SIEVEWELL

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# SIEVEWELL: Versatile Single-Cell Arraying Technology Using a Microwell Array Filter

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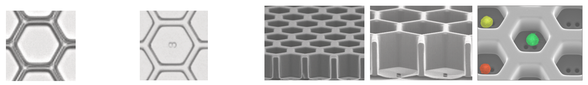
## Introduction

Single-cell arraying is essential for high-throughput and cost-effective biological screening but is fundamentally limited by Poisson statistics, where higher cell loading increases multi-cell occupancy. We developed SIEVEWELL, a nanowell array filter with precisely fabricated through-holes at the well bottom, enabling single-cell distribution beyond Poisson statistics and achieving over 95% single-cell occupancy at 50% microwell occupancy.

## Structure of SIEVEWELL Filter

SIEVEWELL consists of nanowells with 2–3 µm through-holes positioned within the single-cell capture area at the well bottom (**Fig. 1**). Once a cell occupies a nanowell, local fluid flow is reduced, biasing subsequent cells toward unoccupied wells (**Fig. 2**). The nanowell array filter is fabricated by photolithography with photoresist exhibiting lower autofluorescence than conventional one, and no detectable cytotoxicity.



Typical nanowell **SIEVEWELL** SEM images of the SIEVEWELL filter

Fig. 1. Structure of SIEVEWELL filter.

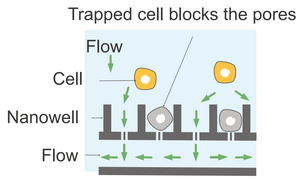



Fig. 2 Single-cell trapping guided by liquid flow through the SIEVEWELL filter.

## Single Cell Arraying Device SIEVEWELL Slide

The SIEVEWELL Slide incorporates a 17 × 17 mm SIEVEWELL filter containing either 20 µm or 50 µm nanowells ($3.7 \times 10^5$ or $9 \times 10^4$ wells, respectively), with a flow channel beneath the filter and fluidically connected aspiration ports (**Fig. 3**). Aspirating liquid from the aspiration port guides suspended cells into nanowells, where they are captured predominantly as single cells and can be directly observed using an inverted microscope. Physical separation of the loading chamber and aspiration port allows solution exchange with minimal cell loss during multiple staining and washing procedures (**Fig. 4**).

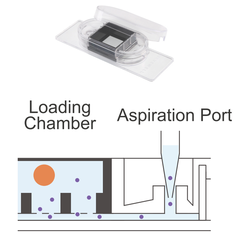



Fig. 3. SIEVEWELLTM Slide and its cross-sectional structure.

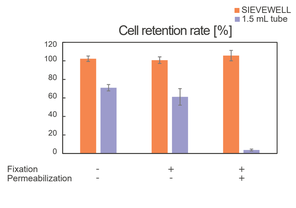



Fig. 4. Cell retention rate after various treatments for cell staining.

## Single Cell Capturing with SIEVEWELL Slide

Single-cell rate, defined as the fraction of occupied nanowells containing a single cell against those occupied any number of cells, exceeded 95% for both 20 µm and 50 µm nanowells across all occupancy levels and cell lines tested, demonstrating high-density single-cell arraying beyond Poisson statistics (**Fig. 5, 6**).

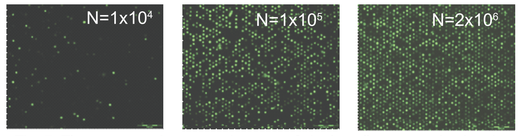



Fig. 5. Fluorescent microscopy images of single cells captured using the 20 µm nanowell array in the SIEVEWELL Slide at different nanowell occupancy levels.

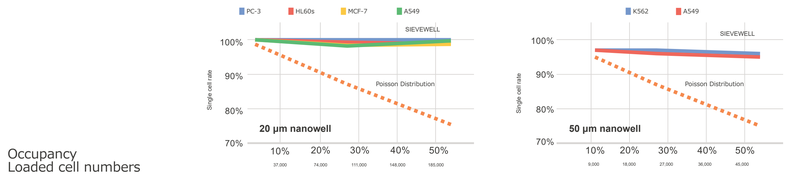



Fig. 6. Single cell rate of 20 and 50 µm nanowell determined using the SIEVEWELL Slide.

## Applications

### Stable Positioning of Non-Adherent Cells

One advantage of single-cell capture in nanowells is the stable positioning of non-adherent suspension cells, such as immune cells. To demonstrate stable positioning and compatibility with multiple on-chip staining steps, we applied the SIEVEWELL™ Slide to Cell Painting of K562 cells. Cell Painting typically requires multiple sequential staining steps and is most commonly applied to adherent cells. Using the SIEVEWELL™ Slide, we successfully stained distinct cellular organelles in non-adherent K562 cells with different fluorescent dyes (**Fig. 7**).

Captured single cells could be cultured within individual nanowells. Distinct growth responses of individual K562 cells to imatinib exposure were observed (data not shown).

We further paired two different types of suspension cells - an effector NK cell and a target K562 cell - within one nanowell. Cytotoxic interactions between an NK and a K562 cell were directly observed by time-lapse imaging (**Fig. 8**). This approach was also applied to evaluate the killing activity of human type 2 innate lymphoid cells [1].

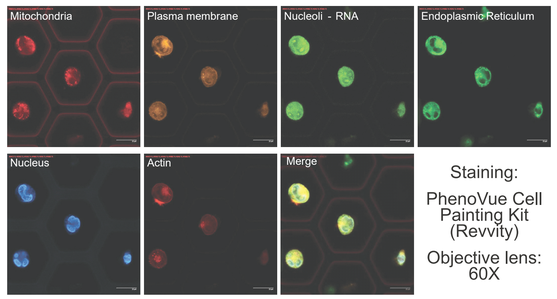



Fig. 7. Cell Painting of suspension cell K562 using the SIEVEWELL Slide.

### Rare Cell Detection

The large number of nanowells enables efficient detection of rare cells, such as circulating tumor cells (CTCs), through multiplex immunostaining. This method was applied to the detection of CTCs in pre-enriched CTC fractions from blood samples of breast cancer patients [2].

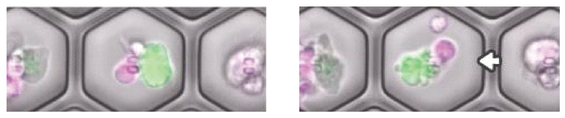


Fig. 8. Cell-killing assay between an effector NK cell, KHYG-1, and a target K562 cell in a 50 µm nanowell.

### Single Cell Pick Up

Captured single cells can be retrieved from individual nanowells using a glass capillary–based pickup system (**Fig. 9**). This single-cell pickup approach was applied to single-cell metabolomic analysis of medicinal plant protoplasts [3, 4].

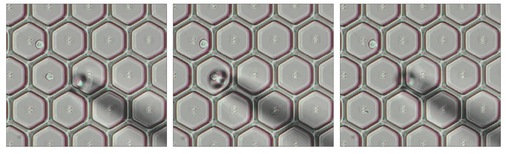


Fig. 9. Retrieval of a captured single cell using a 20 mm glass capillary from a 50 µm nanowell.

**References**

[1] Z. Li et al., *Cell* **187**, 1-18 (2024).
[2] L. Yang et al., *Cytometry A* **101**, 1057-1067 (2022).
[3] C. Li et al., *Nat. Chem. Biol.* **19**, 1031-1041 (2023).
[4] A. H. Vu et al., *J. Am. Chem. Soc.* **146**, 23891-23900 (2024).

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