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High-content screening: getting more from less

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Abstract

A parallel microfluidic cytometer combines low-pixel-count, one-dimensional images with parallel-channel flow cytometry for high-speed, high-throughput screening of cells.

We often hear the adage ‘he who dies with the most [toys], wins’, but in recent times the opposite saying ‘he who needs the least, wins’ has become popular. These sayings summarize two very different approaches to life based on either maximizing or minimizing acquisition. Likewise, our approaches to science tend to follow divergent paths. On one hand, instruments and technologies are developed to capture as much data as possible with the need for complex data analysis and/or subsequent data reduction to identify the salient data that can be used to address the pertinent questions. On the other hand, formulating focused, minimalistic approaches to gather only the most pertinent data for specific questions can be a powerful approach as well. In this issue of *Nature Methods*, Daniel Ehrlich and colleagues provide an example of the latter by using low-pixel-count, one-dimensional (1D) imaging (Fig. 1) combined with parallel microfluidics¹. With less information acquired from each cell, data files are more manageable, analysis is easier, and throughput can be increased beyond traditional high-content screening (HCS) analysis.

Contemporary approaches to drug discovery frequently use a combination of high-throughput screening and HCS or both. High-throughput screening measures the fluorescence of cellular features tagged with fluorochrome-conjugated antibodies or other fluorescent labels in a very rapid manner and is quite often performed by flow cytometry, a technique particularly well suited to this endeavor. Traditional flow cytometry can easily capture emissions from ten or more different fluorochromes on a cell at rates exceeding 25,000 cells per second. Although this is appropriate for many assays, flow cytometry cannot provide adequate information on morphology or subcellular localization of fluorescence. Manual microscopic examination of fluorescence staining can provide information of these features but suffers from both subjectivity and low throughput.

HCS is a technique that relies on automated analysis of cell images to facilitate drug discovery and other biological research and, in general, is designed to capture as much image information regarding a cell as possible. The availability of commercial imaging systems has made HCS increasingly practical to perform and has catapulted HCS into the mainstream of drug discovery². The value of this approach is beyond question, with a vast number of features of individual cells captured in large data-rich files, although the throughput of HCS is constrained by the size and complexity of these files.

Over the past decade advances in hardware, software and biologic probes have led to very practical automated image analysis with throughput far above that possible by manual analysis. Several commercial instruments are now available for HCS, including the BD Pathway (BD Biosciences), IN Cell Analyzer (GE Healthcare), ImageXpress Ultra (Molecular Devices) and Scan[^]R (Olympus) to name a few. The Imagestream (Amnis) is a relatively new instrument that combines aspects of flow cytometry with image analysis, resulting in a device capable of imaging cells in multiple fluorescent colors with a throughput of hundreds of cells per second³. In HCS devices, there are tradeoffs among the quantity (and quality) of data acquired and throughput as well as file sizes and intricacy of data analysis. The Imagestream, for example, trades ultrahigh image resolution for speed, and, as many cellular images are captured in one data file, the size of one data file can easily be 3 gigabytes or greater. The complexity and volume of the data is often the limiting factor in HCS⁴.

McKenna *et al.*¹ offer a new and intriguing approach to HCS by combining elements of flow cytometry, microfluidics and 1D imaging of single cells into a device termed a parallel microfluidic cytometer. Using photo-multiplier tubes rather than charge-coupled device (CCD) imagers, the authors measure fluorescence at one-micrometer increments across a flow channel and construct a 1D image of the cell using less than 0.001% of the amount of data from a typical CCD image. They can make simultaneous measurements of four fluorochromes, thus producing a multi-color 1D image of each cell when overlaying these measurements. Through the use of 384 parallel channels and confocal laser scanning, the throughput of this device can be several thousand cells per second.

Although the device captures much sparser images of cells than are obtained with CCD technology, the reduced amount of data is advantageous in terms of file handling, storage and analysis. The key question is this: does sufficient information remain to provide information regarding patterns of the fluorescent probes? Eighty two scan features can be calculated for each 1D image, including those showing maximum signal strength, maximum signal position, distribution of the signal on each side of the center and ratios of various features relative to one another.

McKenna *et al.*¹ provide compelling evidence that the 82 features calculated via 1D imaging provide sufficient information to be useful in specific HCS assays such as monitoring the translocation of NF- κ B⁵ and aggregation of α -synuclein-GFP⁶. For example, in the translocation assay, cells are stained with one fluorochrome to stain the entire cell and with a second fluorochrome to stain NF- κ B specifically. Similarly, the α -synuclein assay uses two fluorochromes to study whether the α -synuclein-GFP is diffuse and membrane-associated or present as focal aggregates. By overlapping the 1D images of two colors to compare their distribution, one can determine whether NF- κ B or α -synuclein is distributed diffusely or is focal.

Clearly the use of this parallel microfluidic cytometer device will not supplant more detailed 2D or even 3D imaging in all applications, but in others, 1D imaging provides sufficient information regarding the distribution of fluorescence staining in cells for meaningful conclusions to be drawn. Each application for this device will require careful assessment of the number of ambiguous images obtained as well as the development of assay-specific algorithms. However, this device should prove highly useful for HCS where carefully designed assays are constructed to 'live with the least'.

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Figure 1. Reducing the dimensionality of image data results in sparser images with smaller data files and simplified analysis. Coupled with parallel microfluidics, this approach allows high-throughput, high-content screening.