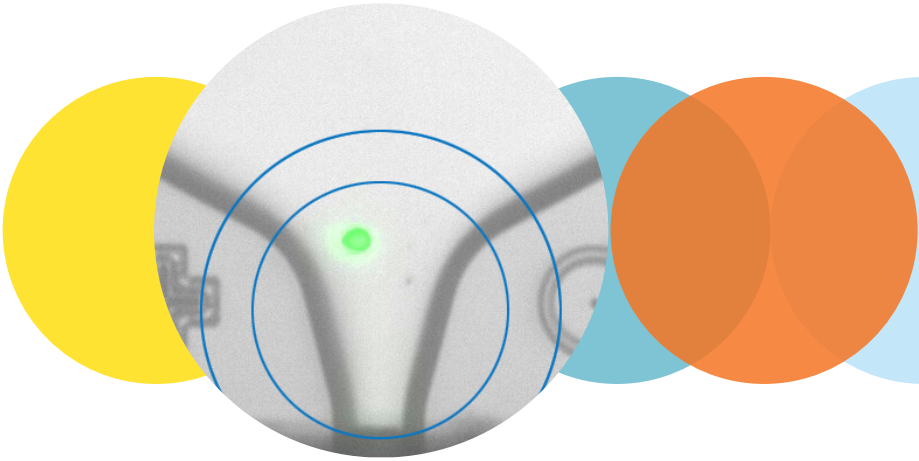


CloneSelect Single-Cell Printer Series

Eyewitness proof of monoclonality

KEY FEATURES

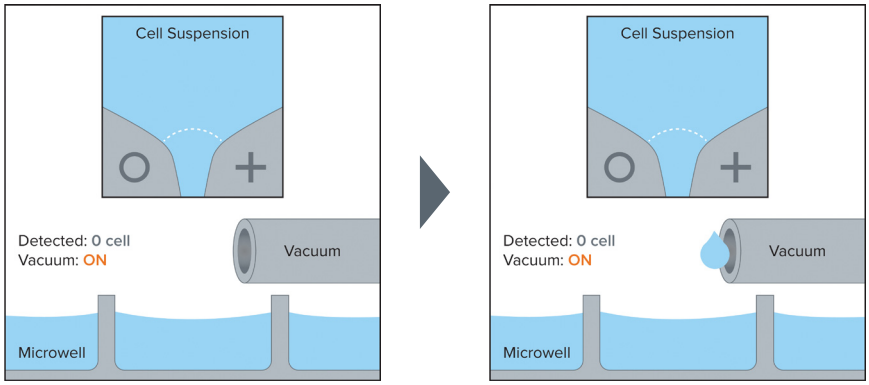
- **Cell isolation proof captured for 96- or 384-well plate formats**
- **Clonal outgrowth improved up to 8x**
- **Cells stay healthy and clean with sterile, disposable microfluidic cartridge**
- **Cells sorted in brightfield or fluorescence**

The CloneSelect™ Single-Cell Printer™ Series deposit single cells gently and with high efficiency using a patented, inkjet-like disposable, one-way dispensing cartridge. Sort cells using high-res imaging in either brightfield or optional fluorescence and capture 5 images per each single cell deposit. Prove monoclonality, improve your efficiency, maintain and enrich your viability, and prevent cross-contamination.

MOLECULAR DEVICES | cytena single cells on demand

Working principles of the CloneSelect Single-Cell Printer Series

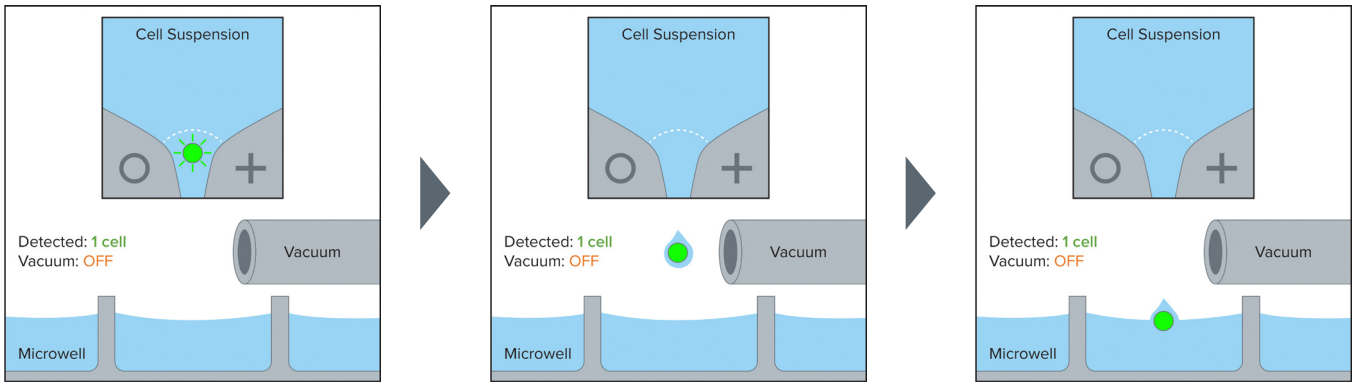
Droplet without a cell



No cells are detected in the nozzle.

The empty droplet is ejected and immediately deflected into waste. No cells are deposited.

Droplet with a single cell

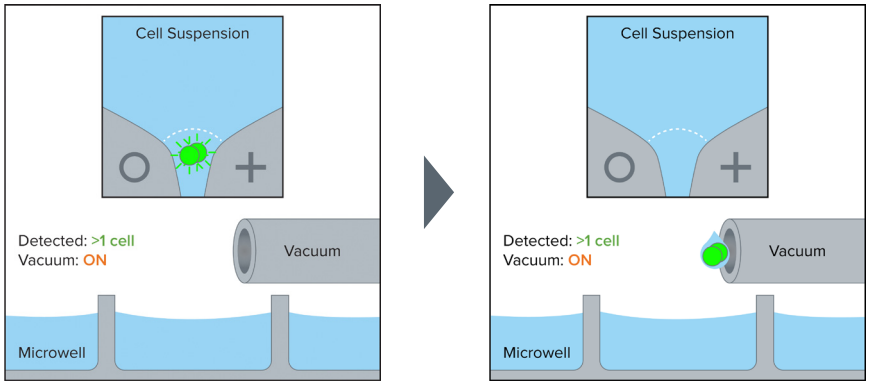


A single cell is detected.

The droplet with a single cell is ejected while the vacuum is inactive.

It is then deposited into the well.

Droplet with more than one cell



The system detects > 1 cell in the nozzle.

The droplet with > 1 cell is ejected and immediately vacuumed into the waste. No cells are deposited.

Figure 1. How the single cell isolation and deposition works.

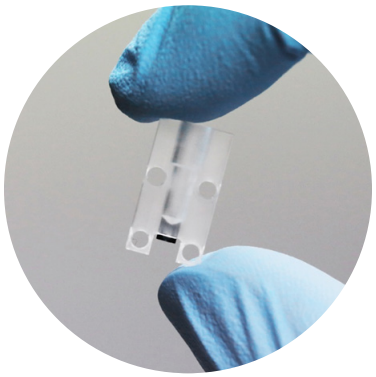


Figure 2. The disposable, sterile cartridge for the Single-Cell Printer Series.

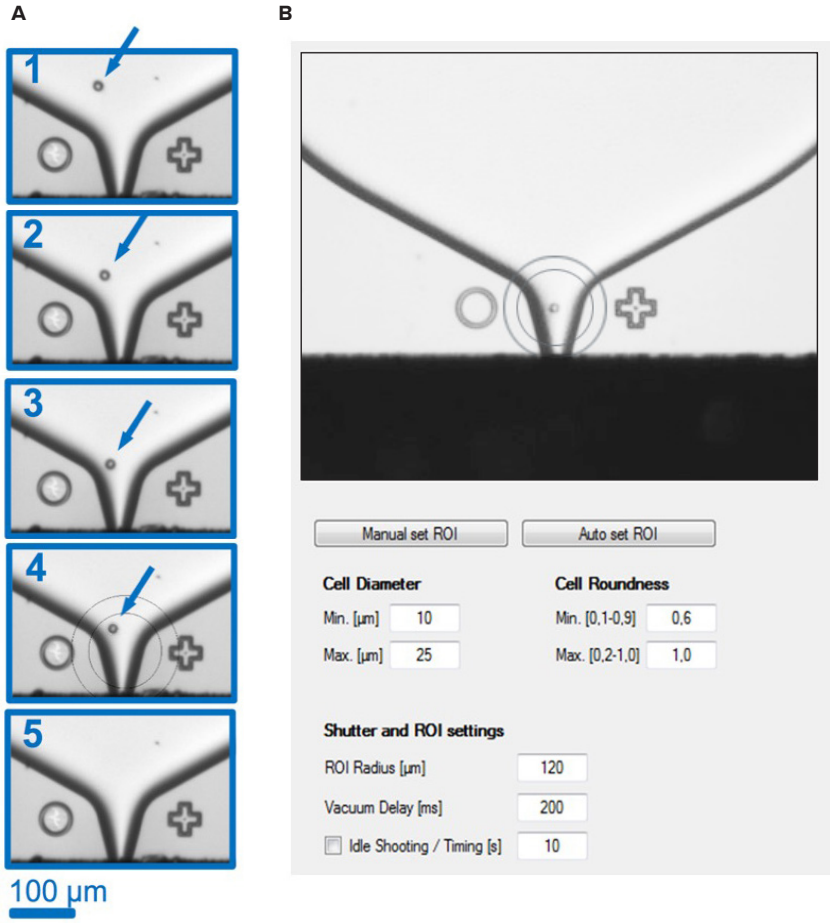


Figure 3. (A) For each single cell a sequence of 5 images are captured at the cartridge nozzle, providing evidence of single cell deposition. These images describe the path taken by a single cell prior to and following ejection from the nozzle tip. Images 1-3 show the cell approaching the nozzle. Image 4 shows the detection of a single cell (inner circle) and the absence of any cells in the vicinity (outer circle). Finally, Image 5 shows the nozzle after droplet ejection to provide evidence that the single cell was expelled from the nozzle. **(B) The software interface for cell selection is shown.** Users can select cells based on the cell size and morphology, ensuring that non-desired objects (debris, dividing cells, unhealthy cells) are not deposited into the wells.

Disposable cartridge at the heart of the CloneSelect Single-Cell Printer Series

The core of all the CloneSelect Single-Cell Printer systems is the patented single-cell isolating cartridge technology, which enables automated deposition of single cells into standard 96- and 384-well plate formats. The system uses an inkjet-like principle featuring a sterile and disposable one-way flow cartridge (Figure 2).

Up to 80 µL of cell sample at concentrations ranging from 0.5 to 1 x 10^6 cells/mL can be hand pipetted into a cartridge. Its silicon microfluidic chip generates gently-dispensed microdroplet that contain single cells. A single cartridge is sufficient to deposit single cells into wells of several microplates. After use, these cartridges can easily be discarded, preventing cross contamination and time associated with handling and sterilizing reusable components.

A high magnification and large depth-of-field camera captures a sequence of events to determine the cell number in each droplet. When multi-cell or zero cell events are automatically detected, the droplets are immediately siphoned away with a vacuum. Once a single cell event with desired and preset size and roundness is observed, a fast shutter mechanism closes access to the vacuum, enabling droplets containing a single cell to be deposited into the microplate (Figure 1).

Captured proof of cell isolation

The cell isolation cartridge is imaged at the nozzle using a high depth-of-field camera which ensures cells appear in focus. An intelligent image analysis algorithm detects single cell events accurately and on the fly. A sequence of five images are captured to track a single cell as it descends down the nozzle tip and into the microplate, providing added evidence of monoclonality over a single snapshot (Figure 3A).

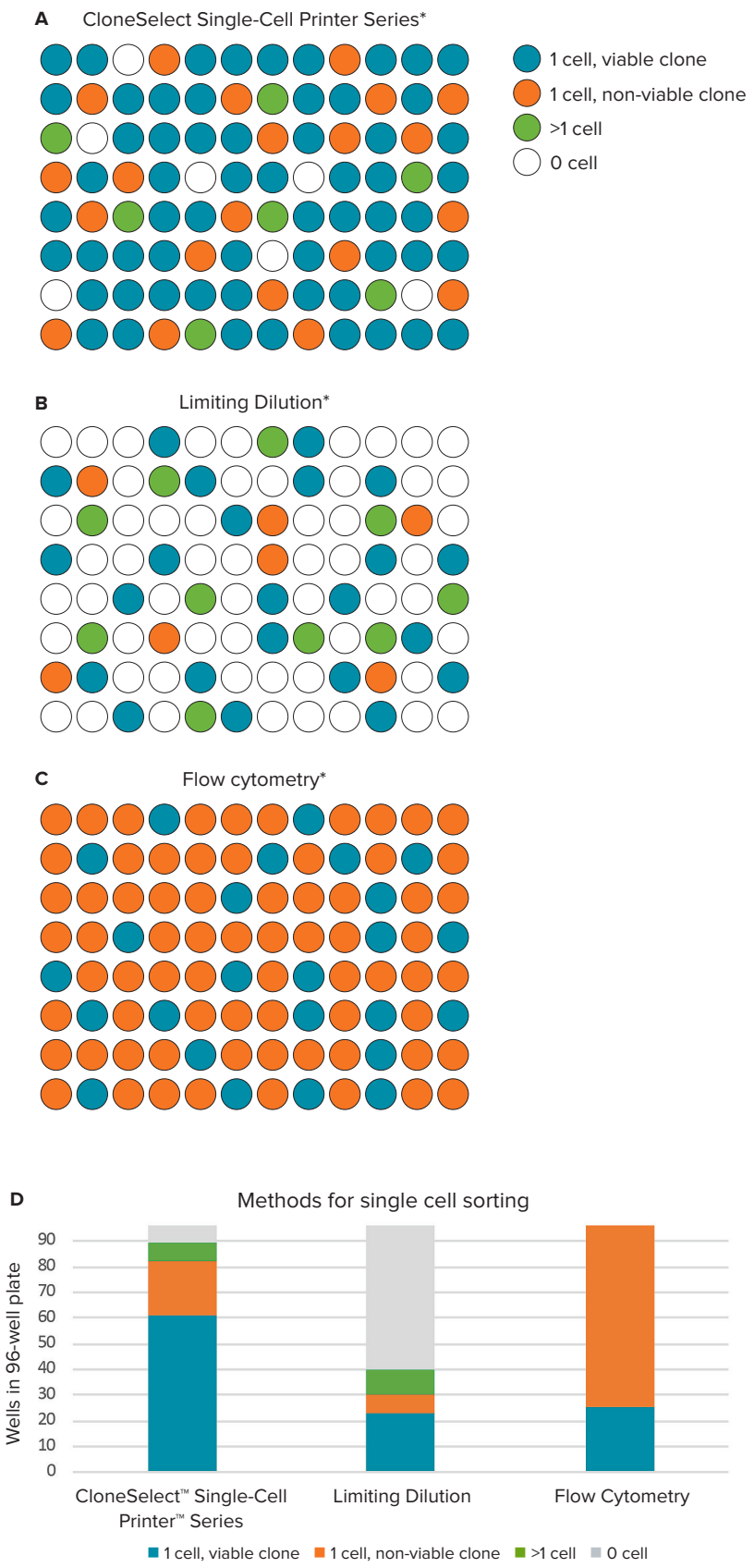


Figure 4. Traditional methods of single cell isolation are compared with that of the CloneSelect Single-Cell Printer Series. The Single-Cell Printer Series provide significant advantages over those techniques in efficiency of deposition and viability of clones.

*Based on predicted values. For limiting dilution, the single cell deposit efficiency was determined based on theoretically seeding 0.5 cells/well. For flow cytometry, viabilities can vary significantly but is usually reported between 5-50%. Thus, a 25% average viability was chosen.

Improved single-cell isolation efficiency

Current workflows for single cell isolation within cell line development have major drawbacks such as inefficient single cell deposition and a lack of image evidence for single cell-containing wells.

Limiting dilution, the standard technique most often used to isolate single cells, relies on statistical probability to favor single cell deposition. The best approaches using limiting dilution yield only 30%, single cell-containing wells with a majority of void and doublet wells. CloneSelect Single-Cell Printer Series significantly improves the efficiency by vacuuming the droplets identified as no cells or multiple cells to waste.

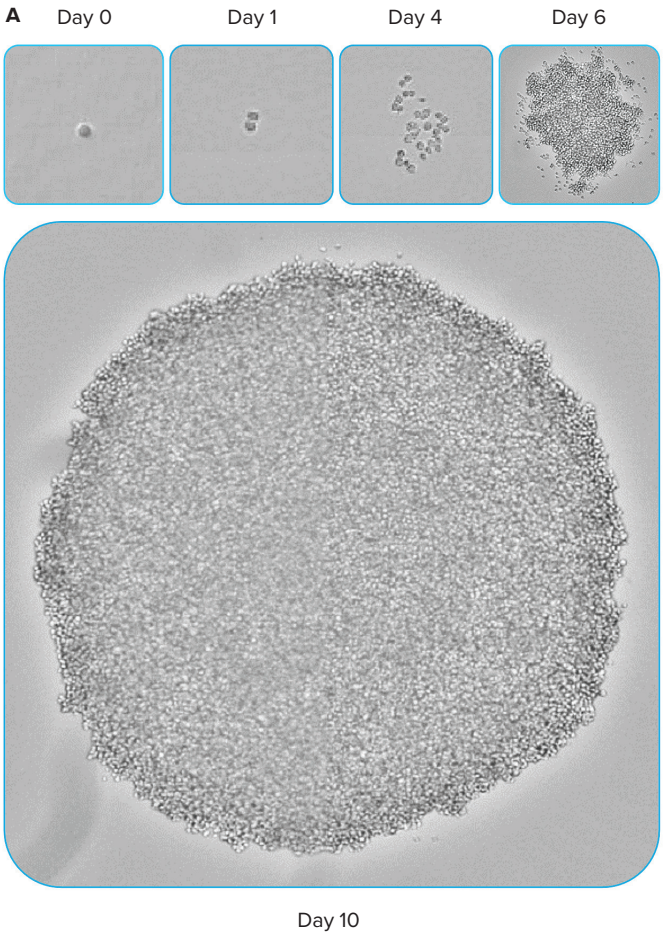
Isolate more viable clones with confidence

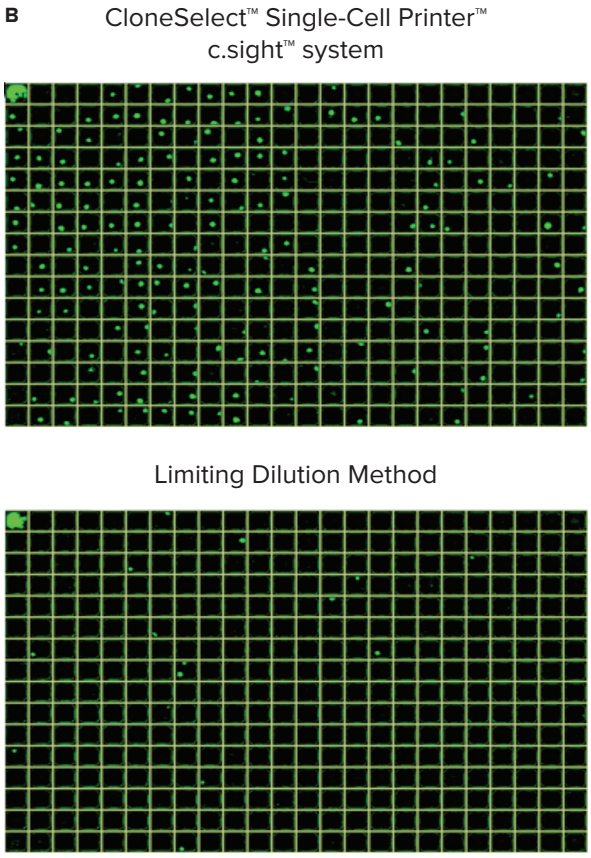
Another drawback of current methods is low cell viability post sorting and isolation. For example, it is common to observe <25% cell viability with flow cytometry-based methods.

The CloneSelect's unique microfluidics-based cell isolation technology is as gentle as pipetting. The cell-containing microdroplets are dispensed slowly and delicately in the absence of any pressure, electric fields, or lasers observed in other technologies. Additionally, the ability to screen cell populations by gating for size and roundness offers the potential to exclude imperfect non-viable cells, making the CloneSelect Single-Cell Printer Series performance on par with limiting dilution when it comes to isolating healthy cells.

After being deposited into microplates, the single cells are monitored and imaged for several days using the CloneSelect™ Imager to follow the growth characteristics. The cells remain viable and form monoclonal colonies within days, indicating high cell viability after deposition (Figure 5).

Improve clonal outgrowth up to 8x





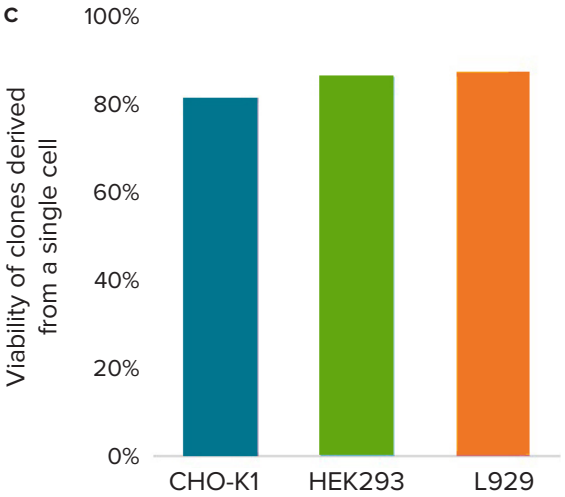
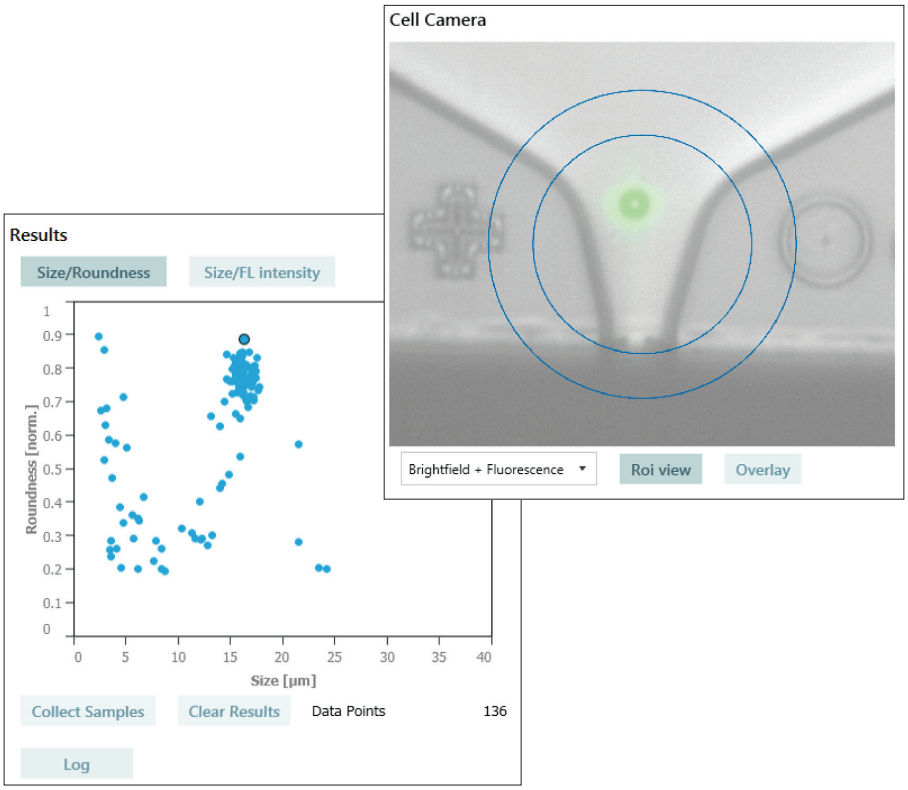


Figure 5. (A) Following single-cell deposition with the CloneSelect Single-Cell Printer system, the single cells remain viable and form a monoclonal colony within a span of several days. Example image of a single cell deposited with c.sight and imaged on the CloneSelect Imager for several days post deposition. **(B) Single cell colonies (shown in green) derived from limiting dilution (bottom panel) are compared to equivalent colonies from the c.sight (top panel) by imaging on day 6 with the CloneSelect Imager.** A representative 384-well plate imaged on the CloneSelect Imager is shown for limiting dilution and c.sight. A total of 13 single-cell clones resulted from limiting dilution, while 167 colonies resulted from single-cell printing. The higher efficiency of single-cell deposition along with similar viabilities allow researchers to characterize more clones per plate, significantly increasing throughput and reducing time. **(C) The viabilities of single-cell wells obtained from different cell types are compared.** Single-Cell Printer Series can be used to isolate and deposit a wide variety of cells, resulting in high viability. Note that cell viability can vary based on cell type and sample preparation, cell media, gating parameters, and other external factors.

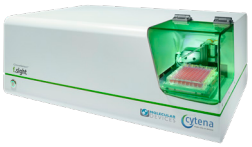


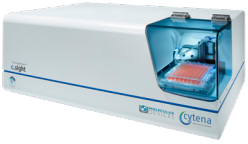
Screen for more than just clonality

Cells can also be screened on the basis of size and morphology using the image analysis algorithm (Figure 3B). Screening for cells based on size may allow for isolating a specific cell type from a heterogeneous population of cells or may allow for screening of viability since dying cells tend to swell and then shrivel. Selecting cells based on cell roundness provides another parameter to screen for healthy cells by ensuring that asymmetrical structures like dividing or unhealthy cells are not deposited into the wells.

Fluorescent cell sorting

The f.sight™ sorts both unlabeled and fluorescent cells with the highest efficiency using a novel dual camera system allowing you to capture brigthfield and fluorescence information simultaneously at full resolution. Use fluorescent dyes like GFP, FITC, Calcein AM, CellTracker Green, and others to label your products right in or on the cells producing them.

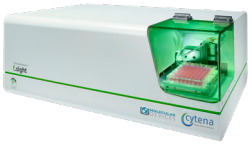


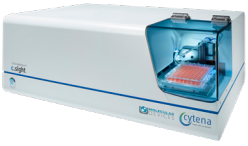




Key system differentiators	f.sight™	c.sight™	scp™
Fluorescent single cell sorting and depositing	✓		
Brightfield single cell sorting and depositing	✓	✓	✓
Computer	Embedded	Embedded	External
Destination plate capacity (96- and 384-well)	1	1	2
Automation ready with API			✓

Cartridge specifications	
Cartridge dimensions (mm)	ca. 8.8 (W) x ca. 16 (H) x ca. 5 (D)
Reservoir volume	100 µl
Cartridge volumes	10–80 µL, 50 µL is the recommended sample volume
Cartridge compatibility	water, PBS and cell growth media*
Optimal cell concentration	1e6 cells/mL
Recommended cell/bead sizes	40x40 micron nozzle (5–35 micron beads or cells)
Cartridge sterility	UV light (CWL 254 nm) TSE/BSE free: Yes Animal component free: Yes

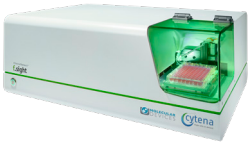


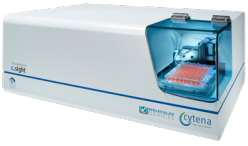




Specifications	f.sight™	c.sight™	scp™
Performance			
Dispensing speed	<5 min in brightfield and <10 min in fluorescent sorting*	<5 min in brightfield sorting*	5-10 min brightfield sorting*
Conformity	CE, CB, QPS laser class 1. laser safety acc. to IEC 60825-1 and 21 CFR 1040.10	CE, CB, QPS	CE, QPS
System robustness	Can deposit into 5, 96 well plates consecutively with equivalent efficiencies		
Operating system	Win7 (x64)		
Data storage	Hard drive: 240 GB SSD, M2		
Networking	External Interfaces: 1x DVI, 1x Displayport, 2x GigE LAN, 4x USB-3.0		2x GigE network slots
Handheld barcode scanner	Supported code standards: Codabar, Code 11, Code 128, Code 39, Code 93 EAN-8, EAN-13, ISBN, ISSN, MSI, UPC-A, UPC-E, IATA 25, Industrial 25 Standard 25, Interleaved 25, Matrix 25, China Postal Code, FedEx, USPS, VIN		Supported code standards: Codabar, Code 11, Code 128, Code 39, Code 93 EAN-8, EAN-13, ISBN, ISSN, MSI, UPC-A, UPC-E, IATA 25, Industrial 25 Standard 25, Interleaved 25, Matrix 25, China Postal Code, FedEx, USPS, VIN
Data			
Image tracking	Total 15 per well. Respectively 3 prior to deposit, 1 deposit, 1 post deposit per channel. Channels: 1 bright field, 1 fluorescence, 1 overlay	Total 5 per well. 3 prior to deposit, 1 deposit, 1 post deposit	
Image format, size	Bright field: JPEG, 1280x1024 pixels, 8-bit greyscale, 300 kb per image Fluo channel: JPEG, 1280x1024 pixels, 8-bit greyscale, 70 kb per image Overlay channel: JPEG, 1280x1024 pixels, 24-bit greyscale, 320 kb per image	JPEG, 1280x1024 pixels, 8-bit greyscale, 200 kb per image	
Data size	310 MB per 96- well plate	110 MB per 96- well plate	
Instrument specifications			
Instrument dimensions (mm)	600 mm (W) 240 mm(H) 400 mm (D)		550 (W) x 510 (H) x 400 (D)
Instrument weight	35 kg	32 kg	30 kg
Computer dimensions (mm)	Embedded		270 (W) x 325 (H) x 270 (D)
Computer weight	Embedded		6.5 kg
Additional space considerations	Fits in standard BSCs		
Power requirements	Two power sockets. Input Voltage (VAC): 100–240 Input Voltage Frequency (Hz): 50/60 Max. input current (A): 1.4 Max. power consumption (W): 133		Three power sockets with 100–240 VAC, 50-60 Hz Maximum power consumption: Lab Robot: ca. 1.34–0.55 A (100–240 VAC); max. 135 W Computer: ca. 0.41–0.21 A (100–240 VAC); max. 41W

*Can address a cell culture 96-well plate with a suspension of 10 µm beads in sterile filtered water (concentration about $5x10^5$ to $1x10^6$ beads/ml) by using optimal parameters for fast sorting by the user.







Specifications	f.sight™	c.sight™	scp™
Instrument specifications, continued			
Light sources	LED source 1: white, 1 Watt LED source 2: red, 3 Watt Laser source 1: 473 nm, 100 mW, class 3B f.sight is declared laser class 1 for standard operation	LED source 1: white, 1 Watt LED source 2: red, 3 Watt	LED source 1: white, 5 Watt LED source 2: blue, 3 Watt
Fluorescence detection	Excitation: 473 nm Emission: 525/20 nm Laser power: 1- 100 mW (freely tunable) Exposure Times: 0.1 – 20 ms (freely tunable) Fluorophores tested and compatible: GFP, Cell-Tracker Green, FITC, Calcein-AM, DyLight	LED source 1: white, 1 Watt LED source 2: red, 3 Watt	LED source 1: white, 5 Watt LED source 2: blue, 3 Watt
Environmental conditions	Temperature: 10°–30° Celsius Humidity: 10–80% (noncondensing)		
Cell camera	8-bit grayscale Type: CMOS Sensor: monochrome		
Image resolution	1280x1024 (bright field camera) and 1936x1216 (fluorescence camera)	1280x1024	1280–1024 pixels
Magnification and numerical aperture	10x (0.14 NA)	11x (0.01 NA)	11x (0.01 NA)
Microplate compatibility	SBS or SLAS standard cell culture well plates (96- and 384-well format*) * May be customizable with additional formats (6-well, 24-well, slides)		
	Not compatible with: deep well plates, or skirt-less/frameless plates.		Not compatible with: skirt-less/frameless plates.
Disposable compatibility	SCP cartridges and Eppendorf 200 mL pipette tips and Mettler Toledo adapter enabling most tip compatibility.		
Instrument sterility	Resistant against 70% Ethanol, Resistant against UV (254 nm CWL)		
Component sterility	Metal piston guide, tubing (agitation and shutter), and Luer-Lock connectors are resistant to ethanol and isopropanol. Piston guide and tubing can be autoclaved (121°C).		

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