

CellCelector™ Application Note

Automatic Transfer of Bacteria Colonies

Introduction

The **CellCelector™** 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 different targets for subsequently performed analysis. 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 an intuitive software.

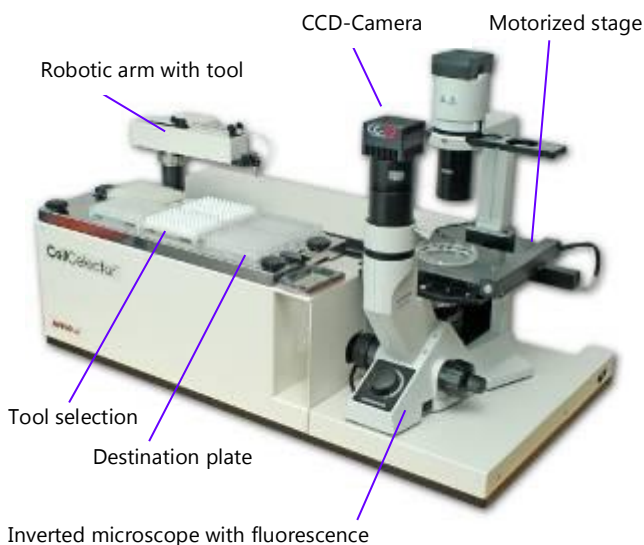


Fig.1 The ALS **CellCelector** - overview

Bacteria may serve as an object for producing organic and inorganic molecules like antibodies and ethanol, respectively, to mention just two. This note describes the picking of genetic engineered *Deinococcus* cells as a clone. Because of the dividing behaviour by binary fission in two planes and the production of high levels carotinoids the cells appear as red tetrads and form on solid medium smooth and convex colonies (Fig. 2).

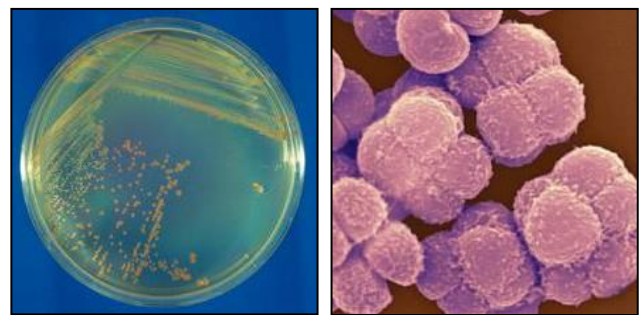


Fig.2 *Deinococcus* cultured on agar (left); microscopic view of *Deinococcus durans* (right)

Deinococcus is a bacteria genus having high resistance to numerous kind of stress (radiation, oxidation, heat, drought, cold, solvents, alkaline, acid, ethanol, buthanol etc.). Additionally to that this Bacterium has a short generation time (approximately 80 minutes) and the ability to utilize a vast range of sugars, amino and organic acids as a carbon source for catabolism which makes it interesting for industrial purposes. Moreover *Deinococcus* cells are able to integrate large fragments of DNA from bacteria, fungi and plants in a highly stable manner and so they are able to produce highly complex compounds like carotinoids, enzymes, antibiotics etc..

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. 3 A). The entire collection of single images during the scan is combined by the software into an overview image (Fig. 3 B). Integrated fluorescence filters can be very useful in terms of immunochemical labelling and staining and allow the user in-plate cell sorting before picking.

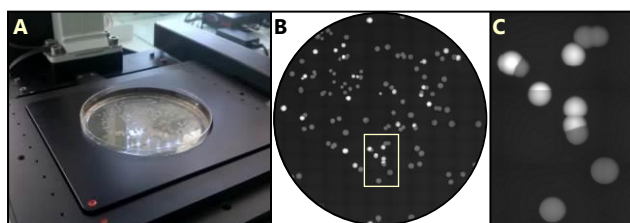


Fig. 3 Scanning process of a petri dish with bacteria colonies on the motorized stage (A); overview image after scanning (B); enlarged area in fluorescence illumination (C).

2. Selection & Targeting

According to grey value thresholds and selection parameters (size, diameter, shape factor, etc.) predefined by the user the software detects and selects the targeted cell colonies (Fig. 4 upper panel). Overlay views from images taken in different illumination methods can be created (Fig. 4 lower panel).

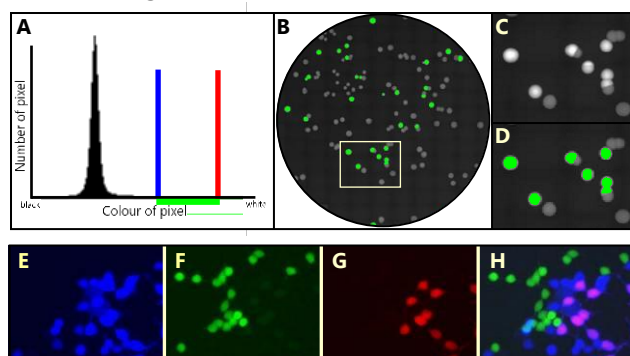


Fig. 4 Colony detection by defining grey value thresholds interactively: Graphical view of grey values (A) of the reference image (C). By moving the upper (blue bar) and lower (red bar) limit a range of grey values (green bar) can be defined and used for detection. Detected colonies on reference image (D); Detected Colonies at the total scanned area (overview image

B); Images (here of HEK cells) taken in fluorescence illumination to detect signals for the fluorescent proteins Cerulean (blue; [E]), Venus (green; [F]) and mCherry (red; [G]); Overlay view (H)

3. Automatic Harvest

The application-specific harvesting module (Fig.5) takes the bacteria colonies up and the robotic arm automatically transfers them to the destination plate according to a user-defined chart.

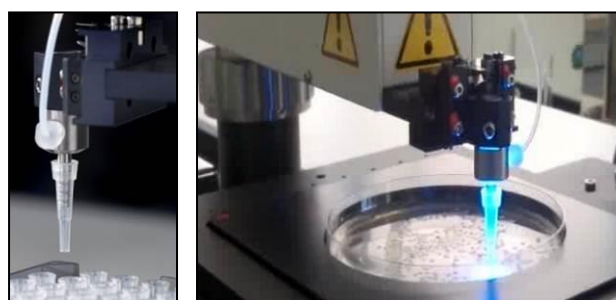


Fig. 5 Methylcellulose-tool - developed for picking cell colonies from semi liquid medium (e.g. Methylcellulose) and from solid media (e.g. Agar). Picking tool right after taken up a fresh tip from a rack (left); picking tool performing a picking process within a fluorescence illuminated agar plate with *Deinococcus* colonies (right)

4. Documentation

In order to keep the whole process reproducible and documented an overview scan image, the picking list with all alphanumeric data and an image of each colony before and after picking is stored in the database (Fig. 6). The images are labelled and can be assigned to the corresponding deposit position in the destination plate.

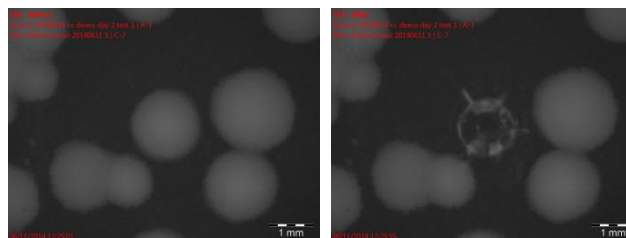


Fig. 6 Documentation of the isolation of a *Deinococcus* colony out of an agar plate: It is shown the region of a culture dish before (left) and after (right) the colony isolation process.

Software

The **CellSelector Software** (Fig. 7) is running with two monitors using the left monitor as user interface and the right monitor for displaying the microscopic images. It 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.

The selection of cell colonies to pick can be done either automatically by using a colony specific detection algorithm or individually by mouse click on-screen. Driving the microscopic motor stage allows the search within the culture dish for target cells and their marking by mouse click, either for instant picking or for collection of multiple targets into a picking list.

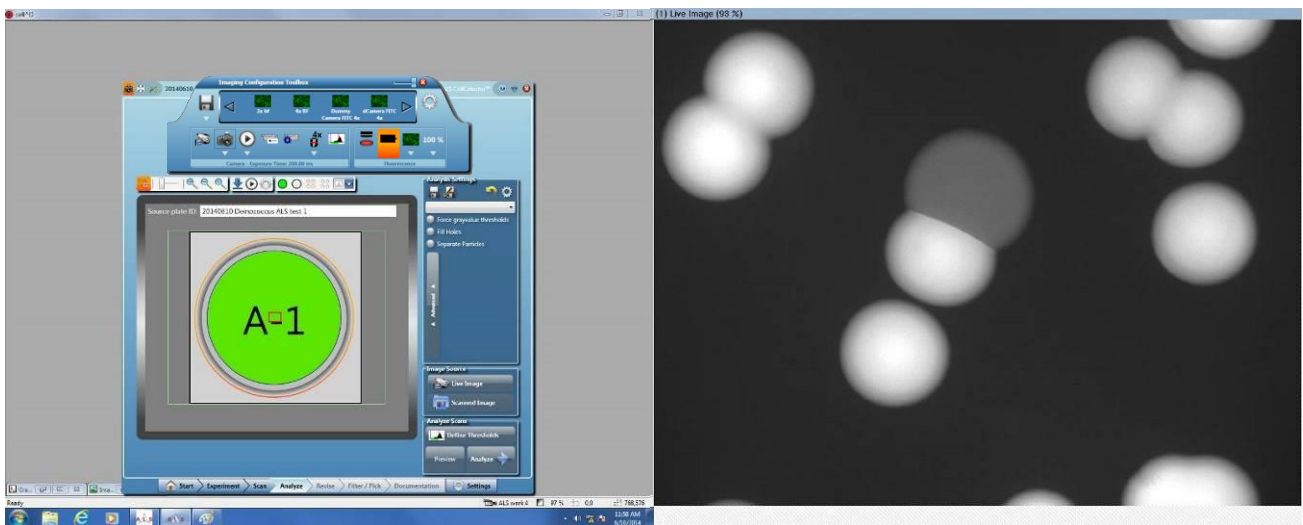


Fig. 7 Screenshot of the analysis part of the software – one of a number of tabs within the straight forward wizard (left) and microscopic image of fluorescence positive and negative bacteria colonies (right).

The *measurement bar* provides several tools for measurement of cells, colonies or structures on-screen like diameter, area, distance to adjacent colonies etc. (Fig. 8). It is helpful for identification of targets as well as for the right choice of the picking tool diameter.

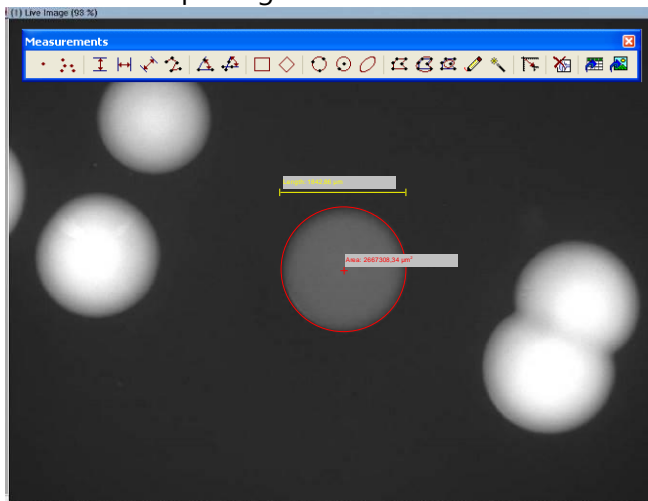


Fig. 8 Using features of the measurement bar the colony can be measured – here for diameter and area

The *imaging configuration toolbox* (Fig. 9) contains all parameter (exposure time, shading correction image, light intensity, selected fluorescence filter set, magnification etc.) to optimize the microscopic view on the sample. Light source and magnification specific settings can be saved within individual camera channels enabling both the fast switching between different camera channel as well as their automatic usage.

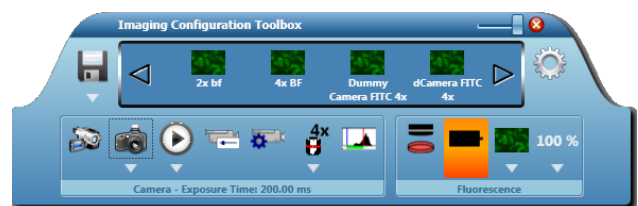


Fig. 9 With the Imaging Configuration Toolbox: all settings for an optimal view on the sample can be selected and optimized (lower part) and saved within individual camera channels (upper part).

The *well navigator* (Fig. 10) 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 motorized stage is connected to the navigator and will be driven to the selected point of interest. Special formats of plates and dishes can be easily configured by the user and saved for later use (Fig.10).

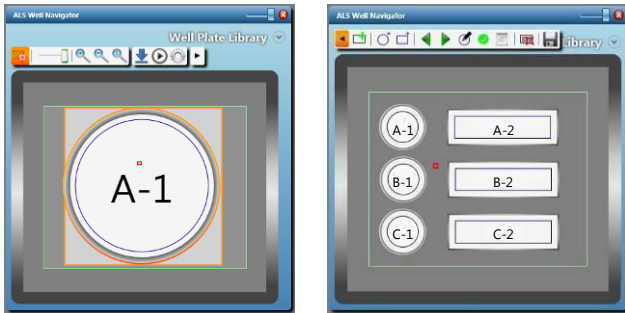


Fig. 10 The well navigator can be used for loading standard plate formats (left) as well as for the creation of user defined scan areas (right)

The special *Revise Mode* (Fig. 11) enables the user to modify the list of detected colonies after automatical scan either by adding or deleting single positions manually or by filtering and ranking the scanning result automatically.

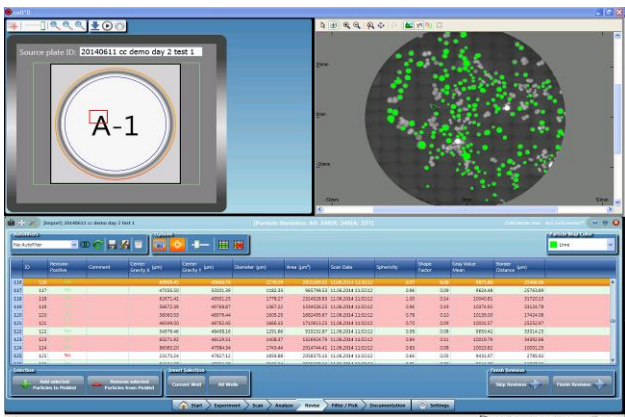


Fig. 11 Revise Mode with plate model and marked actual position (top left), overview image with all automatically detected green labelled colonies (top right) and the revise list with numbers of functions to modify it (down).

As special feature within the software is the possibility to switch between the "Live Image Mode" and the "Scanned Image Mode". The latter enables the user to see the scanned area from real image size to total view in different

views. In case of multiple overview scans the actual selected part of the scanned area can be switched between the different illumination methods (e.g. DAPI, FITC and PI) without having the samples illuminated. A preview mode uses the scanned image mode to highlight these colonies which will be detected by using the actual selected detection algorithm.

Readjusting the position in case of colony movement

Due to the semi liquid nature of less concentrated agar or using other semi solid 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. 12).

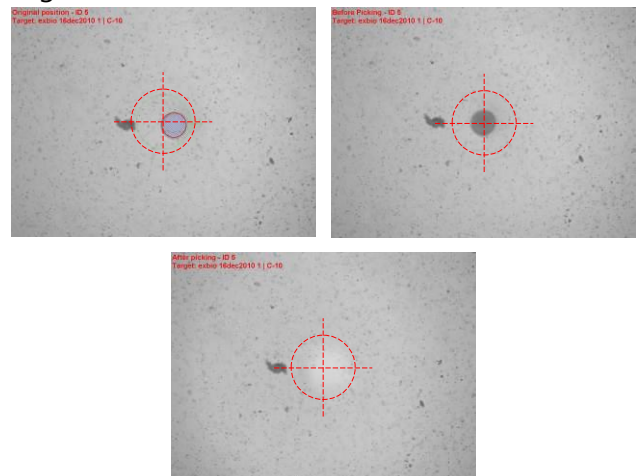


Fig. 12 Readjusting the position (here as example) of a Hybridoma colony: Colony moved slightly from their original position at the centre of the image (A); Hybridoma colony after readjusting into the centre of the image by driving the motorized stage (B); Image after successful picking of the re-centred Hybridoma colony.

Precise isolation of *Deinococcus* colonies out of agar plates

PetriDishes (100mm) with cultures of *Deinococcus* cells were scanned in fluorescence illumination using a 2x objective. Detected colonies were filtered by the parameter for size, shape and next nearest neighbour distance and ranked by the fluorescence intensity. The colonies were picked one by one meaning that for each colony the CellCelector used a fresh picking tip. The wells of the 96well destination plate were pre-filled with 100µl medium. Before each picking process the CellCelector was taken up from the destination well 10µl medium which was used after picking to rinse the tip. Then the robot arm moved into the plate and aspirated with 0,5 µl cells from the colony. During the aspiration process the robot arm moved the tip for 100 µm upwards to compensate height differences within the plate and so to make sure that the tip everywhere in the plate touches the colony to be able to aspirate cells. After picking the aspirated cells were transferred into the destination plate where the tip was rinsed with medium for two times with 8 µl. Figure 13 shows the images for five picked colonies before and after the picking process, the picked cells within the destination well and the position before the pick within the overview scanned image.

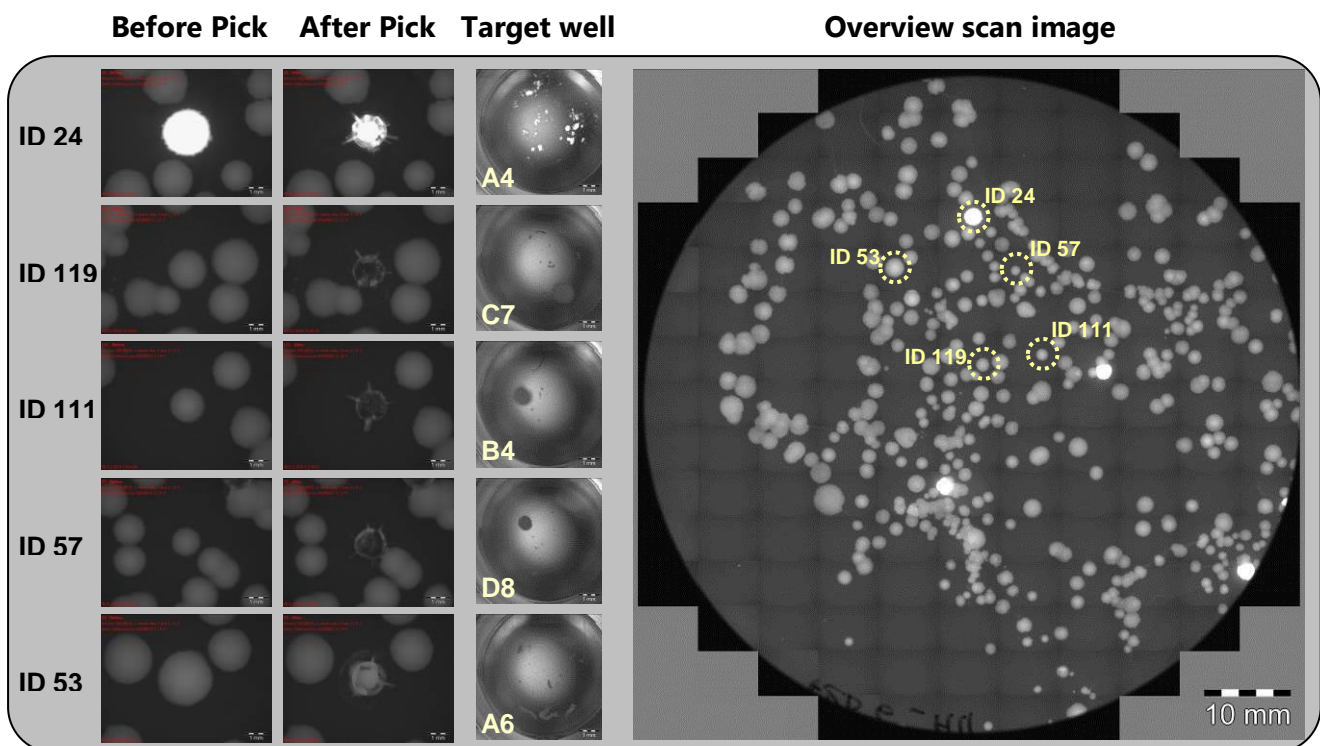


Fig. 13 Summary of a picking experiment of *Deinococcus* colonies from agar plates: As example there are shown the images of five colonies (with the ID of 24, 119, 111, 57 and 53) before and after picking, the colonies within the destination well and the position of the colonies in the source plate. The before and after pick images as well as the overview image of the entire PetriDish were taken and saved automatically by the CellCelector system.

Conclusions

Efficiency

The **CellCelector** is an efficient and highly selective tool for a safe transfer of single cells and cell colonies which allows 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

Several parameters can be combined individually to apply the right and most gentle aspiration force to reduce mechanical stress and increase the viability of cells after transfer. Picking series with sensitive pluripotent stem cell colonies revealed after automated picking and re-plating for several times a high surviving rate as well as their maintained pluripotency status (Haupt, S.; nature methods; June 2009; p iii-iv).

Flexibility

The **CellCelector** also enables the scientist to select cell colonies precisely according to their pattern of expressed proteins using different fluorescence excitations and markers at the same time. For working with bacteria growing within a special atmospherical environment the **CellCelector** and the ALS Incubator **FlowBox** (Fig. 15) are recommended since physiological conditions like temperature, humidity and the gas phase (e.g. CO₂) can be easily and precisely adjusted.

Security

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. The **CellCelector** is placed under a sterile hood (Fig 14 and 15) and is resistant against intense surface sterilization using Ethanol and UV-light.



Fig.14 The CellCelector can be placed in a flow box for increased safety of cell cultures



Fig. 15 The CellCelector is placed in the **ALS Incubator FlowBox** with high CO₂-atmosphere and heated environment (37°C), especially useful for long-term experiments and primary cell cultures.

Acknowledgement

Experiments were carried out in the labs of the Deinove company, Montpellier, France. We like to thanks all responsible persons especially to Raphaël Calbrix for using his cell samples and the possibility to use his data for that note.



ALS Automated Lab Solutions headquarters in Jena, Germany

ALS Automated Lab Solutions GmbH is located in Jena, a dynamic city famous for microscopy and material science. ALS is a specialist for the development of innovative technological solutions for cell biology research and molecular biology. We lift cell culture to a new level of choice and control on the leading edge in cell biology, cell therapy research, regenerative medicine and drug discovery. With automation and standardization of laborious manual procedures, ALS supports science and research for more efficiency and the creation of new methods for the science of tomorrow.

ALS Automated Lab Solutions is partner of:



Please do not hesitate to contact us for further information:

Jens Eberhardt

ALS Automated Lab Solutions GmbH
Otto-Eppenstein-Str. 30
07745 Jena
Germany

Phone: +49 (0) 3641 4820-0
Fax: +49 (0) 3641 4820-11
E-Mail: info@als-jena.com