Chapter 3
Nanomolecular Diagnostics

Introduction

Clinical application of molecular technologies to elucidate, diagnose, and monitor human diseases is referred to as molecular diagnosis. It is a broader term than DNA diagnostics and refers to the use of nucleic acid technologies that employ DNA, RNA, genes, or proteins as bases for diagnostic tests. The term “biotechnology diagnostics” also includes the use of monoclonal antibodies (MAbs) and enzyme-linked immunosorbent assay (ELISA). The term “genomic diagnostics” is used for application of molecular diagnostic technologies in genomics, which is the study of all of the genes in an organism—their sequences, structure, regulation, interaction, and products. A more detailed description of molecular diagnostics is presented elsewhere (Jain 2007a).

Because of the small dimension of nanoparticles, most of the applications of nanobiotechnology in molecular diagnostics fall under the broad category of biochips/microarrays but are more correctly termed nanochips and nanoarrays. Nanotechnology-on-a-chip is a general description that can be applied to several methods. Some of these do not use nanotechnologies but merely have the capability to analyze nanoliter amounts of fluids.

Biochips, constructed with MEMS on a micron scale, are related to micromanipulation, whereas nanotechnology-based chips on a nanoscale are related to nanomanipulation. Even though microarray/biochip methods employing the detection of specific biomolecular interactions are now an indispensable tool for molecular diagnostics, there are some limitations. DNA microarrays and ELISA rely on the labeling of samples with a fluorescent or radioactive tag—a highly sensitive procedure that is time-consuming and expensive.

The chemical modification and global amplification of the nucleic acid samples are achieved by polymerase chain reaction (PCR), which can introduce artifacts caused by the preferential amplification of certain sequences. Alternative label-free methods include SPR and quartz crystal microbalance, which rely on mass detection. Nanotechnologies also provide label-free detection. Nanotechnology is thus being applied to overcome some of the limitations of biochip technology. This chapter focuses on the application of nanotechnologies to nucleic acid as well as protein diagnostics.
Nanodiagnostics

Nanomolecular diagnostics is the use of nanobiotechnology in molecular diagnostics and can be termed “nanodiagnostics” (Jain 2003a). Numerous nanodevices and nanosystems for sequencing single molecules of DNA are feasible. It seems quite likely that there will be numerous applications of inorganic nanostructures in biology and medicine as biomarkers. Given the inherent nanoscale of receptors, pores, and other functional components of living cells, the detailed monitoring and analysis of these components will be made possible by the development of a new class of nanoscale probes. Biological tests measuring the presence or activity of selected substances become quicker, more sensitive, and more flexible when certain nanoscale particles are put to work as tags or labels. Nanotechnology will improve the sensitivity and integration of analytical methods to yield a more coherent evaluation of life processes.

It is difficult to classify such a wide variety of technologies, but various nanotechnologies with potential applications in molecular diagnostics are listed in Table 3.1. Nanotechnology-on-a-chip was described earlier in this Chapter 2. Some of the other technologies will be described briefly in the following text using examples of commercial products. A more detailed description is given in a special book on this topic (Jain 2006b).

Rationale of Nanotechnology for Molecular Diagnostics

Numerous nanodevices and nanosystems for sequencing single molecules of DNA are feasible. It is likely that there will be numerous applications of inorganic nanostructures in biology and medicine as markers:

- Nanoscale probes would be suitable for a detailed analysis of receptors, pores, and other components of living cells that are on a nanoscale.
- Nanoscale particles, used as tags or labels, increase the sensitivity, speed, and flexibility of biological tests measuring the presence or activity of selected substances.
- Nanotechnology will improve the sensitivity and integration of analytical methods to yield a more coherent evaluation of life processes.

Nanoarrays for Molecular Diagnostics

Several nanoarrays and nanobiochips are in development (Jain 2007a). Some of these will be reviewed here.
Table 3.1 Nanotechnologies with potential applications in molecular diagnostics

| Nanotechnology to improve polymerase chain reaction (PCR)          |
|---------------------------------------------------------------|
| Nanotechnology-on-a-chip                                      |
| Microfluidic chips for nanoliter volumes: NanoChip            |
| Optical readout of nanoparticle labels                         |
| NanoArrays                                                    |
| Protein nanoarrays                                            |
| Nanotechnology-based cytogenetics                             |
| Study of chromosomes by atomic force microscopy               |
| Quantum dot fluorescent in situ hybridization (FISH)          |
| Nanoscale single-molecule identification                      |
| Nanoparticle technologies                                     |
| Gold particles                                                |
| Nanobarcodes                                                  |
| Magnetic nanoparticles: ferrofluids, supramagnetic particles combined with MRI |
| Quantum dot technology                                        |
| Nanoparticle probes                                           |
| Nanowires                                                     |
| Nanopore technology                                           |
| Measuring length of DNA fragments in a high-throughput manner |
| DNA fingerprinting                                             |
| Haplotyping                                                   |
| DNA nanomachines for molecular diagnostics                    |
| Nanoparticle-based immunoassays                               |
| DNA–protein and nanoparticle conjugates                       |
| Nanochip-based single-molecular interaction force assays      |
| Resonance light scattering technology                         |
| Nanosensors                                                   |
| Cantilever arrays                                             |
| Living spores as nanodetectors                                |
| Nanopore nanosensors                                          |
| Quartz nanobalance DNA sensor                                 |
| PEBBLE (Probes Encapsulated by Biologically Localized Embedding) nanosensors |
| Nanosensor glucose monitor                                    |
| Photostimulated luminescence in nanoparticles                |
| Optical biosensors: e.g., SPR technology                      |

Source: Jain PharmaBiotech.

**NanoPro™ System**

The NanoPro™ System (BioForce Nanosciences Inc, Ames, IA, USA) consists of three separate components:

1. The NanoArrayer™ embodies proprietary instrumentation and methods for creating a broad spectrum of NanoArray™-based biological tests. This device places molecules at defined locations on a surface with nanometer spatial resolution. The arrays of molecules (NanoArrays™, see below) are unique to BioForce and can only be created with a NanoArrayer™.
2. NanoArrays™ are ultraminiaturized biological tests with applications in many areas. The company’s first NanoArray™ products are presently being evaluated for commercial utilization by potential users and are targeted toward the proteomics/genomics and diagnostics markets. These products include a custom nucleic acid NanoArray™ for RNA expression profiling as well as virus detection NanoArray™ called the ViriChip™. NanoArray™ chips are for protein expression profiling and immunodiagnostics.

3. The NanoReader™ is a customized AFM optimized for reading NanoArray™ chips. Using the AFM as a readout method optimizes analysis, with the following advantages:

- No need for secondary reporter systems such as fluorescence, radioactivity, or enzyme-linked detection schemes
- Reductions in materials used as several thousand molecules can be covered with one test
- Ultimately an increased sensitivity with single-molecule detection

Nanofluidic/Nanoarray Devices to Detect a Single Molecule of DNA

One of the more promising uses of nanofluidic devices is isolation and analysis of individual biomolecules, such as DNA, which could lead to new detection schemes for cancer. This is now closer to realization with the development of a simple nanofluidic device within a silicon nanotube for a relatively long time (Fan et al 2005). This procedure entails first constructing silicon nanowires (SiNWs) on a substrate, or chip, using standard photolithographic and etching techniques, followed by a chemical oxidation step that converts the NWs into hollow nanotubes. Using this process, the investigators can reliably create nanotubes with diameters as small as 10 nm, though for their biomolecule isolation device they used nanotubes with a diameter of 50 nm. To trap DNA molecules, the investigators built a device consisting of a silicon nanotube connecting two parallel microfluidic channels. Electrodes provide a source of current used to drive DNA into the nanotubes. Each time a single DNA molecule moves into the nanotube, the electrical current increases suddenly. The current returns to its baseline value when the DNA molecule exits the nanotube. On average, a DNA molecule remains within the nanotube for ∼7.5 ms, which should be sufficient to make a variety of analytical measurements that could reveal cancer-associated mutations. The investigators are now adding optical and electrical circuitry to probe the trapped DNA molecules.

The nanochannel array technology (BioNanomatrix, Philadelphia, PA, USA) is designed to enable direct visualization and linear analysis of multimegabase genomic DNA at the single-molecule level with high feature resolution in massive parallel fashion. The platform is also anticipated to significantly reduce the cost and time needed for the extensive data and integrative analyses that have hindered widespread use of whole-genome studies to date. It is expected to have broad
application in systems biology, personalized medicine, pathogen detection, drug
development, and clinical research.

A 2D method for MS in solution is based on the interaction between a nanometer-scale pore and analytes (Robertson et al 2007). The technique involves creating a lipid bilayer membrane similar to those in living cells, and “drilling” a pore in it with a protein (\(\alpha\)-HL) produced by the \textit{Staphylococcus aureus} bacteria specifically to penetrate cell membranes. Charged molecules (such as single-stranded DNA [ssDNA]) are forced one at a time into the nanopore, which is only 1.5 nm at its smallest point, by an applied electric current. As the molecules pass through the channel, the current flow is reduced in proportion to the size of each individual chain, allowing its mass to be easily derived. In this experiment, various-sized chains in solution of the uncharged polymer polyethylene glycol (PEG) were substituted for biomolecules. Each type of PEG molecule reduced the nanopore’s electrical conductance differently as it moved through, allowing the researchers to distinguish one size of PEG chain from another. Because the dimensions of the lipid bilayer and the \(\alpha\)-HL pore, as well as the required amount of electrical current, are at the nanoscale level, the single-molecule MS technology may one day be incorporated into “lab-on-a-chip” molecular analyzers and ssDNA sequencers. This single-molecule analysis technique could prove useful for the real-time characterization of biomarkers (i.e., nucleic acids, proteins, or other biopolymers). With automated, unsupervised analytical and statistical methods, this technique should prove viable as a generalized analytical technique with nanopore arrays containing nanopores both with specific affinities for single biomarkers and with nonspecific transducers. In situ monitoring of cellular metabolism with such arrays should provide the sensitivity to monitor subtle changes observed through the release of biomarkers.

\section*{Self-Assembling Protein Nanoarrays}

Protein microarrays provide a powerful tool for the study of protein function. However, they are not widely used, in part because of the challenges in producing proteins to spot on the arrays. Protein microarrays can be generated by printing cDNAs onto glass slides and then translating target proteins with mammalian reticulocyte lysate (Ramachandran et al 2004). Epitope tags fused to the proteins allowed them to be immobilized in situ. This obviates the need to purify proteins, avoided protein stability problems during storage, and captured sufficient protein for functional studies. This technology has been used to map pairwise interactions among 29 human DNA replication initiation proteins, recapitulate the regulation of Cdt1 binding to select replication proteins, and map its geminin-binding domain.

Lumera’s NanoCapture technology is now being combined with nucleic acid programmable protein array (NAPPA) technology to create high-density protein arrays with 10,000 spots. The 10,000-spot array is a very important step toward the ultimate goal of producing a whole-proteome biochip. The current protein arrays are
limited to ~800 spots. The NAPPA technology starts with a printed cDNA array and generates a self-assembled protein array using a cell-free expression mix to produce proteins from the printed genes.

**Fullerene Photodetectors for Chemiluminescence Detection on Microfluidic Chips**

Solution-processed thin-film organic photodiodes have been used for microscale chemiluminescence (Wang et al 2007). The active layer of the photodiodes comprised a blend of the conjugated polymer poly(3-hexylthiophene) and a soluble derivative of fullerene C60. The devices had an active area of $1\text{ mm} \times 1\text{ mm}$ and a broadband response from 350 to 700 nm, with an external quantum efficiency of $>50\%$ between 450 and 550 nm. The photodiodes have a simple layered structure that allows integration with planar chip-based systems. To evaluate the suitability of the organic devices as integrated detectors for microscale chemiluminescence, a peroxyoxalate-based chemiluminescence reaction (PO-CL) was monitored within a poly(dimethylsiloxane) (PDMS) microfluidic device. Quantitation of hydrogen peroxide indicated excellent linearity and yielded a detection limit of 10 $\mu$M, comparable with previously reported results using micromachined silicon microfluidic chips with integrated silicon photodiodes. The combination of organic photodiodes with PDMS microfluidic chips offers a means of creating compact, sensitive, and potentially low-cost microscale CL devices with wide-ranging applications in chemical and biological analysis and clinical diagnostics.

**Protein Microarray for Detection of Molecules with Nanoparticles**

A sensitive technique is being developed for optical detection of nanogold particle-labeled molecules on a protein microarray by applying the SPR and specific molecular binding using rolling circle amplification (Hsu and Huang 2004). A new type of protein chip is being developed based on protein-binding silica nanoparticles at the Fraunhofer Institute for Interfacial Engineering and Biotechnology IGB (Stuttgart, Germany). The surface of this minute particle with a diameter of $<100\text{ nm}$ can be configured with many different capture proteins. The particles configured in this way are then applied to silicon carriers in thin, even layers. After contact is made with a sample, the chips can be analyzed using state-of-the-art MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). Knowing the masses of the bound proteins provides a direct indication of their identity.

**Protein Nanobiochip**

Nanotechnology Group of the NEC Corporation has developed a prototype protein analysis technology that can analyze samples ~20 times faster than conventional
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Biomarker proteins as early warning signs for diseases such as cancer can be identified for diagnostic purposes by finding their isoelectric points and their molecular weights. Isoelectric points are chemical features that refer to the electrical state of a molecule when it has no net charge. Conventional protein chips use a gel across which an electric current is applied to find the targeted protein’s isoelectric points. In the new process, instead of being filtered through a block of gel, the protein molecules are separated by their isoelectric points by a capillary action as the proteins flow in a solution along channels in the chip. A test chip by NEC measures 21 mm² and contained four sets of tiny channels in which the capillary action takes place. The protein molecules are then dried and irradiated by a laser. Their molecular weights are then measured by a mass spectrometer. The laser helps the proteins leave the chip, and the mass spectrometer is used to judge the molecular weights of the protein molecules in the samples by measuring how early they reach a detector. In the mass spectrometer, light molecules fly faster than heavy ones in an electric field. The mass spectrometer judges the weight of the molecules by monitoring the timing of when each molecule reaches a detector. In addition to being faster than techniques that use gel blocks, the new method also needs blood samples of ~1 μl compared with those of ~20 μl or more that are needed for gel-based techniques. NEC is now planning clinical trials, which should last 2–3 years. If those trials go well, the company should commercialize the technology around or after 2008. When commercialized, the technique could be used for health checks that might cost as little as $100.

Nanoparticles for Molecular Diagnostics

Gold Nanoparticles

Scientists at Northwestern University’s Institute for Nanotechnology (http://www.nsec.northwestern.edu/~mkngrp/dnasubgr.html) have attached bits of DNA and Raman-active dyes onto gold particles no larger than 13 nm in diameter. The gold nanoparticles assemble onto a sensor surface only in the presence of a complementary target. If a patterned sensor surface of multiple DNA strands is used, the technique can detect millions of different DNA sequences simultaneously (Cao et al 2002). The current nonoptimized detection limit of this method is 20 fM. Gold nanoparticles were found to be particularly good labels for sensors because a variety of analytical techniques can be used to detect them, including optical absorption, fluorescence, Raman scattering, atomic and magnetic force, and electrical conductivity. They have used gold nanoparticles and a Raman spectroscopy technique to detect life-threatening bacteria and viruses such as anthrax and HIV. Raman approach could replace PCR and fluorescent tags commonly used today. The detection system also relies on chips dotted with DNA. If the targeted disease exists in the sample,
its DNA will bind onto the cDNA strands on the chip and gold particle. The chip is treated with silver-based solution, which coats the nanoparticles. When exposed to a light scanner, the coating enhances the signal enough to detect minute amounts of DNA. Since the Raman band is narrower than the fluorescent band, it allows more dyes to detect more targets quickly. If the sequence of interest is present in the sample, it will bind to the DNA and cause the solution to change color. Labeling oligonucleotide targets with gold nanoparticle rather than fluorophore probes substantially alters the melting profiles of the targets from an array substrate. Northwestern University scientists have developed a nanoparticle-based DNA detection system with 10 times more sensitivity and 100,000 times more specificity than current genomic detection systems (Park et al 2002).

Researchers from the University of Dortmund in Germany have devised a way to coax DNA to aggregate and separate gold nanoparticles on demand (Hazarika et al 2004). They used two ssDNA sequences, each of which is attached to a gold nanoparticle, and a third single strand that has three sections. The first two sections of the third strand match up with each of the nanoparticle strands, gluing them together so that the gold nanoparticles they carry are positioned close together. The nanoparticles can be pulled apart again using a third type of DNA strand that matches up with the entire glue strand of DNA. This glue removal strand first attaches to the free third section of the glue strand, then continues until the entire glue strand is pulled free. The method could be used in sensors that detect biological substances. It could also be used in programmable materials whose properties can be changed by the addition of a piece of DNA.

**Quantum Dots for Molecular Diagnostics**

There is considerable interest in the use of QDs as inorganic fluorophores, owing to the fact that they offer significant advantages over conventionally used fluorescent markers. For example, QDs have fairly broad excitation spectra—from ultraviolet to red—that can be tuned depending on their size and composition. At the same time, QDs have narrow emission spectra, making it possible to resolve the emissions of different nanoparticles simultaneously and with minimal overlap. Finally, QDs are highly resistant to degradation, and their fluorescence is remarkably stable. Advantages of QD technology are as follows:

- Simple excitation—Lasers are not required
- Simple instrumentation
- Availability of red/infrared colors enables whole-blood assays
- High sensitivity

QDs have been used as possible alternatives to the dyes for tagging viruses and cancer cells. A major challenge is that QDs have an oily surface, whereas the cellular environment is water-based. Attempts are being made to modify the surface chemistry of QDs so that they interact with water-friendly molecules like proteins.
and DNA. The current goal is to develop QDs that can target a disease site and
light it up. This can someday lead to an integrated system that will also use the
QDs to diagnose as well to deliver drug therapies to the disease site. QDs can be
designed to emit light at any wavelength from the infrared to visible to ultraviolet.
This enables the use of a large number of colors and thus multiplexed assays
can be performed. Potential applications of QDs in molecular diagnostics can be
summarized as follows:

- Cancer
- Genotyping
- Whole-blood assays
- Multiplexed diagnostics
- DNA mapping
- Immunoassays and antibody tagging
- Detection of pathogenic microorganisms

Quantum Dots for Detection of Pathogenic Microorganisms

QDs have been used as fluorescent labels in immunoassays for quantitative detection
of foodborne pathogenic bacteria (Yang and Li 2005). *Salmonella typhimurium* cells
were separated from chicken carcass wash water using anti-*Salmonella*
antibody-coated magnetic beads and reacted to secondary biotin-labeled anti-
*Salmonella* antibody. QDs coated with streptavidin were added to react with biotin
on the secondary antibody. Measurement of the intensity of fluorescence produced
by QDs provided a quantitative method for microbial detection.

QDs can be used for ultrasensitive viral detection of a small number of microorganisms. QD-based tests can detect as low as 100 copies of hepatitis and HIV RNA. Applications of tests based on QDs for clinical diagnosis of viral infections are
described in Chapter 11.

Bioconjugated QDs for Multiplexed Profiling of Biomarkers

Bioconjugated QDs, collections of different-sized nanoparticles embedded in tiny
polymer beads, provide a new class of biological labels for evaluating biomarkers
on intact cells and tissue specimens. In particular, the use of multicolor QD probes
in immunohistochemistry is considered one of the most important and clinically relevant applications. The medical use of QD-based immunohistochemistry has been
limited by the lack of specific instructions, or protocols, for clinicians. Preliminary
results and detailed protocols for QD–antibody conjugation, tissue specimen prepara-
tion, multicolor QD staining, image processing, and biomarker quantification have
been published (Xing et al 2007). The results demonstrate that bioconjugated QDs
can be used for multiplexed profiling of biomarkers, and ultimately for correlation
with disease progression and response to therapy. This will increase the clinician’s
ability to predict the likely outcomes of drug therapy in a personalized approach
to disease management. Bioinformatics and systems biology is used to link each
individual’s molecule profile with disease diagnosis and treatment decisions. The usefulness of these protocols was demonstrated by simultaneously identifying multiple biomarkers in prostate cancer tissue. In general, QD bioconjugation is completed within 1 day, and multiplexed molecular profiling takes 1–3 days depending on the number of biomarkers and QD probes used.

**Imaging of Living Tissue with Quantum Dots**

Tiny blood vessels, viewed beneath a mouse’s skin with a newly developed application of multiphoton microscopy, appear so bright and vivid in high-resolution images that researchers can see the vessel walls ripple with each heartbeat. Cornell researchers have shown that capillaries many hundreds of microns below the skin of living mice were illuminated in unprecedented detail using fluorescence imaging labels, QDs, circulating through the bloodstream (Larson et al 2003). This is a new approach to using QDs for biological studies of living animals. Although there are easier ways to take a mouse’s pulse, this level of resolution and high signal-to-noise illustrate how useful multiphoton microscopy with QDs can become in biological research for tracking cells and visualizing tissue structures deep inside living animals. Monitoring of vascular changes in malignant tumors is a possible application. This research will pave the way for many new noninvasive in vivo imaging methods using QDs.

Carbohydrate-encapsulated QDs can be used for medical imaging. Certain carbohydrates, especially those included on tumor glycoproteins, are known to have affinity for certain cell types and this can be exploited for medical imaging. Conjugating luminescent QDs with target specific glycans permits efficient imaging of the tissue to which the glycans bind with high affinity. Accurate imaging of primary and metastatic tumors is of primary importance in disease management. Second-generation QDs contain the glycan ligands and PEG of varying chain lengths. The PEG modifications produce QDs that maintain high luminescence while reducing nonspecific cell binding.

Procedures have been developed for using QDs to label live cells and to demonstrate their use for long-term multicolor imaging (Jaiswal et al 2003). The two approaches are endocytic uptake of QDs and selective labeling of cell surface proteins with QDs conjugated to antibodies. These approaches should permit the simultaneous study of multiple cells over long periods of time as they proceed through growth and development. Use of avidin permits stable conjugation of the QDs to ligands, antibodies, or other molecules that can be biotinylated, whereas the use of proteins fused to a positively charged peptide or oligohistidine peptide obviates the need for biotinylating the target molecule. A procedure has been described for the bioconjugation of QDs and specific labeling of both intracellular and cell surface proteins (Jaiswal et al 2004). For generalized cellular labeling, QDs not conjugated to a specific biomolecule may be used.

Fluorescent semiconductor QDs hold great potential for molecular imaging in vivo. However, the utility of existing QDs for in vivo imaging is limited because they require excitation from external illumination sources to fluoresce, which
results in a strong autofluorescence background and a paucity of excitation light at nonsuperficial locations. QD conjugates that luminesce by bioluminescence resonance energy transfer in the absence of external excitation have been prepared by coupling carboxylate-presenting QDs to a mutant of the bioluminescent protein Renilla reniformis luciferase (So et al 2006). The conjugates emit long-wavelength (from red to NIR) bioluminescent light in cells and in animals, even in deep tissues, and are suitable for multiplexed in vivo imaging. Compared with existing QDs, self-illuminating QD conjugates have greatly enhanced sensitivity in small animal imaging, with an in vivo signal-to-background ratio of $>10^3$ for 5 pmol of conjugate.

**Magnetic Nanoparticles**

**Magnetic Nanoparticles for Bioscreening**

Iron nanoparticles, 15–20 nm in size, having saturation magnetization, have been synthesized, embedded in copolymer beads of styrene and glycidyl methacrylate (GMA), which were coated with poly-GMA by seed polymerization (Maeda et al 2006). The resultant Fe/St-GMA/GMA beads had diameters of 100–200 nm. By coating with poly-GMA, the zeta potential of the beads changed from $-93.7$ to $-54.8$ mV, as measured by an electrophoresis method. This facilitated nonspecific protein adsorption suppression, as revealed by gel electrophoresis method, which is a requisite for nanoparticles to be applied to carriers for bioscreening.

**Superparamagnetic Nanoparticles for Cell Tracking**

Magnetic nanoparticles are a powerful and versatile diagnostic tool in biology and medicine. It is possible to incorporate sufficient amounts of superparamagnetic iron oxide (SPIONs) into cells, enabling their detection in vivo using MRI (Bulte et al 2004). Because of their small size, they are easily incorporated into various cell types (stem cells, phagocytes, etc.), allowing the cells to be tracked in vivo—for example, to determine whether stem cells move to the correct target area of the body.

Superparamagnetic nanoparticles (CD34 microbeads), used clinically for specific magnetic sorting, can be used as a magnetic cell label for in vivo cell visualization. In one study, human cells from peripheral blood were selected by CliniMACS CD34 Selection Technology (Miltenyi, Bergisch Gladbach, Germany) and implanted into rats with a cortical photochemical lesion, contralaterally to the lesion (Jendelova et al 2005). Twenty-four hours after grafting, the implanted cells were detected in the contralateral hemisphere as a hypointense spot on T2-weighted images; the hypointensity of the implant decreased during the first week. Staining techniques confirmed the presence of magnetically labeled human cells in the lesion 4 weeks after grafting. Thus CD34 microbeads can be used as a magnetic cell label for in vivo cell visualization. The fact that microbeads coated with different commercially
available antibodies can bind to specific cell types opens extensive possibilities for cell tracking in vivo.

**Superparamagnetic Iron Oxide Nanoparticles for Calcium Sensing**

A family of calcium indicators for MRI is formed by combining a powerful superparamagnetic iron oxide nanoparticle-based contrast mechanism with the versatile calcium-sensing protein calmodulin and its targets (Atanasijevic et al 2006). Calcium-dependent protein–protein interactions drive particle clustering and produce up to 5-fold changes in T2 relaxivity, an indication of the sensor’s potency. Robust MRI signal changes are achieved even at nanomolar particle concentrations that are unlikely to buffer calcium levels. When combined with technologies for cellular delivery of nanoparticulate agents, these sensors and their derivatives may be useful for functional molecular imaging of biological signaling networks in live, opaque specimens.

**Magnetic Nanoparticles for Labeling Molecules**

Bound to a suitable antibody, magnetic nanoparticles are used to label specific molecules, structures, or microorganisms. Magnetic immunoassay techniques have been developed in which the magnetic field generated by the magnetically labeled targets is detected directly with a sensitive magnetometer. Binding of antibody to target molecules or disease-causing organism is the basis of several tests. Antibodies labeled with magnetic nanoparticles generate magnetic signals on exposure to a magnetic field. Antibodies bound to targets can thus be identified as unbound antibodies disperse in all directions and produce no net magnetic signal.

A novel nanosensor based on magnetic nanoparticles has been developed for rapid screens of telomerase activity in biological samples (Grimm et al 2004). The technique utilizes nanoparticles that, on annealing with telomerase-synthesized TTAGGG repeats, switch their magnet state, a phenomenon readily detectable by magnetic readers. High-throughput adaptation of the technique by MRI allowed processing of hundreds of samples within tens of minutes at ultrahigh sensitivities. Together, these studies establish and validate a novel and powerful tool for rapidly sensing telomerase activity and provide the rationale for developing analogous magnetic nanoparticles for in vivo sensing. Since elevated telomerase levels are found in many malignancies, this technique provides access to an attractive target for therapeutic intervention and diagnostic or prognostic purposes.

Superparamagnetic iron oxide nanoparticles have been functionalized to identify *Mycobacterium avium* spp. *paratuberculosis* (MAP) through magnetic relaxation (Kaittanis et al 2007). The results indicate that the MAP nanoprobes bind specifically to MAP and can quantify the bacterial target quickly in milk and blood with high sensitivity. The advantage of this approach is that detection is not susceptible to interferences caused by other bacteria. The use of these magnetic nanosensors is anticipated in the identification and quantification of bacteria in clinical and environmental samples.
Ferrofluids

CellTracks™ Technology (Immunicon, Huntington Valley, PA, USA) is based on patented magnetic nanoparticles called ferrofluids. Ferrofluids consist of a magnetic core surrounded by a polymeric layer coated with antibodies for capturing cells. Ferrofluid particles are colloidal, and when mixed with a sample containing the target cells, the antibodies conjugated to the magnetic core bind the antigen associated with the target cells. It is combined with proprietary technologies for cell separation, labeling, and analysis. This is in development to screen, diagnose, stage, and monitor cancer based on circulating cancer cells in the blood. Potential applications include isolation of endothelial cells, which may be useful in the management of cancer and cardiovascular disease, and isolation of fungus or bacteria, which may be useful in the management of patients with serious infections.

Superconducting Quantum Interference Device

Superconducting quantum interference device (SQUID), developed at the University of California (Berkeley, CA), is a technique for specific, sensitive, quantitative, and rapid detection of biological targets by using superparamagnetic nanoparticles and a “microscope” based on a high-transition temperature (Chemla et al 2000). In this technique, a mylar film to which the targets have been bound is placed on the microscope alongside SQUID. A suspension of magnetic nanoparticles carrying antibodies directed against the target is added to the mixture in the well, and 1-s pulses of magnetic field are applied parallel to the SQUID. In the presence of this aligning field the nanoparticles develop a net magnetization, which relaxes when the field is turned off. Unbound nanoparticles relax rapidly by Brownian rotation and contribute no measurable signal. Nanoparticles that are bound to the target on the film are immobilized and undergo a slowly decaying magnetic flux, which is detected by the SQUID. The ability to distinguish between bound and unbound labels allows one to run homogeneous assays, which do not require separation and removal of unbound magnetic particles.

Study of Living Cells by Superparamagnetic Nanoparticles

Technologies to assess the molecular targets of biomolecules in living cells are lacking. A new technology called magnetism-based interaction capture (MAGIC) has been developed that identifies molecular targets on the basis of induced movement of superparamagnetic nanoparticles (MNPs) inside living cells (Won et al 2005). The scientists painted intracellular proteins with fluorescent materials and inserted magnetic nanoparticles-embedded drugs into the cell. These nanoprobes captured the small molecule’s labeled target protein and were translocated in a direction specified by the magnetic field. Use of MAGIC in genome-wide expression screening identified multiple protein targets of a drug. MAGIC was also used to monitor signal-dependent modification and multiple interactions of proteins. It was also shown that internalized MNPs could be moved inside cells by an external magnetic
field, using a luminescent nanocrystal (NC) QD, which does not exhibit magnetism, as a control. The MNPs not only responded to application of the magnetic field but also rapidly dispersed when the magnetic field was removed and reassembled on reapplication of the magnetic field. MAGIC can be useful in the development of diagnostics and biosensors. Its ultimate use would be for the analysis of interactions inside living cells of patients.

**Use of Nanocrystals in Immunohistochemistry**

A method has been described for simple convenient preparation of bright, negatively or positively charged, water-soluble CdSe/ZnS core/shell NCs and their stabilization in aqueous solution (Sukhanova et al. 2004). Single NCs can be detected using a standard epifluorescent microscope, ensuring a detection limit of one molecule coupled with an NC. NC–antibody (Ab) conjugates were tested in dot blots and exhibited retention of binding capacity within several nanograms of antigen detected. The authors further demonstrated the advantages of NC–Ab conjugates in the immunofluorescent detection and 3D confocal analysis of P-glycoprotein (P-gp), one of the main mediators of the multidrug resistance (MDR) phenotype. The labeling of P-gp with NC–Ab conjugates was highly specific. Finally, the authors demonstrated the applicability of NC–Ab conjugates obtained by the method described to specific detection of antigens in paraffin-embedded formaldehyde-fixed cancer tissue specimens, using immunostaining of cyto-keratin in skin basal carcinoma as an example. They concluded that the NC–Ab conjugates may serve as easy-to-do, highly sensitive, photostable labels for immunofluorescent analysis, immunohistochemical detection, and 3D confocal studies of membrane proteins and cells.

**Imaging Applications of Nanoparticles**

There is rapid growth in the use of MRI for molecular and cellular imaging. Much of this work relies on the high relaxivity of nanometer-sized, ultrasmall dextran-coated iron oxide particles. Chemical modifications to nanosized virus particles may improve MRI. Attachment of a large number of gadolinium chelates, the chemical compound used in MRI contrast agents, onto the surface of the viral particles resulted in the generation of a very intense signal in a clinical MRI scanner (Anderson et al. 2006). Magnetic nanoparticles, QDs, and ferrofluids are examples of some of the nanoparticles that have been used along with imaging technologies.

**Magnetic Nanoparticles Combined with MRI**

Highly lymphotropic superparamagnetic nanoparticles measuring 2–3 nm on average, which gain access to lymph nodes by means of interstitial–lymphatic fluid transport, have been used in conjunction with high-resolution MRI to reveal small
Nanoparticles for Molecular Diagnostics

and otherwise undetectable lymph node metastases (Harisinghani et al 2003). The lymphotropic superparamagnetic nanoparticle used in this study was a monocristalline iron oxide (Combidex, Advanced Magnetics, Cambridge, MA, USA). In patients with prostate cancer who underwent surgical lymph node resection or biopsy, MRI with lymphotropic superparamagnetic nanoparticles correctly identified all patients with nodal metastases. This diagnosis was not possible with conventional MRI alone and has implications for the management. In men with metastatic prostate cancer, adjuvant androgen deprivation therapy with radiation is the mainstay of management.

The presence of lymph node metastases is an important factor in breast cancer patient prognosis. Therefore, the precise identification of sentinel lymph nodes in these patients is critical. Draining lymphatic ducts and lymph nodes were clearly visualized in the mammary tissue of normal mice and in spontaneous and xenografted breast tumor models after a direct mammary gland or peritumoral injection of nano-size paramagnetic molecule, G6. Micro-magnetic resonance lymphangiography using the G6 contrast agent revealed the absence of filling in the metastatic foci of affected lymph nodes (Kobayashi et al 2004). Gd-DTPA-dimeglumine, by contrast, failed to depict lymphatic flow from the mammary tissue in normal mice using the same method. The superior temporal and spatial resolution of micro-magnetic resonance lymphangiography using the contrast agent G6 may facilitate the study of tumor lymphatic drainage and lymphatic metastasis in both experimental animals and clinical medicine. In addition, this may be a powerful new method for sentinel lymph node localization in human breast cancer.

Nanoparticles as Contrast Agent for MRI

The determination of brain tumor margins both during the presurgical planning phase and during surgical resection has long been a challenging task in the therapy of brain tumor patients. Multimodal (NIR fluorescent and magnetic) nanoparticles were used as a preoperative MRI contrast agent and intraoperative optical probe in a model of gliosarcoma with stably green fluorescence protein-expressing 9L glioma cells (Kircher et al 2003). Key features of nanoparticle metabolism, namely, intracellular sequestration by microglia and the combined optical and magnetic properties of the probe, allowed delineation of brain tumors both by preoperative MRI and by intraoperative optical imaging. This prototypical multimodal nanoparticle has unique properties that may allow radiologists and neurosurgeons to see the same probe in the same cells and may offer a new approach for obtaining tumor margins.

Alphanu3beta3-targeted paramagnetic nanoparticles have been employed to non-invasively detect very small regions of angiogenesis associated with nascent melanoma tumors (Schmieder et al 2005). Each particle was filled with thousands of molecules of the metal that is used to enhance contrast in conventional MRI scans. The surface of each particle was decorated with a substance that attaches to newly forming blood vessels, which are present at tumor sites. The goal was to create a high density of the glowing particles at the site of tumor growth, so they are easily visible. Molecular MRI results were corroborated by histology. This study
lowers the limit previously reported for detecting sparse biomarkers with molecular MRI in vivo when the growths are still invisible to conventional MRI. Earlier detection can potentially increase the effectiveness of treatment. This is especially true with melanoma, which begins as a highly curable disorder, then progresses into an aggressive and deadly disease. A second benefit of the approach is that the same nanoparticles used to find the tumors could potentially deliver stronger doses of anticancer drugs directly to the tumor site with fewer side effects. Targeting the drugs to the tumor site in this way would also allow stronger doses without systemic toxicity than would be possible if the drug were injected or delivered in some other systemic way. The nanoparticles might also allow physicians to more readily assess the effectiveness of the treatment by comparing MRI scans before and after treatment. Other cancer types might be accessible to this approach as well, because all tumors recruit new blood vessels as they grow.

Manganese Oxide Nanoparticles as Contrast Agent for Brain MRI

A new MRI contrast agent using manganese oxide nanoparticles produces images of the anatomic structures of mouse brain which are as clear as those obtained by histological examination (Na et al 2007). The new contrast agent will enable better research and diagnosis of neurological disorders such as Alzheimer’s disease (AD), Parkinson’s disease (PD), and stroke. Furthermore, antibodies can be attached to the manganese oxide nanoparticles, which recognize and specifically bind to receptors on the surface of breast cancer cells in mouse brains with breast cancer metastases. The tumors were clearly highlighted by the antibody-coupled contrast agent. The same principle should allow other disease-related changes or physiological systems to be visualized by using the appropriate antibodies.

Gadonanotubes for MRI

More than 25 million patients in the United States undergo MRI each year and contrast agents are used in ~30% of these procedures. Gadolinium agents are the most effective and the most commonly used MRI contrast agents. A new class of contrast agents, gadonanotubes, uses the same highly toxic metal gadolinium (Gd^{3+}) that is used in MRI currently, but the metal atoms are encased inside a carbon nanotube (Sitharaman et al 2005). The ultrashort nanotubes are only about 20–100 times longer than they are wide, and once inside the nanotubes, the gadolinium atoms naturally aggregate into tiny clusters of ~10 atoms each. Clustering causes the unexplained increases in magnetic and MRI effects. Gadonanotubes are at least 40–90 times more effective than Gd^{3+}-based MRI agents now in use. Shrouding the toxic metals inside the benign carbon is expected to significantly reduce or eliminate the metal’s toxicity. Currently available methods of attaching disease-specific antibodies and peptides can be applied to gadonanotubes, so they can be targeted to cancerous and other diseased cells.
Quantum Dots for Biological Imaging

Targeted QDs, coated with paramagnetic and pegylated lipids, have been developed for detection by MRI (Mulder et al 2006). The QDs were functionalized by covalently linking v3-specific peptides, and the specificity was assessed and confirmed on cultured endothelial cells. The bimodal character, the high relaxivity, and the specificity of this nanoparticulate probe make it an excellent contrast agent for molecular imaging purposes. Among other applications, those in cancer are most important.

Accurate imaging of diseased cells (e.g., primary and metastatic tumors) is of primary importance in disease management. The NIH has developed carbohydrate-encapsulated QDs with detectable luminescent properties useful for imaging of cancer or other disease tissues. Certain carbohydrates, especially those included on tumor glycoproteins, are known to have affinity for certain cell types. One notable glycan used in this technology is the Thomsen–Friedenreich disaccharide (Galbeta1-3GalNAc) that is readily detectable in 90% of all primary human carcinomas and their metastases. These glycans can be exploited for medical imaging. Encapsulating luminescent QDs with target-specific glycans permits efficient imaging of the tissue to which the glycans bind with high affinity.

Multifunctional nanoparticle probes based on semiconductor QDs have been used for cancer targeting and imaging in living animals. The structural design involves encapsulating luminescent QDs with an ABC triblock copolymer and linking this amphiphilic polymer to tumor-targeting ligands and drug delivery functionalities. In vivo targeting studies of human prostate cancer growing in nude mice indicate that the QD probes accumulate at tumors both by the enhanced permeability and retention of tumor sites and by antibody binding to cancer-specific cell surface biomarkers (Gao et al 2004). Using both subcutaneous injection of QD-tagged cancer cells and systemic injection of multifunctional QD, sensitive and multicolor fluorescence imaging of cancer cells have been achieved under in vivo conditions. These results raise new possibilities for ultrasensitive and multiplexed imaging of molecular targets in vivo.

Gold Nanorods and Nanoparticles as Imaging Agents

Gold nanorods excited at 830 nm on a far-field laser scanning microscope produced strong two-photon luminescence (TPL) intensities, and the TPL excitation spectrum can be superimposed onto the longitudinal plasmon band (Wang et al 2005a). The TPL signal from a single nanorod is 58 times that of the two-photon fluorescence signal from a single rhodamine molecule. Gold nanorods can be used as imaging agents as demonstrated by in vivo imaging of single nanorods flowing in mouse ear blood vessels.

Nanoprobes has reported that 1.9-nm gold nanoparticles may overcome many limitations to traditional x-ray contrast agents. Gold has higher x-ray absorption than iodine with less bone and tissue interference, thus achieving better contrast with lower x-ray dose. Because nanoparticles clear the blood more slowly than iodine
agents, they permit longer imaging times. In studies in mice, a 5-mm tumor growing in one thigh was clearly evident from its increased vascularity and resultant higher gold content. The gold particles thus enable direct imaging, detection, and measurement of angiogenic and hypervascularized regions. The 1.9-nm gold nanoparticles were found to clear through the kidneys: a closer examination of the kidneys revealed a remarkably detailed anatomical and functional display, with blood vessels <100 μm in diameter delineated, thus enabling in vivo vascular casting. Toxicity was also low: mice intravenously injected with the gold nanoparticles survived over 1 year without signs of illness.

**Nanoparticles Versus Microparticles for Cellular Imaging**

Typically, millions of dextran-coated ultrasmall iron oxide particles (USIOPs) must be loaded into cells for efficient detection. A recent study shows that single, micrometer-sized iron oxide particles (MSIOPs) can be detected by MRI in vitro in agarose samples, in cultured cells, and in mouse embryos (Shapiro et al 2004). Experiments studying effects of MRI resolution and particle size from indicated that significant signal effects could be detected at resolutions as low as 200 μm. Cultured cells were labeled with fluorescent MSIOPs such that single particles were present in individual cells. These single particles in single cells could be detected both by MRI and by fluorescence microscopy. Finally, single particles injected into single-cell-stage mouse embryos could be detected at later embryonic stages, demonstrating that even after many cell divisions, daughter cells still carry individual particles. These results demonstrate that MRI can detect single particles and indicate that single-particle detection will be useful for cellular imaging for certain purposes and may be preferable to nanoparticles. MSIOPs will useful in following the division of stem cells and in vivo labeling of cells.

**Intravital Vascular Imaging**

A significant impediment to the widespread use of noninvasive in vivo vascular imaging techniques is the current lack of suitable intravital imaging probes. A new strategy is the use of viral nanoparticles as a platform for the multivalent display of fluorescent dyes to image tissues deep inside living organisms (Lewis et al 2006). The bioavailable cowpea mosaic virus (CPMV) can be fluorescently labeled to high densities with no measurable quenching, resulting in exceptionally bright particles with in vivo dispersion properties that allow high-resolution intravital imaging of vascular endothelium for periods of at least 72 h. CPMV nanoparticles can be used to visualize the vasculature and blood flow in living mouse and chick embryos to a depth of up to 500 μm. Intravital visualization of human fibrosarcoma-mediated tumor angiogenesis using fluorescent CPMV provides a means to identify arterial and venous vessels and to monitor the neovascularization of the tumor microenvironment.
Study of Chromosomes by Atomic Force Microscopy

A better knowledge of biochemical and structural properties of human chromosomes is important for cytogenetic investigations and diagnostics. Fluorescence in situ hybridization (FISH) is a commonly used technique for the visualization of chromosomal details. Localizing specific gene probes by FISH combined with conventional fluorescence microscopy has reached its limit. Also, microdissecting DNA from G-banded human metaphase chromosomes either by a glass tip or by laser capture needs further improvement. Both AFM and SNOM have been used to obtain local information from G-bands and chromosomal probes (Oberringer et al 2003). The final resolution allows a more precise localization when compared with standard techniques, and the extraction of very small amounts of chromosomal DNA by the scanning probe is possible. Besides new strategies toward a better G-band and fluorescent probe detection, this method is focused on the combination of biochemical and nanomanipulation techniques, which enable both nanodissection and nanoextraction of chromosomal DNA.

Applications of Nanopore Technology for Molecular Diagnostics

Nanopore technology can distinguish between and count a variety of molecules in a complex mixture. For example, it can distinguish between hybridized or unhybridized unknown RNA and DNA molecules that differ only by a single nucleotide. Nanopore blockade can be used to measure polynucleotide length. With further improvements, the method could in principle provide direct, high-speed detection of the sequence of bases in single molecules of DNA or RNA. Biosensor elements that are capable of identifying individual DNA strands with single-base resolution have been described. Each biosensor element consists of an individual DNA oligonucleotide covalently attached within the lumen of the α-HL pore to form a “DNA nanopore.” The binding of ssDNA molecules to the tethered DNA strand causes changes in the ionic current flowing through a nanopore. On the basis of DNA duplex lifetimes, the DNA nanopores are able to discriminate between individual DNA strands up to 30 nucleotides in length differing by a single-base substitution. This is exemplified by the detection of a drug resistance-conferring mutation in the reverse transcriptase (RT) gene of HIV. In addition, the approach was used to sequence a complete codon in an individual DNA strand tethered to a nanopore. Studies on single channels reconstituted into planar lipid bilayer membranes suggest that nanometer-scale pores can be used to detect, quantitate, and characterize a wide range of analytes that includes small ions and ssDNA (Kasianowicz 2002).

Nanopore biosensors can enable direct, microsecond-time scale nucleic acid characterization without the need for amplification, chemical modification, surface adsorption, or the binding of probes. However, routine DNA analysis and sequencing will require a robust nanopore. Solid-state nanopores could be ideal, but fabrication methods need to be improved to develop an electrically addressable array of
pores with reproducible diameters in the required $10^{-9}$ m range. A simple method that enables efficient, not too hasty, electrophoretic translocation of DNA strand through the nanopore remains to be devised. This will require a better understanding of the factors that regulate polymer translocation through nanopores.

This technology can also be applied to the analysis of proteins. Scientists at the National Institute of Standards and Technology (Gaithersburgh, MD) believe that nanopore technology for biomolecules can be applied to cancer diagnosis. The speed and simplicity of this technology will facilitate the development of molecular diagnosis and its application to personalized medicine.

Eagle Research and Development (Boulder, CO) platform comprises an array of nanopores with each nanopore containing embedded semiconductors or field-effect transistors (FETs). As single molecules are driven through a nanopore by a voltage differential, the 3D charge profile of a molecule is measured by the FETs, enabling each molecule in the sample to be uniquely identified and precisely quantified. It does not require fluorescent or other labels, thermal cycling, or optics. This technology offers the prospect to eventually correlate DNA and its expressed proteins with specific disease states using an inexpensive, disposable, and portable device. For example, the device has the potential to enable the development of exquisitely targeted treatments using sequencing data both from a patient and from the disease-causing pathogen. Compared with other nanopore-based technologies for measuring molecules using electronic signals, the Eagle approach achieves a 1,000-fold higher sensitivity as a result of the FETs embedded in the nanopores. Applied Biosciences is collaborating with Eagle for development support and feasibility testing for applications in protein identification and detection of protein-binding events. Provided the ability to electronically profile the individual four nucleotides in DNA is further developed, the Eagle technology could potentially be the first to enable the identification and measurement of both DNA and proteins in a single sample at the same time. The technology could have significant implications for advancing personalized medicine based on its potential for faster, more efficient, and less expensive protein and nucleic acid identification, protein–protein and protein–small-molecule interaction measurements, and DNA sequencing.

DNA–Protein and DNA–Nanoparticle Conjugates

Semisynthetic conjugates composed of nucleic acids, proteins, and inorganic nanoparticles have been synthesized and characterized (Niemeyer 2004). For example, self-assembled oligomeric networks consisting of streptavidin and double-stranded DNA (dsDNA) are applicable as reagents in immunoassays. Covalent conjugates of ssDNA and streptavidin are utilized as biomolecular adapters for the immobilization of biotinylated macromolecules at solid substrates via nucleic acid hybridization. This “DNA-directed immobilization” enables reversible and site-selective functionalization of solid substrates with metal and semiconductor nanoparticles or, vice versa, for the DNA-directed functionalization of gold
nanoparticles with proteins, such as immunoglobulins and enzymes. This approach is applicable for the detection of chip-immobilized antigens. Moreover, covalent DNA–protein conjugates allow for their selective positioning along single-stranded nucleic acids, and thus for the construction of nanometer-scale assemblies composed of proteins and/or nanoclusters. Examples include the fabrication of functional biometallic nanostructures from gold nanoparticles and antibodies, applicable as diagnostic tools in bioanalytics.

DNA-modified nanoparticles have been used for colorimetric SNP analysis (Ihara et al 2004). These nanospheres were prepared by anchoring amino-terminated oligodeoxynucleotides (ODNs) with carboxylates onto a colored polystyrene sphere surface through amido bonds. About 220 ODN molecules were immobilized onto a nanosphere 40 nm in diameter. Preliminary studies using the microspheres with 1 μm diameter reveal that the specificity of hybridization was retained after modification. Three kinds of differently colored (RGB, red/green/blue) nanospheres bearing unique ODNs on their surface were prepared for detecting the p53 gene. The study of FRET showed that spheres R and G directly contact each other in the aggregates with the wild type. The RGB color system gave aggregates with specific colors corresponding to the added ODN samples, wild type or mutant. In addition, in the presence of both samples, all of the spheres formed aggregates with white emission as a consequence of mixing three primary colors of light. This means that the technique should enable an allele analysis.

Resonance Light Scattering Technology

Resonance light scattering (RLS) technology, developed at Genicon Sciences Corporation (now acquired by Invitrogen), offers uniquely powerful signal generation and detection capabilities applicable to a wide variety of analytical bioassay formats (Yguerabide and Yguerabide 2001). RLS exploits submicroscopic metallic particles (e.g., gold and silver) of uniform diameter (in the nanometer range), which scatter incident white light to generate monochromatic colored light that appears as highly intense fluorescence. Each RLS particle produces intense light scattering that can be viewed with the naked eye. Under low-power microscope magnification, individual 80-nm gold particles can be readily observed. The scattering produced by these particles creates a “halo” with an apparent 1 μm diameter. As a result, one can conduct ultrasensitive assays to define location and relative frequency of target molecules. RLS signal generation technology is up to 1,000,000 times more sensitive than current fluorescence signaling technology. Other advantages of RLS technology are that RLS signals do not require computer-enhanced imaging of data as they are so intense. Research applications of RLS technology are as follows:

- **Gene expression**—Relative gene expression studies on slide-based cDNA microarrays.
- **DNA sequencing**—RLS-based DNA sequencing on sequence-by-hybridization biochips.
• **Microfluidics**—RLS particles for solution-based assays in nanofluidic flow-through microarrays.

• **Immunohistology**—Rapid in situ localization/quantitation of proteins in tissue sections using RLS-coupled antibodies.

• **Homogeneous**—RLS particles for bimolecular, microvolume studies in solution.

Clinical applications of RLS technology are as follows:

• RLS technology is being used to score SNPs for discrimination of therapeutically relevant alleles.

• RLS technology provides ultrahigh-sensitivity probes for in situ hybridizations to quantitate therapeutically important DNA and RNA molecules.

• Antibody-coupled RLS particles can deliver increased sensitivity for detection of rare analytes in diagnostic assays.

• Nanoparticle-labeled bacterial RNA generates reproducible RLS signals that are at least 50 times more intense than state-of-the-art confocal-based fluorescence signals for detection of bacterial pathogens (Francois et al 2003).

**DNA Nanomachines for Molecular Diagnostics**

Manipulation of DNA has been shown to perform computational operations. Scientists at the MIT Media Laboratory and the Center for Biomedical Engineering have managed to attach a tiny radio antenna to DNA (Hamad-Schifferli et al 2002). When a radio-frequency magnetic field is transmitted to the antenna, the DNA molecule is zapped with energy and responds. The antenna is a cluster of metal, <100 atoms in size and ~1 nm long. A radio signal sent to a piece of dsDNA has been shown to unwind the two strands—a process called “dehybridization.” The switching is reversible and does not affect neighboring molecules. The technique should also work on proteins, peptides, and other large molecules. MIT licensed the technology to EngeneOS Inc (Waltham, MA, USA) in 2001, but the company is no longer in business. Applications of this technology relevant to molecular diagnostics include biomolecular detectors for homogeneous assays and direct electronic readout of biomolecular interactions.

**Nanobarcodes Technology**

Scientists at Oxonica Inc (Oxfordshire, UK) have produced submicrometer metallic barcodes with striping patterns prepared by sequential electrochemical deposition of metal ions. The differential reflectivity of adjacent stripes enables identification of the striping patterns by conventional light microscopy. This readout mechanism does not interfere with the use of fluorescence for detection of analytes bound to particles by affinity capture, as demonstrated by DNA and protein bioassays. Among other applications such as SNP mapping and multiplexed assays for proteomics,
Nanobarcodes can be used for population diagnostics and in point-of-care (POC) handheld devices. SurroMed is using this technology in developing a state-of-the-art phenotyping platform in a clinical setting with access to a large clinical population. This will enable biomarker-based drug development as a basis for personalized medicines. Key performance advantages relative to existing encoded bead technologies include the following:

- The ability to use the widely installed base of optical microscopes for readout.
- The ability to use multiple colors of fluorophores for quantitation.
- The ability to generate hundreds to thousands of unique codes that can be distinguished at high speed.

Nanobarcodes, with various submicrometer striping patterns, may be readily distinguished in an optical microscope (Walton et al 2002). Results from a library of these particles, of which over 100 different striping patterns have been produced, reveal that >70 patterns may be identified with >90% accuracy. The ability to chemically modify the surface of these particles makes them useful for bioanalytical measurements. Improvements in manufacturing and identification processes will lead to both larger numbers of striping patterns and improved identification accuracy.

**Nanobarcode Particle Technology for SNP Genotyping**

Oxonica’s nanobarcode particle technology has been used in universal array for high-throughput SNP genotyping (Sha et al 2006). The particles are encoded submicron metallic nanorods manufactured by electroplating inert metals such as gold and silver into templates and releasing the resulting striped nanoparticles. The power of this technology is that the particles are intrinsically encoded by virtue of the different reflectivity of adjacent metal stripes, enabling the generation of many thousands of unique encoded substrates. Using SNP found within the cytochrome P450 gene family, and a universal short oligonucleotide ligation strategy, simultaneous genotyping of 15 SNPs was demonstrated—a format requiring discrimination of 30 encoded NWs (one per allele). To demonstrate applicability in practice, 160 genotypes were determined from multiplex PCR products from 20 genomic DNA samples.

**Qdot Nanobarcode for Multiplexed Gene Expression Profiling**

Qdot nanobarcode-based microbead random array platform (Invitrogen) is now available for accurate and reproducible gene expression profiling in a high-throughput and multiplexed format (Eastman et al 2006). Four different sizes of Qdots, with emissions at 525, 545, 565, and 585 nm are mixed with a polymer and coated onto the magnetic microbeads (8 μm-diameter) to generate a nanobarcoded QBeads. Twelve intensity levels for each of the four colors are used. Gene-specific oligonucleotide probes are conjugated to the surface of each spectrally
nanobarcoded bead to create a multiplexed panel, and biotinylated cRNAs are generated from sample total RNA and hybridized to the gene probes on the microbeads. A fifth streptavidin Qdot (655 nm or infrared Qdot) binds to biotin on the cRNA, acting as a quantification reporter. The intensity of the 655-nm Qdot reflects the level of biotinylated cRNA captured on the beads and provides the quantification for the corresponding target gene. The system shows a level of sensitivity, which is better than that with a high-density microarray system, and approaches the level usually observed for quantitative PCR. The QBead nanobarc ode system has a dynamic range of 3.5 logs, better than the 2–3 logs observed on various microarray platforms. The hybridization reaction is performed in liquid phase and completed in 1–2 h, at least 1 order of magnitude faster than microarray-based hybridizations. Detectable fold change is lower than 1.4-fold, showing high precision even at close to single copy per cell level. Reproducibility for this proof-of-concept study approaches that of Affymetrix GeneChip microarray. In addition, it provides increased flexibility, convenience, and cost-effectiveness in comparison with conventional gene expression profiling methods.

**Biobarc ode Assay for Proteins**

Scientists in the laboratory of Prof. Chad Mirkin at the Northwestern University have developed an ultrasensitive method for detecting protein analytes (Nam et al 2003). The system relies on magnetic microparticle probes with antibodies that specifically bind a target of interest and nanoparticle probes that are encoded with DNA that is unique to the protein target of interest and antibodies. Magnetic separation of the complexed probes and target followed by dehybridization of the ODNs on the nanoparticle probe surface allows the determination of the presence of the target protein by identifying the oligonucleotide sequence released from the nanoparticle probe. Because the nanoparticle probe carries with it a large number of ODNs per protein-binding event, there is substantial amplification and prostate-specific antigen (PSA) can be detected at 30 aM concentration. Alternatively, a PCR on the oligonucleotide barcodes can boost the sensitivity to 3 aM. Comparable clinically accepted conventional assays for detecting the same target have sensitivity limits of 3 pM, 6 orders of magnitude less sensitive than what is observed with this method. Further development of this technology has resulted in a biobarc ode assay with a 500 zM target DNA sensitivity limit (Nam et al 2004). Magnetic separation and subsequent release of barcode DNA from the gold nanoparticles leads to a number of barcode DNA strands for every target DNA (Fig. 3.1).

A nanoparticle-based biobarc ode assay was used to measure the concentration of amyloid β (Aβ)-derived diffusible ligands (ADDLs) in the cerebrospinal fluid (CSF) as a biomarker for AD (Georganopoulou et al 2005). Commercial ELISAs can only detect ADDLs in brain tissue where the biomarker is most highly concentrated. Studies of ADDLs in the CSF have not been possible because of their low concentration. The biobarc ode amplification technology, which is a million times
more sensitive than ELISA, can detect ADDLs in the CSF where the biomarker is present in very low concentrations. This study is a step toward a diagnostic tool, based on soluble pathogenic markers for AD. The goal is to ultimately detect and validate the marker in blood.

Using the Verigene ID system (Nanosphere Inc, Northbrook, IL, USA), one can quantify the barcodes using the kind of technology found in a flatbed scanner, providing results as clear as an at-home pregnancy strip test. Biobarcode system is extremely sensitive for protein detection. At 30 aM, it is 5 orders of magnitude more sensitive than is ELISA (peak sensitivity of $\sim$3 pM). The system has enormous potential for multiplexing. Hypothetically, it could simultaneously test for 415 different analytes by tagging the different gold beads with different barcode sequences. However, the fundamental issues with antibodies, such as crossreactivity, nonspecific binding, and lot-to-lot variability, remain. Antibodies can distort, fall apart, or cling to the wrong analyte. Verigene® Warfarin Metabolism Nucleic Acid test (Nanosphere Inc), which detects variants of genes, responsible for sensitivity to Warfarin has been approved by the FDA.

**Single-Molecule Barcoding System for DNA Analysis**

Molecular confinement offers new routes for arraying large DNA molecules, enabling single-molecule schemes aimed at the acquisition of sequence information. Such schemes can rapidly advance to become platforms capable of genome analysis if elements of a nascent system can be integrated at an early stage of development. Integrated strategies are needed for surmounting the stringent experimental requirements of nanoscale devices regarding fabrication, sample loading, biochemical labeling, and detection. Disposable devices featuring both micro- and nanoscale features have been shown to greatly elongate DNA molecules when buffer conditions are controlled for alteration of DNA stiffness (Jo et al 2007). Analytical calculations that describe this elongation were presented. A complementary enzymatic labeling scheme was developed that tags specific sequences (barcodes) on elongated molecules within described nanoslit devices that are imaged via FRET. Collectively, these developments enable scalable molecular confinement approaches for genome analysis.
Nanomolecular Diagnostics

Nucleic acid diagnostics is dominated by fluorescence-based assays that use complex and expensive enzyme-based target or signal amplification procedures. Many clinical diagnostic applications will require simpler, inexpensive assays that can be performed in a screening mode. Scientists at Nanosphere Inc have developed a “spot-and-read” colorimetric detection method for identifying nucleic acid sequences based on the distance-dependent optical properties of gold nanoparticles without the need for conventional signal or target amplification (Storhoff et al 2004). In this assay, nucleic acid targets are recognized by DNA-modified gold probes, which undergo a color change that is visually detectable when the solutions are spotted onto an illuminated glass waveguide. They have improved the sensitivity of the spot test by developing a method that monitors scattered light rather than reflected light from 40- to 50-nm-diameter gold particles. This scatter-based method enables detection of zeptomole quantities of nucleic acid targets without target or signal amplification when coupled to an improved hybridization method that facilitates probe–target binding in a homogeneous format. In comparison with a previously reported absorbance-based method, this method increases detection sensitivity by over 4 orders of magnitude and has been applied to the rapid detection of mecA in methicillin-resistant *S. aureus* genomic DNA samples.

Nanosphere Inc launched its Verigene™ platform, an optical detection system, in 2003 for research environments. This device was later automated to enable one-step processing and the system includes sample preparation (for blood), microfluidics, and detection technologies in an integrated system, using simple disposable cartridges. The phase III system will be designed for medical professionals who do not typically use diagnostic systems.

Nanoparticle assemblies interconnected with DNA triple helices can be used to colorimetrically screen for triplex DNA-binding molecules and simultaneously determine their relative binding affinities based on melting temperatures (Han et al 2006). Nanoparticles assemble only when DNA triple helices form between DNA from two different particles and a third strand of free DNA. In addition, the triple helix structure is unstable at room temperature and only forms in the presence of triplex DNA-binding molecules that stabilize the triple helix. The resulting melting transition of the nanoparticle assembly is much sharper and at a significantly higher $T_m$ than the analogous triplex structure without nanoparticles. Upon nanoparticle assembly, a concomitant red-to-blue color change occurs. The assembly process and color change do not occur in the presence of duplex DNA binders and therefore provide a significantly better screening process for triplex DNA-binding molecules when compared with standard methods.

**SNP Genotyping with Gold Nanoparticle Probes**

Nanosphere’s ClearRead™ nanoparticle technology enables a microarray-based method for multiplex SNP genotyping in total human genomic DNA without the
Surface-Enhanced Resonant Raman Spectroscopy

need for target amplification (Bao et al 2005). This direct SNP genotyping method requires no enzymes and relies on the high sensitivity of the gold nanoparticle probes. ClearRead™ technology sandwiches a target DNA SNP segment between two oligonucleotide sequences to greatly increase detection specificity and sensitivity. One segment identifies any mutations in the DNA; the probe, a highly sensitive gold nanoparticle, creates a strong signal accurately, indicating the presence of a specific target SNP. Proof of principle, reproducibility, and the robust, simple, and rapid characteristics of this technology were demonstrated with unamplified DNA samples representing all possible forms of three genes implicated in hypercoagulation disorders. The assay format is simple, rapid, and robust pointing to its suitability for multiplex SNP profiling at the POC.

Nanoparticle-Based Up-Converting Phosphor Technology

Up-Converting Phosphor Technology (UPT™) is a proprietary label detection platform technology of OraSure Technologies Inc (Bethlehem, PA, USA) that can be applied to the detection of minute quantities of various substances such as antigens, proteins, and DNA. UPT particles are small ceramic nanospheres composed of rare earth metals and have been shown to be 1,000 times more sensitive than current fluorescent technologies. The use of OraSure’s particle-based detection provides a stronger signal for each event detected and thereby enhances sensitivity in diagnostic assay systems. UPT has potential in a broad array of DNA testing applications including drug discovery, SNP analysis, and infectious disease testing. It is possible to detect nucleic acid targets in nonamplified DNA samples using easy, inexpensive, amplification-free hybridization-based assays and the ultrasensitive UPT reporters (Zuiderwijk et al 2003). Employment of UPT allows to bypass target amplification and therefore brings genetic-based testing a step closer to the POC environment. Detection of S. pneumoniae with only 1 ng of DNA indicates a potential for applications in the field of infectious diseases.

Surface-Enhanced Resonant Raman Spectroscopy

Surface-enhanced resonant Raman spectroscopy (SERRS)-Beads (Oxonica) brings various components of the technology into a single robust nanosized polymer-bead support with broad applications in molecular and immunodiagnostics. Compounds that show strong affinity for the silver enhancing surfaces and have good spectral resolution are selected experimentally, particularly organic fluorescent dyes, because of their strong excitation cross section. Initially using four dyes, tens to hundreds of unique labels are currently under development. The chosen dyes also have excitation peaks that overlap with the metal plasmon frequency, thereby adding the all-important resonant amplification to the signal intensity.
At the core of the bead is the Raman-active substrate, where silver colloid, with defined physical characteristics, provides the surface-enhancement substrate and is combined with the dye or dyes for specific bead encoding. Control of the various parameters surrounding dye:colloid aggregate permits SERRS response to be modulated as desired.

To protect the SERRS-active complex from degradation, the aggregate is encapsulated in a polymer coating, a process that incorporates a multitude of dye:colloid particles into the same bead. This leads to highly sensitive beads with responses in excess of that achieved using the conformation of single-dye molecules on an enhancing surface.

The polymer coating is treated further with a polymer shell to allow a variety of biologically relevant probe molecules (e.g., antibodies, antigens, nucleic acids) to be attached through standard bioconjugation techniques.

Oxonica is working closely with Avalon Instruments (Boston, MA, USA) to develop its RamanSpec plate reader with the SERRS-Beads configuration. While current development is focused on heterogeneous assays in a 96-well assay sample presentation, other designs include higher plate capacities (384-well) for higher-throughput screening and microarray slide reading for DNA and proteomic analysis.

Enhancement of Raman signal near silver colloidal nanoparticles is exploited to study the Raman spectrum of yeast cytochrome c (YCc) from *Saccharomyces cerevisiae* in a single molecule (Delfino et al 2005). The investigation is performed on proteins both in solution and immobilized onto a glass slide using a quasiresonant laser line as exciting source with low excitation intensity. In both cases, spectra acquired at different times exhibit dramatic temporal fluctuations in both the total spectrum and the specific line intensity, even though averaging of several individual spectra reproduces the main Raman features of bulk YCc. Analysis of the spectral intensity fluctuations from solutions reveals a multimodal distribution of some specific Raman lines, consistent with the approaching of single-molecule regime. Among other results, the statistical analysis of the spectra from immobilized samples seems to indicate dynamical processes involving the reorientation of the heme with respect to the metal surface.

### Near-Infrared (NIR)-Emissive Polymersomes

A team of chemists, bioengineers, and medical researchers based at the University of Pennsylvania and the University of Minnesota has lodged fluorescent materials called porphyrins within the surface of a polymersome, a cell-like vesicle, to image a tumor within a living rodent (Ghoroghchian et al 2005). NIR-emissive polymersomes (50-nm- to 50-μm-diameter polymer vesicles) were generated through cooperative self-assembly of amphiphilic diblock copolymers and conjugated multi (porphyrin)-based NIR fluorophores (NIRFs). When compared with natural vesicles comprised of phospholipids, polymersomes were uniquely capable of incorporating and uniformly distributing numerous large hydrophobic NIRFs exclusively in
Nanobiosensors

Within these sequestered compartments, long polymer chains regulate the mean fluorophore–fluorophore interspatial separation as well as the fluorophore-localized electronic environment. Porphyrin-based NIRFs manifest photophysical properties within the polymersomal matrix akin to those established for these high-emission dipole strength fluorophores in organic solvents, thereby yielding uniquely emissive vesicles. Furthermore, the total fluorescence emanating from the assemblies gives rise to a localized optical signal of sufficient intensity to penetrate through the dense tumor tissue of a live animal. Robust NIR-emissive polymersomes thus define a soft-matter platform with exceptional potential to facilitate deep-tissue fluorescence-based imaging for in vivo diagnostic and drug delivery applications.

Nanobiotechnology for Detection of Proteins

Detection of proteins is an important part of molecular diagnostics. Uses of protein nanobiochips and nanobarcode technology for detection of proteins have been described in preceding sections. Other methods will be included in this section.

Captamers with Proximity Extension Assay for Proteins

Multivalent circular aptamers or “captamers” are formed through the merger of aptameric recognition functions with the DNA as a nanoscale scaffold. Aptamers are useful as protein-binding motifs for diagnostic applications, where their ease of discovery, thermal stability, and low cost make them ideal components for incorporation into targeted protein assays. Captamers are compatible with a highly sensitive protein detection method termed the “proximity extension” assay (Di Giusto et al, 2005). The circular DNA architecture facilitates the integration of multiple functional elements into a single molecule: aptameric target recognition, nucleic acid hybridization specificity, and rolling circle amplification. Successful exploitation of these properties is demonstrated for the molecular analysis of thrombin, with the assay delivering a detection limit nearly 3 orders of magnitude below the dissociation constants of the two contributing aptamer–thrombin interactions. Real-time signal amplification, detection under isothermal conditions, specificity, and sensitivity would suggest potential application as a protein assay required for the further development of personalized medicine.

Nanobiosensors

Nanosensors are devices that employ nanomaterials, exploiting novel size-dependent properties, to detect gases, chemicals, biological agents, electric fields, light, heat, or other targets. The term “nanobiosensors” implies use of nanosensors for detection of
chemical or biological materials. Nanomaterials are exquisitely sensitive chemical and biological sensors (Jain 2003b).

The sensors can be electronically gated to respond to the binding of a single molecule. Prototype sensors have demonstrated detection of nucleic acids, proteins, and ions. These sensors can operate in the liquid or gas phase, opening up an enormous variety of downstream applications. The detection schemes use inexpensive low-voltage measurement schemes and detect binding events directly, so there is no need for costly, complicated, and time-consuming labeling chemistries such as fluorescent dyes or the use of bulky and expensive optical detection systems. As a result, these sensors are inexpensive to manufacture and portable. It may even be possible to develop implantable detection and monitoring devices based on these detectors.

Some of the technologies that can be incorporated in biosensing are already covered in earlier sections. An example is nanopore technology, which can form the basis of nanosensors. Some of the biosensor devices are described in the following sections.

**Cantilevers as Biosensors for Molecular Diagnostics**

Cantilevers (Concentris) are small beams similar to those used in AFM to screen biological samples for the presence of particular genetic sequences. The surface of each cantilever is coated with DNA that can bind to one particular target sequence. On exposure of the sample to beams, the surface stress bends the beams by $\sim 10 \text{ nm}$ to indicate that the beams have found the target in the sample. This is considered biosensing.

Cantilever technology complements and extends current DNA and protein microarray methods because nanomechanical detection requires no labels, optical excitation, or external probes and is rapid, highly specific, sensitive, and portable. The nanomechanical response is sensitive to the concentration of ODNs in solution, and thus one can determine how much of a given biomolecule is present and active. In principle, cantilever arrays also could quantify gene expression levels of mRNA, protein–protein, drug-binding interactions, and other molecular recognition events in which physical steric factors are important. It can detect a single gene within a genome. Furthermore, fabricating thinner cantilevers will enhance the molecular sensitivity further, and integrating arrays into microfluidic channels will reduce the amount of sample required significantly. In contrast to SPR, cantilevers are not limited to metallic films, and other materials will be explored, e.g., cantilevers made from polymers. In addition to surface stress measurements, operating cantilevers in the dynamic mode will provide information on mass changes, and current investigations will determine the sensitivity of this approach. Currently, it is possible to monitor $> 1,000$ cantilevers simultaneously with integrated piezoresistive readout, which in principle will allow high-throughput nanomechanical genomic analysis, proteomics, biodiagnostics, and combinatorial drug discovery.
Cantilevers in an array can be functionalized with a selection of biomolecules. Researchers at IBM, Zurich, Switzerland reported the specific transduction, via surface stress changes, of DNA hybridization and receptor–ligand binding into a direct nanomechanical response of microfabricated cantilevers (Fritz et al 2000). The differential deflection of the cantilevers was found to provide a true molecular recognition signal despite large nonspecific responses of individual cantilevers. Hybridization of complementary ODNs shows that a single-base mismatch between two 12-mer ODNs is clearly detectable. Similar experiments on protein A–immunoglobulin interactions demonstrate the wide-ranging applicability of nanomechanical transduction to detect biomolecular recognition. Microarrays of cantilevers have been used to detect multiple unlabeled biomolecules simultaneously at nanomolar concentrations within minutes (McKendry et al 2002).

A specific test that uses micrometer-scale beams or “microcantilever” can detect PSA. PSA antibodies are attached to the surface of the microcantilever, which is applied to a sample containing PSA. When PSA binds to the antibodies, a change in the surface stress on the microcantilever makes it bend enough to be detected by a laser beam. This system is able to detect clinically relevant concentrations of PSA in a background of other proteins. The technique is simpler and potentially more cost-effective than other diagnostic tests because it does not require labeling and can be performed in a single reaction. It is less prone to false positives, which are commonly caused by the nonspecific binding of other proteins to the microcantilever.

Potential applications in proteomics include devices comprising many cantilevers, each coated with a different antibody, which might be used to test a sample rapidly and simultaneously for the presence of several disease-related proteins. One application is for the detection of biomarkers of myocardial infarction such as creatine kinase at POC. Other future applications include detection of disease by breath analysis, e.g., by analyzing the presence of acetone and dimethylamine (uremia). Detection of a small number of Salmonella enterica bacteria is achieved by a change in the surface stress on the silicon nitride cantilever surface in situ upon binding of bacteria (Weeks et al 2003). SEMs indicate that <25 adsorbed are required for detection.

**Antibody-Coated Nanocantilevers for Detection of Microorganisms**

Researchers at Purdue University have made a discovery about the behavior of nanocantilevers that could be crucial in designing a new class of ultrasmall sensors for detecting viruses, bacteria, and other pathogens (Gupta et al 2006). The cantilevers, coated with antibodies to detect certain viruses, attract different densities or quantity of antibodies per area depending on the size of the cantilever. The devices are immersed into a liquid containing the antibodies to allow the proteins to stick to the cantilever surface. Instead of simply attracting more antibodies, the longer cantilevers also contained a greater density of antibodies. The density is greater toward the free end of the cantilevers. The cantilevers vibrate faster after the antibody attachment if the devices have about the same nanometer-range thickness (∼20 nm) as the protein layer. Moreover, the longer the protein-coated nanocantilever, the
faster the vibration, which could only be explained if the density of antibodies were to increase with increasing lengths.

The cantilever’s vibration frequency can be measured using an instrument called a laser Doppler vibrometer, which detects changes in the cantilever’s velocity as it vibrates. This work may have broad impact on microscale and nanoscale biosensor design, especially when predicting the characteristics of bionanoelectromechanical sensors functionalized with biological capture molecules. The nanocantilevers could be used in future detectors because they vibrate at different frequencies when contaminants stick to them, revealing the presence of dangerous substances. Because of the nanocantilever’s minute size, it is more sensitive than larger devices, promising the development of advanced sensors that detect minute quantities of a contaminant to provide an early warning that a dangerous pathogen is present.

Advantages of Cantilever Technology for Molecular Recognition

Cantilever technology has the following advantages over conventional molecular diagnostics:

- It circumvents the use of PCR.
- For DNA, it has physiological sensitivity and no labeling is required.
- In proteomics, it enables detection of multiple proteins and direct observation of proteins in diseases such as those involving the cardiovascular system.
- It enables the combination of genomics and proteomics assays.
- It is compatible with silicon technology.
- It can be integrated into microfluidic devices.

Cantilevers for Direct Detection of Active Genes

Researchers from the National Centre of Competence in Research at the Swiss Nanoscience Institute (Basel, Switzerland), in collaboration with Roche scientists, have developed an innovative method for the rapid and sensitive detection of disease- and treatment-relevant genes (Zhang et al 2006a). The new method detects active genes directly by measuring their transcripts (mRNA), which represent the intermediate step and link to protein synthesis. Short complementary nucleic acid segments (sensors) are attached to silicon cantilevers which are 450 nm thick and therefore react with extraordinary sensitivity. Binding of the targeted gene transcript to its matching counterpart on one of the cantilevers results in optically measurable mechanical bending.

Differential gene expression of the gene 1-8U, a potential marker for cancer progression or viral infections, could be observed in a complex background. The measurements provide results within minutes at the picomolar level without target amplification and are sensitive to base mismatches. An array of different gene transcripts can even be measured in parallel by aligning appropriately coated cantilevers alongside each other like the teeth of a comb. The new method complements current molecular diagnostic techniques such as the gene chip and real-time PCR. It could
be used as a real-time sensor for continuously monitoring various clinical parameters or for detecting rapidly replicating pathogens that require prompt diagnosis. These findings qualify the technology as a rapid method to validate biomarkers that reveal disease risk, disease progression, or therapy response. Cantilever arrays have potential as a tool to evaluate treatment–response efficacy for personalized medical diagnostics.

**Portable Nanocantilever System for Diagnosis**

BioFinger, a portable nano detection tool being funded by the EU, could be used as a cheap and fast method in the diagnosis of diseases such as cancer. It could also be used for chemical and food analysis. The BioFinger project is being funded by the European Commission’s Information Society Technology Center. The machine detects and analyzes molecules in fluids using nano- and microcantilevers. During trials at Cork University Hospital in Ireland, the system will be used to detect a protein associated with prostate cancer, while the nanocantilever system, which can detect a single molecule, will be used to test blood samples for interleukin-6, a protein associated with inflammation. BioFinger incorporates the cantilevers on a disposable microchip, allowing it to be reconfigured with new on-chip cantilevers to detect different substances. The analysis, which can be performed anywhere, anytime, takes between 15 and 20 min. The system is likely to be considerably cheaper than traditional diagnosis techniques with each disposable chip expected to cost ∼€8 ($10). It is extremely versatile and could be used to detect virtually any disease, as a pregnancy test, or even to determine blood types. Outside of the medical field, it could be used to analyze chemicals, detect bacteria in food, or test for water pollution. The new system, now in final development stages, is undergoing field testing and is expected to be in the market within 2–3 years.

**Carbon Nanotube Biosensors**

Over the years, researchers have sought to tailor carbon nanotubes to detect chemicals ranging from small gas molecules to large biomolecules. The tubes’ small size and unique electronic properties make them especially adept at detecting minute changes in the environment. A new type of optical nanosensor uses SWNTs that modulate their emission in response to the adsorption of specific biomolecules (Barone et al 2005). It has two distinct mechanisms of signal transduction: fluorescence quenching and charge transfer. The nanotube-based chemical sensors developed so far generate an electric signal in the presence of a particular molecule. The basic design is widely applicable for such analytical tasks as detecting genes and proteins associated with diseases.

To test the feasibility of implanting the sensors in the body, oxidase- and ferricyanide-coated nanotubes were placed inside a sealed glass tube 1 cm long and 200 μm thick. The tube is riddled with pores large enough to let glucose enter but small enough to keep the nanotubes inside. The tube was then implanted in a sample
of human skin, and the sensor could be excited with infrared light and it could detect its fluorescence.

Carbon Nanotube Sensors Coated with ssDNA and Electronic Readout

A new class of nanoscale chemical sensors are based on ssDNA as the chemical recognition site and single-walled carbon nanotube FETs (SWNT-FETs) as the electronic readout component (Staïi et al 2005). SWNT-FETs with a nanoscale coating of ssDNA respond to gas odors that do not cause a detectable conductivity change in bare devices. Responses of ssDNA/SWNT-FETs differ in sign and magnitude for different gases and can be tuned by choosing the base sequence of the ssDNA. ssDNA/SWNT-FET sensors detect a variety of odors, with rapid response and recovery times on the scale of seconds. The arrays of nanosensors could detect molecules on the order of 1 ppm. The sensor surface is self-regenerating: samples maintain a constant response with no need for sensor refreshing through at least 50 gas exposure cycles. The nanosensors could sniff molecules in the air or taste them in a liquid. This remarkable set of attributes makes sensors based on ssDNA-decorated nanotubes promising for “electronic nose” and “electronic tongue” applications ranging from homeland security to disease diagnosis.

Carbon Nanotube Sensors Wrapped with DNA and Optical Detection

SWNTs wrapped with DNA can be placed inside living cells and detect trace amounts of harmful contaminants using NIR light (Heller et al 2006). The sensor is constructed by wrapping the dsDNA around the surface of an SWNT, in much the same fashion as a telephone cord wraps around a pencil. The DNA starts out wrapping around the nanotube with a certain shape that is defined by the negative charges along its backbone. Subtle rearrangement of an adsorbed biomolecule can be directly detected by such a carbon nanotube. At the heart of the new detection system is the transition of DNA secondary structure from the native, right-handed “B” form to the alternate, left-handed “Z” form. The thermodynamics that drive the switching back and forth between these two forms of DNA structure would modulate the electronic structure and optical emission of the carbon nanotube. When the DNA is exposed to ions of certain atoms such as calcium or mercury, the negative charges become neutralized and the DNA changes shape in a similar manner to its natural shape-shift from the B to Z form. This reduces the surface area covered by the DNA, perturbing the electronic structure and shifting the nanotube’s natural, NIR fluorescence to a lower energy. The change in emission energy indicates how many ions bind to the DNA. Removing the ions will return the emission energy to its initial value and flip the DNA back to the starting form, making the process reversible and reusable. The viability of this measurement technique was demonstrated by detecting low concentrations of mercury ions in whole blood, opaque solutions, and living mammalian cells and tissues where optical sensing is usually poor or ineffective. Because the signal is in the NIR, a property unique to only a handful of materials, it is not obscured by the natural fluorescence of polymers and
living tissues. The nanotube surface acts as the sensor by detecting the shape change of the DNA as it responds to the presence of target ions. This discovery opens the door to new types of optical sensors and biomarkers that exploit the unique properties of nanoparticles in living systems.

**FRET-Based DNA Nanosensors**

Rapid and highly sensitive detection of DNA is critical in diagnosing genetic diseases. Conventional approaches often rely on cumbersome, semiquantitative amplification of target DNA to improve detection sensitivity. In addition, most DNA detection systems (e.g., microarrays), regardless of their need for target amplification, require separation of unhybridized DNA strands from hybridized stands immobilized on a solid substrate and are thereby complicated by solution–surface binding kinetics. An ultrasensitive nanosensor is based on FRET capable of detecting low concentrations of DNA in a separation-free format. This system uses QDs linked to DNA probes to capture DNA targets (Zhang et al 2005b). The target strand binds to a dye-labeled reporter strand, thus forming a FRET donor–acceptor ensemble. The QD also functions as a concentrator that amplifies the target signal by confining several targets in a nanoscale domain. Unbound nanosensors produce near-zero background fluorescence, but on binding to even a small amount of target DNA (∼50 copies or less), they generate a very distinct FRET signal. A nanosensor-based oligonucleotide ligation assay has been demonstrated to successfully detect a point mutation typical of some ovarian tumors in clinical samples.

**Ion Channel Switch Biosensor Technology**

The Ion Channel Switch (ICS™), developed by Ambri Ltd (Chatswood, Australia), is a novel biosensor technology based on a synthetic self-assembling membrane. The membrane acts like a biological switch and is capable of detecting the presence of specific molecules, and signaling their presence by triggering an electrical current (Cornell 2002). It has the ability to detect a change in ion flow upon binding with the target molecule, resulting in a more rapid result than that currently achievable using existing technologies. The Ambri ICS™ biosensor is one of the first true nanobiosensor devices and is the basis of SensiDx™ System that has been designed for POC testing in critical care environments in hospitals. By delivering precise, quantitative test results in an immediate timeframe, the SensiDx™ System may assist in reducing the time of emergency diagnoses from hours down to minutes. This has a positive impact on both clinical decision-making and treatment costs.

**Electronic Nanobiosensors**

The Biodetect™ system (Integrated Nano-Technologies, Henrietta, NY, USA) works by electronically detecting the binding of a target DNA molecule to sensors on a
microchip. The target molecules form a bridge between two electrically separated wires. In order to create a strong clear signal, the bound target molecules are chemically developed to form conductive DNA wires. These DNA wires “turn on” a sensor much like an on/off switch. Each chip contains multiple sensors, which can be independently addressed with capture probes for different target DNA molecules from the same or different organisms. Each sensor has hundreds of interdigitated wires that are electrically separated from its neighbors. A proprietary DNA Lithography™ process is used to attach capture probes to each of the sensors on the chip. These chips now have billions of capture probes per sensor, which greatly improves sensitivity. To form detectable DNA wires, target DNA molecules first must form a DNA bridge spanning the gap between the sensor wires. DNA bridge formation has been observed by fluorescent imaging techniques. The final step in the detection process is to metalize the DNA bridge to form a DNA wire. Various metalization chemistries have been developed, which enable metalization of the DNA bridges with very low levels of background deposition. After metalization, bridges can be readily detected by measuring the resistance or other electrical properties of the sensor. DNA wires can be seen using electron microscopy. This portable system is used for rapid detection of microorganisms. This technology will also form the basis of site-specific drug delivery and high-resolution image arrays using nanoscale electronic components.

A signal-on, electronic DNA biosensor has been described that is label-free and achieves a subpicomolar detection limit (Xiao et al 2006). The sensor, which is based on a target-induced strand displacement mechanism, is composed of a “capture probe” attached by its 5′ terminus to a gold electrode and a 5′ methylene blue-modified “signaling probe” that is complementary at both its 3′ and 5′ termini to the capture probe. In the absence of target, hybridization between the capture and signaling probes minimizes contact between the methylene blue and electrode surface, limiting the observed redox current. Target hybridization displaces the 5′ end of the signaling probe, generating a short, flexible ssDNA element and producing up to a 7-fold increase in redox current. The observed signal gain is sufficient to achieve a demonstrated (not extrapolated) detection limit of 400 fM, which is among the best reported for single-step electronic DNA detection. Moreover, because sensor fabrication is straightforward, the approach appears to provide a ready alternative to the more cumbersome femtomolar electrochemical assays described to date.

Capacitators are critical elements in electrical circuits and nanocapacitors are capacitors with electrodes spacing in the nano order. When used with ssDNA probes, target hybridization produces a measurable change in capacitance. When used in arrays, nanocapacitors can enable simultaneous detection of nucleic acids without labeling (Fortina et al 2005).

**Electrochemical Nanobiosensors**

An electrochemical biosensor combining microfluidics and nanotechnology has been developed by GeneFluidics (Monterey Park, CA, USA) with 16 sensors in
the array, each consisting of three single-layer gold electrodes: working, reference, and auxiliary. Each of the working electrodes contains one representative from a library of capture probes, which are specific for a clinically relevant bacterial urinary pathogen. The library included probes for *Escherichia coli*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Enterococcus* spp., and the *Klebsiella–Enterobacter* group. A bacterial 16S rRNA target derived from single-step bacterial lysis was hybridized both to the biotin-modified capture probe on the sensor surface and to a second, fluorescein-modified detector probe. Detection of the probe–target hybrids is achieved through binding of a horseradish peroxidase (HRP)-conjugated antifluorescein antibody to the detector probe. Amperometric measurement of the catalyzed HRP reaction is obtained at a fixed potential of –200 mV between the working and reference electrodes. Species-specific detection of as few as 2,600 pathogenic bacteria in culture, inoculated urine, and clinical urine samples can be achieved within 45 min from the beginning of sample processing. In a feasibility study of this amperometric detection system using blinded clinical urine specimens, the sensor array had 100% sensitivity for direct detection of gram-negative bacteria without nucleic acid purification or amplification (Liao et al 2006). Identification was demonstrated for 98% of gram-negative bacteria for which species-specific probes were available. When combined with a microfluidics-based sample preparation module, the integrated system could serve as a POC device for rapid diagnosis of urinary tract infections.

**Quartz Nanobalance Biosensors**

ssDNA-containing thin films are deposited onto quartz oscillators to construct a device capable of sensing the presence of the cDNA sequences, which hybridize with the immobilized ones. DNA, once complexed with aliphatic amines, appears as a monolayer in a single-stranded form by small angle x-ray scattering. A quartz nanobalance is then utilized to monitor mass increment related to specific hybridization with a cDNA probe. The crystal quartz nanobalance, capable of high sensitivity, indeed appears capable of obtaining a prototype of a device capable of sensing the occurrence of particular genes or sequences in the sample under investigation.

**Viral Nanosensors**

Virus particles are essentially biological nanoparticles. Scientists at the Massachusetts General Hospital (Boston, MA) have used herpes simplex virus (HSV) and adenovirus to trigger the assembly of magnetic nanobeads as a nanosensor for clinically relevant viruses (Perez et al 2003). The nanobeads had a superparamagnetic iron oxide core coated with dextran. Protein G was attached as a binding partner for antivirus antibodies. By conjugating anti-HSV antibodies directly to nanobeads using a bifunctional linker to avoid nonspecific interactions between medium components and protein G and using a magnetic field, the scientists
could detect as few as five viral particles in a 10-ml serum sample. This system is more sensitive than ELISA-based methods and is an improvement over PCR-based detection because it is cheaper, faster, and has fewer artifacts. Upon target binding, these nanosensors cause changes in the spin-spin relaxation times of neighboring water molecules, which can detect specific mRNA, proteins, and enzymatic activity by (NMR/MRI) techniques (Perez et al 2004).

**PEBBLE Nanosensors**

Scientists at the University of Michigan (Ann Arbor, MI) are developing PEBBLE (Probes Encapsulated by Biologically Localized Embedding) nanosensors, which consist of sensor molecules entrapped in a chemically inert matrix by a microemulsion polymerization process that produces spherical sensors in the size range of 20–200 nm (Sumner et al 2002). These sensors are capable of real-time inter- and intracellular imaging of ions and molecules and are insensitive to interference from proteins. PEBBLE can also be used for early detection of cancer.

PEBBLE nanosensors also show very good reversibility and stability to leaching and photobleaching, as well as very short response times and no perturbation by proteins. In human plasma they demonstrate a robust oxygen sensing capability, little affected by light scattering and autofluorescence (Cao et al 2004). PEBBLE has been developed further as a tool for diagnosis as well as treatment of cancer.

**Microneedle-Mounted Biosensors**

NanoSense (NanoPass Technologies Ltd, Haifa, Israel) is a MicroPyramid™ chip that is integrated with biosensors for nanoliter-scale ion diagnostics for congestive heart failure and renal failure. This technology will be integrated with microneedles to provide reliable, inexpensive, and simple to operate transdermal device for ion diagnoses, in POC settings. This work is conducted in collaboration with MESA (Micro Electronics, Materials Engineering, Sensors and Actuators) Laboratories at the University of Twente, the Netherlands (http://www.mesaplus.utwente.nl/).

**Optical Biosensors**

Many biosensors that are currently marketed rely on the optical properties of lasers to monitor and quantify interactions of biomolecules that occur on specially derived surfaces or biochips. An integrated biosensor, based on phototransistor integrated circuits, has been developed for use in medical detection, DNA diagnostics, and gene mapping. The biochip device has sensors, amplifiers, discriminators, and logic circuitry on board. Integration of light-emitting diodes into the device is also possible. Measurements of fluorescent-labeled DNA probe microarrays and hybridization experiments with a sequence-specific DNA probe for HIV-1 on nitrocellulose substrates illustrate the usefulness and potential of this DNA biochip. A number
of variations of optical biosensors offer distinct methods of sample application and detection in addition to different types of sensor surface. SPR technology is the best-known example of this technology.

**Laser Nanosensors**

In a laser nanosensor, laser light is launched into the fiber, and the resulting evanescent field at the tip of the fiber is used to excite target molecules bound to the antibody molecules. A photometric detection system is used to detect the optical signal (e.g., fluorescence) originating from the analyte molecules or from the analyte–bioreceptor reaction (Vo-Dinh 2005). Laser nanosensors can be used for in vivo analysis of proteins and biomarkers in individual living cells.

Physicists at the University of Rochester have assembled a simple laser system to detect nanoparticles. They split a laser beam in two, sending one half to a sample. When the light hits a small particle, it is scattered back and recombines with the reserve half of the laser beam, producing a detectable interference pattern detectable only when a moving particle is present. This laser method works where others do not because it relies on the amplitude rather than the intensity of light. The amplitude is the square root of intensity, so it decays much less than intensity as the particles get smaller. Single particles as small as 7 nm in diameter have been detected.

Researchers at the University of Twente have developed an ultrasensitive sensor that could potentially be used in a handheld device to detect various viruses and measure their concentration within minutes. It requires only a tiny sample of saliva, blood, or other body fluid. The device uses a silicon substrate containing channels that guide laser light. Light enters into the substrate at one end and is split into four parallel beams. When these beams emerge at the other end, they spread out and overlap with one another, creating a pattern of bright and dark bands, known as an interference pattern, which are recorded. A commercial version of the biosensor is being developed in collaboration with Paradocs Group BV (The Netherlands). Although the sensor has been shown to detect only the HSV virus, it could be used to quickly screen people at hospitals and emergency clinics for control of outbreaks of diseases such as severe acute respiratory syndrome (SARS) and avian flu.

**Nanoshell Biosensors**

Nanoshells can enhance chemical sensing by as much as 10 billion times. That makes them ~10,000 times more effective at Raman scattering than traditional methods. When molecules and materials scatter light, a small fraction of the light interacts in such a way that it allows scientists to determine their detailed chemical makeup. This property, known as Raman scattering, is used by medical researchers, drug designers, chemists, and other scientists to determine what materials are made of. An enormous limitation in the use of Raman scattering has been its extremely weak sensitivity. Nanoshells can provide large, clean, reproducible enhancements of this effect, opening the door for new, all-optical sensing applications.
Scientists at the Rice University’s Laboratory of Nanophotonics have found that nanoshells are extremely effective at magnifying the Raman signature of molecules, each individual nanoshell acting as an independent Raman enhancer. That creates an opportunity to design all-optical nanoscale sensors—essentially new molecular-level diagnostic instruments—that could detect as little as a few molecules of a target substance, which could be anything from a drug molecule or a key disease protein to a deadly chemical agent.

The metal cover of the nanoshell captures passing light and focuses it, a property that directly leads to the enormous Raman enhancements observed. Furthermore, nanoshells can be tuned to interact with specific wavelengths of light by varying the thickness of their shells. This tunability allows for the Raman enhancements to be optimized for specific wavelengths of light. The finding that individual nanoshells can vastly enhance the Raman effect opens the door for biosensor designs that use a single nanoshell, something that could prove useful for engineers who are trying to probe the chemical processes within small structures such as individual cells, or for the detection of very small amounts of a material, like a few molecules of a deadly biological or chemical agent. Nanoshells are already being developed for applications including cancer diagnosis, cancer therapy, testing for proteins associated with AD, drug delivery, and rapid whole-blood immunoassays.

**Plasmonics and SERS Nanoprobes**

Surface plasmons are collective oscillations of free electrons at metallic surfaces. These oscillations can give rise to the intense colors of solutions of plasmon resonance nanoparticles and/or very intense scattering. While the use of plasmonic particle absorption based bioaffinity sensing is now widespread throughout biological research, the use of their scattering properties is relatively less studied. Plasmon scatter can be used for long-range immunosensing and macromolecular conformation studies (Aslan et al 2005).

A variety of sensors, metallic nanostructured probes, metallic nanoshells and half-shells, and nanoarrays for SERS sensing have been developed at the Oak Ridge National Laboratory. The SERS technology can detect the chemical agents and biological species (e.g., spores, biomarkers of pathogenic agents) directly. A DNA-based technique based on surface-enhanced Raman gene (SERGen) probes can be also used to detect gene targets via hybridization to DNA sequences complementary to these probes. Advanced instrumental systems designed for spectral measurements and for multiarray imaging as well as for field monitoring (RAMiTS technology) have been constructed. Plasmonics and SERS nanoprobes are useful for biological sensing.

**Novel Optical mRNA Biosensors**

Drs. Phillipe Haas and A. Wild of the NCCR Nanoscale Science (Basel, Switzerland) have developed a novel optical mRNA biosensor for application in pathology. The
scheme of this biosensor is shown in Fig. 3.2. Molecular beacons that are highly sequence specific are used as molecular switches. This biosensor detects single molecules in fluids and can be used to search for molecular markers to predict the prognosis of disease.

**Optonanogen Biosensors**

Currently available commercial biosensing systems are large and designed to be used in laboratories. The Optonanogen program, coordinated by Centro Nacional de Microelectronica (CNM, Madrid, Spain), aims to apply the new micro- and nanotechnologies to DNA array production and analysis to develop a fully integrated biosensor system on a small scale. The aim is to achieve both miniaturization of the biochip format and an increase in the sensitivity of the assays performed. A prototype of the system will initially be used to detect mutations of the BRCA1 gene that are responsible for breast cancer in a small percentage of women. The final system, however, could be used to detect virtually any genetic anomaly as well as proteins linked to viruses, chemical contamination in food, or water pollution. The final device will be roughly the size of a human hand, allowing it to be used in physician’s offices to determine the genetic predisposition of a patient to certain diseases in a matter of minutes. That compares to the hours or even days it can take to carry out the same analysis in a laboratory, which is generally only used to test high-risk groups such as women with a family history of breast cancer.
To detect genetic mutations, the Optonanogen system uses an array of 20 microcantilevers coated in nucleic acid that react when they come into contact with a DNA sample displaying the genetic anomaly. The sample is injected into the device via a microfluidic header and the deflection of the cantilevers—by as little as 0.1–0.5 nm—is picked up by a photodetector array based on the reflection of light off the cantilevers from VCSELs. The cantilever array and microfluidic header are low-cost components that would be disposable if used for medical analysis but which could be cleaned and reused for other applications. After evaluation trials later in 2005, a commercial version of the system is likely to be produced within 1 or 2 years by Sensia, a spin-off company from the CNM.

**Surface Plasmon Resonance Technology**

SPR is an optical–electrical phenomenon involving the interaction of light with the electrons of a metal. The optical–electronic basis of SPR is the transfer of the energy carried by photons of light to a group of electrons (a plasmon) at the surface of a metal. In Biacore systems, Uppsala, Sweden (part of GE Healthcare), SPR arises when light is reflected under certain conditions from a conducting film at the interface between two media of different refractive index. The media are the sample and the glass of the sensor chip, and the conducting film is a thin layer of gold on the chip surface. SPR causes a reduction in the intensity of reflected light at a specific angle of reflection (the SPR angle). When molecules in the sample bind to the sensor surface, the concentration and therefore the refractive index at the surface changes and an SPR response is detected. Plotting the response against time during the course of an interaction provides a quantitative measure of the progress of the interaction. This plot is called a sensorgram.

HTS Biosystems (Hopkinton, MA, USA) and Applied Biosystems Group (Foster City, CA, USA) are now codeveloping a next-generation microarray-based SPR system that is designed to help researchers profile and characterize biomolecular interactions in a parallel format. Applied Biosystems has introduced SPR in its 8500 Affinity Chip Analyzer. This instrument cannot match Biacore’s variety of chip surface chemistries—Biacore offers nine different surfaces to Applied Biosystems’ three—the new system targets the drug discovery market with its high-throughput format. The key strength of the system is that it can measure binding to all the different ligands under exactly the same conditions. The 8500 Affinity Chip Analyzer can simultaneously examine up to 400 binding interactions on a single chip during a 2-h run and can measure binding constants in the micromolar to picomolar range; the minimum analyte size for kinetic measurements is 8 kDa.

Miniature optical sensors that specifically identify low concentrations of environmental and biological substances are in high demand. Currently, there is no optical sensor that provides identification of the aforementioned species without amplification techniques at naturally occurring concentrations. Triangular silver nanoparticles have remarkable optical properties, and their enhanced sensitivity to their nanoenvironment has been used to develop a new class of optical sensors using localized SPR spectroscopy (Haes and Duyne 2004).
Surface-Enhanced Micro-Optical Fluidic Systems

The aim of the surface-enhanced micro-optical fluidic systems (SEMOFS) Europeans project is to develop a new concept for biosensors: a polymer-based card-type integrated “plasmon-enhanced SPR” sensor. The card will combine biologically active surfaces with integrated optics (light source, detection) and bio-compatible multichannel microfluidics. The project aims to achieve a significant breakthrough, since all functions will be totally integrated on a single polymer-based chip. The final product shall be manufactured with large-scale, mass production techniques. The card will therefore be extremely low cost and disposable while providing increased sensitivity and diagnosis possibilities. The project will focus on the following:

- Increasing detection sensitivity and access to new information of the biological sample
- Microfluidics on polymer substrate enabling multichanneling (further enhancing sensitivity by parallel analysis) and integrated fluid actuators
- Integrated optical detection concept based on organic light emitting display (OLED)/waveguide/miniatuorized spectrometer enabling card-type integrated solution and multichanneling
- Hybrid micromachining to ensure compatibility of the mastering and replication protocols with constraints of industrial-scale manufacturing
- Validation of expected applications and evaluation in clinical cancer diagnosis

Nanowire (NW) Biosensors

Since their surface properties are easily modified, NWs can be decorated with virtually any potential chemical or biological molecular recognition unit, making the wires themselves independent of the analyte. The nanomaterials transduce the chemical binding event on their surface into a change in conductance of the NW in an extremely sensitive, real-time, and quantitative fashion. Boron-doped SiNWs have been used to create highly sensitive, real-time electrically based sensors for biological and chemical species. Biotin-modified SiNWs were used to detect streptavidin down to at least a picomolar concentration range. The small size and capability of these semiconductor NWs for sensitive, label-free, real-time detection of a wide range of chemical and biological species could be exploited in array-based screening and in vivo diagnostics.

A novel approach to synthesizing NWs allows their direct integration with microelectronic systems for the first time, as well as their ability to act as highly sensitive biomolecule detectors that could revolutionize biological diagnostic applications. An interdisciplinary team of engineers in Yale University’s Institute for Nanoscience and Quantum Engineering has overcome hurdles in NW synthesis by using a tried-and-true process of wet-etch lithography on commercially available silicon-on-insulator wafers. These NWs are structurally stable and demonstrate an
unprecedented sensitivity as sensors for detection of antibodies and other biologically important molecules. According to researchers, not only can the NWs detect extremely minute concentrations (as few as 1,000 individual molecules in a cubic millimeter), they can do it without the hazard or inconvenience of any added fluorescent or radioactive detection probes. The study demonstrated ability of the NWs to monitor antibody binding, and to sense real-time live cellular immune response using T-lymphocyte activation as a model. Within \( \sim 10 \) s, the NW could register T-cell activation as the release acid to the device. The basis for the sensors is the detection of hydrogen ions or acidity, within the physiological range of reactions in the body. Traditional assays for the detection of immune system cells such as T cells or for antibodies usually take hours to complete.

When biological molecules bind to their receptors on the NW, they usually alter the current moving through the sensor and signal the presence of the substance of interest. This direct detection dispenses with the time-consuming labeling chemistry and speeds up the detection process considerably. NW biosensors are used for the detection of proteins, viruses, or DNA in a highly sensitive manner. They can be devised to test for a complex of proteins associated with a particular cancer and used for diagnosis as well as monitoring the progress of treatment.

**Nanowires for Detection of Genetic Disorders**

The surfaces of the SiNW devices have been modified with peptide nucleic acid (PNA) receptors designed to recognize wild type versus the F508 mutation site in the CF transmembrane receptor gene (Hahm and Lieber 2004). Conductance measurements made while sequentially introducing wild-type or mutant DNA samples exhibit a time-dependent conductance increase consistent with the PNA–DNA hybridization and enabled identification of fully complementary versus mismatched DNA samples. Concentration-dependent measurements show that detection can be carried out to at least the tens of femtomolar range. It provides more rapid results than do current methods of DNA detection. This NW-based approach represents a step forward for direct, label-free DNA detection with extreme sensitivity and good selectivity and could provide a pathway to integrated, high-throughput, multiplexed DNA detection for genetic screening.

**NW Biosensors for Detecting Biowarfare Agents**

Researchers at the Lawrence Livermore National Laboratory have developed a multi-stripped biosensing NW system for detecting biowarfare agents in the field (Tok et al 2006). It is constructed from submicrometer layers of different metals including gold, silver, and nickel that act as “barcodes” for detecting a variety of pathogens ranging from anthrax, smallpox, and ricin to botulinum. Antibodies of specific pathogens are attached to the NWs producing a small, reliable, sensitive detection system. The system could also be used during an outbreak of an infectious disease.
Concluding Remarks and Future Prospects of NW Biosensors

A review has shown that NW biosensors modified with specific SURFACE receptors represent a powerful nanotechnology-enabled diagnostic/detection platform for medicine and the life sciences (Patolsky et al 2006). Key features of these devices include direct, label-free, and real-time electrical signal transduction, ultrahigh sensitivity, exquisite selectivity, and potential for integration of addressable arrays on a massive scale, which set them apart from other sensor technologies that are currently available. NW biosensors have unique capabilities for multiplexed real-time detection of proteins, single viruses, DNA, enzymatic processes, and small organic molecule binding to proteins. Apart from their value as research tools, they have a significant impact on disease diagnosis, genetic screening, and drug discovery. They will facilitate the development of personalized medicine. Because these NW sensors transduce chemical/biological binding events into electronic/digital signals, they have the potential for a highly sophisticated interface between nanoelectronic and biological information processing systems in the future.

Nanoscale Erasable Biodetectors

Scientists at the Duke University’s Pratt School of Engineering are designing biodetectors and structures scaled in nanoscale. The proposed erasable detectors are made of artificial elastin-like polypeptides (ELPs), which are short segments of proteins normally soluble in water. Crafted through genetic engineering with the aid of bacteria, such ELPs have the useful property of coming out of a solution to form a solid whenever a slight temperature increase or other alterations to the water induce a phase change. An ELP could also be chemically linked with another protein so that both “fusion proteins” leave solution together after such phase changes. This method could be used to create a “reversible” protein sensor on a glass slide. After dotting such a slide with microscopic amounts of surface-bound ELPs, the researchers discovered that dissolved fusion proteins would selectively attach to those microdots upon leaving the solution. They also found the “captured” fusion proteins could pull other select proteins from solution, so those could be chemically identified. Finally, they confirmed that microdot array could then be wiped clean of all attached proteins simply by “reversing the phase transition.” In this case, the researchers added salt to the solution to induce the same kind of phase changes as does raising the water temperature. It is possible to create a surface for a sensor, do a binding reaction, detect a signal, then release everything. Then the same process can be repeated with the same fusion protein. An AFM is used that can deposit nanoscale amounts of material through a DPN. Instead of using a glass slide, a gold surface was fabricated on which to bind ELP nanodots because DPN works well on gold. A major reason for their improved success is that the gold surface was specially modified to prevent stray proteins from attaching to the experimental array. The Duke Group has also built a “nonfouling” platform by inducing methyl methacrylate molecules to grow into tall stalks from a gold surface through a self-assembly process known
as “atom transfer radical polymerization.” In the same process, molecules of PEG were also induced to form fuzzy branches extending from those stalks, creating the overall look of bottle brushes. In this case, the PEG branches formed a protective barrier that kept unwanted proteins from coming out of solution and sticking to the platform. The scientists are now exploring a method to build nanotowers of DNA block by block from the surface. They have described how the enzyme terminal deoxynucleotidyl transferase (TdTase) could be used to induce short DNA strands to form extensive chains. Those “polymerizing” chains, growing vertically from nanodots of gold patterned onto silicon, assembled into tower-like structures (Chow et al. 2005). The process worked in a solution of enzyme and DNA building blocks, called nucleotides, with the TdTase grabbing floating nucleotides and pulling those into the extending structure. TdTase-catalyzed surface-initiated polymerization of DNA is expected to be a useful tool for the fabrication of complex biomolecular structures with nanoscale resolution.

**Future Issues in the Development of Nanobiosensors**

New biosensors and biosensor arrays are being developed using new materials, nanomaterials, and microfabricated materials including new methods of patterning. Biosensor components will use nanofabrication technologies. Use of nanotubes, Buckminsterfullerenes (buckyballs), silica, and its derivatives can produce nanosized devices. Some of the challenges are listed below:

- Development of real-time noninvasive technologies that can be applied to the detection and quantitation of biological fluids without the need for multiple calibrations using clinical samples.
- Development of biosensors utilizing new technologies that offer improved sensitivity for detection with high specificity at the single-molecule level.
- Development of biosensor arrays that can successfully detect, quantify, and quickly identify individual components of mixed gases and liquid in an industrial environment.

It would be desirable to develop multiple integrated biosensor systems that utilize doped oxides, polymers, enzymes, or other components to give the system the required specificity. A system with all the sensor components, software, plumbing, reagents, and sample processing is an example of an integrated sensor. There is also a need for reliable fluid handling systems for “dirty” fluids and for relatively small quantities of fluids (nanoliter to attoliter quantities). These should be low cost, disposable, reliable, and easy to use as part of an integrated sensor system. Sensing in picoliter to attoliter volumes might create new problems in the development of microreactors for sensing and novel phenomenon in very small channels.

The University of Ulster (UU, Jordanstown, UK) has extensive experience and expertise in the design, fabrication, and characterization of a wide range of flexible electrode/substrate systems suitable for both 20 mA stimulation/sensing of nerve
bundles and in vivo biosensing. Requirements for such a thin-film sensing device include high substrate/metal adhesion, long-life durability, mechanical stability, and the ability to be patterned and also to exhibit full biocompatibility. UU’s future objectives to research suitable thin-film coatings and processes with relevant characterization techniques that will permit the development of long-life in vivo sensor devices. This involves understanding the bioresponse of the body to various forms of thin-film and plasma surface modification processes. Also the thin-film sensing materials of interest such as platinum, gold, iridium/oxide, titanium, and various polymers require characterization. Cell and platelet growth studies will be correlated with surface science studies in order to develop optimal plasma modification-or deposition-based processes.

Applications of Nanodiagnosics

Applications of nanotechnologies in clinical diagnostics have been reviewed recently (Jain 2005b). Although applications of individual technologies are mentioned in the preceding section, some important areas of clinical application will be identified here.

Nanotechnology for Detection of Biomarkers

A biomarker is a characteristic that can be objectively measured and evaluated as an indicator of a physiological as well as a pathological process or pharmacological response to a therapeutic intervention. Classical biomarkers are measurable alterations in blood pressure, blood lactate levels following exercise, and blood glucose in diabetes mellitus. Any specific molecular alteration of a cell on DNA, RNA, metabolite, or protein level can be referred to as a molecular biomarker. From a practical point of view, the biomarker would specifically and sensitively reflect a disease state and could be used for diagnosis as well as for disease monitoring during and following therapy. Currently available molecular diagnostic technologies have been used to detect biomarkers of various diseases such as cancer, metabolic disorders, infections, and diseases of the central nervous system (CNS). Nanotechnology has further refined the detection of biomarkers. Some biomarkers also form the basis of innovative molecular diagnostic tests.

One project in this area draws together the expertise of a team of researchers from the Australian Institute for Bioengineering and Nanotechnology at The University of Queensland (UQ), the Fred Hutchinson Cancer Research Center (Seattle, WA), and the Seattle Biomedical Research Institute. This project is supported by a contribution of $2 million from the Queensland State Government through the National and International Research Alliances Program. In addition to Alliances funding, the project will receive support from the participating institutes and UQ spin-off company Nanomics Biosystems (Brisbane, Australia).
DNA Y-junctions have been used as fluorescent scaffolds for EcoRII methyltransferase–thioredoxin fusion proteins and covalent links were formed between the DNA scaffold and the methyltransferase at preselected sites on the scaffold containing 5FdC (Singer and Smith 2006). The resulting thioredoxin-targeted nanodevice was found to bind selectively to certain cell lines but not to others. The fusion protein was constructed so as to permit proteolytic cleavage of the thioredoxin peptide from the nanodevice. Proteolysis with thrombin or enterokinase effectively removed the thioredoxin peptide from the nanodevice and extinguished cell line-specific binding measured by fluorescence. Potential applications for devices of this type include the ability of the fused protein to selectively target the nanodevice to certain tumor cell lines, suggesting that this approach can be used to probe cell surface receptors as biomarkers of cancer and may serve as an adjunct to immunohistochemical methods in tumor classification.

**Perfluorocarbon Nanoparticles to Track Therapeutic Cells In Vivo**

Using perfluorocarbon nanoparticles 200 nm in size to label endothelial progenitor cells taken from human umbilical cord blood (UCB) enables their detection by MRI in vivo following administration (Partlow et al 2007). The MRI scanner can be tuned to the specific frequency of the fluorine compound in the nanoparticles, and only the nanoparticle-containing cells are visible in the scan. This eliminates any background signal, which often interferes with medical imaging. Moreover, the lack of interference means one can measure very low amounts of the labeled cells and closely estimate their number by the brightness of the image. Since several perfluorocarbon compounds are available, different types of cells potentially could be labeled with different compounds, injected, and then detected separately by tuning the MRI scanner to each one’s individual frequency. This technology offers significant advantages over other cell-labeling technologies in development. Laboratory tests showed that the cells retained their usual surface markers and that they were still functional after the labeling process. The labeled cells were shown to migrate to and incorporate into blood vessels forming around tumors in mice. These nanoparticles could soon enable researchers and physicians to directly track cells used in medical treatments using unique signatures from the ingested nanoparticle beacons. They could prove useful for monitoring tumors and diagnosing as well as treating cardiovascular problems.

**Monitoring of Implanted Neural Stem Cells Labeled with Nanoparticles**

Noninvasive monitoring of stem cells, using high-resolution molecular imaging, will be important for improving clinical neural transplantation strategies. Labeling of human neural stem cells (NSCs) grown as neurospheres with magnetic nanoparticles
was shown to not adversely affect survival, migration, and differentiation or alter neuronal electrophysiological characteristics (Guzman et al 2007). Using MRI, the authors demonstrated that human NSCs transplanted to the neonatal, the adult, or the injured rodent brain respond to cues characteristic for the ambient microenvironment, resulting in distinct migration patterns. Nanoparticle-labeled human NSCs survive long term and differentiate in a site-specific manner identical to that seen for transplants of unlabeled cells. The impact of graft location on cell migration and MRI characteristics of graft cell death and subsequent clearance were also described. Knowledge of migration patterns and implementation of noninvasive stem cell tracking might help to improve the design of future clinical NSC transplantation.

### Nanobiotechnologies for Single-Molecule Detection

Various nanobiotechnologies for single-molecule detection are listed in Table 3.2. These have been described in preceding sections.

**Protease-Activated Quantum Dot Probes**

QDs have been programmed to glow in presence of enzyme activity and give off NIR light only when activated by specific proteases (Chang et al 2005). Altered expression of particular proteases is a common hallmark of cancer, atherosclerosis, and many other diseases. NIR light also passes harmlessly through skin, muscle, and cartilage, so the new probes could detect tumors and other diseases at sites deep in

**Table 3.2 Nanobiotechnologies for single-molecule detection**

| **Visualization of biomolecules by near-nanoscale microscopy** |
|---------------------------------------------------------------|
| Atomic force microscope                                      |
| Scanning probe microscope                                    |
| 3D single-molecular imaging by nanotechnology                |
| Near-field scanning optical microscope                       |
| Spectrally resolved fluorescence lifetime imaging microscopy |

**Nanolaser spectroscopy for detection of cancer in single cells**

**Nanoproteomics**

Study of protein expression at the single-molecule level
Detection of a single molecule of protein

**Erenna™ Bioassay System: digital single-molecule detection platform**

**Nanofluidic/nanoarray devices: detection of a single molecule of DNA**

**Carbon nanotube transistors for genetic screening**

**Nanopore technology**

**Portable nanocantilever system for diagnosis**

**Nanobiosensors**

Quantum-dots-FRET nanosensors for single-molecule detection

Source: Jain PharmaBiotech.
the body without the need for a biopsy or invasive surgery. The probe’s design makes use of a technique called “quenching” that involves tethering a gold nanoparticle to the QD to inhibit luminescence. The tether, a peptide sequence measuring only a few nanometers, holds the gold close enough to prevent the QD from giving off its light. The peptide tether used is one that is cleaved by the enzyme collagenase. The luminescence of the QDs is cut by >70% when they are attached to the gold particles. They remain dark until the nanostructures were exposed to collagenase after which the luminescence steadily returns. The ultimate aim of the research is to pair a series of QDs, each with a unique NIR optical signature, to an index of linker proteases. This probe would be important for understanding and monitoring the efficacy of therapeutic interventions, including the growing class of drugs that act as protease inhibitors. An important feature of the protease imaging probes described in this study is the combination of the contrast enhancement achievable through a probe that can be activated and is combined with the brightness, photostability, and tunability of QDs.

**Nanotechnology for Point-of-Care Diagnostics**

POC or near-patient testing means that diagnosis is performed in the doctor’s office or at the bedside in case of hospitalized patients or in the field for several other indications including screening of populations for genetic disorders and cancer. POC involves analytical patient testing activities provided within the healthcare system, but performed outside the physical facilities of the clinical laboratories. POC does not require permanent dedicated space but includes kits and instruments, which are either hand-carried or transported to the vicinity of the patient for immediate testing at that site. The patients may even conduct the tests. After the laboratory and the emergency room, the most important application of molecular diagnostics is estimated to be at the POC. Nanotechnology would be another means of integrating diagnostics with therapeutics. Nanotechnology-based diagnostics provides the means to monitor drugs administered by nanoparticle carriers.

**Nanoprobes for POC Diagnosis**

A number of devices based on nanotechnology are among those with potential applications in POC testing. Researchers from Northwestern University’s Institute for Nanotechnology (Evanston, IL) describe a new method of DNA detection that uses gold nanoparticle probes and microarrays of electrodes (Park et al 2002). It is 10 times more sensitive (causing fewer false negatives) and 100,000 times more selective (causing far fewer false positives) than current methods. The nanoprobes are coated with a synthesized string of nucleotides that complement one end of a target sequence in the sample being analyzed, so they can “grab” it if it is there. Another set of nucleotides, complementing the other end of the target, is attached to a surface between two electrodes. If the target sequence is present in the sample, it attaches to both the nanoprobes and the sequences on the surface between the electrodes,
so that the nanoprobes are anchored to the surface like a cluster of little balloons. When they are treated with a silver solution, they create a bridge between the electrodes and produce a charge. The technology could theoretically be used to detect any disease or condition with a unique genomic fingerprint. For example, it could differentiate between various antibiotic-resistant strains of streptococci, or detect cancerous cells, or quickly identify HIV or biological warfare agents like anthrax. A single chip could contain electrode pairs to test for thousands of biological targets at once. And because an electrical charge is either present or absent, there is no ambiguity in the results. Nanosphere Inc’s Verigene™ platform will be suitable for the development of POC testing.

**Carbon Nanotube Transistors for Genetic Screening**

Carbon nanotube network FETs (NTNFETs) have been reported that function as selective detectors of DNA immobilization and hybridization (Star et al 2006). NTNFETs with immobilized synthetic ODNs have been shown to specifically recognize target DNA sequences, including H63D SNP discrimination in the HFE gene, responsible for hereditary hemochromatosis, a disease in which too much iron accumulates in body tissues. The electronic responses of NTNFETs upon ssDNA immobilization and subsequent DNA hybridization events were confirmed by using fluorescence-labeled ODNs and then were further explored for label-free DNA detection at picomolar to micromolar concentrations. A strong effect of DNA counterions on the electronic response was observed, suggesting a charge-based mechanism of DNA detection using NTNFET devices. Implementation of label-free electronic detection assays using NTNFETs constitutes an important step toward low-cost, low-complexity, highly sensitive, and accurate molecular diagnostics. Label-free electronic detection of DNA has several advantages over state-of-the-art optical techniques, including cost, time, and simplicity. The sensitivity of the new device is good enough to detect a single-base mutation in an amount of DNA present in 1 ml of blood. This technology can bring to market handheld POC devices for genetic screening, as opposed to laboratory methods using labor-intense labeling and sophisticated optical equipment. This device will be commercially developed by Nanomix Inc (Emeryville, CA, USA).

**Nanocytometer**

The nanocytometer is a pocket-sized device based on “pore-on-a-chip” technology that can rapidly identify diseases by testing a single drop of blood using an inexpensive disposable cartridge. The cartridges contain a silicon chip laden with artificial nanopores that mimic the filtration system of human cells. The nanocytometer enables work at the intersection of a number of disciplines, from biology and mechanical engineering to solid-state physics and chemical engineering (Carbonaro et al 2006). The tool has the potential to boost survival chances for leukemia, prostate cancer, or breast cancer patients, particularly in patients where
the cancer has recurred by offering early detection of rare, isolated cancer cells. The device is currently in the pipeline for commercial development.

**Nanodiagnostics for the Battle Field**

Researchers at MIT’s Institute for Soldier Nanotechnologies have taken a major step toward making an existing miniature lab-on-a-chip fully portable, so the tiny device can perform hundreds of chemical experiments in any setting including the battlefield. This will make testing soldiers to see if they have been exposed to biological or chemical weapons much faster and easier. Neither of the previous approaches, mechanically force fluid through microchannels or capillary electro-osmosis, offers portability. Within the lab-on-a-chip, biological fluids such as blood are pumped through channels ~ 10 μm wide. Each channel has its own pumps, which direct the fluids to certain areas of the chip, so they can be tested for the presence of specific molecules. In the new system, known as a 3D AC electro-osmotic pump, tiny electrodes with raised steps generate opposing slip velocities at different heights, which combine to push the fluid in one direction, like a conveyor belt. Simulations predict a dramatic improvement in flow rate, by almost a factor of 20, so that fast (mm/s) flows, comparable to that of pressure-driven systems, can be attained with battery voltages.

If exposure to biological or chemical weapons is suspected, the device can automatically and rapidly test a miniscule blood sample, rather than sending a large sample to a laboratory and waiting for the results. The chips are so small and cheap to make that they could be designed to be disposable, or they could be made implantable.

**Nanodiagnostics for Integrating Diagnostics with Therapeutics**

Molecular diagnostics is an important component of personalized medicine. Improvement of diagnostics by nanotechnology has a positive impact on personalized medicine. Nanotechnology has potential advantages in applications in POC diagnosis: on patient’s bedside, self-diagnostics for use in the home and integration of diagnostics with therapeutics. All of these will facilitate the development of personalized medicines.

Scientists at the University of Texas Medical Branch (Galveston, TX) are developing nanoparticles with bound thioaptamers for diagnostics and therapeutics in biodefense. RNA and DNA ODNs can act as “aptamers,” (i.e., as direct in vivo binders selected from large combinatorial libraries) for a number of proteins. Both in vitro enzymatic combinatorial selection and split-synthesis bead-based chemical combinatorial methods have been developed to identify phosphorothioate-modified oligonucleotide “thioaptamers” to a number of different infectious disease targets for detection, diagnostics, and therapeutics. Importantly, it has been noted that
sulfurization of the phosphoryl oxygens of ODNs often leads to their enhanced binding to numerous proteins. Monothiophosphate and dithiophosphate backbone-modified thioaptamers bind to proteins involved in the immune response, as well as to other proteins of the proteome. Bead-based high-throughput screening of thioaptamer bead libraries is used to select thioaptamers for the development of a thioaptamer as well as thioaptamer-gold nanoparticle-based proteomics arrays to identify and quantify toxins, viruses, proteins, and protein complexes relating to biodefense. Furthermore, selected thioaptamers delivered with liposomal nanoparticles can modulate the immune response and show promise as therapeutic agents targeting viruses such as West Nile virus and hemorrhagic fever arenaviruses. The lead thioaptamer, R12-2, shows specific binding to HIV-1 RT and inhibits the RNase H activity of intact HIV-1 RT. Suppression of virus was comparable with that seen with AZT (Somasunderam et al 2005).

Concluding Remarks About Nanodiagnosticstics

It is now obvious that direct analysis of DNA and protein could dramatically improve speed, accuracy, and sensitivity over conventional molecular diagnostic methods. Since DNA, RNA, protein, and their functional subcellular scaffolds and compartments are in the nanometer scale, the potential of single-molecule analysis approach would not be fully realized without the help of nanobiotechnology. Advances in nanotechnology are providing nanofabricated devices that are small, sensitive, and inexpensive enough to facilitate direct observation, manipulation, and analysis of single biological molecule from single cell. This opens new opportunities and provides powerful tools in the fields such as genomics, proteomics, molecular diagnostics, and high-throughput screening. A review of articles published over the past 10 years investigating the use of QDs, gold nanoparticles, cantilevers, and other nanotechnologies concluded that nanodiagnostics promise increased sensitivity, multiplexing capabilities, and reduced cost for many diagnostic applications as well as intracellular imaging (Azzazy et al 2006). Further work is needed to fully optimize these diagnostic nanotechnologies for clinical laboratory setting and to address the potential health and environmental risks related to QDs.

Various nanodiagnostics that have been reviewed will improve the sensitivity and extend the present limits of molecular diagnostics. Numerous nanodevices and nanosystems for sequencing single molecules of DNA are feasible. It seems quite likely that there will be numerous applications of inorganic nanostructures in biology and medicine as biomarkers. Given the inherent nanoscale of receptors, pores, and other functional components of living cells, the detailed monitoring and analysis of these components will be made possible by the development of a new class of nanoscale probes. Biological tests measuring the presence or activity of selected substances become quicker, more sensitive, and more flexible when certain nanoscale particles are put to work as tags or labels. Nanoparticles are the most versatile material for developing diagnostics.
Nanomaterials can be assembled into massively parallel arrays at much higher densities than is achievable with current sensor array platforms and in a format compatible with current microfluidic systems. Currently, QD technology is the most widely employed nanotechnology for diagnostic developments. Among the recently emerging technologies, cantilevers are the most promising. This technology complements and extends current DNA and protein microarray methods, because nanomechanical detection requires no labels, optical excitation, or external probes and is rapid, highly specific, sensitive, and portable. This will have applications in genomic analysis, proteomics, and molecular diagnostics. Nanosensors are promising for detection of bioterrorism agents that are not detectable with current molecular diagnostic technologies and some have already been developed.

**Future Prospects of Nanodiagnostics**

Within the next decade, measurement devices based on nanotechnology, which can make thousands of measurements very rapidly and very inexpensively, will become available. The most common clinical diagnostic application will be blood protein analysis. Blood in systemic circulation reflects the state of health or disease of most organs. Therefore, detection of blood molecular fingerprints will provide a sensitive assessment of health and disease. Another important area of application will be cancer diagnostics. Molecular diagnosis of cancer including genetic profiling would be widely used by the year 2015. Nanobiotechnology would play an important part, not only in cancer diagnosis but also in linking diagnosis with treatment.

In the near future, nanodiagnostics would reduce the waiting time for the test results. For example, the patients with sexually transmitted diseases could give the urine sample when they first arrive at the outpatient clinic or physician’s practice; the results could then be ready by the time they go in to see the doctor. They could then be given the prescription immediately, reducing the length of time worrying for the patient and making the whole process cheaper.

Future trends in diagnostics will continue in miniaturization of biochip technology to nano range. The trend will be to build the diagnostic devices from bottom up starting with the smallest building blocks. Whether interest and application of nanomechanical detection will hold in the long range remains to be seen. Another trend is to move away from fluorescent labeling as miniaturization reduces the signal intensity, but there have been some improvements making fluorescent viable with nanoparticles.

Molecular electronics and nanoscale chemical sensors will enable the construction microscopic sensors capable of detecting patterns of chemicals in a fluid. Information from a large number of such devices flowing passively in the bloodstream allows estimates of the properties of tiny chemical sources in a macroscopic tissue volume. Estimates of plausible device capabilities have been used to evaluate their performance for typical chemicals released into the blood by tissues in response to localized injury or infection (Hogg and Kuekes 2006). These indicate that the
devices can readily discriminate a single cell-sized chemical source from the background chemical concentration in vivo, providing high-resolution sensing in both time and space. With currently used methods for blood analysis, such a chemical source would be difficult to distinguish from background when diluted throughout the blood volume and withdrawn as a blood sample.