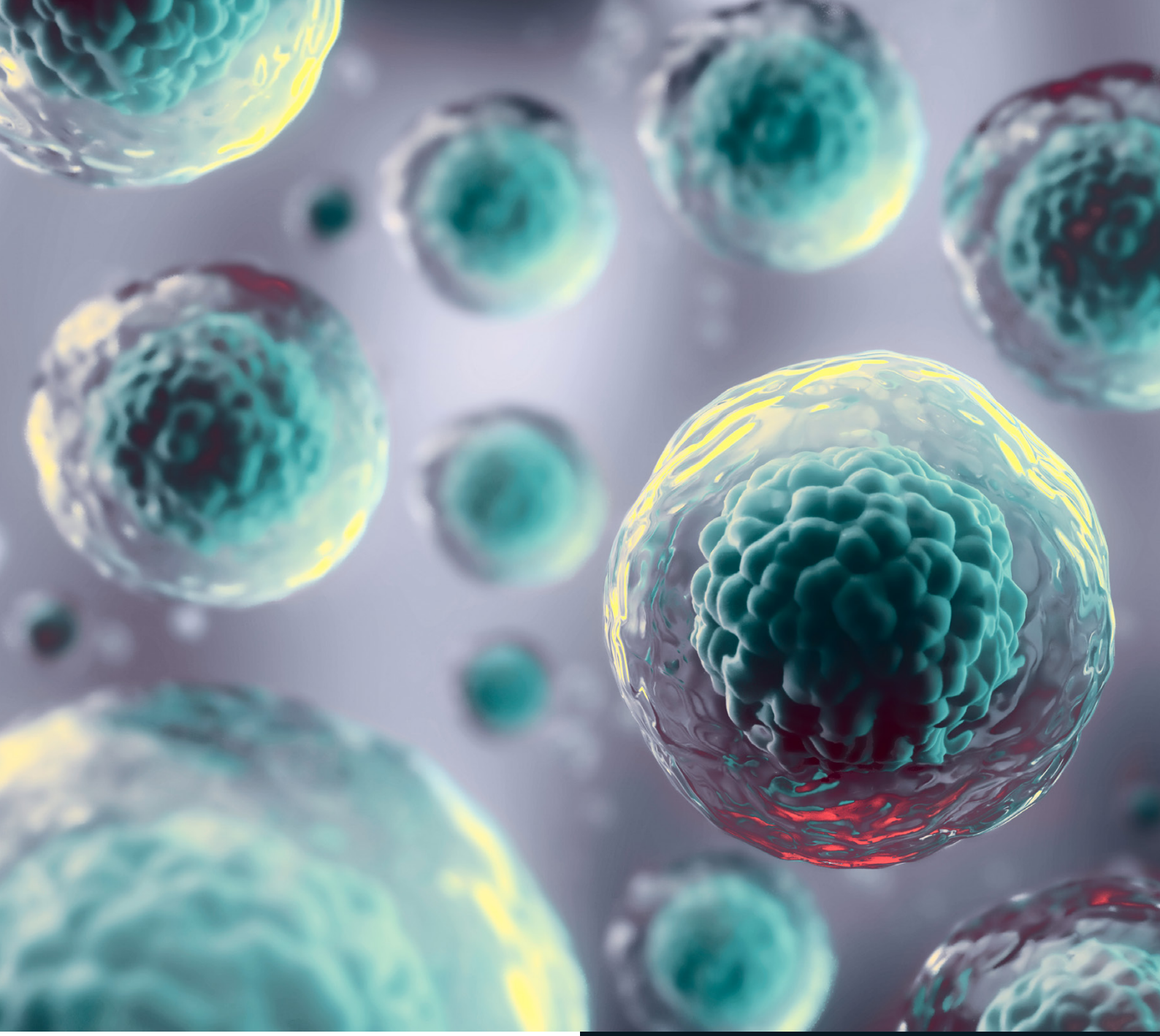


Fully Automated Image-Based Single Cell and Colony Picking for Stem Cells

CellCelector Flex

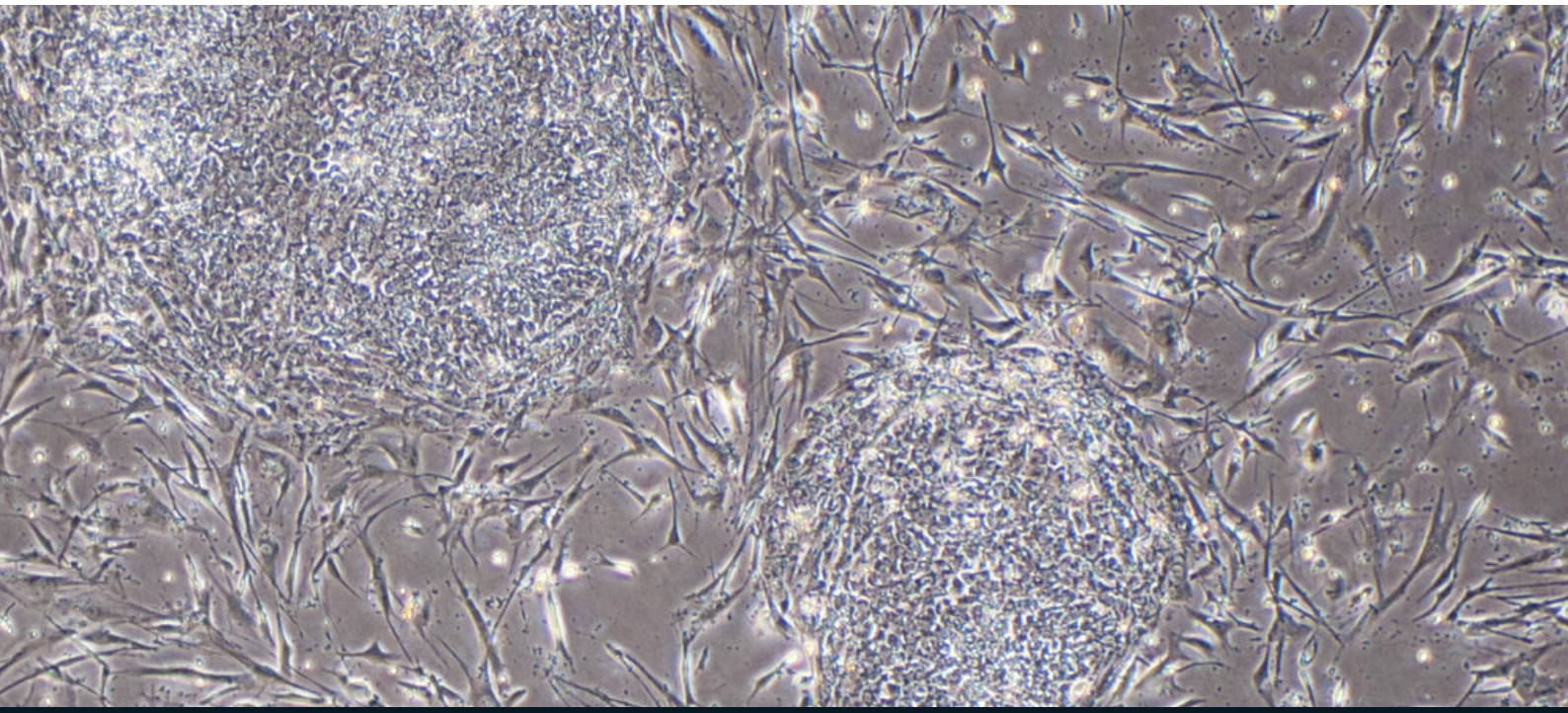
Simplifying Progress

SARTORIUS

Wide Range of Stem Cell Applications

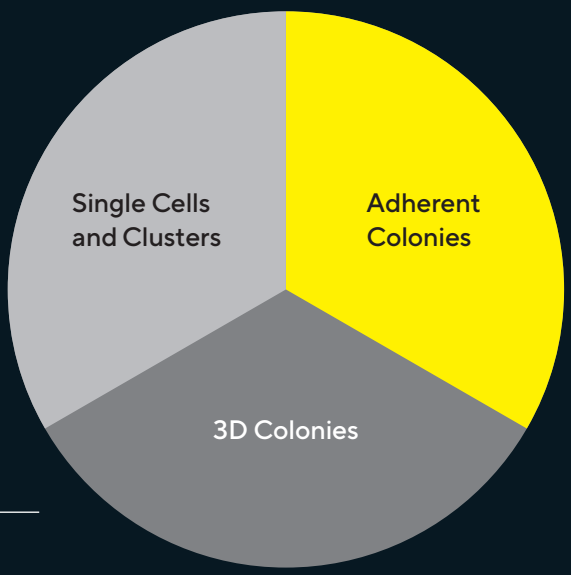
Stem cells play an important role in the field of regenerative medicine as their high self-renewal and differentiation potential makes them particularly suitable for a wide range of biomedical and pharmaceutical research applications. The CellCelector Flex with its specifically designed picking modules for adherent cells and cell colonies is extremely gentle, highly specific and therefore ideally suited for the clonal passaging of stem cells, stem cell colonies as well as isolating specific parts of a stem cell colony. It will support your stem cell research with:

- Isolation of stem cells for single cell cloning or heterogeneity studies
- Clonal picking of newly derived iPS colonies
- Colony picking for genome editing, using CRISPR | Cas9
- Colony splitting with transfer (creation of replica plates)
- Isolation of differentiated stem cell colonies
- Scanning and isolation of hematopoietic stem cell colonies from methylcellulose
- Isolation of HSC daughter cells or "Doublets splitting"
- Removal of unwanted cells (e.g. differentiated areas in stem cell cultures)



Isolation of single cells like mesenchymal stem cells, neuronal stem cells etc.

Complete or partial isolation of induced pluripotent or embryonic stem cell colonies



Isolation of 3D hematopoietic stem cell clusters, neurospheres, embryonic bodies etc.

CellCelector Flex:
A Useful Tool in Stem Cell Applications

The transfer of stem cell colonies is often a stressful procedure resulting in a great number of dead cells influencing their living neighbors. Using Trypsin or similar enzymatic digestion methods to facilitate the transfer of colonies can have distinct effects on the cell phenotype, especially freshly reprogrammed stem cells, and could result in unintentional differentiation. Furthermore, it can lead to the cross-contamination of different colonies resulting in clonality being lost. The incorporation of a manual mechanical transfer (scraping with pipette tips or cell scrapers) is laborious and time consuming. Therefore it is crucial to implement a gentle-mechanical transfer method with high specificity maximizing both the viability as well as the clonality of picked colonies while maintaining their pluripotent characteristics.

Picking Module
- Single cell picking module
- Adherent colony picking module
- Semi-solid media picking module

Deck Tray for Destination Plates and Buffers
Temperature control for destination plates (4 °C–40 °C)

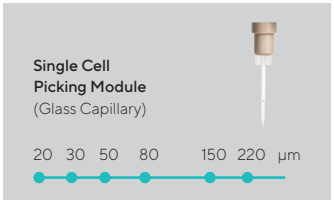
Inverted Microscope with CCD Camera
- Objectives 2X to 40X
- Bright-field (BF)
- Phase contrast (PhC)
- Fluorescence with 6 excitation channels and up to 14 colors using compatible fluorophores

Motorized High-Precision X | Y -Stage with Autofocus for Source Plates

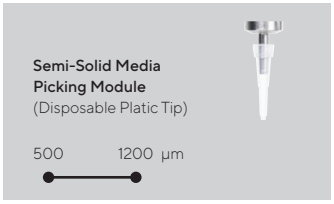
Find out more
www.sartorius.com/cellcelector

Modules for Picking of Single Stem Cells, Stem Cell Colonies or Partial Colonies

The adherent colony picking module is the tool of choice for picking entire colonies or large parts of it. Different diameters allow the operator to use the most suitable ScrapeTip for each colony.

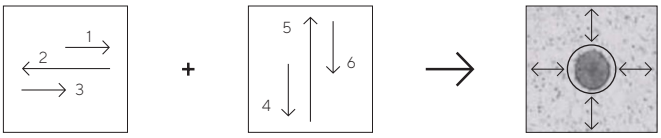


For the precise isolation of specific colony parts e.g. undifferentiated areas within a stem cell colony, as well as for the isolation of single stem cells, the single cell picking module can be used. This module utilizes a glass capillary, which is available in diameters from 20 µm up to 220 µm.



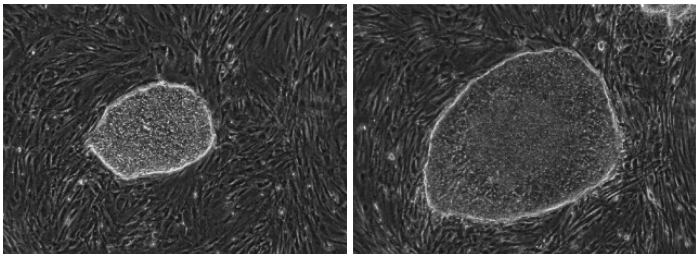
For isolation of 3D colonies, like embryoid bodies, spheroids, organoids or hematopoietic stem cell colonies the semi-solid media picking module is the perfect tool. Depending on the colony size two different diameters are available.

Very Gentle Picking Ensures Cell Survival and Proliferation ...

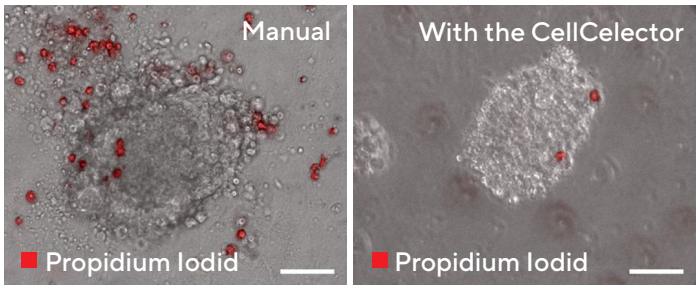


For picking of adherent colonies the CellCelector Flex combines a very gentle, crosswise scrape movement with a simultaneous aspiration of the colony. This gently loosens the colony from the base of the culture plate or feeder cell layer.

The morphology of a colony can provide important information about its current condition. A phase contrast image of a representative colony was taken 3 days (left) and 5 days (right) after automated passage into a new culture dish. The colony shows normal growth.
This indicates that stem cell colonies picked automatically with the CellCelector Flex are not significant different to manually picked colonies of the same passage.



Automated picking is gentler to the cells, thereby causing a much lower rate of dead cells in comparison to manual picking.

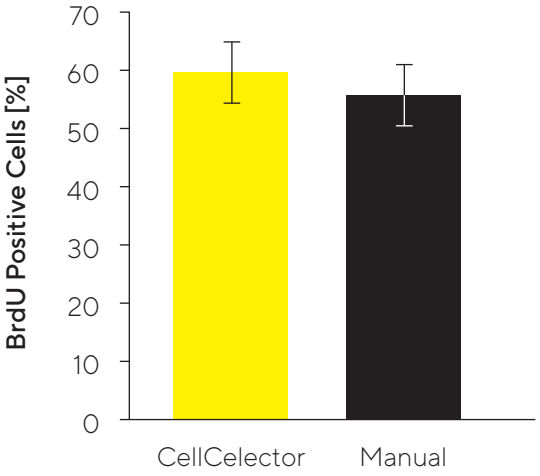


Images were kindly provided by Oliver Brüstle & Simone Haupt, Life & Brain GmbH, Bonn, Germany

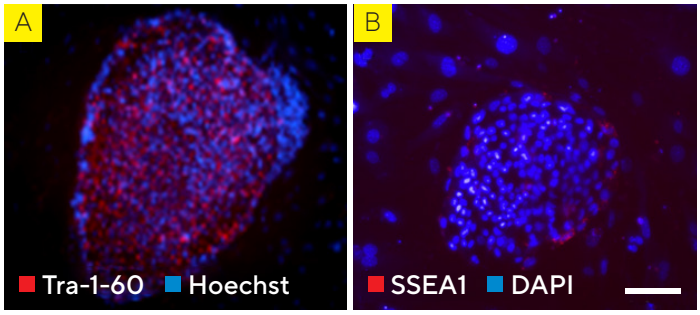
Clusters of hESC after automated picking using the CellCelector Flex (right) or manual picking with a pipette tip as a control (left). The propidium iodide (PI) staining shows dead human embryonic stem cells (red).

The active proliferation of cells is not affected by the automatic picking with the CellCelector Flex.

Mean BrdU (Bromodeoxyuridine) incorporation measured by FACS shows the active proliferation of human embryonic stem cells (hESC) in %, error bars depict standard deviation



... and Retained Pluripotency and Differentiation Potential



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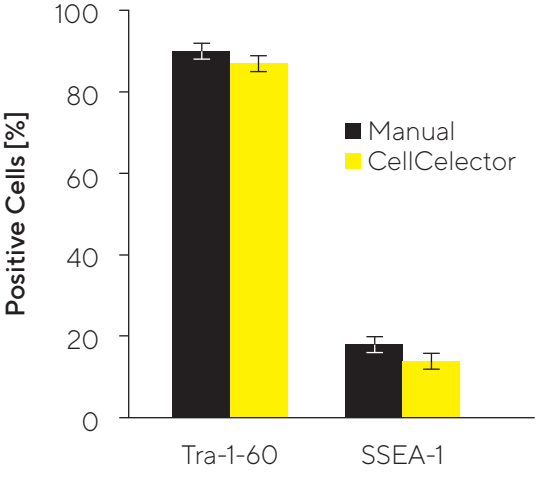
Assessment of the pluripotency-associated surface marker Tra-1-6 0(A) and the differentiation-state marker SSEA-1 of hESC after automated picking (B). Mean Tra-1-60 and SSEA-1 positive cells measured by FACS shows the differentiation state of hESC in %, Error bars depict standard deviation (C).

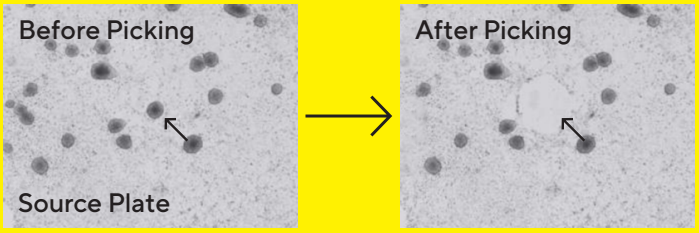
Immunochemical expression analysis of pluripotency-associated markers of hESCs passaged with the CellCelector Flex shows expression levels comparable to those of conventionally propagated hESCs.

The automated picking by the CellCelector Flex does not affect the pluripotency and in-vitro differentiation potential of hESCs.



Successful Transfer and High Cell Growth Rates

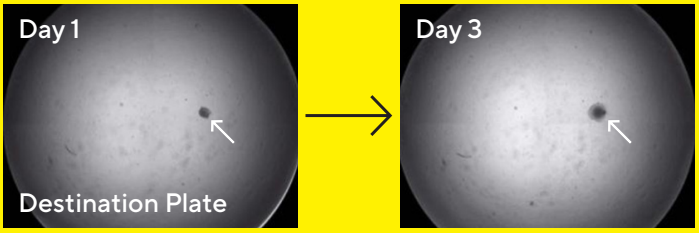
A murine embryonic stem cell colony cultivated on feeder cells was solely isolated and transferred into a 96 well destination plate using the CellCelector Flex.



The CellCelector Flex specifically isolates the selected stem cell.

Transfered by CellCelector Flex

The isolated colony was re-detected after re-scanning the destination well by the CellCelector Flex (Day 1 and Day 3). The colony grew well over a time course of three days.



The CellCelector Flex does not influence the growth of stem cell colonies.

Images were kindly provided by Ute Schaefer & Annette Schneider, Institute for Research in Operative Medicine, Cologne, Germany

Clear Workflow & User-Friendly Software

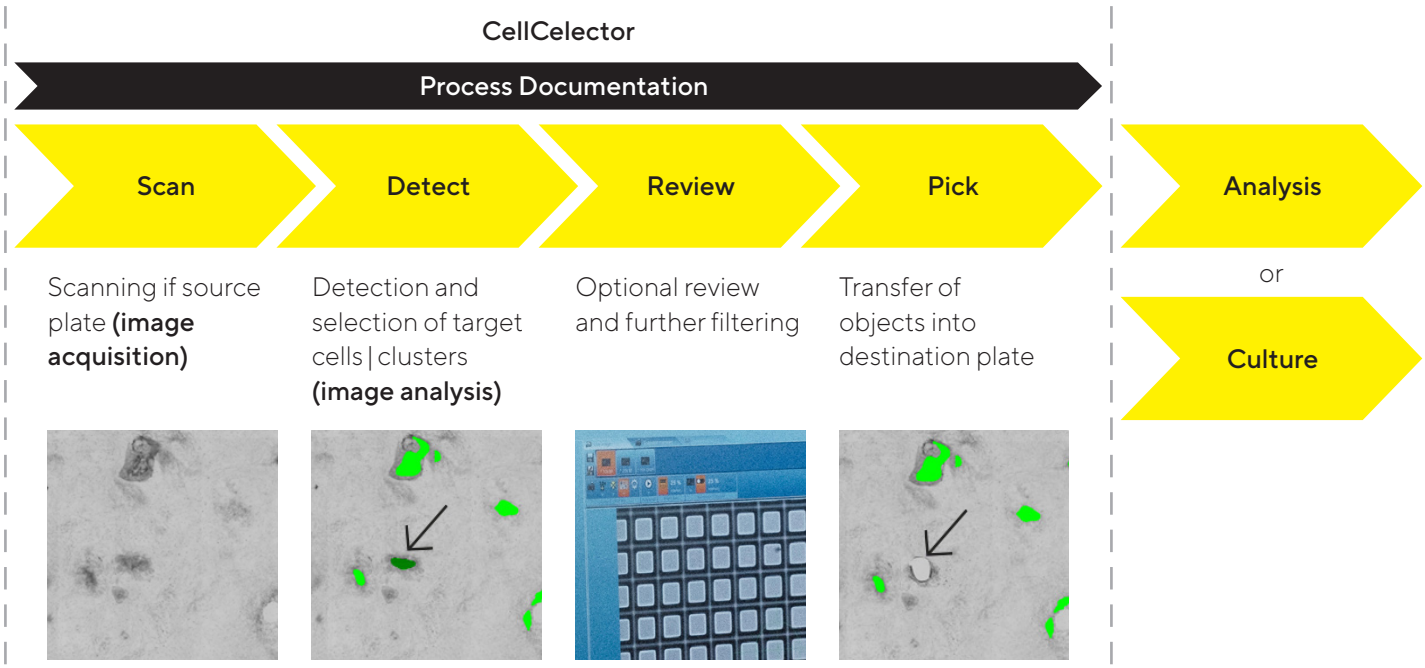
The whole workflow is fully automated and documented by providing a live image during picking, cell tracking data from source to destination plate as well as high-quality before and after picking images.

Depending on the application and user requirements, the cells to be picked can be detected, identified and selected fully automatically either label-free based on the target cell size and morphology or through positive | negative fluorescence markers. As an alternative, semi-automated or manual on-screen selection by the operator is possible.

The standard experiment workflow of the system is separated in up to 5 different workflow steps:

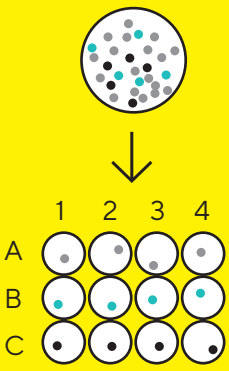
- **Scanning** the source in bright field, phase contrast and fluorescence to acquire and store images in all required channels and magnifications
- **Analysis** of those images and detection of cells or colonies of interest according to user-defined criteria
- **Review** of the analysis results by the operator (optional)
- **Picking** of the detected single cells and colonies
- **Documentation** and export of the results

Different user access levels allow easy access to the software without intensive training for new operators while offering advanced features, open access to all parameters, calibrations and customization capabilities for power users. This enables simple day-to-day use as well as performing sophisticated and complex experiments.



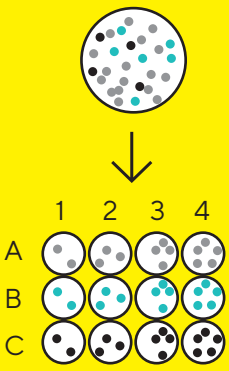
Multiple Deposition Possibilities for Downstream Culturing

Single Deposit



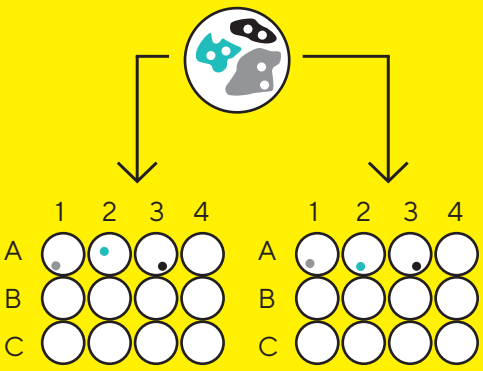
In order to analyze or cultivate single cells, the CellCelector Flex deposits each picked object in one well.

Pooling



Multiple single cells or parts of one colony can be pooled in one well.

Replicate Generation



A combination of colony cultivation and analysis can be reached by generating replica wells of one colony.

Benefits of Using the CellCelector for Stem Cell Applications...

Fast & Easy to Use	▪ Short experimental hands-on time without complex sample preparation ▪ ~30 sec / single cell ▪ No routine maintenance and low consumable cost
Extremely Versatile & Flexible	▪ Precise isolation of individual single cells, clusters, single cell clones, spheroids, small organoids, embryoid bodies ▪ Primary cells and cell lines, Living and fixed cells ▪ Bright field, phase contrast and fluorescence imaging ▪ Automated, semi-automated or manual cell selection for picking ▪ Any standard or custom source and destination format, including microplates, dishes, or PCR tubes ▪ Low aspiration, dispensing and buffer volumes (down to ~1 nl) ▪ Compatible with upstream and downstream applications such as immunomagnetic enrichment, size based separation, Single cell PCR, NGS, genome editing, or cell cloning
Reliable	▪ Accuracy of picking > 95% of selected specific sub-populations ▪ Automatic re-location of moving objects ▪ Possible re-picking of failed picking events ▪ Software with integrated quality control for automatic detection of successful picking
Gentle	▪ No influence on characteristic properties of cells ▪ Isolation of pure intact cells ready for molecular characterization or cell culture (low shear stress; <10 seconds in the capillary) ▪ High cell integrity and outgrowth rates with ≥95% viability
Documented	▪ Complete workflow documentation compliant to GLP and GMP standards ▪ Quality control by using live-tracking and high-quality real-time images during each picking ▪ Unique ID for each detected | picked object, tracking from source to destination well ▪ Easy export of all imaging and numeric data

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