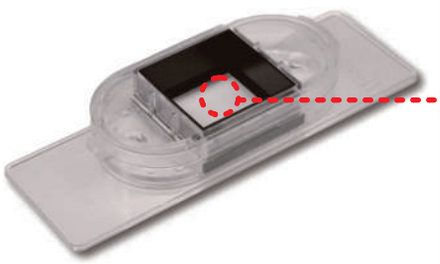
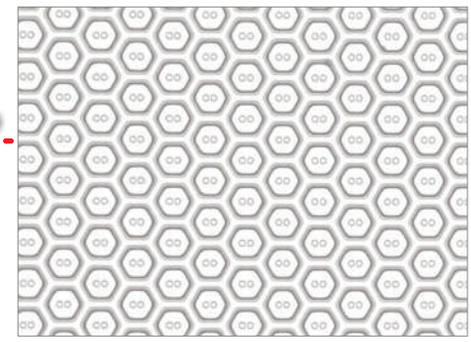
SIEVEWELL®

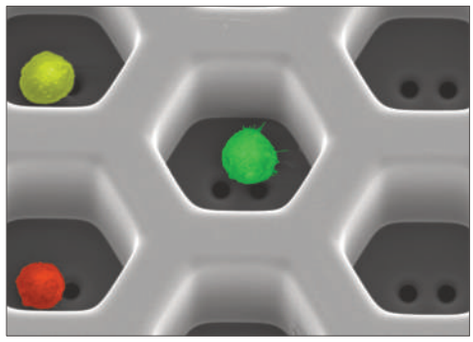
High Density Cell Arraying Device

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





20 µm nanowell, bright field

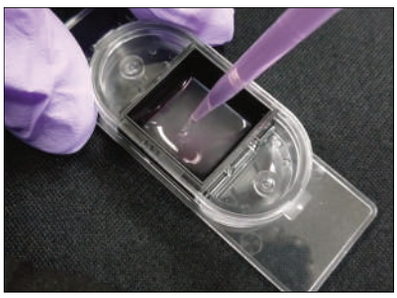


20 µm nanowell, MCF-7, SEM

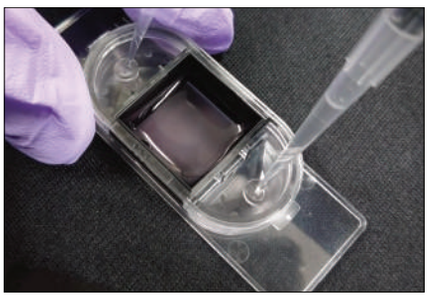
Easy to Use

Fluidic system or instrument is not required.

Add fluid to center chamber

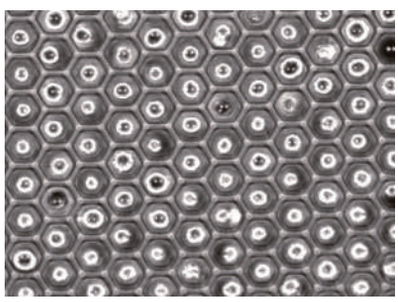


Aspirate fluid from side port



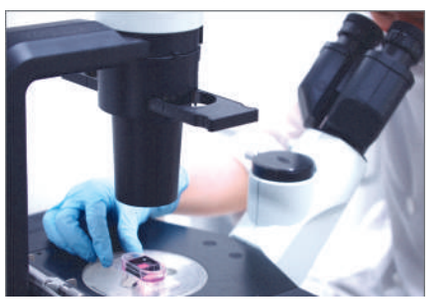
Compatible with 8 channel pipette

Generation of single cell array

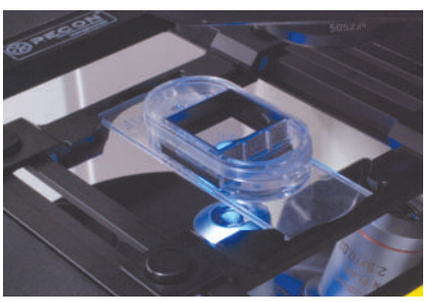


20 µm nanowell, 3T3-L1

Standard chamber slide format
Compatible with conventional microscope



Olympus CKX53



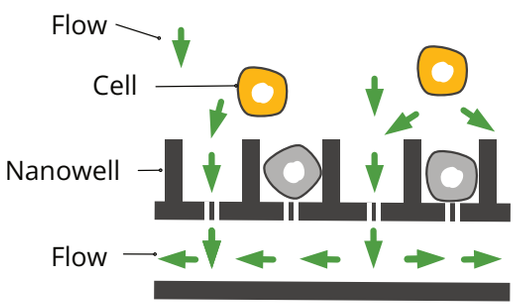
Leica DMI6000B

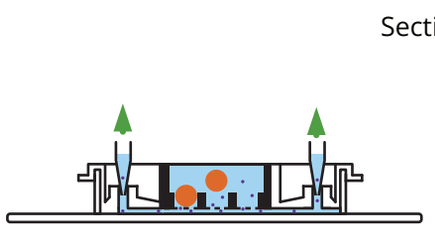
www.sievewell.com

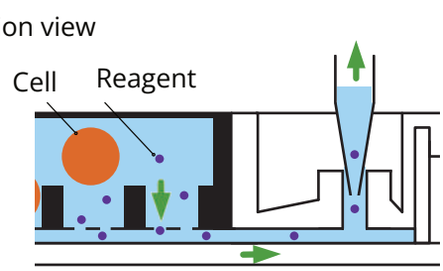
Mechanism of Single Cell Capture

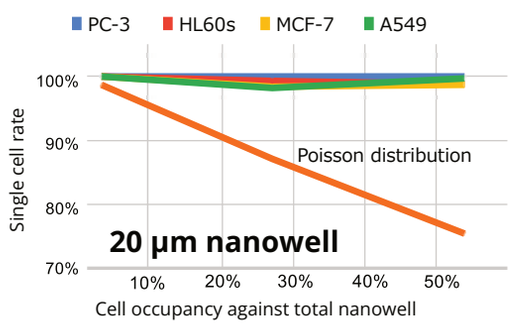
Arraying cells is an important step for single cell analysis. For example, cell overlapping is unfavorable for single cell imaging and isolation. Microcavity array is used to trap singe cell. In general, the distribution of cells into a cavity is by sedimentation and single cell rate obeys Poisson distribution. Its single cell capture rate is relatively low, especially loading cells at higher amounts.

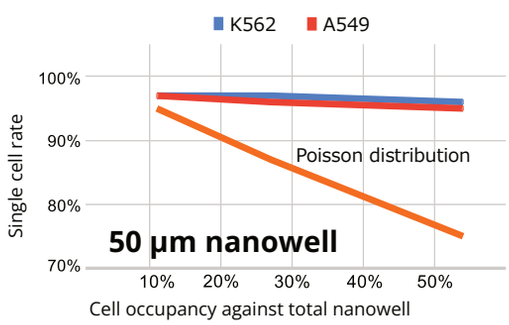
SIEVEWELL® is designed to trap cells at a high single cell rate. Two pores are positioned on the bottom of the nanowell. After loading the cell suspension, directional fluid flow from the inner liquid chamber to the side ports can be generated by aspirating liquid from side ports with a standard pipette. The cells will follow the liquid flow and are trapped in the nanowell. When a cell is entered into a nanowell, it will block the pores, reducing the liquid flow through that nanowell. Other cells are therefore redirected towards other, empty nanowells. This mechanism enables higher single cell rate than that in Poisson distribution.







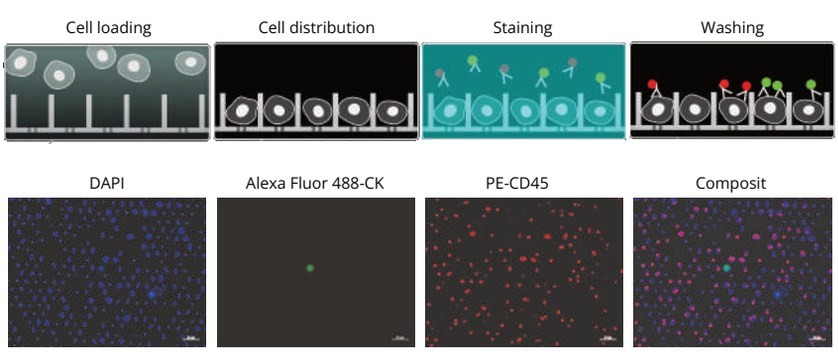




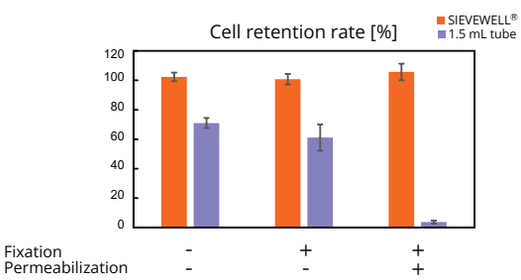
How it works

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. 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.



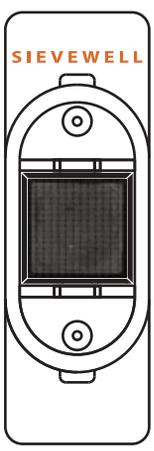
Direct comparison of staining procedure.
A549 cells were stained using 1.5 mL tube or SIEVEWELL® 20 µm.

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

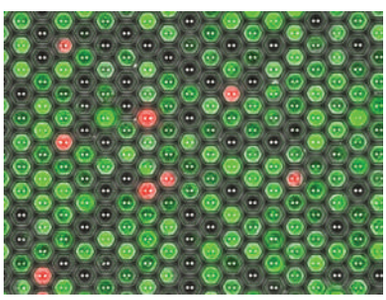
Arraying Cells at High-Density

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.

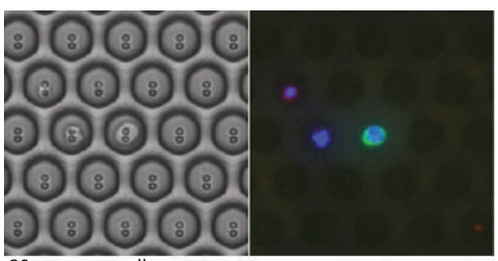


Number of nanowells
370,000（20 µm）
90,000（50 µm）

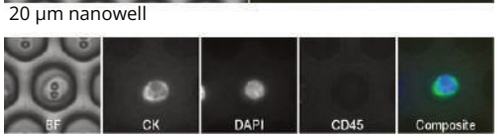


20 µm nanowell

Circulating tumor cell from patient blood



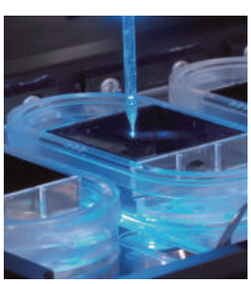
20 µm nanowell

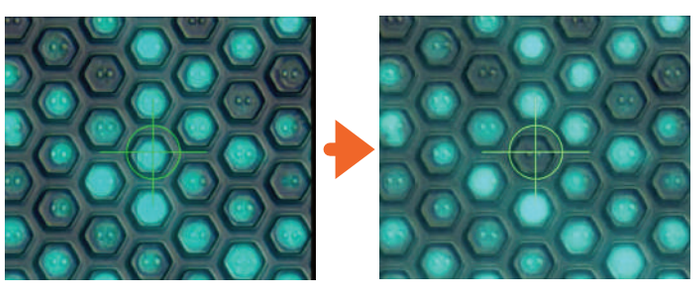


Data provided by Professor Hans Neubauer
Department of Obstetrics and Gynaecology, University Hospital and Medical Faculty of the Heinrich Heine University Düsseldorf, Düsseldorf, Germany

Support for Single Cell Isolation

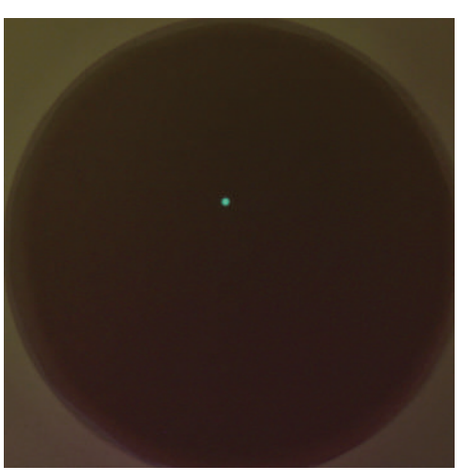
SIEVEWELL® is top-open chamber for accessing glass capillary from top side. SIEVEWELL® and glass capillary-based cell pick up system is an ideal combination for single cell isolation.



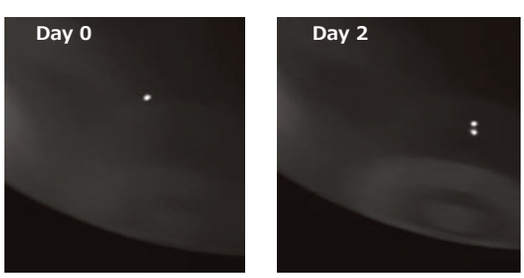


20 µm nanowell

Isolated A549 single cell



Single cell culture



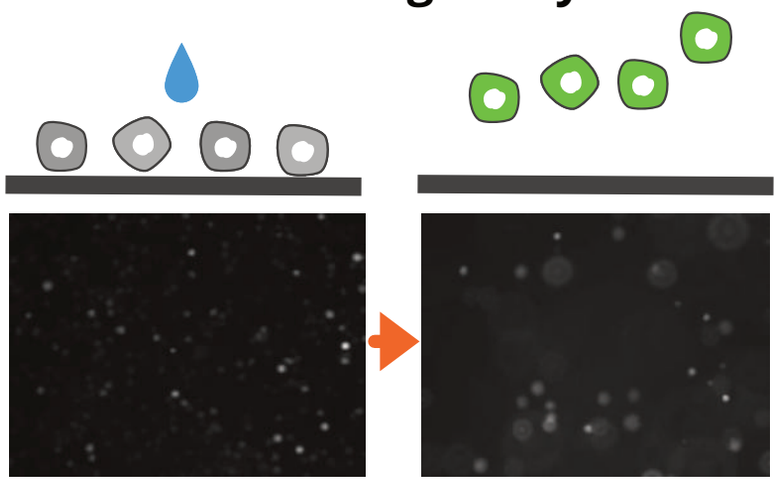
Single cell RT-PCR

PC (Positive Control) : total RNA from A549 (1x10⁶ cells) with RT reaction, NC (Negative Control) : total RNA from A549 (1x10⁶ cells) without RT reaction.

Stable Positioning of Suspension Cells During Assay

Real time imaging, e.g. Ca^{2+} imaging, of floating cells, such as blood or immune cells, is quite challenging. It is important to keep cells at the same position during assay for fluorescence signal based quantitative analysis. However, when using a conventional microwell plate, cells float due to turbulent flow of buffer or reagent addition.

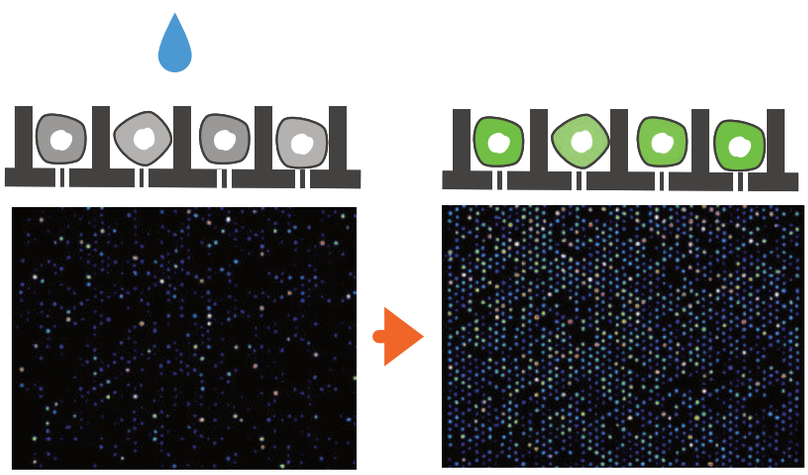
SIEVEWELL® has nanowells to trap single cell and enables stable positioning of each cells during assay.



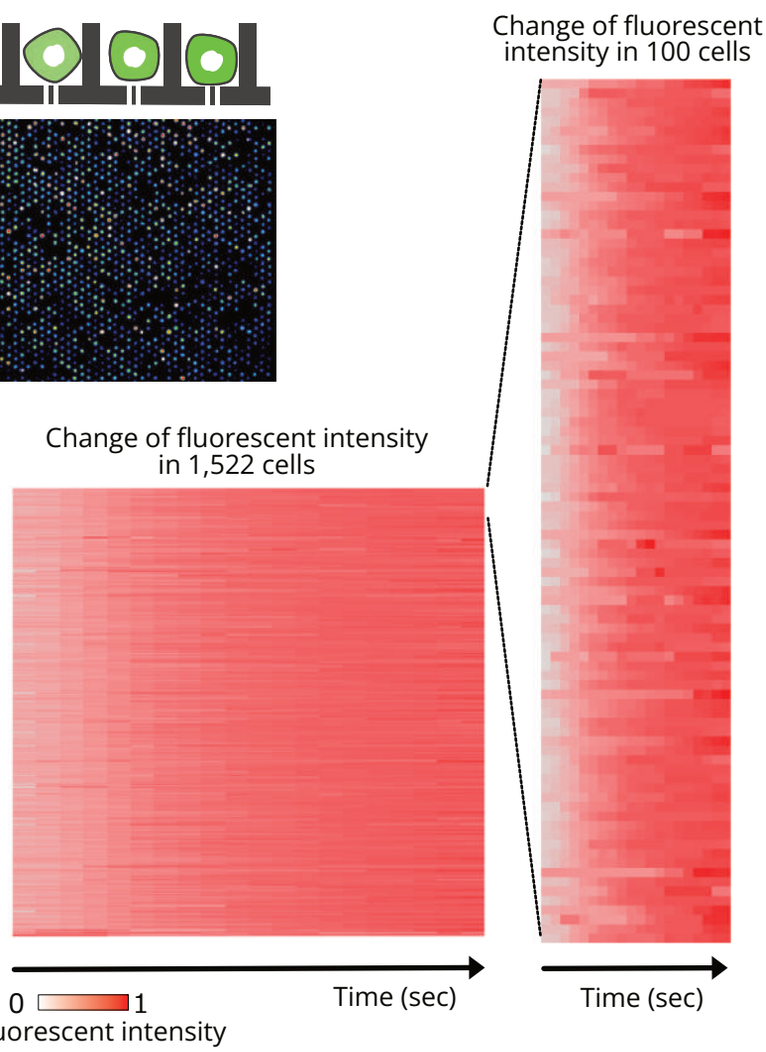
Calcium Flux Assay in Suspension Cells

Calcium response is rapid phenomena that occur within a few seconds duration. Cells captured in SIEVEWELL® keep its position even after addition of reagent, so rapid cell response like Ca^{2+} flux assay is possible in suspension cells. Moreover, high density cell array is ideal for monitoring of >1,000 cells per image.

Ca^{2+} imaging



20 µm nanowell



K562 cells were loaded with 5 µM of Fluo 4-AM for 1 hour and washed 3 times with PBS. Cells were loaded into SIEVEWELL® 20 µm, then the medium was aspirated from top side. The video was taken during addition of 5 µM of Ionomycin with Keyence BZ-9000 fluorescence microscope. Images were analyzed with CALciumIMagingAnalyser (CALIMA) * software to measure the change of fluorescence intensity of individual cells.

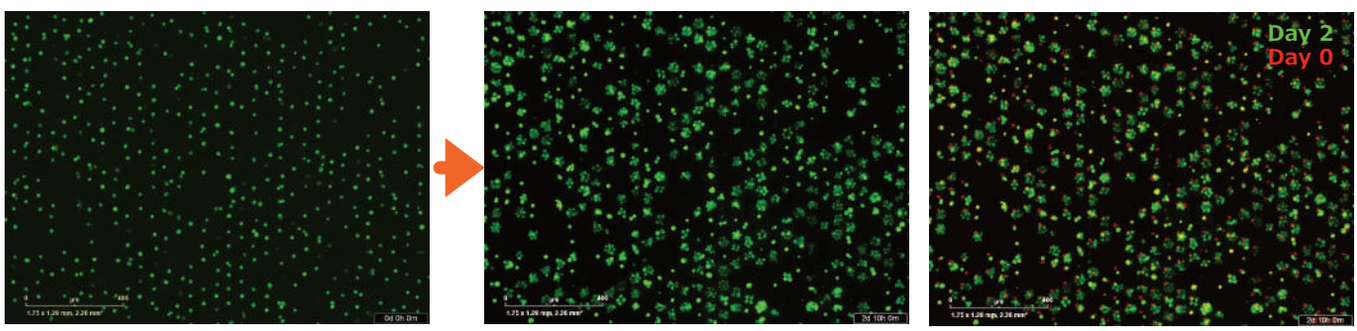
* https://aethelraed.nl/calciumimaginganalyser/index.html
Comput Methods Programs Biomed 2019 Oct;179:104991.
doi: 10.1016/j.cmpb.2019.104991.

Monitoring of Single Cell Growth

For cell growth monitoring from single cell, limiting dilution or sorting with cell sorter is well known method in order 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.

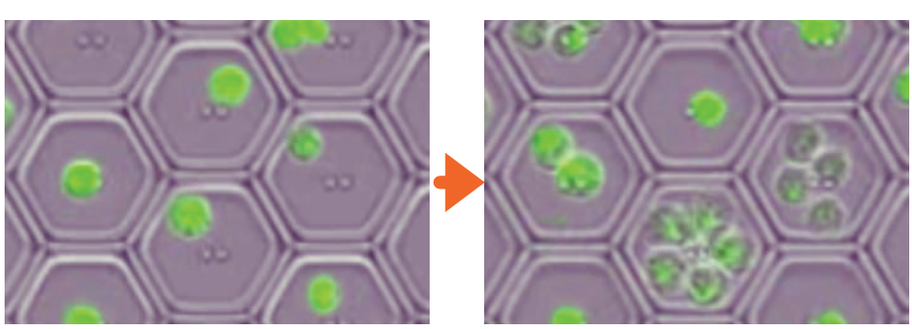
SIEVEWELL®, high density cell arraying device, is suitable for growth monitoring from single cell. Suspension cells are captured in each nanowell.

Day 0 Day 2 Composite



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.

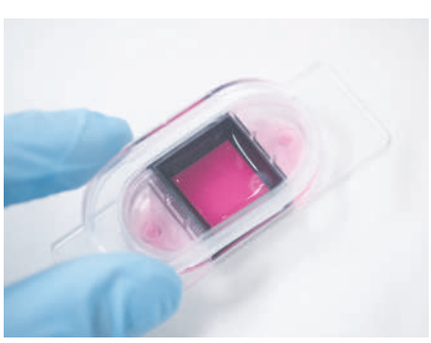
Day 0 Day 2



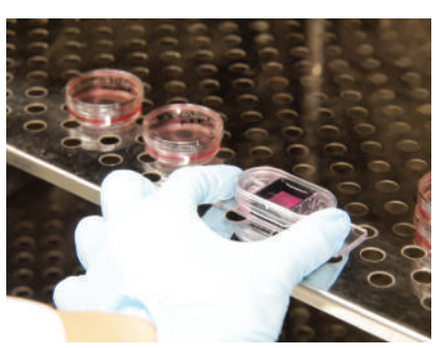
50 µm nanowell, K562 cells

Single cell culture

Detachable lid



Designed for cell cultue



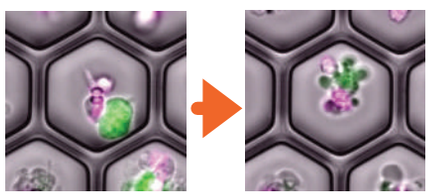
Cell-to-Cell Interaction Assay

SIEVEWELL® 50 µm is suitable for trapping pairs of two different cells to study cell-to-cell interactions.

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.

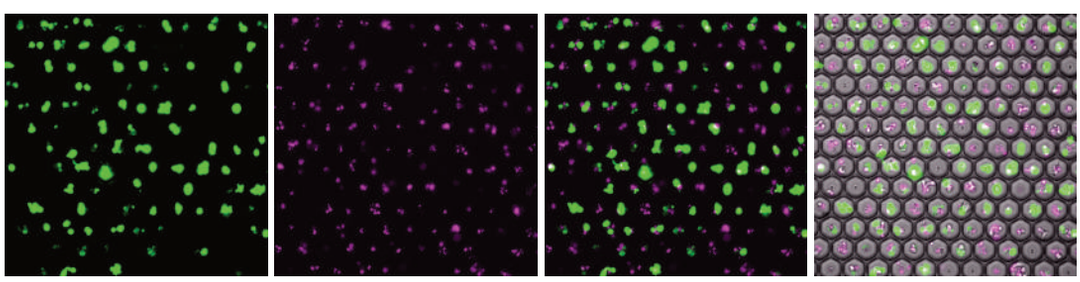
Apoptosis induced by NK cell



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

The erythroleukaemia 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.

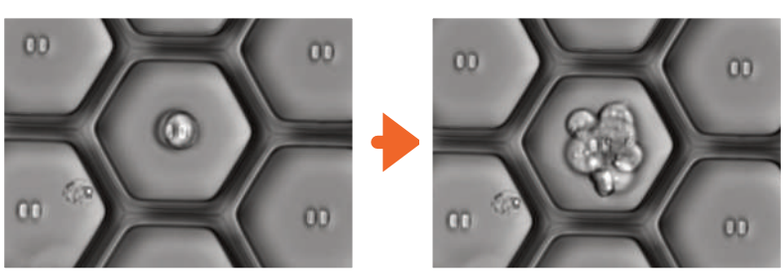
Target: K562 | Effector: KHYG-1 | Composite | Composite



Killing assay

Spheroid Formation from Single Cell

The ability of a single cell to form a spheroid is thought to be potential self-renewal ability. SIEVEWELL® 50 µm enables monitoring of spheroid formation from single cell.



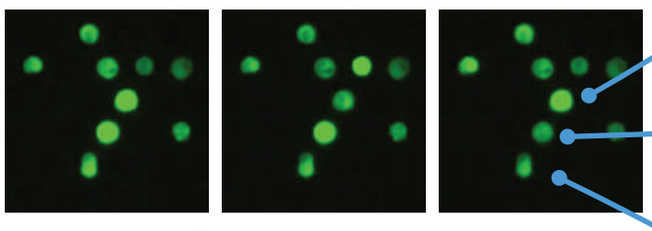
50 µm nanowell, HepG2

Cell Culture Examples

Cardiomyocytes Derived from Human iPS Cells

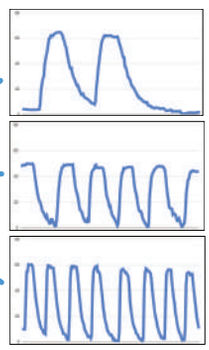
CarmyA-GCaMP is human iPS cell derived cardiomyocyte expressing GCaMP, calcium indicator. Cells are cultured both in suspension and adherent state to visualize Ca^{2+} oscillations.

Cardiomyocytes, single cell susension



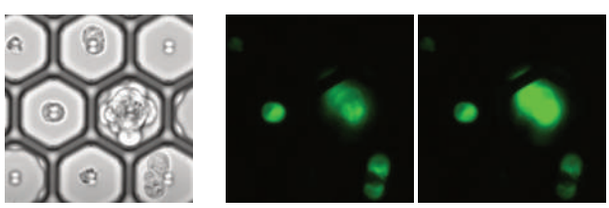
20 µm nanowell

Ca^{2+} oscillations



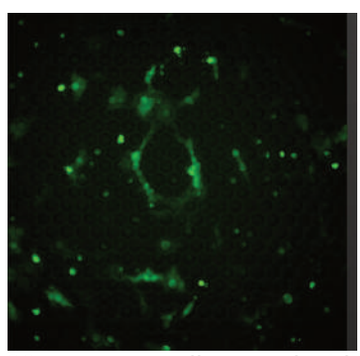
Cardiomyocytes culture

Spheroids of cardiomyocytes



50 µm nanowell

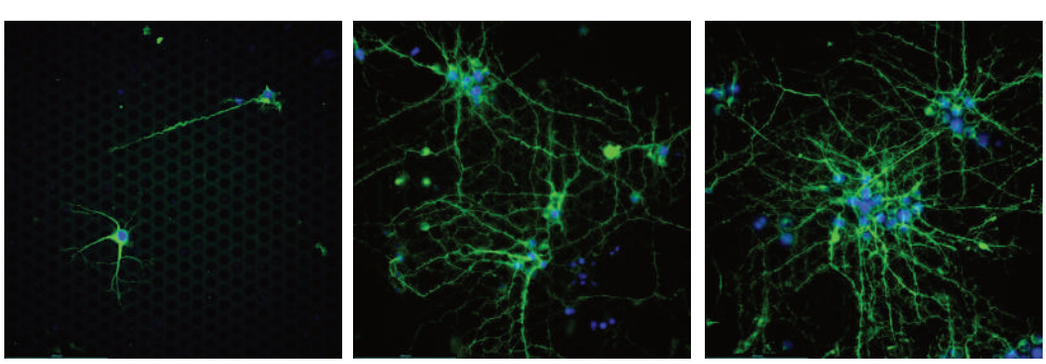
Cardiomyocyte adherent culture



20 µm nanowell, coated with iMatrix 511 silk, laminin *1

On-Chip Neural Differentiation of PC12 Cells

SIEVEWELL® 20 µm was coated with Cellmatrix® Type IV (Collagen Type IV, Nitta Gelatin Inc)*2. 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).

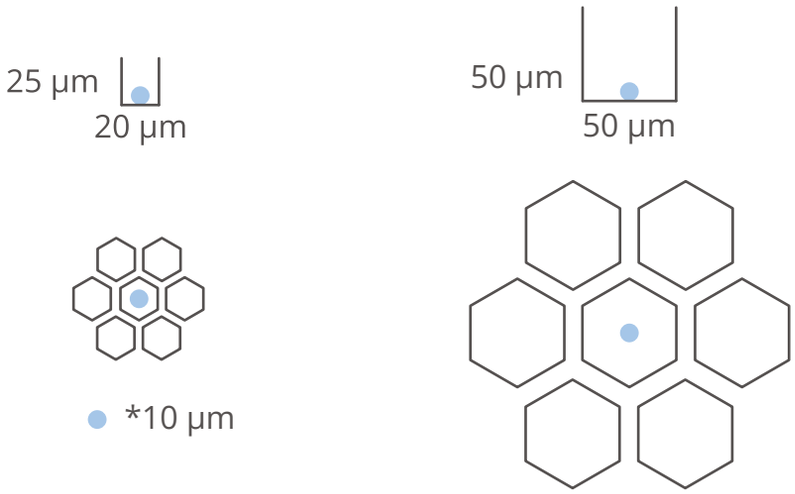


20 µm nanowell

*1, 2 Surface of nanowell is coated with polymer to prevent cell attachment. Protein binding surface is required for coating with ECM e.g. laminin, collagen. Please contact us for more details.

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

Single-cell multi-omics enabled discovery of alkaloid biosynthetic pathway genes in the medical plant *Catharanthus roseus*
bioRxiv, 04 Jul 2022
doi: https://doi.org/10.1101/2022.07.04.498697.

Implementing microwell slides for detection and isolation of single circulating tumor cells from complex cell suspensions
Cytometry. 2022;1–11.
https://doi.org/10.1002/cyto.a.24660.

Validation of Cell-Free RNA and Circulating Tumor Cells for Molecular Marker Analysis in Metastatic Prostate Cancer
Biomedicines. 2021 Aug; 9(8): 1004.
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Improvement of single circulating tumor cells isolation with sievewell slides
Geburtshilfe Frauenheilkd 2020; 80(10): e212
doi: https://doi.org/10.1055/s-0040-1718200.

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