

Fully Automated  
Image-Based Single Cell  
Isolation and Picking

CellCelector Flex

Simplifying Progress

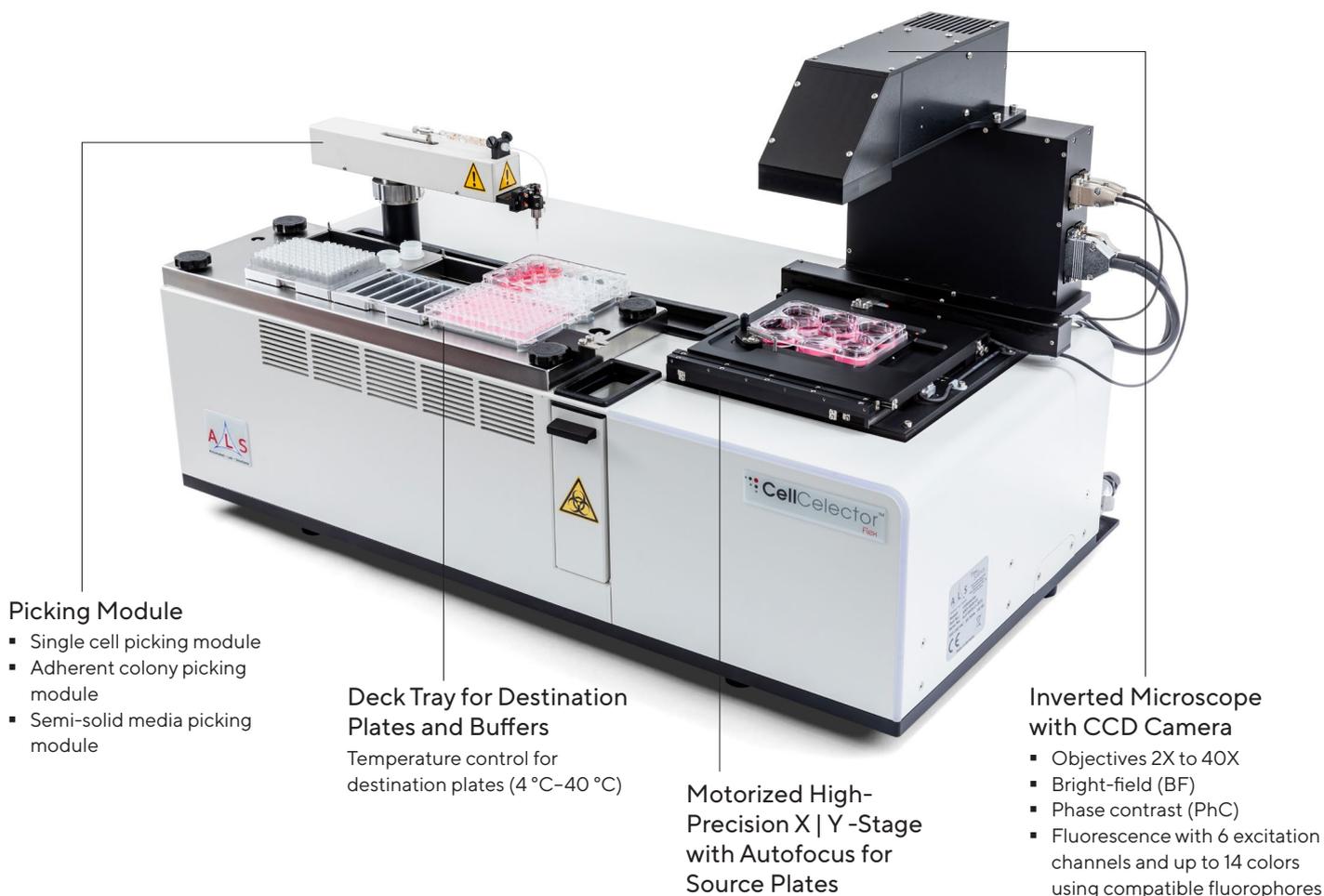
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# Precise Single Cell Picking

Nearly all cellular systems are heterogeneous, contributing to specialized functionality and improved survival. A profound comprehension of single cell heterogeneity on genomic, epigenomic, transcriptomic, and proteomic levels is critical for understanding its impact on the functioning of organisms in both healthy and diseased condition. However, most of our current knowledge about different cell and tissue types comes from bulk assays where hundreds to millions of cells are analyzed together, resulting in substantial underestimation of the true spectrum of cellular heterogeneity.

Modern technologies allow for analysis on a single cell level, which provides greater insights into cellular heterogeneity and its implications. For instance, single cell heterogeneity analysis can help clarify developmental pathways—such as how stem cells make their fate decisions, leading to the development and maintenance of differentiated tissue. This is important for understanding malignancies caused by missing or misdirected differentiation, and also, more generally, for the field of regenerative medicine.

## CellCelector Flex Specifications



It is well known that tumor heterogeneity plays a crucial role both in cancer evolution and in clinical treatment; it is a major contributor to therapy resistance and disease progression. Understanding tumors on the single cell level, and unravelling tumor heterogeneity, may have a huge impact on cancer survivability. It is not only the heterogeneity of the tumor influencing the individual effectiveness of tumor therapy, but also mutations within the circulating tumor cells (CTCs). CTCs are tumor cells that have left the primary tumor to enter the blood stream and drive metastasis.

In order to analyze cellular heterogeneity on the single cell level (e.g., by whole genome amplification [WGA], single nucleotide polymorphism [SNP] detection, or sequencing), these target cells have to be selected and isolated in high purity from a heterogeneous background. The CellCelector is the perfect tool for the automated identification and isolation of 100% pure single cells, offering many advantages over other methods.

## A Wide Range of Single Cell Applications

<b>Circulating Tumor Cells</b>	▪ Isolation of circulating tumor cells (CTC) for oncology applications
<b>Fetal Cell Isolation</b>	▪ Isolation of single fetal cells for cell based non-invasive prenatal testing (cbNIPT)
<b>HT-NIC</b>	▪ High-throughput nanowell-based single cell cloning with integrated monoclonality proof for pharmaceutical cell line development
<b>Single Cell Secretion Screening</b>	▪ Single cell secretion screening and picking (e.g., individual plasma B cells or hybridoma cells for antibody discovery)
<b>CRISPR Single Cell Cloning</b>	▪ Single cell cloning of gene edited iPS cells (or other stem cell lines)
<b>Isolation of Sperm Cells</b>	▪ Forensic applications (e.g., picking of sperms for genetic analysis)
<b>Heterogeneity Analysis</b>	▪ Purification of samples for genetic single cell analysis (single cell PCR, RNA-seq, NGS)
<b>Precise Cell Number Isolation</b>	▪ Preparation of high-quality spike-ins for reference samples

## Precise Single Cell Picking With the CellCelector Flex

The CellCelector Flex Single Cell Picking Module utilizes liquid buffered single-use glass capillaries providing gentle aspiration with extremely high precision down to the nanoliter range. This module can be used for precise isolation of individual single cells, clusters, single cell clones, spheroids, small organoids, embryoid bodies, etc.

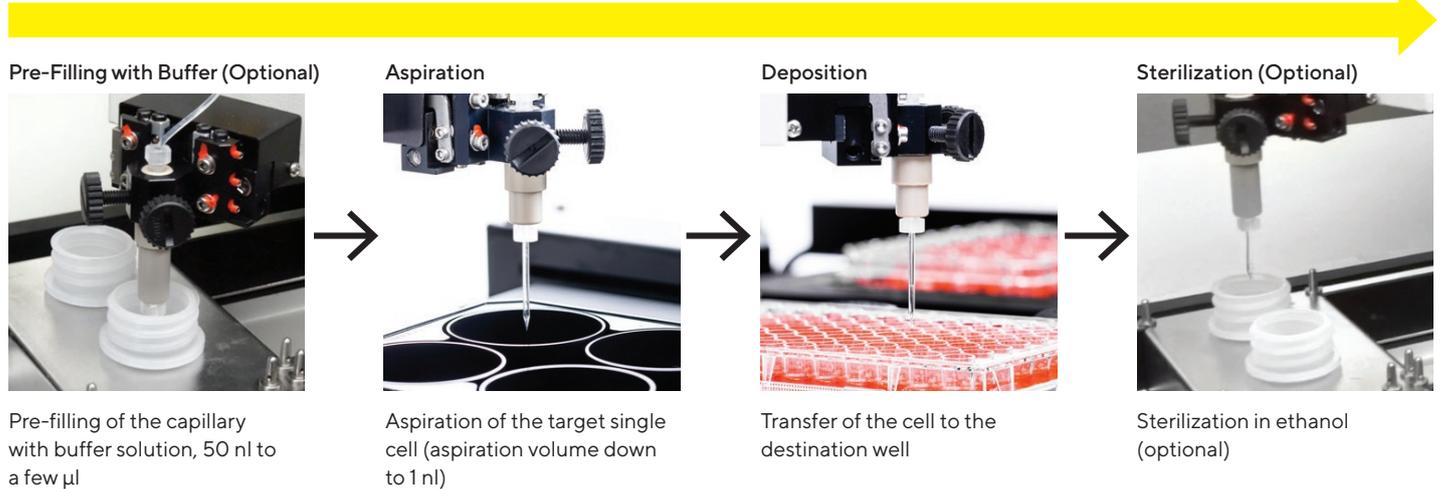
Contrary to manual or semi-automatic picking setups in traditional micro manipulators, the vertical capillary orientation and precision of the CellCelector Flex allows for easy picking from various culture lab ware as well as deposition into small destination wells.



# Single Cell Picking Workflow

The isolation of single cells with the CellSelector Flex single cell picking module is fully automated and can be divided into 4 steps:

## Workflow



## Various Source and Destination Plate Formats

The single cell picking module can be used with almost any kind of standard lab ware: well plates (1 to 96 wells), petri dishes of all sizes, microscope slides, and custom consumables. This flexibility is given for both source and destination plates.

### Source Plates

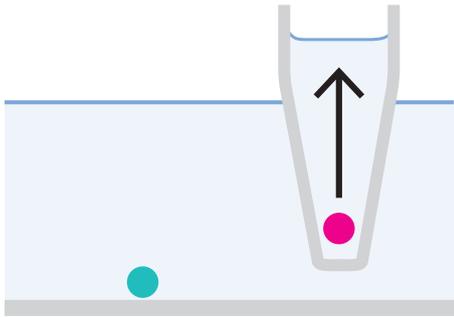
- CellSelector nanowell arrays (both slides and plates) for single cell cloning and secretion assays
- Sievewell slides for rare single cell isolation workflows
- Magnetpick slides for isolation of immunomagnetically enriched rare single cells
- Cell culture microplates (from 1 to 96 wells)
- Microscope slides (including multiwell slides)
- Petri dishes (up to 100 mm)
- Custom plates/chips like filters or membranes

### Destination Plates

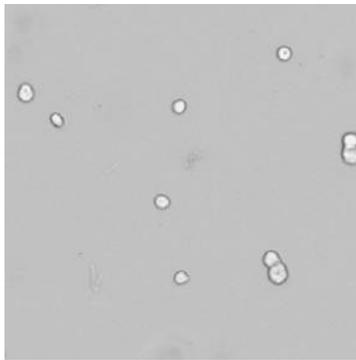
- PCR tubes, strips, or plates (96- or 384-well)
- Cell culture microplates (up to 96 wells)
- Multiwell slides
- Petri dishes
- Custom plates

# Cell Capture Methods for Automated Single Cell Isolation

Localization on Standard Plastic or Glass Surface

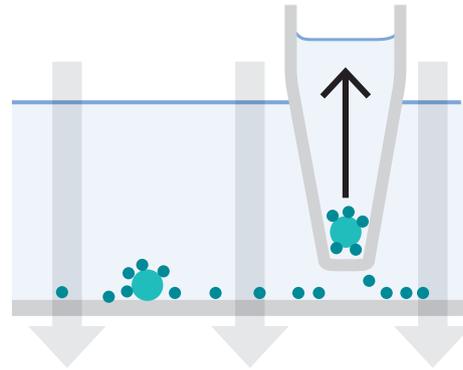


Standard dish or plate

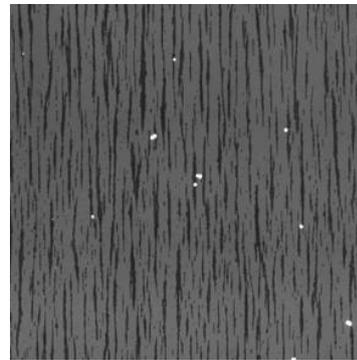


Adherent or immobilized suspension cells

Retention by Magnetic Field

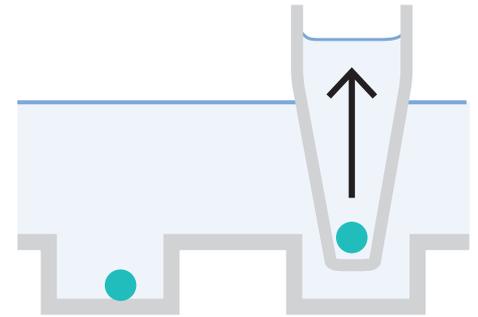


Glass slide plus magnetic field

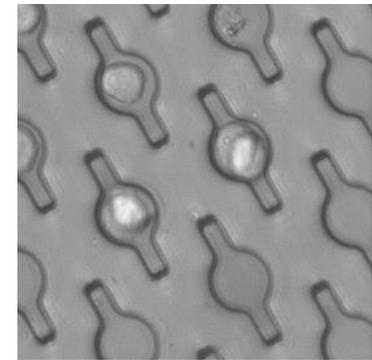


Immunomagnetically enriched cells not attached but localized by the magnetic field

Capture in Nanowells



CellCelector nanowell arrays with low attachment surface



Cells are captured but not attached

## Examples of Precise Isolation of Single Cells

### Human Rare Cell Isolation From Blood

Rare cell isolation and subsequent characterization are technically challenging due to the low cell numbers among an abundance of white and red blood cells. A wide range of analytical methods for rare cell detection, enrichment, and isolation has been developed. They exploit specific properties such as surface marker expression or, for example, size, density, or deformability.

We have developed a unique and complete solution for detection and isolation of circulating tumor cells (CTCs) after enrichment and staining steps. Isolated cells can then be used for DNA, RNA, or proteome analysis. This solution is centered around the CellCelector Flex system utilizing specifically developed consumables (e.g., CellCelector Nanowell arrays, Magnetpick slides) and validated protocols.

1 CTC/10<sup>7</sup> WBCs

Enriched CTCs

100% Pure CTCs

Blood drawing  
RBC lysis

Enrichment and  
IF staining

Imaging, detection and  
recovery of pure CTCs

Single CTC  
characterization



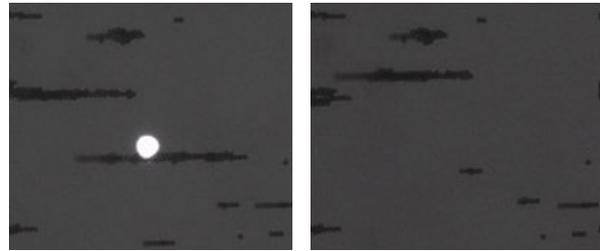
CellSelector



The CellSelector Flex platform is compatible with a variety of upstream enrichment technologies:

- Positive immuno-magnetic enrichment based on EpCAM surface markers and others (e.g., CellSearch®, Isoflux™, MagSweeper™)
- Negative depletion of white blood cells (WBCs) based on CD45 surface markers (e.g., Dynabeads™, RosetteSep™)
- Label-free separation based on microfluidics or filters (e.g., Parsortix™, Clearbridge™, Vortex™, ScreenCell™, RareCells™, Circulogix™ etc.)

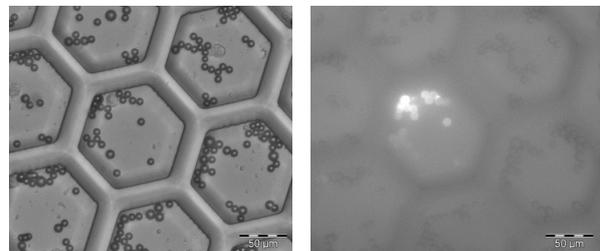
The workflows and consumables we have developed allow handling of up to several hundred thousand of blood cells per sample without need for sample volume reduction. The CellSelector Flex technology is capable of isolating pure single tumor cells from liquid biopsies of a variety of cancer types.



SK-BR-3 cells (DAPI +) attached to magnetic beads; imaging 20 x DAPI before and after picking

## Plasma B Cells

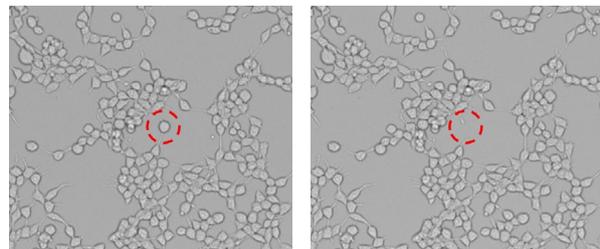
Using our novel nanowell technology, antibody-secreting plasma B cells can be gently isolated for subsequent cultivation or analysis. After seeding the cells into the nanowell wells and performing secretion assays at the single cell level, the cells of interest are identified and transferred to the appropriate target vessels.



Bead-based antigen specificity assay in Hex 100 μm nanowells; bright-field and FITC images

## Adherent Single Cells (e.g. HEK 293T)

HEK cells have been widely-used in cell biology research. They are easy to grow and used for transfections in research and for therapeutic protein production in the biotechnological industry.



Picking of single HEK 293T cells with a 30 μm capillary; bright-field images before and after picking

# Benefits of the CellCelector Flex for Single Cell Applications...

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<b>Easy to Use</b>	<ul style="list-style-type: none"><li>▪ No complex sample preparation and no expensive consumables required</li><li>▪ No routine maintenance necessary</li></ul>
<b>Extremely Versatile</b>	<ul style="list-style-type: none"><li>▪ Precise isolation of individual single cells, clusters, single cell clones, spheroids, small organoids, embryoid bodies</li><li>▪ Primary cells and cell lines; living and fixed cells</li></ul>
<b>Flexible</b>	<ul style="list-style-type: none"><li>▪ Bright-field, phase contrast, and fluorescence imaging</li><li>▪ Automated, semi-automated, or manual cell selection for picking</li><li>▪ Any standard or custom source and destination vessels (nanowell arrays, microplates, dishes, slides, filter, chips, PCR plates   tubes)</li></ul>
<b>Reliable</b>	<ul style="list-style-type: none"><li>▪ Accuracy of picking &gt; 95% of selected specific sub-populations</li><li>▪ Automatic re-location of moving objects</li><li>▪ Possible re-picking of failed picking events</li><li>▪ Software automatically detects successful picking</li><li>▪ Automated picking quality control (picked   failed)</li></ul>
<b>Gentle</b>	<ul style="list-style-type: none"><li>▪ No influence on characteristic properties of cells</li><li>▪ Isolation of pure intact cells ready for molecular characterization or cell culture (low shear stress: &lt; 10 seconds in the capillary)</li><li>▪ High cell integrity and outgrowth rates after picking (including up to 95% and more viability in single cell cloning applications)</li><li>▪ Automated picking quality control (picked   failed)</li></ul>
<b>Downstream Compatible</b>	<ul style="list-style-type: none"><li>▪ Low aspiration, dispensing, and buffer volumes (down to ~1 nl)</li><li>▪ Single cell PCR, NGS, WGA, RNA-Seq, genome editing, cell cloning, heterogeneity studies, etc.</li></ul>
<b>Documented</b>	<ul style="list-style-type: none"><li>▪ Complete workflow documentation compliant to GLP and GMP standards</li><li>▪ Quality control by using live-tracking and high-quality real-time images taken before and after each picking event</li><li>▪ Unique ID for each detected   picked object, tracking from source to destination well</li><li>▪ Easy export of all imaging and numeric data</li></ul>

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