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CellCelector™ Application Note

Automatic Transfer of Hybridoma Clones

CellCelector is protected by US patent 9,822,331 B2

Rev. 12 / 24.09.2018

Introduction

Automated harvesting of cells utilizing the CellCelector

The **Cell**Celector™ from **ALS** is a flexible multiplatform system for precise isolation and secure transport of cell colonies, specific parts of colonies or even single cells into a new culture environment as well as into a number of targets for subsequently performed analysis [2, 3, 4, 5]. The instrument consists of an inverted microscope equipped with a CCD camera and a motorized stage holding the cell culture dish, a high-precision robotic arm, multiple racks for consumables and a heated holder for a reagent vial (Fig. 1). The robotic arm is mountable with different harvesting tools holding plastic tips, metal tips or glass capillaries with different diameters each fitting to the specific application. The harvesting tips are connected via a tube to a motor driven syringe pump and an automatism for system liquid re-filling. The entire unit is housed in a laminar flow hood providing a sterile atmosphere and is controlled by a personal computer via intuitive software (Fig. 2) [4].

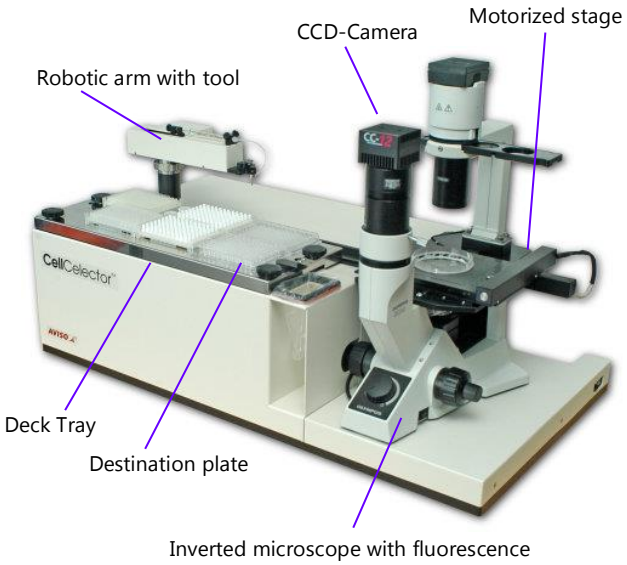

Fig.1 The **Cell**Celector - Overview

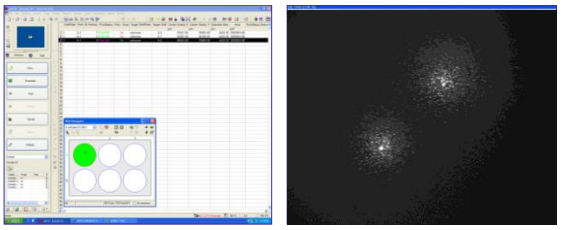

Fig.2 Two screens of the computer: for software operation control (left monitor) and for real-time imaging or selection of cells (antibody secreting Hybridoma colonies) on-screen per mouse click (right monitor).

Nowadays antibodies (Fig. 3) belong to the essential tools in a wide spectrum of biological and medical research and diagnostic applications.

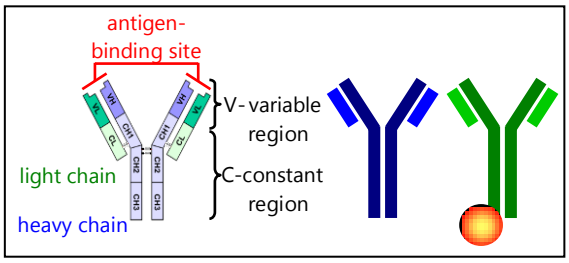

Fig. 3 Structure of a type IgG antibody schematically (left); simplified illustration of a secreted IgG (middle) and of a fluorochrome labelled antibody (right).

The in vitro generation of IgG antibodies with a defined specificity by a stable cell line can be achieved via the fusion of an antibody producing B-cell with an immortal tumour cell. The result is an immortal antibody producing cell line (Fig. 4).

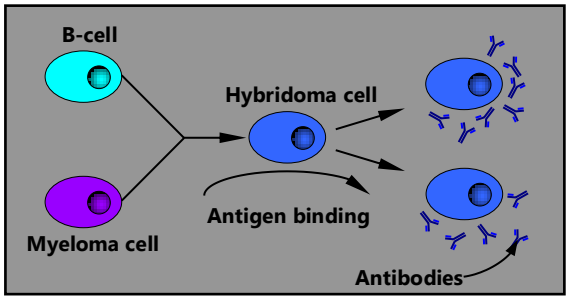

Fig. 4 Schematically illustration of generation a stable antibody producing cell line. Fusion of a B-cell with an immortal cell type (e.g. myeloma cell) results in a hybridoma cell. After binding antigens the hybridoma cell starts the generation of antibodies and their release into the environment.

Technology and work flow

1. Scan & Imaging

The culture dish with the region of interest is scanned automatically by a high resolution camera employing the motorized stage (Fig. 5 A). The entire collection of single images during the scan is combined by the software into an overview image (Fig. 5 B). Integrated fluorescence filters can be very useful in terms of immunochemical labelling and staining and allows the user in-plate cell sorting before picking.

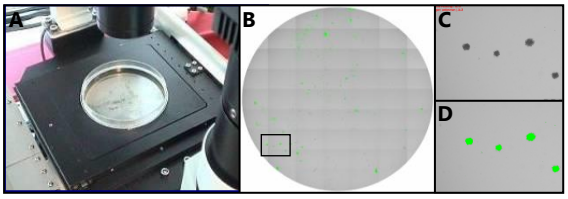

Fig. 5 Scanning process of a petri dish with cell culture on the motorized stage (A); overview image after scanning (B); enlarged area in bright field illumination (C) with detected green labelled target colonies (D).

2. Selection & Targeting

According to selection parameters (size, diameter and shape factor of the cell etc.) predefined by the user the software detects and selects the targeted cells or colonies (Fig. 6). Selection can be done using the great variety of analysis methods provided by the microscope (fluorescence, bright field, phase contrast) and the CELL*D software providing 3D imaging, overlays and movies. An overlay of phase contrast and fluorescence image can be created.

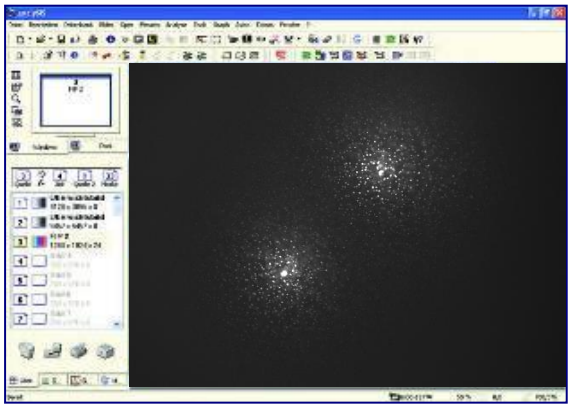

Fig. 6 Cell culture in the imaging software for selection of a specific target region or cell

3. Automatic Harvest

Application-specific harvesting modules (Fig. 7) take the cells or colonies up and the robotic arm automatically transfers them to the destination plate according to a user-defined chart.

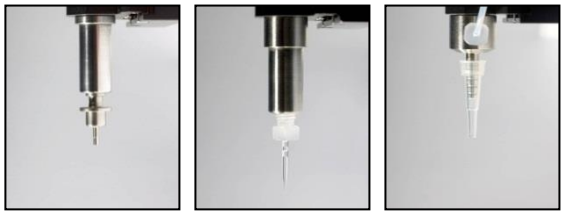

Fig.7 Different tools for automatic cell and colony transfer Modules (le. to ri.): Scrape Module, SingleCell Module, MC Module

The Scrape-Module is convenient for transfer of whole or parts of cell colonies. Single cells can be taken up most sensitive by the SingleCell-Module using glass capillaries with a diameter of 20 to 220 µm. The MC-Module uses plastic tips with a diameter of 500 or 1200 µm for harvest of Hybridoma colonies out of methylcellulose medium.

4. Documentation

In order to keep the whole process reproducible and documented an image of each cell or colony before and after harvesting is stored in the database (Fig. 8) and assigned to the deposit position in the destination plate. That allows comparing growth progression of colonies or cells and later check-up during downstream processes (PCR, Arrays etc.).

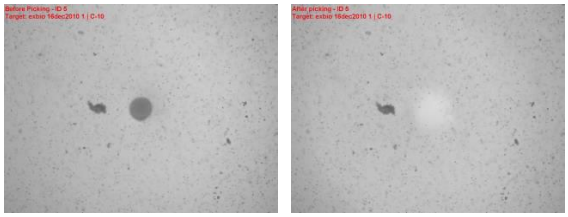

Fig. 8 Documentation of the isolation of a cell colony out of medium containing 1.2% methyl cellulose. Region of a culture dish before (left) and after (right) the cell isolation process.

Software

The **Cell**Celector Software (Fig. 9) contains numerous features for individual analysis of cells and cell colonies either automatically or on-screen. The software combines imaging facilities (camera control, fluorescence, overlay etc.) with the robot control for the cell harvest.

It is possible to select specific cells of interest on-screen. Driving the microscopic motor stage allows for searching within the culture dish for target cells and to mark them by mouse click, either for instant picking or for collection of multiple targets into a picking list.

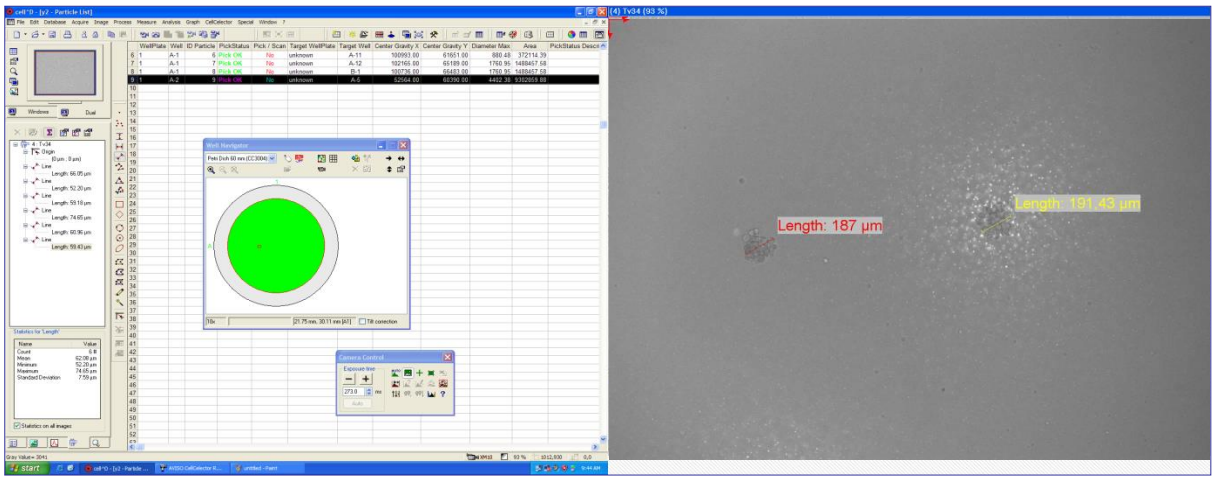

Fig. 9 Screenshot of the software showing software control with well navigator (left) and microscopic image of hybridoma cell colonies with measured cell diameter.

The **measurement bar** provides several tools for measurement of cells, colonies or structures on-screen like diameter, area, distance to adjacent cells etc. (Fig. 10). It is helpful for identification of targets as well as for the right choice of capillary diameters.

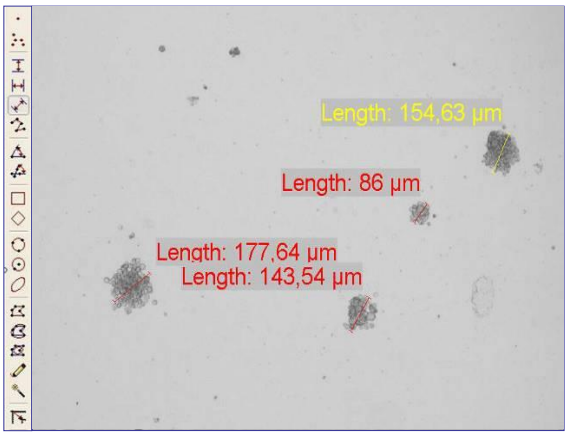

Fig. 10 Measurement bar (left) and close image from screenshot above showing application of the measurement tools on-screen (right)

The **well navigator** (Fig. 11) indicates where the camera focuses on. Wells can be addressed for cell harvest and focus by mouse click and will be shown in green colour. The microscope motor stage is connected to the navigator and will be driven to the selected point of view. Special formats of plates and dishes can be easily configured by the user and saved for later use.

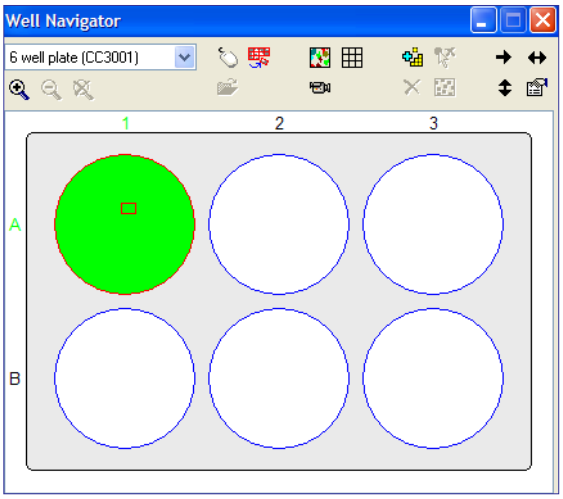

Fig. 11 Well navigator showing a 6 well plate

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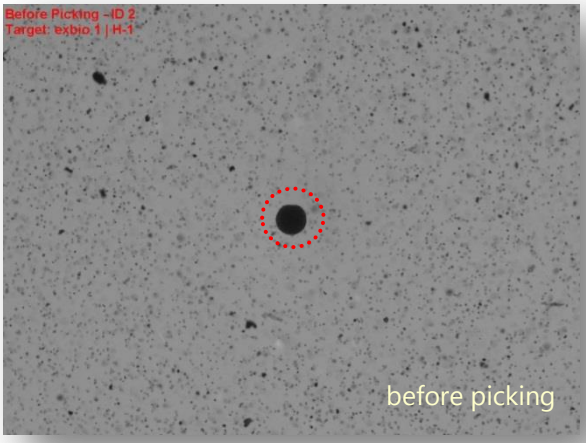
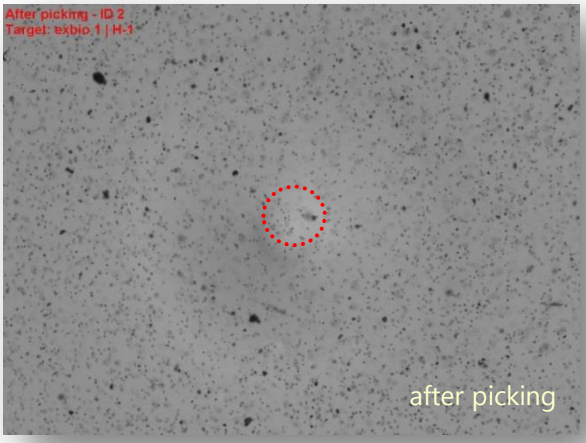
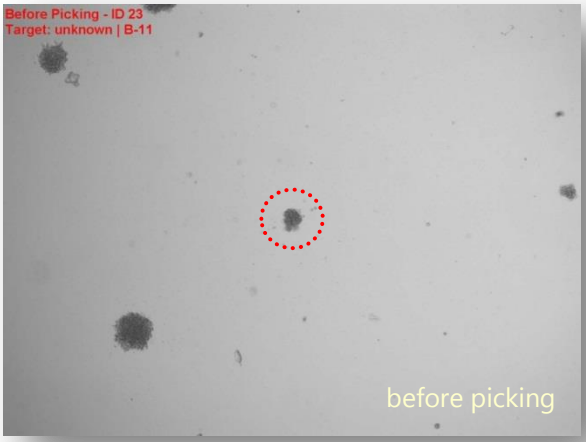
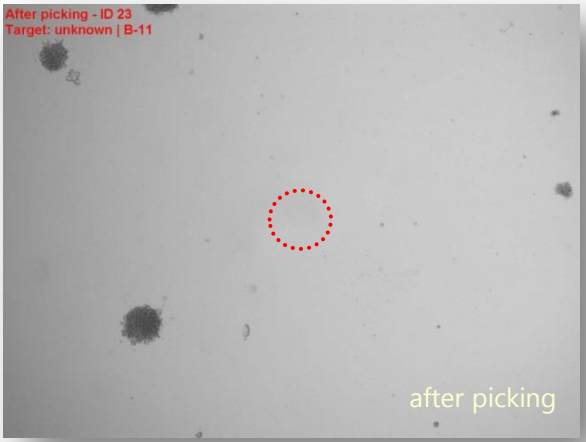
Precise isolation of Hybridoma colonies out of methyl cellulose containing medium…

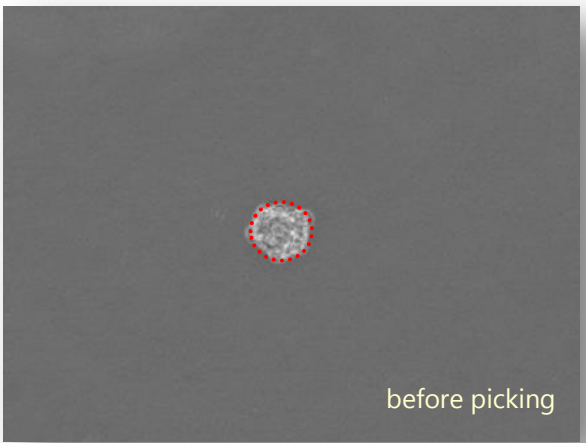
… in bright field

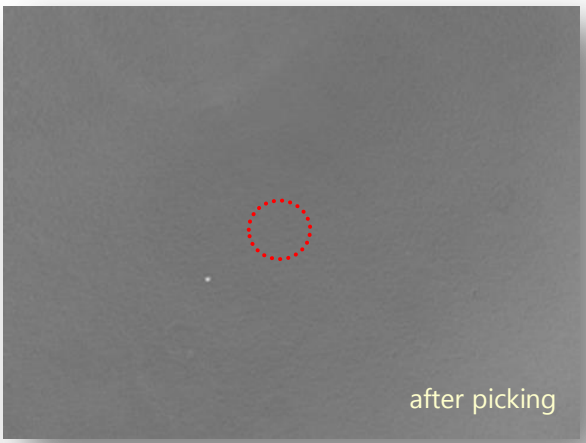




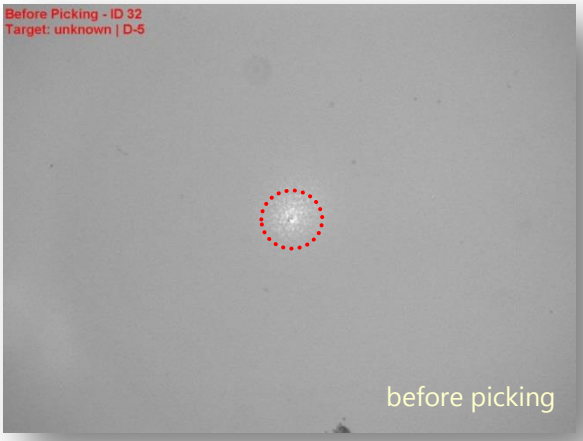


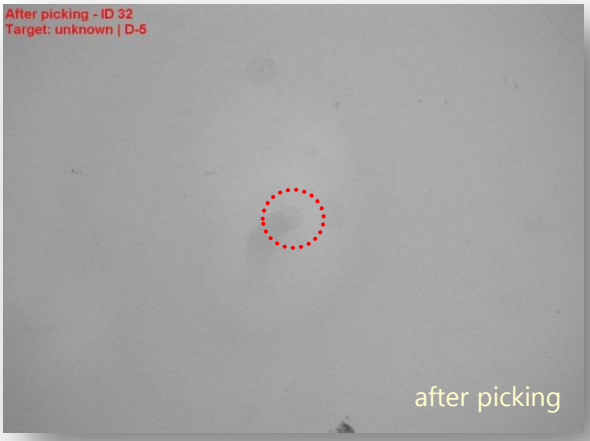




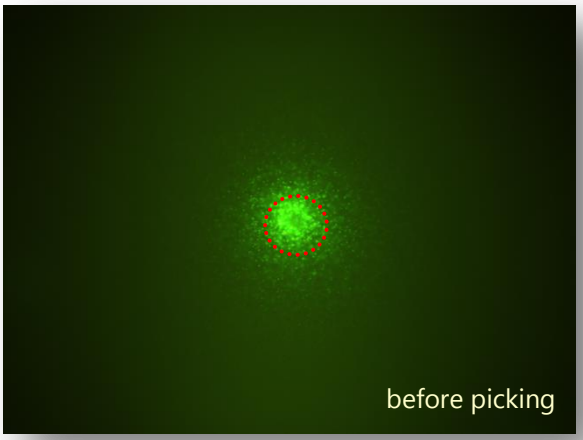


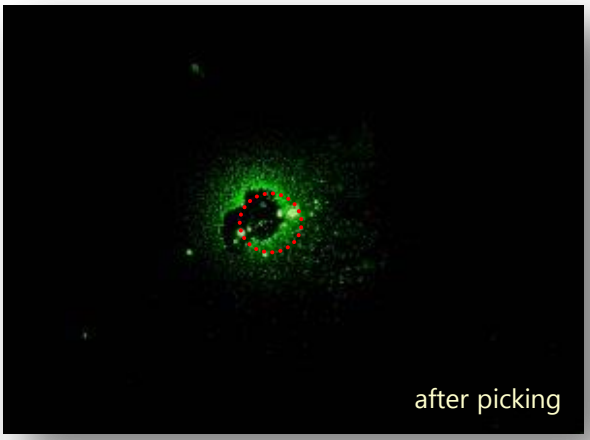
... in bright field and fluorescence illumination combined





... in fluorescence illumination





Selection of target cell colonies

For the automatic detection and selection of cell colonies of interest a set of target specific characteristic parameters can be used and defined. Cell colonies can be selected qualitative (e.g. all fluorescence positive colonies) or based on the intensity of antibody generation (e.g. the top ten high producer).

Qualitative selection of antibody secreting cell colonies

An overview scan in fluorescence illumination might be sufficient to detect the fluorescence positive colonies. The basic detection process is working with grey values. By defining a range of grey values (Fig. 12 B) that correlates with the cells of interest (Fig. 12 C) allows the user to separate the fluorescence positive target cells from the background (Fig. 12 D). By changing the range of grey values via moving the red and blue threshold bars (Fig. 12 B) the area to detect (visualized as green coloured areas on the reference image; Fig. 12 D) can be specified. By combining the grey value based detected signals with additional parameters like size, diameter, shape factor etc. the quality of the scanning result can be increased significantly. After scanning the detected cells are displayed as overview map and listed in a data sheet.

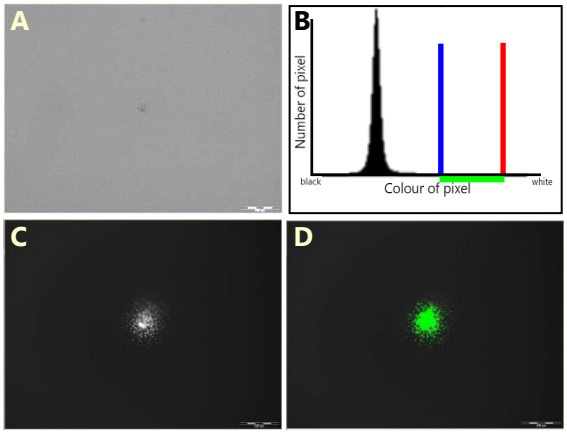


Fig. 12 Detection of an antibody secreting Hybridoma colony by defining grey value thresholds: Original image (A); graphical display to set thresholds (B); fluorescence positive halo around the Hybridoma clone (C); detected parts of the halo surrounding the Hybridoma clone (D)

To further improve the scanning result it might be reasonable to process the original images during the scan as illustrated in Fig. 13. Antibody secreting Hybridoma colonies that are detected by fluorochrome labelled antibodies might appear as cloud-like conglomerate on the original image (Fig. 13 C). This leads to the detection of multiple single dots per colony and results in many different picking positions (Fig. 13 D). Using filter features (Fig. 14) the dotted halo surrounding the cell colony can be processed into one area (Fig. 13 E) which is detectable as an individual colony (Fig. 13 F) with a single picking position in its central part.

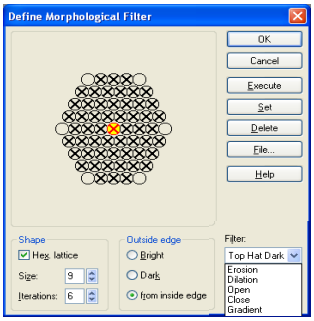


Fig. 14 Dialog window to define morphological filter settings

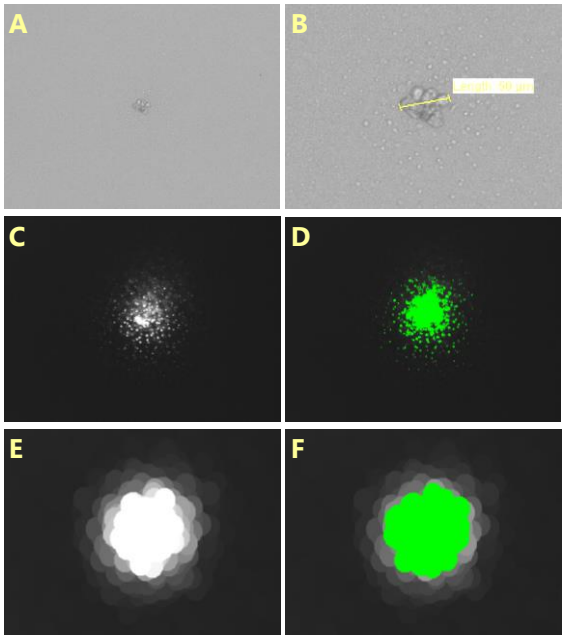


Fig. 13 Detection of antibody secreting Hybridoma colonies. The Hybridoma clone is located within the central part of the detectable dotted halo. Original image (A); Original image enlarged: Diameter of the Hybridoma clone (50µm) measured by specific software features (B); The fluorescence positive halo around the Hybridoma clone (C) is detectable as dotted area resulting in a number of potential picking positions (D); Processing the original image in fluorescence mode (E) results in a homogeneous detectable area (F).

Selection of high producers

For downstream processes it might be essential to select Hybridoma colonies producing antibodies at a maximal rate - so called "high producer" colonies.

Antibodies which are secreted into the medium can become visible by fluorochrome labelled secondary antibodies as fluorescent halo around the cell clone. The antibody generation between Hybridoma clones can vary dramatically (Fig. 15). By comparing the size of the cell colony (visible in bright field illumination) with the size of the cell colony surrounding halo (visible in fluorescence illumination) the rate of the antibody production and can be calculated as so called quality factor. The cell clone with the best quality factor is the top antibody producer inside the culture plate. By scanning your samples that way it is even possible to find out a low number of high producers from a stack of culture plates.

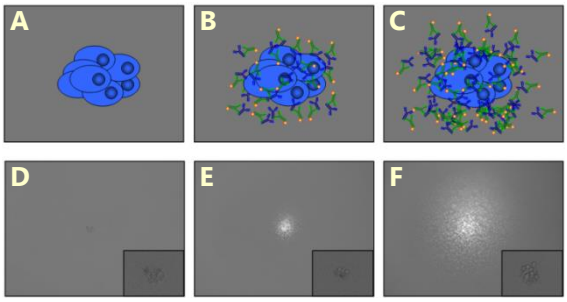


Fig. 15 Detection of antibody high producing Hybridoma clones: Schematic view of Hybridoma clones (light blue) and the detection of secreted antibodies (dark blue) by fluorochrome labelled secondary antibodies (green with yellow circle) (A-C); Microscopic view (overlay of bright field fluorescence illumination) of Hybridoma clones after incubation with fluorochrome labelled secondary antibodies (D-F); The intensity of antibody production correlates with the diameter of the fluorescent halo around the colony: colony producing no antibodies (A and D), colony producing medium amounts of antibodies (B and E); colony producing high amounts of antibodies = high producer (C and F). The size of the colonies is similar (see enlarged area in bright field observation D-F).

Readjusting the position in case of colony movement

Due to the semi liquid nature of methyl cellulose containing medium a cell colony can slightly change the position inside the culture plate. For successful picking events it is essential that the cell colony stays at the position where it was detected during the scanning process. Low distance movements can be corrected by special software features. The colony will be searched around the formerly detected position in a predefined area and will be driven back by the motorized stage to the original pick up position (Fig. 16).

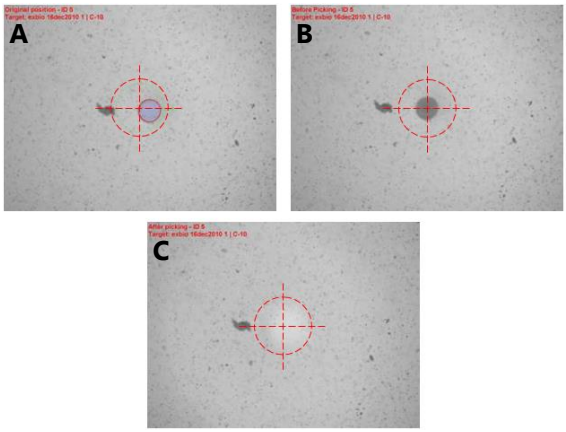


Fig. 16 Readjusting the position of a Hybridoma colony: Colony moved slightly from their original position at the centre of the image (A); Hybridoma colony after readjusting into the centre of the image by driving the motorized stage (B); Image after successful picking of the re-centred Hybridoma colony.

High rate of cell survival after transfer

Transfer of colonies is often a stressful procedure to the cells resulting in a great number of dead cells influencing their living neighbours [2]. Therefore, it is crucial to use a sensitive mechanical transfer method causing the least amount of destroyed cells [1]. Using the CellCelector is gentle and reliable way to isolate and transfer Hybridoma colonies out of methyl cellulose containing media. The cells continue to grow in their new environment rapidly (Fig. 17).

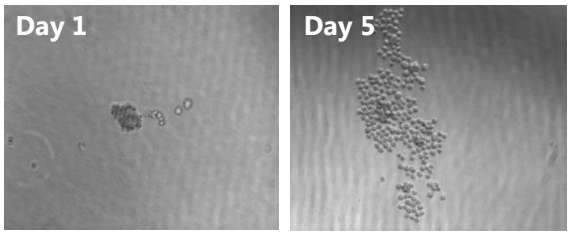


Fig. 17 Antibody producing CHO Hybridoma colony harvested with the Methylcellulose Module. Images kindly provided by Antoine Caron, Biotechnology Research Institute, Montreal, QC, Canada

Efficiency of the transfer and surviving rate was determined with human embryonic stem cells (hESC) which are even more sensitive in their behaviour to changes in their surrounding environment. Compared to manual picking the transfer rate was in the same range. Quantification of re-plated colonies was performed 4 days after transfer (Fig. 18).

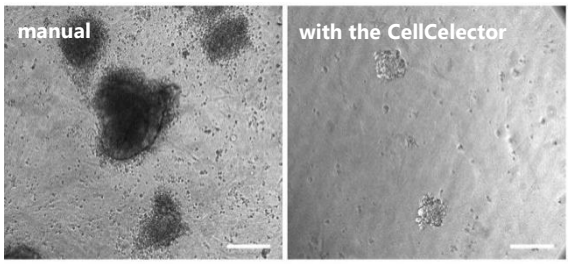


Fig. 18 Phase contrast images of picked hESC clusters after manual transfer with a 100 µl pipette tip (left) and a 220 µm glass capillary (right; scale bar 200 µm) with the CellCelector. Automated picking results in a more standardized cell cluster size.

Propidium iodide (PI) incorporation (Fig. 19) was used to assess cell survival of manually (left) and automatically (right) picked hESCs after transfer.

Phase contrast images of those hESCs were merged with the corresponding fluorescent images of PI staining (scale bar 50 µm). The images (Fig. 19) show the high sensitivity of the transfer method using the **Cell**Celector due to the usage of more precise tools for picking.

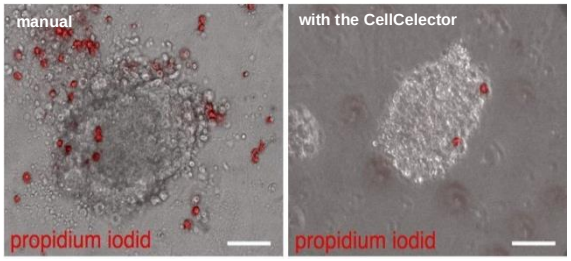


Fig. 19 Propidium Iodide staining for imaging dead cells after manual (left) and automatic transfer with the **Cell**Celector (right).

DNA content as well as DNA synthesis is a reliable indicator of the cell density, activity and viability of the transferred colony and therefore testifies the reaction of the cells to the method of transfer. BrdU is a nucleoside analogue (thymidine). Proliferating cells with DNA *de novo* synthesis will integrate it instead of dTTP. Conjugated antibodies were used to label these cells and thereby providing visual evidence of cell division. BrdU incorporation was analyzed (Fig. 20) by FACS using the fluorescent dye Alexa 488 to measure BrdU and Hoechst to determine total DNA content. Gates were set for cells in the S-phase, G1-phase and G2-phase to determine the percentage of proliferating cells. In the result it turned out that a passage using the **Cell**Celector is slightly better tolerated by the cells than the manual way.

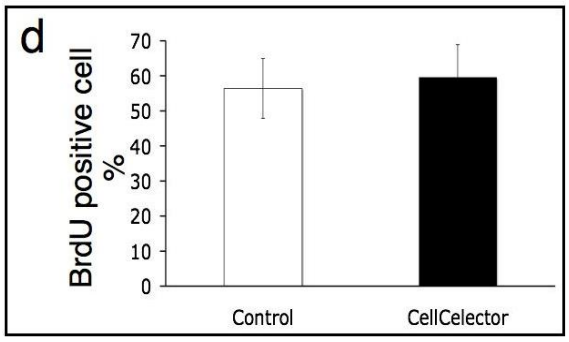


Fig. 20: BrdU positive cell counted after manual transfer of hESC and using the **Cell**Celector.

Conclusions

Efficiency

The **Cell**Celector is an efficient and highly selective tool for a safe transfer of single cells and cell colonies without interfering with important properties of the cells such as pluripotency and viability. Several experiments proofed that an automated process can improve the quality of transferred single cells or cell colonies and allow to set standardized condition for the entire cell picking process. Of special importance is the cell sorting capacity in the culture plate directly to target cell subpopulations before picking the cells.

Sensitivity

An assessment of pluripotency-associated markers and differentiating cells after automated picking and re-plating for several times confirmed their pluripotency status and a lower number of dead cells compared to picking by hand. Several parameters can be combined individually to apply the right and most gentle resolving aspiration force. By that and using the heatable destination positions (37°C) the mechanical stress is reduced and the viability of cells after transfer is increased. Highly precise tools allow for a safe transfer of even single cells provide new possibilities in stem cell research. With a reproducibly small amount of aspiration (below 0.1 µl) quantitative single cell RT-PCR and PCR analysis becomes a standard method.

Flexibility

The **Cell**Celector also enables the scientist to select cells precisely according to their state of differentiation using different fluorescence excitations and markers at the same time. Hence, the integration of a state-of-the-art microscope which is widely used in laboratories provides an innovative and time-saving combination of various analysis methods and a direct transfer into a new culture environment or wells for further genetic analysis (PCR). For working with primary cells and tissue the **Cell**Celector and the ALS Incubator **Flow**Box (Fig. 22) are recommended since physiological conditions like temperature and CO_2 atmosphere can easily and precisely be adjusted.

Security

When working with cells determined for transmission to patients contamination with pathogens is an important issue. The complete automation of the picking process decreases the need of manual intervention (dish positioning) and therefore increases the security of valuable cell material from contamination with retroviruses or other pathogens [2].

The **Cell**Celector is placed under a sterile hood and resistant against intense surface sterilization using Ethanol and UV-light.

Fig.21 The CellCelector can be placed in a flow box for increased safety of cell cultures

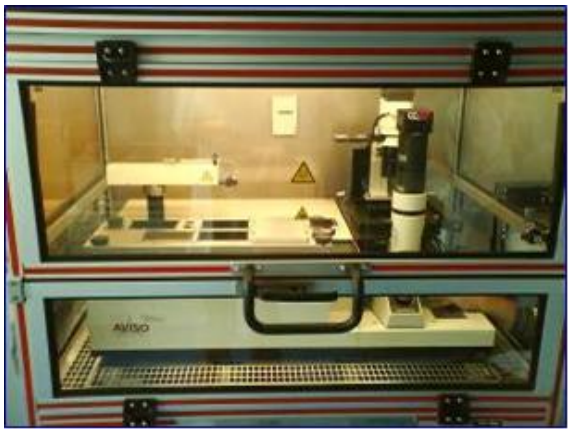


Fig. 22 The CellCelector is placed in the **ALS Incubator FlowBox** with high CO_2-atmosphere and heated environment (37°C), especially useful for long-term experiments and primary cell cultures.

Glossary

Propidium iodide (PI) – is impermeant to cell membranes and generally excluded from living cells. It can be used for identifying dead cells in a population and as a counterstain in multicolor fluorescent techniques. Propidium iodide is an intercalating agent and a fluorescent molecule that can be used to stain DNA. When excited by 488 nm of laser light, it can be detected with 562-588 nm band pass filter. It can be useful for differentiation of necrotic, apoptotic and normal cells.

BrdU – Bromodeoxyuridine is a synthetic nucleoside analogue to Thymidine and can be used for tagging of proliferating cells in viable tissues. BrdU can be taken up by the cell and gets integrated into the new DNA during S-phase instead of dTTP nucleotide. Antibodies can be used for immunochemical visualization of DNA containing integrated BrdU.

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References

[1] Shin Yong Moon, Sun Kyung Oh (2005)
'Methods for expansion of Human Embryonic Stem Cells',
Stem Cells, Vol. **23**, 605–609

[2] Schaefer U, Schneider A, (2008)
'"The Good into the Pot, the Bad into the Crop" – A New technology to Free Stem Cells from Feeder Cells',
PloS ONE, Vol. **3**, Issue 11

[3] Caron AW et. al.(2009)
'Fluorescent labeling in semi-solid medium for selection of mammalian cells secreting high-levels of recombinant proteins',
BMC Biotechnology, Vol. **9**:42

[4] Peterbauer T, Heitz J, Olbrich M, Hering S, (2006)
'Simple and versatile methods for the fabrication of arrays of live mammalian cells',
Lab Chip, Vol. **6**, 857–863

[5] Love C, Choi, J H, (2010)
"Development and optimization of a process for automated recovery of single cells identified by microengraving"
Biotechnology progress, Vol. 26, Issue 3, 888-895

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