JOURNAL HIGHLIGHT

A new approach to cell-based multiplexing expands the drug screening capabilities of flow cytometry

Krutzik, P.O. and Nolan, G.P. (2006) Fluorescent Cell Barcoding in Flow Cytometry Allows High-Throughput Drug Screening and Signaling Profiling. *Nature Methods* 5:361–368.

Can high-throughput, high-content flow cytometry be economically applied to large-scale drug screening? Flow cytometry is a widely utilized and powerful method for the analysis of multiple antigens in cell populations. However, the use of flow cytometry in drug screening applications, which can involve hundreds or thousands of samples, can quickly become cost and time prohibitive, due to the amounts of antibodies required and the throughput limitations of cytometers. The authors present a cell-based multiplexing approach—fluorescent cell barcoding (FCB)—that uses varied staining intensities to allow the analysis of complex samples in a single flow cytometry run.

In this technique (based on standard phospho flow protocols), cell samples that have undergone an initial treatment (e.g., unstimulated, stimulated, and stimulated in the presence of an inhibitor drug candidate) are "barcoded" by general staining with different levels of a reactive fluorophore (Pacific Blue™, Alexa Fluor® 488, Alexa Fluor® 700, and Alexa Fluor® 750 fluorophores were all shown to be effective barcoding dyes). Following barcoding, the samples are then recombined, stained with fluorescently labeled antibodies to detect the effects of the treatment, and analyzed as a single sample. Deconvolution of the results clearly resolves the differentially treated cells into discrete, quantifiable populations. The authors successfully demonstrate the utility of the method for real-world drug screening applications. In an inhibitor-titration experiment using the Pacific Blue™ fluorophore as the barcoding dye, U937 monocyte cells were pretreated with four small-molecule inhibitors of JAK kinases, then stimulated to induce pStat1, pStat3, and pStat5 production. The effect of these inhibitors on the degree of phosphorylation of the three Stat transcription factors was clearly revealed in a single flow cytometry run.

In a separate experiment—a 96-well plate–based drug candidate screening application—the authors employed a three-dye FCB barcoding scheme to label 96 samples. They used this scheme to screen a library of 70 small-molecule inhibitors for their effect on T cell receptor–mediated ERK phosphorylation/Stat1 phosphorylation in response to interferon-γ (IFN-γ) treatment. This screening experiment was completed in a single 5 minute flow cytometry run, and identified two compounds that selectively inhibited one or the other pathway and three compounds that nonselectively affected both pathways.

Overall, the authors report up to 100-fold reduction in antibody consumption, with significantly less acquisition time required for complex sample analyses. Owing to its improved throughput and greatly reduced consumption of antibodies, the FCB methodology may prove useful for drug candidate screening as well as for clinical monitoring of patient samples during late-stage drug trials.

Product	Quantity	Cat. no.
Alexa Fluor® 488 carboxylic acid, succinimidyl ester *mixed isomers*	1 mg	A20000
Alexa Fluor® 488 carboxylic acid, succinimidyl ester *mixed isomers*	5 mg	A20100
Alexa Fluor® 700 carboxylic acid, succinimidyl ester *mixed isomers*	1 mg	A20010
Alexa Fluor® 700 carboxylic acid, succinimidyl ester *mixed isomers*	5 mg	A20110
Alexa Fluor® 750 carboxylic acid, succinimidyl ester *mixed isomers*	1 mg	A20011
Alexa Fluor® 750 carboxylic acid, succinimidyl ester *mixed isomers*	5 mg	A20111
Pacific Blue™ succinimidyl ester	5 mg	P10163

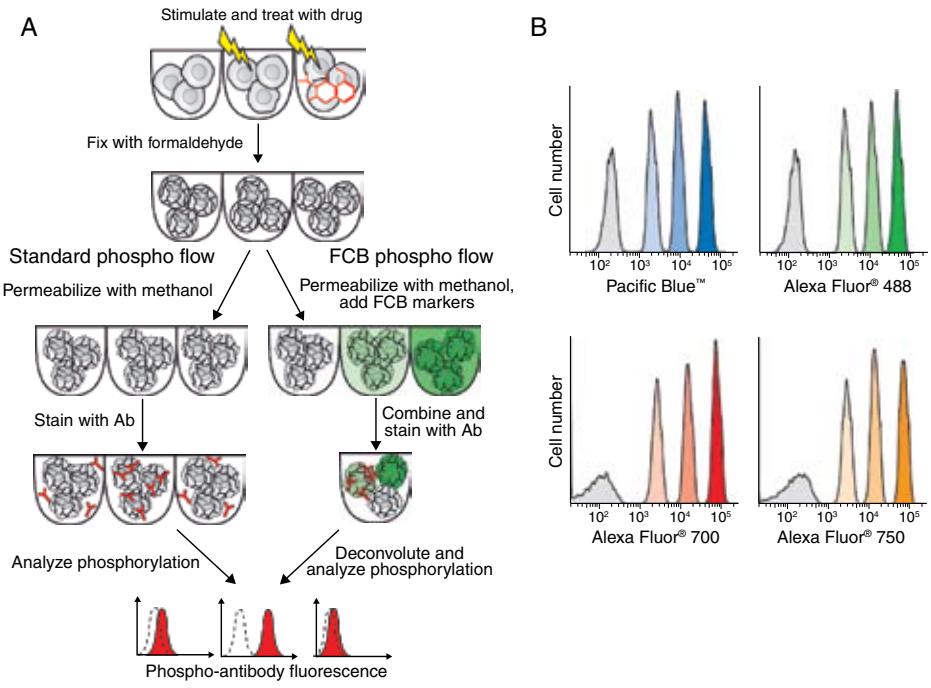

Figure 1—The fluorescent cell barcoding (FCB) technique. (A) Sample one was unstimulated, sample two was stimulated, and sample three was treated with a small-molecule inhibitor before stimulation. After fixation, cells in standard phospho flow (left) were permeabilized with cold methanol, washed, and stained with phospho-specific antibodies. In the FCS technique (right side), each sample was permeabilized with 20–25°C methanol containing a different concentration of amine-reactive fluorescent dyes (FCB markers), yielding a unique fluorescence signature for each sample. Samples were then washed, combined into one tube, and stained with antibodies. During software analysis of the acquired data, the samples were deconvoluted back to the original samples based on their FCB signature. In both standard and FCB phospho flow techniques, fluorescence of the phospho-specific antibody in each sample was measured. In the plots, dotted lines indicate autofluorescence and red histograms represent sample fluorescence. (B) Efficient labeling of four samples per marker with the FCB technique. U937 cells were fixed, then permeabilized in methanol containing 0, 0.04, 0.2, or 1 µg/ml Pacific Blue™–NHS, Alexa Fluor® 488–NHS, Alexa Fluor® 700–NHS, or 0, 0.4, 2, or 10 µg/ml Alexa Fluor® 750–NHS for 15 minutes at 20–25°C. After washing twice, samples stained with each FCB marker were combined and analyzed. Shown are histograms identifying the four original samples barcoded with each FCB marker. Gray peaks represent unlabeled samples (zero FCB marker). Colored peaks represent samples receiving low, medium, and high amounts of the FCB marker, with color intensity correlating to FCB marker staining level. Image reproduced from *Nature Methods* 3:361–368 (2005); used with permission.