IMMUNOLOGY

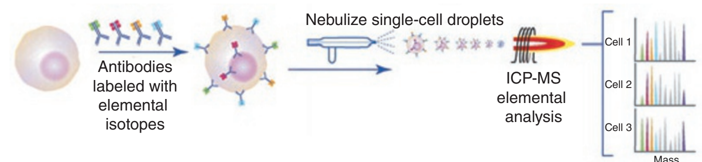
A flow cytometry revolution

A new flow cytometry technology based on mass detection allows a very large number of parameters to be simultaneously measured in single cells.

The flow cytometer is probably the most important tool in an immunologist's toolbox. This high-throughput technique allows multiple markers to be analyzed in thousands of single cells in just seconds. Antibodies targeting markers of interest are tagged with different color fluorophores, allowing the markers to be quantified and thus yielding insights into what defines a cell subtype or activation of a specific pathway in a cell subtype.

The number of markers that can be simultaneously analyzed, however, is inherently limited by the problem of fluorescence spectral overlap. Beyond three fluorophores, corrections for spectral overlap are needed. Corrections for cellular autofluorescence are also needed. Even with such corrections, the practical multiplexing limit of flow cytometry is about ten markers.

A new technology called mass cytometry overcomes these limitations. Developed by Scott Tanner of the University of Toronto, mass cytometry uses mass, rather than fluorescence, as the readout. Antibodies are labeled with chelators that bind specific heavy-metal isotopes. These isotopes are detected and quantified with a highly sensitive readout technique called inductively coupled plasma mass spectrometry (ICP-MS). This has several important advantages. The problem of spectral overlap is completely eliminated by detecting tags with distinct isotope masses, and the method is more quantitatively accurate. Also, heavy metal isotopes are not naturally found in biological systems, so there is no need for background correction. Up to 100 different markers can, in theory, be simultaneously measured, so a much higher density of information can be gathered in an experiment. The technology is commercially available as an instrument called CyTOF. "It's the easiest machine I've ever worked with," says Garry Nolan of Stanford University. "It makes 35-parameter experiments as easy to do as 3-parameter ones."



Schematic of mass cytometry. Antibodies labeled with elemental isotopes bind to cell markers. The single cells are vaporized by ICP-MS, and the antibody labels are detected and quantified for each cell. Reprinted with permission from the American Association for the Advancement of Science.

Nolan has long studied immune signaling, in which flow cytometry is a crucial tool for identifying distinct cell lineages and tracking how molecular markers change during differentiation. After being invited by Tanner to test the CyTOF, Nolan was convinced that mass cytometry would revolutionize the field. Nolan and colleagues now report a comprehensive proof of principle of mass cytometry, using it to analyze immune signaling in healthy human bone marrow samples. They simultaneously measured 34 parameters in single bone marrow cells, including 13 core surface markers, 18 subset-specific cell-surface markers or 18 intracellular epitopes reflecting signaling states and three general cell features—for a total of 52 unique parameters. The ability to monitor many parameters simultaneously allowed them to obtain much more detailed insights into human hematopoiesis than previously possible.

Although using the CyTOF may be simple, analyzing the data that comes out of it is far from trivial. "You're measuring millions of cells, and you could measure a hundred parameters per each of those cells, but those millions of cells are not homogenous," explains Nolan. "That leaves you with a representational problem that no one had to deal with before." Two-dimensional dot plots were not sufficient to capture the extreme complexity of the datasets. Nolan's team therefore adopted a method originally developed by Sylvia Plevritis, also at Stanford University, for ordering information in gene expression datasets. This approach, spanning-tree progression analysis of density-normalized events, (SPADE), allowed the multidimensional data to be displayed in a visually intuitive two-dimensional tree-like structure, which shows the relationships between cell clusters based on marker expression.

The researchers also looked at the signaling dynamics of the 18 intracellular markers in response to various stimuli and kinase inhibitors. This showed that mass cytometry should be useful for identifying drug off-target effects and determining new therapeutic strategies.

There is still room for improvement of mass cytometry technology. Whereas a head-to-head comparison with fluorescence-based flow cytometry showed that the results were comparable, mass detection is not quite as sensitive as the best fluorophores. The technique is also highly dependent on having good antibodies, which take time to validate and may not be available for all targets. It also cannot replace fluorescence-activated cell sorting because the cells are vaporized in the ICP-MS detection process.

Still, future developments will likely address the sensitivity and antibody availability issues. Because the technique is so simple and so powerful at the same time, Nolan believes that in the near future, researchers in all labs currently using fluorescence-based flow cytometry will switch to mass cytometry. "If the people in my lab are any indication," he says, "the traditional fluorescence flow cytometers we have in the lab are sitting unused."

Allison Doerr

RESEARCH PAPERS

Bendall, S.C. *et al.* Single-cell mass cytometry of differential immune and drug responses across a human hematopoietic continuum. *Science* **332**, 687–696 (2011).

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