroplet is also carried out at the dispensing step. The movement of the microtitre well plate is synchronized with each dispensing event and the location of the dispensed picodroplet/cell in the well is also be recorded by the system. After each experimental run, the system will provide a data pack of: 1) a map of monoclonality in each wells 2) images of each picodroplet prior to dispensing and 3) fluorescence intensity reading for that picodroplet. Single cells can be differentiated from zero or two cells. The Cyto-Mine® work flow is biofriendly. No loss in cell viability was observed and the CHO cells dispensed into wells were still alive and can proliferate into colonies.

Cyto-Mine® System Specifications:

Detection mode	Laser induced fluorescence	**Screening throughput**	10,000-5,000,000 cells in 2-6 hours
Number of Lasers	One (λ=488 nm)	**Speed of dispensing**	1 picodroplet per second
Detection colours	Two	**Max. number of dispenses**	10,000
Picodroplet volume	300 pL	**Consumables**	Cyto-Cartridge®
Operation modes	Assay, Monoclonality, Stability		Cyto-Surf®
Screening capacity	Up to 2,000,000 picodroplets/run	**Compatible cell types**	CHO, B Cells, Hybridoma

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.

Conclusions:

By encapsulating single cells in picolitre volume water-in-oil droplets, 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 system is animal origin free, ISO 9001 and GLP compliant.

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