Review Article

Theme: Emerging Technologies in the BioAnalytical Space Applied to Large Molecule Determinations
Guest Editors: Johanna Mora and Binodh Desilva

Next Generation Ligand Binding Assays—Review of Emerging Real-Time Measurement Technologies

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ABSTRACT. Over the last few years, numerous ligand binding assay technologies that utilize real-time measurement have been introduced; however, an assemblage and evaluation of these technologies has not previously been published. Herein, we describe six emerging real-time measurement technologies: Maverick™, MX96 SPR™, NanoDLSay™, AMMP®/ViBE®, SoPrano™, and two Lab-on-a-Chip (LoC) microfluidic devices. The development stage gate of these technologies ranges from pre-commercial to commercially available. Due to the novelty, the application and utility of some of the technologies regarding bioanalysis are likely to evolve but it is our hope that this review will provide insight into the direction the development of real-time measurement technologies is moving and the vision of those that are taking us there. Following the technology discussions, a comprehensive summary table is presented.

KEY WORDS: acoustic wave mass dampening; dynamic light scattering; integrated microfluidic systems; localized surface plasmon resonance; microring array.

INTRODUCTION

The Real-time Measurement team assesses emerging ligand binding assay (LBA) technologies for their potential practical applications in real-time quantification of biologics and observation of binding events (*e.g.* protein-protein interaction) at the time-point they are detected. The team is comprised of members from different pharmaceutical, instrument, and consulting companies with experience in the bioanalysis of biotherapeutics.

In an effort to increase awareness and share knowledge within the LBA bioanalytical community, the team identified six emerging technologies that have the potential to positively impact LBA that deliver results in real-time. These technologies include: Maverick™, MX96 SPR™, NanoDLSay™, AMMP®/ViBE®, SoPrano™, and two Lab-on-a-Chip (LoC) microfluidic devices. Due to the emergent nature of these single sourced technologies, available literature is limited. In order to provide a

balanced and un-biased report on their potential utility, the team collected the information presented here based on discussions with users and the vendor.

THE MAVERICK™ DETECTION SYSTEM BY GENALYTE

Technology Background

The Maverick, from Genalyte, is a scanning detector that utilizes a proprietary disposable microring array constructed using silicon photonic technology to attain a real-time measurement of ligand binding. The refractive index-sensitive silicon photonic devices comprised of 128 microfabricated rings are arrayed on a single disposable chip in pattern of four per analyte resulting in 32 clusters.

The technology is based upon light being trapped by the sensor and circulated around the ring resonator. This sensor is placed next to a linear waveguide that directs light produced by a laser. The light goes past the ring resonator onto a photodetector. Tuning of the wavelength results in the removal of light by the ring resonator and a subsequent loss of signal to the photodetector. As molecules bind to the ring, this signal shifts the resonant frequency of the microring sensor, which results in the capture of a longer wavelength to maintain resonance. The continuous scanning detects these changes. Use of known calibrants allows for the establishment of concentration-dependent spectral shifts. In turn, this enables detection of unknown concentrations of molecules in samples.

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Samples are prepared in 96-well plates and then placed into the instrument where an automated fluidics system draws 2–5 μL of sample into the specific array, followed by presentation of the specific array to the Maverick detection system. The system is a low labor effort compared to traditional ligand binding assays, with a complete walk away process once the preparatory steps are completed.

Demonstrated Applications of Technology

Genalyte markets a number of kits and offers a custom application service. Applications include a multi-tier anti-drug antibody array, semi-quantitative detection of autoantibodies against extractable nuclear antigens, and a type I diabetes assay. In this latter example, the microrings are initially coupled to pancreatic islet autoantigens which play a number of diverse roles within type I diabetes (see Fig. 1).

Figure 1 outlines the ligand binding format applied to detect the various autoantigens that are bound onto separate microrings (large blue circle). Genalyte claims the set-up is rapid with only two pipetting steps and less than 5 min preparatory time. The detection process occurs over 15 min with the real-time recording of relative shifts as first the autoantigen binds, followed by the antibody (sample), and finally confirmation of that secondary molecule being an antibody through the use of either protein A or G.

Numerous recent reports in the literature have listed the application of this system. In particular, this technology has been embraced by Professor Ryan C. Bailey of the University of Illinois in the Department of Chemistry, resulting in a number of applications and subsequent publications including the detection of specific microRNAs through the use of DNA:RNA heteroduplexes (1); the quantitation of carcinoembryonic antigen in serum with (2); the detection of interleukins 2, 4, and 5 and tumor necrosis factor in cell culture matrix (3); and the detection of multiple full-length mRNA transcripts (4). The detection of biological interactions has been demonstrated utilizing a zwitterionic modified silica microring resonator (5).

Summary

The Maverick Detection Systems by Genalyte is a real-time measurement technology that can be applied to the quantitative detection of a biologic. It provides walk away

operation of detection and analysis with a non-label format using very low volumes of sample, and the ability to multiplex for biomarker applications. Further development, expansion, and other demonstrations of this technology will determine its future roles within the diagnostic and pharmaceutical discovery fields.

MX96™ SURFACE PLASMON RESONANCE IMAGER AND CONTINUOUS FLOW MICROSPOTTER ARRAY PRINTER BY WASATCH MICROFLUIDICS

Technology Background

Wasatch Microfluidics (known as IBIS Technologies in Europe) manufactures a real-time system for the detection of molecular interactions. The system includes a continuous flow microspotter (CFM) array printer, MX96 array imager, and SPRint software to perform surface plasmon resonance (SPR) array imaging.

The CFM is a patented microfluidic printing technology that uses continuous flow to deposit up to 96 biomolecule spots onto the sensor surface as a distinct array. Unlike competing systems which deposit droplets of material at one time, the CFM uses a continuous flow to cycle molecules back and forth over the surface, yielding optimal binding from crude and dilute solutions. IBIS reports this technology will provide significant enhancements in sensitivity and will print at concentrations much more dilute than competing technologies.

The MX96 Imager utilizes the scanning angle principle to overcome spot to spot sensitivity problems and linearity issues related to fixed-angle instruments. In fixed-angle instruments, the applicable range can be limited, and spot to spot sensitivity may vary considerably when ligand or analyte panels having different molecular weights are being monitored. Use of a scanning angle system allows for a more accurate detection of angle shifts which corresponds to mass change more linearly, thus allowing for the comparison of the magnitude and affinity of biomolecular interactions across 96 spots at one time. The fluidic system is comprised of a flow-cell and two syringe pumps for generating back and forth flow to minimize the amount of sample and reagents needed for a measurement.

The MX96 requires the use of SensEye sensors. These sensors are available in a variety of SPR surface chemistries ranging from gold to pre-activated surfaces and can be used for covalent binding, biotinylated ligands, and directional antibody binding (Protein A or G).

Demonstrated Applications of the Technology

Utilizing the many sensor surfaces provided by Wasatch Microfluidics, along with the evaluation software (SPRint), a researcher can perform several applications from simple affinity and kinetic studies to complex epitope binning and mapping experiments (6). The system has also been shown to be helpful with the development and optimization of biomarker assays (7).

Kinetic analysis of antibodies and other proteins is critical and unlike the estimates of kinetic information derived from IC₅₀ values obtained from ELISA experiments;

Fig. 1. Schematic representation of the structure formed on microring for the type I diabetes assay

real-time kinetic measurements obtained using the SPR imager offer a direct depiction of these molecular interactions (7). Specifically, the Wasatch system offers a complete affinity determination, enabling the end user to accurately obtain kinetic constants, including k_a , k_d , and k_D in a high-throughput fashion across the 96 spots on the sensor surface.

An important step in the development of biotherapeutics and bioreagents is to characterize groups of monoclonal antibodies by regions of epitope binding within target antigens and bin antibodies that target similar epitopes. Wasatch has developed a high-throughput SPR array that allows for 96×96 binning with small sample requirements of 150 μ L per sample. The software tool allows for complete data management, heat map generation, and bin allocations.

Design of Experiment (DoE) is a relatively common approach to developing novel ligand binding assays. The MX96 is uniquely positioned to support well defined DoE experiments because it is able to simultaneously monitor the binding interactions in a 96×96 matrix. A single overnight run can generate up to 9,216 individual sensorgrams (6) significantly reducing optimization time as well as improving assay performance and robustness.

Summary

Surface plasmon resonance as a detection method for molecular interactions has been applied previously with limited throughput and sensitivities. Wasatch Microfluidics has developed emerging real-time methods and instrumentation that allow for the simultaneous analysis of thousands of interactions. Such new instrumentation (MX96 array Imager, CFM array printer, and powerful analysis software) aligned with new real-time methodology to deliver tools that enable the monitoring of 96 ligands with up to 96 analytes, delivering nearly 10,000 sensorgrams in one automated overnight run.

LOCALIZED SURFACE PLASMON RESONANCE INCLUDING SOPRANO™

Technology Background

Light-matter interactions can be measured with plasmonics, a technology that has potentially many applications in sensing biological interactions. Plasmonic materials are already found in commercially available instruments such as Biacore®, which uses thin metal films as the sensing substrate. Although Biacore® has been used to detect the concentration of protein analytes in biological samples (8–10), the technology is not (yet) established as a routine technique to determine concentrations of endogenous analytes in biological samples.

As the fabrication of nanostructures has significantly advanced in recent years, a related technology, localized surface plasmon resonance (LSPR), represents an attractive platform to detect biological interactions and determine concentration of biological analytes. Nanoparticles exhibit LSPR at visible and near infrared wavelength, resulting in strong light scattering at the resonance wavelength which can be detected by a change in the optical density (11). The absorption maxima and intensity of the absorption is highly dependent on the material (*e.g.* gold, silver, or platinum), and

size and shape of the nanostructures (Fig. 2). Additionally, the environment that surrounds the particle has an impact on the absorption maxima. As these particles can be functionalized with biological molecules like DNA or proteins, the nanoparticles become sensors to measure binding to these molecules. Two general applications can be used for LSPR sensing: Localized Surface Plasmon Resonance Wavelength-Shift sensing and Surface-Enhanced Raman Scattering Sensing (12). Soprano™ is a commercial platform in development by Pharmadiagnostics that utilizes LSPR and provides kits to functionalize gold nano rods. The readout is the wavelength-shift induced by binding events on the surface of the nano rod. The shift is quantified by the ratio between $\lambda_{\max+80}$ before and after the binding event. By using different concentrations of analyte, the ratio can be used to compare the relative affinity of different proteins (Fig. 3). The technology can provide real-time data of the binding reaction, enabling determination of K_D and k_a determination.

The Soprano technology for endpoint analysis needs little initial investment in hardware and common lab spectrophotometers that feature a wavelength scanning function suffice to read samples. Ratiometric data collection in real-time can be automated by software and a spectrophotometer available from BMG Labtech, making kinetic data acquisition possible. The technology is currently in development and kits cannot be obtained to date.

Demonstrated Applications of the Technology

Technologies based on LSPR that detect the wavelength-shift have recently been applied to sense biological interactions. Mayer *et al.* used gold nano rods functionalized with rabbit IgG and detected a red-shift when incubated with 30 nM goat anti-rabbit IgG (13). When the substrate was rinsed, the dissociation of the anti-rabbit IgG caused a blue-shift. By continuously monitoring the peak wavelength, both K_{on} and K_{off} were determined and found consistent with typical antigen-antibody interactions.

Fig. 2. Scanning electron microscopy of gold nano rods. *Inset:* Characteristic absorption spectrum of gold nano rod. Adapted from Tang *et al.* 2012, analytical chemistry

Shifts in spectra depicted are for illustrative purposes only

Fig. 3. Schematic representation of the Soprano™ technology. Gold nano rods are conjugated with protein of choice. Upon specific interaction with the protein ligand with the conjugated protein, the LSPR effect leads to a concentration-dependent red shift in the wavelength with maximal absorption ($\lambda_{\max}$) and thus a change in the OD at $\lambda_{\max}+80$. Figure kindly provided by Pharma Diagnostics

The assay sensitivity could be refined by using gold nano rods in conjunction with magnetic nanoparticles (14). The authors focused on building a sensitive assay to human cardiac troponin I (cTnI). Gold nano rods were functionalized with an antibody to cTnI, and magnetic nanoparticles were labeled with a second, non-competing, cTnI antibody. The magnetic nanoparticle was mixed with human plasma, and the cTnI-magnetic nanoparticle complex was extracted by a magnet. The washed and enriched complexes of the magnetic nano rod and cTnI were incubated with the gold nano rods and the absorption spectrum was measured with a standard UV-Vis spectrophotometer. The group demonstrated that compared with gold nano rods only, the combination of gold nano rods with magnetic nanoparticles lead to a 6-fold amplification in LSPR response, resulting in a sensitive assay with a limit of detection of approximately 30 pM in plasma samples (Fig. 4). As the assay included a standard curve, quantification in patient samples might be possible. Due to the use of only standard lab instrumentation, the principle might be amenable to implementation in clinical laboratories with a sample turnaround in less than 1 day. The magnetic extraction appears attractive as blood might be used directly without preparation of plasma or serum.

Summary

Surface plasmon resonance is an attractive technology to collect kinetic information of binding events and to determine analyte concentration. No label is necessary. So far, the technology has been commercialized in the Biacore® platform; however, its use to determine analyte concentration in biological samples is not widespread, probably due to cost and sample throughput. Localized surface plasmon resonance utilizes nanoparticles to sense binding events and is scalable to 96-well or 384-well plates. Since the required hardware is only a standard laboratory spectrophotometer, the technology could be adopted without much upfront investment. Amplification of the signal by secondary antibodies labeled with magnetic particles is feasible, possibly making the technology attractive in high sensitivity applications.

THE NANODLSAY™ BY NANO DISCOVERY, INC.

Technology Background

NanoDLSay is a label-free analytical technique for chemical and biological detection and analysis that monitors

Fig. 4. Effect of magnetic particle enhanced LSPR on sensitivity, dynamic range, and precision of cTnI assay in 40% diluted human blood plasma. **a** Standard curve of LSPR shift as a function of cTnI concentrations without magnetic nano particles (MNPs). **b** Standard curve calibration for MNP-enhanced LSPR assay, showing an improved linear relationship between the cTnI concentrations and the LSPR shift resulting from specific binding of the magnetic nanoparticle-cTnI complexes. Adapted from Tang *et al.* 2012, analytical chemistry

changes in size of a gold nanoparticle that occurs with binding of target analyte (Fig. 5). The name of the technology comes from “gold *nanoparticle* coupled with dynamic light scattering (DLS) for bimolecular assay.” A sample in solution is mixed with gold nanoparticles and a surface probe coated with

biomolecules or chemical ligands is introduced that specifically recognizes the target analyte. The binding of the target analyte to the probe will cause an individual particle size increase or nanoparticle cluster formation. This change in size is detected by DLS and will provide relative size information

Fig. 5. The NanoDLSay technology illustrating size changes around gold nanoparticles and complexity of analysis types. Light scattering detects differences in the complexity of aggregation

about target analyte molecules. Nano Discovery, Inc. also provides the NDS1200, a DLS system specifically designed and optimized for the NanoDLSay.

Demonstrated Applications of the Technology

Commonly used techniques for protein complex detection and analysis are co-immunoprecipitation (Co-IP) followed by immunoblotting. Co-IP and immunoblotting takes hours to days to complete and requires the use of a large amount of sample. Co-IP is often plagued by a lack of specificity, and the analysis does not provide information about how multiple protein binding partners are complexed together. NanoDLSay provides an alternative method for the detection and analysis of protein complexes. Using a two-step assay (Fig. 6), it can be determined if a target protein is in a complexed state and if it is what binding partners are in the complex (15). In the first step, a specific monoclonal antibody-conjugated gold nanoparticle probe is used to capture the target protein.

The target protein is determined to exist in a complex if the net increase of the average particle size is substantially larger than twice of the diameter of the target protein. In the second step of the assay, which is conducted without isolating the first-step assay product, polyclonal antibodies for the potential protein binding partners are added to the assay solution. If a binding partner is present in the complex, the binding of the antibody to the gold nanoparticle probe complex will result in a further increase of the average particle size.

Protein oligomerization and aggregate formation detection can pose a significant challenge in biopharmaceutical formulation and manufacturing. Available techniques for protein aggregate detection and analysis include size exclusion chromatography (SEC), ultracentrifugation, fluorescence assays, and light scattering. These techniques have limitations that include low sensitivity,

narrow applicable concentration range, requirements of target protein labeling, and low throughput. NanoDLSay provides a new tool for protein oligomer and aggregate detection (16). It requires no labeling and can be applied to essentially any protein. It is a single step homogeneous solution assay. The assay requires small sample volumes (1–5 μL), and results can be obtained in minutes. Using DLS, the NanoDLSay reveals the protein concentration as the aggregation starts to occur. NanoDLSay can be used for both rapid non-quantitative screening and quantitative comparison analysis.

NanoDLSay can also be used for protein-protein interaction studies (17). It allows for real-time monitoring of protein-protein binding kinetics and provides binding affinity information. These investigations can be performed using pure protein solutions or biological samples. Different from other existing techniques, NanoDLSay allows for the real-time study of protein-protein interactions involving multiple binding partners. NanoDLSay has also been demonstrated to be useful in quality control programs for monoclonal antibody isotyping (18) and commercial antibody evaluation. Studies reported in literature have shown that it can be extended to other bimolecular binding partners such as carbohydrate-protein interaction (19), carbohydrate-carbohydrate interaction (18), and DNA-DNA interactions (20).

While not necessary for the NanoDLSay, the NDS1200 has been designed to support NanoDLSay. The NDS1200 system holds up to twelve samples and can analyze approximately 120 samples an hour in high-throughput mode or 12 samples per hour in a time controlled kinetic analysis.

Summary

NanoDLSay is an appealing label-free technology that provides for increased sensitivity and specificity over traditional DLS technologies. The NanoDLSay can be applied to a

Fig. 6. A two-step assay for protein complex detection and binding partner analysis. In the first step, a specific antibody-AuNP conjugate is used to catch the target protein. The binding of a protein complex to the AuNP probe will lead to an average particle size increase significantly larger than what can be caused by a protein monomer. In the second step assay, a specific antibody for suspected protein binding partner is added to the assay solution. The binding of the antibody to the protein partner will lead to a further increase of the average particle size

wide range of applications that include protein-protein interactions, bimolecular binding kinetics, protein complex analysis, and general assays for protein detection and analysis that are similar to ELISA.

THE VIBE® PLATFORM AND AMMP® (ACOUSTIC MEMBRANE MICROPARTICLE) TECHNOLOGY BY BIOSCALE

Technology Background

BioScale's ViBE platform and AAMP technology exploit mass dampening of acoustic waves to determine analyte concentrations (Fig. 7). Samples, standards, and reagents are

loaded onto the ViBE workstation in standard 96-well plates and then automatically transferred to the bioanalyzer to collect and process data. Samples are incubated with antibody-coated magnetic microparticles and tagged antibodies. Following incubation, the mixture is delivered by column-8 channels to the anti-tag coated biosensor membrane under timed continuous flow. The membrane sensor is vibrating at a baseline or internal, natural frequency. A magnetic field attracts all of the microparticles, with or without analyte, to the sensor surface, enhancing binding by the tag moieties. After a set interval determined for each assay, the magnetic field is removed. Only the biologically tagged and anti-tag bound microparticles (with the analyte of interest) remain affixed to the sensor; un-bound beads are washed away. A

Real-Time Frequency Shift & Measurement

Fig. 7. Relationship between the bead binding, membrane vibration, and signal. (1) Signal due to natural vibration. (2) Signal at maximum binding (magnetic and ligand). (3) Signal after release of magnetic field

continuous flow of buffer over the membrane sensors washes the microparticles away in accordance with the strength of binding to the membrane sensor surface, which is directly correlated to their analyte load. The bound microparticles cause a mass change on the vibrating membrane decreasing the vibration frequency. As the microparticles are removed from the membrane surface, the system detects the resulting increase in frequency in a highly precise and quantitative way, and yields a dose–response curve (Fig. 8). When no additional change is noted, regeneration buffer flows over the surface to prepare for the next assay.

Demonstrated Applications of Technology

BioScale provides labeling kits for *de novo* assays as well as prepared kits for cell signaling, cytokines, and endocrines. Applications include analysis of host cell proteins, identification and analysis of His-Tag fusion proteins, and residuals from processing proteins (21). Bioscale reports that its lower limit of quantification (LLOQ) is a fraction of those for electrochemiluminescence (ECL) assays and has a reduced processing time.

The AAMP technology has demonstrated utility across several biological matrices including serum, plasma, urine, ascites, and tissue extracts. Performance with cell culture supernatants and production buffers has been reported (22). Bioscale claims that Enzyme Linked Immunoabsorbent and ECL assays may be easily adapted to AMMP ultimately reflecting a decrease in processing and complexity by reducing processing while providing greater range and lower LLOQs.

Summary

BioScale's AAMP technology applied with their ViBE workstation and software provide unattended analysis for up to three plates (after manual sample preparation). The incubation time is approximately 10 min, and typically no additional gain is noted with an extension of this incubation. The eight channel sensor has been validated for up to three ninety six well plates and is nearly complete for six plates. A twelve plate validation is targeted for the end of 2014; no

material changes will be made for the extended validations. The software can reduce data and provide sample results, and an interface with Watson is in development. The sample preparation (incubation with detection), then introduction of the mixture to the detector is simple and can be managed manually or using the ViBE workstation.

MICROFLUIDIC LAB-ON-A-CHIP TECHNOLOGIES UNDER DEVELOPMENT

Technology Background

Recent advances in real-time measurements using lab-on-a-chip (LOC) technologies with integrated microfluidic systems are enabling rapid, low cost isolation and analysis of cell populations. These miniaturized versions of macroscopic cell sorter platforms represent simple to use, small volume platforms that require less personnel training and can be incorporated into point-of-care diagnostics. Furthermore, the potential cost savings associated with the LOC platforms may allow the technologies to be impactful in underserved, developing countries.

Demonstrated Applications of Technology

Many microfluidic LOC separation and sorting devices are label-free. Separations are based upon intrinsic physical properties such as the hydrodynamic signature (size, density, and deformability) or dielectrophoretic signatures (a non-uniform electric field used to polarize cells). Label-free sorting has proven effective for several applications; for example, sorting live cells from dead cells and normal cells from malaria-infected cells (23,24). While these separations are low cost and fast, they are ultimately limited by selectivity due to the overlap in physical properties of multiple cell types (25).

Miniaturized fluorescence-activated cell sorters (μ FACS) are more complex LOC devices that can overcome the selectivity limitations of label-free separations. A variety of publications are available describing these particle separation platforms (26–28); however, most of these technologies display drawbacks ranging from a lack of robustness to harsh

Fig. 8. Steps in AMMP analysis: 1 Sample is mixed with anti-hapten antibodies and magnetic beads coated with analyte capture antibodies. 2 Beads are directed over membrane, binding is enhanced by the magnetic field. 3 Beads without anti-hapten antibodies are released with removal of magnetic field. 4 As the particles wash off the membrane the system detects a specific change in vibrational frequency which generates a dose–response curve. 5 The membrane is regenerated

Table 1. Technology Summary

Technology attributes	Maverick	MX96 SPR	NanoDL-Say	AMMP/Vibe	Localized plasmon resonance/SoPrano	LoC
Stage gate	Commercially available	Commercially available	Commercially available	Commercially available	In development	In development
Assay sensitivity	Single digit ng/mL	N/A	N/A	Low pg/mL	N/A	N/A
Automation	Automated sample processing	No	N/A-this technology can be used in conjunction with standard DLS systems	Automated sample processing	N/A	N/A
LIMS connectivity	Yes, custom software application	No	N/A	To be released Q4 2014 or 2015	N/A	N/A
Multiplexing capacity	128 sensors/chip	Yes, up to 96	Not capable of multiplexing	Not capable of multiplexing	No	Yes, dependent on configuration
Throughput	24 results in 15 min	9,216 results in overnight run	N/A-this technology can be used in conjunction with standard DLS systems	96 results in 130 min	N/A	N/A
Batch sizing	24 samples per chip	2 × 48 spots	N/A-this technology can be used in conjunction with standard DLS systems	96 samples per plate, up to 3 plates per run	N/A	N/A
Sample volume requirement	5–10 µL	100 µL	5 µL	120 µL in well, at least 80 µL for fluid path. This consists of sample plus diluent capture mix	100	N/A
Performance	Specificity and precision equal to or better than ELISA	Similar to other SPR	Sensitivity equal to or better than DLS	Specificity and precision equal to or better than ELISA	Sensitivity equal to or better than LSPR	Specificity and precision similar to flow cytometry
Application-biomarkers, immunogenicity, PK	ADA, autoantibodies	Biomarkers, PK	Biomarkers	Biomarkers, analysis of high-tagged fusion proteins	Biomarkers, PK	Biomarker
Regulatory fit	21 CFR Part 11	RUO only	Could be validated as assay format	Target 21CFR11 by Q42014	Could be validated as assay format	RUO
Secondary detector label technology	Label-free	Label-free	Gold nanoparticle	Label-free	Label-free	Fluorescent
Sample type (<i>e.g.</i> , serum, CSF, tissue, <i>etc.</i>)	Serum/Plasma	All biological fluids	All biological fluids	All biological fluids	All biological fluids	Whole blood, tissue culture, individualized cells from tissue
Instrument technology cost (\$)	80,000	300,000	N/A-this technology can be used in conjunction with standard DLS systems	100,000 for VIBE	N/A-this technology can be used in conjunction with Biacore systems	50,000
Novel reagents or consumables and cost (~\$)	Yes	Sensors (150)	Gold nanoparticles (100)	AMMP kits	Functionalized gold nanorods	N/A
Technology flexibility—open/closed format	Limited flexibility; assay development services available	Open flexible format; assay development flexibility	Open flexible format; assay development flexibility	Open flexible format. Universal assay kit for custom assay development is available	Open flexible format; assay development flexibility	Open

Information provided in this table has been obtained from the manufacturers' and should be verified by the user

N/A information not available

methods that diminish post-sorting cell viability. Using surface acoustic waves (SAW) to move cells down a microchannel is a technology under investigation by several groups because the acoustic waves are gentle to the cells and do not negatively impact post-sorting cell viability. Johansson *et al.* have reported on the development of a μ FACS system using an ultrasonic transducer to shift the positioning of cells in a continuous flow (29). At present, this is not a fully integrated system; it requires an external mercury lamp and charge-coupled device camera to excite and detect fluorescent events, respectively. Once an event is detected, the transducer is triggered, shifting the alignment of the cell passing through the sorting region of the chip. Evaluation of this technology shows it to have an acceptable percent identification (94%); however, an operational sorting speed of only three cells/s is a significant limitation (29).

Another acoustic-driven LOC μ FACS system utilizes standing surface acoustic waves (SSAW) (30). The SSAW devices utilize the constructive interference of two sound waves to separate cells based on size, density, and compressibility. In brief, acoustic radiation force is used to directly manipulate cells (not the fluid in which the cells are embedded) into pressure nodes (PN) or anti-nodes (AN) depending on the cell's material properties. To sort the cells, the position of the PN and AN are "tuned" through use of a chirped interdigital transducers (IDT). Cells are positioned in the center channel by the net forces applied to them (acoustic force, viscous force, gravity, and buoyancy) and separated (31). The separation requirements, or the distance between cells can be adjusted by tuning the surface acoustic wave. Post-sorting cell viability and proliferation does not appear to be impacted. Tune-ability of the SSAW LOC as well as the ability to sort cells into five separate channels has been published; however, Dr. Huang indicated the number of channels for sorting is not fixed and could be expanded (personal communication). Presently, the ability to sort into five or more outlet channels provides the SSAW LOC a distinct advantage as most LOC sorters are limited to only two outlet channels. The SSAW LOC can be used independently to sort based on physical parameters or coupled to a fluorescent detection module and high-speed electrical feedback module for use as a μ FACS system (30).

Summary

Broad interest in the development of LOC technologies is primarily driven by the potential to develop low cost platforms that may be developed to provide diagnostic solutions in underserved developing economies. At this time, μ FACS platforms are being investigated as platforms for intracellular screening for malaria or parasites (24), isolation of circulating tumor cells to inform on cancer treatment (32), and cytostatic immunity screening for cancer patients (33), indicative of their broad potential use. While most of these technologies are pre-commercial, the ever growing need to develop global diagnostic solutions will likely drive further development and commercialization (Table I).

CONCLUSION

With today's limited Capital budgets, technology developers and vendors alike find themselves in a challenging

position; how to drive innovative technology buy-up and deliver cutting edge advantages to the groups that can benefit from them most. As scientists, our limited budgets present us with numerous challenges, not the least of which is identifying technology investments that will provide us the greatest opportunities/results over time. In compiling this paper on real-time measurement technologies, it was our goal to highlight information on several new technologies that we believe have potential utility to support bioanalysis and might, after further development, have significant bioanalytical value.

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