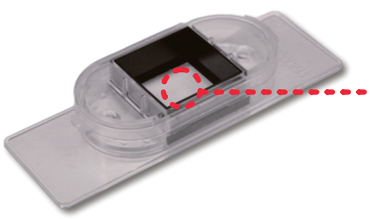
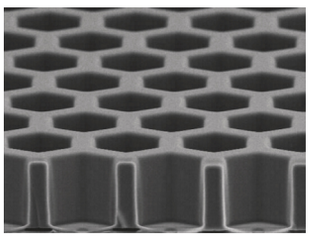
SIEVEWELL

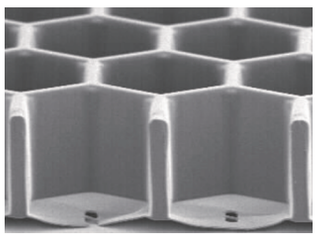
High Density Cell Arraying Device

- **Membrane with nanowells for single cell trapping**
- **Generate high density cell array**
- **Single cell culture, staining, imaging and assay**

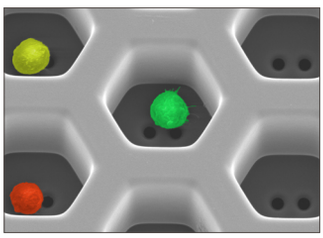




20 µm nanowell



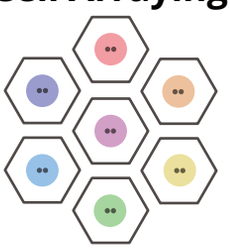
50 µm nanowell

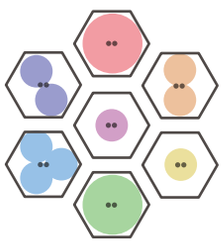


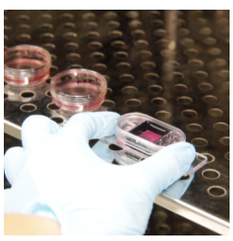
20 µm nanowell, MCF-7

The SIEVEWELL single cell arraying platform brings new insights to your research.

Cell Arraying



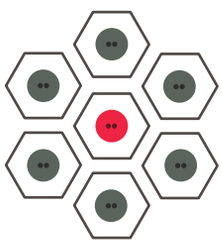


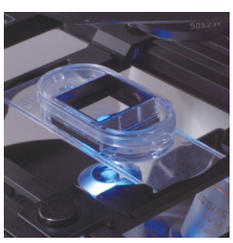


Cell Culture

Suspension cell culture

Spheroids generation

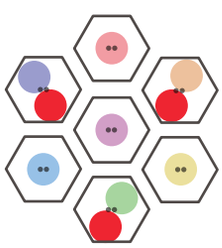


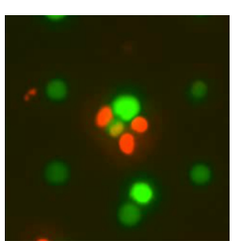


Imaging

On-chip staining

Rare cell detection





Assay

Cell-to-cell interaction

Immune Cell Killing Assay

Mechanism of Single Cell Capture

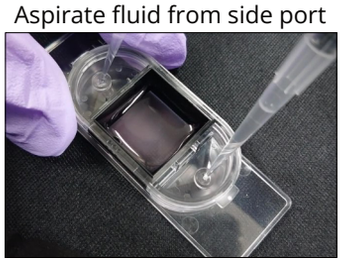
Arraying cells is an important step for single cell analysis. For example, cell overlap is unfavourable for single cell imaging and isolation. Microcavity arrays are used to capture single cells. In general, the distribution of cells in a cavity is by sedimentation and the single cell rate follows a Poisson distribution. Its single cell capture rate is relatively low, especially when loading cells in higher quantities.

SIEVEWELL is designed to capture cells at a high single cell rate. Two pores are positioned at the bottom of the nanowell. After loading the cell suspension, a directional liquid flow can be created from the inner liquid chamber to the side ports by aspirating liquid from the side ports with a standard pipette. The cells follow the liquid flow and are captured in the nanowell. When a cell enters a nanowell, it blocks the pores, reducing the flow of liquid through that nanowell. Other cells are then redirected to other empty nanowells. This mechanism allows a higher single cell rate than a Poisson distribution.

Easy to use. Fluidic system or instrument is not required.

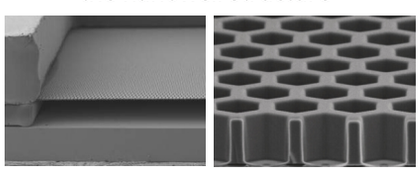
Add fluid to center chamber

Aspirate fluid from side port

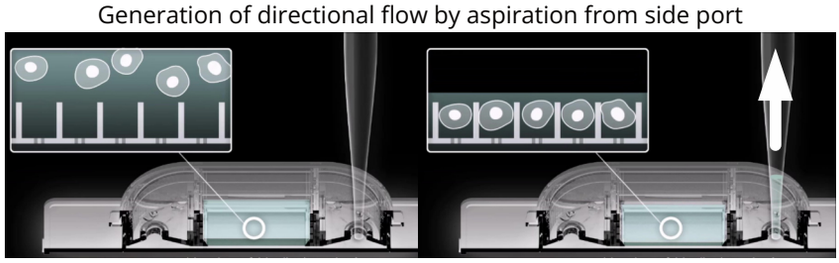


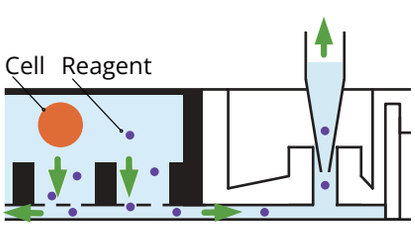
Compatible with 8 channel pipette

Micro-gap situated under the nanowell structure

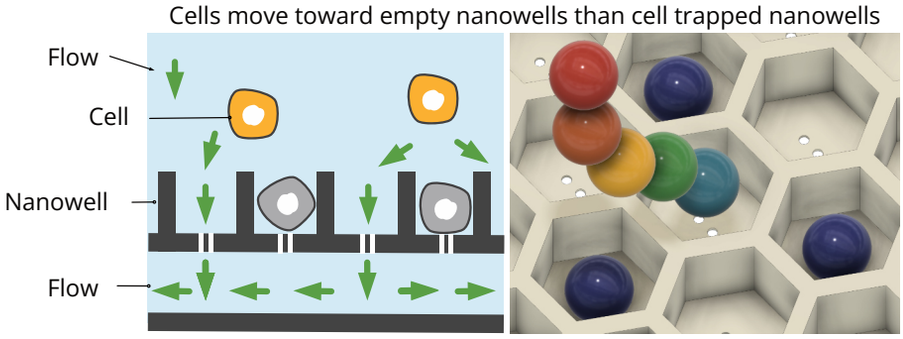


Generation of directional flow by aspiration from side port

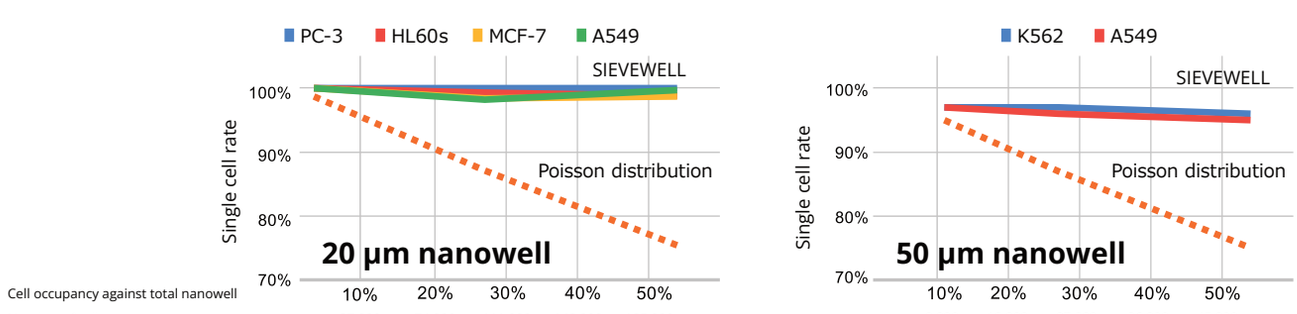




Cells move toward empty nanowells than cell trapped nanowells



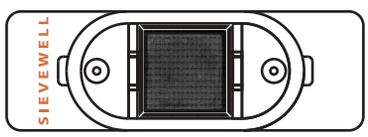
Single Cell Capture Rate



Arraying Cells at High-Density

Seeding cells at low density is one option to avoid cell overlapping for single cell imaging. This requires not only more glass slide, microplate and reagents but also a time for taking images and analyzing to detect cell of interest.

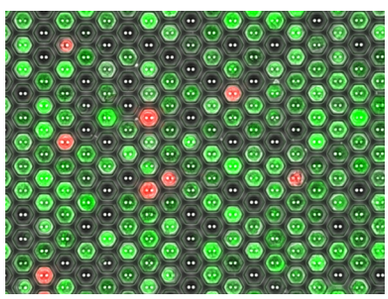
SIEVEWELL has nanowells at high-density in 17 x 17 mm (1/3 of glass slide). This minimizes required number of glass slide or microplate, resulting in reducing the required time for taking images.

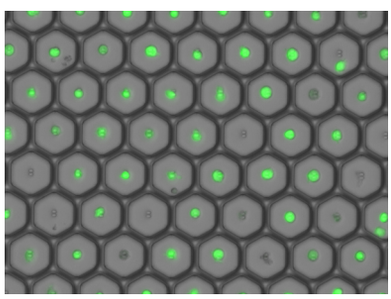


Number of Nanowells

370,000(**20 µm**)

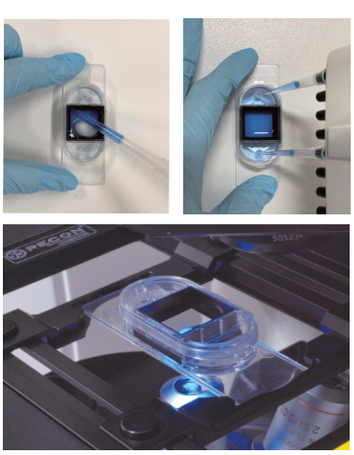
90,000(**50 µm**)

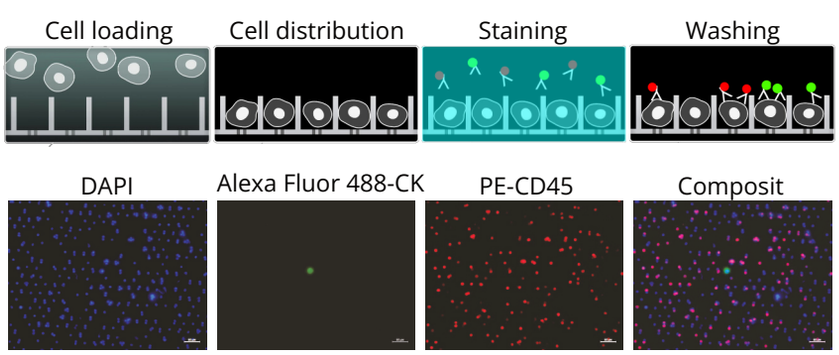

20 µm nanowell


50 µm nanowell

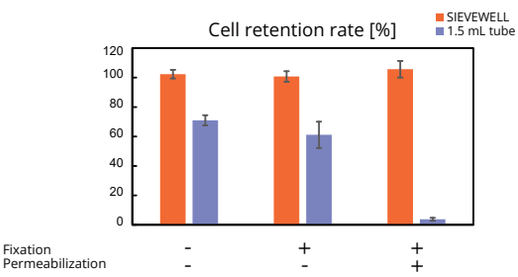
On-Chip Staining

Cell staining is standard procedure to visualize cells and cellular components under microscope. Conventional methods require multiple transfer or wash steps during staining that risk causing loss of the rare cell population of interest, e.g. circulating tumor cells. The pore size of the SIEVEWELL is smaller than typical mammalian cells. Thanks to this design, cell loss can be minimized during on-chip staining in the same way, e.g., fixation, permeabilization, blocking, incubation and washing.





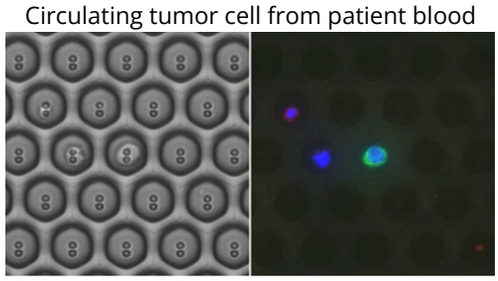
Human PBMC spiked with MCF7 cell were loaded. Cells were fixed with 4% PFA, permeabilized with 0.2 % Triton X-100, blocked with Protein Block, stained with a cocktail of PE mouse anti-human CD45 antibody, Alexa Fluor 488 mouse anti-human CK antibody and DAPI, then washed.

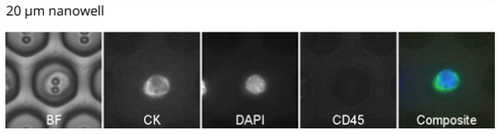


Direct comparison of staining procedure.
A549 cells were stained using 1.5 mL tube or SIEVEWELL 20 µm.

Case 1: no fixation, no permeabilization
Case 2: fixation with 4 % PFA, no permeabilization
Case 3: fixation with 4 % PFA, permeabilization with 0.2 % Triton X-100

Circulating tumor cell from patient blood


20 µm nanowell



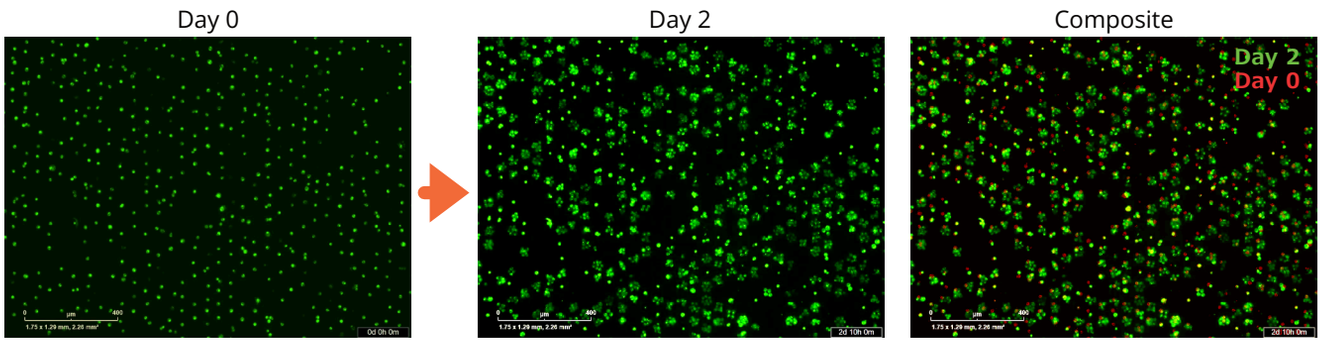
Data provided by Professor Hans Neubauer
Department of Obstetrics and Gynaecology, University Hospital and Medical Faculty of the Heinrich Heine University Düsseldorf, Düsseldorf, Germany

Monitoring of Cell Growth from Single Cells

Suspension Cell Culture from Single Cells

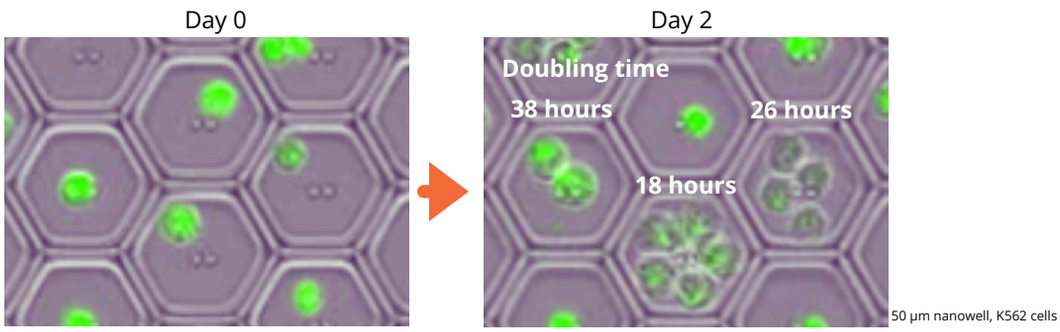
For cell growth monitoring from single cell, limiting dilution or sorting with cell sorter is well known method to generate single cell state in microwell plate. However, many microplates and medium is required to analyze single cell growth, and it is laborious to check if each well contains single cell or not. Also it takes time for imaging of many microplates. Suspension cells are floating in the culture medium and roaming freely, so it is difficult to track growth from single cell.

SIEVEWELL, high density cell arraying device, is suitable for growth monitoring from single cell. Suspension cells are captured in each nanowell during proliferation.

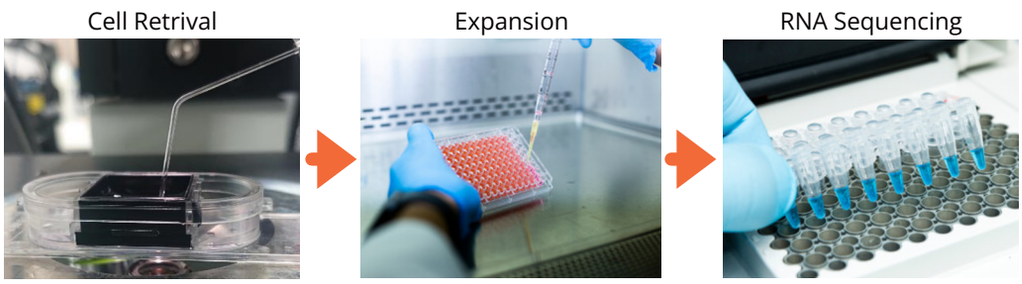


K562 cells (stained with CellBrite Green) were cultured in SIEVEWELL® Slide 50 µm. Images were taken every 2 hours with IncuCyte S3 (10x objective lens). Images of day 0 and day 2 were ovelayed using ImageJ.

Monitoring of cell growth from a single cell clarify that even cell line is a mixture of different growth rate of cells. K562 cells are a mixture of cells with different growth rates, 18 hours, 26 hours and 38 hours. Cells in the nanowell were retrieved using micromanipulator and transferred into 96 well microwell plates for further expansion, and RNA sequencing was conducted.

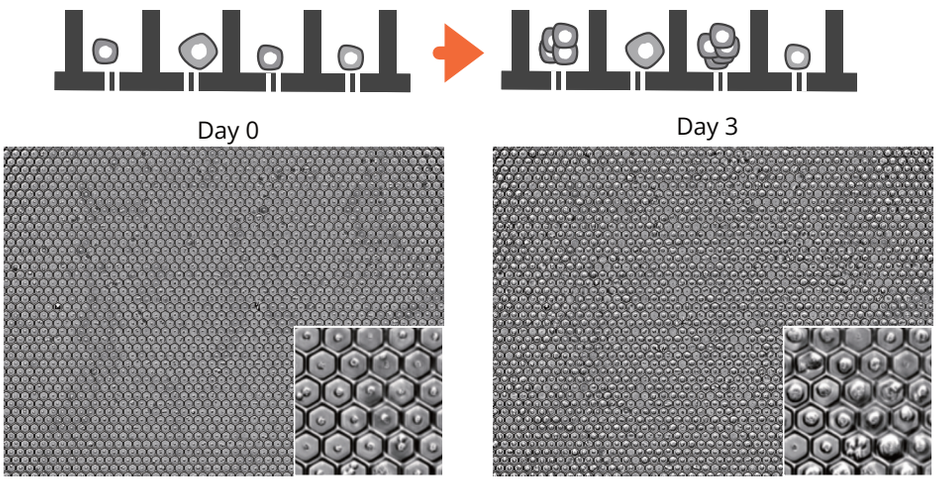


50 µm nanowell, K562 cells



Spheroid Generation from Single Cells

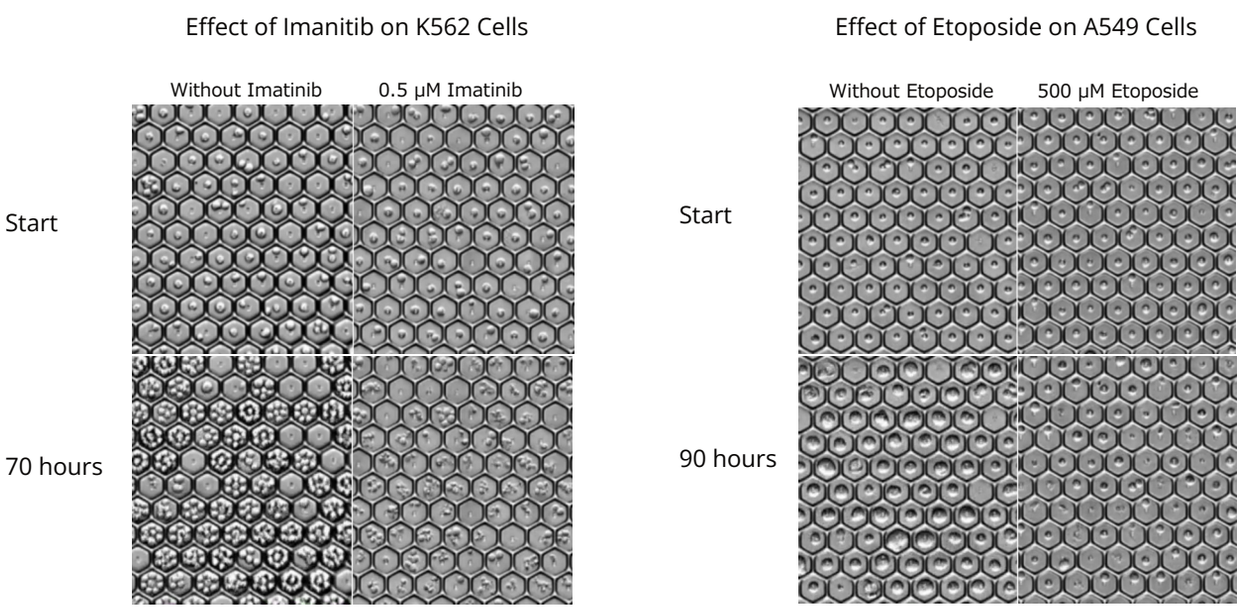
The ability of a single cell to form a spheroid is considered a potential self-renewal ability. SIEVEWELL 50 µm makes it possible to monitor the formation of spheroids from a single cell. Over 50,000 spheroids could be generated on a single chip.



A549 cells were cultured in SIEVEWELL Slide 50 µm. Images were taken every 1 hour with CM30 Incubation Monitoring System, Evident.

Monitoring of Drug Response

Culture in SIEVEWELL allows the behaviour of cells exposed to drugs to be monitored from individual cells. High-density cell arrays could capture many individual cell images simultaneously. For example, over 1,000 cells could be tracked in a single image. This will be useful to identify rare drug-resistant cells and provide clues to elucidate drug resistance mechanisms and develop better drugs.



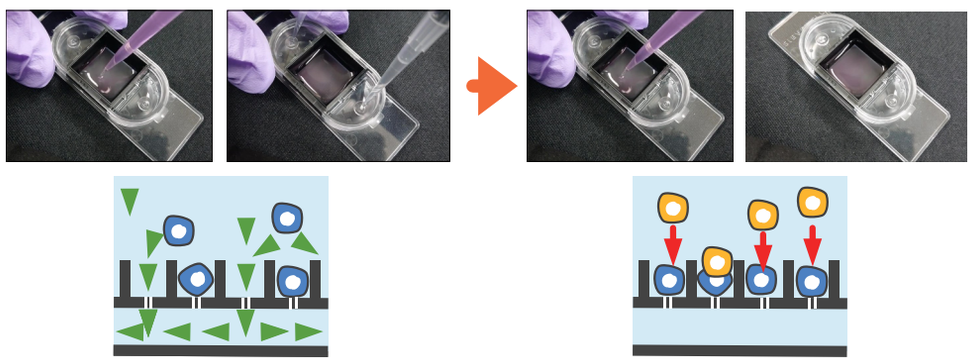
Cells were cultured in SIEVEWELL Slide 50 µm. Images were taken every 1 hour with CM30 Incubation Monitoring System, Evident.

Cell-to-Cell Interaction Assay

SIEVEWELL 50 µm is suitable for capturing pairs of two different cells to study cell-to-cell interactions. Combination of flow based capture and gravitational sedimentation enables to create a large amount of 1:1 cell-to-cell pairs in a single device.

1st Cell Type Loading
Flow based procedure

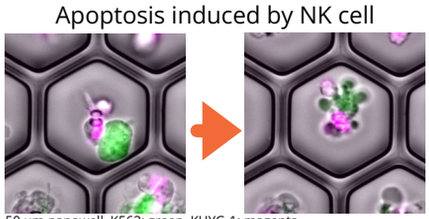
2nd Cell Type Loading
Sedimentation based procedure



Immune Cell Killing Assay

Cytotoxic immune cells can recognize and kill target cancer cells. Immune cell killing assays are a valuable tool for immuno-oncology research projects for in vitro assessment of these cells. With SIEVEWELL, the dynamic interactions of immune and cancer cells can be visualized.

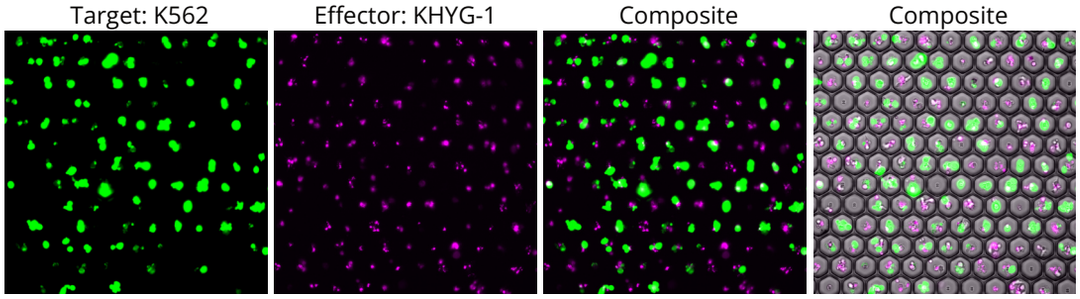
Apoptosis induced by NK cell



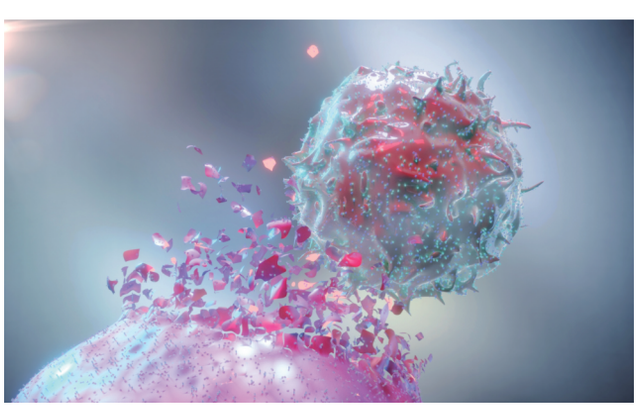
50 µm nanowell, K562; green, KHYG-1; magenta

The erythroleukemia K562 cell is known as a NK-cell sensitive target. Calcein AM-stained K562 cells were loaded into SIEVEWELL 50 µm, then KHYG-1 cells, NK leukemia cell line, were loaded by sedimentation. Time-lapse images were taken every 3 minutes.

Target: K562 | Effector: KHYG-1 | Composite | Composite

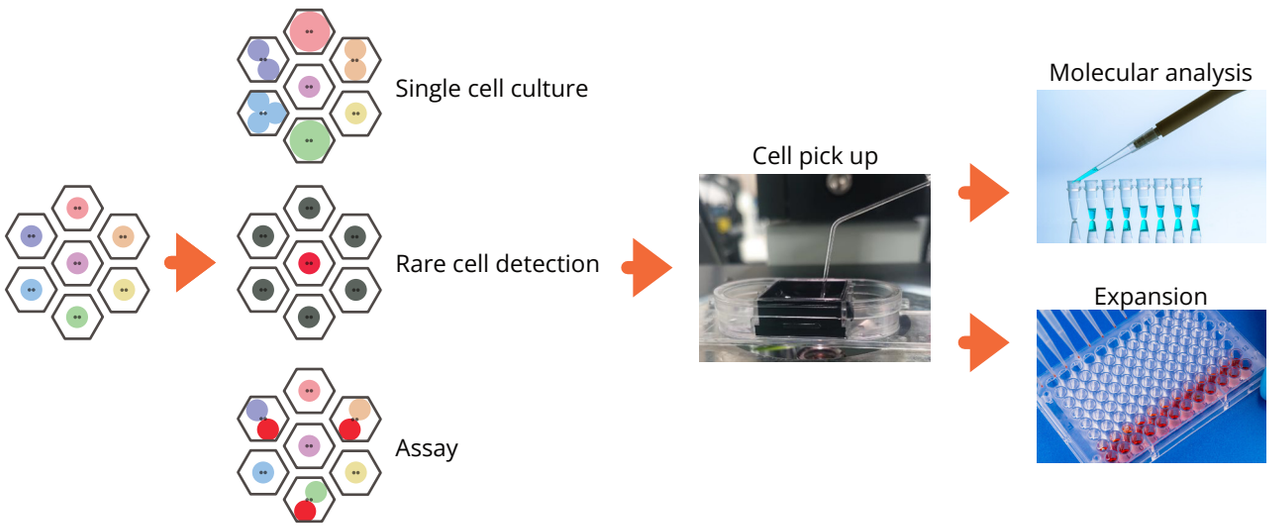


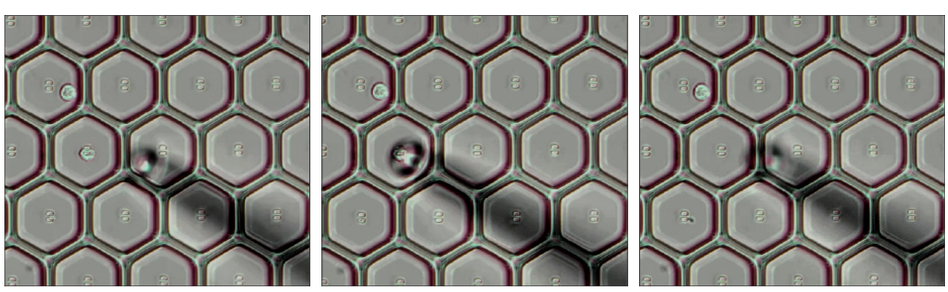
Killing assay video



Support for Cell Isolation

SIEVEWELL is top-open chamber for accessing glass capillary from top side. SIEVEWELL and glass capillary based cell pick up tool is an ideal combination for cell isolation, not only for single cell cloning, but also for picking up spheroids generated from single cells and cells of interest after assay.

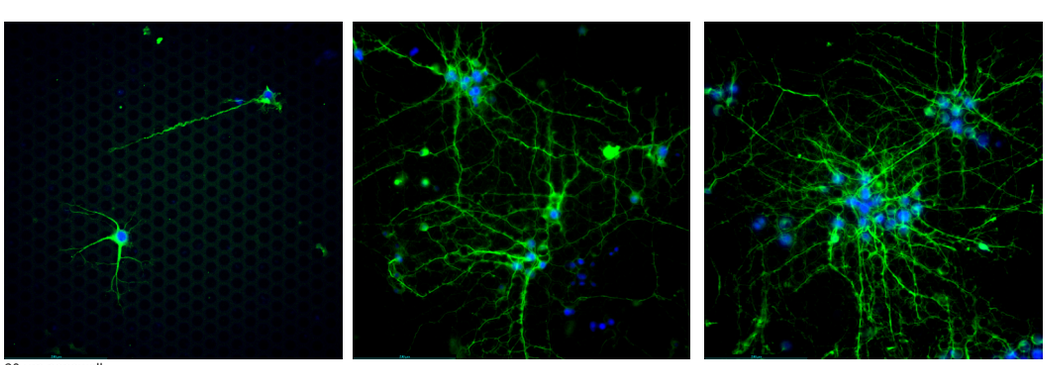




Examples of Surface Modification

Surface of nanowell is coated with polymer to prevent cell attachment. However, for adherent cell culture, protein binding surface type is available for coating with ECM e.g. laminin, collagen by request.

On-Chip Neural Differentiation of PC12 Cells

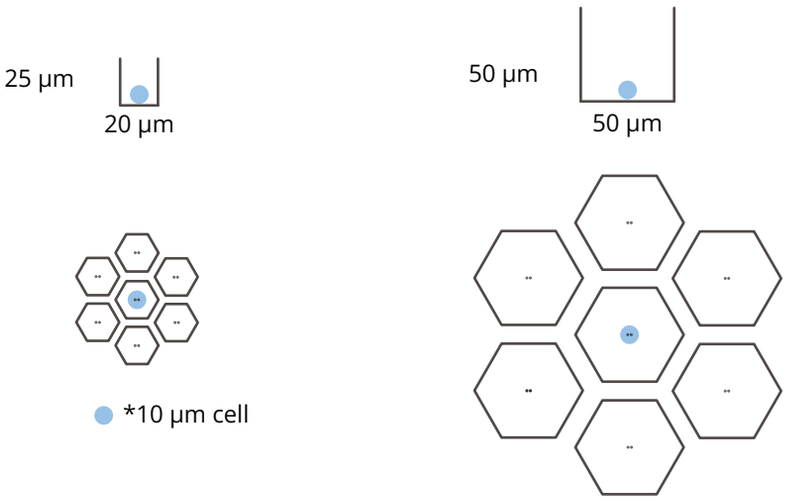


20 µm nanowell

SIEVEWELL 20 µm was coated with Cellmatrix Type IV (Collagen Type IV, Nitta Gelatin Inc). Rat pheochromocytoma cell line PC12 cells were loaded into SIEVEWELL 20 µm. Cells were cultured with RPMI 1640/10% horse serum/5% fetal bovine serum containing 10 ng/µL NGF. After 7 days, cells were fixed with PFA, permeabilized with 0.05% Tween 20/PBS, blocked with 1%BSA/PBS, stained with mouse anti-rat Tubulin β3 (TUBB3) antibody (Clone, TUJ1) followed by staining with Alexa Fluor Plus 488 labelled anti-mouse IgG antibody and DAPI. Images were taken with THUNDER Imaging Systems (Leica Microsystems).

Specifications　SIEVEWELL Slide

Product code	SWS 2001-5	SWS 5001-5
Nanowell size	Width 20 μm、Depth 25 μm	Width 50 μm、Depth 50 μm
Number of nanowell	370,000	90,000



Dimension	25 mm x 75 mm x 12 mm
Chamber size	17 mm x 17 mm, Cell repellent surface
Working volume	0.3 - 2 mL
Material	PS, PC, Biocompatible polymer
Package	5 slides per box（sterilized）

References

Therapeutic application of human type 2 innate lymphoid cells via induction of granzyme B-mediated tumor cell death
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Cytometry A. 2022 Dec;101(12):1057-1067.
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Contact

contact@sievewell.com

Tokyo Ohka Kogyo Co., Ltd.
New Business Division

1590 Tabata, Samukawa, Koza, Kanagawa
253-0114, Japan

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www.sievewell.com

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