NEXT GENERATION SINGLE-CELL DISPENSING

FOR CELL LINE DEVELOPMENT AND SINGLE-CELL GENOMICS

ASSURANCE OF CLONALITY

cytena
A CELLINK COMPANY

We believe that automation and microtechnology will bring the understanding and control of individual cells to a whole new level and empower our customers to serve patients faster and better.

index

Next-generation Single-cell Dispensing in

Assurance of clonality

by an image series for each isolated single cell

Very high cell viability

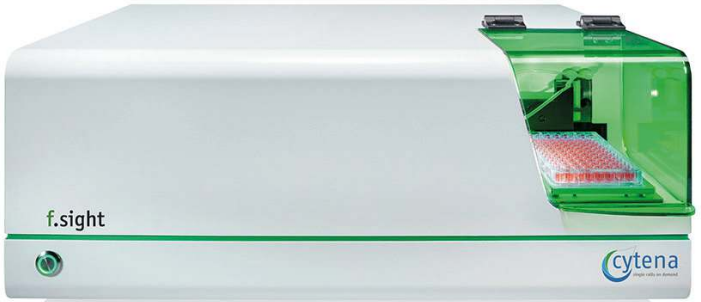
as high as standard handpipetting

Easy to use

after a short introduction all your lab staff can easily use our devices

Isolate single cells in well plates within minutes.
Brightfield nozzle imaging with 10x magnification.
Cell line development and single-cell genomics.

c.sight™



Isolate green fluorescent eukaryotic cells in well plates.
Brightfield and fluorescence imaging.
Fully tunable to enable even low-intensity samples.

f.sight™

Cell Line Development and Single-cell Genomics

No cross-contamination

thanks to the
disposable cartridges

Very high efficiency

enabling time- and costeffective
workflows

Flexibility

enabled by 96- and 384-plates
and additional substrates

Isolate prokaryotic cells in well plates. Brightfield nozzle imaging with 20x magnification. Sort single bacteria for culture, genomics or mass spec.

b.sight™

Single-cell isolation by brightfield imaging.
Two separate carriers for well plates.
Automation-ready with open deck and software API.

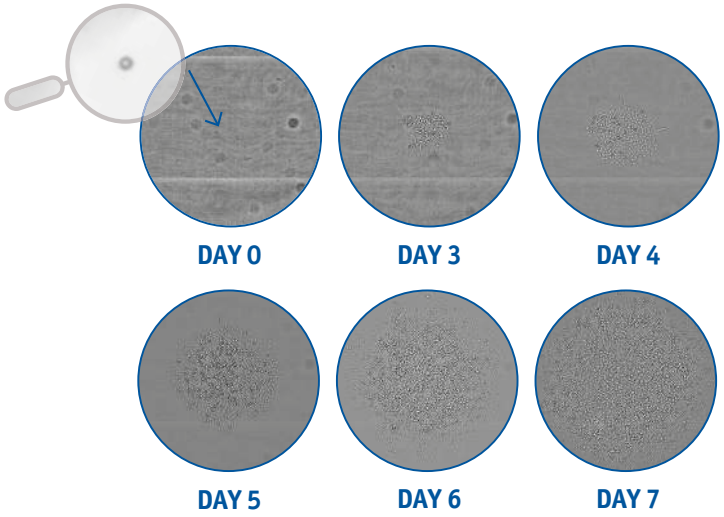
single-cell printer™

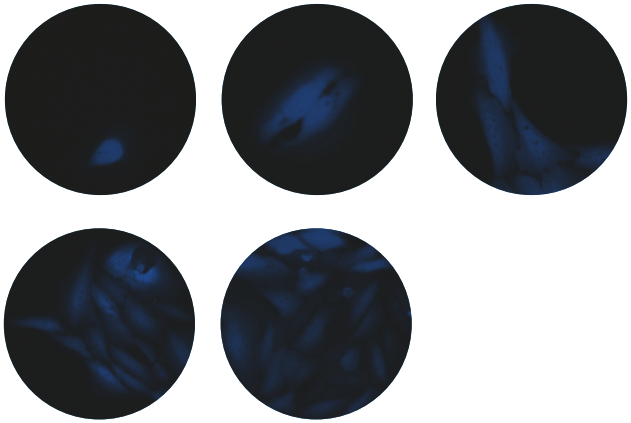
cell line development

Clonal cell line development is a crucial step in many applications including generating biopharmaceuticals (e.g. monoclonal antibodies). Current workflows in cell line development have major drawbacks such as missing proof of clonality, inefficient single-cell isolation and reduced cell viability.

The single-cell printer™ technology enables documented **assurance of clonality**, providing efficient and fast single-cell seeding combined with excellent cell viability and zero risk of crosscontamination.

All cytena products supports SLAS/SBS format 96-well and 384-well plates. You can process a great variety of typical cell lines used in cell line development, such as CHO-K1, HEK 293 and L929.



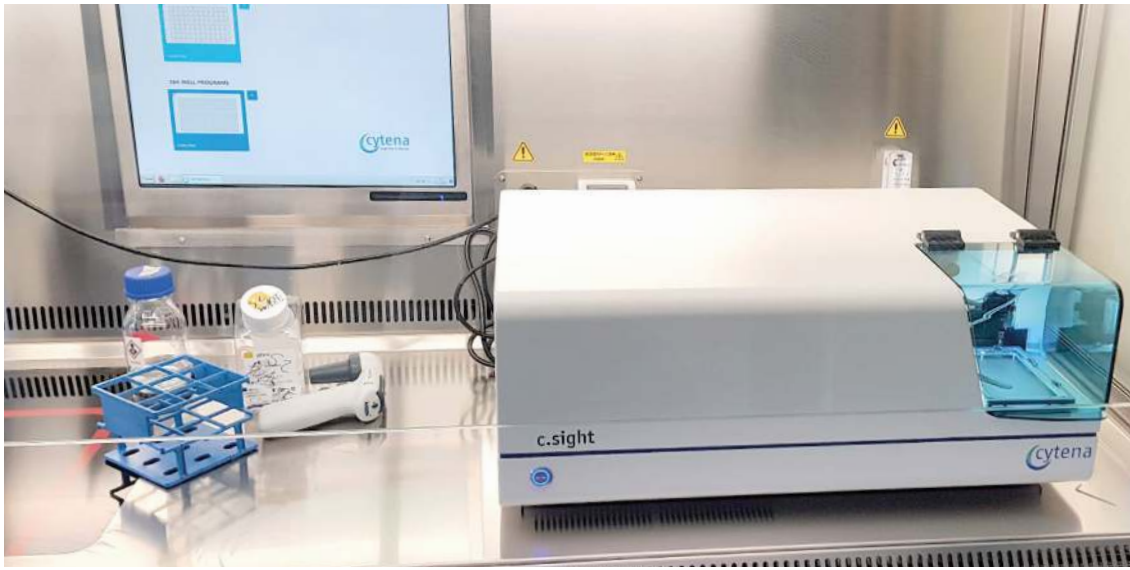


Workflow

enabled by instruments from cytena

Transfection >>> **Single-Cell isolation** >>> **Assurance of clonality** >>>

single-cell printer™ in a standard biosafety cabinet at a customer site



c.sight™ in a standard biosafety cabinet at a customer site

>>> **Select for productivity** >>> **Screen for quality** >>> **Screen for stability**

single-cell genomics

Isolating single cells remains a challenging task in single-cell genomics. Current methods can not provide the evidence needed to ensure that only a single cell has been isolated in the analysis vessel.

To preserve their DNA, it is essential to maintain cell integrity before lysis. Cells should also undergo as little stress as possible before lysis in order to preserve their RNA and their expression level. The single-cell printer™ deposits single cells in a very gentle manner, guaranteeing high purity and high viability.

This system provides an optimized foundation for downstream single-cell genomic analysis.

application example

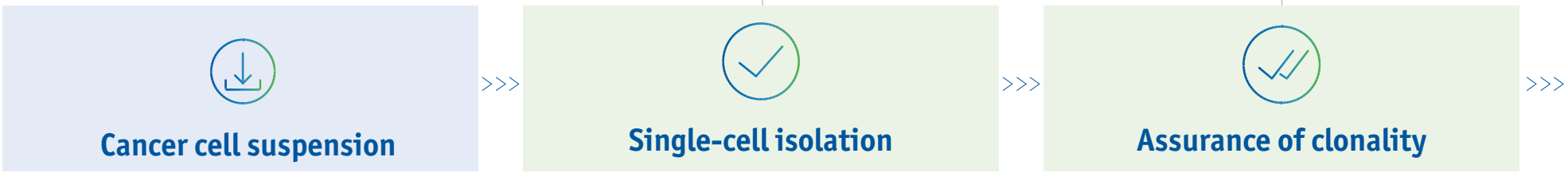
Single cells of the osteosarcoma cell line U2OS were dispensed in wells of a 384-well microliter plate and preloaded with one microliter of PBS. Additional cells were dispensed in dry wells, resulting in 98% single-cell dispensing efficiency.

Whole genome amplification (WGA) was performed on the cells and comparable DNA yields were achieved for dry and PBS wells. The WGA DNA was evaluated by a multiplex PCR on repetitive LINE1 transposons and revealed positive results in all WGA samples.

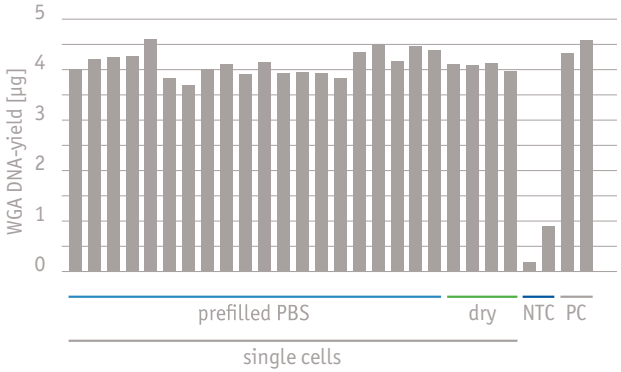
In addition, U2OS-specific mutations in SLC34A2 (c.1538G>T) and TET2 (c.1394C>T) were detected in representative WGA samples of single cells dispensed in PBS.

Workflow

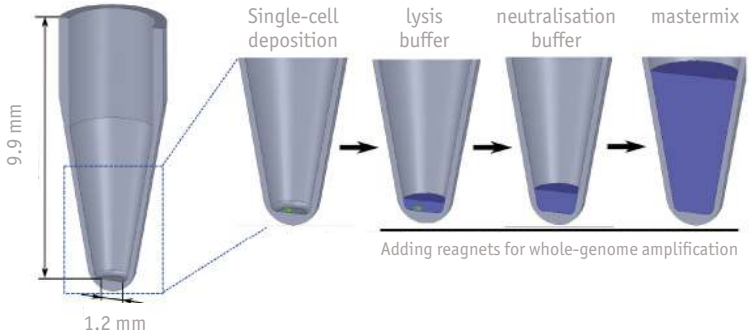
enabled by instruments from cytena



whole genome amplification

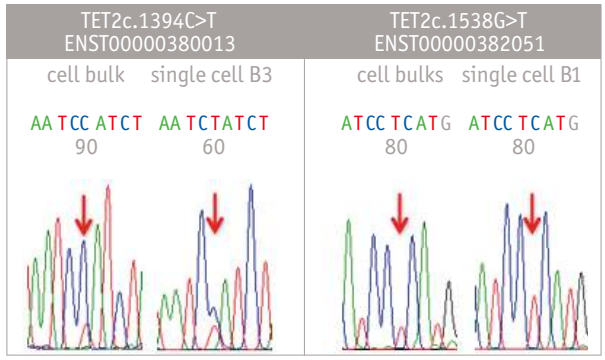


precise droplet deposition for single-cell genomics applications

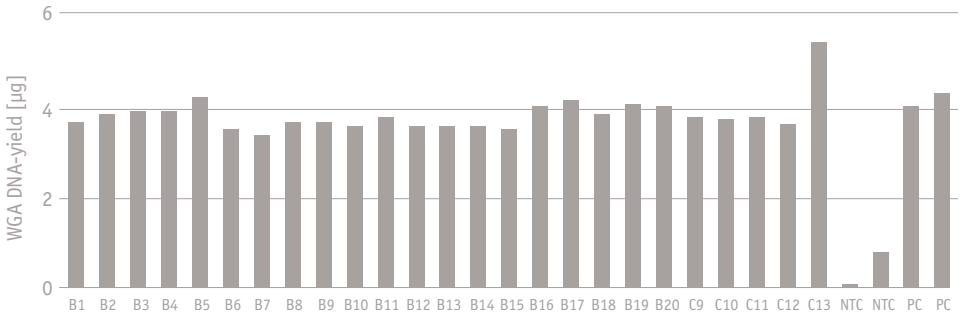


The fully automated drop positioning ensures that every cell is deposited with millimeter precision. This enables minimal quantities of prestored medium — ideal for low-volume assays and single-cell genomics.

mutational analysis by sequencing



empty droplets as negative template controls



Empty droplets are dispensed as negative template controls, enabling detection of potential free-floating DNA. We have demonstrated WGA-reagent reductions between four and 20-fold.

1 J. Riba et al., Biospektrum, May 2017, Volume 23, Issue 3, pp 298–300

>>> **Extract gDNA/RNA** >>> **Amplify gDNA/RNA** >>> **Sequencing of gDNA/RNA**

c.sight™

We designed the **c.sight™** to meet the specific requirements of both cell line development and single-cell genomics. Our system offers high efficiencies, fast plate processing and reliable image and data storage — along with assurance of clonality by an image series of the single-cell dispensing event for each well.

tailored for:

- cell line development.
- single-cell genomics applications.
- low-volume assays (WGA, PCR, NGS setups).

benefits:

- assurance of clonality by series of nozzle images.
- high cell viability and high recovery enabled by gentle dispensing technology.
- no cross-contamination thanks to the disposable cartridges.
- easy to use.

features:

- integrated plate deionizer eliminates electrostatic charge.
- high-precision droplet deposition unit delivers your cell in even the smallest volumes of prestored media or reagents for low volume assays.
- compatible with a large variety of PCR-plates including nonskirted formats.

The embedded deionizer efficiently removes any electrostatic charge from your 96- and 384-well plates. 96- and 384-PCR well plates can also be used, and our plate chiller keeps your reagents at the required temperature throughout dispensing. Our high-precision droplet deposition ensures that every cell is deposited with millimeter precision. This enables minimal quantities of prestored medium or reagents — ideal for low-volume assays and single-cell genomics.

Thanks to its new design, the **c.sight™** can now be operated outside of a biosafety cabinet to support specific applications. The hatch and flap protect your sample and substrate from environmental influences while dispensing.

The **c.sight™** comes with our all-new software which is easy and intuitive to use. The interactive wellplate enables you to freely define your experiment.
You can save and load templates and experiments directly from the start screen — your experiment is just one click away.

Our proven and reliable single-cell dispensing technology has a new look.

f.sight™

The f.sight™ dispenses both unlabeled and fluorescent cells with the highest efficiency. The innovative dual-camera system enables you to simultaneously capture brightfield and fluorescence information at full resolution.

You can control both cameras independently and leverage the powerful built-in and free adjustable blue laser. Combined, their high dynamic range visualizes even the weakest fluorophores.

Want to use fluorescent dyes to label your products right in or on the cells producing them? Not a problem. The f.sight™ can visualize them, even when there are only a few of them.

tailored for:

- single-cell applications based on fluorescent sorting.
- selection of best-producers for CLD.
- rare cell research and discovery.

benefits:

- separate camera systems for brightfield and fluorescence imaging.
- supports most common green dyes (GFP, FITC, CT-Green, DyLight, Calcein AM and more).
- detects even low-intensity dye and low copy numbers.

features:

- high-resolution objectives.
- freely adjustable blue laser.
- integrated plate deionizer eliminates electrostatic charge.

Use standard dyes like GFP, FITC, Calcein AM, CellTracker Green and more. Thanks to our lightblocking flap, your dyes and cells are always protected from ambient light. Worried about bleaching or phototoxic effects from the laser? You don't need to worry. The laser's spot only illuminates the nozzle, and you can freely adjust its intensity. When running sensitive dyes, just reduce the laser's intensity and increase the camera's exposure time to keep your sample safe.

Want to process non-fluorescent cells? Simply switch off the laser and use the **f.sight's™** brightfield-sorting mode. Just like its smaller brother — the **c.sight™** — the cells can be sorted afterward according to morphological criteria.

Our all-new software is fast and intuitive. Design your individual fluorescence experiments within minutes. You get separate full-resolution brightfield and fluorescence imaging along with an overlay image. Everything is always recorded and saved for future analysis and assurance of clonality.

Meet our new flagship!

b.sight™

For the first time, you can dispense single bacteria using validated single-cell dispensing technology. The b.sight™ isolates prokaryotic cells in the submicron range. Its extremely high-resolution optics and in-line illumination make even the smallest cells visible. No staining or labeling is required to isolate single bacteria.

Isolation is made simple with our disposable cartridge. Just pipette your sample into its reservoir, mount the cartridge to the b.sight™ and start sorting right away.

A new dimension of cells also requires a new dimension of dosing chips. Our new cartridges designed for bacteria dispensing produce even smaller droplets to ensure stable and accurate bacteria encapsulation.

tailored for:

- single-bacteria applications.
- single strain isolation and culture.
- research and discovery.

benefits:

- leverages brightfield imaging to isolate single bacteria from suspension samples.
- disposable cartridge eliminates risk of cross-contamination.
- fully functional in anaerobic conditions.

features:

- high-resolution objective and special cartridge enable sorting of bacteria at the sub-micrometer scale
- integrated plate deionizer eliminates electro-static charge

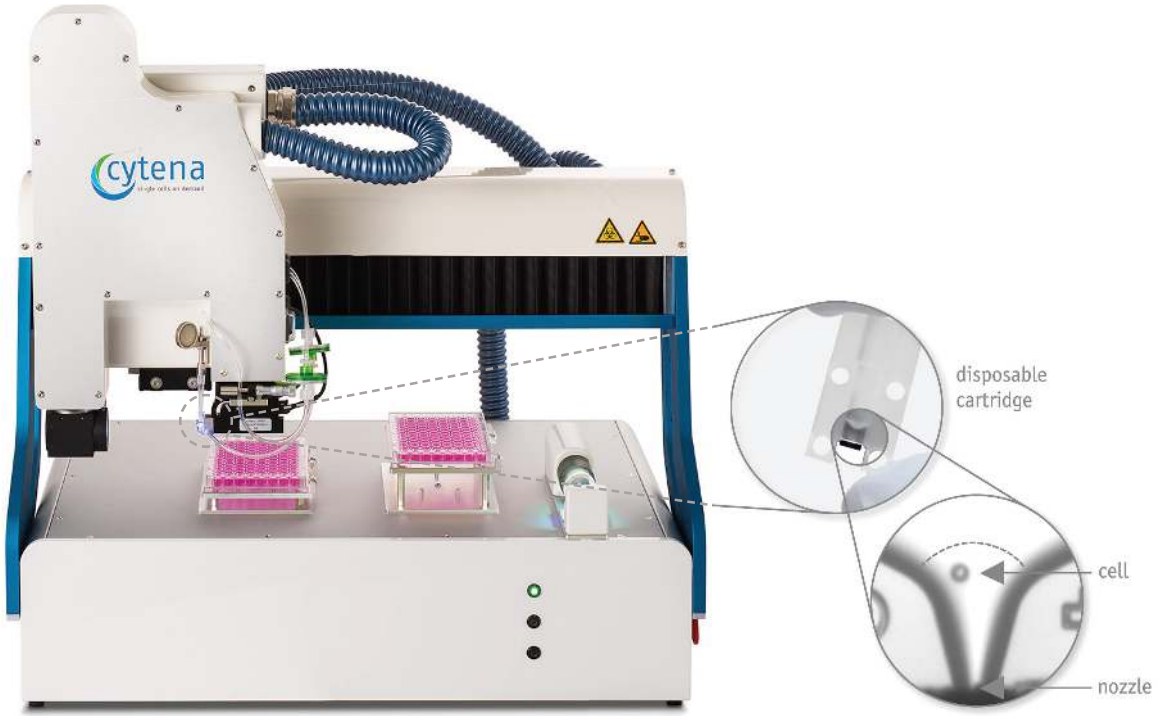
Small, smaller, smallest

Our all-new software is easy and intuitive to use. The interactive well plate enables you to design your own individual bacteria-dispensing experiment. Plus, you can visualize how bacteria are sorted in the cartridge and dispensed into your 96- or 384-well plate in real-time. Images of the singlebacteria dispensation are safely stored to provide assurance of clonality.

The b.sightTM can be operated at 37 degrees Celsius and under nitrogen atmosphere. Enjoy its small footprint, low-particle emission and low-heat emission. These specifications provide the ideal basis for its use in anaerobic conditions. You can also control the device from outside the cabinet — just connect a monitor, keyboard and mouse, or use a remote control terminal.

single-cell printer™

cytena's **single-cell printer™** (scp™) is a benchtop-sized automated laboratory instrument. The open deck contains two separate carriers for loading 96- and 384-well plates. The dispensing head's dispensing unit and optics can be moved freely along a three-axes robotic stage.



throughput:

- dispense single cells in 96- or 384-well plates.
- one cartridge feeds multiple plates.
- fast and consistent processing times.

integration:

- low footprint (550 x 430 mm) and external PC.
- open deck architecture.
- deal for plate-handling robots.

automation:

- automation-ready API.
- sample client and documentation.
- drivers from various integrators available.

Thanks to its open deck, our system is ideal for an automation environment. Plate-handling robots can freely access the deck to load and remove plates. The scp™ comes with a ready-to-use API so you can leverage our sample client and extensive API manual. Develop your own drivers or let your integrator do this job for you.

The system is user-friendly and can be operated by every member of your lab after a short introduction.

Our proven single-cell technology

since 2014

cartridge

Our internally developed and patented single-cell dispensing technology enables fully automated isolation of single cells into standard microwell plate formats. The instrument uses an inkjet-like principle featuring a disposable, one-way dispensing cartridge.

The cartridge holds your sample in a small reservoir. From there, the cells are transported into the microfluidic chip. Once loaded, the cartridge attaches easily to the dispenser. The dispenser's piezo-electric plunger generates tiny droplets of cell suspension from the cartridge without ever coming into direct contact with the sample.

sample:

- single-cell suspensions.
- 5 - 80 µl sample volume.
- <1 µl dead volume.

safety:

- single-use disposable.
- noncontact dispensing.
- no cross-contamination.

usability:

- UV-sterilized.
- individually packed.
- sample load by pipetting.

certifications:

- animal-component free.
- BSE / TSE free.
- no cytotoxic materials.

Every droplet that contains a single cell of interest can be sorted into your desired substrate. Dispense as many single cells as you need with one cartridge. After changing samples or isolating sufficient cells, just detach the cartridge and dispose of it.

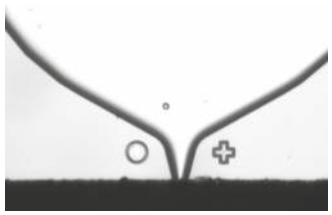
cytena's disposable, one-way cartridge takes up to 80 microliters of cell sample. The individually-packed, sterilized cartridges are easily loaded by hand-pipetting. Its silicon microfluidic chip generates free-flying picoliter droplets to act as transport vessels for the cells. One cartridge can fill many well plates with single cells. After use, the cartridge is disposed of and replaced. This innovative approach prevents cross-contamination and eliminates the need for extensive cleaning steps.

assurance of clonality

Our systems provide assurance of cell line clonality for each dispensed cell.

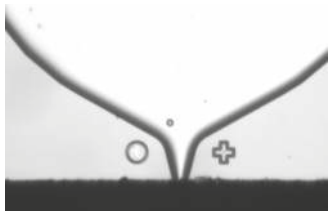
Each single cell for each well is monitored, automatically generating and storing a sequence of images on the hard drive. This documentation enables you to confirm every single cell ejection.

1



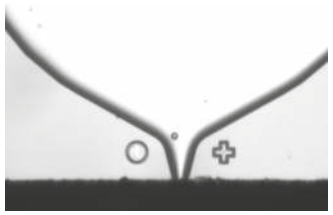
before cell dispensing tracking ...

2



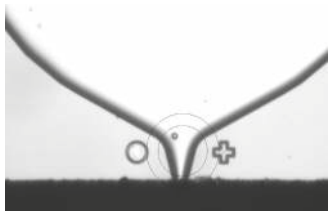
... a cell's path toward the nozzle

3



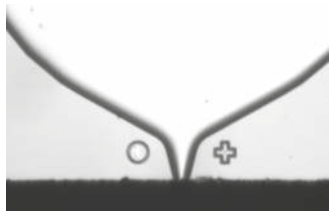
targeted single cell

4



targeted single cell with contour overlay

5



imaging after cell dispensing confirms the single cell has left the nozzle

Leverage the pharmaceutical industry's most trusted technology for assuring monoclonality!


cytena

working principle

Our technology is based on an inkjet-like principle. We generate tiny, free-flying droplets from a microfluidic chip (1). Your sample cells are stored inside this chip, which is part of our dispensing cartridge (2). The system works as a direct displacement dispenser. This means we can generate a droplet at any point in time, also known as dropon-demand. We use a microscope objective and a camera to look into the chip directly at your cells. We take an image of them in the nozzle. The nozzle is the lower part of the chip where droplets are ejected from.

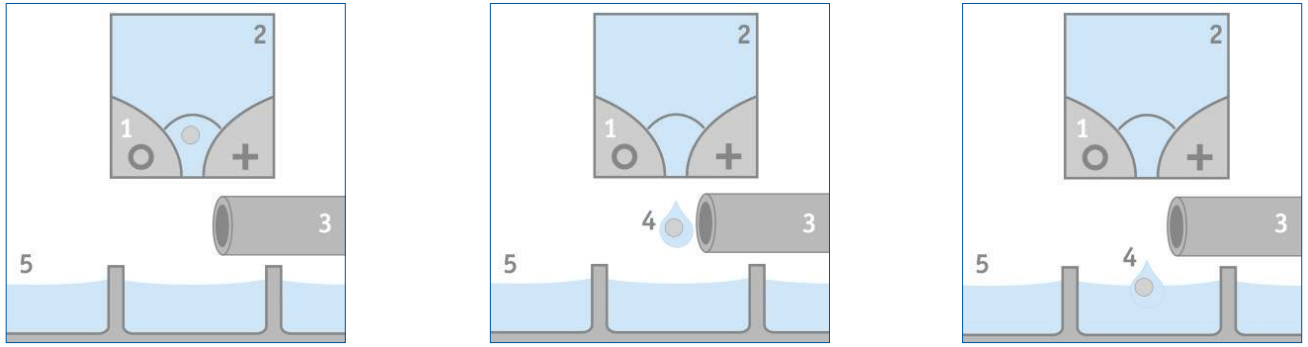
After taking an image, our lightning-fast image-processing algorithm detects all cells captured, counting and classifying them according to morpho-logical criteria like size and roundness. The system then generates a droplet to eject everything inside the nozzle. In this way, the system functions by confirming — not controlling — what the droplet contains.

To sort the droplets, we have developed a pneumatic shutter system (3) located directly underneath the nozzle.

After generating a droplet that contains a single cell (4), the system allows this specific droplet to pass onto your substrate (5). The shutter handles all droplets that do not contain one cell, including void and multicell droplets, as waste.

droplet with one cell

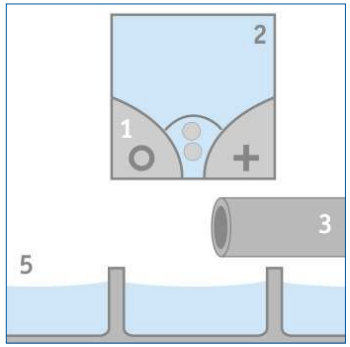
1 cartridge
2 cell suspension
3 pneumatic shutter
4 free-flying droplet
5 micro-well plate



high-resolution optics and software algorithm detect a single cell in the nozzle >>> droplet with a single cell is ejected while the pneumatic shutter is closed >>> droplet with a single cell lands in the dedicated well

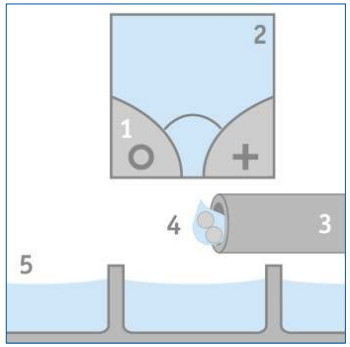
droplet with more than one cell

1 cartridge
2 cell suspension
3 pneumatic shutter
4 free-flying droplet
5 micro-well plate



high-resolution optics and software algorithm detect more than one cell in the nozzle

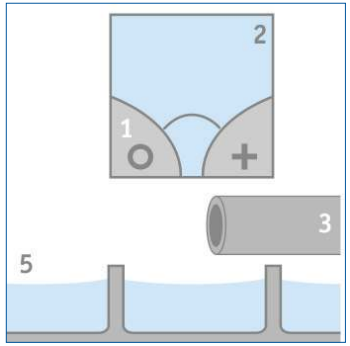
>>>



droplet with more than one cell is ejected and immediately deflected into waste by pneumatic shutter

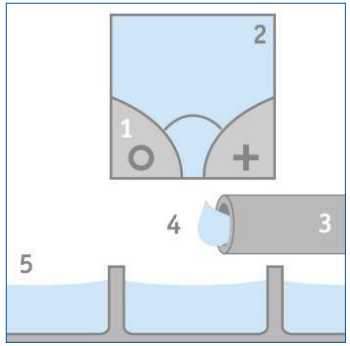
droplet without a cell

1 cartridge
2 cell suspension
3 pneumatic shutter
4 free-flying droplet
5 micro-well plate



high-resolution optics and software algorithm detect no cell in the nozzle

>>>



empty droplet is ejected and immediately deflected into waste by pneumatic shutter

approved cell types*

human cancer cell lines	human cell lines	human primary cells	animal cell lines
NCI-H460** (non-small cell lung) A549** (non-small cell lung) HCT-116** (colon) MDA-MB-231** (breast) U-2 OS (human osteosarcoma) HeLa (cervix adenocarcinoma) Ca Ski (cervix epidermoid carcinoma) SiHa (cervix squamous cell carcinoma) C-33 A (cervix carcinoma) Jurkat (Acute T-cell Leukemia) Kasumi-1 (human myeloblast) THP-1 (Acute Monocytic Leukemia) Raji (Burkitt's Lymphoma)	B-cells T-cells HEK 293	fibroblasts keratinocytes	CHO-K1 RBL L929 SP2/0-Ag14 NIH-3T3

* These cell types have been successfully dispensed and postdispensing viability is confirmed by successful proliferation
** NCI60: human tumor cell lines used in the US National Cancer Institute (NCI) 60 human tumor cell line anticancer drug screen

sample requirements

instrument	sample type	prior filtering (mesh size)	cell type	min. cell diameter	max. cell diameter	min. concentration	optimal concentration	max. concentration
scp™ c.sight™ f.sight™	suspension	40 µm	eukaryotic	5 µm	35 µm	10^4 cells/ml	10^5–10^6 cells/ml	10^7 cells/ml
b.sight™	suspension	10–15 µm	prokaryotic	~ 1 µm	10 µm	10^4 cells/ml	10^5–10^6 cells/ml	10^7 cells/ml

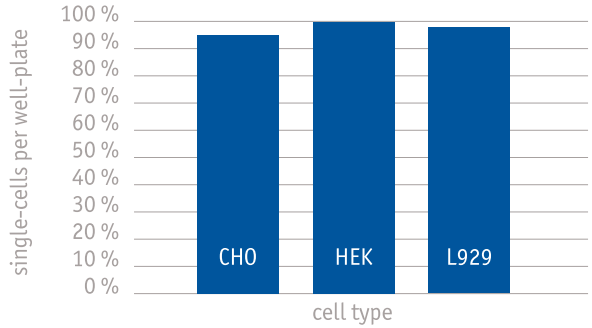
cartridge characteristics

instrument	chip	reservoir	min. sample volume	max. sample volume	dead volume	microfluidic outlet (nozzle)	droplet volume	dispensation mode
scp™ c.sight™ f.sight™	silicon-glass	polymer	5 µl	80 µl	< 1 µl	40 x 40 µm	150 pl	noncontact
b.sight™	silicon-glass	polymer	5 µl	80 µl	< 1µl	20 x 20 µm	35 pl	noncontact

single-cell dispensing efficiency

- dispensing efficiency* >90%
- confirmed and proven by single-cell genomics
- tested on many common cell lines
- throughput >5 min per 96-well plate (one cell per well)

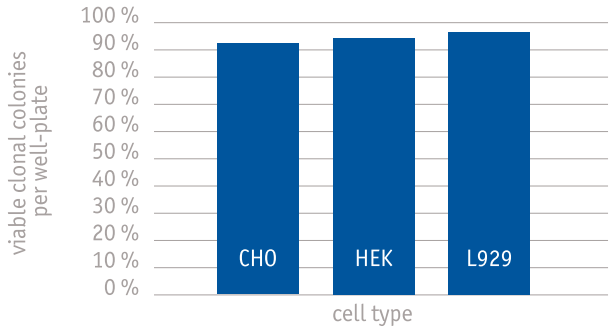
*dispensing efficiency = number of wells containing a single cell per total numbers of wells addressed.



single-cell viability (clonal recovery rate)

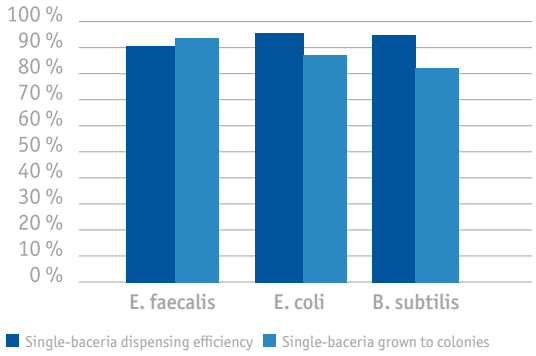
- excellent cell viability
- high clonal recovery rates*
- no pressure, no cross-contamination, no electric fields
- as gentle as hand-pipetting

*clonal recovery rate = number of viable colonies derived from a confirmed single cell.

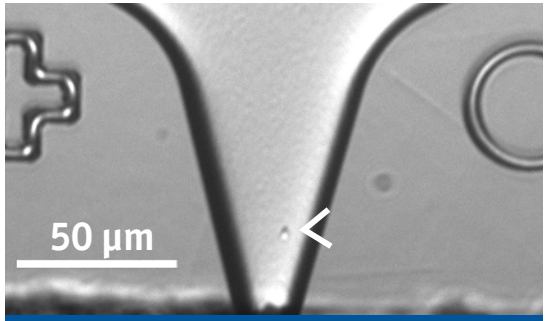


single-bacteria dispensing and growth

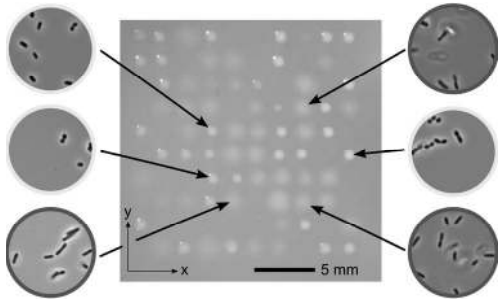
The b.sight™ was verified with different bacterial species. Very high single-bacteria isolation efficiencies (above 90%) and very high rates of colony growth from individual bacteria (between 75% and 92%) have consistently been achieved.



Single-bacteria dispensing and growth



Example picture of the 20 µm nozzle opening with a single bacteria indicated by the arrow.



1. Riba, J (2016). Label-free isolation and deposition of single bacterial cells from heterogeneous samples for clonal culturing. Scientific Reports

Bacteria colony array grown from 10 × 10 dispensed single bacteria on LB-agar. Single bacteria cells were dispensed from a heterogeneous culture of E. coli and E. faecalis previously mixed in a ratio of 1:1. Two clearly distinguishable colony morphologies can be found for the two different types of bacteria. Visual inspection by light microscopy revealed that shiny sharped edge colonies were grown from E. faecalis while matte colonies with diffuse edges could be assigned to E. coli.

validation of fluorescence intensity based sorting

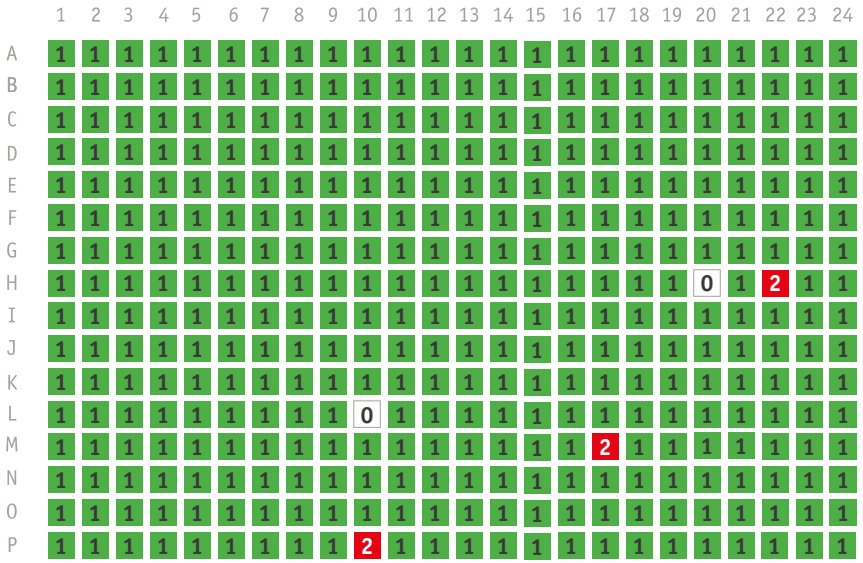
Validated with industry partner.
Multiple 384 well plates were filled with fluorescent single beads and subsequently additional plates were filled with fluorescent single CHO cells.

Very high dispensing efficiencies with beads: 98% (n = 1’152 wells) and CHO-cells: 97% (n = 1’536 wells) was demonstrated. Single-cell deposition efficiency determined by nozzle images and additionally confirmed by plate imaging.

Example of one of the 384 well plates filled with fluorescent beads.

White = 0 bead per well
Green = 1 bead per well
Red = 2 beads per well

The number of all the dispensed beads was determined using our nozzle images and achieved 100% correlation with results obtained by additional plate imaging.



Example of one of the 384 well plates filled with fluorescent-labeled CHO cells.

White = 0 cell per well
Green = 1 cell per well
Red = 2 cells per well

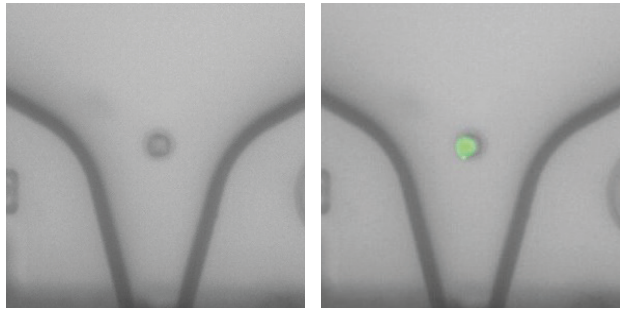
The number of all the dispensed cells was determined using our nozzle images and achieved 100% correlation with results obtained by additional plate imaging.

application example – cold capture assay

We conducted experiments with our industrial partner in order to isolate well-producing CHO cells after transfection. Cold capture assay was applied in accordance with N. Borth[1].

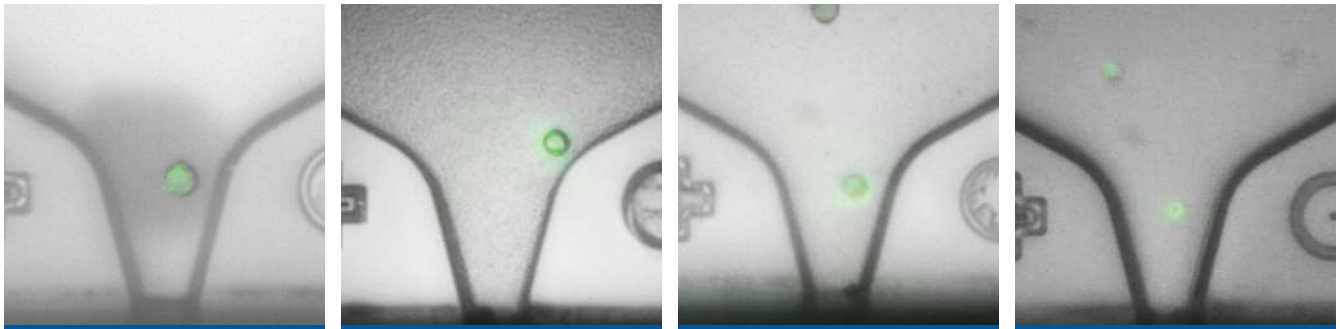
f.sight's™ fluorescence sensitivity is sufficient for the low-fluorescence intensities of the cold capture application. Most fluorescent plate imaging instruments do not have the sensitivity needed to detect these low intensities. The cold capture assay enables users to sort antibody-producing cells from non-producers to dispense only antibody-producing cells in your well plate.

The impressive results demonstrate the highfluorescence sensitivity of the f.sight™.



The f.sight™ provides full-resolution brightfield (left) and fluorescence images, as well as an overlay image (right). All images are recorded and stored for future analysis.

example of validated dyes



Cold capture | Cell tracker green | Live-or-dye | Nuclear Green DCS1

(dispensing isolated single nuclei from cells)

1. Pichler J, Hesse F, Wieser M, Kunert R, Galosy SS, Mott JE, Borth N., A study on the temperature dependency and time course of the cold capture antibody secretion assay, J Biotechnol. 2009 Apr 20;141(1-2)

customer feedback and testimonials

Beyond many biotechnology companies, CMOs, CROs and CDMOs, the majority of the world's top-ten pharmaceutical companies are leveraging cytena's instruments.

More than 15 publications have been published by our customers so far, all focusing on cytena's **single-cell printer™** technology. The authors include Novartis, Bayer, Genentech, AstraZeneca, Selexis, MedImmune, Novimmune, Octapharma, ExcellGene and many more.

Yim M., Shaw D., Achieving greater efficiency and higher confidence in single-cell cloning by combining cell printing and plate imaging technologies, Biotechnol Prog. 2018

Novimmune

"... By use of a scp, the number of monoclonal cell lines increased six-fold compared to the LD process therefore increasing the number of pools that can be cloned."

"Consequently by implementing both of these technologies the CLC process can be decreased from 9 months to 6 months."

Mahé A., et al. (2017). Optimization of the cell line construction process for manufacturing purposes using a novel single cell printing technology. Poster presentation at ESACT 2017 in Lausanne.

Genentech

"It has also been demonstrated that the SCP™ can accurately and precisely deposit one cell in a well."

"Single-cell images from SCP™ and Celigo® can be used as documented evidence of a viable clonally derived cell line in a regulatory submission package."

Yim M., Shaw D., Achieving greater efficiency and higher confidence in single-cell cloning by combining cell printing and plate imaging technologies, Biotechnol Prog. 2018

notes

NEXT GENERATION SINGLE-CELL DISPENSING

FOR CELL LINE DEVELOPMENT AND SINGLE-CELL GENOMICS

contact us

cytena GmbH | Neuer Messplatz 3 | 79108 Freiburg | Germany
+49 (0)761 / 708890 - 0 | info@cytena.com | www.cytena.com

single-cell isolation
proven | viable | pure

cytena GmbH | Neuer Messplatz 3 | 79108 Freiburg | Germany | +49 (0)761 / 708890 - 0 | info@cytena.com | www.cytena.com

cytena
A CELLINK COMPANY