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Particles and microfluidics merged: perspectives of highly sensitive diagnostic detection

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Abstract

There is a growing need for diagnostic technologies that provide laboratories with solutions that improve quality, enhance laboratory system productivity, and provide accurate detection of a broad range of infectious diseases and cancers. Recent advances in micro- and nanoscience and engineering, in particular in the areas of particles and microfluidic technologies, have advanced

the “lab-on-a-chip” concept towards the development of a new generation of point-of-care diagnostic devices that could significantly enhance test sensitivity and speed. In this review, we will discuss many of the recent advances in microfluidics and particle technologies with an eye towards merging these two technologies for application in medical diagnostics. Although the potential diagnostic applications are virtually unlimited, the most important applications are foreseen in the areas of biomarker research, cancer diagnosis, and detection of infectious microorganisms.

Keywords

Diagnostic detection; Particle technologies; Microfluidic; Biomarker research; Cancer diagnosis; Detection of infectious microorganisms; Bead based diagnostics

Introduction

The “lab-on-a-chip” (LOC) concept for micro-total analysis system, proposed first by Manz et al., in 1990s [1], has been expanded and enhanced to include molecular and cellular analyses [2, 3] biological and chemical assays [4–13], combinatorial chemistry [12–19] and drug discovery [19]. Recent developments in micro- and nanoscience and engineering have further advanced the LOC concept towards the development of new generation of point-of-care diagnostic devices [20]. In particular, recent progress in particle-based arrays [18, 21, 22] and microfluidic technologies [22, 23] have resulted in significant enhancements in sensitivity and detection speed. Furthermore, the synergism between microfluidics and particle-based technologies in micro-total analysis systems may provide laboratories with solutions that enhance laboratory system productivity and test accuracy for the detection of a broad range of disease markers.

Microfluidic systems have been introduced into the field of medical diagnostics to allow rapid data collection from distinct biological samples acquired daily at hospitals [23]. Materials used for soft lithography, typically polydimethylsiloxane (PDMS), have made the microfluidics compatible with many biological assays. These microfluidic devices allow manipulation and analysis of biological samples in micrometer-size channels. Besides the obvious advantages of low sample volumes and less reagent consumption needed to perform the analysis in these microfluidic channels, features like high surface area-to-volume ratio and short diffusion distances, hence rapid analysis, support the further development of miniaturized analytical devices [23]. While several research groups have already employ microfluidic systems for simultaneous detection of different analytes [20–22, 24–27], companies such as Agilent Technologies and Fluidigm provide custom design services to produce microfluidic devices and entire measuring systems making the LOC approaches feasible for future translation into the clinic.

Currently, most microfluidic lab-on-a-chip devices are based on continuous-flow in microchannels utilizing pumps based on electrokinetic phenomena, external pressure sources (syringe pumps), or passive mechanisms (capillary or gravity) for activation. An alternative approach towards continuous flow microfluidics is to manipulate the liquid as unit-sized discrete *microdroplets* [23]. Recently, this type of microfluidic droplet system has

attracted significant interest in chemical and biochemical screening, single-cell analysis, protein crystallization, enzyme kinetics, and cellular bio-assays [28]. Such well-defined miniaturized systems allow pico- or nanoliter sample volumes and reagents to form extremely high-density microreactors preventing sample loss and cross-contamination, achieving fast mixing, and enhancing thermal transfer and chemical reaction rates. The microdroplet technology offers several advantages over continuous-flow systems, the most important being compatibility with biosamples, scalability, and reconfiguration for point-of-care testing [28].

Multiplexed analyses for simultaneous detection of different analytes, employed already by research laboratories, are promising tools for clinical diagnostics [20–22, 24–27]. The particle-based arrays have already demonstrated their versatility in numerous multiplexed analyses including genotyping, gene expression [29], enzyme assays [30] and protein immunoassays [30, 31]. One of the advantages of particle-based arrays is that different sensors can be integrated by encoding each probe-functionalized particle via chemical, spectrometric, or physical means creating particle arrays [22]. Furthermore, multiple microparticle-based assay kits, such as Luminex xMAP (Luminex, TX, USA), Cytometric Bead Array, CBA (BD Bioscience, CA, USA) and VeraCode™/BeadXpress (Illumina CA, USA), are now commercially available for measuring cytokines, cell signaling molecules and antibodies for screening human serum samples, cerebrospinal fluid and synovial fluid, all of which are highly medically-relevant.

An alternative approach towards particle-based arrays is multiplexing in the microfluidic format [11, 12, 16]. This is accomplished by surface modification with capture molecules specific to analytes of interest in different microchannels of a microfluidic device. Recently, implementation of particles for multiplexing in microfluidic format was introduced [22]. Similar to the advantages offered by microchannels, the particle-array in microfluidic format benefit from fast reaction kinetics in liquid phase, high binding capacity due to their larger surface area-to-volume ratios [22]. In particle-array in microfluidic format, additional targets are possible via the addition of extra particles conjugated with probes to those analytes, while a new surface-treated microfluidic device has to be fabricated for a new analyte. Furthermore, the low cost and better quality control of the particle fabrication compared to microarrays or surface modification of microfluidic channels, offers a more flexible selection of probe sets for analytes [22, 24, 25]. We therefore, believe that the synergy between particles and microfluidics technologies will make a big impact in enhancing detection sensitivities and up-scaling multiplexed analyses for medical applications. The goal of this review is to survey the latest progress on these three technological fronts with relation to bioanalytical and cellular analysis platforms.

Bioanalytical analysis technologies

Particle-based diagnostics in homogenous fluid systems

Non-magnetic particles—There is an increasing demand for the ability to make multiple measurements from the same biological sample with a single test. To this end, microparticles with different surface chemistries that can accommodate multiple assays have shown promise through several demonstrations. Flow cytometry presents a powerful

platform for multiplexed microsphere-based assays. Additional microscope reading platforms, such as optical reading platforms and fiber optic platforms, have been introduced for microparticle analysis and decoding. Substantial contributions from Walt group to the microsphere-based detection arrays on optic fiber platforms demonstrated the feasibility of this approach for genomic and proteomic analysis for clinical samples [32–40]. In this approach, microsphere arrays were assembled on the distal end of a fiber's core, which were selectively etched relative to the cladding to create wells of defined depth for each microsphere. Employing fiber bundles comprised of thousands of individually addressable fibers enables massively parallel detection capabilities in this approach. Both DNA [32, 36, 38, 39] and protein [34, 40] detection protocols were developed for multiplexed analysis of human saliva utilizing this approach. For the DNA detection protocol, a high-density array was fabricated to detect and quantify the hybridization of fluorescently labeled targets. Each specific hybridization event was detected by emission of a fluorescent signal localized to the probe positions complementary to the targets. The versatility of this technology has been demonstrated for immunoassays, where target-specific monoclonal antibodies were immobilized on the microparticles to create a fiber optic array that is capable of simultaneous measurements of multiple proteins [34, 40].

Li et al. demonstrated another clinically-relevant example of microparticle-based detection by quantifying single-nucleotide polymorphisms (SNPs) [41]. Detecting SNPs is of high diagnostic interest, as SNPs have been shown to correlate with disease development, response to pathogens, chemicals, drugs, vaccines, and other agents. The authors utilized a combination of flow cytometry of microparticles and a DNA amplification technique to detect specific SNP recognition events on the surface of microparticles (Fig. 1). This technology can be further advanced by incorporating the assay into a microfluidic chip with an integrated fluorescence detector, to benefit from low reaction volumes and associated reduction in costs.

This work inspired the development of a high-density microarray for simultaneous detection of proteins and DNA within a single test [38]. In this approach, a two-layer sandwich assay was performed on microparticle surfaces to test for the combined presence of a protein and DNA using rolling circle amplification (RCA) (Fig. 2). Detection limits down to 10 fM and 1 pM were achieved for proteins and target DNA, respectively. In addition to this new approach for detecting both protein and DNA in a single test using RCA, the limit of detection for IL-8 and IL-6 cytokines was improved by three orders of magnitude compared to similar microparticle-based immunoassays. Furthermore, this tool offers the ability to measure various combinations of analytes and simultaneously detect both proteomic and genomic information, thereby giving a more comprehensive description of the sample. This sensor may be useful for analyzing serum or saliva samples collected from patients with chronic obstructive pulmonary disease (COPD) and/or asthma.

Doyle et al. devised another interesting approach involving soft flow lithography to produce barcoded hydrogel microparticles containing capture antibodies [42]. In order to functionalize the microparticles, pressure driven streams of fluorescent marker and capture antibodies were passed below a photomask for creating UV-polymerized hydrogels with mask-defined shapes (Fig. 3). The hydrogel microparticles were then coded with a

fluorescent marker and three different cytokine capture antibodies (interleukin-2, interleukin-4 and tumor necrosis factor alpha) by poly (ethylene glycol)-based covalent functionalization. The antibody functionalized hydrogel particles were then incubated with the sample to capture the analyte and assayed using fluorescent detector antibodies for quantification. Authors reported detection limits in the range of 1–8 pg.mL⁻¹ for all three cytokines.

Magnetic particles—Magnetic particles offer unique advantages, such as low production cost, physiochemical stability, biocompatibility, and negligible background noise. To date, numerous methods have been developed to sense biomolecules using magnetic labels [43]. In addition, biological samples exhibit virtually no magnetic background, and thus highly sensitive measurements can be performed in turbid or otherwise visually obscured samples without further processing. Optical techniques, on the other hand are often affected by scattering, absorption, fluorescence quenching and/or autofluorescence within the sample.

There are three main types of magnetic particle-based biosensors that employ different magnetic sensing principles. The first type consists of magnetic relaxation switch assay-sensors, which are based on the effects that the magnetic particles exert on water proton relaxation rates. The second type is magnetoresistive sensors, which detect the presence of magnetic particles on the surface of electronic devices that are sensitive to changes in magnetic fields on their surface. The third type consists of magnetic particle relaxation sensors, which determine the relaxation of the magnetic moment within the magnetic particle. Here we discuss the first two types of magnetic particle sensors as they have been more frequently integrated into microfluidic devices.

Magnetic relaxation switches (MRS)—Superparamagnetic particles made of iron oxide and polymeric coatings are clinically proven magnetic resonance (MR) contrast agents and widely used in targeted molecular imaging applications [44, 45]. Surface-modified nanoparticles (NPs, diameter 5–300 nm) bind specific molecules producing local inhomogeneities due to the applied magnetic field in tissues where molecular targets are present. These inhomogeneities result in decreases in the T₂ relaxation time (the transverse or spin-spin relaxation), which in turn, lead to changes in the contrast of MP images. Building upon this observation, Josephson et al. exploited the change in T₂ produced by magnetic NPs to obtain MR based assays called Magnetic Relaxation Switches. In this method, dispersed magnetic NPs form an aggregate upon binding with target analytes. The aggregated form of the NPs dephases (causing loss of phase coherence) the spins of the surrounding protons of water molecules more efficiently than NPs present as the dispersed state. This effect results in a decrease in spin-spin relaxation time T₂ (so called type I NP-based systems), [46]. However, when larger magnetic particles (MPs, diameter 300–5,000 nm) are employed the T₂ increases with aggregation (type II MP-based systems). The detailed discussion of the mechanism of this phenomenon is described elsewhere [47–49]. Because the sensing step is not dependent on the separation of bound and unbound reagents or on the use of light, measurements can be carried out in turbid samples and suspensions, which is highly advantageous for sensors designed to measure analytes in bodily fluids [50]. The dispersed and aggregated states of NPs (or MPs) can be reversed by factors such as

temperature, pH, and a high concentration of competing analytes, and therefore these systems are referred to as “relaxation switches”. Josephson et al. reasoned that MRS assays could be employed for the continuous monitoring of analytes if: i) equilibrium exists between dispersed nanoswitches and a binding-protein-mediated microaggregate state, and ii) the equilibrium position is dependent on analyte concentrations. Such continuous analyte sensing/equilibrium-based nanoswitches might find use as implantable sensors for the following reasons. First, the sensor signal is due to the radiofrequency stimulation and emission of water protons, which can be measured through biological samples without light-based interferences. When placed in an MR imager (or other device that can distinguish the T_2 of sensor water from that of bulk water), the sensor sends out a radio frequency signal reflecting its chemical environment. Second, since the analyte is detected via its ability to alter the size of nanoswitch microaggregates in solution, a solid phase is not employed. The sensor does not require an immobilized enzyme, immobilized binding protein, or electrode, which are found on most conventional sensor designs. Third, the three components of the sensor (the semipermeable membrane, the nanoparticle, and the binding protein) offer excellent prospects for long-term stability when implanted [51]. “Clickable” cross-linked iron oxide (CLIO) NPs are also appropriate for MRS applications because they are sufficiently stable to permit a variety of surface chemistries [51, 52]. CLIO surfaces have been designed to detect ions [53], DNA [46, 50, 54], proteins [50, 54, 55] and bacteria, as well as mammalian cells [56]. A particularly valuable system for the study of MRS is the reaction of NPs displaying the Tag peptide and reacting to a monoclonal antibody (anti Tag) binding to the peptide [48]. The formation of NP aggregates with anti-Tag antibodies has been shown to be analogous to the interaction between antibodies and antigens, with a maximum complex formation occurring at the temperatures at the equivalence point as the concentration of analyte was increased. A variation of the type I MRS aggregation/dispersion method is found with the miniaturized NMR system (Diagnostic Magnetic Resonance, DMR). This system achieves high assay sensitivity by reducing sample volume to 5 μ L and by using filtration methods [56]. Incorporation of microfluidic system with a filter unit into miniaturized NMR system permitted the detection of as few as 20 colony-forming units per mL of sputum being detected [57]. Another important application of the type I MRS is its use in implantable MR-based, water relaxation sensor. A semipermeable membrane was employed with a size cutoff that permitted small analytes like glucose, to diffuse in and out while the larger CLIO NPs were retained within the sensor [51]. Continuous monitoring of the T_2 values of the solution inside the membrane showed a competitive assay type-response of glucose-functionalized CLIO to glucose [51, 58]. Similar concept was translated to an implantable water relaxation sensor detecting hCG as a cancer biomarker [59, 60].

Magnetoresistance-based biosensors—Magnetoresistance-based sensors, conventionally used as read heads in hard disk drives, have been used in combination with biochemically-functionalized magnetic particles to enable molecular recognition. This technology is based on the detection of micrometer or nanometer-sized functionalized magnetic particles, using high-sensitivity microfabricated magnetic-field sensors. Magnetoresistive biosensor platform offers several key advantages over other sensing modalities. First, the biological samples (blood, urine, serum, etc.) naturally lack any

detectable magnetic content, providing a sensing platform with a very low background level and thus lower detection limit of analytes. Second, the sensors can be arrayed and multiplexed to perform analysis on a panel of proteins or nucleic acids in a single assay. Lastly, the sensors can be manufactured cheaply, in mass quantities, to be deployed in a disposable format. For these reasons, magnetic biosensors are an attractive alternative to optical techniques. Magnetoresistive sensors are based on the binding of magnetic particles to a sensor surface while the magnetic fields of the particles alter the magnetic fields of the sensor which result in electrical current changes within the sensor. There are two methods through which magnetic particles bind to the sensor surface: (i) direct labeling and (ii) indirect labeling (sandwich-type binding). In the direct labeling case, the biomolecule to be detected (target or analyte) is magnetically labeled (immobilized on a magnetic label) and passed over an array of specifically patterned complementary or noncomplementary (probe) molecules, which are immobilized over on-chip magnetic field sensors. In direct labeling, magnetic labels bind to the surface via streptavidin-biotin interaction or complementary DNA sequence recognition [61]. On the other hand, indirect labeling uses the principle of sandwich immunoassay in ELISA. Antibodies that bind to the target protein are immobilized onto the surface. After the surface is exposed to the sample solution containing the target proteins, biotinylated secondary antibodies are added to the system. Finally, streptavidin-coated magnetic particles are applied for tagging the biotinylated antibodies. In both direct and indirect binding, the sensors detect the presence of the magnetic labels via a change in sensor resistance at a fixed bias current. The unbound target biomolecules are washed away and residual sensor signals are obtained at sensor sites, where complementary magnetically labeled target- and surface-bound probe molecules have successfully interacted. Giant magnetoresistance (GMR) spin valve (SV) or magnetic tunnel junction (MTJ) sensors have also been successfully used for sensing MPs. These sensors are composed of multiple layers of ferromagnetic materials, coated with gold or silicon dioxide to facilitate conjugation of biomolecules. A comprehensive description of the magnetoresistive sensor structure can be found elsewhere [61, 62]. Another type of particle used for magnetoresistive biosensing is super-paramagnetic particles. Earlier applications used relatively large magnetic particles, with diameters between 0.1 and 3 μm [61]. Micrometer-sized particles have the advantage of facile observation under a light microscope and a higher particle-based magnetic moment that allows for the detection of very small numbers of particles. However, magnetic nanoparticles have recently replaced the larger particles because the nanoparticles are more stable in suspension and are less prone to particle clustering in an applied magnetic field [62–65]. Streptavidin-coated MPs were applied to spin valve sensors in the protein marker detection at 27 $\text{pg}\cdot\text{mL}^{-1}$ level of sensitivity [66]. By using 50 nm magnetically-activated cell sorting (MACS) of magnetic nanoparticles, Wang et al. demonstrated cancer marker detection in 50% serum at sub-picomolar concentrations [64]. Multiplexed sensing of different protein markers in serum was demonstrated on a single chip by carefully selecting antibodies and by employing the signal enhancement strategy using multiple layers of NPs. Wang et al. used nanoimprint lithography to synthesize antiferromagnetic nanoparticles of 100 nm size with high magnetic moment and zero remanence [67]. The disk-shaped antiferromagnetic nanoparticles were composed of multiple layers of ferromagnetic material separated by a nonmagnetic interlayer. NPs with high magnetic moments were

functionalized with streptavidin and permitted the detection of DNA at concentrations as low as 10 pM [63].

Microfluidic-based detection in continuous flow modalities

Identification of particular pathogen species and virus strains is important for appropriate intervention as well as devising infection control strategies such as the development of vaccines. Development of sensitive and effective platforms that can unambiguously identify virus species and pathogen strains is of high importance for research and clinic. In this section, we will discuss representative platforms and their applications in analysis of various biological pathogens.

DNA-based screening via target-specific nucleic acid amplification in diagnostic technologies has been the gold standard for identifying biological pathogens. Despite the sensitivity and specificity associated with polymerase chain reaction (PCR) amplification, its limited throughput has impeded the potential of this approach. A promising alternative analytical platform is microfluidics-based amplification of specific regions of the pathogen DNA. Miniaturized microfluidics-based PCR reactions or equivalent amplification methods, such as rolling circle amplification (RCA) and solid-state PCR, allows for fast reaction rates in microchambers with the added advantage of less sample requirement. In a recent report, Wulff-Burchfield et al. demonstrated microfluidic PCR for detection of *Mycoplasma pneumonia* in clinical samples. This approach was shown to be equally sensitive and up to three times faster than conventional PCR[68], with the capability to run twenty real-time PCR reactions, consisting of four reactions each on five separate chips. The microfluidic cartridge used consisted of an 86 mm×86 mm printed circuit board (PCB) chip, polymer spacer/gasket, and glass top plate with drilled holes. In addition, the instrument contained the power supply, control electronics, fluorometer module, heaters, and the cartridge deck. For detection of the amplicon, a light emitting diode (LED)-based photodiode fluorometer was used to measure the fluorescence at the end of each extension cycle.

Apart from the amplification-based methods, a few novel approaches that involve microfluidics have been used to identify or detect pathogens. Frisk et al. reported a sensor that uses self-assembled monolayers (SAMs) for the detection of *botulinum* neurotoxin type A (BoNT/A) [71]. A neurotoxin by nature, *botulinum* neurotoxin type A, causes fatal poisoning if allowed to contaminate meat products. In this paper, the SAMs consisted of an immobilized synthetic peptide (that resembled one of the toxin's protein substrate) on gold layers enclosed by microfluidic channels. As the peptide SAMs were exposed to BoNT/A or its catalytic light chain, the peptide substrate was enzymatically cleaved from the substrate and detected fluorescently. The method is sensitive to detect the toxin down to 20 fM in buffer and 3 nM in food. Since the design of this sensor is very versatile, the microfluidic platform could be formatted to detect other enzyme toxins such as the BoNT serotypes (B-G), *tetanus* toxin, or *anthrax*, thus having implications for a multiplexed, on-site toxin sensor. Another attractive approach to detect both bacteria and virus is electrochemical biosensors. Liao et al. described species-specific detection of bacterial pathogens in human clinical fluid samples using a microfabricated electrochemical 16-gold electrode sensor array [72]. For this design, each sensor consisted of working, reference, and auxiliary

electrodes. Each working electrode was functionalized with one capture probe from a probe library for both bacteria and virus pathogens. A bacterial 16s rRNA target isolated from bacterial lysate was allowed to hybridize with both the biotin-modified capture probe on the sensor surface and a fluorescein-modified detector probe. Detection of the target-probe hybrids was achieved through binding of horseradish peroxidase (HRP)-conjugated anti-fluorescein antibody to the detector probe. Species-specific detection of as few as 2,600 uropathogenic bacteria in culture, inoculated urine, and clinical urine samples was achieved using this sensor approach in as low as 45 min. In a blinded study, the sensor array directly detected gram-negative bacteria without nucleic acid purification or amplification. On a different front, Einav et al. have conducted a high-throughput microfluidic screen involving protein expression and affinity analysis to identify 18 compounds that substantially inhibit replication of Hepatitis C Virus (HCV) RNA [73]. A significant advantage of their microfluidic device was the ability to preserve the natural protein structure via microsomal membranes embedded in the device. In addition, the device consisted of hundreds of fluidically-configurable miniature reaction chambers (10 nL volume each), allowing high-throughput assays. A different approach by Zordan et al. utilized surface plasmon resonance (SPR) in a microfluidic biochip for multiplexed detection of single cell pathogens. The PDMS device captures pathogens (>95% efficiency) by binding them onto a 4×4 array of gold spots which are functionalized with biomolecules specific to the pathogen. With the aid of a dual detection modality, the captured pathogens were imaged by SPR and epi-fluorescence simultaneously. In another example, Mannoer et al. illustrated the detection of *E. coli* and *Salmonella* via antimicrobial peptide functionalized microcapacitive electrode arrays. The antimicrobial peptide magainin I was immobilized on gold microelectrodes, which was exposed to various concentration of pathogenic *E. coli*, revealing a detection limit of 1 bacterium/μL.

Microfluidic-based detection in microdroplet modalities

The use of nano-liter reaction volumes and parallel sample processing offered by droplet-based microfluidic devices make them ideally suited for total chemical and bioassay analyses, ultra-high throughput screening applications, and other cases where samples and reagents are available in limited quantities [74–79]. The microdroplet technology was previously described for analysis of DNA samples providing targeted sequence enrichment to prepare samples for next-generation sequencing. Previously, picoliter (pL) volume PCR platform was developed by Griffiths and coworkers in batch generated micro-droplets through water-in-oil emulsion [80] to amplify complex genomic libraries. It was Beer et. al. who first demonstrated an LOC system for picoliter droplet generation and PCR amplification with real-time fluorescence detection [81]. Their silicon microfluidic device consisted of a shearing T-junction that was capable of generating uniformly-sized 10 pL droplets encapsulated in oil-phase carrier, which were then thermally cycled and continuously monitored. Their system allowed real-time PCR in isolated picoliter droplets containing single-copy nucleic acids with reduced thermal cycle thresholds and extremely low reagent usage compared to commercial instruments.

For almost all viral infections, including human papillomavirus (HPV), seasonal influenza, and avian influenza virus (AIV), it is important to not only detect the infection, but also to

identify the viral strain. Detection and pathotyping of AIV is traditionally carried out in the clinic by reverse transcription PCR (RT-PCR). Initial RT-PCR assays were complicated and time-consuming due to multi-step amplifications. Even though newer multiplexed RT-PCR targets multiple genes in a single assay, this technique has limited sensitivity because of uncontrollable primer-primer interferences and preferential amplification of one target sequence over another. One method that minimizes interferences in multiplexed PCR is solid-phase PCR, in which the primers are immobilized on a solid support while remaining PCR components are in liquid phase [69]. The advantage of this method is that the primers targeting specific analytes are spatially separated, which minimizes steric hindrance. In addition, amplification and sequence detection are performed in a single chamber that eliminates the need for post-PCR step in this technique. The solid phase can be flat surfaces (such as microtiter plates) or microparticles. Inclusion of microparticles in the PCR assay, may be conducive to high-throughput analysis, as the particles can be combined with microdroplets or microfluidic enzymatic assays [70]. For instance, assays can be carried out on microparticles that are encapsulated in droplets as has been demonstrated by Martino et al. in which cell lysate was encapsulated with antibody-conjugated beads in water-in-oil droplets to measure binding of intracellular proteins. In this approach, a droplet microfluidic system was integrated with an on-chip cell lysis system to perform a quantitative immunoassay on as little as 1000 cells. This demonstrates the method as a valuable tool for analyzing rare cell populations (Fig. 4).

Tewhey et al. combined microdroplet PCR with flow-cell technologies to perform large-scale targeted sequencing of human genome (Fig. 5) [82]. A microfluidic chip was used to encapsulate fragmented genomic DNA and PCR primers respectively and subsequently merge droplets for PCR reaction. They fully exploited the capacity of microdroplet technology for multiplexed analysis on small amount of genomic samples. By coupling second generation sequencing with microdroplet PCR, they demonstrated parallel characterization of thousands of targeted sequences. They demonstrated that the coverage and genotyping accuracy of the new method is comparable to that of conventional PCR, while the microdroplet-based approach captured less non-specific DNA than other strategies.

Microparticle-based detection in microfluidic format—the merge

Even though a flow cytometer can rapidly process optically-, physically-, or electronically-encoded particles, its high cost and lack of portability prevent this approach to be efficiently translated into clinic. This limitation can be overcome by introducing encoded particles into micro-devices, utilizing the microfluidic technology advantages. In particular, large analytical surface of microspheres, mixing ability in the channel, sorting enriched molecules of interest as well as multiplexing allows them to become a powerful tool for analysis in microfluidic channels.

Particle immunoassays in microfluidic format—Immunoassays, such as the enzyme-linked immunosorbent assay (ELISA), have become one of the most commonly utilized techniques in medical diagnosis. Traditionally, these assays are carried out without sample replenishment in microplates having submilliliter-volume wells. Various strategies to

perform immunoassays within microfluidic devices have been tested. These microfluidic-based immunoassays employed various detection methods such as fluorometric and colorimetric measurement, thermal lensing, electrochemical detection, and surface plasmon resonance (SPR). In addition, the microbead-based assays have an additional advantage for multiplexing using color-coded particles to expand the specificity of multicolor flow cytometry [83].

One approach of performing an immunoassay in the microfluidic format is by direct functionalization of the device surface with antibodies to capture the biomolecules from the sample [84]. In this approach, for multiplexing, multiple functionalized surfaces conjugated to the distinct antibodies are required to enable the detection of distinct targets within the same sample solution. Microfluidic immunoassay coupled with optically encoded microspheres overcomes this need, thus making the multiplexing and mixing in the microfluidic channels more amicable for diagnostic analysis. In addition, the smaller dimensions of the materials enable enhanced sensitivity by at least two orders of magnitude with the capability to manipulate and analyze small sample volumes (10^{-9} – 10^{-18} L) thereby minimizing reagent consumption [84].

Taking this approach, Aderem et al. developed microbead-based assays in microfluidic devices for multiplexed protein detection of TNF- α , CXCL2, IL-6 and IL-1b [84]. With this method authors claim highly sensitive detection of the proteins (less than 1000 pg.mL $^{-1}$) in a small volume of 4.7 nL. Furthermore, these assays can be easily modified and expanded for detecting other biomolecules by encoding additional microspheres with pre-functionalized microbeads (such as Luminex beads). Joos et al. have adapted a capillary-based system (~ 100 μ m diameter) to perform bead-based multiplexed sandwich immunoassays to detect protein expression of receptor tyrosine kinase in breast cancer tissues. In this approach antibody-coated beads were captured on a 0.5 μ m filter while washing and incubation steps were done by manipulating the flow across the beads. Quantitative analysis of the beads was performed using a flow cytometer or collecting the beads in a microwell plate [85] (Fig. 6).

Microfluidic devices in combination with microparticles can be applied to detect small molecules using enzyme-based readouts to produce a fluorescent product with a sensitive detection range. For instance, Kim et al. have developed a microfluidic device incorporating glass microbeads with an immobilized enzyme to detect glucose in the range of 1–10 mM. In this approach, the enzyme reaction between glucose (analyte) and glucose oxidase (enzyme) produces hydrogen peroxide which could then be quantified by Amplex Red reagent to yield a fluorescent readout [86].

Recently, magnetic microparticles have been introduced into immunoassays. This approach gives an added advantage of capturing the beads with the target analyte and consequently performing the wash, concentration, and other procedural steps within microfluidic devices by applying external magnetic fields. Peyman et al. have demonstrated the use of magnetic particles as magnetically-actuated vehicles within microfluidic channels. The device is a continuous-flow reactor consisting of multi-laminar streams of solutions for consecutive washing and binding steps (Fig. 7). In this approach, the position of the magnetic particles

(biotinylated) is controlled via an external magnetic field, and the particles are sequentially exposed to various flow streams, including sample(streptavidin), buffer, and detector analyte (FITC-biotin). This approach displayed a detection limit of $20 \text{ ng}\cdot\text{mL}^{-1}$ of streptavidin using biotinylated beads and FITC-Biotin to capture streptavidin in solution in a sandwich ELISA assay in as short as 60 s [87].

Particle based DNA detection in microfluidic format—Nucleic acid analyses, including those of DNA and RNA, have been widely used in applications such as clinical diagnostics, food safety, forensics, and bio-defense. Microfluidics-based nucleic acid analysis provides many advantages over traditional procedures, including reduction of sample and reagent consumption, shortening analysis time, parallelization of analysis, and elimination of cross-contaminations. Furthermore, microspheres have been used to perform nucleic acid sample preparation prior to sequencing, in the form of solid phase extraction (SPE). SPE of DNA samples is a process in which DNA samples are usually absorbed onto an insoluble material (solid phase, e.g. silica) to remove impurities, and eluted later with a buffer. In less common cases, the solid phase can also be used to bind impurities while DNA is left in the sample. SPE has largely replaced conventional DNA purification methods such as CsCl gradient and phenol-chloroform phase separation, which involve time-consuming ultracentrifugation or toxic reagents. SPE of DNA samples was first implemented in microfluidic devices by Christel et al. [88]. Their device contained a large region of high aspect-ratio pillars of oxidized silica for nucleic acid binding, which was fabricated by deep reactive ion etching. The same concept was followed by the work of Cady et al. [89], with improved DNA binding capacity by increasing surface area of the device. However, these systems usually require complex etching protocols that come with high fabrication costs. Alternatively, Tian et al. employed a capillary-based device that contained nanograms of silica microparticles and was capable of adsorption and de-sorption of pictogram to nanogram amounts of DNA samples [90]. Their device was capable of extracting DNA from white blood cells with an efficiency of 70% while removing 80% of proteins. Their choice of silica microparticles significantly reduced the complexity and cost of microdevice fabrication.

Recently, SPE has become integrated with other forms of microfluidic manipulations and downstream applications. Lehmann et al. demonstrated a platform where magnetic beads were combined with droplet technology to allow extraction of genomic DNA from as low as 10 cells in a $10 \mu\text{L}$ volume [91]. In their design, magnetic particles with silica-modified surface were used both as force mediators and mobile substrates. A printed circuit board with a switchable matrix of coils generated an adjustable magnetic field. Aqueous droplets containing binding/lysis, washing, and elution buffers were immobilized at separate locations on the substrate submerged in silicone oil phase. Magnetic beads were manipulated to merge or separate from those droplets through modulation of a magnetic field. Their system proved to yield good DNA recovery and an order of magnitude higher sensitivity compared to microscopic bead-based systems.

Yeung et al. integrated magnetic particle-based isolation of genomic DNA, asymmetric PCR, and electrochemical sequence-specific detection into a single device that was capable of on-the-spot multiplexed pathogen identification [92]. Ko et al. devised a method named

LIMBS (Laser-Irradiated Magnetic Bead System) that combined laser irradiation and magnetic beads to rapidly and efficiently extract DNA from biological samples [93]. They demonstrated its use for DNA isolation from various pathogens, and integrated it on a centrifugal microfluidic device to perform one-step pathogen DNA extraction from a whole blood sample [94].

In genetics, one of the most important issues is the analysis of the genetic differences. The so-called polymorphisms are on average only 0.1% between two unrelated individuals, and mostly found in the form of single-nucleotide polymorphisms (SNPs) [95]. There is a range of genetic diseases associated with SNP, such as sickle cell anemia, cystic fibrosis, etc. Lien et al. built an automated magnetic bead-based microfluidic platform that integrated leukocyte purification, genomic DNA extraction and on-chip PCR [57]. They utilized two types of magnetic particles in their system, one for leukocyte purification and another for DNA purification, which greatly facilitated the automation and integration of microfluidic components. They showed its successful use in SNP that encodes an enzyme MTHFR that has been associated with genetic diseases such as congenital abnormalities, cardiovascular disease, renal failure, and cancers. Although their final detection still relied on gel electrophoresis, the integration of the system with existing on-chip detection techniques such as capillary electrophoresis (CE) can be readily envisioned.

In addition, application of microfluidic devices and microspheres for miniaturized analytical strategies for toxicological evaluation was previously shown by Wolfbeis and Köhler. In their approach, sequences of microfluid segments behave as artificial compartments for complete separation of small reaction or cultivation volumes for a large spectrum of different targets for toxicological studies. In this work the authors present the simultaneous measurement of the metabolic activity readout by the fluorescence signals of pH-dependent microbeads and the microorganism as well as the growth of the *E. coli* culture by scattering measurement inside micro fluid segments [96].

Vogelstein et al. developed a digital technology called BEAMing, based on four of the principal components (beads, emulsion, amplification, and magnetics) [97], to analyze human genetic variations. This could benefit areas such as studying the role of inheritance in physiologic states, detecting pre-symptomatic cancer, and identifying stages of diseases related to somatic mutations. Oligonucleotides acting as primers were bound to streptavidin-coated magnetic beads, mixed with template DNA and PCR components, and trapped into microemulsions, with each emulsion compartment containing less than one template and one bead on average. PCR reactions were carried out by thermal cycling of the microemulsions, which were subsequently broken and collected by the magnetic beads. Following incubation with detection oligonucleotides and fluorescently labeled antibodies, the signals on magnetic beads were analyzed by flow cytometry (Fig. 8). In this approach the use of magnetic beads not only facilitated sample handling, but also the recovery of desired target through flow sorting. The microdroplets created through emulsion provided a stable compartment for single DNA template entrapment and PCR. The authors realized the limitation of microemulsion through simple stirring of water/oil/detergent mixture, which generated non-uniform aqueous compartment sizes and resulted in encapsulation of two or more DNA template molecules in the same compartments, which limits the accuracy of this technology.

Cellular analysis technologies

Microfluidic devices and other miniaturization technologies offer powerful techniques for cell interrogation with rapid processing speed and low cost [98]. Further, these techniques can be combined with monoclonal antibodies (mAb) developed against cellular markers to identify and capture cells with high specificity [99]. Another thrust for microfluidic devices is to understand the cellular heterogeneity; such as in the case of autoimmunity and intercellular communication between rare leukocytes [84]. Inhere we will introduce several previously developed microfluidic approaches for rare cell detection and analysis.

Microfluidic flow-based approaches for cell diagnostics

The discovery of “cancer stem cells” and circulating tumor cells (CTCs) and their characterization is essential for the advancement of cancer research, diagnosis and treatments [99]. CTCs constitute a very small fraction of a blood sample (as small as one cell per 10^9 cells) and consequently their separation and characterization present an enormous challenge. Toner et al. demonstrated the ability to screen for circulating tumor cells (CTCs) in human blood. This approach can be translated into clinic for screening a variety of cells from the blood of patients with common epithelial tumors [100]. In their work, Toner et al. utilized microscopic pillars functionalized with antibodies for specific surface markers expressed by CTCs that can effectively bind to these cells from whole blood samples without any pre-dilution, pre-labelling, pre-fixation or other processing steps [100] (Fig. 9). Similar strategies have been applied to screen for various cells, such as the circulating lung cancer cells [101] and label-free detection of CD4+ T cell in HIV patients [102]

Another thrust for microfluidic devices is to understand the cellular heterogeneity. Detection techniques in microfluidic format that enable monitoring changes in cellular function and secretion over time can provide insights into various mechanisms of cell function.

Microfluidic devices can be tailored to detect very low number of secreted biomolecules (down to few thousand copies) either from a group of cells or from single cells. Clark and co-workers have demonstrated the use of such microfluidic devices to monitor the secretion of glycerol from adipocytes ($\sim 50,000$ cells) to a limit of detection (LOD) of $4\mu\text{M}$ detecting changes in glycerol concentration every 90 s [103]. The setup consisted of two microfluidic devices, a perfusion chip for cell seeding and incubation and an enzyme assay chip capable of performing on-line mixing of reagents and detection of the enzymatic product using fluorescence. Cells in the perfusion chip were stimulated and the media from the perfusion chip was pumped into the enzyme assay chip. The concentration of glycerol in the media was detected using fluorescence by the inclusion of the hydrogen peroxide sensitive dye Amplex UltraRed.

Singhal et al. demonstrated the ability to detect antibodies secreted from hybridoma cells utilizing previously conjugated microspheres on microfluidic platform [104]. The authors in this article were able to demonstrate antigen-antibody kinetics using a bead based assay and then applied the system to detect biomolecules secreted from hybridoma cells at a rate as low as 200 antibodies per second [104]. In this approach, the authors used two versions of this assay, allowing for association and dissociation rate measurements directly, using

fluorescently labeled antigen and dissociation rate indirectly using unlabeled antigen [104]. The coupled use of microfluidics and microbead-based assay enabled the measurement of antigen-antibody kinetics in as little as 8×10^4 antibody molecules (~ 132 zeptomoles) immobilized on a single bead and less than 2×10^6 antibodies (~ 3 attomoles) loaded into the microfluidic device. Further, the use of beads allowed the measurement of multiple antibody-antigen binding kinetics by optical and spatial multiplexing using fluorescent microscopy. Using this system, the authors have measured association and dissociation kinetics of three distinct mAbs (HyHEL-5, D1.3, and LGB-1 mAb) interacting with two different antigens (HEL-Dylight633 conjugate and eGFP). Using the low detection limits of the system, the authors have extended this approach to measure the antigen binding kinetics released from single cells. To this end, rabbit anti-mouse pAb coated protein A beads were co-incubated adjacent to single D1.3 hybridoma cells and subsequently measured antibody-antigen binding kinetics by recording the fluorescence of a single bead washed with buffer and successively higher concentrations of fluorescent antigen. The authors infer that the minimum incubation time (~ 5 min) and detection limit of the assay ($\sim 8 \times 10^4$ antibodies), suggests that the single hybridoma cells secreted at least 200 antibodies per second when incubated at room temperature in the microfluidic device. Another soft lithography-based technique to monitor and characterize single cell function was designed to implement microarrays to hold single cells and assay for a variety of functional properties [105]. Such microwell-based methods have been used to detect gene expression [106], cytokine secretion [107], and presence of antibodies [105].

Detection of cell types and their quantification within a blood sample is essential for diagnosing a variety of diseases, such as HIV. Adopting microfluidic devices as flow cytometry instruments for detecting cells using simple detection techniques is advantageous for pre-screening purposes. McDevitt et al. have developed a microfluidic system that incorporates semiconductor quantum dots (QD) for the isolation and enumeration of CD4+ cells, which has potential applications in point-of-care and resource-scarce settings [108]. In this approach, the microfluidic device (nano-bio-chip) contains a *nano-net* generated in agarose microspheres enabling the entrapment of cells. The QD-CD3+ (red) and QD-CD4+ (green) conjugated with antibodies were allowed to bind to white blood cells captured on the membrane in a microfluidic device. The resulting cell-QD conjugates were imaged to obtain fluorescence from the quantum dots. A digital overlap of the fluorescence images allowed the detection of cells expressing a) CD3+ (red), b) CD4+ (green) and c) both CD4+ and CD3+ (yellow, which is an overlap of red and green).

A major challenge in detection of soluble molecules is the ability to monitor time-varying molecular concentrations in real-time. Such a technology would have a big impact on understanding biological processes, which are inherently dynamic. For example, cells secrete signaling molecules (e.g., cytokines, growth hormones, etc.) at varying concentrations in many diseases, including obesity. The temporal profile of the molecules is at least as important as molecule type and concentration in establishing intercellular signaling. In order to understand the underlying mechanisms of a disease, particularly using in vitro models, it is necessary to accurately and simultaneously monitor independent molecule levels. Motivated with this necessity, our group has initiated the development of a microfluidic platform that involves a bead-based protein detection scheme, as illustrated in

Fig. 10. The operation principle is simply a flow-through ELISA system. First, sampled solution containing the signaling molecules mixes with a suspension of fluorescently-encoded beads that are functionalized with antibodies against the molecule (antigen) to be detected. The solution can be administered externally or delivered from a cell culture chamber connected to the detection structure. As the bead-antibody and antigen solution flows through the serpentine channel and diffusively mixes, antigens are captured by the antibody-coated beads. The high surface area-to-volume ratio of the bead, as well as the diffusive mixing generated within the serpentine structure, reduces incubation time to a few minutes, thereby enabling continuous flow-through detection. The bead-antibody-antigen complex consequently meets the secondary antibodies against the antigen. These antibodies are conjugated with a specific fluorophore. Following the incubation of the antibody-antigen-antibody complex, a localized fluorescent signal is attained around the bead that is more intense than the background. This reduces the need for a wash step to remove unbound secondary antibodies. The fluorescence from the beads and the reporter antibodies are simultaneously detected via a photo-multiplier-tube (PMT) downstream to the second serpentine mixing region. At the detection region adjacent to the PMT, the co-localized fluorescence from the bead and the secondary antibody indicates the presence of the molecule to be detected. Since the medium from the cells and the assay constituents are continuously replenished, it is possible to observe the changes in levels of cytokines/metabolites in real time. Beads and antibodies encoded with different fluorophores enable the facile multiplexing and scale-up of this technology to accommodate a vast array of molecules.

Droplet-based microfluidic devices for cell interrogation

Single-cell droplet technology provides a cost-effective method to gain sequence information from individual cells that have been sorted for phenotype. Thus, both phenotypic and genomic information can be obtained from droplet-encapsulated individual cells. For cell encapsulation, droplet microfluidics uses a two-phase system in which live cells and assay reagents can be compartmentalized in an aqueous microdroplet (of 1 pL to 10 nL in size) surrounded by immiscible oil [74–79]. The advantages of this droplets-based technique include the physical and chemical isolation of droplets eliminating the risk of cross-contamination, the fast and efficient mixing of the reagents within droplets, the ability to digitally manipulate droplets at a very high-throughput, the ability to incubate stable droplets off-chip and reintroduce them into the microfluidic environment for further processing and analysis, and the absence of moving parts such as on-chip valves or pumps [74–79]. More importantly, this nano-liter in vivo-like microenvironment allows appropriate gas exchange which is necessary to ensure viability and functionality of encapsulated cells. In addition, this system can operate as a droplet-based fluorescence-activated cell sorting (FACS), interrogating the entire reaction volume and sorting cells based on the results. However, unlike a traditional FACS, the cells remain encapsulated in droplets and therefore can be identified individually post-sorting.

Previously, Konry et al. developed a robust, highly specific and sensitive screening protocol for the detection of protein markers in a microfluidic nanoliter-reaction-droplet system (Fig. 11). For identifying low-abundance proteins on cell surfaces, RCA reaction was integrated

inside monodisperse aqueous-emulsion nanoliter droplets containing single- PC3 tumor cell and linear RCA reaction mix. This allowed identifying a tumor specific marker, EpCAM, on live tumor-cell surface in miniaturized nanoliter reaction droplets [109].

Another, microfluidic droplet based system was recently introduced for analyzing single cell secretion within the droplets. In this system each cell was encapsulated in its own defined liquid microenvironment within a single droplet together with fluorescent detection antibodies and microspheres previously conjugated with an analyte-specific antibody [110]. To demonstrate the ability of this system to assess secretions from a complex cell population, in prior work the capacity of CD4+CD25 regulatory T (Treg) cell clones to secrete IL-10 was demonstrated. CD4+CD25+ regulatory T cells were encapsulated in nanoliter droplets along with fluorescently labeled detection antibodies and SPHERO™ Avidin-coated particles (0.9 µm) conjugated with 40 µg biotinylated IL-10 monoclonal capture antibodies in PBS suspension according to previously developed protocol. The single-cell secretion of IL-10 was detected after 15 min of incubation in the restricted droplet volume (Fig. 12).

Using a custom designed optical system for interrogation of fluorescent signal within the droplets, one can then determine the secretion pattern in the nanoliter droplets in a time-dependent fashion and sort the cells that secrete specific molecules to establish the heterogeneity in the population. Thus, live cell secretion and surface monitoring can be carried out in distinct microenvironments utilizing microfluidic approach merged with microsphere sensors, which previously was only possible using complicated and multi-step in vitro and in vivo live-cell microscopy, together with immunological studies of the outcome secretion of cellular interactions.

Conclusions

Recent breakthroughs in microfluidic and particle-base technologies, described in this review, demonstrate the feasibility of both microfluidics and particle-based platforms in detection of various clinically relevant agents. We believe that the merger of these two technologies can create powerful LOC devices that may overcome the current limitations of diagnostic strategies such as portability, low cost fabrication and deliberate time to perform the analysis to generate low cost portable devices to be efficiently implemented in clinic. The future success of multiplexed particle analysis in microfluidic devices greatly depends on the integration of optics and electronics on a chip for particle detection in microchannels. The accomplishment of this integration will fulfill the lab-on a chip concept and allow its translation to clinic reducing the cost and efficiency of the diagnostic process. There is no doubt that additional efforts should be placed on fundamental research for development of multiplexed particle-based LOC in order to bring this concept closer to clinical diagnostics. We believe that with the fast-paced development of nanobiotechnology, LOC devices will one day be incorporated for point-of-care assessment.

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Fig. 1. Schematic drawing of the microsphere-based rolling circle amplification (RCA) assay for SNP detection

Fig. 2.

Microsphere-based RCA assay for the detection of a target DNA sequence and IL-8. **a**

Capture of the target protein and binding of secondary biotin-labeled antibodies. **b** Capture

of biotinylated DNA probe via an avidin bridge, and hybridization of the padlock probe. **c**

Hybridization of the target gene brings padlock probe termini in proximity. **d** Joining of the

padlock probe ends by DNA ligase, followed by capture probe extension by polymerase. **e**

Rolling circle amplification of the ligated probe. **f** Hybridization of the Cy3-tagged detection

probes to the RCA product

Fig. 3.

Figure showing the schematic for protein detection using barcoded hydrogel particles. 1) A three probe hydrogel particle is formed in a microfluidic device by combining pressure driven streams into a single channel and inducing polymerization by UV exposure, defined by a transparency mask. The bar-coded microparticles are collected and washed before storage and use. 2) For the assay, pre-made barcoded particles are incubated with target protein (shown as purple pentagon), washed and exposed to a biotinylated reporter antibody (orange) to form a sandwich with the protein target. The reporter antibody bound microparticles are washed and incubated Streptavidin phycoerythrin (SAPE, yellow stars) to bind to the complexes after incubation. A final wash step removes unbound SAPE and the particles are scanned in another flow focusing microfluidic device and protein concentration quantified using fluorescence measurement and analyzed using MATLAB

Fig. 4.

The device architecture showing Inlet A to inject oil–surfactant solution, inlet B to inject a suspension of functionalized beads, and inlet C to inject calibration solution, lysate, or cell suspension. Water/Oil droplets were generated at the T-junction and were stored in a microfluidic chamber (F). Interdigitated square-shaped microelectrodes were used to electrically lyse flowing cells. **b** Droplet formation at the T-junction. **c, d** Fluorescence (panel c) and bright-field (panel d) images showing laminar flow at the Y-junction. The fluorescent phase flowing from inlet C was a fetal serum albumin solution. White arrows indicate functionalized beads within the aqueous stream

Fig. 5.

Microdroplet PCR workflow. **a** Primer library generation. (1) Identify targeted sequences of interest in the genome. (2) Design and synthesize primer pairs for targeted sequences. (3) Generation of primer pair droplets for each library element. (4) Primer pair droplets are equally mixed together. **b** Genomic DNA (gDNA) template mix preparation. (5) gDNA is biotinylated (red dots), broken into 2- to 4-kb fragments and purified. (6) Purified gDNA is mixed with all of the PCR components except for the primers. **c** Droplet merge and PCR. (7) Primer library droplets are dispensed to the microfluidic chip. (8) gDNA template droplets are formed in the microfluidic chip. The primer pair droplets and template droplets are paired together in a 1:1 ratio. (9) Paired droplets merge into a single PCR droplet under electric field in the merge area of microfluidic chip. ~1.5 million PCR droplets are collected into a single 0.2 ml PCR tube and thermocycled. PCR amplicons are released into solution by breaking the emulsion of droplets, for gDNA removal, purification and sequencing

Fig. 6. Schematic illustration showing the microfluidic bead-based immunoassay (μFBI), using a capillary and controlling the flow in the capillary using syringe pump. Antibody-coated beads are captured on a filter (0.5 μm pore diameter) to capture target analytes and react with detection reagents. The beads are collected and analyzed on a Luminex 100 IS instrument for fluorescence and quantified

Fig. 7. Schematic showing the principle of a continuous flow reactor with magnetic particles. Functionalized magnetic particles are introduced in a laminar flow stream into the chamber and are continuously deflected across the width of the chamber (using a magnetic field perpendicular to the direction of the flow), passing through each of the laminar flow streams incorporating assay reagents and washing steps. The beads were then collected and fluorescence was quantified by imaging the beads using a CCD camera and analyzed using Image J

Fig. 8.

Schematic of BEAMing. 1: biotinylated oligonucleotides (oligos) are bound to magnetic beads through streptavidin. 2: oligo-bound beads, PCR components, and template DNA are stirred with an oil-detergent mixture to create microemulsions. On average, each aqueous compartment contains less than one template and one bead. 3: The microemulsions are temperature-cycled to perform PCR in compartments with both bead and DNA template. 4: The beads are purified with a magnet after breaking emulsions. 5: After denaturation, the beads are incubated with oligos that can distinguish between the sequences of templates. The oligos are then labeled with fluorescent antibodies. 6: Fluorescent beads are counted with flow cytometry [97], Copyright 2003 National Academy of Sciences, USA

Fig. 9. Isolation of CTCs from whole blood using a microfluidic device. **a** The workstation setup for CTC separation. The sample is continually mixed on a rocker, and pumped through the chip using a pneumatic pressure-regulated pump. **b** The CTC-chip with microposts etched in silicon. **c** Whole blood flowing through the microfluidic device. **d** Scanning electron microscope image of a captured NCI-H1650 lung cancer cell spiked into blood (pseudo coloured red). The inset shows a high magnification view of the cell [100]

Fig. 10. Schematic of the microfluidic device to monitor molecular secretions in real-time

Fig. 11.

Microfluidic nano-liter platform for cell screening and sorting (1) Droplet-based microfluidic device which was applied to encapsulate PC3 cells in distinct nL-sized droplets of RCA reagents, (2) The RCA reaction droplets are conveyed by the oil focused in the channel for amplification of cell surface marker and optical interrogation, (3– 5) Incubation channel, (6) Shows the interactive 3D surface plot of fluorescence intensity distribution in image 5. **a** Schematic top view of the device. Water drops formed by flow focusing in the continuous phase of oil flow into the waste channel since the resistance of the waste channel is smaller than that of the collect channel. **b** Schematic cross section of the device. The molded PDMS microfluidic channel is aligned to the PDMS layer which is spin-coated on the patterned ITO electrodes

Fig. 12.

a Sequential images showing the droplet formation (1,2) and incubation channel (3,4). **b** Schematic illustration of the configuration of droplet-based microfluidic platform. The center stream contains a mixture of microspheres, cells and secondary antibodies (Mix), while the two opposing side streams contain the oil phase. The encapsulated cells proceed into the downstream incubation region for cell analysis and then can be sorted based on secretion of the interrogated analyte, if the sorting system similar to the one presented in the literature will be added