B Cell Screening Using TROVO

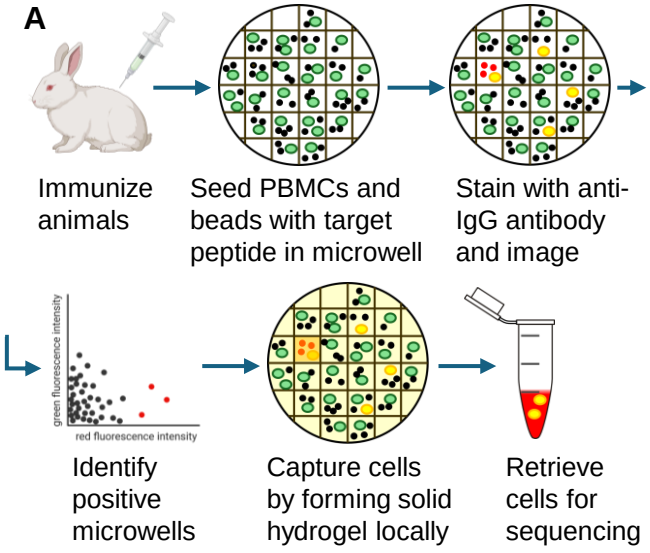
Introduction

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 lost, 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. Coat streptavidin beads with biotinylated target peptide
3. Seed PBMCs and beads with target peptide into microwells. PBMCs are pre-stained with green fluorescence. Incubate for 2 hours. Positive antibody secreted by B cells will bind to the target peptide on beads.
4. Remove media with excess antibody. Add anti-rabbit IgG antibody with red fluorescence for staining. Image the plate with TROVO. Beads that bind to positive antibody will be stained red along with B cells.
5. Identify microwells with positive antibody production in the TROVO 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.



21 Business Park Drive, Suite 4, Branford, CT 06405

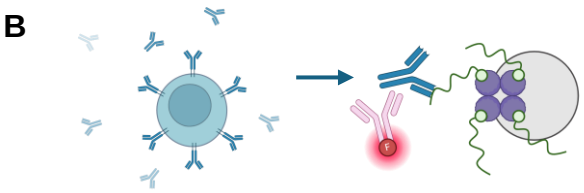


Figure 1. antibody screening process by TROVO system

A, a schematic of workflow. **B**, primary antibody secreted by B cells binds to biotinylated target peptides on streptavidin beads. Fluorescent secondary antibody against primary antibody is then added for detection.

Example Results

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 the 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 cell in all B cells in this experiment is around 0.04%.

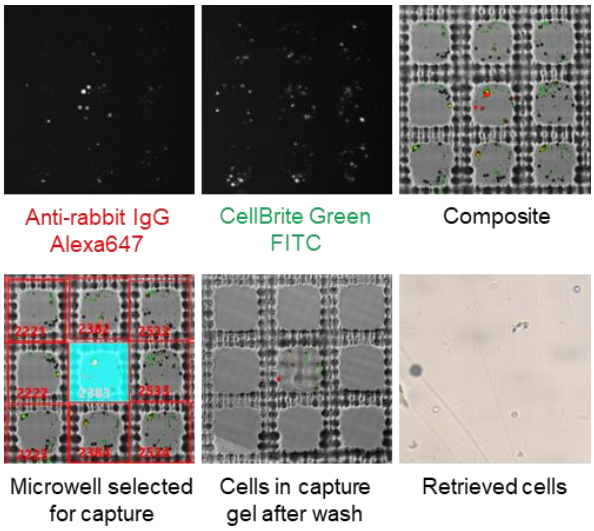


Figure 2. PBMCs that produce antibodies against a specific target were screened for, captured, and retrieved

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.

Input 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 cell (Sensitivity)	0.039%	0.032%	0.044%

Conclusions

- TROVO can select of B cells secreting antibody of interest in a fast and easy way;
- TROVO enables B cells imaging, analysis and isolation all in one instrument;

Advanced Screening

- Using live cells expressing target protein instead of beads to screen for antibody secretion;
- Adding more than one type of target beads or cells to include negative control or target mutation for specificity testing;
- Isolated B cells can be RTPCR and sequenced as a small pool. Or serial diluted, RTPCR, and Sanger sequencing.

21 Business Park Drive, Suite 4, Branford, CT 06405