B Cell Screening Using TROVO

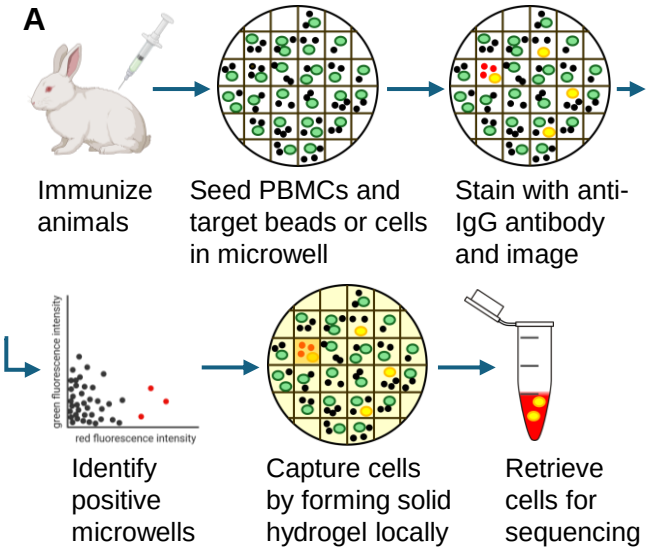
Introduction

While T cells have been at the forefront of immunotherapies, our understanding of the important role and therapeutic benefits of B lymphocytes is gaining ground. B lymphocytes have demonstrated that they play a key role in a patient's response to a therapy and therefore their benefits for long term patient outcomes[9-12]. However, B lymphocyte populations have a lot of heterogeneity even in apparent homologous subpopulations[4,7,8]. This has made it difficult to link secretion functionality to an individual cell. Traditional systems of detecting secretion from B lymphocytes are limited to looking at overall population trends, and not the differences between individual cells[1,5,6]. This prevents any rapid changes amongst smaller populations of cells to be detected, making it challenging to see critical time points[2]. Furthermore, these systems require a large cell input and can be labor intensive[2]. More recently, microfluidic platforms like the nanopen by Berkley Lights have become of interest as it allows coupling of secretion to be linked to an individual cell[2,3]. However, within the field adaptation of this workflow has been slow due to its complexity, time, and cost[2]. Within the field there is a need for a high throughput cost effective, simple to use platform and our microfluidics free platform TROVO aims to solve these issues.

TROVO is an "all in one" high throughput imaging system capable of monitoring individual cell behavior over time with the ability to then recover cells of interest. TROVO can print thousands of hydrogel based microwells on a single well of a 6 well plate, allowing microenvironments to be established. B cells can easily be incubated in our microwells with antigen coated beads, or target cells (suspension/adherent) that can be labeled with a secondary antibody to detect secretion within just 2 hours. These sequestered cells can be tracked over time, captured, and recovered for expansion or sequencing.

Approach and Methods

1. Immunize animals and obtain PBMCs
2. Prepare target cells that express the target protein on cell surface or target beads by coating streptavidin beads with biotinylated target peptide.
3. Seed PBMCs and target beads or target cells into microwells. PBMCs are pre-stained with green fluorescence. Incubate for 2 hours or overnight. Positive antibody secreted by B cells will bind to the target peptide on beads or target protein on cells.
4. Remove media with excess antibody. Add anti-rabbit IgG antibody with red fluorescence for staining and image with TROVO. Beads and target cells that bind to positive antibody will be stained red along with B cells.
5. Identify microwells with positive antibody production in TROVO's software by selecting microwells with high red fluorescent signal intensity on a distribution chart and/or looking at the images.
6. Capture cells in positive microwells with TROVO: a liquid capture gel (gelatin-based hydrogel) is added onto cells. In selected microwells, the capture gel is solidified locally by photo-crosslinking to immobilize cells of interest. Uncaptured cells and beads are then washed away.
7. Captured cells are retrieved by dissolving solid capture gels in a tube and are ready for downstream analysis such as sequencing.



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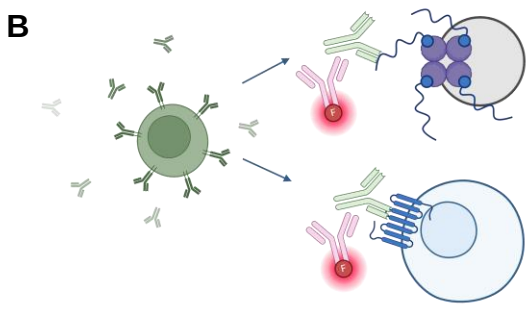

Figure 1. B cell screening process by TROVO system
A, a schematic of workflow. **B**, primary antibody secreted by B cells binds to biotinylated target peptides on streptavidin beads or target proteins expressed on the surface of target cells. Fluorescent secondary antibody against primary antibody is then added for detection.

Example Results

Screening for B cells which secret antibodies that bind to target peptide

6.4X10^4 PBMCs were stained with CellBrite Green and then seeded into 3200 hydrogel microwells in one well of a 6-well plate, resulting in a density of 20 PBMCs per microwell on average. Streptavidin beads coated with target peptides were then added into the microwells. PBMCs and beads were co-incubated for 2 hours for beads to capture secreted antibodies. Then the cells and beads were stained with anti-rabbit IgG Alexa647 antibody and imaged on TROVO. Beads that bind to positive antibodies secreted by B cells were stained red together with the B cells. TROVO can measure the cell count and fluorescence intensity in each microwell based on the image. The microwells containing strong red fluorescent signal from beads were selected for capture. During capture, a liquid hydrogel was added onto the microwells. TROVO identified the position of selected microwells and shone a beam of light onto those spots to locally photo-crosslink the hydrogel. The uncaptured cells and beads were then washed away, leaving only cells and beads in selected microwells in the solid capture gels. The capture gels were eventually dissolved enzymatically to release the captured cells in a tube for downstream application such as sequencing.

Different PBMC densities were also tested and the percentage of positive B cells were estimated. Based on anti-IgG staining, it was estimated that about 36% of the PBMCs were B cells. Assuming that in each positive microwell there is one B cell that produces the positive antibody, the average percentage of positive B cells which secret antibodies that bind to target peptide in all B cells is around 0.04% in this experiment .

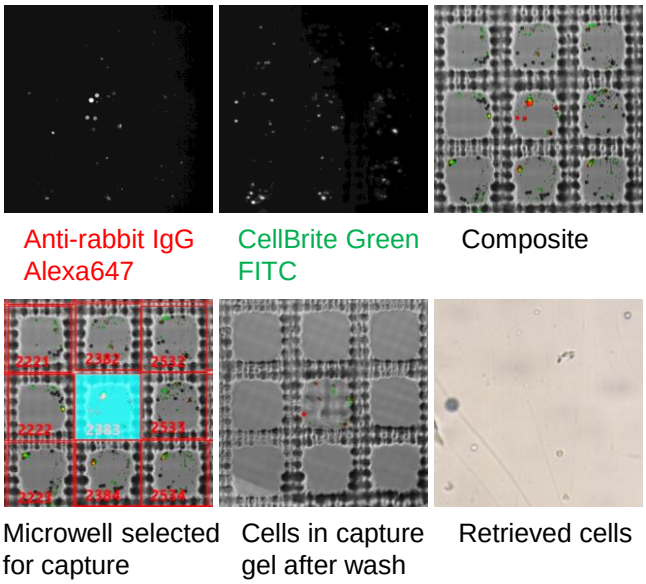

Figure 2. PBMCs that produce antibodies to bind target peptide were screened for, captured and retrieved
PBMCs (stained with CellBrite Green) and beads coated with target peptide were co-incubated in microwells for 2 hours and then stained with anti-rabbit IgG Alexa647 antibody. Beads in the microwell with positive antibody production were stained red. The positive microwell was selected and captured by TROVO. The captured cells were then retrieved.

PBMC number per microwell	5	20	50
Estimated B cell number per microwell	1.6	8.2	18.5
Number of positive microwells	2	12	26
Average percentage of positive B cells	0.039%	0.032%	0.044%

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Facile multi-signal screening for B cells which secret antibodies that bind to both target peptides on beads and target proteins on cells

Single readout screening suffers from high false-positive rate, i.e. sticky targets or IgGs. TroVo enables facile consecutive validation steps to identify double-positive or triple-positive binding events within one screening round before B cell clone retrieval. PBMCs stained with CellBrite Green were seeded into 3200 hydrogel microwells in one well of a 6-well plate at a density of 50 PBMCs per microwell. Target cells expressing the target protein on their cell surface were added into the microwells and co-cultured with PBMCs overnight. Afterward, the cells were stained with anti-rabbit IgG Alexa647 antibody and imaged on TROVO. Beads coated with target peptide were then added to the same microwells to co-incubate with the PBMCs overnight, after which staining with anti-rabbit IgG Alexa647 antibody and imaging with TROVO were performed again. Target cells and target beads that bind to positive antibodies secreted by B cells were stained red with weak and strong signal, respectively. Microwells with both positive bead staining and target cell staining were selected based on red fluorescent signal intensity and looking at the images by eye. 4 out of 3200 microwells showed positive staining for both target cells and beads, therefore containing double-positive B cells and were subject to capture and retrieval. This two-round screening not only ensures binding specificity but also enables the identification of antibodies that bind to both target peptide and full-length protein in its native conformation.

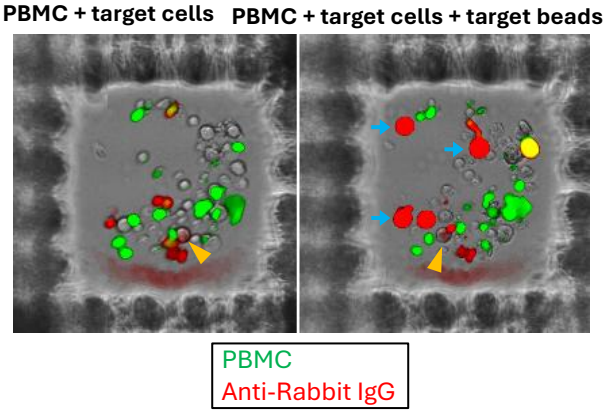

Figure 3. Double positive B cells were identified for by a two-round screen with both target cells and target beads
PBMCs (stained with CellBrite Green) were co-cultured with target cells (bigger and not green) and target beads consecutively. Target cells and beads in the microwell with positive antibody production were stained red.
▲ indicates positively stained target cell
→ indicates positively stained target bead

Conclusions

- TROVO can select B cells secreting antibody of interest in a fast and easy way
- TROVO enables B cells imaging, analysis and isolation all in one machine
- TROVO can perform two-round screening to identify B cells which produce antibodies that bind to both target peptide and full-length, native protein.

Advanced screening

- Adding more than one type of target beads or cells to include negative control or target mutation for specificity testing

Downstream

Isolated B cells can be RTPCR and sequenced as a small pool (Fluent BIo). Or serial diluted, RTPCR, and Sanger sequencing.

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