THE FUTURE: BIOMARKERS, BIOSENSORS, NEUROINFORMATICS, AND E-NEUROPSYCHIATRY

Christopher R. Lowe

Department of Chemical Engineering and Biotechnology, Institute of Biotechnology, University of Cambridge, Cambridge, United Kingdom

Abstract
I. Introduction
II. Current Diagnostic Tools for Mental Illness
III. The Emergence of Molecular Biomarkers
IV. Clinical Impact of Biomarkers
V. Point-of-Care Testing
VI. Biosensors
VII. Biosensors in Neuroscience
VIII. Neuroinformatics
IX. e-Neuroscience and e-Neuropsychiatry
Acknowledgments
References

Abstract

The emergence of molecular biomarkers for psychological, psychiatric, and neurodegenerative disorders is beginning to change current diagnostic paradigms for this debilitating family of mental illnesses. The development of new genomic, proteomic, and metabolomic tools has created the prospect of sensitive and specific biochemical tests to replace traditional pen-and-paper questionnaires. In the future, the realization of biosensor technologies, point-of-care testing, and the fusion of clinical biomarker data, electroencephalogram, and MRI data with the patient's past medical history, biopatterns, and prognosis may create personalized bioprofiles or fingerprints for brain disorders. Further, the application of mobile communications technology and grid computing to support data-, computation- and knowledge-based tasks will assist disease prediction, diagnosis, prognosis, and compliance monitoring. It is anticipated that, ultimately, mobile devices could become the next generation of personalized pharmacies.

INTERNATIONAL REVIEW OF NEUROBIOLOGY, VOL. 101
DOI: 10.1016/B978-0-12-387718-5.00015-8

0074-7742/11 $35.00

I. Introduction

A mental illness is a psychological or behavioral pattern associated with subjective distress or disability which lies outside normal development or culture of the afflicted individuals. There are multiple types of mental illness where different facets of human behavior, emotion, and personality can become disordered (Gazzaniga and Heatherton, 2006). Psychological, psychiatric, and neurodegenerative disorders are three manifestations of this diverse group of debilitating behavioral and mental health diseases which can strike at various ages and gravely impair the sufferer's quality of life, social well-being, and productivity. Psychological disorders lie at the mild end of the spectrum and include atypical expressions of anxiety, panic, obsession, compulsion, personality, and behavior and are generally treated by psychopharmacy and psychotherapy. They can strike at any age, whereas the more severe neuropsychiatric disorders, such as schizophrenia, bipolar disorder, major depression, and autism, afflict the young and can be ameliorated to some extent with prescription medicines. For example, major depression is a severe neuropsychiatric disorder which affects approximately 10% of the global population and is characterized by low mood and self-esteem, misguided guilt, harboring thoughts of death and suicide, reduced concentration, anhedonia, and disturbance of sleep and appetite. Only 60% of patients respond to current lengthy antidepressant regimes with a high rate of relapse and marked resistance to treatment (Martins-de-Souza *et al.*, 2010). Similarly, schizophrenia and bipolar affective disorder affect at least 2% of the population worldwide and cost hundreds of billions of dollars in health-care provision, treatments, and lost earnings. The World Health Organization (WHO) found schizophrenia to be the world's fourth leading cause of disability accounting for 1.1% of the total DALYs (disability adjusted life years) and 2.8% of YLDs (years of life lived with disability). These severe psychiatric disorders often manifest themselves with psychotic states characterized by disruption of basic perceptual, cognitive, affective, and judgmental processes. Typically, schizophrenia has its onset in late adolescence or early adulthood and presents as a constellation of positive (hallucination, delusions, disorganization of thought, and bizarre behavior), negative (loss of motivation, restricted range of emotional experience and expression, and reduced hedonic capacity), and cognitive impairments with extensive variation between individuals. Like many neuropsychiatric disorders, no single symptom is unique to schizophrenia and/or is present in every case. Psychotic episodes, for example, are also not uncommon in cases of brain injury, learning disability, substance abuse, and a range of metabolic disorders, and may occur after chronic psychological stress and vary in duration between individuals. In addition, many patients with schizophrenia experience comorbid difficulties with depression and substance abuse contributing to the 10–15% lifetime incidence of suicide. Not surprisingly, up to 90% of schizophrenia patients are unemployed.

Neurodegenerative disorders, on the other hand, are characterized by senile dementia and include Alzheimer's (AD), Huntington's (HD), and Parkinson's diseases (PD) plus a plethora of other medical or neurological disorders characterized by chronic inflammation of the brain. It is estimated that nearly 25 million people currently suffer from dementia with the number of sufferers projected to increase to more than 80 million by 2040 (Ferri *et al.*, 2005). The most common dementia, AD, is an age-related and insidious onset neurodegenerative disease which afflicts approximately 12.5% of people over 65 years (Song *et al.*, 2009). Disease pathogenesis may affect the brains of patients possibly years or decades prior to expression of the clinical symptoms (Snowdon *et al.*, 1996).

II. Current Diagnostic Tools for Mental Illness

In conventional medical practice, the physician renders a diagnosis based on observation of particular overt combinations of symptoms that patients present, identifies an underlying disorder, and prescribes a specific treatment. However, with mental illness, psychologists, psychiatrists, and other mental health workers are confronted with a plethora of ethereal and even covert cognitive, behavioral, and emotional symptoms which collectively may form a particular syndrome. Diagnosis of mental disorders is generally based on judgment of the patient's appearance and behavior, self-reported symptoms, mental health history, and current lifestyle. Psychiatric diagnosis is usually performed via paper-and-pen or computerized questionnaires based on a standard diagnostic proforma guided by ICD-10, Chapter V: "Mental and behavioral disorders, part of the International Classification of Diseases" produced by the WHO, and the Diagnostic and Statistical Manual of Mental Health (DSM-IV) produced by the American Psychiatric Association (APA). However, in reality, these diagnostic evaluations are often incomplete, unstructured, open-ended, and prone to inaccuracy or misdiagnosis of the patient (Shear *et al.*, 2000).

These current diagnostic protocols for mental disorders rely on phenomenological and behavioral paradigms which systematically exclude observations from biological or pathophysiological sources. However, recent advances in clinical neuroscience are beginning to unravel the neurobiochemical underpinnings of such disorders. Advances in molecular imaging techniques, such as positron emission tomography (PET), magnetic resonance imaging (MRI), functional magnetic resonance imaging (fMRI), ultrasound, and single photon emission computerized tomography (SPECT), have made enlightening contributions to the understanding of the pathophysiology of neuropsychiatric disorders (Bressan *et al.*, 2007; see chapter "Imaging brain microglial activation using positron

emission tomography and translocator protein specific radioligands" by Owen and Matthews). These minimally invasive technologies exploit radiolabeled tracers based most commonly on ^{11}C ($t_{1/2} = 20$ min), ^{13}N ($t_{1/2} = 10$ min), ^{15}O ($t_{1/2} = 2$ min), and ^{18}F ($t_{1/2} = 110$ min) which when injected *in vivo* undergo positron emission decay to produce a pair of photons moving in approximately opposite directions and detected with a photomultiplier or silicon avalanche photodiode in a dedicated PET scanner. The use of 2-[^{18}F]-fluorodeoxy-D-glucose (2FDG), a nonmetabolizable analogue of glucose, permits regional glucose uptake, and hence tissue metabolic activity to be assessed, in 3D or 4D space (Young *et al.*, 1999). PET neuroimaging is based on the assumption that areas of high radioactivity are associated with brain activity and this has been widely used in aiding early diagnosis of AD and for differentiating AD from other dementias (Klunk *et al.*, 2004; see Chapter "Imaging brain microglial activation using positron emission tomography and translocator protein-specific radioligands" by Owen and Matthews). Similarly, numerous compounds that bind selectively to neuroreceptors of interest, including dopamine (D1, D2), serotonin (5HT1A, 5HT2A), and opioid (μ) receptors, have been labeled with ^{11}C or ^{18}F and used in human subjects for investigating the differences between patients and healthy controls in dementia, schizophrenia, substance abuse, mood disorders, and other psychiatric disorders (Bressan *et al.*, 2001; Ravina *et al.*, 2005; Small *et al.*, 2006). Molecular imaging techniques have furnished compelling pathophysiological evidence of dopaminergic dysfunction *in vivo* in psychiatric disorders such as schizophrenia (Bressan *et al.*, 2001). Several studies showed increased DOPA decarboxylase activity using [^{18}F]-fluoro-DOPA or [^{11}C]-DOPA and higher dopamine release after amphetamine α-methyl-paratyrosine challenges (Bressan *et al.*, 2001). However, despite being of interest scientifically, such techniques still have limited clinical utility due to the complexity of psychiatric disorders and the low sensitivity and specificity of the current data. Nevertheless, a more powerful strategy of combining several diagnostic techniques at the same time is beginning to emerge. For example, coregistration of PET scans alongside CT or MRI scans allows both anatomic and metabolic information to be spatiotemporally colocated, while inclusion of genetic algorithms, structural neuroimaging, neuropsychology, neuroendocrinology, and molecular imaging allows a more confident interpretation of clinical data (Bressan *et al.*, 2007).

III. The Emergence of Molecular Biomarkers

Current dogma on the diagnosis and follow up of most psychological, neuropsychiatric, and neurodegenerative disorders is based on the aggregation of a cluster of symptoms and scales, which makes identification of individuals at risk, the severity of the disorder, and even an accurate diagnosis quite difficult. This is

primarily due to the fact that the fundamental lesions underlying diseases of the central nervous system (CNS) are ill established and likely to be accentuated by complex perturbations and dysregulation of regulatory mechanisms, protein expression profiles, and metabolic pathways. Consequently, any understanding of these disorders should be accompanied with a systems level view of the dysregulated factors contributing to the pathogenesis. It is imperative to construct disease- or symptom-specific molecular fingerprints or panels of molecular biomarkers by integrating multiple "omics" databases established from genomics, proteomics, and metabolomics studies on disease versus control samples (see chapters "The utility of gene expression in blood cells for diagnosing neuropsychiatric disorders" by Woelk *et al.* and "Proteomic technologies for biomarker studies in psychiatry: advances and needs" by Martins-de-Souza *et al.*).

A molecular biomarker is a chemical, physical, or biological parameter that can be objectively measured and evaluated quantitatively as an indicator of normal or pathogenic states, to measure the onset or progress of disease, of compliance or response to a therapeutic intervention (Quinones and Kaddurah-Daouk, 2009). Biomarkers can be specific cells, genes, gene products, such as mRNA transcripts, proteins, and their posttranslational modifications, enzymes, hormones, peptides, or metabolites (Craig-Shapiro *et al.*, 2008).

The discovery of authenticated biomarkers for psychological, neuropsychiatric, and neurodegenerative disorders could dramatically change the future delivery of mental health care if they are incorporated into standard operating systems and clinical decision making. Appropriate genomic, proteomic, and metabolomic biomarkers should allow precise prediction of disease susceptibility and risk, diagnosis and prognosis, patient and therapeutic stratification, patient response, and adverse drug reactions, and ensure compliance (Fig. 1).

Genes abnormally regulated during neuropsychiatric disorders or affected by drug treatments may help to define aberrant cellular processes and serve as diagnostics and novel druggable targets. Several recent studies using cDNA microarray and differential display techniques have investigated changes in gene expression in AD (Hata *et al.*, 2001; Pasinetti, 2001), seizures and epilepsy (Aronica *et al.*, 2001; French *et al.*, 2001), PD (Chun *et al.*, 2001), schizophrenia (Mirnics *et al.*, 2000), bipolar disorder (Sun *et al.*, 2001; Le-Niculescu *et al.*, 2007), and other neurological disorders (Kontkanen and Castrén, 2002). However, despite progress on functional genomics for neuropsychiatric disorders, significant challenges remain before expression profiling can form a reliable basis for specific and sensitive biomarkers. Neuropsychiatric diseases are complex polygenic disorders with variable penetrance, whose phenotypic heterogeneity, overlap, and interdependence are not well understood and which are greatly influenced by epigenetic modifications, stress, lifestyle, infections, and medications (Danziger *et al.*, 2005; Crow, 2007 (Fig. 2).

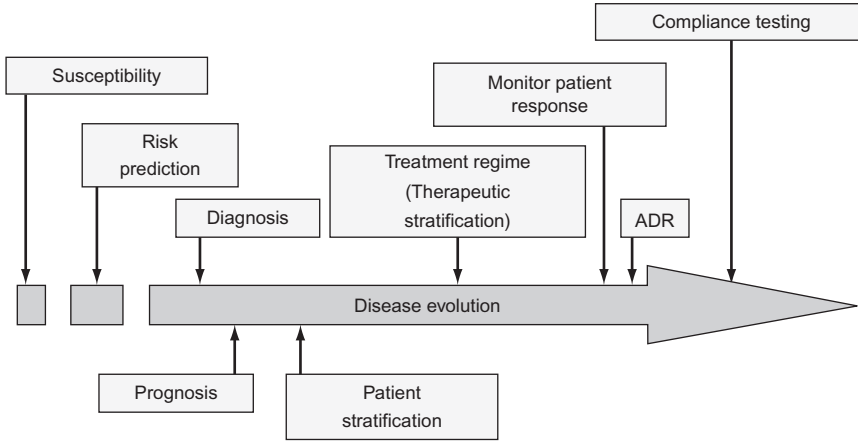

FIG. 1. Implication of biomarkers and biosensors in the disease evolution of psychological, neuropsychiatric, and neurodegenerative disorders.

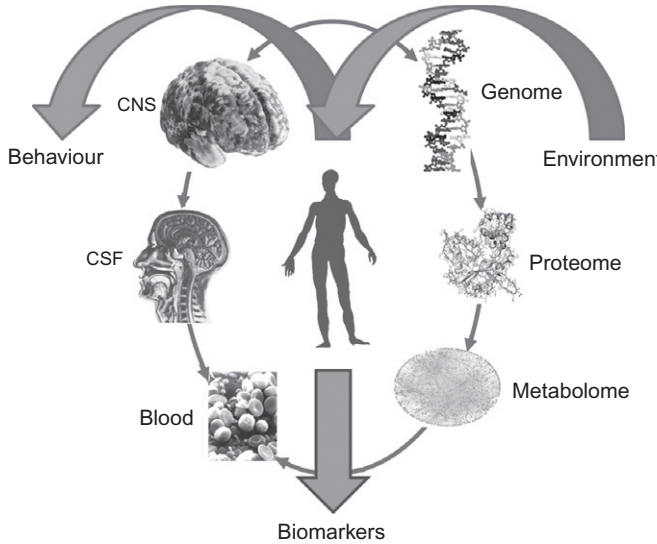

FIG. 2. Schematic of the interactions between genetic and epigenetic factors and behavior and their effect on the discovery of genomic, proteomic, and metabolomic biomarkers from brain biopsies, cerebrospinal fluid (CSF), and blood.

Genomic studies are able to identify genes conferring susceptibility to a particular disease, although the functional abnormality is ultimately reflected in the posttranslational proteome. In recent years, proteomics has been used as a tool for the

discovery of biomarkers for diagnosis, monitoring disease progression, treatment response, and for the identification of novel therapeutic targets (Martins-de-Souza *et al.*, 2010). However, for proteome analysis of CNS disorders, the brain is not readily accessible for invasive diagnostic purposes and sources such as cerebrospinal fluid (CSF), serum, plasma, saliva, urine, and peripheral blood cells are more appropriate (Schwarz and Bahn, 2008; Taurines *et al.*, 2011). CSF is of particular relevance to biomarker discovery in psychiatric disorders since it is in direct contact with the brain and most likely reflects aberrations in brain function more closely than any other fluid (Rohlff, 2001). The protein concentration (0.18–0.58 mg ml^{-1}) in CSF is two to three orders of magnitude lower than in serum ($\sim$75 mg ml^{-1}; Schwarz and Bahn, 2008) and has presented problems with analytical techniques.

Serum presents the most accessible fluid for proteome analysis, although it is inherently complex, has a dynamic range exceeding 10 orders of magnitude, and the 22 most abundant proteins account for 99% of the proteome. The concentration of proteins in plasma ranges from 5 pg ml^{-1} (interleukin-6) to 50 mg ml^{-1} (albumin; Anderson and Anderson, 2002). Proteomics platforms must cope with multiple samples, high throughput, substantial complexity, a wide dynamic range, and a plethora of posttranslational modifications. Key techniques with promise include traditional 2-DE MS, 2D-DIGE, SELDI-TOF MS, iTRAQ-based LC–MALDI–MS/MS, and label-free LC–MS/MS which balance accuracy and high throughput with varying degrees of success (Anderson and Anderson, 2002; see Chapter "Proteomic technologies for biomarker studies in psychiatry: Advances and needs" by Martins-de-Souza et al.). Application of these platforms in CSF and serum has identified multiple up- and downregulated protein biomarkers in AD (Song *et al.*, 2009; Flood *et al.*, 2011), schizophrenia (Schwarz and Bahn, 2008; Madaan *et al.*, 2010; Stanta *et al.*, 2010), depression (Gudmundsson *et al.*, 2010; Martins-de-Souza *et al.*, 2010), and Asperger's syndrome (Schwarz *et al.*, 2010a,b).

The study of metabolism at the global level also promises to impact on the identification of biomarkers for neuropsychiatric diseases (Quinones and Kaddurah-Daouk, 2009). Several metabolic aberrations have been observed in CNS disorders; for example, impairments in neuronal survival, neurotransmitters, oxidative stress, free radical ratios, membrane composition, mitochondrial function, and immune function. Thousands of low molecular weight metabolites can be resolved and quantified in CSF, plasma, or urine samples using a variety of platforms such as a nuclear magnetic resonance (NMR) spectroscopy, gas chromatography– and liquid chromatography–mass spectroscopy (GC–MS, LC–MS), and liquid chromatography with electrochemical array detection (LCECA). However, there is no universal platform which can capture the entire metabolome, and generally speaking, combinations of such techniques will give a more complete picture of metabolite changes in the patient versus the healthy controls. Metabolic signatures have been identified in PD (glutathione, uric acid; Bogdanov *et al.*, 2008) and HD (glycerol, malonate; Underwood *et al.*, 2006), depression

(fatty acids, 3-hydroxybutanoic acid, glycerol, γ-aminobutyric acid; Paige *et al.*, 2007), and schizophrenia (structural and energetic lipids, glucose; Holmes *et al.*, 2006; Wolf and Quinn, 2008).

IV. Clinical Impact of Biomarkers

In principle, simultaneously capturing biomarker data from genomic, proteomic, and metabolomic platforms should assist in determining how gene-expression patterns result in specific protein translation, pathway up -or downregulation, and changes in the metabolome. Such an approach has demonstrated compromised brain metabolism in bipolar disorder and schizophrenia (Prabakaran *et al.*, 2004; Khaitovich *et al.*, 2008). However, currently, clinicians capture only a small fraction of the information contained in the global bionome, usually via a standard set of blood analytes, including gases, ions, metabolites, enzymes, and antigens/antibodies, to define healthy and diseased states. In the near future, it is conceivable that such a restricted blood analysis will be replaced with a much more extensive bionomic signature that captures global biochemical changes in health, disease, and upon medication. A carefully selected multiparameter diagnostic should enhance the sensitivity (the ability of a diagnostic to identify all patients afflicted with the illness, i.e., few false negatives) and specificity (the ability to identify all patients without the illness, i.e., few false positives) of the test. Ideally, to be clinically relevant to diagnose a particular disorder, a multiparameter biomarker panel should have a sensitivity and specificity of $>85\%$ (Quinones and Kaddurah-Daouk, 2009). Multiplexed biomarker assays have proven useful in diagnosing breast cancer using gene-expression signatures (Sotiriou and Pusztai, 2009).

The avalanche of candidate biomarkers discovered by MS-based proteomics has created a market for high-throughput multiplexed immunoassays that allow simultaneous quantification of the analytes. Two basic assay formats have been developed to facilitate quantification of multiple antigens: planar array assays and microbead assays (Fu *et al.*, 2010). In the first format, different capture antibodies are spotted at defined positions on a 2D array. In the second, the capture antibodies are conjugated to different populations of microbeads, which can be distinguished by their fluorescence intensity in a flow cytometer. Commercial assay platforms such as MULTI-ARRAY (Meso Scale Discovery), Bio-Plex (Bio-Rad Laboratories), A^2 (Beckman Coulter), FAST Quant (Whatman Schleicher & Schuell BioScience), and FlowCytomix (Bender MedSystems) represent examples of these technologies currently used for high-throughput immunoanalysis (Fu *et al.*, 2010).

Recently, a serum-based diagnostic test has been developed to aid in the confirmation of a diagnosis of schizophrenia (Schwarz *et al.*, 2010a,b; see chapters "The application of multiplexed assay systems for molecular diagnostics" by Schwarz *et al.* and "Algorithm development for diagnostic biomarker assays" by Izmailov *et al*). In preliminary studies using a multiplexed immunoassay platform, the luminex xMAP technology, a disease signature comprising 51 metabolites, peptides, and proteins which could differentiate schizophrenia ($n = 250$) from control ($n = 230$) samples. These analytes were then used to construct a diagnostic decision rule by developing a refined 51-plex immunoassay panel, validated using a large independent cohort of samples from schizophrenia ($n = 577$) and matched control ($n = 229$) subjects, and shown to display an overall sensitivity of 83% and specificity of 83% while implemented in a Clinical Laboratory Improvement Amendments (CLIA)-certified laboratory in the United States (Schwarz *et al.*, 2010a,b). It is anticipated that a multiplexed immunoassay platform will eventually be able to distinguish between various neuropsychiatric diseases such as schizophrenia, bipolar disorder, and major depressive disorder.

The luminex technology comprises 100 color-coded microsphere sets which can be coated with a reagent specific to a particular bioassay, allowing the capture and detection of specific analytes from a sample. Lasers within the compact analyzer excite the internal dyes that identify both each microsphere particle and any reporter dye captured during the assay. In this way, the xMAP technology allows rapid and precise multiplexing of up to 100 unique assays within a single sample. Focused, flexible multiplexing in the range of 1–100 analytes meets the needs of a wide variety of applications, including protein and gene-expression profiling; autoimmune, genetic, and molecular infectious disease monitoring; and human leukocyte antigen (HLA) testing.

V. Point-of-Care Testing

A typical centralized hospital laboratory has evolved over the past 100 years into a fully automated system of bar-coded patient identification, sample collection, sample pretreatment, and passage through high-throughput multiplexed clinical chemistry and immunoassay platforms, with results becoming available minutes, hours, or days later. Such delays can hamper timely diagnosis, impede decision making, and affect outcomes (see chapter "Challenges of introducing new biomarker products for neuropsychiatric disorders into the market" by Bahn *et al.*). However, in recent years, there has been a quiet revolution in diagnostics practice, with a perceptible trend in taking tests to the patient, rather than the patient to the tests. Substantial technological advances in assay chemistry, sensor and transducer configurations, electronic processing and data manipulation,

instrumentation and miniaturization have seen an upsurge of interest in "alternate site" diagnostic testing in ward, outpatients, physician's office, workplace, and home. This type of patient-proximal, or point-of-care (POC) testing as it is often referred to, can reduce the cost per test by as much as 35% with additional savings in skilled manpower. POC testing simplifies the steps involved in sample handling and pretreatment and can reduce the turnaround time from hours to minutes with proportionately rapid clinical decisions (Price *et al.*, 2010). These trends are illustrated schematically in Fig. 3 which highlights the movement toward making health care more patient centric as part of global health-care reforms which are being driven by the steady growth in health-care expenditure as a proportion of GDP and concerns about the quality of health care, particularly the increasing burden of chronic conditions such as psychiatric and neurodegenerative disorders, and their effective management (Price *et al.*, 2010).

The question is what technology can accommodate multiparameter diagnostic tests to generate rapid, precise, reliable, and foolproof data suitable for clinical decision rules for psychiatric and neurodegenerative patients. POC testing devices include a spectrum of systems covering *in vivo* implanted, wearable, and handheld

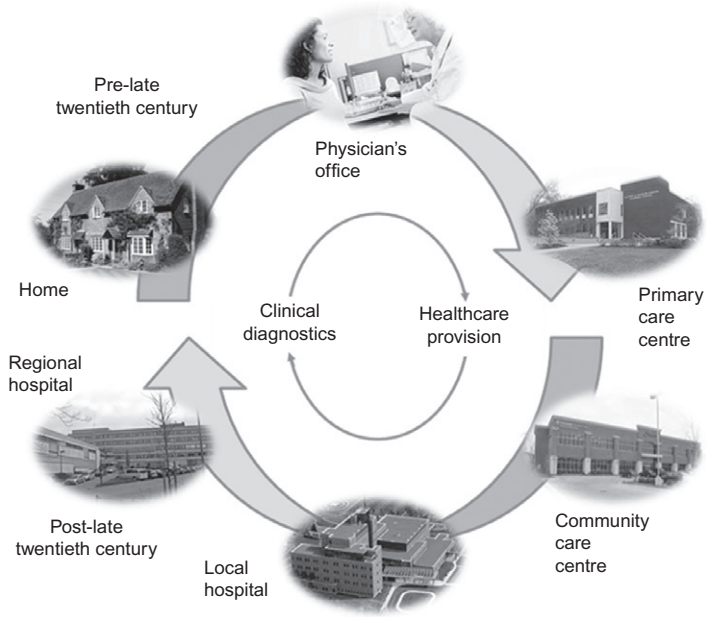

FIG. 3. Evolution of health-care provision from the home in the late twentieth century to the regional hospital via various community and local services in the late twentieth century and anticipated full circle back to the home in the early twenty-first century. Clinical diagnostics are following a similar pattern of development.

devices and the larger table- or bench-top instruments. The majority of handheld systems comprise a disposable reagent prefilled strip incorporated into a cartridge or cassette to facilitate addition of the sample, whence following development of the test, the resulting signal is interpreted visually or measured using an inexpensive reader or meter. Current POC tests cover critical care (blood gases, ions), emergency medicine (troponin), risk assessment (cholesterol), diabetes management (glucose, hemoglobin A1c), personal well-being (pregnancy, fertility), lifestyle (alcohol, drugs of abuse), and infectious diseases (human immunodeficiency virus, sexually transmitted infections) in a market estimated to be worth $18.7B by 2013 (Kricka and Thorpe, 2010). Such tests usually comprise a reagent-impregnated paper strip or pad, lateral flow devices, dipsticks, cards, slides, flow-through tubes, cartridges, and cassettes with inbuilt meters and displays. A good example of state-of-the-art consumer electronics technology for POC testing is the introduction of the Clearblue Digital Pregnancy test® by SPD, which provides both an unambiguous readout of the result (pregnant/not pregnant) and an indication of the number of weeks since conception.

VI. Biosensors

A key element for the development of POC systems is the biosensor. Biosensor technology is an analytical platform which can empower physicians or psychiatrists with the ability to confirm a suspected diagnosis on the spot when the patient presents him or herself and thereby obviate the requirement to attend hospital. A biosensor is an analytical device which combines a biorecognition system with a suitable physicochemical transducer to convert the recognition of the target analyte directly into an electrical signal (Lowe *et al.*, 1992; Gizeli and Lowe, 1996; Lowe, 2007a,b). Figure 4 shows a schematic biosensor comprising (i) a biorecognition system, such as a natural or an engineered enzyme, antibody, receptor, nucleic acid, aptamer, peptide, or other synthetic biomimetic, microorganism, organelle, or tissue, in close conjunction with (ii) a transducer, which transforms the signal resulting from the interaction of the analyte with the biorecognition system into an electrical system, and (iii) the associated electronics and signal processing to display the results in a user-friendly fashion. The transducer can exploit any physical principle based on electrochemical, optical, acoustic, magnetic, thermal, or microegineered devices (Lowe, 1999, 2007a,b).

Classic examples of biosensors include amperometric glucose sensors for diabetes management (Foulds and Lowe, 1988; Wolowacz *et al.*, 1992), conductimetric devices (Cullen *et al.*, 1990), surface plasmon resonance (SPR; Cullen *et al.*, 1987/1988), the resonant mirror (Buckle *et al.*, 1993; Davies *et al.*, 1994;

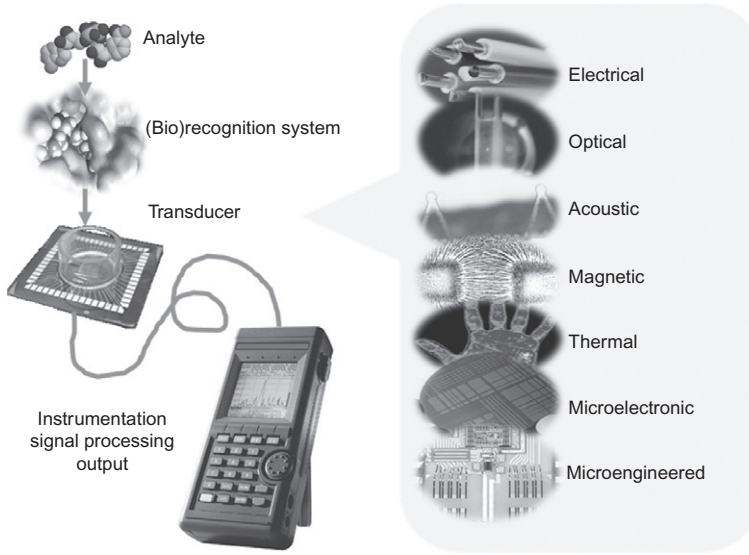

FIG. 4. Schematic of a generalized biosensor showing the (bio)recognition system identifying the analyte in a complex biological sample, the physic-chemical transducer for conversion of the recognition into an electrical signal, and the instrumentation for outputting the signal in the desired user-friendly fashion.

Watts *et al.*, 1994), fiber optic sensors (Carlyon *et al.*, 1992; Tubb *et al.*, 1995, 1997; Hale *et al.*, 1996; Schipper *et al.*, 1997), Mach-Zehnder interferometry (Schipper *et al.*, 1997), planar waveguides (Mayr *et al.*, 2009), and various optical grating (Erdélyi *et al.*, 2007) and acoustic and microcantilever devices (Gizeli *et al.*, 1992; Stevenson and Lowe, 1999; Sindi *et al.*, 2001; Stevenson *et al.*, 2001, 2003, 2006; Haefliger and Boisen, 2007). More recent trends in biosensor technology include the use of aptamers (Brody and Gold, 2000; Strehlitz *et al.*, 2008; Abe *et al.*, 2011), peptides (Huang and Koide, 2010), molecularly imprinted polymers (Haupt and Belmont, 2007), and genetically engineered binding proteins (Ge *et al.*, 2003) and enzymes (Campas *et al.*, 2009) as more durable, selective, and higher affinity recognition elements, the use of electropolymerization to immobilize biomolecules in thin films on sensor surfaces (Cosnier, 2007), metal (Elghanian *et al.*, 1997) and magnetic nanoparticles (Yellen and Erb, 2007), quantum dots (Abramowitz, 2007) to amplify signals, and conducting polymer nanowire- (Wanekaya *et al.*, 2007) and carbon nanotube-based sensors (Barone *et al.*, 2007) to aid in miniaturization of sensor formats.

Perhaps the largest single advance in biosensor technology for clinical and POC analysis involves microfluidics and lab-on-a-chip platforms (Li, 2010). This technology was first demonstrated with a fully integrated GC and thermal

conductivity detector on a 4-in. silicon wafer in 1979 (Terry *et al.*, 1979), while the first liquid separation was exemplified in 1989 (Sethi *et al.*, 1989) and a high-performance liquid chromatography (HPLC) and conductimetric detector was fabricated on Si-Pyrex in 1990 (Manz *et al.*, 1990). A miniaturized chemical analysis platform based on capillary electrophoresis (CE) linked to laser-induced fluorescence (LIF) detection was fabricated on a glass chip with channels 10 μm deep and 30 μm wide and used to resolve biomolecules in 6 min (Manz *et al.*, 1992). Since then, a plethora of different clinical, cellular, protein, and nucleic acid analytes have been resolved and quantified using these techniques in devices fabricated in silicon, glass, plastics, and polydimethylsiloxane using on-chip CE, dielectrophoresis, optical trapping, filters, pumps, and valves (Li, 2010; Napoli *et al.*, 2010). For example, DNA extraction; amplification by thermal, isothermal, and rolling circle polymerase chain reaction (PCR); and subsequent hybridization, sequencing, and genotyping have been facilitated by the use of a microfluidic system implemented on a plastic monolithic platform (Liu *et al.*, 2002). Similarly, microfluidic devices have been actively developed for clinical diagnostics, protein separation and functional assay, proteomics and immunoassay (Li, 2010). Thus, homogeneous (Chen *et al.*, 2002), competitive (Schmalzing *et al.*, 1997), and heterogeneous (Ko *et al.*, 2003) immunoassays and multiplexed label-free assays (Carlborg *et al.*, 2010) have been developed.

VII. Biosensors in Neuroscience

The long cherished ambition to witness biosensors displacing all other analytical technologies within the health-care and biomedical sectors has yet to be realized after nearly three decades of intensive research and the introduction of a vast armamentarium of new devices and techniques. The most attractive features of the biosensor, that is, its small size, ruggedness, inexpensiveness, low power burden, rapid and real-time response, use by lay personnel, biocompatibility, and ready interface with computer and mobile technology has yet to be translated into reality. However, biosensors are beginning to emerge as a solution to resolving challenging issues in fundamental neuroscience and as a means to reduce the complexity of multiplexed genomic and proteomic biomarker assays and bring the diagnosis of multigenic neurological diseases closer to the patient (Bell and Kornguth, 2007).

Chemical signaling plays a key role in neural function although few investigators directly measure the concentrations of neurotransmitters and modulators in the extracellular space. Biosensors offer the prospect of measuring neurotransmitter production with adequate temporal (milliseconds) and spatial (100 nm–10 μm) resolution (Dale *et al.*, 2005). Key targets such as pH, pO_2, glutamate,

dopamine, GABA, ATP, adenosine, acetylcholine, glucose, pyruvate, lactate, hydrogen peroxide (H_2O_2), and nitric oxide (NO) can be measured with single or cascade oxidase-based microamperometric electrodes, with direct electrochemical assay with carbon fiber microelectrodes (Llaudet *et al.*, 2003, 2005; Dale *et al.*, 2005), or by using additional enzymes to ameliorate interferences or amplify the sensor signal by introducing substrate or coenzyme recycling (Georganopoulou *et al.*, 2000; Llaudet *et al.*, 2003). Amperometric biosensors were developed originally in the early 1960s, although it is only the miniaturization of these electrodes that has made the study of chemical signaling in the brain possible, since they are less invasive than microdialysis sensors (Larsson *et al.*, 1998; Mitchell, 2004; Bell and Kornguth, 2007; Broderick *et al.*, 2008; Broderick and Kolodny, 2009). More recently, an *in vivo* biosensor for neurotransmitter release and *in situ* receptor activity has been devised which uses cell-based neurotransmitter fluorescent engineered reporters (CNiFERs) to address this challenge and monitor *in situ* neurotransmitter receptor activation (Quoc-Thang Nguyen *et al.*, 2010).

However, despite these advances in fundamental understanding of brain metabolism, metabolites alone offer poor definition for the diagnosis of psychological, neuropsychiatric, and neurodegenerative disorders. Thus a key challenge is to circumvent the fact that for most neurological disorders there is currently no single test that can provide an unequivocal diagnosis; instead, a combined multiparametric psychoanalytic, genomic, proteomic, and metabolomic approach is suggested with a panel of 1–100 biomarkers to obviate the well-established conundrum that biomarker signatures overlap various diseases, syndromes, and symptoms (Dale *et al.*, 2005). Protein- and peptide-based chips from Ciphergen Biosystems have been used to identify biomarker panels for AD from CSF samples using SELDI-TOF MS (Choe *et al.*, 2002; Carrette *et al.*, 2003). A panel of four biomarkers was able to distinguish mild AD from unaffected controls in a blinded test set with high sensitivity and specificity (Carrette *et al.*, 2003).

A second challenge facing the early detection of mental disorders is the low concentration of key biomarkers in accessible biological fluids. Biomarker panels can be interrogated via bead assays, as in the case of the schizophrenia diagnostic (Schwarz *et al.*, 2010a,b), or by various particulate systems designed to enhance sensitivity. For example, the biobarcode system is a nonenzymatic amplification protocol capable of detecting low concentrations of nucleic acid, protein, small molecules, and metal ions. The biobarcode system was able to detect the amyloid-β-derived AD marker in CSF at a concentration of 100 fM–100 aM (Georganopoulou *et al.*, 2005) and, when used in conjunction with a PCR amplification step, was able to detect prostate specific antigen (PSA) at a concentration six orders of magnitude higher sensitivity than conventional ELISA, that is, at 3 aM versus 3 pM, respectively (Nam *et al.*, 2004). The biobarcode assay has also proven useful for the detection of the tau protein AD biomarker (Bell and Kornguth, 2007).

The most popular platform for the detection and quantitation of neurodegenerative biomarkers uses the phenomenon of SPR (Cullen *et al.*, 1987/1988). For example, a nanoscale optical sensor based on SPR has been exploited to quantitate the interaction between the amyloid-β-derived diffusible ligands (ADDLs) and specific anti-ADDL antibodies (Haes *et al.*, 2005), while the Biacore® SPR system has been used to characterize the polyglutamine tracts as threshold biomarkers for the onset of AD (Bennett *et al.*, 2002). Similarly, fiber optic and SPR array technologies have been suggested as ways to multiplex multiple assays on the same biological sample (Gašparac and Walt, 2007; Suzuki *et al.*, 2007). More recently, a flexible new grating-coupled SPR sensor system that combines the joint advantages of discretely functionalized, encoded microparticles and label-free detection has been reported (Kastl *et al.*, 2008). This system offers the prospect of simultaneously investigating the real-time binding kinetics of a variety of molecular interactions in a single multiplexed platform; thus, one multiplexed assay could employ a wide range of immobilization chemistries, surface preparation methods, and formats. The new system offers a very high level of assay conformability to the end user, particularly when compared to fixed microarrays and permits the contemporaneous assay of both genomic and proteomic biomarkers (Kastl *et al.*, 2010).

The third, and most challenging option, for the diagnosis of psychological, neuropsychiatric, and neurodegenerative disorders, is to reduce the complexity and cost of these multiparametric assay panels to permit POC testing in the hands of frontline physician's and psychiatrists. There are a variety of simplified tests being developed for field use, that is, outside the controlled laboratory environment. One example of such a test is the development of simple reflection holograms that combine an analyte-selective "smart" polymer with optical interrogation by eye or instrumentation and a reporting diffraction grating transducer (Blyth *et al.*, 1996). They are fabricated by passing a single collimated laser beam through a silver halide emulsion coated onto a glass or plastic substrate backed with a silvered mirror. Interference between the ingoing and outgoing beams creates a standing wave interference pattern, which after development and fixing, forms a series of fringes of silver grains ~20 nm in diameter separated by a distance equal to $1/2\lambda$ of the laser light used in their construction and distributed within the thickness of the emulsion (~5–10 μm). Under white light illumination, the fringes act like a Bragg diffraction grating and reflect a narrow band of wavelength governed by Bragg's law:

$$m\lambda = 2n\partial\cos\theta$$

where m is the diffraction order, λ is the wavelength of light *in vacuo*, n is the average refractive index of the system, ∂ is the spacing between the fringes, and θ is the angle between the incident light and the diffracting planes. Any physical, chemical, or biological interaction which alters the fringe spacing (∂) by swelling

or contraction, the average refractive index (n) or the total number or distribution of the fringes within the thickness of the film will result in observable changes in the wavelength (color) or intensity (brightness) of the hologram. It has been demonstrated that through the rational design of the hydrogel, which incorporates suitable receptors, that volumetric changes can be induced on binding the complementary target analyte. Thus, responsive holograms for gases, ions, metabolites, inhibitors, drugs, enzymes, antigens, antibodies, and whole cells have been created based on this principle (Mayes *et al.*, 1999, 2002; Marshall *et al.*, 2003, 2004; Lee *et al.*, 2004; Sartain *et al.*, 2006; Lowe, 2007a,b; Tan and Lowe, 2009; Martinez-Hurtado *et al.*, 2010). Responsive holograms of this type are ideally suited to POC applications since they are designed react to specific stimuli; are inexpensive to manufacture by mass producible techniques; are miniaturizable; generate a readout in real time; can be configured as planar, fiber optic, or particulate arrays; and display low or no power burden (Martinez-Hurtado *et al.*, 2010). It is anticipated that these devices will be configured as a handheld card for use by the physician or psychiatrist to aid in the diagnosis of psychological, neuropsychiatric, and neurodegenerative disorders.

Microfluidic devices also offer the prospect of inexpensive POC tests suitable for execution by lay personnel with small sample sizes (Whitesides, 2006). Such devices have been fabricated on thin and flexible films (Focke *et al.*, 2010) and have used manual torque-operated valves for sandwich immunoassays (Weibel *et al.*, 2005) and thread as a versatile material for low-cost microfluidic diagnostics (Li *et al.*, 2010; Reches *et al.*, 2010).

VIII. Neuroinformatics

Advances in experimental protocols have given neuroscientists and psychiatrists an increasingly powerful arsenal for acquiring data across multiple spatiotemporal scales, from the level of single biomarker molecules, cellular architectures, neural connectivity to complex, and interdependent metabolic pathway, physiological, and behavioral data (Kotter, 2001; Martone *et al.*, 2004). It is also evident that combining multiple "omics" data with matching detailed imaging, microscopic, physiological, behavioral, and psychiatric codata for complex multigeneic neuropsychiatric and neurodegenerative disorders is a task beyond even the best funded research groups. Thus, government agencies have promoted the concept of data sharing via schemes such as the Human Brain Project (Shepherd *et al.*, 1998) in order to support the development of computational tools and algorithms for visualizing, analyzing, and modeling of neuroscience and neuropsychiatric data. In addition, the Neuroscience Trust of the

National Science Foundation (NSF) supported an initiative, the National Partnerships for Advanced Computational Infrastructure (NPACI) which encouraged neuroinformatics and computer scientists to collaborate on new database architectures, grids, and networking algorithms (www.npaci.edu). Such initiatives are not without challenges since, aside from the difficulties in representing, storing, and accessing substantial quantities of nonstandardized complex data, there is a range of sociological, ethical, and commercial issues associated with sharing hard won data from different sponsoring organizations. However, if these issues could be resolved, a universal database would go some distance toward realizing the promise of a "global neuroscientific and neuropsychiatric forum" (Martone *et al.*, 2004). Nevertheless, even if this lofty goal could be achieved, it is important to realize that relatively unstructured data does not necessarily enhance knowledge or understanding of these complex disorders and thus data will have to be transmitted to competent interpreters in order to gain maximum value out of its content.

IX. e-Neuroscience and e-Neuropsychiatry

The final part of the jigsaw relates to mobile communication and the internet which will allow unstructured data to be organized, interpreted, evaluated, and reformatted for facile presentation to the end user, be they the physician, psychiatrist, or patient. Frontline analysis, that is, near or on-patient collection of data via real-time or multiplexed sensor arrays, coupled with artificial intelligence and mobile communication systems could bring diagnosis, application, and internet-based services back to the patient (Fig. 5; Ryhänen *et al.*, 2010). The prospect of ambient intelligence, in which sensing, computation, and communication are universally available and ready to serve the end user in an intelligent way, is predicated on intelligent mobile devices embedded in all aspects of the human environment, home, office, and public places. The concept of remote health care is particularly relevant to psychological, neurodegenerative, and neuropsychiatric disorders, since these are long-term debilitating conditions which require constant monitoring and treatment and afflict many of the more developed regions of the world. Telepsychiatry is already an established approach where the patient is in his own home or office, particularly in rural or underserved regions, and can access the psychiatrist via Webcam or high-speed internet. Similarly, there have been developments in emergency e-psychiatry to provide consultation for suicidal, homicidal, violent, psychotic, depressed, manic, and acutely anxious psychiatric patients (Shore *et al.*, 2007). Such emergency e-psychiatry services can be provided to hospital A&E departments, jails, community mental health centers, substance abuse clinics, and schools.

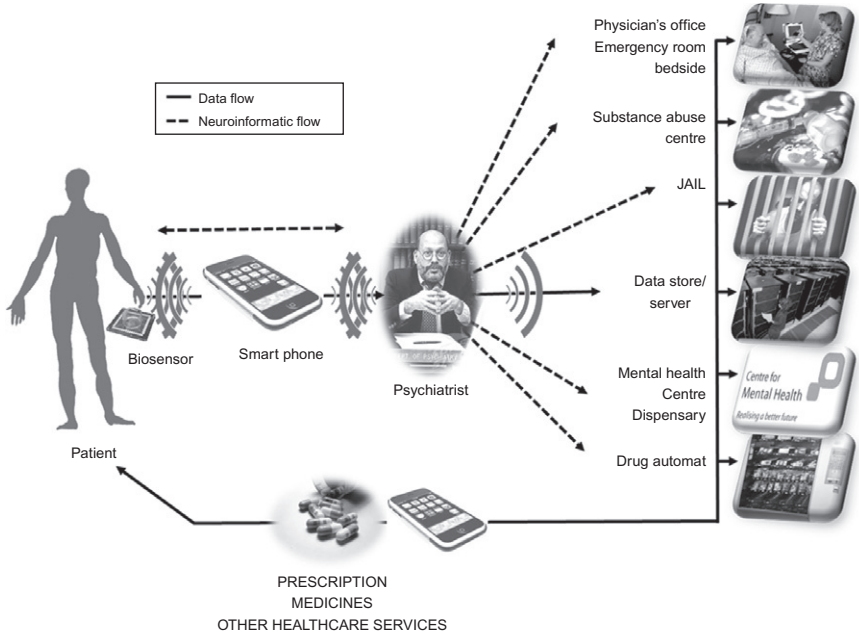

FIG. 5. e-Neuroscience: A future scenario in which psychiatric and neurodegenerative patients are monitored with selective wireless biosensors and the data is transmitted to the psychiatrist for interpretation and onward passage to key service providers and drug automats prior to release of prescription medicines and other health-care services to the patient.

There is an upsurge in interest in individualization of health care and the application of grid computing to support data-, computation-, and knowledge-based tasks to assist disease prediction, diagnosis, prognosis, and compliance. There is effort aimed at creating bioprofiles or personal fingerprints for brain disorders derived from fusing genomics, proteomics, electroencephalogram, and MRI data with the patient's past medical history, biopatterns, and prognosis (Sun *et al.*, 2006). It has been shown that the grid could be used for individual biopattern analysis and bioprofiling for the early detection of dementia and brain injury assessment.

Mobile technologies are likely to offer the most pervasive approach to e-psychiatry and e-neuromedicine, since such mobile integrated cognitive systems could include sensing, perception, cognition, learning, and in some cases where medicines need to be prescribed and delivered, actuation. Mobile devices could thus become the next generation of personalized pharmacies, which sample an accessible fluid from the patient, measure key analytes, communicate the unstructured data to a central interpretation point, return the diagnosis with an encrypted key to allow access to an automated drug dispenser, and then dose

the appropriate drug treatment on an individualized basis. However, for this concept to become a reality, a key step is to ensure a correct diagnosis with reliable data coming from sensitive and selective sensors for analytes of interest to psychological, neurodegenerative, and neuropsychiatric disorders.

Acknowledgments

This work was partially supported by a grant awarded to C. R. Lowe by the BBSRC and the Home Office (CBRN).

References

Abe, K., Ogasawara, D., Yoshida, W., Sode, K., and Ikebukuro, K. (2011). Aptameric sensors based on structural change for diagnosis. *Faraday Discuss.* **149**, 93–106.

Abramowitz, S. (2007). Quantum dots: their use in biomedical research and clinical diagnostics. In: R. S. Marks, D.C. Cullen, I. Karube, C.R. Lowe, and H. Weetall (Eds.), *Handbook of Biosensors and Biochips*. Wiley Interscience, Sussex, UK, pp. 793–798.

Anderson, N.L., and Anderson, N.G. (2002). The human plasma proteome: history, character, and diagnostic prospects. *Mol. Cell. Proteomics* **1**, 845–867.

Aronica, E., Van Vliet, E.A., Hendricksen, E., *et al.* (2001). Cystatin C, a cysteine protease inhibitor, is persistently upregulated in neurons and glia in a rat model for mesial temporal lobe epilepsy. *Eur. J. Neurosci.* **14**, 1485–1491.

Barone, P.W., Jeng, E.S., Heller, D.A., and Strano, M.S. (2007). Biosensors based on single-walled carbon nanotube near-infrared fluorescence. In: R.S. Marks, D.C. Cullen, I. Karube, C.R. Lowe, and H. Weetall (Eds.), *Handbook of Biosensors and Biochips*. Wiley Interscience, Sussex, UK, pp. 843–854.

Bell, K.M., and Kornguth, S.E. (2007). Biosensors for neurological disease. In: R.S. Marks, D.C. Cullen, I. Karube, C.R. Lowe, and H. Weetall (Eds.), *Handbook of Biosensors and Biochips*. Wiley Interscience, Sussex, UK, pp. 1084–1098.

Bennett, M.J., Huey-Tubman, K.E., Herr, A.B., West, A.P., Ross, S.A., and Bjorkman, P.J. (2002). Inaugural article: a linear lattice model for polyglutamine in CAG-expansion diseases. *Proc. Natl. Acad. Sci. USA* **99**, 11634–11639.

Blyth, J., Millington, R.B., Mayes, A.G., Frears, E.R., and Lowe, C.R. (1996). A holographic sensor for water in solvents. *Anal. Chem.* **68**, 1089–1094.

Bogdanov, M., Matson, W.R., Wang, L., Matson, T., Saunders-Pullman, R., Bressman, S.S., and Flint Beal, M. (2008). Metabolomic profiling to develop blood biomarkers for Parkinson's disease. *Brain* **131**, 389–396.

Bressan, R.A., Bigliani, V., and Pilowsky, L.S. (2001). Neuroimaging of D2 dopamine receptors in schizophrenia. *Rev. Bras. Psiquiatr.* **23**, 46–49.

Bressan, R.A., Shih, M.C., Hoexter, M.Q., and Lacerda, A.L.T. (2007). Can molecular imaging techniques identify biomarkers for neuropsychiatric disorders? *Rev. Bras. Psiquiatr.* **29**, 102–104.

Broderick, P.A., and Kolodny, E.H. (2009). Real time imaging of biomarkers in the Parkinson's brain using mini-implantable biosensors. II. Pharmaceutical therapy with bromocriptine. *Pharmaceuticals* **2**, 236–249.

Broderick, P.A., Ho, H., Karyn Wat, K., and Vivek Murthy, V. (2008). Laurate biosensors image brain neurotransmitters *in vivo*: can an antihypertensive medication alter psychostimulant behaviour? *Sensors* **8**, 4033–4061.

Brody, E.N., and Gold, L. (2000). Aptamers as therapeutic and diagnostic agents. *Rev. Mol. Biotechnol.* **74**, 5–13.

Buckle, P.E., Davies, R.J., Kinning, T., Yeung, D., Edwards, P.R., Pollard-Knight, D.V., and Lowe, C.R. (1993). The resonant mirror: a novel optical sensor for direct sensing of biomolecular interactions part II: applications. *Biosens. Bioelectron.* **8**, 355–363.

Campas, M., Prieto-Simon, B., and Marty, J.-L. (2009). A review of the use of genetically engineered enzymes in electrochemical biosensors. *Semin. Cell Dev. Biol.* **20**, 3–9.

Carlborg, C.F., Gylfason, K.B., Kaźmierszak, A., Dortu, F., Banuls Polo, M.J., Maquieira Catala, A., Kresbach, G.M., Sohlström, H., Moh, T., Vivien, L., Popplewell, J., Ronan, G., *et al.* (2010). A packaged optical slot waveguide ring resonator sensor array for multiplex label-free assays in lab-on-chips. *Lab Chip* **10**, 281–290.

Carlyon, E.E., Lowe, C.R., Reid, D., and Bennion, I. (1992). A single-mode fibre optic evanescent wave biosensor. *Biosens. Bioelectron.* **7**, 141–146.

Carrette, O., Demalte, I., Scherl, A., Yalkinoglu, O., Corthals, G., Burkhard, P., Hochstrasser, D.F., and Sanchez, J.C. (2003). A panel of cerebral spinal fluid potential biomarkers for the diagnosis of Alzheimer's disease. *Proteomics* **3**, 1486–1494.

Chen, S., Lin, Y., Wang, L., Lin, C., and Lee, G. (2002). Flow-through sampling for electrophoresis-based microfluidic chips and their applications for protein analysis. *Anal. Chem.* **74**, 5146–5153.

Choe, L.H., Dutt, M.J., Relkin, N., and Lee, K.H. (2002). Studies on potential cerebral spinal fluid molecular markers for Alzheimer's disease. *Electrophoresis* **23**, 2247–2251.

Chun, H.S., Gibson, G.E., Degiorgio, L.A., Zhang, H., Kidd, V.J., and Son, J.H. (2001). Dopaminergic cell death induced by MPP+, oxidant and specific neurotoxicants shares the common mechanism. *J. Neurochem.* **76**, 1010–1021.

Cosnier, S. (2007). Immobilization of biomolecules by electropolymerised films. In: R.S. Marks, D. C. Cullen, I. Karube, C.R. Lowe, and H. Weetall (Eds.), *Handbook of Biosensors and Biochips*. Wiley Interscience, Sussex, UK, pp. 237–249.

Craig-Shapiro, R., Fagan, A.M., and Holtzman, D.M. (2008). Biomarkers of Alzheimer's disease. *Neurobiol. Dis.* .

Crow, T.J. (2007). How and why genetic linkage has not solved the problem of psychosis: review and hypothesis. *Am. J. Psychiatry* **164**, 13–21.

Cullen, D.C., Brown, R.G.W., and Lowe, C.R. (1987). Detection of immuno-complex formation on goldcoated diffraction gratings. *Biosensors* **3**, 211–225.

Cullen, D.C., Sethi, R.S., and Lowe, C.R. (1990). A multi-analyte miniature conductance biosensor. *Anal. Chim. Acta* **231**, 33–40.

Dale, N., Hatz, S., Tian, F., and Llaudet, E. (2005). Listening to the brain: microelectrode biosensors for neurochemicals. *Trends Biotechnol.* **23**, 420–428.

Danziger, R.S., You, M., and Akil, H. (2005). Discovering the genetics of complex disorders through integration of genomic mapping and transcriptional profiling. *Curr. Hypertens. Rev.* **1**, 21–34.

Davies, R.J., Edwards, P.R., Watts, H.J., Lowe, C.R., Buckle, P.E., Yeung, D., Kinning, T.M., and Pollard-Knight, D.V. (1994). The resonant mirror: a versatile tool for the study of biomolecular interactions. In: J.W. Crabb (Ed.), *Techniques in Protein Chemistry V.* Academic Press, New York, pp. 285–292.

Elghanian, R., Storhoff, R.C., Mucic, R., Letsinger, R.L., and Mirkin, C.A. (1997). Selective colorimetric detection of polynucleotides based on the distance-dependent optical properties of gold nanoparticles. *Science* **277**, 1078–1081.

Erdélyi, K., Frutos, A.G., Ramsden, J.J., and Szendrö, I. (2007). Grating-based optical biosensors. In: R.S. Marks, D.C. Cullen, I. Karube, C.R. Lowe, and H. Weetall (Eds.), *Handbook of Biosensors and Biochips*. Wiley Interscience, Sussex, UK, pp. 569–586.

Ferri, C.P., Prince, C.P., Brayne, C., Brodaty, H., Fratiglione, L., Ganguli, M., Hall, K., Hasegawa, K., Hendrie, H., Huang, Y., Jorm, A., Mathers, C., *et al.* (2005). Global prevalence of dementia: a Delphi consensus study. *Lancet* **366**, 2112–2117.

Flood, D.G., Marek, G.J., and Williams, M. (2011). Developing predictive CSF biomarkers: a challenge critical to success in Alzheimer's disease and neuropsychiatric translational medicine. *Biochem. Pharmacol.* **81**, 1422–1434. Available on-line 03 February 2011.

Focke, M., Kosse, D., Müller, C., Reinecke, H., Zengerle, R., and von Stetten, F. (2010). Lab-on-a-Foil: microfluidics on thin and flexible films. *Lab Chip* **10**, 1365–1386.

Foulds, N.C., and Lowe, C.R. (1988). Immobilisation of glucose oxidase in ferrocene-modified pyrrole polymers. *Anal. Chem.* **60**, 2473–2478.

French, P.J., O'Connor, V., Voss, K., Stean, T., Hunt, S.P., and Bliss, T.V. (2001). Seizure-induced gene expression in the area CA1 of the mouse hippocampus. *Eur. J. Neurosci.* **14**, 2037–2041.

Fu, Q., Jie Zhu, J., and Van Eyk, J.E. (2010). Comparison of multiplex immunoassay platforms. *Clin. Chem.* **56**, 314–318.

Gašparac, R., and Walt, D.R. (2007). Fibre-optic array biosensors. In: R.S. Marks, D.C. Cullen, I. Karube, C.R. Lowe, and H. Weetall (Eds.), *Handbook of Biosensors and Biochips*. Wiley Interscience, Sussex, UK, pp. 895–916.

Gazzaniga, M.S., and Heatherton, T.F. (2006). Psychological Science. W.W. Norton & Co., Inc., New York.

Ge, X., Tolosa, L., Simpson, J., and Rao, G. (2003). Genetically engineered binding proteins as biosensors for fermentation and cell culture. *Biotechnol. Bioeng.* **84**, 723–731.

Georganopoulou, D.G., Carley, R., Jones, D.A., and Boutelle, M.G. (2000). Development and comparison of biosensors for *in-vivo* applications. *Faraday Discuss.* **116**, 291–303.

Georganopoulou, D.G., Chang, L., Nam, J.M., Thaxton, C.S., Mufson, E.J., Klein, W.L., and Mirkin, C.A. (2005). Nanoparticle-based detection in cerebral spinal fluid of a soluble pathogenic biomarker for Alzheimer's disease. *Proc. Natl. Acad. Sci. USA* **102**, 2273–2276.

Gizeli, E., and Lowe, C.R. (1996). Immunosensors. *Curr. Opin. Biotechnol.* **7**, 66–79.

Gizeli, E., Stevenson, A.C., Goddard, N.J., and Lowe, C.R. (1992). A love-plate biosensor utilising a polymer layer. *Sens. Actuators* **6**, 131–137.

Gudmundsson, P., Skoog, I., Waern, M., Blennow, K., Zetterberg, H., Rosengren, L., and Gustafson, D. (2010). Is there a CSF biomarker profile related to depression in elderly women? *Psychiatry Res.* **176**, 99–102.

Haefliger, D., and Boisen, A. (2007). Microcantilever array devices. In: R.S. Marks, D.C. Cullen, I. Karube, C.R. Lowe, and H. Weetall (Eds.), *Handbook of Biosensors and Biochips*. Wiley Interscience, Sussex, UK, pp. 949–959.

Haes, A.J., Chang, L., Klein, W.L., and Van Duyne, R.P. (2005). Detection of a biomarker for Alzheimer's disease from synthetic and clinical samples using a nanoscale optical biosensor. *J. Am. Chem. Soc.* **127**, 2264–2271.

Hale, Z.M., Payne, F.P., Marks, R.S., Lowe, C.R., and Levine, M.M. (1996). The single mode tapered optical fibre immunosensor. *Biosens. Bioelectron.* **11**, 137–148.

Hata, R., Masumara, M., Akatsu, H., Li, F., Fujita, H., Nagai, Y., Yamamoto, T., Okada, H., Kosaka, K., Sakanaka, M., and Sawada, T. (2001). Upregulation of calcineurin Aβ mRNA in the Alzheimer's disease brain: assessment by cDNA microarray. *Biochem. Biophys. Res. Commun.* **284**, 310–316.

Haupt, K., and Belmont, A.-S. (2007). Molecularly imprinted polymers as recognition elements in sensors. In: R.S. Marks, D.C. Cullen, I. Karube, C.R. Lowe, and H. Weetall (Eds.), *Handbook of Biosensors and Biochips*. Wiley Interscience, Sussex, UK, pp. 199–215.

Holmes, E., Tzang, T.M., Huang, J.T.-J., Leweke, F.M., Koethe, D., Gerth, C.W., Nolden, B.M., Gross, S., Schreiber, D., Nicholson, J.K., and Bahn, S. (2006). Metabolic profiling of CSF: evidence that early intervention may impact on disease progression and outcome in schizophrenia. *PLoS Med.* **3**, e327.

Huang, J., and Koide, S. (2010). Rational conversion of affinity reagents into label-free sensors for peptide motifs by designed allostery. *ACS Chem. Biol.* **5**, 273–277.

Kastl, K.F., Lowe, C.R., and Norman, C.E. (2008). A novel encoded and multiplexed SPR sensor platform. *Anal. Chem.* **80**, 7862–7869.

Kastl, K.F., Lowe, C.R., and Norman, C.E. (2010). Label-free genetic and proteomic marker detection within a single flow-cell assay. *Biosens. Bioelectron.* **26**, 1719–1722.

Khaitovich, P., Lockstone, H.E., Wayland, M.T., Tsang, T.M., Jayatilaka, S.D., Guo, A.J., Somel, M., Harris, L.W., Holmes, E., Pääbo, S., and Bahn, S. (2008). Metabolic changes in schizophrenia and human brain evolution. *Genome Biol.* **9**, R124.

Klunk, W.E., Engler, H., Nordberg, A., Wang, Y., Blomqvist, G., Holt, D.P., Bergstrom, M., Savitcheva, I., Huang, G.F., Estrada, S., Ausen, B., Debnath, M.L., *et al.* (2004). Imaging brain amyloid in Alzheimer's disease with Pittsburgh compound-B. *Ann. Neurol.* **55**, 306–319.

Ko, J.S., Yoon, H.C., Yang, H., Pyo, H.B., Chung, K.H., Kim, S.J., and Kim, Y.T. (2003). A polymer-based microfluidic device for immunosensing biochips. *Lab Chip* **3**, 106–113.

Kontkanen, O., and Castrén, E. (2002). Functional genomics in neuropsychiatric disorders and in neuropharmacology. *Expert Opin. Ther. Targets* **6**, 363–374.

Kotter, R. (2001). Neuroscience databases: tools for exploring brain structure–function relationships. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **356**, 1111–1120.

Kricka, L.J., and Thorpe, G.H.G. (2010). Technology for handheld devices for point-of-care testing. In: C.P. Price, A. St John, and L.J. Kricka (Eds.), *Point-of-Care Testing: Needs, Opportunity and Innovation*. AACC Press, Washington DC, USA, pp. 27–41.

Larsson, N., Ruzgas, T., Gorton, L., Kokaia, M., Kissinger, P., and Csoregi, E. (1998). Design and development of an amperometric biosensor for acetylcholine determination in brain microdialysates. *Electrochim. Acta* **43**, 3541–3554.

Lee, M.-C., Kabilian, S., Hussain, A., Yang, X., Blyth, J., and Lowe, C.R. (2004). Glucose-sensitive holographic sensors for monitoring bacterial growth. *Anal. Chem.* **76**, 5748–5755.

Le-Niculescu, H., McFarland, M.J., Mamidipalli, S., Ogden, C.A., Kuczensli, R., Kurian, S.M., Salomon, D.R., Tsuang, M.T., Nurnberger, J.I., and Niculescu, A.B. (2007). Convergent functional genomics of bipolar disorder: from animal model pharmacogenomics to human genetics and biomarkers. *Neurosci. Biobehav. Rev.* **31**, 897–903.

Li, P.C.H. (2010). Fundamentals of Microfluidics and Lab on a Chip for Biological Analysis and Discovery. CRC Press, Taylor & Francis Group, Boca Raton, FL.

Li, X., Tian, J., and Shen, W. (2010). Thread as a versatile material for low cost microfluidic diagnostics. *Appl. Mater. Interfaces* **2**, 1–6.

Liu, Y., Rauch, C.B., Stevens, R.L., Lenigk, R., Yang, J., Rhine, D.B., and Grodzinski, P. (2002). DNA amplification and hybridization assays in integrated monolithic plastic devices. *Anal. Chem.* **74**, 3063–3070.

Llaudet, E., Botting, N.P., Crayston, J.A., and Dale, N. (2003). A three-enzyme microelectrode sensor for detecting purine release from central nervous system. *Biosens. Bioelectron.* **18**, 43–52.

Llaudet, E., Hatz, S., Droniou, M., and Dale, N. (2005). Microelectrode biosensor for real-time measurement of ATP in biological tissue. *Anal. Chem.* **77**, 3267–3273.

Lowe, C.R. (1999). Chemoselective biosensors. *Curr. Opin. Chem. Biol.* **3**, 106–111.

Lowe, C.R. (2007a). Overview of biosensor and bioarray technologies. In: R.S. Marks, D.C. Cullen, I. Karube, C.R. Lowe, and H. Weetall (Eds.), *Handbook of Biosensors and Biochips*. Wiley Interscience, Sussex, UK, pp. 7–22.

Lowe, C.R. (2007b). Holographic sensors. In: R.S. Marks, D.C. Cullen, I. Karube, C.R. Lowe, and H. Weetall (Eds.), *Handbook of Biosensors and Biochips*. Wiley Interscience, Sussex, UK, pp. 587–596.

Lowe, C.R., Cullen, D.C., Gizeli, E., Goddard, N.J., Gray Stephens, L.D., Maynard, P., and Yon Hin, B.F.Y. (1992). Biosensors. *Clin. Biochem. Rev.* **13**, 22–26.

Madaan, V., Bestha, D.P., and Kolli, V.B. (2010). Biological markers in schizophrenia: an update. *Drugs Today* **46**, 661–669.

Manz, A., Miyahara, Y., Miura, J., Watanabe, Y., Miyagi, H., and Sato, K. (1990). Design of an open-tubular column liquid chromatograph using silicon chip technology. *Sens. Actuators B* **1**, 249–255.

Manz, A., Harrison, J.D., Verpoorte, E.M.J., Fettinger, J.C., Paulus, A.L.H., and Widmer, H.M. (1992). Planar chip technology for miniaturization and integration of separation techniques into monitoring system. *J. Chromatogr.* **593**, 253–258.

Marshall, A.J., Blyth, J., Davidson, C.A.B., and Lowe, C.R. (2003). pH-sensitive holographic sensors. *Anal. Chem.* **75**, 4423–4431.

Marshall, A.J., Young, D.S., Blyth, J., Kabilan, S., and Lowe, C.R. (2004). Metabolite-sensitive holographic sensors. *Anal. Chem.* **76**, 1518–1523.

Martinez-Hurtado, J.L., Davidson, C.A.B., Blyth, J., and Lowe, C.R. (2010). Holographic sensors for hydrocarbon gases and other volatile organic compounds. *Langmuir* **26**, 15694–15699.

Martins-de-Souza, D., Harris, L.W., Guest, P.C., Turck, C.W., and Bahn, S. (2010). The role of proteomics in depression research. *Eur. Arch. Psychiatry Clin. Neurosci.* **260**, 499–506.

Martone, M.E., Gupta, A., and Ellisman, M.H. (2004). e-Neuroscience: challenges and triumphs in integrating data from molecules to brains. *Nat. Neurosci.* **7**, 467–472.

Mayes, A.G., Blyth, J., Kyröläinen-Reay, M., Millington, R.B., and Lowe, C.R. (1999). A holographic alcohol sensor. *Anal. Chem.* **71**, 3390–3396.

Mayes, A.G., Blyth, J., Millington, R.B., and Lowe, C.R. (2002). Metal ion-sensitive holographic sensors. *Anal. Chem.* **74**, 3649–3657.

Mayr, T., Abel, T., Enko, B., Borisov, S., Konrad, C., Köstler, S., Lamprecht, B., Sax, S., List, E.J.W., and Klimant, I. (2009). A planar waveguide optical sensor employing simple light coupling. *Analyst* **134**, 1544–1547.

Mirnics, K., Middleton, F.A., Marquez, A., Lewis, D.A., and Levitt, P. (2000). Molecular characterization of schizophrenia viewed by microarray analysis of gene expression in prefrontal cortex. *Neuron* **28**, 40–48.

Mitchell, K.M. (2004). Acetylcholine and choline amperometric enzyme sensors characterised in vitro and in vivo. *Anal. Chem.* **76**, 1098–1106.

Nam, J.M., Stoeva, S.I., and Mirkin, C.A. (2004). Biobarcode-based DNA detection with PCR-like sensitivity. *J. Am. Chem. Soc.* **126**, 5932–5933.

Napoli, M., Eijkel, J.C.T., and Pennathur, S. (2010). Nanofluidic technology for biomolecule applications: a critical review. *Lab Chip* **10**, 957–985.

Paige, L.A., Mitchell, M.W., Krishman, R.R., Kaddurah-Daouk, R., and Steffens, D.C. (2007). A preliminary metabolomic analysis of older adults with and without depression. *Int. J. Geriatr. Psychiatry* **22**, 418–423.

Pasinetti, G.M. (2001). Use of cDNA microarray in the search for molecular markers involved in the onset of Alzheimer's disease dementia. *J. Neurosci. Res.* **65**, 471–476.

Prabakaran, S., Swatton, E., Ryan, M.M., Huffaker, S.J., Huang, J.T., Griffin, J.L., Freeman, T., Dudbridge, F., Lilley, K.S., Karp, N.A., Hester, S., Tkachev, D., *et al.* (2004). Mitochondrial dysfunction in schizophrenia: evidence for compromised brain metabolism and oxidative stress. *Mol. Psychiatry* **9**, 684–697.

Price, C.P., St John, A., and Kricka, L.J. (2010). Putting point-of-care testing into context: moving beyond innovation to adoption. In: C.P. Price, A. St John, and L.J. Kricka (Eds.), *Point-of-Care Testing: Needs, Opportunity and Innovation*. AACC Press, Washington DC, USA, pp. 1–20.

Quinones, M.P., and Kaddurah-Daouk, R. (2009). Metabolomics tools for identifying biomarkers for neuropsychiatric diseases. *Neurobiol. Dis.* **35**, 165–176.

Quoc-Thang Nguyen, Q.-T., Schroeder, L.F., Mank, M., Muller, A., Taylor, P., Griesbeck, O., and Kleinfeld, D. (2010). An *in vivo* biosensor for neurotransmitter release and *in situ* receptor activity. *Nat. Neurosci.* **13**, 127–132.

Ravina, B., Eidelberg, D., Ahlskog, J.E., Albin, R.L., Brooks, D.J., Carbon, M., Dhawan, V., Feigin, A., Fahn, S., Guttman, M., Gwinn-Hardy, K., McFarland, H., *et al.* (2005). The use of radiotracer imaging in Parkinson disease. *Neurology* **64**, 208–215.

Reches, M., Mirica, K.A., Dasgupta, R., Dickey, M.D., Butte, M.J., and Whitesides, G.M. (2010). Thread as a matrix for biomedical assays. *Appl. Mater. Interfaces* **2**, 1722–1728.

Rohlff, C. (2001). Proteomics in neuropsychiatric disorders. *Int. J. Neuropsychopharm.* **4**, 93–102.

Ryhänen, T., Uusitalo, M.A., and Kärkkäinen, A. (2010). When everything is connected. In: T. Ryhänen, M.A. Uusitalo, O. Ikkala, and A. Kärkkäinen (Eds.), *Nanotechnologies for Future Mobile Devices*. Cambridge University Press, Cambridge, UK.

Sartain, F., Yang, X., and Lowe, C.R. (2006). A holographic lactate sensor. *Anal. Chem.* **78**, 5664–5670.

Schipper, E.F., Brugman, A.M., Dominguez, C., Lechuga, L.M., Kooyman, R.P.H., and Greve, J. (1997). The realisation of an integrated Mach-Zehnder waveguide immunosensor in silicon technology. *Sens. Actuators B* **40**, 147–153.

Schmalzing, D., Koutny, L.B., Taylor, T.A., Nashabeh, W., and Fuchs, M. (1997). Immunoassay for thyroxine (T4) in serum using capillary electrophoresis and micromachined devices. *J. Chromatogr. B* **697**, 175–180.

Schwarz, E., and Bahn, S. (2008). Biomarker discovery in psychiatric disorders. *Electrophoresis* **29**, 2884–2890.

Schwarz, E., Guest, P.C., Rahmoune, H., Wang, L., Levin, Y., Ingudomnukul, E., Ruta, L., Kent, L., Spain, M., Baron-Cohen, S., and Bahn, S. (2010a). Sex-specific serum biomarker patterns in adults with Asperger's syndrome. *Mol. Psychiatry* Original article.

Schwarz, E., Izmailov, R., Spain, M., Barnes, A., Mapes, J.P., Guest, P.C., Rahmoune, H., Pietsch, S., Leweke, F.M., Rothermindt, M., Steiner, J., Koethe, D., *et al.* (2010b). Validation of a blood-based laboratory test to aid in the confirmation of a diagnosis of schizophrenia. *Biomark. Insights* **5**, 39–47.

Sethi, R.S., Brettle, J., and Lowe, C.R. (1989). Chromatographic microseparation and detection on a silicon wafer. Proceedings of the Fifth International Conference on Solid State Sensors and Actuators and Eurosensors III, Montreux, Switzerland, p. 45.

Shear, M.K., Greeno, C., Kang, J., Ludewig, D., Frank, E., Swartz, H.A., and Hanekamp, M. (2000). Diagnosis of non-psychotic patients in community clinics. *Am. J. Psychiatry* **157**, 581–587.

Shepherd, G.M., Mirsky, J.S., Healy, M.D., Singer, M.S., Skoufos, E., Hines, M.S., Nadkarni, P.M., Miller, P.L., and Shepherd, G.M. (1998). The human brain project: neuroinformatics tools for integrating, searching and modeling multidisciplinary neuroscience data. *Trends Neurosci.* **21**, 460–468www.nimh.nih.gov/neuroinformatics.

Shore, J.H., Hilty, D.M., and Yellowlees, P. (2007). Emergency management guidelines for telepsychiatry. *Gen. Hosp. Psychiatry* **29**, 199–206.

Sindi, H.S., Stevenson, A.C., and Lowe, C.R. (2001). A strategy for chemical sensing based on frequency tunable acoustic devices. *Anal. Chem.* **73**, 1577–1586.

Small, G.W., Kepe, V., Ercoli, L.M., Siddarth, P., Bookheimer, S.Y., Miller, K.J., Lavretsky, H., Bruggren, A.C., Cole, G.M., Vinters, H.V., Thompson, P.M., Huang, S.C., *et al.* (2006). PET of brain amyloid and tau in mild cognitive impairment. *N. Engl. J. Med.* **355**, 2652–2663.

Snowdon, D.A., Kemper, S.J., Mortimer, J.A., Greiner, L.H., Wekstein, D.R., and Markesbery, W.R. (1996). Linguistic ability in early life and cognitive function and Alzheimer's disease in late life. Findings from the Nun study. *JAMA* **275**, 528–532.

Song, F., Poljak, A., Smythe, G.A., and Sachdev, P. (2009). Plasma biomarkers for mild cognitive impairment and Alzheimer's disease. *Brain Res. Rev.* **61**, 69–80.

Sotiriou, C., and Pusztai, L. (2009). Gene-expression signatures in breast cancer. *N. Engl. J. Med.* **19**, 790–800.

Stanta, J.L., Saldova, R., Struwe, W.B., Byme, J.C., Leweke, F.M., Rothemund, M., Rathmoune, H., Levin, Y., Guest, P.C., Bahn, S., and Rudd, P.M. (2010). Identification of N-glycosylation changes in the CSF and serum in patients with schizophrenia. *J. Proteome Res.* **9**, 4476–4489.

Stevenson, A.C., and Lowe, C.R. (1999). Magnetic-acoustic-resonator sensors (MARS): a new sensing methodology. *Sens. Actuators A* **72**, 32–37.

Stevenson, A.C., Mehta, H.M., Sethi, R.S., Cheran, L.-E., Thompson, M., Davies, I., and Lowe, C.R. (2001). Gigaherz surface acoustic wave probe for chemical analysis. *Analyst* **126**, 1619–1624.

Stevenson, A.C., Araya-Kleinsteuber, B., Sethi, R.S., Mehta, H.M., and Lowe, C.R. (2003). The acoustic spectrophonometer: a novel bioanalytical technique based on multifrequency acoustic devices. *Analyst* **128**, 1222–1227.

Stevenson, A.C., Araya-Kleinsteuber, B., Kioupritzi, E., Roque, A.C.A., and Lowe, C.R. (2006). Non-contact excitation of quartz crystal resonator chips. *Appl. Phys. Lett.* **89**, 083516.

Strehlitz, B., Nikolaus, N., and Stoltenburg, R. (2008). Protein detection with aptamer biosensors. *Sensors* **8**, 4296–4307.

Sun, Y., Zhang, L., Johnston, N.L., Torrey, E.F., and Yolken, R.H. (2001). Serial analysis of gene expression in the frontal cortex of patients with bipolar disorder. *Br. J. Psychiatry* **178**, S137–S141.

Sun, L., Hu, P., Goh, C., Hamadicharef, B., Ifeachor, E., Barbounalis, I., Zervakis, M., Nurminen, N., Varri, A., Fontanelli, R., Di Bona, S., Guerri, D., *et al.* (2006). Bioprofiling over grid for healthcare. InfoScale '06 Proceedings of the First International Conference on Scalable Information Systems, ACM, New York, NY, USA1-59593-428-6.

Suzuki, M., Iribe, Y., and Tobita, T. (2007). Surface plasmon resonance array devices. In: R.S. Marks, D.C. Cullen, I. Karube, C.R. Lowe, and H. Weetall (Eds.), *Handbook of Biosensors and Biochips*. Wiley Interscience, Sussex, UK, pp. 917–924.

Tan, E.V., and Lowe, C.R. (2009). Holographic enzyme inhibition assays for drug discovery. *Anal. Chem.* **81**, 7579–7589.

Taurines, R., Dudley, E., Grassl, J., Wamke, A., Gerlach, M., Googan, A.N., and Thome, J. (2011). Proteomic research in psychiatry. *J. Psychopharmacol.* **25**, 151–196.

Terry, S.C., Jerman, J.H., and Angell, J.B. (1979). A gas chromatographic air analyser fabricated on a silicon wafer. *IEEE Trans. Electron Devices* **26**, 1880–1886.

Tubb, A.J.C., Payne, F.P., Millington, R.B., and Lowe, C.R. (1995). Single mode optical fibre surface plasma wave chemical sensor. *Electronics Lett.* **31**, 1770–1771.

Tubb, A.J.C., Payne, F.P., Millington, R.B., and Lowe, C.R. (1997). Single-mode optical fibre surface plasma wave chemical sensor. *Sens. Actuators B Chem.* **41**, 71–79.

Underwood, B., Broadhurst, D., Dunn, W.B., Ellis, D.I., Michell, A.W., Vacher, C., Mosedale, D.E., Kell, D.B., Barker, R.A., Grainger, D.J., and Rubinsztein, D.C. (2006). Huntington disease patients and transgenic mice have similar pro-catabolic serum metabolite profiles. *Brain* **129**, 877–886.

Wanekaya, A.K., Chen, W., Myung, N.V., and Mulchandani, A. (2007). Conducting polymer nanowire-based biosensors. In: R.S. Marks, D.C. Cullen, I. Karube, C.R. Lowe, and H. Weetall (Eds.), *Handbook of Biosensors and Biochips*. Wiley Interscience, Sussex, UK, pp. 831–842.

Watts, H.J., Lowe, C.R., and Pollard-Knight, D.V. (1994). An optical sensor for monitoring whole microbial cells. *Anal. Chem.* **66**, 2465–2470.

Weibel, D.B., Kruithof, M., Potenta, S., Sia, S.K., Lee, A., and Whitesides, G.M. (2005). Torque-operated valves for microfluidics. *Anal. Chem.* **77**, 4726–4733.

Whitesides, G.M. (2006). The origins and the future of microfluidics. *Nature* **442**, 368–373.

Wolf, C., and Quinn, P.J. (2008). Lipidomics: practical aspects and applications. *Prog. Lipid Res.* **47**, 15–36.

Wolowacz, S.E., Yon Hin, B.F.Y., and Lowe, C.R. (1992). Covalent electropolymerisation of glucose oxidase in polypyrrole. *Anal. Chem.* **64**, 1541–1545.

Yellen, B.B., and Erb, R.M. (2007). Manipulation and detection of magnetic nanoparticles for diagnostic applications. In: R.S. Marks, D.C. Cullen, I. Karube, C.R. Lowe, and H. Weetall (Eds.), *Handbook of Biosensors and Biochips*. Wiley Interscience, Sussex, UK, pp. 799–809.

Young, H., Baum, R., Cremerius, U., Herholtz, K., Hoekstra, O., Lammertma, A.A., Pruim, J., and Price, P. (1999). Measurement of clinical and subclinical tumour response using [18F]-fluorodeoxyglucose and positron emission tomography: review and 1999 EORTC recommendations. *Eur. J. Cancer* **35**, 1773–1782.