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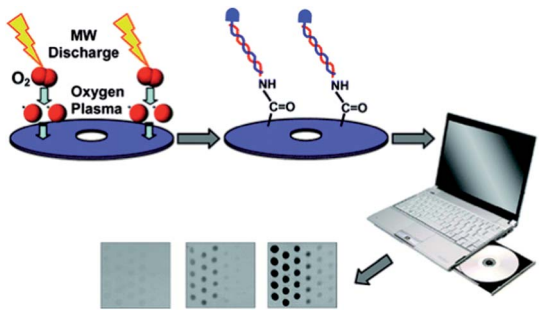
CRITICAL REVIEW

Emerging technologies for biomedical analysis

Christine F. Woolley* and Mark A. Hayes

Emerging technologies for biomedical analysis is a critical review of recent representative publications aimed at improving medical diagnostic capabilities.



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Options for biomedical analysis continue to evolve from many fields of study, employing diverse detection and quantification methods. New technologies in this arena focus on improving the sensitivity of analysis and the speed of testing, as well as producing systems at low cost which can be used on site as a point-of-care device for telemedicine applications. In this article, the most important original experimental platforms as well as current commercial approaches to biomedical analysis are critically chosen and reviewed, covering January 2010 to January 2014. While literature is quite broad and numerous, there is clear emphasis on biological recognition and imaging for the most impactful works. The analytical approaches are discussed in terms of their utility in diagnostics and biomedical testing.

Introduction

Early disease diagnosis is vital so that more effective intervention strategies may be employed. Because early intervention is essential for improving disease prognosis, in recent years there has been an emphasis on increasing the sensitivity of biomedical analysis methods.[1–6] Stemming from this quest for superior sensitivity, several new technologies for biomedical analysis have emerged on both experimental and commercial platforms. These technologies, in general, take place in one of two formats; biological recognition or imaging.

The area of biological recognition is focused on specifically and sensitively quantifying low-abundance species that are indicators of disease pathways. The platforms are highly diverse, ranging from the immobilization of antibodies on hydrogels[1] or unmodified plastics,[7,8] to CD disks for microarray printing[2,9–11] and assays taking place in the traditional microwell format.[3] While some of these assays focus on reducing the non-specific binding associated with long incubation times in traditional assays,[3] the primary trends over the last few years have been toward the development of multiplex assays or rapid point-of-care devices.[8,11,12]

Work also continues towards improving traditional imaging methods used in disease diagnosis. Imaging approaches analyze the information-rich spectra obtained from tissues to identify characteristic absorptions revealing the underlying chemical composition of the sample and identifying cellular biomarkers.[13] Using these methods both labeled and label-free detection methods have been utilized.[13–20] A main contributions

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Dr Mark A. Hayes holds a Professorship in the Department of Chemistry and Biochemistry at Arizona State University, where he serves as an active researcher, mentor, teacher and colleague. His academic career has produced significant results across several disciplines within the analytical and physical chemistry community that includes aspects of engineering, physics, biology and medicine. His under graduate degree in chemistry was from Humboldt State and doctorate from Penn State. While contributing to the knowledge base, he has energetically and creatively supported the wider profession at local, regional, national and international levels.

in this area have been the improvement of resolution in infrared spectroscopy (IR) to overcome the usual limitations imposed by diffraction.[13–17]

Although methods that can be used for biomedical analysis in a traditional lab setting continue to be investigated, a primary focus over the last several years has been the development of mobile health (mHealth) platforms that could be used in telemedicine applications or resource-poor settings where access to quality healthcare is limited.[21–26] Mobile health devices have largely been created on one of two platforms: CD discs that have been used as the solid phase for immunorecognition assays and can be read using an unmodified CD player,[8,27] and those that take place *via* a smartphone attachment.[9,12,21–23,25,26] In both cases an emphasis is placed on using simple fabrication procedures, as well as reusable materials, to keep costs low. There is also an emphasis on producing user-friendly applications that allow patients to perform tests independently and upload the results for experts to interpret and determine the best course of treatment.[21–23,26] Advances in this capacity are vital to rapidly identify and address emerging public health threats, as well as to treat chronic diseases that require persistent monitoring.[26]

While both imaging techniques and biological recognition assays have been the subject of recent reviews,[28–31] the focus of this review will be the application of those new techniques to biomedical analysis and the improvement of early disease intervention published during the time span from January 2010 to January 2014, initiated with literature keyword searches associated with biomedical analysis as well as their references and the later citations of found works. Articles were chosen based on their contribution to new technologies in the area of biomedical analysis and offering improvements both in terms of shorter test duration and higher sensitivity, as well as improving the mobility of testing platforms to reach resource poor areas. While many tests were described in the context of identifying a particular molecule or disease pattern, they could easily be adapted for the testing of many biological species. The topics addressed are divided into techniques used for biological recognition [Section 1] and those used for imaging analysis [Section 2]. These sections are further subdivided into laboratory or clinic-based testing and platforms intended for point-of-care diagnostics.

Biological recognition

Techniques utilizing biological recognition may further be divided into two main subcategories: (i) biorecognition assays that take place within a laboratory or clinical setting and (ii) portable microarrays allowing point-of-care diagnostic testing. Some of the techniques discussed in this section include magnetic bead-based assays, assays taking place on unmodified plastic substrates (Fig. 1), and those utilizing a compact disc (CD) as a solid support. CD-based assays have gained popularity for a variety of reasons including the ease of fabrication and established detection methods. The use of computer drives/disc players adapted as a precise optical reading mechanism and employed as a detection instrument allows the assays to be accomplished at low-cost and away from specialized laboratories. Users are able to fabricate high-density microarrays on a CD disc and perform tests for a variety of different targets including DNAzyme assays, antibody–antigen binding, and microorganisms. The recent expansion of this technique to Blu-ray technology has allowed a reduction in feature sizes and a subsequent improvement in assay sensitivity.

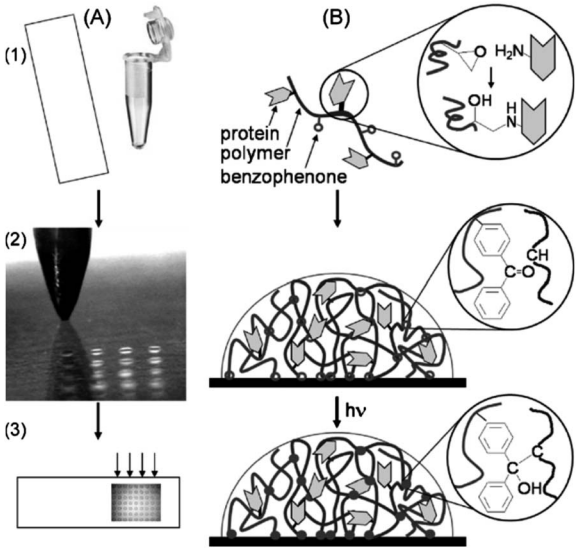

Fig. 1 Illustration of the process for printing microarrays with mixed polymer protein solutions and immobilization through UV-cross-linking both in the macroscopic (A) and microscopic (B) views.[7] Macroscopic view images show (1) a solution is mixed of buffer and polymer (2) a microarray of the solution is printed onto the provided plastic slide and (3) the chip is irradiated using UV light to crosslink the polymers and immobilize them on the surface. On a microscopic level (B) it is observed that (1) epoxide side groups of the polymer react with primary amino acids, (2) droplets containing these formed complexes form on the surface, and (3) the UV photoreaction crosslinks the polymer, attaching it to the surface and immobilizing the proteins in the network of polymers.

Laboratory-based bio-recognition assays

Traditional biological recognition assays employing a static solid support continue to have widespread use both on commercial and experimental platforms.[1,3,7,32–36] Their high sensitivity, versatility in detection methods, and adaptability for the quantification of a myriad of targets continue to make their replacement by other testing platforms a challenge (summary of techniques shown in Table 1). While these methods employ diverse tactics, a primary focus over the last several years has been to address a common pitfall of these assays: non-specific binding (NSB) and its limitation of the assays' potential sensitivity.[1,3] Efforts continue to be made toward increasing sensitivity and reducing the characteristically long incubations associated with these techniques so they may eventually be adapted to portable care diagnostics.

The reduction in non-specific binding for assays having long incubations was investigated by Farajollah *et al.*[3] They describe that the most common problem in microwell assays is the

Table 1

Technique	Applications	Sensitivity	Analysis time	Fabrication/equipment requirements	Reference(s)
Microwell ELISA	Serum antibody immunoassays, protein detection	—	10 min	Micro-well plate pre-incubation procedure, optical density detection	3
Microarray assays	Protein detection, diagnostics	1 pM	3+ hours	Protein printing, UV-exposure crosslinking; Biodetect 645® read-out system	1 and 7
Colorimetric detection	Sandwich bioassays, DNA and protein detection	0.67–10 nM	2+ hours	UV/ozone activation of plastic sheet, 1-ethyl-3-(3′-dimethylaminopropyl)-cabodiimide/*N*-hydroxy-succinimide coupling; fluorescent microscope	8
Rolling circle amplification assay	Low-abundance protein monitoring	38 fM	5+ hours	Overnight microplate preparation; electrochemical workstation	37
pH and metabolic monitoring of live cells	Personalized medicine applications	50 cells	Real-time monitoring	Chemical modification of nanowires; extracellular pH probe	38
Amperometric biosensor	Simultaneous drug detection	1.2–5.5 fM	35 minutes	Fabrication of biosensor probe; electrochemical detector	39
Magnetic bead separation assay	Biomolecule detection	7.1 nM	15 min	Soft lithography fabrication; hemocytometer and microscope apparatus	40
Optically switched dielectrophoretic force	Tissue engineering	—	Days	Photolithography using SU-8 photoresist; CCD equipped microscope	41

detection antibody binding directly to the solid phase. This can occur if undiluted serum is applied even when using a well that has been chemically or biochemically blocked. To minimize the signal arising from NSB while continuing to provide sufficient signal for the detection of low-abundance species, this work introduces a pre-incubation procedure. Using this technique, a biotinylated capture reagent is first incubated with the serum sample and introduced in a secondary step to a streptavidin-coated well. Detection is enabled with labeled anti-species antibodies. The utility of this technique was investigated and findings show that NSB is time-dependent and both serum as well as purified IgG would bind non-specifically to plastic wells. Blocking provided a slight reduction in NSB, but the blocking agents could be displaced after lengthy incubations. Utilizing the pre-incubation method, along with rapid capture times have allowed for improved sensitivities compared with traditional ELISA (enzyme-linked immunosorbent assay) testing and could potentially help to solve the background noise issue associated with those tests. However, because pre-incubation takes place prior to antibody fixation this technique would be difficult to adapt to the parallel detection of analytes since signals could not be spatially isolated.

The issue of NSB was also addressed by the Ruhe group.[1] In earlier work the single step production of protein microarrays on unmodified plastic substrates is presented.[7] Proteins, along with a terpolymer, were printed at high concentration in surface-attached hydrogels. A single UV-exposure step both covalently immobilizes the protein and modifies the surface, inducing swelling to a 3D surface and increasing its binding capacity. The swelling strongly influences the accessibility of the proteins in the hydrogel. Analyzing this method over a series of analyte standards, it was discovered that analyte capture increases linearly with antibody concentration up to an asymptotic limit of $\sim 10^8$ antibodies per spot while achieving a signal-to-noise value of more than 200 at a concentration of 9×10^7 antigens per spot. This technique, through employing more complicated fabrication procedures than assays achieving detection through use of a microwell plate, enables parallel detection of analytes due to the pre-printing of capture proteins prior to analyte incubation and detection. If the fabrication and swelling of the 3D surfaces in the device could be achieved at low cost and with a reasonable shelf life this technique could transition from use in the lab to a portable device.

In later work the Ruhe group evaluated assay sensitivity and the extent of NSB observed using these hydrogels.[1] Compared to traditional methods of preventing NSB through blocking procedures the hydrogel has an intrinsically weak binding capacity for proteins. While purified capture antibody is covalently linked to the structure during the UV-exposure step, NSB is essentially eliminated on these surfaces. This method proved effective for the quantification of bovine serum albumin (BSA) between 1 and 500 nM. It was detected to a limit of 1 pM using this technique. However, at this level the concentration dependence of the signal is too low for quantification. While this limitation is sufficient for the detection of BSA, for adaptation to the detection of other species more sensitive quantitation to the low pM range would be necessary.

Work has also been done on the development of highly sensitive assays by Zhang *et al.* using alpha-fetoprotein (AFP) as a model protein.[37] This was accomplished by coupling metal surface nanolabels to a silver nanocluster (AgNC)-based rolling circle amplification strategy. Under optimal assay conditions results show a dynamic range of 0.14 fM–2.9 nM with a detection limit of 0.11 fM and a limit of quantification found at

38 fM. These levels are able to completely meet the clinical diagnostic requirements for AFP. However, the long duration of incubations and specialized detection methods required for the assay prevents its easy adaptation for use outside the clinic.

In an effort to move biochip technology away from labs and hospitals and enable its use as a point-of-care device, Wen *et al.* describe the development of a novel plastic biochip.[8] The work shows its utility for the sensitive colorimetric detection of both human IgG and DNA. After UV/ozone activation of the plastic substrate probe biomolecules are covalently attached. Signal reporting units are introduced to complete a sandwich-style assay and achieve sensitive detection. Using this label-free recognition system detection limits of 67 pM and 10 nM were achieved for IgG and DNA, respectively. These limits are dependent on staining time and could be adjusted according to assay needs, providing an easy and flexible approach to a portable biochip.

Beyond assays employing a static support, several unique laboratory-based approaches have been developed.[38] Quantitative bioanalysis was accomplished using a sensitive pH sensor by the Patolsky group. This detection method was performed by evaluating the ratio of electrical signals in ground and excited states to determine the concentration of target species. The device was applied to the real-time monitoring of both intra- and extracellular metabolic activity, with sensitivities down to the signal produced by less than 50 cells, or in the vicinity of single-cell metabolic measurements. Although the assay is not currently carried out on a mobile platform, its ability to provide sensitive and rapid monitoring gives this approach potential for the expansion to detecting specific biological species and its utilization in personalized medicine-oriented diagnostics. However, since detection is based on ratios of electrical signals, modifications to allow the parallel detection of biomarker panels represent a significant hurdle for this technique.

Amperometric biosensors were used in a microfluidic device by Chandra *et al.* for the sensitive detection of several anticancer drugs.[39] Sensing was accomplished through the integration of preconcentration and separation steps prior to detection. Results show that the detection limit for all four drugs tested was between 1.2–5.5 fM with a linear response over the 2–60 pM range. This work represents a rapid and sensitive microscale total analysis system whose adaptation to the detection of biomarkers would be beneficial in diagnostics as well as disease monitoring.

Detection on the microscale was also accomplished by Wang *et al.* who demonstrated the capture and separation of biomolecules using magnetic beads.[40] Taking place on a microchip consisting of two reservoirs connected by a tapered channel, assays were performed in one well using the beads as a solid surface and separated for detection in the second well by an external permanent magnet. Results show the transfer could be accomplished within two minutes and that carryover was less than 0.002%. This separation was achieved without the use of a pump, giving it potential as a point-of-care device. While this method allows for rapid detection and in the absence of excess detection antibody, the current setup only allows for the quantification of one target molecule at a time. Alterations of the assay to detect markers in parallel would require drastic changes to the chip design as species would need to be separated prior to capture and detection to avoid cross reactivity and allow differentiation of signals.

Microbeads were also utilized alongside and optically switched dielectrophoretic (ODEP) force in bottom-up tissue engineering.[41] Cell-encapsulating alginate microbeads with three different densities were assembled and manipulated using an ODEP force-based mechanism. Manipulations allowed for the formation of a sheet-like cell structure imitating the cell distribution of articular cartilage. Cells encapsulated remained viable to a rate of 96 ± 2%. This system holds promise for the engineering of tissue with a tunable cell distribution and may aid in efforts for developing biological substitutes for the repair of damaged or diseased tissues.

Many commercial methods for bio-recognition assays employing a solid support are available. These include Whatman's FAST® slides, Oncyte-Avid slides, and Unisart slides.[6,32–36] FAST® slides can be used to perform reverse phase protein arrays, traditional protein arrays and antibody arrays utilizing less sample than a traditional ELISA test. They also allow for the parallel quantitation of many samples to a limit of 1 pg mL^{-1}.[6] This platform has recently been utilized as a point of reference for new experimental techniques as well as tested in comparison to other commercially available slides.[32–34] The ONCYTE® nitrocellulose slides provide a three dimensional microporous film designed in three formulations to diversify its use for higher binding capacities, low-fluorescence intensity, or both.[35] This diversity allows for the use of ONCYTE slides in biomarker discovery as well as studies of protein function. The Unisart membranes are utilized in lateral flow immunoassays (LFIAs) by binding the antibodies or capture molecules while preserving their reactivity.[36] These membranes currently enable the analysis of over 60 markers.

Point-of-care assays

The compact disc assay is a diagnostic platform that functions without the use of sophisticated laboratory equipment. Utilizing this technique, a microfluidic device is created by modifying a CD so that quantitative biological assays can be performed and detection can take place using a standard (or modified) disc reader or the CD drive in a personal computer (Fig. 2 and Table 2). Typically, CDs are prepared by activating the polycarbonate surface with UV/ozone treatment followed by the use of a PDMS stamp to apply surface patterning.[2,9,27] Following CD disc preparation, samples are applied and allowed to incubate for extended periods of time. Once assays are complete, the disc is loaded into a CD-drive where the extent and location of errors in disc reading directly detect and quantify compounds of interest. In addition to their high sensitivity and relatively simplistic fabrication processes, assays using this platform have become increasingly popular due to their low-cost and portability. This makes them an attractive diagnostic option for remote settings as well as use in areas with limited resources.

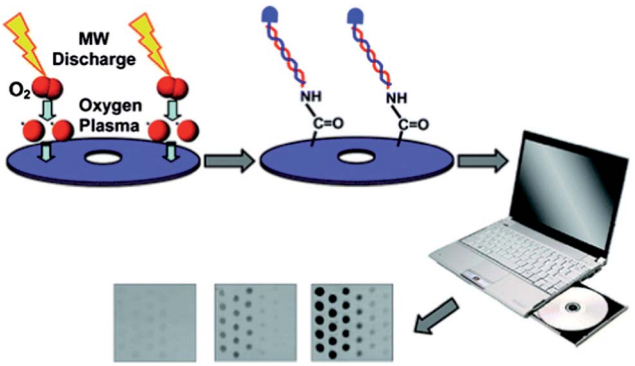

Fig. 2 Is an illustration showing the overall process for performing a DVD-based assay. On the polycarbonate surface of the DVD, activation with oxygen plasma generates carboxylic acid groups which are covalently attached to amino-modified oligionucleotide probes. The use of an unmodified disc reader on a laptop allows for the visualization of spots where binding events occurred and the quantitation of species being investigated. Reproduced from ref. 11 with permission from The Royal Society of Chemistry.

In one experimental incarnation of this technique, Wang *et al.* have demonstrated the detection of lead at the parts-per-billion (ppb) level.[2] Lead detection was quantified using a DNAzyme assay where a DNAzyme strand was hybridized to a substrate strand and immobilized on CD. In the presence of lead the substrate strand was cleaved preventing reporter strand binding and reducing CD reading-error rates. The results of this work demonstrate a direct correlation between lead concentrations and error reading signals in the range of 10 nM to 1 mM, with a lead detection limit of 10 nM (2 ppb). This high sensitivity for lead is more than is required for the routine monitoring of its presence in environmental samples. However, while the disc-based assay is adaptable for other targets the detection setup reflects the approach used in a competitive assay, where signal decreases as concentrations of target analyte increase. This method may therefore not prove to be as sensitive as those for non-competitive immunoassay where signal is directly proportional to sample concentration and could result in an assay with insufficient sensitivity for other low-abundance species. Additionally, the method requires long assay times in excess of two hours, which should ideally be reduced for its application as a point-of-care device.

A similar example of a reading error detection-based disc assay is demonstrated by Pallapa *et al.* in the development of a quantitative biotin-streptavidin binding assay utilizing IsoBuster software for the more specific detection of erroneous bits in a frame as opposed to total error number alone.[27] Identifying where errors took place on disc allows the most direct approach for the modification of a CD to biomedical diagnostics and allows various data formats to be used. Using the IsoBuster analysis software, the results depict a clear dependence of reading error on streptavidin concentration. This allowed for a quantitative assay over the tested range of 5.8–29 nM with high spatial accuracy. Detection was accomplished using an unmodified conventional optical drive, increasing its potential in point-of-care applications. While quantitation was achieved for the tested samples, to adaptto other targets the dynamic range should be expanded and fully evaluated, allowing clinically relevant concentrations of various biomarkers to be detected.

This same approach was used to analyze three trial systems: DNA hybridization, antibody–antigen binding, and ultrasensitive lead detection.[9] The CD-quality diagnostic program is used for detection, which allows a relationship to be generated displaying the reading error on disc as a function of CD playtime. This enables the specific position of the error on disc to be identified, corresponding to the position of the binding event. The results show that this method is sensitive for all systems tested achieving a 25 nM detection limit of DNA from a sample volume of 2.0 μL (50 fmol of DNA), a 0.17 nM detection limit for IgG, and a 10 nM (2 ppb) detection limit for lead. This platform has been shown to be highly diverse in terms of applicable target molecules and portable for on-site applications due to its use of an unmodified disc reader for detection.

Error reading detection was also employed in microparticle and cell counting,[10] providing development of a health diagnostic compact disc (HDCD) aimed at providing rapid and affordable point-of-care diagnostics. While the detection methods employed are simplistic and accessible, the device

Table 2

Technique	Applications	Sensitivity	Analysis time	Fabrication/equip-ment requirements	Reference(s)
CD disc bioassay	Lead detection, streptavidin binding, DNA detection, antibody–antigen detection	10–25 nM	45–65 min	Soft lithography fabrications; unmodified disc reader/computer drive and error analysis software	2, 9 and 27
CD disc micro-immunoassay	Agrochemical residue quantification	37 pM–0.28 nM	30 min	Direct attachment of binding groups to disc; silver enhancement/optical disc drive detection	11
CD disc microparticle counting	Measure biomolecules/cells	1×10^6 cells mL^{-1}	2.5 hours	Soft lithography fabrication; unmodified disc drive	10
Blu-ray microarray	Competitive microcystin array	0.4 nM	1 hour	Blu-ray disc drive and Nero disc speed software	42

fabrication is more complicated than that used in similar assays. First, a trench was machined in the polycarbonate surface of the CD and transparency was restored through wet sanding. A PDMS microfluidic layer was fabricated *via* soft lithography, embedded into the CD trench, and bonded to the CD through an additional PDMS adhesive layer. After sample application, a focused laser beam reflected on the CD data layer is interfaced by the sample suspension. Because the sample is placed directly above the CD data layer, the original digital information is changed through optical interference. The alteration of interaction between the laser and data layer directly relates to the shape, concentration, and optical density of the sample being analyzed. Results show a clear trend with increasing error rates as sample concentration increases. However, incubations of two hours were required between sample introduction and detection. This represents an obstacle to be overcome in the development of a truly mobile device, which would benefit from rapid assay times to increase end user ease of operation.

An alternative CD assay approach was presented by Tamarit-Lopez *et al.*[11] Haptens were attached to the polycarbonate surface of the CD by direct covalent attachment. Assays were based on an indirect competitive format and utilized silver enhancement solution to display the immunoreaction. Compared to related methods, this assay occurs rapidly, lasting about 30 minutes from sample application to detection. The detection limits for the tested compounds chloropyrifos, atrazine, and 2-(2,4,5-trichlorophenoxy)propionic acid were found to be 0.1 nM, 37 pM and 0.28 nM, respectively, an order of magnitude better than classical methods. Along with the use of a conventional optical detector, this illustrates the potential of this assay to be a useful point-of-care diagnostic tool even though it is demonstrated on non-biological samples.

The disc-based assay platform was expanded by this group through its extension to the use of the underlying physicality of DVD technology as the solid support.[4] The disc was activated with oxygen plasma and used to detect the PCR products of *Salmonella* spp. through attenuated analog signal detection. Similar to their previous work the assay time was short, with an 18 minute amplification time to achieve a detection limit of 2 nM with unmodified DVD drive detection.

A further evolution of this technique is illustrated by the introduction of Blu-ray technology in the recent work by Arnandis-Chouer *et al.*[42] The use of Blu-ray discs presents several advantages over a DVD-based assay: (1) blue laser light is used, so the range of optical detection is expanded and (2) a higher numerical aperture lens is used for greater focusing precision allowing for smaller spot sizes and more information to be stored on disc. As a proof-of-concept experiment Blu-ray discs were compared to DVDs for the same assay, Blu-ray assays were detected by a drive attached to a personal computer through a USB and analyzed using Biodisk software. Results show the Blu-ray disc has a 6.2 fold improved detection limit *versus* DVD assays, achieving levels of 0.4 nM for microcystin LR and 10^0 and 10^1 cfu mL^{-1} for *Salmonella typhimurium* and *Cronobacter sakazakii* respectively. While these assays have achieved clinically relevant detection limits for the targets investigated, to become a versatile point-of-care device increased sensitivity should be achieved for the detection of varied proteins.

Several commercial methods utilizing mobile platforms are also available through IMTEK, GenePOC, and Gyros.[43–45] These products have been utilized extensively for biomedical analysis investigations. IMTEK is working to develop an automated, user-friendly platform that can integrate micro, nano, and bio components into a multifunctional point-of-care device.[43] The core of this lab-on-a-chip device is its foil-based centrifugal cartridge that can assist in the integration of all operations allowing raw sample to be injected, purified and analyzed at low cost. Although use of this device has not been widespread to date, it's continued investigation and fine-tuning promises to result in a valuable tool for point-of-care diagnostics and personalized medicine.

The Gyros lab has developed a range of Gyrolab Bioaffy™ CD's that are used for nanoliter-scale immunoassays that allow results to be read in under an hour.[44] This technique was recently validated for the quantification of rituximab in human serum by Liu *et al.*[46] Here, the Gyrolab™ technology was tested and results show validation of the quantification of rituximab between concentrations of 0.62 nM–0.41 μM. This platform allows for fully automated assays to take place utilizing small reagent and sample volumes. While there are limited examples of fully validated Gyrolab assays, this method holds many advantages that make it an attractive option for point-of-care diagnostics. While it also lacks significant examples of test validation in literature, the platform developed by GenePOC diagnostics was designed to be user-friendly with only four steps required for performance.[45] This fully automated system generates results in less than an hour with minimal hands-on time.

Imaging

Imaging-based techniques can be similarly divided into subcategories: (i) those utilizing modified traditional instrumentation and taking place in the laboratory or clinic and (ii) portable platforms allowing remote diagnostics. Some of the techniques discussed in this section include modified Fourier-Transform Infrared Spectroscopy (FTIR) detection, the development of multimodal optical probes, and mHealth monitoring applications. The mHealth platform and others similar to it have gained interest over the last several years because they allow for rapid, sensitive and affordable testing to be accessible in remote settings. It also allows quality healthcare to be possible through telemedicine in regions where access is limited.

Laboratory-based imaging technologies

Infrared (IR) spectroscopy has been used extensively for imaging-based biomedical analysis. A major focus in this area has been the improvement of FTIR for high definition imaging.[13–20] Conventional FTIR microscopy has been limited by trade-offs between signal-to-noise ratios (SNRs) and data

Table 3

Technique	Applications	Resolution	Duration	Fabrication/equipment requirements	Reference(s)
ATR-FTIR	Endometriosis detection	4–8 cm^{-1}	32–45 scans per sample	ATR-FTIR, Bruker Vector 22 FTIR spectrometer, Thermo Nicolet Continuum FTIR microscope	13
Synchrotron FTIR	Lipid detection, label-free imaging	Diffraction limited – 0.54 × 0.54 μm^2	From <1 min for 30 × 30 μm	Mid-infrared beamline IRENI; multiple synchrotron beam source and wide field detection FTIR	16 and 17
Nanoscale imaging	FTIR, AFM-IR	100–200 nm	From 10 min for 100 × 100 pixel image	AFM-IR, novel FTIR system based on s-SNOM	15 and 19
Photothermally induced resonance	Organometallic conjugate detection	20–50 nm; 10 μM	1 hour	AFM and tunable pulsed laser	20
Multifunctional probes	Detection of cancer cells, estrogen	From 10 cells mL^{-1}; 25 μM	2 hours – overnight	TEM, UV-Vis-near infrared laser; synchrotron UV spectromicroscope, FTIR	47 and 48

acquisition times, as well as spatial resolution.[17,18] While IR spectroscopy has long been recognized as a potentially valuable diagnostic tool due to its coverage of regions encompassing characteristic biomolecule absorptions,[13] its utility has been limited by these trade-offs and the lower size boundary imposed by the diffraction limit for the relatively long wavelengths. New experimental techniques have worked over the past several years to overcome those limitations so its full diagnostic potential may be realized (Table 3).

Work by Nasse *et al.* introduces the use of multiple synchrotron beams into FTIR.[17] This was able to extend the IR abilities to truly diffraction-limited imaging over the whole mid-infrared spectrum by combining the multiple beams with wide-field detection. This approach was based on the strategy of wide-field imaging with the use of multichannel focal plane array detectors. Results show the successful measurement of ~1 μm polystyrene beads to a limit of 6 ± 1 fmol in a single pixel (0.54 μm^2). In addition to vast improvement in acquisition time (30 minutes to scan a 280 μm × 310 μm area compared to over 11 days using diffraction-limited resolution raster-scanning), this modification to IR holds great promise as a diagnostic imaging technique.

Synchrotron FTIR (sFTIR) was further used to examine lipids in and around amyloid plaques associated with Alzheimer's disease.[16] The primary motivation was to test for elevated lipid presence near recently formed plaques using sFTIR in transmission mode. To archive acceptable SNR ratios, between 64 and 256 scans were co-added. From this analysis a lipid membrane-like signature was found in and around dense core plaques in both advanced and early stage plaque. While analysis suggests that lipid is a common feature of the plaque structure, there are several potential explanations for its origin, which remains unknown.

In addition to the analysis of Alzheimer's disease-related tissue FTIR was recently used by Cheung *et al.* to discriminate spectral signatures of endometriosis.[13] In this work, both transmission FTIR and attenuated total reflection Fourier-transform IR (ATR-FTIR) were coupled with subsequent computational analysis in an attempt to discern endometrial tissue-specific biochemical-cell fingerprints. Through detailed spectral analysis biochemical differences were identified between healthy tissue and tissue with endometriosis present. While spectral signatures have been observed, this technique requires highly specialized analysis which may limit its utility as a widespread clinical diagnostic tool.

Beyond diffraction-limited resolution lies an interest in the application of FTIR to nanoimaging.[15] As recently discussed by Huth *et al.*, an approach has been developed based on the superfocusing of thermal radiation with an infrared antenna, detection of the scattered light and signal enhancement using an asymmetric FTIR spectrometer. A semiconductor device was used as the sample and imaging was accomplished within a few minutes. Results show that 10 nm spatial resolution can be achieved. For even more rapid applications spectra with 25 cm^{-1} resolution and an SNR of 10 : 1 were captured in only 2 minutes. While demonstrated here for the mapping of a semiconductor, imaging of this resolution could become a powerful tool for chemical/biochemical sample analysis. However, as with many imaging techniques, results must be interpreted by an expert adding to overall analysis time. Additionally, the use of highly specialized lab equipment prevents adaptation of this method to a point-of-care device.

Pita *et al.* also demonstrated simulations aimed at improving the spatial resolution of IR techniques.[14] Results of these simulations suggest that the difference in transmitted and reflected IR energy between a Gaussian reference and a vortex-shaped beam using a confocal microscope could be mapped. This would result in vibrational absorption images with a spatial resolution better than $\lambda/10$. This resolution would enable detection sensitivities great enough for the imaging of organic nanoparticles and would indicate great improvement over classical IR for diagnostic imaging.

In related work toward nanoscale IR spectroscopy, Marcott *et al.* have coupled an atomic force microscope (AFM) and a tunable IR laser source.[19] Using samples of stratum corneum (SC), results show a spatial resolution of ~200 nm. This technique enables the SC to be spectroscopically characterized in more detail than ever before. These studies may prove useful in

diagnostic testing as they are enabling use of this technique for the understanding of penetration pathways for topically applied drugs. However, data capture requires sophisticated laboratory equipment and data interpretation is performed by specialized personnel limiting its capability for widespread use in diagnostics or disease monitoring.

Another technique for the coupling of AFM to IR to improve near-field resolution is described by Policar *et al.*[20] It focusses on the development of photothermally induced resonance (PTIR), where AFM is coupled with a tunable pulsed IR laser. IR is attractive for bioimaging because IR probes are stable in biological environments, they are small, and have intense absorption in the 1800–2200 cm^{-1} region where biological samples are transparent. Using PTIR the spatial resolution is improved to 20–50 nm, which is sensitive enough for subcellular mapping. This was demonstrated using an organometallic conjugate whose uptake by breast cancer cells could be monitored. While not currently used in diagnostic biomedical analysis, imaging at this sensitive level illustrates a powerful improvement to traditional IR imaging and could provide valuable diagnostic data in a sophisticated laboratory setting.

Recent work by Guo *et al.* describes the development of an optical probe used for cancer cell detection.[47] Localized surface plasmon resonance (LSPR) absorption, as well as the fluorescence properties of folic acid-conjugated gold nanorods (F-GNRs) were used as detection systems for the multifunctional probe. The absorption capabilities were explored through the quantification of human cervical carcinoma (HeLa) cells *versus* African green monkey (Vero) control cells. Results indicate a detection limit using fluorescence detection of 70 cells mL^{-1} for HeLa cells with a quantitative range covering 100–5000 cells mL^{-1}. Using the absorption mode, the probe reduced the detection limit to 10 cells mL^{-1} while maintaining the quantitative range for the technique. While this technique achieves low detection limits for whole cells, the same approach could not be used for the detection of proteins without alteration of the assay procedures prior to detection. Additionally, major adjustments would need to be made to the assay platform in order to achieve parallel target detection.

Another unique multimodal probe was investigated by Clède *et al.*[48] This probe, called SCoMPI (single core multimodal probe for imaging), was used for the detection of two breast cancer cell lines. Resolution at the subcellular level was achieved, allowing information about the location of the metal conjugated probe within the cell. This capability allows reliable information to be gathered using many imaging techniques. The diverse and sensitive, rapid imaging platform may prove to be a valuable tool for the biomedical analysis of tissues used in diagnostics.

Experimental adaptations to traditional imaging equipment have also been pursued by Neaspec.[49] The NeaSNOM microscope utilized a new technology allowing imaging in the visible, infrared and terahertz spectral regions to a spatial resolution of 10 nm. This technology has been utilized to map insulin fibrils,[50] as well as determine the local dielectric permittivity of a PMMA film,[51] among other applications. With a scan speed of up to 20 $\mu m\ s^{-1}$ and capability for the analysis of sample up to $40 \times 50 \times 15$ mm, this technique offers a desirable balance of sensitivity and speed for the imaging of biological tissues.

Point-of-care and mHealth platforms

While improvements to traditional imaging techniques have garnered attention over the past several years, mHealth imaging efforts are growing just as quickly (Table 4). This technique takes place with a variety of imaging detection formats attached to the camera lens of a smartphone. By using the camera to capture data, results can be sent to a central hub in a clinic or hospital so care providers can make treatment decisions without patients having to commute from remote locations. This is also valuable for monitoring health in resource-poor settings where access to healthcare is limited and telemedicine is the only real viable option.

Table 4

Technique	Applications	Resolution	Duration	Fabrication/equipment requirements	Reference(s)
mHealth	Fluorescein detection	1–10 nM	10–15 s data capture (~85 min sample prep)	Capillary array, fluorescence detector, multiple wavelength LED, computational image stacking program	12 and 25
Cell-phone microscope	Detection of micro-particles, red blood cells, white blood cells, parasites	~1–2 μm	5 minutes	Specially designed microscope attachment	21
Fluorescent imaging cytometry on cellphone	White blood cell density measurement	2 μm resolution	6 minutes	Specialized optofluidic cellphone attachment	23
Label-free smartphone biosensor	Protein detection	4.25 nM	20 minutes	Photonic crystal biosensor, application for automated data interpretation	24
Rapid diagnostic test reader on cellphone	Immune-chromatographic assays	4× dilution of whole blood	<1 minute, (0.2 seconds per image)	Specialty rapid diagnostic test reader attachment	26

The Rasooly group has contributed a several studies in the area of mHealth over the last few years. In a first example a capillary tube array was developed to improve the sensitivity of smart phones.[25] An increase in sensitivity was needed since common cellphone cameras are generally not able quantify the weak fluorescent signals present in many mHealth applications. An array using 36 capillaries was developed and illuminated using a multi-wavelength LED directed horizontally to the capillary axis. Fluorescein dilutions were tested between 0–10 000 nM, and a limit of detection was found to be ~10 nM in water. This represents roughly a 100-fold increase in sensitivity over the unmodified phone as well as a vast increase in sensitivity compared to 36 well plates whose LOD was 1000 nM.

In a secondary work from this group, mHealth diagnostic sensitivity was further improved using computational image stacking.[12] This was accomplished by capturing data in video mode, stacking the collected images and averaging the intensity of each pixel to reduce or eliminate random noise. To demonstrate the ability of this system to quantify disease-related biomarkers, adenovirus DNA was labeled with SYBR green or fluorescein. Using computational image stacking signal sensitivity was improved, reducing the LOD to 1 nM. While this technique currently demonstrates sensitivity similar to a standard well-plate reader, its portability, ease of use, and the potential for further increasing the sensitivity while utilizing minute sample volumes makes it an attractive and promising mHealth platform. However, before this technique could be used in diagnostic applications the platform would need to be evaluated for signal arising from cross-reactivity or non-specific binding in samples which are not present in the current model system and have not been considered in its limit of detection.

Cost-effective microscopy for telemedicine has also been studied extensively by the Ozcan group. The primary focus of this group has been the development of lens-free microscopy (Fig. 3).[21–23,26] A microscope-based on digital in-line holography was developed using an light-emitting-diode (LED) and compact opto-electric sensor-array, which allows imaging without the need for lenses or bulky optical equipment.[21] The microscope was tested for imaging performance using a variety of cells and particles. Results show that, in addition to making the platform robust and cost-effective, lens-free imaging was able to achieve subcellular resolution. This work was extended by the imaging of micro-particles, red-blood cells, white blood cells, platelets and a waterborne parasite.[22] The spatial resolution in this application was limited by the pixel size of the sensor.

Further expansion of these efforts was observed by the integration of imaging cytometery and fluorescent microscopy as an optofluidic attachment.[23,26] This is achieved by inserting a disposable microfluidic channel above the existing cellphone camera to deliver targets of interest to the imaging volume. The captured images are then processed through a smart application that both validates the test and automatically presents the diagnostic results. This provided an imaging resolution of ~2 μm, which highlights this technique's potential as a valuable rapid imaging test for the routine remote monitoring of chronic conditions as well as disease screening in resource-poor areas.

The rapid-diagnostic-test platform was expanded to work with various lateral flow immuno-chromatographic assays to sense a target analyte.[26] In order to accomplish high-contrast imaging, diffused LED arrays were incorporated prior to data processing. The platform was experimentally tested using malaria, tuberculosis (TB) and HIV through detection with gold-labeled antibody-antigen complexes. This technique is capable of rapid, high-resolution screening while providing instant testing results. However, because its format only allows for the analysis of one target species at a time, its use as a diagnostic point-of-care device would require the transport of a library of microfluidic channel inserts which could decrease its ease of use, leaving it better suited to the monitoring of chronic diseases in remote locations as opposed to a diagnostic tool.

Label-free detection on a smart phone was recently demonstrated by Gallegos *et al.*[24] Broadband light entered through a pinhole and was collimated prior to passing through a photonic crystal biosensor fabricated on a plastic substrate. A custom software application was able to convert the resulting images to transmission spectra and perform curve-fitting analysis. Results

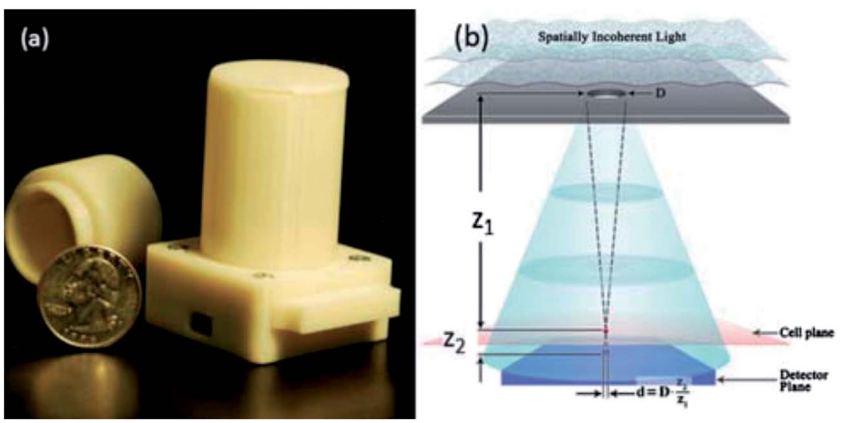

Fig. 3 Is reproduced from ref. 21 with permission from The Royal Society of Chemistry. The figure shows the platform for the attachment of a lens-free microscopy application to an existing cellphone camera. (A) Shows an image of the actual device used. Shown in (B) is a schematic of the microscope shown in (A).

show peak wavelength value shifts could be measured with 0.009 nm spectral accuracy, allowing the detection of an immobilized protein monolayer as well as selective, concentration-dependent antibody binding to a limit of 4.25 nM. The measurement of wavelength shifts above the sensor-specific background noise suggests the LOD is controlled by variations within the assay as opposed to the detector resolution. The development of label-free biodetection on an affordable, sensitive, and mobile platform represents a valuable potential tool for point-of-care diagnsotics, but similar to many cellphone-based techniques is hampered by its limitation to the detection of a single analyte.

The concept of a rapid, affordable point-of-care device has also been explored commercially.[52–55] The most common commercial platform thus far is that traditionally used in glucose monitoring.[52,53] One recent study compares the Stat-Strip (SS) and SureStrep Flexx (SF) for glucose testing.[52] Results of this show the that the sensitivity of SS was 94.7%, compared to 100% for SF and negative predictive values were found to be 86.1% in both cases. These results were achieved using venous blood samples compared to plasma glucose traditionally monitored, and it was determined that they were of limited use compared to the reading of plasma samples.

The development of point-of-care testing for diverse disease biomarkers has also been explored through a partnership between Texas Instruments and Cnoga Medical.[54,55] Together they have investigated the use of video cameras to measure vital signs like blood oxygen, carbon dioxide levels, blood pressure and pulse rate through skin analysis. Current research by these companies aims to expand this technology toward having capabilities to noninvasively identify biopatterns for diverse diseases.

Conclusions

In examining the publications over this time span, there was a large emphasis on the development of mobile diagnostic devices both utilizing imaging and biological recognition detection platforms. This focus has evolved from the need to monitor global health issues, as well as treat patients in remote or resource limited locations. While platforms in this arena range from microarrays printed on CD or DVD discs, to the use of a cellular phone camera to image or count cells and interface with applications that allow data to be analyzed centrally through telemedicine, importance has been placed on the need to perform tests rapidly, at low cost, with sensitivity comparable to technologies used in a permanent laboratory, and with user-friendly interfaces. While mHealth and related cellphone applications do not currently share the advantage of parallel biomarker analysis, alterations in the design of the microfluidic cartridges used for testing prior to detection through the cell phone camera should not detract from the sensitivity of detection.

Another broad area of research is modifying traditional infrared spectroscopy equipment to overcome the traditional diffraction-limited resolution capabilities while maintaining reasonable analysis times. These approaches are valuable as they allow for subcellular imaging, pinpointing label locations within a cell, and determination of characteristic spectral signatures for related tissues that may help in the evolution of imaging biomarkers for disease. However, the ultimate limitation to these techniques is the singleplex analysis format they are confined to. While improvements in resolution and reduction in analysis time make these valuable testing platforms, often a great deal of post image-capture analysis is required for the interpretation of results, and only one tissue may be imaged at a time, presenting a bottleneck to diagnosis of conditions compared to techniques that allow many tests to be performed in parallel in a similar time frame.

Finally, laboratory or clinic-based biorecognition assays have continued to evolve in terms of their sensitivity and capability for the analysis of many compounds simultaneously. While these techniques are diverse, they often require moderate sample volumes and longer incubation times than many of the point-of-care testing devices. In terms of these testing devices, the greatest potential for providing an optimized biomedical analysis device comes from those assays being developed as part of a microfluidic analysis system, where traditional incubation methods are forgone *in lieu* of convective mixing, as well as sample pre-concentration and purification prior to detection which both reduces cross-reactivity and allows for relatively complete capture of analyte from a sample volume enabling low sample volumes and reduced analysis times.

While many of the biomedical analysis techniques described here have been successful in improving upon on diagnostic capabilities or disease monitoring, there does not currently exist one definitive optimal technique. Progress has been made in the ability of various platforms to be user-friendly, rapid, and sensitive while achieving high resolution and using low sample sizes. However, challenges still remain in adapting many laboratory-based biological recognition assays to a point-of-care format without compromising sensitivity or requiring specialists to interpret results. Future techniques, in addition to being adaptable for the testing of a variety of diseases, should require minimal sample and be capable of quantifying multiple targets in parallel.

While various analysis approaches have specific advantages, combining the rapid analysis capabilities and user-friendly data presentation of the cellphone-based platforms with the high sensitivities and parallel analyte analysis achieved in laboratory-based biological recognition assays appears to present the most realistic path toward optimizing diagnostic and disease monitoring capabilities. Although more work needs to be done toward optimizing microfluidic attachments that may be used in remote locations for multi-analyte detection, developments in this arena over the next few years hold promise in providing optimize tests for disease monitoring tailored to the needs of their specific targets, as well as versatile platforms that may be adapted to the early detection and diagnosis of varied diseases.

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