STOCHASTIC BARCODING FOR SINGLE-CELL TRACKING

Marc Castellarnau, Gregory L. Szeto, Darrell J. Irvine, J. Christopher Love and Joel Voldman*

Massachusetts Institute of Technology, USA

ABSTRACT

We present stochastic barcoding (SB), a method for tracking cell identity across analytical platforms. SB uses a randomly generated code based on number, color and position of beads encapsulated together with a set of cells of interest. We demonstrate SB use in an application where cells are transferred from a microwell array into a microtitre plate while keeping their identity, and obtain an average identification accuracy of 96% for transfer of 100 blocks. Finally, we model scaling of the method up to 1000 blocks and show that SB is able to achieve ~90% accuracy.

KEYWORDS: barcoding, single-cell tracking, cell encapsulation

INTRODUCTION

Acquiring multiparametric data from single cells is critical for assessing phenotype in heterogeneous populations, and is increasingly used across biology. As a result, a diverse set of academic and commercial platforms have been developed to obtain single-cell data (e.g., cytokine secretion, gene expression, function, etc.) (Figure 1). However, because the academic platforms do not necessarily interface with standard microtitre plates, transferring cells between platforms while maintaining cell identity is challenging. Here, we present a simple, scalable method for tracking cell identity across assay platforms.

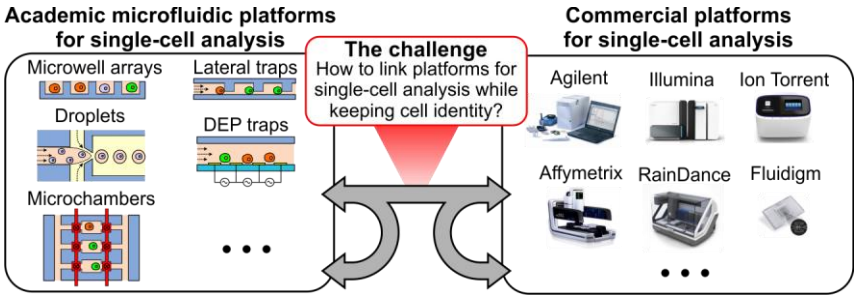

Figure 1: Overview. Many platforms are being developed for assaying single cells, but tracking cells across platforms is challenging because academic platforms do not often interface with microtitre plates.

Current methods for tracking identity across assay platforms mainly rely on direct tracking or deterministic codes. Direct tracking by manually picking cells with micromanipulators [1] has limited throughput and requires open access to the cells, while deterministic fluorescent labeling is limited by multiplexing depth (i.e., number of colors that can be detected [2],[3]) and the specificity of the label [4],[5]. Instead, we developed stochastic barcoding (SB), a method that uses bead location and color within a block photo-polymerized around cells to enable high multiplexing depth (1000's) without needing physical access to cells.

EXPERIMENTAL

Stochastic barcoding uses a randomly generated code determined by the number, color, and position of beads added to and polymerized in a hydrogel block around a set of cells (Figure 2). Because we use these three parameters, we get high coding depth using a modest number of beads, with little likelihood of overlapping codes. For cell and code encapsulation, we prepare a polymer solution consisting of 20% PEGDA (MW1000) and 1% Irgacure 2959 as photo-initiator, to obtain fast polymerization. We use a simple UV direct-writing approach through a microscope objective to photopolymerize the regions of interest. Imaging of the hydrogel blocks after photopolymerization *assigns* the code, and imaging after transfer to the recipient container *reads* the code. We implemented a custom Matlab script to identify the codes from the images and find the best candidate to match images of pre- and post-transferred blocks. Finally, we also developed a model for the stochastic barcoding method.

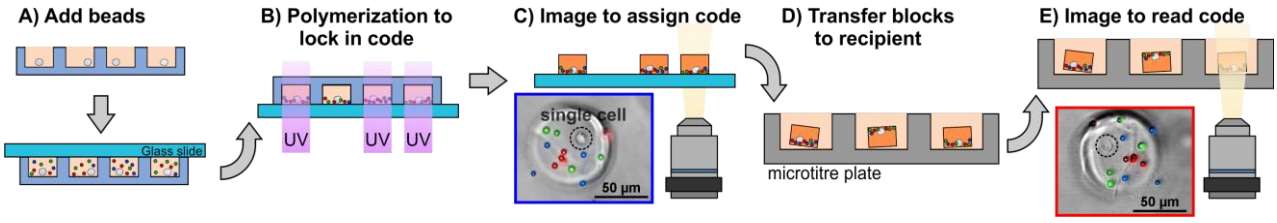

Figure 2: Stochastic barcoding (SB). A) Addition of PEGDA polymer solution with fluorescent beads into single-cell microwell array. B) Encapsulation of all or selected (shown) cells and beads by photopoylmerization of the hydrogel. Encapsulated beads constitute a random code based on their color, number, and relative positions. C) Imaging of blocks to assign the code. D) Transfer of blocks into a microtitre plate. E) Imaging blocks after transfer to read the code.

RESULTS AND DISCUSSION

We demonstrate SB in an application where one wishes to assay cells in a microwell array and then transfer those cells to another container (e.g., microtitre plate) while maintaining their identity. Code matching requires correlating images of blocks prior to transfer with those after transfer (Figure 3). We first process the block images to determine the contour of the blocks, so then we can identify the number, color, and location of the beads within each block, assigning the code (Figure 3A). To match the post-transfer images to the pre-transfer images, we account for the fact that blocks can rotate, flip, lose beads, and lose blocks (Figure 3B). We compare the positions for all beads/block between the two sets of images to determine a score that is minimized for the best-matching blocks, and then global optimization determines the overall set of best matches (Figure 3C). Implementing this method to select subpopulations of 100 blocks, we obtain an average identification accuracy of 96% (Figure 3D) when using an average of 15 beads/block (k).

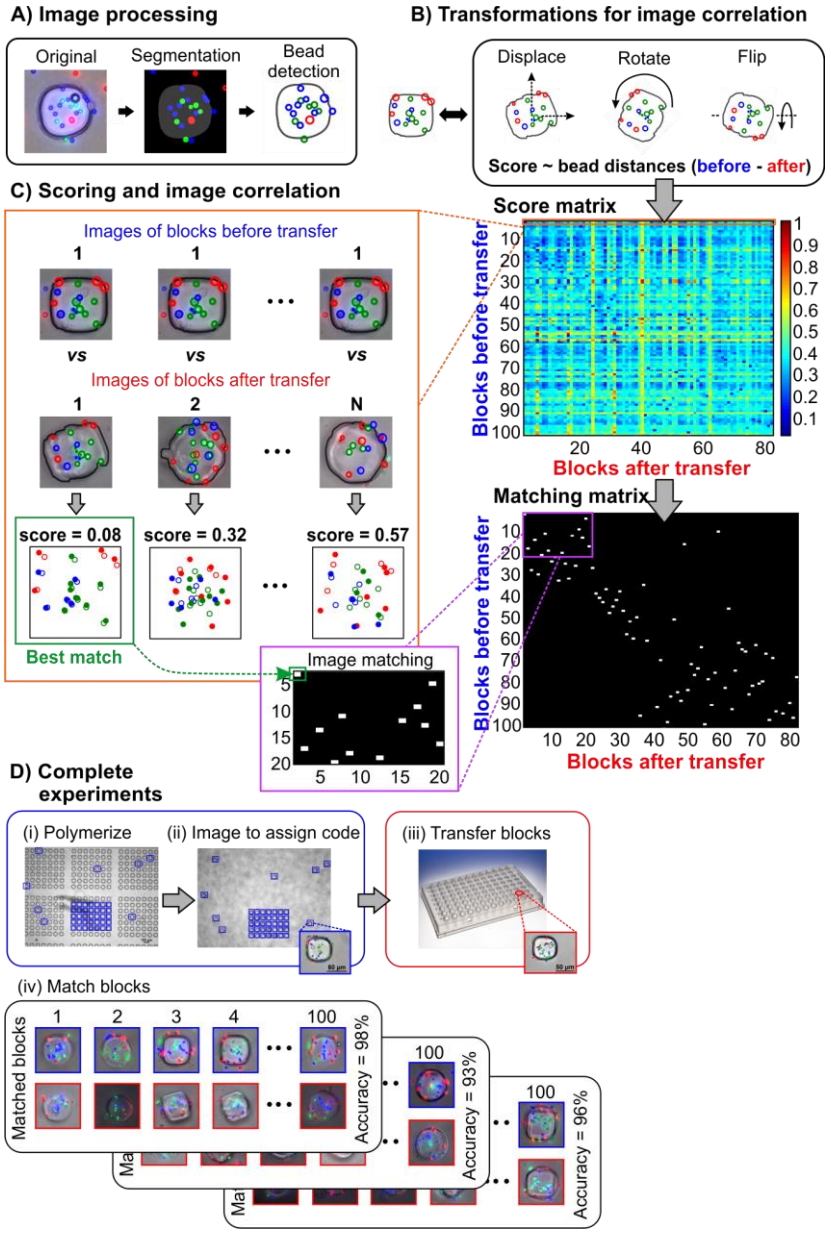

Figure 3: Image correlation for cell identity recovery. A) Images are first processed to contour the blocks and detect beads in the blocks, generating the code. B) To match the processed block codes, we apply transformations (i.e., XY displacement, rotation and flip) during the image correlation process. C) To perform the correlation, we compare the bead positions between the before\after image codes while applying the transformations in (B) to one of the blocks. (Here we show the actual images, but the processing is on the codes). Each pre-transfer block image is compared to all the images from the blocks after transfer, from which we generate the score matrix. Finally, we use the Hungarian algorithm to determine the global set of best image matches and generate the matching matrix to recover cell identity. D) Results from complete 100-block experiments. (i-iii) Representative images of blocks during steps B-D of Fig. 2. (iv) Block-matching accuracy from three experiments, with an overall accuracy of 96±2%.

This is consistent with stochastic modeling, which shows that ~100 blocks can be uniquely coded with k=8 beads/block, and that k=15 beads/block is able to achieve ~100% matching accuracy while tolerating up to 20% block loss (Figure 4A). Finally, we modeled scaling of the method up to 1000 blocks, and found that k=15 beads/block is able to achieve ~90% accuracy (Figure 4B). Scaling to even larger numbers of blocks merely requires increasing the number of beads/block, and thus SB provides a simple, scalable approach to maintaining identity of cells across platforms.

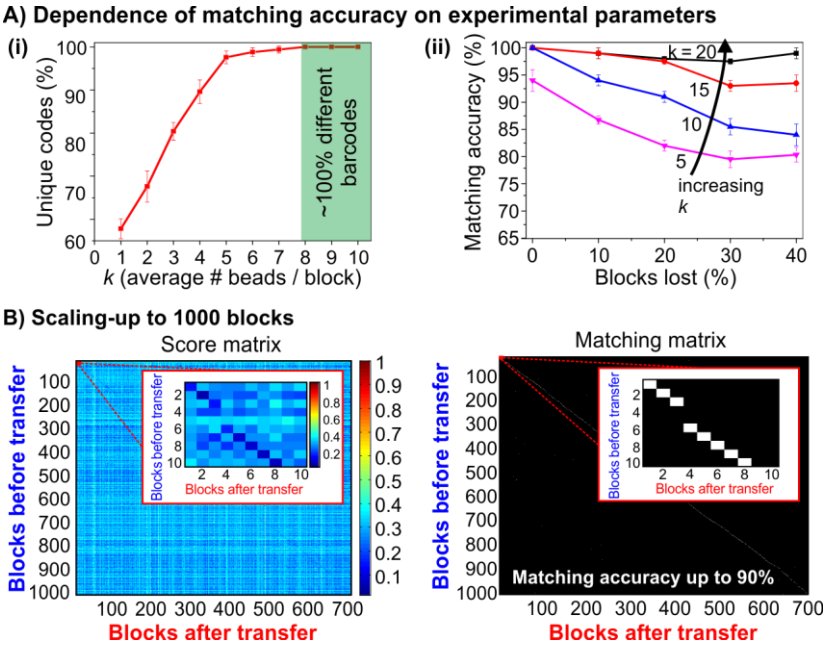

Figure 4: SB modeling. A) We have implemented stochastic simulations of block matching to understand how experimental parameters affect matching accuracy. (i) Simulations show that an average number of beads/block k=8 is able to attain ~100% unique codes for 100 blocks. (ii) Simulations can account for bead and block loss during the experiments. For example, matching accuracy decreases if blocks are lost during transfer, but increasing k can restore the accuracy. B) Simulation of 1000-block matching with k=15 beads/block shows that ~90% accuracy can be attained even with 30% block loss.

CONLUSION

In summary, we introduced stochastic barcoding as a simple and scalable method for tracking cell identity across assay platforms. This approach may enable performing multiparametric studies at a single-cell level for improving our understanding of cellular heterogeneity within populations, in fields including immunology, cancer research and developmental biology.

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CONTACT

*Joel Voldman, +1 617.253.2094 or voldman@mit.edu