Flow cytometer with mass spectrometer detection for massively multiplexed single-cell biomarker assay*

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Abstract: This paper describes the development and application of new metal-tagging reagents and a novel mass spectrometer (MS) detector for a flow cytometer that enables highly multiplexed measurement of many biomarkers in individual cells. A new class of tagging reagents, based on an acrylic polymer backbone that incorporates a reproducible number of lanthanide elements, has been developed. When linked to antibodies that specifically recognize target proteins of interest, determination of the tag elements is diagnostic for the presence and quantification of the antigen. The use of enriched stable isotope tags provides the opportunity for multiparametric assay. The new instrument uses inductively coupled plasma (ICP) to vaporize, atomize, and ionize individual cells that have been probed using the metal-labeled antibodies. The elemental composition, specifically of the metal tags, is recorded simultaneously using a time-of-flight (TOF)-MS that has been specifically designed for high-speed analysis during the short transient corresponding to the individual cell event. The detector provides for well-resolved atomic fingerprints of many elemental and isotopic tags, with little overlap of neighboring signals (high abundance sensitivity) and wide dynamic range both for a single antigen and between antigens.

Keywords: flow cytometer; ICP-MS; metal tags; leukemia cell lines; multiparametric analysis; element-tagged immunoassay.

INTRODUCTION

Astonishing advances in the understanding, and sometimes treatment, of diseases have been enabled with the application of biotechnologies that were developed to advance the Human Genome Project and its postgenome fall-out. It is becoming increasingly apparent that delineation of cell signaling pathways and the genesis of disease states require the simultaneous knowledge of the many genes and proteins that are the machinery of the cell.

Flow cytometry (FC) is an analytical art that recognizes a limited number (typically 4, though as many as 17 have been reported [1] with heroic effort) of proteins in single cells at high throughput (typically 600, but up to 30 000, cells per second). The method requires the availability of a suite of non-cross-reactive antibodies that are specific for each target antigen, and these antibodies are tagged with...
fluorescent moieties. Cells are caused to flow individually through an interrogation region where they are exposed to laser excitation. Forward and side scatter of the laser light informs on the size and granularity of the cells, and capture of the fluorescent emission from the tags reports on the presence and, at least qualitatively, on the number of each antigen probed. In the fluorescence-activated cell sorting (FACS) configuration, this information is rapidly and automatically assessed to determine if the cell signature corresponds to a predetermined characteristic, whereupon the cell is ejected from the flow stream, charged and deflected into a capture cuvette, whereby purification of the cells having the desired protein signature is achieved. The conventional FC method is limited in the number of simultaneous antigens that can be probed because of mis-match of the laser and excitation wavelengths and, more importantly, because of the overlap of the emission spectra. An increase in the number of fluorophores used requires rigorous, complex, and increasingly uncertain compensation (mathematical correction). Quantum dots [2] have been promoted as a solution to this conundrum, but in fact technological limitations result in emission spectra that are only slightly improved, if at all, from organic fluorophores.

Massively multiparameter assay of genes and proteins averaged over an ensemble of cells is provided by array technologies, and these have found enormous acceptance in biological research laboratories. A sample whose average biomarker signature is of analytical interest is lysed and applied to a 2D array of many (e.g., 60,000) spots, each of which contains an antibody (for proteins) or anti-sense oligonucleotide (for genes) that captures the biomarkers of interest. Secondary staining with a fluorescently labeled probe yields a 2D fluorescent image that can be deconvoluted to report on the presence and at least qualitative copy-count of the target biomarkers. The method is expensive (largely because of the rigor of cross-referencing the array during production) and time-consuming to read.

It is evident that a less-expensive and fast-reading analog of the array technologies would be of interest. A number of commercial platforms (e.g., from Luminex and BD Biosciences) provide an approach to this goal through the use of fluorescently labeled beads. Imbibing the beads with two fluorophores in quantized concentrations that can each be distinguished at 10 concentration levels allows for the preparation of 100 distinguishable beads. If each bead is functionalized to bind a different antibody or oligonucleotide, and the beads are mixed with a cell lysate, the beads will bind the corresponding biomarker that is identified by the bead signature. Sandwiching the biomarker with a secondary probe that carries a (third) reporter fluorophore allows determination of the presence and at least qualitative assay of the copy count of the biomarker. The beads thus treated can be analyzed by a three-color FC at rates up to several 1000 per second. Because production of the beads is less expensive than cross-referencing the 2D array, the method provides a step toward the goal of a cheaper and faster array analog.

Of even more interest would be a technology that offers the capability to perform absolute quantitative analysis of many proteins simultaneously in a single cell, at high throughput. Such a device will provide a dramatic improvement for the determination of cell transformation pathways. Further, the detailed biomarker signature of individual cells allows a diseased (or healthy) cell to be distinguished and identified within a complex sample matrix of other cells, much as the integral of a fingerprint pattern distinguishes one person from another. As this dream comes to reality, it can be projected that diagnosis of disease will be able to be performed earlier and with better confidence through recognition of the rare disease cells (e.g., cancer stem cells) in a patient’s blood sample.

A novel element-tagged immunoassay technology [10–14] that employs inductively coupled plasma-mass spectrometry (ICP-MS) detection of element-containing tags appears poised to answer the multiplex need. Some immunochemical tags containing elements are commercially available. Results for immunoassay linked with ICP-MS detection have been reported using nanogold particles (Nanoprobes Inc.; usually used for microscopic detection) [3,4], lanthanide-enhanced chelation tags (DELFIA, Perkin Elmer; used for fluorescence assays) [5–7] and diethylenetriaminepentaacetic acid (DTPA)-chelated elements (commonly used for delivery of radio-isotopes for radio-immunochemistry, imaging, or therapeutics) [8]. Each of these has a deficiency for sensitive ICP-MS assay. Nanogold particles, while containing many atoms of the element (approximately 67 for a 1.4-nm particle), provides...
a challenge for washing and memory in the sample introduction system, resulting in elevated background signals. The DELFIA products, while offering improvement in optical detection due to enhanced fluorescence of the metal chelate, and typically containing 6–8 atoms of the element, provide less than optimum sensitivity because the element linkage is designed to allow transfer to the chelator, and thus is somewhat less resistant to the rigorous washing required for low backgrounds in the ICP-MS application. DTPA (or DOTA, 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) are strong chelators ($K_D \sim 10^{-16}$), but provide only one atom of tag per attached chelator: this limitation has been addressed by Bettmer et al. [9] through attachment of many DTPA chelators through binding to cysteine, but this approach is not universal and requires validation of the retention of antigen binding efficiency.

We report here on the current status of developments in our laboratory to produce reagents and instrumentation that enable massively multiparametric FC analysis of individual cells at throughputs approaching 1000 cells per second. The approach takes advantage of the many available enriched stable isotopes as tags, combined with the high resolution of mass detection of the ICP-MS [more than 6 orders of magnitude abundance sensitivity for quadrupole instruments, and approximately 4 orders of magnitude for time-of-flight (TOF) analyzers]. Our method uses a universal polymeric tag linked to individual antibodies, where the polymer has the ability to strongly bind multiple atoms of a given enriched stable isotope. Addition of a different isotope to each antibody, combination of the distinctly tagged antibodies, complexation of the probe suite with the sample, and removing unbound tagged antibodies associates a given isotope with a specific antigen. Quantification of the stable isotope using ICP-MS detection provides simultaneous quantitative determination of antigens. Two formats of analysis are facilitated. Where the average biomarker signature of the ensemble of cells is of interest, the tagged cells are degraded in acid and the elemental composition, specifically of the tag isotopes, of the resultant homogeneous solution is measured by conventional ICP-MS (bulk analysis). Where individual cell analysis is required, the whole tagged cells are introduced individually into the ICP and the transient signals corresponding to each cell ionization event are recorded using a novel high-speed ICP-MS in the TOF configuration (flow cytometry with mass spectrometer detection, FC-MS).

The new approach allows for the simultaneous analysis of many biomarkers in single cells, but clearly does not offer the opportunity for enrichment or purification: the cells are consumed in the analysis. However, simultaneous tagging with fluorophore and element tags does permit pre-purification by conventional FACS followed by multiparametric analysis by FC-MS, a tandem approach that in some instances may improve the overall throughput of the method.

### MATERIALS AND REAGENTS

Phosphate-buffered saline (PBS) with calcium and magnesium (PBS; 150 mM NaCl, 1.2 mM Ca$^{2+}$; 0.8 mM Mg$^{2+}$; 20 mM sodium phosphate, pH 7.4); concentrated 34 % HCl (Seastar Chemicals Inc.); 37 % formaldehyde (Sigma); Triton X-100 (Sigma); Ir (iridium) and In (indium) diluted from stock 1000 µg ml$^{-1}$ solutions (SPEX) to 1 ng ml$^{-1}$ in 1 % HCl v/v. All solutions were prepared in deionized water (DIW) (Elix/Gradient water purification system, Millipore).

**Antibodies**

Primary monoclonal and polyclonal antibodies to cell surface antigens were obtained from commercial suppliers (BD Biosciences, Biomeda, Immunotech, and Leinco). Species-specific isotype immunoglobulins were used for negative controls. Antibodies were labeled with the prototype MAXPAR™ reagents (DVS Sciences, Inc., Richmond Hill, Ontario, Canada; <www.DVSsciences.com>), based on metal-conjugated polymer tags described in detail by Lou et al. [7]. Chloride hydrates of La, Pr, Tb, Ho, and Tm (Sigma-Aldrich) were used for naturally high abundant isotopes. Oxides of the enriched (>98 %) isotopes $^{142}$Nd, $^{144}$Nd, $^{145}$Nd, $^{146}$Nd, $^{147}$Sm, $^{152}$Sm, $^{151}$Eu, $^{153}$Eu, $^{156}$Gd, $^{164}$Dy, $^{166}$Er, $^{170}$Er,
171Yb, 174Yb, 176Yb) (Trace Sciences International Corp., Richmond Hill, Ontario, Canada) were converted to chlorides for use in labeling protocols.

Cell lines
Model cell lines representing early and late human acute myeloid leukemias, KG1a and THP-1, were used for biomarker analysis. Cells were obtained from the American Type Culture Collection (Manassas, VA) and propagated under standard tissue culture conditions.

Metallointercalator
Numerous studies have been devoted to the investigation of binding of transition-metal polypyridyl complexes with DNA molecules. The Ir- and Rh-containing metallointercalators were prepared in accordance with the published procedure [15] and are indefinitely stable in the solid state and in aqueous solution. Their use and application has been previously reported [16].

Analytical method
Two assay types are employed here. For relatively homogeneous samples, for which the concentration of each biomarker averaged over the ensembles of cells is desired, solution analysis by conventional ICP-MS is appropriate. Where the analysis of each cell or particle is to be conducted, a novel high-speed ICP-TOF-MS instrument, FC-MS, has been developed. The sample preparation for both assay types is similar and has been reported [16–19]. Briefly, an acrylic polymer is functionalized to covalently attach approximately 30 DTPA chelators, and is conjugated to a bismaleimide linker [7]. The disulfides in the Fc fragment of antibodies are reduced under mild conditions with TCEP [(tris(2-carboxyethyl)phosphine hydrochloride; Pierce], which allows attachment of 2–4 polymer tags. Each tagged antibody is mixed with a different isotope chloride to chelate approximately 30 atoms of the isotope to each polymer tag (60–120 isotopes per antibody). The panel of tagged antibodies against cell surface markers are combined and allowed to react with the suspension of sample cells, after which stringent washing removes unbound tagged antibodies. The cells are then fixed (formaldehyde) and permeabilized (methanol) and mixed with the panel of intracellular-staining antibodies and metallointercalator. The sample is thoroughly washed and pelletized. For solution analysis, the pellet is dissolved in 34 % HCl and an equal volume of 1 ppb In internal standard in 10 % HCl, and the homogeneous solution is analyzed by ICP-MS. For cytometric analysis, the pellet is resuspended in buffer, and the resultant slurry is nebulized into the FC-MS, typically using a TR-30 concentric nebulizer at 25 µl/min uptake combined with a low-volume on-axis spray chamber. A flow diagram for the immunochemical procedure is provided in Fig. 1.
Inductively coupled plasma-mass spectrometry

Solution analysis was performed on a commercial ICP-MS instrument ELAN DRC™ Plus (Perkin Elmer SCIEX) described elsewhere [20] and operated under normal plasma conditions (plasma power: 1400 W; nebulizer Ar flow 0.95 L/min; plasma gas Ar flow 17 L/min; auxiliary gas Ar flow 1.2 L/min; CeO+/Ce⁺ ratio in 10 % HCl < 3 %). The sample uptake rate was adjusted depending on the particular experiment and sample size, typically 100 µl/min. A MicroFlow PFA-ST concentric nebulizer (Elemental Scientific, Inc) was used in all instances. ICP-MS measurements were performed using an autosampler (Perkin Elmer AS 91) modified for operation with Eppendorf 1.5-ml tubes. Sample size varied from 150 to 300 µl. Standards were prepared from 1000 µg/mL PE Pure single-element standard solutions (Perkin Elmer, Shelton, CT) by sequential dilution with high-purity DIW produced using an Elix/Gradient (Millipore, Bedford, MA) water purification system. The analyte signal was normalized to the signal of the internal standard of 1 ng ml⁻¹ In added during sample preparation or to the Ir-intercalator signal.

Flow cytometer mass spectrometer

For analysis of individual particles, the FC-MS instrument was operated at 55 kHz spectrum generation frequency. Data from 30 000 consecutive single spectra were collected within ~0.5 s, then compressed and recorded within further 0.5 s, after which the system was ready for the next batch of 30 000 consecutive single spectra. After recording the compressed data for 1000 such cycles (e.g., 16 min. compressed single spectra were collected in ~30 min), data were further processed off-line for cell event detection and outputting integrated per cell responses for selected isotopes. The characteristics of the prototype instrument include: resolution (full width at half maximum) at m/z = 159 m/Δm = 600, sensitivity in standard sample aspiration mode of 8 × 10⁷ counts per second per µg ml⁻¹ of Tb, abundance sensitivity of 2 × 10⁻⁴ [the ratio of the ion current recorded at mass (m ± 1) to that recorded at the nominal mass m of an element]. The dynamic range of affinity reagent detection per cell is currently limited by the microchannel plate (MCP) detector comprised of a chevron assembly of two microchannel
plates (40/12/8 60:1, BURLE Electro-Optics, Inc., Sturbridge, MA) and the signal digitizer, and is approximately $10^3$.

RESULTS AND DISCUSSION

A major goal of our work has been to develop a universal antibody tag that is optimized for ICP-MS determination. One successful approach has been reported [7]. Briefly, a linear acrylic acid polymer, having low chain-length dispersity and containing a reproducible number of activated NHS ($N$-hydroxysuccinimide) ester groups is produced, for example, by reversible addition-fragmentation chain transfer (RAFT) or anionic polymerization. Functionalized DTPA or DOTA are reacted with the polymer to attach a reproducible number (e.g., 30 for the DTPA polymer) of chelators to the polymer chain. The polymer is end-functionalized with a thiol that reacts with bismaleimide, yielding a chelate-containing polymer that can be linked to antibodies through reduced disulfides in the Fc fragment. After linking to the antibody, a solution of a lanthanide chloride is added, resulting in the strong chelation of the lanthanide isotope. The metal-labeled antibody thus produced is highly resistant to cross-exchange with other labeled antibodies (storage of the labeled antibody in PBS buffer for 9 days resulted in no detectable leaching of the element tags into solution) that is resistant to rigorous washing, and contains a reproducible number of metal atoms per polymer tag. The major steps in the synthetic protocol for the polymer tag are shown in Fig. 2, and a flow diagram for the antibody labeling method is shown in Fig. 3. Typically, between 2 and 4 polymers, each containing typically 30 metal atoms (for the DTPA polymer) may be linked to the Fc fragment of a given antibody, resulting in 60–120 tag isotope atoms per antibody.

![Fig. 2 Synthesis of the element tags. An acrylic acid polymer having low chain-length dispersity is functionalized with NHS reactive groups at regular intervals. Functionalized DTPA appends chelators to the reactive groups. An end-terminus thiol allows linkage to bismaleimide which in turn complexes the polymer to reduced thiols in the Fc fragment of the antibody. The unlabeled Ab-tag can be stored and labeled with enriched lanthanide elements prior to application to the sample.](image-url)
The recognition and quantification of DNA in whole cells is an important tool in bioanalysis. Conventional means include incorporation of BrdU (5-bromo-2'-deoxyuridine) and 3H-thymidine into replicating live cells. Intercalating dyes, such as Hoechst 33258, propidium iodide, 4',6-diamidino-2-phenylindole (DAPI), and acridine orange, which insert between the base pairs of DNA, have been successfully employed in fluorescent FC to enumerate cells. Metallointercalators, which incorporate a metal such as Rh, Ru, Os, Co, Re, or Ir, have been used as sensitive molecular probes and as therapeutics, and have a rich presence in the literature [21–37]. We have reported on Ir- and Rh-containing metallointercalators that can be used in conjunction with ICP-MS analysis to enumerate cells (act as an internal standard in bulk assay) or as trigger for data acquisition corresponding to the vaporization of whole cells [16]. The structures of a Rh-intercalator, bis(phenanthrenequinonedimine)(bipyridyl) rhodium(III), and an Ir-intercalator are shown in Fig. 4. Also shown in that figure is a collection of transient Rh signals from the intercalator associated with separate cells. Two important points are evident. First, the duration of the transient from a cell ionization event is of the order of 200–400 µs. This places a considerable constraint on the data acquisition speed of an ICP-MS detector that is intended to measure many elements simultaneously during that transient; where it is important to recognize the shape of the transient (point 2 following), it is apparent that at least 10 (or preferably 20) full mass spectra should be recorded and archived during the transient, which proposes a scan rate of at least 50 000 (preferably at least 100 000) per second. Secondly, the shape of the metallointercalator transient informs on the integrity of the cell vaporization event: a whole cell event appears to be characterized by a more-or-less
Gaussian transient, whereas cell fragments may appear as an extended or even discontinuous transient (e.g., cell #5 in Fig. 4). Further, except in the instance of complete concomitance, the appearance of a duplex cell event (two or more cells agglomerated or appearing together) can be expected to be evidenced by an overlap of two or more Gaussian transient profiles; in this instance, conventional FC wisdom advises to disregard such data.

We report here for the first time the results of 20-parameter measurements in the bulk analysis mode. KG1a cells were stained for 20 cell surface markers, and the DNA was stained with the Rh-metallointercalator. Degradation of the sample in HCl with Ir internal standard provided a homogeneous solution for introduction to a conventional ICP-MS instrument. Each 200-µl sample was aspirated at 100 µl/min, and analyte signals corresponding to the tag and internal standard elements were measured for approximately 1 min during the plateau of the transient signal. All analyte signals were normalized to the intercalator signal, which effectively accounts for variability of the number of cells in each sample. The results are presented in Fig. 5 in the form of a logarithmic polar plot, formatted for increasing cell surface marker response (normalized to the intercalator) for the KG1a cell line. Of significant interest is the relatively high concentration of the CD34 cell surface marker, which is characteristic of undifferentiated cells having stem cell character. A similar experiment was conducted for the more differentiated THP-1 cell line, also presented in Fig. 5, which clearly shows the relative suppression (by about a factor of 50) of the CD34 antigen. Results for a patient sample, indicated in Fig. 5 as BCLQ (Quebec Leukemia Cell Bank), show further suppression of the CD34 marker and other characteristic antigen expression differences. While there is little basis for a direct comparison of such simul-
Simultaneous analysis, because this is the enabling technology for such assays, a good correlation for the BCLQ sample was observed with data for separate antigen measurements by conventional FC provided by the BCLQ.

Also presented here for the first time are data obtained with the prototype FC-MS instrument. Figure 6 shows a screen capture of 5.4 ms of raw digitized data for slurry nebulization of KG1a cells stained against seven cell surface markers (anti-CD7, -CD13, -CD44, -CD45, -CD38, -CD34, and -CD49d) and with Ir metallointercalator. The vertical scale displays scan number for sequential single TOF scans recorded at 18 µs intervals, and the horizontal scale displays ion TOF for each orthogonal extraction pulse which corresponds to ion mass. The presence of a pixel indicates the appearance of an ion above a preselected signal at a particular TOF in a particular single scan. Lighter-color vertical bars highlight the mass channels corresponding to the metal tags used. It is clear that the registration of ion signals appears in groups, usually consisting of approximately 20 consecutive scans (about 350 µs) and with most tags appearing in the same scan window. An exception is the intercalator, for which a significant pseudo-continuous background is observed for nonspecific binding punctuated with significantly greater density correlated to the appearance of the metal tags. The concomitance of the metal tag and intercalator signals and the similarity of the duration of the ion groups to the period of a single cell event (per Fig. 4), suggests that these clusters of ions correspond to a single-cell ionization event. The lighter-colored horizontal bars highlight two such events in this screen capture. Off-line analysis confirms that the metallointercalator signals bear the Gaussian trademark of a single-cell ionization event, and this triggers the collection and compression of the data.

Fig. 5 Simultaneous 20-parameter analysis of cell surface antigens in bulk mode. Cells from cultures of KG1a and THP-1 lines, and a patient sample, were separately probed with a 20-member antibody panel tagged with the enriched isotopes shown. Raw signals for the tag elements are normalized to the metallointercalator signal and displayed in a logarithmic polar plot. The tag fingerprint allows distinction of the cell types.
corresponding to each cell event. The N-parameter data thus collected is processed off-line as a series of \((N - 1)^2/2\) 2D scatter plots correlating antigen signals in pairs as is common in FC, as shown in Fig. 7. At the left of each series of plots is the histogram representation of the frequency distribution of individual cell events for the marker that is displayed vertically in each row. A novel display format for the same data is given in the inset of Fig. 7 in the form of a multidimensional parallel-axis plot; each line corresponds to the antigen fingerprint measured for a single cell. Ultimately, it is intended that groups of ions in the scatter plots can be selected (e.g., by drawing a circle around a region of interest), and the selected cells will be highlighted in all scatter plots and in the multidimensional plot, allowing visual distinction of the biomarker fingerprint for those cells from other cells.
The element-tagging approach also allows for a high-throughput cytometric analog of a 2D gene/protein array [38]. Beads can be constructed containing a number of elements in distinctive concentration ratios. Thus, encoding beads with \( n \) elements, for each of which there are \( k \) distinguishable concentrations, yields \( k^n - 1 \) distinguishable beads. For the reasonable values of \( n = 5, k = 8 \), a total of 59 048 distinguishable beads can be constructed; for \( n = 6, k = 8 \) there are 262 143 distinguishable beads. Conceivably, the beads can be functionalized such that each distinguishable bead attaches a different oligonucleotide (for genes) or antibody (for proteins) probe, where the identity of the probe is associated with the bead composition. Exposing the mixture of beads to a cell lysate sample will cause the corresponding gene or antigen to bind to the bead. The biomarker-bead sample can then be counterstained with a metal-tagged “reporter” probe, where the reporter tag element can be the same for all secondary probes. Concomitance of the bead encoding and reporter tag elements for individual beads recorded in cytometric mode reports on the identity (bead composition) and quantity (reporter tag) of each biomarker. Measured at 1000 beads per second, a 30 000 point array equivalent (e.g., 1000 genes with 30-fold redundancy) can be recorded in 30 s. The concept is shown in Fig. 8 for the determination of...

Fig. 7 15 min of data collected in the form of Fig. 6 are compressed into integral signals for the \( N = 7 \) cell-surface marker tag isotopes (all cells are Ir-positive) and displayed in \((N - 1)^2/2\) 2D plots of correlation, where each pair of tag signals for an individual cell are represented by a pixel in scatter plot format. Such displays hide the density distribution of the cell data, so histograms are provided at the left of each row.
The identity of the probe attached to the bead is determined from the bead composition. mRNA will bind to complementary oligonucleotide sequences attached to the bead. The mRNA can be universally recognized through its 5'-poly(A) sequence, allowing a universal reporter construct of a metal-tagged poly(dT) sequence to quantify the hybridized transcript.

Feasibility for the determination of element-tagged beads is shown in the scatter plots of Fig. 9. Beads (1.8 µm amino-functionalized polystyrene, Bangs Laboratories, Fishers IN, part #PA04N/7603) were modified to bind DTPA chelators on their surface. Exposure of the beads to various mixtures of elements encodes the beads according to the concentration and binding efficiency of the elements used. Thus, we prepared two groups of beads, one containing Pr, Eu, and Tb and the other containing Ho and Tm. The beads were analyzed on the prototype FC-MS instrument. The bead signatures largely group into high/low levels corresponding to the bead composition (note that the density of “zero” measurements for absent elements is much greater than for “low” measurements, but scatter plots such as that shown inherently discriminate against zero measurements—perhaps reflecting that fluorescent measurements almost always have a non-zero background, unlike elemental analysis).
CONCLUSIONS

We have shown feasibility for a novel immunochemical assay using stable metal isotope tags combined with ICP-MS analysis. The method can be applied using a conventional ICP-MS detector for bulk assay of cell samples homogenized by acid degradation. A fast-reading ICP-TOF-MS configured to optimize sensitivity for the elemental tags has been prototyped and can be used for the determination of rare individual cells or as a convenient analog of gene/protein arrays. The sensitivity and universality of all of these applications benefit from the use of polymeric tag constructs that bind multiple copies of the tag isotope. Further work will focus on the optimization of the tagging constructs, efficiency of particle (cell or bead) introduction, data compression and archiving, and software for presentation and interpretation of multidimensional data.

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