Image-based screening of high-performing clones: single cell phenotyping, prediction and sorting

Zeeee Teeee and Geee Leeee
Massachusetts Institute of Technology, Cambridge, USA
Email: teeee@mit.edu; leeeee@mit.edu

Biologics (e.g., recombinant antibodies) are an increasing proportion of therapeutics, and are used to treat diseases ranging from arthritis to cancer. The process of producing a biologic entails introducing the specific gene into a host cell system, typically Chinese Hamster Ovary (CHO) cells, followed by screening hundreds of cell clones to hopefully find one producing enough biologic (Fig. 1A). Methods that select better-performing clones earlier in the process reduce development time and cost and are of great interest to the biotech industry. Strategies such as GFP-tagging the biologic to make clones flow sortable1-3 have been explored to improve clone selection but have not been widely adopted by industry. Instead, here we present a clone screening approach that combines microfluidic photopolymerization-based cell sorting with quantitative image-based clone prediction (Fig. 1B).

We first developed a method to visually distinguish low- and high-producing cells. Although cells had never been visually distinguished based on secretion, we wondered whether the secretion performance of a cell could be computationally predicted. We stained and imaged intracellular organelles (Fig. 2A) of low (CHO_L) and high (CHO_H) biologic-secreting CHO cell lines obtained from collaborators at Pfizer. We then used quantitative image analysis to translate the imaged cellular phenotypes into a multidimensional cell signature matrix (Fig. 2B), and used support vector machine, a machine learning method, to generate a classifier to distinguish the cell lines. The resulting classifier can accurately classify the two cell populations (Fig. 2C). To test the robustness of the classifier, we performed the classification across independent experiments (Fig. 2D), showing that the classifier is robust.

To use the imaged information for cell line development, we next developed a method to retrieve classified high-producing clones. Existing approaches to cell sorting, such as flow sorting, are inadequate as they do not image. To enable image-based sorting, we developed a photoactivated sorting technology to retrieve the desired cells that uses two sequential photopolymerization steps using complementary photopolymers (Fig. 3A). We first photo-pattern a microwell array out of an optical photopolymer for massively parallel single-cell trapping (Fig. 3B). Using this microwell array we are able to load and image biologic-producing cells. To sort desired cells, we add a second pre-polymer solution onto the microwell array and use spatially patterned ultraviolet light to selectively polymerize the second photopolymer (PEGDA) and encapsulate undesired cells. Desired cells are isolated in the unpolymerized region (Fig. 3C). To retrieve desired cells, we simply sort by washing (Fig. 3D).

1. Booom, Y. G, et. al., Green fluorescent protein as a second selectable marker for selection of high producing clones from transfected CHO cells. Gene 2000, 242, 201-207.
2. Zaaaap, E. G., et. al., Automated in situ measurement of cell-specific antibody secretion and laser-mediated purification for rapid cloning of highly-secreting producers. Biotechnol. Bioeng. 2005, 91 (7), 872-876.
3. Dulll, C. T., et. al., Accelerated clone selection for recombinant CHO cells using a FACS-based high-throughput screen. Biotechnol. Prog. 2007, 23, 465-472.

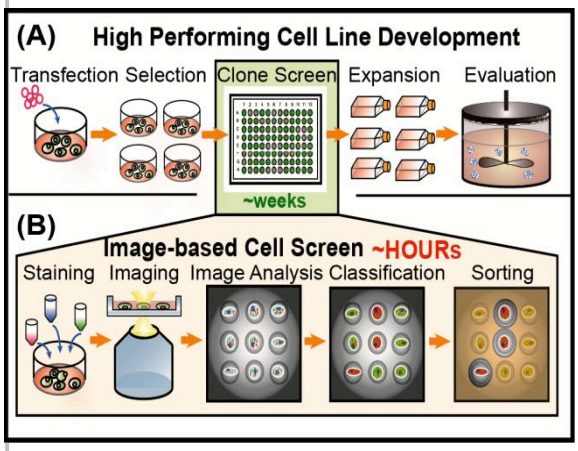

Figure 1: High-performing recombinant antibody clone selection. (A) Industrial cell line development process, requiring 4-6 months. The ability to pick up the ''best'' clones early in the process can decrease development time. (B)To speed up the clone selection, we developed an image-based cell screen method, involving live cell staining and imaging, quantitative image analysis, machine learning classification and microfluidic single cell sorting.

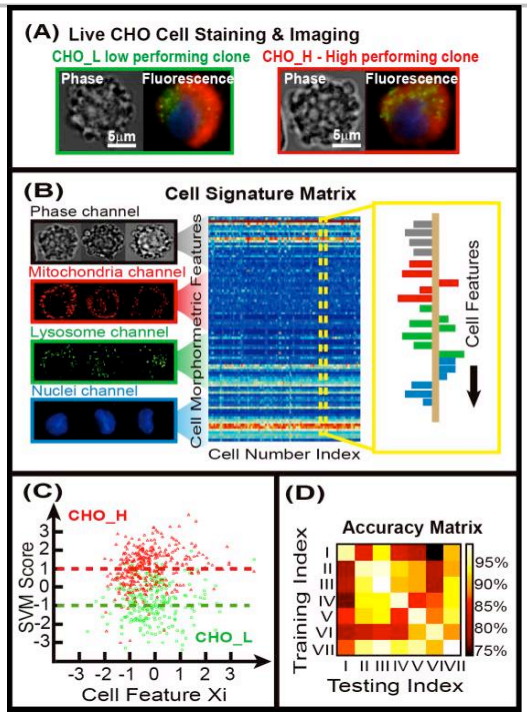

Figure2. Live-cell staining, imaging and classification. (A) Images of CHO cells (B)Image analysis to extract quantitative information from imaged cell organells. (C) Scatter plot showing classification of independent pure CHO_L and CHO_H samples. (D) Accuracy matrix showing the classification accuracy across multiple independent experiments, demonstrating the roubustness of the method.

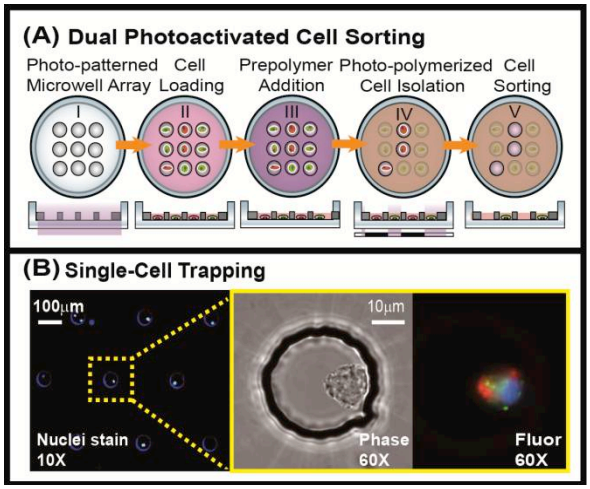

Figure 3. Microfluific single-cell sorting. (A)Dual photoactivated cell sorting pipeline. (B)Image showing trapped single CHO cells in photo-patterned microwell arrays

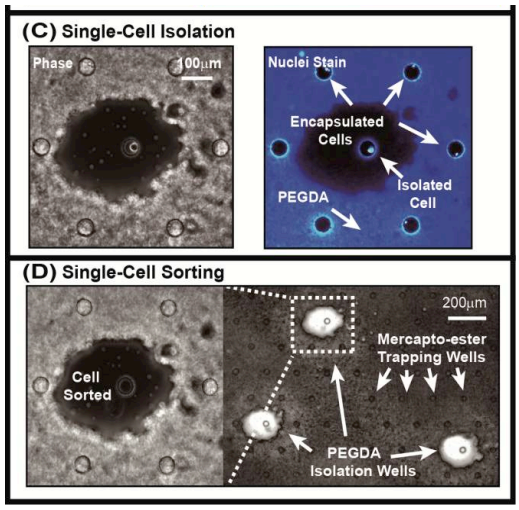

Figure 4. The size of the figures and of the box of . (C) Images showing targeted cells isolated from surrounding cells after photopolymerization. (D) Closed-up and zoomed-out images of PEGDA isolation wells after cell sorting