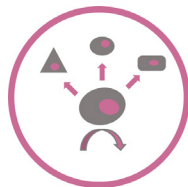


Automate cloning steps with cellenONE®

Single cell dispenser based on **acoustic** handling



mAb



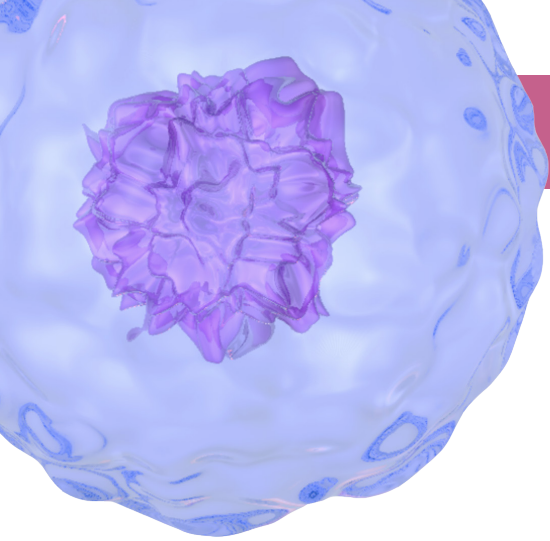
Stem Cells



CRISPR/CAS9

LOW VOLUME, HIGH VIABILITY & PRECISION SINGLE CELL ISOLATION
FOR CELL LINE DEVELOPMENT... FOR SEQUENCING... AND MUCH MORE

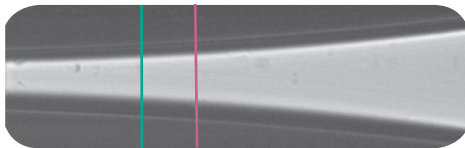
About the technology



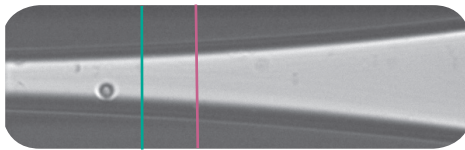
cellenONE, an automated single cell dispensing system based on patented piezo-acoustic technology, allows precise cell deposition on a wide range of microplates (96, 384, 1536) and microwell substrates.

Most dispensing and microfluidic technologies follow Poisson distribution, which leads to multiple cells per position and low efficiency. cellenONE uses software-integrated visual feedback to ensure only single cells are deposited in every position.

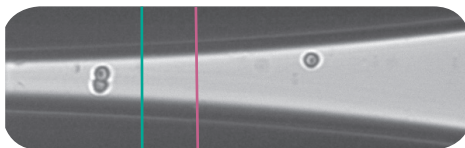
With cellenONE, cell sample is divided into droplets of identical volume. Each drop can contain:



(A) no cell



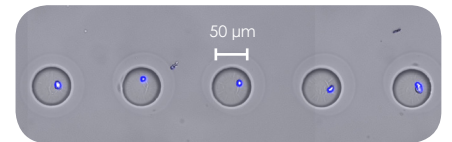
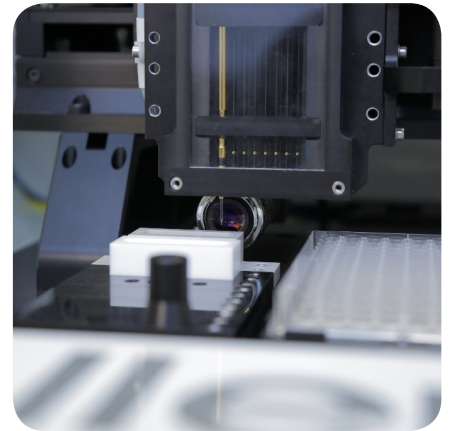
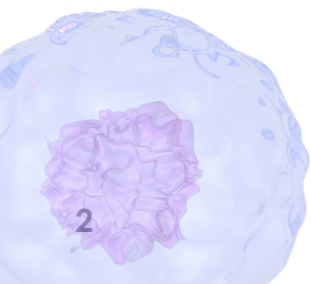
(B) one cell,



(C) multiple cells.

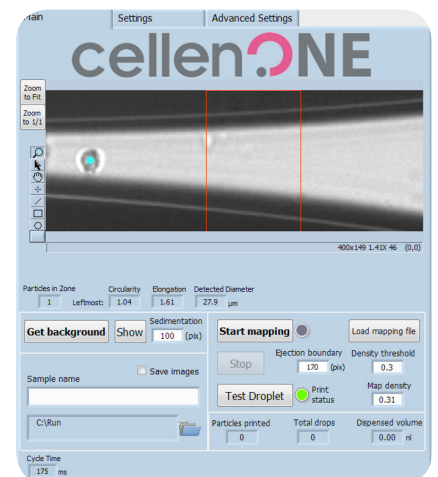
cellenONE only dispenses single-cell containing drops into the microplate. All remaining drops will be dispensed into a recovery tube, resulting in no sample wastage.

Samples are analyzed live and pictures recorded for monoclonality documentation



Single cell deposited in 50 µm microwells

Thanks to visual feedback and software mapping process, only single cells are dispensed



Workflow



Cell Sample Preparation

Place sample in holder
Aspirate sample

- Choose culture media (DMEM, RPMI, Ham's F12) or buffer (PBS)
- Prepare a minimum volume of 2 μL , ideally around 10 μL
- Optimal cell concentration below 200 cells/ μL

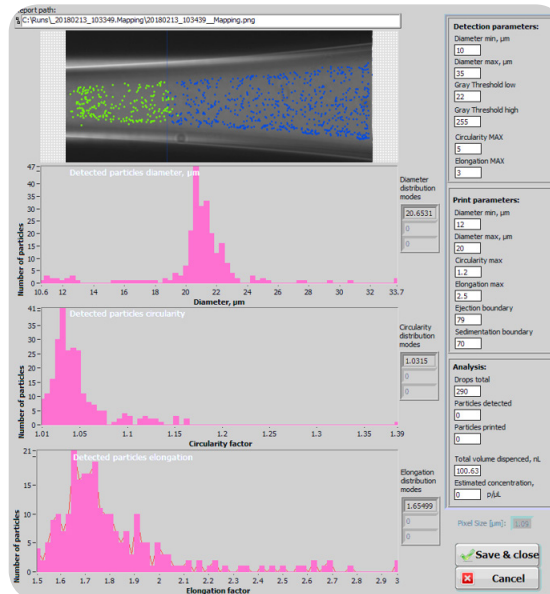


Cell Mapping

System setup only takes a few minutes

Users can walk away during isolation process

- Automated cell tracking
- Automated report generation
- Choose isolation parameters
- Choose type of target (96,384,1536 wp)



A mapping report is automatically generated, it contains:

- Evaluation of ejection zone boundary
- Detected cell diameter, elongation and circularity distribution
- Average cell concentration

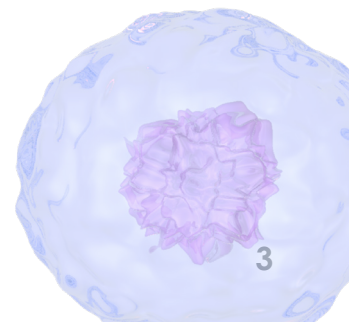


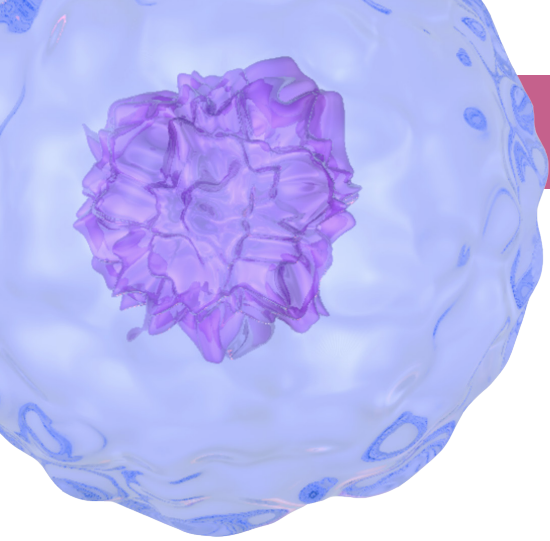
Automated Cloning

- Up to 2 microplates per run
- 96 single cells in ~ 4 min (or 384 in ~ 16 min)
- Walk-away operation

Sample will be processed autonomously until all programmed positions have been filled with a single cell.

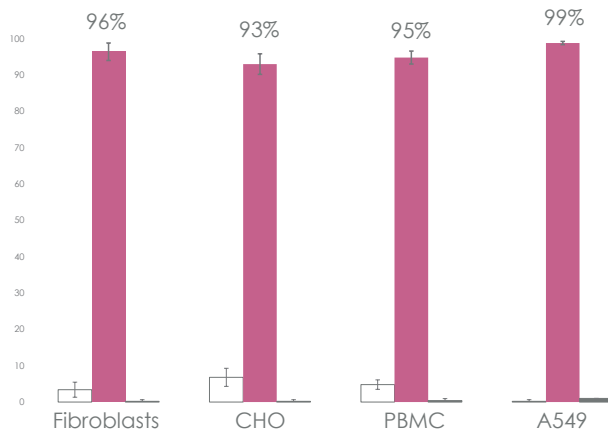
* per 96 well plate at optimal cell concentration



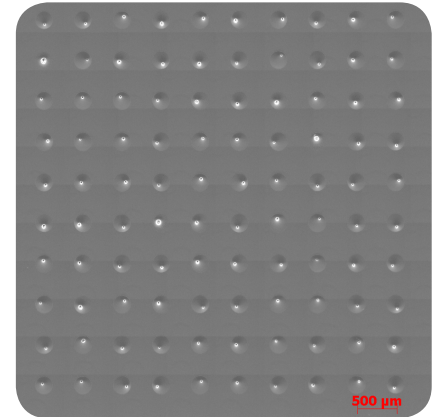


Up to 100% single cells!

Accurate



Results from 5x100 positions filled with single cells from four different cells samples. Single cells rate is indicated in pink, up to 99% for A549 cells; in white are empty positions and in grey, multiple cells positions.



Single cells from dissociated lung cancer spheroids successfully isolated onto a microscope slide. Every position contains one single cell.

Key Features

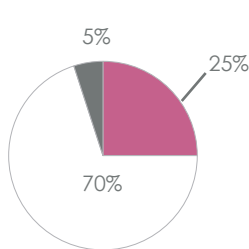
cellenONE®

Only single cells dispensed

High viability

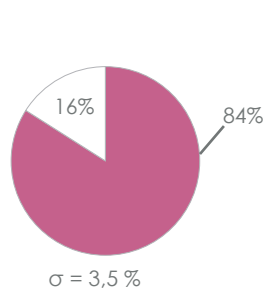
Suitable for most cell types

Outstanding cell viability



By Limiting Dilution

vs.



With

cellenONE®

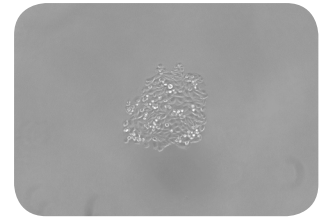
Limiting dilution achieves approx. 25% of wells with a single colony; but several wells will contain multiple colonies which then forces a second round of cloning to ensure mono-clonality.



Single CHO were dispensed into 96-well plates and colonies were counted after 4 days and 7 days of culture. An average of 84% of wells (403 out of 480) contained single colonies after 7 days of culture.

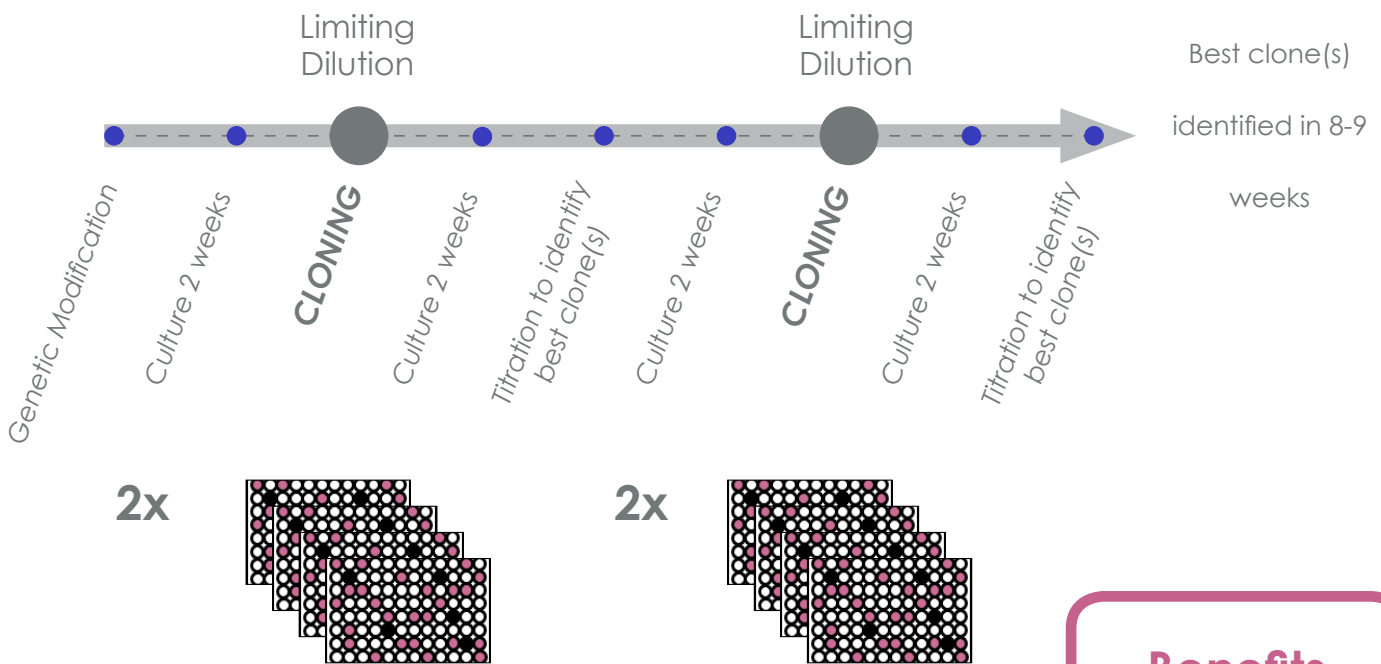
Accelerate Cell Line Development

The main challenges associated with cell line development include maintaining good cell viability and ensuring mono-clonality of isolated cells.

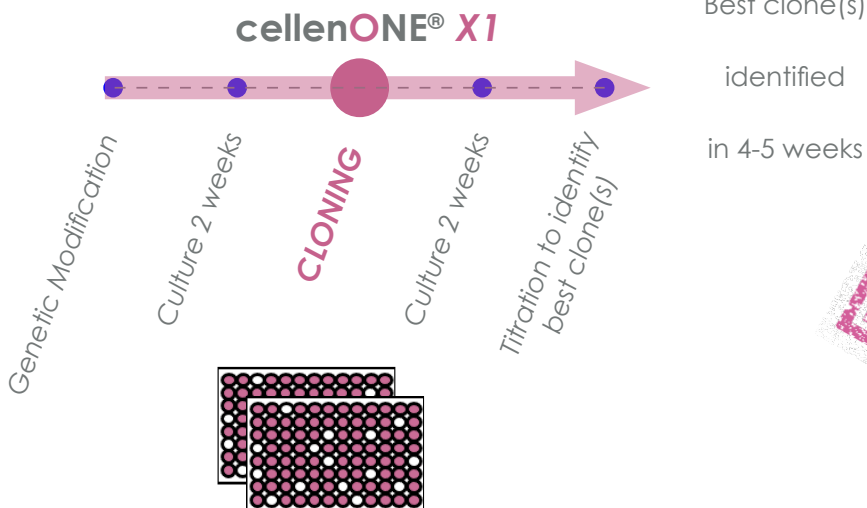


Cloning experiment with cellenONE® showing a single CHO colony after 4 days of culture

TRADITIONAL DILUTION PROCESS



WITH cellenONE® X1



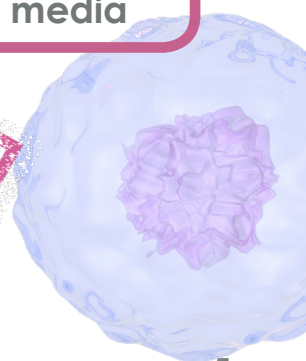
Benefits cellenONE®

Avoid Recloning

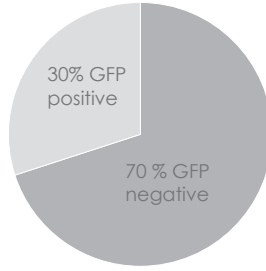
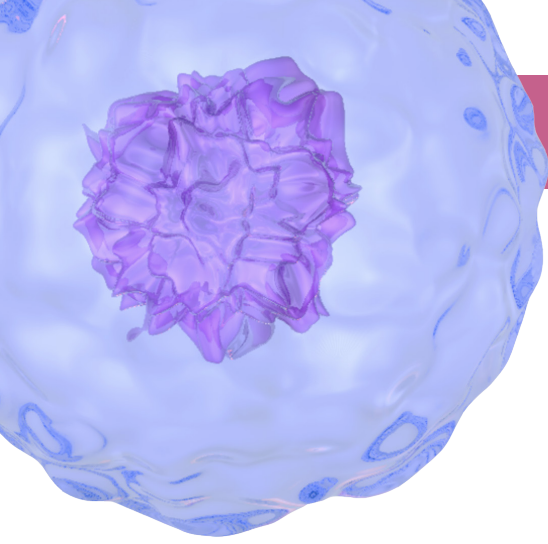
Save time

Save on consumables and media

Save 4 weeks



cellenONE® vs. FACS

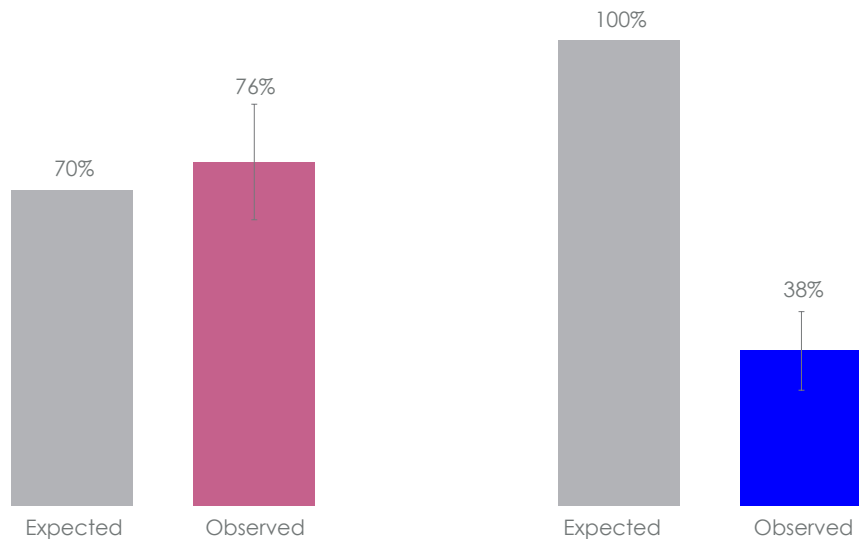
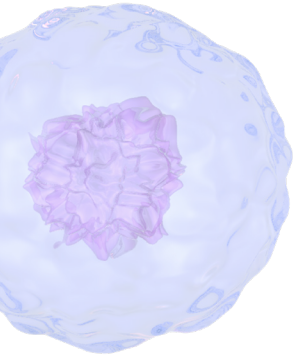


Initial cell composition

Aim was to isolate only single GFP negative cells (70%)

cellenONE® X1

FACSria™ III

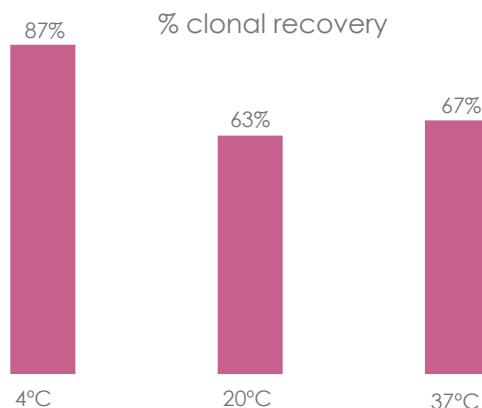


Comparative cloning experiment: cellenONE® X1 vs. FACSria™ III. The aim of this experiment was to clone GFP negative cells from a mixed CHO cell suspension. Cells were isolated into antibiotic containing media that selects only cells of interest (GFP negative fraction). After 5 days of culture, colonies were counted and results summarized as shown above.

FACSria™ III used fluorescent detection to select only GFP negative cells, as a result, theoretically, up to 100% of wells should contain single cell. However, after 5 days of culture, only 38% of wells contained single cells due to high shear stress involved in this technology.

In comparison, with cellenONE® X1, a maximum of clonal recovery of about 70% was expected. After 5 days of culture, the piezo-acoustic technology resulted in 76% of wells containing single growing colonies.

cellenONE®
ensures most
isolated single
cells are
maintained
alive!



In addition, cellenONE® can be equipped with a temperature controlled unit to improve clonal recovery.

For CHO and HEK cells, optimal cloning results were obtained at 4°C!

CHO Cloning experiment in 384wp at different temperature.

Specifications



Technical information

Dispensing technology: piezo acoustic drop-on-demand
Dispense volume: 50-800 pL per drop
Linear drives for X/Y and spindle drive for Z
Resolution: 1 μm
Accuracy (Absolute Position): < 10 μm
Precision (Repeat Position): < 3 μm
Max. speed: 100 cm/sec
Spottable area (mm): x=180; y=120 (2 microtiter plates)
Dimensions LxWxH (mm): 740 x 750 x 1580
-> including monitor's arm L = 1260 mm
-> with door open H = 1970 mm
Weight: 205 kg
Voltage: 110 V; 220 V

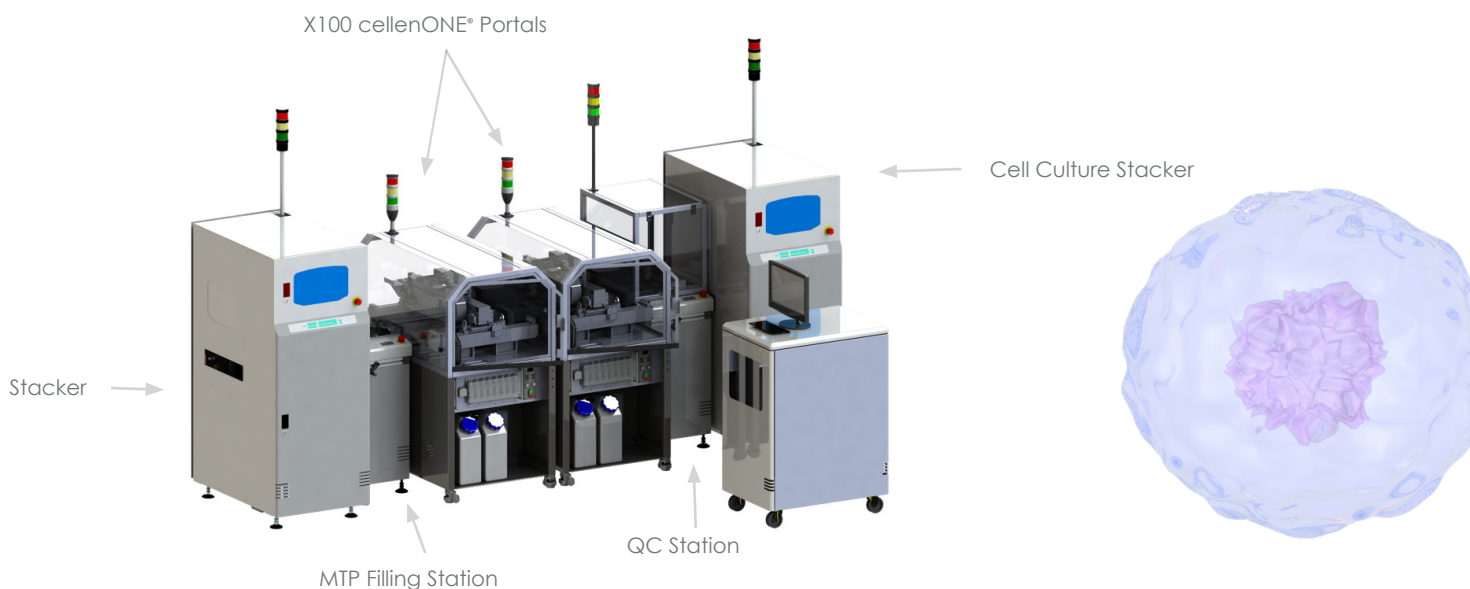
Options & Software

cellenONE® HD Vision upgrade for cells or particles < 5 μm
Temperature, humidity and dew point control
2nd channel for nanoliter dispensing
Customized holders for microwells chips
Fiducial recognition and automated target alignment

Related Products & Services

cellenBEADS for calibration
cellenWASH for sterilization
cellenVIALS for recovery
cellenSERVICES for application support

If high throughput is required, **cellenONE®** can be implemented on SCIENION S100 systems with automated cell culture stackers.



"We have been using cellenONE® in combination with our latest cell sorter, the MACSQuant® Tyto®. These two technologies offer a great combination for cell line development. The MACSQuant® Tyto® Sorter is an easy to operate instrument that gently enriches the cell population of interest, while cellenONE®'s accuracy allows for highly efficient clone selection from the enriched population. Used together, these two technologies reduced our monoclonal antibody development protocols by nearly 4 weeks."



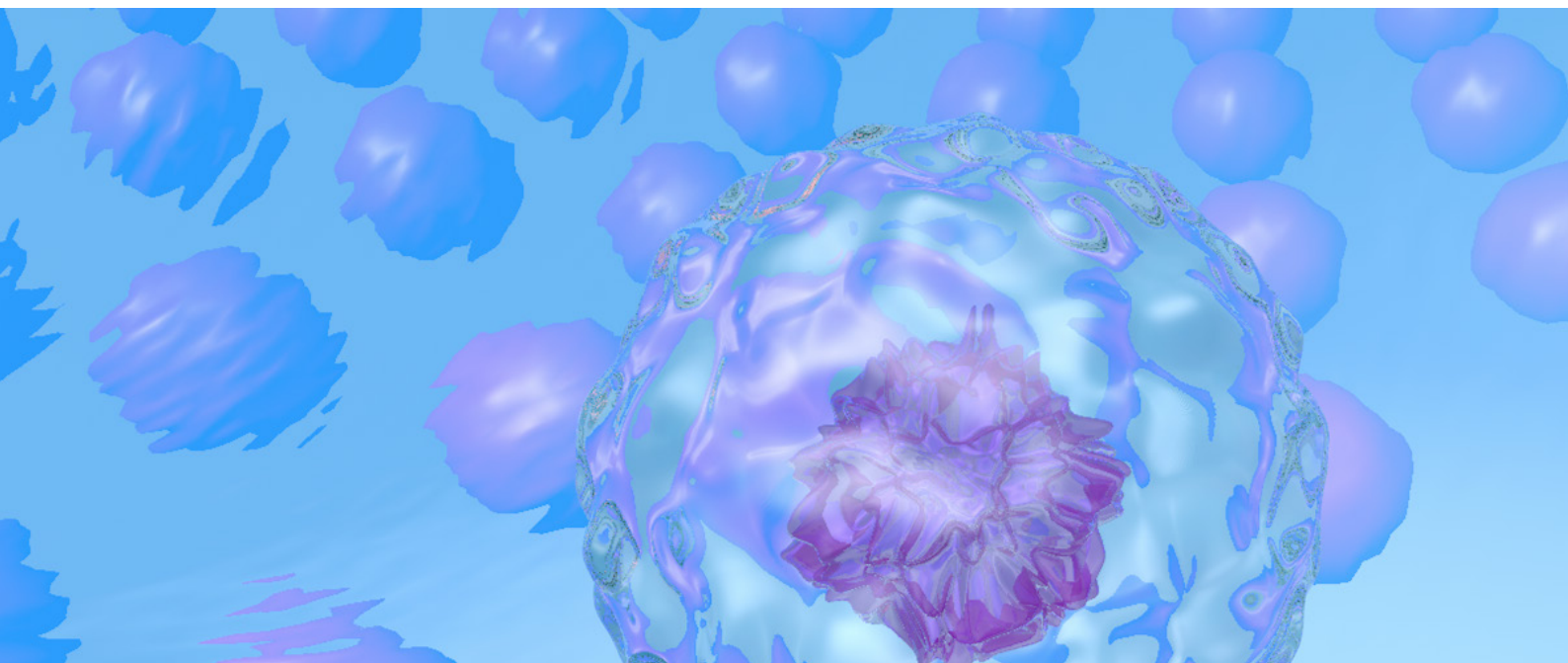
Bastian Ackermann,
Global Product Manager MACSQuant® Tyto®



"HuBBBTM technology is a very powerful B cell immortalization system. However, data were limited due to successful cloning of only 10% of immortalized B cells.

The combination of HuBBBTM and CellenONE® dramatically increases cloning efficiency up to 90%, thus providing significant time saving and ensuring identification of the very best clones."

Jean-Jacques Pin,
CEO Dendritics



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