SIEVEWELL

SIEVEWELL: High Throughput Secretion Based Single Cell Screening for Antibody Discovery

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Introduction

We have developed a SIEVEWELL-based platform for high-throughput screening of antibody-secreting cells (ASCs), which utilizes both bead-based and cell-based assays for secreted antibody detection and functional antibody identification. Using PD-1/PD-L1 blocking and non-blocking antibodies as a model, we demonstrate its potential for accelerating therapeutic antibody development through efficient hit discovery.
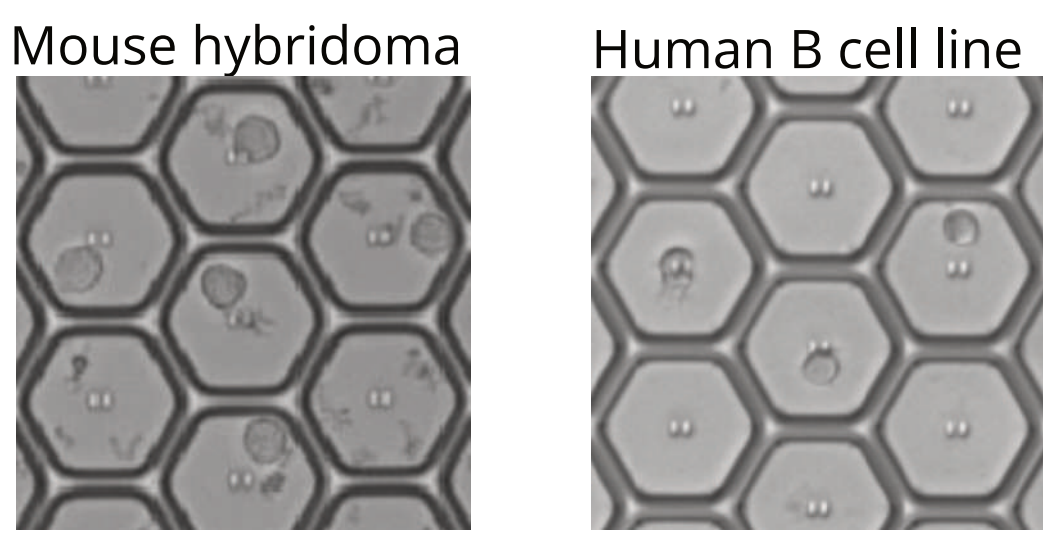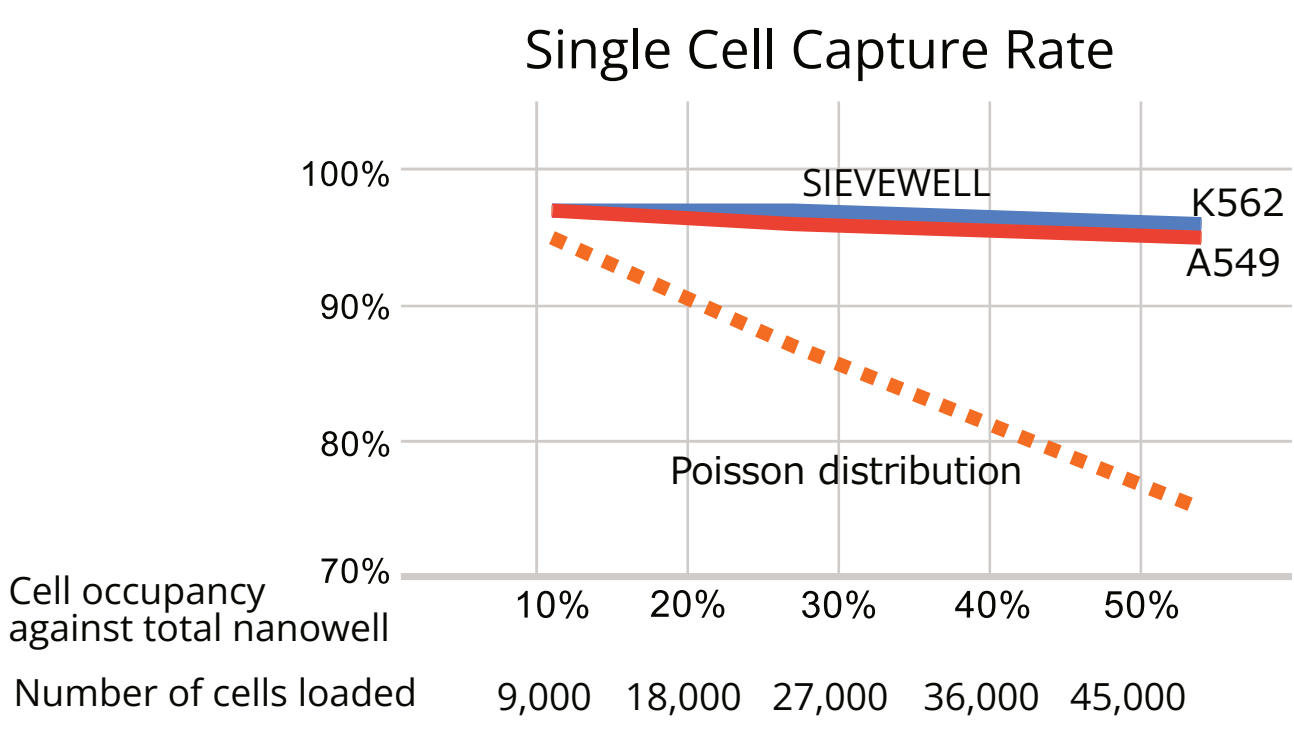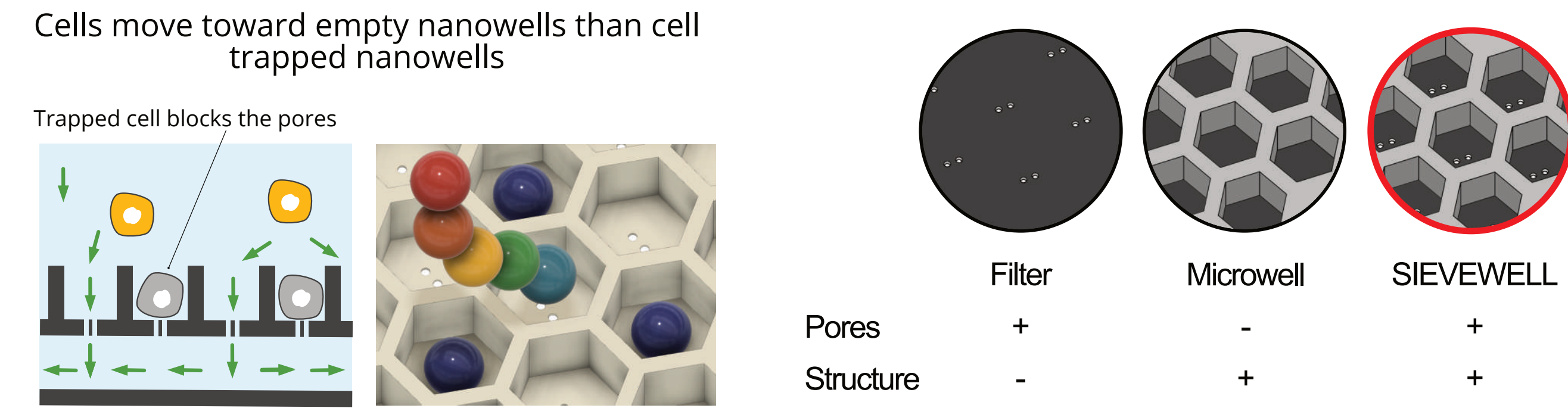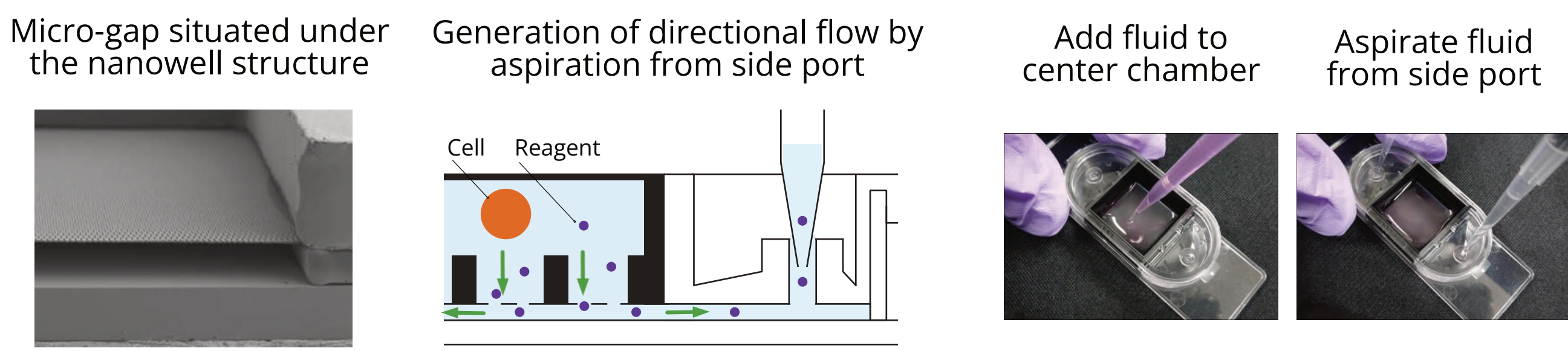
Materials and Methods

Screening Workflow

Preparation of ASCs → ASCs loading → Beads/cell loading → Incubation → Hit detection
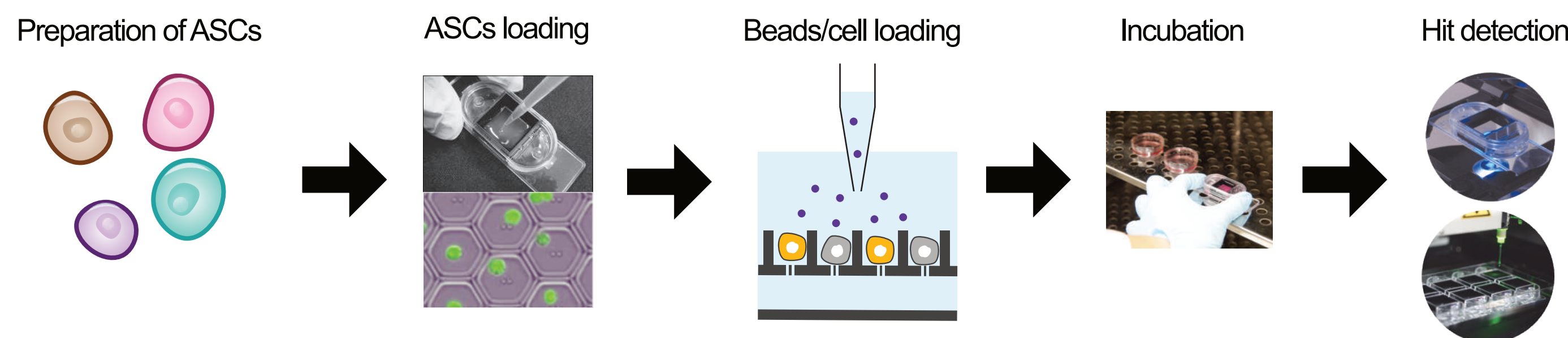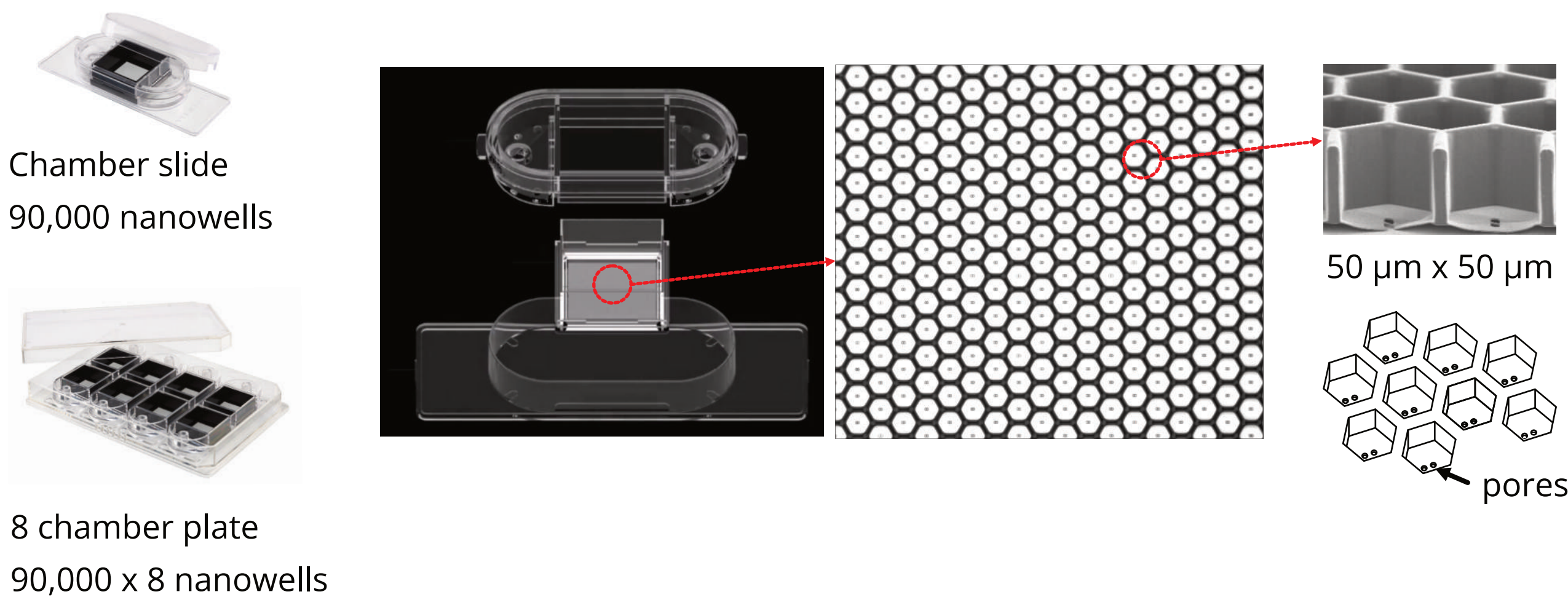
SIEVEWELL High Density Cell Arraying Device

Precise cell arraying is critical for single-cell analysis, as cell overlap impedes accurate imaging and isolation. Microcavity arrays, commonly used for single-cell capture, typically rely on sedimentation, resulting in a Poisson distribution of cells within cavities. This method yields a limited single-cell capture rate, particularly with increased cell loading.

SIEVEWELL addresses this limitation by employing a novel design that significantly enhances single-cell capture rates. Each nanowell features two basal pores, enabling directional liquid flow from an inner chamber to side ports upon aspiration. This flow directs cells into nanowells. Upon cell entry, pore blockage reduces flow, diverting subsequent cells to unoccupied nanowells. This active flow mechanism achieves a single-cell capture rate exceeding that of a Poisson distribution, optimizing single-cell analysis.

Chamber slide
90,000 nanowells

8 chamber plate
90,000 x 8 nanowells

50 µm x 50 µm

pores

Micro-gap situated under the nanowell structure

Generation of directional flow by aspiration from side port

Cell   Reagent

Add fluid to center chamber

Aspirate fluid from side port

Cells move toward empty nanowells than cell trapped nanowells

Trapped cell blocks the pores

	Filter	Microwell	SIEVEWELL
Pores	+	-	+
Structure	-	+	+

Single Cell Capture Rate

SIEVEWELL
K562
A549
Poisson distribution

Cell occupancy against total nanowell

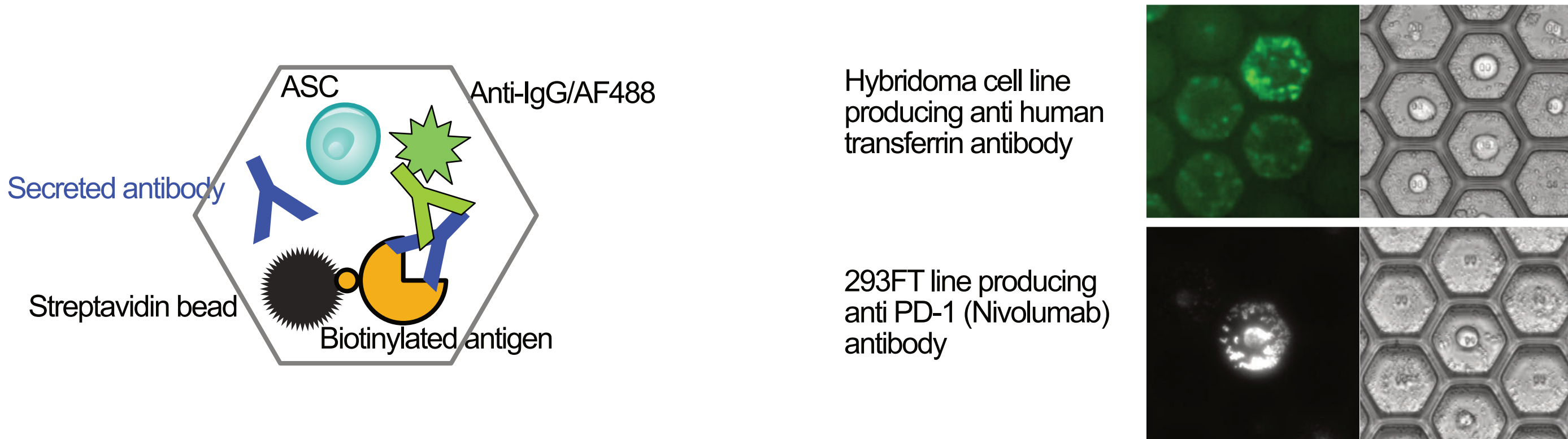| Number of cells loaded | 9,000 | 18,000 | 27,000 | 36,000 | 45,000 |

Mouse hybridoma

Human B cell line

Results

Single cell ELISA

- The nanowell surfaces were coated with recombinant cytomegalovirus pp65 protein via physical adsorption
- EBV-transformed human B cell line XYFMGG was seeded into the SIEVEWELL device
- The cells were cultured in a medium containing AF488-labeled Anti-Human IgG

Anti-IgG/AF488 (detection Ab)
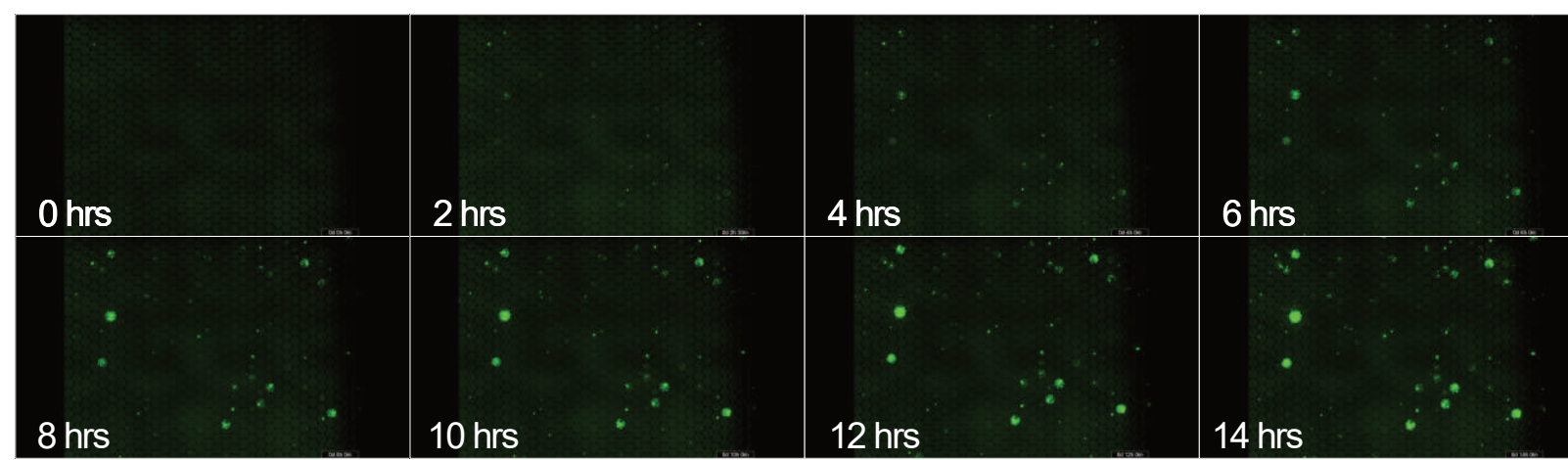
Secreted antibody
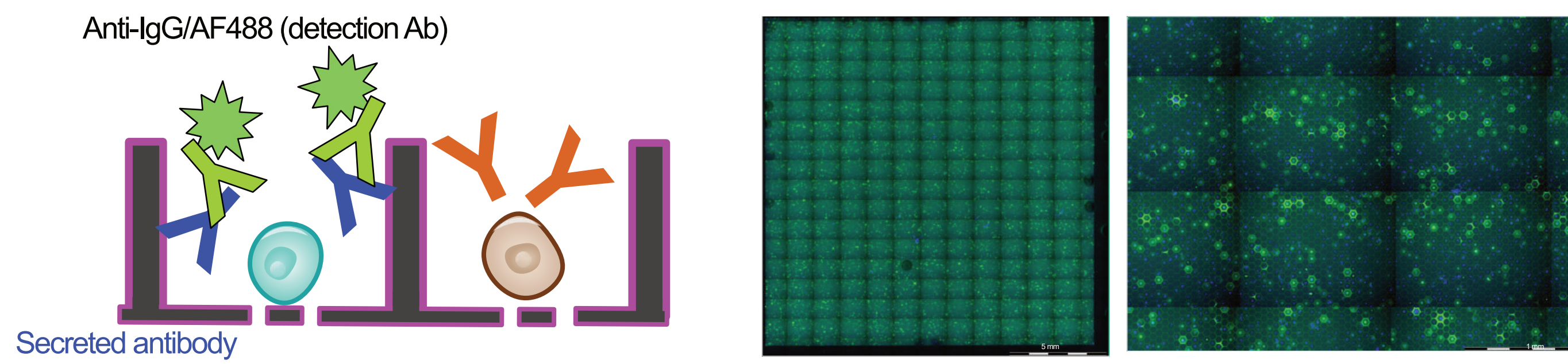
Beads-based Assay

- Biotinylated antigen was conjugated to streptavidin-labeled beads
- ASCs were seeded into the SIEVEWELL device
- The cells were cultured in a medium containing AF488-labeled Anti-Human IgG
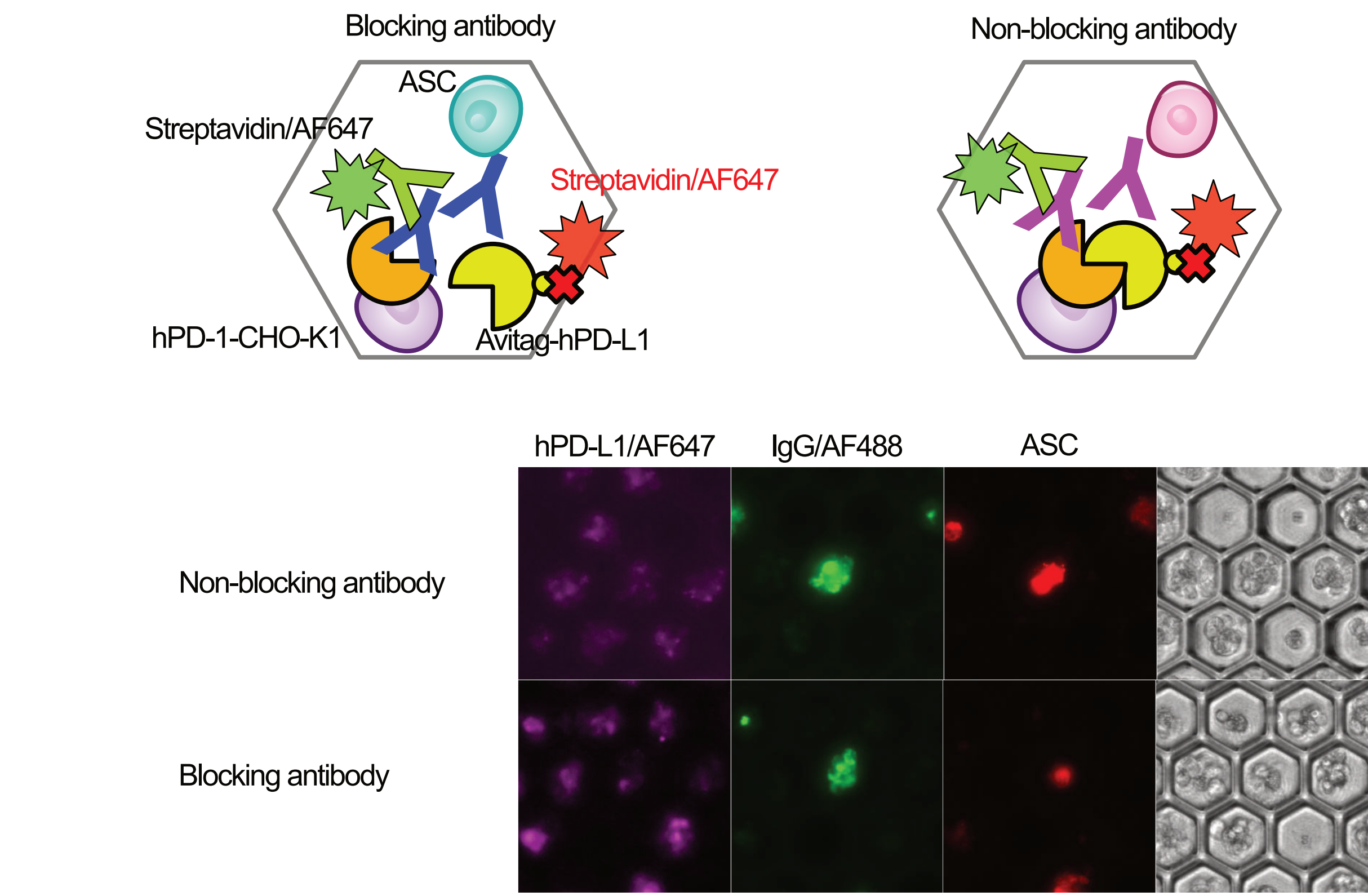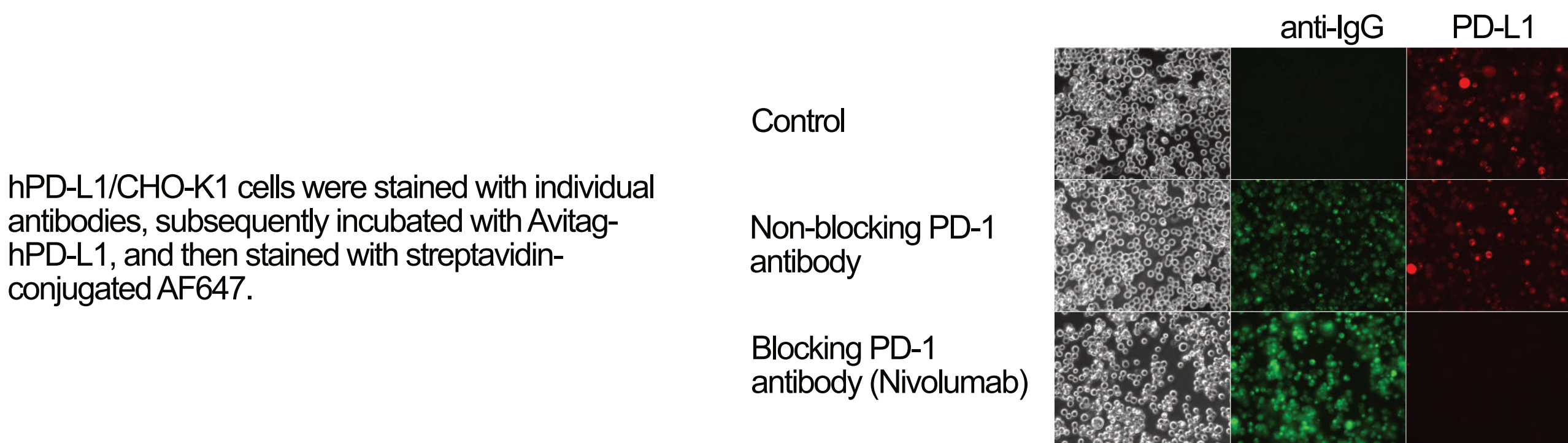

ASC   Anti-IgG/AF488

Secreted antibody

Streptavidin bead   Biotinylated antigen

Hybridoma cell line producing anti human transferrin antibody

293FT line producing anti PD-1 (Nivolumab) antibody

The time-course formation of AF488-labeled detection antibody and antigen-secreted antibody complexes was monitored using IncuCyte S3.

0 hrs   2 hrs   4 hrs   6 hrs
8 hrs   10 hrs   12 hrs   14 hrs

Cell-based Assay

- We established 293FT cell lines that secrete blocking and non-blocking anti-PD1 antibodies
- Anti-PD1 antibody producing 293FT cells were seeded into the SIEVEWELL device
- hPD1-expressing CHO-K1 cells were seeded into the SIEVEWELL device
- Cells were cultured in AF488-labeled Anti-human IgG medium
- The cells were incubated with Avitag-hPD-L1 in PBS
- The cells were incubated with AF647-labeled streptavidin in PBS

hPD-L1/CHO-K1 cells were stained with individual antibodies, subsequently incubated with Avitag-hPD-L1, and then stained with streptavidin-conjugated AF647.

anti-IgG   PD-L1

Control

Non-blocking PD-1 antibody

Blocking PD-1 antibody (Nivolumab)

Blocking antibody

Streptavidin/AF647

ASC

hPD-1-CHO-K1   Avitag-hPD-L1

Non-blocking antibody

Streptavidin/AF647

hPD-L1/AF647   IgG/AF488   ASC

Non-blocking antibody

Blocking antibody

Conclusions

SIEVEWELL high-density nanowell devices enable efficient antibody discovery with high single cell occupancy in high-throughput screening of antibody-secreting cells (ASCs). In particular, it accelerates the development of therapeutic antibodies by facilitating the discrimination and isolation of blocking and non-blocking antibodies. SIEVEWELL is a powerful platform for streamlining single-cell antibody analysis and contributing to the development of next-generation antibody therapeutics.