DEPArray™ Technology

Sorting and Recovery of Rare Cells

silicon
biosystems
THE LIVING-CELL COMPANY®

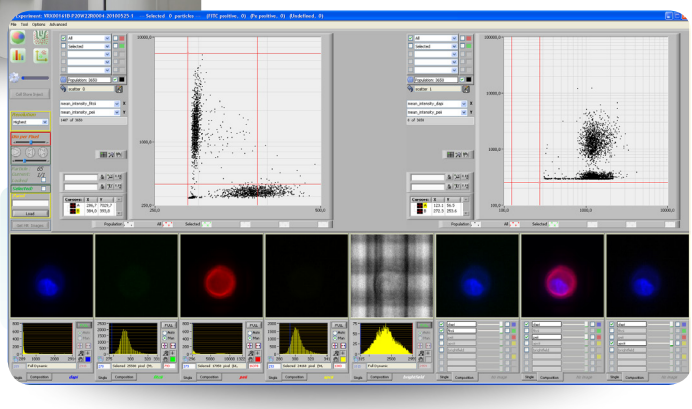
Delivering pure, single, viable cells

The DEPArray™ system from Silicon Biosystems is the only automated instrument that can identify, quantify, and recover pure individual rare cells, or cell groups, for culture or molecular analysis.

The DEPArray™ instrument for imaged-based cell selection and collection.

The DEPArray™ instrument

combines high quality, image-based selection of individual cells with automated cell routing and recovery technology. A six-channel fluorescent microscope and CCD camera are used to capture images and identify the fluorescently labeled cells of interest in the sample. The CellBrowser™ software allows multiple parameters from fluorescence and bright field images to be used for cell selection. Only DEPArray™ technology can provide single cell sorting resolution while delivering the cell purity needed for stringent downstream applications.



The system optics provide 10x and 20x magnification with 0.64 and 0.32 micron/pixel resolution, respectively.

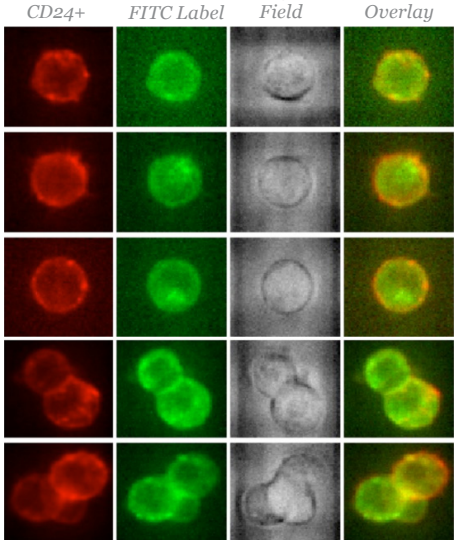
Single-use microfluidic cartridges enable individual cells to be separated and collected.

The DEPArray™ sample cartridge

controls the manipulation and collection of cells. Each microfluidic cartridge is a single-use consumable that channels the sample into a chamber with an array of individually controllable electrodes that create electronic "cages" around the cells. After imaging, cages with cells of interest are electronically programmed to move to a recovery area where the cells can be dispensed to slides, collection tubes, or culture plates.

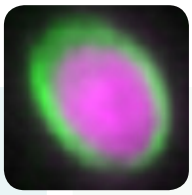
Pure

Image-based selection ensures that cells identified by multiparametric sorting are intact, individual cells that meet the selection criteria. The visual inspection of cells, trapped in the electronic cages, allows elimination of non-specific fluorescent "events" that might be false positives, cell fragments, or clusters of singly-labeled cells that meet the selection criteria only as a group. This ensures that only the target cells of interest are collected in a separate, clean buffer solution, eliminating impurities and contaminants that could otherwise confound downstream analyses.



Imaged-based inspection provides the ability to differentiate single intact cells from cell clusters co-labeled with fluorescent markers

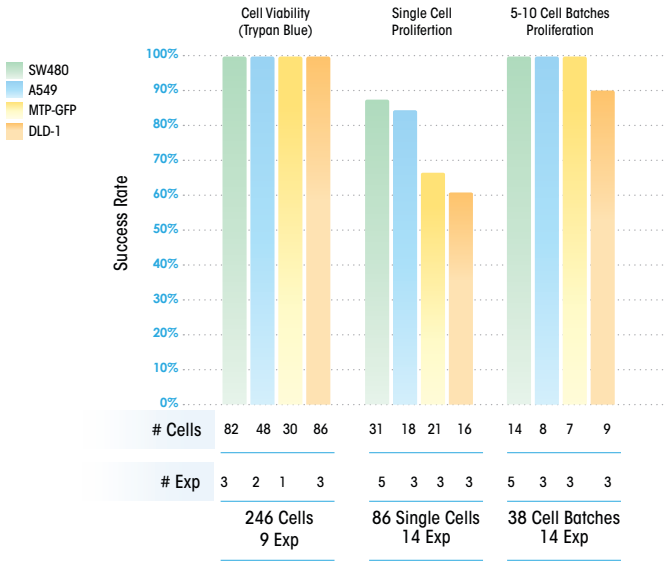
Single



The DEPArray™ system provides automated isolation and collection of individual cells. This unique feature enables molecular analysis of specific cells and cell subpopulations, which is critical to understanding the biological significance of rare cell subtypes within biological samples. By ensuring recovery of individual cells of interest, DEPArray™ technology allows genomic and expression analysis down to the single cell level.

Viable

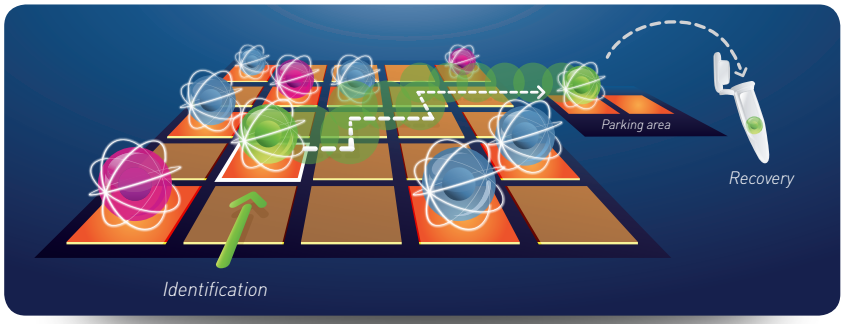
Dielectrophoretic (DEP) cages are formed by the differential application of a very low voltage at megahertz frequencies. Capture and movement of cells in DEP cages is gentle; cells are not subject to shear force, strain, or potential damage from cell-cell adhesion. Cells do not need to be permeabilized or fixed, so even live cells in culture media can be analyzed and isolated. Cells can be recovered from the DEPArray™ cartridge directly to cell culture plates.



Various cancer cell lines sorted using DEPArray™ technology showed 100% viability (n=246 cells). Cloning success rate on 86 single cells across 14 experiments ranged between 60% and 87% depending on cell type. All recoveries of 5 or 10 cell batches show proliferation success rate of 100%, except for DLD-1 cell line (89%).

How DEPArray™ technology works

The DEPArray™ system takes advantage of the dielectrophoretic force that is exerted on neutral particles, such as cells, when non-uniform electric fields are present. DEP "cages" are formed on the cartridge when the electric fields created above some electrodes are in "counter" phase with the electric fields of surrounding electrodes. Cells are trapped within these DEP cages. When a DEP cage is moved by a change in the electrode pattern, the trapped cell moves with it.



The electrodes are individually programable so only DEP cages with cells of interest are moved; others remain in suspension

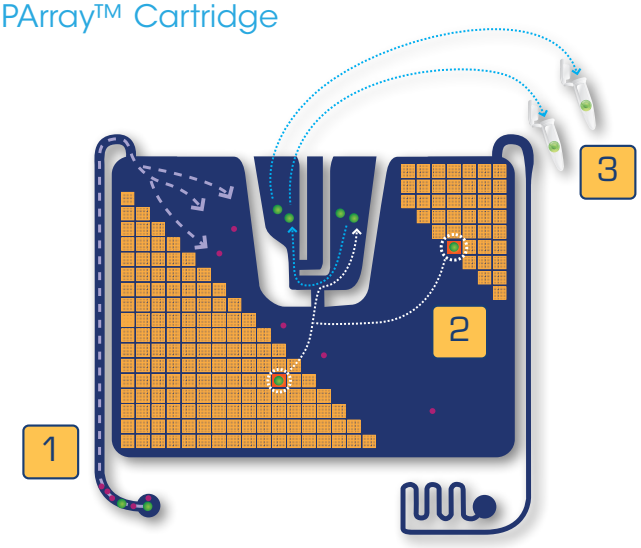
The electrodes in the main chamber of the cartridge form ~40,000 DEP cages. Thus, cell suspensions containing tens of thousands of cells, typical of enriched samples, will result in individual cells being isolated in separate DEP cages.

Selecting and collecting cells

A suspension of labeled cells is pipetted into the DEPArray™ cartridge and allowed to randomly distribute in the main chamber. The array of electrodes is activated to form DEP cages, causing the cells to be trapped. The main chamber is scanned in each of the fluorescent channels to identify cells meeting the selection criteria. DEP cages are then programmed to move the selected cells to a "parking" area of the cartridge.

The "parked" cells are held in a separate, clean buffer solution to eliminate carry-over of impurities from the original sample matrix. Individual cells or groups of cells can then be selected for collection and moved to a recovery area, where they are dispensed to the collection vessel of choice.

The DEPArray™ Cartridge



1

Inject, trap and image cells

2

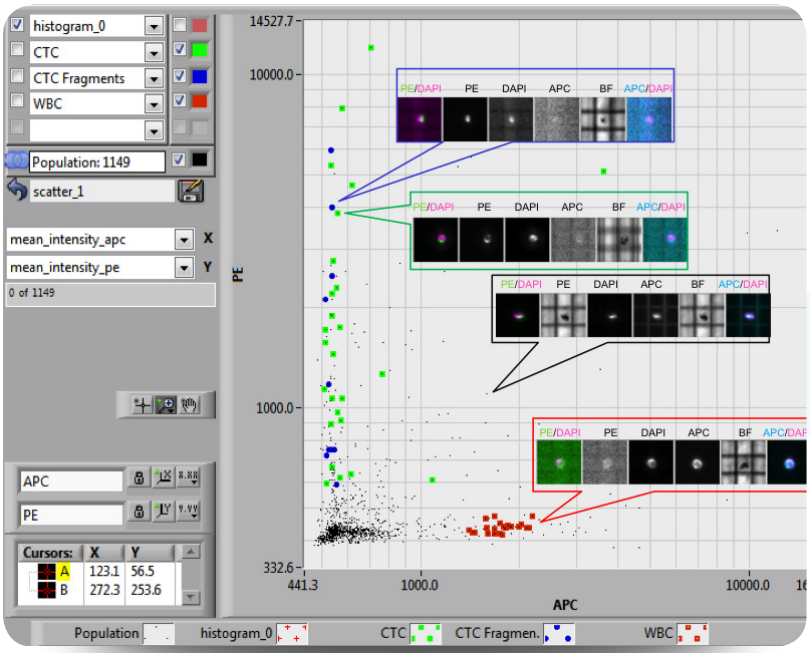
Move cells of interest into parking chamber

3

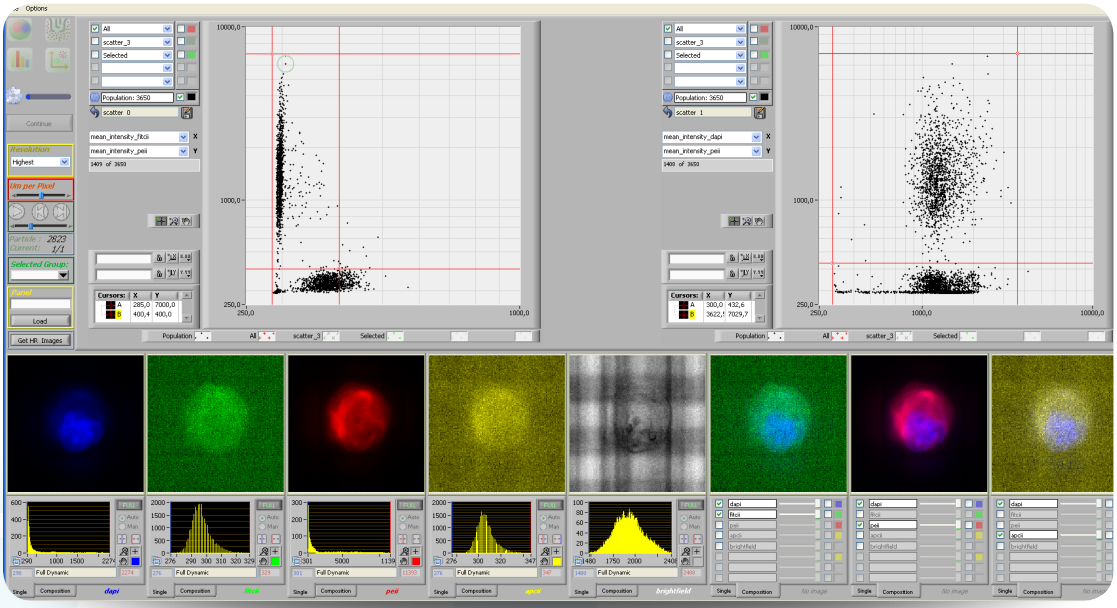
Move individual or multiple cells into recovery chamber and flush

CellBrowser™ Software

The DEPArray™ system's CellBrowser™ software is a powerful tool enabling the review and selection of multiple cell populations from the sample based on multi-parametric fluorescence and bright field criteria. For each channel scanned, multiple values, such as peak and mean fluorescence intensity and background, are collected and can be graphically represented by scatter plots and histograms. Images of individual events can be viewed on the image bar, allowing specific cells of interest to be easily identified and confirmed. Cell perimeter, diameter, and circularity measures can be viewed with the bright field channel ensuring recovery of whole intact cells exhibiting the desired fluorescence patterns.



Subpopulations of cells and cell fragments can be identified and color-coded. Here, green dots are CTCs while blue dots appear to be CTC fragments. Black dots are cell debris and red squares are white blood cells. The cell images shown here have been captured from the image bar and overlain on the scatter plot for illustrative purposes.

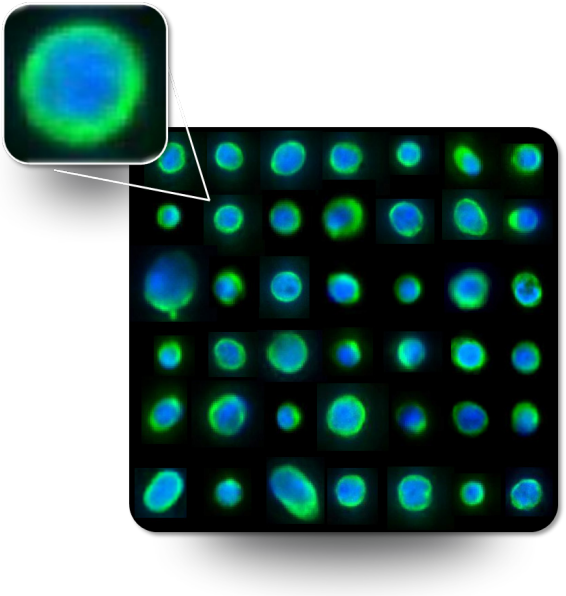


Scatter plots provide an easy way to visualize the distribution of cellular fluorescence across different channels. X-axis and Y-axis criteria are user selectable from drop down lists. Upper and lower gating thresholds can be adjusted by sliding the gating bar on the graph. Individual events can be viewed on the image bar by hovering the mouse over a spot on the scatter plot. Color selection and overlays are completely user selectable.

Flexibility of Sample Type

Researching biological processes over time, such as mechanisms of disease progression and development of drug resistance, often requires characterization and comparison of cells from different types of samples. The DEPArray™ system is designed to recover pure cells from a wide range of rare cell suspensions:

- Live cells
- Fixed cells, e.g. cells in 2% PFA
- Samples with small cell loads, e.g. fine needle aspirates
- Whole blood enriched by CellSearch® or other immunomagnetic and filter-based systems
- Cells labeled with intracellular or extracellular fluorescent probes

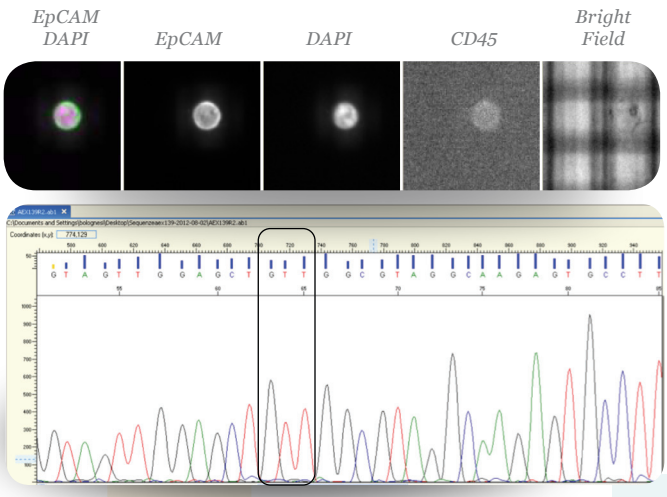


Flexibility in Downstream Analysis

Heterogeneity in biological samples can confound downstream analysis by masking important molecular characteristics present in a small subpopulation of rare cells. Similarly, contaminants, cell fragments, and false positives can obscure results and waste resources. The DEPArray™ platform enables isolation of intact, pure cells that are suitable for even the most challenging single cell downstream applications:

- Whole genome amplification*
- Array CGH
- Whole genome sequencing
- Next generation sequencing
- Mutation and CNV analysis
- Expression analysis

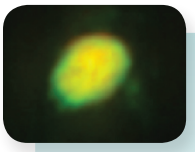
**For whole genome amplification optimized for single cells, Silicon Biosystems offers the Ampli1™ WGA kit.*



Isolation of individual cells enables recovery of pure subpopulations of specific cell subtypes and ensures robust, reproducible results from downstream analyses. Here we show a single CTC and the detection of the KRAS mutation on codon 12. No wild type allele was detected, clearly showing that only the single pure cell was sequenced.

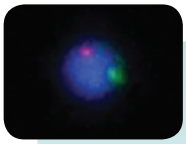
Enabling rare cell analysis for life sciences applications

DEPArray™ technology can be used for isolation and recovery of any rare cell type that can be identified by combining positive and negative selection of fluorescent markers and morphological features. The system is ideally suited for any application that would benefit from analysis of individual cells or homogeneous cell populations that are free of contaminants, nonspecific fluorescent particles, and admixtures of cells.



Oncology Research

- Analyze individual circulating tumor cells (CTC)
- Investigate the epithelial-mesenchymal transition (EMT)
- Analyze tumor clonal subpopulations



Fetal Cell Biology

- Isolate fetal trophoblasts and nucleated erythrocytes from maternal blood
- Discover and validate fetal cell biomarkers

Stem Cell Research

- Recover primary cells from tissues for ex-vivo cell culture and expression analysis

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DEPArray™ Rare Cell Isolation Technology

- Pure, Single, Viable Cells
- Multiparametric Image-Based Selection
- Automated Sorting and Recovery

CORPORATE

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